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Study of Drought Tolerance Responses in Root and Tuber Crops Using NIRS Technology and Biochemical Markers Model Assessment

DOCTORAL THESIS

Carla Susana Silva Gouveia
DOCTORATE IN BIOLOGICAL SCIENCES

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“Above all, don't fear difficult moments.
The best comes from them.”

Rita Levi Montalcini (1909-2012)
Nobel Prize in Physiology or Medicine, 1986

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Resumo

Estudo das respostas de tolerância à seca em culturas tuberosas usando a tecnologia NIRS e avaliação de modelos por marcadores bioquímicos

Batata-doce (*Ipomoea batatas* L.) e inhame (*Colocasia esculenta* L.) representam a segunda fonte alimentar mais importante do mundo. Atualmente, as alterações climáticas ameaçam a produtividade agrícola, principalmente devido à escassez de água, cujo efeito não é ainda suficientemente compreendido. As principais lacunas são a perceção do efeito da seca, através da eficiência no uso da água (WUE), bases bioquímicas e fisiológicas de produtividade e qualidade. Aqui, apresentamos os modelos das respostas de sete acessos de inhame e oito de batata-doce às condições de insuficiência hídrica, seguindo-se a avaliação da tolerância à seca, através da criação de modelos de previsão rápidos e precisos baseados na espectroscopia próxima do infravermelho (NIRS). Os resultados forneceram informações relevantes sobre o desenvolvimento destas culturas em ambiente de stress. A escassez de água conduziu a mecanismos de redução da perda de água, com diminuição da biomassa total (TPB) em todos os acessos. Respostas distintas na flexibilidade fenotípica foram explicadas pela razão parte tuberosa/parte vegetativa e variação do índice de stress no crescimento dos órgãos. Acessos com resposta mais favorável à seca melhoraram a WUE, com menor redução de TPB. Os parâmetros de composição isotópica de carbono ($\delta^{13}C$) e azoto ($\delta^{15}N$) atuaram como integradores fisiológicos de resposta ao stress ambiental. Maior teor de $\delta^{13}C$ representou maior fixação de carbono, e maior variação de $\delta^{15}N$ indicou realocação generalizada de azoto entre os órgãos sob stress. O teor de oxalatos relacionou-se significativamente com o amido, índice de clorofila e proteína. O desenvolvimento da técnica NIRS demonstrou ser uma excelente ferramenta para detetar as respostas destas culturas à seca, através de parâmetros de qualidade e fisiológicos, que poderá ser aplicada na investigação, pelos agricultores e empresas. Esta informação também auxiliará na seleção de características a serem utilizadas nos programas de melhoramento de inhame e batata-doce, para adaptação às mudanças climáticas.

Palavras-chave: Ácido abscísico, batata-doce (*Ipomoea batatas* (L.) Lam.), espectroscopia próxima do infravermelho, inhame (*Colocasia esculenta* (L.) Schott), integradores fisiológicos de seca, qualidade das culturas sob seca

Abstract

Sweet potato (*Ipomoea batatas* L.) and taro (*Colocasia esculenta* L.) represent the world's second most important food crop, as crucial carbohydrate source and good substitute for local imported cereals. Its productivity is currently threatened by climatic changes, mainly through the scarce of water availability, and affects tuber crops in a way that's insufficiently understood. Major gaps are understanding how drought affects the sweet potato and taro whole-plants, throughout their water use efficiency (WUE), biochemical and physiological basis of productivity and quality. Here, we show seven taro and eight sweet potato accessions responses to water insufficiency conditions, following with the accurate models' creation for drought tolerance assessment, through the rapid and precise prediction models based on Near-Infrared Spectroscopy (NIRS). Therefore, the results have provided new insights and relevant information about the taro and sweet potato whole-plant development under scarcity environment. The water shortage led to a drought avoidance response, with total plant biomass (TPB) loss in all accessions. Distinct phenotypic flexibility was also explained by the root-to-shoot ratio and stress index variation of the organs' growth. Accessions with more favorable response to drought had improved WUE, with higher TPB. The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition traits acted as physiological integrators of response to environmental stress. The ones with heaviest $\delta^{13}C$-values shown a greater carbon fixation, and $\delta^{15}N$ variation indicating a generalized nitrogen reallocation between whole-plant organs under drought. The oxalate content was significantly related to starch content, chlorophyll content index, and protein. NIRS demonstrated to be an excellent tool to detect the crops drought responses, through physiological and quality traits, that could be applied for research purposes, or by farmers and agriculture companies. The information generated could also aid the selection of traits to be used in taro and sweet potato breeding programs, to adapt them to climate change.

Keywords: Abscisic acid, drought physiological integrators, near-infrared spectroscopy, quality of drought-stressed crops, sweet potato (*Ipomoea batatas* (L.) Lam.), taro (*Colocasia esculenta* (L.) Schott)

Abbreviations

ABA – abscisic acid
Acc. – accession number
ADF – acid-detergent fiber
ANOVA – analysis of variance
CAN – Canary Islands
CaOx – calcium oxalate
CCI – chlorophyll content index
CK – phytohormone cytokinin
DF – dietary fiber
DHNs – dehydrins or group II LEA proteins
DW – dry weight basis
$\Delta^{13}C$ – carbon isotope discrimination
E – nitrogen efficiency of utilization
ELISA – enzyme-linked immunosorbent assay
Fb – crude fiber
FW – fresh weight
$\delta^{13}C$ – carbon isotopic composition
$\delta^{15}N$ – nitrogen isotopic composition
GUI – Guinea-Bissau
H – Mahalanobis distance values
KOx – potassium oxalate
M – total mineral
MAD – Madeira Island
MSC – Multiplicative Scatter Correction
N – nitrogen
NDF – neutral-detergent fiber
NER – nitrogen efficiency ratio
NHI – nitrogen harvest index
NIR-HSI – near-infrared hyperspectral imaging
NIRS – near-infrared spectroscopy
NRA – nitrate reductase activity
OA – oxalic acid
Pt – protein
PAR – photosynthetic active radiation
PCA – principal component analysis
PCR – principal component regression
PLS – partial least squares regression model
R – isotope (abundance) ratios
r^2 – determination coefficient
ROS – reactive oxygen species
RPD – ratio of performance to deviation, with RPD = SD/SECV
R:S – root-to-shoot ratio
RuBisCo – ribulose-1,5-bisphosphate carboxylase/oxygenase
St – starch
SD – standard deviation
SEC – standard error of calibration
SECV – standard error of cross-validation
SEP – standard error of prediction
SI – whole-plant stress index
SNV – standard normal variate
S-Ox – soluble oxalates
SPC – South Pacific community
SSP – starch swelling power
SWS – starch solubility in water
T – cross-validation residuals
TC – total carbon
T-Ox – total oxalates
TPB – total plant biomass
WP – whole-plant
WUE – water use efficiency

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Thesis Publications

- **CHAPTER 2: Gouveia, C.S.S.**, Ganança, J.F.T., de Nóbrega, H.G.M., de Freitas, J.G.R., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Phenotypic flexibility and drought avoidance in taro (*Colocasia esculenta* (L.) Schott). *Emirates Journal of Food and Agriculture*, 32 (2): 150–159 (2020).
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- **CHAPTER 3: Gouveia, C.S.S.**, Ganança, J.F.T, Lebot, V. and Pinheiro de Carvalho, M.Â.A. Quantitation of oxalates in corms and shoots of *Colocasia esculenta* (L.) Schott under drought conditions. *Acta Physiologiae Plantarum*, 40 (214): 1–11 (2018).
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- **CHAPTER 4: Gouveia, C.S.S**., Ganança, J.F.T, Slaski, J., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) and carbon-water relations as drought stress mechanism response of taro (*Colocasia esculenta* L. Schott). *Journal of Plant Physiology*, 232: 100–106 (2019).
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- **CHAPTER 5: Gouveia, C.S.S.**, Ganança, J.F.T., Slaski, J., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Involvement of abscisic acid and other stress indicators in taro (*Colocasia esculenta* (L.) Schott) response to drought conditions. Acta Physiologiae Plantarum, 42 (173): 1–11 (2020).
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- **CHAPTER 6: Gouveia, C.S.S.**, Ganança, J.F.T., de Nóbrega, H.G.M., de Freitas, J.G.R., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Drought avoidance and phenotypic flexibility of sweet potato (*Ipomoea batatas* (L.) Lam.) under water scarcity conditions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47 (4): 1037–1046 (2019).
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- **CHAPTER 7: Gouveia, C.S.S.**, Ganança, J.F.T, Lebot, V. and Pinheiro de Carvalho, M.Â.A. Changes in oxalate composition and other nutritive traits in root tubers and shoots of sweet potato (*Ipomoea batatas* L. [Lam.]) under water stress. *Journal of the Science of Food and Agriculture*, 100: 1702–1710 (2020).
  DOI: 10.1002/jsfa.10185 [JIF (2019) = 2.614]

- **CHAPTER 8: Gouveia, C.S.S**., Ganança, J.F.T, Slaski, J., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Variation of carbon and isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) of whole-plant sweet potato (*Ipomoea batatas* L.) subjected to prolonged water stress. *Journal of Plant Physiology*, 243: 153052 (2019).
  DOI: 10.1016/j.jplph.2019.153052 [JIF (2019) = 3.013]

- **CHAPTER 9: Gouveia, C.S.S.**, Ganança, J.F.T., Slaski, J., Lebot, V. and Pinheiro de Carvalho, M.Â.A. Abscisic acid phytohormone estimation in tubers and shoots of *Ipomoea batatas* subjected to drought stress using competitive immunological assay. *Physiologia Plantarum*, advanced online publication (2020).
  DOI: 10.1111/ppl.13192 [JIF (2019) = 4.148]

- **CHAPTER 10: Gouveia, C.S.S.**, Lebot, V. and Pinheiro de Carvalho, M.Â.A. NIRS estimation of drought stress on chemical quality constituents of taro (*Colocasia esculenta* L.) and sweet potato (*Ipomoea batatas* L.) flours. *Applied Sciences*, 10: 8724 (2020).
  DOI: 10.3390/app10238724 [JIF (2019) = 2.474]

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CHAPTER 1

General Introduction

The general introduction explains the taro (*Colocasia esculenta* (L.) Schott) and the sweet potato (*Ipomoea batatas* (L.) Lam.) importance as food and nutritional security. We followed the plant problems associated with drought stress, such as lack of productivity, change of quality, and the drought avoidance and phenotypic flexibility response to drought. This evolves the analysis of nutritional and biochemical traits in the plant response to drought, as well as the use of Near-Infrared Spectroscopy (NIRS) as a rapid and reliable technique for quality traits prediction in plant material. The aims and outline of the present doctoral dissertation were also addressed in this chapter.

1.1 Plant Material

1.1.1 Taro (*Colocasia esculenta* (L.) Schott)

Tropical tuber crops contribute significantly for food and nutritional security, being the second-most important group of cultivated species after cereals (Sharma and Kaushal 2016).

Taro, *Colocasia esculenta* (L.) Schott, belongs to the Araceae family. The Araceae is composed by 117 plant genera, that includes 8,106 scientific plant names, of those 3,368 are accepted species. The genus *Colocasia* contains a total of 99 species, of those 8 species have the accepted status (8.08%) (The Plant List 2013).

Taro (*Colocasia esculenta*, var. *esculenta*, dasheen) is an herbaceous plant, with vegetative propagation. The taro's plant height usually ranges between 0.5–1.5 m tall (Figure 1.1A) and owns a large cylindrical central corm (corm tuber) (Figure 1.1B). This crop is originated from the Indo-Malayan region (or crop domestication center) and is probably one of the oldest crops on earth, grown on irrigated terraces in tropical Asia for more than 10,000 years (Sharma and Kaushal 2016; Singh et al. 2016; Lebot 2009).

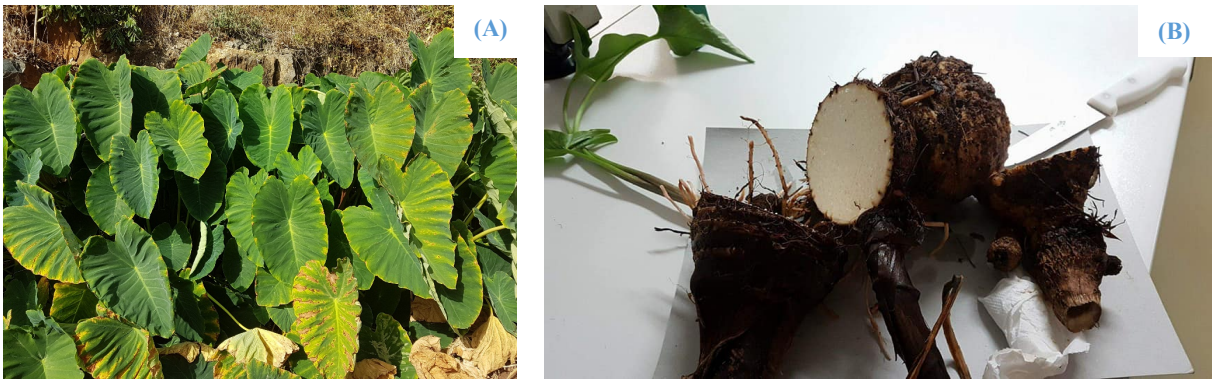

Figure 1.1
Taro plants at Preces (Câmara de Lobos, Madeira, Portugal) (A), and detail of a cross section of the taro corm (B). Photos by: ISOPlexis Genebank, University of Madeira.

In 1832 was published the key species descriptors for taro, in "*Colocasia esculenta* (Linnaeus) Schott in Schott & Endlicher, *Melet. Bot.* 18 (1832)", with the following content:

"Rhizome vertical to horizontal, tuberous, 3–5 cm or more (up to 15 cm) in diam. Stolons long or absent. Leaves 2 or 3 or more; petiole green, 25–80 cm, sheathing for 1/3–2/3 length; leaf blade adaxially matte waxy-glaucous and water-shedding (water sometimes forming "mercury droplets"), oblong-ovate to suborbicular, 13–45 × 10–35 cm, base shallowly cordate (sinus 1–4 cm), apex broadly and shortly cuspidate. Peduncle usually solitary, 16–26 cm. Spathe tube green, 3.5–5 × 1.2–1.5 cm; limb open proximally, cream-colored to golden yellow, lanceolate or elliptic, 10–19 × 2–5 cm, apex acuminate. Spadix: female zone conic, 3–3.5 × ca. 1.2 cm; ovary 1–3 mm in diam.; stigma subsessile, narrower than apex of ovary; sterile zone narrowly cylindric, 3–3.3 cm; sterile flowers (pistils) seen from above elongate, ca. 0.5 mm in diam.; male zone cylindric, 4–6.5 cm × ca. 7 mm; appendix narrowly conic, 15–45 × ca. 2 mm. Berry green, ca. 4 mm. Seeds few; synandria ca. 1 mm high, ca. 0.8 mm in diam. Fl. Feb–Apr (Yunnan), or Aug–Sep (Qin Ling area). 2n = 26, 28, 30, 36, 38, 42, 44, 46, 48, 52, 58, 84, 116."

The International Plant Genetic Resources Institute (IPGRI) published the key morphological descriptors of taro, which are currently used to aid the selection of the agronomical features that describe efficiently each taro genetic resources, supported by the Consultative Group on International Agricultural Research (CGIAR) (IPGRI 1999).

The worldwide taro production increased from 9.46 Mt in 2010 to 10.14 Mt in 2015. In 2015, Africa registered almost 72% of worldwide production in taro production, near 7.34 Mt (FAOSTAT 2016). It is extensively cultivated mostly near farmhouses or even in water fields. The corms, petioles, and inflorescences are used as a vegetable, as an important source of food by local populations and feed for livestock. In developing countries, the taro corms were also used for medicinal treatments, such as swellings, abscesses, snake and insect bites, and swollen lymph nodes in the neck (Fang and Staples 1995).

1.1.2 Sweet Potato (*Ipomoea batatas* (L.) Lam.)

Possibly originated from Central/South America, the sweet potato has been cultivated at least for 5,000 years (Chaïr et al. 2016; Mohan et al. 2016; Srisuwan et al. 2006). It has been transferred from the tropical America accompanying the migrations of Polynesian to Oceania (kumara line) in pre-Columbian age, and later with Spanish travelers to Europe and Philippines (camote line), and with Portuguese explorers from Brazil and Mediterranean to Africa and India (Montenegro et al. 2008; Zhang et al. 2000). This crop shows an enormous capability of adaptation to a wide range of agrosystems and environmental conditions. Thus, co-exists three major crop gene pools, corresponding to the continent of origin (American gene pool) and Polynesian gene pool (the kumara line), the African and Asian gene pool (the batata line), and one smaller gene pool for Canary Islands and Philippines (the camote line) (Zhang et al. 2000).

Sweet potato belongs to the Convolvulaceae family and is scientifically known as *Ipomoea batatas* (L.) Lam. (1793). The Convolvulaceae contains 67 plant genera, including 6,014 plant names, of those 1,296 are accepted species names. The genus *Ipomoea* contains a total of 1,901 species, of those 468 species have the accepted status (24.62%) (The Plant List 2013).

In 1793 was described the key species descriptors for sweet potato, in "*Ipomoea batatas* (Linnaeus) Lamarck, *Encycl.* 1: 465 (1793)", with the following quote:

"*Herbs annual, with ellipsoid, fusiform, or elongated subterranean tubers; sap milky; axial parts glabrous or pilose. Stems prostrate or ascending, rarely twining, green or purplish, much branched, rooting at nodes. Petiole 2.5–20 cm; leaf blade broadly ovate to circular, 4–13 × 3–13 cm, margin entire or palmately 3–5(–7)-lobed, herbaceous; lobes broadly ovate to linear-lanceolate, sparsely pilose or glabrous. Inflorescences 1- or 3–7-flowered; peduncle 2–10.5 cm, stout, angular; bracts early deciduous, lanceolate, 2–4 mm. Pedicel 2–10 mm. Sepals oblong or elliptic, ± unequal, glabrous or pilose abaxially, margin ciliate, apex acute, mucronulate, outer 2 sepals 7–10 mm, inner 3 sepals 8–11 mm. Corolla pink, white, pale purple to purple, with a darker center, campanulate to funnelform, 3–4 cm, glabrous. Stamens included. Pistil included; ovary pubescent or glabrous. Capsule rarely produced, ovoid or de-pressed globose. Seeds glabrous. 2n = 84, 90*."

Nowadays, the list of sweet potato crop descriptors performed by the CIP/AVRDC/IBPGR consortium has been used to help the selection of the key morphological descriptors that agronomically describes effectively each accession (CIP/AVRDC/IBPGR 1991). The International Potato Center (CIP) is a scientific, autonomous, and non-profit institution devoted to improving and spread knowledge for the better use of tuber and root crops as basic foods in the developing world. The Asian Vegetable Research and Development Center (AVRDC) is an international center established in 1971, with the main objective to promote the research and development of vegetative crops in the humid and sub-humid tropics. The International Board for Plant Genetic Resources (IBPGR) is an international scientific organization, with the main focus to promote and coordinate an international network of genetic resources, contributing to the living and welfare of people throughout the world (CIP/AVRDC/IBPGR 1991).

As a brief description, the sweet potato is a vine-like perennial herb, having as main physiological characteristics long surface stems (ranging from 1 to 5 m) (Figure 1.2A), with nodes that produce storage roots

(root tubers) when in contact with the soil. The tubers can offer a range of diverse skin and flesh color (Figures 1.2B and 1.2C), from white to purple (Singh et al 2016; Sharma and Kaushal 2016; Lebot 2009).

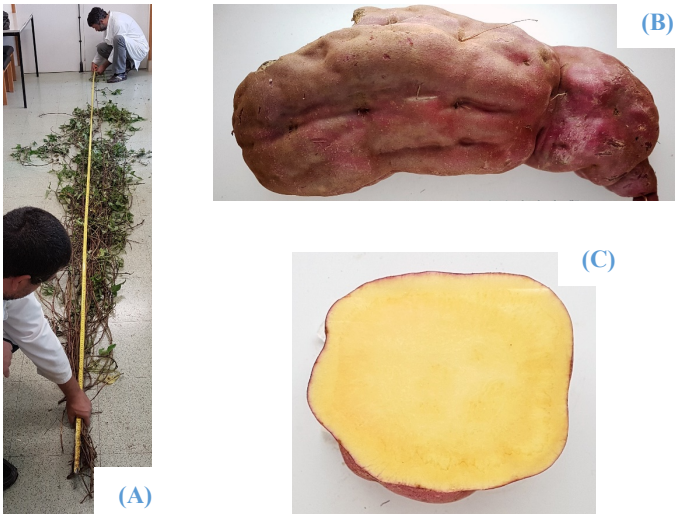

Figure 1.2
Measurement of the sweet potato stems (A). Example of a sweet potato storage root with red skin (B) and dark yellow flesh (C).
Photos by: ISOPlexis Genebank, University of Madeira.

Cultivated worldwide, the sweet potato is a productive and adaptable crop. Its tubers are an important source of food, starch, and raw material for producing alcohol. The stems and leaves can be used as livestock forage (Fang and Staples 1995). The worldwide sweet potato production increased from 106.27 Mt in 2010 to 108.33 Mt in 2015. In 2015, Asia registered approximately 73% of sweet potato worldwide production with 78.95 Mt (FAOSTAT 2016).

1.2 Drought

Tropical tuber crops contribute significantly to nutritional security, with the main abiotic stresses (cold, heat, salinity, and drought) pressuring the survival, biomass production, and yields of these staple food crops, jeopardizing food security worldwide (Mantri et al. 2012). Climate change is expected to aggravate in the next 30-50 years, through more extreme occurrences in temperature and precipitation, increased evaporative demand of water, and arising of the carbon dioxide (CO_2), whose degree of impact will distinctly affect all agricultural areas worldwide (Hatfield 2014). To obtain optimal yields, the taro plant needs a very good water supply, so the water scarcity and severe weather events are expected to impact negatively this important subsistence crop (Ganança et al. 2015). The sweet potato can be moderately tolerant to drought conditions due to low plant growth habit and extensive root system, and its production is usually done well with relatively low inputs (Motsa et al. 2015a; Ekanayake and Collins 2004; Smittle et al. 1990). However, the most certain is that agriculturalists will have to adjust crop production to cope with the changing climate (Hatfield 2014).

The tolerance to the abiotic stress is quantitative and multigenic in nature, so the actual trend is to comprehend the key molecular mechanisms for advanced selective breeding purposes, conjugating the physiological, biochemical, and gene regulatory network knowledge to develop or select stress-tolerant and high-yielding crop cultivars, using the field cultural practices to mitigate drought abiotic stress (Mantri et al. 2012).

The crop biochemical composition, and consequently the quality of food products from crop plants

exposed to extended periods of stress conditions are affected due to physiological changes (Wang and Frei 2011). Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by evapotranspiration (Motsa et al. 2015b). The agronomic understanding is that the stress occurs due to the decrease of leaf water potentials into a point that occurs crop physiological damage and yield reduction (Oliver et al. 2010).

Drought changes the soil composition and structure, and thus affects nutrient cycling (Jaradat 2014). Since nitrogen (N) is a critical plant nutrient, the water stress can influence the soil supply of N, which is usually absorbed by the roots in whether ammonium or nitrate inorganic form, interfering mainly in the carbohydrate metabolism and nitrogen assimilation in plant roots and shoots organs (Goodger and Schachtman 2010). Other changes can occur when crop plants are subjected to stress, evolving the gas exchange and assimilates allocation, nutrient uptake and allocation, antioxidant effects, and changes in the enzyme activity and gene expression (Wang and Frei 2011). If plants are towards a slow water loss, it could allow the increment of cell-protective compounds (such as sugars, proteins, and antioxidants), through a slower regulation of their metabolism, thus lessening the increase of reactive oxygen species (ROS) production (Oliver et al. 2010). An additional important factor in agricultural production is the study of root-to-shoot (R:S) signaling, including the abscisic acid (ABA) phytohormone stress-signal, and the water use efficiency (WUE) of crop plants under drought (Goodger and Schachtman 2010; Rock et al. 2010).

Nonetheless, the main crop quality constituents contemplate the determination of the nitrogen-protein, carbohydrates (total sugars and starch), and total minerals content (Wang and Frei 2011). Other quality constituents, such as crude fiber, carbon, and nitrogen isotopic compositions are increasing in compound feeds and in plant tissues grown under non-stress conditions. These quality constituents usually are measured in biological samples by costly and length laboratory analysis, with the other fast and accurate technological resources usage is increasing, such as the Near-Infrared Spectroscopy (NIRS) technique (Kleinebecker et al. 2009; Lebot et al. 2009; Ferrio et al. 2001; Bruno-Soares et al. 1998; de Boever et al. 1995). Still, whether the quality constituents and the NIRS application in tropical tuber crops under drought are rather limited. Similarly, no studies are modeling the influence of drought stress on their quality constituents, considering both underground and aboveground organs. In the following introductory points, these parameters were addressed, by detailing the drought avoidance and phenotypic flexibility responses, the physiological and biochemical traits, and the NIRS technique, as important components to aid the evaluation of the impact of abiotic environmental stresses on crop quality, based in the available information and thus with the relevant contribution for this dissertation.

1.3 Drought Avoidance and Phenotypic Flexibility

1.3.1 Water Use Efficiency, Root-to-Shoot Ratio and Stress Index

Tolerant plants rely on morphological mechanisms to avoid biomass loss, such as drought avoidance and/or phenotypic flexibility, as natural protection against drought stress (Farooq et al. 2009).

The plant drought avoidance strategy consists of supporting the equilibrium between the water loss and the rate of water uptake through stomatal conductance and WUE maintenance (Oliver et al. 2010). The WUE is measured as the weight unit of dry biomass produced per weight unit of water consumed and gives the relationship between productivity and water use (Hatfield 2014; Mengel et al. 2001). WUE is also expressed as transpiration, evaporation, or total water input to the environment, given as the ratio of the crop CO_2 assimilation, total biomass, or gain yield to water consumption (Kaushik and Kumar 2014). The plants with improved WUE could be considered the most drought-tolerant when compared with drought-sensitive ones (Farooq et al. 2009). That is, the higher WUE could result in a slight increase in dry biomass production (biomass allocation), usually due to the presence of increasing CO_2 effects on stomatal conductance that

reduces the water loss through transpiration processes and increases the water conservation at the leaf level (Hatfield 2014; Mengel et al. 2001). Still, the WUE provides a form of evaluation on how climate change could impact crop production, for being critical about the availability of the soil water to the plant (Hatfield 2014).

In the case of continue water deficit, some plants will also try to increase the root growth, showing a higher root-to-shoot ratio (R:S), as a phenotypic flexibility strategy for adaptation to drought (Oliver et al. 2010). The increase of the R:S could be a good indication of the decline of the plant leaf growth in detriment of the improvement of the root growth and performance under water-limited conditions (Fageria and Moreira 2011; Hubick and Gibson 1993; Laureti et al. 1993; Farooq et al. 2009). This strategy is important due to the interdependency of shoot and root for plant growth and development, once the shoot depends on the root for water and nutrients, while the roots depend on the shoot for carbohydrates and biomolecules supply (Fageria and Moreira 2011). Even with an increased biomass allocation to roots as a strategy to cope with drought, the total plant biomass loss is still considerable under water scarcity conditions (Salehi-Lisar and Bakhshayeshan-Agdam 2016). This flexibility strategy could imply a stress effect on plant growth, evaluated through the stress index (SI) obtained by the dry biomass weight present in both stress and non-stress conditions. The lower the SI value, then the less is the difference between the plant biomass obtained in both environmental conditions, being a good indicator of plant resistance and growth capacity under stress conditions (Robinson et al. 2000).

The WUE was determined in potato (Obidiegwu et al. 2015) and sweet potato (van Heerden and Laurie 2008) under drought conditions, and also in sweet potato under nitrogen and potassium supplementation (Prabawardani and Suparno 2015). The R:S was applied in several works to seek the dry matter distribution between the roots and shoots of tomato plants supplied with nutrients (Claussen 2015), or even for the root functioning of cotton and tea plants subjected to drought stress (Farooq et al. 2009). The SI was also determined to seek the stress responses in wild barley plants (Robinson et al. 2000). Hitherto, this information is still scarce for tropical tuber crops.

1.4 Physiological traits

1.4.1 Photosynthesis, Stomatal Conductance and Isotopes of Carbon and Nitrogen

Stress conditions could lead to downregulation of the photosynthesis as a result of the stomatal closure to avoid water loss according to the leaf intracellular CO_2 availability. The decrease of photosynthesis leads to less excitation of photosystem II (PSII) by light photons, by lowering the number of ionized chlorophyll molecules (Salehi-Lisar and Bakhshayeshan-Agdam 2016; van Heerden and Laurie 2008). The photosynthetic rate decrease can be also associated with other factors, such as oxidative damage in chloroplasts through the photooxidation of chlorophyll, as a nonstomatal limitation when drought-stressed (Prasad et al. 2008). Another factor that can contribute to a reduced photosynthetic activity under stress is the downregulation of genes that are involved in chlorophyll biosynthesis as a mechanism to avoid the overhoarding of electrons delivered from the $NADP^+$ electron acceptors transport chain (Lau et al. 2018). The chlorophyll content index (CCI) could give the relative chlorophyll value that is proportional to the amount of chlorophyll in the leaf tissue, as a direct field method for plant health indicator applied by the agronomists. The leaf chlorophyll can be associated with photosynthetic capacity, with the canopy greenness usually related to the photosynthetic efficiency of the plant, according to Tiwari and Mamrutha (2013).

The atmospheric CO_2 also acts as a signaling molecule in stomatal responses, whereas increased CO_2 concentrations in leaf induce stomatal closure, leading to a reduction in the photosynthesis rate in leaf and consequently affecting the WUE (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Osakabe et al. 2014). The enhancement of photorespiration during drought could be essential for the plant growth regulation and survival when photosynthesis is down-regulated, helping to protect the chloroplasts from photoinhibition and

subsequent oxidative damage (Prasad et al. 2008; van Heerden and Laurie 2008; Igamberdiev et al. 2004; Igamberdiev et al. 2001). The photosynthesis and photorespiration occur only in green plant tissues, where the rate of photorespiration during daylight is usually lower than during the night (Igamberdiev et al. 2004). The decline of the leaf intracellular CO_2 availability during stomatal closure changes the carboxylase activity of RuBisCo (ribulose-1,5-bisphosphate carboxylase/oxygenase). As RuBisCo is also the key enzyme of photorespiration, its oxygenase function became active with the lowering of the internal CO_2/O_2 ratio. The photorespiration increases at low internal CO_2 concentrations, resulting from stomatal closure (decrease of photosynthesis rate), and decreases with an increase of internal CO_2 concentrations when gas exchange occurs freely through open stomata, during photosynthesis increase rate (Igamberdiev et al. 2004; Igamberdiev et al. 2001).

Crop plants with the Calvin-Benson photosynthetic pathway (C3), such as sweet potato and taro, converts the atmospheric CO_2 into plant biomass, through the efficient incorporation of carbon isotopes during photosynthesis, usually with carbon isotopic composition ($\delta^{13}C$) values ranging between -23‰ to -30‰ (Bayala et al. 2015; Lomax et al. 2012; Farquhar et al. 1982). The leaf $\delta^{13}C$ can be associated with stomatal aperture, photosynthesis carboxylation, and changes in WUE under water scarcity stress (Igamberdiev et al. 2004; Robinson et al. 2000; O'Leary 1993; Farquhar et al. 1989).

Plants with relatively open stomata show an increase of the photosynthesis activity with a RuBisCo oxygenase activity decreasing and improving the carboxylation fractionation due to an equilibrate CO_2 diffusion in leaf chloroplasts, reaching $\delta^{13}C$ values close to -38‰ through a higher ^{13}C depletion (O'Leary 1993). During drought, the photosynthesis activity reduction due to stomatal closure restricts the CO_2 uptake into leaves by decreasing CO_2 assimilation, reaching $\delta^{13}C$ values near -12‰ (O'Leary 1993). The $\delta^{13}C$ usually is converted into carbon discrimination ($\Delta^{13}C$) for field-grown plants to display positive isotopic discrimination values (Farquhar et al. 1989, Farquhar et al. 1982).

The nitrogen isotopic composition ($\delta^{15}N$) could also provide important insights regarding the nitrogen assimilation or dissimilation during drought (Robinson et al. 2000; Farquhar et al. 1989). The changes of $\delta^{15}N$ during a drought can indicate how plant genotypes retain nitrogen (N) in their tissues, where the more the ^{15}N-enriched plant show more positive $\delta^{15}N$ (Robinson 2001). Both $\delta^{13}C$ and $\delta^{15}N$ could act as physiological integrators to assess the plant responses to stress environments, including water scarcity (Robinson et al. 2000; Laureti et al. 1993).

Additionally, some works used the $\delta^{13}C$ values as a successful tool to access the WUE and yield stability in sunflower plants (Laureti et al. 1993), tomato, wheat, and rice (Obidiegwu et al. 2015). They observed that plant genotypes exhibiting higher dry biomass production, higher $\delta^{13}C$, lower $\Delta^{13}C$, and higher WUE values show the best drought tolerance response. Hitherto, there is no published information about the $\delta^{13}C$ and $\delta^{15}N$ serving as physiological indicators of taro and sweet potato drought response. However, one work used the $\Delta^{13}C$ to study the dry mass accumulation and allocation of one variety of sweet potato during drought stress (Zhang et al. 2015).

1.4.2 Abscisic Acid (ABA)

Abscisic acid (ABA) is an isoprenoid metabolite known as stress phytohormone, produced in the plastidial 2-C methyl-D-erythritol-4-phosphate pathway (Wani et al. 2016). ABA plays a fundamental role in plant adaptation to stress. It has been hypothesized that it acts as a long-distance stress signal between the root and the shoot plant organs (Rock et al. 2010). Considered one of the fastest responses of the plant to abiotic stress, the ABA synthesis can reach up to a 40-fold increase in just a few hours of drought, with the ability to fast decrease the ABA content after the water balance is restored (Peleg and Blumwald 2011; Rock et al. 2010).

The ABA increase content influences several plant physiological processes, such as water-saving antitranspirant activity with stomata closure and reduce leaf expansion, being generally considered a plant

growth inhibitor under stress conditions (Wani et al. 2016). The stomatal closure is induced due to an ABA root-to-shoot signal through the root detection of water availability decrease in the soil. The water status reduction leads to an ABA content increase in leaf guard cells to trigger stomatal closure (Osakabe et al. 2014). That is, the low water potential sensed by the roots contributes to ABA signaling, allows the water influx and ion flux into the root tissues, by regulating the turgor due to lower transpiration in the leaf area (Sah et al. 2016). Other approaches were made, repositioning the shoots as the primary ABA source synthesis, considering, in this case, the shoots as the first organ to sense drought, with the leaves being the predominant location for ABA biosynthesis during drought stress (McAdam et al. 2016). With this, ABA from leaves was considered to have a greater effect on root development, and even if there is no ABA synthesis in the underground organs, it was found that plants could have normal increases in foliar ABA level, with normal stomatal responses to drought (McAdam et al. 2016).

However, it was observed in non-stressed conditions, that the ABA changes in sweet potato accessions during tuber formation and thickening were positively correlated with early plant development (Nakatani and Komeichi 1991). The sweet potato leaf sugar and phytohormones (cytokinin (CK) or ABA) levels present in the process of tuberous root formation of sweet potato plays an important role in gene expression of D3-type Cyclins and ADP-Glucose Pyrophosphorylase genes regulation, and thus promoting the cell division activity (Nagata and Saitou 2009). However, the CK promotes the stomatal opening, acting as an antagonist to ABA, with CK levels usually being reduced with exposure to water stress conditions (Peleg and Blumwald 2011).

ABA can be quantitated by whether physical-chemical methods through mass spectrometry technique or by immunochemical methods that comprehend the utilization of radioisotopes (radioimmunoassay) or enzymes (enzyme-linked immunosorbent assay, ELISA) (Setter 2012). The immunochemical systems have been widely used for ABA quantification, with ELISA being used as a sensitive method for detecting low concentrations of this phytohormone (Huang et al. 2014). It is one of the most affordable techniques that allow fast detection of ABA without the need for complex purification steps and uses the combination of the specificity of Antigen-Antibody reaction with the sensitivity of enzymatic assays (Huang et al. 2014; Setter 2012). The sample reacts inside the microplate wells pre-coated with an antibody specific to ABA, added to Horseradish Peroxidase (HRP)-conjugated ABA. The competitive inhibition reaction occurs between HRP labeled ABA and unlabeled ABA with the antibody.

Still, only a few works investigated the effects of ABA on sweet potato cultivars grown in non-stress conditions, with absent data on how much this phytohormone variates under drought stress conditions. Those works were performed in the ABA determination and their relation to the weight of the tubers grown in fully irrigated pots (Nakatani and Komeichi 1991), in the inhibitory growth effects of ABA added to *in vitro* plantlets (Jarret and Gawel 1991), and in the sink activity through leaf-petiole cuttings from one accession grown in a growth chamber and treated with ABA solution (Nagata and Saitou 2009). Similarly, recent studies explored the effects of several concentration levels of ABA on *in vitro* grown taro plantlets (Acedo et al. 2017). However, very little is known about ABA in taro at the whole-plant level and even less about the role of ABA in taro's response to drought.

1.4.3 Nutrient Allocation

1.4.3.1 Mineral Nitrogen

Minerals are composed of at least 13 chemical inorganic elements found in the soil. These elements, when dissolved in water, allows the plant's uptake through its roots. Plants absorb minerals that are essential for their development and subsistence (Duman 2012; Hajiboland 2012). Plants are a good source for daily mineral intake for humans and animals, playing a key role in the structure of various hormones, biomolecules,

and enzymes in the body, although with some bioavailability limitation in the type of food ingested (Navarra 2004).

The macronutrients are the mineral nutrients that plants require in higher quantities for their biochemical and physiological processes and growth. These elements include nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur (Duman 2012). On the other hand, the micronutrients are required in lower amounts and are evolved directly in the plant metabolism (*e.g.*, component of an enzyme, or metabolic step such as enzyme reaction), consisting of elements such as copper, iron, manganese, molybdenum, zinc, etc. (Hajiboland 2012).

Minerals are essentially determined by incineration at 550ºC, which leads to the destruction of the organic compounds, leaving only the inorganic residue in the form of total ash (Katoch 2011). This ashing process leads to a loss of volatile mineral elements, such as selenium and iodine. Yet, it is generally used for the crude mineral content quantification without performing a mineral refined analysis, representing practically all the inorganic matter in the studied sample (Katoch 2011; deMan 1999).

Several works reported the determination of total mineral content through ashes in taro and sweet potato underground and aboveground organs, under non-stress conditions. The taro corms from the Philippines presented an ash content of 2.78 g/100g DW (Alcantara et al. 2013), from West African 4.07 g/100g DW (Amon et al. 2011), from Thailand 0.1 – 0.28 g/100g DW (Tattiyakul et al. 2007; Tattiyakul et al. 2006), from Ethiopia an average of 3.5 g/100g DW (Mulugeta and Tebeka 2017), and the Vanuatu germplasm collection showed 1.47 – 8.85 g/100g DW (Lebot et al. 2011a; Lebot et al. 2009). Meanwhile, the shoots ash content was also registered, with 10 g/100g DW for taro samples from Nigeria (Fai et al. 2013) and 9.8 g/100g DW from Cameroon (Ejoh et al. 1996).

Tubers of distinct sweet potato accessions from the Pacific showed an ash content range from 0.6 to 8.22 g/100g DW (Lebot et al. 2011b; Lebot 2009; Lebot et al. 2009), with the ones from Nigeria reaching between 1.30 – 1.70 g/100g (Ukom et al. 2009), from Japan between 1.08 – 1.43 g/100g DW (Ishida et al. 2000), and from the Sri Lankan ranging among 2.34 – 4.19 g/100g DW (Ravindran et al. 1995). The shoots ash content of the accessions from Japan ranged between 3.31 – 4.83 g/100g DW (Ishida et al. 2000), and the ones from the Pacific reached 1.3 g/100g DW (Lebot 2009).

Nitrogen comprises around 80% of the plant's total absorbed nutrients, mainly as nitrate (NO_3^-) in the anion form, or either in the cation form as ammonium (NH_4^+) (Kaur et al. 2017; Sahoo et al. 2010; Wang et al. 2009). The nitrogen content in taro's corm could range between 2.3 – 14.79 g/100g DW, meanwhile the nitrogen content of sweet potato's tuber can show values between 2.67 – 10.2 g/100g DW (Lebot et al. 2009).

However, environmental factors, such as drought conditions, can interfere with the plant uptake, allocation, and segregation of this mineral nutrient, due to the change of the ion concentrations in the plant tissues. With this, when the water content decreases, it reduces the nitrate availability and absorption from the soil matrix to the plant roots, compromising the diffusion of nutrients along with the plant tissues (Duman 2012; Goodger and Schachtman 2010). Proteins are the major nitrogen source found in living things, occurring also in amino acids, nucleotides and nucleic acids, vitamins, and secondary metabolites, including alkaloids (Katoch 2011).

Beyond the quantification of plant total mineral content, it is important to understand how efficiently the plant uses the nitrogen (NUE) in biomass allocation, whether in non-stress or stress conditions (Yuan and Peng 2017). The NUE could be calculated based on the nitrogen harvest index (NHI), nitrogen efficiency ratio (NER) and nitrogen utilization efficiency (E) by the plant (Lammerts van Bueren and Struik 2017; Mathur and Goel 2017; Siddiqi and Glass 1981). Usually, the NER is used to differentiate accessions into efficient and inefficient nutrient use (Mathur and Goel 2017; Good et al. 2004). The E allows comparing the increase of the produced biomass with whole-plant NER (Siddiqi and Glass 1981). Normally, when a plant shows a decrease in the NER, the nitrogen accumulation is higher in whole-plant, which could mean a lower biomass production

(Siddiqi and Glass 1981). Finally, the NHI describes the share of nitrogen accumulated in the tuber in relation to total plant nitrogen content (Kołodziejczyk 2014).

Though, NUE has been mostly used in grain crops, with very few works addressing the nutrient absorption ability and nutrient use for maximum biomass accumulation in tuber crops, such as potato, sweet potato, and taro (Lammerts van Bueren and Struik 2017; Kołodziejczyk 2014; Hartemink et al. 2000). Yet, the association of mineral allocation with nutrient use for these crops subjected to drought conditions is still very limited, as well as their nitrogen and total mineral content variation to stress.

1.4.3.2 Protein

Protein content has a significant role in determining the crops' nutritional quality and value (Wang and Frei 2011). Several methods are used to determine the protein content in food crops, including the spectrophotometric UV/Visible method by Biuret (1849) and Lowry (1951), the combustion method by Dumas (1831), and the digestion, distillation, and sample titration method by Kjeldahl (1883), among others (Katoch 2011; Owusu-Apenten 2002; Simonne et al. 1997).

In food analysis, the Biuret and Lowry methods are less sensitive due to the presence of complex protein mixtures with other components, in which case the Dumas and Kjeldahl methods are more appropriate (Owusu-Apenten 2002; Simonne et al. 1997). The use of the Kjeldahl technique is common in the determination of protein content in biological samples. It calculates the amount of nitrogen in an organic sample (S_N, g/100g of sample) from the conversion into ammonium sulfate by H_2SO_4 during digestion. Kjeldahl's method assumes that nitrogen recovered during digestion is mostly nitrogen from protein amino acids (total organic nitrogen) and that the contribution of inorganic nitrogen (nitrate, nitrite, and ammonium) or other organic nitrogen (nucleotides, nucleic acids) is practically negligible (Simonne et al. 1997). In the further steam-distillation, the ammonium sulfate salt liberates ammonia ions in the boric acid receiving solution and is titrated against standard hydrochloric acid. The titration quantifies the nitrogen content present in the sample, which is then converted to crude protein (Pt) using a Kjeldahl conversion factor (F_k) (Katoch 2011; Owusu-Apenten 2002):

$$Pt\ (\%) = S_N \times F_K$$

The conversion factor for plant protein ($F_K = 5.7$) is distinct from animal protein ($F_K = 6.25$), which included non-protein nitrogen, and may lead to an overestimation of protein content. These factors assume that animal protein contains about 16% of nitrogen, and plant protein contains around 17.5% of nitrogen (Katoch 2011; Owusu-Apenten 2002). Ideally, a conversion factor for each sample type should be calculated from the ratio of the weight of total amino acids to nitrogenous amino acids (Hui 2006). Similarly, the protein quality depends on amino acid composition and amino acid bioavailability. The most limiting amino acids are methionine and cysteine in sweet potato cultivars. Lysine, tyrosine, and isoleucine are "rare" in some cultivars, with the remaining amino acids being abundant (Hal 2000).

Numerous works used the Kjeldahl method to quantify the protein content in tuber crops around the world. The protein content of taro's corm represented 4.5 g/100g DW in Ethiopia (Mulugeta and Tebeka 2017), 8.07 g/100g DW in the Philippines (Alcantara et al. 2013), 5.88 g/100g DW in West Africa (Amon et al. 2011), 2.13 – 14.79 g/100g DW in the Vanuatu germplasm collection (Lebot et al. 2011a), and 0.9 – 1.9 g/100g DW in Thailand (Tattiyakul et al. 2007; Tattiyakul et al. 2006). Taro's shoot protein was also evaluated in accessions from Ethiopia with 25.061 – 26.069 g/100g DW (Temesgen et al. 2016), Nigeria with 5.57 g/100g DW (Fai et al. 2013), and Cameroon with 30.7 g/100g DW (Ejoh et al. 1996).

The protein content of sweet potato tuber accessions ranged between 2.53 – 6.93 g/100g DW in samples from South Africa (Magwaza et al. 2016), 1.2 – 10 g/100g DW from Pacific (Lebot 2009), 3.28 – 5.47 g/100g DW from Nigeria (Ukom et al. 2009), 0.237 g/100g DW from Peru (Ekanayake and Collins 2004), 1.28 – 2.13 g/100g DW from Japan (Ishida et al. 2000), and 2.95 – 6.32 g/100 g DW from Sri Lankan

(Ravindran et al. 1995). The protein content of sweet potato shoots was 4.0 g/100g DW in accessions from Pacific (Lebot 2009) and ranged between 5.15 – 5.81 g/100g DW in Japanese accessions (Ishida et al. 2000).

Although, the protein content could be affected by both genetic and environmental factors (Wang and Frei 2011; Toyama et al. 2006). The protein present in the leaves (aboveground organs) could be related to photosynthesis, meanwhile, the protein in tubers (underground organs) could be associated with the regulation of cell defense and detoxification, also with meaningful variations in expression during stress (Salehi-Lisar and Bakhshayeshan-Agdam 2016). If the protein content increase under scarcity conditions, it could be related to the synthesis of specific high molecular proteins, to minimize the stress effect. Proteins can act as a donor of natural osmoprotectants amino acids (*e.g.*, proline) or as cellular compartment space fillers (*e.g.*, Late Embryogenesis Abundant (LEA) proteins) (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Osuagwu and Edeoga 2013). The dehydrins (DHNs) or group II LEA proteins are fundamental for the plant response and adaptation to abiotic stress. When the plant cells lose water, can occur the denaturation of the proteins. The DHNs could act as space-fillers in several cellular complexes during dehydration, avoiding the cellular collapse. DHNs binds to the partly dehydrated surface of other proteins, enhancing the amphipathic α-helices in a DHN molecule, conferring protection to other proteins from further loss of water, and thus protein denaturation and loss of functions (Hanin et al. 2011). The proline accumulation protects the subcellular and molecular structures under osmotic stress. It has the function of a molecular chaperone to keep protein integrity and improve the activities of diverse enzymes (Szabados and Savour 2010).

The quantification of the protein content is an important tool to define the nutritional quality and value of crop plants, under normal or stress conditions. Usually, the presence of stress condition leads to higher protein content in plant tissues or, more rarely, it could also have no effect or even lower the protein content (Wang and Frei 2011). One of the few examples of protein determination in sweet potato tubers subjected to drought showed a decrease in its content under stress, from 0.237 – 0.201 g/100g DW (Ekanayake and Collins 2004). However, until now, it is still limited the information about the variation of the protein content of taro and sweet potato exposed to water scarcity stress.

1.4.3.3 Starch

Starch (St) is a polysaccharide, and the largest carbohydrate energy storage in plants (Kays 1985). Other carbohydrates are found in animal and plant tissues and have distinct functions, such as storage (glycogen, fructans), structural (cellulose, xylan), protective (polysaccharides that induce protein synthesis), and cell recognition (glycoproteins, glycolipids) (Izydorczyk 2005).

Starch is synthesized during photosynthesis, involving complex biological processes, and is stored as granules (relatively dense and insoluble in water) in chloroplasts (leaves) or amyloplasts (root parenchyma), tubers, endosperm, or seed (cotyledons) (Amaral 2007; Liu 2005). Shoot starch can have a significant function in the operation of the stomatal guard cells because its hydrolysis produces sugars (such as sucrose, a non-reducing sugar) acting as osmoregulators during drought (Santelia and Lunn 2017). It can occur fast starch degradation during the day to release sugars to keep the osmotic potential within the guard cells, which contributes to stomatal opening during drought conditions (Santelia and Lunn 2017; Preiss and Sivak 1996). Normally, the shoot starch could be temporarily held in granules in the leaf chloroplasts during daylight active photosynthesis CO_2 fixation, to be broken at night for sucrose synthesis (Zeeman et al. 2010; Preiss and Sivak 1996). Sugars can increase the cell pressure potential by fulfilling the cell bilayer interfaces during osmotic stress (Santelia and Lunn 2017; Leshem and Kuiper 1996). Similarly, the sucrose transport from the shoots to the tuber organs could be transformed into storage starch for long-term storage (Zeeman et al. 2010). However, very few works quantified the starch content in taro and sweet potato whole-plants subjected to drought conditions (Ekanayake and Collins 2004).

The main sources of starch are tubers (taro 90%), tuberous roots (sweet potato 75%), cereals (wheat 67%; rice 89%; corn 57%), and legumes (beans 42%) (Liu 2005). Several works determined the starch content in taro and sweet potato underground organs under non-stress conditions. Taro corms from the Pacific showed a range in the starch content between 55.88 – 89.46 g/100g DW (Lebot et al. 2011a; Lebot et al. 2009). Meanwhile, the starch content of sweet potato's tubers from Taiwan shows 24.35 – 46.72 g/199g DW (Lai et al. 2016), from Pacific 30 – 85 g/100g DW (Lebot et al. 2011b; Lebot 2009; Lebot et al. 2009), and finally from Sri Lankan with 63.13 – 77.34 g/199g DW (Ravindran et al. 1995). The methods commonly used to determine the starch content are physical (polarimetric, specific gravity), chemical (hydrochloric acid or perchloric acid), and enzymatic hydrolysis (Wrolstad et al. 2005). The chemical method is based on the previous removal of the sugars present in the sample, using hot alcohol treatment, with starch being extracted in a hot acidic medium to hydrolyze the starch into dehydrated glycose monomers that are converted into hydroxymethylfurfural compounds. The chemical reaction between the anthrone with the furfurals present in the sample confers a greenish color product, which is then quantified colorimetrically (Katoch 2011). The enzymatic method converts selectively the starch into glycose, through the combination of amylase enzymes, with the glucose colorimetric quantification. The final mixture shows a pink color in the presence of glucose (Wrolstad et al. 2005). The enzymatic method is more sensitive than the chemical method, though the chemical method is most affordable to determine the starch content in plant tissues (Wrolstad et al. 2005).

Beyond the starch quantification, it is also important to determine the quality of the starch granules, either to understand the plant biological processes or to evaluate its bakery potential (Kusumayanti et al. 2015). Starch is a polysaccharide composed of α-D-glucopyranosyl units, *i.e.*, by a glucose biopolymer bonded together by a glycosidic linkage (Sajilata et al. 2006; Wrolstad et al. 2005). The starch macromolecules, both amylose (polysaccharide with amorphous linear structure, *i.e.*, with no defined structure) and amylopectin (polysaccharide with crystalline-branched structure) provide the configuration of the starch granules (Wrolstad et al. 2005). Generally, amylopectin represents the major starch fraction (approx. 75%), with amylose representing the lower fraction (approx. 25%) (Lai et al. 2016; Sinnott 2007; Sajilata et al. 2006; Kays 1985). The starches composed largely of amylopectin are easily gelatinized and more effective for starch conversion (Rendleman Jr. 1999). In the presence of heat and water, starch gelatinization occurs, starting to swell the amylopectin granules, with some of the amylose fractions being leached into the water (Lai et al. 2016).

It is known that the amylose to amylopectin ratio can reduce the swelling power due to inhibition of water diffusion into the starch granules (Kumoro et al. 2014). Few works were published about the starch gelatinization properties of taro (Tattiyakul et al. 2007; Tattiyakul et al. 2006) and sweet potato (Lai et al. 2016; Kusumayanti et al. 2015) grown under normal conditions. They also mentioned that the changes in starch gelatinization determined by the amylose to amylopectin ratio can be expressed through the starch solubility in water (SWS) and the starch swelling power (SSP) properties. These parameters are obtained by gravimetry, with the flour homogenization in water and poured in a hot water bath until the starch gelatinization, followed by centrifugation. The SSP values are given through the weight of the swollen starch granules present in the sediment, and the SWS are given through the soluble starch granules present in the supernatant dehydrated until constant weight (Tattiyakul et al. 2006). The Indonesian taro corms had an average of 0.060 g/g SWS, and the Thailand ones had a range between approx. 0.070 – 0.14 g/g SWS, suggesting that taro starch has low solubility in water (Kumoro et al. 2014; Tattiyakul et al. 2007; Tattiyakul et al. 2006). Thailand's taro corms show approximately a range between 11 – 17.4 g/g SSP, and Indonesian ones an average of 14.5 g/g SSP, also indicating that taro has low starch swelling power (Kumoro et al. 2014; Tattiyakul et al. 2007; Tattiyakul et al. 2006). Meanwhile, the flour from sweet potato tubers from Indonesia had 0.086 – 0.096 g/g SWS and 3.40 – 3.67 g/g SSP (Kusumayanti et al. 2015). Other sweet potato tubers cultivated in Taiwan show 0.4031 – 0.6187 g/g SWS and 20.01 – 28.87 g/g SSP (Lai et al. 2016). Hitherto, no works were published mentioning the changes in the amylose to amylopectin ratio, through the variation of starch water solubility and swelling power in a drought environment.

1.4.3.4 Fiber

Crude fiber (Fb) is the insoluble residue (or indigestible polysaccharides) of the plant total carbohydrate, present in the cell walls, with 97% of its constitution in hemicellulose (non-cellulosic polysaccharide), cellulose and lignin, that provides the plant's stems rigidity and stiffness (Katoch, 2011; Yoshida and Kuwano 1989).

The fiber content of the plant cell wall is usually estimated through a gravimetric process, based in successive boiling with dilute acid and alkali, simulating the monogastric digestion of ruminants (Möller 2014). Möller (2014) referred to several analytical methods that could be applied for the obtaining of the crude fiber content, either with a general method (ISO 5498:1981) or modified Scharrer method (ISO 6541:1981) for agricultural food products, with intermediate filtration for animal feeding stuff (ISO 6865:2000), or with near-infrared spectrometry for animal feeding stuff, cereals and milled cereal products (ISO 12099:2010).

The crude fiber estimation is usually performed through a neutral detergent (NDF) procedure for cell wall hemicellulose, cellulose, and lignin constituents in vegetable feedstuffs. The acid detergent fiber (ADF) procedure provides a fast determination of lignocellulose in feeds stuff, with the difference between the NDF and ADF estimate directly the hemicellulose content (Möller 2014; Katoch 2011). All the polysaccharides and lignin present in the plant cell that is not digested by the human digestive tract is defined as dietary fiber (DF) (Selvendran et al. 1989). These indigestible components of plant foods are broken down into formic, acetic and galacturonic acids in the human large intestine, which are poorly absorbed, being transferred to intestinal microflora (Katoch 2011). The DF serves as a substrate to the intestinal microflora, and influences the nutrient absorption, such as sugars, lipids, and proteins, aiding in weight maintenance by reducing their absorption (Kritchevsky 2001; Morris 2001).

For example, some works determined the crude fiber in taro plants, with the corms showing 5 g/100g DW from Ethiopia (Mulugeta and Tebeka 2017), 3.10 g/100g DW from the Philippines (Alcantara et al. 2013), 1.22 g/100g DW from West Africa (Amon et al. 2011), and 0.3 – 0.94 g/100g DW from Thailand (Tattiyakul et al. 2007; Tattiyakul et al. 2006). Meanwhile, the taro shoots had 4.037 – 4.597 g/100g DW from Ethiopia (Temesgen et al. 2016) and 1.0 g/100g DW from Nigeria (Fai et al. 2013). However, these crude fiber determinations were performed in taro plants grown in normal conditions. Hitherto, fiber content determination as a nutritional quality parameter of taro plants submitted to water scarcity conditions is absent.

1.4.4 Oxalates

The insoluble raphide crystals of calcium oxalate (CaOx) present in raw taro corms, petioles, and leaves, and the insoluble druse crystals of CaOx present in raw sweet potato (root tubers and shoots), produces acridity and kidney disorders if consumed fresh (Woolfe 1992; Franceschi and Horner 1980; Schadel et al. 1980). The combination of soluble oxalates (*e.g.*, oxalic acid, OA) with calcium (Ca^{2+}) generates the insoluble CaOx crystals. Conversely, when OA combines with sodium (Na^{+}), potassium (K^{+}), and iron (Fe^{2-}) forms soluble salts (Franceschi and Horner 1980). The CaOx crystal formation in plants could reach about 90% of the total calcium content and plays important functions in tissue calcium regulation, protection from herbivory, and metal detoxification (Nakata 2003). These crystals commonly occur inside the vacuoles of specialized cells, *i.e.*, crystal idioblasts, and could participate in the storage of calcium as CaOx (Nakata 2003; Franceschi and Horner 1980). Genetic and drought factors can change the overall intensity of plant CaOx accumulation (Sharma and Kaushal 2016).

The plant photosynthesis process and carbohydrate metabolism are related to plant oxalate production (Franceschi and Horner 1980). Still, the most evident way of oxalate accumulation is the incomplete oxidation of photosynthetic assimilates (Igamberdiev and Eprintsev 2016). Tooulakou et al. (2016) observed that CaOx crystals degradation during daylight on drought pigweed plants provided carbon for photosynthetic assimilation, with CaOx acting also as biochemical reservoir through the collection of non-atmospheric carbon

during the night. They also suggested that the carbon in the CaOx crystals is not only derived from the photosynthetic CO_2 fixation, indicating the dark respiration as another probable source. Nevertheless, the oxidation of glyoxylate formed during photorespiration could accumulate oxalate by the reaction catalyzed by glycolate oxidase, or by isocitrate lyase. Although, the oxalate accumulation could not be correlated with the photorespiration processes (Igamberdiev and Eprintsev 2016). The oxalate accumulation in leaves acts as an osmotically active molecules pool and shows the capacity to inhibit the ABA-induced stomatal closure in *Arabidopsis thaliana* plants (Guimarães and Stotz, 2004).

According to Fatoki et al. (1994), the HPLC, GC, and even enzymatic methods currently used on the determination of oxalates in vegetables are very laborious, with the advantage of being a faster and sensitive technique, but have the disadvantage of having more additional steps (*e.g.*, esterification) and for being over expensive. The oxalate quantitation by the classical precipitation method passes through steps of digestion, oxalate precipitation, and permanganate titration and is still a cheap and accurate analytical technique available through a fast, explicit and observable reaction that allow the quantitative determination of oxalates in taro crops (Kumoro et al. 2014; Adeniyi et al. 2010; Iwuoha and Kalu 1995).

Some reviews about organic acids in tropical root crops referred to 32 mg/100g FW of CaOx for sweet potato tubers (Lebot 2009; Holloway et al. 1989; Bradbury and Holloway 1988). Meanwhile, several works determined the taro corm CaOx content, with samples from Indonesia showing 770 mg/100g DW (Kumoro et al. 2014), from Thailand showing 185.2 – 198.3 mg/100g FW (Tattiyakul et al. 2007; Tattiyakul et al. 2006), and from Nigeria with values between 367 – 710 mg/100g DW (Iwuoha and Kalu 1995). Taro shoots from New Zealand presented 110 – 147 mg/100g FW in CaOx (Oscarsson and Savage 2007). Additionally, taro reviews showed 43 mg/100g FW for corm CaOx, and 400 mg/100g FW for shoots (Lebot 2009; Bradbury and Holloway 1988). However, none of these works quantified the CaOx under drought conditions, regarding only the acridity in raw and cooked leaves, processed flour, and chips.

1.5 Near-Infrared Spectroscopy

Regular laboratory analysis has high costs, high waste production, and usually it is performed by lengthy wet chemistry techniques (Workman and Weyer 2008). The Near-Infrared Spectroscopy (NIRS) technique, compared to the wet chemistry techniques, has numerous advantages in terms of speed, reliability, and versatility (Williams 2007). This technique is recognized as a practically unlimited source of analytical information for the most diverse analytical purposes and types of samples, due to its universal response features (Pasquini 2018). The ease of sample preparation and multiple parameters determination in a single non-destructive analysis can be applied, *e.g.*, to fruits, tubers, and grains (Hacisalihoglu et al. 2010; Lebot et al. 2009; Nicolai et al. 2007; Schimleck 2007). NIRS was used to predict the quality constituents of taro and sweet potato underground organs [carbohydrates, N, Pt, and M] (Magwaza et al. 2016; Lebot et al. 2011a; Lebot et al. 2011b; Lebot et al. 2009). Other components, such as crude fiber and carbon isotope discrimination ($\Delta^{13}C$) in cereals (Ferrio et al. 2001; Bruno-Soares et al. 1998), tubers and shoots of sweet potato (Zhang et al. 2015), and compound feeds (de Boever et al. 1995), as well as carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in plant tissues (Kleinebecker et al. 2009), were also predicted by NIRS. The NIR spectrophotometer is relatively expensive, although it generates highly reproducible predictions in a huge number of biologic samples, showing analogous accuracy respectively to the analytical reference tests obtained by wet chemistry, which is its biggest technical limitation to depend sometimes on these less precise reference methods (Hacisalihoglu et al. 2010; Osborne 2000).

At the beginning of NIRS development, in 1881, the spectra from the near-infrared region had an absence of defined peaks, multiple overlapping of the absorption bands, and a combination of molecular vibrations, which at the time was considered irrelevant structural information (Workman and Weyer 2008; Schimleck 2007). This irrelevant information in the NIR electromagnetic spectral zone was a result of multiple

absorption bands of the vibrational molecular transition of the overlap and combination of axial deformation or stretching vibrations of the XHn (OH, NH, CH, and SH) functional groups, between 1100 to 2500 nm (Hacisalihoglu et al. 2010; Schimleck 2007; Siesler et al. 2002). If this zone is above 2500 nm, the bands become too intense, while beneath 1200 nm occurs low band absorption, making reflectance recording difficult (Schimleck 2007). It was observed that the greatest deviation of vibration results from the chemical bond between the molecule atoms, and the greatest anharmonicity of the chemical bonds between a lighter atom (Hydrogen) and a heavier atom (Carbon, Nitrogen or Oxygen) (Workman and Weyer 2008; Osborne 2000).

NIR region uses algorithms to interpret the sample absorbance data, based on Lambert-Beer's law, relating the sample absorbance (χ) at a specific wavelength concerning the analyte concentration:

$$\chi = \varepsilon \times d \times \gamma$$

The differences between transmission and reflection are due to the difference in the optical path traveled by the light. In the 1100 to 2500 nm region, the light beam scatters are unable to penetrate samples larger than 1 cm in transmittance mode. Thus, much of the incident radiation is reflected, with diffuse reflectance radiation (R) empirically related to the concentration (γ) of the substances in the sample. The absorbance χ can be described by $log\ (1/R) = k\gamma$, where k is the factor that incorporates both the molar absorptivity (ε) of each sample and the optical path of radiation (d), in reflectance mode (Figure 1.3) (Osborne 2000). Radiation dispersion fluctuates with variations in water content, particle size, and temperature, making larger the absorbed radiation, and increasing the $log\ (1/R)$ values (Berzaghi et al. 2005). The complexity of the organic constituents of food and biological samples leads to large absorbance peaks, requiring multivariate linear regression chemometrics to calibrate the NIR spectrum concerning the chemical composition (Hacisalihoglu et al. 2010; Osborne 2000). The principal component analysis (PCA), principal component regression (PCR), and Partial Least Squares (PLS) are the multivariate techniques forms of data treatment at NIRS (Pasquini 2018). The PLS regression model is one of the quantitative regression algorithms used for linear data (Williams 2007). PLS is the favorite multivariate regression technique used to obtain calibration equations, suitable for calibration of complex samples with low interference signal between the analyte and the spectrum characteristics, detecting outliers in global sampling (Pasquini 2018; Siesler et al. 2002; Burns and Ciurczak 2001). Chemometrics is a mathematical approximation applied to the chemical sciences to develop a model that defines the mean and standard deviation of each type of sample under study in a multidimensional space, to further test and identify the group to which an unknown sample belongs (Osborne 2000).

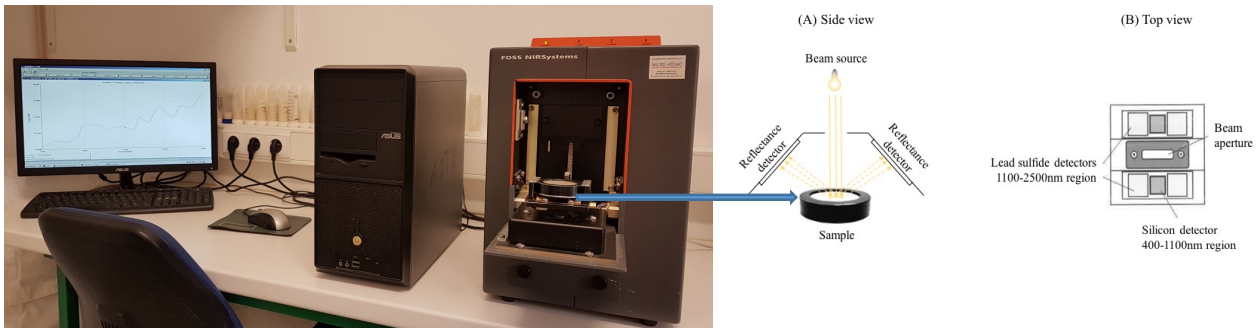

Figure 1.3
Example of conventional NIRS analytical technique equipment (NIRSystems 5000-M, FOSS, U.S.A.). Scheme shows the side view (A) and top view (B) of NIR absorbance recorded in reflectance mode (log 1/R) between 1100-2500 nm. Photo by: ISOPlexis Genebank, University of Madeira.
Scheme adapted from: Foss (2003) and Osborne (2000).

The development of a calibration model depends on the complexity of the spectra created in NIRS, with the need to use several corrections based on algorithms to reduce the complexity of the calibration model, making it more linear for simplified interpretation (Siesler et al. 2002). The calibration development requires the analysis of calibration samples from reference values obtained for each parameter of the sample. Then, it is necessary to obtain the NIR spectrum corresponding to each sample calibration. Additionally, determine the mathematical model between NIR spectra and values of corresponding references for each parameter. And finally, the validation of calibration with independent samples to involve both reference and NIRS values, to verify if both are statistically correlated (Burns and Ciurczak 2001).

All the chemometric procedures for the calibration model robustness goes through the spectral pretreatment, improvement of quantitative and qualitative methods, sample selection for the calibration set, and model validation excluding outliers. The pretreatment facilitates the calibration process by reducing, eliminating or standardizing spectral variations and oscillations from sample light scattering and intermolecular interactions between components. It consists of reducing interfering potentials, such as particle size, smooth, detrend, and improved definition of overlapping peaks (by derivatives) (Siesler et al. 2002). The Standard Normal Variate (SNV) improves the accuracy of the results by minimizing the sampling error relative to the difference in density and particle size of the sample. SNV centers each spectrum around zero by subtracting the mean, with subsequent division of each signal by the standard deviation of the entire spectrum, without requiring additional information on the analyzed product (Siesler et al. 2002). The Multiplicative Scatter Correction (MSC) allows the simplification of the calibration model by correcting for light scattering in solid samples, averaging the calibration set points, expressed as the sum of the squared difference between the transformed spectrum data and the target spectrum. This treatment allows the use of linear regression to estimate the parameters of the spectrum set. This increases the linearity by simplifying the mathematical model, which implies choosing the most appropriate target spectrum that correctly represents the sample composition (Nicolai et al. 2007; Siesler et al. 2002). The derivation transforms the NIR spectrum to remove baseline oscillations and overlapping peaks in the spectrum, improving the original peak resolution, demonstrating greater efficacy in eliminating additive and particle diameter interfering effects over MSC and SNV (Nicolai et al. 2007). The MSC and SNV are very popular because of their prompt availability in commercial user-friendly chemometrics software packages (Pasquini 2018). Still, the derivate is the most suitable treatment for prediction by the PLS model (Siesler et al. 2002). The first derivative is the most used for this purpose, emphasizing more clearly the less prominent signals in the spectrum by amplifying the spectral noise (Siesler et al. 2002; Osborne 2000). Each derivative reshapes the spectrum with linear transformation in constant terms, where the second derivative can transform maximum peaks to minimums and vice-versa.

Furthermore, the calibration models are also subjected to a validation process along with the prediction process. This validation consists primarily in spectral differences evaluation between the calibration and prediction steps, followed by the calculating of the standard parameters to correct the estimated differences (Burns and Ciurczak 2001). The data should be divided into two subsets to make the model more robust to system variations (operator, environmental conditions, and/or sample temperature). One subset corresponds to the samples used for calibration, and the other subset of samples corresponds to external validation, which is generally used to increase the robustness of the applied model (Siesler et al. 2002).

When the number of data is limited, *leave-one-out* cross-validation is often used for the samples used for calibration. In a series of samples used for calibration, a new sample is always left apart until all samples have been excluded at least one time. Thus, is predicted at a time the concentration of each sample that was not used in the calibration set. Also, the cross-validation can be used to determine the optimal number of factors used in the calibration model (Siesler et al. 2002). The prevailing model will be the one with the smallest Root Mean Squared Error of Cross Validation (RMSECV) or better known as Standard Error of Cross-

Validation (SECV) (Siesler et al. 2002). The external validation requires the use of independent samples, *i.e.*, unknown to the calibration group, and used when the model is sufficiently robust (Burns and Ciurczak 2001).

With this, the calibrated model of the analyzed parameters relates the spectra of the samples to the reference values obtained in the laboratory by scanning new samples, using the calibration as a reference for the prediction. The quality of calibration, prediction, and validation, is mainly determined through standard statistical tests:

a) Standard Error of Calibration (SEC) is useful for selecting a specific set of wavelengths to use in the development of a calibration model. The SEC is the standard deviation of the residuals obtained by the difference between the laboratory reference values and the NIRS predictions (Burns and Ciurczak 2001).
b) Standard Error of Prediction (SEP) gives the variability as the difference between the prediction and the reference values, when a specific equation is applied to the validation data series, *i.e.*, to samples that are outside of the data set calibration (Burns and Ciurczak 2001). In some publications, SEP is reported instead of Root Mean Square Error of Prediction (RMSEP) (Nicolai et al. 2007).
c) Standard Error of Cross-Validation (SECV) is a repetition algorithm, which is based on the variability recorded in the difference between prediction and reference values when an equation is applied to a subset of the calibration set. SECV is generally used in various equations, where the equation with the lowest SECV value is selected as the best calibration (Burns and Ciurczak 2001).
d) The correlation coefficient or multiple determination coefficient (r^2) allows the determination of variability in the reference data, using the regression equation. The closer the value to the unit, the greater the modeling ability of the calibration data (Burns and Ciurczak 2001).
e) Bias, defined as the difference between the predicted and reference mean values, allows the calculation of systematic variations in prediction and calibration errors, through the standard error corrected by Bias for calibration and prediction, *i.e.*, SEC (C) and SEP (C), respectively (Siesler et al. 2002; Burns and Ciurczak 2001).
f) Ratio of Performance to Deviation (RPD = SD/SECV) gives the model prediction accuracy. It classifies the equations predictive potential with RPD < 1.5 as inadequate; 1.5 < RPD < 2.0 as rough; 2.0 < RPD < 2.5 as quantitatively approximated; 2.5 < RPD < 3.0 as good; and RPD > 3.0 as excellent (Ceballos et al. 2006).

The complexity of the spectral model increases as samples differ in common chemical characteristics. However, the sampling requires a wide range of laboratory reference values to be representative of the studied biological material (Burns and Ciurczak 2001). The accuracy correlates with the robustness of the model, as it is restricted to spectral variation to obtain a more accurate calibration equation, taking into account spectral variability to obtain an equation with extended spectral variability, and apply it to diverse spectral material (Gillon et al. 1999). In fact, previous NIRS models were used to predict the quality constituents of biological material grown under non-stress conditions, such as taro and sweet potato (Magwaza et al. 2016; Zhang et al. 2015; Lebot et al. 2011a; Lebot et al. 2011b; Lebot et al. 2009), cereals (Ferrio et al. 2001; Bruno-Soares et al. 1998), compound feeds (de Boever et al. 1995), and other plant tissues (Kleinebecker et al. 2009), as previously mentioned. Likewise, a review on the last decade NIRS general publishing in another area of interest, such as soil, fuel, pharmaceuticals, also refers to other recent NIR instrumentation inputs, such as miniaturization (MicroNIR, NanoNIR) and imaging (spectral image with spatial resolution, pixel) (Pasquini 2018). These inputs added to the conventional NIRS analytical technique allowed its expansion over time. Nevertheless, the evolution of the NIR technique to be a cheaper and portable miniaturized spectrophotometer, still leads to inaccurate results, due to the deficient representativeness of the measurement, *e.g.*, small probing area and the use of non-homogeneous samples. However, the input of spectral camera devices in conventional NIRS,

known as near-infrared hyperspectral imaging (NIR-HSI), allows the single sample properties analysis through its surface imaging as pixel coordinates, being faster and more sensitive than the classical NIRS technique. Also, chemometrics algorithms for pre-processing and multivariate data analysis of NIR-HSI can be employed mostly in the same way as the conventional NIRS. The main disadvantages of NIR-HSI are that the sample must remain static during the image crop and the price of the HSI cameras (Pasquini 2018).

Considering the maturity of NIRS over time, we still did not find its application in the evaluation of the quality changes related to the physiological response of taro and sweet potato crops to stress conditions when subjected to water scarcity.

1.6 Background of the Doctoral Dissertation and Aims

Taro was introduced in Madeira Island around 1640, with cultivation made up to the present day in terrains under rain feed or under regular water irrigation (da Silva and de Meneses 1946a). It is an important Madeiran traditional dish during the Easter, that coincides with the Spring annual harvest (between March and April) (DGADR 2001a; da Silva and de Meneses 1946a). The taro production in Madeira registered 628 tons in 31 hectares between 2012 and 2015 (INE 2016; INE 2013). A previous work in the INEA Project framework (EuropeAid/128-500/C/ACT/TPS, DCI-Food/2009/45 - *Adapting clonally propagated crops to climatic and commercial changes*), evaluated the drought resistance in taro elite cultivars. One of the tasks consisted of the evaluation of taro behavior using morpho-agronomic and multi-criteria indices. Thirty-three accessions were submitted to drought conditions, including 10 accessions from Madeira Island.

The sweet potato was introduced in Madeira Island near the 17th century, whose cultivation can reach two to three vegetative cycles per year (da Silva and de Meneses, 1946b). In Madeira, it is a traditional food and is an economic and socially important crop. Sweet potato is a traditional ingredient in the regional bread, usually baked or roasted, that complements the wheat flour (DGADR 2001b). The sweet potato production increased in Madeira Island, from 10,920 tons in 520 hectares in 2012 to 13,194 tons in 546 hectares in 2015 (INE 2016, INE 2013). The demand for innovative products, reduced production of regional varieties of sweet potatoes, limitation of sweet potatoes throughout the year, and the need to avoid wastage in agricultural production, led to the BATATINPAN project (MADFDR-01-0190-FEDER-000012). This project performed the nutritional, physicochemical, technological, rheological, microbiological, organoleptic, and agro-morphological evaluation of six local sweet potato accessions. It allowed the selection of the ones with the most appropriate characteristics for baking, and the creation of a new local product, resulting from the combination of composite wheat and sweet potato flours. The industrial process developed the production of sweet potato flour and has been later registered and protected as a national patent (https://patents.google.com/patent/PT107526B/en). It allowed Madeiran local sweet potato production to flow, contributing to add value to the food chain, and renewed the interest for production and marketing of the local varieties most suited to flour processing. The BATATINPAN project also helps to increase the incomes for regional farmers and the bakery industry and contributed to the development of local microeconomics.

However, there is a need soon for the implementation of breeding programs in Madeira, to adapt both taro and sweet potato crops to climate change, through the selection of the accessions with the best quality traits and yield under climate constraints. But this will require new fast tools for phenotypic recurrent selection of traits that can confer the physiological stress response. Still, no studies are modeling the influence of drought stress on the quality constituents in the underground and aboveground organs of these crops.

Considering the background and the doctoral dissertation timeline, the taro development (Ganança et al. 2015) and sweet potato quality (Zhang et al. 2015) grown under drought conditions were recently addressed. Protein, fiber, starch, nitrogen, total mineral, and calcium oxalate were the main quality traits studied in these crops. Several works selected these traits for the study of sweet potato tubers (Ukom et al. 2009, Ekanayake and Collins 2004, Holloway et al. 1989) from Tenerife (Suárez et al. 2016), Taiwan (Lai et al. 2016), South

Africa (Magwaza et al. 2016), Vanuatu (Lebot et al. 2011b, Lebot et al. 2009), Sri Lankan (Ravindran et al. 1995), and for both tuber and shoot organs (Lebot 2009, Ishida et al. 2000; Bradbury and Holloway 1988). Taro was also studied for the same quality traits, either for the corm from Ethiopia (Mulugeta and Tebeka 2017), Indonesia (Kumoro et al. 2014), Philippines (Alcantara et al. 2013), West Africa (Amon et al. 2011), Vanuatu (Lebot et al. 2011a, Lebot et al. 2009), Thailand (Tattiyakul et al. 2007, Tattiyakul et al. 2006), or the shoot from Ethiopia (Temesgen et al. 2016), Nigeria (Fai et al. 2013), New Zealand (Oscarsson and Savage 2007), Cameroon (Ejoh et al. 1996), and both organs (Lebot 2009, Bradbury and Holloway 1988). NIRS has been widely used to characterize these quality traits in biological samples, developing a high-precision spectroscopic technique with complex computations, involving multivariate analyses in both taro and sweet potato underground organs (Magwaza et al. 2016; Cabrera-Bosquet et al. 2012; Lebot et al. 2011a; Lebot et al. 2011b; Lebot et al. 2009). However, beyond the quantification of the crop quality traits, it's essential to link complex phenotypes and chemotypes to plant breeding in changing environments, such as drought (Champagne et al. 2009; Desjardins 2008). Drought stress is not static and affects crops with variable length and severity over their life cycle (Hatfield 2014). Still, NIRS application to root and tuber crops is quite limited, with no studies aiming to model the influence of drought stress in the quality constituents of both underground and aboveground organs of taro and sweet potato crops. It became necessary the NIRS technique use to complement the physiological response of these cultures to drought stress. This fast tool could be applied for research, and by farmers and agriculture companies in the fast screening of production and could also aid in the selection of traits to be used in taro and sweet potato monitoring, during breeding programs.

The main objective of this Ph.D. research project (December 2015-2019) was the assessment of the biomass allocation, adaptation, and response to water stress in taro and sweet potato crops, using seven taro and eight sweet potato accessions. These studies were conducted during two full-growth vegetative cycles. The taro accessions from Madeira Island, Canary Islands, and South Pacific Community collection were selected based on the preliminary data of the morpho-agronomic and multi-criteria indices to drought assessment, carried out in a previous project to the present dissertation (EuropeAid/128-500/C/ACT/TPS, DCI-Food/2009/45). The Madeiran sweet potato accessions were selected based on a previous project to the present dissertation (MADFDR-01-0190-FEDER-000012). Sweet potato accessions from the Canary Islands and Guinea-Bissau were provided by locals, all elected as the ones with the best features for the local economy, food security importance, and farmer preferences. The specific objectives were:

1. Implement the water stress agronomic trials on both crops in study, and collect relevant morpho-agronomic data, namely CCI, PAR, WUE, TPB, R:S, and SI.
2. Process the plant material (underground and aboveground organs) cultivated under drought and control conditions.
3. Obtain the data of nutritional and biochemical parameters by wet chemistry, namely N, Pt, St, Fb, M, T-Ox, OA, CaOx, $\delta^{13}C$, $\Delta^{13}C$, $\delta^{15}N$ and TC content; and the data of the physiological parameters, specifically NER, E and NHI.
4. Develop the NIRS calibration equations with the evaluation of variation of the nutritional and biochemical parameters, including the elaboration of spectra database associated with chemometrics.
5. Analyze the robustness of predictive model for estimation of the crops parameter(s) variation(s) in drought stress response(s).
6. Compare the morpho-agronomic data with nutritional and biochemical information to identify specific traits, allowing the classification of accessions as tolerant or susceptible, and to confirm the marker reliability, and drought model trustworthiness.
7. Realize the data statistical analysis to elaborate a model that define the markers that allow to screen and identify drought tolerant accessions in a timely manner, to contribute to a better understanding of the biochemical and physiological basis of drought tolerance in these crops, and to facilitate the identification of new material for breeding purposes.

1.7 Outline of the Doctoral Dissertation

This doctoral dissertation was designed in the form of scientific articles. The present research is reported in the form of manuscripts that have been published, accepted, or submitted in peer-reviewed scientific journals of the field. A general introduction and bibliographical research (Chapter 1) approached the different subjects of this dissertation. The taro (Chapters 2-5) and sweet potato (Chapters 6-9) manuscripts were written independently, as the taro drought agronomic assay was performed in a pot experiment, while the sweet potato assay was performed in a field experiment. Both the agronomic assays were performed in this way because of the field logistic available for this thesis project, taking in consideration the ability of the target culture to develop and literature recommendations. Those manuscripts were placed following the main objectives of this dissertation. Some similarities, mostly in the introduction and methods sections, could be detected. The NIRS predictive models for taro and sweet potato crops were joined together in the same manuscript (Chapter 10). Final considerations and future perspectives (Chapter 11) comprises an overview of all issues addressed in this dissertation, bringing together the main conclusions discussed in the previous chapters. To aid the interpretation of the agronomic assays in the material and methods of the manuscripts, additional information was provided regarding the main physiological descriptions (based on the IPGRI descriptors) and origin of taro accessions (Annex A), and sample processing into flour for analysis (Annex B). Similarly, the main physiological descriptions (based in the CIP/AVRDC/IBPGR descriptors) and origin of sweet potato accessions (Annex C), and sample processing into flour for analysis (Annex D), were also provided.

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CHAPTER 2

Phenotypic flexibility and drought avoidance in taro (*Colocasia esculenta* (L.) Schott)

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RESEARCH ARTICLE

Phenotypic flexibility and drought avoidance in taro (*Colocasia esculenta* (L.) Schott)

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2.1 Abstract

Taro (*Colocasia esculenta* (L.) Schott) is a substantial staple food in most of the tropical regions. Prolonged exposure to drought impairs crop production worldwide. Tolerant crops have the best capability to cope and avoid drought, through phenotypic flexibility mechanisms. The water use efficiency (WUE) is well known in taro crops, but very scarce information is available relating to their nutrient efficiency (NER) in drought conditions. Our work provided pertinent information about the physiological variation of seven taro accessions subjected to seven months of drought, by recording the differences for nutrient allocation, chlorophyll canopy, biomass loss, and stress intensity. Significant relationships between control and drought treatments on WUE (+85%), total plant biomass (TPB, -26.8%), chlorophyll content index (CCI, +1.8%), and nutrient harvest index (NHI, +0.2%) were detected. Drought led to a generalized loss of TPB as drought avoidance strategy, although distinct phenotypic flexibility was observed through the root:shoot ratio (R:S) and stress index (SI) from the corm and shoot organs. The nutrient allocation from the corms to shoots, with NER increase registered in drought conditions, can be a valuable tool to complement the TPB and WUE productivity traits, to be used in taro breeding programs.

Keywords
Biomass; drought; nutrient efficiency; root:shoot ratio; stress index

Abbreviations
Acc. accession, *CAN* Canary Islands, *CCI* chlorophyll content index, *DW* dry weight basis, *E* nitrogen efficiency of utilization, *M* total mineral content, *MAD* Madeira Island, *N* nitrogen content, *NER* nitrogen efficiency ratio, *NHI* nitrogen harvest index; *R:S* root-to-shoot ratio, *SI* whole-plant stress index, *TPB* total plant biomass, *SPC* South Pacific Community, *WUE* water use efficiency.

2.2 Introduction

Taro (*Colocasia esculenta* (L.) Schott) is one of the most consumed tropical crops in the world. Corms contribute significantly to food and nutrients intake, being the second-most group of cultivated species after cereals (Sharma and Kaushal, 2016). In 2018, Africa registered 74% of worldwide taro production, near 7.9 Mt (FAOSTAT, 2020).

Water scarcity is currently one of the most devastating abiotic stresses with a great impact on crop productivity, and populations' food security and subsistence, which is expected to be aggravated with ongoing climatic changes (Ganança et al., 2018; Ganança et al., 2015). Taro needs a high water supply to obtain optimal

yields, about 2,500 mm rainfall per year (Ganança et al., 2018). Therefore, water scarcity and severe weather events were expected to affect negatively this crop productivity (Ganança et al., 2015). The drought stress occurs with the soil water availability reduction, and with the plant water loss through evapotranspiration due to the atmospheric conditions (Motsa et al., 2015).

The adaptive plant response to water scarcity could be associated with their physiological and biochemical resistance mechanisms, where the wider their adaptation capability, the greater is their protection toward different stresses (Zlatev and Lidon, 2012). Plants rely on intrinsic physiological mechanisms, such as phenotypic flexibility and/or drought avoidance to tolerate water scarcity (Farooq et al., 2009).

Drought avoidance mechanisms reduces the water loss through transpiration, maintaining the water uptake and root biomass accumulation under water scarcity (Farooq et al., 2009). Water use efficiency (WUE) is essential for plant drought tolerance discrimination. The most tolerant taro accessions can increase or maintain WUE under drought, improving or showing a small decrease in total plant biomass (TPB) and yield (Gouveia et al., 2019a; Ganança et al., 2018). Drought can lead to a nutrient deficiency, since the amount of water availability and nitrogen (N) absorption are strongly correlated (Duman, 2012). Tolerant accessions exhibit higher yield and nitrogen use efficiency (NUE), under low input or drought conditions (Yuan and Peng, 2017). NUE can be calculated according to the crop and its harvest (Good et al., 2004). NUE is the result of the nutrient uptake efficiency (NUpE), harvest index (NHI), incorporation efficiency (efficiency ratio, NER) and plant utilization efficiency (E), differentiating accessions by their ability to nutrient absorption and use to obtain maximum yields (Lammerts van Bueren and Struik, 2017; Mathur and Goel 2017; Siddiqi and Glass 1981). NUE has been mostly used in grain crops, with very few studies realized on root crops, such as taro, sweet potato and cassava (Gouveia et al., 2019b; Lammerts van Bueren and Struik, 2017; John et al., 2016; Hartemink et al., 2000).

Major food crops could have distinct magnitude in the range of their phenotypic flexibility, varying according to the intensity of the abiotic stress (Jaradat 2018). The phenotypic flexibility consists in the plant growth capacity during drought, where the roots and leaves are the main affected organs, playing both key roles in the adaptation or responses to drought (Farooq et al., 2009). Usually, the roots are the key organ in the plant adaptation to drought. Cotton and tea accessions improved the root functioning and growth under drought, allowing them to maintain the leaf area and growth, during prolonged stress (Farooq et al., 2009). Rundel and Sharifi (1993) hypothesized that root:shoot ratio (R:S) retains an appropriate balance allowing the maintenance of WUE during the variation of water availability along the plant life. The lack of water could reduce the leaf area development and thus can increase the R:S, whose ratio usually is greater in water limited plants (Hubick and Gibson, 1993; Laureti et al., 1993). Leshem and Kuiper (1996) postulated that under water-limiting conditions, plants can promote osmoregulation through GAS (general adaptation syndrome), ceasing completely the shoot growth, but still displaying availability to root elongation. Root crops can also have a typical R:S ratio increase through age, due to the investment of carbon in the underground organs (Atwell et al., 1999). The increase of photosynthetic rate is another indicator of the plant's ability to tolerate this abiotic stress, where the higher plant resistance to drought appears correlated with the highest values of chlorophyll index (Gouveia et al., 2018; Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Pereira et al., 2015, Mabhaudhi and Modi, 2015; Tiwari and Mamrutha, 2013).

To better understand how these strategies of phenotypic flexibility and stress avoidance encompass the taro's capacity to cope with drought, we assessed the nutrient, carbon and water allocation, chlorophyll rate, and stress intensity of taro whole-plants submitted to prolonged water scarcity stress. Thus, we aimed to increase our knowledge of how abiotic stress affects the taro's intrinsic physiological mechanisms under drought conditions.

2.3 Materials and Methods

2.3.1 Experimental sites and drought management

Taro (*Colocasia esculenta* L.) experimental design and watering regimes was performed during a 9 months' full plant growth cycle in 2015, according to Gouveia et al. (2018), in an open greenhouse in the Preces experimental station (32º39'N, 16º58'W, 188 m a.s.l., Câmara de Lobos, Madeira, Portugal) (Figure 2.1). Seven taro accessions from Madeira and Canary Islands, and Pacific Community (SPC, Fiji) collection (Table 2.1) where used. During seven months, three rows (replicates) from control were maintained at field capacity, and another three rows for drought received 40.2% of water applied to control. Four plants per accession were distributed in each row. The greenhouse average temperature was 25.12 ºC, with an average relative humidity of 49.94%, during the drought assay. All the experiment was implement in a soil free of chemical contaminants, without addition of any fertilizers or phytopharmaceutical products. Weeds were removed manually at regular intervals, to prevent interference in the crops yield.

2.3.2 Harvest and sample preparation

At the end of the assay, 336 corms and shoots (considering petioles and leafs) samples of control and drought experimental rows were harvest, washed, sliced, oven-dried at 65ºC until constant weight during approximately 48 h (Memmert UF260, Germany), and milled (IKA-Werke M20, USA). The flour was stored in bags (Termofilm PA/PE) sealed by vacuum (Audionvac VMS153, Netherlands) at -35ºC (Liebherr ProfiLine GGPV6570, Germany) until analysis.

2.3.3 Soil chemical and physical properties

Air-dried soil samples were grinded, sieved (2 mm) and analysed by the Agriculture Quality Laboratory at the Directory of Laboratory and Agro-Food Research Services, in Camacha, Madeira, Portugal. The soil chemical and physical properties were evaluated for: pH H_2O (1:2.5 w/v); pH KCl (1:2 w/v); organic matter according to Walkley and Black method; ammonia and nitrate content by continuous-flow auto analyser (3:15 w/v); the soil particle-size and texture were classified following the World Reference Base for Soil Resources (IUSS, 2015).

2.3.4 Chlorophyll content index (CCI)

A chlorophyll fluorescence technique (Opti-Sciences CCM-200 PLUS, USA) determined the CCI in taro fresh leaves. An uniform reading along the adaxial leaf surface (left, centre and right sides) was made, avoiding the branching veins. A mean CCI value per main plant leaf in each row was determined.

2.3.5 Nitrogen content (N)

The nitrogen content of the sample flours was determined by the Kjeldahl method AOAC 945-18-B (AOAC, 2005), through a distillation and titration automatic unit (Velp Scientifica UDK 152, Italy). All analyses were performed in triplicate, and the values expressed in g/100 g dry flour.

2.3.6 N efficiency ratio (NER)

NER was calculated as a nutrient efficiency ratio (Steenbjerg and Jakobsen, 1963):

$$NER = \frac{W}{N}, \quad (1)$$

where *W* is the plant dry biomass, and *N* is the plant nitrogen uptake. Calculations were performed in triplicate, and the values expressed in kg/kg of dry flour.

2.3.7 N efficiency of utilization (E)

The E was calculated according to Siddiqi and Glass (1981):

$$E = W \times NER, \quad (2)$$

where *W* the product of the absolute biomass production, and *NER* is the nutrient efficiency ratio. Calculations were performed in triplicate, and the values expressed in kg of dry flour.

2.3.8 N harvest index (NHI)

The NHI was calculated according with Kołodziejczyk (2014):

$$NHI = \frac{Nt}{N}, \quad (3)$$

where the *Nt* is the N uptake in corm, and *N* is the N uptake by the whole-plant. Calculations were performed in triplicate, and the values expressed in % of dry flour.

2.3.9 Root-to-shoot ratio (R:S)

The ratio between the corms and shoots dry biomass was calculated for both control and drought experimental conditions, according to Laureti et al. (1993).

2.3.10 Total mineral content (M)

Total mineral was gravimetrically determined by sample flour calcination in a furnace (Vulcan Model 3-550, NEY, USA) at 550 ± 10°C during 5h, according to the method AOAC 923.03 (AOAC, 2005). The analyses were performed in triplicate, and the values expressed in g/100g of dry flour.

2.3.11 Total plant biomass (TPB)

TPB represents the average of total dry weight from corms and shoots obtained from the replicates, that were dehydrated in an air oven (Memmert UF260, Germany) (Undersander et al., 1993). Results are expressed in g of dry flour.

2.3.12 Water use efficiency (WUE)

WUE was the ratio between the total plant dry biomass and total water used per plant, expressed in g/L (Ganança et al., 2018).

2.3.13 Whole-plant stress index (SI)

The whole-plant stress index (SI) was calculated (Robinson et al. 2000):

$$SI = \frac{W_{unstressed} - W_{stressed}}{W_{unstressed}}, \quad (4)$$

where the *W* represents the mean dry weight of the whole-plant. The SI ranges from 0 to 1, conferring the effect of the environment on plant growth. The plant SI values tend toward 0 when less sensitive to stress (SI $\rightarrow$ 0), and to 1 with the increase of stress sensitivity (SI $\rightarrow$ 1).

2.3.14 Data analysis

The results represent the mean ± standard deviation of corms and shoots of 3 control *vs* 3 drought replicates, expressed in a dry weight basis. All samples were statistically evaluated with IBM SPSS Statistics 24.0 for Mac, for one-way analysis of variance (One-Way ANOVA), Tukey's Honestly Significance Difference Post Hoc test (Tukey's HSD), and Pearson correlations, signalizing the significant differences found ($p \leq 0.05$).

2.4 Results

2.4.1 WUE, biomass, chlorophyll and nutrient use interactions during drought

All taro accessions decreased their biomass content, when under drought, showing a stress avoidance mechanism. Although, they had distinct physiological responses for the water allocation, chlorophyll content rate and nutrient use at the whole-plant level. The analysis of variance showed a significant difference between control and stress conditions for CCI, TPB, WUE, E, and NHI, with Tukey's HSD test signalizing the accessions that were significantly different from which others, presented in Table 2.2.

Total plant biomass (TPB) of taro accessions (acc.) in average decreased from 61.6 to 45.1 g (-26.8%),

with acc. 2210 and 2061 showing the lowest significant weight loss. All taro acc. increased WUE, registering an average increase from 0.20 to 0.37 g/L (+85.0%), minimizing the water loss through transpiration, specially for the acc. 2056, 2061 and 2216 that registered the highest significant WUE increase. Chlorophyll content index (CCI) in average was near 33. The acc. showed an average increase of CCI from 32.7 to 33.3 (+1.8%), with acc. 2210, 2061 and 2216 reaching the highest significant values.

N efficiency ratio (NER) decreased from 15.3 to 12.2 kg/kg (-20.3%), without the observation of significant differences between accessions. The acc. 2234 and 2061 were the exceptions by increasing NER.

The variation of N efficiency of utilization (E) decreased in average from 5.7 to 4.3 kg (-24.6%). The acc. 2056 registered the highest E decrease, while acc. 2061 slightly increased E, without showing significant differences between them. The average N harvest index (NHI) slightly increased from 29.3 to 29.5% (+0.2%), with acc. 2216 and 2239 showing the highest significant NHI values, meanwhile the acc. 2232 and 2239 were the ones that presented a NHI decrease due to drought.

2.4.2 Nutrient efficiency ability during drought

Drought modified the taro nutrient allocation between underground corms and aboveground shoots organs, through the mineral (M), nitrogen (N), NER and E traits. The analysis of variance showed a significant difference between control and stress conditions for M-corm, N-corm, NER-corm, E-corm, N-shoot and NER-shoot, with Tukey's HSD test signalizing the accessions that were significantly different from which others, present in Table 2.3.

Taro corms showed a lower M content in comparison with shoots, in both experimental variants. Although, drought increased the corm and shoot M content of all accessions. The acc. 2210 and 2216 shoots were exceptions, as they slightly diminished the M-shoot content to water stress. In average, taro M-shoot increased from 9.2 to 10.0 g/100g (+0.8%), with acc. 2232, 2234 and 2239 showing the highest content, but without significant differences between accessions. The taro M-corm, in average, increased between 4.1 and 4.7 g/100g (+0.6%), with acc. 2239 and 2216 having significantly higher content during drought.

Taro shoots exhibited a higher N content relatively to corms, in both experimental variants. Although, both taro corms and shoots slightly increased the N content, under water scarcity. In average, N-corm slightly increased from 0.8 to 0.9 g/100g (+0.1%), and the N-shoot from 1.8 to 2.0 g/100g (+0.2%), between control and drought conditions, respectively. When in drought, acc. 2216 presented the highest significant N-corm content, meanwhile acc. 2210 had the highest significant N-shoot content.

This nutrient variation registered among taro organs have different efficiency in their use. We observed that both NER and E were higher in the corms than in the shoots, but decreased on both organs, under drought. Water scarcity decreased the NER-corm from 40.1 to 30.0 kg/kg (-25.2%) and NER-shoot from 6.7 to 5.7 kg/kg (-14.9%), respectively. Meanwhile, E-corm decreased from 11.2 to 7.3 kg (-34.8%), and shoot E did not vary (0.7 kg) under stress. The corm from acc. 2234 showed the highest significant NER and E content in drought conditions.

To help the further discussion about this nutrient variation in the taro accessions, we registered at 0.2 m of pot soil sampling depth, a slightly acidic pH 5.9, silt clay loam texture and high inorganic mineral content (NO_3^- and NH_4^+) (data not shown).

2.4.3 Stress index and root-to-shoot relationship to drought

The TPB loss in all accessions was registered as a consequence of water shortage, but distinct responses in the plant organs growth can be explained by the phenotypic flexibility expressed through the R:S and SI estimation (Figure 2.2).

The R:S was used to evaluate the plant ability to maintain a dynamic balance between the functionally interdependent organs, such as corm and shoots, during drought. In control conditions, taro R:S ranged from 1:4 to 1:18, for acc. 2216 and 2210, respectively. Taro accessions showed a natural difference between the organs weight, with shoots being 4 to 18 times lighter than corms. In general, drought decreased the R:S due to an increase investment in the shoot development. The exception was for taro acc. 2056 (1:6 to 1:8, +39.5%)

and 2210 (1:18 to 1:28, +56.1%). These accessions showed a R:S increase, indicating that they decreased their shoot development in detriment of corm growth. The acc. 2210 showed the highest biomass variation between organs, both in control and drought conditions. On the other hand, acc. 2239 (1:6 to 1:3, -49.4%) showed the highest investment in shoot development, and the biggest R:S ratio reduction.

The SI was used to explain the differences of drought stress level in terms of its impact on accessions innate growth aptitude ($0 < SI < 1$). Acc. 2061, 2210 and 2234 registered the lowest SI values, around 0.1 ($SI \rightarrow 0$), showing to be the lowest drought-stressed acc., with a higher growth capacity and the best tolerance response to water scarcity. In opposition to this, the acc. 2239 was the most stressed, with SI value 0.5 ($SI \rightarrow 1$).

2.4.4 Pearson correlation coefficients between traits

Forty-five significant correlations were identified between the 16 traits in study, of which 25 are considered strong with $r \geq 0.50$ (Table 2.4). The TPB and WUE had a strong positive correlation. CCI showed negative moderate correlations with NER-shoot and M-shoot. R:S had negative moderate associations with NHI, NER-shoot and E-shoot. The N-corm showed positive correlations with NHI and M-corm, and negative correlations with E, NER-corm and E-corm. The whole-plant NER also had a negative moderate correlation with taro N-corm. SI was strong negative correlated with CCI and N-shoot.

2.5 Discussion

2.5.1 Relation between water scarcity and plant development

The present study provided important information about the physiological responses of taro accessions, when submitted to drought stress. The water shortage led to biomass loss in all studied taro acc., a drought avoidance response related with phenotypic flexibility in organs' growth. Along with the loss of biomass, the variation of CCI and WUE showed similarities among the accessions.

During drought, on average more than a quarter of the TPB was lost. According to Atwell et al. (1999), plants can adjust constantly their shoot and root growth rate according to available resource capture, in order to increase their biomass content. The taro acc. maintained a dynamic balance between both underground and aboveground organs during drought, showing a R:S ratio decrease, which indicates the generalized trend for developing the shoot rather than corm. Accessions 2056 and 2210 were exceptions, presenting a R:S increase and preferential corms development instead of investing energy in the shoots growth. Possibly, these acc. showed an increased trend for sustained carbon investment in underground structures, like Atwell et al. (1999) proposed to be possible for root plants growth. Being the acc. with the smallest canopies, they showed the capacity to prioritize corm over shoot development, reducing the shoot area as a way to prevent shoot water deficits, increasing its WUE and photosynthesis efficiency (Motsa et al., 2015; van den Boogaard et al., 1995). This behaviour can be associated to an osmoregulation mechanism as GAS response to drought, according to Leshem and Kuiper (1996). Although these acc. invested the carbon in underground corms, works in cotton and peanut accessions also showed a leaf area reduction when the plant had water or nutrient deficit, leading likewise to a greater R:S (Hubick and Gibson, 1993; Laureti et al., 1993; Harris, 1992; Atwell et al., 1999). Despite the generalized biomass loss in all taro acc., low SI was recorded in acc. 2061, 2210 and 2234. The low SI indicated a small TPB difference between control and drought, showing the best ability to grow under drought.

The CCI indirectly measured the difference of the plant photosynthetic rate, throughout the comparison of the intensity of photosynthetic electron transport (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). This index varies with the plant tolerance to drought, where the higher the CCI value indicates greater plant capacity to cope with stress (Gouveia et al., 2018; Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Tiwari and Mamrutha, 2013). The taro accessions showed a 1.8% increase in chlorophyll content towards drought. According to Pereira et al. (2015), it corresponds to an increase of photosynthesis and consequently an increase

in plant production potential and vigour. Gouveia et al. (2019a, 2018) noticed that the CCI increase of taro under drought conditions, was an indication of the taro capacity to keep partially open stomata, allowing a higher CO_2 absorption and electron transport from the available H_2O. The excitation of photosystem PSII by light photons can allow the ionization of chlorophyll molecules and electron transport, through electron transport chain with generation of ATP and NADPH (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). The decrease of photorespiration is another probable factor with enhance of CCI during drought. A partially open stomata could allow the increase of the leaf intracellular CO_2/O_2 ratio and the carboxylase activity, with inhibition of oxygenase function of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), and consequently decreasing photorespiration (Igamberdiev et al., 2004; Igamberdiev et al., 2001). As CCI increased, the photorespiration was unlikely to occur during the daylight, with no need for chloroplasts protection from photoinhibition (Prasad et al., 2008; Igamderdiev et al., 2004; Igamderdiev et al., 2001). Nonetheless, Mabhaudhi and Modi (2015) used the same chlorophyll fluorescence measurement technique, but registered a decrease of CCI in South African taro landraces when drought-stressed.

WUE is an usual measure of the plant drought resistance, with the more tolerant accessions usually showing higher WUE (Gouveia et al., 2019a; Ganança et al., 2018). Linked to stomatal aperture, WUE could represent the ratio of biomass (produced by CO_2 assimilation in photosynthesis) to water loss by transpiration (Igamberdiev et al., 2004). When drought-stressed, all taro acc. increased WUE, in average by 85%, with acc. 2216 showing the highest value. This increase can be due to the partial reduction of the stomatal aperture in transpiration. Gouveia et al. (2019a) indicated that in spite of taro showing partially open stomata under drought, they were able to maintain leaf turgidity, by minimizing water loss through transpiration and improving water use in metabolic and physiological processes. Therefore, strong correlation of WUE with CCI and TPB obtained under drought supports these finds. According to Farooq et al. (2009), plants that allocate nutrients and improve WUE are more drought-tolerant, than that one's not showing such behaviour.

2.5.2 The role of plant nutrient use during drought

N is a primary mineral nutrient that comprises about 80% of the total nutrients absorbed by plants (Kaur et al., 2017; Duman, 2012). Plants usually uptakes the N in the inorganic form, either as nitrate (NO_3^-) or ammonium (NH_4^+), where NO_3^- is usually the major source of N for plants (Kaur et al., 2017; Sahoo et al., 2010; Wang et al., 2009).

Duman (2012) reported that the soil water availability was strongly related with the plant nitrogen absorption. The plant nutrient transport from the roots to the shoots can decrease due to the lack of soil moisture during drought, hindering the N availability and absorption by roots, and its uptake and utilization by plant (Duman, 2012). However, we did not observe that behaviour. In overall, drought changed the nutrient allocation in taro, by registering a slight increase of N in both organs. In fact, the accessions showed a higher M and N content in shoots in both environmental conditions. The soil pH can also be an important factor to determine the nutrient bioavailability, were the ideal pH is close to neutral (6.5 to 7.5) to allow a better root absorption (Jensen, 2010). The soil pH of the taro experimental pots was slightly lower than the mentioned above, and with the high inorganic mineral content, it facilitated the nutrient allocation from the underground corms to the aboveground shoots, whose nutrients were used mainly for leaf and corm growth.

The N-shoot accumulation can be related with the plant use of N to increase the rate of photosynthesis, while the N-corm can be used for the synthesis of proteins for the regulation of cellular defence and detoxification processes (Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Van den Boogaard et al., 1995). The relation between the N allocation and use was assessed by NER, to differentiate accessions into efficient and inefficient nutrient use (Mathur and Goel, 2017; Good et al., 2004); by E, to allow a comparison between the increase of the produced biomass with NER (Siddiqi and Glass, 1981); and by NHI, that describes the share of nitrogen accumulated in corm yield in relation to total plant nitrogen uptake (Kołodziejczyk, 2014). We observe that the increase of N-shoot and N-corm was significantly correlated with NER and E decrease. Siddiqi and Glass (1981) mentioned that NER decrease could be due to the higher accumulation of nutrients in whole-plant instead of biomass production. As taro accessions preferred to accumulate N in both organs, they use it

for increase photosynthesis and protein synthesis. Van den Boogaard et al. (1995) also mentioned that the N-shoot increase could be associated with an increase in the rate of photosynthesis.

The N accumulation in both taro organs also decreased the N efficiency of utilization (E), in relation to the decrease of NER into TPB production. With drought, the N-corm was negatively correlated with E, NER-corm and E-corm, and positively with M-corm and NHI, confirming a good nutrient allocation between whole-plant and corm. Kaur et al. (2017) also registered a positive correlation between NHI and N content among wheat accessions.

2.6 Conclusion

Taro accessions with the best capability to cope and avoid drought increased their R:S ratio, lowered their SI and reduced their TPB loss. The increase of NER, WUE, CCI and N content also allowed the improvement of the photosynthesis rate and support metabolic and physiological processes, under drought conditions. The acc. 2216 and 2210 showed to be the most tolerant ones, being good candidates for taro breeding programs, due to their phenotypic flexibility and drought avoidance response in prolonged stress conditions.

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2.8 Authors' contribution

Carla Gouveia participated on the drought assay and samples preparation, performed the N and M analysis, obtained the NER, E, NHI, R:S and SI, interpreted and summarized all data generated from those experiments, and wrote the manuscript. José Ganança designed the drought assay, quantified WUE and contributed to CCI determination. José de Freitas e Humberto de Nóbrega significantly contributed with the greenhouse assay management and with meaningful support in the harvesting and preparation of samples, and obtaining TPB. Vincent Lebot and Miguel Carvalho coordinated the work and revised the manuscript.

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Tables

Table 2.1
Name and origin of taro (*Colocasia esculenta* L.) accessions selected for this study.

Acc. ID[a]	Accession local name	Origin
2056	Listado	Canary Islands – La Palma
2061	Blanco Saucero	Canary Islands – La Palma
2210	Roxo	Madeira Island
2216	Branco	Madeira Island
2232	PExPH 15-6 BL/HW/08	SPC, Fiji
2234	C3-22 BL/PNG/11	SPC, Fiji
2239	Karang CE/MAL/10	SPC, Fiji

[a] Accession identification number code used by the ISOPlexis Genebank.

Table 2.2

Chlorophyll content index (CCI), total plant biomass (TPB), water use efficiency (WUE), N efficiency ratio (NER), N efficiency utilization (E) and N harvest index (NHI) of taro (*C. esculenta*) whole-plant accessions.

			CCI§§	TPB§§ (g)	WUE§§ (g/L)	NER (kg/kg)	E§ (kg)	NHI§§ (%)
***Colocasia esculenta* L.**								
CAN	2056	Control	37.4 ± 7.5 ^{abcd}	87.5 ± 23.6 ^{cd}	0.29 ± 0.08 ^{abc}	17.9 ± 3.3 ^{a}	6.9 ± 1.5 ^{ab}	27.8 ± 4.0 ^{abcde}
		Drought	38.7 ± 15.6 ^{abcd}	46.9 ± 14.5 ^{abc}	0.39 ± 0.12 ^{bc}	11.0 ± 1.7 ^{a}	3.2 ± 0.7 ^{ab}	27.3 ± 4.9 ^{abcde}
	2061	Control	40.3 ± 2.0 ^{abcd}	73.8 ± 17.8 ^{bcd}	0.23 ± 0.03 ^{ab}	14.5 ± 4.7 ^{a}	5.1 ± 2.3 ^{ab}	26.6 ± 1.5 ^{abcd}
		Drought	43.4 ± 6.8 ^{cd}	69.9 ± 17.3 ^{bcd}	0.48 ± 0.16 ^{cd}	15.0 ± 1.2 ^{a}	5.7 ± 0.8 ^{ab}	32.0 ± 2.4 ^{bcde}
MAD	2210	Control	38.3 ± 7.9 ^{abcd}	37.8 ± 10.5 ^{ab}	0.12 ± 0.04 ^{a}	11.5 ± 3.1 ^{a}	3.7 ± 1.8 ^{ab}	23.0 ± 2.9 ^{ab}
		Drought	46.9 ± 13.4 ^{d}	33.4 ± 10.4 ^{ab}	0.28 ± 0.09 ^{abc}	9.1 ± 0.6 ^{a}	2.7 ± 0.6 ^{a}	26.2 ± 3.1 ^{abc}
	2216	Control	41.3 ± 4.3 ^{bcd}	95.7 ± 35.3 ^{d}	0.32 ± 0.12 ^{abc}	11.3 ± 1.8 ^{a}	4.1 ± 0.8 ^{ab}	36.7 ± 3.9 ^{def}
		Drought	44.8 ± 13.7 ^{cd}	75.3 ± 9.1 ^{bcd}	0.67 ± 0.11 ^{d}	9.8 ± 0.5 ^{a}	3.4 ± 0.3 ^{ab}	37.4 ± 3.1 ^{ef}
SPC	2232	Control	18.6 ± 7.0 ^{abcd}	40.5 ± 4.2 ^{ab}	0.14 ± 0.01 ^{a}	18.6 ± 4.3 ^{a}	7.7 ± 3.0 ^{b}	27.7 ± 2.2 ^{abcde}
		Drought	16.1 ± 6.1 ^{abc}	23.2 ± 5.6 ^{a}	0.19 ± 0.05 ^{ab}	11.8 ± 4.3 ^{a}	4.2 ± 2.3 ^{ab}	26.7 ± 3.5 ^{abcd}
	2234	Control	40.7 ± 16.5 ^{abcd}	52.3 ± 0.3 ^{abcd}	0.18 ± 0.00 ^{ab}	15.4 ± 3.2 ^{a}	6.9 ± 1.7 ^{ab}	18.6 ± 1.6 ^{a}
		Drought	30.8 ± 10.0 ^{abcd}	46.5 ± 12.3 ^{abc}	0.39 ± 0.10 ^{bc}	16.8 ± 1.2 ^{a}	6.7 ± 1.2 ^{ab}	20.5 ± 1.0 ^{a}
	2239	Control	12.4 ± 3.6 ^{a}	43.3 ± 12.6 ^{abc}	0.15 ± 0.04 ^{ab}	18.0 ± 6.7 ^{a}	5.8 ± 1.4 ^{ab}	44.5 ± 6.6 ^{f}
		Drought	12.7 ± 5.2 ^{ab}	20.4 ± 5.3 ^{a}	0.17 ± 0.04 ^{ab}	11.9 ± 2.2 ^{a}	4.0 ± 1.7 ^{ab}	36.2 ± 2.6 ^{cdef}
	Mean	Control	32.7	61.6	0.20	15.3	5.7	29.3
		Drought	33.3	45.1	0.37	12.2	4.3	29.5
	Min	Control	12.4	37.8	0.12	11.3	3.7	18.6
		Drought	12.7	20.4	0.17	9.1	2.7	20.5
	Max	Control	41.3	95.7	0.32	18.6	7.7	44.5
		Drought	46.9	75.3	0.67	16.8	6.7	37.4

Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent replications per accession, with total mean, minimum and maximum per trait.

§,§§ Significant differences between control and drought stress conditions (One-way ANOVA, §$p \leq 0.05$; §§$p \leq 0.01$).

Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$).

Control is well-watered, drought is water scarcity.

Table 2.3

Mineral (M), nitrogen (N), N efficiency ratio (NER) and N efficiency utilization (E) from the corms and shoots of taro (*C. esculenta*) accessions.

			M (g/100g)		N (g/100g)		NER (kg/kg)		E (kg)	
Colocasia esculenta L.			**Corm§§**	**Shoot**	**Corm§§**	**Shoot§§**	**Corm§§**	**Shoot§**	**Corm§§**	**Shoot**
CAN	2056	Control	3.9 ± 0.1 ^{abcd}	8.0 ± 0.6 ^{a}	0.6 ± 0.2 ^{ab}	1.6 ± 0.2 ^{ab}	49.4 ± 14.6 ^{cde}	6.2 ± 0.9 ^{ab}	14.2 ± 4.7 ^{bcd}	0.6 ± 0.1 ^{a}
		Drought	5.1 ± 0.5 ^{cdef}	9.2 ± 0.5 ^{a}	0.7 ± 0.2 ^{abc}	1.9 ± 0.2 ^{abcd}	28.6 ± 9.5 ^{abc}	4.7 ± 0.4 ^{ab}	5.7 ± 2.2 ^{ab}	0.4 ± 0.0 ^{a}
	2061	Control	4.1 ± 0.3 ^{abcde}	8.4 ± 0.6 ^{a}	0.6 ± 0.1 ^{ab}	1.8 ± 0.3 ^{abcd}	37.5 ± 11.0 ^{abcde}	6.1 ± 2.0 ^{ab}	9.1 ± 3.9 ^{abc}	0.7 ± 0.3 ^{a}
		Drought	4.5 ± 0.6 ^{bcdef}	9.2 ± 0.3 ^{a}	0.8 ± 0.0 ^{abc}	1.7 ± 0.1 ^{abc}	32.9 ± 3.8 ^{abcd}	6.7 ± 1.3 ^{ab}	8.7 ± 1.9 ^{abc}	0.8 ± 0.3 ^{a}
MAD	2210	Control	4.0 ± 0.5 ^{abcde}	9.7 ± 1.3 ^{a}	0.6 ± 0.2 ^{ab}	2.0 ± 0.3 ^{abcd}	40.3 ± 13.2 ^{abcde}	3.3 ± 1.5 ^{a}	10.0 ± 4.7 ^{abcd}	0.3 ± 0.2 ^{a}
		Drought	4.1 ± 1.2 ^{abcde}	9.6 ± 0.5 ^{a}	0.9 ± 0.2 ^{abc}	2.4 ± 0.1 ^{cd}	25.6 ± 1.6 ^{abc}	3.1 ± 0.7 ^{a}	5.8 ± 1.8 ^{ab}	0.2 ± 0.1 ^{a}
	2216	Control	5.6 ± 0.8 ^{def}	10.3 ± 2.2 ^{a}	1.2 ± 0.2 ^{bc}	2.0 ± 0.1 ^{abcd}	21.7 ± 5.3 ^{ab}	5.5 ± 0.6 ^{ab}	5.4 ± 1.5 ^{ab}	0.6 ± 0.1 ^{a}
		Drought	5.7 ± 0.5 ^{ef}	9.9 ± 0.3 ^{a}	1.3 ± 0.1 ^{c}	2.2 ± 0.1 ^{bcd}	17.7 ± 2.0 ^{a}	5.1 ± 0.3 ^{ab}	4.1 ± 0.5 ^{ab}	0.6 ± 0.1 ^{a}
SPC	2232	Control	3.6 ± 0.5 ^{abc}	10.2 ± 0.7 ^{a}	0.6 ± 0.0 ^{ab}	1.6 ± 0.2 ^{ab}	46.0 ± 7.0 ^{bcde}	8.0 ± 2.9 ^{ab}	13.0 ± 3.4 ^{abcd}	1.1 ± 0.7 ^{a}
		Drought	4.3 ± 0.5 ^{abcde}	11.2 ± 2.0 ^{a}	0.8 ± 0.0 ^{abc}	2.0 ± 0.3 ^{bcd}	30.8 ± 8.6 ^{abcd}	4.8 ± 2.1 ^{ab}	7.6 ± 3.7 ^{abc}	0.5 ± 0.3 ^{a}
	2234	Control	2.7 ± 0.2 ^{a}	8.3 ± 0.8 ^{a}	0.6 ± 0.1 ^{ab}	2.5 ± 0.3 ^{d}	60.5 ± 17.2 ^{e}	5.2 ± 1.0 ^{ab}	19.8 ± 6.4 ^{d}	0.7 ± 0.2 ^{a}
		Drought	3.3 ± 0.0 ^{ab}	10.5 ± 0.6 ^{a}	0.5 ± 0.0 ^{a}	1.9 ± 0.1 ^{abcd}	57.1 ± 6.8 ^{de}	6.4 ± 0.5 ^{ab}	16.0 ± 4.3 ^{cd}	0.8 ± 0.1 ^{a}
	2239	Control	4.7 ± 0.6 ^{bcdef}	9.6 ± 1.8 ^{a}	1.2 ± 0.6 ^{bc}	1.4 ± 0.4 ^{a}	25.5 ± 8.6 ^{abc}	12.4 ± 5.6 ^{b}	6.6 ± 2.2 ^{abc}	1.4 ± 0.6 ^{a}
		Drought	6.1 ± 0.8 ^{f}	10.2 ± 0.2 ^{a}	1.0 ± 0.1 ^{abc}	1.7 ± 0.1 ^{abcd}	17.3 ± 2.9 ^{a}	9.0 ± 4.1 ^{b}	2.9 ± 0.6 ^{a}	1.6 ± 1.5 ^{a}
	Mean	Control	4.1	9.2	0.8	1.8	40.1	6.7	11.2	0.7
		Drought	4.7	10.0	0.9	2.0	30.0	5.7	7.3	0.7
	Min	Control	2.7	8.0	0.6	1.4	21.7	3.3	5.4	0.3
		Drought	3.3	9.2	0.5	1.7	17.3	3.1	2.9	0.2
	Max	Control	5.6	10.3	1.2	2.5	60.5	12.4	19.8	1.4
		Drought	6.1	11.2	1.3	2.4	57.1	9.0	16.0	1.6

Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent replicates per accession, with total mean, minimum and maximum per trait.

§,§§ Significant differences between control and drought stress conditions (One-way ANOVA, $^{§}p \leq 0.05$; $^{§§}p \leq 0.01$).

Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$).

Control is well-watered, drought is water scarcity.

Table 2.4

Pearson correlation coefficients of the analysed traits of taro (*C. esculenta*) in control and drought stress conditions.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. CCI	-														
2. TPB	**0.48****	-													
3. WUE	**0.48****	**0.55****	-												
4. NER	-0.29	0.26	-0.10	-											
5. E	-0.18	0.27	-0.06	**0.88****	-										
6. NHI	-0.22	0.10	0.14	-0.04	-0.14	-									
7. R:S	0.19	-0.26	-0.24	-0.26	-0.27	**-0.34***	-								
8. SI	**-0.76***	-0.39	-0.35	0.69	0.69	0.62	-0.47	-							
9. M-corm	-0.12	0.08	0.28	**-0.38***	**-0.53****	**0.53****	-0.23	-0.01	-						
10. M-shoot	**-0.36***	-0.08	0.09	0.11	-0.01	0.04	-0.13	-0.15	**0.34***	-					
11. N-corm	0.01	0.04	0.22	**-0.44****	**-0.34***	**0.83****	-0.24	0.43	**0.43****	-0.08	-				
12. N-shoot	**0.45****	-0.16	0.10	**-0.69****	**-0.44****	**-0.35***	0.21	**-0.80***	-0.17	-0.14	0.19	-			
13. NER-corm	-0.02	0.17	-0.12	**0.68****	**0.74****	**-0.70****	0.07	-0.27	**-0.68****	-0.03	**-0.76****	-0.15	-		
14. NER-shoot	**-0.43****	0.03	-0.09	**0.64****	**0.65****	**0.42****	**-0.49****	**0.78***	0.05	0.12	0.11	**-0.63****	0.08	-	
15. E-corm	-0.01	0.22	-0.09	**0.69****	**0.79****	**-0.60****	0.02	-0.20	**-0.71****	-0.06	**-0.63****	-0.10	**0.98****	0.12	-
16. E-shoot	**-0.37***	-0.09	-0.12	**0.46****	**0.51****	**0.40****	**-0.35***	0.65	0.04	0.12	0.20	**-0.41****	-0.04	**0.94****	0.01

CCI chlorophyll content index of shoots; *TPB* total plant biomass (g, DW); *WUE* water use efficiency (g/L, DW); *NER* nitrogen efficiency ratio (Kg/Kg DW); *E* nitrogen efficiency utilization (Kg, DW); *NHI* nitrogen harvest index (%, DW); *R:S* root-to-shoot ratio; *SI* whole-plant stress index; *M-corm* total mineral content of corms (g/100g, DW); *M-shoot* total mineral content of shoots (g/100g, DW); *N-corm* total nitrogen content of corms (g/100g, DW); *N-shoot* total nitrogen content of shoots (g/100g, DW); *NER-corm* corm nitrogen efficiency ratio (Kg/Kg DW); *NER-shoot* shoot nitrogen efficiency ratio (Kg/Kg DW); *E-corm* corm nitrogen efficiency utilization (Kg, DW); *E-shoot* shoot nitrogen efficiency utilization (Kg, DW).

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Figures

Figure 2.1
Taro greenhouse development at the beginning (left) and end (right) of the drought assay.

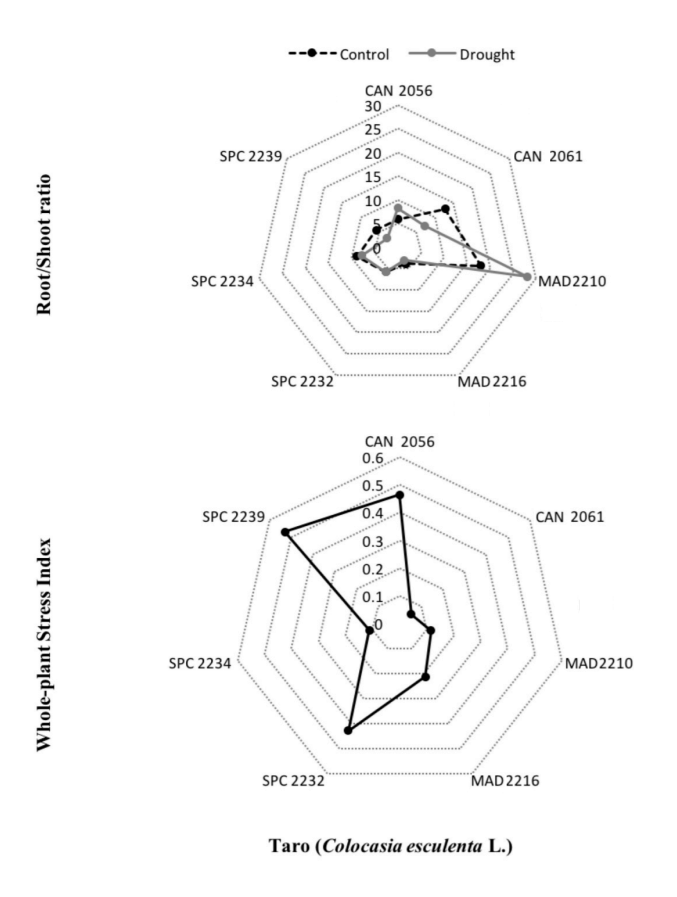

Figure 2.2
Stress index (SI) and root-to-shoot ratio (R:S) in taro (*C. esculenta*) accessions, under control and drought conditions.
Taro accessions with ISOPlexis Genebank identification number code, from *CAN* Canary Islands, *MAD* Madeira Island, *SPC* Pacific community. Data are expressed in dry weight basis, and represents the mean of three independent replicates per accession. Control is well-watered, drought is water scarcity.

CHAPTER 3

Quantitation of oxalates in corms and shoots of *Colocasia esculenta* (L.) Schott under drought conditions

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ORIGINAL ARTICLE

Quantitation of oxalates in corms and shoots of *Colocasia esculenta* (L.) Schott under drought conditions

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3.1 Abstract

Oxalate (calcium oxalate) accumulation in taro plants (*Colocasia esculenta* (L.) Schott) impacts their nutritional quality, producing acridity, causing lips, mouth and throat tissues swelling if consumed fresh. The oxalate content is related to photosynthesis, through the glycolate–glyoxylate oxidation pathway. The plant's photosynthetic rate usually increases in non-stressed conditions. Differences in photosynthetic rate are indirectly related to the chlorophyll content index. Protein accumulation and starch variation are also important traits to understand the taro oxalate synthesis caused by drought and how they affect corm quality. The purpose of this study was to quantitate oxalates in taro corms and shoots submitted to drought conditions and to evaluate how stress response can affect the nutritional quality of taro whole-plant. Seven taro genotypes from Madeira, Canaries and Pacific Community (SPC) collections were grown in greenhouse conditions and submitted to different watering regimes for drought tolerance screening. Corms and shoots were harvested and evaluated for oxalates (soluble, insoluble and total), chlorophyll content index (CCI), crude protein, starch, starch solubility in water and starch swelling power. All accessions had very high calcium oxalate content. Drought-tolerant genotypes showed good osmotic response by oxalate precipitation and mobilization through shoot to corm tissues, photosynthesis adaptation by increase of CCI, protein accumulation, and very low starch hydrolysis. Sensitive-drought genotypes showed less mobilization of calcium oxalate, decreased photosynthetic rate and protein synthesis, and slight increase of starch hydrolysis. Variation in taro oxalate content is consistent and significantly correlated with the photosynthetic rate, carbohydrate metabolism and protein synthesis.

Keywords
Carbohydrate metabolism; chlorophyll content index; *Colocasia esculenta* (L.) Schott; drought tolerance; photosynthesis; soluble and insoluble oxalates.

Abbreviations
Acc. Accession
ANOVA Analysis of variance
CCI Chlorophyll content index
CaOx Calcium oxalate
KOx Potassium oxalate
PCA Principal Component Analysis
SPC Pacific Community
S-Ox Soluble oxalates [oxalic acid]
SSP Starch swelling power
SWS Starch solubility in water
T-Ox Total oxalates

3.2 Introduction

Taro, *Colocasia esculenta* (L.) Schott (Araceae), is probably one of the oldest crops on earth that has been grown on irrigated terraces in tropical Asia for more than 10,000 years (Lebot 2009). It is a staple food in tropical and developing countries, being an important source of carbohydrates, in the form of starch stored in the corms (Sharma and Kaushal 2016; Lebot 2009). According to FAOSTAT database (http://www.fao.org, 2018), taro has the lowest yield of all root crops (cassava, sweet potato and yam). However, its worldwide yield had a slight decrease from 6.98 t/ha in 2010 to 6.06 t/ha in 2016, still demanding for food security. To obtain optimal yields, the taro plant needs a very good water supply, but the water scarcity and severe weather events are expected to have a growing negative impact on this important subsistence crop (Ganança et al. 2015; Farooq et al. 2009).

Raw taro corms, petioles and leaves are considered toxic due to the presence of calcium oxalate (CaOx) crystals (or raphides), which negatively affects their nutritional value and quality (Franceschi and Horner 1980).

Oxalate accumulation by plants is used as a protection mechanism against foraging animals, by producing acridity, causing lips, mouth and throat swelling, and kidney disorders if consumed fresh (Sharma and Kaushal 2016; Franceschi and Horner 1980). The calcium absorption and oxalic acid synthesis is important for the plant ion balance and osmoregulation, through the regulation of excess calcium ions by precipitation with oxalic acid, in the form of CaOx crystals. Generally, the intensity of the plants tissues acridity changes during the growing season. The acridity can be connected to drought, nutrient or genetic factors (Prasad and Shivay 2017; Sharma and Kaushal 2016; Libert and Franceschi 1987).

The plant oxalate synthesis is related to the photosynthetic glycolate–glyoxylate oxidation by light stimulation. The oxalate concentration usually increases during the hours of peak photosynthesis and decreases during the evening and night (Franceschi and Horner 1980). The glyoxylate can also be converted in oxalate via iso-citrate during acid metabolism in the dark, and by the Krebs cycle from sugars and certain protein amino acids (Burgess and Huang 2016; Franceschi and Horner 1980).

The leaf chlorophyll content measurement with optic detection is one of the common traits used for drought stress assessment. Differences in the photosynthetic rate can be indirectly assessed by the chlorophyll content index (CCI), where the canopy greenness is directly related with the plant's photosynthetic efficiency. During the day light, the glycolic acid is converted into oxalate, leading to an increase of oxalate production, which can be associated with the increase of the photosynthetic rate and chlorophyll content (Tiwari and Mamrutha 2013; Franceschi and Horner 1980). The photosynthetic rate increase is an indicator of the plant's ability to tolerate abiotic stress, where the better the plant resistance to drought, the higher will be the plant chlorophyll index (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Mabhaudhi and Modi 2015; Tiwari and Mamrutha 2013).

The plant starch content variation and protein accumulation are important traits linked with oxalate metabolism, and are also the main traits for the corm nutritional quality assessment. During the photosynthesis, the carbohydrate synthesis supplies the plant tissues with metabolites needed for growth, energy production and signaling processes. The carbohydrate maintenance depends on the strength of the stress factor on the carbon metabolic pathways (Burgess and Huang 2016). The carbohydrates oxidation provides energy for the nitrates reduction into protein nitrogen, where the oxalate is a direct subproduct (Franceschi and Horner 1980). Throughout drought stress, there is a reduction of the carbohydrate metabolism relative to photosynthesis, with accumulation of soluble sugars in detriment of increased starch hydrolysis, as a way to protect the subcellular structures against the negative effects of water deficit (Burgess and Huang 2016; Osuagwu and Edeoga 2013; Epron and Dreyer 1996). The drought affects the quantity and quality of plant proteins, and generally results in an increase of the crude protein content under stress. Plant tissues usually synthetizes specialized high molecular proteins as a resistance response to drought. The stress effect is minimized by proteins acting as cellular compartment space fillers (e.g., LEA proteins), or as natural osmoprotectants amino acids (e.g., proline) (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Osuagwu and Edeoga 2013).

There are several studies on the biochemical composition of taro, but there is scarce information about drought stress effects and how the oxalate, protein and starch variation under stress can be reflected on its

nutritional quality. There is data regarding the acridity and nutritional aspects (Kaushal et al. 2015; Temesgen and Retta 2015; Tattiyakul et al. 2006, 2007), the oxalates quantitation in raw and cooked leaves (Oscarsson and Savage 2007), processed flour (Iwuoha and Kalu 1995) and in corm chips (Kumoro et al. 2014) of non-stressed taro plants. The main research on drought tolerance of this crop is based on physiological traits and plant productivity assays, performed mainly under rain feed conditions (Mabhaudhi and Modi 2015). Recently, a genetic and phenotypic approach was used to screen taro stress sensitivity or tolerance to drought stress, by applying morpho-agronomic and yield stress indexes. There is the possibility of using the identified genetic diversity in breeding programs to adapt this crop to climatic change (Ganança et al. 2015, 2018; Lebot et al. 2017).

The aim of the present study was to improve our understanding on how drought-stressed taro plants changes their nutritional quality and biochemical composition, by assessing oxalate mechanisms and their interactions with the CCI, protein-nitrogen accumulation and starch variation to water shortage.

3.3 Materials and Methods

3.3.1 Plant material

7 from 33 cultivars of *Colocasia esculenta* (L.) Schott were selected to assess the variation of plant biochemical composition under drought conditions. These seven cultivars, with origin in Madeira and Canary Islands, and from the Pacific Community (SPC) collection, were selected based on their morpho-agronomic and multi-criteria indices performance under drought conditions (Table 3.1) performed by Ganança et al. (2018) in 2014.

3.3.2 Experimental drought conditions

The present study was conducted during a plant full-growth cycle in 2015, under controlled conditions, in an open greenhouse in the Preces experimental station, Câmara de Lobos, Madeira, Portugal (32°39′N; 16°58′W). Plants were individually grown in 30 × 30 cm pots, filled with 15 kg of dried soil. The pots were arranged in six rows, spaced 90 cm apart, and 30 cm in row separation. Twenty-four plants per accession, four per row, were submitted to two different watering regimes to assess the influence of drought conditions in biochemical parameters. Three rows were maintained at field capacity (control), and another three were submitted to water deficit (drought stress, 40.2% of water applied to control) from April to November 2015. Each row was considered a replicate. During drought assay, the greenhouse average temperature was 25.12 °C, with an average relative humidity of 49.94%. Experimental design and watering regimes were adapted from Ganança et al. (2018). During full-growth cycle, no pesticide or fertilizer was used and weeds were manually removed as necessary.

3.3.3 Sample preparation

Three hundred and thirty-six corm and shoot (petioles and leafs) samples of control and drought rows were harvested at the end of the agronomic assay. All samples were cleaned with running water, measured with a caliper rule (Mitutoyo solar digimatic caliper CD-S15C, Japan), weighed with a scale (Sartorius Basic BA2100S, Germany), sliced (2–3 mm thick) with a mandolin slicer, dehydrated using an air oven at 65 °C, for 48 h (Memmert UF260, Germany) and finely milled (IKA-Werke M20, USA). The flour was placed into bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153, Netherlands) and stored at -35 °C (Liebherr ProfiLine GGPV6570, Germany) until analysis.

3.3.4 Chlorophyll content index (CCI)

The CCI was determined in fresh taro leaves, with a chlorophyll content meter (Opti-Sciences CCM-200 PLUS, USA). The CCM-200 calculates the CCI as the ratio between leaf transmission percentages at 932 nm (reference wavelength) and 653 nm (absorbed by molecules, other than chlorophyll). The measurements were done between 9.00 and 11.00 a.m., in the main plant's fully unfolded leaf. The reading was made

uniformly along the adaxial leaf surface (left, center and right sides), avoiding the branching veins, covering 0.71 cm^2 of leaf area per reading. A mean CCI value per main plant leaf was determined in each growth row.

3.3.5 Oxalic acid content

Total oxalates, including water-soluble and water-insoluble oxalates, were quantified on all the dry corm and shoot flours. The oxalic acid [$(COOH)_2$] is a dicarboxylic acid, and it can be oxidized to carbon dioxide and water by potassium permanganate and by ferric and ceric salts. Oxalic acid can form acidic and neutral soluble salts when combined with sodium (Na^+), potassium (K^+) and iron (Fe_2^-), or insoluble salts when combined with calcium (Ca^{2+}) (Franceschi and Horner 1980). When treated with hydrochloric acid, the oxalic acid from flour is reduced to glyoxylic acid, and then into glycolic acid, being precipitated and titrated with a standard potassium permanganate solution, as described below according to AOAC (1990), Oke (1965) and Dye (1956) method modifications, and with Fatoki (1994) recommendations.

Exactly 0.4 g of corm and shoot flours were solubilized in 30 mL of water, extracted with 2 mL of hydrochloric acid (HCl, 6.0M), digested at 100 °C, during 1 h in a water bath (Julabo SW22, Germany), made up to final volume of 50 mL and filtered with Whatman paper. The filtrate was divided into two portions, methyl red indicator was added ($C_{15}H_{15}N_3O_2$) and titrated with ammonium hydroxide (NH_4OH). Then, the filtrate was heated until 90 °C, and subsequently cooled and filtered to remove the ferrous ion precipitate. The oxalic acid [$(COOH)_2$] was precipitated as CaOx by adding 2 mL of calcium chloride ($CaCl_2$, 5%), and was left overnight at 5°C. The filtrate was centrifuged (Eppendorf Centrifuge 5430R, Hamburg) at 1438*g* for 5 min, the supernatant was decanted, and the precipitate was treated with boiling sulfuric acid (H_2SO_4, 20%). Oxalate concentration was quantified by titration with a standard potassium permanganate solution ($KMnO_4$, 0.05M). The extraction with boiling H_2SO_4 gave the insoluble CaOx content, and the decanted supernatant gave the total acid soluble oxalates (*S*–Ox), whose calculations were based according to Dye (1956) formulation (Eq. 1). The total oxalates (*T*–Ox) was quantified according to Holloway et al. (1989) (Eq. 2). Each sample was analyzed in triplicate and all data are expressed as mg/100 g dry flour.

$$Oxalate\ content\ (CO,\ mg/100\ g) = (T \times Vme \times 3 \times 10^5) / (5 \times mf) \quad (1)$$

where *T* is the titer of $KMnO_4$ (mL), *Vme* is the volume-mass equivalent between 0.05M $KMnO_4$ and anhydrous oxalic acid (i.e., 1 mL of 0.05M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), *3* is the dilution factor, *5* is the molar equivalent of KMnO4 redox reaction in oxalate, and *mf* is the mass of flour used.

$$T\text{-}Ox\ (mg/100\ g) = CaOx + S\text{-}Ox \quad (2)$$

The method accuracy was validated through recovery index percentage of the oxalic acid (Van Reeuwijk et al. 1998). The samples were spiked with 2 and 5 mg of oxalic acid (*S*–Ox) in 400 mg of corm and shoot flours, respectively. All the samples, as well the spikes, were analyzed five times. The recovery index ranged between 90 and 95% for the corms, and between 99 and 102% for the shoots. Fatoki (1994) reported oxalic acid recoveries of 97–99% from dry, finely ground vegetables, using a similar precipitation method.

3.3.6 Nitrogen and crude protein content

Total protein content was determined for all the dry corm and shoot flours by the Kjeldahl method with the quantification of total nitrogen, using a distillation and titration automatic unit (Velp Scientifica UDK 152, Italy). The factor Nx6.25 was applied to convert the total nitrogen to crude protein content (AOAC 2005). The analyses were performed in triplicate, and the values were expressed in g/100 g dry flour.

3.3.7 Starch content

Starch was extracted from all the dry corm and shoot flours, according to Hodge and Hofreiter (1962). Starch soluble (Merck, Germany) was used as standard for quantitative estimation at 630 nm, using a spectrophotometer UV/Vis (Shimadzu, 2401 PC, Japan) with the UVProbe 2.52 software. The analyses were performed in triplicate, and the values were expressed in g/100 g dry flour.

3.3.8 Swelling power and solubility of starch content

The quality of the grain starch was determined according to Tattiyakul et al. (2006), with minor changes. Exactly 0.2 g of corm flours (M_0) were homogenized in water and poured into a shaking water bath (Julabo SW22, Germany) at 80 °C, until obtaining a gelatinized dispersion (approximately 45 min). The gelatinized dispersion was centrifuged (Eppendorf Centrifuge 5430R, Hamburg) at 3000*g* during 15 min. The soluble starch granules (supernatant) were decanted and dried until a constant weight (MS) using an air oven at 100 °C (Memmert UF260, Germany). The swollen starch granules (MSW) (pellet) were weighted (Precisa ES225 SM-DR, Switzerland). The water solubility (WS) and swelling power (SP) were calculated according to the following Eqs. 3 and 4, in triplicate, and the values were expressed in g/g dry flour.

$$WS = MS / M_0 \quad (3)$$

$$SP = MSW / [M_0 \text{ x } (1 - WS)] \quad (4)$$

3.3.9 Statistical analysis

The results are represented as the main average of taro corms and shoots of three control vs three drought individual rows, expressed in a dry weight basis. All samples were statistically evaluated with SPSS version 23.0 for Mac, for Pearson correlations and one-way ANOVA ($p \leq 0.05$). The MVSP version 3.1 for Windows was used for principal component analysis (PCA).

3.4 Results and Discussion

3.4.1 Whole-plant oxalate content

About 90% of the total calcium in the plant tissue can be found as oxalate salt (Nakata 2003). The overall intensity of root crops acridity by CaOx can change depending on exposure to drought and genetic factors (Sharma and Kaushal 2016; Libert and Franceschi 1987). The mean values of Total-Ox (*T*-Ox), Soluble-Ox (*S*-Ox) and CaOx on drought-stressed accessions increased slightly in the corms (Table 3.2) and decreased in the shoots (Table 3.3). The corms had a *T*-Ox average value of approximately 259 mg/100 g in control conditions, with 27% increase to 328 mg/100 g in drought conditions (Table 3.2). The main fraction of *T*-Ox value was essentially composed by CaOx, which increased 28% from 223 to 285 mg/100 g between control and stress conditions. The CaOx content was higher than the 182–200 mg/100 g range obtained by Tattiyakul et al. (2006), and lower than the 691 mg/100 g registered by Iwuoha and Kalu (1995), both evaluated in non-stressed corm flours. On the other hand, the *S*-Ox had the lowest content in all corms, ranging between approximately 36 and 44 mg/100 g, for control and stress conditions (Table 3.2).

The *T*-Ox average value in shoots shows the opposite variation, decreasing 17% from approximately 322 mg/100 g in non-stress conditions to 268 mg/100 g in drought conditions. The mean CaOx content decreased 17% from 268 to 222 mg/100 g between control and stress conditions. The *S*-Ox had also the lowest content in shoots, decreasing 15% between approximately 54 and 46 mg/100 g for control and drought conditions (Table 3.3).

Different accessions showed different behavior in oxalate production, accumulation and mobilization between corm and shoot organs. The *S*-Ox (as oxalic acid) and CaOx increase in corms and decrease in shoots during drought stress can be associated with the regulation of plant osmotic pressure by oxalate precipitation, with CaOx storage as insoluble oxalate salt at the root organs, and subsequent oxalate excretion into the environment, according to Nakata (2003). In general, the biochemical most tolerant showed less CaOx in the corms, in both control and drought conditions. During drought, they increased CaOx very slightly and

mobilized it from shoots to corms as the better osmotic balance response, relatively to the sensitive ones. The regulation of the *S*-Ox and CaOx equilibrium allows changing tissues turgidity, and increasing the efficiency of water absorption under water scarcity. The most sensitive ones had naturally higher CaOx, with a higher accumulation in both tissues during drought, which is more difficult for the plant to equilibrate/eliminate this insoluble salt during stress, thus compromising the efficiency of water absorption. Although, this role of the *S*-Ox and CaOx system in the tissue should be understood as complementary to other plant osmoprotectant systems.

The corms from acc. 2056 showed the highest increase of CaOx accumulation under drought, from 271 to 469 mg/100 g. Corms of acc. 2216 practically unchanged the CaOx, standing in the 185 mg/100 g, meanwhile acc. 2061 and 2234 slightly decreased the CaOx content in response to water scarcity. Accessions 2232 and 2210 had the lowest CaOx content variation, 125–190 mg/100 g and 187–232 g/100 g in corms under non-stress and stress conditions, respectively (Table 3.2).

The shoots showed a *S*-Ox and CaOx decrease in response to drought. Possibly, during stress, the oxalate was mobilized from the shoots to the corms to be excreted as response to drought by osmotic pressure regulation. The shoots from acc. 2234 had the highest CaOx decrease, from 446 to 300 mg/100 g. Meanwhile, in acc. 2061 and 2239 shoots, the CaOx content increased with drought, from 136 to 179, and 172 to 226 mg/100 g, respectively.

The acc. 2210 showed the best drought response, according to the amount of CaOx precipitation and its mobilization between organs, for further excretion in case of plant need. Acc. 2234 presented loss of CaOx in both organs, which suggests that it does not have the osmotic regulation by CaOx precipitation as drought response.

All the studied taro plants were not safe for raw consumption because of the high CaOx content, exceeding the allowed limit of 71 mg/100 g in food (Kumoro et al. 2014). The insoluble oxalate values are 3 and 4 times higher than the allowable value for the corms and shoots, which affects negatively the taro nutritional quality.

3.4.2 Crude protein accumulation

Drought stress leads to the crude protein content increasing, due to synthesis of high molecular weight proteins as a drought resistance response, according to Osuagwu and Edeoga (2013). Accordingly, taro plants slightly increased the protein content from the corms and shoots, when drought-stressed. The corms had the biggest increase of protein accumulation during stress, although the protein content in control conditions was 7% higher in the shoots than in the corms (Tables 3.2, 3.3). Sharma and Kaushal (2016) only registered 4% higher protein content in the leaves, relatively to non-stressed corms. The corm average protein content increased from 4 g in control to 5 g/100 g in drought conditions. The accession 2210 had the highest protein increase, from 3 to 5 g/100 g. Meanwhile, acc. 2216 showed the highest protein content, that increased from 7 to 8 g/100 g in response to drought. On the other hand, in acc. 2234, the drought seemed to induce a slight decrease of the corm protein content, from 3.5 to 3 g/100 g (Table 3.2). These results are in accordance with the Tattiyakul et al. (2006) work, which registered 5.1–8.7 g/100 g, and are higher than the Tattiyakul et al. (2007) study, which only had 1.6–1.9 g/100 g, both data in non-stressed dry corm flour, whose protein variation can be related with the different origin and environmental growth conditions.

The average crude protein of the stressed shoots increased from approximately 11 to 12 g/100 g, relatively to control. Accession 2210 had the highest shoot protein content, increasing from 13 to 15 g/100 g, approximately. Accession 2232 had the higher shoot protein increase, from approximately 10 to 13 g/100 g. Meanwhile, acc. 2234 was again the only one that presented a decreased protein content, from 15 to 12 g/100 g (Table 3.3). Sharma and Kaushal (2016) reported a lower protein content, only 4 g/100 g in non-stressed taro leaves.

The effect of drought stress in corms and shoots leads to the synthesis of different drought-inducible proteins, like high molecular weight proteins, enabling the increase of the crude protein content (Osuagwu and Edeoga 2013). The corm proteins are associated with the regulation of cell defense and detoxification,

meanwhile the proteins of the leaves are related to photosynthesis, with significant changes in expression during stress (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Therefore, acc. 2210 had a better drought response, which could be related with higher drought-inducible proteins synthesis, and acc. 2234 had the weakest drought response, which could be related with the decrease of protein synthesis in both plant organs.

3.4.3 Chlorophyll content index

The studied taro accessions showed an increase in CCI values, when submitted to drought, with a mean value of 33. The CCI measurement of the leaves was done using a chlorophyll fluorescence measurement technique, which can indirectly imply the difference of the plant photosynthetic rate. This index was proven efficient and reproducible for the plant evaluation susceptibility to drought. It was used among cultivars with contrasting drought tolerance, by the comparison of the photosynthetic electron transport among them, where the higher the plant chlorophyll index, the better the plant resistance to drought (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Tiwari and Mamrutha 2013). Accessions that increased the photosynthesis under severe drought conditions maintained their photosynthetic electron transport from water molecules, through light excitation of photosystem PSII (one of the major sources of ROS in plants). The more excitation of photosystem PSII through photons of light, the greater are the number of ionized chlorophyll molecules, and the more ATP and NADPH are generated (Salehi-Lisar and Bakhshayeshan-Agdam 2016).

Accession 2210 had the highest photosynthesis rate, with a CCI increase from 38 to 47. On the other hand, acc. 2232 and 2234 showed a decrease of their CCI content from nearly 19 to 16, and 41 to 31, respectively (Table 3.3). Mabhaudhi and Modi (2015) evaluated the CCI of taro landraces, using the same technique, and observed that under drought stress, taro plants lowered down the CCI to down-regulate the photosynthesis in response to decreased intracellular CO_2 availability resulting from stomatal closure.

As the CCI varies accordingly with the plant drought stress tolerance, acc. 2210 has the best photosynthetic adaptation to drought, followed by acc. 2061 and 2216, respectively. Accessions 2232 and 2234, on the other hand, decreased the CCI, presenting a more sensitive response to drought.

3.4.4 Starch content and grain quality

The taro corm is the main plant energy storage organ (through starch), and it shows highest content variation under stress, when compared with the shoots. During drought stress, there is an increase of starch hydrolysis, due to the need to supply energy and metabolites to protect the subcellular structures against water deficit (Burgess and Huang 2016). In average, the drought environment slightly decreased the taro starch content, from 48 to 45 g/100 g for the corms, and about 11 g/100 g for the shoots (Tables 3.2, 3.3). At corm level, acc. 2056 and 2232 had the highest starch decrease, between 53–45 and 48–41 g/100 g, respectively. Among the analyzed samples, acc. 2210 was the only one that showed a slight increase of the starch content due to drought stress, from 44 to 45 g/100 g, approximately (Table 3.2). The starch content is in accordance with Kaushal et al. (2015) for non-stressed taro corm flour, between 41.27 and 61.44 g/100 g.

The shoots had a starch decrease due to drought, with acc. 2056 going from nearly 18 to 14 g/100 g, followed by acc. 2210 with 13–10 g/100 g. The acc. 2239 was the only accession registering a starch increase in shoots, from 9 to 11 g/100 g, approximately (Table 3.3).

We also evaluated the quality of the corm starch grain variation under water scarcity. According to Kaushal et al. (2015) and Tattiyakul et al. (2007), the corm starch forms a hard coating layer, with high starch swelling power (SSP), and usually is composed by four-fifths of amylopectin (22 glucose units per molecule) and one-fifth of amylose (490 glucose units per molecule). The loss of starch content and increase of amylose to amylopectin ratio leads to starch solubility in water (SWS) and SSP decrease, which can compromise the quality and functional properties of the corm starch grain.

Accessions 2210, 2232 and 2234 increased the SWS content between control and stress conditions, from 0.19 to 0.20, 0.18–0.21 and 0.14–0.20 g/g, respectively. The acc. 2061 had the highest SWS decrease, between 0.21 and 0.16 g/g (Table 3.2). Works of Tattiyakul et al. (2007) and Tattiyakul et al. (2006) registered a range of 0.06–0.13 g/g SWS of dry taro flour, respectively, which corresponds to a low starch solubility in

water.

The SSP alteration due to drought was very low, and the main changes were observed at acc. 2239, who increased from nearly 11 to 13 g/g, and acc. 2056 who decreased from nearly 16 to 14 g/g, corresponding to a low starch swelling power in water. Tattiyakul et al. (2006) and Kumoro et al. (2014) observed also a low starch swelling power, with 11.0–17.4 g/g and 14.5 g/g SSP in dry non-stressed taro flour.

Overall, the accessions had a small variation in the corm starch content and quality grain under drought. We observed that some starch hydrolysis occurred because of reserves mobilization, as a way to supply energy and metabolites for protection of the cellular structures against water deficit. The grain starch of the corms had also a low SWS and SSP. Accessions 2056 and 2232 applied this strategy of starch mobilization, as a mechanism of response to drought and metabolism maintenance under stress conditions, leading to a higher starch loss. However, acc. 2061 and 2210 showed a different trend: not lose corm starch by hydrolysis under stress; were able to maintain the photosynthesis and nutrients allocation efficiency; they supplied the energy and metabolites without recourse to starch hydrolysis. Despite this, only acc. 2210 maintained the quality and functional properties of the corm starch grain.

3.4.5 Whole-plant variation

To better understand the taro whole-plant response to water scarcity, by the role of the oxalate mechanism and its relation with the chlorophyll content index, protein accumulation and starch variation, the one-way ANOVA analysis was applied to evaluate their variance. Significant differences were found between accessions ($p \leq 0.01$) and experimental variants (control and drought-stress conditions, $p \leq 0.05$) (Tables 3.2, 3.3).

Statistically significant correlations were found among the variables, demonstrating the consistency of the variation of oxalate content with the photosynthetic rate, carbohydrate metabolism and protein synthesis. The corm traits showed nine significant correlations. The strongest trait associations were observed between protein and SSP (− 0.60). Moderate correlations were found between protein and starch (− 0.46), and protein and SWS (− 0.44) (Table 3.4). The shoots showed seven significant correlations. The strongest correlations were observed between starch and *T*-Ox (0.53), and starch and CaOx (0.52). Moderate correlations were found between CCI and protein (0.48), and starch and *S*-Ox (0.37) (Table 3.5).

The PCA analysis, based in the average values of biochemical and CCI analysis, showed little variation of the shoots between control and drought cases (Fig. 3.1b), with the corms displaying a higher dissimilarity between control and drought groups (Fig. 3.1a). The principal components explain 88.8% of variance observed in corms traits, with 76.0% at first and 12.8% at second axes, eigenvalues of 0.07 and 0.01, respectively (Fig. 3.1a). The principal components explain 93.2% of cumulative variance of shoots traits, with 75.1% at first and 18.2% at second axes, eigenvalues of 0.23 and 0.06, respectively (Fig. 3.1b). The accessions distribution agrees with observed variability of plant answers under drought stress.

The whole-plant multivariate analysis, analysis of variance and correlations among the traits variables, showed that the corm was the main organ affected by stress, displaying a higher variation in response to drought, with the leaves showing little variation. We observed that oxalic acid could be mainly a derivative product of the photosynthesis oxidative processes or carbohydrate metabolism, and was precipitated to CaOx for osmoregulation as insoluble oxalate. Overall, the *T*-Ox, *S*-Ox and CaOx synthesis, which expression increased in corms and decreased in shoots, had positive significant correlations. The shoots CaOx and *S*-Ox had a positive significant correlation with starch, which in turn was negatively correlated with protein content. According to Franceschi and Horner (1980), the oxidation of carbohydrates provided the energy for the reduction of nitrates into protein nitrogen, with oxalate synthesis as a direct subproduct. The increase in CCI and protein content had a positive moderate correlation, leading to an increase of plant tolerance to abiotic stress, as Salehi-Lisar and Bakhshayeshan-Agdam (2016), Mabhaudhi and Modi (2015) and Tiwari and Mamrutha (2013) mentioned.

The seven taro accessions in this study were previously classified as drought sensitive, moderate or tolerant, through stress indices calculated from agro-morphologic and yield screening (Ganança et al. 2018)

(Table 3.1). The biochemical drought response could classify them as tolerant (acc. 2061, 2210 and 2216), moderately tolerant (acc. 2056 and 2232), and sensitive (acc. 2234 and 2239).

The acc. 2210, in this study, shows the better drought tolerance response: had the bigger CaOx precipitation and mobilization as osmotic response; the best photosynthetic adaptation with the highest CCI content and protein increase; the less starch hydrolysis, with less quality loss by SWS and SSP. However, this accession was classified as moderately tolerant by the previous assay of Ganança et al. (2018) (Table 3.1). Accessions 2216 and 2061 also showed to be tolerant to water scarcity, agreeing with the Ganança et al. (2018) classification (Table 3.1). The drought tolerance was mainly supported through CCI increase, high protein content and osmoregulation by CaOx precipitation. Accessions 2056 and 2232 were, from a biochemical point of view, moderately tolerant. However, acc. 2232 was previously classified as sensitive by Ganança et al. (2018) (Table 3.1). The moderate tolerance response was mainly observed through the starch hydrolysis ratio and oxalate osmoregulation. The acc. 2234 and 2239 had the most sensitive response to drought, being acc. 2234 previously classified as moderately tolerant by Ganança et al. (2018) (Table 3.1). These accessions showed less CaOx mobilization, decreased photosynthetic rate, with a slight increase of starch hydrolysis or protein synthesis as strategy for drought mitigation.

3.5 Conclusion

Variation in oxalate content by the taro plant was consistent with the photosynthetic rate, carbohydrate metabolism and protein synthesis, with significant correlations observed between parameters. Drought-tolerant accessions had good osmotic response by oxalate precipitation and mobilization from the shoots to the corms to be excreted as plant response to drought by osmotic pressure regulation, had photosynthetic adaptation by the highest CCI content and protein increase, and less starch hydrolysis and quality loss. Drought sensitive presented less mobilization of CaOx, decreased photosynthetic rate, and slight increase starch hydrolysis or protein synthesis. All the corms in this study had high CaOx content, three times higher than the allowable value for the corms, and four times for the shoots, which affects negatively the whole-plant nutritional quality. Taro accessions were classified according to the biochemical drought response as tolerant for the accessions 2061, 2210 and 2216, followed by accessions 2056 and 2232 as moderate tolerant, and 2234 and 2239 as sensitive. The biochemical drought classification is in accordance for acc. 2061, 2216, 2056 and 2239, with the previous work from Ganança et al. (2018).

3.6 Acknowledgements

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3.7 Author contribution statement

CSSG performed the biochemical experiments, analyzed, interpreted, summarized all data generated from those experiments, and wrote the manuscript. JFTG designed the study for the drought assay, and helped in statistical analysis. VL and MAAPC coordinated the work and revised the manuscript.

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Tables

Table 3.1

Taro (*Colocasia esculenta* (L.) Schott) accessions submitted to different watering regimes, to assess the plant's biochemical responses to drought conditions.

Accession ID[a]	Variety local name	Origin	Drought response[b]
2056	Listado	Canary Islands – La Palma	Moderate
2061	Blanco Saucero	Canary Islands – La Palma	Tolerant
2210	Roxo	Madeira Island	Moderate
2216	Branco	Madeira Island	Tolerant
2232	PExPH 15-6 BL/HW/08	SPC, Fiji	Sensitive
2234	C3-22 BL/PNG/11	SPC, Fiji	Moderate
2239	Karang CE/MAL/10	SPC, Fiji	Sensitive

[a] Accessions identification number code, used by the ISOPlexis Genebank.

[b] Taro classification by drought sensitive, moderate or tolerant response, obtained through their agro-morphologic screening, according to previous work of Ganança et al. (2018) performed in 2014.

Table 3.2
Variation of biochemical traits in the taro corms submitted to different irrigation regimes.

Corms		T-Ox[a,b]	S-Ox[a,b]	CaOx[a,b]	CP[a,b]	St[a,b]	SWS[a]	SSP[a]
2056	Control	312.2 ± 42.2	41.1 ± 12.6	271.2 ± 44.1	3.8 ± 0.1	53.1 ± 1.0	0.20 ± 0.0	15.5 ± 1.1
	Drought	528.1 ± 64.9	59.4 ± 20.4	468.8 ± 69.3	4.5 ± 0.1	45.4 ± 0.8	0.19 ± 0.0	14.4 ± 0.8
2061	Control	327.1 ± 135.4	56.9 ± 3.9	270.2 ± 135.8	4.0 ± 0.1	46.3 ± 0.9	0.21 ± 0.0	17.8 ± 0.4
	Drought	295.8 ± 61.0	31.0 ± 0.2	264.8 ± 60.8	5.0 ± 0.1	46.6 ± 1.5	0.16 ± 0.0	17.7 ± 0.1
2210	Control	203.3 ± 25.1	16.6 ± 4.2	186.7 ± 25.7	3.9 ± 0.1	43.7 ± 1.1	0.19 ± 0.0	16.4 ± 1.1
	Drought	260.4 ± 56.5	28.7 ± 6.3	231.7 ± 52.2	5.4 ± 0.0	44.7 ± 1.0	0.20 ± 0.0	15.6 ± 0.6
2216	Control	210.5 ± 52.1	25.8 ± 11.6	184.7 ± 44.7	7.4 ± 0.1	45.6 ± 2.2	0.14 ± 0.0	11.8 ± 0.6
	Drought	224.4 ± 50.5	39.6 ± 13.8	184.7 ± 41.5	8.2 ± 0.2	43.3 ± 1.8	0.14 ± 0.0	12.0 ± 1.5
2232	Control	153.2 ± 36.4	28.4 ± 4.9	124.8 ± 39.7	3.8 ± 0.2	48.1 ± 2.1	0.18 ± 0.0	17.7 ± 1.0
	Drought	226.1 ± 25.2	36.0 ± 4.3	190.1 ± 25.4	4.7 ± 0.0	40.7 ± 1.1	0.21 ± 0.1	16.9 ± 1.0
2234	Control	353.4 ± 23.3	55.7 ± 4.5	297.7 ± 27.7	3.5 ± 0.0	54.6 ± 0.9	0.14 ± 0.0	17.0 ± 0.7
	Drought	379.2 ± 113.1	84.1 ± 37.5	295.1 ± 84.6	3.0 ± 0.0	52.6 ± 0.6	0.20 ± 0.0	16.9 ± 1.4
2239	Control	251.4 ± 55.7	27.0 ± 7.9	224.4 ± 54.8	5.0 ± 0.0	47.7 ± 1.3	0.15 ± 0.0	11.3 ± 0.6
	Drought	384.9 ± 88.7	25.8 ± 3.1	359.1 ± 91.0	6.1 ± 0.0	43.0 ± 2.1	0.15 ± 0.0	12.9 ± 1.1
Mean	Control	258.7	35.9	222.8	4.5	48.4	0.17	15.4
	Drought	328.4	43.5	284.9	5.3	45.2	0.18	15.2
Min	Control	153.2	16.6	124.8	3.5	43.7	0.14	11.3
	Drought	224.4	25.8	184.7	3.0	40.7	0.14	12.0
Max	Control	353.4	56.9	297.7	7.4	54.6	0.21	17.8
	Drought	528.1	84.1	468.8	8.2	52.6	0.21	17.7

Control is well-watered, drought is severe stress.

Data are expressed in dry weight basis, and represents the mean±SD of three independent lines replications per acc., with total mean, minimum and maximum per trait.

T-Ox total oxalates (mg/100 g), S-Ox soluble oxalates (mg/100 g), CaOx calcium oxalate (mg/100 g), CP crude protein (g/100 g), St starch content (g/100g), SWS starch solubility in water (g/g), SSP starch swelling power (g/g).

[a]Significant differences between accessions (ANOVA, $p \leq 0.01$).

[b]Significant differences between control and drought stress conditions (ANOVA, $p \leq 0.05$).

Table 3.3
Variation of biochemical traits in the taro shoots submitted to different irrigation regimes.

Shoots		T-Ox[a]	S-Ox[a,b]	CaOx[a,b]	CP[a,b]	St[a]	CCI[a]
2056	Control	427.3 ± 195.9	84.1 ± 20.4	343.2 ± 179.4	9.8 ± 0.0	17.6 ± 0.6	37.4 ± 7.5
	Drought	358.8 ± 149.4	62.4 ± 1.0	296.3 ± 150.4	11.7 ± 0.3	14.3 ± 1.1	38.7 ± 15.6
2061	Control	187.4 ± 31.7	51.7 ± 3.4	135.7 ± 28.3	11.2 ± 0.1	9.4 ± 0.8	40.3 ± 2.0
	Drought	204.1 ± 27.3	25.6 ± 9.9	178.6 ± 20.2	10.7 ±0.1	9.6 ± 0.5	43.4 ± 6.8
2210	Control	354.1 ± 66.6	52.8 ± 6.0	301.3 ± 70.6	12.9 ± 0.1	12.8 ± 0.5	38.3 ± 7.9
	Drought	237.3 ± 30.6	27.0 ± 6.4	210.3 ± 37.1	15.1 ± 0.0	10.0 ± 0.9	46.9 ± 13.4
2216	Control	193.7 ± 43.1	40.3 ± 9.0	153.3 ± 35.1	12.6 ± 0.0	7.5 ± 0.5	41.3 ± 4.3
	Drought	154.3 ± 9.5	34.6 ± 4.4	119.7 ± 7.2	13.8 ± 0.0	7.3 ± 0.5	44.8 ± 13.7
2232	Control	363.5 ± 29.8	37.8 ± 2.9	325.7 ± 32.2	9.9 ± 0.1	12.4 ± 1.6	18.6 ± 7.0
	Drought	275.0 ± 24.5	50.7 ± 14.7	224.3 ± 13.9	13.2 ± 0.0	11.8 ± 0.6	16.1 ± 6.1
2234	Control	528.2 ± 33.9	82.6 ± 42.2	445.6 ± 23.2	15.2 ± 0.1	11.1 ± 0.8	40.7 ± 16.5
	Drought	388.7 ± 100.5	89.2 ± 29.0	299.5 ± 75.6	11.8 ± 0.1	10.6 ± 0.3	30.8 ± 10.0
2239	Control	200.7 ± 94.2	29.0 ± 7.5	171.7 ± 91.8	8.6 ± 0.3	9.2 ± 0.8	12.4 ± 3.6
	Drought	260.0 ± 106.9	34.4 ± 12.2	225.6 ± 94.7	10.8 ± 0.0	10.6 ± 2.1	12.7 ± 5.2
Mean	Control	322.1	54.0	268.1	11.5	11.4	32.7
	Drought	268.3	46.3	222.0	12.4	10.6	33.3
Min	Control	187.4	29.0	135.7	8.6	7.5	12.4
	Drought	154.3	25.6	119.7	10.7	7.3	12.7
Max	Control	528.2	84.1	445.6	15.2	17.6	41.3
	Drought	388.7	89.2	299.5	15.1	14.3	46.9

Control is well-watered, drought is severe stress.

Data are expressed in dry weight basis, and represents the mean±SD of three independent lines replications per acc., with total mean, minimum and maximum per trait.

T-Ox total oxalates (mg/100 g), S-Ox soluble oxalates (mg/100 g), CaOx calcium oxalate (mg/100 g), CP crude protein (g/100 g), St starch content (g/100g), SWS starch solubility in water (g/g), SSP starch swelling power (g/g).

[a]Significant differences between accessions (ANOVA, $p \leq 0.01$).

[b]Significant differences between control and drought stress conditions (ANOVA, $p \leq 0.05$).

Table 3.4
Pearson correlation coefficients of the biochemical traits from taro corms in control and drought stress conditions.

Variables	1	2	3	4	5	6	7
1. S-Ox	-						
2. CaOx	0.40[a]	-					
3. T-Ox	0.56[a]	0.99[a]	-				
4. CP	-0.31[b]	-0.10	-0.15	-			
5. St	0.38[b]	0.02	0.09	-0.46[a]	-		
6. SWS	0.17	0.07	0.09	-0.44[a]	0.01	-	
7. SSP	0.19	-0.06	-0.01	-0.60[a]	0.11	0.42[a]	-

S-Ox soluble oxalates (mg/100g), *CaOx* calcium oxalate (mg/100g), *T-Ox* total oxalates (mg/100g), *CP* crude protein (g/100g), *St* starch content (g/100g), *SWS* starch solubility in water (g/g), *SSP* starch swelling power (g/g).

[a]Correlation is significant at the 0.01 level (two-tailed).

[b]Correlation is significant at the 0.05 level (two-tailed).

Table 3.5

Pearson correlation coefficients of the biochemical and CCI traits from taro shoots in control and drought stress conditions.

Variables	1	2	3	4	5	6
1. S-Ox	-					
2. CaOx	0.57[a]	-				
3. T-Ox	0.70[a]	0.99[a]	-			
4. CP	0.15	0.19	0.19	-		
5. St	0.37[b]	0.52[a]	0.53[a]	-0.25	-	
6. CCI	0.15	0.09	0.11	0.48[a]	-0.01	-

S-Ox soluble oxalates (mg/100g), *CaOx* calcium oxalate (mg/100g), *T-Ox* total oxalates (mg/100g), *CP* crude protein (g/100g), *St* starch content (g/100g), *CCI* chlorophyll content index.

[a]Correlation is significant at the 0.01 level (two-tailed).

[b]Correlation is significant at the 0.05 level (two-tailed).

Figure

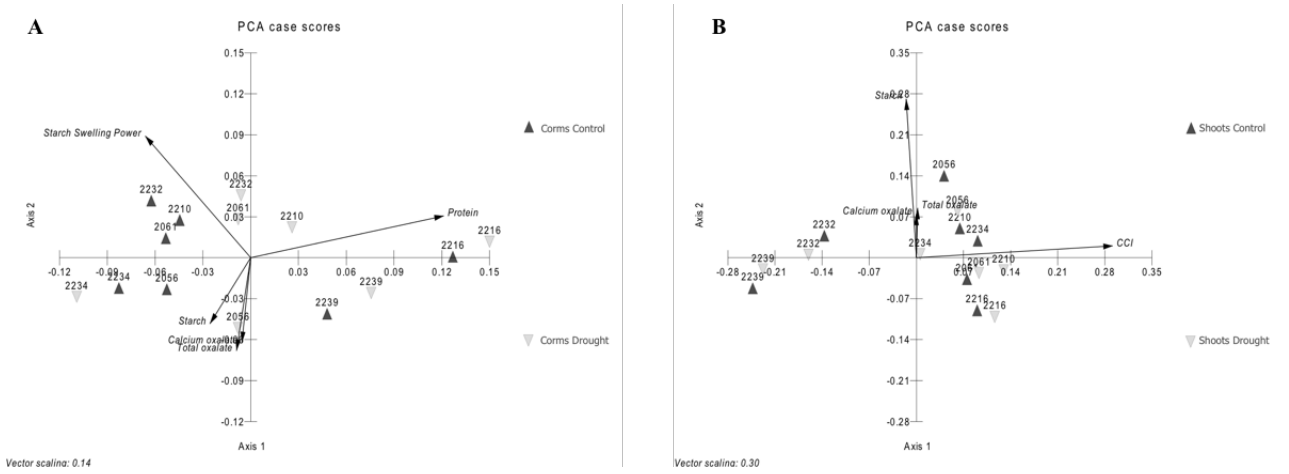

Figure 3.1

Representation of the Euclidean biplot by principal component analysis (PCA), with spatial distribution of the *C. esculenta* accessions based in the average values of biochemical and CCI analysis in drought-stressed conditions.

a The scattering distribution of the variables (traits) and case scores (taro accessions) evaluated in corms in two PCA axes.

b The representation of the spatial distribution of the variables (traits) and case scores (taro accessions) studied in shoots in two PCA axes.

Data *loge* transformation was applied to all the traits (variables) in analysis.

Control is well-watered, drought is severe stress.

CHAPTER 4

Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) and carbon-water relations as drought stress mechanism response of taro (*Colocasia esculenta* L. Schott)

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Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) and carbon-water relations as drought stress mechanism response of taro (*Colocasia esculenta* L. Schott)

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4.1 Abstract

Taro (*Colocasia esculenta* L. Schott) is an important staple food crop in tropical and developing countries, having high water requirements. The purpose of this study was to evaluate the feasibility of using carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) as a physiological indicator of taro response to drought, and elucidation of the relationship between the water use efficiency (WUE) under drought conditions and carbon isotope discrimination ($\Delta^{13}C$). As an alternative to WUE determination, obtained by measuring plant growth and water loss during an entire vegetative cycle, we have used $\Delta^{13}C$ to determine the tolerance of C_3 taro plants to drought. Seven taro accessions from Madeira, Canary Islands and the Secretariat of the Pacific Community (Fiji) collections were grown under greenhouse conditions and subjected to different watering regimes during a one-year cycle. Total plant biomass (TPB), WUE and $\delta^{15}N$ were determined at the whole-plant level (WP). Corms and shoots were evaluated separately for nitrogen content (N), $\delta^{13}C$, $\Delta^{13}C$ and $\delta^{15}N$. WUE showed positive correlation with TPB ($r = 0.4$) and negative with $\Delta^{13}C$ ($r = -0.3$); Corm $\delta^{15}N$ showed positive correlations with WP $\delta^{15}N$ ($r = 0.6$) and corm N ($r = 0.3$). Accordingly, the taro plants with enhanced WUE exhibited low $\Delta^{13}C$ and $\delta^{15}N$ values as a physiological response to drought stress. The approach used in the present study has developed new tools that could be used in further research on taro response to environmental stresses.

Keywords

Colocasia esculenta; taro; drought; $\delta^{15}N$; $\Delta^{13}C$; WUE

Abbreviations

Acc.	Taro accession number
Δ	Carbon isotope discrimination
δ	Natural abundance or isotopic composition
N	Nitrogen
PCA	Principal component analysis
R	Isotope (abundance) ratios
SPC	South Pacific Community
TPB	Total plant biomass
WP	Whole-plant
WUE	Water use efficiency

4.2 Introduction

Taro (*Colocasia esculenta* L. Schott) is considered to be one of the oldest crops, currently, playing an important role as a staple food in tropical and developing countries. For optimal yields taro requires a very high water supply, about 2,500 mm rainfall per year (Ganança et al., 2018; Sharma and Kaushal, 2016; Lebot, 2009). In our previous work we evaluated the impacts of drought stress on taro based on morpho-agronomic and yield stress indexes (Ganança et al., 2018; Lebot et al., 2017; Ganança et al., 2015). The feasibility of using the identified genetic diversity in breeding programs to adapt this crop to climate change was also addressed.

Carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) can provide insights regarding the chemical, physical and metabolic processes involved in carbon transformations and nitrogen processes (Robinson et al., 2000; Farquhar et al., 1989). The $\delta^{13}C$ and $\delta^{15}N$ were used as integrators in several studies addressing plant-mycorrhizal interactions (Hobbie et al., 2000), ecological and environmental stresses (Kohzu et al., 1999), plant responses to salt (Romero-Trigueros et al., 2014) and drought (Robinson et al., 2000; Laureti et al., 1993). Hitherto, there is no published information about the $\delta^{13}C$ and $\delta^{15}N$ serving as physiological indicators of taro drought response or the relationship between taro water use efficiency (WUE) and carbon isotope discrimination ($\Delta^{13}C$) under drought stress.

The isotopes of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) are natural stable isotopes. The carbon most abundant isotope is ^{12}C (98.9%), while ^{13}C (1.1%) is far less abundant one (Farquhar et al., 1989). Likewise, ^{14}N is more abundant than ^{15}N (Robinson 2001).

The isotopic composition of a sample is usually expressed in δ-notation. The $\delta^{13}C$ represents the difference between the carbon from a sample and the internal standard Vienna Pee Dee Belemnite (VPDB) in parts per thousand (per mille units, ‰). The VPDB refers to a previous nomenclature PDB Marine Carbonate Standard, which is a Cretaceous limestone fossil *Belemnitella americana* from the Pee Dee formation in South Carolina, USA (Boutton 1991; O'Leary 1981).

The isotopic compositions are denoted as δ-values, according to Farquhar et al., (1982),

$$\delta(‰) = (R_{(sample)} / R_{(standard)} - 1) \times 1000, \quad (1)$$

where R is the isotope ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Generally, the abundance of ^{13}C relative to ^{12}C in plant tissue is lower than in the atmospheric CO_2, conferring them negative $\delta^{13}C$ values (Farquhar et al., 1989). The $\delta^{13}C$ values in biological carbon compounds can fluctuate from 0‰ to ~-110‰, relative to the VPDB standard, because organic matter is invariably depleted in ^{13}C compared to VPDB (Lomax et al., 2012; O'Leary 1981). The terrestrial plants $\delta^{13}C$ is predominantly controlled by two main photosynthetic reaction pathways including the Calvin-Benson (or C3), and the Hatch-Slack (or C4), denoting that the carbon isotope discrimination happens by the assimilation of CO_2 into plant biomass (Lomax et al., 2012). Most terrestrial plants are C3, comprising over 80% of crop plants, which includes taro (*C. esculenta*) (Bayala et al., 2015). C3 plants can highly fractionate the carbon isotopes during photosynthesis, by the conversion of atmospheric CO_2 (or δ_a) into a phosphoglycerate compound with three C atoms, with $\delta^{13}C$ values ranging between -23‰ to -30‰ (Bayala et al., 2015; Lomax et al., 2012; Farquhar et al., 1982).

Farquhar et al. (1989, 1982) proposed a more direct measure through the isotope carbon discrimination ($\Delta^{13}C$) for field-grown plants. As the isotopic difference between the source and the product reflects the carbon isotope fractionations, the $\Delta^{13}C$ was determined from the known carbon isotope composition of plant material (δ_p) and the source of atmospheric CO_2 carbon (δ_a = -8‰, in the absence of industrial activity), according to Farquhar et al. (1989), as:

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p), \quad (2)$$

where the $\Delta^{13}C$ is defined as the depletion of ^{13}C from δ_a and δ_p (Farquhar et al., 1982).

The field-grown plants show always a positive isotope carbon discrimination. If the plants are grown in a closed system, there is no isotope effect, since all CO_2 is fixed (Tiwari and Mamrutha 2013; O'Leary 1993).

Conversely, $\delta^{15}N$ acts as a plant physiological integrator of stress responses by the fractionations of ^{15}N and ^{14}N during the nitrogen cycle processes (Serret et al., 2018; Evans 2001; Robinson 2001; Robinson et al., 2000). $\delta^{15}N$ levels in live organisms can range between -5‰ to +10‰, where the standard is atmospheric N_2 ($\delta^{15}N$ = 0‰). For most naturally occurring nitrogen (N), $\delta^{15}N$ can range from −30‰ to +30‰ (Robinson 2001). The plants differ in their ^{15}N values, because the ^{15}N is more abundant in the soil than in the atmosphere (Robinson 2001).

The $\delta^{13}C$ value has been used as a standard method to determine the resistance and improvement of the C3 genotypes to drought. However, $\delta^{15}N$ is less explored as a plant physiological integrator of stress responses (Robinson et al., 2000). Usually drought decreases the leaf $\delta^{13}C$ abundance, which is associated with stomatal aperture, photosynthesis effects by carboxylation and changes in water use efficiency (WUE) (Igamberdiev et al., 2004; Robinson et al., 2000; O'Leary 1993; Farquhar et al., 1989). Meanwhile, the genotypic differences in whole-plant $\delta^{15}N$ values can reveal how plants retain N in their tissues during drought stress, where the more positive $\delta^{15}N$ is, the more the plant is ^{15}N-enriched. On the other hand, the more negative $\delta^{15}N$ reflects the better ability of the plant to fix N (Robinson 2001). Farooq et al. (2009) suggested that drought-tolerant genotypes could have improved WUE by reducing the water loss and nutrients allocation when compared to drought-sensitive ones.

Laureti et al. (1993) documented the negative correlation between WUE and $\Delta^{13}C$ in C3 plants. This finding was a significant breakthrough as it overcomes disadvantages of time-consuming WUE determination based on measurements of plant growth and water loss over extended vegetative cycles. Genotypes with higher dry matter production associated with low $\Delta^{13}C$ and high WUE values exhibited higher drought tolerance (Tiwari and Mamrutha 2013; Johnson and Tieszen 1993).

The objectives of the present study were: i) to determine the relationship between carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) and the whole-plant biomass, WUE and $\Delta^{13}C$ in taro grown under water deficit, and ii) to validate $\Delta^{13}C$ measurements as a rapid screening tool for WUE and yield stability.

4.3 Materials and Methods

4.3.1 Plant materials

Seven *C. esculenta* accessions originating from Madeira, Canaries and the South Pacific Community (SPC, Fiji) collections were selected to assess the variation of plant biochemical composition under drought conditions. The accessions were selected based on the recently reported information on morpho-agronomic parameters and multi-criteria indices (Ganança et al., 2018) who studied the performance of 33 taro genotypes under drought stress (Table 4.1).

4.3.2 Experimental drought conditions

The present study was conducted under controlled conditions during a plant full-growth cycle in 2015 in an open greenhouse in the Preces experimental station, Câmara de Lobos, Madeira, Portugal (32°39'N; 16°58'W). Plants were individually grown in 30x30 cm pots, filled with 15 kg of dried soil. The pots were arranged in 6 rows, spaced 90 cm apart, and 30 cm in row separation. Twenty-four plants per accession, 4 per row, were submitted to two watering regimes to assess the influence of drought conditions on plant performance. Three rows were maintained at field capacity (control), while three other were submitted to water deficit (drought stress, 40.2% of water applied to control) from April to November 2015. Each row was considered a replicate. Experimental design and watering regimes were adapted from Ganança et al. (2018). The crop was grown under a low input management system with no use of pesticides or fertilizers.

4.3.3 Sample preparation

Two hundred and fifty two corm and shoot (petioles and leafs) samples from control and stressed plants were harvested at the end of the agronomic trial. All samples were cleaned with running water, weighed with a scale (Sartorius Basic BA2100S, Germany), sliced (2-3 millimeters thick) with a mandolin slicer, dehydrated using an air oven at 65ºC for 48 h (Memmert UF260, Germany) and finely milled (IKA-Werke M20, USA). The flour was placed into bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153, Netherlands) and stored at -35ºC (Liebherr ProfiLine GGPV6570, Germany) until analysis.

4.3.4 Total Plant Biomass (TPB)

TPB represented whole-plant biomass (corms and shoots) collected per pot, dehydrated in an air oven (Memmert UF260, Germany) according to Undersander et al. (1993). Each treatment was run in triplicate; results are expressed in $g.pot^{-1}$ of dry flour.

4.3.5 Water Use Efficiency (WUE)

WUE was calculated as the ratio of total fresh plant biomass to total water used per pot expressed in $g.L^{-1}$ (Ganança et al., 2018).

4.3.6 Nitrogen content

Total nitrogen content was determined for all the dry corm and shoot flours by the Kjeldahl method AOAC 945.18-B:2005 using a distillation and titration automatic unit (Velp Scientifica UDK 152, Italy). The analyses were performed in triplicate; the values were expressed in g/100 g dry flour.

4.3.7 Stable Isotope analysis

Taro corms and shoots flours were vacuum packaged and sent for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis performed by the Natural Resources Analytical Laboratory at the University of Alberta, Edmonton, Canada. The sample isotopic composition was determined by the micro-chemical AOAC 972.4:2000 method, using a Delta V Advantage Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS) from Thermo Finnigan Corp, Bremen, Germany. An aliquot of sample was combusted under oxygen where the carbon and nitrogen contained in the sample was converted to gas form. Gases CO_2 & N_2 were separated chromatographically, then analyzed in a CF-IRMS. Intensities of mass 46/45/44 for CO_2 and mass 28/29/30 for nitrogen were measured. Internal standards were calibrated against the International Reference scale (i.e. C13 vs. VPDB and N15 vs. Air). Raw data from the mass spectrometer was then referenced to PDB or air using a linear regression calculated from the internal standard results. The $\delta^{13}C$ results are reported relative to the VPDB standard (δ_a = -8‰) and $\delta^{15}N$ is relative to the standard atmospheric N_2 ($\delta^{15}N$ = 0‰), using the equation (1) by Farquhar et al. (1982). The carbon isotope ratios in this paper have been converted from $\delta^{13}C$ to $\Delta^{13}C$ through the carbon isotope composition of taro shoots and corms (δ_p) and source air CO_2 (δ_a), using the equation (2) by Farquhar et al. (1989). The whole-plant (WP) $\delta^{15}N$ was calculated according to Robinson et al. (2000) as an average of shoot and corm $\delta^{15}N$ multiplied by the total nitrogen (N, mg) of shoots and corms, as:

$$\text{WP } \delta^{15}N\ (‰) = [(\text{Shoot } \delta^{15}N \times \text{Shoot N}) + (\text{Corm } \delta^{15}N \times \text{Corm N})] / (\text{Shoot N} + \text{Corm N}) \quad (3)$$

The analyses were performed in triplicate and all the values were expressed in per mille units.

4.3.8 Statistical analysis

The results are represented as the main average of taro plants corms and shoots in each of the three control vs three drought individual rows, expressed per dry weight basis. All samples were statistically evaluated with IBM SPSS Statistics v. 24 for Mac, for Pearson correlations and Analysis of Variance (ANOVA, $p \leq 0.05$). The MVSP v. 3.1 for Windows was used for principal component analysis (PCA).

4.4 Results and Discussion

4.4.1 Variation of shoots and corms $\delta^{13}C$ under drought conditions

The $\delta^{13}C$ can provide significant information about taro development, where the isotope value reflects the plant isotopic composition of the immediate environment (O'Leary 1981). Taro shoots had a greater $\delta^{13}C$ variation than the corms, both under control and drought stress conditions (Table 4.2). Under drought conditions $\delta^{13}C$ of the shoots and corms became less negative than the controls. A less negative value means richer in ^{13}C, or 'heavier' (O'Leary 1981). The shoots had a more pronounced variation (+2.60‰), ranging between -27.18‰ (acc. 2232) and -24.58‰ (acc. 2061). In corms, it increased to 2.16‰ and ranged between -26.98‰ (acc. 2239) and -24.82‰ (acc. 2056) (Table 4.2). The acc. 2216 and 2239 slightly increased the $\delta^{13}C$ negativity in the shoots and corms under drought conditions. The remaining acc. maintained or decreased the $\delta^{13}C$ negativity, with acc. 2061 registering the highest negativity loss (+1.26‰) (Table 4.2).

Each acc. isotope fractionations might change due to variety, temperature, CO_2 concentration or other natural variables (O'Leary 1993). According to Igamberdiev et al. (2004), another important factor for determining carbon isotope fractionation in plants is the stomatal conductance. In this work, all the taro $\delta^{13}C$ values under both control and drought conditions (Table 4.2) pointed to plants with relatively open stomata, since according to O'Leary (1993) $\delta^{13}C$ values for C3 plants are near the -38‰. The response of acc. 2216 and 2239 to drought can be attributed to higher stomatal aperture and photosynthesis effects by carboxylation (O'Leary 1993). The stomatal aperture increased the intracellular CO_2 uptake under drought and maintained their photosynthetic electron transport from water molecules, through light excitation of photosystem PSII (one of the major sources of ROS in plants), increasing the number of ionized chlorophyll molecules (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Igamberdiev et al., 2004).

Thus, in acc. 2216 and 2239, the higher stomatal aperture lead to a decrease of shoot $\delta^{13}C$ into a more negative δ-value under drought, which remains with accordance with Robinson et al. (2000), O'Leary (1993) and Farquhar et al. (1989). The remaining shoot samples, for instance acc. 2061, had less open stomata, leading to a bigger negativity decrease of $\delta^{13}C$ (i.e. more positive and heavier) under drought. Robinson et al. (2000) also observed a high loss of negativity of $\delta^{13}C$ in the shoots of wild barley exposed to drought and associated it with a better response to drought.

4.4.2 Whole-plant (WP) $\delta^{15}N$ as a physiological integrator of drought

The genotypic differences in whole-plant (WP) $\delta^{15}N$ values revealed how the taro plants retained N in their tissues under drought stress. The $\delta^{15}N$ acts as a plant potential indicator of the N metabolism and the growing conditions (Serret et al., 2018). We observed a physiological transformation of N within the taro plant, since the $\delta^{15}N$ abundance can be affected at the whole-plant level due to water scarcity (Romero-Trigueros et al., 2014). The plant $\delta^{15}N$ content is usually linked to N fractionation, resulting from plant absorption, assimilation, allocation and loss of N (Evans 2001). Assimilation of the inorganic N forms (NO_3^- and NH_4^+) allows the plants to synthesize organic N compounds: nitrate (NO_3^-) is converted to nitrite (NO_2^-) by the cytoplasmic enzyme nitrate reductase, and then to ammonium (NH_4^+) through nitrite reductase (Romero-Trigueros et al., 2014; Sahoo et al., 2010; Pike et al., 2002; Robinson 2001). In higher plants, N is usually taken up as NO_3^- (Sahoo et al., 2010). Relatively to our controls, all stressed taro plants decreased WP $\delta^{15}N$ (Table 4.3), with the shoot $\delta^{15}N$ abundance exhibiting a greater variation than the corms (Table 4.2). Robinson et al. (2000) also reported that WP $\delta^{15}N$ of wild barley subjected to drought stress was always more negative than the controls, implying an effective drought response mechanism.

Acc. 2210 had the highest WP $\delta^{15}N$ values under control and drought conditions reaching 9.59‰ and 8.88‰, respectively (-0.71‰) (Table 4.3). Drought decreased shoot $\delta^{15}N$ from 11.22‰ to 10.20‰ (-1.02‰), while corm $\delta^{15}N$ registered a slight increase from 4.28‰ to 5.31‰ (+1.02‰). This acc. was the most ^{15}N-enriched sample in the study having the most positive $\delta^{15}N$ abundance, which may indicate a good whole-plant N retention during drought stress as reported by Robinson (2001). N-shoot content in acc. 2210 increased from

20.69% to 24.24%, while N-corm also increased from 6.25% to 8.71% under drought (Table 4.2). Acc. 2216 had also exhibited substantial increase of whole-plant N content under drought, with N-shoot ranging from 20.25% to 22.13%, and N-corm ranging from 11.90% to 13.22%. The WP $\delta^{15}N$ values slightly decreased from 7.63‰ to 6.70‰ (-0.93‰) (Tables 4.2 and 4.3). The most significant WP $\delta^{15}N$ decrease of 3.29‰ (from 9.00‰ to 5.71‰) under drought was observed in acc. 2234. The shoot $\delta^{15}N$ decreased from 10.10‰ to 6.32‰ (-3.79‰), while the corm also decreased from 4.05‰ to 3.38‰ (-0.67‰). On the other hand, acc. 2239 had lower WP $\delta^{15}N$ content, with only a -0.15‰ difference under drought, ranging from 6.23‰ to 6.08‰. The shoot $\delta^{15}N$ registered an increase from 7.05‰ to 7.25‰ (0.20‰), and the corm $\delta^{15}N$ registered a slight decrease from 4.90‰ to 4.67‰ (-0.24‰). Among all tested taro lines, the lowest ^{15}N-enrichement was noted in acc. 2239; it had the lowest $\delta^{15}N$ abundance, and the weakest ability to retain N in the tissues. Its N-shoot content of the fully watered controls of 13.84% increased to 17.31% (+3.47%) under drought (Table 4.2).

The corms $\delta^{15}N$ values were weakly correlated ($r = 0.31$) with N whereas a high correlation of $r = 0.65$ with WP $\delta^{15}N$ was found (Table 4.4). No significant correlations between $\delta^{15}N$ and $\delta^{13}C$ in taro shoots or corms (Tables 4.4 and 4.5) could be reported, which remains in conformity with Robinson et al. (2000) work on wild barley under drought. We observed a greater variation on the shoot-$\delta^{15}N$ abundance than in the corms (Table 4.2). This variation was most likely because of the plant nitrate reductase big dependence on NO_3^- flux from the underground organs to the shoots (Sahoo et al., 2010; Werner and Schmidt 2002). The observed effect of corm-$\delta^{15}N$ increase and shoot-$\delta^{15}N$ decrease on acc. 2210 and 2216, with N accumulation during drought, can be potentially related with greater assimilatory nitrate reductase reaction in corms. The restriction of NO_3^- flux during drought maybe contributed for the overall decrease of WP $\delta^{15}N$ (Table 4.3), due to the potential reduction of the activity of nitrate reductase in stress conditions (Sahoo et al., 2010). These variations of $\delta^{15}N$ values between the organs could be attributed to tissue reallocation of N under drought, and to external conditions (environment and N source availability) that led to the escalation of N consumption by ^{15}N and ^{14}N isotope fractionations, with $\delta^{15}N$ acting as a plant physiological integrator of stress responses (Romero-Trigueros et al., 2014; Robinson 2001).

4.4.3 Relationships among $\Delta^{13}C$, WUE and biomass

During drought, the increase of photosynthesis and decreased rate of photorespiration is regulated by the stomata, which aperture can increase the CO_2 plant intercellular spaces (C_i) (Igamberdiev et al., 2004). When drought-stressed, the stomatal regulated reduction in transpiration provides an opportunity to increase plant WUE. According to Black et al. (2015) a higher stomatal aperture can lead to a higher loss of water due to transpiration. WUE is linked to stomatal aperture, calculated as the ratio of plant biomass through assimilation of CO_2 by photosynthesis, to the loss of water by transpiration (Igamberdiev et al., 2004). Under drought conditions, we observed a significant variability with regard to WUE, ranging from 0.64 to 4.57 $g.L^{-1}$, among taro accessions included in our study. Regardless of water supply, the taro accessions displayed exactly the same variability. Three acc. (2056, 2061, 2216) had high WUE, presumably featuring a more drought-tolerant mechanism. Meanwhile, the remaining acc. had lower WUE probably indicating a moderate susceptibility to drought (Table 4.3). According to Farooq et al. (2009) genotypes with improved WUE and nutrients allocation are the most drought-tolerant, when compared to drought-sensitive ones. Acc. 2232 recorded the lowest WUE increase of 0.49 $g.L^{-1}$ between control and drought, 0.64 to 1.13 $g.L^{-1}$, respectively. On the other hand, acc. 2216 had the highest WUE in control and under drought conditions, 1.93 to 4.57 $g.L^{-1}$, respectively (2.64 $g.L^{-1}$ increase) (Table 4.3). Acc. 2216 showed the highest partial stomata aperture ($\delta^{13}C$ abundance ~ -26.27‰) among all acc. included in the study, which should lead to a higher water loss by transpiration (Table 4.2). In spite of the partially open stomata under drought, acc. 2216 was able to maintain leaf turgidity, minimized water loss through transpiration and improved water use for vital activities, maintaining high photosynthesis rate under drought. One can speculate that taro could have some phenotypic flexibility and morphological mechanisms of drought avoidance through selective biomass loss to prevent the water loss (Farooq et al., 2009). Water shortage during drought period led to a different biomass loss in all studied accessions. Acc. 2061 suffered the smaller biomass loss of only 3.88g under stress, while the highest

biomass loss of 40.57g was reported for acc. 2056 (Table 4.3). This mechanism of drought avoidance reduced the taro biomass relatively to water availability, enhancing the WUE, and allowing to maintain plant turgidity and vital activities. As each acc. enhanced WUE with the biomass decrease, in an overall similar way, it led to a moderate correlation between WUE and TPB (r = 0.43) (Tables 4.4 and 4.5). In contrast, in a previous drought study of Ganança et al. (2018), these taro cultivars grown under full water conditions showed higher WUE and higher fresh TPB.

A fairly small differences in the $\Delta^{13}C$ values reaching 17.39‰ in acc. 2061 and 19.53‰ in acc. 2232 were found (Table 4.3). The $\Delta^{13}C$ variation among the accessions is seemingly a reflection in their genetic variation, as no differences between well-watered and water-deprived environments could be identified (Boutton et al., 1993). Ivlev (2015) mentioned that lower $\Delta^{13}C$ values leads to higher carbon isotope fractionation, which can be related with the photosynthesis through CO_2 assimilation and photorespiration. For C3 plants, the $\Delta^{13}C$ values could be directly estimated by photorespiration, through stomatal conductance and carbon isotope fractionation. Although, this estimation depends on the growth conditions (Lanigan et al., 2008; Igamberdiev et al., 2004). During photosynthesis, plants discriminate against the heaviest carbon isotope. It changes according to the ratio of the plant intercellular spaces (C_i) *vs* atmospheric CO_2 (C_a), depending on the balance between photosynthetic activity and stomatal conductance (Serret et al., 2018; Farquhar et al., 1989; Farquhar et al., 1982). Farquhar et al. (1989) suggested that the richer the C3 plants are in $\delta^{13}C$ (with lower $\Delta^{13}C$ values), the greater WUE is. We observed that relationship as a significantly negative correlation between taro shoots $\Delta^{13}C$ and plant WUE (r = -0.33) was found (Tables 4.3 and 4.5). Laureti et al. (1993) also observed the same correlation in C3 sunflower plants grown under drought. They suggested that the significant negative relationship between $\Delta^{13}C$ and plant WUE can be used for assessing the WUE in breeding programs. Indeed, the results obtained during the course of our study seem to confirm that $\Delta^{13}C$ values could serve a possible substitute of the time-consuming direct WUE measurements of taro plants.

4.4.4 Whole-plant transformation processes

To better understand the relationship of $\delta^{15}N$, $\Delta^{13}C$, WUE and TPB in seven taro plants subjected to water stress, the one-way ANOVA analysis was applied. The variance and significant differences between accessions ($p \leq 0.01$) and experimental variants (control and drought-stress conditions, $p \leq 0.01$) were found (Tables 4.2 and 4.3). Multiple comparisons by the Tukey HSD (data not shown) revealed significant differences between control and drought shoots-$\delta^{15}N$, while no noteworthy differences in the corms-$\delta^{15}N$ could be identified. The corms-N content was significantly different from shoots-N under control and drought conditions. The WUE also differed significantly between control and drought.

The PCA analysis based in the average values of WUE, biomass and isotopic analysis showed discrimination between the corms (Figure 4.1A) and the shoots (Figure 4.1B) from control and the drought sets. The parameters that contributed the most to the main component were the WUE, TPB and $\delta^{15}N$. The principal components explained 93.2% of cumulative variance observed in the shoots, with 65.6% at first and 27.6% at the second axes, with eigenvalues of 0.25 and 0.11, respectively (Figure 4.1B). The accessions distribution corresponded to the observed variability of plant responses under drought stress.

The whole-plant multivariate analysis, analysis of variance and correlations between the traits variables, showed that the acc. 2061, 2216 and 2210 exhibited the best drought-tolerant response that was expressed as the ability to: maintain biomass production under stress; maintain leaf turgidity; minimize water loss through transpiration and improving the WUE, even with the partially open stomata; reallocate N and decrease WP $\delta^{15}N$ during drought; and had better assimilation of CO_2 into plant biomass, having the lowest $\Delta^{13}C$ values.

4.5 Conclusion

This study determined how taro $\delta^{13}C$ and $\delta^{15}N$ is related with the whole-plant biomass, WUE and $\Delta^{13}C$ under conditions of water deficit. Accessions 2061, 2216 and 2210 appeared to be the most drought-tolerant

genotypes showing the highest WUE and nutrients acquisition. Under drought, $\delta^{13}C$ of shoots and corms became less negative than the controls, with the shoots displayed a greater variation than the corms, increasing by 2.60‰. All $\delta^{13}C$ values pointed to relatively open stomata for C_3 plants. Improved WUE under drought conditions was achieved by minimizing water loss through evapotranspiration and employing phenotypic flexibility and morphological mechanisms of drought avoidance leading to a selective biomass loss. The $\delta^{15}N$ acted as a physiological integrator of stress responses in taro plants. The decrease of WP $\delta^{15}N$ due to stress relative to control acted as a good drought response mechanism. The corms $\delta^{15}N$ exhibited a fair correlation with [N] ($r = 0.31$), while was strongly correlated with WP $\delta^{15}N$ ($r = 0.65$). However, no significant correlations between $\delta^{15}N$ and $\delta^{13}C$ in taro shoots or corms could be identified. We observed a significant negative correlation between taro shoots $\Delta^{13}C$ and plant WUE ($r = -0.33$), similarly to previously reported findings for the C_3 plants. Information presented herein suggest that $\Delta^{13}C$ could be a plausible tool for screening for WUE in taro breeding programs, while $\delta^{15}N$ could serve as a physiological integrator of stress responses in taro plants.

4.6 Acknowledgements

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4.7 Author contribution

CSSG participated on the drought assay and samples preparation, performed the nitrogen analysis, interpreted and summarized all data generated from those experiments, and wrote the manuscript. JFTG designed the study for the drought assay, and helped in WUE quantification. JS coordinated the $\delta^{13}C$ and $\delta^{15}N$ analysis. VL and MAAPC coordinated the work and revised the manuscript.

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Tables

Table 4.1

Taro (*C. esculenta*) accessions subjected to different watering regimes, for the assessment of the plant biochemical responses to drought stress.

Accession ID[a]	Variety local name	Origin	Drought response[b]
2056	Listado	Canary Islands – La Palma	Moderate
2061	Blanco Saucero	Canary Islands – La Palma	Tolerant
2210	Roxo	Madeira Island	Moderate
2216	Branco	Madeira Island	Tolerant
2232	PExPH 15-6 BL/HW/08	SPC, Fiji	Sensitive
2234	C3-22 BL/PNG/11	SPC, Fiji	Moderate
2239	Karang CE/MAL/10	SPC, Fiji	Sensitive

[a] Accession identification number code used by the ISOPlexis Genebank.

[b] Classification of drought sensitive, moderate or tolerant accessions based on agro-morphologic screening according to Ganança et al. (2018).

Table 4.2

Mean value of $\delta^{15}N$ and $\delta^{13}C$ (‰), and total nitrogen concentrations (mg, DW) of shoots and corms, in control and drought taro plants.

Accession			Control	Drought	Variation
2056	$\delta^{15}N$	Shoot	8.72 ± 2.36	6.75 ± 1.00	-1.97
		Corm	4.63 ± 0.51	4.77 ± 1.04	+0.14
	$\delta^{13}C$	Shoot	-26.58 ± 0.59	-25.49 ± 0.97	+1.08
		Corm	-25.07 ± 0.26	-24.82 ± 0.14	+0.25
	[N]	Shoot	15.72 ± 1.68	18.73 ± 1.81	+3.01
		Corm	6.15 ± 1.72	7.25 ± 1.73	+1.09
2061	$\delta^{15}N$	Shoot	8.89 ± 1.05	7.40 ± 0.56	-1.49
		Corm	5.08 ± 0.71	4.06 ± 0.21	-1.02
	$\delta^{13}C$	Shoot	-25.84 ± 0.47	-24.58 ± 0.51	+1.26
		Corm	-25.33 ± 0.25	-25.32 ± 0.06	0.00
	[N]	Shoot	17.99 ± 3.48	17.23 ± 1.18	-0.76
		Corm	6.48 ± 0.95	8.07 ± 0.36	+1.59
2210	$\delta^{15}N$	Shoot	11.22 ± 0.95	10.20 ± 0.95	-1.02
		Corm	4.28 ± 1.11	5.31 ± 1.41	+1.02
	$\delta^{13}C$	Shoot	-27.53 ± 0.40	-26.46 ± 0.36	+1.07
		Corm	-26.33 ± 0.19	-25.76 ± 0.50	+0.57
	[N]	Shoot	20.69 ± 3.23	24.24 ± 1.20	+3.55
		Corm	6.25 ± 1.74	8.71 ± 1.79	+2.46
2216	$\delta^{15}N$	Shoot	9.14 ± 1.71	7.62 ± 0.19	-1.52
		Corm	4.89 ± 1.15	5.12 ± 0.23	+0.23
	$\delta^{13}C$	Shoot	-25.82 ± 1.20	-26.27 ± 1.52	-0.45
		Corm	-26.10 ± 1.69	-26.21 ± 0.36	-0.11
	[N]	Shoot	20.25 ± 1.51	22.13 ± 0.98	+1.88
		Corm	11.90 ± 2.77	13.22 ± 1.29	+1.32
2232	$\delta^{15}N$	Shoot	7.81 ± 1.99	7.09 ± 0.33	-0.72
		Corm	5.10 ± 0.53	4.70 ± 0.46	-0.41
	$\delta^{13}C$	Shoot	-27.24 ± 0.38	-27.18 ± 1.10	+0.07
		Corm	-26.75 ± 0.27	-26.76 ± 0.30	-0.01
	[N]	Shoot	15.94 ± 2.04	21.11 ± 2.85	+5.17
		Corm	6.09 ± 0.27	7.61 ± 0.49	+1.52
2234	$\delta^{15}N$	Shoot	10.10 ± 4.09	6.32 ± 0.38	-3.79
		Corm	4.05 ± 0.94	3.38 ± 0.29	-0.67
	$\delta^{13}C$	Shoot	-26.12 ± 1.55	-25.81 ± 1.05	-0.31
		Corm	-26.32 ± 0.84	-26.53 ± 0.15	-0.21
2234	[N]	Shoot	24.40 ± 3.46	18.83 ± 1.22	-5.56
		Corm	5.64 ± 1.35	4.85 ± 0.15	-0.79
2239	$\delta^{15}N$	Shoot	7.05 ± 1.27	7.25 ± 2.51	+0.20
		Corm	4.90 ± 1.17	4.67 ± 1.17	-0.24
	$\delta^{13}C$	Shoot	-26.69 ± 0.65	-26.84 ± 0.60	-0.15
		Corm	-26.47 ± 0.89	-26.98 ± 0.41	-0.51
	[N]	Shoot	13.84 ± 4.45	17.31 ± 0.72	+3.47
		Corm	9.01 ± 1.64	12.51 ± 5.52	+3.50
Total	$\delta 15N$ *ab*	Shoot	8.99 ± 1.28	7.52 ± 1.17	-1.47
		Corm	4.71 ± 0.40	4.57 ± 0.66	-0.13
	$\delta^{13}C$ *a*	Shoot	-26.55 ± 0.67	-26.09 ± 0.88	+0.46
		Corm	-26.05 ± 0.62	-26.05 ± 0.79	0.00
	[N] *ab*	Shoot	18.40 ± 1.10	19.94 ± 0.71	+1.54
		Corm	7.36 ± 2.29	8.89 ± 2.98	+1.53

$\delta^{15}N$ nitrogen isotopic composition (‰); *$\delta^{13}C$* carbon isotopic composition (‰), *[N]* total nitrogen (mg, DW); *a* significant differences between accessions (ANOVA, p≤0.01), *b* significant differences between control and drought stress conditions (ANOVA, p≤0.01). Control is well-watered, drought is severe stress. Variation is the difference between control and drought per trait. Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent lines replications per accession.

Table 4.3

Mean value of $\delta^{15}N$ and $\Delta^{13}C$ (‰), total WUE (g/L) and biomass concentrations (g/pot, DW) in control and drought taro whole-plant.

Accession		Control	Drought	Variation
2056	WP $\delta^{15}N$	7.53 ± 1.60	6.20 ± 0.92	-1.33
	$\Delta^{13}C$	18.30 ± 1.11	17.60 ± 0.50	-0.70
	WUE	1.27 ± 0.50	3.05 ± 0.98	+1.79
	TPB	87.47 ± 23.56	46.90 ± 14.46	-40.57
2061	WP $\delta^{15}N$	7.87 ± 0.94	6.33 ± 0.32	-1.54
	$\Delta^{13}C$	18.05 ± 0.38	17.39 ± 0.55	-0.66
	WUE	1.28 ± 0.19	2.87 ± 0.85	+1.58
	TPB	73.81 ± 17.75	69.94 ± 17.30	-3.88
2210	WP $\delta^{15}N$	9.59 ± 0.43	8.88 ± 0.67	-0.71
	$\Delta^{13}C$	19.45 ± 0.89	18.59 ± 0.52	-0.86
	WUE	0.64 ± 0.12	1.46 ± 0.44	+0.83
	TPB	37.78 ± 10.53	33.36 ± 10.43	-4.42
2216	WP $\delta^{15}N$	7.63 ± 1.27	6.70 ± 0.18	-0.93
	$\Delta^{13}C$	18.44 ± 0.20	18.73 ± 0.05	+0.29
	WUE	1.93 ± 0.61	4.57 ± 0.88	+2.64
	TPB	95.72 ± 35.31	75.26 ± 9.09	-20.46
2232	WP $\delta^{15}N$	7.07 ± 1.48	6.45 ± 0.25	-0.62
	$\Delta^{13}C$	19.53 ± 0.36	19.49 ± 0.31	-0.03
	WUE	0.64 ± 0.07	1.13 ± 0.06	+0.49
	TPB	40.54 ± 4.17	23.17 ± 5.61	-17.37
2234	WP $\delta^{15}N$	9.00 ± 3.55	5.71 ± 0.27	-3.29
	$\Delta^{13}C$	18.71 ± 0.15	18.66 ± 0.53	-0.05
	WUE	0.74 ± 0.10	1.63 ± 0.33	+0.89
	TPB	52.30 ± 0.33	46.47 ± 12.27	-5.83
2239	WP $\delta^{15}N$	6.23 ± 1.08	6.08 ± 1.34	-0.15
	$\Delta^{13}C$	19.09 ± 0.16	19.43 ± 0.11	+0.34
	WUE	0.76 ± 0.22	1.33 ± 0.44	+0.58
	TPB	43.25 ± 12.59	20.36 ± 5.29	-22.89
Total	WP $\delta^{15}N$	7.84 ± 1.14	6.62 ± 1.04	-1.22
	$\Delta^{13}C$ [a]	18.79 ± 0.58	18.56 ± 0.81	-0.24
	WUE [ab]	1.04 ± 0.48	2.29 ± 1.26	+1.26
	TPB [ab]	61.55 ± 23.86	45.07 ± 21.45	-16.49

WP $\delta^{15}N$ whole-plant nitrogen isotopic composition (‰); *$\Delta^{13}C$* whole-plant carbon isotope discrimination (‰); *WUE* whole-plant water use efficiency ($g.L^{-1}$); *TPB* total plant biomass ($g.pot^{-1}$, DW); *a* significant differences between accessions (ANOVA, p≤0.01), *b* significant differences between control and drought stress conditions (ANOVA, p≤0.01). Control is well-watered, drought is severe stress. Variation is the difference between control and drought per trait. Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent lines replications per accession.

Table 4.4

Pearson correlation coefficients of the biochemical traits from taro corms in control and drought stress conditions.

Variables	1	2	3	4	5	6
1. $\delta^{13}C$	-					
2. $\Delta^{13}C$	-1.00**	-				
3. $\delta^{15}N$	0.26	-0.26	-			
4. [N]	0.02	-0.02	0.31*	-		
5. WP $\delta^{15}N$	-0.01	0.01	0.65**	0.17	-	
6. WUE	0.25	-0.25	-0.02	0.30	-0.15	-
7. TPB	0.27	-0.27	-0.04	0.04	-0.22	0.43**

$\delta^{13}C$ carbon isotopic composition (‰); *$\Delta^{13}C$* carbon isotope discrimination (‰); *$\delta^{15}N$* nitrogen isotopic composition (‰); *[N]* total nitrogen (g, DW); *WP $\delta^{15}N$* whole-plant nitrogen isotopic composition (‰); *WUE* whole-plant water use efficiency ($g.L^{-1}$); *TPB* total plant biomass ($g.pot^{-1}$, DW); **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

Table 4.5

Pearson correlation coefficients of the biochemical traits from taro shoots in control and drought stress conditions.

Variables	1	2	3	4	5	6
1. $\delta^{13}C$	-					
2. $\Delta^{13}C$	-1.00**	-				
3. $\delta^{15}N$	-0.11	0.11	-			
4. [N]	0.05	-0.05	0.29	-		
5. WP $\delta^{15}N$	-0.18	0.18	0.10	-0.11	-	
6. WUE	0.33*	-0.33*	-0.25	0.13	-0.15	-
7. TPB	0.28	-0.28	0.04	-0.16	-0.22	0.43**

$\delta^{13}C$ carbon isotopic composition (‰); *$\Delta^{13}C$* carbon isotope discrimination (‰); *$\delta^{15}N$* nitrogen isotopic composition (‰); *[N]* total nitrogen (g, DW); *WP $\delta^{15}N$* whole-plant nitrogen isotopic composition (‰); *WUE* whole-plant water use efficiency ($g.L^{-1}$); *TPB* total plant biomass ($g.pot^{-1}$, DW); **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

Figure

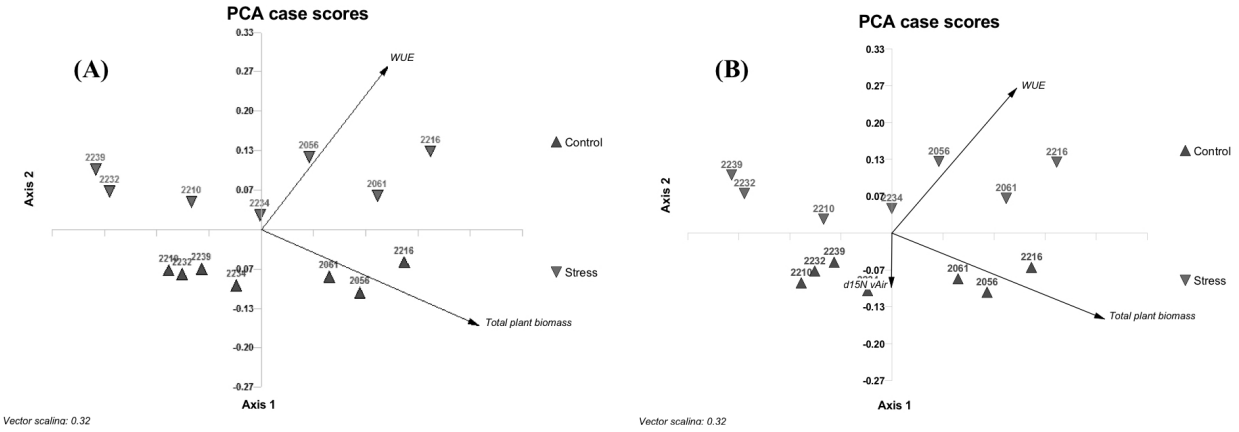

Figure 4.1
Representation of the Euclidean biplot by principal component analysis (PCA), with spatial distribution of the *C. esculenta* accessions based in the average isotope values in drought-stressed conditions.

(A) Scattering distribution of the variables (traits) and case scores (taro accessions) evaluated in corms in two PCA axes.
(B) Representation of the spatial distribution of the variables (traits) and case scores (taro accessions) studied in shoots in two PCA axes.
Data log_e transformation was applied to all the traits (variables) in analysis.
Control represents well-watered plants; stress - severe drought.

CHAPTER 5

Involvement of abscisic acid and other stress indicators in taro (*Colocasia esculenta* (L.) Schott) response to drought conditions

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ORIGINAL ARTICLE

Involvement of abscisic acid and other stress indicators in taro (*Colocasia esculenta* (L.) Schott) response to drought conditions

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5.1 Abstract

Taro (*Colocasia esculenta* (L.) Schott) is a staple food and represents an important food security role in most tropical regions. It is, unfortunately, susceptible to prolonged drought conditions. Abscisic acid (ABA) is a well-documented stress-induced phytohormone that tolerant crops usually accumulate in leaves to induce stomatal closure, preventing water loss through inhibition of transpiration. Hitherto, exists very scarce information regarding the ABA role in taro response to drought. Here, we determined the ABA content in the shoots and corms of taro subjected to seven months of water scarcity and linked ABA to other drought resilience traits, including carbon isotopic discrimination ($\Delta^{13}C$), oxalic acid (OA), chlorophyll content index (CCI), water use efficiency (WUE), and biomass (B). The $\Delta^{13}C$-shoot content showed partially open stomata in all accessions, and significant correlation with $\Delta^{13}C$-corm, CCI, and WUE. The osmotically active OA-shoot decrease seemed not to interfere with the stomatal aperture. The tolerant accessions subjected to drought stress had higher B-corm, ABA-shoot, $\Delta^{13}C$-shoot, CCI, OA, and WUE. However, the observed under drought conditions increase of ABA in the shoots, and its decrease in the corms were not significantly correlated, nor with other traits, suggesting that ABA was not the main regulator of taro physiological processes under stress. The information gained should be considered in breeding programs to predict taro's response to climate change.

Keywords
Carbon isotope; Chlorophyll content index; Drought stress; Enzyme-linked immunosorbent assay; Oxalic acid; Water use efficiency.

Abbreviations

ABA Abscisic acid
Acc Accession number
B Biomass
CCI Chlorophyll content index
$\delta^{13}C$ Carbon isotope composition
$\Delta^{13}C$ Carbon isotope discrimination
ELISA Enzyme-linked immunosorbent assay
OA Oxalic acid
PCA Principal component analysis
SPC Pacific community
WUE Water use efficiency

5.2 Introduction

Taro (*Colocasia esculenta* L. Schott) is an important crop for food security in developing countries, as an important source of carbohydrates in the form of starch stored in the corms (Sharma and Kaushal 2016). In 2018, taro production in Africa alone reached almost 7.9 Mt, representing 74% of the worldwide production (FAOSTAT Statistical Database 2018). Taro requires nearly 2,500 mm rainfall per year, which is considered a high-water volume to achieve optimal yields. As its productivity highly depends on water availability, this crop is more exposed to long periods of water shortage, regarding the expected events in climate change (Ganança et al. 2018).

Crop plants use abscisic acid (ABA), a sesquiterpene with an α,β-unsaturated ketone in the ring and a conjugated diene side-chain, as an important isoprenoid stress-induced phytohormone to signalize the development and growth physiological processes, such as stomatal closure under drought conditions (Wani et al. 2016; Huang et al. 2014; Ma and Qin 2014; Mengel et al. 2001).

Literature reports a general plant root-to-shoot ABA signalization during drought that leads to a generalized stomatal closure to prevent water loss by inhibiting transpiration, largely due to ABA accumulation at the chloroplast level (Wani et al. 2016; Osakabe et al. 2014; Mengel et al. 2001). Leaves could be the major location for ABA biosynthesis when under drought-stressed conditions, showing a greater effect over root development (McAdam et al. 2016). Other traits, such as the chlorophyll content index (CCI), carbon isotopic discrimination ($\Delta^{13}C$), oxalic acid (OA), water use efficiency (WUE), and biomass (B) have been recently identified as important parameters, reflecting taro's resilience to water scarcity (Gouveia et al. 2019; Ganança et al. 2018; Gouveia et al. 2018). Plants with the greatest resistance to drought, usually accumulate ABA in the shoots, have higher chlorophyll content, improved WUE, and less biomass loss (Black et al. 2015; Tuberosa 2012; Tardieu and Davies 1992; Farooq et al. 2009).

The oxalic acid (OA) can inhibit the ABA-induced stomatal closure, and thus plays pivotal role in the plant ion balance and osmoregulation (Guimarães and Stotz 2004; Franceschi and Horner 1980). Increased atmospheric CO_2 can also lead to stomatal closure and subsequent reduction of the photosynthesis rate, while measurements of chlorophyll content were successfully used as a plant health indicator during drought stress (Gouveia et al. 2018; Salehi-Lisar and Bakhshayeshan-Agdam 2016; Shao et al. 2015; Osakabe et al. 2014). The reduction of photosynthesis inhibits transpiration and increases plant WUE for vital activities during scarcity conditions, with subsequent attenuation of the biomass loss to water availability (Gouveia et al. 2019; Ganança et al. 2018; Salehi-Lisar and Bakhshayeshan-Agdam 2016). The photosynthetic depletion of ^{13}C, determined as the plant carbon isotopic discrimination ($\Delta^{13}C$), has been reported to correspond with the closure of stomata during drought (Gouveia et al. 2019; Farquhar et al. 1989). The biomass (B) expresses the whole-plant growth under stress, explaining the energy investment and the individual growth of the organs. The shooting area usually is the most affected with water scarcity, with higher biomass decay to prevent water loss by evapotranspiration (Atwell et al. 1999; van den Boogaard et al., 1995).

Hitherto, very little is known about ABA in taro at the whole-plant level and even less about the role of ABA in taro's response to drought. Recent studies explored the effects of several concentration levels of ABA on *in vitro* grown taro plantlets (Acedo et al. 2017), and the impacts of foliar spray of ABA on field-grown taro, under different irrigation regimes (Abuzeed et al. 2019). In our study, we used a collection of seven accessions from distinct geographical provenances, aiming to quantify their endogenous ABA-corm and ABA-shoot content after long exposure to water scarcity conditions. Hereupon, to seek ABA connection to the taro stress response, we related this phytohormone to other drought resilience indicators, including biomass, CCI, $\Delta^{13}C$, OA, and WUE.

5.3 Materials and Methods

5.3.1 Plant materials and experimental setup

Seven taro (*Colocasia esculenta* L. Schott) accessions (acc.) originating from Madeira Island, Canary Islands and from the Pacific Community (SPC) germplasm collection (Table 5.1), were submitted to seven months of water scarcity, according to Gouveia et al. (2019), in an open greenhouse (April to November 2015, Câmara de Lobos, Madeira, Portugal, 32°39'N; 16°58'W). Twenty-four plants per accession were individually grown in 30×30 cm pots, filled with 15 kg of dried soil. The pots were arranged in six rows, spaced 90 cm apart, and 30 cm in row separation. Control pots were maintained at field capacity while experimental (drought stress) pots received 40.2% of the water applied to the controls. No pesticide or fertilizer were used and weeds were manually removed as needed. In total, 336 corms and shoots (petioles and leaves) samples from control and drought pots were collected, washed, chopped, oven-dried for 48h at 65ºC (Memmert UF260, Germany) and grounded into flour with a universal mill (IKA-Werke M20, USA). The flour was placed in bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153, Netherlands) and stored at -35ºC (Liebherr ProfiLine GGPV6570, Germany) until analysis.

5.3.2 Abscisic acid (ABA)

The ABA was determined in corms and shoots flours by the Enzyme-Linked Immunosorbent Assay (ELISA) technique. We used a plant hormone ABA ELISA Kit, 96 tests (MyBioSource Inc., USA, Cat. Nº MBS282218) according to the kit instructions. A microplate reader (Tecan Sunrise Remote A-5082, Austria; software MagellanTM V7.1, Tecan, Austria) was used for ABA quantitation at 450 nm corrected at 620 nm. The data obtained were calculated using the logarithmic transformation. The analyses were performed in triplicate and the values were expressed in ng/g of dry flour.

5.3.3 Carbon isotope discrimination ($\Delta^{13}C$)

The flours from corms and shoots were vacuum packaged and sent to the Natural Resources Analytical Laboratory at the University of Alberta (Edmonton, Canada) for carbon isotope composition ($\delta^{13}C$) analysis. The $\delta^{13}C$ was determined by the micro-chemical AOAC 972.43:2000 method using a Delta V Advantage Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS, Thermo Finnigan Corp, Germany). The $\delta^{13}C$ was converted to $\Delta^{13}C$ from the obtained carbon isotope composition of corm and shoot plant material ($\delta^{13}C_p$) and the source of atmospheric CO_2 carbon ($\delta^{13}C_a$ = -8‰), with Farquhar et al. (1989) equation:

$$\Delta^{13}C\ (‰) = (\delta^{13}C_a - \delta^{13}C_p) / (1 + \delta^{13}Cp)$$

The analysis was made in triplicate, and the results were expressed as ‰ units of dry flour.

5.3.4 Oxalic acid (OA)

OA from corms and shoots flours was determined according to Gouveia et al. (2018) by a titrimetric method with potassium permanganate solution ($KMnO_4$, 0.05M). The oxalic acid content was obtained according to the Dye (1956) calculation:

$$OA\ (mg/100\ g) = (T \times V_{me} \times 3 \times 105) / (5 \times mf)$$

with *T* titer of $KMnO_4$ (mL); *Vme* volume-mass equivalent between 0.05M $KMnO_4$ and anhydrous oxalic acid; *3* dilution factor; *5* molar equivalent of $KMnO_4$ redox reaction in oxalate; *mf* mass of flour used. The analyses were made in triplicate, and the values were expressed as mg/100 g of dry flour.

5.3.5 Chlorophyll content index (CCI)

CCI was measured with a chlorophyll content meter (Opti-Sciences CCM-200 PLUS, USA) and represents the relative chlorophyll content of the taro fresh leaves. The CCI values represent an average of three measurements per plant taken in the morning avoiding the branching veins at the adaxial leaf surface.

5.3.6 Biomass (B)

The fresh biomass of corms and shoots was collected from each pot and dehydrated until constant weight, using an air oven (Memmert UF260, Germany) (Undersander et al. 1993). The values were expressed as g/pot of dry flour.

5.3.7 Water Use Efficiency (WUE)

Productivity WUE was measured as an average value per replicate of the ratio between the whole-plant dry biomass and the total water used, expressed in g/L (Ganança et al. 2018; Mengel et al. 2001).

5.3.8 Statistical methods

The results were expressed on a dry weight basis as the main average of taro corms and shoots samples from control and drought replicates. The variables in our study that had non-normal distribution were normalized according to Templeton (2011) and were submitted to One-way ANOVA, Tukey HSD test and Pearson correlations by IBM SPSS Statistics V24 for Mac, expressing the statistically significant differences at $p \leq 0.05$. Principal component analysis (PCA) was performed with MVSP V3.1 for Windows.

5.4 Results

5.4.1 Variation of abscisic acid from taro whole-plants subjected to water scarcity

The quantitation values for ABA obtained for taro corms and shoots samples, under control and drought conditions, are presented in Table 5.2.

Control well-watered plants contained four times more ABA in the shoots than in the corms. However, under drought conditions ABA-shoot was almost 5-fold higher in comparison to ABA-corm, with ABA-shoots showing high variability. A 12% decrease in the ABA-corm was observed under drought, while a slight 4% increase of ABA was reported in the shoot.

Relatively to shoot tissues, drought led to a register of a wide range of ABA content when compared to control, in the following order: acc. 2056 (-18.3 ng/g); 2234 (-15.3 ng/g); 2061 (-9.9 ng/g); 2239 (-7.5 ng/g); 2210 (+12.2 ng/g); 2232 (+30.3 ng/g); and 2216 (+38.5 ng/g). The acc. 2239 had significantly lower ABA-shoot content under both experimental conditions, decreasing from 21 to 14 ng/g (-33%). Conversely, the acc. 2232 showed significantly highest ABA-shoot content, with an increase from 189 to 219 ng/g (+16%) under drought. The acc. 2216 reached the upper range of the ABA-shoot content, increasing from 119 to 158 ng/g (+32%) under drought.

Relatively to ABA-corm, the acc. 2239 had the lowest ABA-corm content under control conditions, but registered the highest content under drought, increasing from 4 to 33 ng/g (+725%). Acc. 2232 had the lowest ABA-corm difference between control and drought, decreasing from 25 to 24 ng/g (-2%). That is, under drought conditions, the variation in ABA content in corm tissues was registered in the following order when comparing with the control: acc. 2234 (-27.5 ng/g); 2210 (-7.3 ng/g); 2061 (-6.6 ng/g); 2216 (-4.9 ng/g); 2056 (-1.0 ng/g); 2232 (-0.6 ng/g); and 2239 (+28.0 ng/g).

5.4.2 Carbon isotope discrimination and oxalic acid in drought-stressed taro' whole-plants

The Table 5.2 shows the $\Delta^{13}C$ and OA values obtained from the taro corm and shoot samples, under control and drought conditions.

The $\Delta^{13}C$ differences between control and drought were practically negligible. On average, water scarcity decreased $\Delta^{13}C$-shoot by only 3%, at 19‰, and kept the $\Delta^{13}C$-corm content at 18‰. The acc. 2061 had the lowest $\Delta^{13}C$-shoot (17‰) under drought. All acc. faintly decreased the $\Delta^{13}C$-corm, with the exception of acc. 2216, 2234 and 2239. Acc. 2239 logged the highest significant $\Delta^{13}C$-corm content (20‰).

The differences of the OA content between control and drought were similar for both organs, although in opposite ways. Drought decreased the OA-shoot from 54 to 46 mg/100g (-8%), meanwhile the OA-corm

increased from 36 to 44 mg/100g (+8%). Acc. 2061 and 2239 were the exceptions, since the OA-corm decreased under drought conditions with highest difference registered in acc. 2061 (reduction of 46%, from 57 to 31 mg/100g). Acc. 2232, 2234, and 2239 increased the OA-shoot under drought, with acc. 2232 recording the highest growth from 38 to 51 mg/100g (+34%).

5.4.3 Variation in water use efficiency, chlorophyll content index, and biomass under drought conditions

Table 5.2 contains the taro's CCI, WUE, and biomass values in response to drought. Under water scarcity, the average CCI and WUE values increased, meanwhile the biomass decreased.

A slight 2% increase in CCI content during water scarcity, with a mean value of 33 was recorded. Acc. 2210 had significantly the highest chlorophyll content under drought conditions, with CCI ranging from 38 to 47 (+22%). Contrariwise, acc. 2232 and 2234 decreased their CCI content under water scarcity. Although the acc. 2234 suffered the highest CCI loss, from 41 to 31 (-24%), it managed to keep a high chlorophyll content under both experimental conditions. Acc. 2239 showed the lowest CCI among all acc., registering a slight insignificant increase from 12 to 13 (+2%), under drought conditions.

On average, an 85% increase of WUE (from 0.20 to 0.37 g/L) was found in all acc. under drought. The lowest WUE increase, from 0.14 to 0.19 g/L (+36%) was recorded in acc. 2232, while the highest increase in WUE, from 0.32 to 0.67 g/L (+109%) was found in acc. 2216.

Regarding the biomass (B), the water scarcity led to an average 29% reduction in corms (from 53 to 38 g/pot) and 18% in shoots (from 8 to 7 g/pot). Acc. 2061 and 2216 were the ones that showed the highest B-corm and B-shoot content under drought, with 61 and 57 g/pot, and with 9 and 18 g/pot, respectively. Acc. 2061 was the only one which increased the B-shoot content under drought, from 5 to 9 g/pot (+60%), demonstrating smaller biomass loss at the whole-plant level. The acc. 2210 had a smaller loss at the corm level (-10%), losing only 4 g/pot to stress, from 36 to 32 g/pot. Meanwhile, the highest biomass loss in both organs was reported for acc. 2056, with the B-corm decreasing from 75 to 42 g/pot (-44%), and the B-shoot from 13 to 5 g/pot (-60%).

5.4.4 Statistical variance, correlation and component analysis

The ABA, CCI, $\Delta^{13}C$, OA, WUE and B traits (variables) were submitted to One-way ANOVA, Tukey HSD test (Table 5.2), Pearson correlation (Table 5.3) and PCA analysis (Figure 5.1).

The ABA-corm and $\Delta^{13}C$-shoot had a non-normal distribution according to the Kolmogorov-Smirnov non-parametric test. Pairwise comparisons performed by Kruskal-Wallis non-parametric test retained the null hypothesis, *i.e.*, showed no differences between the accessions for ABA-corm and $\Delta^{13}C$-shoot variables. Then, ABA-corm and $\Delta^{13}C$-shoot were normalized according to Templeton (2011). Subsequently, all variables were independently subjected to one-way analysis of variance (One-Way ANOVA) to verify if there were significant differences between experimental conditions. The Tukey HSD test was also performed to identify the samples that differ significantly at each variable. We verified that the ABA-corm did not show statistically significant differences between accessions ($p > 0.05$) and experimental conditions, probably due to the great variability registered among the accession's row replicates. The $\Delta^{13}C$-shoot also showed no significant differences among accessions and experimental conditions ($p > 0.05$). One-Way ANOVA and Tukey HSD multiple comparisons performed at the remaining traits showed significant differences ($p \leq 0.01$) between accessions, and between control and drought environments (Table 5.2).

Using the Pearson's correlation coefficient, we found 12 significant correlations among the six variables in study ($p \leq 0.05$). The strongest significant and positive correlations were observed within B-shoot and B-corm (r = 0.61), followed by OA-corm and OA-shoot (r = 0.57), WUE within B-corm (r = 0.51) and B-shoot (r = 0.51), and finally negative between $\Delta^{13}C$-corm and CCI (r = -0.50). Moderate positive correlations were also observed among WUE and CCI (r = 0.48); CCI within B-corm (r = 0.48) and B-shoot (r = 0.33); $\Delta^{13}C$-shoot and $\Delta^{13}C$-corm (r = 0.41). Moderate negative correlations were registered between

Δ^{13}C-shoot and CCI (r = -0.34) and WUE (r = -0.32). The B-corm also had moderate negative correlation with Δ^{13}C-corm (r = -0.33) (Table 5.3).

Finally, a principal component analysis (PCA) was performed using these variables (Figure 5.1). The first two principal components accumulated 74.7% of variance. Component 1 explained 41.3% of variance, with eigenvalues of 0.78, while component 2 explained 33.4% with eigenvalues of 0.63. The ABA-shoot was strongly correlated with component 1, meanwhile B-shoot and ABA-corm showed a higher correlation with component 2. Almost all the accessions grouped closely together, with little difference between control and drought environments, representing low variability of the measured variables in response to drought. However, acc. 2234 and 2239 exhibited the highest spatial difference between test conditions, denoting a greater variability of the traits in response to the environment. This suggests that both of these accessions apparently are the most sensitive to drought. However, acc. 2239 is located far-off from other samples, and is in opposed direction from the main variable vectors, suggesting perchance a different type of response and metabolic composition, as they were the ones with the lowest ABA-corm content under control, and the lowest ABA-shoot content under drought conditions.

5.5 Discussion

5.5.1 ABA-corm and ABA-shoot performance under water scarcity

ABA is typically detected in plants at early growth stages, contributing slightly to the increase of taro's corm yield under non-stress conditions (Abuzeed et al. 2019; Nakatani and Komeichi 1991). ABA has also a meaningful function as a plant stress response hormone (Abuzeed et al. 2019; Nakatani and Komeichi 1991). When plants are exposed to adverse environmental conditions, such as drought, ABA is accumulated to regulate their growth and water status during water scarcity conditions (Nakatani and Komeichi 1991).

In non-stress conditions, an average of 4-fold higher ABA-shoot relatively to ABA-corm content was recorded. This ABA difference between organs remains in accordance with Li and Jia (2014) who argued that plants usually accumulate more ABA in chloroplast cells when compared to other tissues, such as roots and stems. We observed almost 5-fold increase in ABA-shoot under drought in comparison to ABA-corm. It could be a good indicator of root-to-shoot signaling because despite facing a long period of drought, the most resistant ones were still able to increment ABA-shoot content (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Ma and Qin 2014; Osakabe et al. 2014; Mengel et al. 2001). However, the observed differences in ABA between taro organs under stress were not significantly correlated. The ABA-shoot increase could be considered a good response to drought, suggesting an ABA signaling from corms to accumulate ABA in leaf chloroplasts (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Osakabe et al. 2014; Mengel et al. 2001). However, under water scarcity, only acc. 2210, 2216, and 2232 enhanced their ABA-shoot content, while the ABA-corm content decreased in practically all accessions (except for acc. 2239). McAdam et al. (2016) explained that when the underground organs are unable to synthesize ABA during drought conditions, plants could have normal increases in foliar ABA level, with normal stomatal responses to drought. Still, ABA content decreased in acc. 2056, 2061, and 2234 in both organs, a potential unraveling of the dynamic equilibrium between the ABA biosynthesis and catabolism. To accumulate ABA as in acc. 2210, 2216, and 2232, the remain accessions needs to keep the depletion and delivery of the ABA precursors (xanthophyll) between their corms and shoots during drought (Li and Jia 2014).

The ABA increase can regulate the plant's water status associated with a better plant response to absorbing water and nutrients, such as enhanced root elongation in maize crops (Sah et al. 2016; Duman 2012; Tuberosa 2012). Although, Abuzeed et al. (2019) have shown that when field-grown taro shoots are sprayed with ABA at low irrigation levels, the foliar absorption of ABA did not show tissue growth. Acedo et al. (2017) has also shown that taro plantlets subjected to a minimum of 0.5 mg/L (500 ng/g) of ABA solution resulted in hindrance of the root and shoot development at in vitro conditions. Our highest drought ABA value registered for corms (33.2 ng/g for acc. 2239) and shoots (219 ng/g for acc. 2232) were above the

one reported by Acedo et al. (2017). However, all tested taro accessions showed biomass loss under water scarcity that was not significantly correlated with ABA content. Hereupon, the lack of significant associations between ABA and biomass indicates that this phytohormone does not contribute directly to the loss of taro's tissue growth during water scarcity conditions.

5.5.2 ABA relationship to other drought stress indicators

ABA has been reported to regulate the stomata function under non-stress conditions, allowing the photosynthesis and transpiration processes in plants (Osakabe et al. 2014; Tuberosa 2012; Tardieu and Davies 1992). However, abiotic stress factors, such as nutrient depletion and drought, can increase ABA content in leaves, which induces the stomata closure as a way to regulate the water status by lowering the transpiration processes (Osakabe et al. 2014; Tardieu and Davies 1992). The stomatal closure regulates the plant growth and development under adverse environmental conditions (Ramakrishna and Ravishankar 2014). In our study, the taro's ABA-shoot content increased under drought, but did not affect the stomata closure (discussed below). Likewise, the ABA content did not show significant correlations with the other measured traits, namely the Δ^{13}C-shoot, CCI, OA-shoot, WUE, and biomass.

The average Δ^{13}C-shoot content under control (Δ^{13}C = 19.1‰) was indicative of near open stomata (Δ^{13}C = 31‰, or δ^{13}C = -38‰), as referred by O'Leary (1993), exhibiting partially open stomata under non-stress conditions. As the Δ^{13}C derives from the plant carbon isotope fractionation, it is a very useful tool that can reflect the plant stomatal conductance and carbon assimilation in leaves, which is linked with the photosynthesis through CO_2 assimilation and photorespiration (Ivlev 2015). Under the closed stomata scenario, the Δ^{13}C-shoot content would be around 4‰ (or δ^{13}C= -12‰) due to a lower photosynthesis activity derived from the limitation of leaf CO_2 uptake, and a lower carboxylation fractionation (O'Leary 1993). Since the Δ^{13}C-shoot only faintly decreased, and remained at 19‰ under both control and drought conditions, no considerable changes in the plant stomatal conductance and carbon assimilation should be expected (Lanigan et al. 2008). Likewise, both organs had practically the same ^{13}C fixation, with Δ^{13}C values fairly similar between the treatments (Wegener et al. 2015).

We registered a higher CCI content during drought, inferring a slight increase of photosynthesis activity (except for acc. 2232 and 2234). The Δ^{13}C-shoot appeared to be significantly correlated with CCI content, which could be considered as another evidence of partially open stomata during stress. The partial stomatal aperture allowed the intracellular CO_2 uptake under stress, maintaining their photosynthetic electron transport from water molecules, with chlorophyll molecules ionization through light excitation of photosystem PSII (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Igamberdiev et al. 2004). Contrariwise, the CCI decrease observed in acc. 2232 and 2234 remains in accordance with a study performed on South-African taro landraces subjected to drought, reporting stomatal closure in response to decreased intracellular CO_2 availability (Mabhaudhi and Modi 2015).

As a result of partially open stomata under both conditions, all the acc. managed to improve the WUE during drought, with the acc. 2216 exhibiting the highest increase. The WUE increase was foremost caused by lower transpiration during drought, leading to increased water availability for plant vital activities (Black et al. 2015). The WUE improvement was significantly correlated with the loss of corm and shoot biomass, with the slight CCI increase and minute decrease of Δ^{13}C-shoot content. Usually, drought mainly affects the aboveground organs biomass relatively to underground organs, to limit evapotranspiration and improve WUE (Atwell et al. 1999; van den Boogaard et al., 1995). We observed that pattern, with the acc. decrease of root-to-shoot ratio, showing a generalized trend for developing the shoot rather than corm (except for acc. 2056 and 2210) (Gouveia et al. 2020). However, the acc. 2216 maintained a high chlorophyll content, minimized the water loss despite the partially open stomata (Δ^{13}C-shoot = 18.8‰), and showed one of the best phenotypic flexibility and morphological mechanisms of drought avoidance with lower B-shoot and B-corm loss (Gouveia et al. 2020; Farooq et al. 2009). This one also had higher ABA-shoot content during drought, showing the second-highest content among all accessions in the study. According to

Osakabe et al. (2014) and Tardieu and Davies (1992), improved WUE is usually attributed to stomatal closure, which could be mediated by ABA content accumulated in shoots, with the most tolerant plants showing elevated ABA-shoot production. However, in our study, ABA was not correlated with these traits, implying that this accumulation was not significant for WUE improvement.

Nonetheless, the partially open stomata allowed for a high CCI, and biosynthesis of organic osmolytes such as the oxalic acid (OA) (Gouveia et al. 2018; Tuberosa 2012). OA can be synthesized from the photosynthetic glycolate-glyoxylate oxidation by light stimulation (Igamberdiev and Eprintsev 2016; Franceschi and Horner 1980). The photosynthetic rate was not influenced during drought, but still we reported a decrease of the OA-shoot, with an accumulation of OA-corm content. According to Gouveia et al. (2018), this could be due to the precipitation of the oxalic acid present in the leaves into calcium oxalate, with the possible mobilization of this insoluble salt from the shoots to the roots for plant osmotic regulation, to be further excreted to environment. According to Guimarães and Stotz (2004), the OA-shoot acts as osmotically active molecules, which increment leads to a stomatal opening, and can consequently counteract the stomatal closure induced by ABA-shoot accumulation under drought. Besides these two traits were not significantly correlated, the OA-shoot decrease did not affect the ABA-shoot signal to induce stomatal closure under stress.

The ABA content detected in our taro accessions was much lower than reported by Abuzeed et al. (2019) and Acedo et al. (2017). Abuzeed et al. (2019) sprayed ABA on the taro leaves and referred that ABA mediated the stomata closure under low irrigation regime by reducing the photosynthesis rate. However, in our study, the slight increase of ABA-shoot did not influence directly the stomatal conductance. We indeed registered an ABA-shoot increase with ABA-corm decrease but the correlation was not significant. Otherwise, it could be related to root-to-shoot signaling, leading to a generalized stomatal closure to prevent water loss by inhibiting transpiration (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Wani et al. 2016; Ma and Qin 2014; Osakabe et al. 2014; Mengel et al. 2001). For a significant root-to-shoot ABA signaling, our taro accessions need to accumulate additional ABA-shoot in the chloroplasts to improve the signalization of stomata closure, with the need for a higher depletion and delivery of ABA precursors between their organs, as proposed by Li and Jia (2014).

Plants with increased resilience to drought feature accumulation of ABA-shoot, improved WUE, chlorophyll and biomass content when compared with sensitive ones (Black et al. 2015; Tardieu and Davies 1992; Tuberosa 2012). The accessions with the best capability to cope and avoid drought showed a lower stress index due to a smaller difference between the corm and shoot biomass during stress (Gouveia et al. 2020). Therefore, among all taro accessions in the study, acc. 2216 appears to be the most resistant to water scarcity conditions. This accession showed a good carboxylation fractionation during photosynthesis resulting from an increase of Δ^{13}C-shoot, with one of the best CCI content; and transport of photosynthetic assimilates from shoots to corms, leading to an increase of Δ^{13}C-corm, required for plant growth improvement during drought stress (Wegener et al. 2015). It also displayed the highest significant WUE content in both treatments, and the highest biomass content in both organs. And lastly, the acc. 2216 presented a good plant osmotic regulation with the lowest loss of OA-shoot, and the highest ABA-shoot accumulation. Acc. 2239 appeared to be the most sensitive to drought, by showing significantly lower corm and shoot biomass, CCI, WUE, and ABA-shoot content.

5.6 Conclusion

The present study showed ABA-shoot accumulation and ABA-corm decreased content in taro submitted to drought. This points to a good stress response through root-to-shoot ABA signaling, according to the literature. However, the observed relationship did not show significant correlations with any other indicator traits. With this, and the low ABA content reported at the taro whole-plant level in both non-stress and stress conditions, indicates that ABA does not participate actively in the physiological processes of the studied taro accessions. Nevertheless, this study substantiates that the loss of osmotically active OA-shoot was correlated with OA-corm increase at low irrigation conditions, and not interfere with ABA signal for

stomatal closure. Under both experimental conditions, all accessions showed partially open stomata, according to the stomatal conductance and carbon assimilation in the leaves, given by Δ^{13}C-shoot content. The increase of Δ^{13}C-shoot, CCI, and WUE was significantly correlated and were the main indicators of partially open stomata during the drought assay. Taro plants used the phenotypic flexibility and morphological mechanisms of drought avoidance to minimize the biomass loss, instead of using ABA as the main regulator of stomatal activity and plant growth. Acc. 2216 possesses the best combination of the traits mentioned above, and consequently exhibited the best tolerance response to water stress, and is selected as a potential parent for drought tolerance breeding programs.

5.7 Acknowledgments

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5.8 Author contribution statement

CSSG participated at the drought assay and samples preparation, performed the ABA and oxalic acid analysis, interpreted and summarized all data generated from those experiments, and wrote the manuscript. JFTG quantified the WUE and helped CSSG in CCI recording. JJS coordinated the δ^{13}C analysis and revised the manuscript. VL and MAAPC coordinated the overall work and revised the manuscript.

5.9 Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 5.1
Name and origin of taro (*Colocasia esculenta* L.) accessions selected for this study.

Accession ID[†]	Variety local name	Origin
2056	Listado	La Palma – Canary Islands (CAN)
2061	Blanco Saucero	La Palma – Canary Islands (CAN)
2210	Roxo	Madeira Island (MAD)
2216	Branco	Madeira Island (MAD)
2232	PExPH 15-6 BL/HW/08	Fiji – Pacific Community (SPC)
2234	C3-22 BL/PNG/11	Fiji – Pacific Community (SPC)
2239	Karang CE/MAL/10	Fiji – Pacific Community (SPC)

[†]Accessions identification number code, used by the ISOPlexis Genebank.

Table 5.2

Abscisic acid (ABA), carbon isotope discrimination ($\Delta^{13}C$), oxalic acid (OA), chlorophyll content index (CCI), water use efficiency (WUE), and biomass (B) of taro (*Colocasia esculenta* L.) organs under both control and drought experimental conditions.

			ABA		$\Delta^{13}C$		OA		CCI **	WUE **	B	
			Corm	Shoot **	Corm **	Shoot	Corm **	Shoot **			Corm **	Shoot **
			---------- ng/g ----------		---------- ‰ ----------		-------- mg/100g ----------		---	---- g/L ----	---------- g/pot ----------	
CAN	2056	Control[A]	12.3 ± 6.9 [a]	141.7 ± 15.4 [abc]	17.5 ± 0.3 [ab]	19.1 ± 0.6 [a]	41.1 ± 12.6 [ab]	84.1 ± 20.4 [cd]	37.4 ± 7.5 [abcd]	0.29 ± 0.08 [abc]	74.8 ± 17.8 [de]	12.7 ± 5.9 [abc]
		Drought[B]	11.3 ± 15.0 [a]	123.4 ± 63.8 [abc]	17.2 ± 0.1 [a]	18.0 ± 1.0 [a]	59.4 ± 20.4 [bc]	62.4 ± 1.0 [abcd]	38.7 ± 15.6 [abcd]	0.39 ± 0.12 [bc]	41.8 ± 12.7 [abcde]	5.1 ± 2.0 [a]
		Variation[C]	-1.0	-18.3	-0.3	-1.1	+18.3	-21.7	+1.3	+0.10	-33.0	-7.6
	2061	Control	17.0 ± 7.0 [a]	42.5 ± 23.6 [ab]	17.8 ± 0.3 [abc]	18.3 ± 0.5 [a]	56.9 ± 3.9 [bc]	51.7 ± 3.4 [abcd]	40.3 ± 2.0 [abcd]	0.23 ± 0.03 [ab]	68.5 ± 16.0 [cde]	5.3 ± 1.8 [a]
		Drought	10.4 ± 4.0 [a]	32.6 ± 44.3 [ab]	17.8 ± 0.1 [abc]	17.0 ± 0.5 [a]	31.0 ± 0.2 [ab]	25.6 ± 9.9 [a]	43.4 ± 6.8 [cd]	0.48 ± 0.16 [cd]	61.4 ± 14.5 [cde]	8.5 ± 4.6 [abc]
		Variation	-6.6	-9.9	0.0	-1.3	-25.9	-26.1	+3.1	+0.25	-7.1	+3.2
MAD	2210	Control	35.3 ± 14.2 [a]	109.6 ± 51.8 [abc]	18.8 ± 0.2 [abc]	20.1 ± 0.4 [a]	16.6 ± 4.2 [a]	52.8 ± 6.0 [abcd]	38.3 ± 7.9 [abcd]	0.12 ± 0.04 [a]	35.8 ± 8.6 [abcd]	2.0 ± 2.2 [a]
		Drought	28.0 ± 20.7 [a]	121.8 ± 17.9 [abc]	18.2 ± 0.5 [abc]	19.0 ± 0.4 [a]	28.7 ± 6.3 [ab]	27.0 ± 6.4 [a]	46.9 ± 13.4 [d]	0.28 ± 0.09 [abc]	32.2 ± 10.1 [abc]	1.1 ± 0.3 [a]
		Variation	-7.3	+12.2	-0.6	-1.1	+12.1	-25.8	+8.6	+0.16	-3.6	-0.8
	2216	Control	20.8 ± 12.7 [a]	119.3 ± 28.1 [abc]	18.6 ± 1.8 [abc]	18.3 ± 1.3 [a]	25.8 ± 11.6 [ab]	40.3 ± 9.0 [abcd]	41.3 ± 4.3 [bcd]	0.32 ± 0.12 [abc]	76.2 ± 29.9 [e]	19.5 ± 8.3 [c]
		Drought	15.2 ± 5.8 [a]	157.8 ± 65.3 [abc]	18.7 ± 0.4 [abc]	18.8 ± 1.6 [a]	39.6 ± 13.8 [ab]	34.6 ± 4.4 [ab]	44.8 ± 13.7 [cd]	0.67 ± 0.11 [d]	56.9 ± 6.9 [bcde]	18.3 ± 8.0 [bc]
		Variation	-4.9	+38.5	+0.1	+0.5	+13.8	-5.7	+3.5	+0.35	-19.4	-1.1
SPC	2232	Control	25.0 ± 3.6 [a]	188.5 ± 66.5 [bc]	19.3 ± 0.3 [bc]	19.8 ± 0.4 [a]	28.4 ± 4.9 [ab]	37.8 ± 2.9 [abc]	18.6 ± 7.0 [abcd]	0.14 ± 0.01 [a]	34.6 ± 4.9 [abc]	5.9 ± 0.8 [a]
		Drought	24.4 ± 9.1 [a]	218.8 ± 127.9 [c]	19.3 ± 0.3 [bc]	19.7 ± 1.2 [a]	36.0 ± 4.3 [ab]	50.7 ± 14.7 [abcd]	16.1 ± 6.1 [abc]	0.19 ± 0.05 [ab]	19.8 ± 6.4 [ab]	3.4 ± 2.8 [a]
		Variation	-0.6	+30.3	0.0	-0.1	+7.6	+12.9	-2.5	+0.05	-14.8	-2.5
	2234	Control	54.6 ± 38.3 [a]	81.1 ± 83.4 [abc]	18.8 ± 0.9 [abc]	18.6 ± 1.6 [a]	55.7 ± 4.5 [abc]	82.6 ± 42.2 [bcd]	40.7 ± 16.5 [abcd]	0.18 ± 0.00 [ab]	47.0 ± 3.6 [abcde]	5.3 ± 3.8 [a]
		Drought	27.1 ± 7.6 [a]	65.8 ± 16.2 [abc]	19.0 ± 0.2 [abc]	18.3 ± 1.1 [a]	84.1 ± 37.5 [c]	89.2 ± 29.0 [d]	30.8 ± 10.0 [abcd]	0.39 ± 0.10 [bc]	41.2 ± 13.9 [abcde]	5.3 ± 2.5 [a]
		Variation	-27.5	-15.3	+0.2	-0.3	+28.4	+6.6	-9.9	+0.21	-5.8	0.0
	2239	Control	4.2 ± 1.8 [a]	21.2 ± 2.9 [a]	19.0 ± 0.9 [abc]	19.2 ± 0.7 [a]	27.0 ± 7.9 [ab]	29.0 ± 7.5 [a]	12.4 ± 3.6 [a]	0.15 ± 0.04 [ab]	36.9 ± 12.1 [abcde]	6.4 ± 1.4 [ab]
		Drought	33.2 ± 48.1 [a]	13.7 ± 10.1 [a]	19.5 ± 0.4 [c]	19.4 ± 0.6 [a]	25.8 ± 3.1 [ab]	34.4 ± 12.2 [ab]	12.7 ± 5.2 [ab]	0.17 ± 0.04 [ab]	15.2 ± 4.2 [a]	5.2 ± 2.7 [a]
		Variation	+28.0	-7.5	+0.5	+0.2	-1.2	+5.4	+0.3	+0.02	-21.7	-1.2
	Mean	Control	24.2	100.6	18.5	19.1	35.9	54.0	32.7	0.20	53.4	8.2
		Drought	21.4	104.8	18.5	18.6	43.5	46.3	33.3	0.37	38.4	6.7
		Variation	-2.8	+4.2	0.0	-0.5	+7.6	-7.7	+0.6	+0.17	-15.0	-1.4

Data are expressed on dry weight basis (DW), and represent the mean ± SD of three independent replications per accession, with means not sharing the same letters between the columns are significantly different (Tukey HSD, $p \leq 0.05$).

** Significant differences between control and drought stress conditions (One-way ANOVA, $p \leq 0.01$)

[A] Control is fully irrigated.

[B] Drought is water scarcity.

[C] Variation is the difference between control and drought per trait.

Table 5.3

Pearson correlation coefficients between the water use efficiency (WUE), biomass (B), abscisic acid (ABA), oxalic acid (OA), carbon isotope discrimination ($\Delta^{13}C$), and chlorophyll content index (CCI), evaluated in taro (*Colocasia esculenta* L.) organs subjected to control and drought conditions.

	1	2	3	4	5	6	7	8	9
1. WUE	-								
2. B-corm	0.51[a]	-							
3. ABA-corm	-0.25	-0.16	-						
4. OA-corm	0.25	0.10	0.08	-					
5. $\Delta^{13}C$-corm	-0.24	-0.33[b]	0.25	-0.19	-				
6. CCI	0.48[a]	0.48[a]	-0.11	0.13	-0.50[a]	-			
7. B-shoot	0.51[a]	0.61[a]	-0.27	-0.16	0.02	0.33[b]	-		
8. ABA-shoot	-0.01	-0.11	-0.13	-0.11	-0.04	0.09	0.12	-	
9. OA-shoot	-0.06	0.12	0.08	0.57[a]	-0.20	0.15	-0.05	0.13	-
10. $\Delta^{13}C$-shoot	-0.32[b]	-0.24	0.29	-0.16	0.41[a]	-0.34[b]	-0.30	0.21	-0.10

[a]Correlation is significant at the 0.01 level (2-tailed)

[b]Correlation is significant at the 0.05 level (2-tailed).

Figure

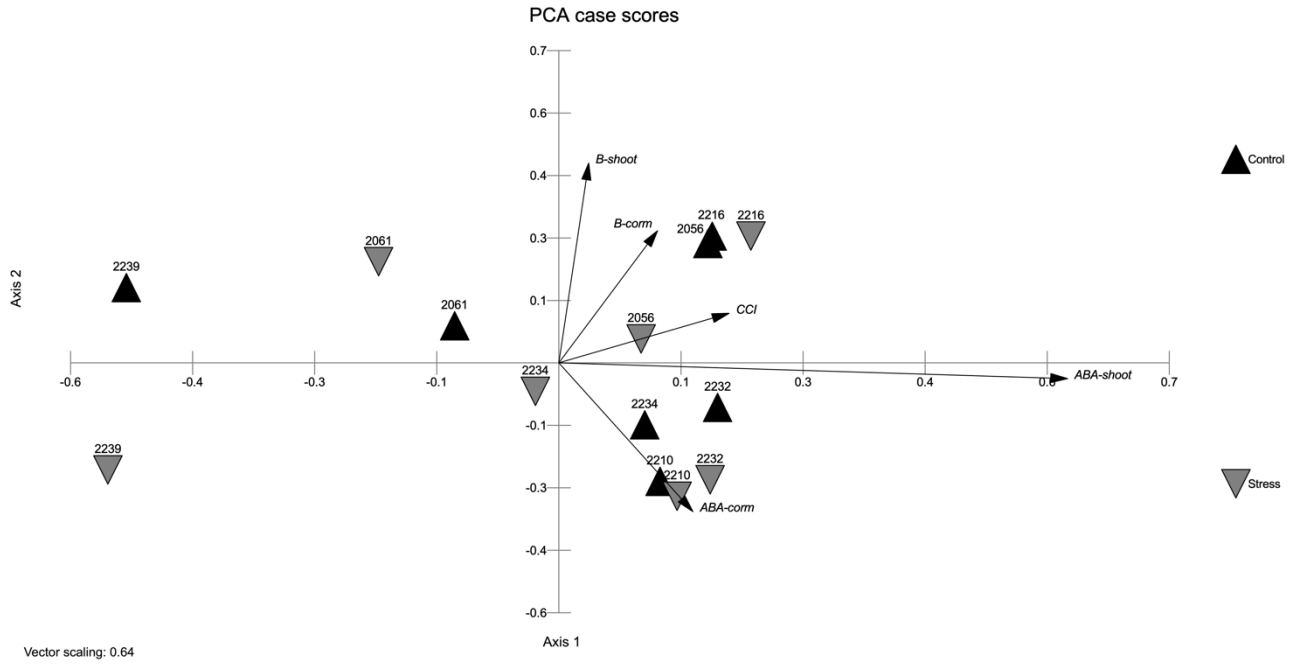

Figure 5.1

Principal component analysis (PCA), represented by euclidean biplot spatial distribution of taro (*Colocasia esculenta* L.) accessions, using corm and shoot measurements of the studied variables (ABA, CCI, $\Delta^{13}C$, OA, WUE).

All the variables were converted by $\log_e$. Control - fully irrigated. Stress - water scarcity.

CHAPTER 6

Drought avoidance and phenotypic flexibility of sweet potato (*Ipomoea batatas* (L.) Lam.) under water scarcity conditions

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Original Article

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Drought Avoidance and Phenotypic Flexibility of Sweet Potato (*Ipomoea batatas* (L.) Lam.) Under Water Scarcity Conditions

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6.1 Abstract

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important staple food in several regions of the world. Water scarcity is the most devastating abiotic stress, with a great impact on crop productivity, food security, and subsistence. Drought restricts the nutrient intake and transport into the plant. Tolerant crops have morphological mechanisms of drought avoidance and/or phenotypic flexibility, showing also good water and nutrient efficiency. However, that information is scarce for sweet potato, which is usually based on physiological traits of plant productivity. Here, we show the physiological responses of eight sweet potato accessions subjected to a 3 months' drought period, by recording their differences for nutrient and leaf chlorophyll content, biomass and stress level. Our results showed that the differences in water use efficiency (WUE, +68.1%), chlorophyll content index (CCI, -5.3%), total plant biomass (TPB, -55.4%), nutrient efficiency (NER, +38.1%) and nutrient harvest index (NHI, +2.9%) where significantly correlated with the water regime. The water shortage led to a drought avoidance response, with TPB loss in all accessions. Distinct phenotypic flexibility responses were also recorded and explained by the root:shoot ratio (R:S) and stress index (SI) variation of the storage root and shoot growth. This information could be relevant for the development of sweet potato breeding programs, adapting this crop to climate change.

Keywords

Biomass; chlorophyll content index; drought; nitrogen; nutrient efficiency; root:shoot ratio; stress index

Abbreviations

Acc. Accession
CAN Canary Islands
CCI Chlorophyll content index
DW Dry weight basis
E Nitrogen efficiency of utilization
GUI Guinea-Bissau
M Total mineral content
MAD Madeira Island
N Nitrogen content
NER Nitrogen efficiency ratio
NHI Nitrogen harvest index

R:S Root-to-shoot ratio
SI Whole-plant stress index
TPB Total plant biomass
WUE Water use efficiency

6.2 Introduction

Roots and tubers are the second-most cultivated group of species after cereals, contributing significantly to food and nutritional security. Sweet potato (*Ipomoea batatas* (L.) Lam.) is a tropical crop tuber, and a worldwide important staple food (Sharma and Kaushal, 2016). Asia had the highest sweet potato production in 2017, with 79.6 Mt, representing 71% of worldwide production (FAOSTAT database, www.fao.org, 2019).

Water scarcity is one of the main collateral abiotic consequences of climate change, which is increasingly affecting worldwide crop production. This lack of water decreases productivity, jeopardizing subsistence and food security (Lebot, 2009; Ganança *et al*., 2015; Ganança *et al*., 2018). Cultivars are considered to be under drought stress when they face water limitation in the soil, and when they are subjected to the constant loss of water through evapotranspiration triggered by atmospheric conditions (Motsa *et al*., 2015a).

Sweet potato can be moderately tolerant to drought conditions due to low plant growth habit and extensive root system, and its production is usually done under relatively low input conditions (Smittle *et al*., 1990; Ekanayake and Collins, 2004; Motsa *et al*., 2015b). The most tolerant plants show inherent morphological mechanisms of drought avoidance and/or phenotypic flexibility, as a natural defense towards water scarceness conditions (Farooq *et al*., 2009).

The main drought avoidance response aims to reduce the plant water loss through transpiration, keeping the water uptake through the root system and improving the root biomass yield under drought (Farooq *et al*., 2009). Water use efficiency (WUE) is a fundamental trait for the distinction of plant drought tolerance. The more tolerant accessions usually display higher WUE by minimizing water loss, through the reduction of stomatal aperture, improving water use for vital activities and plant production (Ganança *et al*., 2018; Gouveia *et al*., 2019). In sweet potato, the water deficit reduces root yield and nitrogenous compounds (Ekanayake and Collins, 2004; Sharma and Kaushal, 2016).

Drought reduces the diffusion of nutrients (minerals) from the soil matrix to the roots, and with reduced transpiration rates, the nutrient transport from the roots to the shoots is compromised (Duman, 2012). The nitrogen use efficiency (NUE) can discriminate the accessions according to their aptitude for nutrient absorption and use for yield increase (Mathur and Goel, 2017). Tolerant varieties can display higher yield and NUE in a drought environment or under low input condition (Yuan and Peng, 2017). NUE can be calculated according to the crop and its harvest product, based on the nutrient uptake efficiency (NUpE), harvest index (NHI), incorporation efficiency (efficiency ratio, NER) and utilization efficiency (E) (Siddiqi and Glass, 1981; Good *et al*., 2004; Lammerts van Bueren and Struik, 2017; Mathur and Goel, 2017). The NER is generally utilized to differentiate accessions into efficient and inefficient nutrient use (Good *et al*., 2004; Mathur and Goel, 2017). Although, very few NUE studies were made on tuber crops (such as cassava, sweet potato, and taro) being mostly done in grain crops (Hartemink *et al*., 2000; John *et al*., 2016; Lammerts van Bueren and Struik, 2017).

The phenotypic flexibility comprises the plant growth behavior during scarcity conditions. The underground (roots) and aboveground (leaves) tissues are the main affected organs, but both can have different mechanisms of adaptation to drought. Plants can improve root performance and growth through drought, allowing them to preserve the leaf area and root development under extensive stress (Farooq *et al*., 2009). According to Rundel and Sharifi (1993), the variation of root:shoot ratio (R:S) during the plants' life cycle allows them to keep the WUE according to the water availability. The water-limited plants can increase the R:S, because of the decline of their leaf growth under water scarcity (Hubick and Gibson, 1993; Laureti *et al*.,

1993). When the plant shoots development stops and root growth continues under drought stress, can be associated with a general adaptation syndrome (GAS) to water scarcity evolving osmoregulation control (Leshem and Kuiper, 1996). Root crop accessions can show an R:S increase throughout their aging, due to carbon accumulation in the underground tissues (Atwell *et al*., 1999). The increment of plant photosynthesis during abiotic stress is another good indicator of their capacity to defy drought, where best plant's resistance appears related with higher plant chlorophyll index, and consequently a greater production and plant vigor (Tiwari and Mamrutha, 2013; Mabhaudhi and Modi, 2015; Pereira *et al*., 2015; Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Gouveia *et al*., 2018).

The goals of the present study were i) to assess the chlorophyll rate, nutrient, carbon, and water allocation, as well as stress levels of sweet potato accessions submitted to drought, and ii) to correlate these parameters with mechanisms of drought avoidance and phenotypic flexibility.

6.3 Materials and Methods

6.3.1 Experimental sites and drought management

The sweet potato assay was performed in randomly split-plot field design, established at the ISOPlexis experimental field (32° 39' N, 16° 55' W, 174 m a.s.l., Funchal, Madeira, Portugal), during a 5 months' cycle. Eight accessions of sweet potato from Madeira and Canary Islands, and Guinea-Bissau (Table 6.1) were grown in two independent blocks, one under regular open field conditions (control) and the other under a rain shelter (water deficit). Each accession was then planted in 3 plots (replicates), in eight independent rows, 30 plants per accession in total, with 70 × 80 cm in and between the rows, respectively. Three vines were added per plot as blind samples, in both open and shelter environments, with full irrigation.

After stress imposition, two distinct water regimes were applied through a drip irrigation system, 1.6 mm three times a week for control (based on normal irrigation in Madeiran agricultural practices) and 0.9 mm three times a week for water deficit, per plot, for 3 months. During this period, control plots received about 77 mm and stressed plots received 54 mm of water. Control obtained 117.5 mm from rainfall, during this period. During rain periods, the irrigation of control plots was suspended. Both control and drought plots were assessed periodically for the: photosynthetic active radiation (PAR, 400-700 nm) with a ceptometer (AccuPAR LP-80, USA); volume water content of soil (VWCs) with a soil moisture sensor (WaterScout SM100, USA); air temperature (Ta) and relative air humidity (RHa) with a data logger (Testo 174H, Germany). During the assay, we registered a 24.6% PAR decrease under the rain shelter when compared to control, on average with 1,514.5 μmol m^{-2} s^{-1} for control and 1,142.0 μmol m^{-2} s^{-1} for drought. At 10 cm of depth of homogenized field soil, we registered an average of 12.8% VWCs for control, representing 35% of field capacity; and 3.5% VWCs for drought, representing 10% of field capacity. On average, control showed 19.46 °C for Ta and 68.07% for RHa; drought showed 22.25 °C for Ta and 66.40% for RHa.

All experiments were implemented in a soil-free of chemical contaminants, without adding any fertilizers or phytopharmaceutical products. Weeds were manually removed at regular intervals, to prevent interference in the crop yield.

6.3.2 Harvest and sample preparation

Three hundred eighty-four sweet potato storage roots and shoots (considering stem, stalk, and leaves) samples of control and drought replicates were harvested, at the end of the agronomic trial.

All samples were cleaned with running water, weighed with a scale (Sartorius Basic BA2100S, Germany), sliced 2-3 mm thickness with a mandolin slicer, oven-dried at 65 °C during 48 h (Memmert UF260, Germany), and finely milled (IKA-Werke M20, USA). The flour was placed into bags (Termofilm PA/PE), vacuum-sealed (Audionvac VMS153, Netherlands) and stored at -35 °C (Liebherr ProfiLine GGPV6570, Germany) until analysis.

6.3.3 Soil chemical and physical properties

Air-dried soil samples were grinded, sieved (2 mm) and analyzed by the Agricultural Quality Laboratory at the Directory of Laboratory Services and Agrifood, in Camacha, Madeira, Portugal. Soil chemical and physical properties were analyzed as following: pH H_2O (1:2.5 w/v); pH KCl (1:2 w/v); organic matter by Walkley and Black method; nitrate and ammonia content by continuous-flow auto analyzer (3:15 w/v); soil texture and particle-size were classified according to the World Reference Database for Soil Resources (IUSS, 2015).

6.3.4 Chlorophyll content index (CCI)

The CCI in sweet potato fresh leaves was determined, through the chlorophyll fluorescence measurement, using a chlorophyll content meter (Opti-Sciences CCM-200 PLUS, USA). Three readings were made uniformly along the adaxial leaf surface (left, center and right), avoiding the branching veins. A mean CCI value per main plant leaf was determined in each growth row.

6.3.5 Nitrogen content (N)

Nitrogen concentration in sample flours was determined by the Kjeldahl method AOAC 945-18.B (AOAC 2005), using a distillation and titration automatic unit (Velp Scientifica UDK 152, Italy). Analyses were performed in triplicate, and the average values expressed in g 100 g^{-1} of dry flour.

6.3.6 N efficiency ratio (NER)

The nutrient efficiency ratio was calculated as the ratio between the plant dry biomass (W) and nitrogen plant uptake (N), according to Steenbjerg and Jakobsen (1963):

$$NER = W / N \quad (1)$$

The calculations were done in triplicate, and the values expressed in kg kg^{-1} of dry flour.

6.3.7 N efficiency of utilization (E)

The E was calculated according to the modified approach by Siddiqi and Glass (1981) as whole-plant nutrient use efficiency (NER), allowing to compare the increase of the produced biomass with NER. They take into account the absolute amount of biomass production increases (W) and NER, given as the ratio of biomass (W) per plant nitrogen (N) uptake capacity (plant N accumulated):

$$E = W \times NER = W \times (W / N) \quad (2)$$

The calculations were performed in triplicate, and the values expressed in kg of dry flour.

6.3.8 N harvest index (NHI)

The NHI describes the share of nitrogen accumulated in storage roots, concerning the total plant nitrogen uptake (Kołodziejczyk, 2014). It was calculated as the ratio of N in storage root (Nt) to N uptake by the plant (N):

$$NHI = Nt / N \quad (3)$$

The calculations were performed in triplicate, and the values expressed in % of dry flour.

6.3.9 Root-to-shoot ratio (R:S)

The ratio between the storage roots dry weight and respective shoots was calculated for both control and drought conditions (Laureti *et al*., 1993).

6.3.10 Total mineral content (M)

Total mineral content or ashes was determined gravimetrically by sample flour calcination method AOAC 923.03 (AOAC, 2005), using a furnace (Vulcan Model 3-550, NEY, USA) at 550 ± 10 °C, for 5 h. The analyses were performed in triplicate, and the values expressed in g $100g^{-1}$ dry flour.

6.3.11 Total plant biomass (TPB)

TPB represented the whole-plant biomass calculated from the storage roots and shoots weight of four plants per replicate, dehydrated in an air oven according to Undersander *et al*. (1993). Each treatment was run in triplicate and results expressed in g of dry flour.

6.3.12 Water use efficiency (WUE)

WUE was the ratio between total plant dry weight and total water used per plant, and expressed in g L^{-1} (Ganança *et al.*, 2018).

6.3.13 Whole-plant stress index (SI)

The whole-plant stress index (SI) for each accession was calculated to measure the dimension of the natural response to drought among accessions, using the average dry weight (W) of the whole-plant according to the Robinson *et al*. (2000) equation:

$$SI = (W_{unstressed} - W_{stressed}) / W_{unstressed} \quad (4)$$

The SI ranges from 0 to 1, in terms of the effect of the environment on plant growth. Plant SI values tend toward 0 when less stressed ($SI \rightarrow 0$), and tend to 1 with increasing stress conditions ($SI \rightarrow 1$).

6.3.14 Data analysis

The results are represented as the main average of storage roots and shoots of 3 control *vs* 3 drought replicates, expressed in a dry weight basis. All samples were statistically evaluated with SPSS version 23.0 for Mac, for Pearson correlations, one-way ANOVA and Tukey HSD. The statistically significant differences were expressed with a *p*-value lower than 0.05.

6.4 Results

6.4.1 WUE, biomass, chlorophyll and nutrient use interactions during drought

All sweet potato accessions decreased their biomass, showing a drought avoidance behaviour during stress. However, they showed a variation in their physiological responses, according to water allocation, chlorophyll rate and nutrient use at the whole-plant level (Table 6.2).

On average, total plant biomass (TPB) of sweet potato decreased from 533.1 to 237.6 g (-55.4%). Acc. 3124 and 3125 showed limited weight loss variation, while acc. 2937 and 2938 had the lowest biomass content in all drought assays.

Relatively to water use efficiency (WUE), the sweet potato acc. increased the WUE from 4.7 to 7.9 g L^{-1} (+68.1%) on average, with the acc. 3124 and 3125 showing the highest significant WUE values. The acc. 3126 and 2938 were the exception, by decreasing WUE, with both of them having the highest water loss through transpiration.

Slight variations of chlorophyll activity, *i.e.* chlorophyll content index (CCI), were registered in drought-stressed sweet potato accessions. Their CCI decreased from 30.4 to 28.8 (-5.3%), by lowering the photosynthetic rate, except for acc. 1036 and 3126.

N efficiency ratio (NER) for all the sweet potato acc. increased, from 11.3 to 15.6 kg kg^{-1} (+38.1%), with acc. 2937 and 2938 showing the highest significant NER. N efficiency of utilization (E) increased from 4.6 to 7.1 kg (+54.4%), where acc. 2937 and 2938 shows a significant E increase. Meanwhile, E content of acc. 1036 decreased.

N harvest index (NHI), on average, increased from 27.9 to 30.8% (+2.9%), with acc. 2927 and 2937 showing significantly higher NHI content, and 1038 acc. being the only one where an NHI decrease was observed.

6.4.2 Nutrient efficiency ability during drought

The sweet potato acc. varied their nutrient allocation, between underground storage roots and aboveground shoots, along with the mineral (M), nitrogen (N), NER and E traits (Table 6.3). The soil of the experimental plot, at 0.2 m sampling depth, showed moderate inorganic mineral content (NO_3^- and NH_4^+), with a slightly acidic pH 6.6, and silt clay texture (data not shown).

In general, the sweet potato acc. showed a lower M content in storage roots relatively to shoots, in both experimental conditions. They had a slight increase of M-root during drought stress, from 4.2 to 4.3 g $100g^{-1}$ (+0.1%), but registered a decrease in the M-shoot, from 12.2 to 10.5 g 100 g^{-1} (-1.7%). Acc. 3125 had the highest M-root content (4.9 to 5.0 g 100 g^{-1}), while acc. 3126 had the highest M-shoot content (14.2 to 11.9 g 100 g^{-1}). Only the acc. 2927 and 2937 showed a decrease in the mineral content in both plant organs.

The sweet potato acc. exhibited a higher N content in shoots when compared to storage roots, in both experimental conditions. Although, under water scarcity, sweet potato decreased the N content in both organs. In average, the N-root slightly decreased from 1.0 to 0.9 g 100 g^{-1} (-0.1%), and the N-shoot dropped from 2.6 to 2.1 g 100 g^{-1} (-0.5%). Acc. 3126 showed the highest decrease of N-shoot (3.3 to 2.7 g 100 g^{-1}). The acc. 1036 had the highest N-root content (1.0 to 1.5 g 100 g^{-1}) and was the exception by showing an N increase in both organs during drought.

Both NER and E were higher in the underground organs. Under stress, both NER-root and NER-shoot of the sweet potato acc. increased from 26.8 to 32.6 kg kg^{-1} (+21.6%) and from 5.6 to 8.9 kg kg^{-1} (+58.9%), respectively. The acc. 2938 showed the highest NER-root content, with 32.4 kg kg^{-1} for control and 51.1 kg kg^{-1} for stress conditions. The E-root and E-shoot had an overall increase from 7.1 to 9.1 kg (+28.2%), and from 0.8 to 1.6 kg (+100%), respectively. The highest E-root content was registered at acc. 2938 (9.3 to 14.8 kg).

6.4.3 Drought stress index and root-to-shoot relationship

All accessions showed a decrease of TPB, as an effect of low water availability, exhibiting differences within the plant organs development, which can be described by the phenotypic flexibility through the R:S and SI (Fig. 6.1).

The R:S shows the plant capacity to maintain an active balance between the underground storage roots and aboveground shoots, under drought. In control conditions, the R:S ranged from 1:0.6 to 1:7 for acc. 1036 and 1038, respectively. The acc. 1038 showed the highest variation between the weight of the organs, where shoots were 7 times lighter than storage roots, in control conditions. Overall, drought decreased the sweet potato R:S due to the investment in shoot development. The acc. 1036 showed the highest investment in shoot production, and the biggest R:S decrease during drought (1:0.6 to 1:0.1, -81.9%). The exception was for sweet potato acc. 2938 (1:1.6 to 1:2, +43.8%), that was the only one which showed an R:S increase. This can indicate that it was the only one that decreased shoot development during drought, in favor of tuber growth.

The SI was used to assess the differences in stress strength for each acc., showing the impact of drought conditions on their innate growth ($0 < SI < 1$). The acc. 3124 and 3125 registered the lowest SI value, around 0.1 ($SI \rightarrow 0$), being the less drought-sensitive, by showing higher growth capacity and a more tolerant response to water scarcity. On the other hand, acc. 3126, 2937 and 2938 showed to be the most stress-sensitive, with SI values around 0.8 ($SI \rightarrow 1$).

6.4.4 Pearson correlation coefficients between traits

Seventy-seven significant correlations were found between 16 sweet potato traits, of which 34 were strong, with $r \geq 0.50$ (Table 6.4). TPB and WUE had a moderate positive correlation, while CCI showed a negative correlation with NER-shoot and a significant positive correlation with M-shoot. R:S had negative correlations with NHI, NER-shoot, and E-shoot. N-root showed positive correlations with NHI and M-root, and negative correlations with E, NER-root, and E-root. NER also had a positive correlation with N-root. The sweet potato acc. with the highest TPB showed also the highest CCI, WUE, R:S, M-shoot, and N-shoot

contents. We also observed that lower NER and NHI corresponded to greater CCI, TPB and WUE values.

6.5 Discussion

6.5.1 Relation between water scarcity and plant health development

The water scarcity is one of the most threatening environmental stresses for crop yield, with undesirable impacts in the main staple food crops (Lebot, 2009; Ganança *et al*., 2015). In this study, all acc. decreased their biomass under water scarcity, a drought avoidance response. They also showed variations in organ growth between treatments, demonstrating phenotypic flexibility. Besides the loss of biomass, changes amongst the CCI and WUE contents were also recorded.

Sweet potato lost on average more than half of the TPB due to drought. Motsa *et al*. (2015b) and Smittle *et al*. (1990) reported that sweet potato cultivars had good ability to alter their growth features, mainly the development of an extensive root system. They adapt to low input conditions, earning moderate tolerance to drought stress through greater phenotypic plasticity. Plants can improve their biomass content through the constant adjustment of shoot and root growth rates, according to the resources uptake (Atwell *et al*., 1999). Overall, the sweet potato acc. showed a dynamic balance between underground and aboveground organs, during drought, leading to an R:S ratio decrease, which implies a generalized trend for developing the shoot rather than the storage roots. One sweet potato accession (2938) was the exception, registering an R:S increase dictated by tubers growth instead of providing energy for the development of the shoots. Probably, this acc. had a bigger tendency for carbon investment in underground structures, as Atwell *et al.* (1999) proposed to be possible on roots development. Being this the acc. with the smallest canopy, it could favor the storage root growth over shoot development, reducing the shoot area as a way to avoid shoot water loss (van den Boogaard *et al*., 1995; Motsa *et al*., 2015a). This can be a plant response, related to GAS, with water scarcity involving osmoregulation and WUE increase (Leshem and Kuiper, 1996). In cotton and peanut cultivars, the leaf area reduction occurs when they are submitted to lack of water or nutrients, inducing a higher R:S (Harris 1992; Hubick and Gibson, 1993; Laureti *et al*., 1993; Atwell *et al*., 1999). Notwithstanding the biomass loss in all accessions, we also registered low SI in two sweet potato accessions (3124 and 3125). The low SI is related to low TPB variation between control and drought environments, being the best ones in maintaining their growth under water scarcity.

The CCI can indirectly infer the plant photosynthetic rate, through the association of the photosynthetic electron transport between shoots (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). It can indicate the plant drought stress tolerance, where the bigger the plant chlorophyll index, the better the plant resistance to water scarcity (Tiwari and Mamrutha, 2013; Salehi-Lisar and Bakhshayeshan-Agdam 2016; Gouveia *et al*., 2018). Although, drought lowered the sweet potato CCI content (-5.3%). The lower CCI values indicate a lower photosynthetic activity (Shao *et al*., 2015). According to Mabhaudhi and Modi (2015) and Osakabe *et al*. (2014), the photosynthesis down-regulation resulted from the decrease of the intracellular CO_2 availability, leading to a relative stomatal closure to avoid water loss during drought.

The decrease of CCI was more associated with water scarcity than with the PAR decrease (-24.6%) observed inside the shelter. Under fully irrigated conditions, the blind samples had higher chlorophyll content inside the shelter, relatively to open field conditions (data not shown). The CCI decrease inside the shelter was mainly due to water limitation, which could have led to a lower excitation of photosystem II (PSII) through photons of light, through a lower number of ionized chlorophyll molecules (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). The increase of photorespiration rates is another consequence of photosynthesis inhibition that could be related to the CCI decrease, during drought. The decrease of leaf CO_2/O_2 ratio, due to stomata closure led to inhibition of carboxylase and activation of oxygenase functions of the Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) (Igamberdiev *et al*., 2001; Igamberdiev *et al*., 2004). The photorespiration is an essential process for the plant growth and survival, when photosynthesis was down-regulated, protecting the chloroplasts and photosystems from photoinhibition and oxidative activity of ROS

(Igamberdiev *et al*., 2001; Igamberdiev *et al*., 2004; Prasad *et al*., 2008). The acc. 1036 and 3126 were the exceptions, managing to maintain partially open stomata, allowing photosynthesis activity and nutrient allocation during drought. This is in accordance with Lebot (2009), by referring that the sweet potato root growth, maintenance, and dry matter production is mainly from the carbon fixed in photosynthesis.

WUE is another important trait to determine if a plant has drought resistance, with the most tolerant ones normally showing higher WUE (Ganança *et al*., 2018; Gouveia *et al*., 2019). The WUE is usually related to stomata aperture and is estimated as the ratio of biomass from CO_2 assimilation in the photosynthesis, relatively to the water loss by transpiration (Igamberdiev *et al*., 2004). In sweet potato, drought increased in average 68% the WUE for most accessions, with acc. 3124 and 3125 showing significantly the highest WUE. The WUE increase can be due to the partial reduction of the stomatal aperture, where acc. were able to maintain leaf turgidity, by minimizing the water loss through transpiration and improving water use for vital activities (Gouveia *et al*., 2019). This partial reduction of the stomatal aperture still kept good photosynthetic rates under drought. Therefore, as the WUE increase derives from the reduction of water loss by transpiration, it would constitute a drought avoidance response. According to Prabawardani and Suparno (2015), the reduction of WUE in the acc. 3126 and 2938 and the overall sweet potato dry matter, can be related to nitrogen deficiency and transpiration increase. Duman (2012) reported that drought could lead to an overall plant nutrient deficiency, which is strongly related to the soil water availability and plant nitrogen absorption.

6.5.2 The role of plant nutrients during drought

The mineral nutrients that are found in the soil are classified as macro and micronutrients. Nitrogen, phosphorous and potassium are the primary macronutrient for plant growth and survival. According to Duman (2012), drought conditions can decrease the N availability from the soil matrix, limiting its uptake and transport from the roots to the shoots. N represents approximately 80% of the plant's total absorbed nutrients. It is an essential constituent of amino acids, chlorophyll, other metabolites and cellular structures (Duman 2012; Kaur *et al*., 2017). Usually, the major part of plant N is taken in the nitrate (NO_3^-) form and less in the ammonium (NH_4^+) form (Wang *et al*., 2009; Sahoo *et al*., 2010; Kaur *et al*., 2017).

Besides water availability, nutrient availability also depends on soil pH. The pH close to neutral (6.5 to 7.5) is ideal to enhance plant growth (Jensen, 2010). The pH values from the soil of both experimental sites are in this favorable range. Overall, the soil water availability and pH can be related to the good nutrient uptake and allocation from the underground storage roots to the aboveground shoots. The nutrient uptake from soil and allocation in plant organs can be mainly used for leaf and storage roots growth (Duman, 2012). We observed that the sweet potato shoots showed always-higher mineral content relatively to storage roots. Although, drought increased M-root content and led to an M-shoot decrease. It could be related to a greater limitation in the transport of nutrients from the underground to aboveground tissues, since the lack of water reduces the diffusion of minerals to the shoots, according to Duman (2012). Although, beyond the M-shoot decrease, the accessions showed a greater N content in shoots when compared to storage roots, in both experimental conditions. The registered N-shoot accumulation could be related to the plant use of N to increase the rate of photosynthesis, while the N-root can be used for the synthesis of proteins for the regulation of cell defense and detoxification (van den Boogaard *et al*., 1995; Salehi-Lisar and Bakhshayeshan-Agdam, 2016). The sweet potato registered a decrease of N-shoot under water scarcity that led to a significant NER and E increase, implying that the N content in the shoots was applied more efficiently into biomass production, rather than into photosynthesis increase rate. According to Siddiqi and Glass (1981), the NER increase observed in sweet potato can be due to the low accumulation of the nutrient in the whole-plant, in detriment of biomass production. Although not as productive as in control, sweet potato showed to be more efficient in their N-shoot use under drought stress, which is in accordance with Mathur and Goel (2017). The decrease of sweet potato N-root also decreased NER and increased E, which can indicate that although showing a lower N-root accumulation, the acc. displayed a more efficient use of N for biomass production during drought. The N-root was negatively correlated with E, NER-root, and E-root, under drought. N-root showed a positive correlation between M-root and NHI, confirming a good nutrient relation between the whole-plant and the storage roots.

Kaur *et al*. (2017) also registered a positive correlation between NHI and N content among wheat accessions.

6.6 Conclusions

This study presented meaningful information about the physiological responses of sweet potato accessions when subjected to water scarcity conditions. The sweet potato accessions that allocate nutrients and had improved WUE are the most drought-tolerant which agrees with Farooq *et al.* (2009). Accessions 3124 and 3125 were the ones that showed the best physiological response to drought stress, namely: higher R:S ratio, lower TPB loss, and lower SI as drought avoidance strategies. Both accessions also showed a good phenotypic flexibility response, with a better WUE and NER for growth and vital functions, and higher M-root, CCI and N-shoot. This information can be helpful for the overall screening of the sweet potato sensitivity or tolerance to drought, and to the adaptation of this crop to climatic change through breeding programs.

6.7 Acknowledgements

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6.8 Author contribution statement

CSSG participated on the drought assay and samples preparation, performed the nitrogen and total mineral content analysis, calculated NER, E, NHI, R:S, SI, interpreted and summarized all data generated from those experiments, and wrote the manuscript. JFTG quantified the WUE and helped in CCI measurement. HGMN and JGRF contributed with the field assay management, supported the preparation of samples and harvesting, and had meaningful support in the manuscript conceptualization. VL and MAAPC coordinated the work and revised the manuscript.

6.9 Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

6.10 References

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Tables

Table 6.1

Accession code, local name, and origin of the eight sweet potatoes (*Ipomoea batatas* L.) used in this study.

Acc. ID[a]	Accession local name	Origin
1036	Brasileira	Madeira Island
1038	5 Bicos	Madeira Island
2927	de Flor	Madeira Island
3126	Inglesa	Madeira Island
2937	Roja	Canary Islands – Tenerife
2938	Cubana	Canary Islands – Tenerife
3124	Vermelha	Guinea-Bissau – Bafatá
3125	Branca	Guinea-Bissau – Bafatá

[a] Accession identification number code used by the ISOPlexis Genebank.

Table 6.2

Chlorophyll content index (CCI), total plant biomass (TPB), water use efficiency (WUE), N efficiency ratio (NER), N efficiency utilization (E) and N harvest index (NHI) of sweet potato (*I. batatas*) whole-plant accessions.

Ipomoea batatas L.			CCI ††	TPB †† (g)	WUE †† (g L^{-1})	NER †† (kg kg^{-1})	E †† (kg)	NHI †† (%)
MAD	1036	Control	18.5 ± 2.4 ^{ab}	377.5 ± 62.9 ^{abcd}	3.6 ± 0.9 ^{abc}	11.6 ± 2.3 ^{abcd}	4.5 ± 1.4 ^{abcd}	30.0 ± 1.8 ^{bcdef}
		Drought	31.3 ± 7.2 ^{abcde}	231.2 ± 70.4 ^{abc}	6.2 ± 1.3 ^{abcd}	9.8 ± 0.0 ^{ab}	3.9 ± 0.0 ^{abc}	36.1 ± 0.0 ^{efg}
	1038	Control	37.9 ± 5.3 ^{de}	1038.6 ± 176.6 ^{e}	9.3 ± 2.2 ^{cde}	9.8 ± 0.3 ^{ab}	3.7 ± 0.2 ^{ab}	28.1 ± 1.9 ^{bcde}
		Drought	28.6 ± 3.5 ^{abcde}	342.4 ± 106.8 ^{abcd}	11.1 ± 3.4 ^{def}	15.3 ± 2.4 ^{d}	6.4 ± 1.4 ^{cde}	25.0 ± 0.6 ^{abc}
	2927	Control	41.6 ± 4.8 ^{e}	512.8 ± 229.0 ^{cd}	4.8 ± 1.8 ^{abc}	10.8 ± 2.1 ^{abc}	4.0 ± 1.0 ^{abc}	28.5 ± 1.5 ^{bcdef}
		Drought	35.9 ± 0.7 ^{de}	268.4 ± 125.8 ^{abc}	7.8 ± 3.7 ^{cd}	14.5 ± 1.2 ^{cd}	5.9 ± 0.6 ^{bcde}	40.5 ± 1.0 ^{g}
	3126	Control	25.8 ± 1.5 ^{abcd}	731.4 ± 367.6 ^{de}	6.9 ± 3.0 ^{bcd}	9.3 ± 0.7 ^{a}	3.9 ± 0.4 ^{abc}	26.5 ± 3.0 ^{abcd}
		Drought	31.5 ± 5.6 ^{abcde}	135.8 ± 25.1 ^{abc}	4.6 ± 0.8 ^{abc}	13.8 ± 0.7 ^{bcd}	7.0 ± 0.6 ^{de}	27.1 ± 3.1 ^{abcd}
CAN	2937	Control	21.0 ± 2.5 ^{abc}	106.4 ± 36.8 ^{abc}	0.8 ± 0.3 ^{a}	15.2 ± 2.4 ^{d}	7.2 ± 1.8 ^{e}	34.7 ± 4.2 ^{defg}
		Drought	18.3 ± 2.4 ^{a}	22.9 ± 21.0 ^{a}	1.1 ± 0.4 ^{a}	24.6 ± 0.0 ^{e}	12.9 ± 0.0 ^{g}	36.7 ± 0.0 ^{fg}
	2938	Control	32.3 ± 3.7 ^{bcde}	523.5 ± 116.5 ^{cd}	1.7 ± 0.8 ^{ab}	12.5 ± 1.8 ^{abcd}	5.3 ± 0.9 ^{abcde}	26.0 ± 0.3 ^{abc}
		Drought	25.6 ± 0.8 ^{abcd}	58.1 ± 28.4 ^{ab}	1.6 ± 1.2 ^{ab}	20.8 ± 1.5 ^{e}	9.9 ± 0.7 ^{f}	26.4 ± 8.7 ^{abcd}
GUI	3124	Control	31.2 ± 6.7 ^{abcde}	507.2 ± 108.1 ^{cd}	5.0 ± 1.5 ^{abc}	9.2 ± 1.0 ^{a}	3.1 ± 0.5 ^{a}	19.0 ± 5.0 ^{a}
		Drought	26.8 ± 3.0 ^{abcd}	449.9 ± 23.6 ^{bcd}	16.5 ± 2.2 ^{f}	10.8 ± 1.1 ^{abc}	3.8 ± 0.4 ^{ab}	22.4 ± 2.3 ^{ab}
	3125	Control	35.1 ± 8.2 ^{de}	467.8 ± 173.3 ^{bcd}	5.1 ± 1.2 ^{abc}	12.0 ± 1.9 ^{abcd}	5.2 ± 1.1 ^{abcde}	30.8 ± 1.4 ^{bcdef}
		Drought	32.5 ± 5.7 ^{cde}	391.8 ± 45.4 ^{abcd}	14.2 ± 1.3 ^{ef}	15.2 ± 1.1 ^{d}	7.1 ± 0.3 ^{e}	32.4 ± 3.3 ^{cdefg}
	Mean	Control	30.4	533.1	4.7	11.3	4.6	27.9
		Drought	28.8	237.6	7.9	15.6	7.1	30.8
	Min	Control	18.5	106.4	0.8	9.2	3.1	19.0
		Drought	18.3	22.9	1.1	9.8	3.8	22.4
	Max	Control	41.6	1038.5	9.3	15.2	7.2	34.7
		Drought	35.9	449.9	16.5	24.6	12.9	40.5

Data are expressed in dry weight basis (DW) and represent the mean ± SD of three independent replications per accession. Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$).

†† Significant differences between control and drought stress conditions (One-way ANOVA, $^{**}p \leq 0.01$).

Control is well-watered, drought is water scarcity.

Table 6.3

Mineral (M), nitrogen (N), N efficiency ratio (NER) and N efficiency utilization (E) from the storage roots and shoots of sweet potato (*I. batatas*) accessions.

Ipomoea batatas L.			M (g 100g^{-1})		N (g 100g^{-1})		NER (kg kg^{-1})		E (kg)	
			Root ††	Shoot ††	Root ††	Shoot ††	Root ††	Shoot ††	Root ††	Shoot ††
MAD	1036	Control	4.2 ± 0.2 ^{abc}	11.1 ± 0.6 ^{bcde}	1.0 ± 0.1 ^{abcd}	2.3 ± 0.0 ^{cd}	23.8 ± 6.2 ^{abc}	6.4 ± 0.8 ^{bcd}	5.7 ± 2.3 ^{abc}	1.0 ± 0.2 ^{bcd}
		Drought	4.5 ± 0.1 ^{abc}	11.0 ± 0.2 ^{bcde}	1.5 ± 0.0 ^{e}	2.6 ± 0.1 ^{cde}	14.1 ± 0.0 ^{a}	7.4 ± 0.0 ^{cde}	2.9 ± 0.0 ^{a}	1.4 ± 0.0 ^{def}
	1038	Control	4.0 ± 0.3 ^{abc}	13.8 ± 0.1 ^{fg}	1.1 ± 0.1 ^{bcd}	2.8 ± 0.1 ^{def}	24.1 ± 2.0 ^{abc}	4.2 ± 0.1 ^{ab}	6.4 ± 0.6 ^{abc}	0.5 ± 0.0 ^{ab}
		Drought	4.1 ± 0.4 ^{abc}	11.8 ± 0.7 ^{cdefg}	0.7 ± 0.1 ^{a}	2.0 ± 0.2 ^{bc}	39.0 ± 6.2 ^{bcd}	7.4 ± 1.3 ^{cde}	10.3 ± 2.1 ^{bcde}	1.1 ± 0.3 ^{cd}
	2927	Control	4.6 ± 0.6 ^{abc}	11.6 ± 1.4 ^{cdef}	1.0 ± 0.1 ^{abcd}	2.5 ± 0.3 ^{cde}	20.8 ± 3.1 ^{ab}	6.7 ± 1.6 ^{cd}	4.2 ± 0.9 ^{ab}	1.1 ± 0.4 ^{cd}
		Drought	4.5 ± 0.1 ^{abc}	9.9 ± 0.7 ^{abcd}	1.1 ± 0.1 ^{cde}	1.7 ± 0.1 ^{ab}	20.9 ± 1.4 ^{ab}	10.1 ± 1.2 ^{fg}	4.9 ± 0.3 ^{abc}	1.7 ± 0.3 ^{efg}
	3126	Control	3.7 ± 0.3 ^{a}	14.2 ± 1.3 ^{g}	1.2 ± 0.2 ^{de}	3.3 ± 0.1 ^{f}	26.0 ± 4.8 ^{abc}	3.4 ± 0.3 ^{a}	7.9 ± 1.7 ^{abcd}	0.4 ± 0.1 ^{a}
		Drought	4.0 ± 0.1 ^{abc}	11.9 ± 0.5 ^{cdefg}	1.0 ± 0.1 ^{abcd}	2.7 ± 0.1 ^{cde}	33.3 ± 1.5 ^{bcd}	6.7 ± 0.3 ^{cd}	10.8 ± 0.2 ^{cde}	1.2 ± 0.1 ^{cde}
CAN	2937	Control	4.0 ± 0.4 ^{abc}	9.6 ± 1.0 ^{abc}	1.1 ± 0.2 ^{bcd}	2.0 ± 0.0 ^{bc}	29.4 ± 8.8 ^{abc}	8.0 ± 0.2 ^{def}	9.3 ± 3.8 ^{bcde}	1.3 ± 0.1 ^{cdef}
		Drought	3.7 ± 0.0 ^{a}	7.6 ± 0.0 ^{a}	0.8 ± 0.0 ^{abc}	1.5 ± 0.2 ^{a}	41.0 ± 0.0 ^{cd}	15.1 ± 0.0 ^{h}	13.2 ± 0.0 ^{de}	3.1 ± 0.0 ^{h}
	2938	Control	4.4 ± 0.2 ^{abc}	11.7 ± 0.3 ^{cdef}	0.9 ± 0.1 ^{abcd}	2.5 ± 0.2 ^{cde}	32.4 ± 5.3 ^{abc}	5.5 ± 0.4 ^{abc}	9.3 ± 2.2 ^{bcde}	0.8 ± 0.0 ^{abc}
		Drought	4.4 ± 0.0 ^{abc}	8.9 ± 1.0 ^{ab}	0.6 ± 0.2 ^{a}	1.7 ± 0.1 ^{ab}	51.1 ± 21.2 ^{d}	11.2 ± 0.9 ^{g}	14.8 ± 6.4 ^{e}	2.1 ± 0.2 ^{g}
GUI	3124	Control	3.9 ± 0.8 ^{ab}	12.9 ± 1.1 ^{efg}	0.7 ± 0.1 ^{a}	3.0 ± 0.3 ^{ef}	33.6 ± 11.7 ^{bcd}	3.9 ± 0.6 ^{a}	7.7 ± 3.5 ^{abcd}	0.5 ± 0.1 ^{ab}
		Drought	4.1 ± 0.2 ^{abc}	12.9 ± 0.9 ^{efg}	0.7 ± 0.0 ^{ab}	2.6 ± 0.3 ^{cde}	33.9 ± 1.1 ^{bcd}	4.2 ± 0.8 ^{ab}	8.4 ± 0.5 ^{abcd}	0.4 ± 0.1 ^{ab}
	3125	Control	4.9 ± 0.6 ^{bc}	12.3 ± 1.4 ^{defg}	1.1 ± 0.1 ^{cde}	2.5 ± 0.2 ^{cde}	24.3 ± 2.4 ^{abc}	6.5 ± 1.4 ^{bcd}	6.5 ± 0.8 ^{abc}	1.1 ± 0.3 ^{cd}
		Drought	5.0 ± 0.3 ^{c}	10.3 ± 0.5 ^{bcd}	1.0 ± 0.2 ^{abcd}	2.1 ± 0.1 ^{bc}	27.6 ± 4.2 ^{abc}	9.4 ± 0.8 ^{efg}	7.6 ± 1.0 ^{abcd}	1.8 ± 0.2 ^{fg}
	Mean	Control	4.2	12.2	1.0	2.6	26.8	5.6	7.1	0.8
		Drought	4.3	10.5	0.9	2.1	32.6	8.9	9.1	1.6
	Min	Control	3.7	9.6	0.7	2.0	20.8	3.4	4.2	0.4
		Drought	3.7	7.6	0.6	1.5	14.1	4.2	2.9	0.4
	Max	Control	4.9	14.2	1.2	3.3	33.6	8.0	9.3	1.3
		Drought	5.0	12.9	1.5	2.7	51.1	15.1	14.8	3.1

Data are expressed in dry weight basis (DW) and represent the mean ± SD of three independent replicates per accession.

Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$).

†† Significant differences between control and drought stress conditions (One-way ANOVA, $^{**}p \leq 0.01$).

Control is well-watered, drought is water scarcity.

Table 6.4

Pearson correlation coefficients of the analyzed traits of sweet potato (*I. batatas*) in control and drought stress conditions.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. CCI	-														
2. TPB	**0.43****	-													
3. WUE	0.24	**0.44****	-												
4. NER	**-0.39****	**-0.65****	**-0.33***	-											
5. E	**-0.40****	**-0.65****	**-0.37****	**0.99****	-										
6. NHI	-0.09	**-0.29***	-0.16	**0.31***	**0.33***	-									
7. R:S	**0.43****	**0.50****	**0.38****	-0.27	**-0.32***	**-0.53****	-								
8. SI	-0.05	0.00	-0.36	0.10	0.18	0.20	-0.28	-							
9. M-root	**0.30***	0.07	0.21	-0.14	-0.16	0.28	-0.08	0.25	-						
10. M-shoot	**0.34***	**0.76****	**0.42****	**-0.85****	**-0.82****	**-0.54****	**0.46****	-0.11	-0.04	-					
11. N-root	0.16	0.22	0.01	**0.45****	**-0.37****	**0.62****	**-0.35***	0.31	**0.33***	0.19	-				
12. N-shoot	0.27	**0.63****	0.21	**-0.86****	**-0.80****	**-0.55****	**0.29***	-0.057	-0.10	**0.90****	0.27	-			
13. NER-root	**-0.31***	**-0.38****	-0.18	**0.65****	**0.63****	**-0.47****	0.13	-0.21	**-0.39****	**-0.33***	**-0.84****	**-0.35***	-		
14. NER-shoot	**-0.29***	**-0.68****	**-0.32***	**0.91****	**0.89****	**0.60****	**-0.40****	-0.13	0.06	**-0.92****	-0.16	**-0.91****	**0.33***	-	
15. E-root	**-0.37***	**-0.42****	-0.27	**0.73****	**0.74****	**-0.33***	0.01	0.13	**-0.42****	**-0.39****	**-0.72****	**-0.38****	**0.96****	**0.40****	-
16. E-shoot	-0.28	**-0.68****	**-0.32***	**0.88****	**0.88****	**0.60****	**-0.42****	-0.18	0.08	**-0.90****	-0.11	**-0.85****	**0.30***	**0.99****	**0.38****

CCI chlorophyll content index of shoots; *TPB* total plant biomass (g, DW); *WUE* water use efficiency (g L^{-1}, DW); *NER* nitrogen efficiency ratio (kg kg-1 DW); *E* nitrogen efficiency utilization (kg, DW); *NHI* nitrogen harvest index (%, DW); *R:S* root-to-shoot ratio; *SI* whole-plant stress index; *M-root* total mineral content of storage roots (g $100g^{-1}$, DW); *M-shoot* total mineral content of shoots (g $100g^{-1}$, DW); *N-root* total nitrogen content of storage roots (g $100g^{-1}$, DW); *N-shoot* total nitrogen content of shoots (g $100g^{-1}$, DW); *NER-root* nitrogen efficiency ratio of storage roots (kg kg^{-1} DW); *NER-shoot* shoot nitrogen efficiency ratio (kg kg^{-1} DW); *E-root* nitrogen efficiency utilization of storage roots (kg, DW); *E-shoot* shoot nitrogen efficiency utilization (kg, DW).

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Figure

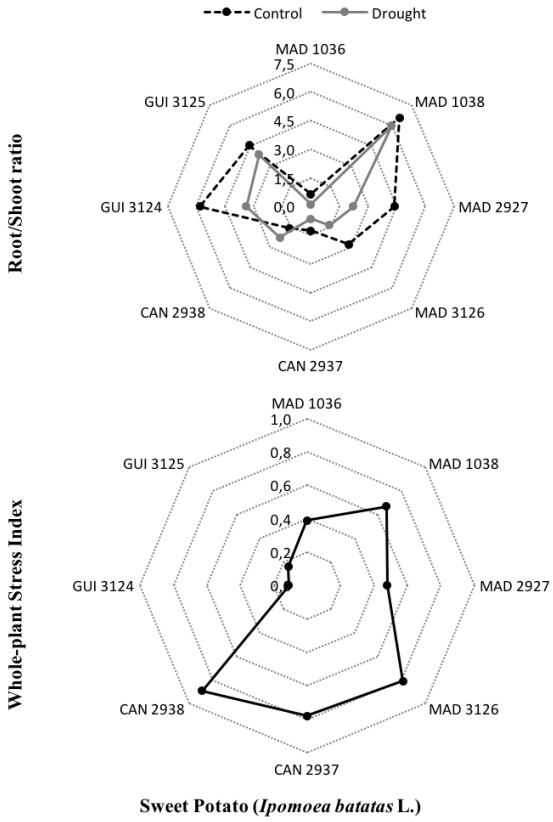

Figure 6.1

Stress index (SI) and R:S in sweet potato (*I. batatas*) accessions, under control and drought conditions.

Sweet potato accessions with ISOPlexis Genebank identification number code, from CAN Canary Islands, MAD Madeira Island and GUI Guinea-Bissau.
Data are expressed in dry weight basis and represent the mean of three independent replicates per accession.
Control is well-watered, drought is water scarcity.

CHAPTER 7

Changes in oxalate composition and other nutritive traits in root tubers and shoots of sweet potato (*Ipomoea batatas* L. [Lam.]) under water stress

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Changes in oxalate composition and other nutritive traits in root tubers and shoots of sweet potato (*Ipomoea batatas* L. [Lam.]) under water stress

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7.1 Abstract

BACKGROUND: The presence of insoluble calcium oxalate druse crystals (CaOx) in sweet potato (*Ipomoea batatas*) can negatively affect its nutritional quality. Photosynthesis, starch and protein composition are linked with oxalate synthesis and tuber quality under water scarcity. Our main objective was the oxalate quantitation of sweet potato tubers and shoots and also to assess how drought changes their nutritional value. Eight sweet potato accessions from Madeira, the Canaries and Guinea-Bissau were analyzed for their response to drought stress. Tubers and shoots were analyzed for total (T-Ox), soluble (S-Ox) and insoluble (CaOx) oxalates, protein, chlorophyll content index (CCI), soluble starch, starch swelling power and starch solubility in water.
RESULTS: The S-Ox and CaOx contents was higher in shoots. Six accessions were above maximum CaOx levels for raw consumption. Accessions with more favorable responses to drought had decreased CaOx with S-Ox increase content for osmoregulation. They also presented slightly decreased CCI and protein contents. These accessions also had an increased shoot starch content, for further tuber storage starch hydrolysis, and maintained the quality and functional properties of the tuber starch grain. Those with a less favorable response to drought had a higher T-Ox and CaOx content in both organs, hindering water absorption. They also had decreased protein and CCI, with a slight increase in tuber starch hydrolysis.
CONCLUSION: Oxalate content was significantly related with carbohydrate metabolism, CCI and protein synthesis. This study significantly contributed to the screening of the sweet potato stress response to drought, to adapt this crop to climatic change through breeding programs.

Keywords
Chlorophyll content index; drought; *Ipomoea batatas*; protein; soluble and insoluble oxalates; starch.

Abbreviations

Acc.	Accession
CCI	Chlorophyll content index
CaOx	Calcium oxalate
S-Ox	Soluble oxalates [oxalic acid]
SSP	Starch swelling power
SWS	Starch solubility in water
T-Ox	Total oxalates

7.2 Introduction

Sweet potato, *Ipomoea batatas* L. [Lam.] (Convolvulaceae), has been cultivated for over 5,000 years, probably originating in Central/South America.[1,2] The starchy root tubers are a major source of food in tropical and developing countries, with the leaves also being used as a vegetable source in some countries.[2,3] After potatoes and cassava root tubers, sweet potatoes were the most important source of starch for the food supply.[4] Worldwide, sweet potato production increased from 106.27 Mt in 2010 to 112.84 Mt in 2017. Asia is the main sweet potato producer, supplying 79.6 Mt in 2017, and representing 71% of worldwide production.[4] Sweet potatoes are grown in a wide range of environments in temperate climates, from sea level to 2700 m altitude.[1-3] Sweet potato can be moderately tolerant to drought, having a low plant growth habit and an extensive root system. Production is usually achieved with relatively low inputs.[5-7]

Raw sweet potato (root tubers and shoots) can present potential nutritional toxicity due to the presence of insoluble druse crystals of calcium oxalate (CaOx) and soluble oxalates (S-Ox, e.g., oxalic acid).[8] The oxalic acid [$(COOH)_2$] can form insoluble salts when combined with calcium (Ca^{2+}).[9] The CaOx content can vary during the plant growth period, usually associated with plant genetics, nutrient assimilation, or drought.[1,10-13]

Widely distributed in plants, oxalates under the form of CaOx and soluble oxalates (S-Ox) can confer negative nutritional quality.[8,9] Sweet potato CaOx crystals result from the precipitation of excess calcium ions with synthetized oxalic acid, for plant osmoregulation and ion balance[8,9,14]. The oxalic acid can also help plant tolerance to heavy metals and plant pathogens, binding with iron to form iron oxalate.[14] When animals are fed with raw plants that contain high percentages of oxalates, this can induce chronic poisoning. The CaOx can lead to renal health complications due to accumulation in the kidneys.[1,8,9]

Oxalic acid is often considered a result of the incomplete oxidation of photosynthetic products, related to CO_2 fixation both during daylight hours and at night.[15,16] The accumulation of oxalic acid can be due to: (i) photosynthetic glycolate-glyoxylate oxidation increase during hours of intense daylight, and/or (ii) through the conversion of glyoxylate during the night.[9,15,16] Thus, CaOx acts as a biological plant dynamic carbon pool storage. If the stomata are partially or totally closed during drought conditions, the degradation of CaOx can provide CO_2 for photosynthetic assimilation under carbon starvation conditions. The reduction of photosynthetic activity can lead to lower evapotranspiration, helping to keep cell turgor during drought.[17] The leaf chlorophyll concentration can be associated with photosynthetic capacity, which is one of the most common parameters used for plant drought stress appraisal.[10,18-20]

Besides photosynthesis, carbohydrate metabolism is also related to plant oxalic acid production.[9] Photosynthetic activity allows carbohydrate synthesis providing metabolites for plant growth, energy, and signalling pathways.[15] Under water-scarcity conditions, plants can increase starch hydrolysis to gather soluble sugars, or they can increase their crude protein content with the synthesis of specific high molecular proteins as strategies for surviving water scarcity.[15,18,21,22] The oxidation of carbohydrates supplies energy for the reduction of nitrates in protein, with oxalate formation as a direct reaction sub-product.[9] The variation in both sweet potato protein and starch content can be important for understanding the role of oxalates in the sweet potato's response and tolerance to drought and also how it affects the quality of the tuber.

Some biochemical and nutritional assessments were done to study sweet potato responses under different irrigation,[6] low input,[5] rain-fed,[23-25] drought and other environmental conditions.[2,3,26] There is limited information on plant oxalate variation, protein synthesis and starch allocation under stress, which can affect the nutritional quality of the sweet potato. The main objective of our study was therefore to increase our knowledge of how water scarcity affects the sweet potato's nutritional quality and biochemical composition, through the assessment of oxalate content and its relationship with chlorophyll content index (CCI), protein, and starch content variation under drought.

7.3 Materials and Methods

7.3.1 Sweet potato accessions

The biochemical composition of eight accessions of sweet potato (*Ipomoea batatas* L.) were assessed when submitted to a water-deficit environment. These accessions were originated from Madeira, the Canary Islands, and Guinea-Bissau (Table 7.1).

7.3.2 Experimental field assay

The sweet potato field assay was plotted in a randomly split-plot field design, established at the ISOPlexis experimental field (32º 39' N, 016º 55' W, Funchal, Madeira, Portugal), during a 5-month cycle (from August to December 2017). Sweet potato accessions grew in two independent blocks: the first under ordinary open field conditions (control) and the other under a rain shelter, with limited irrigation (water deficit). Thirty vine cuttings per accession were planted in three plots (replicates), in eight independent rows, 30 plants per accession in total, with 70 x 80 cm in between the rows. Three vines per plot were also added as test samples, being fully irrigated in both open and sheltered environments, not subjected to water stress.

At the storage root bulking stage (3rd month), stress was imposed with two distinct water regimes, through a drip irrigation system. Approximately 1.6 mm of water was applied three times a week to the control plots while 0.9 mm was applied three times a week for water deficit variants, per plot, over the next 3 months. During this period, control plots received approximately 77 mm of water and stressed plots received approximately 54 mm. Control was also subject to 117.5 mm of rainfall per plot during this period. During raining periods, irrigation was suspended on control plots.

Both control and drought stress environments were assessed periodically for: photosynthetic active radiation (PAR, 400-700 nm) with a ceptometer (AccuPAR LP-80, Washington, USA); volume water content of soil (VWC_s) with a soil moisture sensor (WaterScout SM100, Illinois, USA, from 0% to 54% VWC_s from dry to saturated); air temperature (T_a) and relative air humidity (RH_a) with a data logger (Testo 174H, Lenzkirch, Germany). Over the course of the experiment, we registered a 24.6% PAR decrease under the rain shelter relative to the control environment, on average, with 1514.5 µmol m^{-2} s^{-1} for control and 1142.0 µmol m^{-2} s^{-1} for drought. At 10 cm of depth of homogenized field soil, we registered, on average, 12.8% VWC_s for control, representing 35% of field capacity; and 3.5% VWC_s for drought, representing 10% or less of field capacity. During the assay, a 19.46ºC average T_a and 68.07% average RH_a were observed for control; a 22.25ºC average T_a and 66.40% average RH_a were registered for drought.

Throughout the experiment, neither fertilizers nor pesticides were applied, and weeds were regularly removed, manually.

7.3.3 Preparation of sweet potato whole-plant flour

At the end of the agronomic experiment, 384 samples of root tubers and shoots (stem, stalk, and leaves) from control and drought replicates were collected. All the samples were washed in water, weighed (Sartorius Basic BA2100S, Göttingen, Germany), chopped on a mandolin slicer (2-3 mm thick), distributed in an air oven to dehydrate during 48h at 65ºC (Memmert UF260, Schwabach, Germany) and ground into 200 mesh flour with a universal mill (IKA-Werke M20, Staufen, Germany). The flour was placed in bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153, Weesp, Netherlands) and stored at -35ºC (Liebherr ProfiLine GGPV6570, Schwabach, Germany) until analysis.

7.3.4 Analysis of biochemical composition and nutritional quality

7.3.4.1 Chlorophyll content index

The chlorophyll content index (CCI) was obtained from sweet potato fresh leaves as described by Gouveia *et al.*,[10] using a chlorophyll content meter (Opti-Sciences CCM-200 PLUS, New Hampshire, USA). Three measurements were performed early in the morning, through the adaxial leaf surface, avoiding the branching veins. An average CCI value was recorded for each replicate.

7.3.4.2. Soluble, insoluble and total oxalates

Flour from root tubers and shoots was analyzed for total oxalates (T-Ox), water-soluble oxalates (oxalic acid, S-Ox), and water-insoluble oxalates (calcium oxalate, CaOx). This method was optimized by Gouveia *et al.*,[10] as described by Fatoki,[27] AOAC,[28] Oke,[29] and Dye.[30] Precisely 0.4 g of flour was extracted with hydrochloric acid (HCl, 6.0 mol L^{-1}) to allow the reduction of oxalic into glyoxylic acid, with a further reduction into glycolic acid. The insoluble CaOx content was obtained by boiling sulfuric acid (H_2SO_4, 20%). A potassium permanganate solution ($KMnO_4$, 0.05 mol L^{-1}) was used to precipitate and titrate the sample extracts for oxalate quantitation. We used Dye´s[30] calculation for the total acid soluble oxalates (S-Ox) and the method described by Holloway *et al.*[25] for total oxalate (T-Ox) quantifications. The analysis was performed in triplicate, with results presented as g kg^{-1} dry flour.

7.3.4.3 Protein

The total nitrogen content of root tubers and shoots flour was determined by an automatic distillation and titration unit (Velp Scientifica UDK 152, Milan, Italy). The protein content was obtained by the conversion of nitrogen with the factor N X 6.25, according to the AOAC method.[31] The analysis was performed in triplicate, with results presented as g kg^{-1} dry flour.

7.3.4.4 Soluble starch

The content of soluble starch from the root tubers and shoot flour was spectrophotometrically quantified at 630 nm, using the method described by Hodge and Hofreiter,[32] with a UV/Vis spectrophotometer (Shimadzu, 2401 PC, Kyoto, Japan; UVProbe 2.52 software). The analysis was performed in triplicate, with results presented as g kg^{-1} dry flour.

7.3.4.5 Quality of the starch grain

The quality of grain starch in root tuber flour was assessed with the Gouveia *et al.*[10] method, with starch water solubility (SWS) and starch swelling power (SSP) calculations according to Tattiyakul *et al.*[33] The analysis was performed in triplicate with results presented as g g^{-1} dry flour.

7.3.4.6 Statistical methods

The results were computed on a dry weight basis, as the average of three control versus three drought replicates of sweet potato root tubers and shoots. SPSS V23 for Mac was used for one-way ANOVA ($P \leq 0.05$), Tukey HSD test ($P \leq 0.05$), and Pearson correlations; and MVSP V3.1 for Windows for principal component analysis (PCA).

7.4 Results

7.4.1 Variation of oxalate composition according to drought

The sweet potato accessions mean values of T-Ox and CaOx decreased slightly in the root tubers, while the S-Ox increased under drought stress (Table 7.2). In the shoots, an average T-Ox and S-Ox increase and an approximately equal CaOx were recorded (Table 7.3).

The root tubers had a T-Ox average value of approximately 0.79 g kg^{-1} in control conditions, with a 15% decrease to 0.67 g kg^{-1} under drought conditions. The main fraction of T-Ox value was essentially composed by CaOx, which decreased by 22% from 0.68 to 0.53 g kg^{-1} between control and drought stress. The S-Ox and CaOx showed lower content in tubers. S-Ox ranged from approx. 0.11 to 0.14 g kg^{-1}, with a 28% increase from control to stress. The tubers from accession (acc.) 2938 showed the highest increase on CaOx accumulation under drought, from 0.47 to 0.85 g kg^{-1} (+81%). Tubers from acc. 1036 had significantly ($P \leq 0.05$) lower CaOx content variation, decreasing from 0.16 to 0.15 g kg^{-1} (–6%) between control and stress conditions (Table 7.2). Acc. 2938 presented a significant ($P \leq 0.05$) fivefold higher CaOx tuber content than

acc. 1036. The tubers of acc. 3124, 3125 and 3126 showed a greater decrease in CaOx content in response to water scarcity.

The T-Ox average value in shoots increased 3% from 1.31 g kg^{-1} in control to 1.35 g kg^{-1} under drought. S-Ox and CaOx content was higher in shoots. The S-Ox increased 16% from approx. 0.32 to 0.37 g kg^{-1} for control and drought conditions. The mean CaOx content was approximately equal in control and stress conditions, at approximately 0.98 g kg^{-1}. The shoots from acc. 1036 showed significantly ($P \leq 0.05$) the highest CaOx decrease from 1.56 to 0.69 g kg^{-1} (–56%), followed by acc. 3125, which decreased from 0.48 to 0.17 g kg^{-1} (–64%), approximately. Meanwhile, the acc. that recorded the biggest shoot CaOx increase under drought was acc. 2938, with a significant ($P \leq 0.05$) increase from 1.45 to 2.46 (+70%), and acc. 3126 from 0.90 to 1.17 g kg^{-1} (+30%), respectively (Table 7.3). Acc. 2938 had a 14-fold higher shoot CaOx content than acc. 3125, during drought.

7.4.2 Protein content variation and water scarcity

The total mean protein content variation indicated that the sweet potato plants slightly decreased protein content on root tubers and shoots under drought stress. The shoots showed the biggest decrease in protein during stress, although the protein content in controls was 10% higher in the shoots than in the tubers (Tables 7.2 and 7.3). The tuber average protein content decreased slightly, at 62 g kg^{-1} in both experimental conditions. Acc. 1038 showed the highest decrease in tuber protein content, from 69 to 43 g kg^{-1} (–43%), which was statistically different ($P \leq 0.05$). On the other hand, acc. 1036 and 2927 were the only ones where the drought seemed to induce a slight increase in the tuber protein content, with acc. 1036 showing the highest significant ($P \leq 0.05$) protein accumulation, from 62 to 93 g/100 g (+50%) (Table 7.2).

The average crude protein of the stressed shoots decreased by 19%, from approximately 163 to 130 g kg^{-1}, relatively to control. Accession 3126 had the highest shoot protein content, decreasing 19% from 205 to 166 g kg^{-1}, approximately. Accessions 2938 and 2927 showed the highest significant ($P \leq 0.05$) shoot protein decrease, from approximately 157 to 105 g kg^{-1} (–31%), and from 155 to 104 g kg^{-1} (–33%), respectively. Meanwhile, acc. 1036 was the only one that showed a shoot increase in protein content during drought, from 145 to 164 g kg^{-1} (+6%) (Table 7.3).

7.4.3 Shoot chlorophyll content index variation to drought conditions

The sweet potato accessions showed a slight decrease in total average CCI values, when submitted to drought, from 30 to 29 (–3%), approximately. Accessions 1038, 2927, 3124, and 3125 had the highest CCI values in both assay conditions, but still showed a CCI decrease under drought. Accession 1038 had the highest CCI decrease during drought, from 38 to 29 (–24%). Accession 2937 had the lowest CCI content among all accessions, ranging between 21 and 18 (–14%). In contrast, acc. 1036 and 3126 were the only ones that increased CCI during drought, from 19 to 31 (+63%) and from 26 to 32 (+23%), respectively (Table 7.3).

7.4.4 Tuber starch content and grain gelatinization changes to drought

On average, tuber starch decreased slightly under drought, from 434 to 423 g kg^{-1} (–2%), while the shoot starch content increased from 37 to 71 g kg^{-1} (+75%) (Tables 7.2 and 7.3). Regarding tubers, acc. 3126 and 1036 had the highest starch decrease, from 465 to 413 (–13%) and 442 to 382 g kg^{-1} (–14%), respectively. Accession 3124 was the only one showing an increase in tuber starch content under drought stress, ranging significantly ($P \leq 0.05$) from 382 to 484 g kg^{-1} (+26%), approximately (Table 7.2). Regarding shoots, all accessions had increased starch content, except acc. 3124, which maintained constant starch content levels. Accession 2937 showed the highest shoot starch accumulation due to drought, ranging significantly ($P \leq 0.05$) from 66 to 169 g kg^{-1} (+143%), followed by acc. 2938, ranging significantly ($P \leq 0.05$) from 41 to 86 g/100g (+125%), respectively (Table 7.3).

The quality of tuber starch grain under water scarcity was assessed through starch solubility in water (SWS) and starch swelling power (SSP) (Table 7.2). Overall, tubers maintained or slightly increased SWS

during drought, from 0.36 to 0.38 g g^{-1} (+6%). Accession 3126 had the highest SWS increase under drought, from 0.39 to 0.43 g g^{-1} (+10%). Meanwhile, acc. 1038 and 2937 did not change their SWS, registering 0.34 and 0.37 g g^{-1}, respectively. The change in SSP due to drought was also very low, registering a very slight increase on average. The main variation was observed for acc. 3124, which had an increased SSP from nearly 14 to 15 g g^{-1} (+7%), and acc. 1036 was the only one that showed a decreased SSP, from nearly 15 to 14 g g^{-1} (–7%).

7.4.5 Variance and parameter associations

Statistically significant associations among variables (parameters) were detected (Tables 7.2 and 7.3). The tuber variables had ten significant correlations, with the strongest ones observed between starch and S-Ox (r = –0.57), starch and SSP (r = 0.56), and protein and S-Ox (r = 0.55). Moderate correlations were observed between protein and SSP (r = –0.46), protein and CaOx (r = –0.40), and S-Ox and CaOx (r = –0.34) (Table 7.4). The shoot variables showed 12 significant correlations, with the strongest observed between starch and protein (r = –0.71), CCI and CaOx (r = –0.63), and starch and T-Ox (r = 0.55). Moderate correlations were found between CCI and starch (r = –0.41), S-Ox and CaOx (r = 0.36), and protein and T-Ox (r = –0.35) (Table 7.5).

The average values obtained from biochemical and CCI variables, for both experimental variants, were submitted to principal component analysis (PCA), to transform the case and variable data into a spatial coordinate system. The PCA analysis allowed us to observe a slightly higher dissimilarity in shoot variables (Fig. S1(B)) than between the tubers (Fig. S1(A)), when in drought stress. The tuber PCA analysis showed 96.4% of cumulative variance: 71.1% at the first axis, with eigenvalues of 0.59; and 25.3% at second axis, with eigenvalues of 0.21 (Fig. S1(A)). The shoot PCA showed 87.0% of cumulative variance: 72.4% at the first axis, with eigenvalues of 1.43; and 14.6% at second axis, with eigenvalues of 0.29 (Fig. S1(B)). The CaOx and T-Ox variables were strongly correlated with the first axis, whereas the S-Ox variable was highly correlated with second axis, in both tuber and shoot organs.

7.5 Discussion

7.5.1 The influence of water scarcity on the nutritional value of oxalates

The present study provided significant information on the variation of the nutritional quality of sweet potato root tubers and shoots when submitted to drought stress. Oxalates can negatively affect their nutritional value and quality.[9] Our accessions showed different behaviour towards drought, both in oxalate production and accumulation, between tuber and shoot organs. In drought conditions, the study showed lower S-Ox and CaOx content in tubers, with an average decrease in druse CaOx crystals content in both tuber and shoot organs. The T-Ox content in the control and stressed tubers was lower than the 116 mg/100g registered by Ravindran *et al.*[24] for rain-fed sweet potato accessions, except acc. 3124, which had relatively higher T-Ox content. Likewise, the oxalate content (T-Ox, S-Ox and CaOx) determined for acc. 1036 and 2927, in both experimental conditions, was lower than that reported for non-stressed sweet potatoes from the South Pacific region.[3,25] Only tubers and shoots from acc. 1038 and 2927 were safe for raw consumption, in both control and drought conditions, seeing as they were below the maximum recommended levels for food, 0.71 g kg^{-1} CaOx.[34]

In both sweet potato organs, the T-Ox was mainly composed of insoluble CaOx, and very low S-Ox was detected. This agrees with Nakata[35], indicating that nearly 90% of the plant's total calcium can be detected as insoluble oxalate salt. Calcium absorption and oxalic acid synthesis can play an important role in sweet potato ion balance and osmoregulation. This is achieved through the regulation of excess calcium ions by their precipitation with oxalic acid, in the form of druse CaOx crystals – i.e., spherical aggregate of individual crystals.[1,11–13,35] These crystals commonly occur inside the vacuoles of specialized cells, i.e., crystal idioblasts, and can participate in the storage of calcium as CaOx.[9,35] Schadel and Walter[13] reported that sweet potato could increase CaOx because of the plant's mechanism isolating surplus calcium accumulation. Our findings are in

accordance with the studies mentioned above, as we observed oscillations in tuber and shoot S-Ox (as oxalic acid) and CaOx content during drought. According to Sharma and Kaushal[1] and Libert and Franceschi,[12] genetic and drought factors can change the overall intensity of root-crop CaOx accumulation. The observed oscillation in CaOx formation and oxalic acid content possibly resulted from ion balance, due to a dynamic fluctuation of druse crystals formation according to the availability of free calcium.[9,35] Drought could have limited the free calcium in acc. 1036, 2927, and 3125, leading to an average decrease of druse CaOx crystals content in both organs, presumably freeing up the calcium for plant osmoregulation, and thus slightly increasing the S-Ox. These accessions possibly showed the best biochemical response to drought, because the CaOx and S-Ox equilibrium during drought led to the lowest biomass loss content during water scarcity.[36] Similarly, Nakata[35] reported the loss of these crystals in plant tissues under conditions of calcium deficiency and active growth. Gouveia *et al.*[10] also reported that less sensitive taro (*Colocasia esculenta*) accessions had lower CaOx accumulation during drought. However, acc. 2938 can be considered one of the most sensitive to drought, displaying a decrease in S-Ox and naturally higher CaOx in both tissues during drought, with higher CaOx whole-plant accumulation and lower biomass content, even though the synthesis and mobilization of CaOx and S-Ox in the plant tissues should complement other systems of osmoprotection.[10,36]

7.5.2 Protein and chlorophyll content variation to drought

According to Osuagwu and Edeoga[21], drought can lead to the increase of plant crude protein content. When in drought, they can induce high molecular-weight protein synthesis as an adaptive response to stress. However, we recorded an overall decrease in crude protein content in both organs, with shoots presenting the highest protein content, but also with the highest content loss during drought. Our average protein content was higher than the ones reported by Ravindran[24] for rain-fed sweet potato tubers, which had an average content of 44.1 g kg^{-1}. Ekanayake and Collins[6] obtained a much lower tuber protein content under different irrigation conditions, ranging from 2.4 to 2.0 g kg^{-1} for control and drought. We also had a higher protein content in both organs than Ishida *et al.,*[23] who recorded an average content of 21.3 g kg^{-1} in tubers and 51.5 g kg^{-1} in shoots, under non-stressed conditions. Besides an overall loss of protein content in both sweet potato organs, we also recorded a CCI decrease during drought. However, our average CCI content was slightly higher than that of Motsa *et al.*,[5] who recorded a CCI content of 29.4 for South African sweet potatoes, grown in low-input conditions.

According to Salehi-Lisar and Bakhshayeshan-Agdam[18], shoot protein content can be directly connected to photosynthesis rate, and the tuber protein can be directly related to plant defence and regulation. Acc. 1036 and 2927 were the only ones that showed increased protein synthesis in tubers (+17% and +50%, respectively), as a response to drought. The remaining accessions did not show the need for protein synthesis as a response mechanism to water scarcity. The sweet potato accessions also decreased their CCI under drought, which could lead to a decrease in protein content in shoots, although they are not correlated.

Most of the sweet potato accessions showed a CCI decrease with drought, according to Shao *et al.*,[19] with a feasible decrease of the leaf intracellular CO_2 availability as a result of the relative stomatal closure to avoid water loss during drought.[37] That is, drought interfered with the photosynthetic carbon (^{13}C) depletion due to partial stomatal closure, with an increase registration in the carbon isotope abundance and the ^{13}C fixation in sweet potato shoots, according to the previous work of Gouveia *et al.*[36] The 24.6% PAR decrease inside the shelter had the potential to influence the CCI. According to fully irrigated test samples located inside the shelter and in open field, those inside the shelter accumulated significantly more chlorophyll – on average more than 9% relative to the open field (data not shown). The CCI decrease inside the shelter was therefore associated with water limitation, which possibly led to less excitation of the photosystem II (PSII) through photons of light, lowering the number of ionized chlorophyll molecules.[18,38] Accessions 1036 and 3126 were the exception, which may have been due to more open stomata during drought, allowing photosynthetic activity to be maintained.[36] Along with stomatal closure, the CCI decrease in the remaining accessions can also be also associated with other factors, such as oxidative damage in chloroplasts through the photo-oxidation of chlorophyll as a nonstomatal limitation when drought stressed, as a way to help to protect the chloroplasts

from photoinhibition and subsequent oxidative damage.[37–41]

7.5.3 Starch hydrolysis and grain quality during water shortage

Starch is the major form of biomass (carbon) and energy storage in the root tubers of sweet potatoes.[42] Under drought stress, this organ showed lower content variation than the shoots. According to Zeeman *et al.*,[43] starch can be synthesized in the plastids of both photosynthetic (leaves) and nonphotosynthetic (e.g., tubers) cell tissues. Preiss and Sivak[44] denoted that the biosynthesis and degradation of starch in the leaf are more dynamic than its metabolism in reserve tissues (tubers). The increase of shoot starch content in all accessions, except in acc. 3124, which was constant, can be related to the photosynthesis and stomatal activity during drought. Santelia and Lunn[45] and Preiss and Sivak[44] reported that shoot starch can have an important function in the operation of stomatal guard cells, through rapid starch degradation during the day to release sugars in order to maintain osmotic potential within the guard cells, which can contribute to stomatal opening during water deficit. Our sweet potato accessions showed an overall starch accumulation in the shoot during daylight, suggesting sufficient photosynthetic activity to avoid starch degradation into sugars during the day. According to Zeeman *et al.*[43] and Preiss and Sivak[44], the shoot starch is considered transitory, due to its deposition in granules in the leaf chloroplasts during daylight active photosynthesis carbon dioxide fixation, and then is broken down for sucrose synthesis (non-reducing sugar) during the night. The sucrose can then be mobilized from shoots to the underground tuber organs, to be converted to storage starch for long-term storage.[43]

The distinct carbohydrate content registered between accessions was probably due to the different supply needs in energy and metabolites during drought. The tuber starch hydrolysis was possibly due to the need for tissue energy and for a supply of metabolites through the mobilization of reserves, aiming to protect their structures against water deficit.[15] Starch hydrolysis produces sugars (such as sucrose) as osmoregulators during drought. Sugars can increase the cell-pressure potential by fulfilling the cell bilayer interfaces during drought osmotic stress.[45,46] The exceptions were acc. 2938, which practically did not change the tuber starch content, and acc. 3124, which increased the tuber starch content (+26%). As acc. 3124 maintained shoot starch and increased tuber starch content, this suggests that the photosynthesis rate was sufficient to lead to constant shoot starch synthesis and its mobilization to the tuber as storage starch during night, ensuring its biomass allocation. Conversely, acc. 2937 registered the highest shoot starch accumulation (+143%) and still had slight tuber starch loss (–8%). The fact that there is an accumulation of starch in shoots with no starch mobilization from shoots to the tubers, compromises plant growth and health.[43] However, acc. 1036 applied another mechanism of response to drought. It was the only acc. that registered an increment of both chlorophyll content and protein content, which could have contributed to the shoot starch increase (+88%), but still showed starch mobilization through tuber starch hydrolysis (–14%) as a cellular filler during drought. The starch content obtained for the tuber flour is in accordance with Lai *et al.*,[47] which recorded between 24.35 and 46.72 g/100g of starch for non-stressed sweet potatoes. Ekanayake and Collins[6] obtained a much lower tuber starch content under different irrigation conditions, ranging from 122.3 to 136.3 g kg^{-1} for control and drought conditions, respectively. However, the present study showed lower starch content than Ravindran *et al.*,[24] which obtained between 631.3 and 773.4 g kg^{-1} for rain-fed, non-stressed sweet potato tubers.

According to Kays[42] and Artschwager[48], who studied the physiological anatomy of the sweet potato root tuber, the starch grains are stored in a central core of storage parenchyma cells, mainly at the normal bundle parenchyma. The shape of the sweet potato starch granules is typically oval, round, or polygonal, with a central hilum, the size of which can significantly fluctuate within the same cultivar.[8] Two starch polymers composed by glucose monomers are present in sweet potato: amylopectin (crystalline-branched structure, 70–80%) and amylose (amorphous linear structure, 20–30%).[42,47] The SWS and SSP expresses the starch gelatinization properties influenced by the amylose and amylopectin features.[49] The starch gelatinization occurs in the presence of heat and water, with hydration and starch swelling due to amylopectin water uptake. The granules thereby lose organization and some of the amylose granules leach into the water.[47] During water scarcity, an overall increase in SWS and SSP was observed, with the exception of acc. 1036 and 2927. They still showed lower values than the 0.4031–0.6187 g g^{-1} of SWS and 20.01–28.87 g g^{-1} of SSP obtained on non-

stressed sweet potato tubers cultivated in Taiwan.[47] Lower values, between 0.086–0.096 g g^{-1} of SWS and 3.40–3.67 g g^{-1} of SSP, were also obtained for sweet potato flour purchased from a local market in Indonesia.[49] The SWS and SSP increase (except for acc. 1036 and 2927) can be related to a decrease in the amylose-to-amylopectin ratio during drought. While the starch content decreased, the quality and functional properties of the tuber starch grain gelatinization increased. Accession 3124 was the only one that increased tuber starch content (+26%) and maintained a good tuber starch grain quality with increased SWS (+8%) and SSP (+7%). Overall, and unlike Kusumayanti *et al.*,[49] our study showed that drought slightly improved the SSP of acc. 2938 (+7%), 3126 (+2%), 3124 (+7%), and 3125 (+5%), showing potential as a bakery product, due to better starch quality. However, acc. 1036 had the highest starch content loss (–14%) and was the only one that showed a loss of grain quality with a decreased SSP (–8%) under drought.

7.5.4 Whole-plant mechanism response to drought

In the present study, the effect of water shortage on root and shoot oxalates, protein and starch content, CCI and starch grain quality were evaluated. This shortage leads to significant differences between plant organs ($P \leq 0.05$). Overall, drought triggered an oxalate and starch mobilization / allocation, a decrease in photosynthesis, and a slight decrease in protein synthesis on both organs. The whole-plant multivariate analysis, variance, and correlation analysis showed that shoots displayed higher variability than tubers among the measured parameters, in response to drought. The distribution of accessions (cases) along the PCA axis (Fig. S1(A) and (B)), also demonstrated the variability of plant responses under drought stress.

Overall, the shoot S-Ox and CaOx synthesis had significant positive correlations, meanwhile the tubers showed a CaOx significant negative correlation with S-Ox. Drought could have limited the free calcium in most accessions, which led to a decreased tuber CaOx content, as a feasible calcium release for plant osmoregulation, thus increasing the S-Ox.[9] This promoted a better balance of this insoluble salt, leading to a higher active growth, and reducing the biomass loss due to stress.

The oxalic acid content could mainly be a derivative product from both the carbohydrate metabolism and the photosynthesis oxidative processes.[9] According to Igamberdiev and Eprintsev[16] and Franceschi and Horner[9], the most common form of oxalic acid plant accumulation is due to the glycolate oxidized in glyoxylate, derived from photosynthesis, and then oxidised in oxalic acid (S-Ox). Plants can precipitate the excess calcium ions with synthetized oxalic acid, forming CaOx insoluble druse crystals, as calcium storage, ion balance, and osmoregulation.[9,13,35] An average CCI slight decrease was observed in our sweet potato accessions during drought stress conditions, except for acc. 1036 and 3126. The CCI was not correlated with S-Ox but showed a relatively strong negative correlation with CaOx. On average, drought showed an increase of S-Ox and decrease in druse CaOx crystals content in both tubers and shoots organs. Drought could have limited the plant free calcium, as in acc. 1036, 2927, 3125, leading to an average decrease in druse CaOx crystals content in both organs, presumably freeing up the calcium for plant osmoregulation, and thus slightly increasing the S-Ox. Tooulakou *et al.*,[17] observed that decreased photosynthesis during daylight in drought pigweed (*Amaranthus hybridus*) plants by limiting the leaf CO_2 fixation, compensated the lack of photosynthetic carbon with CaOx druse crystals degradation. We observed that with a slight reduction in CCI, there was a CaOx decrease and a S-Ox increase. This indicates that drought mainly freed the shoot sweet potatoes calcium for osmoregulation, increasing S-Ox, instead of using CaOx as a carbon source to compensate the decrease in CO_2 due to partial stomatal closure.[36]

During daylight, the carbon dioxide fixation by photosynthesis also synthetises starch, which is deposited in granules in the leaf chloroplasts.[43,44] We observed that sweet potato shoots managed to increase starch content during daylight, while there was a slight CCI decrease, and then mobilized the starch to the tubers as storage starch, to be further hydrolyzed for energy and growth. The shoots CaOx and S-Ox had a significant positive correlation with starch, which in turn was negatively correlated with protein content. According to Franceschi and Horner[9], the oxidation of carbohydrates can provide energy for the reduction of nitrates into protein nitrogen, with oxalate synthesis as a direct sub-product. According to Burgess and Huang,[15] Salehi-Lisar and Bakhshayeshan-Agdam,[18] Osuagwu and Edeoga,[21] and Epron and Dreyer,[22] plants

can increase starch hydrolysis for accumulation of soluble sugars, or they can also increase crude protein content due to synthesis of specific high molecular proteins as a resistance response to water scarcity. The CCI and protein content have not shown any significant correlation, but the CCI has shown a negative correlation with CaOx and starch content. While the CCI decreased slightly due to potential partial stomatal closure, starch was still accumulated in the shoots. We also spotted a generalized appetence to starch tuber hydrolysis as a response mechanism. It possibly occurred for the increase of the cell pressure potential, through the fulfilling of the cell bilayer interfaces with sugars during drought osmotic stress. A direct correlation between the SWS and SSP increase was registered, with acc. 2938, 3126, 3124, and 3125 showing better bakery potential due to starch quality. This direct correlation shows that accessions with greater swelling power had higher solubility, thus agreeing with Kusumayanti *et al.*[49]

Accessions 3124 and 3125 showed a better balance between all biochemical processes that were studied, which may have contributed to the highest total plant biomass content with less loss in drought stress conditions.[36] Accession 3125 applied a mechanism that responded to drought by starch mobilization, through a slight hydrolysis of tuber starch, and an increase in shoot starch content, while maintaining the quality and functional properties of the tuber starch grain. Accession 3124 did not lose tuber and shoot starch under stress, supplying energy and metabolites without recourse to starch hydrolysis, and maintaining a good tuber starch grain quality, with an increase in SWS and SSP. Both accessions showed a CCI decrease but still had the highest CCI content in both assay conditions. They also show a greater decrease in CaOx content in response to drought, with one of the best plant osmoregulations by CaOx and S-Ox equilibrium in both organs. Their shoots were safe for raw consumption in both experimental conditions, as they were below the 0.71 g kg^{-1} maximum recommended level of CaOx for food.[34] The tubers also became safe for raw consumption in drought conditions. These accessions therefore presented the best trait response to drought, and are potential candidates for breeding programs.

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7.7 Author contribution statement

CSSG performed the biochemical experiments, analysed, interpreted, summarized all data generated from those experiments, and wrote the manuscript. JFTG designed the study for the drought assay, and helped in statistical analysis. VL and MAAPC coordinated the work and revised the manuscript.

7.8 Conflict of interest: none

7.9 References

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Tables

Table 7.1

Identification code, variety name and origin of the eight sweet potato (*Ipomoea batatas* L.) accessions submitted to water-scarcity conditions.

Accession ID[a]	Variety local name	Origin
1036	Brasileira	Madeira Island
1038	5 Bicos	Madeira Island
2927	de Flor	Madeira Island
3126	Inglesa	Madeira Island
2937	Roja	Canary Islands – Tenerife
2938	Cubana	Canary Islands – Tenerife
3124	Vermelha	Guinea-Bissau – Bafatá
3125	Branca	Guinea-Bissau – Bafatá

[a] Accession identification number code, used by the ISOPlexis Genebank.

Table 7.2

Biochemical parameters of sweet potato root tubers subjected to control and water-scarcity conditions.

Tubers		T-Ox	S-Ox	CaOx	CP	St	SWS	SSP
1036	Control	0.30 ± 0.07^{a}	0.14 ± 0.00^{c}	0.16 ± 0.08^{a}	62.0 ± 5.8^{abcd}	452.2 ± 20.9^{bcde}	0.35 ± 0.0^{abc}	14.7 ± 0.3^{b}
	Drought	0.42 ± 0.06^{ab}	0.27 ± 0.05^{d}	0.15 ± 0.01^{a}	92.7 ± 1.7^{e}	382.4 ± 14.0^{ab}	0.37 ± 0.0^{abc}	12.9 ± 0.1^{ab}
1038	Control	0.51 ± 0.19^{abc}	0.07 ± 0.02^{abc}	0.44 ± 0.18^{abcd}	68.6 ± 5.4^{bcd}	466.5 ± 24.6^{de}	0.34 ± 0.0^{ab}	13.6 ± 0.3^{ab}
	Drought	0.40 ± 0.07^{ab}	0.04 ± 0.01^{a}	0.36 ± 0.06^{abc}	42.8 ± 4.9^{a}	456.7 ± 4.5^{cde}	0.34 ± 0.0^{ab}	13.7 ± 0.4^{ab}
2927	Control	0.51 ± 0.08^{abc}	0.13 ± 0.01^{bc}	0.38 ± 0.06^{abc}	61.6 ± 7.9^{abcd}	382.8 ± 31.6^{ab}	0.33 ± 0.0^{a}	12.7 ± 0.3^{a}
	Drought	0.57 ± 0.14^{abc}	0.29 ± 0.06^{d}	0.28 ± 0.19^{ab}	71.0 ± 6.2^{cd}	375.4 ± 6.8^{a}	0.36 ± 0.0^{abc}	12.7 ± 0.3^{a}
3126	Control	0.87 ± 0.12^{cd}	0.11 ± 0.04^{abc}	0.76 ± 0.12^{cde}	74.2 ± 11.0^{de}	465.1 ± 20.2^{de}	0.39 ± 0.0^{cd}	13.8 ± 0.4^{ab}
	Drought	0.75 ± 0.07^{bcd}	0.15 ± 0.00^{c}	0.60 ± 0.07^{abcde}	61.4 ± 6.3^{abcd}	412.8 ± 20.4^{abcd}	0.43 ± 0.0^{d}	14.1 ± 1.5^{ab}
2937	Control	0.64 ± 0.13^{abcd}	0.05 ± 0.01^{ab}	0.59 ± 0.13^{abcde}	68.2 ± 13.6^{bcd}	481.8 ± 20.7^{e}	0.37 ± 0.0^{abc}	15.0 ± 0.4^{b}
	Drought	0.86 ± 0.11^{cd}	0.08 ± 0.00^{abc}	0.79 ± 0.11^{cde}	49.0 ± 0.6^{ab}	440.6 ± 41.4^{bcde}	0.37 ± 0.0^{abc}	14.9 ± 0.1^{b}
2938	Control	0.59 ± 0.18^{abc}	0.12 ± 0.03^{abc}	0.47 ± 0.19^{abcde}	53.9 ± 5.2^{abcd}	430.1 ± 4.4^{abcde}	0.38 ± 0.0^{bc}	13.8 ± 0.6^{ab}
	Drought	0.92 ± 0.32^{cd}	0.07 ± 0.04^{abc}	0.85 ± 0.28^{de}	50.0 ± 0.8^{abc}	437.9 ± 28.2^{abcde}	0.38 ± 0.0^{bcd}	14.5 ± 0.2^{b}
3124	Control	1.84 ± 0.21^{e}	0.10 ± 0.02^{abc}	1.73 ± 0.22^{f}	44.7 ± 9.4^{a}	382.1 ± 30.0^{ab}	0.36 ± 0.0^{abc}	13.6 ± 0.8^{ab}
	Drought	0.80 ± 0.03^{bcd}	0.13 ± 0.04^{abc}	0.67 ± 0.06^{bcde}	45.7 ± 0.6^{a}	484.4 ± 15.8^{e}	0.39 ± 0.0^{cd}	15.0 ± 0.5^{b}
3125	Control	1.04 ± 0.17^{d}	0.15 ± 0.02^{c}	0.89 ± 0.18^{e}	69.2 ± 5.5^{bcd}	430.4 ± 5.6^{abcde}	0.37 ± 0.0^{abc}	13.5 ± 0.2^{ab}
	Drought	0.64 ± 0.12^{abcd}	0.10 ± 0.03^{abc}	0.54 ± 0.15^{abcde}	63.3 ± 11.7^{abcd}	396.0 ± 5.9^{abc}	0.38 ± 0.0^{bc}	14.2 ± 0.2^{ab}
Mean	Control	0.79	0.11	0.68	6.3	433.9	0.36	13.8
	Drought	0.67	0.14	0.53	5.9	423.3	0.38	14.0
Min	Control	0.30	0.05	0.16	4.4	382.1	0.33	12.7
	Drought	0.40	0.04	0.15	4.3	375.4	0.34	12.7
Max	Control	1.84	0.15	1.73	7.4	481.8	0.39	15.0
	Drought	0.92	0.29	0.85	9.3	484.4	0.43	15.0

T-Ox total oxalates (g kg^{-1}), *S-Ox* soluble oxalates (g kg^{-1}), *CaOx* calcium oxalate (g kg^{-1}), *CP* crude protein (g kg^{-1}), *St* starch content (g kg^{-1}), *SWS* starch solubility in water (g g^{-1}), *SSP* starch swelling power (g g^{-1}). Accessions not sharing the same letters between columns are significantly different (Tukey HSD, $P \leq 0.05$). Data are expressed on a dry weight basis and represent the means ± SD of three independent replications per accession, with total mean, minimum and maximum per trait. Control is fully irrigated; drought is water scarcity.

Table 7.3

Biochemical and CCI parameters of sweet potato shoots subjected to control and water-scarcity conditions.

Shoots		T-Ox	S-Ox	CaOx	CP	St	CCI
1036	Control	2.03 ± 0.24^{ef}	0.47 ± 0.07^{hij}	1.56 ± 0.17^{ef}	145.1 ± 13.3^{cde}	40.4 ± 7.4^{bcde}	18.5 ± 2.4^{ab}
	Drought	1.50 ± 0.04^{de}	0.81 ± 0.09^{k}	0.69 ± 0.12^{abc}	164.1 ± 0.1^{cdef}	75.2 ± 7.7^{fg}	31.3 ± 7.2^{abcde}
1038	Control	0.51 ± 0.13^{ab}	0.08 ± 0.01^{ab}	0.44 ± 0.16^{ab}	175.6 ± 5.8^{efg}	17.3 ± 3.0^{ab}	37.9 ± 5.3^{de}
	Drought	0.42 ± 0.09^{a}	0.04 ± 0.01^{a}	0.37 ± 0.09^{ab}	127.9 ± 12.9^{bcd}	49.2 ± 10.5^{cdef}	28.6 ± 3.5^{abcde}
2927	Control	0.50 ± 0.10^{ab}	0.13 ± 0.02^{abc}	0.37 ± 0.08^{ab}	154.7 ± 23.0^{cdef}	29.3 ± 5.2^{abc}	41.6 ± 4.8^{e}
	Drought	0.58 ± 0.15^{abc}	0.29 ± 0.05^{abc}	0.29 ± 0.20^{a}	104.2 ± 5.7^{ab}	44.9 ± 2.9^{bcde}	35.9 ± 0.7^{de}
3126	Control	1.14 ± 0.12^{cd}	0.24 ± 0.01^{cdef}	0.90 ± 0.12^{bcd}	205.0 ± 6.5^{g}	11.2 ± 4.1^{a}	25.8 ± 1.5^{abcd}
	Drought	1.48 ± 0.11^{de}	0.31 ± 0.02^{defg}	1.17 ± 0.10^{cde}	165.9 ± 9.4^{def}	48.4 ± 13.1^{cdef}	31.5 ± 5.6^{abcde}
2937	Control	2.59 ± 0.21^{fg}	0.37 ± 0.03^{efgh}	2.22 ± 0.20^{g}	127.3 ± 2.5^{bc}	65.7 ± 14.9^{efg}	21.0 ± 2.5^{abc}
	Drought	2.64 ± 0.35^{g}	0.49 ± 0.07^{hij}	2.15 ± 0.29^{fg}	84.6 ± 0.5^{a}	169.3 ± 9.8^{h}	18.3 ± 2.4^{a}
2938	Control	1.99 ± 0.38^{e}	0.54 ± 0.03^{j}	1.45 ± 0.41^{de}	157.1 ± 11.8^{cdef}	41.2 ± 0.8^{bcde}	32.3 ± 3.7^{bcde}
	Drought	2.85 ± 0.06^{g}	0.39 ± 0.06^{ghi}	2.46 ± 0.12^{g}	105.4 ± 3.6^{ab}	86.1 ± 14.1^{g}	25.6 ± 0.8^{abcd}
3124	Control	1.03 ± 0.20^{bcd}	0.53 ± 0.09^{ij}	0.49 ± 0.28^{ab}	188.7 ± 23.6^{fg}	35.0 ± 4.3^{abcd}	31.2 ± 6.7^{abcde}
	Drought	0.94 ± 0.29^{abcd}	0.38 ± 0.05^{fgh}	0.56 ± 0.30^{ab}	159.7 ± 19.7^{cdef}	34.8 ± 3.5^{abcd}	26.8 ± 3.0^{abcd}
3125	Control	0.67 ± 0.02^{abc}	0.19 ± 0.05^{abcd}	0.48 ± 0.04^{ab}	155.8 ± 16.8^{cdef}	58.1 ± 13.2^{defg}	35.1 ± 8.2^{de}
	Drought	0.39 ± 0.01^{a}	0.22 ± 0.02^{bcde}	0.17 ± 0.04^{a}	131.3 ± 9.2^{bcd}	59.6 ± 15.3^{defg}	32.5 ± 5.7^{cde}
Mean	Control	1.31	0.32	0.99	163.4	37.3	30.4
	Drought	1.35	0.37	0.98	130.4	70.9	28.8
Min	Control	0.50	0.08	0.37	127.3	11.2	18.5
	Drought	0.39	0.04	0.17	84.6	34.8	18.3
Max	Control	2.59	0.54	2.22	205.0	65.7	41.6
	Drought	2.85	0.81	2.46	165.9	169.3	35.9

T-Ox total oxalates (g kg^{-1}), *S-Ox* soluble oxalates (g kg^{-1}), *CaOx* calcium oxalate (g kg^{-1}), *CP* crude protein (g kg^{-1}), *St* starch content (g kg^{-1}), *CCI* chlorophyll content index. Accessions not sharing the same letters between columns are significantly different (Tukey HSD, $P \leq 0.05$). Data are expressed on a dry weight basis and represent the means ± SD of three independent replications per accession, with total mean, minimum and maximum per trait. Control is fully irrigated; drought is water scarcity.

Table 7.4

Pearson correlation coefficients (r) of biochemical parameters of sweet potato root tubers subjected to control and water-scarcity conditions.

Variables	1	2	3	4	5	6
1. S-Ox	-					
2. CaOX	**-0.34***	-				
3. T-Ox	-0.16	**0.98****	-			
4. CP	**0.55****	**-0.40****	**-0.31***	-		
5. St	**-0.57****	-0.04	-0.15	-0.26	-	
6. SWS	0.10	0.18	0.21	-0.01	0.07	-
7. SSP	**-0.50****	0.14	0.05	**-0.46****	**0.56****	**0.34***

S-Ox soluble oxalates (mg/100g), *CaOx* calcium oxalate (mg/100g), *T-Ox* total oxalates (mg/100g), *CP* crude protein (g/100g), *St* starch content (g/100g), *SWS* starch solubility in water (g g^{-1}), *SSP* starch swelling power (g g^{-1}).

** Correlation is significant at the 0.01 level (two-tailed).

* Correlation is significant at the 0.05 level (two-tailed).

Table 7.5

Pearson correlation coefficients (r) of biochemical and CCI parameters of sweet potato shoots submitted to control and water scarcity conditions.

	1	2	3	4	5
1. S-Ox	-				
2. CaOx	**0.36***	-			
3. T-Ox	**0.56****	**0.98****	-		
4. CP	0.02	**-0.39****	**-0.35***	-	
5. St	**0.34***	**0.53****	**0.55****	**-0.71****	-
6. CCI	-0.28	**-0.63****	**-0.63****	0.28	**-0.41****

S-Ox soluble oxalates (mg/100g), *CaOx* calcium oxalate (mg/100g), *T-Ox* total oxalates (mg/100g), *CP* crude protein (g/100g), *St* starch content (g/100g), *CCI* chlorophyll content index.

** Correlation is significant at the 0.01 level (two-tailed).

* Correlation is significant at the 0.05 level (two-tailed).

7.10 Supporting information

Supporting information may be found in the online version of this article:
https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fjsfa.10185&file=jsfa10185-sup-0001-FigureS1.docx

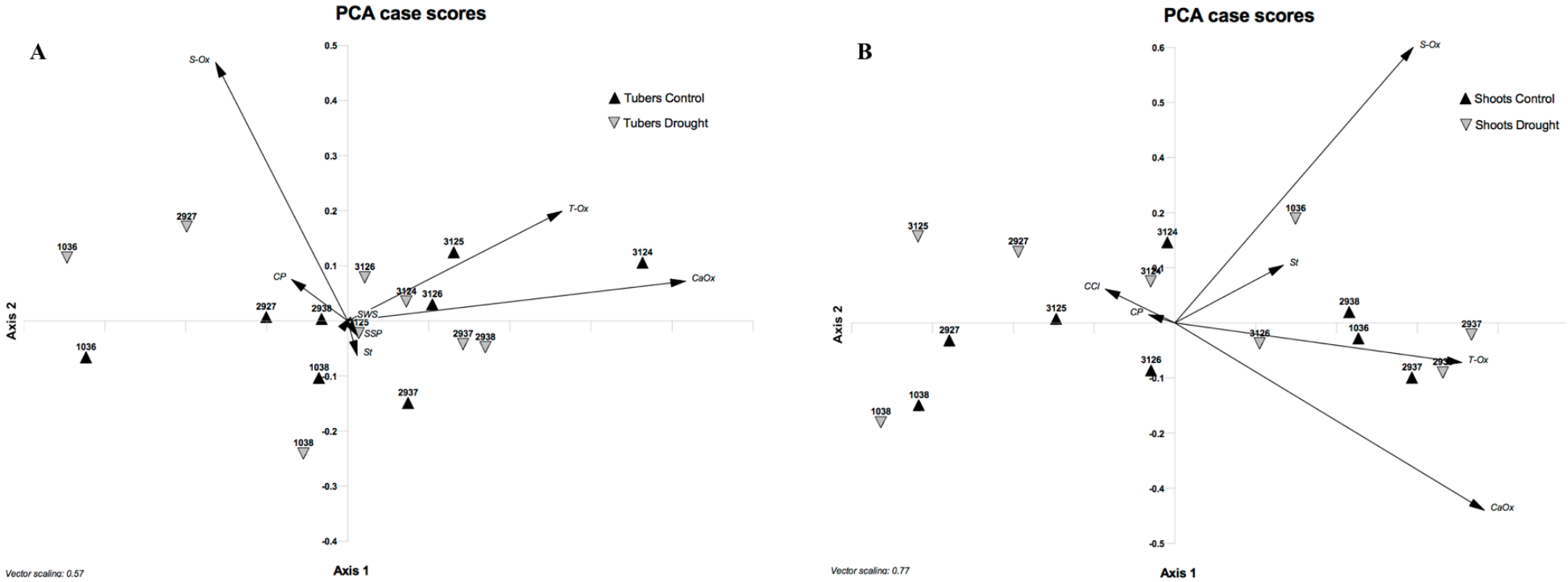

Figure S1

Euclidean biplot with variables (biochemical and CCI) spatial distribution of the (A) root tubers and (B) shoots case scores from sweet potato accessions, by principal component analysis (PCA).
All the variables were converted by $\log_e$.
Control is fully irrigated; drought is water scarcity.

CHAPTER 8

Variation of carbon and isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) of whole-plant sweet potato (*Ipomoea batatas* L.) subjected to prolonged water stress

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Variation of carbon and isotope natural abundances (δ^{15}N and δ^{13}C) of whole-plant sweet potato (*Ipomoea batatas* L.) subjected to prolonged water stress

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8.1 Abstract

Sweet potato (*Ipomoea batatas* L.) is an important crop in the world, cultivated in temperate climates under low inputs. Drought changes the plant biomass allocation, together with the carbon and nitrogen isotopic composition (δ^{13}C and δ^{15}N), whose changes are faintly known in sweet potato crops. Here, we show the biomass allocation of eight sweet potato accessions submitted to drought during 3 months, using the δ^{13}C, δ^{15}N, carbon isotope discrimination (Δ^{13}C), total carbon (TC) and water use efficiency (WUE) traits. The tolerant accessions had improved WUE, with higher TPB and TC. Storage roots and shoots had a heavier δ^{13}C content under drought stress, with greater ^{13}C fixation in roots. The Δ^{13}C did not show a significant association with WUE. The δ^{15}N values indicated a generalised N reallocation between whole-plant organs under drought, as a physiological integrator of response to environmental stress. This information can aid the selection of traits to be used in sweet potato breeding programs, to adapt this crop to climate change.

Keywords

Biomass allocation; drought physiological integrator; drought stress; *Ipomoea batatas*; stable isotope abundances

Abbreviations

Acc.	Sweet potato accession identification number
Δ	Isotope discrimination
δ	Natural abundance or isotopic composition
N	Nitrogen
NRA	Nitrate reductase activity
PCA	Principal component analysis
R	Isotope (abundance) ratios
TC	Total carbon
TPB	Total plant biomass
WP	Whole-plant
WUE	Water use efficiency

8.2 Introduction

Carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) could provide important insights regarding the plant chemical, physical and metabolic processes involved in carbon transformations and nitrogen processes during drought. Water scarcity decreases the leaf $\delta^{13}C$ abundance and changes the plant water use efficiency (WUE), both associated to photosynthesis effects by carboxylation (Robinson et al., 2000; O'Leary 1993; Farquhar et al., 1989). Meanwhile, the changes of $\delta^{15}N$ during drought can indicate how genotypes retain nitrogen (N) in their tissues (Robinson, 2001).

While both the $\delta^{13}C$ and $\delta^{15}N$ can be a useful physiological integrators of stress responses, $\delta^{13}C$ is the most commonly used for drought assessment of C3 plants, with the $\delta^{15}N$ being less explored (Gouveia et al., 2019; Robinson et al., 2000). C3 plants, such as sweet potato (*Ipomoea batatas* L.), convert the atmospheric CO_2 through efficient incorporation of carbon isotopes during photosynthesis into plant biomass (Bayala et al., 2015; Lomax et al., 2012; Farquhar et al., 1982). The negative $\delta^{13}C$ values can be simplified into a positive isotopic carbon discrimination ($\Delta^{13}C$) for field-grown plants according to Farquhar et al. (1989, 1982).

The WUE is often based on measurements of plant growth and water loss (Johnson and Tieszen, 1993). The association of both WUE and $\Delta^{13}C$ in C3 plants are important because they can provide pertinent information about plant biomass production and allocation during drought (Gouveia et al., 2019; Tiwari and Mamrutha, 2013; Johnson and Tieszen, 1993; Laureti et al., 1993). No publication concerns both $\delta^{13}C$ and $\delta^{15}N$ as physiological indicators of sweet potato drought response. Albeit, one work used the $\Delta^{13}C$ to study the dry mass accumulation and allocation of one variety of sweet potato under drought stress (Zhang et al., 2015). Our main objectives were i.) to assess drought response with sweet potato biomass allocation through stress $\delta^{13}C$ and $\delta^{15}N$ physiological integrators, and ii.) to assess $\Delta^{13}C$ as a potential fast tool for improvement of WUE determination.

8.3 Materials and Methods

8.3.1 Plant material and experimental setup

Eight accessions (acc.) of sweet potato (*Ipomoea batatas*) (designated in Supplement 1), originating from Madeira and Canary Islands, and from Guinea-Bissau, were simultaneously submitted to control and drought environments. The experimental split-plot design is detailed in Supplement 2. Both control and drought stress environments were assessed periodically for the photosynthetic active radiation (PAR, 400–700 nm) with a ceptometer (AccuPAR LP-80, USA), volume water content of soil (VWC_s) with a soil moisture sensor (WaterScout SM100, USA), air temperature (T_a) and relative air humidity (RH_a) with a data logger (Testo 174H, Germany). Along the assay, the PAR decreased 24.6% under the rain shelter relative to control, on average 1514.5µmol/m^2/s for control and 1142.0µmol/m^2/s for drought. At 10 cm of depth of homogenized field soil, 12.8% VWC_s was registered for control, indicating 35% of field capacity and 3.5% VWC_s for drought indicating equal or less than 10% of field capacity, in average. Control had a 19.46 °C T_a and 68.07% RH_a, while drought had a 22.25 °C T_a and 66.40% RH_a, in average.

8.3.2 Preparation of sweet potato sample flours

At the end of the agronomic assay, we collected 384 storage roots (hereafter designed as tubers) and shoots (stem, stalk and leaves) from control and drought subplots. The samples were washed to remove soil residues, weighed (Sartorius Basic BA2100S, Germany), sliced on a mandolin slicer (2-3 mm thick), oven-dried during 48h at 65ºC (Memmert UF260, Germany) and finely grounded (IKA-Werke M20, USA). The flour was placed into bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153, Netherlands) and stored at -35ºC (Liebherr ProfiLine GGPV6570, Germany) until analysis.

8.3.3 Total Plant Biomass (TPB)

TPB was quantified as the dry matter of the whole-plant replicate (tubers and shoots), from dried biomass values obtained by air oven, according to Undersander et al. (1993). Each treatment was triplicated, with results expressed as g/plant dry flour.

8.3.4 Water use efficiency (WUE)

WUE was calculated as the ratio of TPB to total water used per subplot, expressed in g/L.

8.3.5 Nitrogen (N)

N content was quantified in dried tuber and shoot flours using the Kjeldahl method AOAC 945.18-B: 2005, through a distillation and titration automatic unit system (Velp Scientifica UDK 152, Italy). The analysis was triplicated, with results expressed as g/100 g dry flour.

8.3.6 Carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$)

The sweet potato tuber and shoot flours were vacuum packaged and sent to the Natural Resources Analytical Laboratory at the University of Alberta, Edmonton, Canada, for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis and total carbon (TC) content. The isotopic compositions were determined by the micro-chemical AOAC 972.43:2000 method, using a Delta V Advantage Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS, Thermo Finnigan Corp, Bremen, Germany). The conversion of $\delta^{13}C$ into $\Delta^{13}C$ by Farquhar et al. (1989), and whole-plant (WP) $\delta^{15}N$ calculation by Robinson et al. (2000). The analysis was triplicated, and results expressed in per mill (‰).

8.3.7 Statistical methods

The results were expressed on a dry weight basis, as the main average of sweet potato tubers and shoots, for control *vs* drought plots. IBM SPSS Statistics V24 for Mac was used for One-way ANOVA, Tukey HSD test, and Pearson correlations; MVSP V3.1 for Windows was used for principal component analysis (PCA).

8.4 Results

8.4.1 $\delta^{13}C$, $\delta^{15}N$ and N variation between plant organs under drought

Table 8.1 shows the data obtained for $\delta^{13}C$, $\delta^{15}N$ and N contents of tubers and shoots, under both control and drought environments. On average drought increased $\delta^{13}C$ and decreased $\delta^{15}N$ and N contents in both organs. Acc. 3124 was the only one presenting a $\delta^{13}C$-shoot content decrease during drought.

Under drought conditions, the shoots showed the lower $\delta^{13}C$ content, compared to the tubers. On average $\delta^{13}C$ content in the shoots increased more, from −27.33‰ to −25.93‰ (+1.40‰), as compared to tubers that increased from −25.64‰ to −24.34‰ (+1.30‰). Acc. 3126 had significantly higher $\delta^{13}C$ content for both organs in both experimental variants. The lowest variability in whole-plant $\delta^{13}C$ content under water scarcity was reported in acc. 1038, 3124 and 3125.

The content of $\delta^{15}N$-shoots decreased slightly from 5.25‰ to 3.46‰ (−1.78‰), and $\delta^{15}N$-tubers also showed a small decrease from 5.23‰ to 3.65‰ (−1.59‰). Acc. 1038 exhibited the highest significant $\delta^{15}N$ decrease in both organs and experimental variants. Acc. 3124 had the highest $\delta^{15}N$-shoot and $\delta^{15}N$-tuber content, while it had the lowest $\delta^{13}C$ content in both organs, on drought environments.

The N content had substantially higher variation in shoots compared to tubers, with the N-shoot content on average was twice the N-tuber content. Still, drought led to a greater loss of N-shoot content. On average, drought decreased the N-shoot from 26.30 mg to 20.95 mg (−5.35mg), with N-tuber registering only a slight decrease from 10.08 mg to 9.54 mg (−0.55 mg). Acc. 3126 registered a significantly higher N-shoot content in both experimental variants. Acc. 2938 had the highest N-shoot content loss due to drought. Acc. 1036 was the only acc. that increased N in both organs, and registered the significantly highest N-tuber content and range

during drought. Acc. 3124 and 2927 have also accumulated N in the shoots, although in smaller quantities.

8.4.2 Drought variation of $\delta^{15}N$, $\Delta^{13}C$, TC, WUE and TPB at whole-plant level

On average, the sweet potato whole-plant (tubers and shoots) acc. decreased WP $\delta^{15}N$ (−1.73‰), WP $\Delta^{13}C$ (−1.46‰) and TPB (−295.56 g), and increased WUE (+3.23 g/L) and WP TC (+0.18%) under drought (Table 8.2). The WUE and TPB showed significant variability under drought conditions, with WUE ranging from 1.09 g/L (acc. 2937) to 16.45 g/L (acc. 3124), and TPB from 22.90 g (acc. 2937) to 449.91 g (acc. 3124).

Acc. 2937 had the lowest WP $\delta^{15}N$, WUE and TPB content in both experimental environments. On the other hand, acc. 3124 was the exception since it slightly decreased WP TC and exhibited higher WP $\delta^{15}N$ (4.30‰), WP $\Delta^{13}C$ (19.27‰), WUE (16.45 g/L) and TPB (449.91 g) content under drought. Acc. 3124 also showed the lowest loss of WP $\delta^{15}N$, $\Delta^{13}C$ and TPB under stress. Acc. 3125 had the second highest WUE and TPB content. However, acc. 1038 showed significantly higher WP $\delta^{15}N$, WUE and TPB under control conditions, and the most pronounced loss of WP $\delta^{15}N$ and TPB during water scarcity.

8.4.3 Variance and traits associations

One-Way ANOVA, Tukey HSD and Pearson correlations were used to better understand the impact of water deficit in all the traits (variables) and their relation with the whole-plant response to drought stress conditions. Variables with significant differences ($p \leq 0.01$) were recorded between the eight sweet potato acc. (cases) by the One-Way ANOVA and Tukey HSD multiple comparisons, in both control and drought environments (Tables 8.1 and 8.2), with the $\delta^{13}C$-tuber and $\delta^{13}C$-shoot showing the highest variability.

Nineteen significant Pearson correlations were found between the variables in tubers, of which 13 were strong correlations, with r greater to 0.50. Twenty-three significant correlations were found in shoots, of which 16 were also greater than r = 0.50. $\delta^{15}N$-shoot and N-shoot decrease was strongly correlated (r = 0.56). Tubers and shoots $\delta^{13}C$ increase showed a relatively strong correlation with $\delta^{15}N$ decrease (r = −0.56 and r = −0.57, respectively). TPB was the variable with the most correlations. At the whole-plant level, strong correlations were registered for TPB and WP $\delta^{15}N$ decrease (r = 0.72), and for the TPB decrease and WP TC increase (r = −0.58). Modest correlations were also observed for TPB and WP $\Delta^{13}C$ decrease (r = 0.47). The enhanced WUE with the TPB decrease, in an overall similar way, also showed a modest correlation (r = 0.44) (Table 8.3).

The PCA analysis was performed using the whole-plant WUE, TPB, TC, and N, $\delta^{15}N$, $\Delta^{13}C$ variables from the tubers (Fig. 8.1A) and shoots (Fig. 8.1B). Two principal components (PC) explained 68.5% of cumulative variance in the tubers, and 76.0% in the shoots. The tubers PC1 showed 42.8% of variance with eigenvalues of 2.6, while PC2 had 25.7% of variance and eigenvalues of 1.5. TPB, $\delta^{15}N$ and $\Delta^{13}C$ were strongly correlated with PC1, while TC and N were correlated with PC2 (Fig. 8.1A). The shoots PC1 showed 55.2% of variance with eigenvalues of 3.3, while PC2 explained 20.8% with eigenvalues of 1.2. TPB and N were correlated with PC1, while $\Delta^{13}C$ and WUE were correlated with PC2 (Fig. 8.1B).

8.5 Discussion

8.5.1 $\delta^{13}C$ value as plant development integrator during drought

Drought led to an approx. 5% decrease of the sweet potato acc. chlorophyll content. The blind samples support that the decrease of the chlorophyll content inside the shelter was due to the lack of water. As the blind samples were fully irrigated in both environments, they showed a 9% significantly higher accumulation of chlorophyll at the shelter than at open field, even with the PAR, T and HR difference between environments (data not shown). The decrease of chlorophyll in the acc. at drought conditions can be in agreement with van Heerden and Laurie, 2008 and Igamberdiev et al. (2004) works, referring to a lower photosystem II (PSII) excitation by partial stomatal closure, or oxidative damage in chloroplast by chlorophyll photo-oxidation. The decrease of PSII under stress environments can slightly reduce the photosynthetic capacity as a reversible photo-protective mechanism. This strategy dissipates the excess excitation energy through heat loss as non-

photochemical quenching mechanism within the light-harvesting complex of PSII (Dahal et al., 2014).

The sweet potato photosynthetic minor down-regulation could have interfered with ^{13}C depletion, leading to a slight increment of $\delta^{13}C$-shoot values during drought. We observed that $\delta^{13}C$-shoot values for all acc. were related to those observed in C3 plants with relatively open stomata in non-stress environments. Drought slightly increased the $\delta^{13}C$-shoot into a more positive and heavier δ-value in all acc., indicating less open stomata as a stress response. Similarly, Robinson et al. (2000) found that in wild barley exposed to drought, $\delta^{13}C$-shoots can be associated with a better response to stress. Moreover, acc. 1038, 3124 and 3125 showed lower $\delta^{13}C$-shoot content, with less variation caused by water scarcity. Possibly they also decreased the stomata aperture, but with less intensity than the remaining acc., by keeping the highest photosynthetic ^{13}C fractionation during water scarcity. Robinson et al. (2000); O'Leary (1993) and Farquhar et al. (1989) also argued that higher stomatal aperture leads to a decreased shoot $\delta^{13}C$ under drought. As the sweet potato tubers and shoots presented a less negative $\delta^{13}C$ value compared to control, we denoted a heavier ^{13}C content under stress environments, which concurs with O'Leary (1981) findings. The overall $\delta^{13}C$ decrease content was also correlated with TPB decrease, as previously mentioned by Igamberdiev et al. (2004). Although, the sweet potato $\delta^{13}C$-tubers had greater ^{13}C fixation during drought, with higher values compared to $\delta^{13}C$-shoots, which is in accordance with Wegener et al. (2015). This generalized greater ^{13}C fixation in tubers may have occurred due a carbohydrate photoassimilate transport from source to sink tissues (Zhang et al., 2015).

8.5.2 WP $\delta^{15}N$ as a drought physiological integrator

During drought, N physiological transformations were observed within the sweet potato whole-plant. Both organs lost N and $\delta^{15}N$ content in a significantly correlated manner. The WP $\delta^{15}N$ loss implies an effective drought response mechanism, as observed by Robinson et al. (2000) in wild barley, and by Gouveia et al. (2019) in taro. The decrease of whole-plant N content and fractionation can also be due to soil water scarcity. The lack of water limits the soil N availability, comprising the plant N uptake and transport from the roots to the shoots, and thus limiting their fractionation during N cycle processes (Duman, 2012). The nitrate (NO_3^-) is converted into nitrite (NO_2^-) for further plant N assimilation, through the cytoplasmic enzyme nitrate reductase activity (NRA) (Romero-Trigueros et al., 2014; Sahoo et al., 2010; Pike et al., 2002; Robinson, 2001). Probably the observed $\delta^{15}N$ loss was due to limited soil N availability that restricted the NO_3^- flux from underground to aboveground organs (Sahoo et al., 2010). Usually, the NRA decreases at whole-plants subjected to stress, being highly dependent of NO_3^- content from the soil (Kaur et al., 2017; Pike et al., 2002). The sweet potato WP $\delta^{15}N$ decrease was greater on the $\delta^{15}N$-shoot compared to $\delta^{15}N$-tuber in all acc. subjected to drought. The N-shoot that was twice the N-tuber content could be due to a higher NRA at the shoot level, which remains in accordance with the Robinson et al. (1998) theory. This could be possible due to the N cycling between the under and aboveground plant organs (Robinson et al., 1998). The N reallocation and $\delta^{15}N$ variation between the organs could be caused by external environmental factors, such as water scarcity and N source availability (Romero-Trigueros et al., 2014; Robinson, 2001).

Robinson et al. (2000) also explained that wild barley that contained less N during stress expressed the smallest $\delta^{15}N$ content and appeared to be less productive due to lower capacity to retain N in the tissues. We also observed that pattern in sweet potato. For example, acc. 2937 showed the lowest $\delta^{15}N$-shoot and N-shoot content in both experimental environments, and was the less ^{15}N-enriched with less N retention, which is in accordance with Robinson (2001). $\delta^{15}N$-shoot and WP $\delta^{15}N$ were positively correlated with N-shoot content. Acc. 2937 was also the lowest yielding acc., exhibiting the lowest TPB content under both experimental conditions. On the other hand, acc. 3124 had the highest $\delta^{15}N$-shoot and $\delta^{15}N$-tuber content under drought. It was the most ^{15}N-enriched acc. in study, showing a good whole-plant N retention and registering the highest TPB content under drought stress. WP $\delta^{15}N$ and TPB were positively correlated. The $\delta^{15}N$ values indicated a generalized N reallocation between whole-plant organs under drought, leading to the increment of N consumption by ^{15}N and ^{14}N isotope fractionation, with $\delta^{15}N$ acting as a sweet potato physiological integrator of drought stress responses (Romero-Trigueros et al., 2014; Robinson, 2001).

8.5.3 Whole-plant carbon-water relationship to drought

Drought resulted in a significant variability in the sweet potato carbon-water relationship, with WP $\Delta^{13}C$ and TPB decrease in all acc., and WUE and WP TC increase.

The ^{13}C fixation and TPB are converted from the light energy harvested by photosynthesis, with WP TC representing that energy allocation (Dahal et al., 2014). The TPB decrease and WP TC increase were significantly correlated. Although, the slight increment of WP TC during drought, except for acc. 3124, does not reveal significant differences in WP TC assimilation, along with the observed TPB loss difference. The WP TC slight increase during drought may be due to a higher ^{13}C fixation, which reduced the ^{13}C depletion required for plant growth processes (Warembourg and Kummerow, 1991).

According to Igamberdiev et al. (2004), partial stomata closure decreased CO_2 plant intercellular spaces, regulating the decrease of photosynthesis and increasing the rate of photorespiration during drought stress. The $\delta^{13}C$-shoot slight increase into a more positive and heavier δ-value under stress suggests a partial stomata closure in all acc. under drought. The acc. down-regulated photosynthesis activity under stress, interfering with the carbon isotope fractionation, leading to a less negative $\delta^{13}C$ value by a WP $\Delta^{13}C$ decrease (Ivlev, 2015). Zhang et al. (2015) also observed that pattern in sweet potato, noting also a higher level of ^{13}C fixation. Acc. 3126 showed the lower WP $\Delta^{13}C$ content in both experimental conditions, with acc. 3124 showing the highest WP $\Delta^{13}C$ content under drought. Since all acc. shared the same growth conditions, the $\Delta^{13}C$ variation can be due to genotypic differences (Lanigan et al., 2008; Igamberdiev et al., 2004).

The partial stomatal closure also reduces the water loss by decreasing transpiration and leading to WUE improvement (Black and Randhawa, 2015). Along the increase of WUE, the sweet potato TPB loss under water scarcity allowed them to maintain vital activities during stress. Both the WUE and WP $\Delta^{13}C$ are also directly linked to leaf stomatal aperture (Black and Randhawa, 2015; Igamberdiev et al., 2004; Farquhar et al., 1989). According to Farquhar et al. (1989), the C3 plants that have greater WUE, showed lower $\Delta^{13}C$ values (richer in $\delta^{13}C$). Gouveia et al. (2019) and Laureti et al. (1993) found significant negative relationship between WUE and $\Delta^{13}C$ in taro and sunflower plants grown under drought, respectively. We have also observed a similar, although not significant, relation in sweet potato.

WUE and TPB displayed significant variability under drought conditions, with acc. 3124 and acc. 2937 showing the highest and the lowest content for both traits, respectively. Following the Farooq et al. (2009) hypothesis, we can infer that acc. 3124 was the most drought-tolerant, with improved WUE and nutrient allocation, when compared to drought-sensitive ones, such as acc. 2937. Since acc. 3124 had the highest partial stomata aperture ($\delta^{13}C$ abundance ~ -27.73‰) during drought, one could expect a greater water loss by transpiration. However, this acc. only decreased 0.08‰ $\delta^{13}C$ during stress, which highly improved the WUE by minimizing the transpiration water loss, with minor down-regulation of the photosynthetic efficiency rate. This water loss avoidance also led to a differential biomass loss between acc. during drought (Gouveia et al., 2019). Acc. 3124 was one of the most drought tolerant, with the highest WUE and TPB, lowest weight loss and highest WUE improvement compared to the control. Meanwhile, acc. 2937 was one of the most sensitive, with the lowest TPB and WUE under both experimental conditions. Enhanced WUE allowed the plant turgidity improvement and other vital activities, with different efficiency among the acc., in accordance with Gouveia et al. (2019) and Farooq et al. (2009). We observed a direct association between the TPB and WP $\Delta^{13}C$ content loss under drought. The overall decrease of TPB was significantly correlated with the WP $\Delta^{13}C$ decrease, leading to more positive $\delta^{13}C$ content due to a greater ^{13}C fixation, which was also observed by Igamberdiev et al. (2004).

In conclusion, stressed $\delta^{13}C$ tubers and shoots appeared to be less negative than the controls, with the shoots displaying the higher increase. All $\delta^{13}C$ values pointed to relatively open stomata as expected for C3 plants. Drought increased WUE by minimizing evapotranspiration through photosynthesis downregulation, leading to a selective biomass loss, suggesting a drought avoidance strategy. Shoots-$\delta^{15}N$ shown a strong correlation with N ($r = 0.56$), while it was most strongly correlated with WP $\delta^{15}N$ ($r = 0.98$), as an efficient drought response mechanism. Negative correlation between $\Delta^{13}C$ and WUE was observed, although not significant. The $\delta^{15}N$ was a good physiological integrator of drought response for sweet potato plants, as one

of the potential tools to be applied in breeding programs of climate change adaptation.

8.6 Author contribution statement

CSSG participated on the drought assay and samples preparation, performed the nitrogen analysis, interpreted and summarized all data generated from those experiments, and wrote the manuscript. JFTG designed the study for the drought assay, and helped in WUE quantification. JS coordinated the TC, $\delta^{13}C$ and $\delta^{15}N$ analysis. VL and MAAPC coordinated the work and revised the manuscript.

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Tables

Table 8.1
Nitrogen isotopic composition, carbon isotopic composition and total nitrogen content of storage roots (tubers) and shoots, under control and drought conditions.

			$\delta^{15}N$		$\delta^{13}C$		N	
			Tuber	**Shoot**	**Tuber**	**Shoot**	**Tuber**	**Shoot**
MAD	1036	Control	4.68 ± 0.41 ^{abcdef}	4.70 ± 0.11 ^{bcdef}	-26.77 ± 0.62 ^{a}	-27.89 ± 0.40 ^{a}	9.99 ± 0.96 ^{bcd}	23.32 ± 2.12 ^{cd}
		Drought	2.96 ± 0.00 ^{a}	3.63 ± 0.00 ^{abcd}	-24.19 ± 0.00 ^{efg}	-25.22 ± 0.00 ^{e}	14.95 ± 0.28 ^{e}	26.31 ± 0.01 ^{de}
		Variation	-1.72	-1.07	+2.58	+2.67	+4.97	+2.99
	1038	Control	6.00 ± 0.57 ^{ef}	6.17 ± 0.03 ^{f}	-25.66 ± 0.38 ^{bc}	-27.29 ± 0.31 ^{ab}	11.06 ± 0.88 ^{cd}	28.20 ± 0.94 ^{def}
		Drought	3.67 ± 0.20 ^{abc}	3.10 ± 0.54 ^{ab}	-25.18 ± 0.87 ^{bcde}	-26.81 ± 0.91 ^{abcd}	6.56 ± 1.19 ^{a}	20.58 ± 2.04 ^{bc}
		Variation	-2.33	-3.06	+0.48	+0.48	-4.50	-7.63
	2927	Control	5.86 ± 1.09 ^{def}	5.30 ± 0.96 ^{cdef}	-25.42 ± 0.05 ^{bcd}	-27.37 ± 0.26 ^{ab}	9.91 ± 1.28 ^{abcd}	24.86 ± 3.69 ^{cde}
		Drought	4.08 ± 0.18 ^{abcde}	3.37 ± 0.25 ^{abc}	-24.37 ± 0.34 ^{defg}	-25.72 ± 0.34 ^{de}	11.43 ± 1.01 ^{de}	16.74 ± 0.90 ^{ab}
		Variation	-1.78	-1.92	+1.05	+1.65	+1.52	-8.12
	3126	Control	4.90 ± 0.84 ^{bcdef}	4.96 ± 0.80 ^{bcdef}	-24.63 ± 0.28 ^{cdef}	-26.51 ± 0.25 ^{abcde}	11.93 ± 1.77 ^{de}	32.95 ± 1.03 ^{f}
		Drought	3.32 ± 0.53 ^{ab}	2.97 ± 0.32 ^{ab}	-22.51 ± 0.17 ^{h}	-23.63 ± 0.47 ^{f}	9.89 ± 1.00 ^{bcd}	26.67 ± 0.17 ^{cde}
		Variation	-1.59	-1.99	+2.12	+2.89	-2.04	-6.29
CAN	2937	Control	3.96 ± 0.46 ^{abcd}	4.20 ± 0.43 ^{abcdef}	-25.33 ± 0.49 ^{bcd}	-27.48 ± 0.54 ^{ab}	10.97 ± 2.17 ^{cd}	20.43 ± 0.37 ^{bc}
		Drought	3.13 ± 0.00 ^{ab}	2.53 ± 0.00 ^{a}	-24.05 ± 0.00 ^{fg}	-26.12 ± 0.00 ^{bcde}	7.90 ± 0.09 ^{abc}	13.59 ± 0.09 ^{a}
		Variation	-0.83	-1.66	+1.28	+1.37	-3.07	-6.84
	2938	Control	4.79 ± 0.68 ^{abcdef}	5.46 ± 0.38 ^{def}	-25.55 ± 0.15 ^{bc}	-27.21 ± 0.21 ^{abcd}	8.66 ± 0.83 ^{abcd}	25.24 ± 1.89 ^{cde}
		Drought	3.61 ± 0.73 ^{abc}	3.98 ± 1.45 ^{abcde}	-23.43 ± 0.02 ^{gh}	-25.79 ± 1.23 ^{cde}	8.04 ± 0.13 ^{abc}	16.96 ± 0.57 ^{ab}
		Variation	-1.18	-1.48	+2.13	+1.42	-0.62	-8.28
GUI	3124	Control	5.31 ± 1.30 ^{cdef}	5.37 ± 0.83 ^{cdef}	-26.14 ± 0.18 ^{ab}	-27.65 ± 0.43 ^{a}	7.04 ± 1.51 ^{ab}	30.33 ± 3.72 ^{ef}
		Drought	4.36 ± 0.02 ^{abcde}	4.27 ± 0.29 ^{abcdef}	-25.78 ± 0.27 ^{ab}	-27.73 ± 0.35 ^{a}	7.35 ± 0.09 ^{ab}	25.66 ± 3.18 ^{cde}
		Variation	-0.94	-1.10	+0.37	-0.08	+0.31	-4.67
	3125	Control	6.37 ± 0.92 ^{f}	5.81 ± 1.37 ^{ef}	-25.59 ± 0.37 ^{bc}	-27.22 ± 0.26 ^{abc}	11.13 ± 0.88 ^{cd}	25.05 ± 2.65 ^{cde}
		Drought	4.06 ± 0.23 ^{abcd}	3.83 ± 0.44 ^{abcde}	-25.19 ± 0.30 ^{bcde}	-26.40 ± 0.34 ^{abcde}	10.18 ± 1.87 ^{bcd}	21.11 ± 1.50 ^{bc}
		Variation	-2.32	-1.98	+0.40	+0.82	-0.94	-3.94
	Total	Control	5.23 ± 0.80	5.25 ± 0.62	-25.64 ± 0.62	-27.33 ± 0.40	10.08 ± 1.58	26.30 ± 4.00
		Drought	3.65 ± 0.49	3.46 ± 0.58	-24.34 ± 1.05	-25.93 ± 1.20	9.54 ± 2.73	20.95 ± 4.95
		Variation	-1.59 **	-1.78 **	+1.30 **	+1.40 **	-0.55	-5.35 **

$\delta^{15}N$ nitrogen isotopic composition (‰); *$\delta^{13}C$* carbon isotopic composition (‰); *N* total nitrogen (mg). Control is fully irrigated; drought is water scarcity. Variation is the difference between control and drought per trait. Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$). ** Significant differences between control and drought stress conditions (One-way ANOVA, $^{**}p \leq 0.01$). Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent replications per accession.

Table 8.2

Sweet potato whole-plant nitrogen isotopic composition, carbon isotope discrimination, total carbon, water use efficiency and total plant biomass variation to control and drought conditions.

			WP $\delta^{15}N$	WP $\Delta^{13}C$	WP TC	WUE	TPB
	1036	Control	4.70 ± 0.17 ^{bcdef}	19.88 ± 0.53 ^{h}	41.16 ± 0.10 ^{b}	3.62 ± 0.88 ^{abc}	377.48 ± 62.94 ^{abcd}
		Drought	3.39 ± 0.00 ^{abc}	17.13 ± 0.00 ^{bc}	41.21 ± 0.00 ^{b}	6.25 ± 1.29 ^{abcd}	231.22 ± 70.36 ^{abc}
		Variation	-1.31	-2.75	+0.05	+2.63	-146.26
	1038	Control	6.11 ± 0.15 ^{f}	18.98 ± 0.31 ^{efgh}	40.26 ± 0.33 ^{a}	9.34 ± 2.24 ^{cde}	1038.55 ± 176.61 ^{e}
		Drought	3.24 ± 0.46 ^{ab}	18.48 ± 0.93 ^{defg}	40.61 ± 0.16 ^{ab}	11.13 ± 3.35 ^{def}	342.44 ± 106.78 ^{abcd}
MAD		Variation	-2.88	-0.50	+0.35	+1.79	-696.11
	2927	Control	5.46 ± 0.99 ^{ef}	18.90 ± 0.15 ^{efgh}	40.93 ± 0.15 ^{ab}	4.82 ± 1.84 ^{abc}	512.77 ± 228.98 ^{cd}
		Drought	3.66 ± 0.15 ^{abcd}	17.48 ± 0.13 ^{bcd}	40.98 ± 0.14 ^{ab}	7.85 ± 3.65 ^{cd}	268.35 ± 125.84 ^{abc}
		Variation	-1.80	-1.41	+0.04	+3.03	-244.42
	3126	Control	4.94 ± 0.81 ^{cdef}	18.04 ± 0.27 ^{cde}	40.60 ± 0.18 ^{ab}	6.94 ± 2.99 ^{bcd}	731.40 ± 367.62 ^{de}
		Drought	3.06 ± 0.36 ^{ab}	15.43 ± 0.19 ^{a}	40.81 ± 0.14 ^{ab}	4.58 ± 0.83 ^{abc}	135.83 ± 25.08 ^{abc}
		Variation	-1.88	-2.61	+0.21	-2.36	-595.57
	2937	Control	4.11 ± 0.33 ^{abcde}	18.91 ± 0.51 ^{efgh}	41.14 ± 0.03 ^{b}	0.77 ± 0.28 ^{a}	106.39 ± 36.79 ^{abc}
		Drought	2.75 ± 0.00 ^{a}	17.52 ± 0.00 ^{bcd}	41.31 ± 0.00 ^{b}	1.09 ± 0.43 ^{a}	22.90 ± 21.01 ^{a}
CAN		Variation	-1.36	-1.38	+0.17	+0.32	-83.50
	2938	Control	5.29 ± 0.37 ^{def}	18.88 ± 0.04 ^{efgh}	40.59 ± 0.04 ^{ab}	1.74 ± 0.83 ^{ab}	523.46 ± 116.47 ^{cd}
		Drought	3.86 ± 1.11 ^{abcde}	16.66 ± 0.02 ^{b}	41.00 ± 0.35 ^{ab}	1.59 ± 1.25 ^{ab}	58.14 ± 28.44 ^{ab}
		Variation	-1.44	-2.22	+0.41	-0.15	-465.33
	3124	Control	5.37 ± 0.85 ^{ef}	19.42 ± 0.24 ^{gh}	40.93 ± 0.64 ^{ab}	5.03 ± 1.55 ^{abc}	507.19 ± 108.09 ^{cd}
		Drought	4.30 ± 0.23 ^{abcde}	19.27 ± 0.20 ^{fgh}	40.78 ± 0.23 ^{ab}	16.45 ± 2.24 ^{f}	449.91 ± 23.62 ^{bcd}
GUI		Variation	-1.07	-0.15	-0.16	+11.42	-57.28
	3125	Control	6.00 ± 0.74 ^{f}	18.91 ± 0.16 ^{efgh}	40.82 ± 0.55 ^{ab}	5.06 ± 1.18 ^{abc}	467.82 ± 173.28 ^{bcd}
		Drought	3.90 ± 0.37 ^{abcde}	18.27 ± 0.34 ^{def}	41.14 ± 0.12 ^{b}	14.24 ± 1.34 ^{ef}	391.80 ± 45.37 ^{abcd}
		Variation	-2.10	-0.64	+0.32	+9.19	-76.03
	Mean	Control	5.25 ± 0.66	18.99 ± 0.52	40.80 ± 0.31	4.66 ± 2.73	533.13 ± 269.00
		Drought	3.52 ± 0.50	17.53 ± 1.18	40.98 ± 0.24	7.90 ± 5.65	237.57 ± 155.60
		Variation	-1.73 **	-1.46 **	+0.18	+3.23 *	-295.56 **

WP $\delta^{15}N$ whole-plant nitrogen isotopic composition (‰); *WP $\Delta^{13}C$* whole-plant carbon isotope discrimination (‰); *WP TC* whole-plant total carbon (%); *WUE* whole-plant water use efficiency (g/L); *TPB* total plant biomass (g). Means not sharing the same letters between columns are significantly different (Tukey HSD, $p \leq 0.05$). *,** Significant differences between control and drought stress conditions (One-way ANOVA, *$p \leq 0.05$; **$p \leq 0.01$). Control is fully irrigated; drought is water scarcity. Variation is the difference between control and drought per trait. Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent replications per accession.

Table 8.3
Pearson correlation coefficients of the analyzed traits of sweet potato (*Ipomoea batatas*) storage roots (tubers) and shoots, in control and drought stress conditions.

Tubers	1	2	3	4	5	6	7	8
1. $\delta^{13}C$	-							
2. $\Delta^{13}C$	**-1.00****	-						
3. WP $\Delta^{13}C$	**-0.98****	**0.98****	-					
4. WP TC	0.10	-0.10	-0.13	-				
5. $\delta^{15}N$	**-0.56****	**0.56****	**0.55****	**-0.48****	-			
6. [N]	0.14	-0.14	-0.19	0.04	0.10	-		
7. WP $\delta^{15}N$	**-0.58****	**0.58****	**0.58****	**-0.46****	**0.89****	0.14	-	
8. WUE	-0.23	0.23	0.19	-0.22	0.11	-0.04	0.03	-
9. TPB	**-0.49****	**0.49****	**0.47****	**-0.58****	**0.71****	0.18	**0.72****	**0.44****

Shoots	1	2	3	4	5	6	7	8
1. $\delta^{13}C$	-							
2. $\Delta^{13}C$	**-0.97****	-						
3. WP $\Delta^{13}C$	**-0.95****	**0.98****	-					
4. WP TC	0.18	-0.15	-0.13	-				
5. $\delta^{15}N$	**-0.57****	**0.53****	**0.55****	**-0.44****	-			
6. [N]	-0.17	0.22	0.25	**-0.46****	**0.56****	-		
7. WP $\delta^{15}N$	**-0.58****	**0.56****	**0.58****	**-0.46****	**0.98****	**0.55****	-	
8. WUE	-0.11	0.15	0.19	-0.22	0.00	0.21	0.03	-
9. TPB	**-0.40****	**0.45****	**0.47****	**-0.58****	**0.68****	**0.63****	**0.72****	**0.44****

$\delta^{13}C$ carbon isotopic composition (‰); *$\Delta^{13}C$* carbon isotope discrimination (‰); *WP $\Delta^{13}C$* whole-plant carbon isotope discrimination (‰); *WP TC* whole-plant total carbon (g/100g); *$\delta^{15}N$* nitrogen isotopic composition (‰); *N* total nitrogen (g, DW); *WP $\delta^{15}N$* whole-plant nitrogen isotopic composition (‰); *WUE* whole-plant water use efficiency (g/L); *TPB* total plant biomass (g, DW); **Correlation is significant at the 0.01 level (2-tailed).

Figure

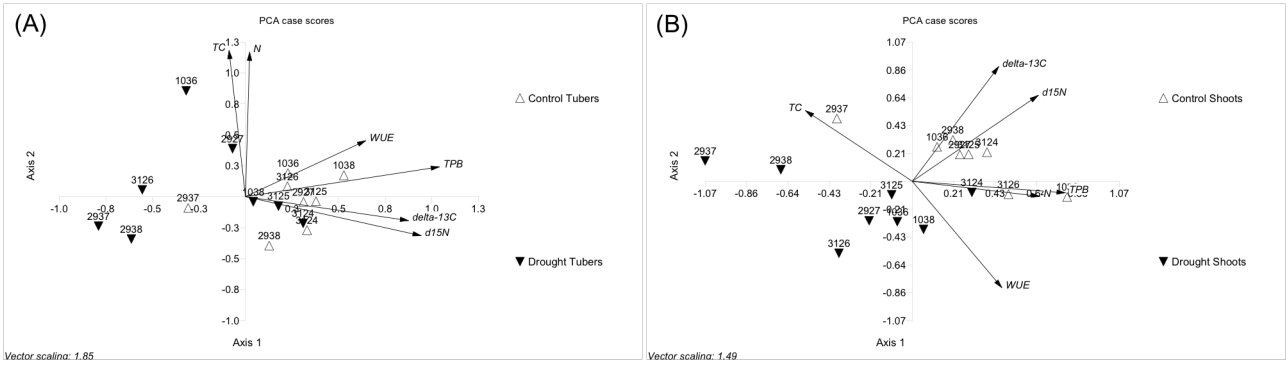

Figure 8.1
Euclidean biplot obtained from principal component analysis (PCA), showing variables contribution to the spatial distribution on sweet potato accessions in two principal components (PC), with storage roots (tubers) (**A**) explaining 68.5% of cumulative variance and shoots (**B**) explaining 76.0% of cumulative variance.

All the variables were standardized by $\log_e$.
Control is fully irrigated; drought is water scarcity.

8.9 Supplementary data

Supplementary material related to this article can be found, in the online version at:
https://doi.org/10.1016/j.jplph.2019.153052

Supplement 1

Designation and origin of sweet potato (*Ipomoea batatas* L.) accessions (acc.) selected for this study.

Acc. ID[a]	Accession local name	Origin
1036	Brasileira	Madeira Island
1038	5 Bicos	Madeira Island
2927	de Flor	Madeira Island
3126	Inglesa	Madeira Island
2937	Roja	Canary Islands – Tenerife
2938	Cubana	Canary Islands – Tenerife
3124	Vermelha	Guinea-Bissau – Bafatá
3125	Branca	Guinea-Bissau – Bafatá

[a] Accession identification number code used by the ISOPlexis Genebank

Supplement 2

Experimental setup

The present study was performed in a split-plot design during a growing season (from August to December 2017), in the ISOPlexis experimental field (32º39' N, 16º55' W, 174 m a.s.l., Funchal, Madeira, Portugal). The experimental field assay occurred simultaneously on two independent plots, one under ordinary open field environment (control) and one under a rain shelter for water deficit conditions (drought). Each accession was planted in three plots (replicates) with eight independent rows per block, 30 plants per accession in total, with 70 x 80 cm in and between the rows. Three vines were also added in each row per plot as blind samples, in both open and shelter environments, with full irrigation during all experimental assay. To introduce variable stress conditions, each plot was submitted to the two different irrigation regimes using a pressure compensating drip irrigation system, with control accessions receiving 1.6 mm/day (based on normal irrigation in Madeiran agricultural practices) and drought accessions receiving 0.9 mm/day (43.7% of water applied to control), three times per week, over three months. During this period, the stressed plots received 54 mm of water from drip irrigation, while control plots received 77 mm of water from drip irrigation and 117.5 mm from rainfall. The drip irrigation on control plots ceased during the raining periods. During full-growth cycle, no pesticides or fertilizers were used and weeds were manually removed as necessary.

CHAPTER 9

Abscisic acid phytohormone estimation in tubers and shoots of *Ipomoea batatas* subjected to long drought stress using competitive immunological assay

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Abscisic acid phytohormone estimation in tubers and shoots of *Ipomoea batatas* subjected to long drought stress using competitive immunological assay

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9.1 Abstract

Sweet potato (Ipomoea batatas L.), typically cultivated in temperate climates under low inputs, is one of the most important crops worldwide. Abscisic acid (ABA) is an important plant stress-induced phytohormone. Hitherto, few works analyzed the ABA function in sweet potato tissue growth. Very scarce information is available concerning the ABA role in sweet potato response to water scarcity conditions. Here, we show the ABA content variation in shoots and tubers of eight sweet potato accessions subjected to drought stress. ABA was also related to other resistance traits, such as chlorophyll content index (CCI), carbon isotopic discrimination ($\Delta^{13}C$), oxalic acid (OA) and water use efficiency (WUE), to assess stress response mechanisms to water deficit between their organs. The most resilient drought-stressed sweet potato plants accumulated ABA-shoot, and significantly decreased the ABA-tuber content. ABA signaling was related to $\Delta^{13}C$ and CCI decrease and WUE increment, as an attempt to cope with water stress by partially closing the stomata. The partial closure of stomata could be in part due to the presence of OA-shoots, known to affect the intensity of the ABA-shoot signal in stomatal closure. Higher CCI content and minimal $\Delta^{13}C$-shoot differences indicated good carboxylation fractionation, with higher $\Delta^{13}C$-tuber content as an indicator of efficient tuber ^{13}C fixation and growth. Our work demonstrated that ABA could be used in conjunction with the other traits studied for the assessment of sweet potato whole-plant responses to environmental stresses, and thus aid the selection of the best drought tolerant genotypes for breeding programs.

Keywords
Abscisic acid; drought; carbon isotope discrimination; ELISA; sweet potato.

Abbreviations
ABA, abscisic acid; Acc., accession; CAN, Canary Islands; CCI, chlorophyll content index; $\delta^{13}C$, carbon isotope composition; $\Delta^{13}C$, carbon isotope discrimination; GUI, Guinea-Bissau; HRP, Horseradish peroxidase; MAD, Madeira Island; OA, oxalic acid; PCA, principal component analysis; WUE, water use efficiency.

9.2 Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam.], after potatoes (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz) root tubers, is one of the most important staple crops in the world (Lebot 2009; FAOSTAT Statistical Database 2018). Sweet potato is an important food source supply in tropical and developing countries owing to the storage of 80–90% carbohydrates in root dry matter content (Lebot 2009). Some countries also use the sweet potato leaves as a vegetable source, providing high content in protein, soluble dietary fiber, minerals (iron), polyphenols and vitamins (Bradbury and Holloway 1988, Ishida et al. 2000).

The major sweet potato production occurs in Asia, with 60.7 Mt in 2018, representing 66% of worldwide production (FAOSTAT Statistical Database 2018). Its production is usually done in temperate climates, under low input conditions. With the characteristic low plant growth habit and extensive root system, the sweet potato can be moderately tolerant to water scarcity (Smittle et al. 1990, Ekanayake and Collins 2004, Motsa et al. 2015a,b). The exposure to an extended period of abiotic stress leads to plant physiological modifications, changing the crop quality and biochemical composition to cope and survive stress (Wang and Frei 2011).

Abscisic acid (ABA) is an important isoprenoid stress-induced phytohormone present in sweet potato (Nakatani and Komeichi 1991, Nagata and Saitou 2009, Wani et al. 2016, Lau et al. 2018). This isoprenoid plays a pivotal role in numerous plant biochemical and physiological processes, including stomatal closure, lipid synthesis, protein storage and starch accumulation, related to development, growth and signaling pathways (Mengel et al. 2001, Firon et al. 2009, Danquah et al. 2013, Wani et al. 2016, Saddhe et al. 2017, Vishwakarma et al. 2017). Proline and ethylene synthesis, which have a protective function under both water and saline stress, are also stimulated by ABA (Mengel et al. 2001). ABA is considered one of the most effective plant hormones due to its quick synthesis under abiotic stresses, such as drought, salinity, temperature, and nutrient imbalance (Mengel et al. 2001, Firon et al. 2009, Osakabe et al. 2014, Sah et al. 2016, Salehi-Lisar and Bakhshayeshan-Agdam 2016). Plants also abscises organs, such as leaf abscission, to promote senescence as stress response (Mengel et al. 2001, Sah et al. 2016). ABA can be quantitated by the Enzyme-Linked Immunosorbent Assay (ELISA), a sensitive method that can detect low concentrations of this phytohormone in plant tissues. Currently, it is one of the most affordable techniques that allow fast detection of ABA without the need for complex purification steps and uses a combination of the specificity of antigen–antibody reaction with the same sensitivity of enzymatic assays (Huang et al. 2014).

Under a tenacious dry environment, root ABA signalizes the plant shoots that they are facing stressful conditions around the roots, leading to stomatal closure to avoid water loss (Wani et al. 2016). Drought could induce the stomatal closure through a root-to-shoot signaling with an ABA synthesis occurring mainly in the chloroplasts (Mengel et al. 2001, Osakabe et al. 2014, Salehi-Lisar and Bakhshayeshan-Agdam 2016). Other works also indicate that the leaves are the predominant location for ABA biosynthesis during drought stress, and also that the ABA from leaves has a greater effect over root development (McAdam et al. 2016). The cellular dehydration that triggers ABA biosynthesis in the chloroplast mesophyll tissues involves various enzymes and the utilization of β-carotene (Mengel et al. 2001, Wani et al. 2016, Vishwakarma et al. 2017). The stomatal responses are closely related to soil water potential, with its closure inhibiting transpiration and increasing the water flow in the plant through hydraulic conductivity (Mengel et al. 2001, Ma and Qin 2014, Osakabe et al. 2014, Salehi-Lisar and Bakhshayeshan-Agdam 2016). The plasma membrane and tonoplast use the ion and water transport systems to control turgor pressure changes in the guard cell. The guard cells detect the increase of ABA levels, leading to a subsequent reduction of turgor and volume to avoid water loss, and trigger the stomatal closure (Ma and Qin 2014, Osakabe et al. 2014).

The atmospheric CO_2 also acts as a signaling molecule in stomatal responses, whereas increased CO_2 concentrations in leaves induce the stomatal closure, leading to a reduction in the photosynthesis rate in leaves, and affecting the water use efficiency (WUE; Salehi-Lisar and Bakhshayeshan-Agdam 2016, Osakabe et al. 2014). WUE reflects how plants manage the water use for vital activities and plant production during scarcity conditions, with the most tolerant ones usually displaying higher WUE (Mengel et al. 2001, Ganança et al. 2018, Gouveia et al. 2019). The chlorophyll measured in the full-grown leaves could be associated with the rate of photosynthesis, which is one of the most common parameters used as indicator of plant performance under drought stress (Shao et al. 2015, Salehi-Lisar and Bakhshayeshan-Agdam 2016, Gouveia et al. 2020). The carbon isotopic discrimination ($\Delta^{13}C$), that represents the photosynthetic depletion of ^{13}C in field-grown plants, is linked with stomatal aperture and is commonly used in C3 plants for the assessment of drought resilience (Farquhar et al. 1989, Zhang et al. 2015, Gouveia et al. 2019). Oxalic acid (OA) is an ethanedioic acid that inhibits ABA-induced stomatal closure in *Arabidopsis thaliana* plants (Guimarães and Stotz 2004). OA is a free acid that can form an equilibrium between soluble (potassium or sodium oxalate) or insoluble

(calcium oxalate) salts, which variation plays an important role in the plant ion balance and osmoregulation under drought conditions (Franceschi and Horner 1980, Gouveia et al. 2020).

The CCI, $\Delta^{13}C$, OA and WUE traits were previously used in the evaluation of the crop resilience to water scarcity environments (Gouveia et al. 2019, Gouveia et al. 2020). To date, a few works reported the ABA effects on sweet potato cultivars, including tuber yield of two accessions grown in fully irrigated pots (Nakatani and Komeichi 1991), the inhibitory growth effects of exogenous application of ABA to in vitro plantlets of one accession (Jarret and Gawel 1991), sink activity through leaf-petiole cuttings from one accession grown in a growth chamber and treated with ABA solution (Nagata and Saitou 2009), and growth of in vitro shoots of two accessions exposed to osmotic pressure induced by polyethylene glycol (Lau et al. 2018). These experiments revealed that ABA was one of the factors that regulated the sweet potato sink activity and strength. The higher the sink activity, the higher sugar uptake and metabolic activity observed in the growing tuberous roots (Nagata and Saitou 2009). The thickening of the tuberous root diameter by cell division activity in the secondary cambium of sweet potato was mainly promoted by ABA located around the primary cambium and meristem in the xylem (Nagata and Saitou 2009, Ravi and Saravanan 2012). The heaviest tubers that show the highest ABA content under control conditions had a decreased tissue growth under osmotic pressure (Nakatani and Komeichi 1991, Nagata and Saitou 2009, Lau et al. 2018). These experiments were performed on a model scale to assess ABA importance in the growth of sweet potato plantlets without involving the real field conditions, and determining ABA throughout the whole plant subjected to water stress. Therefore, our study aimed to better elucidate the ABA involvement in the responses of field full-grown sweet potatoes subjected to long water scarcity conditions. We used a set of eight accessions from diverse geographical provenances to seek if drought leads to endogenous ABA accumulation due to stress as a physiological response to drought stress conditions. The specific goals were: (1) determining the ABA content in shoots and tubers of field-grown sweet potato plants subjected to long-term drought stress, and (2) relating ABA with other resistance traits (CCI, $\Delta^{13}C$, OA and WUE) evolved in crop tolerance to better understand the sweet potato responses to water deficit.

9.3 Materials and Methods

9.3.1 Sweet potato accessions

Eight accessions of sweet potato [*Ipomoea batatas* (L.) Lam.] from Madeira Island (MAD), Canary Islands (CAN) and Guinea-Bissau (GUI) (Table 9.1) were submitted to water scarcity conditions in the present study.

9.3.2 Experimental field assay

Five months of field trials were established in 2017 at the ISOPlexis experimental field (32º39' N, 16º55' W, at Funchal, Madeira, Portugal), using a split-plot design. In the main plot, the sweet potato accessions were grown in two independent blocks, one under regular open field conditions (control) and the other under an open rain shelter (experimental variant). The shelter aimed to avoid rain feed and ensure the water deficit conditions. Each accession was planted in three subplots (replicates): eight independent rows per block, 30 vine cuttings per accession in total, with 70 x 80 cm in and between the rows. This makes five vine cuttings per accession, added in each row per subplot, that were fully irrigated in both open and shelter environments until the beginning of the third month of the trial. Three vines were also added as test samples, in both open and shelter blocks, exempt from water stress throughout the trial duration by keeping full irrigation. Two distinct water regimes were then applied, through drip irrigation system, with 1.6 mm for control and 0.9 mm for water deficit variants, per subplot, three times a week during the remaining 3 months. During this period, control block received approximately 77 mm of water, while the drought block received approximately 54 mm. Controls were also feed with 117.5 mm of rainfall during this period. During rain periods, irrigation was suspended on control block. Both control and drought variants were also assessed periodically for the photosynthetic active radiation (PAR, 400-700 nm) with a ceptometer (AccuPAR LP-80),

volume water content of soil (VWC_s) with a soil moisture sensor (WaterScout SM10), air temperature (T_a) and relative air humidity (RH_a) with a data logger (Testo 174H). During the assay, we registered a 24.6% PAR decrease under the rain shelter relative to open field environment, on average, with 1514.5 µmol m^{-2} s^{-1} for control and 1142.0 µmol m^{-2} s^{-1} for drought. The VWC_s measured at 10 cm of depth of homogenized field soil shown on average 12.8% VWC_s for control, representing 35% of field capacity, and 3.5% VWC_s for drought, representing equal or less than 10% of field capacity. During the assay, an average of 19.46ºC T_a and 68.07% RH_a were observed for control; an average 22.25ºC T_a and 66.40% RH_a were registered for drought. Throughout the entire agronomic trial, neither fertilizers nor pesticides were applied, and manual weeding was performed regularly.

9.3.3 Preparation of sweet potatoes whole-plant flour samples

At the end of the agronomic trial, 384 root tubers and shoots (stem, stalk and leaves) samples from control and drought plots were collected. All samples were washed, weighed (Sartorius Basic BA2100S), chopped on a mandolin cutter (2 to 3 mm thick), subsequently placed in an air oven to dehydrate until constant weight during approximately 48h at 65ºC (Memmert UF260), and finally ground into flour (IKA-Werke M20). The flour was placed in bags (Termofilm PA/PE), vacuum sealed (Audionvac VMS153) and stored at -35ºC (Liebherr ProfiLine GGPV6570) until analysis.

9.3.4 Quantitative detection of abscisic acid in plant tissues

ABA, a weak acid, is a sesquiterpene with an α,β-unsaturated ketone in the ring and a conjugated diene side-chain (Huang et al. 2014). Its content was determined in the root tuber and shoot flours by the ELISA technique, using the ABA ELISA Kit (MBS 282218, 96 tests, MyBioSource Inc.) and a microplate reader (Tecan Sunrise Remote A-5082; software Magellan™ V7.1, Tecan). Flour samples were previously extracted with 0.940 µl 1X PBS, and centrifuged at 9838 *g* during 5 min, with supernatant collected for analysis. Lyophilized ABA standard was diluted (from 1.56 to 100 ng ml^{-1}) and used as calibration standards. Specifically, 50 µl of standards and samples were individually added to the microplate wells pre-coated with an antibody specific to ABA, followed by 50 µl Horseradish Peroxidase (HRP)-conjugated ABA, and incubated for 1 h at 37ºC, protected from light. The competitive inhibition reaction occurs between HRP-labelled ABA and unlabelled ABA with the antibody. One hundred microliter of substrate solution was added to the wells protected from light and incubated for 20 min at 37ºC. The reaction was terminated by adding 50 µl of stop solution. The obtained data at 450 nm, corrected at 620 nm, were subjected to logarithmic transformations. The analyses were performed in triplicate and the values were expressed in ng g^{-1} of dry flour.

The method accuracy was validated through the recovery index percentage of ABA (Van Reeuwijk et al. 1998). Three distinct flour samples were spiked with 25 ng ml^{-1}. The samples and the spikes were analyzed five times, showing 102% of recovery index, a high sensitivity and excellent specificity for ABA detection and quantification in sweet potato tissues.

9.3.5 Carbon isotope discrimination

The sweet potato root tuber and shoot flours were vacuum packaged and sent to the Natural Resources Analytical Laboratory at the University of Alberta (Edmonton, Canada) for carbon isotope composition ($\delta^{13}C$) analysis (Gouveia et al. 2019). The $\delta^{13}C$ was determined by the micro-chemical AOAC 972.43:2000 method, using a Delta V Advantage Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Finnigan Corp CF-IRMS). The $\delta^{13}C$ was converted into $\Delta^{13}C$, from the obtained carbon isotope composition of plant material ($\delta^{13}C_p$) and the source of atmospheric CO_2 carbon ($\delta^{13}C_a$ = -8‰), according to Farquhar et al. (1989) equation:

$$\Delta^{13}C\ (‰) = (\delta^{13}C_a - \delta^{13}C_p) / (1 + \delta^{13}C_p)$$

The analysis was made in triplicate with results presented in ‰ units of dry flour.

9.3.6 Chlorophyll content index

The relative chlorophyll content of the sweet potato fresh leaves was measured and expressed as the CCI. We employed a non-destructive measurement method using a chlorophyll content meter (Opti-Sciences CCM-200 PLUS). The CCI values were obtained through the absorbance in transmittance mode, at 653 and 931 nm, whose values were proportional to the amount of chlorophyll in the tissue. Three measurements were performed in the morning on the adaxial leaf surface, avoiding the branching veins. An average CCI value was recorded for each replicate.

9.3.7 Oxalic acid

OA was quantitated in flour samples from root tubers and shoots (Gouveia et al. 2020). Exactly 0.4 g of flour was extracted with hydrochloric acid (HCl, 6.0 M) to allow the reduction of oxalic into glyoxylic acid, with a further reduction into glycolic acid. A potassium permanganate solution ($KMnO_4$, 0.05 M) was used to precipitate and titrate the sample extracts for OA quantitation. We used Dye (1956) calculation for the total OA quantification. The analyses were made in triplicate, with values presented as mg $100g^{-1}$ of dry flour.

9.3.8 Water Use Efficiency

WUE was calculated as the ratio of the whole-plant dehydrated biomass to total water used per subplot and expressed in g l^{-1} (Ganança et al. 2018).

9.3.9 Statistical methods

The results were expressed on a dry weight basis, as the main average of sweet potato root tubers and shoots, for control vs drought plots. IBM SPSS Statistics V24 for Mac was used for one-way ANOVA, Tukey HSD test and Pearson correlations. The statistically significant differences were expressed with *P*-value lower than 0.05. The MVSP V3.1 for Windows was used to perform the principal component analysis (PCA).

9.4 Results

9.4.1 ABA variation in root tubers and shoots during drought

A wide range of strategies of ABA synthesis and accumulation in both organs was observed among the eight sweet potato accessions. The data of ABA variation in root tubers and shoots for both control and drought experimental variants are shown in Table 9.2.

Overall, the ABA mean content in the shoots was almost 5-fold higher than in the tubers for control and reached almost 11 folds under drought. At the same time, the ABA mean content decreased by 37% in the tubers, from 45 ng g^{-1} to 28 ng g^{-1}, meanwhile it increased by 41% in the shoots, from 215 to 304 ng g^{-1}, under water scarcity.

The variation of ABA-shoot content submitted to drought ranged from 175.1 (acc. 3125) to 483.5 ng g^{-1} (acc. 1036). The variations of ABA-tuber content were between 11.5 (acc. 1038) and 50.5 ng g^{-1} (acc. 3125). The highest values of ABA content were recorded in the shoots, but the highest variations were observed in the tubers, with 4.4 folds (drought) and 16.9 folds (control), respectively.

The analysis of ABA variation in tubers shows that acc. 3125 holds a significant highest content under both control and stress variants, decreasing during stress from 98 to 51 ng g^{-1} (-48%). However, the highest decrease was observed in the acc. 1038, where ABA tuber content changes from 59 to 12 ng g^{-1} (-80%) under stress. That is, under stress, the ABA content varied according to the following series: acc. 3125 (-48 ng g^{-1}), 1038 (-47 ng g^{-1}), 2938 (-29 ng g^{-1}), 1036 (-12 ng g^{-1}), 2937 (-9 ng g^{-1}), 3126 (-9 ng g^{-1}), with acc. 2927 (+8 ng g^{-1}) and 3124 (+15 ng g^{-1}) as exceptions. These last two accessions had the lowest ABA-tuber content in control conditions, but were the only ones that recorded an ABA increase when submitted to drought, from 6 to 14 ng g^{-1} (133%), and 7 to 22 ng g^{-1} (214%), respectively.

Still, in the shoots, the acc. 1036 had significantly the highest variation of ABA content between control and stress conditions, increasing from 298 to 484 ng g^{-1} (+62%). Meanwhile, the ABA-shoot suffers the lowest variation in acc. 3124, only ranging from 288 to 295 ng g^{-1} (+2%). Contrariwise, acc. 3125 showed

the lowest ABA-shoot content under stress, with 175 ng g^{-1}. The ABA variation in shoots, due to stress, increased according to the following series: acc. 3124 (+7.8 ng g^{-1}), 3125 (+42.6 ng g^{-1}), 2937 (+46.8 ng g^{-1}), 1038 (+59.5 ng g^{-1}), 2937 (+71.9 ng g^{-1}), 3126 (+145.0 ng g^{-1}), 2938 (+153.6 ng g^{-1}), and 1036 (+185.7 ng g^{-1}).

9.4.2 $\Delta^{13}C$ and OA in root tubers and shoots during drought

Table 9.2 shows the variation of carbon isotope discrimination ($\Delta^{13}C$) and OA content in the control and experimental (stress) variants.

Water scarcity slightly decreased the $\Delta^{13}C$ content in both organs. On average, drought decreased $\Delta^{13}C$-shoot from 20 to 18‰ (-10.0%), while the $\Delta^{13}C$-tuber decreased from 18 to 17‰ (-6%). The acc. 3124 was the exception, by slightly increasing the $\Delta^{13}C$-shoot during drought, but remaining at 20‰ under both experimental conditions. However, acc. 1036 had the highest $\Delta^{13}C$-tuber content but also exhibited the highest loss due to drought, ranging from 19 to 17‰ (-11%). Conversely, both the acc. 1038 and 3124 showed the lowest difference and significantly higher $\Delta^{13}C$ content in both organs under both experimental conditions. The acc. 3126 showed the lowest $\Delta^{13}C$ content in both organs under drought stress, displaying 15‰ for $\Delta^{13}C$-tuber and 16‰ for $\Delta^{13}C$-shoot.

The OA content in shoots was about threefold higher than in the tubers, under both experimental conditions. Also, the OA variation on average is higher in tubers than in the shoots. On average, the OA in tubers and shoots increased, respectively, from 11 to 14 mg $100g^{-1}$ (+27%), and from 32 to 37 mg $100g^{-1}$ (+16%), between control and drought stress. However, in the acc. 1038, the OA content decreased to the same extend in both organs, clocking the same response to drought at 4 mg $100g^{-1}$. Inversely, acc. 1036, 2927, 3126, and 2937 increased the OA content in both organs under both conditions. The acc. 1036 showed significantly higher OA content, reaching 27 mg $100g^{-1}$ for OA-tuber and 81 mg $100g^{-1}$ for OA-shoot under water scarcity conditions.

9.4.3 WUE and CCI content variation to drought

Data regarding the whole-plant WUE and the CCI from shoots are presented in Table 9.2. On average, drought led to a 60% increase of WUE, from 5 to 8 g l^{-1}. The acc. 1038 showed significantly higher WUE (9.34 g l^{-1}) under control conditions. The acc. 3124 exhibited the highest WUE range, from 5 to 17 g l^{-1} (+240%) under drought. Contrariwise, acc. 2937 had the lowest WUE under both experimental environments, ranging between 0.77 and 1.09 g l^{-1} (+42%). Meanwhile, acc. 3126 and 2938 were the only ones that decreased WUE under drought conditions.

Drought decreased the CCI by 3% on average, from 30 to 29. The acc. 1036 and 3126 were the only ones that increased the CCI during drought, from 19 to 31 (+63%) and 26 to 32 (+23%), respectively. However, acc. 2927, 3124 and 3125 decreased the CCI under drought, but they managed to keep the highest chlorophyll content under both experimental conditions. Accession 1038 recorded the highest CCI decrease under drought, from 38 to 29 (-24%), meanwhile acc. 2937 showed the smallest CCI among all acc., ranging from 21 to 18 (-14%).

9.4.4 Variance and parameters associations

Significant differences ($P \leq 0.01$) were recorded between the eight sweet potato accessions (cases) by the one-Way ANOVA and Tukey HSD multiple comparisons between control and drought environments for ABA, CCI, $\Delta^{13}C$, OA and WUE (Table 9.2). Nine significant correlations ($P \leq 0.05$) were detected among the five variables in the study, using the Pearson correlation coefficient (Table 9.3). One strong significant correlation was observed between $\Delta^{13}C$-shoot and $\Delta^{13}C$-tuber ($r = 0.92$). Modest correlations were registered between ABA-shoot with $\Delta^{13}C$-shoot ($r = -0.45$), $\Delta^{13}C$-tuber ($r = -0.40$), OA-shoot ($r = 0.36$) and with ABA-tuber ($r = -0.30$). Other moderate correlations between OA-shoot with OA-tuber ($r = 0.39$) and WUE ($r = -$

0.33) were also detected. Similarly, the OA-tuber with CCI (r = 0.32) and with Δ^{13}C-shoot (r = -0.32) were moderately correlated.

The data from cases (samples) and variables (traits) were transformed into linearly uncorrelated variables through a PCA (Fig. 9.1). The PCA analysis explained 84.4% of total cumulative variance along with four principal components, i.e. axis. The first two axis explained 54.6% of the cumulative variance. The axis 1 had 34.1% of variance with eigenvalues of 2.7, while the axis 2 had 20.5% of variance with eigenvalues of 1.6. The ABA-shoot, Δ^{13}C-shoot and Δ^{13}C-tuber were the main variables that show a strong correlation relative to axis 1; meanwhile, the CCI and WUE variables were highly correlated with axis 2. The accessions spatially distributed near to the end of the main variable vectors, as referred above for both axis, are the ones with the highest variability. When the main variable vectors are in opposed directions, it can show a reverse relation between them. For example, the drought-stressed acc. 1036 and 3126 were situated at the end of the ABA-shoot variable vector, which was in the opposed direction relative to the Δ^{13}C-shoot and Δ^{13}C-tuber vectors. It showed that their spatial distribution associated them, among all acc., as the ones with the highest ABA-shoot, and with the lowest Δ^{13}C-shoot and Δ^{13}C-tuber for drought conditions. As both accessions showed the highest spatial distance between the control and drought variants, they apparently also denoted the highest sensitivity to drought. On the other hand, acc. 3124, 3125 and 1038 shared practically the same spatial distribution between control and drought environments, displaying a more efficient response to drought.

9.5 Discussion

9.5.1 ABA-shoot and ABA-tuber interaction at drought

ABA naturally accumulates during plant growth and its accumulation is enhanced under stress conditions (Nakatani and Komeichi 1991). When plants experience water scarcity, they respond with ABA-shoot accumulation by initiating an adaptive response through the regulation of the plant water status as a strategy to survive under the hostile conditions (McAdam et al. 2016). In fact, we observed an ABA-shoot accumulation in all accessions tested in our study.

Under control conditions, the content of ABA-shoot was approximately fivefold higher than ABA-tuber, which agrees with Li and Jia (2014) findings. These authors argued that under stress-free conditions, plant leaves usually show a much higher ABA content than the roots and stems, due to a higher ABA content located in the chloroplasts (up to 68% of total ABA). The 5-fold difference registered between the shoot and tuber organs increased up to 11-fold under drought stress. This increase could be attributed to the general ABA accumulation in sweet potato shoots in detriment to the decrease of ABA in tubers as a response to drought. In case the underground organs are unable to synthesize ABA during drought conditions, either because of biochemical inability or collapse of carotenoid precursor reserves (β-carotene), it was found that plants could have a normal increase in foliar ABA level, with normal stomatal responses to drought (Danquah et al. 2013, McAdam et al. 2016). The significant negative correlation between ABA in both organs allow us to corroborate the presence of a root-to-shoot ABA signaling path in sweet potato during water privation (Mengel et al. 2001, Osakabe et al. 2014, Salehi-Lisar and Bakhshayeshan-Agdam 2016). The drought is signalized to the shoots, where the signal is amplified in the leaf chloroplasts to accumulate ABA (Mengel et al. 2001, Osakabe et al. 2014, McAdam et al. 2016, Salehi-Lisar and Bakhshayeshan-Agdam 2016, Wani et al. 2016,). However, the increase of the ABA-shoot biosynthesis can lead to a higher ABA catabolism due to the depletion of the ABA xanthophyll precursors. Drought may compromise the delivery of ABA precursors from the shoots to the roots, which could be one of the reasons for the decrease of ABA-tubers content (Li and Jia 2014, McAdam et al. 2016). We reported that only two accessions (2927 and 3124) increased the ABA-tuber during stress. This suggests that both exhibit a dynamic equilibrium between ABA biosynthesis and catabolism, which remains in agreement with the conclusions of Li and Jia (2014). Taking into consideration reports of Duman (2012) and Tuberosa (2012), we hypothesize that the ABA-tuber increase in acc. 2927 and 3124 could have facilitated the plant water uptake into the roots during drought, which also led to a better diffusion of nutrients taken from

the soil into the plant. Such a pattern of response to drought was not present in the remaining accessions which show a decreased ABA-tuber content. However, the increasing levels of ABA in maize roots during drought conditions stimulated root elongation and hence facilitated the pursuit of water and nutrients in surrounding soil environments (Sah et al. 2016). However, our observations do not support Sah et al. (2016) findings in maize plants, since the reduction of sweet potato total biomass appeared to be a way for them to cope with water stress (Gouveia et al. 2019). Likewise, Lau et al. (2018) observed a downregulation of tissue growth with higher ABA accumulation in in vitro sweet potato plantlets. Jarret and Garrel (1991) also demonstrated that sweet potato plantlets exposed to four concentrations of ABA, ranging from 0.01 to 10 mg l^{-1} (i.e. 10-10 000 ng g^{-1}), had lag development of root and axillary buds from nodal segments. Considering that we dealt with fully grown tubers and shoots that were subjected to a long water scarcity period, the ABA values naturally varied among the accessions and their organs. Although, the highest ABA values obtained for the shoots (484 ng g^{-1} for acc. 1036) and for tubers (51 ng g^{-1} for acc. 3125) under drought were not significantly correlated with the total plant biomass loss (data not shown). The ABA accumulation in response to water scarcity stress ought to be considered merely a conjecture of the mechanism of plants' resilience to water stress. We incline to hypothesize that ABA was predominantly involved in the regulation of plant water status in association with the other traits investigated in this study rather than playing a direct role in the downregulation of tissue growth during drought. However, our results also show that ABA evolvement depends on accessions skills, because they demonstrated to have differentiated behavior under drought.

9.5.2 ABA variation and its relationship with other drought tolerance traits

ABA modulates the plant responses including the regulation of stomatal opening, growth and development, and contributes to the stress signal transduction pathways during drought (Ramakrishna and Ravishankar 2014). Under normal conditions of ample water availability, stable ABA content delays leaf senescence and allows cumulative photosynthesis and transpiration processes over the crop cycle (Tardieu and Davies 1992, Tuberosa 2012, Osakabe et al. 2014). Open stomata allows higher photosynthesis activity and higher CCI content, which improves the carboxylation fractionation due to an equilibrate CO_2 diffusion in leaf chloroplasts, with $\Delta^{13}C$ values reaching near 31‰ (or $\delta^{13}C$= -38‰; O'Leary 1993, Osakabe et al. 2014, Shao et al. 2015). The higher the plant transpiration, the greater is the water potential gradient between the root tubers and shoot cells. The scarce water availability in the roots reduces the water status in shoots, i.e. the ABA increased content in the leaves induces stomata closure to reduce transpiration as a water-conserving mechanism (Tardieu and Davies 1992, Osakabe et al. 2014). Reduced transpiration rates compromise nutrient transport from the roots to the shoots, while nutrient stress could be another possible factor stimulating the accumulation of ABA (Firon et al. 2009, Duman 2012). Lower transpiration reduces water loss and leads to WUE increase (Black et al. 2015). Nonetheless, the stomatal closure restricts the photosynthetic activity and constrains the leaf CO_2 uptake, which leads to a decrease of carboxylation fractionation and drives to $\Delta^{13}C$ values near to 4‰ (or $\delta^{13}C$= -12‰; O'Leary, 1993).

We observed that the ABA-shoot increase was significantly correlated with $\Delta^{13}C$-shoot decrease, and both factors were connected to stomatal closure. The available data of $\Delta^{13}C$, CCI, and WUE point to a partial closure of the stomata in sweet potato as a response to drought. Lau et al. (2018) also showed that in vitro sweet potato plantlets registered an ABA level increment during drought, leading to stomatal closure to avoid water loss by transpiration. The CCI decrease could indicate that drought slightly restricted the sweet potato photosynthesis activity, while the $\Delta^{13}C$ decrease indicates that the carboxylation fractionation was lowered due to lower leaf CO_2 availability (Farquhar et al. 1989, O'Leary 1993, Shao et al. 2015, Zhang et al. 2015, Gouveia et al. 2019). Both organs decreased $\Delta^{13}C$ due to drought, with the amount of $\Delta^{13}C$-shoot slightly greater than $\Delta^{13}C$-tuber in both control and drought conditions, which was also observed by Zhang et al. (2015). This indicates that the $\Delta^{13}C$-tuber still had a greater ^{13}C fixation relatively to $\Delta^{13}C$-shoot as a result of the transport of photo-assimilates from shoots to tubers, which improved plant growth during drought stress (Wegener et al. 2015, Zhang et al. 2015).

The partial closure of stomata could be also connected to the increment of oxalic acid, affecting the ABA signal to induce stomatal closure. With the increase of oxalic acid content, the shoot accumulates osmotically active molecules that induce the stomatal opening instead of closing it as signaled by ABA (Guimarães and Stotz 2004). We found that OA-shoot increase during drought, which was significantly correlated with ABA-shoot accumulation. It may suggest that the ABA capacity to induce stomata closure was affected. The biosynthesis of OA organic osmolytes is the key for plant resistance to drought, helping to resist prolonged harsh water scarcity and permitting a better recovery upon rehydration (Tuberosa 2012). Plant accumulation of OA occurs from the glycolate oxidized in glyoxylate derived from photosynthesis activity, which is then oxidized into oxalic acid (Igamberdiev and Eprintsev 2016). However, drought slightly downregulated photosynthesis due to the CCI decrease, perchance to protect the chloroplasts from photoinhibition and subsequent oxidative damage (Prasad et al. 2008, van Heerdena and Laurie 2008, Osakabe et al. 2014). Nonetheless, the partial stomatal closure still allowed for reasonably high CCI and organic osmolytes synthesis and permitted the increase of WUE during drought to minimize biomass loss.

The plants that were the most resilient to drought had greater ABA-shoot production, higher photosynthesis activity, higher chlorophyll content and increased WUE (Tardieu and Davies 1992, Tuberosa 2012, Black et al. 2015, Lau et al. 2018). Taking into consideration all studied traits, the acc. 3124 could be considered the most resilient to drought, followed by accessions 3125 and 1038. The acc. 3124 managed to retain high CCI content and a good carboxylation fractionation resulting from an equilibrate CO_2 diffusion in leaf chloroplasts, with minimal $\Delta^{13}C$ difference. This may point to a good photosynthesis rate under both experimental conditions. This acc. also had the highest $\Delta^{13}C$-tuber content, an indicator of efficient tuber ^{13}C fixation and growth during drought. It maintained a balanced OA equilibrium in both organs and showed the best WUE under both experimental conditions. Finally, a high ABA production sustains in both organs in response to stress, allowing for a better osmotic and nutrient equilibrium.

9.6 Conclusions

Using multivariate analysis, analysis of variance and correlations, it was shown how the differences of ABA content in eight sweet potato accessions grown under water scarcity were related to the CCI, $\Delta^{13}C$, OA and WUE. Drought triggered ABA-shoots biosynthesis, while it significantly decreased the ABA-tubers content. The ABA-shoot accumulation was correlated with $\Delta^{13}C$-shoot and OA decrease. The ABA-shoot increase seemed to be a root-to-shoot ABA signaling attempt to cope with water stress by stomatal closure, which was directly related to the $\Delta^{13}C$ and CCI decrease, and higher WUE. The presence of OA-shoot may have affected the intensity of the ABA-shoot signal in stomatal closure, contributing to only a partial stomatal closure during the harsh water scarcity conditions. These combined factors appeared to be suitable tools to identify sweet potato accessions with higher resilience to drought. Among all accessions in the study, acc. 3124 exhibited the best trait combination in response to water scarcity. Therefore, it should be considered as a potential candidate for sweet potato drought tolerance improvement programs, that can be used in culture adaptation to climate changes.

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9.8 Author contributions

C.G. participated to the drought assay and samples preparation, performed the ABA and oxalic analysis, interpreted and summarized all data generated from those experiments, and wrote the manuscript. J.G. quantified the WUE and helped C.G. in CCI. J.S. coordinated the $\delta^{13}C$ analysis. V.L. and M.C. coordinated the work and revised the manuscript.

9.9 Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 9.1

Identification code, variety name and origin of eight sweet potato (*Ipomoea batatas* L.) accessions subjected to water scarcity conditions. Each accession has an identification number code used by the ISOPlexis Genebank.

Accession ID[a]	Variety local name	Origin
1036	Brasileira	Madeira Island (MAD)
1038	5 Bicos	Madeira Island (MAD)
2927	de Flor	Madeira Island (MAD)
3126	Inglesa	Madeira Island (MAD)
2937	Roja	Canary Islands – Tenerife (CAN)
2938	Cubana	Canary Islands – Tenerife (CAN)
3124	Vermelha	Guinea-Bissau – Bafatá (GUI)
3125	Branca	Guinea-Bissau – Bafatá (GUI)

Table 9.2

Abscisic acid (ABA), carbon isotope discrimination ($\Delta^{13}C$), oxalic acid (OA), whole-plant water use efficiency (WUE) and chlorophyll content index of shoots (CCI), in control and drought sweet potato (*Ipomoea batatas*) tubers and shoots organs from Madeira Island (MAD), Canary Islands (CAN) and Guinea-Bissau (GUI). Control is fully irrigated, drought is water scarcity. Variation is the difference between control and drought per trait. Means not sharing the same letters between columns are significantly different (Tukey HSD, $P \leq 0.05$). †† Significant differences between control and drought stress conditions (one-way ANOVA, $^{\dagger\dagger}P \leq 0.01$). Data are expressed in dry weight basis (DW), and represents the mean ± SD of three independent replications per accession.

			ABA content (ng g^{-1})		$\Delta^{13}C$ content (‰)		OA content (mg $100g^{-1}$)		WUE †† (g l^{-1})	CCI ††
			Tuber ††	Shoot ††	Tuber ††	Shoot ††	Tuber ††	Shoot ††		
MAD	1036	Control	38.8 ± 3.6^{a}	297.8 ± 51.5^{bcd}	19.3 ± 0.7^{h}	20.5 ± 0.4^{g}	13.8 ± 0.3^{c}	47.0 ± 7.1^{hij}	3.6 ± 0.9^{abc}	18.5 ± 2.4^{ab}
		Drought	26.9 ± 0.0^{a}	483.5 ± 14.5^{d}	16.6 ± 0.0^{bcd}	17.7 ± 0.0^{b}	26.8 ± 4.8^{d}	80.7 ± 8.5^{k}	6.3 ± 1.3^{abcd}	31.3 ± 7.2^{abcde}
		Variation	-11.9	+185.7	-2.7	-2.8	+13.0	+33.8	+2.6	+12.9
	1038	Control	58.8 ± 38.8^{ab}	231.2 ± 30.2^{ab}	18.1 ± 0.4^{fg}	19.8 ± 0.3^{efg}	7.4 ± 1.5^{abc}	7.5 ± 1.2^{ab}	9.3 ± 2.2^{cde}	37.9 ± 5.3^{de}
		Drought	11.5 ± 9.4^{a}	290.6 ± 68.2^{abc}	17.6 ± 0.9^{defg}	19.3 ± 1.0^{cdefg}	4.4 ± 1.4^{a}	4.4 ± 1.2^{a}	11.1 ± 3.4^{def}	28.6 ± 3.5^{abcde}
		Variation	-47.3	+59.43	-0.5	-0.5	-3.1	-3.1	+1.8	-9.3
	2927	Control	5.8 ± 3.2^{a}	180.6 ± 11.0^{ab}	17.9 ± 0.1^{efg}	19.9 ± 0.3^{efg}	13.3 ± 1.4^{bc}	12.9 ± 2.1^{abc}	4.8 ± 1.8^{abc}	41.6 ± 4.8^{e}
		Drought	13.5 ± 4.4^{a}	227.4 ± 57.5^{ab}	16.8 ± 0.1^{bcde}	18.2 ± 0.4^{bc}	29.1 ± 5.9^{d}	29.0 ± 5.3^{abc}	7.9 ± 3.7^{cd}	35.9 ± 0.7^{de}
		Variation	+7.7	+46.8	-1.1	-1.7	+15.8	+16.1	+3.0	-5.7
	3126	Control	53.3 ± 8.0^{ab}	301.2 ± 41.1^{bcd}	17.1 ± 0.3^{cdef}	19.0 ± 0.3^{cdef}	10.6 ± 3.5^{abc}	23.6 ± 0.9^{cdef}	6.9 ± 3.0^{bcd}	25.8 ± 1.5^{abcd}
		Drought	44.3 ± 33.6^{ab}	446.2 ± 60.3^{cd}	14.9 ± 0.2^{a}	16.0 ± 0.5^{a}	15.2 ± 0.2^{c}	31.4 ± 1.9^{defg}	4.6 ± 0.8^{abc}	31.5 ± 5.6^{abcde}
		Variation	-9.1	+145.0	-2.2	-3.0	+4.7	+7.8	-2.4	+5.7
CAN	2937	Control	40.2 ± 27.2^{a}	178.9 ± 38.1^{ab}	17.8 ± 0.5^{efg}	20.0 ± 0.6^{efg}	5.0 ± 1.1^{ab}	36.5 ± 3.2^{efgh}	0.8 ± 0.3^{a}	21.0 ± 2.5^{abc}
		Drought	31.0 ± 0.0^{a}	250.8 ± 6.8^{ab}	16.4 ± 0.0^{bc}	18.6 ± 0.0^{bcd}	7.7 ± 0.0^{abc}	49.2 ± 6.5^{hij}	1.1 ± 0.4^{a}	18.3 ± 2.4^{a}
		Variation	-9.2	+71.9	-1.3	-1.4	+2.7	+12.7	+0.3	-2.7
	2938	Control	54.8 ± 24.1^{ab}	107.4 ± 14.7^{a}	18.0 ± 0.2^{fg}	19.7 ± 0.2^{defg}	12.3 ± 3.3^{abc}	54.4 ± 2.7^{j}	1.7 ± 0.8^{ab}	32.3 ± 3.7^{bcde}
		Drought	26.3 ± 7.1^{a}	261.0 ± 44.4^{abc}	15.8 ± 0.0^{ab}	17.5 ± 0.1^{b}	7.1 ± 3.6^{abc}	39.2 ± 5.5^{ghi}	1.6 ± 1.3^{ab}	25.6 ± 0.8^{abcd}
		Variation	-28.6	+153.6	-2.2	-2.2	-5.2	-15.2	-0.2	-6.7
GUI	3124	Control	6.6 ± 0.5^{a}	287.5 ± 96.1^{abc}	18.6 ± 0.2^{gh}	20.2 ± 0.5^{fg}	10.1 ± 1.6^{abc}	53.2 ± 9.1^{ij}	5.0 ± 1.6^{abc}	31.2 ± 6.7^{abcde}
		Drought	21.5 ± 1.7^{a}	295.2 ± 145.4^{abcd}	18.3 ± 0.3^{gh}	20.3 ± 0.4^{g}	12.5 ± 3.7^{abc}	38.2 ± 5.1^{fgh}	16.5 ± 2.2^{f}	26.8 ± 3.0^{abcd}
		Variation	+14.9	+7.8	-0.4	+0.1	+2.4	-15.0	+11.4	-4.4
	3125	Control	98.1 ± 13.5^{b}	132.5 ± 78.2^{ab}	18.1 ± 0.4^{fg}	19.8 ± 0.3^{defg}	15.2 ± 1.9^{c}	18.9 ± 4.9^{abcd}	5.1 ± 1.2^{abc}	35.1 ± 8.2^{de}
		Drought	50.5 ± 28.7^{ab}	175.1 ± 73.2^{ab}	17.6 ± 0.3^{defg}	18.9 ± 0.4^{cde}	9.8 ± 3.3^{abc}	22.0 ± 2.4^{bcde}	14.2 ± 1.3^{ef}	32.5 ± 5.7^{cde}
		Variation	-47.7	+42.6	-0.4	-0.9	-5.4	+3.2	+9.2	-2.6
	Mean	Control	44.6	214.6	18.1	19.9	11.0	31.8	4.7	30.4
		Drought	28.2	303.7	16.7	18.3	14.1	36.8	7.9	28.8
		Variation	-16.4	+89.1	-1.4	-1.6	+3.1	+5.0	+3.2	-1.6

Table 9.3

Sweet potato (*Ipomoea batatas*) tubers and shoots Pearson correlation coefficients for WUE (whole-plant water use efficiency, g l^{-1}), ABA (abscisic acid ng g^{-1}), OA (oxalic acid, mg $100g^{-1}$), $\Delta^{13}C$ (carbon isotope discrimination, ‰) and CCI (chlorophyll content index of shoots), for control and drought stress conditions. **correlation is significant at the 0.01 level (2-tailed), *correlation is significant at the 0.05 level (2-tailed).

	1	2	3	4	5	6	7
1. WUE	-						
2. ABA-tuber	-0.11	-					
3. OA-tuber	-0.12	-0.09	-				
4. $\Delta^{13}C$-tuber	0.23	0.03	-0.19	-			
5. CCI	0.24	-0.04	**0.32***	0.01	-		
6. ABA-shoot	0.07	**-0.30***	0.26	**-0.40****	-0.12	-	
7. OA-shoot	**-0.33***	-0.15	**0.39****	-0.08	-0.28	**0.36***	-
8. $\Delta^{13}C$-shoot	0.15	0.01	**-0.32***	**0.92****	-0.06	**-0.45****	-0.15

Figure

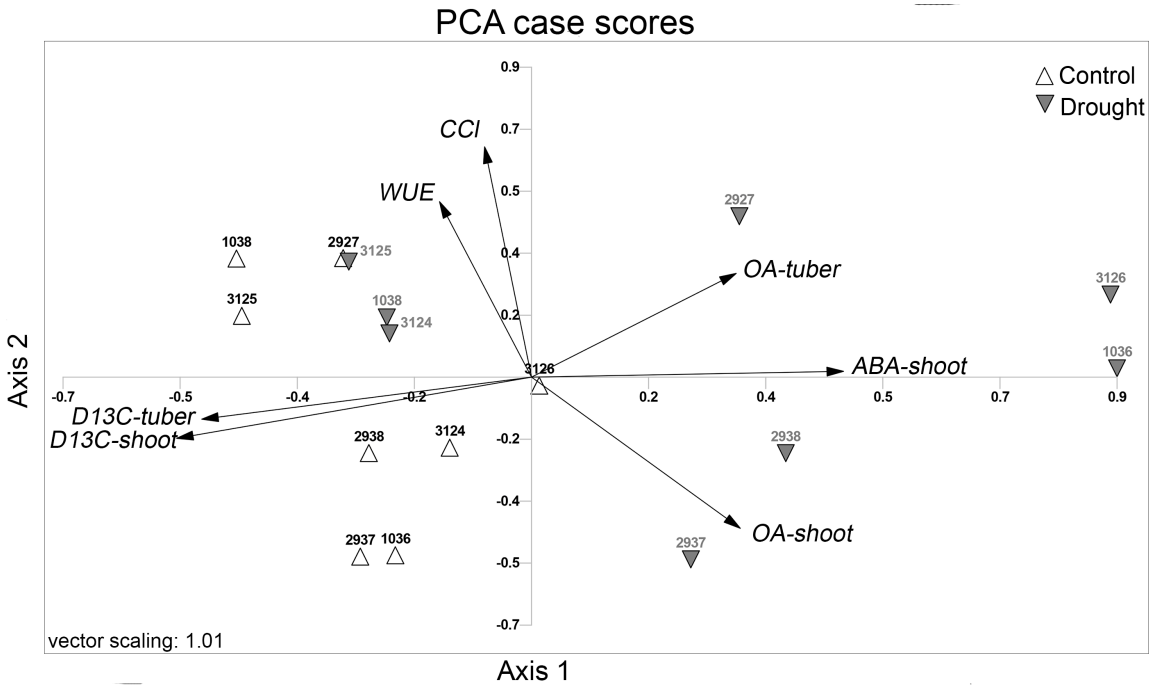

Figure 9.1

Euclidean biplot with variables (ABA, CCI, $\Delta^{13}C$, OA, WUE) spatial distribution of the root tubers and shoots case scores from sweet potato accessions, using the principal component analysis (PCA).

All the variables were converted by $\log_e$.
Control is fully irrigated; drought is water scarcity.

CHAPTER 10

NIRS estimation of drought stress on chemical quality constituents of taro (*Colocasia esculenta* L.) and sweet potato (*Ipomoea batatas* L.) flours

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NIRS Estimation of Drought Stress on Chemical Quality Constituents of Taro (*Colocasia esculenta* L.) and Sweet Potato (*Ipomoea batatas* L.) Flours

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10.1 Abstract

Taro (*Colocasia esculenta* (L.) Schott) and sweet potato (*Ipomoea batatas* (L.) Lam.) are important food crops worldwide, whose productivity is threatened by climatic constraints, namely drought. Data calibration, validation, and model development of high-precision near-infrared spectroscopy (NIRS) involving multivariate analyses are needed for the fast prediction of the quality of tubers and shoots impacted by drought stress. The main objective of this study was to generate accurate NIRS models for quality assessment of taro and sweet potato accessions (acc.) subjected to water scarcity conditions. Seven taro and eight sweet potato acc. from diverse geographical origins were evaluated for nitrogen (N), protein (Pt), starch (St), total mineral (M), calcium oxalate (CaOx), carbon isotope discrimination ($\Delta^{13}C$), and nitrogen isotopic composition ($\delta^{15}N$). Models were developed separately for both crops underground and aboveground organs. N, Pt, St, and M models could be used as quality control constituents, with a determination coefficient of prediction (r^2_{pred}) between 0.856 and 0.995. $\delta^{13}C$, $\delta^{15}N$, and CaOx, with r^2_{pred} between 0.178 and 0.788, could be used as an informative germplasm screening tool. The approach used in the present study demonstrates NIRS's potential for further research on crop quality under drought.

Keywords

calcium oxalate; carbon and nitrogen isotopic compositions; near-infrared spectroscopy; nitrogen protein; starch; sweet potato; taro; total minerals; water scarcity

Abbreviations

Acc., accession; CaOx, calcium oxalate; $\delta^{13}C$, carbon isotopic composition; $\Delta^{13}C$, carbon isotope discrimination; Fb, crude fiber; $\delta^{15}N$, nitrogen isotopic composition; DW, dry weight; H, Mahalanobis distance values; M, total minerals; N, nitrogen; NIRS, near-infrared spectroscopy; PLS, partial least squares regression model; Pt, crude protein; r, Pearson correlation coefficient; r^2, determination coefficient; RPD, ratio of performance to deviation (RPD = SD/SECV); St, starch; SD, standard deviation; SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of prediction; SNV, standard normal variate; T, cross-validation residuals; TC, total carbon.

10.2. Introduction

Tropical tuber crops, such as taro [*Colocasia esculenta* (L.) Schott] and sweet potato [*Ipomoea batatas* (L.) Lam.], are widely consumed, with significant food and nutritional security contribution in developing countries [1–4].

Taro belongs to the family Araceae and needs relatively high-water availability (up to 3500 mm) to achieve optimum growth and production [2,3,5]. Taro corms could be processed into fresh or fermented paste, flour, chips, flakes, and beverages, and contain beneficial nutritional components, such as a good amount of dietary fiber, highly digestible starch granules, and mucilage [2,3]. Taro leaves are also a good source of protein and minerals, consumed as a steamed or boiled vegetable [2,5].

Sweet potato, belonging to the family Convolvulaceae, requires up to 1000 mm of rainfall or irrigation water supply for the good development of the storage root tubers. Sweet potato leaves and storage roots (often called tubers) have remarkable importance in diverse uses from feed consumption and industrial sectors, such as animal feed, flour, starch, glucose, candy, noodles, natural colorants, and alcohol [2,5]. The leaves are a good source of protein and minerals, and the tubers are mainly composed of carbohydrates consisting of starch and sugars [5].

The raw consumption of both taro and sweet potato crops is avoided as they contain different oxalic acid concentration as crystals of calcium oxalate (CaOx) in their tissues, especially under stress conditions (e.g., drought), which negatively affects their nutritional value and quality [6–8].

Protein (Pt), nitrogen (N), starch (St), total minerals (M), crude fiber (Fb), and CaOx are the major quality constituents analyzed by laboratories for both crops, either grown under non-stress conditions [7,9–18], or low-input [19], different irrigation [20], rain-fed [21–23], or other environmental conditions [4,5,24–26]. Taro growth [27,28] and sweet potato quality under drought conditions have recently been studied [29]. However, the analytical process for the major crop quality constituents is still constrained by cumbersome laboratory protocols.

Near-infrared spectroscopy (NIRS) is a fast and reliable spectral technique that allows the screening of numerous varieties, recommended for plant breeding and improvement programs [18,30,31]. Due to its speed of analysis, the accuracy of results, versatility, ease of sample preparation, multiple sample determination in a single operation, without using any chemical reagents allied to a low-cost technique, NIRS has been widely used to characterize several parameters in biological samples [30,31]. The introduction of several new features over the conventional NIR spectroscopy in the last decade led to its development, passing from the optical light beam scatters to the recent inputs of hyperspectral sample surface imaging [32]. NIR spectroscopy uses high-precision spectral techniques that evolve complex computations, operating chemometric algorithms for preprocessing and multivariate data analysis [32,33].

Beyond quality control through the quantification of major chemical constituents, it is essential to link complex phenotypes and chemotypes to plant breeding in changing environments, especially under drought stress [34,35]. Drought stress is not static and affects crops with variable length and severity over their life cycle as one of the main collateral consequences of climate change, leading to changes in chemical composition, productivity, and quality of harvest products as a physiological crop response to stress [36,37]. Depending on drought conditions, there are direct correlations between major quality constituents and phenotype [37].

The quality constituents of taro and sweet potato underground organs [starch and total sugars (as carbohydrates), N, Pt, and M] have been predicted by conventional NIRS [10,16–18]. Other components, such as crude fiber and carbon isotope discrimination ($\Delta^{13}C$) in cereals [38,39] and compound feeds [40], as well as carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in plant tissues [41], were also predicted by conventional NIRS. Hitherto, the application of NIRS modeling to register the influence of drought stress on the quality constituents of both the under and aboveground crop plant organs is rather limited. No study has shown its application in the evaluation of the quality changes related to the physiological response of taro and sweet potato crops to stress conditions, when subjected to water scarcity. In current breeding programs, it is crucial to use fast tools for phenotyping the recurrent selected quality traits, linked with a fast-

physiological response to stress. NIRS can be used for the prediction analysis of major quality constituents and can replace the costly laboratory analysis, and contribute to predicting crop physiological responses to drought. The development of NIRS technological application for such purposes could ease the detection of plant response to water scarcity through physiologic and quality traits that could be used for research, or breeders, and agri-food companies.

The main objectives of the present study were (1) to develop accurate NIR prediction models of chemical quality constituents (N, Pt, St, Fb, M, CaOx, $\delta^{13}C$, $\Delta^{13}C$, $\delta^{15}N$, and total carbon (TC)), using the flour of the underground and aboveground organs of taro and sweet potato crops subjected to drought stress; and (2) to clarify the relationships between phenotype drought response, chemotype, and other major chemical quality constituents, using the generated NIR models to help monitor their tolerance or susceptibility to drought stress.

10.3 Materials and Methods

10.3.1 Sample Preparation and Chemical Analysis

Seven taro and eight sweet potato accessions (acc.) were cultivated in six plots (three experimental replicates for control, and three experimental replicates for drought) to determine their physiological response to water scarcity (Table 10.1).

The taro assay (Figure 10.1) was performed in an open greenhouse at the Preces experimental station (32°39′ N, 16°58′ W, 188 m a.s.l., Câmara de Lobos, Madeira, Portugal), with the implementation of drought conditions for seven months, for two agronomic trials in 2015 and 2017 [42]. Similarly, the sweet potato assay (Figure 10.1) was established at the ISOPlexis experimental field (32°39′ N, 16°55′ W, 174 m a.s.l., Funchal, Madeira, Portugal), in randomly split-plot field design, with water scarcity conditions applied for three months, for two trials in 2017 and 2018 [43]. The trials were established on soil free of chemical contaminants, without the addition of any fertilizers or phytopharmaceutical products.

At the end of each trial, 336 taro corm and shoot (petioles and leaves) and 384 sweet potato storage root and shoot (stem, stalk, and leaves) samples were harvested from the control and drought plots. All samples were cleaned under running water, weighed with a scale (Sartorius Basic BA2100S, Göttingen, Germany), sliced (2–3 mm thick) with a mandolin slicer, dehydrated using an air oven at 65 °C during 48 h (Memmert UF260, Schwabach, Germany), and finely milled (IKA-Werke M20, Staufen, Germany). The flour was placed into bags (Termofilm PA/PE), vacuum-sealed (Audionvac VMS153, Weesp, The Netherlands), and stored at −35 °C (Liebherr ProfiLine GGPV6570, Schwabach, Germany) until analysis.

The flours from the 1st trial were chemically evaluated and used as reference values for the above and underground organs, for the following constituents: nitrogen (N), protein (Pt), starch (St), crude fiber (Fb), total minerals (M), calcium oxalate (CaOx), carbon isotopic composition ($\delta^{13}C$), carbon isotope discrimination ($\Delta^{13}C$), nitrogen isotopic composition ($\delta^{15}N$), and total carbon (TC) content.

Nitrogen and protein content were determined by the Kjeldahl method, using a distillation and titration automatic unit (Velp Scientifica UDK 152, Milan, Italy), with the factor Nx6.25 applied to convert the total nitrogen to crude protein content [44]. Starch was determined according to Hodge and Hofreiter [45] using a spectrophotometer UV/Vis (Shimadzu, 2401 PC, Kyoto, Japan) with the UV Probe 2.52 software. Total mineral or ash content was gravimetrically determined using a furnace at 550 °C (Ney Vulcan Model 3-550, Yucaipa, CA, USA) [44]. Crude fiber was determined by a raw fiber extractor (Velp Scientifica FIWE 6, Usmate, Italy), according to the modified Scharrer method [46]. Calcium oxalate was obtained by extraction with boiling sulfuric acid (H_2SO_4, 20%) [47] and calculated from consumption of permanganate potassium ($KMnO_4$, 0.05M) [23,48]. The isotopes $\delta^{13}C$, $\Delta^{13}C$, $\delta^{15}N$, and TC were determined at the Natural Resources Analytical Laboratory at the University of Alberta (Edmonton, Canada), using the micro-chemical method [49], and the Delta V Advantage Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS, Thermo Finnigan Corp, Bremen, Germany).

10.3.2 NIRS Measurements, Data Pretreatment and Analysis

Flour samples from the above and underground organs of both crops studied were inserted into a spectrometer cell (small ring cup, 50 mm diameter) and tightly compressed with the cell disposable backs to remove the air between the flour particles. The NIR spectra were recorded using a NIRSystems 5000-M (FOSS, Silver Spring, MD, USA), through the WinISI II software version 1.5e (FOSS, USA). The absorbance values were recorded between 1100 and 2498.2 nm in reflectance mode, as log 1/R (where R is the reflectance of the sample), at every 2 nm. All samples' spectra were recorded in triplicate, with 25 scans on average per sample.

Partial least squares (PLS) multivariate data analysis was performed between NIR spectra and reference values to develop prediction models for each trait in the study. Chemical quality analysis data, randomly selected from 75% of the sample flours from the 1st trial, were used as references to construct the calibration models. A spectral pretreatment, based on standard normal variate (SNV) and detrend corrections, was performed with the PLS regression model using the 1st spectrum derivative. PCA was also used to define the spectral boundaries of both crop sample spectra, through their average structure and distribution, with spectral outlier removal based on Mahalanobis distance (H) > 3.

Full cross-validation was performed as leave-one-out for all calibration models to determine the number of factors for model calibration. One sample prediction was repeated until all the calibration samples were predicted, measuring the difference between actual and predicted values calculated throughout whole cross-validation calibrations. The calibration models were evaluated by the standard error of calibration (SEC), standard error of cross-validation (SECV), determination coefficient of cross-validation (r^2_{cv}), and ratio performance to deviation (RPD = SD/SECV).

The calibration models were then validated with an independent set of samples. The performance for each calibration model by predicting the remaining 25% of the sample flours from the 1st trial was evaluated through the standard error of prediction (SEP) and the determination coefficient of prediction (r^2_{pred}). This indicates whether the reference values and NIR prediction performance is reliable enough to be used with good accuracy to predict the quality constituents of the sample flours obtained from the 2nd trial. A robust fitting prediction model indicates higher r^2_{pred} and RPD, associated with the lower SEC, SECV, and SEP values [10,18,50].

10.3.3. Statistical Analysis

The results were expressed on a dry weight basis (DW) as the mean ± standard deviation (SD) for control vs. drought plots of three independent experimental replicates per accession. The reference and predicted values obtained for taro and sweet potato organs for both control and drought conditions were submitted to: a Kolmogorov–Smirnov non-parametric normality test to examine if the variables in the study are normally distributed; Levene's test or equal variance test to examine the data homoscedasticity—both tests were performed with an assumption of normal distribution to apply the one-way analysis of variance (ANOVA) to determine statistically significant differences ($p \leq 0.05$) between the means of control and drought independent groups; and the Pearson correlation coefficient (r) to measure of the strength and direction of the association between the variables (SPSS Statistics software version 26 for Mac).

10.4. Results and Discussion

10.4.1 NIRS Calibration and Validation of PLS Models

10.4.1.1 Taro

The actual or reference values of taro flour samples from the first trial for control and drought conditions were obtained by chemical quality analysis (and are presented in Table S1, as the variation in primary constituents within each acc.). Overall, an average increase in the N, Pt, Fb, and M content was recorded in both shoot and corm organs under stress. The M-corm increase under drought conditions was

significantly different from the control ($p \leq 0.05$). An average slight decrease in St, $\Delta^{13}C$, and $\delta^{15}N$ content was also registered in both organs under such conditions. In this particular case, drought significantly decreases ($p \leq 0.05$) the $\delta^{15}N$-shoot content when compared with control conditions. Still, from these primary chemical constituents, St is the most abundant constituent produced in corms, followed by Pt, M, and Fb. On the contrary, the shoots have Pt as the main abundant constituent, followed by St, M, and Fb. Therefore, the St-corm and Pt-shoot are chemotypes for representing the most abundant constituents produced by taro organs in both control and drought conditions, ranging from 48.44 to 45.18 g/100g DW, and from 11.48 to 12.44 g/100g DW, respectively [34].

The Pearson correlation coefficient (r) from the reference values is shown in 35 significant correlations overall among these quality constituents, of which 12 were strongly correlated with $|r| \geq 0.50$ (Table S2). These correlations observed between primary chemical constituents allow the quality identification between acc. under drought, which could be important for selecting the best ones for breeding purposes [17].

The strongest significant correlations were observed between St-corm and Fb-corm (−0.87), followed by Pt-corm and N-corm (0.85). The St-corm also showed a moderate correlation with Pt-corm (−0.46) and M-corm (−0.48), whose relationship between constituents agrees with previous studies on 315 taro acc. from various geographical origins [17]. On the other hand, the CaOx-shoot had a positive significant correlation with St-shoot (0.52), which in turn was negatively correlated with Pt-shoot content (−0.39), also confirming previous studies on taro's oxalate quantitation [47]. The $\Delta^{13}C$-corm showed a moderate correlation with CaOx-corm (0.40) and $\Delta^{13}C$-shoot (0.41). Meanwhile, $\delta^{15}N$-corm showed a weak correlation with N-corm (0.31).

This set of correlation coefficients indicates that increased chemotype St-corm contents under drought will reduce fiber, protein, and minerals. Therefore, the higher chemotype Pt-shoot content will consequently present higher CaOx-shoot and lower St-shoot content with drought conditions. This could assist taro breeders in their choice and selection of the best ones for taro breeding programs [39]. However, the chemotypes St-corm and Pt-shoot presented distinct correlations (r) according to their origin (Table S2). The St-corm from the Canary Islands acc. showed negative correlation with Pt-shoot (−0.78) and Fb-corm (−0.76), while the St-corm from Madeira Island acc. had a negative correlation with Fb-corm (−0.93). The Pt-shoot only showed correlation with CaOx-shoot (0.64) for the SPC accessions. This is a strong indication that we might observe a distinct genetic variation to stress response, as no differences between control and drought conditions could be identified.

The chemical reference values for each plant organ from the first trial were used to implement the calibration set. Another set of flour samples from the first trial were held and used as an independent validation set. NIRS spectra were obtained for corms (Figure 10.2A) and shoots (Figure 10.2B) calibration sets, and for the independent validation test sets for corms (Figure 10.2C) and shoots (Figure 10.2D). The spectra were composed mainly of groups of peaks located, respectively, at 1200, 1465, 2050, 2140, 2314, and 2350 nm. These peaks are associated with C-H, O-H, and N-H stretching-bending vibrational modes, mainly related to starch and proteins [17].

The comparison between the calibration models created from the chemical analysis and the NIR spectra allowed the implementation of PLS equations and the prediction of the ten quality constituents with results presented in Table 10.2.

The St-corm model showed the highest SEC, SECV, and SEP values of 0.982, 1.048, and 0.767, respectively. Meanwhile, the r^2_{cv} (0.964), r^2_{pred} (0.977), and RPD (5.22) values indicate good predictive potential for the starch model in corm flours. The $\Delta^{13}C$-corm model had an excellent prediction application, according to the r^2_{pred} (0.977) and RPD (2.83) values. However, the $\Delta^{13}C$-shoot model showed a rough prediction with RPD (1.94) and r^2_{cv} (0.740), still with r^2_{pred} (0.910) value showing a satisfactory prediction [17,42]. Hence, we could identify the equations with the poorest performance, showing the lowest r^2_{cv}, r^2_{pred}, and RPD values, with 0.564, 0.717, and 1.53 for $\delta^{15}N$-corm, and with 0.574, 0.873, and 1.53 for $\delta^{13}C$-shoot, respectively. Even with the r^2_{pred} slightly higher than 0.71, the RPD (1.53) values indicate that both $\delta^{15}N$-

corm and δ^{13}C-shoot constituents could not be satisfactorily predicted [16]. The CaOx acridity models had low and quite similar SEC and SECV values, with good RPD values in both corm and shoot models, with 0.027, 0.028, 3.58, and with 0.038, 0.040, 2.70, respectively. While the CaOx-shoot model showed the lower r^2_{pred} (0.498), and even an acceptable RPD value (2.70), this validation set was not robust enough to have a good determination coefficient for acridity prediction. These δ^{15}N-corm, δ^{13}C-shoot and CaOx-shoot prediction constituents should be only used as an informative tool in germplasm screening, and not for quantitation.

The remaining models—moisture, Pt, Fb and M—showed good quantitative predictions, with r^2_{cv} and r^2_{pred} higher than 0.863. They also showed high RPD values, ranging between 2.14 and 11.56 for the corm moisture and protein models, and between 2.21 and 5.97 for the shoot moisture and protein models. The simultaneous prediction of the St, Pt, Fb, and M chemical constituents on a single taro sample, could allow a fast estimation of the variety chemotype and quality with acceptable accuracy [17].

10.4.1.2 Sweet Potato

The sweet potato reference values of the first trial, for both control and water scarcity conditions, were chemically determined (Table S3). On average, drought determined the decrease in the tuber and shoot Pt, N, CaOx, Δ^{13}C and δ^{15}N content. However, this decrease was significant ($p \leq 0.05$) for Pt-shoot, N-shoot, and for Δ^{13}C and δ^{15}N in both organs. Contrariwise, δ^{13}C and TC increased slightly with drought in both organs. The δ^{13}C increase under stress conditions was significantly different ($p \leq 0.05$) from the control. However, M and St showed an opposite variation between the sweet potato organs. Still, from these major constituents, St is the most abundant constituent produced in tubers, followed by the Pt and M. Instead, the shoots show Pt as the main abundant constituent, followed by M and St. The St-tuber and Pt-shoot are chemotypes because they are the constituents with the highest concentration in sweet potato organs [34]. St-tuber showed an average range of 43.39 and 42.33 g/100 g DW, while Pt-shoot ranged significantly ($p \leq 0.05$) between 16.36 and 13.04 g/100 g DW when under stress.

The overall Pearson correlations displayed 62 significant correlations in the first trial, among the quality constituents of sweet potato's tuber and shoot organs, of which 29 were highly correlated ($|r| \geq 0.50$) (Table S4). For example, the highest significant correlations were registered for δ^{13}C-tuber with Δ^{13}C-shoot (−0.93) and δ^{13}C-shoot (−0.88), and for M-shoot with Pt-shoot (0.89) and St-shoot (−0.79). Still, Pearson correlations performed according to the acc.'s locality show differences (Table S4) due to different genotypic responses to drought. The chemotype Pt-shoot had greater correlations than St-tuber chemotype, with the St-tuber not showing significant correlation among the acc. of Canary Islands and Guinea-Bissau. Instead, the ones from Madeira Island had significant and negative correlation for St-tuber with M-tuber (−0.76), TC-shoot (−0.59), and St-shoot (−0.48), and positive to M-shoot (0.58). On the other hand, the Pt-shoot had greater significate correlations in those three locations, among them with M-shoot for Canary Islands (0.94), Guinea-Bissau (0.86), and Madeira Island (0.82).

The creation of PLS equations from both the calibration models and chemical analysis (used as a reference method) from the sweet potato flour organs was performed through NIRS spectra collected from tubers (Figure 10.3A) and shoots (Figure 10.3B) calibration sets. NIRS spectra of the validation sets were also obtained for the tubers (Figure 10.3C) and shoots (Figure 10.3D) independent samples from the first trial. Overall, NIR spectra peaks for both sweet potato plant organs lead mainly to starch (1460, 1930, 1966, and 2286 nm) and proteins (2176, 2296, and 2466 nm). These peaks result from overlapping absorption by mostly overtones and combinations of vibrational modes comprising C-H, O-H, and N-H stretching-bending vibrations [10,18].

Good fitting prediction models amongst the quality constituents were recorded in this study, with low SEC and SECV, and with high r^2_{cv}, $r^2_{pred,}$ and RPD values (Table 10.3), which agrees with previous studies [10,16,18,51]. The TC-tuber and δ^{15}N-tuber models were the exceptions, showing the lowest prediction performance through the smallest r^2_{cv} (0.687, 0.727, respectively), r^2_{pred} (0.705, 0.769), and RPD (1.79, 1.92) values. The δ^{13}C-tuber and δ^{13}C-shoot models had the lowest discrimination in the prediction of

the independent samples, showing only 17.8% and 57.9% prediction confidence, with r^2_{pred} of 0.178 and 0.579, respectively. The $\Delta^{13}C$ also showed low discrimination in the prediction of the validation set for both organs, showing r^2_{pred} of 0.629 and 0.580 for tubers and shoots, respectively. Low discrimination was expected, since NIRS is hardly efficient for constituents representing less than 1% of the dry matter weight. In contrast, the M-tuber and M-shoot equations proved to be excellent predictive models. They show high r^2_{cv} and r^2_{pred} values, similar SEC and SECV values, and RPD values of 5.40 and 8.16 for M-tuber and M-shoot, respectively.

The nitrogen and protein models were also demonstrated to be excellent predictive models in both organs, with very high r^2_{cv}, r^2_{pred}, and RPD values, and low average difference between SEC, SECV, and SEP values, which indicates a robust fitting. Previous studies [10,16] also observed good predictive performance for nitrogen and protein constituents in sweet potato tubers.

The acridity prediction by CaOx constituent was shown to be a good model for CaOx-shoot, and an approximate model for CaOx-tuber, with RPD values of 2.74 and 2.41, respectively. It also showed SEC, SECV, and SEP similarity within the models, and quantitatively approximate r^2_{cv} and r^2_{pred} values. Lastly, the starch models showed a better prediction performance in St-shoot than in St-tuber, with RPD values of 5.03 and 2.56, respectively. This difference in prediction performance among the tissues could be due to the starch content being almost ten times lower in shoots than in tubers, allowing improved discrimination [18].

The models generated allow the reading of one unique sweet potato sample to predict the St, Pt, and M major chemical constituents and thus provide a quick assessment of the variety, chemotype, and quality with good accuracy [16].

10.4.2 Variability of Chemical Constituents

10.4.2.1 Taro

Taro's reference values from the first trial allowed the creation of prediction equations by NIRS, with the immediate and simultaneous estimation for each acc. quality and chemotype from the second trial (Table S1).

Overall, drought leads to the increase in Pt, N, Fb, and M in both taro corm and shoot organs for both trials, with different levels of significance (Table S1). However, these primary constituents showed stronger correlations in the second trial (Table S5), compared to the first trial (Table S2). Taking into consideration the most abundant constituents, the chemotype St-corm was highly correlated with Fb-corm (−0.93), M-corm (−0.72), and Pt-corm (−0.70), while the chemotype Pt-shoot was strongly correlated with Fb-shoot (−0.76) and moderately correlated with St-shoot (0.48) (Table S5).

The corms from the second trial show the highest significant ($p \leq 0.01$) Fb accumulation compared to the control. Fb is the indigestible portion of the plant's total carbohydrate content, which consists mainly of cellulose and lignin present in the cell walls, whose provides the plant stems with rigidity and stiffness during drought [52]. The corms from both trials had the significantly highest M accumulation ($p \leq 0.05$), compared with shoots. Meanwhile, N increase was only significative ($p \leq 0.05$) in corms from the second trial. M and N increase photosynthesis and protein synthesis as plant responses to drought, allocating nutrients between the plant organs [42,53].

Drought increased CaOx-corm in both trials, but a CaOx-shoot increase only occurred in the second trial. The increase in this insoluble oxalate could help the plant osmoregulation under water scarcity conditions, through the adjustment of oxalic acid synthesized by photosynthesis oxidative processes or carbohydrate metabolism [47].

However, we also observed the decrease in St, $\Delta^{13}C$, and $\delta^{15}N$ in both taro organs, and in both trials, with significance for the $\delta^{15}N$-shoot ($p \leq 0.05$) in the first trial, and St-corm ($p \leq 0.01$) from the second trial. The slight decrease in St may be due to the mobilization of the reserves in a plant attempting to provide energy and metabolites to subcellular structure protection against water deficit to avoid the need to allocate biomass reserves [47]. The reduction in $\Delta^{13}C$ and $\delta^{15}N$ could be related to physiological integrators of stress

in taro subjected to drought scarcity [54–57]. The obtained Δ^{13}C-shoot content indicates relative stomata opening during stress. Since the Δ^{13}C-shoot content fell minimally, no considerable changes should be expected in the ^{13}C depletion, related to CO_2 fixation (carboxylation), which in turn leads to only a slightly heavier or richer ^{13}C content during drought, in both trials, with a less negative δ^{13}C value [55]. The δ^{15}N indicates the plant N metabolism and the growing conditions by the fractionations of ^{15}N and ^{14}N during the nitrogen trial processes, whose variation between the organs could be attributed to tissue reallocation of N under drought [54,56]. A decrease in the whole-plant δ^{15}N abundance in both trials was observed. This could indicate a good drought response, with the most ^{15}N-enriched plants having the most positive δ^{15}N abundance [57].

According to the geographical origin of acc. (Table 10.1), acc. 2216 from Madeira Island had the most resilient response to drought during the first trial, showing higher productive capacity in both environments, with greater total plant biomass and water use efficiency [54]. This resiliency could be due to its chemical constitution, by showing the highest whole-plant Pt, Fb, and M content, and the lowest St content loss to cope with drought [42,47]. Advantageously, NIRS models applied for these major quality constituents allowed a good accuracy with fast and explicit predictive quantitation for the classification of the studied taro acc. according to their tolerance or susceptibility to water scarcity, at both the under and aboveground organ level. The prediction of the second trial estimates that acc. 2016 had the best tolerant response to drought, by keeping practically the same variation within those primary constituents (Table S1). This could indicate a plausible link within the taro phenotype's response to stress with its chemotype and other major chemical quality constituents.

10.4.2.2 Sweet Potato

The quality traits varied between the sweet potato organs, under influence of water scarcity, either in the reference values of the first trial, or in the predicted values by NIRS of the second trial (Table S3).

Taking into consideration the major constituents, M, St and Pt, in both trials, the water scarcity conditions triggered opposite responses. Pearson correlation shown a weaker relationship between these constituents in the second trial compared to the first trial (Table S6 and Table S4, respectively). Still, both trials registered an accumulation of M-tuber and St-shoot, and a decrease in M-shoot and St-tuber, the variation of which was only significant ($p \leq 0.01$) in the shoots. Pt also decreased in both trials and organs, with the exception of the Pt-tuber, which increased in the 2nd trial (Table S3).

The average M-tuber increase and M-shoot significant ($p \leq 0.01$) decrease in both trials may have been due to the limitation of macro and micronutrient uptake from water-scarce soil. In such conditions, the mineral allocation into the shoots could be reduced, with lower M-shoot allocation registered in the first trial [43,58].

The scarcity significantly decreased the N-shoot and Pt-shoot in both trials. However, the N-tuber and Pt-tuber also decreased during the first trial but registered an average significant ($p \leq 0.05$) increase in the second trial. N is one of the essential primary macronutrients, the uptake of which, as a result of primary assimilation, is strongly compromised during drought stress, with δ^{15}N indicating the N fractionation during the N cycle processes [57–59]. The average content of the shoots δ^{15}N and Δ^{13}C had the same pattern, by significantly ($p \leq 0.05$) decreasing its content in both trials. The reduction in δ^{15}N-shoot is a signal of less N retention in aboveground tissue due to drought [59]. The lower Δ^{13}C-shoot indicates a low ^{13}C depletion, through partial stomatal closure, leading to a slight variation in the TC as ^{13}C fixation due to stress [59].

Both trials showed that stress increased St-shoot and consequently decreased St-tuber. Starch is deposited in the leaf chloroplasts during daylight carbon dioxide fixation in photosynthesis to be broken down to sucrose during the night, which is then mobilized and converted into storage starch in the tubers [60]. The slight downregulation of photosynthesis, possibly due to the partial stomatal closure, still allowed an accumulation of starch at the shoot level, with the second trial registering significantly ($p \leq 0.01$) greater St-shoot accumulation. It still allowed good photosynthesis activity and avoided the starch degradation into sugars, reducing its mobilization into tubers. However, in such conditions, the carbohydrate metabolism and

photosynthesis oxidative processes can produce oxalic acid, forming insoluble salts when combined with free calcium, as CaOx insoluble druse crystals [61]. We observed that the whole-plant CaOx average content decreased in the first trial, with an increase in the second trial. The CaOx increase in the second trial could be due to the plant needing to isolate the excess of calcium accumulated in its tissues [62]. The oscillation in the CaOx formation in stress was probably due to the plant ion balance, leading to the fluctuation of druse crystal formation, according to the availability of free calcium [8,63].

Acc. 3124 from Guinea-Bissau had one of the best physiological responses to water scarcity during the first trial, namely the highest total plant biomass content and the best water use efficiency [52]. The fact that it showed the combination of high St-tuber, M-shoot, and Pt-shoot content indicates that it had one of the best nutrient allocations into the shoot and tuber starch storage under stress. Excellent predictive NIR models were generated for the major quality constituents St, Pt, and M for the whole-plant sweet potato acc., which could be a fast tool to be used in the continuous monitoring of drought response, according to its tolerance or susceptibility. Acc. 3124 showed good to moderate stress tolerance response in the second trial, mainly due to lesser M and Pt shoot content, indicating a more limiting nutrient diffusion into plants, but still keeping the best starch accumulation in tubers. Still, we could find a reasonable connection within the sweet potato phenotype response and its chemotype and other major chemical quality constituent values obtained for drought.

10.5 Conclusions

NIRS represents a rapid and cost-effective analytical tool when the predictive models are accurate enough. The models for nitrogen, protein, starch, fiber, and total minerals quality constituents of taro and sweet potato showed good prediction accuracy. The TC-tuber in sweet potato and CaOx-shoot in taro, the $\delta^{15}N$ in the underground organs and the $\delta^{13}C$ in the vegetative parts of both crops showed the poorest prediction performances. However, these last constituent predictions could be used as an informative tool in germplasm screening. The present models could be applied in further research on crop quality subjected to water scarcity conditions. Additional work is needed to improve the existing models with new data from new drought trials. The development of more robust NIRS models for all constituents will improve their efficiency and reliability for assessing crop quality and phenotyping plants subjected to water scarcity.

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10.7 Author Contributions

Conceptualization, V.L. and M.P.d.C.; methodology, C.S.S.G.; investigation, C.S.S.G.; data curation, C.S.S.G.; writing—original draft preparation, C.S.S.G.; writing—review and editing, V.L., M.P.d.C. and C.S.S.G.; software, C.S.S.G.; supervision, V.L. and M.P.d.C.; funding acquisition, C.S.S.G. and M.P.d.C. All authors have read and agreed to the published version of the manuscript.

10.8 Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

10.9 References

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Tables

Table 10.1
Accessions analyzed for quality traits as actual reference (calibration) and near-infrared spectroscopy (NIRS) prediction.

Accession ID [1]	Name	Locality	Origin	Category
Taro [*Colocasia esculenta* (L.) Schott]				
2056	Listado	La Palma	Canary Islands—Spain (CAN)	Local
2061	Blanco Saucero	La Palma	Canary Islands—Spain (CAN)	Local
2210	Roxo	Porto Moniz	Madeira Island—Portugal (MAD)	Local
2216	Branco	Santa Cruz	Madeira Island—Portugal (MAD)	Local
2232	PExPH 15-6 BL/HW/08	Hawaii	Fiji—Pacific Community (SPC)	Breeding lines
2234	C3-22 BL/PNG/11	Papua New Guinea	Fiji—Pacific Community (SPC)	Breeding lines
2239	Karang CE/MAL/10	Malaysia	Fiji—Pacific Community (SPC)	Breeding lines
Sweet potato [*Ipomoea batatas* (L.) Lam.]				
1036	Brasileira	Câmara de Lobos	Madeira Island—Portugal (MAD)	Local
1038	5 Bicos	Santana	Madeira Island—Portugal (MAD)	Local
2927	de Flor	Porto Moniz	Madeira Island—Portugal (MAD)	Local
3126	Inglesa	Porto Moniz	Madeira Island—Portugal (MAD)	Local
2937	Roja	Tenerife	Canary Islands—Spain (CAN)	Local
2938	Cubana	Tenerife	Canary Islands—Spain (CAN)	Local
3124	Vermelha	Bafatá	Guinea-Bissau (GUI)	Local
3125	Branca	Bafatá	Guinea-Bissau (GUI)	Local

[1] Identification number code used by the ISOPlexis Genebank (Madeira, Portugal).

Table 10.2
Quality constituent statistics for calibration and independent validation models of taro (*Colocasia esculenta* (L.) Schott) flour accessions subjected to water scarcity.

Calibration Set										Validation Independent Set		
Taro Models	n	Outliers	Mean	SD	PLS Terms	SEC	SECV	r^2_{cv}	RPD	n	r^2_{pred}	SEP
Moisture, g/100g DW												
Corm	105	6	7.584	1.352	9	0.592	0.633	0.783	2.14	21	0.894	0.433
Shoot	102	11	7.894	2.352	9	1.011	1.063	0.795	2.21	18	0.838	0.837
Protein, g/100g DW												
Corm	105	5	4.705	1.460	9	0.119	0.126	0.993	11.56	21	0.995	0.191
Shoot	102	4	11.542	2.145	9	0.363	0.359	0.972	5.97	18	0.883	0.693
Nitrogen, g/100g DW												
Corm	105	3	0.754	0.231	9	0.020	0.021	0.992	11.07	21	0.995	0.031
Shoot	102	5	1.856	0.340	9	0.059	0.059	0.970	5.79	18	0.953	0.058
Starch, g/100g DW												
Corm	105	3	47.021	5.469	9	0.982	1.048	0.964	5.22	21	0.977	0.767
Shoot	102	3	10.955	2.999	9	0.483	0.524	0.969	5.72	18	0.984	0.805
Crude Fiber, g/100g DW												
Corm	105	6	0.435	0.108	9	0.019	0.019	0.968	5.59	21	0.985	0.018
Shoot	102	14	1.646	0.227	9	0.053	0.057	0.939	4.01	18	0.980	0.031
Minerals, g/100g DW												
Corm	105	4	4.483	1.026	9	0.170	0.176	0.970	5.83	21	0.986	0.122
Shoot	102	5	9.707	1.049	9	0.285	0.334	0.898	3.14	18	0.950	0.245
CaOx, g/100g DW												
Corm	105	9	0.249	0.101	9	0.027	0.028	0.922	3.58	21	0.721	0.046
Shoot	102	4	0.241	0.107	9	0.038	0.040	0.863	2.70	18	0.498	0.056
$\delta^{13}C$, ‰ DW												
Corm	105	3	−26.183	0.788	9	0.250	0.285	0.868	2.76	21	0.977	0.177
Shoot	102	0	−26.304	1.077	9	0.642	0.704	0.574	1.53	18	0.873	0.420
$\Delta^{13}C$, ‰ DW												
Corm	105	3	18.662	0.830	9	0.263	0.293	0.874	2.83	21	0.977	0.156
Shoot	102	7	18.804	1.074	9	0.503	0.552	0.740	1.94	18	0.910	0.411
$\delta^{15}N$, % DW												
Corm	105	0	4.634	0.864	9	0.473	0.564	0.575	1.53	21	0.717	0.488
Shoot	102	4	0.241	0.107	8	0.038	0.040	0.863	2.70	18	0.711	0.549

Table 10.3
Quality constituent statistics for calibration and independent validation models of sweet potato (*Ipomoea batatas* (L.) Lam.) accessions flour under drought conditions.

Calibration Set										Validation Independent Set		
Sweet Potato Models	n	Outliers	Mean	SD	PLS Terms	SEC	SECV	r^2_{cv}	RPD	n	r^2_{pred}	SEP
Moisture, g/100g DW												
Tuber	105	7	5.066	1.539	9	0.670	0.761	0.755	2.02	24	0.532	0.822
Shoot	111	0	6.114	1.083	9	0.616	0.701	0.584	1.54	24	0.495	0.945
Protein, g/100g DW												
Tuber	105	9	6.038	1.262	9	0.112	0.114	0.992	11.10	24	0.994	0.099
Shoot	111	9	14.480	3.160	9	0.218	0.244	0.994	12.96	24	0.995	0.274
Nitrogen, g/100g DW												
Tuber	105	11	0.973	0.203	9	0.022	0.024	0.986	8.47	24	0.994	0.016
Shoot	111	9	2.318	0.506	9	0.035	0.039	0.994	12.96	24	0.995	0.044
Starch, g/100g DW												
Tuber	105	3	42.509	4.122	9	1.430	1.608	0.847	2.56	24	0.856	0.944
Shoot	111	4	4.944	2.599	9	0.487	0.517	0.961	5.03	24	0.981	0.323
Minerals, g/100g DW												
Tuber	105	8	4.336	0.482	9	0.082	0.089	0.966	5.40	24	0.868	0.158
Shoot	111	8	11.266	1.800	9	0.215	0.221	0.985	8.16	24	0.992	0.171
CaOx, g/100g DW												
Tuber	105	8	0.058	0.034	9	0.012	0.014	0.826	2.41	24	0.865	0.020
Shoot	111	3	0.099	0.079	9	0.027	0.029	0.866	2.74	24	0.960	0.014
Total carbon, g/100g DW												
Tuber	105	6	40.341	0.333	9	0.173	0.185	0.687	1.79	24	0.705	0.176
Shoot	111	6	41.510	0.572	9	0.220	0.238	0.826	2.40	24	0.914	0.232
$\delta^{13}C$, ‰ DW												
Tuber	105	6	−25.065	1.161	9	0.306	0.364	0.901	3.19	24	0.178	0.602
Shoot	111	6	−26.611	1.257	9	0.257	0.265	0.956	4.75	24	0.579	0.311
$\Delta^{13}C$, ‰ DW												
Tuber	105	6	17.505	1.211	9	0.319	0.380	0.901	3.18	24	0.629	0.174
Shoot	111	6	19.121	1.314	9	0.269	0.277	0.956	4.75	24	0.580	0.325
$\delta^{15}N$, % DW												
Tuber	105	0	4.586	1.120	9	0.533	0.584	0.727	1.92	24	0.769	0.499
Shoot	111	5	4.185	1.097	9	0.260	0.274	0.938	4.01	24	0.788	0.345

Figures

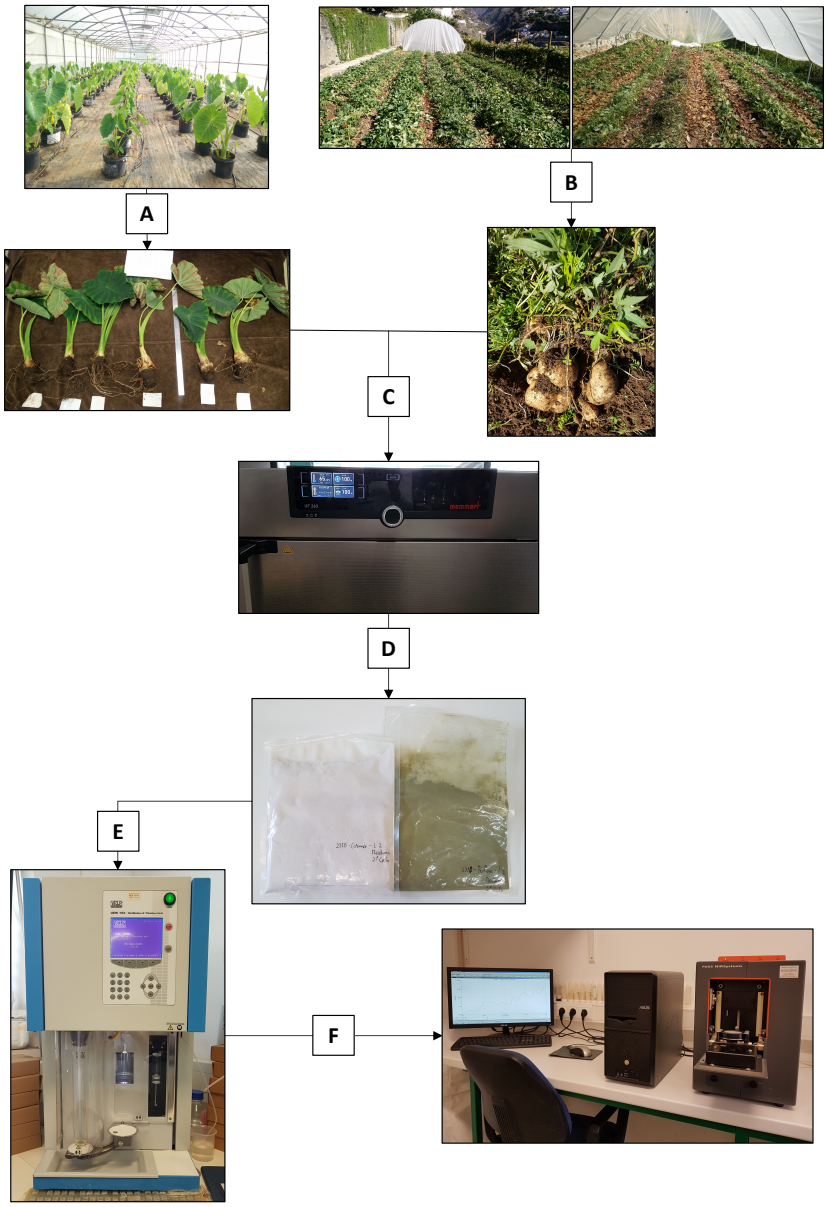


Figure 10.1
Taro and sweet potato crops were submitted to drought stress for two agronomic trials in distinct experimental sites, but they share the same steps from harvest until analysis.

A – harvest of the plants of the taro drought assays performed in an open greenhouse in 2015 and 2017, B – harvest of the plants of the sweet potato drought trials implemented in a split-plot field design in 2017 and 2018, C – oven-drying the underground and aboveground plant organs at 65 °C for 48 h, D – milling the dried samples and placing them into vacuum-sealed bags stored at -35 °C, E – flours from the 1st drought assay were chemically evaluated for quality constituents, F – NIR prediction of the quality constituents of the sample flours obtained from the 2nd drought assay.

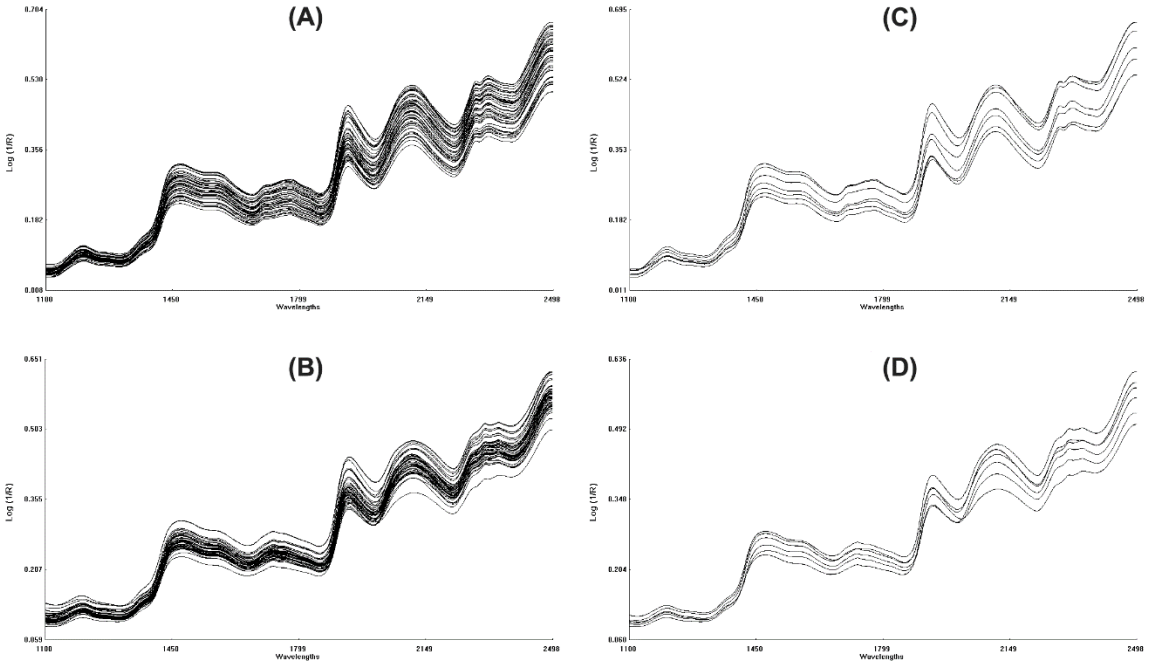


Figure 10.2
NIR spectra of the seven taro accessions, with 126 corm (**A**) and 120 shoot (**B**) samples, and external set validation with 21 samples for corms (**C**) and 18 shoot samples (**D**). Wavelengths from 1100 to 2498.2 nm of the NIR range.

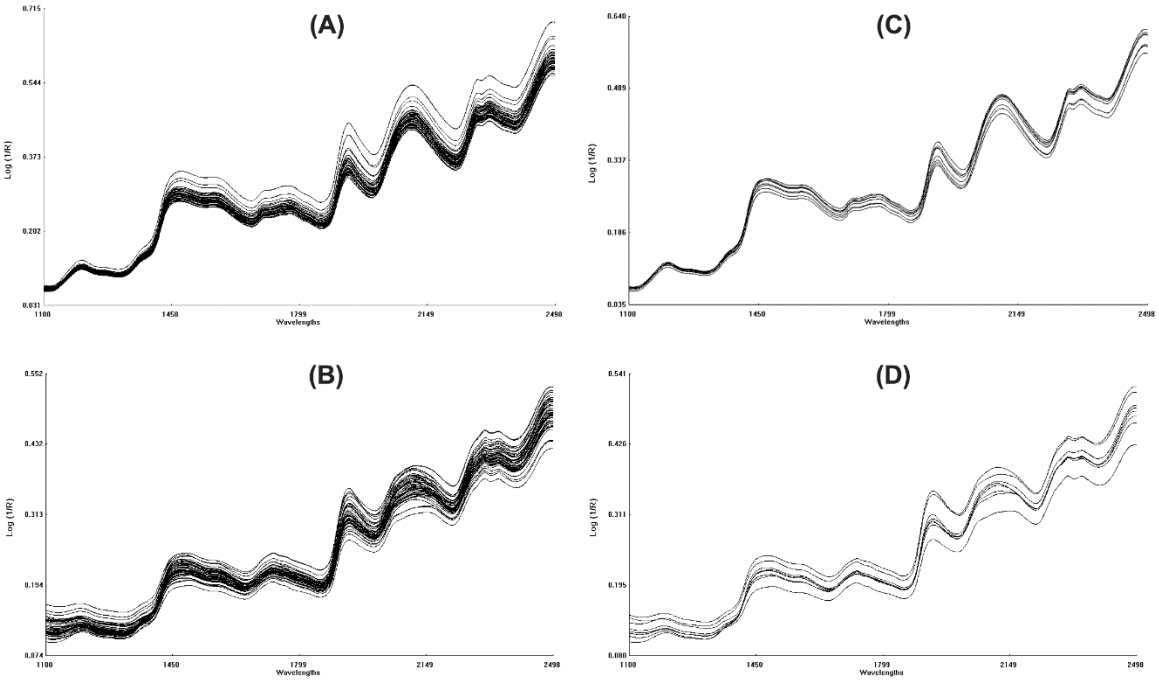


Figure 10.3
NIR spectra of the eight sweet potato accessions, with 129 root (**A**) and 135 shoot (**B**) samples, and 24 external set validation for both root (**C**) and shoot (**D**) samples. Wavelengths from 1100–2498.2 nm of the NIR range.

10.10 Supplementary materials

The following are available online at www.mdpi.com/2076-3417/10/23/8724/s1.

Table S1

Taro quality constituents of the flour obtained by laboratory reference analysis (1st trial) and NIRS prediction (2nd trial) for both corms and shoots submitted to control and drought conditions.

		Corm organs				Shoot organs			
		1st trial 2015		2nd trial 2017		1st trial 2015		2nd trial 2017	
Taro acc.		Control	Drought	Control	Drought	Control	Drought	Control	Drought
					Moisture, g/100g DW				
CAN	2056	6.65 ± 1.26	6.66 ± 0.59	6.56 ± 0.23	8.79 ± 0.91	6.04 ± 0.54	5.94 ± 2.52	5.81 ± 0.90	7.37 ± 0.57
	2061	5.72 ± 0.79	6.18 ± 1.76	7.34 ± 0.64	**8.95 ± 1.22**	**10.58 ± 3.04**	5.46 ± 1.05	4.84 ± 1.69	7.35 ± 0.42
MAD	2210	6.56 ± 0.15	5.89 ± 4.58	7.42 ± 0.59	8.12 ± 0.30	5.55 ± 1.15	4.39 ± 0.00	6.24 ± 3.02	7.59 ± 0.52
	2216	6.85 ± 0.80	7.12 ± 0.99	**9.10 ± 0.43**	8.37 ± 0.73	7.58 ± 1.95	6.90 ± 0.44	7.83 ± 0.17	**10.40 ± 0.40**
SPC	2232	8.24 ± 1.72	7.36 ± 1.59	5.52 ± 0.61	5.39 ± 0.68	9.87 ± 1.79	9.57 ± 2.04	**8.47 ± 0.44**	9.68 ± 1.05
	2234	**8.56 ± 0.58**	**8.58 ± 0.59**	6.64 ± 0.76	6.56 ± 0.34	9.75 ± 1.90	**10.50 ± 1.23**	3.79 ± 1.42	6.73 ± 0.74
	2239	7.91 ± 1.63	7.93 ± 1.36	7.10 ± 0.53	8.20 ± 0.37	9.21 ± 2.27	7.75 ± 3.03	3.63 ± 1.13	5.77 ± 0.38
	Mean	7.21	7.10	7.10	7.77	8.37	7.22	5.80	7.84
	Variation (%)	-1.53		+9.44		-13.74		+35.17 **	
					Protein, g/100g DW				
CAN	2056	3.83 ± 0.05	4.51 ± 0.08	4.22 ± 0.28	4.72 ± 0.19	9.80 ± 0.02	11.68 ± 0.34	6.44 ± 1.82	6.98 ± 1.93
	2061	4.04 ± 0.07	5.03 ± 0.05	4.12 ± 0.04	5.14 ± 0.24	11.23 ± 0.06	10.75 ± 0.06	6.72 ± 2.21	7.57 ± 0.69
MAD	2210	3.89 ± 0.09	5.42 ± 0.04	5.00 ± 0.28	5.58 ± 0.63	12.90 ± 0.12	**15.14 ± 0.01**	**9.29 ± 1.09**	9.29 ± 1.54
	2216	**7.42 ± 0.11**	**8.23 ± 0.20**	**6.23 ± 0.17**	7.29 ± 0.50	12.63 ± 0.01	13.78 ± 0.01	7.72 ± 1.98	**10.09 ± 1.26**
SPC	2232	3.79 ± 0.17	4.74 ± 0.03	4.92 ± 0.47	6.38 ± 0.09	9.94 ± 0.07	13.17 ± 0.00	7.62 ± 1.63	8.96 ± 1.94
	2234	3.51 ± 0.04	3.02 ± 0.02	3.96 ± 0.08	4.41 ± 0.10	**15.22 ± 0.06**	11.75 ± 0.06	4.33 ± 2.26	6.55 ± 1.13
	2239	5.02 ± 0.01	6.14 ± 0.04	5.24 ± 0.45	**7.60 ± 0.83**	8.63 ± 0.25	10.79 ± 0.04	4.73 ± 0.46	5.02 ± 2.85
	Mean	4.50	5.30	4.81	5.87	11.48	12.44	6.69	7.78
	Variation (%)	+17.78		+22.04 **		+8.36		+16.29	
					Nitrogen, g/100g DW				
CAN	2056	0.61 ± 0.15	0.72 ± 0.16	0.67 ± 0.05	0.75 ± 0.03	1.57 ± 0.15	1.90 ± 0.15	1.02 ± 0.31	1.13 ± 0.31
	2061	0.65 ± 0.09	0.81 ± 0.03	0.66 ± 0.01	0.82 ± 0.04	1.80 ± 0.31	1.72 ± 0.11	1.03 ± 0.35	1.20 ± 0.11
MAD	2210	0.62 ± 0.16	0.90 ± 0.16	0.80 ± 0.05	0.89 ± 0.10	1.99 ± 0.25	**2.42 ± 0.12**	**1.44 ± 0.16**	1.46 ± 0.25
	2216	**1.19 ± 0.25**	**1.32 ± 0.12**	**1.00 ± 0.03**	1.17 ± 0.08	2.03 ± 0.15	2.19 ± 0.09	1.22 ± 0.30	**1.60 ± 0.20**
SPC	2232	0.61 ± 0.04	0.77 ± 0.04	0.79 ± 0.07	1.02 ± 0.01	1.59 ± 0.18	2.05 ± 0.26	1.18 ± 0.26	1.40 ± 0.31
	2234	0.56 ± 0.12	0.48 ± 0.01	0.63 ± 0.01	0.71 ± 0.02	**2.51 ± 0.27**	1.88 ± 0.11	0.62 ± 0.37	0.97 ± 0.19
	2239	1.16 ± 0.55	0.98 ± 0.11	0.84 ± 0.07	**1.22 ± 0.13**	1.38 ± 0.40	1.74 ± 0.06	0.70 ± 0.08	0.78 ± 0.45
	Mean	0.77	0.85	0.77	0.94	1.84	1.99	1.03	1.22
	Variation (%)	+10.39		+22.08 **		+8.15		+18.45	
					Starch, g/100g DW				
CAN	2056	53.09 ± 1.02	45.42 ± 0.80	50.82 ± 0.65	44.75 ± 5.70	**17.60 ± 0.62**	**14.35 ± 1.07**	5.72 ± 2.85	4.92 ± 1.35
	2061	46.35 ± 0.87	46.57 ± 1.53	51.74 ± 1.61	46.61 ± 2.76	9.41 ± 0.80	9.60 ± 0.46	5.69 ± 2.23	2.66 ± 0.83
MAD	2210	43.69 ± 1.06	44.65 ± 1.02	**52.28 ± 3.36**	**48.78 ± 0.71**	12.84 ± 0.5	10.01 ± 0.86	7.90 ± 2.91	6.77 ± 2.67
	2216	45.59 ± 2.18	43.35 ± 1.83	44.59 ± 1.62	40.40 ± 0.83	7.50 ± 0.46	7.33 ± 0.50	2.80 ± 1.39	2.99 ± 0.81
SPC	2232	48.07 ± 2.09	40.70 ± 1.10	42.12 ± 3.32	39.35 ± 4.26	12.35 ± 1.60	11.80 ± 0.59	**9.08 ± 1.50**	**8.13 ± 2.81**
	2234	**54.62 ± 0.93**	**52.59 ± 0.64**	50.72 ± 2.99	48.85 ± 1.85	11.08 ± 0.84	10.57 ± 0.30	3.68 ± 2.19	1.06 ± 0.17
	2239	47.69 ± 1.28	43.00 ± 2.13	48.12 ± 3.79	40.12 ± 3.13	9.20 ± 0.80	10.56 ± 2.05	5.89 ± 2.09	4.07 ± 2.09
	Mean	48.44	45.18	48.63	44.12	11.43	10.60	5.82	4.37
	Variation (%)	-6.73		-9.27 **		-7.26		-24.91	

Table S1 (continued).

					Crude Fiber, g/100g DW				
CAN	2056	0.30 ± 0.03	0.43 ± 0.09	0.28 ± 0.03	0.47 ± 0.18	1.36 ± 1.45	1.45 ± 0.09	2.13 ± 0.33	2.33 ± 0.23
	2061	0.45 ± 0.05	0.37 ± 0.04	0.25 ± 0.05	0.44 ± 0.07	1.28 ± 0.63	1.71 ± 0.15	2.13 ± 0.27	2.32 ± 0.04
MAD	2210	**0.54 ± 0.18**	0.51 ± 0.12	0.31 ± 0.07	0.37 ± 0.02	1.38 ± 0.00	-	1.93 ± 0.26	2.08 ± 0.23
	2216	0.48 ± 0.04	**0.54 ± 0.02**	0.41 ± 0.04	0.52 ± 0.03	1.80 ± 0.07	1.62 ± 0.07	2.26 ± 0.26	2.28 ± 0.17
SPC	2232	0.37 ± 0.01	0.52 ± 0.18	**0.48 ± 0.08**	0.50 ± 0.12	1.78 ± 0.49	1.57 ± 0.23	2.14 ± 0.23	2.12 ± 0.30
	2234	0.32 ± 0.09	0.31 ± 0.08	0.30 ± 0.06	0.37 ± 0.05	1.81 ± 0.43	**1.84 ± 0.12**	**2.33 ± 0.20**	2.33 ± 0.09
	2239	0.41 ± 0.07	**0.54 ± 0.03**	0.41 ± 0.10	**0.61 ± 0.04**	**1.91 ± 0.07**	1.60 ± 0.08	**2.33 ± 0.20**	**2.50 ± 0.33**
	Mean	0.41	0.46	0.35	0.47	1.62	1.63	2.18	2.28
	Variation (%)		+12.20		+34.29 **		+0.62		+4.59
					Minerals, g/100g DW				
CAN	2056	3.86 ± 0.15	5.15 ± 0.50	4.99 ± 0.25	7.16 ± 1.87	7.98 ± 0.58	9.20 ± 0.50	9.64 ± 0.19	10.33 ± 0.65
	2061	4.10 ± 0.28	4.50 ± 0.58	4.89 ± 0.77	7.59 ± 1.11	8.40 ± 0.62	9.16 ± 0.34	9.63 ± 0.24	10.95 ± 0.79
MAD	2210	4.05 ± 0.51	4.13 ± 1.17	5.02 ± 0.86	5.75 ± 0.29	9.75 ± 1.30	9.58 ± 0.46	10.47 ± 0.48	10.84 ± 0.40
	2216	**5.58 ± 0.76**	5.72 ± 0.50	**6.89 ± 0.67**	7.92 ± 0.91	**10.31 ± 2.22**	9.89 ± 0.29	10.71 ± 0.28	11.08 ± 0.64
SPC	2232	3.58 ± 0.53	4.32 ± 0.55	5.35 ± 0.43	5.82 ± 1.50	10.16 ± 0.69	**11.18 ± 2.00**	10.40 ± 0.32	11.78 ± 0.42
	2234	2.70 ± 0.17	3.29 ± 0.02	3.32 ± 0.07	4.58 ± 0.74	8.29 ± 0.82	10.48 ± 0.60	**11.15 ± 0.46**	**12.70 ± 0.19**
	2239	4.69 ± 0.61	**6.08 ± 0.84**	5.87 ± 0.67	**8.45 ± 0.28**	9.63 ± 1.77	10.20 ± 0.24	10.59 ± 0.49	12.29 ± 0.61
	Mean	4.08	4.74	5.19	6.75	9.22	9.95	10.37	11.42
	Variation (%)		+16.18 *		+30.06 **		+7.92		+10.13 **
					CaOx, mg/100g DW				
CAN	2056	271.17 ± 44.13	**468.75 ± 69.35**	240.74 ± 47.00	439.58 ± 188.33	343.24 ± 179.44	296.33 ± 150.40	**304.98 ± 17.47**	**450.90 ± 83.82**
	2061	270.20 ± 135.84	264.77 ± 60.80	207.37 ± 57.05	**506.97 ± 34.75**	135.68 ± 28.33	178.60 ± 20.20	183.33 ± 35.30	230.44 ± 40.83
MAD	2210	186.69 ± 25.71	231.66 ± 52.23	117.94 ± 104.02	36.86 ± 22.19	301.33 ± 70.62	210.34 ± 37.05	157.22 ± 97.01	217.64 ± 35.40
	2216	184.66 ± 44.70	184.74 ± 41.48	67.64 ± 5.04	114.12 ± 73.68	153.32 ± 35.06	119.65 ± 7.19	120.48 ± 71.47	140.17 ± 78.27
SPC	2232	124.83 ± 39.66	190.12 ± 25.39	**274.59 ± 96.83**	312.35 ± 108.05	325.67 ± 32.17	224.30 ± 13.89	263.87 ± 5.60	283.25 ± 14.75
	2234	**297.74 ± 27.75**	295.05 ± 84.62	80.79 ± 6.63	172.81 ± 60.68	**445.63 ± 23.17**	**299.46 ± 75.62**	145.90 ± 56.87	127.79 ± 34.39
	2239	224.37 ± 54.84	359.13 ± 91.01	220.40 ± 72.79	278.91 ± 78.08	171.68 ± 91.75	225.57 ± 94.71	73.67 ± 7.27	52.86 ± 33.02
	Mean	222.81	284.89	172.78	265.94	268.08	222.04	178.49	214.72
	Variation (%)		+27.86 *		+53.92		-17.17		+20.30
					$\delta^{13}C$, ‰ DW				
CAN	2056	**-25.07 ± 0.26**	**-24.82 ± 0.14**	-24.41 ± 0.38	-24.18 ± 0.26	-26.58 ± 0.59	-25.49 ± 0.97	**-21.92 ± 1.37**	**-19.77 ± 1.90**
	2061	-25.33 ± 0.25	-25.32 ± 0.06	**-24.30 ± 0.14**	**-23.85 ± 0.13**	-25.84 ± 0.47	**-24.58 ± 0.51**	-23.25 ± 1.26	-20.31 ± 0.42
MAD	2210	-26.33 ± 0.19	-25.76 ± 0.50	-25.32 ± 0.35	-24.62 ± 0.58	-27.53 ± 0.40	-26.46 ± 0.36	-26.97 ± 0.78	-25.78 ± 0.84
	2216	-26.10 ± 1.69	-26.21 ± 0.36	-25.10 ± 0.28	-25.76 ± 0.30	**-25.82 ± 1.20**	-26.27 ± 1.52	-24.18 ± 0.62	-25.03 ± 0.38
SPC	2232	-26.75 ± 0.27	-26.76 ± 0.30	-25.59 ± 0.27	-25.37 ± 0.45	-27.24 ± 0.38	-27.18 ± 1.10	-26.52 ± 0.53	-25.61 ± 1.04
	2234	-26.32 ± 0.84	-26.53 ± 0.15	-25.62 ± 0.12	-25.52 ± 0.21	-26.12 ± 1.55	-25.81 ± 1.05	-24.48 ± 0.64	-23.78 ± 0.99
	2239	-26.47 ± 0.89	-26.98 ± 0.41	-26.06 ± 0.70	-26.62 ± 0.40	-26.69 ± 0.65	-26.84 ± 0.60	-27.38 ± 1.46	-25.86 ± 0.58
	Mean	-26.05	-26.05	-25.20	-25.13	-26.55	-26.09	-24.96	-23.74
	Variation (%)		0.00		+0.28		+1.73		+4.89
					$\Delta^{13}C$, ‰ DW				
CAN	2056	17.51 ± 0.27	17.25 ± 0.14	17.11 ± 0.41	16.80 ± 0.33	19.08 ± 0.61	17.95 ± 1.01	15.02 ± 1.25	13.15 ± 1.75
	2061	17.78 ± 0.26	17.78 ± 0.07	16.99 ± 0.13	16.28 ± 0.13	18.31 ± 0.49	17.00 ± 0.53	16.70 ± 1.43	14.06 ± 0.46
MAD	2210	18.83 ± 0.20	18.23 ± 0.52	18.38 ± 0.38	17.51 ± 0.68	**20.08 ± 0.42**	18.96 ± 0.38	19.70 ± 0.84	18.72 ± 0.82
	2216	18.58 ± 1.76	18.70 ± 0.37	18.00 ± 0.23	18.67 ± 0.27	18.30 ± 1.26	18.77 ± 1.59	17.13 ± 0.86	17.86 ± 0.41
SPC	2232	19.27 ± 0.28	19.28 ± 0.32	18.50 ± 0.35	18.07 ± 0.54	19.78 ± 0.40	**19.71 ± 1.15**	19.15 ± 0.46	18.33 ± 0.96
	2234	18.82 ± 0.88	19.04 ± 0.15	18.76 ± 0.15	18.50 ± 0.25	18.61 ± 1.62	18.29 ± 1.10	17.52 ± 0.62	16.98 ± 0.80
	2239	**18.98 ± 0.93**	**19.51 ± 0.43**	**19.13 ± 0.78**	**19.55 ± 0.39**	19.20 ± 0.68	19.36 ± 0.63	**20.47 ± 1.49**	**18.90 ± 0.59**
	Mean	18.54	18.54	18.13	17.91	19.05	18.58	17.96	16.86
	Variation (%)		0.00		-1.21		-2.47		-6.12

Table S1 (continued).

		δ^{15}N, ‰ DW							
CAN	2056	4.63 ± 0.51	4.77 ± 1.04	5.42 ± 0.47	5.98 ± 0.81	8.72 ± 2.36	6.75 ± 1.00	8.54 ± 1.41	9.98 ± 0.47
	2061	5.08 ± 0.71	4.06 ± 0.21	5.32 ± 0.56	5.92 ± 0.57	8.89 ± 1.05	7.40 ± 0.56	9.50 ± 0.82	9.38 ± 0.52
MAD	2210	4.28 ± 1.11	**5.31 ± 1.41**	**7.53 ± 0.62**	**6.67 ± 0.41**	**11.22 ± 0.95**	**10.20 ± 0.95**	10.75 ± 0.98	10.16 ± 0.66
	2216	4.89 ± 1.15	5.12 ± 0.23	5.71 ± 0.50	5.12 ± 0.28	9.14 ± 1.71	7.62 ± 0.19	9.25 ± 1.51	9.55 ± 1.61
SPC	2232	**5.10 ± 0.53**	4.70 ± 0.46	5.86 ± 0.54	5.16 ± 0.16	7.81 ± 1.99	7.09 ± 0.33	9.26 ± 1.24	8.90 ± 0.52
	2234	4.05 ± 0.94	3.38 ± 0.29	4.34 ± 0.58	3.29 ± 0.77	10.10 ± 4.09	6.32 ± 0.38	9.82 ± 1.36	7.91 ± 0.96
	2239	4.90 ± 1.17	4.67 ± 1.17	5.68 ± 0.22	5.60 ± 1.38	7.05 ± 1.27	7.25 ± 2.51	**13.96 ± 1.04**	**13.35 ± 1.20**
	Mean	4.71	4.57	5.69	5.39	8.99	7.52	10.15	9.89
	Variation (%)	-2.97		-5.27		-16.35 *		-2.56	

Data are expressed in dry weight basis (DW) and represents the mean ± SD of three independent lines replications per accession.
Variation is the difference between control and drought per constituent.
*,** Significant differences between control and drought conditions (One-way ANOVA, *$p \leq 0.05$; **$p \leq 0.01$).
Bold signalizes the maximum value. Underline signalizes the minimum value.
CAN, Canary Islands; MAD, Madeira Island; SPC, South Pacific Community.

Table S2

Pearson correlations of taro corms and shoots, from the 1st agronomic trial.

Origin	Constituents	Pt-corm	N-corm	St-corm	Fb-corm	M-corm	CaOx-corm	δ^{13}C-corm	Δ^{13}C-corm	δ^{15}N-corm	Pt-shoot	N-shoot	St-shoot	Fb-shoot	M-shoot	CaOx-shoot	δ^{13}C-shoot	Δ^{13}C-shoot
CANARY ISLANDS	N-corm	**1.00****	-															
	St-corm	**-0.72****	**-0.72****	-														
	Fb-corm	0.13	0.14	**-0.76****	-													
	M-corm	0.22	0.22	-0.56	**0.63***	-												
	CaOx-corm	0.24	0.23	-0.51	0.50	**0.72****	-											
	δ^{13}C-corm	0.20	0.19	-0.28	0.13	0.51	**0.70***	-										
	Δ^{13}C-corm	-0.20	-0.19	0.28	-0.13	-0.51	**-0.70***	**-1.00****	-									
	δ^{15}N-corm	0.18	0.18	-0.12	-0.08	-0.27	0.06	0.15	-0.15	-								
	Pt-shoot	0.42	0.42	**-0.78****	**0.62***	0.50	0.52	0.57	-0.57	0.13	-							
	N-shoot	0.45	0.45	**-0.79****	**0.61***	0.51	0.57	**0.59***	**-0.59***	0.19	**0.99****	-						
	St-shoot	-0.23	-0.25	0.45	-0.44	-0.12	0.24	0.50	-0.50	0.02	-0.16	-0.14	-					
	Fb-shoot	0.20	0.20	0.05	-0.24	0.10	-0.36	-0.30	0.30	-0.18	-0.42	-0.43	-0.44	-				
	M-shoot	0.04	0.04	-0.10	0.17	**0.60***	0.19	0.03	-0.03	-0.29	0.08	0.11	-0.31	0.31	-			
	CaOx-shoot	0.26	0.25	0.22	-0.51	-0.14	0.14	0.52	-0.52	0.40	-0.12	-0.06	**0.72****	-0.13	-0.17	-		
	δ^{13}C-shoot	0.44	0.45	-0.55	0.31	0.41	0.08	-0.12	0.12	-0.37	0.38	0.36	**-0.65***	0.44	0.45	**-0.59***	-	
	Δ^{13}C-shoot	-0.44	-0.45	0.55	-0.31	-0.41	-0.08	0.12	-0.12	0.37	-0.38	-0.36	**0.65***	-0.44	-0.45	**0.59***	**-1.00****	-
	δ^{15}N-shoot	0.24	0.24	0.02	-0.29	**-0.72****	-0.46	-0.13	0.13	0.56	-0.02	-0.03	0.12	-0.19	**-0.72****	0.40	-0.43	0.43
MADEIRA ISLAND	N-corm	**1.00****	-															
	S-corm	-0.03	-0.03	-														
	F-corm	0.01	0.01	**-0.93****	-													
	M-corm	0.36	0.36	-0.03	-0.05	-												
	CaOx-corm	-0.16	-0.17	0.00	0.04	-0.11	-											
	δ^{13}C-corm	0.31	0.31	-0.10	0.07	-0.20	0.55	-										
	Δ^{13}C-corm	-0.31	-0.31	0.10	-0.07	0.20	-0.55	**-1.00****	-									
	δ^{15}N-corm	0.19	0.19	-0.07	0.06	0.22	**0.70***	**0.58***	**-0.58***	-								
	P-shoot	0.20	0.20	-0.18	0.37	-0.45	0.40	0.42	-0.43	0.22	-							
	N-shoot	0.20	0.21	-0.19	0.37	-0.45	0.39	0.42	-0.42	0.22	**1.00****	-						
	S-shoot	**-0.80****	**-0.80****	0.08	-0.16	-0.47	0.02	-0.05	0.05	-0.28	-0.31	-0.31	-					
	F-shoot	0.70	0.70	-0.10	0.18	0.63	-0.07	0.17	-0.17	0.14	0.30	0.31	**-0.83***	-				
	M-shoot	-0.23	-0.23	0.24	-0.19	0.47	-0.31	**-0.73****	**0.73****	-0.08	-0.38	-0.37	-0.19	0.14	-			
	CaOx-shoot	**-0.58***	**-0.58***	-0.17	0.25	**-0.78****	-0.04	0.12	-0.12	-0.34	0.20	0.20	**0.59***	-0.63	-0.26	-		
	δ^{13}C-shoot	0.43	0.43	0.13	-0.13	0.26	-0.05	0.18	-0.18	0.31	0.11	0.10	-0.46	0.33	-0.00	-0.43	-	
	Δ^{13}C-shoot	-0.43	-0.43	-0.13	0.13	-0.26	0.05	-0.18	0.18	-0.31	-0.11	-0.11	0.46	-0.33	0.00	0.43	**-1.00****	-
	δ^{15}N-shoot	**-0.67***	**-0.67***	-0.11	0.20	**-0.60***	-0.07	0.06	-0.07	-0.33	0.08	0.08	**0.69***	-0.49	-0.21	**0.78****	-0.12	0.12

Table S2 (continued).

SOUTH PACIFIC COMMUNITY	N-corm	**0.66****	-															
	St-corm	**-0.60****	**-0.50***	-														
	Fb-corm	**0.60****	0.40	**-0.90****	-													
	M-corm	**0.78****	0.41	**-0.56***	**0.66****	-												
	CaOx-corm	0.30	0.23	0.10	**0.03**	0.13	-											
	δ^{13}C-corm	-0.11	0.03	0.32	**-0.55***	-0.34	0.16	-										
	Δ^{13}C-corm	0.11	-0.03	-0.32	**0.55***	0.34	-0.16	**-1.00****	-									
	δ^{15}N-corm	0.32	0.36	-0.29	0.22	0.28	-0.19	0.11	-0.11	-								
	Pt-shoot	-0.22	-0.14	-0.01	-0.03	-0.46	0.33	0.18	-0.18	-0.43	-							
	N-shoot	-0.22	-0.14	-0.01	-0.02	-0.46	0.33	0.18	-0.18	-0.43	**1.00****	-						
	St-shoot	-0.12	-0.25	0.08	-0.16	-0.12	-0.38	-0.16	0.16	-0.05	0.06	0.06	-					
	Fb-shoot	-0.36	-0.09	0.40	-0.24	-0.21	0.00	-0.10	0.10	0.28	-0.34	-0.34	**-0.49***	-				
	M-shoot	0.05	-0.24	-0.14	0.10	0.34	-0.27	-0.03	0.03	0.27	-0.41	-0.41	-0.07	0.12	-			
	CaOx-shoot	**-0.50***	-0.29	0.40	-0.42	**-0.64****	0.22	0.28	-0.28	-0.12	**0.64****	**0.64****	0.26	-0.01	**-0.52***	-		
	δ^{13}C-shoot	-0.29	-0.22	0.12	-0.08	-0.16	0.15	0.27	-0.27	-0.46	0.20	0.20	-0.43	0.19	-0.15	0.08	-	
	Δ^{13}C-shoot	0.29	0.22	-0.12	0.08	0.16	-0.15	-0.27	0.27	0.46	-0.20	-0.20	0.43	-0.19	0.15	-0.08	**-1.00****	-
	δ^{15}N-shoot	-0.20	-0.04	0.10	0.10	-0.37	0.13	-0.21	0.21	0.24	0.20	0.20	-0.24	**0.52***	-0.28	0.36	0.03	-0.03
OVERALL	N-corm	**0.85****	-															
	St-corm	**-0.46****	**-0.46****	-														
	Fb-corm	**0.45****	**0.39***	**-0.87****	-													
	M-corm	**0.55****	**0.43****	**-0.48****	**0.51****	-												
	CaOx-corm	-0.10	-0.06	0.02	-0.07	0.11	-											
	δ^{13}C-corm	0.07	0.02	0.07	-0.20	-0.05	**0.40****	-										
	Δ^{13}C-corm	-0.07	-0.02	-0.07	0.20	0.05	**-0.40****	**-1.00****	-									
	δ^{15}N-corm	0.29	**0.31***	-0.24	0.19	0.23	-0.02	0.26	-0.26	-								
	Pt-shoot	0.25	0.19	-0.27	**0.33***	-0.17	0.07	0.04	-0.04	-0.08	-							
	N-shoot	0.25	0.19	-0.27	**0.33***	-0.17	0.08	0.05	-0.05	-0.07	**1.00****	-						
	St-shoot	**-0.49****	**-0.46****	0.27	**-0.35***	-0.24	0.20	0.22	-0.22	-0.12	-0.25	-0.24	-					
	Fb-shoot	0.04	0.11	0.22	-0.13	-0.10	-0.29	**-0.38***	**0.38***	0.03	-0.18	-0.18	**-0.51****	-				
	M-shoot	0.03	-0.08	-0.09	0.13	**0.34***	-0.29	**-0.51****	**0.51****	0.06	-0.14	-0.14	-0.28	0.32	-			
	CaOx-shoot	**-0.39***	-0.30	0.30	**-0.37***	**-0.56****	0.19	0.03	-0.03	-0.09	0.18	0.20	**0.52****	-0.02	-0.27	-		
	δ^{13}C-shoot	0.06	0.00	0.01	-0.10	0.07	0.23	**0.41****	**-0.41****	-0.15	0.05	0.05	-0.27	0.05	-0.20	-0.25	-	
	Δ^{13}C-shoot	-0.06	0.00	-0.01	0.10	-0.07	-0.23	**-0.41****	**0.41****	0.14	-0.05	-0.05	0.27	-0.05	0.20	0.25	**-1.00****	-
	δ^{15}N-shoot	-0.01	0.00	-0.08	0.23	-0.29	-0.19	-0.02	0.02	0.21	0.29	0.29	-0.02	0.12	-0.23	0.25	-0.11	0.11

Pt, protein (g/100g, DW); N, nitrogen (g/100g, DW); St, starch (g/100g, DW); Fb, crude fiber (g/100g, DW); M, total minerals (g/100g, DW); CaOx, calcium oxalate (g/100g, DW); δ^{13}C, carbon isotopic composition (‰, DW); Δ^{13}C, carbon isotope discrimination (‰, DW); δ^{15}N, nitrogen isotopic composition (‰, DW).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table S3

Sweet potato quality constituents of the flour obtained by laboratory reference analysis (1st trial) and NIRS prediction (2nd trial) for both tuber and shoot organs, under control and drought conditions.

		Tuber organs				Shoot organs			
		1st trial 2017		2nd trial 2018		1st trial 2017		2nd trial 2018	
Sweet potato Acc.		Control	Drought	Control	Drought	Control	Drought	Control	Drought
					Moisture, g/100g DW				
MAD	1036	3.60 ± 0.24	3.18 ± 0.00	4.50 ± 0.24	4.52 ± 0.12	6.21 ± 2.36	6.41 ± 0.00	4.74 ± 0.44	4.50 ± 0.11
	1038	4.23 ± 0.91	3.29 ± 0.24	5.74 ± 0.22	4.12 ± 0.72	5.36 ± 1.81	6.47 ± 0.86	6.04 ± 0.60	**7.07 ± 1.03**
	2927	5.04 ± 0.59	5.27 ± 1.62	5.70 ± 0.42	**5.87 ± 0.43**	4.82 ± 0.97	**6.64 ± 1.23**	4.17 ± 0.28	4.57 ± 0.62
	3126	**6.16 ± 1.19**	5.67 ± 1.34	4.22 ± 0.94	3.53 ± 0.66	6.02 ± 1.74	5.23 ± 1.05	4.33 ± 0.60	5.45 ± 0.16
CAN	2937	5.41 ± 1.61	4.82 ± 0.00	**7.43 ± 0.54**	4.91 ± 1.55	**6.86 ± 1.12**	5.61 ± 0.00	3.73 ± 1.00	6.48 ± 0.39
	2938	5.37 ± 0.93	4.93 ± 0.17	4.15 ± 0.59	4.32 ± 1.25	5.49 ± 0.61	6.36 ± 0.40	3.02 ± 1.17	4.07 ± 1.07
GUI	3124	3.94 ± 1.22	5.63 ± 2.37	3.92 ± 0.19	3.16 ± 0.62	5.78 ± 1.17	5.85 ± 0.50	4.08 ± 0.35	4.27 ± 0.72
	3125	6.01 ± 1.23	**7.35 ± 1.67**	6.03 ± 1.19	3.73 ± 1.17	6.35 ± 0.88	**6.64 ± 0.68**	**7.04 ± 0.86**	5.03 ± 0.40
	Mean	4.97	5.02	5.21	4.27	5.86	6.15	4.64	5.18
	Variation (%)	+1.01		-18.04 *		+4.95		+11.64	
					Protein, g/100g DW				
MAD	1036	6.20 ± 0.58	**9.27 ± 0.17**	4.91 ± 0.81	**10.10 ± 0.03**	14.51 ± 1.33	16.41 ± 0.01	15.08 ± 1.89	17.18 ± 0.05
	1038	6.86 ± 0.54	4.28 ± 0.49	**5.49 ± 1.03**	4.11 ± 0.44	17.56 ± 0.58	12.79 ± 1.29	17.80 ± 0.87	15.10 ± 1.30
	2927	6.16 ± 0.79	7.10 ± 0.62	4.34 ± 0.42	6.78 ± 0.30	15.47 ± 2.30	10.42 ± 0.57	15.07 ± 1.16	11.06 ± 0.36
	3126	**7.42 ± 1.10**	6.14 ± 0.63	5.02 ± 0.27	6.54 ± 0.75	**20.50 ± 0.65**	**16.59 ± 0.94**	**18.76 ± 0.45**	17.08 ± 1.34
CAN	2937	6.82 ± 1.36	4.90 ± 0.06	5.33 ± 0.85	5.68 ± 0.56	12.73 ± 0.25	8.46 ± 0.05	16.31 ± 0.55	10.22 ± 1.20
	2938	5.39 ± 0.52	5.00 ± 0.08	4.23 ± 0.37	8.76 ± 1.63	15.71 ± 1.18	10.54 ± 0.36	15.92 ± 1.87	**18.72 ± 1.18**
GUI	3124	4.37 ± 0.94	4.57 ± 0.06	2.89 ± 0.16	4.56 ± 0.16	18.87 ± 2.36	15.97 ± 1.97	15.21 ± 0.79	13.11 ± 0.71
	3125	6.92 ± 0.55	6.33 ± 1.17	5.20 ± 1.48	4.80 ± 0.55	15.58 ± 1.68	13.13 ± 0.92	15.88 ± 1.27	14.10 ± 1.05
	Mean	6.27	5.95	4.68	6.42	16.36	13.04	16.25	14.57
	Variation (%)	-5.10		+37.18 **		-20.29 **		-10.34 *	
					Nitrogen, g/100g DW				
MAD	1036	0.99 ± 0.09	**1.48 ± 0.04**	0.78 ± 0.13	**1.62 ± 0.01**	2.32 ± 0.00	2.58 ± 0.05	2.41 ± 0.30	2.75 ± 0.01
	1038	1.10 ± 0.09	0.68 ± 0.08	**0.88 ± 0.17**	0.66 ± 0.07	2.81 ± 0.08	2.05 ± 0.19	2.85 ± 0.14	2.42 ± 0.21
	2927	0.99 ± 0.13	1.14 ± 0.10	0.69 ± 0.07	1.09 ± 0.05	2.48 ± 0.33	1.67 ± 0.08	2.41 ± 0.19	1.77 ± 0.06
	3126	**1.19 ± 0.18**	0.98 ± 0.10	0.80 ± 0.04	1.05 ± 0.12	**3.28 ± 0.10**	**2.65 ± 0.14**	**3.00 ± 0.07**	2.73 ± 0.21
CAN	2937	1.09 ± 0.22	0.78 ± 0.01	0.85 ± 0.14	0.91 ± 0.09	2.04 ± 0.04	1.50 ± 0.17	2.61 ± 0.09	1.64 ± 0.19
	2938	0.86 ± 0.08	0.61 ± 0.24	0.67 ± 0.06	1.41 ± 0.26	2.51 ± 0.22	1.69 ± 0.07	2.55 ± 0.30	**3.00 ± 0.19**
GUI	3124	0.70 ± 0.15	0.73 ± 0.01	0.46 ± 0.03	0.73 ± 0.03	3.02 ± 0.34	2.56 ± 0.28	2.43 ± 0.13	2.10 ± 0.11
	3125	1.11 ± 0.09	1.01 ± 0.19	0.84 ± 0.24	0.77 ± 0.09	2.49 ± 0.24	2.10 ± 0.13	2.54 ± 0.20	2.26 ± 0.17
	Mean	1.00	0.93	0.75	1.03	2.62	2.10	2.60	2.33
	Variation (%)	-7.00		+37.33 **		-19.85 **		-10.38 *	
					Starch, g/100g DW				
MAD	1036	44.22 ± 2.01	38.24 ± 1.40	**47.90 ± 0.43**	**47.90 ± 0.04**	4.04 ± 0.74	7.52 ± 0.77	1.80 ± 0.49	2.65 ± 0.01
	1038	46.65 ± 2.46	45.67 ± 0.45	41.32 ± 4.06	38.13 ± 6.63	1.73 ± 0.30	4.92 ± 1.05	2.22 ± 0.37	3.55 ± 1.25
	2927	38.28 ± 3.16	37.54 ± 0.68	40.85 ± 3.50	32.77 ± 1.20	2.93 ± 0.52	4.49 ± 0.29	4.41 ± 0.55	2.55 ± 1.56
	3126	46.51 ± 2.02	41.28 ± 2.04	44.67 ± 1.21	32.67 ± 3.63	1.12 ± 0.41	4.84 ± 1.31	2.92 ± 0.52	1.41 ± 0.84
CAN	2937	**48.18 ± 2.07**	44.06 ± 4.14	42.72 ± 5.46	44.38 ± 2.29	**6.57 ± 1.49**	**16.93 ± 0.98**	2.45 ± 0.21	5.35 ± 0.68
	2938	43.01 ± 0.44	43.79 ± 2.82	43.72 ± 2.40	33.54 ± 4.50	4.12 ± 0.08	8.61 ± 1.41	2.20 ± 0.48	3.19 ± 0.28
GUI	3124	38.21 ± 3.00	**48.44 ± 1.58**	40.17 ± 1.66	44.26 ± 1.60	3.50 ± 0.43	3.48 ± 0.35	5.01 ± 0.38	5.28 ± 1.54
	3125	42.04 ± 0.56	39.60 ± 0.59	31.15 ± 2.18	31.88 ± 2.19	5.81 ± 1.32	5.96 ± 1.53	**6.05 ± 0.66**	**6.75 ± 1.56**
	Mean	43.39	42.33	41.56	38.19	3.73	7.09	3.38	3.84
	Variation (%)	-2.44		-8.11		+90.08 **		+13.61	

Table S3 (continued).

Minerals, g/100g DW									
MAD	1036	4.17 ± 0.20	4.52 ± 0.06	3.69 ± 0.15	4.07 ± 0.03	11.12 ± 0.64	10.98 ± 0.22	13.67 ± 0.56	12.85 ± 0.04
	1038	4.03 ± 0.28	4.07 ± 0.39	**4.29 ± 0.20**	4.10 ± 0.25	13.80 ± 0.13	11.77 ± 0.66	14.56 ± 0.27	12.71 ± 0.31
	2927	4.58 ± 0.59	4.46 ± 0.06	3.95 ± 0.14	4.08 ± 0.15	11.63 ± 1.44	9.89 ± 0.65	12.70 ± 0.45	11.32 ± 0.20
	3126	3.71 ± 0.32	3.96 ± 0.06	3.64 ± 0.13	3.69 ± 0.27	**14.18 ± 1.26**	11.86 ± 0.46	**15.14 ± 0.23**	13.05 ± 0.11
CAN	2937	4.01 ± 0.37	3.70 ± 0.02	3.94 ± 0.07	3.83 ± 0.16	9.57 ± 1.01	7.58 ± 0.00	13.97 ± 0.25	10.46 ± 0.78
	2938	4.39 ± 0.17	4.40 ± 0.03	3.82 ± 0.21	3.98 ± 0.28	11.70 ± 0.34	8.89 ± 1.01	13.98 ± 0.58	**13.20 ± 0.38**
GUI	3124	3.93 ± 0.80	4.07 ± 0.22	3.38 ± 0.15	3.56 ± 0.14	12.91 ± 1.06	**12.88 ± 0.87**	11.52 ± 0.77	10.84 ± 0.11
	3125	**4.89 ± 0.57**	**5.04 ± 0.28**	4.09 ± 0.50	**4.16 ± 0.05**	12.32 ± 1.43	10.30 ± 0.51	12.27 ± 0.48	11.32 ± 0.42
	Mean	4.21	4.28	3.85	3.93	12.15	10.52	13.48	11.97
	Variation (%)	+1.66		+2.08		-13.42 **		-11.20 **	
CaOx, mg/100g DW									
MAD	1036	15.83 ± 7.56	15.34 ± 1.81	27.16 ± 11.57	39.61 ± 19.08	155.75 ± 17.00	69.42 ± 12.33	-	40.26 ± 24.24
	1038	43.73 ± 17.56	35.84 ± 6.26	23.85 ± 11.87	8.39 ± 3.09	43.81 ± 16.12	36.72 ± 8.82	-	40.50 ± 2.95
	2927	37.75 ± 6.25	28.26 ± 19.37	16.59 ± 9.69	31.91 ± 7.81	36.70 ± 8.19	28.53 ± 20.20	**70.01 ± 31.82**	-
	3126	76.13 ± 11.98	59.59 ± 6.48	16.17 ± 5.17	66.11 ± 26.84	89.95 ± 12.21	116.76 ± 10.07	25.80 ± 18.33	32.68 ± 2.90
CAN	2937	59.37 ± 12.90	78.59 ± 10.52	46.18 ± 15.89	29.16 ± 25.62	**221.98 ± 20.10**	214.77 ± 28.51	26.83 ± 3.09	47.84 ± 2.20
	2938	47.02 ± 18.76	**85.19 ± 28.12**	8.63 ± 13.13	**74.60 ± 30.18**	144.69 ± 40.82	**245.57 ± 11.87**	39.50 ± 2.17	**105.27 ± 17.89**
GUI	3124	**173.42 ± 22.01**	66.97 ± 5.57	101.54 ± 2.18	54.66 ± 12.72	49.44 ± 27.68	56.12 ± 30.04	68.36 ± 31.53	70.16 ± 58.35
	3125	88.77 ± 17.84	54.17 ± 14.57	**102.75 ± 5.70**	71.72 ± 12.88	47.75 ± 4.38	17.02 ± 3.53	32.76 ± 15.96	57.71 ± 20.32
	Mean	67.75	52.99	42.86	47.02	98.76	98.11	43.87	56.35
	Variation (%)	-21.79		+9.71		-0.66		+28.45	
Total carbon, % DW									
MAD	1036	**40.89 ± 0.17**	**41.15 ± 0.00**	40.72 ± 0.20	**41.40 ± 0.03**	41.44 ± 0.18	41.28 ± 0.00	40.72 ± 0.35	40.15 ± 0.04
	1038	40.51 ± 0.22	40.59 ± 0.18	40.34 ± 0.05	40.33 ± 0.19	40.02 ± 0.76	40.63 ± 0.25	40.43 ± 0.14	40.87 ± 0.19
	2927	40.38 ± 0.42	40.80 ± 0.31	40.57 ± 0.30	40.84 ± 0.17	41.49 ± 0.15	41.15 ± 0.05	40.69 ± 0.23	40.10 ± 0.49
	3126	40.13 ± 0.25	40.31 ± 0.11	40.63 ± 0.18	40.73 ± 0.18	41.07 ± 0.13	41.32 ± 0.27	40.41 ± 0.12	40.73 ± 0.12
CAN	2937	40.29 ± 0.20	40.39 ± 0.00	39.93 ± 0.06	40.51 ± 0.17	**41.98 ± 0.16**	42.23 ± 0.00	40.63 ± 0.07	41.09 ± 0.63
	2938	39.91 ± 0.28	40.06 ± 0.01	**40.80 ± 0.32**	41.12 ± 0.27	41.27 ± 0.20	41.94 ± 0.70	40.76 ± 0.44	40.28 ± 0.32
GUI	3124	40.34 ± 0.26	40.12 ± 0.08	40.55 ± 0.23	40.69 ± 0.09	41.53 ± 1.10	41.44 ± 0.38	**41.83 ± 0.41**	**41.80 ± 0.16**
	3125	40.24 ± 0.27	39.94 ± 0.38	40.41 ± 0.16	41.06 ± 0.11	41.39 ± 0.82	**42.34 ± 0.29**	40.98 ± 0.28	40.98 ± 0.40
	Mean	40.34	40.42	40.49	40.83	41.27	41.54	40.81	40.75
	Variation (%)	+0.20		+0.84		+0.65		-0.15	
$\delta^{13}C$, ‰ DW									
MAD	1036	-26.77 ± 0.62	-24.19 ± 0.00	-27.60 ± 0.60	-27.93 ± 0.07	-27.89 ± 0.40	-25.22 ± 0.00	-29.14 ± 1.12	-25.89 ± 0.02
	1038	-25.66 ± 0.38	-25.18 ± 0.87	**-26.19 ± 0.19**	-26.26 ± 0.61	-27.29 ± 0.31	-26.81 ± 0.91	-27.48 ± 0.40	-25.54 ± 0.13
	2927	-25.42 ± 0.05	-24.37 ± 0.34	-27.56 ± 0.31	-26.33 ± 0.25	-27.37 ± 0.26	-25.72 ± 0.34	-26.56 ± 0.94	-26.59 ± 0.64
	3126	**-24.63 ± 0.28**	**-22.51 ± 0.17**	-26.41 ± 0.41	**-26.01 ± 1.03**	**-26.51 ± 0.25**	**-23.63 ± 0.47**	-27.05 ± 0.23	-24.87 ± 0.61
CAN	2937	-25.33 ± 0.49	-24.05 ± 0.00	-27.82 ± 0.60	-26.89 ± 0.51	-27.48 ± 0.54	-26.12 ± 0.00	-28.44 ± 0.53	-27.63 ± 0.46
	2938	-25.55 ± 0.15	-23.43 ± 0.02	-26.41 ± 0.44	-28.97 ± 0.32	-27.21 ± 0.21	-25.79 ± 1.23	-28.77 ± 0.66	-24.83 ± 0.24
GUI	3124	-26.14 ± 0.18	-25.78 ± 0.27	-26.54 ± 0.40	-27.05 ± 0.74	-27.65 ± 0.43	-27.73 ± 0.35	-26.59 ± 0.15	-27.15 ± 0.90
	3125	-25.59 ± 0.37	-25.19 ± 0.30	-27.01 ± 0.74	-26.85 ± 0.64	-27.22 ± 0.26	-26.40 ± 0.34	**-24.89 ± 0.10**	**-24.60 ± 0.40**
	Mean	-25.64	-24.34	-26.94	-27.03	-27.33	-25.93	-27.37	-25.89
	Variation (%)	+5.07 **		-0.33		+5.12 **		+5.41 **	

Table S3 (continued).

		$\Delta^{13}C$, ‰ DW							
MAD	1036	**19.29 ± 0.65**	16.59 ± 0.00	20.15 ± 0.63	20.50 ± 0.07	**20.47 ± 0.42**	17.67 ± 0.00	**21.77 ± 1.18**	18.36 ± 0.02
	1038	18.12 ± 0.40	17.62 ± 0.91	18.68 ± 0.20	18.75 ± 0.63	19.83 ± 0.32	19.33 ± 0.95	20.03 ± 0.42	18.00 ± 0.14
	2927	17.88 ± 0.05	16.78 ± 0.05	20.11 ± 0.33	18.83 ± 0.26	19.92 ± 0.27	18.19 ± 0.36	19.06 ± 0.98	19.10 ± 0.67
	3126	17.06 ± 0.29	14.85 ± 0.17	18.91 ± 0.43	18.49 ± 1.08	19.02 ± 0.26	16.00 ± 0.49	19.58 ± 0.24	17.31 ± 0.64
CAN	2937	17.78 ± 0.51	16.44 ± 0.00	**20.39 ± 0.63**	19.41 ± 0.53	20.03 ± 0.56	18.60 ± 0.00	21.04 ± 0.55	**20.18 ± 0.48**
	2938	18.01 ± 0.15	15.80 ± 0.02	18.91 ± 0.47	**21.58 ± 0.33**	19.74 ± 0.22	17.52 ± 0.07	21.38 ± 0.69	17.26 ± 0.25
GUI	3124	18.63 ± 0.19	**18.25 ± 0.28**	19.04 ± 0.42	19.58 ± 0.77	20.21 ± 0.46	**20.29 ± 0.37**	19.10 ± 0.16	19.68 ± 0.94
	3125	18.05 ± 0.38	17.63 ± 0.31	19.53 ± 0.78	19.36 ± 0.67	19.76 ± 0.27	18.90 ± 0.36	17.32 ± 0.10	17.01 ± 0.47
	Mean	18.10	16.74	19.46	19.56	19.87	18.31	19.91	18.36
	Variation (%)	-7.51 **		+0.51		-7.85 **		-7.79 **	
		$\delta^{15}N$, ‰ DW							
MAD	1036	4.68 ± 0.41	2.96 ± 0.00	4.41 ± 0.84	4.87 ± 0.14	4.70 ± 0.11	3.63 ± 0.00	3.24 ± 0.48	3.29 ± 0.02
	1038	6.00 ± 0.57	3.67 ± 0.20	4.01 ± 0.48	3.67 ± 0.27	**6.17 ± 0.03**	3.10 ± 0.54	**5.27 ± 0.13**	2.64 ± 0.50
	2927	5.86 ± 1.09	4.08 ± 0.18	4.03 ± 0.33	4.37 ± 0.34	5.30 ± 0.96	3.37 ± 0.25	4.04 ± 0.28	1.55 ± 0.48
	3126	4.90 ± 0.84	3.32 ± 0.53	3.95 ± 0.43	5.08 ± 1.51	4.96 ± 0.80	2.97 ± 0.32	3.91 ± 0.04	1.67 ± 0.46
CAN	2937	3.96 ± 0.46	3.13 ± 0.00	4.75 ± 0.15	3.67 ± 0.81	4.20 ± 0.43	2.53 ± 0.00	4.27 ± 0.18	0.95 ± 0.71
	2938	4.79 ± 0.68	3.61 ± 0.73	**5.12 ± 0.29**	**7.38 ± 0.52**	5.46 ± 0.38	3.98 ± 1.45	4.17 ± 0.44	3.16 ± 0.25
GUI	3124	5.31 ± 1.30	**4.36 ± 0.02**	3.83 ± 0.18	4.93 ± 0.49	5.37 ± 0.83	**4.27 ± 0.29**	5.11 ± 0.44	3.76 ± 0.49
	3125	**6.37 ± 0.92**	4.06 ± 0.23	4.05 ± 0.93	3.93 ± 0.28	5.81 ± 1.37	3.83 ± 0.44	4.79 ± 0.46	**3.94 ± 0.42**
	Mean	5.23	3.65	4.27	4.74	5.25	3.46	4.35	2.62
	Variation (%)	-30.21 **		+11.01		-34.10 **		-39.77 **	

Data are expressed in dry weight basis (DW) and represents the mean ± SD of three independent lines replications per accession.
Variation is the difference between control and drought per constituent.
*,** Significant differences between control and drought conditions (One-way ANOVA, *$p \leq 0.05$; **$p \leq 0.01$).
Bold signalizes the maximum value. Underline signalizes the minimum value.
CAN, Canary Islands; MAD, Madeira Island; GUI, Guinea-Bissau.

Table S4

Pearson correlations of sweet potato tubers and shoots, from the 1st agronomic trial.

Origin	Constituents	Pt-tuber	N-tuber	St-tuber	M-tuber	CaOx-tuber	δ^{13}C-tuber	Δ^{13}C-tuber	δ^{15}N-tuber	TC-tuber	Pt-shoot	N-shoot	St-shoot	M-shoot	CaOx-shoot	δ^{13}C-shoot	Δ^{13}C-shoot	δ^{15}N-shoot
MADEIRA ISLAND	N-tuber	**0.99****	-															
	St-tuber	-0.39	-0.38	-														
	M-tuber	0.31	0.31	**-0.76****	-													
	CaOx-tuber	-0.08	-0.05	0.39	**-0.48***	-												
	δ^{13}C-tuber	0.18	0.19	-0.32	-0.10	0.32	-											
	Δ^{13}C-tuber	-0.18	-0.19	0.32	0.10	-0.32	**-1.00****	-										
	δ^{15}N-tuber	-0.05	-0.06	0.15	0.16	0.18	**-0.54****	**0.54****	-									
	TC-tuber	0.31	0.31	-0.23	0.22	**-0.78****	-0.17	0.17	**-0.47***	-								
	Pt-shoot	0.35	0.35	0.35	-0.28	**0.57****	0.06	-0.06	0.33	**-0.47***	-							
	N-shoot	0.34	0.34	0.34	-0.27	**0.58****	0.04	-0.04	0.35	**-0.49***	**1.00****	-						
	St-shoot	0.20	0.19	**-0.48***	0.34	**-0.56****	0.35	-0.35	**-0.76****	**0.63****	**-0.47***	**-0.50***	-					
	M-shoot	0.10	0.08	**0.58****	-0.35	**0.65****	-0.10	0.10	**0.54****	**-0.60****	**0.82****	**0.82****	**-0.69****	-				
	CaOx-shoot	0.05	0.09	0.21	-0.27	0.10	-0.09	0.09	-0.16	0.00	0.32	0.32	0.02	0.05	-			
	δ^{13}C-shoot	0.21	0.22	-0.38	-0.05	0.20	**0.94****	**-0.94****	**-0.62****	-0.05	0.01	-0.01	**0.47***	-0.22	0.07	-		
	Δ^{13}C-shoot	-0.21	-0.22	0.38	0.05	-0.20	**-0.94****	**0.94****	**0.62****	0.05	-0.01	0.01	**-0.47***	0.22	-0.07	**-1.00****	-	
	δ^{15}N-shoot	0.20	0.19	0.26	0.12	0.18	**-0.57****	**0.57****	**0.90****	-0.31	**0.53****	**0.54****	**-0.68****	**0.63****	-0.02	**-0.64****	**0.64****	-
	TC-shoot	0.17	0.18	**-0.59****	0.34	-0.12	0.15	-0.15	-0.21	0.04	-0.06	-0.05	0.26	**-0.43***	0.40	0.26	-0.26	-0.27
CANARY ISLANDS	N-tuber	**0.99****	-															
	St-tuber	0.48	0.50	-														
	M-tuber	0.14	0.11	-0.29	-													
	CaOx-tuber	-0.24	-0.23	-0.24	-0.12	-												
	δ^{13}C-tuber	**-0.63***	**-0.65***	-0.24	-0.10	**0.66***	-											
	Δ^{13}C-tuber	**0.63***	**0.64***	0.24	0.11	**-0.66***	**-1.00****	-										
	δ^{15}N-tuber	0.36	0.34	-0.13	0.48	-0.40	**-0.66***	**0.66***	-									
	TC-tuber	0.04	0.07	0.18	**-0.59***	0.41	0.25	-0.25	**-0.64***	-								
	Pt-shoot	0.35	0.33	-0.03	0.57	**-0.64***	**-0.79****	**0.79****	**0.82****	**-0.66***	-							
	N-shoot	0.39	0.37	-0.04	**0.59***	**-0.65***	**-0.79****	**0.79****	**0.85****	**-0.59***	**0.98****	-						
	St-shoot	-0.37	-0.35	-0.04	**-0.62***	0.45	**0.58***	**-0.59***	**-0.78****	**0.64***	**-0.88****	**-0.88****	-					
	M-shoot	0.40	0.39	-0.14	**0.62***	-0.47	**-0.78****	**0.78****	**0.87****	**-0.63***	**0.94****	**0.94****	**-0.83****	-				
	CaOx-shoot	0.05	0.00	0.20	-0.21	0.24	**0.58***	**-0.58***	-0.50	0.12	**-0.58***	**-0.58***	0.34	**-0.65***	-			
	δ^{13}C-shoot	**-0.59***	**-0.61***	-0.35	0.01	0.50	**0.78****	**-0.78****	-0.48	0.11	-0.57	-0.53	0.50	-0.48	0.38	-		
	Δ^{13}C-shoot	**0.64***	**0.67***	0.38	-0.15	**-0.61***	**-0.95****	**0.95****	0.51	-0.02	**0.61***	**0.62***	-0.45	**0.58***	-0.50	**-0.810****	-	
	δ^{15}N-shoot	0.30	0.28	-0.15	**0.65***	-0.35	-0.57	0.58	**0.78****	**-0.65***	**0.85****	**0.80****	**-0.82****	**0.82****	-0.45	**-0.64***	0.38	-
	TC-shoot	-0.02	-0.02	0.37	-0.50	0.16	0.44	-0.44	**-0.67***	0.41	**-0.68***	**-0.64***	**0.62***	**-0.69***	**0.68***	0.56	-0.29	**-0.88****

Table S4 (continued).

GUINEA-BISSAU	N-tuber	**0.99****	-														
	St-tuber	-0.29	-0.34	-													
	M-tuber	**0.87****	**0.88****	-0.34	-												
	CaOx-tuber	-0.45	-0.42	-0.42	-0.54	-											
	δ^{13}C-tuber	**0.62***	**0.61***	0.02	**0.68***	**-0.68***	-										
	Δ^{13}C-tuber	**-0.62***	**-0.61***	-0.02	**-0.68***	**0.68***	**-1.00****	-									
	δ^{15}N-tuber	0.46	0.49	-0.30	0.26	0.32	-0.28	0.28	-								
	TC-tuber	-0.26	-0.25	0.03	-0.45	0.40	-0.36	0.36	0.05	-							
	Pt-shoot	-0.51	-0.48	-0.05	**-0.64***	**0.81****	**-0.63***	**0.63***	0.22	0.29	-						
	N-shoot	-0.49	-0.46	-0.04	**-0.63***	**0.80****	**-0.62***	**0.62***	0.23	0.29	**1.00****	-					
	St-shoot	0.54	0.52	-0.24	0.47	-0.42	0.46	-0.46	0.10	-0.34	**-0.64***	**-0.65***	-				
	M-shoot	-0.39	-0.36	0.31	-0.49	0.49	-0.45	0.44	0.23	0.29	**0.86****	**0.87****	**-0.73****	-			
	CaOx-shoot	-0.15	-0.15	0.25	-0.18	0.19	-0.52	0.51	0.40	0.16	0.24	0.25	-0.35	0.40	-		
	δ^{13}C-shoot	**0.69***	**0.68***	-0.48	**0.69***	-0.52	**0.67***	**-0.66***	-0.07	-0.35	**-0.69***	**-0.68***	**0.63***	**-0.78****	-0.55	-	
	Δ^{13}C-shoot	**-0.69***	**-0.68***	0.48	**-0.69***	0.52	**-0.67***	**0.66***	0.07	0.35	**0.68***	**0.68***	**-0.63***	**0.78****	0.54	**-1.00****	-
	δ^{15}N-shoot	0.19	0.23	-0.29	0.17	0.33	-0.17	0.17	**0.58***	0.29	0.33	0.34	-0.06	0.41	0.44	-0.20	0.20
	TC-shoot	-0.01	-0.06	-0.18	0.13	-0.28	0.16	-0.16	-0.52	0.05	-0.58	**-0.59***	0.43	**-0.74****	-0.36	0.44	-0.44
OVERALL	N-tuber	**0.99****	-														
	St-tuber	-0.26	-0.28	-													
	M-tuber	**0.36***	**0.35***	**-0.51****	-												
	CaOx-tuber	**-0.40****	**-0.38****	-0.04	-0.21	-											
	δ^{13}C-tuber	0.12	0.14	-0.15	-0.08	-0.07	-										
	Δ^{13}C-tuber	-0.12	-0.14	0.15	0.08	0.07	**-1.00****	-									
	δ^{15}N-tuber	0.11	0.10	-0.10	**0.31***	0.21	**-0.56****	**0.56****	-								
	TC-tuber	**0.34***	**0.36***	-0.17	-0.16	**-0.40****	-0.01	0.01	**-0.29***	-							
	Pt-shoot	0.23	0.22	0.01	-0.10	0.24	-0.28	0.28	**0.47****	-0.11	-						
	N-shoot	0.24	0.23	0.01	-0.09	0.23	-0.28	0.28	**0.48****	-0.11	**1.00****	-					
	St-shoot	-0.15	-0.13	-0.01	-0.07	0.06	**0.39****	**-0.39****	**-0.53****	0.05	**-0.71****	**-0.72****	-				
	M-shoot	0.16	0.15	0.10	-0.04	0.16	**-0.36***	**0.36***	**0.57****	-0.10	**0.89****	**0.90****	**-0.79****	-			
	CaOx-shoot	-0.13	-0.11	**0.34***	-0.26	0.05	0.26	-0.26	**-0.34***	-0.17	**-0.39****	**-0.40****	**0.53****	**-0.56****	-		
	δ^{13}C-shoot	0.24	0.26	**-0.33***	0.02	-0.16	**0.88****	**-0.88****	**-0.51****	0.11	-0.16	-0.17	0.28	-0.26	0.09	-	
	Δ^{13}C-shoot	-0.20	-0.22	**0.32***	-0.03	0.14	**-0.93****	**0.92****	**0.53****	-0.07	0.21	0.22	**-0.31***	**0.32***	-0.16	**-0.97****	-
	δ^{15}N-shoot	0.16	0.16	-0.01	**0.29***	0.19	**-0.55****	**0.55****	**0.80****	-0.24	**0.56****	**0.56****	**-0.55****	**0.59****	-0.20	**-0.57****	**0.53****
	TC-shoot	-0.15	-0.16	-0.14	0.13	0.17	0.11	-0.11	**-0.33***	-0.23	**-0.41****	**-0.41****	**0.51****	**-0.60****	**0.39****	0.12	-0.11

Pt, protein (g/100g, DW); N, nitrogen (g/100g, DW); St, starch (g/100g, DW); M, total minerals (g/100g, DW); CaOx, calcium oxalate (g/100g, DW); δ^{13}C, carbon isotopic composition (‰, DW); Δ^{13}C, carbon isotope discrimination (‰, DW); δ^{15}N, nitrogen isotopic composition (‰, DW); TC, total carbon (%, DW).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table S5

Pearson correlations of taro corms and shoots, from the 2nd agronomic trial.

Origin	Constituents	Pt-corm	N-corm	St-corm	Fb-corm	M-corm	CaOx-corm	δ^{13}C-corm	Δ^{13}C-corm	δ^{15}N-corm	Pt-shoot	N-shoot	St-shoot	Fb-shoot	M-shoot	CaOx-shoot	δ^{13}C-shoot	Δ^{13}C-shoot
CANARY ISLANDS	N-corm	**1.00****	-															
	St-corm	**-0.64***	**-0.63***	-														
	Fb-corm	**0.72****	**0.71***	**-0.97****	-													
	M-corm	**0.79****	**0.78****	**-0.93****	**0.97****	-												
	CaOx-corm	**0.83****	**0.82****	**-0.91****	**0.95****	**0.95****	-											
	δ^{13}C-corm	**0.68***	**0.69***	-0.19	0.26	0.40	0.39	-										
	Δ^{13}C-corm	**-0.79****	**-0.79****	0.37	-0.43	**-0.59***	-0.56	**-0.97****	-									
	δ^{15}N-corm	0.45	0.43	**-0.88****	**0.81****	**0.80****	**0.79****	-0.01	-0.20	-								
	Pt-shoot	0.12	0.11	-0.39	0.41	0.35	0.42	-0.42	0.26	0.53	-							
	N-shoot	0.15	0.13	-0.42	0.43	0.37	0.44	-0.41	0.26	0.52	**1.00****	-						
	St-shoot	**-0.63***	**-0.64***	0.14	-0.15	-0.28	-0.29	**-0.80****	**0.79****	0.01	**0.59***	**0.58***	-					
	Fb-shoot	0.44	0.45	-0.10	0.09	0.16	0.17	**0.77****	**-0.69***	-0.08	**-0.76****	**-0.73****	**-0.89****	-				
	M-shoot	**0.87****	**0.87****	**-0.75****	**0.82****	**0.90****	**0.84****	**0.60***	**-0.75****	0.55	0.06	0.07	-0.57	0.37	-			
	CaOx-shoot	0.08	0.08	-0.13	0.11	0.02	0.02	0.03	0.06	-0.09	-0.24	-0.16	-0.02	0.33	-0.09	-		
	δ^{13}C-shoot	0.56	0.57	-0.13	0.16	0.20	0.24	0.52	-0.47	-0.04	-0.38	-0.32	**-0.66***	**0.72****	0.30	**0.67***	-	
	Δ^{13}C-shoot	-0.49	-0.50	0.11	-0.13	-0.15	-0.19	-0.47	0.41	0.09	0.42	0.35	**0.62***	**-0.71****	-0.24	**-0.73****	**-0.99****	-
	δ^{15}N-shoot	0.33	0.34	-0.21	0.17	0.15	0.23	0.49	-0.42	0.09	-0.48	-0.47	**-0.64***	**0.79****	0.23	0.20	0.49	-0.47
MADEIRA ISLAND	N-corm	**1.00****	-															
	St-corm	**-0.90****	**-0.90****	-														
	Fb-corm	**0.88****	**0.88****	**-0.95****	-													
	M-corm	**0.91****	**0.90****	**-0.93****	**0.94****	-												
	CaOx-corm	0.46	0.46	-0.20	0.21	0.47	-											
	δ^{13}C-corm	**-0.63***	**-0.63***	0.41	-0.51	-0.58	**-0.76***	-										
	Δ^{13}C-corm	0.53	0.53	-0.27	0.38	0.44	**0.74***	**-0.98****	-									
	δ^{15}N-corm	**-0.86****	**-0.86****	**0.90****	**-0.79****	**-0.76****	-0.04	0.23	-0.13	-								
	Pt-shoot	-0.03	-0.03	0.12	0.01	-0.09	0.15	-0.16	0.21	0.22	-							
	N-shoot	0.01	0.01	0.08	0.05	-0.05	0.15	-0.17	0.21	0.18	**1.00****	-						
	St-shoot	**-0.65***	**-0.66***	**.074****	**-0.61***	**-0.59***	0.14	0.16	-0.08	**0.87****	0.57	0.54	-					
	Fb-shoot	**0.62***	**0.62***	**-0.72****	**0.61***	**0.60***	0.04	-0.20	0.07	**-0.72****	**-0.62***	**-0.58***	**-0.82****	-				
	M-shoot	0.48	0.48	-0.57	**0.59***	**0.58***	0.12	-0.08	-0.03	-0.55	-0.48	-0.45	-0.52	0.55	-			
	CaOx-shoot	-0.16	-0.17	0.13	-0.13	-0.03	0.33	0.21	-0.28	0.27	-0.20	-0.20	0.35	0.16	0.25	-		
	δ^{13}C-shoot	**0.62***	**0.63***	**-0.72****	0.55	**0.60***	-0.21	0.00	-0.10	**-0.85****	**-0.62***	**-0.59***	**-0.93****	**0.79****	0.53	-0.20	-	
	Δ^{13}C-shoot	**-0.61***	**-0.62***	**0.71****	-0.54	**-0.58***	0.17	0.02	0.08	**0.84****	**0.67***	**0.64***	**0.95****	**-0.83****	-0.53	0.17	**-0.99****	-
	δ^{15}N-shoot	-0.26	-0.26	0.37	-0.31	-0.29	0.32	-0.19	0.22	0.55	0.25	0.24	0.50	-0.13	**-0.60***	0.37	-0.55	0.50

Table S5 (continued).

	N-corm	**1.00****	-															
	St-corm	**-0.73****	**-0.72****	-														
	Fb-corm	**0.75****	**0.75****	**-0.92****	-													
	M-corm	**0.83****	**0.83****	**-0.73****	**0.90****	-												
	CaOx-corm	0.46	0.46	**-0.79****	**0.76****	**0.64****	-											
	δ^{13}C-corm	-0.47*	**-0.47***	0.32	**-0.59***	**-0.69****	-0.15	-										
	Δ^{13}C-corm	0.28	0.28	-0.13	0.43	**0.54***	0.05	**-0.97****	-									
SOUTH	δ^{15}N-corm	0.33	0.33	-0.20	0.24	0.36	0.44	-0.18	0.14	-								
PACIFIC	Pt-shoot	0.16	0.16	**-0.48***	0.24	0.01	0.32	0.24	-0.36	-0.15	-							
COMMUNITY	N-shoot	0.20	0.19	**-0.52***	0.28	0.05	0.35	0.21	-0.34	-0.12	**1.00****	-						
	St-shoot	0.06	0.06	-0.30	0.09	0.02	0.39	0.32	-0.38	0.45	**0.60***	**0.61***	-					
	Fb-shoot	0.10	0.11	0.16	0.06	0.20	-0.11	-0.26	0.30	0.10	**-0.87****	**-0.85****	**-0.70****	-				
	M-shoot	0.33	0.34	-0.11	0.15	0.25	-0.01	-0.14	0.06	**-0.50***	-0.01	-0.01	**-0.54***	0.28	-			
	CaOx-shoot	-0.16	-0.17	-0.36	0.08	-0.26	0.31	**0.52***	**-0.59***	0.15	**0.58***	**0.58***	0.51	-0.42	-0.34	-		
	δ^{13}C-shoot	-0.29	-0.29	0.25	-0.25	-0.32	-0.40	0.20	-0.14	**-0.67****	-0.12	-0.14	-0.45	0.13	0.44	-0.20	-	
	Δ^{13}C-shoot	0.28	0.28	-0.19	0.25	0.35	0.37	-0.26	0.21	**0.63****	0.03	0.05	0.33	-0.06	-0.41	0.07	**-0.99****	-
	δ^{15}N-shoot	0.40	0.41	0.03	0.19	**0.50***	0.17	**-0.50***	**0.50***	**0.59****	**-0.62****	**-0.59****	-0.17	**0.53***	-0.14	**-0.56***	**-0.53***	**0.59***
	N-corm	**1.00****	-															
	St-corm	**-0.70****	**-0.70****	-														
	Fb-corm	**0.67****	**0.67****	**-0.93****	-													
	M-corm	**0.69****	**0.69****	**-0.72****	**0.82****	-												
	CaOx-corm	-0.01	-0.01	**-0.38***	**0.45****	**0.50****	-											
	δ^{13}C-corm	**-0.48****	**-0.48****	**0.38***	**-0.44****	-0.14	0.25	-										
	Δ^{13}C-corm	**0.41****	**0.41****	-0.28	**0.34***	0.04	**-0.33***	**-0.99****	-									
	δ^{15}N-corm	0.08	0.08	0.09	0.02	0.24	0.16	0.17	-0.16	-								
OVERALL	Pt-shoot	0.23	0.22	-0.22	0.14	0.16	-0.06	0.11	-0.12	0.27	-							
	N-shoot	0.25	0.24	-0.24	0.16	0.20	-0.03	0.13	-0.15	0.28	**1.00****	-						
	St-shoot	-0.15	-0.16	0.05	-0.09	-0.22	0.01	-0.04	0.05	**0.42****	**0.48****	**0.47****	-					
	Fb-shoot	0.18	0.19	-0.17	0.21	0.22	0.11	-0.11	0.08	-0.27	**-0.76****	**-0.74****	**-0.73****	-				
	M-shoot	**0.44****	**0.44****	**-0.43****	**0.49****	0.28	0.04	**-0.46****	**0.41****	**-0.40****	-0.16	-0.17	**-0.35***	**0.35***	-			
	CaOx-shoot	**-0.32***	**-0.32***	0.00	-0.08	-0.06	**0.38***	**0.54****	**-0.57****	0.10	0.04	0.08	0.20	0.01	**-0.36***	-		
	δ^{13}C-shoot	-0.30	-0.30	0.15	-0.17	0.08	**0.40***	**0.66****	**-0.69****	-0.23	-0.21	-0.17	**-0.45****	0.29	-0.20	**0.52****	-	
	Δ^{13}C-shoot	0.28	0.28	-0.13	0.17	-0.07	**-0.38***	**-0.65****	**0.68****	0.23	0.19	0.15	**0.44****	-0.28	0.20	**-0.56****	**-0.99****	-
	δ^{15}N-shoot	0.30	0.30	-0.01	0.16	0.23	0.04	**-0.39***	**0.39***	**0.37***	**-0.42****	**-0.41****	-0.03	**0.40****	0.04	-0.27	**-0.35***	**0.37***

Pt, protein (g/100g, DW); N, nitrogen (g/100g, DW); St, starch (g/100g, DW); Fb, crude fiber (g/100g, DW); M, total minerals (g/100g, DW); CaOx, calcium oxalate (g/100g, DW); δ^{13}C, carbon isotopic composition (‰, DW); Δ^{13}C, carbon isotope discrimination (‰, DW); δ^{15}N, nitrogen isotopic composition (‰, DW).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table S6

Pearson correlations of sweet potato tubers and shoots, from the 2nd agronomic trial.

Origin	Constituents	Pt-tuber	N-tuber	St-tuber	M-tuber	CaOx-tuber	δ^{13}C-tuber	Δ^{13}C-tuber	δ^{15}N-tuber	TC-tuber	Pt-shoot	N-shoot	St-shoot	M-shoot	CaOx-shoot	δ^{13}C-shoot	Δ^{13}C-shoot	δ^{15}N-shoot
MADEIRA ISLAND	N-tuber	**1.00****	-															
	St-tuber	0.10	0.10	-														
	M-tuber	0.16	0.15	-0.21	-													
	CaOx-tuber	-0.05	-0.05	**-0.72****	-0.18	-												
	δ^{13}C-tuber	-0.33	-0.33	**-0.44***	0.07	0.35	-											
	Δ^{13}C-tuber	0.33	0.33	**0.44***	-0.07	-0.35	**-1.00****	-										
	δ^{15}N-tuber	**0.55****	**0.56****	-0.21	-0.13	0.38	-.410*	.410*	-									
	TC-tuber	**0.79****	**0.78****	0.27	-0.09	-0.26	**-0.59****	**0.59****	0.40	-								
	Pt-shoot	0.11	0.12	0.25	-0.11	0.01	-0.07	0.07	0.28	-0.07	-							
	N-shoot	0.12	0.12	0.25	-0.11	0.01	-0.07	0.07	0.28	-0.07	**1.00****	-						
	St-shoot	-0.18	-0.18	0.14	0.03	-0.26	-0.37	0.37	-0.03	-0.08	-0.07	-0.07	-					
	M-shoot	-0.23	-0.23	0.40	-0.16	-0.20	0.11	-0.11	-0.06	-0.32	**0.83****	**0.83****	-0.15	-				
	CaOx-shoot	-0.19	-0.19	-0.23	0.27	0.06	-0.50	0.50	-0.14	-0.02	-0.22	-0.22	0.53	-0.36	-			
	δ^{13}C-shoot	0.29	0.29	**-0.49***	0.04	**0.46***	0.20	-0.20	0.31	0.10	0.15	0.15	0.25	-0.30	0.20	-		
	Δ^{13}C-shoot	-0.29	-0.30	**0.49***	-0.04	**-0.46***	-0.20	0.20	-0.31	-0.10	-0.15	-0.15	-0.25	0.30	-0.20	**-1.00****	-	
	δ^{15}N-shoot	-0.19	-0.19	**0.48***	0.27	**-0.44***	-0.25	0.25	-0.14	-0.27	**0.55****	**0.55****	0.25	**0.67****	0.26	-0.40	0.40	-
	TC-shoot	**-0.49***	**-0.49***	-0.01	-0.16	0.19	-0.05	0.05	-0.08	-0.40	0.13	0.12	0.21	0.08	0.10	0.02	-0.02	0.14
CANARY ISLANDS	N-tuber	**1.00****	-															
	St-tuber	**-0.61***	**-0.61***	-														
	M-tuber	0.49	0.50	-0.25	-													
	CaOx-tuber	**0.79****	**0.79****	**-0.64***	0.51	-												
	δ^{13}C-tuber	**-0.74****	**-0.74****	**0.71****	-0.09	**-0.69***	-											
	Δ^{13}C-tuber	**0.74****	**0.74****	**-0.71****	0.09	**0.69***	**-1.00****	-										
	δ^{15}N-tuber	**0.62***	**0.63***	**-0.73****	0.17	0.44	**-0.73****	**0.73****	-									
	TC-tuber	0.39	0.39	-0.38	-0.11	-0.04	-0.19	0.19	0.48	-								
	Pt-shoot	0.44	0.44	**-0.61***	0.25	0.51	-0.57	0.57	**0.78****	0.18	-							
	N-shoot	0.44	0.44	**-0.61***	0.25	0.50	-0.57	0.57	**0.78****	0.18	**1.00****	-						
	St-shoot	0.19	0.19	0.06	-0.22	0.10	0.02	-0.02	-0.35	0.01	**-0.61***	**-0.61***	-					
	M-shoot	-0.03	-0.03	-0.21	0.11	0.07	-0.21	0.21	0.51	-0.11	**0.83****	**0.83****	**-0.82****	-				
	CaOx-shoot	**0.80***	**0.80***	**-0.79***	0.25	0.67	**-0.73***	**0.73***	**0.96****	**0.71***	0.57	0.57	0.16	-0.23	-			
	δ^{13}C-shoot	**0.89****	**0.89****	**-0.79****	0.22	**0.66***	**-0.77****	**0.77****	**0.74****	0.53	0.45	0.45	0.27	-0.04	**0.97****	-		
	Δ^{13}C-shoot	**-0.89****	**-0.89****	**0.79****	-0.22	**-0.66***	**0.77****	**-0.77****	**-0.74****	-0.53	-0.45	-0.45	-0.27	0.04	**-0.97****	**-1.00****	-	
	δ^{15}N-shoot	-0.12	-0.12	-0.13	0.06	0.09	-0.15	0.15	0.39	-0.20	**0.79****	**0.79****	**-0.81****	**0.96****	-0.41	-0.16	0.16	-
	TC-shoot	-0.51	-0.51	0.38	-0.40	-0.31	0.39	-0.39	**-0.65***	-0.07	**-0.66***	**-0.66***	0.25	**-0.59***	-0.61	-0.51	0.51	-0.40

Table S6 (continued).

GUINEA-BISSAU	N-tuber	**1.00****	-														
	St-tuber	-0.30	-0.31	-													
	M-tuber	**0.68***	**0.69***	-0.43	-												
	CaOx-tuber	-0.44	-0.44	-0.32	0.00	-											
	δ^{13}C-tuber	-0.23	-0.23	-0.28	-0.52	-0.14	-										
	Δ^{13}C-tuber	0.32	0.32	0.21	0.52	0.13	**-0.98****	-									
	δ^{15}N-tuber	0.47	0.46	0.30	0.25	-0.39	-0.35	0.45	-								
	TC-tuber	0.09	0.09	0.37	0.49	0.08	**-0.92****	**0.83****	0.13	-							
	Pt-shoot	-0.25	-0.24	-0.44	-0.16	0.44	**0.64***	**-0.61***	-0.27	**-0.65***	-						
	N-shoot	-0.24	-0.24	-0.45	-0.15	0.44	**0.63***	**-0.61***	-0.27	**-0.64***	**1.00****	-					
	St-shoot	0.28	0.28	-0.41	0.45	0.02	-0.17	0.19	0.10	0.15	0.16	0.15	-				
	M-shoot	-0.12	-0.11	-0.50	-0.14	0.41	**0.64***	**-0.62***	-0.41	**-0.65***	**0.82****	**0.82****	-0.11	-			
	CaOx-shoot	-0.03	-0.04	0.14	-0.04	0.01	-0.26	0.35	0.40	0.06	0.08	0.08	0.33	-0.14	-		
	δ^{13}C-shoot	0.31	0.32	**-0.86****	0.46	0.28	0.16	-0.12	-0.29	-0.18	0.42	0.42	**0.74****	0.40	-0.01	-	
	Δ^{13}C-shoot	-0.31	-0.32	**0.86****	-0.46	-0.28	-0.16	0.12	0.29	0.18	-0.42	-0.42	**-0.74****	-0.41	0.01	**-1.00****	-
	δ^{15}N-shoot	-0.47	-0.47	-0.16	-0.30	.634*	0.43	-0.42	-0.26	-0.45	**0.88****	**0.88****	-0.13	**0.72****	0.19	0.09	-0.09
	TC-shoot	-0.51	-0.52	**0.79****	**-0.59***	-0.25	0.02	-0.07	0.13	0.10	-0.18	-0.18	-0.21	-0.51	0.17	**-0.69***	**0.69***
OVERALL	N-tuber	**1.00****	-														
	St-tuber	0.01	0.00	-													
	M-tuber	**0.38****	**0.38****	-0.17	-												
	CaOx-tuber	-0.11	-0.10	**-0.61****	-0.18	-											
	δ^{13}C-tuber	**-0.42****	**-0.42****	-0.18	-0.28	0.01	-										
	Δ^{13}C-tuber	**0.46****	**0.45****	0.14	0.22	0.02	**-0.98****	-									
	δ15N-tuber	**0.54****	**0.54****	-0.19	0.09	0.14	**-0.53****	**0.59****	-								
	TC-tuber	0.26	0.26	0.23	**0.37***	-0.15	**-0.69****	**0.53****	0.17	-							
	Pt-shoot	0.26	0.26	-0.06	0.04	0.08	-0.09	0.14	**0.42****	-0.11	-						
	N-shoot	0.26	0.26	-0.06	0.04	0.08	-0.09	0.14	**0.42****	-0.11	**1.00****	-					
	St-shoot	**-0.29***	**-0.29***	-0.20	-0.13	**0.42****	0.00	0.04	-0.16	-0.12	**-0.29***	**-0.30***	-				
	M-shoot	0.10	0.10	0.21	0.10	**-0.34***	0.00	0.01	0.20	-0.08	**0.79****	**0.80****	**-0.59****	-			
	CaOx-shoot	0.11	0.11	-0.26	0.05	0.28	**-0.36***	**0.44***	**0.45***	0.07	0.13	0.13	0.28	-0.20	-		
	δ^{13}C-shoot	0.27	0.28	**-0.66****	0.09	**0.52****	0.05	-0.01	0.20	-0.07	0.23	0.23	**0.43****	-0.23	0.33	-	
	Δ^{13}C-shoot	-0.27	-0.28	**0.66****	-0.09	**-0.52****	-0.05	0.01	-0.20	0.07	-0.23	-0.23	**-0.43****	0.23	-0.33	**-1.00****	-
	δ^{15}N-shoot	**-0.33***	**-0.33***	0.06	-0.06	0.20	0.06	-0.01	-0.01	-0.27	**0.52****	**0.52****	0.19	**0.40****	0.08	-0.08	0.08
	TC-shoot	**-0.58****	**-0.58****	0.06	**-0.48****	**0.35***	0.18	-0.17	-0.26	-0.15	**-0.30***	**-0.30***	**0.51****	**-0.52****	0.05	-0.07	0.07

Pt, protein (g/100g, DW); N, nitrogen (g/100g, DW); St, starch (g/100g, DW); M, total minerals (g/100g, DW); CaOx, calcium oxalate (g/100g, DW); δ^{13}C, carbon isotopic composition (‰, DW); Δ^{13}C, carbon isotope discrimination (‰, DW); δ^{15}N, nitrogen isotopic composition (‰, DW); TC, total carbon (%, DW).

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CHAPTER 11

Final considerations and Future perspectives

The final considerations comprise all issues that have been already addressed in detail in the respective chapters of this dissertation, providing only a summary of the main findings and a brief critical review.

11.1 Final considerations

Even though the drought stress effects are currently studied in crop plants, the main research on drought tolerance is based on physiological traits and plant productivity assays. We can find several studies based on the biochemical composition of taro and sweet potato crops, but there is still scarce information about how drought stress affects their metabolism and how the oxalate, protein, and starch variation can reflect its nutritional quality. Similarly, very scarce information is available relating their NER under drought conditions, with no published information about the $\delta^{13}C$ and $\delta^{15}N$ as physiological indicators of osmotic stress, and no ABA phytohormone variation during drought response.

To increase the knowledge in this field, our work had the main goal to conduct crops full-growth vegetative cycles to generate new information about the taro and sweet potato plant drought responses, evolving the analysis of biochemical and nutritional traits, and detect differences in the nutrient allocation, chlorophyll canopy, biomass loss, and stress level. As well, to model the influence of drought stress in the quality constituents at both underground and aboveground organs, with the use of the Near-Infrared Spectroscopy (NIRS) as a rapid and reliable technique for quality traits prediction in plant material submitted to stress conditions. In this final chapter, we compiled these main findings, with a first approach for the taro, followed by the sweet potato, regarding the whole-plant level to assess their physiological responses to drought.

We found that taro responded to drought with a generalized loss of TPB as a drought avoidance mechanism, showing a higher reduction in the shoot area development, and thus increasing the R:S ratio as phenotypic flexibility response to avoid stress. This allowed the WUE increase and plant maintenance according to the water availability, which becomes a good indicator of the plant drought resistance. Also, the taro accessions with great adaptableness to stress had lower SI, which corresponds to a smaller TPB difference between control and drought, showing the best ability to grow under drought. Likewise, the highest NER, CCI, and N contents also support the best taro's plant metabolic and physiological processes, under drought conditions. The CCI content indicates the taro's plant health and represents its relative chlorophyll value, which is considered proportional to the amount of chlorophyll in the leaf tissue. The observed leaf chlorophyll increase in taro shoots can be associated with the increase of the photosynthetic capacity, wherein the canopy greenness is usually related to the photosynthetic efficiency of the plant, throughout the association of the intensity of photosynthetic electron transport. Stress led to N accumulation in both organs, which was significantly correlated with NER and E decrease. This indicated that taro whole-plants are not very efficient in nutrient use in stress conditions, for TPB production. However, N-corm showed a suitable allocation of nutrients between the whole-plant, with a positive correlation with NHI and M-corm. Some accessions increased slightly the NHI, which indicates a potential share of nitrogen accumulated in corm yield concerning total plant N uptake. These results were partially presented at a national scientific meeting (*2nd PhD Students Meeting in Environment and Agriculture*, Évora, Portugal, 16-17 November 2017) and at a local seminar (Seminário de Agricultura Biológica, Funchal, 22 January - 02 February 2018), and published in a peer-reviewed scientific journal (*Emirates Journal of Food and Agriculture*, Chapter 2).

All taro accessions presented very high CaOx crystals content, with the corms showing three times and the shoots with four times the allowable value for food, which affects negatively the whole-plant nutritional quality and value. The oxalate precipitation and mobilization from the shoots to the corms were considered a good drought response, allowing better osmotic pressure regulation. The variation in taro oxalate content was significantly correlated with the CCI, starch, and protein synthesis. The water scarcity slightly decreased the taro starch content in both organs. The starch is the main reserve of energy in corm organs, and it showed the highest content variation under stress when compared with the shoots. The stress conditions increased the starch mobilization, due to the need to supply energy and metabolites to protect the subcellular structures against water deficit, with a small variation in the corm starch quality grain under drought. The taro plants slightly increased the protein content from the corms and shoots, with the corms registering the biggest increase of protein accumulation during stress. These results were presented and received the Best Poster

Award at an international scientific meeting (*3rd Global Summit on Plant Science,* Rome, Italy, 7-9 August 2017) and were published in a peer-reviewed scientific journal (*Acta Physiologiae Plantarum*, Chapter 3).

The evaluation of $\delta^{13}C$ and $\delta^{15}N$ provided insights into the plant carbon and nitrogen processes and their relationship with TPB and $\Delta^{13}C$ in stress conditions. We observed variation in these traits, according to their $\delta^{13}C$-shoot values, that point to a partial openness of stomata in all taro accessions. Some of them show a slight heavier δ-value (with lower $\Delta^{13}C$), which points to a partial closure of stomata, trying to avoid water loss under drought, and consequently promoting the sample ^{13}C enrichment. Meanwhile, the $\delta^{15}N$ value revealed a $\delta^{15}N$-corm increase and $\delta^{15}N$-shoot decrease, with N accumulation during drought. These slight variations of $\delta^{15}N$ values between organs could be attributed to tissue N reallocation under drought. The taro plants with enhanced WUE exhibited low $\Delta^{13}C$ and $\delta^{15}N$ values, showing a good physiological response to drought stress. We observed a significant negative correlation between taro shoots $\Delta^{13}C$ and plant WUE, suggesting that $\Delta^{13}C$ could be a plausible marker for WUE screening and plant material selection for breeding programs. These results were presented at an international meeting (*Plant Abiotic Stress Tolerance V*, Vienna, Austria, 5-6 July 2018) and published in a peer-reviewed scientific journal (*Journal of Plant Physiology*, Chapter 4).

The taro increase of $\Delta^{13}C$-shoot, CCI, and WUE was significantly correlated and the main indicators of partially open stomata during the drought assay. The ABA-shoot accumulation and ABA-corm decreased content in taro submitted to drought did not show significant correlations with any other indicator traits. The loss of osmotically active OA-shoot was correlated with OA-corm increase at low irrigation conditions but seems not to interfere with an ABA signal for stomatal closure. The ABA content in both taro organs apparently has no active participation in signalization of stomatal closure or biomass regulation under drought conditions. The low ABA content registered at the taro whole-plant level in both non-stress and stress conditions may indicate that ABA does not have a relevant role in the mobilization of plant metabolism in response to drought. Usually, ABA increases in leaves of tolerant crops to induce stomatal closure, preventing water loss through the reduction of transpiration. Therefore, taro plants seem to use the phenotypic flexibility and morphological mechanisms of drought avoidance to minimize the biomass loss, instead of active use of ABA as the main regulator of stomatal activity and plant growth. These results were presented at an international meeting (The 8th World Sustainability Forum (Virtual), Switzerland, 15-17 September 2020) and published in a peer-reviewed scientific journal (*Acta Physiologiae Plantarum*, Chapter 5).

The sweet potato accessions lost on average more than half of the TPB due to drought. It was registered a dynamic balance between the plant organs due to stress, preferentially developing the shoot rather than the storage roots. Drought also decreased slightly the CCI, with WUE increase, which indicates a possible partial reduction of the stomatal aperture, to minimize water loss and maintaining the photosynthesis. The sweet potato registered a decrease of N-shoot under water scarcity that led to a significant NER and E increase, implying that the N content in the shoots was applied more efficiently into biomass production. The sweet potato accessions showing higher R:S ratio, lower TPB loss, and lower SI had better drought avoidance strategies, better WUE and NER to ensuring growth and vital functions. They had also higher M-root, CCI, and N-shoot as good phenotypic flexibility responses. These results were partially presented at a local seminar (Seminário de Agricultura Biológica, Funchal, 22 January - 02 February 2018), and at an international meeting (*FloraMac2018*, Funchal, Portugal, 12-15 September 2018), and published in a peer-reviewed scientific journal (*Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, Chapter 6).

We also observed that the presence of insoluble CaOx druse crystals in sweet potato can negatively affect its nutritional quality. Tubers of six accessions were above the maximum CaOx levels accepted for raw consumption. The CaOx content could mainly be a product resulting from both the carbohydrate metabolism and the photosynthesis oxidative processes. The accessions showing the best response to drought, decreased the CaOx for plant osmoregulation, with oxalic acid increase. They also show a slight decrease in CCI and protein content, although with the CCI highest values in both experimental conditions. Additionally, they

showed an increased shoot starch content, deposited in leaf chloroplasts granules due to photosynthesis activity, with mobilization to tuber for further storage starch hydrolysis. These accessions supplied energy and metabolites without recourse to tuber starch hydrolysis and maintained the quality and functional properties of the tuber starch grain. These results were published in a peer-reviewed scientific journal (*Journal of the Science of Food and Agriculture*, Chapter 7).

The sweet potato tubers and shoots show faintly heavier $\delta^{13}C$ content under drought, with slightly lower $\Delta^{13}C$ and greater ^{13}C fixation in tubers, due to partial closure of stomata as a response to stress. Likewise, the TC increased slightly during drought, but it does not reveal significant differences in carbon assimilation. Still, we registered a slight upper ^{13}C fixation, by reducing the ^{13}C depletion required for plant growth processes, and thus decreasing TPB and $\Delta^{13}C$. The $\delta^{15}N$ values indicated a generalized N reallocation between the plant organs under drought, as a physiological integrator of plant response to environmental stress. Also, the highest $\delta^{15}N$-shoot and $\delta^{15}N$-tuber content under drought indicated more ^{15}N-enrichment under drought. These results were published in a peer-reviewed scientific journal (*Journal of Plant Physiology*, Chapter 8).

We observed an ABA-shoot accumulation in sweet potato, which was correlated with OA-shoot increase. The ABA-shoot increase was a root-to-shoot ABA signaling attempt to cope with water stress through stomatal closure, which was directly related to the $\Delta^{13}C$ and CCI decrease and higher WUE. The presence of OA-shoot may affect the intensity of the ABA-shoot signal, contributing to only a partial stomatal closure during the harsh water scarcity conditions. These results were published in a scientific peer-reviewed journal (*Physiologia Plantarum*, Chapter 9).

Finally, these data allowed us to create prediction models for quality traits in taro and sweet potato when submitted to drought stress. The models of the N, Pt, St, Fb, and M quality constituents for taro and sweet potato showed good prediction accuracy. The TC-tuber in sweet potato and CaOx-shoot in taro, the $\delta^{15}N$ in the underground organs, and the $\delta^{13}C$ in the vegetative parts of both crops showed the poorest prediction performance. However, these last constituent predictions could be used as a tool to obtain preliminary information in plant material screening. The present models could be applied for research purposes, or by farmers and agriculture companies, in further fieldwork and evaluation of crop quality subjected to water scarcity conditions. These results were presented at an international meeting (*2nd Global Conference on Plant Science and Microbial Ecology*, Dubai, United Arab Emirates, 14-15 October 2019) and published in a peer-reviewed scientific journal (*Applied Sciences*, Chapter 10).

To conclude, the present dissertation contributed with new insights on taro and sweet potato physiological response and capacity to cope with problems associated with drought stress, such as the changes in composition and quality, the phenotypic flexibility to avoid water scarcity, and the lack of productivity. In the framework of this thesis, new models were created for quality prediction in the under and aboveground organs, in both crops. This information assisted the identification of Madeiran taro accessions 2216 and 2210 and Guinea-Bissau sweet potato accessions 3124 and 3125 as good candidates for further breeding purposes to adapt these crops to climate change. The new knowledge and models could be useful to apply in the study of the behavior of other accessions and vegetatively propagated crops, in drought conditions, to evaluate their whole-plant physiological response to stress.

11.2 Future perspectives

The findings of this thesis should be understood following the explicit characteristics of each crop and different features of studied accessions, as a result of their genetic variability, biochemical diversity, and cultivar molecular memory, resulting from biochemical adaptation to drought events, whilst no other differences between well-watered and water-deprived environments occurred during the experimental assays.

The present dissertation has provided new insights and relevant information about the taro and sweet potato whole-plant biochemical response to water scarcity stress. Those responses were expressed through

physiological traits throughout the growth and development of the plant body. However, further studies should focus on increase our knowledge and deeper study in the contribution of individual biochemical paths and mechanisms in the plant physiology response to drought, and biomass allocation and productivity. To aid in the interpretation of the change in protein content in response to stress, further investigation is needed to calculate the ratio of the weight of total amino acids to nitrogenous amino acids to give the true conversion factor of nitrogen to crude protein content. In particular, the synthesis and accumulation of some amino acids, such as proline, could give good information about the plant's physiological status and stress tolerance, due to its overproduction in an osmotic stress situation, and potential contribution to the increase of the protein content. We optimized the colorimetric technique for proline determination in sweet potato and taro underground and aboveground tissues, but without getting satisfactorily data for the droughted sample tissues. In further studies, it must be increased the frequency of the harvest of plant material samples in the first 21 days of the drought conditions, to avoid proline metabolization and degradation in prolonged drought stress. We suspect that proline plays an important role with ABA in stress signaling and mobilization of plant responses, but their action has been masked by the duration of the experimental drought assays.

Another important parameter that should be specifically analyzed to a deeper understanding of the contribution of the protein variation in sweet potato and taro adaptation to drought, are the dehydrins (DHNs) [or group 2 LEA (Late Embryogenesis Abundant) proteins] that are accumulated during drought stress conditions, acting as a space-fillers in several cellular complexes. The searching of the drought-inducible protein synthesis in response to water scarcity, by profiling protein patterns through electrophoretic analysis, will be interesting to confirm the expression and time accumulation of that drought-related proteins. The Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) enzyme catalyzes the first major step of carbon fixation during photosynthesis, and at the time is responsible for carbon dilapidation through photorespiration. The study of the activity of this enzyme could be another interesting contribution to elucidate the synthesis of protein fraction in the green parts of plants. Photosynthesis provides carbon skeleton for the synthesis of amino acids, and that protein synthesis, which may represent about 50% of the protein total fraction. The study and determination of organic acids pool, such as malate, citrate, and succinate, could be useful to better understand the role of these organic acids in metabolic pathways in the tropical crop adaptation to drought conditions.

Additional work could be done in a new set of drought experimental assays, to measure photosynthesis, respiration, transpiration, stomatal conductance, PAR, and internal CO_2 in the plant leaf, using a portable photosynthesis system (*e.g.*, CI-340 from CID Bio-Science). This leaf spectroscopy tool could highlight and elucidate in real time the data generated in the present dissertation, complementing the data knowledge about the plant physiological response obtained mainly through the measurement of carbon isotopes, chlorophyll content, and ABA traits variation to drought, in further research. The new set of drought experimental assays can be carried out to study the both crops acclimatization to drought, subjecting them to short cycles of water stress, and learning whether drought priming will improve photosynthesis, protein synthesis, water use efficiency, and productivity.

It could be done supplementary work to substantiate and increase the robustness of the actual NIRS models with new data from new drought cycles. The increase of robust fitting model for all the constituents in the study whilst improving the capacity to create a fast tool to be used in crop quality and phenotyping plants subjected to water scarcity. However, we also feel the need to evolve our NIRS models, because the NIR spectra were done on dried flour samples due to our limitation means, that could be limiting for plant breeders that will use it, having the setback to collect a vast number of samples and to process them into fine compact flour into capsules to screen hundreds of progenies in the field. So, we propose to develop NIR calibration of fresh raw flesh using, *e.g.*, the near-infrared hyperspectral imaging (NIR-HSI), one of the evolutions of the conventional NIRS technique through the input of spectral camera devices, being faster and more sensitive, through the immediate sample surface imaging data collection.

ANNEXES

The annexes contain the figures relative to taro and sweet accessions in the study, contemplating the experimental drought assays, the main physiological descriptors that identifies each accession, and the respective world map indicating their origin.

Colocasia esculenta (L.) Schott
var. Listado
Origin: La Palma, Canary Islands, Spain
Acc. 2056

(A) Plant with Y patterned, yellow green leafs, with yellow petiole junction colour and yellow leaf main vein colour, red petiole.

(B) Plant with few (1 to 5) suckers and absent stolons.

(C) Corms and shoots after the harvest.

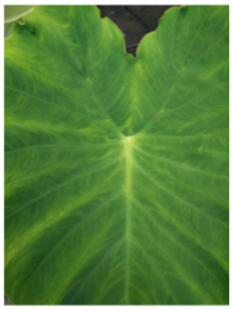
Colocasia esculenta (L.) Schott
var. Blanco Saucero
Origin: La Palma, Canary Islands, Spain
Acc. 2061

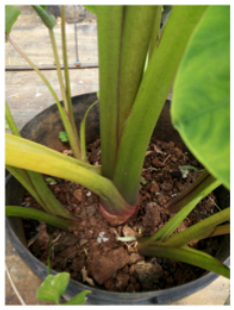
(A) Plant with Y pattern green leafs, yellow petiole junction colour, yellow leaf main vein colour, brown to red petiole.

(B) Plant with few (1 to 5) suckers and absent stolons.

(C) Corms and shoots after the harvest.

Colocasia esculenta (L.) Schott
var. Roxo
Origin: Madeira Island, Portugal
Acc. 2210





(A) Plant with Y pattern, yellow green leafs, yellow petiole junction colour, yellow leaf main vein colour, yellow to red petiole.

(B) Plant with several (6 to 10) suckers and absent stolons.

(C) Corms and shoots after the harvest.

Colocasia esculenta (L.) Schott
var. Branco
Origin: Madeira Island, Portugal
Acc. 2216

(A) Plant with V pattern, yellow green leafs, yellow petiole junction colour, yellow leaf main vein colour, yellow petiole.

(B) Plant with few (1 to 5) suckers and absent stolons.

(C) Corms and shoots after the harvest.

Colocasia esculenta (L.) Schott
var. PExPH 15-6 BL/HW/08
Origin: Fiji, South Pacific Community (SPC)
Acc. 2232

(A) Plant with Y pattern, yellow green leafs, purple petiole junction colour, green leaf main vein colour, brown petiole.

(B) Plant with several (6 to 10) suckers and absent stolons.

(C) Corms and shoots after the harvest.

Colocasia esculenta (L.) Schott
var. C3-22 BL/PNG/11
Origin: Fiji, South Pacific Community (SPC)
Acc. 2234

(A) Plant with Y pattern, dark green leafs, purple petiole junction colour, green leaf main vein colour, brown to red petiole.

(B) Plant with few (1 to 5) suckers and absent stolons.

(C) Corms and shoots after the harvest.

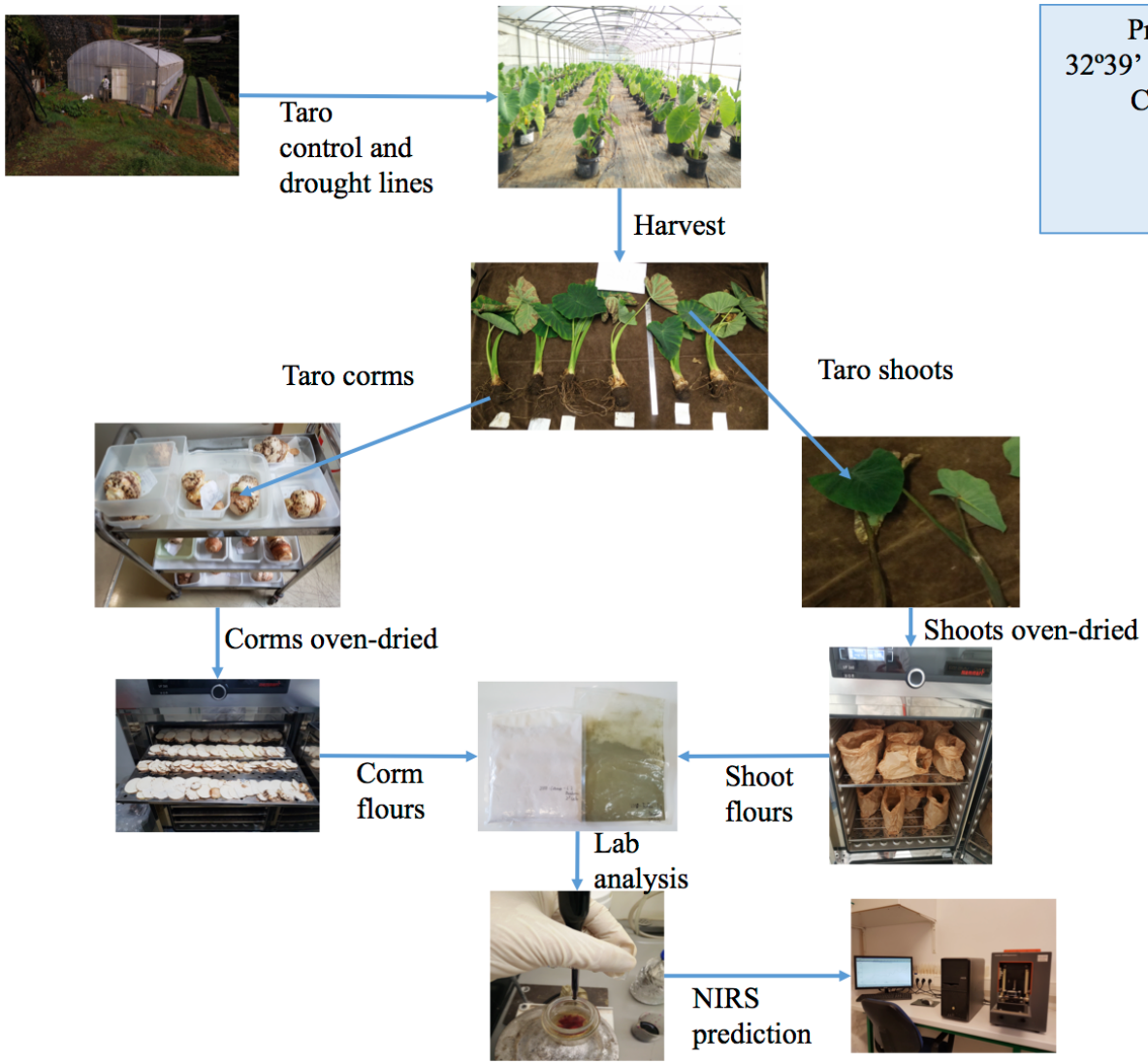
Colocasia esculenta (L.) Schott
var. Karang CE/MAL/10
Origin: Fiji, South Pacific Community (SPC)
Acc. 2239

(A) Plant with Y pattern, yellow green leafs, yellow petiole junction colour, yellow leaf main vein colour, red petiole.

(B) Plant with few (1 to 5) suckers and with medium (6 to 10) stolons.

(C) Corms and shoots after the harvest.

WORLD MAP INDICATING THE TARO ACCESSIONS ORIGIN USED IN THE THESIS

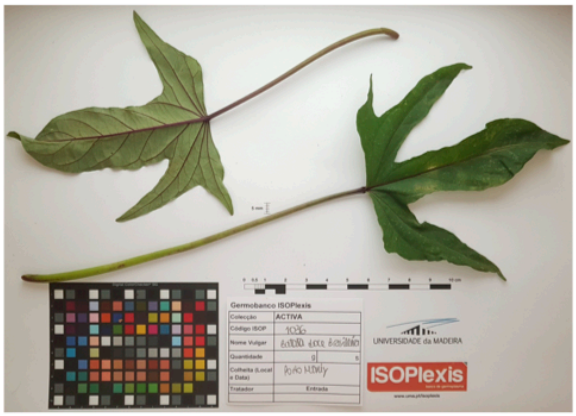


Preces experimental station
32°39' N, 16°58' W, 188 m a.s.l.
Câmara de Lobos, Madeira
Portugal

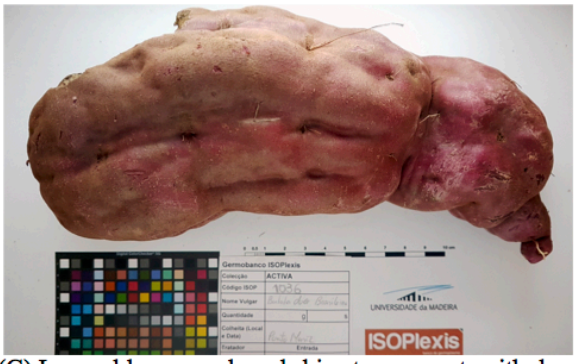
Years: 2015 and 2017

Ipomoea batatas (L.) Lam.
var. Brasileira
Origin: Madeira Island, Portugal
Acc. 1036

(A) Shoots



(B) Deep oblanceolate hastate leaf

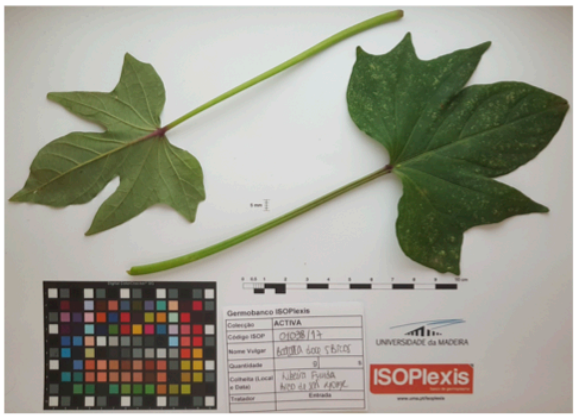


(C) Long oblong purple red skin storage root, with deep longitudinal grooves and deep horizontal constrictions

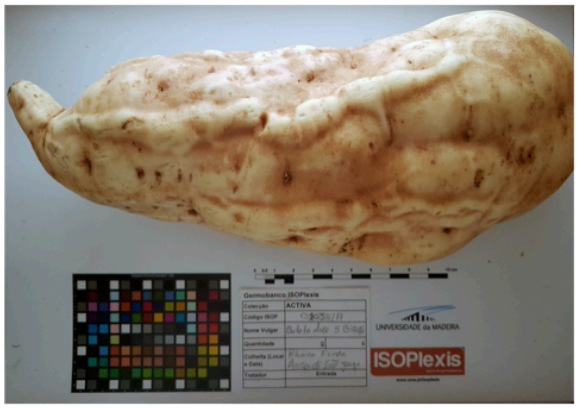
(D) Storage root cross section with dark yellow flesh

Ipomoea batatas (L.) Lam.
var. 5 Bicos
Origin: Madeira Island, Portugal
Acc. 1038

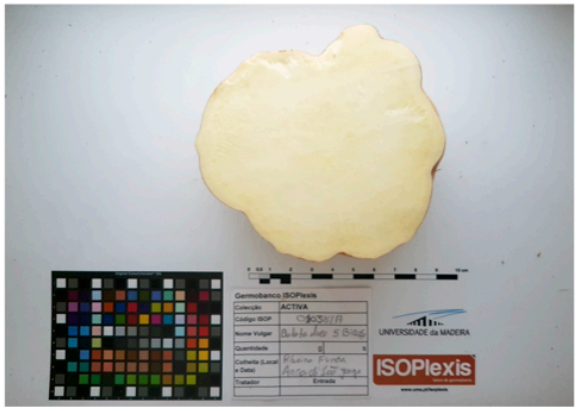
(A) Shoots



(B) Moderate elliptic lobed leaf



(C) Ovate cream skin storage root, with veins and shallow longitudinal grooves

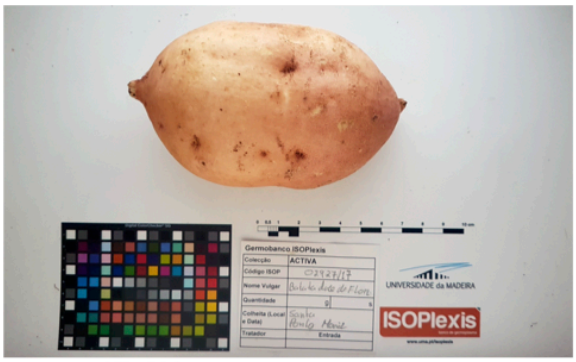


(D) Storage root cross section with cream flesh

Ipomoea batatas (L.) Lam.
var. de Flor
Origin: Madeira Island, Portugal
Acc. 2927

(A) Shoots with flowering

(B) Cordate leaf

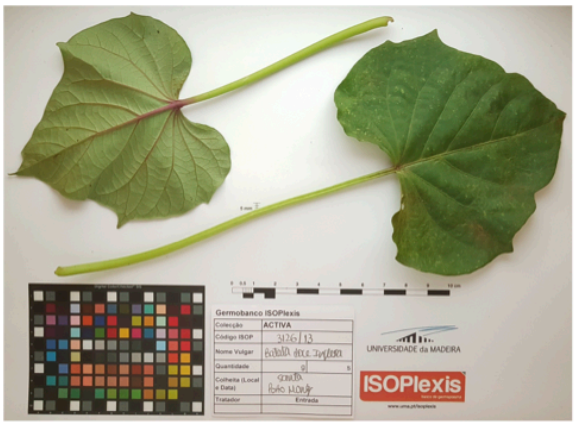


(C) Oblong cream-rose storage root

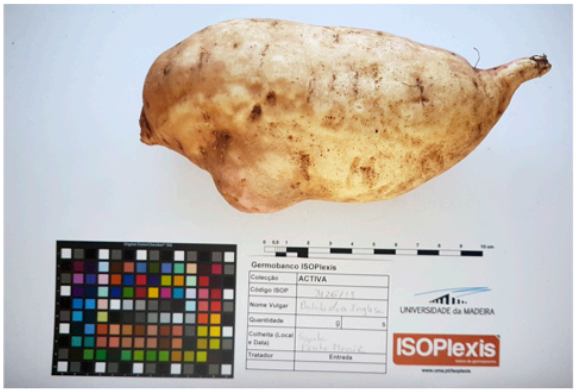
(D) Storage root cross section with yellow flesh

Ipomoea batatas (L.) Lam.
var. Inglesa
Origin: Madeira Island, Portugal
Acc. 3126

(A) Shoots



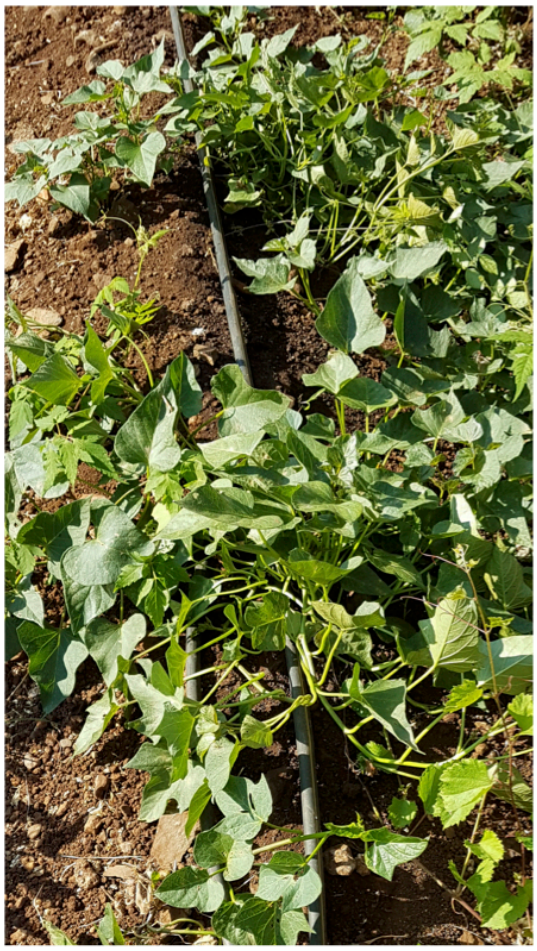
(B) Very slight toothed reniform leaf



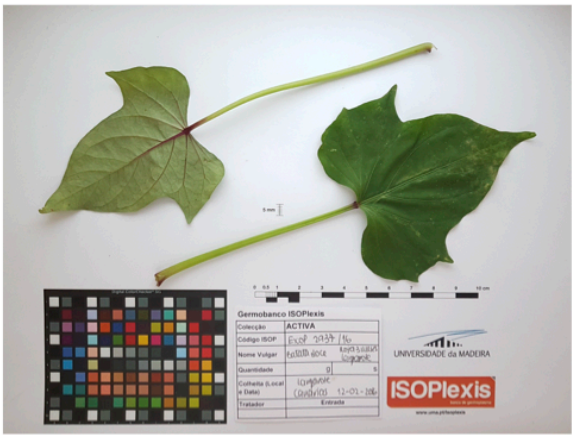
(C) Ovate cream-brown skin storage root, with veins

(D) Storage root cross section with cream-yellow flesh

Ipomoea batatas (L.) Lam.
var. Roja
Origin: Tenerife, Canary Islands, Spain
Acc. 2937



(A) Shoots



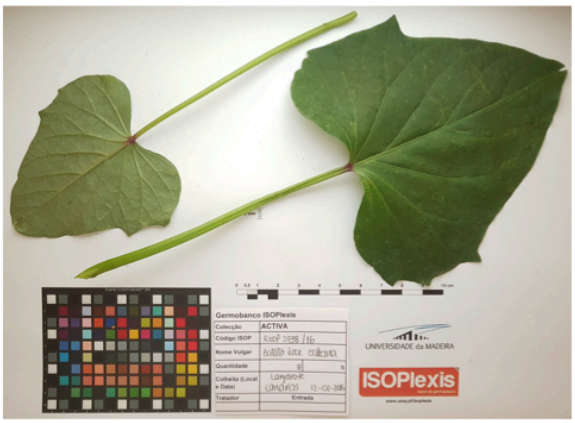
(B) Slight semi-eliptic lobed leaf

(C) Elliptical red-cream storage root, with shallow horizontal constrictions

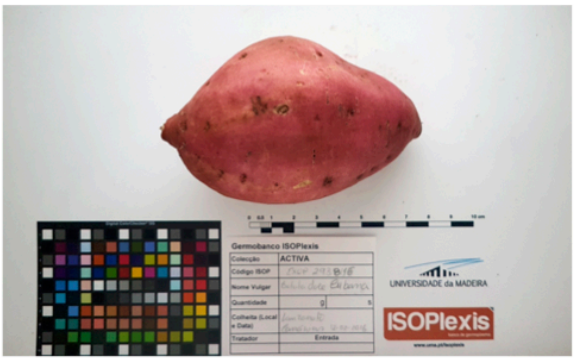
(D) Storage root cross section with dark yellow flesh, and broad ring in the cortex

Ipomoea batatas (L.) Lam.
var. Cubana
Origin: Tenerife, Canary Islands, Spain
Acc. 2938

(A) Shoots



(B) Very slight toothed cordate leaf

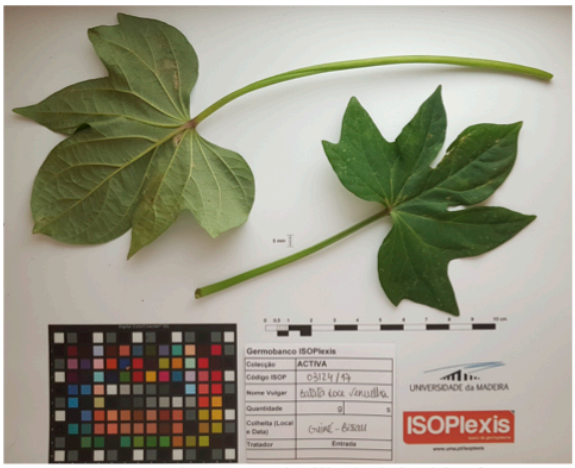


(C) Round elliptic red skin storage root

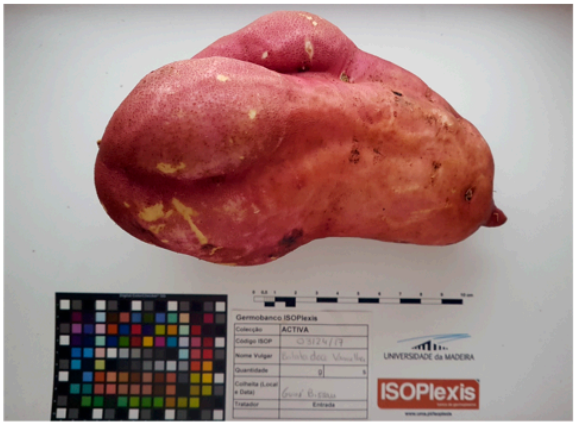
(D) Storage root cross section with cream flesh

Ipomoea batatas (L.) Lam.
var. Vermelha
Origin: Bafatá, Guinea-Bissau
Acc. 3124

(A) Shoots



(B) Moderate semi-elliptic lobed leaf



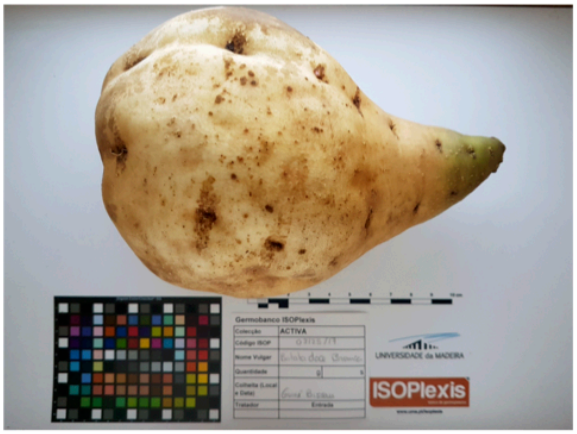
(C) Ovate red-pink skin storage root, with deep longitudinal grooves

(D) Storage root cross section with light yellow flesh

Ipomoea batatas (L.) Lam.
var. Branca
Origin: Bafatá, Guinea-Bissau
Acc. 3125

(A) Shoots

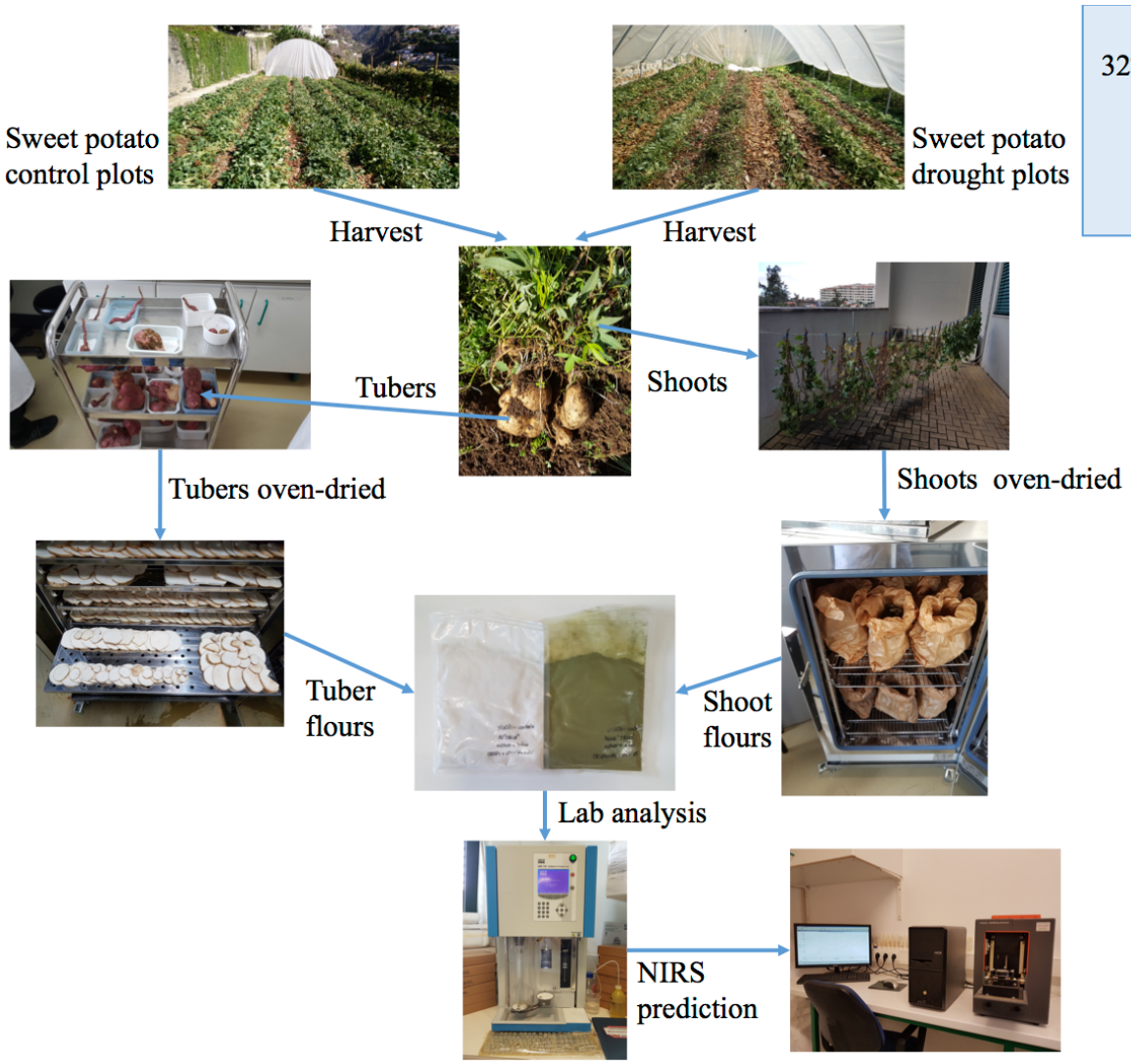
(B) Deep lanceolate lobed leaf



(C) Ovate cream skin storage root, with shallow longitudinal grooves

(D) Storage root cross section, with cream flesh and narrow ring in the cortex

WORLD MAP INDICATING THE SWEET POTATO ACCESSIONS ORIGIN USED IN THE THESIS


Sweet potato control plots
Sweet potato drought plots
ISOPlexis experimental field
32°39' N, 16°55' W, 174 m a.s.l.
Funchal, Madeira
Portugal
Years: 2017 and 2018
Harvest
Harvest
Tubers
Shoots
Tubers oven-dried
Shoots oven-dried
Tuber flours
Shoot flours
Lab analysis
NIRS prediction