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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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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 (Δ^{13}C), oxalic acid (OA), chlorophyll content index (CCI), water use efficiency (WUE), and biomass (B). The Δ^{13}C-shoot content showed partially open stomata in all accessions, and significant correlation with Δ^{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, Δ^{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
δ^{13}C	Carbon isotope composition
Δ^{13}C	Carbon isotope discrimination
ELISA	Enzyme-linked immunosorbent assay
OA	Oxalic acid
PCA	Principal component analysis
SPC	Pacific community
WUE	Water use efficiency

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 et al. 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 2500 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

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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. 2018, 2019; Ganança 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.

Materials and methods

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 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 48 h at

Table 1 Name and origin of taro (*Colocasia esculenta* L.) accessions selected for this study

Accession ID[a]	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)

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

65 °C (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 – 35 °C (Liebherr ProfiLine GGPV6570, Germany) until analysis.

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 Magellan™ 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.

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 (2000) 972.43 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:

$$\Delta^{13}\mathrm{C}\ (‰) = (\delta^{13}\mathrm{C}_\mathrm{a} - \delta^{13}\mathrm{C}_\mathrm{p})/(1 + \delta^{13}\mathrm{C}_\mathrm{p}).$$

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

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 ($KMnO_4$, 0.05 M). The oxalic acid content was obtained according to the Dye (1956) calculation:

$$\mathrm{OA}\ (\mathrm{mg}/100\,\mathrm{g}) = \left(T \times V_\mathrm{me} \times 3 \times 10^5\right)/(5 \times m_\mathrm{f}),$$

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

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.

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.

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).

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.

Results

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 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 fivefold 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

Table 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** g/L	WUE** g/L	B	
	Corm ng/g	Shoot** ng/g	Corm** ‰	Shoot ‰	Corm** mg/100 g	Shoot** mg/100 g			Corm** g/pot	Shoot** 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 ± 6.8cd	0.48 ± 0.16cd	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 ± 13.7cd	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

Table 2 (continued)

	ABA		$\Delta^{13}C$		OA		CCI** g/L	WUE** g/L	B	
	Corm ng/g	Shoot** ng/g	Corm** ‰	Shoot ‰	Corm** mg/100 g	Shoot** mg/100 g			Corm** g/pot	Shoot** g/pot
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 a dry weight basis (DW), and represents 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

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).

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

The Table 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/100 g (−8%), meanwhile the OA-corm increased from 36 to 44 mg/100 g (+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/100 g). Acc. 2232, 2234, and 2239 increased the OA-shoot under drought, with acc. 2232 recording the highest growth from 38 to 51 mg/100 g (+34%).

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

Table 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%).

Statistical variance, correlation and component analysis

The ABA, CCI, Δ^{13}C, OA, WUE and B traits (variables) were submitted to One-way ANOVA, Tukey HSD test (Table 2), Pearson correlation (Table 3) and PCA analysis (Fig. 1).

The ABA-corm and Δ^{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 Δ^{13}C-shoot variables. Then, ABA-corm and Δ^{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 Δ^{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 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 Δ^{13}C-corm and CCI ($r = -0.50$). Moderate positive correlations were also observed among WUE and

Table 3 Pearson correlation coefficients between the water use efficiency (WUE), biomass (B), abscisic acid (ABA), oxalic acid (OA), carbon isotope discrimination (Δ^{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) Δ^{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[b]	–			
(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) Δ^{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)

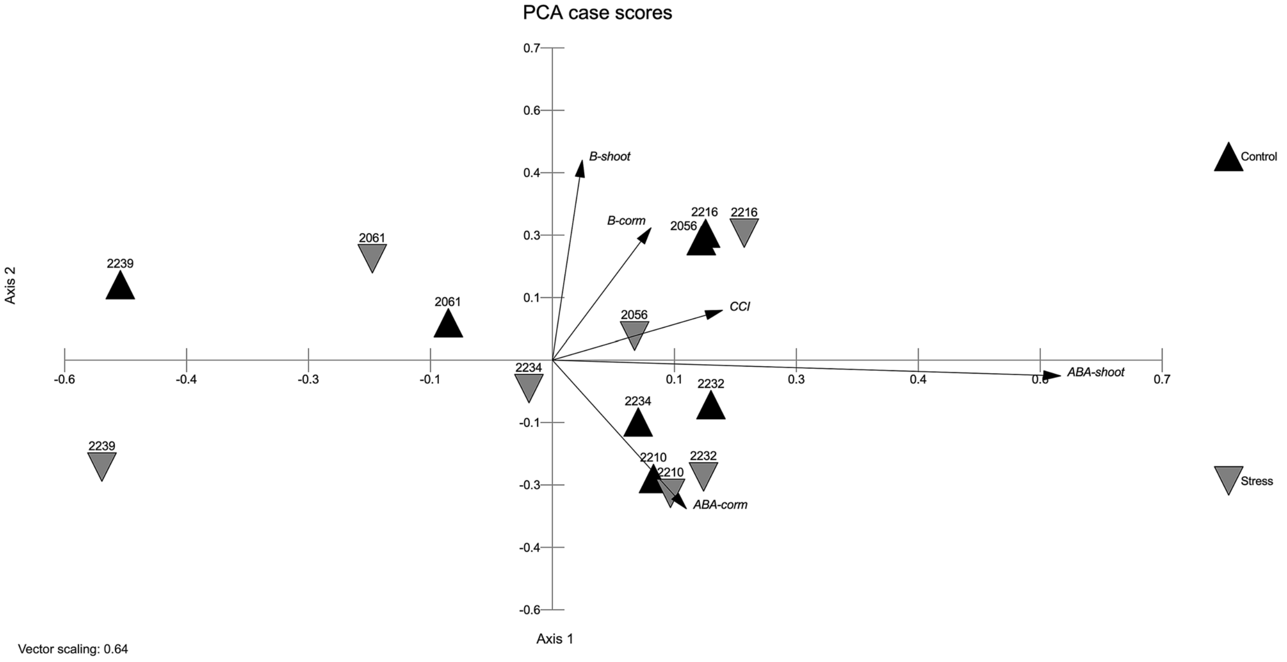

Fig. 1 Principal component analysis (PCA), represented by euclidean biplot spatial distribution of taro (*Colocasia esculenta* L.) accessions, using corm and shoot measurements of the studied variables (ABA, B, CCI, Δ^{13}C, OA, WUE). All the variables were converted by $\log_e$. Control—fully irrigated. Stress—water scarcity

CCI ($r=0.48$); CCI within B-corm ($r=0.48$) and B-shoot ($r=0.33$); Δ^{13}C-shoot and Δ^{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 3).

Finally, a principal component analysis (PCA) was performed using these variables (Fig. 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.

Discussion

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 fourfold 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 fivefold 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.

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 ($\Delta^{13}C = 19.1‰$) was indicative of near open stomata ($\Delta^{13}C = 31‰$, or $\delta^{13}C = -38‰$), 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 $\delta^{13}C = -12‰$) 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.

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.

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 $\delta^{13}C$ analysis and revised the manuscript. VL and MAAPC coordinated the overall work and revised the manuscript.

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