Research Article

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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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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 content 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 to 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.

Supporting information may be found in the online version of this article.

Keywords: chlorophyll content index; drought; *Ipomoea batatas*; protein; soluble and insoluble oxalates; starch

INTRODUCTION

Sweet potato, *Ipomoea batatas* L. [Lam.] (Convolvulaceae), has been cultivated for over 5000 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

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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 signaling 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 the chlorophyll content index (CCI), protein, and starch content variation under drought.

MATERIALS AND METHODS

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 1).

Table 1. Identification code, variety name and origin of the eight sweet potato (*Ipomoea batatas* L.) accessions subjected 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.

Experimental field assay

The sweet potato field assay was plotted in a randomly split-plot field design, established at the ISOPlexis experimental field (32° 39′ N, 016° 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 × 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 $\mu mol\ m^{-2}\ s^{-1}$ for control and 1142.0 $\mu mol\ m^{-2}\ s^{-1}$ 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 19.46 °C average T_a and 68.07% average RH_a were observed for control; a 22.25 °C 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.

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 48 h at 65 °C (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 −35 °C (Liebherr ProfiLine GGPV6570, Schwabach, Germany) until analysis.

Analysis of biochemical composition and nutritional quality

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.

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.

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 × 6.25, according to the AOAC method.[31] The analysis was performed in triplicate, with results presented as g kg^{-1} dry flour.

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-visible 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.

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.

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 a 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).

RESULTS

Variation of oxalate composition according to drought

The sweet potato accession mean values of T-Ox and CaOx decreased slightly in the root tubers, while the S-Ox increased under drought stress (Table 2). In the shoots, an average T-Ox and S-Ox increase and an approximately equal CaOx were recorded (Table 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 2). Accession 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 approximately 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 3). Acc. 2938 had a 14-fold higher shoot CaOx content than acc. 3125, during drought.

Protein content variation and water scarcity

The total mean protein content variation indicated that the sweet potato plants slightly reduced protein content in root tubers and shoots under drought stress. The shoots showed the biggest decrease in protein during stress, although the protein content in the controls was 10% higher in the shoots than in the tubers (Tables 2 and 3). The tuber average protein content decreased slightly, at 62 g kg^{-1} in both experimental conditions. Accession 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 2).

The average crude protein of the stressed shoots decreased by 19%, from approximately 163 to 130 g kg^{-1}, relative to the controls. 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 3).

Table 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.

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 3).

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 2 and 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 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/100 g (+125%), respectively (Table 3).

The quality of tuber starch grain under water scarcity was assessed through starch solubility in water (SWS) and starch swelling power (SSP) (Table 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%).

Variance and parameter associations

Statistically significant associations among variables (parameters) were detected (Tables 2 and 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 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 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 allowed us to observe

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

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

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

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

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

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

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 the 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 the 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.

DISCUSSION

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 behavior 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/100 g 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]

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 those 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 defense and regulation. Accessions 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 in 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 the 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 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]

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] noted 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 a 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 in 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/100 g 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 express 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 in 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.

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 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 oxidized 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 shoot 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 for 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 for the decrease in CO_2 due to partial stomatal closure.[36]

During daylight, the carbon dioxide fixation by photosynthesis also synthetizes 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 the 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 increasing 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 the 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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CONFLICT OF INTEREST

None.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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