



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

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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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), 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 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, carbon isotope discrimination ($\Delta^{13}\text{C}$), 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 $\delta^{13}\text{C}$ content under drought stress, with greater ^{13}C fixation in roots. The $\Delta^{13}\text{C}$ did not show a significant association with WUE. The $\delta^{15}\text{N}$ 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.

1. Introduction

Carbon and nitrogen isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ during drought can indicate how genotypes retain nitrogen (N) in their tissues (Robinson, 2001).

While both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be a useful physiological integrators of stress responses, $\delta^{13}\text{C}$ is the most commonly used for drought assessment of C3 plants, with the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values can be simplified into a positive isotopic carbon discrimination ($\Delta^{13}\text{C}$) 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 $\Delta^{13}\text{C}$ 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; Lauteri et al., 1993). No publication concerns both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as physiological indicators of sweet potato drought response. Albeit, one work used the $\Delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ physiological integrators, and ii.) to assess $\Delta^{13}\text{C}$ as a potential fast tool for improvement of WUE determination.

2. Materials and methods

2.1. Plant material and experimental setup

Eight accessions (acc.) of sweet potato (*Ipomoea batatas*) (designated

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Table 1

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

			$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		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 ^{abcde}	-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 ^{**}	

$\delta^{15}\text{N}$ nitrogen isotopic composition (‰); $\delta^{13}\text{C}$ 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.

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 1514.5 μmol/m²/s for control and 1142.0 μmol/m²/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.

2.2. 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 48 h at 65 °C (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 -35 °C (Liebherr ProfiLine GGPV6570, Germany) until analysis.

2.3. 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.

2.4. Water use efficiency (WUE)

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

2.5. 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.

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

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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ into $\Delta^{13}\text{C}$ by Farquhar et al. (1989), and whole-plant (WP) $\delta^{15}\text{N}$ calculation by Robinson et al. (2000). The analysis was triplicated, and results expressed in per mill (‰).

2.7. 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).

3. Results

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

Table 1 shows the data obtained for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and N contents of tubers and shoots, under both control and drought environments. On average drought increased $\delta^{13}\text{C}$ and decreased $\delta^{15}\text{N}$ and N contents in both organs. Acc. 3124 was the only one presenting a $\delta^{13}\text{C}$ -shoot content decrease during drought.

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

The content of $\delta^{15}\text{N}$ -shoots decreased slightly from 5.25‰ to 3.46‰ (-1.78‰), and $\delta^{15}\text{N}$ -tubers also showed a small decrease from 5.23‰ to 3.65‰ (-1.59‰). Acc. 1038 exhibited the highest significant $\delta^{15}\text{N}$ decrease in both organs and experimental variants. Acc. 3124 had the highest $\delta^{15}\text{N}$ -shoot and $\delta^{15}\text{N}$ -tuber content, while it had the lowest $\delta^{13}\text{C}$ 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 (-5.35 mg), 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.

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

On average, the sweet potato whole-plant (tubers and shoots) acc. decreased WP $\delta^{15}\text{N}$ (-1.73‰), WP $\Delta^{13}\text{C}$ (-1.46‰) and TPB (-295.56 g), and increased WUE ($+3.23\text{ g/L}$) and WP TC ($+0.18\%$) under drought (Table 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 $\delta^{15}\text{N}$, 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 $\delta^{15}\text{N}$ (4.30‰), WP $\Delta^{13}\text{C}$ (19.27‰), WUE (16.45 g/L) and TPB (449.91 g) content under drought. Acc. 3124 also showed the lowest loss of WP $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ and TPB under stress. Acc. 3125 had the second highest WUE and TPB content. However, acc. 1038 showed significantly higher WP $\delta^{15}\text{N}$, WUE and TPB under control conditions, and the most pronounced loss of WP $\delta^{15}\text{N}$ and TPB during water scarcity.

3.3. 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 1 and 2), with the $\delta^{13}\text{C}$ -tuber and $\delta^{13}\text{C}$ -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 $r = 0.50$. $\delta^{15}\text{N}$ -shoot and N-shoot decrease was strongly correlated ($r = 0.56$). Tubers and shoots $\delta^{13}\text{C}$ increase showed a relatively strong correlation with $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\Delta^{13}\text{C}$ 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 3).

The PCA analysis was performed using the whole-plant WUE, TPB, TC, and N, $\delta^{15}\text{N}$, $\Delta^{13}\text{C}$ variables from the tubers (Fig. 1A) and shoots (Fig. 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, $\delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were strongly correlated with PC1, while TC and N were correlated with PC2 (Fig. 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 $\Delta^{13}\text{C}$ and WUE were correlated with PC2 (Fig. 1B).

4. Discussion

4.1. $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ -shoot values during drought. We observed that $\delta^{13}\text{C}$ -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 $\delta^{13}\text{C}$ -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, $\delta^{13}\text{C}$ -shoots can be associated with a better response to stress. Moreover, acc. 1038, 3124 and 3125 showed lower $\delta^{13}\text{C}$ -shoot content, with less variation caused by water scarcity. Possibly they also decreased the stomatal 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 $\delta^{13}\text{C}$

Table 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 $\delta^{15}\text{N}$	WP $\Delta^{13}\text{C}$	WP TC	WUE	TPB
MAD	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}
		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 ^{abcd}	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
CAN	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 ^{abcd}	41.31 ± 0.00 ^b	1.09 ± 0.43 ^a	22.90 ± 21.01 ^a
		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
GUI	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}
		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 $\delta^{15}\text{N}$ whole-plant nitrogen isotopic composition (‰); WP $\Delta^{13}\text{C}$ 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 3Pearson 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. $\delta^{13}\text{C}$	-							
2. $\Delta^{13}\text{C}$	-1.00 ^{**}	-						
3. WP $\Delta^{13}\text{C}$	-0.98 ^{**}	0.98 ^{**}	-					
4. WP TC	0.10	-0.10	-0.13	-				
5. $\delta^{15}\text{N}$	-0.56 ^{**}	0.56 ^{**}	0.55 ^{**}	-0.48 ^{**}	-			
6. N	0.14	-0.14	-0.19	0.04	0.10	-		
7. WP $\delta^{15}\text{N}$	-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. $\delta^{13}\text{C}$	-							
2. $\Delta^{13}\text{C}$	-0.97 ^{**}	-						
3. WP $\Delta^{13}\text{C}$	-0.95 ^{**}	0.98 ^{**}	-					
4. WP TC	0.18	-0.15	-0.13	-				
5. $\delta^{15}\text{N}$	-0.57 ^{**}	0.53 ^{**}	0.55 ^{**}	-0.44 ^{**}	-			
6. N	-0.17	0.22	0.25	-0.46 ^{**}	0.56 ^{**}	-		
7. WP $\delta^{15}\text{N}$	-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 ^{**}

$\delta^{13}\text{C}$ carbon isotopic composition (‰); $\Delta^{13}\text{C}$ carbon isotope discrimination (‰); WP $\Delta^{13}\text{C}$ whole-plant carbon isotope discrimination (‰); WP TC whole-plant total carbon (g/100 g); $\delta^{15}\text{N}$ nitrogen isotopic composition (‰); N total nitrogen (g, DW); WP $\delta^{15}\text{N}$ 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).

under drought. As the sweet potato tubers and shoots presented a less negative $\delta^{13}\text{C}$ value compared to control, we denoted a heavier ^{13}C content under stress environments, which concurs with O'Leary (1981) findings. The overall $\delta^{13}\text{C}$ decrease content was also correlated with TPB decrease, as previously mentioned by Igamberdiev et al. (2004). Although, the sweet potato $\delta^{13}\text{C}$ -tubers had greater ^{13}C fixation during

drought, with higher values compared to $\delta^{13}\text{C}$ -shoots, which is in accordance with Wegener et al. (2015). This generalized greater ^{13}C fixation in tubers may have occurred due a carbohydrate photo-assimilate transport from source to sink tissues (Zhang et al., 2015).

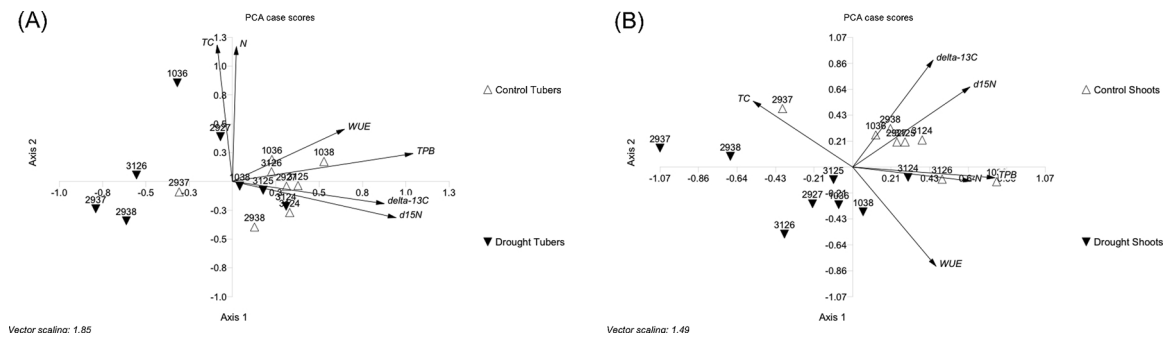


Fig. 1. Euclidean biplot obtained from principal component analysis (PCA), showing variables contribution to the spatial distribution on sweet potato accessions in two principal components (PC), with storage roots (tubers) (A) explaining 68.5% of cumulative variance and shoots (B) explaining 76.0% of cumulative variance. All the variables were standardized by \log_e .

Control is fully irrigated; drought is water scarcity.

4.2. WP $\delta^{15}\text{N}$ as a drought physiological integrator

During drought, N physiological transformations were observed within the sweet potato whole-plant. Both organs lost N and $\delta^{15}\text{N}$ content in a significantly correlated manner. The WP $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ decrease was greater on the $\delta^{15}\text{N}$ -shoot compared to $\delta^{15}\text{N}$ -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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ -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). $\delta^{15}\text{N}$ -shoot and WP $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ -shoot and $\delta^{15}\text{N}$ -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 $\delta^{15}\text{N}$ and TPB were positively correlated. The $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ acting as a sweet potato physiological integrator of drought stress responses (Romero-Trigueros et al., 2014; Robinson, 2001).

4.3. Whole-plant carbon-water relationship to drought

Drought resulted in a significant variability in the sweet potato carbon-water relationship, with WP $\Delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ -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 $\delta^{13}\text{C}$ value by a WP $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ content in both experimental conditions, with acc. 3124 showing the highest WP $\Delta^{13}\text{C}$ content under drought. Since all acc. shared the same growth conditions, the $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ values (richer in $\delta^{13}\text{C}$). Gouveia et al. (2019) and Lauteri et al. (1993) found significant negative relationship between WUE and $\Delta^{13}\text{C}$ 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 ($\delta^{13}\text{C}$ abundance $\sim -27.73\text{‰}$) during drought, one could expect a greater water loss by transpiration.

However, this acc. only decreased 0.08‰ $\delta^{13}\text{C}$ 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 $\text{WP}\Delta^{13}\text{C}$ content loss under drought. The overall decrease of TPB was significantly correlated with the $\text{WP}\Delta^{13}\text{C}$ decrease, leading to more positive $\delta^{13}\text{C}$ content due to a greater ^{13}C fixation, which was also observed by Igamberdiev et al. (2004).

In conclusion, stressed $\delta^{13}\text{C}$ tubers and shoots appeared to be less negative than the controls, with the shoots displaying the higher increase. All $\delta^{13}\text{C}$ values pointed to relatively open stomata as expected for C_3 plants. Drought increased WUE by minimizing evapotranspiration through photosynthesis downregulation, leading to a selective biomass loss, suggesting a drought avoidance strategy. Shoots- $\delta^{15}\text{N}$ shown a strong correlation with N ($r = 0.56$), while it was most strongly correlated with $\text{WP}\delta^{15}\text{N}$ ($r = 0.98$), as an efficient drought response mechanism. Negative correlation between $\Delta^{13}\text{C}$ and WUE was observed, although not significant. The $\delta^{15}\text{N}$ 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.

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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. VL and MAAPC coordinated the work and revised the manuscript.

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Appendix A. Supplementary data

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

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