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

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ABSTRACT

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

1. Introduction

Taro (*Colocasia esculenta* L. Schott) is considered to be one of the oldest crops, currently, playing an important role as a staple food in tropical and developing countries. For optimal yields taro requires a very high water supply, about 2500 mm rainfall per year (Ganança et al., 2018; Sharma and Kaushal, 2016; Lebot, 2009). In our previous work we evaluated the impacts of drought stress on taro based on morpho-agronomic and yield stress indexes (Ganança et al., 2018; Lebot et al., 2017; Ganança et al., 2015). The feasibility of using the identified genetic diversity in breeding programs to adapt this crop to climate change was also addressed.

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

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

Abbreviations: Acc, taro accession number; Δ, carbon isotope discrimination; δ, natural abundance or isotopic composition; N, nitrogen; PCA, principal component analysis; R, isotope (abundance) ratios; SPC, South Pacific Community; TPB, total plant biomass; WP, whole-plant; WUE, water use efficiency

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

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

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

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

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

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

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

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

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

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

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

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

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

2. Materials and methods

2.1. Plant materials

Seven *C. esculenta* accessions originating from Madeira, Canaries and the South Pacific Community (SPC, Fiji) collections were selected to assess the variation of plant biochemical composition under drought conditions. The accessions were selected based on the recently reported information on morpho-agronomic parameters and multi-criteria indices (Ganança et al., 2018) who studied the performance of 33 taro genotypes under drought stress (Table 1).

2.2. Experimental drought conditions

The present study was conducted under controlled conditions during a plant full-growth cycle in 2015 in an open greenhouse in the Preces experimental station, Câmara de Lobos, Madeira, Portugal (32°39'N; 16°58'W). Plants were individually grown in 30 × 30 cm pots, filled with 15 kg of dried soil. The pots were arranged in 6 rows, spaced 90 cm apart, and 30 cm in row separation. Twenty-four plants per accession, 4 per row, were submitted to two watering regimes to assess the influence of drought conditions on plant performance. Three rows were maintained at field capacity (control), while three other were submitted to water deficit (drought stress, 40.2% of water applied

Table 1
Taro (*C. esculenta*) accessions subjected to different watering regimes, for the assessment of the plant biochemical responses to drought stress.

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

[a] Accession identification number code used by the ISOPlexis Genebank.
[b] Classification of drought sensitive, moderate or tolerant accessions based on agro-morphologic screening according to Ganança et al. (2018).

Table 2
Mean value of $\delta^{15}N$ and $\delta^{13}C$ (‰), and total nitrogen concentrations (mg, DW) of shoots and corms, in control and drought taro plants.

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

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

to control) from April to November 2015. Each row was considered a replicate. Experimental design and watering regimes were adapted from Ganança et al. (2018). The crop was grown under a low input management system with no use of pesticides or fertilizers.

2.3. Sample preparation

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

2.4. Total plant biomass (TPB)

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

2.5. Water use efficiency (WUE)

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

2.6. Nitrogen content

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

2.7. Stable isotope analysis

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

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

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

2.8. Statistical analysis

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

3. Results and discussion

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

The $\delta^{13}C$ can provide significant information about taro development, where the isotope value reflects the plant isotopic composition of the immediate environment (O'Leary, 1981). Taro shoots had a greater $\delta^{13}C$ variation than the corms, both under control and drought stress conditions (Table 2). Under drought conditions $\delta^{13}C$ of the shoots and corms became less negative than the controls. A less negative value means richer in ^{13}C, or 'heavier' (O'Leary, 1981). The shoots had a more pronounced variation (+2.60‰), ranging between −27.18‰ (acc. 2232) and −24.58‰ (acc. 2061). In corms, it increased to 2.16‰ and ranged between −26.98‰ (acc. 2239) and −24.82‰ (acc. 2056) (Table 2). The acc. 2216 and 2239 slightly increased the $\delta^{13}C$ negativity in the shoots and corms under drought conditions. The remaining acc. maintained or decreased the $\delta^{13}C$ negativity, with acc. 2061 registering the highest negativity loss (+1.26‰) (Table 2).

Each acc. isotope fractionations might change due to variety, temperature, CO_2 concentration or other natural variables (O'Leary, 1993). According to Igamberdiev et al. (2004), another important factor for determining carbon isotope fractionation in plants is the stomatal conductance. In this work, all the taro $\delta^{13}C$ values under both control and drought conditions (Table 2) pointed to plants with relatively open stomata, since according to O'Leary (1993) $\delta^{13}C$ values for C3 plants are near the -38‰. The response of acc. 2216 and 2239 to drought can be attributed to higher stomatal aperture and photosynthesis effects by carboxylation (O'Leary, 1993). The stomatal aperture increased the intracellular CO_2 uptake under drought and maintained their photosynthetic electron transport from water molecules, through light excitation of photosystem PSII (one of the major sources of ROS in plants), increasing the number of ionized chlorophyll molecules (Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Igamberdiev et al., 2004).

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

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

The genotypic differences in whole-plant (WP) $\delta^{15}N$ values revealed how the taro plants retained N in their tissues under drought stress. The $\delta^{15}N$ acts as a plant potential indicator of the N metabolism and the growing conditions (Serret et al., 2018). We observed a physiological transformation of N within the taro plant, since the $\delta^{15}N$ abundance can be affected at the whole-plant level due to water scarcity (Romero-Trigueros et al., 2014). The plant $\delta^{15}N$ content is usually linked to N fractionation, resulting from plant absorption, assimilation, allocation and loss of N (Evans, 2001). Assimilation of the inorganic N forms (NO_3^- and NH_4^+) allows the plants to synthesize organic N compounds: nitrate (NO_3^-) is converted to nitrite (NO_2^-) by the cytoplasmic enzyme nitrate reductase, and then to ammonium (NH_4^+) through nitrite reductase (Romero-Trigueros et al., 2014; Sahoo et al., 2010; Pike et al., 2002; Robinson, 2001). In higher plants, N is usually taken up as NO_3^- (Sahoo et al., 2010). Relatively to our controls, all stressed taro plants decreased WP $\delta^{15}N$ (Table 3), with the shoot $\delta^{15}N$ abundance exhibiting a greater variation than the corms (Table 2). Robinson et al. (2000) also reported that WP $\delta^{15}N$ of wild barley subjected to drought stress was always more negative than the controls, implying an effective drought response mechanism.

Acc. 2210 had the highest WP $\delta^{15}N$ values under control and drought conditions reaching 9.59‰ and 8.88‰, respectively (−0.71‰) (Table 3). Drought decreased shoot $\delta^{15}N$ from 11.22‰ to 10.20‰ (−1.02‰), while corm $\delta^{15}N$ registered a slight increase from 4.28‰ to 5.31‰ (+1.02‰). This acc. was the most ^{15}N-enriched sample in the study having the most positive $\delta^{15}N$ abundance, which may indicate a good whole-plant N retention during drought stress as reported by Robinson (2001). N-shoot content in acc. 2210 increased from 20.69% to 24.24%, while N-corm also increased from 6.25% to 8.71% under drought (Table 2). Acc. 2216 had also exhibited substantial increase of whole-plant N content under drought, with N-shoot ranging from 20.25% to 22.13%, and N-corm ranging from 11.90% to 13.22%. The WP $\delta^{15}N$ values slightly decreased from 7.63‰ to 6.70‰ (−0.93‰) (Tables 2 and 3). The most significant WP $\delta^{15}N$ decrease of 3.29‰ (from 9.00‰ to 5.71‰) under drought was observed in acc. 2234. The shoot $\delta^{15}N$ decreased from 10.10‰ to 6.32‰ (−3.79‰), while the corm also decreased from 4.05‰ to 3.38‰ (−0.67‰). On the other hand, acc. 2239 had lower WP $\delta^{15}N$ content, with only a −0.15‰ difference under drought, ranging from 6.23‰ to 6.08‰. The shoot $\delta^{15}N$ registered an increase from 7.05‰ to 7.25‰ (0.20‰), and the corm $\delta^{15}N$ registered a slight decrease from 4.90‰ to 4.67‰ (−0.24‰). Among all tested taro lines, the lowest ^{15}N-enrichement was noted in acc. 2239; it had the lowest $\delta^{15}N$ abundance, and the weakest ability to retain N in the tissues. Its N-shoot content of the fully watered controls of 13.84% increased to 17.31% (+3.47%) under drought (Table 2).

Table 3
Mean value of $\delta^{15}N$ and $\Delta^{13}C$ (‰), total WUE (g/L) and biomass concentrations (g/pot, DW) in control and drought taro whole-plant.

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

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

Table 4
Pearson correlation coefficients of the biochemical traits from taro corms in control and drought stress conditions.

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

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

Table 5
Pearson correlation coefficients of the biochemical traits from taro shoots in control and drought stress conditions.

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

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

The corms $\delta^{15}N$ values were correlated (r = 0.31) with N whereas a high correlation of r = 0.65 with WP $\delta^{15}N$ was found (Table 4). No significant correlations between $\delta^{15}N$ and $\delta^{13}C$ in taro shoots or corms (Tables 4 and 5) could be reported, which remains in conformity with Robinson et al. (2000) work on wild barley under drought. We observed a greater variation on the shoot-$\delta^{15}N$ abundance than in the corms (Table 2). This variation was most likely because of the plant nitrate reductase big dependence on NO_3^- flux from the underground organs to the shoots (Sahoo et al., 2010; Werner and Schmidt, 2002). The observed effect of corm-$\delta^{15}N$ increase and shoot-$\delta^{15}N$ decrease on acc. 2210 and 2216, with N accumulation during drought, can be potentially related with greater assimilatory nitrate reductase reaction in corms. The restriction of NO_3^- flux during drought maybe contributed for the overall decrease of WP $\delta^{15}N$ (Table 3), due to the potential reduction of the activity of nitrate reductase in stress conditions (Sahoo et al., 2010). These variations of $\delta^{15}N$ values between the organs could be attributed to tissue reallocation of N under drought, and to external conditions (environment and N source availability) that led to the escalation of N consumption by ^{15}N and ^{14}N isotope fractionations, with $\delta^{15}N$ acting as a plant physiological integrator of stress responses (Romero-Trigueros et al., 2014; Robinson, 2001).

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

During drought, the increase of photosynthesis and decreased rate of photorespiration is regulated by the stomata, which aperture can increase the CO_2 plant intercellular spaces (C_i) (Igamberdiev et al., 2004). When drought-stressed, the stomatal regulated reduction in transpiration provides an opportunity to increase plant WUE. According to Black et al. (2015) a higher stomatal aperture can lead to a higher loss of water due to transpiration. WUE is linked to stomatal aperture, calculated as the ratio of plant biomass through assimilation of CO_2 by photosynthesis, to the loss of water by transpiration (Igamberdiev et al., 2004). Under drought conditions, we observed a significant variability with regard to WUE, ranging from 0.64 to 4.57 $g.L^{-1}$, among taro accessions included in our study. Regardless of water supply, the taro accessions displayed exactly the same variability. Three acc. (2056, 2061, 2216) had high WUE, presumably featuring a more drought-tolerant mechanism. Meanwhile, the remaining acc. had lower WUE probably indicating a moderate susceptibility to drought (Table 3). According to Farooq et al. (2009) genotypes with improved WUE and nutrients allocation are the most drought-tolerant, when compared to drought-sensitive ones. Acc. 2232 recorded the lowest WUE increase of 0.49 $g.L^{-1}$ between control and drought, 0.64 to 1.13 $g.L^{-1}$, respectively. On the other hand, acc. 2216 had the highest WUE in control and under drought conditions, 1.93 to 4.57 $g.L^{-1}$, respectively (2.64 $g.L^{-1}$ increase) (Table 3). Acc. 2216 showed the highest partial stomata aperture ($\delta^{13}C$ abundance ~ −26.27‰) among all acc. included in the study, which should lead to a higher water loss by transpiration (Table 2). In spite of the partially open stomata under drought, acc. 2216 was able to maintain leaf turgidity, minimized water loss through transpiration and improved water use for vital activities, maintaining high photosynthesis rate under drought. One can speculate that taro could have some phenotypic flexibility and morphological mechanisms of drought avoidance through selective biomass loss to prevent the water loss (Farooq et al., 2009). Water shortage during drought period led to a different biomass loss in all studied accessions. Acc. 2061 suffered the smaller biomass loss of only 3.88 g under stress, while the highest biomass loss of 40.57 g was reported for acc. 2056 (Table 3). This mechanism of drought avoidance reduced the taro biomass relatively to water availability, enhancing the WUE, and allowing to maintain plant turgidity and vital activities. As each acc. enhanced WUE with the biomass decrease, in an overall similar way, it led to a moderate correlation between WUE and TPB (r = 0.43) (Tables 4 and 5). In contrast, in a previous drought study of Ganança et al. (2018), these taro cultivars grown under full water conditions showed higher WUE and higher fresh TPB.

A fairly small differences in the $\Delta^{13}C$ values reaching 17.39‰ in acc. 2061 and 19.53‰ in acc. 2232 were found (Table 3). The $\Delta^{13}C$ variation among the accessions is seemingly a reflection in their genetic variation, as no differences between well-watered and water-deprived environments could be identified (Fu et al., 1993). Ivlev (2015) mentioned that lower $\Delta^{13}C$ values leads to higher carbon isotope fractionation, which can be related with the photosynthesis through CO_2 assimilation and photorespiration. For C3 plants, the $\Delta^{13}C$ values could be directly estimated by photorespiration, through stomatal conductance and carbon isotope fractionation. Although, this estimation depends on the growth conditions (Lanigan et al., 2008; Igamberdiev et al., 2004). During photosynthesis, plants discriminate against the heaviest carbon isotope. It changes according to the ratio of the plant intercellular spaces (C_i) *vs* atmospheric CO_2 (C_a), depending on the balance between photosynthetic activity and stomatal conductance (Serret et al., 2018; Farquhar et al., 1989, 1982). Farquhar et al. (1989) suggested that the richer the C3 plants are in $\delta^{13}C$ (with lower $\Delta^{13}C$ values), the greater WUE is. We observed that relationship as a significantly negative correlation between taro shoots $\Delta^{13}C$ and plant WUE (r = −0.33) was found (Tables 3 and 5). Lauteri et al. (1993) also observed the same correlation in C3 sunflower plants grown under drought. They suggested that the significant negative relationship between $\Delta^{13}C$ and plant WUE can be used for assessing the WUE in breeding programs. Indeed, the results obtained during the course of our study seem to confirm that $\Delta^{13}C$ values could serve a possible substitute of the time-consuming direct WUE measurements of taro plants.

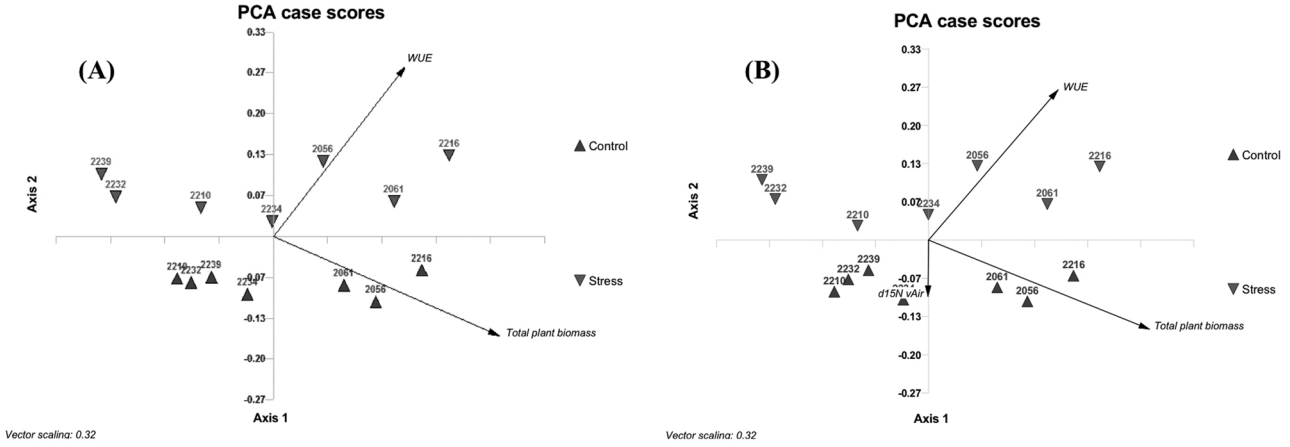

Fig. 1. Representation of the Euclidean biplot by principal component analysis (PCA), with spatial distribution of the *C. esculenta* accessions based in the average isotope values in drought-stressed conditions.
A Scattering distribution of the variables (traits) and case scores (taro accessions) evaluated in corms in two PCA axes.
B Representation of the spatial distribution of the variables (traits) and case scores (taro accessions) studied in shoots in two PCA axes.
Data log_e transformation was applied to all the traits (variables) in analysis.
Control represents well-watered plants; stress - severe drought.

3.4. Whole-plant transformation processes

To better understand the relationship of $\delta^{15}N$, $\Delta^{13}C$, WUE and TPB in seven taro plants subjected to water stress, the one-way ANOVA analysis was applied. The variance and significant differences between accessions ($p \leq 0.01$) and experimental variants (control and drought-stress conditions, $p \leq 0.01$) were found (Tables 2 and 3). Multiple comparisons by the Tukey HSD (data not shown) revealed significant differences between control and drought shoots-$\delta^{15}N$, while no noteworthy differences in the corms-$\delta^{15}N$ could be identified. The corms-N content was significantly different from shoots-N under control and drought conditions. The WUE also differed significantly between control and drought.

The PCA analysis based in the average values of WUE, biomass and isotopic analysis showed discrimination between the corms (Fig. 1A) and the shoots (Fig. 1B) from control and the drought sets. The parameters that contributed the most to the main component were the WUE, TPB and $\delta^{15}N$. The principal components explained 93.2% of cumulative variance observed in the shoots, with 65.6% at first and 27.6% at the second axes, with eigenvalues of 0.25 and 0.11, respectively (Fig. 1B). The accessions distribution corresponded to the observed variability of plant responses under drought stress.

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

4. Conclusion

This study determined how taro $\delta^{13}C$ and $\delta^{15}N$ is related with the whole-plant biomass, WUE and $\Delta^{13}C$ under conditions of water deficit. Accessions 2061, 2216 and 2210 appeared to be the most drought-tolerant genotypes showing the highest WUE and nutrients acquisition. Under drought, $\delta^{13}C$ of shoots and corms became less negative than the controls, with the shoots displayed a greater variation than the corms, increasing by 2.60‰. All $\delta^{13}C$ values pointed to relatively open stomata for C_3 plants. Improved WUE under drought conditions was achieved by minimizing water loss through evapotranspiration and employing phenotypic flexibility and morphological mechanisms of drought avoidance leading to a selective biomass loss. The $\delta^{15}N$ acted as a physiological integrator of stress responses in taro plants. The decrease of WP $\delta^{15}N$ due to stress relative to control acted as a good drought response mechanism. The corms $\delta^{15}N$ exhibited a fair correlation with [N] ($r = 0.31$), while was strongly correlated with WP $\delta^{15}N$ ($r = 0.65$). However, no significant correlations between $\delta^{15}N$ and $\delta^{13}C$ in taro shoots or corms could be identified. We observed a significant negative correlation between taro shoots $\Delta^{13}C$ and plant WUE ($r = -0.33$), similarly to previously reported findings for the C_3 plants. Information presented herein suggest that $\Delta^{13}C$ could be a plausible tool for screening for WUE in taro breeding programs, while $\delta^{15}N$ could serve as a physiological integrator of stress responses in taro plants.

Author contribution

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

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