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Original Article

Screening for Drought Tolerance in Thirty Three Taro Cultivars

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Abstract

Taro [*Colocasia esculenta* (L.) Schott] is a root crop which is an important staple food in many regions of the world, producing 10.5 million tonnes on 1.4 million hectares a year. The crop is cultivated in wet (rain fed) or irrigated conditions, requiring on average 2,500 mm water per year, and in many countries it is cultivated in flooded plots. It is estimated that taro production could decrease by 40% as a result of the increase in drought and other severe events. In this work, thirty three accessions, including local cultivars, selected and hybrid lines were submitted to long duration drought stress and screened for tolerance. Twelve physiological, morphological and agronomic traits were measured at harvest, and subject to multivariate analysis. Stress indices, Water Use Efficiency and Factorial Analysis were useful for discriminating accessions regarding drought tolerance and yield stability, and drought tolerant and susceptible cultivars were identified. Our results confirm that different taro cultivars have different drought avoidance and tolerance strategies to cope with water scarcity. Better yield performers minimised biomass and canopy loss, while tolerance was observed in cultivars that presented low potential yield, but efficiently transferred resources to enhance corm formation. Among the 33 accessions, two local cultivars showed high yield stability and could be considered as suitable parents for breeding programs, while two others are well adapted to drought, but with overall low yield potential.

Keywords: abiotic stress, multicriteria indices, plant water use, taro breeding, yield stability

Introduction

Taro [*Colocasia esculenta* (L.) Schott] is an underutilized root crop, originating from the Southeast Asia (Rao *et al.*, 2010; Mabhaudhi and Modi, 2015), which is an important staple food in many regions of the world, producing 10.5 million tonnes on 1.4 million hectares, with an average yield of 7.5 t/ha (FAOSTAT, 2013). It is grown through the Pacific Islands, Asia, Africa, Europe and the Caribbean Islands, where thousands of cultivars adapted to different agro-ecological conditions are maintained by local farmers.

The crop is cultivated under wet (rain fed) or irrigated conditions, on flooded plots, or on dry fields requiring on average 2,500 mm rainfall per year, to obtain optimal yields (Onwueme, 1999). The taro genetic diversity outside Southeast Asia is considered narrow, which makes the crop vulnerable to a range of damaging biotic factors (Rao *et al.*, 2010). Similarly, it makes taro vulnerable to abiotic constraints, namely increasing frequency and intensity of droughts, resulting from ongoing climate changes affecting the main producing countries (Wairiu *et al.*, 2012).

Under the current scenario, it is estimated that taro production during the next 30 years could decrease by 40% as a result of the increase in drought and other severe weather events (Wairiu *et al.*, 2012). Little is known about the crop performance under drought conditions. Several

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morphological, agronomic, yield and physiological parameters have been used to assess varietal performance of taro cultivars with regard to drought tolerance (Sivan, 1995; Bussel and Bonin, 1998; Manyatsi *et al.*, 2011; Mabhaudhi *et al.*, 2013; El-Zohiri and Abd El-Aal, 2014; Mabhaudhi and Modi, 2015). Unfortunately, no information is available regarding the genetic control of drought tolerance in taro, hindering the possibility of fast screening of the crop towards drought tolerance.

Recently, Mabhaudhi *et al.* (2014) used the AquaCrop model to simulate yield responses to water supply of a South African eddoe type taro landrace. However, the model still showed some limitations in simulating taro growth under moisture stress, and more information is required to develop a comprehensive drought stress model. Moreover, in this crop its difficult to replicate or to maintain stress conditions under controlled environments (Ganança *et al.*, 2015). A major issue to be addressed is still, therefore, the establishment of the crop drought stress conditions, as well as the detection of traits that can specifically discriminate the variation of plant performance under drought. However, from a breeders perspective, yield stability under dry conditions is the main objective (Ganjeali *et al.*, 2011). In fact, genotypes that have high yield under both stressed and non-stressed conditions could be considered drought resistant (Blum, 2005), and plant screening should be conducted based on the comparison of fitness and high performance under these conditions (Ganjeali *et al.*, 2011). Several indices were developed to evaluate crop yield stability under stress conditions. Stress Tolerance Index (STI) (Fernandez, 1992), and Stress Susceptibility Index (SSI) (Fisher and Maurer, 1978) are two of the most frequently used (Ganjeali *et al.*, 2011). These indices were applied to evaluate yield stability under drought stress conditions in mungbean (Fernandez, 1992), chickpea (Ganjeali *et al.*, 2011), and wheat (Farshadfar *et al.*, 2013).

To date, research attempts in taro have focused on describing the effects of drought on morphological and physiological traits. Crop productivity expressed as Harvest Index (HI) has been used to assess and compare a traditional Samoan cultivar and an improved drought resistant cultivar (Bussel and Bonin, 1998), as well as South African cultivars (Mabhaudhi *et al.*, 2015) for their suitability to growth under stress conditions. To the best of our knowledge, no attempts to identify taro genotypes suitable for breeding towards drought tolerance trough evaluations of yield stability under conditions of soil moisture deficit have been reported. The objectives of this study were to screen a large number of taro cultivars for drought tolerance using easy to record morpho-agronomic parameters, and evaluate yield stability as a measure of taro drought tolerance.

Materials and Methods

Plant materials and culture

Evaluation of taro cultivars for drought resistance was conducted in open greenhouses in the Preces experimental station, Câmara de Lobos, Madeira, Portugal (32° 39' N; 16° 58' W) during a full plant growth cycle (from June 2013 to July 2014).

Thirty three taro accessions (Table 1), including 14 breeders lines or elite cultivars from TANSAO core collection provided by the Secretariat of the Pacific Community (SPC, Fiji), and 19 cultivars from Europe (ten from Madeira, six from Canary Islands, two from Azores and one from Cyprus) were studied. Before the drought assay (2012-2013), plants of all cultivars were multiplied, acclimatized and maintained in the experimental greenhouses. Ploidy levels of the studied varieties (whenever available) were obtained from Traoré (2013) and Kreike *et al.* (2004).

Experimental design

In June 2013, a total of 660 plants were established in individual 30×30 cm pots filled with 15 kg of dried soil. The pots were arranged in rows spaced 90 cm apart, with 30 cm in row separation. Each pot was treated as a single soil-plant system and all were laid out in a randomized complete block design. Twenty corms heads per cultivar at the same development stage and size were collected. Ten plants per cultivar were used as control (fully watered) and ten plants were submitted to stress conditions (experimental variant). The experimental design was a factorial experiment with two factors: irrigation level and cultivar.

Experimental conditions

Before potting, soil in pots was amended with compost in a 3:1 proportion, sampled and analysed for its physical and edaphic properties, including major mineral nutrients content (data not shown). The soil was classified as Sandy Clay Loam (USDA taxonomic system). Soil pH (water) was 5.7 and organic mater content was 14.65%. The soil water characteristics hydraulic properties calculator (http://hydrolab.arsusda.gov/soilwater/Index.htm) was used to calculate soil field capacity (37.1%), permanent wilting point (22.6%), and saturation (58.4%). Following soil analyses, fertilizer application during the experiment was not deemed necessary. Manual pot weeding was done regularly.

Irrigation regimes were defined based on a test pilot were evapotranspiration (ET) at field capacity (FC) was monitored during four weeks, in the acclimation year (three weeks in July-August and one week in November) in the real pot plant-soil system, with six fully developed taro plants. Evapotranspiration (ET) was measured daily by weighting the pot, and compensating for water loss (Dwyer, 1987) during each week of the pilot study. Under greenhouse conditions, ET averaged 10.7 ± 3.5 $mm.day^{-1}$ (ranging from 5.6 to 13.7 $mm.day^{-1}$). This value was subsequently used to define water irrigation regimes in the actual experiment. After planting, all pots were brought to full FC and equally irrigated (10.7 $mm.day^{-1}$) until desired experimental conditions were met. Drought conditions were imposed from October 2013 until the harvest 298 days later (July 2014). Control plants (100% water requirement) received in total 3,373.4 $mm.pot^{-1}$ at rate of 10.7 $mm.day^{-1}$. The stress variant (53% water requirement) was provided in total with 1,812.8 $mm.pot^{-1}$ at rate of 5.7 $mm.day^{-1}$. Irrigation was delivered daily using a drip irrigation system with an average discharge rate per dripper of 2.9 $L.h^{-1}$.

Table 1. ISOPlexis Genebank accession, designation and geographical origin, and ploidy levels of the cultivars used in the drought stress assay

Accession	Cultivar name	Origin	Ploidy levels
2056	Listado	Canary Islands - La Palma	n.d.
2057	Colorado	Canary Islands - La Gomera	n.d.
2058	Morado	Canary Islands - La Palma	n.d.
2060	Barranquera	Canary Islands - Gran Canaria	n.d.
2061	Blanco Saucero	Canary Islands - La Palma	n.d.
2062	Barranquera de los Sauces	Canary Islands - La Palma	n.d.
2183	190/05 Branco	Azores Islands - Terceira Island	n.d.
2184	Vermelho	Azores Islands - Terceira Island	n.d.
2186	Kolokasi	Cyprus	n.d.
2207	Roxo	Madeira Island	3*x*
2208	Branco	Madeira Island	n.d.
2209	Branco	Madeira Island	n.d.
2210	Roxo	Madeira Island	3*x*
2211	Branco	Madeira Island	n.d.
2212	Roxo	Madeira Island	n.d.
2213	Branco	Madeira Island	n.d.
2214	Roxo	Madeira Island	n.d.
2215	Roxo	Madeira Island	n.d.
2216	Branco	Madeira Island	3*x*
2232	PE×PH 15-6 BL/HW/08	SPC, Fiji	2*x*
2233	C3-12 BL/PNG/10	SPC, Fiji	2*x*
2234	C3-22 BL/PNG/11	SPC, Fiji	2*x*
2235	Samoa43 BL/SM/43	SPC, Fiji	2*x*
2236	Lepa BL/SM/149	SPC, Fiji	2*x*
2237	Ngerruch CE/PAL/10	SPC, Fiji	n.d.
2238	Karang CE/MAL/08	SPC, Fiji	n.d.
2239	Karang CE/MAL/10	SPC, Fiji	n.d.
2240	Lebak CE/Ind/16	SPC, Fiji	2*x*
2241	Manokwari CE/Ind/31	SPC, Fiji	2*x*
2242	Srisamrong CE/THA/07	SPC, Fiji	2*x*
2244	Boklua CE/THA/24	SPC, Fiji	2*x*
2245	Wasehasuba-Imo CA/JP/02	SPC, Fiji	2*x*
2246	Takenoko-Imo CA/JP/08	SPC, Fiji	n.d.

n.d. - not determined

Data collection and statistical analysis

For each cultivar, seven morphological, one physiological and three agronomic traits were measured at harvest on five randomly selected plants per treatment (Tables 2, 3, 4, 5). Chlorophyll content index was measured using a CCM-200 plus chlorophyll content meter from Opti-Sciences (USA). Leaf Area (LA) was calculated as a function of leaf width and length according to Manyatsi *et al.* (2011). Water Use Efficiency (WUE) was calculated as the ratio of total plant biomass to total water used per pot, and expressed in g.m^{-3}. Harvest Index (HI) was adapted from Mabhaudhi *et al.* (2013). Stress Tolerance Index (STI) and Stress Susceptibility Index (SSI) were adapted from Ganjeali *et al.* (2011) and Farshadfar *et al.* (2013), respectively, using corm biomass as a measure of yield. Statistical analysis, with variance analysis, correlations and factorial analysis, were performed using SPSS for Windows, version 22.

Results

Growth traits

Under controlled conditions, leaf number (LN), plant height (PH) and leaf area (LA), (Tables 2, 3, 4), were significantly higher than under stress ($p \leq 0.001$). Also, the three parameters differed significantly among cultivars ($p \leq 0.001$). A significant treatment × genotype interaction was detected for PH and LA ($p \leq 0.001$), but not to LN ($p \leq 0.05$). PH and LN varied significantly between stress and non stress conditions, but we can not confirm that observed LN variation between the treatments could be attributed to drought conditions. Mean LN under stress was on average 83.20% of the control plants, and the majority of cultivars (27) had equal or less leaves under stress conditions than under control. All cultivars presented a decreased plant height under stress, ranging between -11.62 and -66.36% of the total height. Mean PH under stress was on average

62.34% of the control, and LA under stress was on average 48.66% of the control plants.

These results indicate that PH and LA could be used to discriminate cultivars with regard to drought tolerance, while LN appeared to be an unusable indicator.

Chlorophyll Content Index (CCI)

Chlorophyll Content Index (CCI) showed great variability among the cultivars (Tables 3 and 4). Under stress conditions, 22 cultivars showed higher CCI, while 11 had lower CCI. Mean CCI was significantly higher (p ≤ 0.001) under stress than under control (22.16% higher). Performance among cultivars differed significantly in relation to CCI (p ≤ 0.001). Significant treatment × genotype interaction (≤ 0.001) was observed, revealing that, at least in some cultivars, CCI varied between stress and non stress conditions.

Water Use Efficiency (WUE)

WUE was used to assess taro's capacity to face water scarcity (Table 4). On average, WUE decreased 35.42% under stress, with cultivar 2061 showing the best performance. Mean WUE was significantly higher under control than under stress (p ≤ 0.001). WUE differed significantly among the cultivars, and a significant treatment × genotype interaction (p ≤ 0.001) was detected, showing a significant parameter variation under stress in some genotypes.

Table 2. Data of morphological, physiological, and agronomic parameters obtained under control conditions

Accession	CCI	LN	PH (cm)	LA (cm^2)	TPB (g)	FAGW (g)	DAGW (g)	DCW (g)	FCW (g)
2056	39.57	2.20	48.86	325.76	347.00	83.43	8.28	15.27	185.43
2057	30.98	1.80	57.06	705.95	568.00	175.60	15.18	72.94	335.56
2058	22.67	2.00	67.78	576.16	836.00	171.89	15.74	167.57	610.27
2060	32.98	1.20	52.50	483.49	744.00	100.04	11.99	102.61	442.36
2061	25.77	2.60	66.76	668.20	986.00	293.25	37.29	177.40	621.87
2062	35.98	1.80	53.96	851.04	473.00	154.53	15.54	33.08	230.53
2183	27.99	1.60	49.50	375.62	634.00	119.92	7.70	73.17	444.76
2184	21.47	1.40	58.06	662.23	600.00	139.42	9.33	86.46	407.18
2186	22.71	1.80	54.04	485.60	569.00	118.01	15.57	70.37	381.71
2207	32.16	2.00	56.84	491.63	592.00	113.64	8.42	64.03	381.89
2208	30.97	1.40	48.76	682.28	682.00	134.86	23.09	80.31	493.08
2209	24.03	2.00	58.12	837.46	881.00	194.53	17.05	117.44	585.78
2210	2.20	2.20	41.08	276.71	314.00	76.43	9.13	40.59	191.59
2211	29.07	2.20	67.90	990.61	1,788.00	730.11	65.43	201.09	756.79
2212	41.59	2.20	39.10	219.84	205.00	29.78	3.81	32.18	149.96
2213	29.45	2.00	63.66	1,093.28	967.00	258.68	27.21	112.11	594.49
2214	24.26	2.20	56.96	480.67	665.00	155.09	28.16	112.93	414.60
2215	49.75	2.20	43.94	220.15	244.00	52.04	5.37	34.56	143.52
2216	41.17	2.00	82.10	1,883.19	1,728.00	618.50	47.22	112.51	770.55
2232	22.65	1.60	60.98	378.03	304.00	39.04	11.13	40.30	175.27
2233	28.33	2.50	63.58	446.57	403.75	144.13	14.75	61.68	175.43
2234	28.96	2.60	55.04	298.74	200.00	64.18	9.27	19.61	63.97
2235	27.24	3.00	61.80	355.17	295.00	160.64	11.48	20.05	105.17
2236	22.27	2.60	45.06	157.05	261.00	49.90	10.83	54.97	177.14
2237	33.73	2.60	63.92	588.16	496.00	219.11	18.80	42.49	178.89
2238	32.29	2.40	44.76	223.95	351.00	76.99	8.59	48.28	214.49
2239	17.20	2.80	43.74	307.40	410.00	133.60	12.37	39.84	199.60
2240	24.53	2.20	43.04	299.39	560.00	251.49	21.68	39.10	189.96
2241	29.30	2.80	62.18	520.53	583.00	304.70	20.71	63.53	207.46
2242	41.91	3.40	35.86	215.08	115.00	48.47	5.42	12.17	74.85
2244	17.58	2.00	40.18	146.86	199.00	22.23	3.66	31.61	136.14
2245	55.17	2.80	35.66	230.84	279.00	69.92	8.04	32.75	163.83
2246	49.71	3.00	31.64	54.23	49.00	11.69	2.13	8.19	24.00
Mean	31.35ab	2.22ab	53.16ab	500.97ab	555.42ab	161.09ab	16.07ab	67.31ab	309.94ab
S.E.	1.62	0.09	1.98	61.30	68.28	26.72	2.29	8.40	35.81
Min	17.20	1.20	31.64	54.23	49.00	11.69	2.13	8.19	24.00
Max	55.17	3.40	82.10	1,883.19	1,788.00	730.11	65.43	201.09	770.55

CCI - Chlorophyll Content Index; LN - Leaf Number; PH - Plant Height; LA - Leaf Area (Leaf Width × Leaf Length × 0,85); TPB - Total Plant Biomass; FAGW - Fresh Above Ground Weight; DAGW - Dry Above Ground Weight; DCW - Dry Corm Weight; FCW - Fresh Corm Weight.

a - Significant differences between accessions (ANOVA, $p \leq 0.05$)

b - Significant differences between stress and non stress conditions (ANOVA, $p \leq 0.05$)

Table 3. Data of morphological, physiological, and agronomic parameters obtained under drought conditions

Accession	CCI	LN	PH (cm)	LA (cm^2)	TPB (g)	FAGW (g)	DAGW (g)	DCW (g)	FCW (g)
2056	22.83	1.80	40.18	305.64	330.00	54.38	6.94	44.71	229.41
2057	32.83	1.40	24.76	130.37	104.00	18.80	2.02	11.52	73.55
2058	72.22	1.20	31.28	262.28	331.00	33.46	3.99	25.77	158.62
2060	19.09	1.20	36.82	431.58	296.00	52.44	7.32	14.36	77.06
2061	37.77	1.40	55.46	567.56	565.00	157.25	24.39	87.73	376.98
2062	75.29	2.00	25.80	201.72	85.00	44.14	4.81	3.96	27.39
2183	39.69	2.00	32.24	142.59	223.80	28.28	3.95	16.86	137.91
2184	32.51	2.40	29.62	257.15	115.00	38.14	4.93	7.49	48.90
2186	44.73	1.80	33.30	225.69	150.00	47.44	5.39	20.21	103.47
2207	21.67	2.60	40.42	379.87	306.00	93.35	9.79	33.59	145.65
2208	33.11	1.40	40.96	346.63	108.00	53.76	6.02	6.25	34.58
2209	47.81	1.80	29.62	290.70	123.00	36.52	3.72	9.74	70.63
2210	69.35	1.60	27.54	116.71	105.00	19.95	2.67	15.85	57.96
2211	58.81	1.60	33.70	400.85	276.00	68.36	9.42	25.57	157.27
2212	19.90	1.80	29.98	222.80	149.00	43.20	3.61	22.28	85.34
2213	46.30	1.60	37.74	427.27	244.00	60.56	6.38	27.37	157.22
2214	58.53	1.80	34.24	174.69	259.00	31.50	3.07	50.46	190.60
2215	35.22	2.20	30.44	160.67	139.00	32.52	3.48	23.18	91.56
2216	29.11	2.20	46.56	861.63	451.00	196.96	19.02	29.24	200.13
2232	24.25	1.25	33.48	157.14	51.25	18.51	2.48	4.15	18.13
2233	54.08	1.50	44.50	233.52	179.17	59.54	4.34	34.15	131.46
2234	29.68	1.60	38.12	237.24	214.00	52.41	6.39	40.60	160.94
2235	21.13	1.67	22.40	85.69	18.33	9.25	0.97	0.54	2.94
2236	33.14	2.50	34.15	124.07	124.00	12.39	2.32	22.10	67.57
2237	42.27	1.00	21.50	88.82	15.00	9.06	1.11	0.48	3.19
2238	42.91	2.20	34.92	199.34	126.00	40.001	4.61	13.11	58.14
2239	34.08	2.80	31.74	153.72	292.00	91.20	11.25	31.65	159.95
2240	53.08	2.40	38.04	246.89	264.00	77.68	9.86	29.40	136.43
2241	40.13	2.25	31.20	179.10	273.00	29.10	3.28	29.14	113.22
2242	24.71	2.00	29.30	110.52	139.00	12.91	2.50	34.61	108.84
2244	12.93	1.60	30.30	140.09	171.00	26.72	3.55	26.77	115.71
2245	41.76	2.25	19.80	103.33	98.75	17.54	2.72	14.72	62.75
2246	12.96	2.00	23.58	102.48	31.00	13.48	2.18	2.84	9.56
Mean	38.30c	1.84	33.14c	244.49c	192.62c	47.90c	5.71c	23.04c	108.27c
S.E.	2.84	0.77	1.31	28.02	21.43	7.03	0.85	3.02	13.32
Min	12.93	1.00	19.80	85.69	15.00	9.06	0.97	0.48	2.94
Max	75.29	2.80	55.46	861.63	565.00	196.96	24.39	87.73	376.94

CCI - Chlorophyll Content Index; LN - Leaf Number; PH - Plant Height; LA - (Leaf Width x Leaf Length x 0,85); TPB - Total Plant Biomass; FAGW - Fresh Above Ground Weight; DAGW - Dry Above Ground Weight; DCW - Dry Corm Weight; FCW - Fresh Corm Weight.
c - Significant G×E interaction (ANOVA, $p \leq 0.05$)

Agronomic traits

All five parameters, Total Plant Biomass (TPB), Fresh and Dry Above Ground Biomass (FAGW and DAGW) and Fresh and Dry Corm Weight (FCW and DCW), (Tables 3 and 4), were significantly higher under control than under stress ($p \leq 0.001$), and all of them differed significantly among cultivars ($p \leq 0.001$). Also, a significant treatment x genotype interaction was detected in all of them ($p \leq 0.001$), showing that variation on these parameters can discriminate crop behaviour under stress conditions. Mean TPB under stress was on average 34.68% of control plants. Only two cultivars showed an increase in plant biomass under stress with the largest gain being 20.87% of the control plants biomass in cultivar 2242. For the remaining 31 cultivars, the highest loss (97% of the control plants biomass) was in cultivar 2237.

Three of the tested cultivars increased their FAGW, and cultivar 2216 showed the higher FAGW under drought stress. In contrast, cultivar 2237 showed a 95% loss in relation to control plants. Mean FAGW under stress was on average only 29.74% of the control plants. Mean DAGW under stress was on average only 35.53% of control plants. Two of the tested cultivars slightly increased their DAGW, with cultivar 2207 showing the best performance (more 16.27%). Cultivar 2237 had similar performance in FAGW and DAGW, with a decrease of 94% of biomass. Mean FCW under stress was on average only 34.93% of the control. Only three of the tested cultivars showed an increase of yield under stress, with maximal biomass value reaching 151.57% in cultivar 2234 and maximal biomass loss of 98% in cultivar 2237.

Dry corm biomass (DCW) was recorded as an important measure of corm quality, in which heavier dry corms translate into an increased content of starch. Mean DCW under stress was on average 34.23% of control. Three of the tested cultivars increased their dry yield under stress, with cultivar 2056 presenting the best performance of 192.89% and with cultivar 2237 showing a decrease of 99% of dry corm biomass.

Stress tolerance indices

Fresh corm weight as a measure of taro yield was used to calculate harvest (HI), stress susceptibility (SSI) and tolerance (STI) indices (Tables 5 and 6). Mean HI was slightly lower (7.62%) under stress. There was no statistically significant differences among cultivars, and between treatments for HI. Differences under stress indices are significant among cultivars for SSI and STI ($p \leq 0.001$).

Using corm biomass, WUE and stress tolerance indices, we were able to rank taro cultivars according to their drought tolerance (Table 5). The Madeiran, Canary Islands and Azorean cultivars are among the ten top drought tolerant cultivars as measured by STI (Table 5), and in general also show a better WUE and HI. SPC cultivars dominate in SSI top ranking with some of them showing good WUE.

Multivariate analysis

Factor analysis (FA), using the Principal Component Analysis (PCA) as extraction method and Varimax rotation, was performed to verify if the assay data variation and obtained factors could explain cultivars performance and identify drought tolerance (Fig. 1). Ten variables, showing significant differences among cultivars and treatments were used in the FA including: plant growth (PH, LA) and agronomic (TPB, FCW, DRW, FAGW, DAGW) traits, WUE and stress indices (SSI, STI). In spite that CCI satisfies both previous conditions, it was not used because it decreased the sample adequacy for the analysis. Result of the Kaiser Meyer Olkin Test (KMO) was 0.749, indicating that sampling was adequate. Two factors were extracted, with eigenvalues > 1, explaining 81.73% of total variability, with 1st component representing 63.17% and the 2nd, 18.55% (Fig. 1). Factor 1 appears to be associated with variables for plant growth, agronomic traits and WUE under control, while factor 2 was linked to the same variables for plants under stress. SSI and STI were equally associated with both components. STI scored positively in both components, while SSI scored positively in factor 1 and negatively in factor 2. Taro cultivars distribution along plot components indicates that these agronomic traits and stress indices can be used to discriminate taro cultivars with regard to drought tolerance (Fig. 1).

Table 4. Data for Harvest Index (HI), Water Use Efficiency (WUE), Stress Susceptibility Index (SSI) and Stress Tolerance Index (STI), obtained under control and drought conditions

Accession	HI (%) control	HI (%) drought	WUE (g m^{-3}) control	WUE (g m^{-3}) drought	SSI	STI
2056	52.36	69.66	3,070.52	5,433.89	-6.75	0.34
2057	58.78	60.51	5,026.10	1,712.50	0.69	0.23
2058	73.04	50.91	7,397.58	5,450.35	1.10	0.93
2060	57.99	28.80	6,583.49	4,874.03	1.14	0.43
2061	62.83	68.67	8,724.89	9,303.47	0.60	2.66
2062	49.78	31.75	4,185.47	1,399.64	1.39	0.07
2183	70.17	56.99	5,610.12	3,685.16	0.95	0.77
2184	66.20	34.20	5,309.26	1,893.63	1.38	0.27
2186	67.15	81.35	5,034.95	2,469.95	1.12	0.43
2207	64.39	44.30	5,238.47	5,038.70	0.83	0.70
2208	75.21	32.06	6,034.86	1,778.36	1.43	0.17
2209	70.66	44.61	7,795.77	2,025.36	1.34	0.38
2210	61.10	53.59	2,778.52	1,728.96	1.02	0.13
2211	48.36	49.81	15,821.61	4,544.71	1.27	1.55
2212	72.97	59.73	1,814.00	2,453.48	0.73	0.16
2213	62.57	68.78	8,556.76	4,017.78	1.11	0.98
2214	66.87	68.71	5,884.44	4,264.78	0.85	0.89
2215	55.04	65.82	2,159.10	2,288.82	0.18	0.15
2216	46.16	46.22	15,290.68	7426.31	1.13	1.61
2232	51.59	35.60	2,690.03	675.12	1.12	0.02
2233	45.29	62.20	2,381.80	2,950.22	-0.50	0.09
2234	29.80	70.12	1,769.75	3,523.79	-8.25	0.08
2235	24.90	16.56	2,610.39	301.88	1.01	0.00
2236	67.86	50.69	2,309.53	2,041.82	0.72	0.12
2237	35.76	21.27	4,388.99	49.40	1.50	0.00
2238	61.85	47.99	3,105.92	2074.76	1.11	0.13
2239	49.10	56.61	3,628.00	4,808.17	-0.06	0.38
2240	39.07	52.04	4,955.31	4,347.11	0.03	0.28
2241	74.27	47.82	5,158.84	4,495.31	0.68	0.27
2242	49.90	77.04	1,017.61	2,288.82	-0.02	0.06
2244	66.74	67.38	1,760.91	2,815.74	-2.38	0.16
2245	57.29	62.26	2,468.81	1,300.84	0.53	0.09
2246	46.03	53.78	433.59	510.46	0.95	0.00
Mean	57.00	52.66	4,878.66abc	3,150.71abc	0.24a	0.44a
S.E.	2.26	2.77	607.79	357.07	0.37	0.10
Min	24.90	16.56	433.59	49.40	-8.25	0.00
Max	75.21	81.35	15,821.61	9,303.47	1.50	2.66

a - Significant differences between accessions (ANOVA, $p \leq 0.05$)
b - Significant differences between stress and non stress conditions (ANOVA, $p \leq 0.05$)
c - Significant G×E interaction (ANOVA, $p \leq 0.05$)

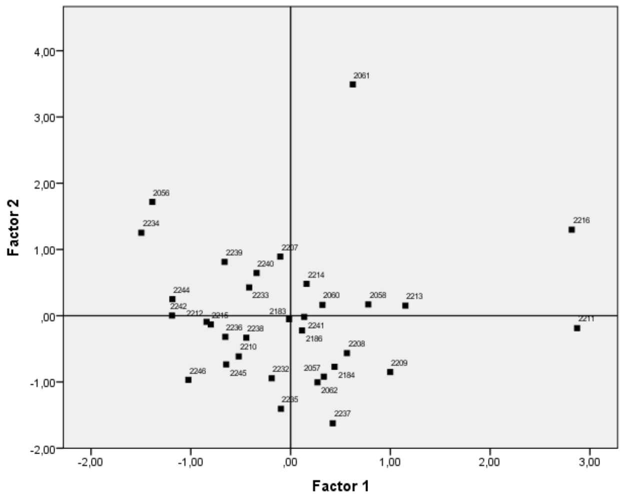

Fig. 1. Factor Analysis, using Principal Component Analysis (PCA) as extraction method, and 10 significant variables to discriminate the behaviour of taro cultivars under drought stress. Factor 1 and 2 explain 63.17% and 18.55 % of observed variability, respectively

Discussion

Screening of taro genotypes for drought tolerance is a challenging task, due to the crop growth characteristics, and cultivation under persistent wet conditions. Previous studies were conducted only on a limited number of cultivars, with more than 25 morphological, agronomic and physiological parameters used to assess taro drought tolerance (Sivan, 1995; Bussel and Bonin, 1998; Manyatsi *et al.*, 2011; Mabhaudhi *et al.*, 2013; El-Zohiri and Abd El-Aal, 2014; Mabhaudhi and Modi, 2015). Sivan (1995) calculated that the crop water use varied between 1,500 and 2,000 mm (3.2 to 5.6 mm.day^{-1}) or 2 to 6.5 mm.day^{-1} in Florida. Onwueme (1999) showed that optimal yields required 2,500 to 3,000 mm of water. Fares (2008) used Kc described by Allen *et al.* (1998) to determine taro water (ETa) needs in 115 to 120% of local evapotranspiration. Mabhaudhi and Modi (2013) used this approach to define irrigation regimes and modulate crop behaviour under drought stress.

Potential evapotranspiration (ETo) during the drought trial on Madeira, measured at open field, was on average 3.8 mm day^{-1} (data not shown). A four week pilot trial with daily

Table 5. Taro cultivars, ranked in decreasing order of drought tolerance, using Harvest index (HI), Water Use Efficiency (WUE), Stress Susceptibility Index (SSI) and Stress Tolerance Index (STI)

HI (%) control	HI (%) drought	WUE (g m^{-3}) control	WUE (g m^{-3}) drought	SSI	STI
2208	2186	2211	2061	2234	2061
2241	2242	2216	2216	2056	2216
2058	2234	2061	2058	2244	2211
2212	2056	2213	2056	2233	2213
2209	2213	2209	2207	2239	2058
2183	2214	2058	2060	2242	2214
2236	2061	2060	2239	2240	2183
2186	2244	2208	2211	2215	2207
2214	2215	2214	2241	2245	2186
2244	2245	2183	2240	2061	2060
2184	2233	2184	2214	2241	2209
2207	2057	2207	2213	2057	2239
2061	2212	2241	2183	2236	2056
2213	2183	2186	2234	2212	2240
2238	2239	2057	2233	2207	2241
2210	2246	2240	2244	2214	2184
2057	2210	2237	2186	2246	2057
2060	2240	2062	2212	2183	2208
2245	2058	2239	2242	2235	2244
2215	2236	2238	2215	2210	2212
2056	2211	2056	2238	2058	2215
2232	2238	2210	2236	2238	2238
2242	2241	2232	2209	2213	2210
2062	2216	2235	2184	2232	2236
2239	2209	2245	2208	2186	2245
2211	2207	2233	2210	2216	2233
2216	2232	2236	2057	2060	2234
2246	2184	2215	2062	2211	2062
2233	2208	2212	2245	2209	2242
2240	2062	2234	2232	2184	2232
2237	2060	2244	2246	2062	2235
2234	2237	2242	2235	2208	2246
2235	2235	2246	2237	2237	2237

Table 6. Pearson's correlations detected between traits, indices and factor 1 and 2 of FA

	SSI	STI	HI C	HI D	FCW (g) C	FCW (g) D	TPB (g) C	TPB (g) D	WUE C	WUE D
SSI	-									
STI	n.s.	-								
HI C	n.s.	n.s.	-							
HI D	-0.420*	n.s.	n.s.	-						
FCW (g) C	0.357*	0.789**	0.361*	n.s.	-					
FCW (g) D	n.s.	0.802**	n.s.	0.560**	0.450**	-				
TPB (g) C	n.s.	0.770**	n.s.	n.s.	0.925**	0.433*	-			
TPB (g) D	n.s.	0.828**	n.s.	n.s.	0.580**	0.921**	0.583**	-		
WUE C	n.s.	0.772**	n.s.	n.s.	0.926**	0.427*	0.998**	0.581**	-	
WUE D	n.s.	0.826**	n.s.	n.s.	0.581**	0.920**	0.582**	0.999**	0.580**	-
Factor 1	0.510**	0.637**	n.s.	n.s.	0.884**	n.s.	0.948**	0.348*	0.947**	0.346*
Factor 2	-0.449**	0.675**	n.s.	0.505**	n.s.	0.943**	n.s.	0.900**	n.s.	0.900**

SSI - Stress Susceptibility Index; STI - Stress Tolerance Index; HI - Harvest Index; FCW - Fresh Corm Weight; TPB - Total Plant Biomass; WUE - Water Use Efficiency. C - Control; D - Drought;
n.s. - not significant; * significant at $p \leq 0.05$; ** significant at $p \leq 0.001$.

monitoring of adult plants evapotranspiration in the assay greenhouses, determined a water use of 10.7±3.5 mm.day^{-1}, and showed that 120% of ETo (4.03 mm day^{-1}) was not enough to maintain plants fully hydrated in the test conditions. Based on these findings, the irrigation regimes for the assay were based in measured ET.

In the present study, 11 traits and four derived variables were used to discriminate plants tolerance to drought, following the methodology developed by Ganança *et al.* (2015). These parameters were selected based on their connection with known strategies to avoid or answer to drought (Langridge and Reynolds, 2015), namely plant effective growth, WUE, nutrients allocation and yield. Drought tolerance was defined as yield stability under stress conditions, and a small number of traits at harvest were used for this assessment, with yield potential defined as the maximum yield obtained under non-stress conditions. Drought resistance is determined by dehydration avoidance or tolerance. Dehydration-avoidant phenotypes normally have smaller plants and leaf area, and are associated with low yield potential (Blum, 2005), and therefore, are not interesting from breeders perspective. Genotypes showing high yield both in stressed and non-stressed conditions were considered drought tolerants (Ganjeali *et al.*, 2011; Farshadfar *et al.*, 2013).

As expected, in the present work all the plant growth traits were reduced under drought, with variance analysis showing significant differences among cultivars, and significant influence of water scarcity in plant development. Traits decrease can be a result of the reduction of nutrients uptake and assimilation, increased water transpiration or energy allocation to face the stress. Reduction of LA is a known drought avoidance strategy used by taro to cope with low water availability, and Mabhaudhi and Modi (2015) associated the reduction of leaf number to a drought escape mechanism involving leaf shedding, phenological plasticity and a shortened crop cycle. The present data reveals different responses among cultivars, suggesting that both avoidance and tolerance strategies could be used by taro. For instance, the three top cultivars (2061, 2216 and 2211) for potential yield are representative of the different approaches used to cope with drought stress. Cultivar 2061 reduced leaf number, but maintained plant height and leaf area, while 2211 and 2216 maintained leaf number, but decreased plant height and leaf area. In 2061, LA was already lower under control conditions, showing potential for lower water loss and transpiration, than the other cultivars. Cultivars bred for water-limited environments normally have a constitutively reduced leaf area. However, some species, such as sorghum, maintain high LA, discarding older leaves and maintaining turgor in younger leafs (Blum, 2005). Similarly, drought tolerant aroids also maintain high leaf water content, reducing LN or LA in less extent than the sensitive ones (Sivan, 1995).

In our work, only one third of the tested cultivars decreased CCI to avoid drought. Leaf chlorophyll content has been shown to decrease with drought stress (Mabhaudhi *et al.*, 2013; Mabhaudhi and Modi, 2015), suggesting that the decrease in chlorophyll content could be a plant strategy to dissipate radiation energy and down regulate photosynthesis, due to the CO_2 scarcity resulting from stomatal closure. The majority of cultivars included in this study did not decrease CCI to compensate drought, and no significant correlations between CCI and other stress indices were observed.

WUE is generally considered a measure of drought resistance. Plants showing higher WUE are considered normally drought tolerant (Blum, 2005). WUE normally decreases under stress conditions, but Mabhaudhi *et al.* (2013) could not find differences in taro cultivars grown under control and stress conditions. However, they found that more tolerant cultivars had higher WUE. Bussel and Bonin (1998) also reported that a tolerant variety had higher WUE. In our work, cultivars showing higher WUE under full watering conditions had higher TPB and yield potential, while the ones that increase or maintain WUE under drought improve or show a small decrease in TPB and yield. Results show that WUE is a essential parameter for discriminating tolerance in taro.

Concerning the agronomic and yield traits, plant biomass, including TPB, FAGW and FCW were also significantly reduced under drought (Tables 3, 4, 5). Our results show that TPB appears highly correlated ($p \leq 0.001$) with corm biomass, both under control or under drought conditions (Table 6). Plants that were able to maintain TPB under drought were also capable to develop and maintain CW, which is also highly correlated with PH and LA (data not shown). These traits alleviate negative influences of fixation of nutrients and allocation of biomass in the corm (CW). Some cultivars (2056, 2234 and 2242), presenting lower yield potential, were able to maintain or slightly increase corm biomass under drought. These cultivars can be classified as expressing drought avoidance mechanisms (Ganjeali *et al.*, 2011; Farshadfar *et al.*, 2013), and efficiently transferred resources to sustain corm formation. However, these results need to be carefully interpreted, as they presented very low yield potential under control conditions and should be highly sensitive to other external variants. Cultivars 2061, 2216 and 2211 showed high potential yield, but suffered a yield reduction under drought. Nevertheless, cultivar 2061 is more efficient in biomass accumulation than cultivars 2211 and 2216. In conclusion, cultivars with high potential yield, heavily reduce canopy size and biomass, leading to decrease of yield under drought. Better performers minimised biomass and canopy loss. Mabhaudhi and Modi (2015) and Mabhaudhi *et al.* (2013) attributed lower yield in all studied taro cultivars to plant energetic investment towards drought avoidance mechanisms, reducing growth and biomass accumulation. In our study, some cultivars showed TPB increase or low reduction, showing that they can avoid or tolerate drought and efficiently transfer resources to corm formation in detriment of canopy formation. HI was not statistically affected by drought stress and appears to be correlated with corm biomass ($p \leq 0.05$), but not with TPB or WUE. However, HI was slightly lower under stress conditions, which remains in agreement with previously reported data (Bussel and Bonin, 1998; Mabhaudhi and Modi, 2015). Mabhaudhi and Modi (2015) found that contribution of corm yield to HI under drought was minimal.

In order to classify cultivars for their drought tolerance, yield stability was expressed as STI and SSI (Ganjeali *et al.*, 2011; Farshadfar *et al.*, 2013). STI identifies cultivars with the best yield stability, hence it is one of the best criterions to identify tolerant genotypes (Farshadfar *et al.*, 2013). STI is highly correlated ($p \leq 0.001$) with corm biomass under both conditions (Table 6), confirming that it can be used for ranking purposes. On the other hand, SSI reflects the difference between yield under stress and non stress conditions (Table 4). The higher SSI values, the most susceptible is the cultivar. SSI had lower, although significant correlations with CW under control ($p \leq 0.001$), and with HI ($p \leq 0.05$) under drought (Table 6). Consequently, SSI can identify cultivars lacking or having less successful strategies to avoid drought (Ganjeali *et al.*, 2011). Both indices should be considered for ranking cultivars for tolerance, but SSI should be used when taking in account potential yields (Farshadfar *et al.*, 2013). According to STI, cultivars 2061, 2216, 2211, 2213, 2058 and 2214 have the highest yield potential under drought stress. Cultivars 2234, 2056, 2244, 2233, 2239 and 2242, according to SSI, presented a efficient response to drought. Cultivar 2061 showed the highest STI with only slightly decreased yield under stress. On the contrary, cultivars 2234 and 2056 have medium and low STI (low yield potential) but showed resistance, ranking 1st and 2nd in SSI indexes. Among yield tolerant cultivars, 2061 was able to sustain WUE under stress, while 2216 and 2211 were less successful. Cultivars 2234 and 2056 increased their WUE under stress conditions, but their yields were average or low. STI was highly correlated with WUE ($p \leq 0.001$), but not with SSI (Table 6), making it a less appropriate trait for ranking cultivars. Our results confirm that WUE is an important trait in taro drought tolerance, but it is not the only determining factor.

Ploidy data were obtained for 12 of the studied cultivars (Table 1) (Kreike *et al.*, 2004; Traore, 2013). A correlation was found between STI and available ploidy levels (data not shown), indicating that in taro triploids can in fact have an advantage in yield and yield stability under drought. However, no correlation was found with SSI, indicating that with the limited set of data we cannot claim that ploidy levels contribute to drought tolerance.

To confirm ranking of taro cultivars for drought tolerance, we developed a model summarizing the traits variation under stress and non stress conditions, using multivariate statistics. Factorial Analysis (Fig. 1) allowed us to resume the information obtained along two factors, useful to discriminate cultivars regarding drought resistance and yield stability. Factor 1 is strongly correlated with cultivars' behaviour (yield potential) under non stress conditions, while factor 2 is strongly correlated with cultivars behaviour (WUE and resistance) under stress (Table 6). According to Ganjeali *et al.* (2011), cultivars can be distinguished for their drought resistance by high or low scores for factor 2. STI was positevely correlated with both factors ($p \leq 0.001$), confirming a good association with potential yield and drought tolerance (Tables 6). SSI was positively correlated with factor 1 and negatively correlated with factor 2 ($p \leq 0.001$), showing that cultivars with low SSI generally show low yield potential but high drought resistance (Table 6). Cultivars of interest showing both medium-high yield potential and drought tolerance have scores higher than 0 for both factors. Cultivars 2061 and 2216 stand out in this classification. Cultivars 2056 and 2234 excel as having good WUE and drought resistance, but showed low yield. Cultivars with scores below 0 for both factors show very low yield potential and are drought susceptible, standing out cultivars such as 2246, 2245 and 2235. Cultivars 2061 and 2216 probably are good candidates for breeding programs targeting crop improvement or as standards for screening for drought tolerance. In contrast, cultivars 2056 and 2234 can be adapted to drought but with overall low yield potential, are less attractive to taro breeders.

In our work, the Macaronesian cultivars were screened regarding drought for first time. Most of the local cultivars (e.a. cultivars 2061 and 2216) showed higher yield potential, but also STI, WUE and HI, when compared with SPC cultivars. At the same time, SPC cultivars 2233, 2234, 2239 and 2240 showed some drought resistance based on WUE, which in our test was not reflected in yield, but demonstrates existence of stress tolerance strategies. Cultivars presenting different strategies to cope with drought could be used as benchmarks for screening other taro cultivars for drought tolerance, as source of genetic variability for breeding programs, or for studying the molecular and biochemical background of taro drought stress and tolerance.

Conclusions

In order to screen and classify a relatively high number of taro cultivars, crop water requirements were analysed, and a model of drought conditions was defined, using a plant-soil system to monitor water use. Frequently used traits to assess the crop behaviour under drought conditions were assessed for their discriminating efficiency. A methodology aiming at creating long term stress and screening cultivars based on a small number of parameters collected at harvest was defined. Significant differences in behaviour between cultivars and treatments were observed. Our results confirm that different taro cultivars have different drought avoidance and tolerance strategies to cope with water scarcity. Cultivars with high potential yield, heavily reduce canopy size and biomass, leading to decrease of yield under drought. Better performers minimised biomass and canopy loss. Drought physiological tolerance was observed in cultivars that presented low potential yield, but efficiently transferred resources to enhance corm formation. Cultivars sustaining or increasing WUE under drought were more tolerant. Drought tolerant and susceptible cultivars were classified based on STI and SSI, respectively. Factorial analysis contributed to discriminate cultivars regarding to their drought tolerance and yield stability, confirming cultivars 2061 and 2216 as the most interesting ones, and the physiological tolerance, based on WUE, of cultivars 2056 and 2234. A group of cultivars that show a compromise between yield potential and tolerance, using WUE or metabolism bioenergetics to face drought stress was also identified, namely cultivars 2207, 2214, 2233, 2239 and 2240. The cultivars can now be used as benchmarks for screening other taro cultivars for drought tolerance, as source of genetic variability for breeding programs or to study mechanisms involved in plant adaptation to drought.

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