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Drought tolerance assessment of melon germplasm searching for adaptation to climate change

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Shortage of irrigation water at critical melon growth stages can be the most important limiting factor in the future due to climate change, especially in the Mediterranean region. Apart from the improvement of irrigation systems and crop management, the development of drought tolerant cultivars by genetic breeding is the best solution to achieve stable yields. Screening germplasm collections is a prerequisite for that. A melon core collection was evaluated in the current work in two assays. Seven morphological traits were assessed at plantlet stage and compared under drought and standard conditions imposed. Significant differences for all traits were recorded among the sixty accessions evaluated. Clustering analysis also grouped the accessions according to their response to drought, detecting some landraces and wild types of interest, mainly of Indian and African origin, although the best behavior under drought was found in a *flexuosus* melon from Irak. Some Spanish *inodorus* landraces also showed better response than the average behavior of commercial types. The employment of this set of traits has allowed screening a large germplasm collection in an easy and non-expensive way, in one of the most sensitive developmental stages.

Key words: *Cucumis melo* germplasm, morphological seedling traits, abiotic stress, response to drought, variability

INTRODUCTION

Melon (*Cucumis melo* L.; 2n=2x= 24), which belongs to Cucurbitaceae family, is one of the most important fruit crops worldwide. Approximately about 31 million tonnes of melons were produced worldwide with more than 1.2 million ha harvested in 2016 (FAOSTAT, 2018). In addition to China, which is the main producer country with nearly 16 million tonnes, Egypt and Spain are also important producers, ranking 4 and 8th position, with about 1 million and 660,000 tonnes produced in 2016, respectively (FAOSTAT, 2018). In fact, in 2016 Spain was the second exporter in the world after Guatemala.

Therefore, due to its economic relevance, the development of new melon cultivars adapted to different biotic and abiotic stresses and with high quality standards is required by global markets. This includes the tolerance or resistance to drought and salinity, as climate change is likely to affect many croplands, particularly in the Mediterranean region where is predicted an important increase of arid areas (Turral et al., 2011). The incidence of this stress and the resulting losses in yield for many

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crops including melon, are a major threat to economic and social stability in many societies, since developing countries probably will suffer the consequences more drastically. In addition, weed invasion can aggravate this problem in croplands with no weed management, since competition for water, light and nutrients takes place, also affecting seed germination (Yigit et al., 2016). Several studies have focused on the determination of optimum irrigation requirements in melon, as water scarcity is a growing problem nowadays in many melon-producer regions (Kusvuran et al., 2011; Lima et al., 2017). Negative effects of excessive watering in melon have been reported such as an increase in the presence of rotten fruits, flesh vitrescence or flesh sweetness loss (Sensoy et al., 2007). However, as previously said, water deficit can seriously affect yield (Sensoy et al., 2007; Sharma et al., 2016), fruit size (Fabeiro et al., 2002; Long et al., 2006), and can cause an important reduction in biomass finally leading to plant death (Kusvuran, 2010). This reduction in growth under drought and salinity is consequence of several physiological responses including modifications of ion balance, mineral nutrition and photosynthetic efficiency. The rate of photosynthetic CO_2 assimilation is reduced and generally implies oxidative stress. Additionally, metabolic disturbances and fruit quality effects due to salt stress have been described in melon, whose fruits usually become soft, wrinkled and turn brown, displaying premature ripening. In contrast, activation of secondary metabolism with positive effects on fruit quality (antioxidant compounds, aroma) also has been reported in several species in response to deficit of water (Ripoll et al., 2014).

Although plants can be affected by drought at any time of their life, presenting unique challenges to growth and productivity, one of the most critical stages are during germination and seedling growth (early-season drought). In fact, recent studies such as the one by Yigit et al. (2016) and Sevik and Cetin (2015) carried out with landscape species, have focused on the germination rate, reporting significant falls under water stress. In addition, early drought stress experienced during the seedling stage, when plants are very sensitive to this deficit, provokes significant inhibition of growth and developmental delay (Blum, 1996).

Drought tolerance is a function of several morphological traits such as reduced leaf area or stomatal density, physiological aspects such as low transpiration rate or cell membrane fluidity (Cetin et al., 2018); and biochemical composition such as proline or trehalose accumulation, which are effective indices for screening in breeding programs (Ashraf and Foolad, 2007; Cha-um and Kirdmanee, 2009). Regarding biochemical indices, studies by Dasgan et al. (2009) and Kusvuran et al. (2013) suggested citrulline as a good indicator of tolerance to salinity and drought as they reported higher accumulation in tolerant melon accessions in comparison to sensitive ones.

Characterization of these drought-tolerance-related traits under water stress conditions have been assessed in several species, including seedling traits like shoot and root weight and lengths, root/shoot ratio and coleoptiles length at seedling stage (Taiz and Zeiger, 2006; Zhang et al., 2011; Zafar and Azhar, 2015; Akinwale et al., 2017).

A few candidate genes for drought tolerance in crops have been characterized to date (Ripoll et al., 2014), and metabolic pathways and differential gene expression related to this stress are still not well understood. Recently, in the model plant *Arabidopsis*, Fàbregas et al. (2018) have overexpressed BRL3, a brassinosteroid receptor, conferring drought tolerance without affecting plant growth, and in melon, Altunoglu et al. (2017) identified several LEA genes which were up-regulated under drought conditions. Nevertheless, routine screening for drought tolerance is nowadays carried out by phenotyping the response under deficiency and not by the analysis of particular drought-related genes.

C. melo is a very polymorphic species that exhibits high levels of diversity regarding morphological, physiological and biochemical aspects, including tolerance to different abiotic and biotic stresses (Esteras et al., 2013; Pitrat, 2017). This species is divided into two subspecies, subsp. *melo* and subsp. *agrestis* (Kirkbride, 1993). Although in the last classification, Pitrat (2017) reported 19 horticultural groups, a simplified version adapted from Pitrat (2008) is usually adopted: *inodorus*, *cantalupensis-reticulatus*, *adana*, *chandalak*, *ameri*, *chate*, *flexuosus*, and *dudaim* (in subsp. *melo*), and *momordica*, *conomon*, *chinensis*, *makuwa*, *acidulus*, *tibish* and wild *agrestis* (in subsp. *agrestis*). The extant diversity in the species for drought tolerance is nowadays underexploited, as few studies have been carried out searching for drought-tolerant accessions (Kusvuran et al., 2013; Pandey et al., 2013, 2018; Ozer et al., 2015; Sharma et al., 2016; Leskovar et al., 2017).

In this context, we present the characterization of a melon germplasm collection representing most of the variability of the species, with several morphological traits easily-measured at seedling stage with the aim to search for new genotypes most adapted to the increasing lack of water. The finding of these resources may provide valuable information about potential crosses in future breeding programs to develop drought-tolerant commercial varieties. Also, the most tolerant landraces reported may be used to develop varieties better adapted to local farming systems in developing countries.

MATERIALS AND METHODS

A set of 60 melon accessions representing the huge diversity of the species and including melon varieties, from diverse origins, maintained at the COMAV's (Institute for the Conservation and Breeding of Agricultural Biodiversity) core collection at Polytechnic University of Valencia (UPV, Spain) (Esteras et al., 2013; Leida et al., 2015), and some wild accessions from India held at Germplasm Resources Information Network-National Plant Germplasm System, USDA (GRIN-NPGS) (Table 1), were analyzed for their response to early-drought in two assays.

Table 1. Accessions included in the study.

Genotype code[1]	Accession name[2]	Reference	Origin	subsp.	Variety/Hort. group	Assay
Ac-TGR1551Zimb	TGR1551	Leida et al. (2015)	Zimbabwe	*agrestis*	acidulus	1
Ac-TGR1843Zimb	PI 482429	Leida et al. (2015)	Zimbabwe	*agrestis*	acidulus	2
Ag-15591Gha	PI 185111	Leida et al. (2015)	Ghana	*agrestis*	wild melon	1
Ag-C38Nig	CO38 (CUM 287)	Leida et al. (2015)	Nigeria	*agrestis*	wild melon	1
Ag-CallInd	Callosus	Leida et al. (2015)	India	*agrestis*	wild melon	2
Ag-CallosusInd	PI 435284	Endl et al. (2018)	India	*agrestis*	wild melon	2
Ag-ChibbarInd	PI 532839	-	India	*agrestis*	wild melon	2
Ag-Cuba	Cuba	Leida et al. (2015)	Cuba	*agrestis*	wild melon	2
Ag-FadSud	Fadasi	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-HumSud	Humaid	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-KSM428Ind	PI 614465	-	India	*agrestis*	wild melon	2
Ag-KSM528Ind	PI 614518	-	India	*agrestis*	wild melon	2
Ag-KSM531Ind	PI 614521	-	India	*agrestis*	wild melon	2
Ag-MelCol	Meloncillo	Leida et al. (2015)	Colombia	*agrestis*	wild melon	1
Ag-SM2Ind_B	PI 381782 B	-	India	*agrestis*	wild melon	2
Ag-SM2Ind_A	PI 381782 A	-	India	*agrestis*	wild melon	2
Ag-TendSud	Tendelti	Leida et al. (2015)	Sudan	*agrestis*	wild melon	2
Ag-USM170Ind	PI 614307	-	India	*agrestis*	wild melon	2
Ag-VelliInd	PI 164320	Leida et al. (2015)	India	*agrestis*	wild melon	2
Ag-WChInd	Wild Chibbar	Leida et al. (2015)	India	*agrestis*	wild melon	2
Am-3584Afg	PI 125951	Leida et al. (2015)	Afghanistan	*melo*	ameri	1
Am-AfrMor	Afr-c-1	Leida et al. (2015)	Morocco	*melo*	ameri	2
Am-ApelRus	Apelsinaja	Leida et al. (2015)	Russia	*melo*	ameri	1
Am-ChandAfg	Chandalack (PI 276660)	Leida et al. (2015)	Afghanistan	*melo*	ameri	1
Am-GalaTun	Galaoui	Leida et al. (2015)	Tunisia	*melo*	ameri	2
Am-HassanTur	Hassanbey (PI 169368)	Leida et al. (2015)	Turkey	*melo*	ameri	1
Am-KafEgy	Kafr Hakim (PI 288233)	Leida et al. (2015)	Egypt	*melo*	ameri	2
Am-KizilUzbe	Kizil-uruk	Esteras et al. (2013)	Uzbekistan	*melo*	ameri	1
Am-KokUzb	Kokcha (ASI-C-5)	Leida et al. (2015)	Uzbekistan	*melo*	ameri	1
Am-NanaGeorg	Nanatri	Leida et al. (2015)	Georgia	*melo*	ameri	1, 2
Am-NesviGeor	Mucha Nesvi	Leida et al. (2015)	Georgia	*melo*	ameri	2
Am-OuzUzb	Ouzbeque	Leida et al. (2015)	Uzbekistan	*melo*	ameri	2
Am-U1715Br	CUM502		Brazil	*agrestis*	ameri	2
Can-NYIsr	Noy Israel	Leida et al. (2015)	Israel	*melo*	cantalupensis	1
Can-U1716Br	Casca de Carvalho		Brazil	*agrestis*	cantalupensis	2
Can-VedFran	Vedrantais	Leida et al. (2015)	France	*melo*	cantalupensis	1

Table 1. Contd.

Con-Co6Chi	Makuwa	Leida et al. (2015)	China	*agrestis*	conomon-makuwa-chinensis	1
Con-Pat81Ko	Pat 81	Leida et al. (2015)	Korea	*agrestis*	conomon-makuwa-chinensis	1
Con-SCKo	Songwhan Charmi (PI 161375)	Leida et al. (2015)	Korea	*agrestis*	conomon-makuwa-chinensis	2
Con-ShiroJa	Shiro Uri Okayama	Leida et al. (2015)	Japan	*agrestis*	conomon-makuwa-chinensis	1
Dud-254Afg	CUM 254	Nunes et al. (2017)	Afganistan	*melo*	dudaim	1
Dud-QPMAfg	Queen's pocket melon	Leida et al. (2015)	Afganistan	*melo*	dudaim	1
Flex-AlficozSp	Alficoz	Leida et al. (2015)	Spain	*melo*	flexuosus	1
Flex-AryaInd	Arya	Leida et al. (2015)	India	*melo*	flexuosus	1
Flex-KhiIrak	Khiar	Leida et al. (2015)	Irak	*melo*	flexuosus	1
In-AmCañSp	Caña Dulce	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-BBescrSp	Blanco Escrito	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-BTempSp	Blanco Tempranillo	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-MaazTun	Maazoon	Leida et al. (2015)	Tunisia	*melo*	inodorus	2
In-PsPiñSp	Piel de sapo Piñonet	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-RoMoch1Sp	Mochuelo	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-StutzUSA	CUM 468, Stutz Supreme	Nunes et al. (2017)	USA	*melo*	inodorus	1
In-TeLVillSp	Largo de Villaconejos	Leida et al. (2015)	Spain	*melo*	inodorus	1
In-TeMollSp	Mollerusa	Leida et al. (2015)	Spain	*melo*	inodorus	1
La-Bol	Bol-84	Leida et al. (2015)	Bolivia	*melo*	indeterminate landrace	1
La-VoaMad	Voatango	Leida et al. (2015)	Madagascar	*agrestis*	indeterminate landrace	1
Mom-KhaInd	Kharbuja	Leida et al. (2015)	India	*agrestis*	momordica	1
Mom-PI124Ind	PI124112	Leida et al. (2015)	India	*agrestis*	momordica	1
	F1_PSxDud				hybrid Inodorus x dudaim	1
	F1_PSxCon				hybrid Inodorus x conomon	2

[1]Some codes employed in previous studies. [2]PI and CUM accessions were kindly provided by NPGS-USDA and IPK genebanks respectively.

Two assays were performed under greenhouse conditions in Valencia (UPV facilities) with the following conditions: average air temperature of 27.8°C and average humidity 61.5%. The first assay was conducted from June to the end of July, while the second assay was conducted from August to the end of September. Seeds were germinated in a pre-germination chamber for 24 h at a temperature 37°C. Afterwards, the uniform-sized seedlings were transferred into plastic pots (one seedling/pot, 55 ×45 cm) with commercial substrate (Huminsubstrat N3®) at the cotyledon stage. A triplicate complete randomized block design (RCBD) was used. The plantlets grew under the same conditions until the three-leaf stage. Subsequently, plants were divided into two groups, and different conditions were applied: drought and standard conditions. For drought condition, the water deficit was achieved by watering the plants with a decrease of 50% of water with respect to standard irrigation (control). The application of drought was accomplished and monitored using ECH_2O EC-5 capacitance sensors connected to an Em50 data logger using the ECH_2O Utility software (Decagon Devices Inc., Pullman WA., USA). When the humidity degree reached 15%, plants were approximately 45 days under this condition and were phenotyped. Plants (5 to 10) were evaluated per accession and condition. The seven morphological traits selected as indicators of drought tolerance at plantlet stage were: first leaf curled score, second leaf curled score, length (cm), fresh weight (g), dry weight (g), number of green leaves, and number of brown leaves. High percentage of curled leaf area is indicative of high susceptibility to drought. The leaf score employed was a 1-5 scale, where 0 is 0%, 1 is 1-5%, 2 is 5-10%, 3 is 10-15%, 4 is 15-20%, and 5 is more than 20% curled in leaf. A decrease in fresh and dry weight, and in the number of green leaves are also traits used as indicators of a decrease in total biomass in the response to drought.

A mixed model was used to analyze the data of each assay. Drought conditions (two levels; drought and standard conditions), genotype and its interaction were included as fixed effect whereas the plant was included as random effect. Significant differences were estimated by least square difference (LSD) method 95%. Correlations between traits were also estimated separately by each drought condition and assay. SAS/STAT 12.1 was used to perform all the analysis.

New parameters were calculated to measure the differences in the traits assessed with respect to control conditions in each genotype. For vine length, fresh weight, dry weight and number of green leaves, the value for control conditions were assumed to be 100% and the decrease percentage was subtracted to obtain a new parameter. For first leaf score, second leaf score and number of brown leaves the difference was calculated directly subtracting control value from drought value. A clustering analysis for each assay was also conducted with these new parameters to determine groupings of accessions with similar responses. The hierarchical dendrogram was performed with JMP v.5.1 using Ward method.

RESULTS AND DISCUSSION

Selection of drought tolerant plant species has been considered to be an economic and efficient means of alleviating agricultural problems especially in dry areas. To achieve this goal, a set of reliable drought-related traits of rapid and relatively inexpensive screening was used to assess a melon core collection which displays high levels of variability.

After the descriptive study of the dataset, a few outlier values were discarded in both assays. The analysis of the seven traits for the two subsets corresponding to both assays, at the beginning (assay 1) and at the end of summer (assay 2), were done separately, as only one accession was characterized in both assays. General data for these two subsets of accessions evaluated in drought and standard conditions are presented in Table 2.

Drought, genotype effect and its interaction were observed for all traits at 95% with the exception of the trait first leaf score which was detected at 90% in the assay performed at the beginning of summer (figures for each trait and genotype in both conditions are shown in Supplementary File 1). In addition, the drought effect for each subset of accessions for each trait is as shown in Figure 1. The effects of water availability on the traits were found significant in both assays. As expected, effects on leaves (first leaf score, second leaf score) increased with application of drought, having scores around 0 in standard conditions and over 2 (assay 1) or between 1 and 2 (assay 2) when drought was applied. The number of brown leaves also increased under drought conditions in both assays. Regarding the remaining traits, their values decreased with drought as fresh weight, dry weight, green leaves and vine length are traits directly related to biomass production. This effect was more evident in the conditions of assay 2, maybe due to the slightly increase in the temperatures in the second assay. Fresh weight was the best indicator of drought damages in both assays, with a decrease of 55.9 and 68.6% with respect to standard conditions in assays 1 and 2, respectively (Figure 2). Dry weight also suffered an important decrease of 51.9 and 65.0%, respectively, while number of green leaves (45.8 and 57.1% for assays 1 and 2, respectively) and vine length (31.2 and 41.5%, respectively) presented much moderate loss. Previous studies about salinity and drought effects on melon genotypes reported that shoot growth differed significantly among the tolerant and sensitive melon genotypes (Kusvuran, 2010; Kusvuran et al., 2011), which is in agreement with our results.

Significant differences were observed among the assayed accessions for seedling traits. As expected, any accession showed higher fresh weight under drought conditions than standard (or no drought) conditions, except for the accession Flex-KhiIrak which behaved better in this condition (Figure 2). Other accessions like Ag-C38Nig, Am-3584Afg, In-BTempSp, In-RoMoch1Sp and La-VoaMad showed similar values in both conditions (Figure 2). These results suggest that these accessions are adapted to semi-arid climates, since for example, Flex-KhiIrak, Am-3584Afg, and In-RoMoch1Sp, are grown in regions of Irak, Afganistan, and Spain, respectively, where precipitations are generally scarce. This group of accessions included a wild type and also landraces, not only from African and Asian origin, but also Spanish ones which are closer to the commercial types. These accessions, therefore, are described as not affected by drought, or even positively affected in the case of Flex-KhiIrak. La-VoaMad, together with Am-KizilUzbe, Am-KokUzb, Can-VedFran, Flex-AryaInd (assay1) and Am-NesviGeor, hybrid F1_PSxCon and Am-OuzUzb (assay 2) presented the highest fresh weight under drought conditions (Figure 2). These results under limited water conditions which inhibit plant growth suggested a sort of tolerance to drought in these high-weighed genotypes, revealing landraces such as La-VoaMad as potential genotypes for breeding. In addition, some of them also displayed one of the highest fresh values under standard conditions (Am-OuzUzb), showing the vegetative growing potential of this accession in both conditions. However, future characterization of the fruit quality will be necessary to use them in breeding programs since effects on organoleptic traits and fruit size have not been assessed in this first approach.

The correlation values (P-value < 0.01) different from zero are presented in Table 3 for each assay and condition. Similar correlations between traits were obtained in each assay. When drought conditions were evaluated, positive correlations among vine length, fresh weight and dry weight were observed in both assays. In fact, Flex-KhiIrak, In-BTempSp, In-RoMoch1Sp, La-VoaMad, Ag-C38Nig, and Am-3584Afg, accessions previously mentioned with no drought effect in fresh weight (or a positive effect in Flex-KhiIrak), presented also no differences in vine length as well as the Indian Flex-AryaInd. In both assays, positive correlation was also observed between first leaf score and second

Table 2. Mean raw effects of drought (1) and standard (2) conditions on the seedling traits analyzed in the two assays.

Assays	First leaf score		Second leaf score		Vine length		Fresh weight		Dry weight		Green leaves		Brown leaves	
	D[1]	S[2]	D	S	D	S	D	S	D	S	D	S	D	S
1	2.25	0.21	3.16	0.36	53.55	77.79	4.78	10.84	0.64	1.33	4.48	8.27	1.71	0.94
2	0.96	0.15	2.18	0.30	71.44	122.06	3.76	11.93	0.56	1.60	6.46	15.05	3.00	1.34

leaf score (Table 3). Flex-KhiIrak, La-VoaMad, Ag-VelliInd and Ag-ChibbarInd were the only accessions displaying 0% of curled leaf area (first and second leaf score) in both conditions (Supplementary file 1), suggesting again the tolerance to drought of these landraces. In addition, these two traits showed negative correlation with vine length and the number of green and brown leaves. When standard conditions were evaluated, most of the positive correlations observed in drought conditions were achieved. In addition, negative correlation between fresh weight and brown leaves were obtained.

Cluster analysis was carried out in both assays and several groups of accessions were detected according to the different response to early-drought (Figure 3). In assay 1, some accessions showed lower to medium-level of damages (red, Figure 3A) displaying lower losses of weight and length and less curling in leaves. This cluster included subsp. *agrestis* types like *momordica*, *acidulus*, *conomon* and wild types, and also some intermediate types between both subspecies like *ameri* or *flexuosus*. They included types mentioned previously like the African Ag-C38Nig or the Asian Am-KizilUzbe, Am-KokUzb, and Am-3584Afg. Flexuosus melons like the Indian landrace Arya (Flex-AryaInd), also in this cluster, have been previously reported to have good adaptability to drought (Ahlawat et al., 2018). In the present work, this accession showed a good behavior although it did not group with accessions with the best response (blue cluster). This blue cluster (Flex-KhiIrak, La-VoaMad, In-BTempSp, In-RoMoch1Sp) included accessions which showed no or small difference under drought in comparison to standard conditions for weight and vine length (Figure 3A). The other two clusters (green, orange; Figure 3A) presented more severe damages and included mainly subsp. *melo* accessions (*inodorus*, *cantalupensis*, *ameri*) with the exception of two *conomon* types. The commercial Charentais and Piel de Sapo types (Can-VedFran, In-PSPiñSp), especially important in Western countries, were included in this group (green cluster), whereas Ag-MelCol and Dud-QPMAfg (orange cluster) were the ones with the higher level of brown leaves in response to drought.

Regarding assay 2, three clusters were observed, one corresponding to accessions with more severe losses in weight and length with respect to the remaining accessions assessed in this assay although a low level of curling in leaves (red, Figure 3B), a second one with intermediate accessions regarding the response to drought (blue, Figure 3B) which mainly included *ameri* types like Am-OuzUzb, and a third one with a lower level of damages (green, Figure 3B). Although most of the Indian *agrestis* accessions were among the most affected accessions (red cluster) together with the two Brazilian landraces assayed, two of them (Ag-KSM428Ind, Ag-KSM528Ind) can be highlighted due to their good response (green cluster) as well as the African wild *agrestis* Ag-HumSud and the Egypcian Am-KafEgy. India has several agro-ecological regions and therefore, maintains huge diversity in melon (Fergany et al., 2011; Roy et al., 2012), which explains the diverse response found in this work. The accessions with more damages on leaves were Am-NanaGeorg, Ag-MelCol and Dud-QPMAfg (assay1), and In-MaazTun and Am-GalaTun from Tunisia (assay 2) (Figure 3). In general, African and Indian accessions showed better results for these traits (La-VoaMad, Ac-TGR1551Zimb and Mon-PI124Ind in assay 1 and Ag-WChInd, Ag-VelliInd, Ag-ChibbarInd and Ag-USM170Ind in assay 2) (Figure 3).

The results of the evaluation of this large melon collection suggest that African and Asian continents retain an important genetic variability which should be further studied, as well as Spanish landraces which seems to be underexploited for drought-adaptation traits. In fact, several accessions were detected with better behavior than typical international commercial types belonging to *inodorus* and *cantalupensis* groups. Traditionally, exotic types belonging to *momordica* and *acidulus* groups from India have been used as sources of resistance genes to biotic stresses, but few studies have evaluated

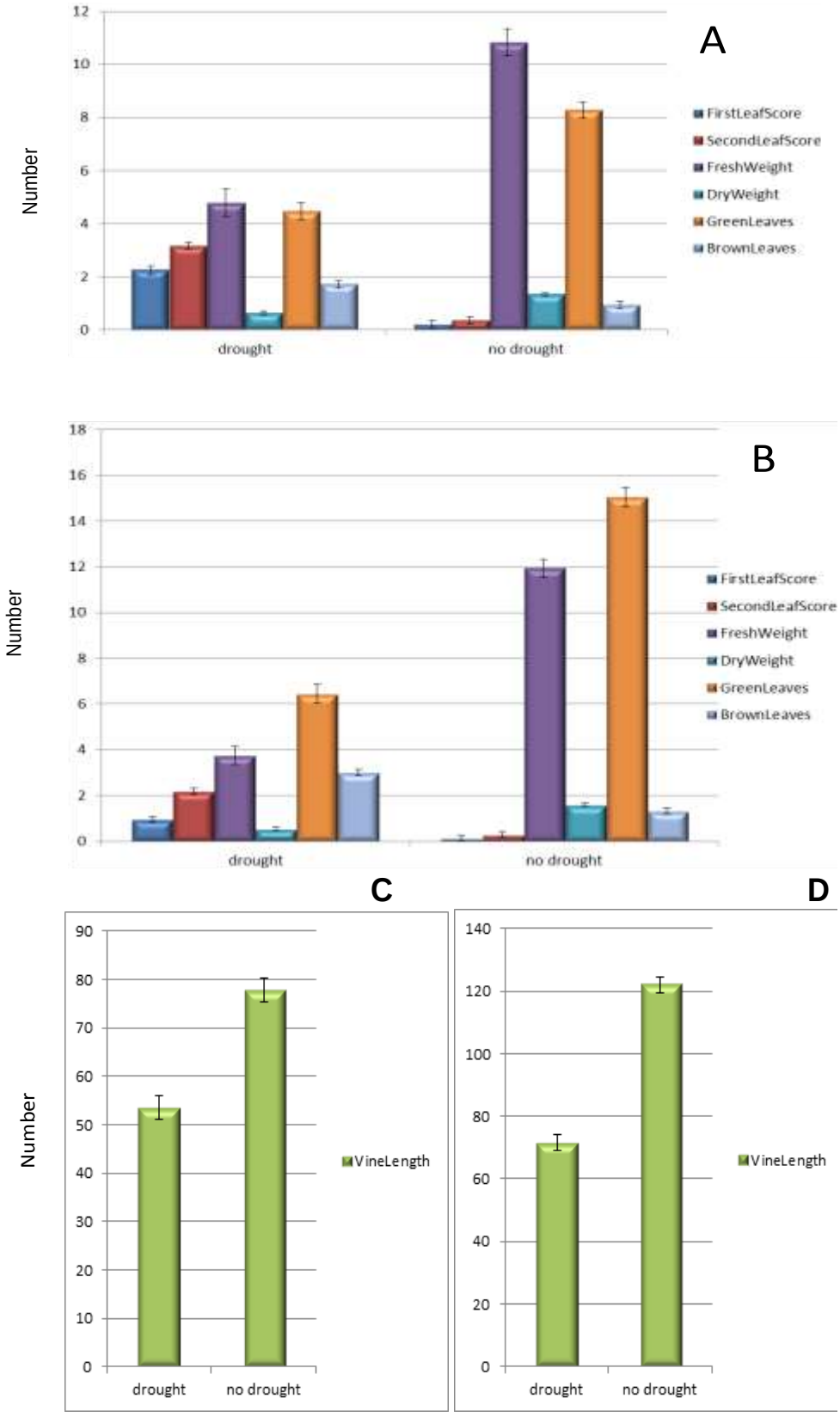

Figure 1. Effect of drought on the traits assessed in the germplasm collection. Means and standard error represented for first leaf score, second leaf score, fresh weight, dry weight, number of green leaves, and number of brown leaves evaluated in assay 1 (A) and 2 (B), and vine length evaluated in assay 1 (C) and 2 (D).

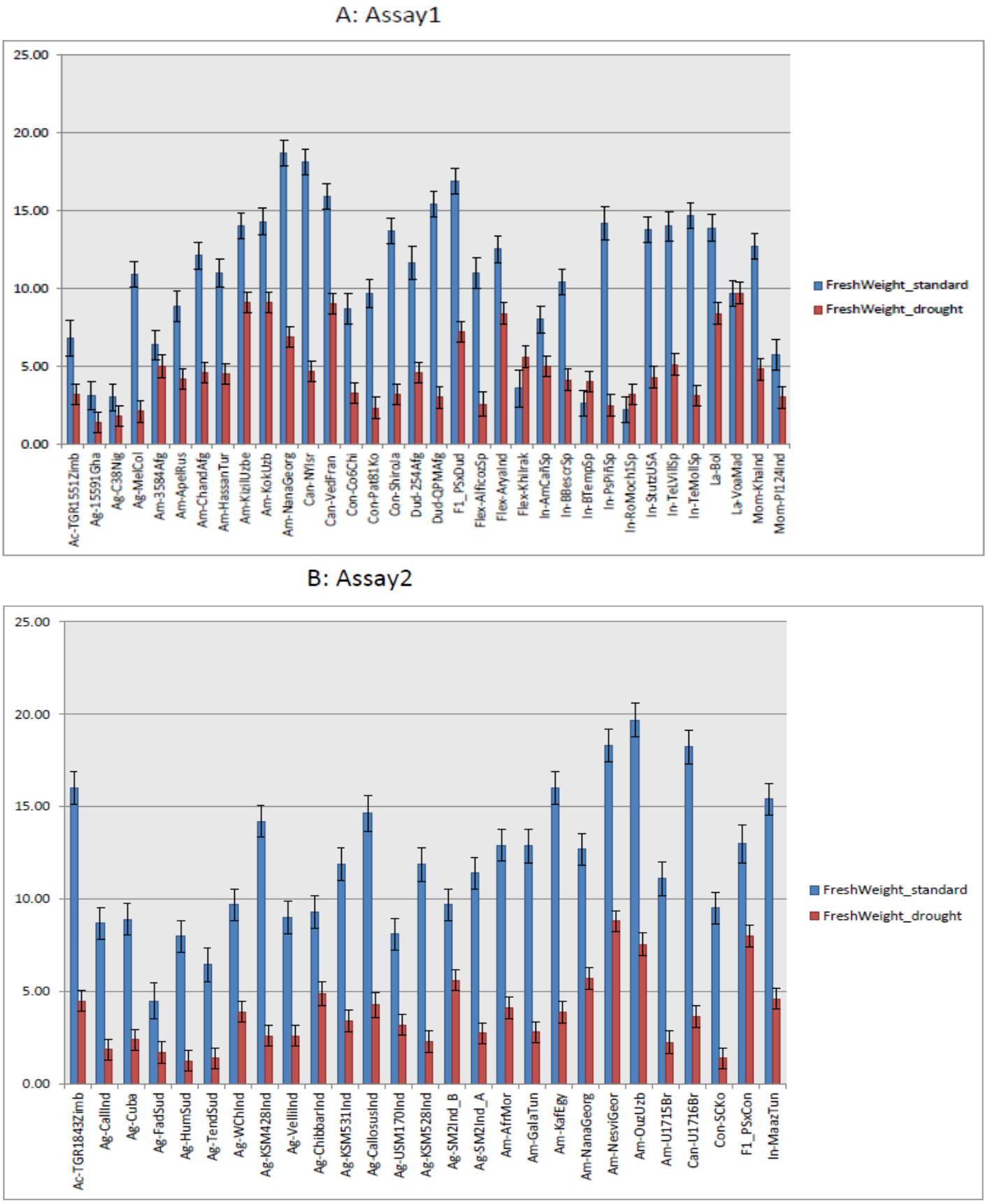

Figure 2. Mean and standard error for fresh weight for every genotype in both conditions (standard and drought). A assay1, B assay2.

Table 3. Correlations among traits evaluated under drought and standard conditions in two assays (assay 1 performed at the beginning of the summer and assay 2 at the end of the summer).

Assay 1	Drought conditions						Standard conditions					
	Second leaf score	Vine length	Fresh weight	Dry weight	Green leaves	Brown leaves	Second leaf score	Vine length	Fresh weight	Dry weight	Green leaves	Brown leaves
First leaf score	0.83	-0.38	-	-	-0.57	-0.36	0.73	-	-	-	-	-
Second leaf score		-0.31	-	-	-0.56	-0.30		-	-	-	-	-
Vine length			0.61	0.69	0.56	-			0.63	0.65	0.74	-
Fresh weight				0.85	0.31	-				0.89	0.36	-0.40
Dry weight					0.44	-					0.43	-
Green leaves						-						-

Assay 2	Drought conditions						No drought conditions					
	Second leaf score	Vine length	Fresh weight	Dry weight	Green leaves	Brown leaves	Second leaf score	Vine length	Fresh weight	Dry weight	Green leaves	Brown leaves
First leaf score	0.70	-0.29	-	-	-0.32	-0.41	0.65	-	-	-	-	0.36
Second leaf score		-0.25	-	-	-0.38	-0.42		-	-	-	-	-0.46
Vine length			0.55	0.52	0.56	-			0.21	0.33	0.55	-
Fresh weight				0.90	0.36	-				0.59	-	-0.34
Dry weight					0.41	-					-	-
Green leaves						-						-

-: Non significant correlations (P value >0.01).

drought tolerance. In fact, to date few studies have focused on the screening for drought tolerance of large melon germplasm collections (Ozer et al., 2015; Sharma et al., 2016; Leskovar et al., 2017; Pandey et al., 2018) and most of them only include *cantalupensis* and *inodorus* types. Regarding exotic types, some accessions like the Turkish Kav-248 have been described as drought-tolerant (Ozer et al., 2015; Torun et al., 2018). Also, some Kachri melons from India, described as semi-domesticated large *agrestis* probably evolved from Wild Chibber and *momordica* melons, have been reported to show drought tolerance (Pareek and Samadia, 2002; Roy et al., 2012; Pitrat, 2017), since they can be grown in semi-desert areas. The present results are coherent with this, as the two Wild Chibbar types tested herein (Ag-WChInd, Ag-ChibbarInd) showed a good behavior (green cluster, Figure 3). Moreover, some other Indian accessions presented a notable good response under this stress, confirming again the importance of Indian germplasm in the genetic breeding of this crop. However, the most adapted melon to drought conditions was a *flexuosus* type from Irak (Flex-KhiIrak), reinforcing Near East as an important area to search for germplasm to use in breeding.

Since the screening of a core collection is the first step for breeding for adaptation to the new scenario with more severe and frequent periods of drought due to climate change (Turral et al., 2011), the results presented in this work can be very useful. As a conclusion, the accessions presented in this work as more tolerant to water scarcity might play a significant role for the incorporation of drought-tolerance genes in landraces to improve production in local-farming systems and under ecological farming, or even in commercial types. Traditionally, the development of such cultivars has been hampered by the complex nature of drought adaptation, genotype × environment interactions and the difficulty of having an effective drought screening method (Verulkar et al., 2010). However, the parameters measured in this work have allowed a first rapid

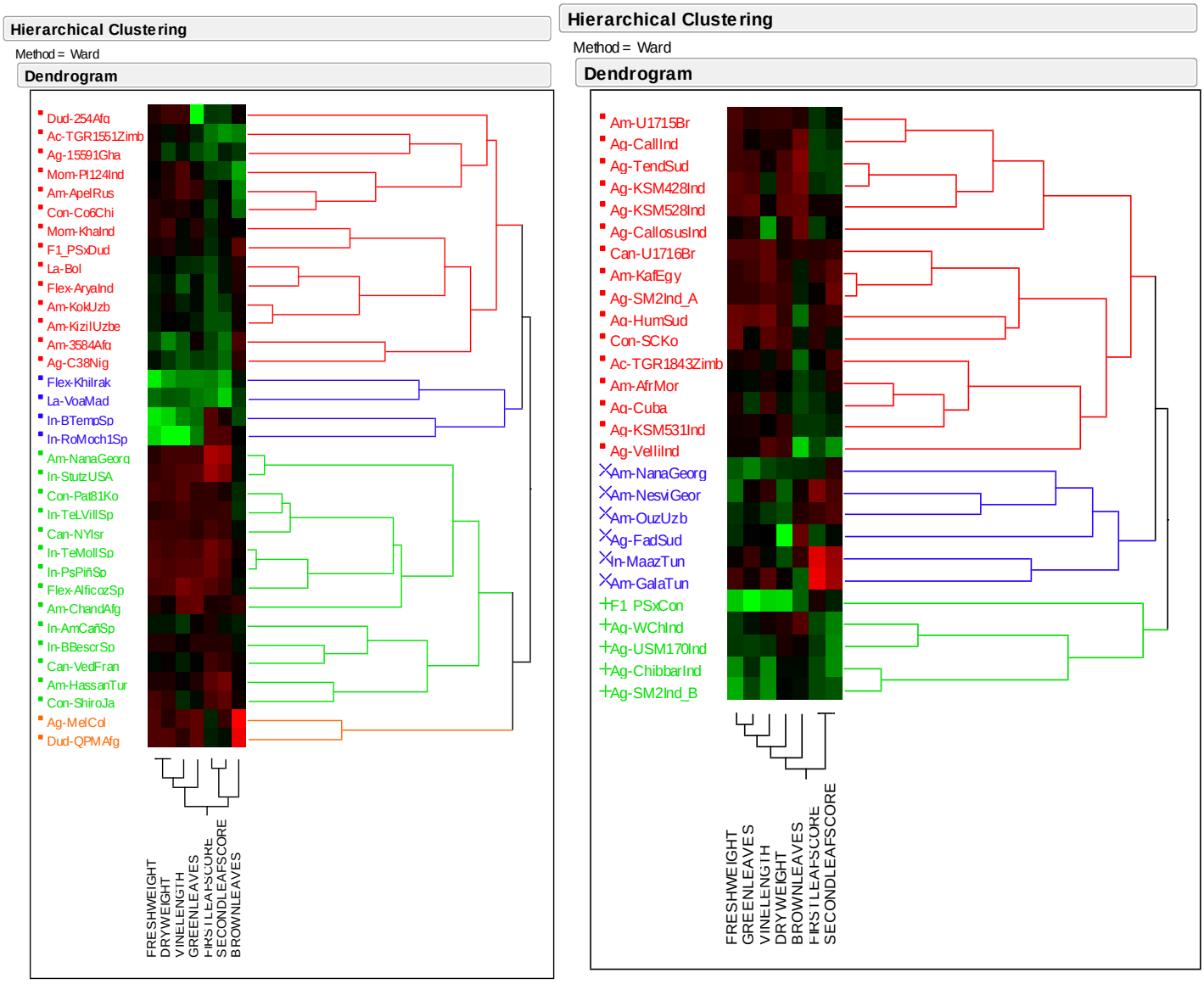

Figure 3. Dendrogram for assay 1 (A) and assay 2 (B) showing several groups based on the drought response of the 60 accessions screened. The new parameters calculated for each trait were used. Hierarchical clustering was performed using JMP V.5.1 and Ward method.

and low-cost screening to select genotypes with the best behavior in early stages of plant growth in order to further assess fruit yield and quality in future assays. Therefore, herein we report, not only some genotypes of potential interest for melon breeding such as some landraces and wild types from India and Africa as well as a *flexuosus* type from Irak, but also a useful and straightforward set of traits for early-drought tolerance screening.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLEMENTARY

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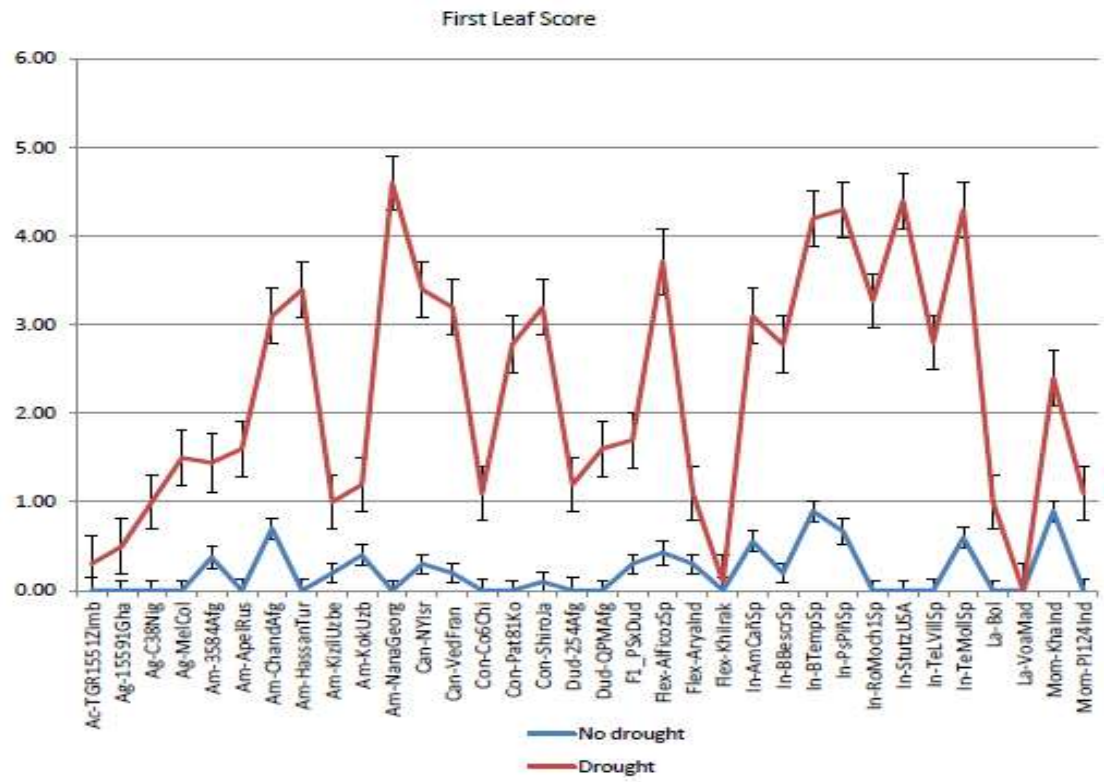

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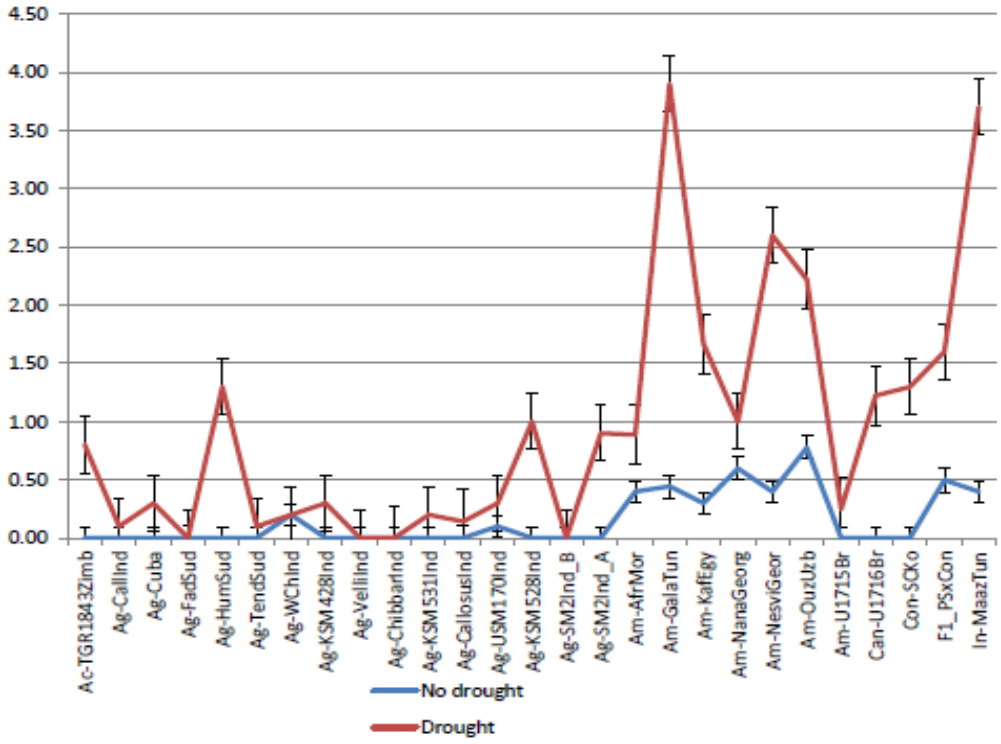

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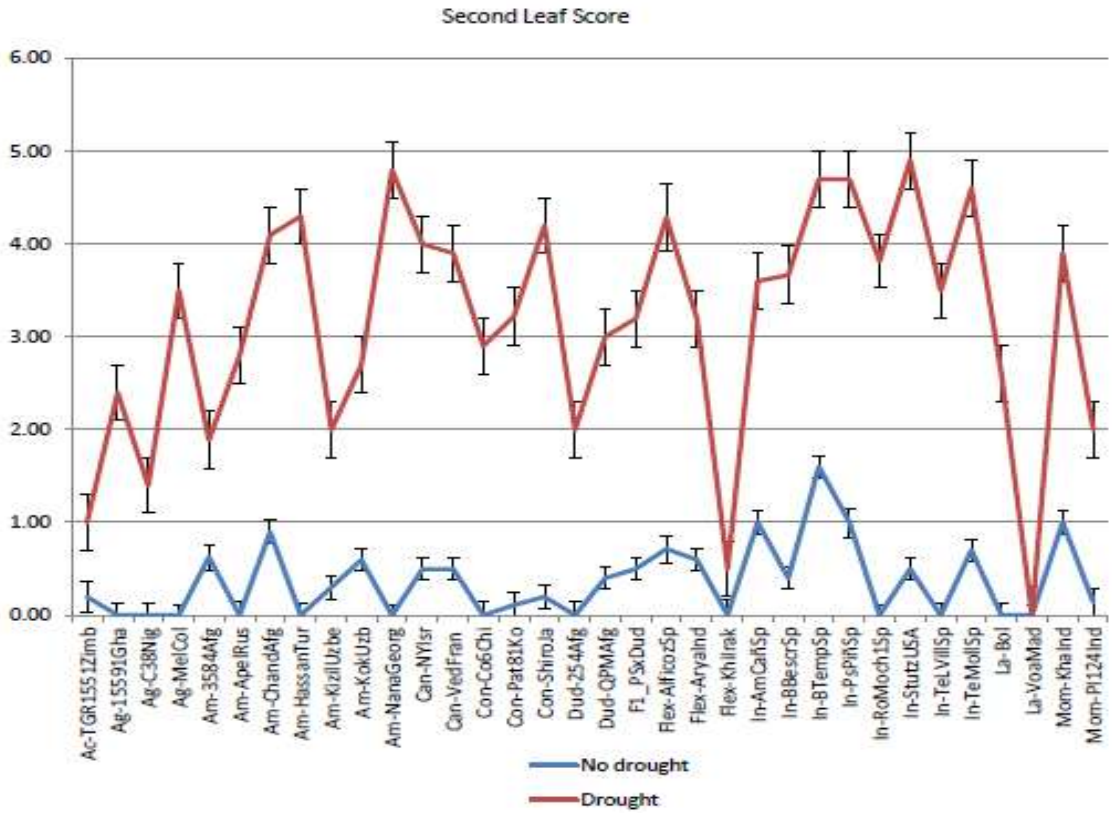

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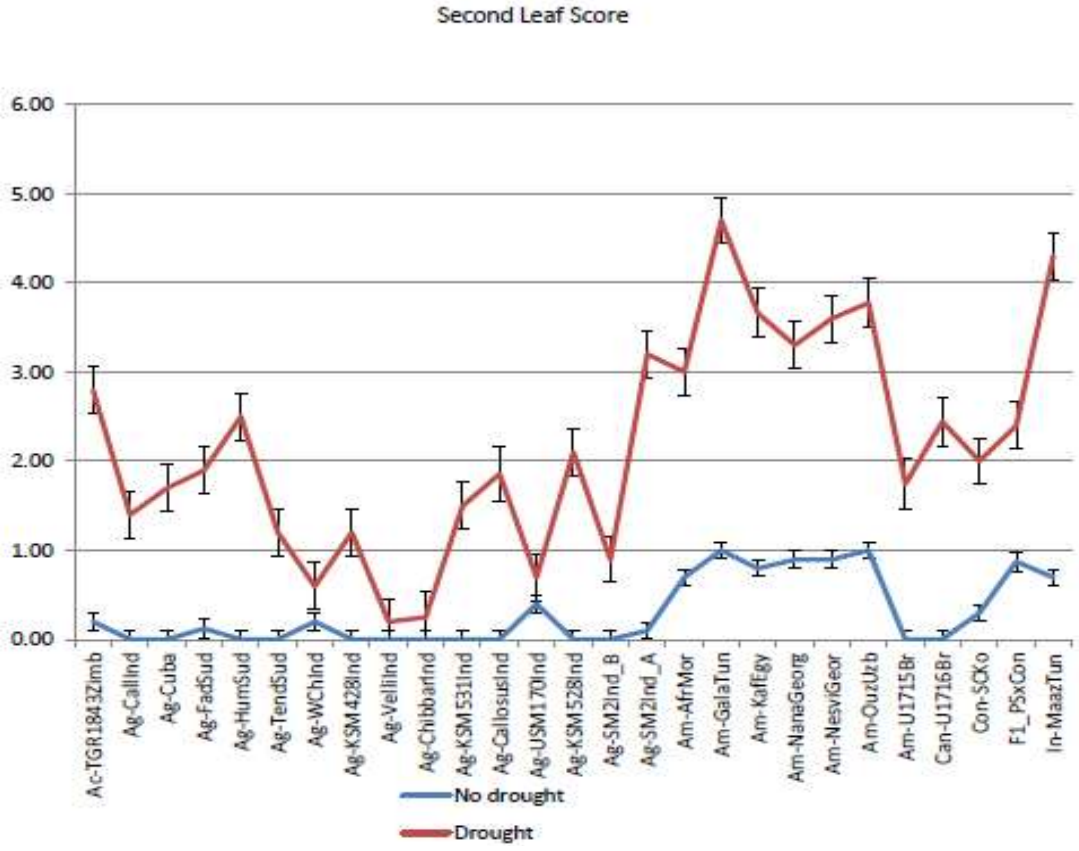

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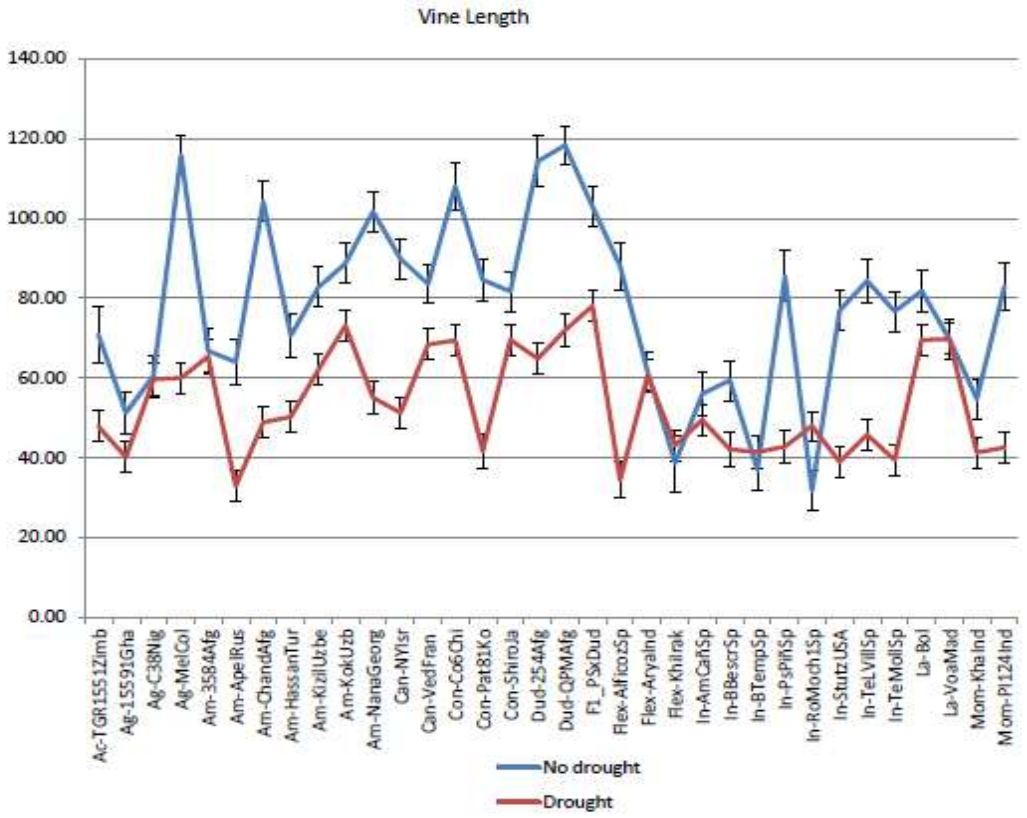

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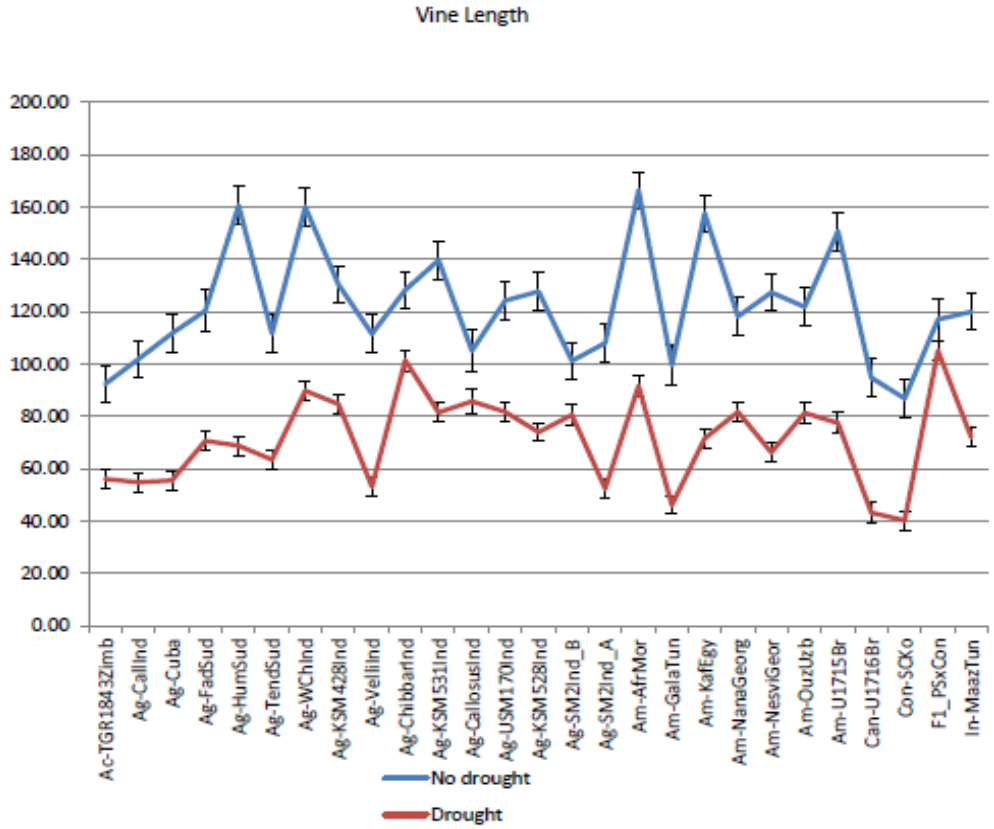

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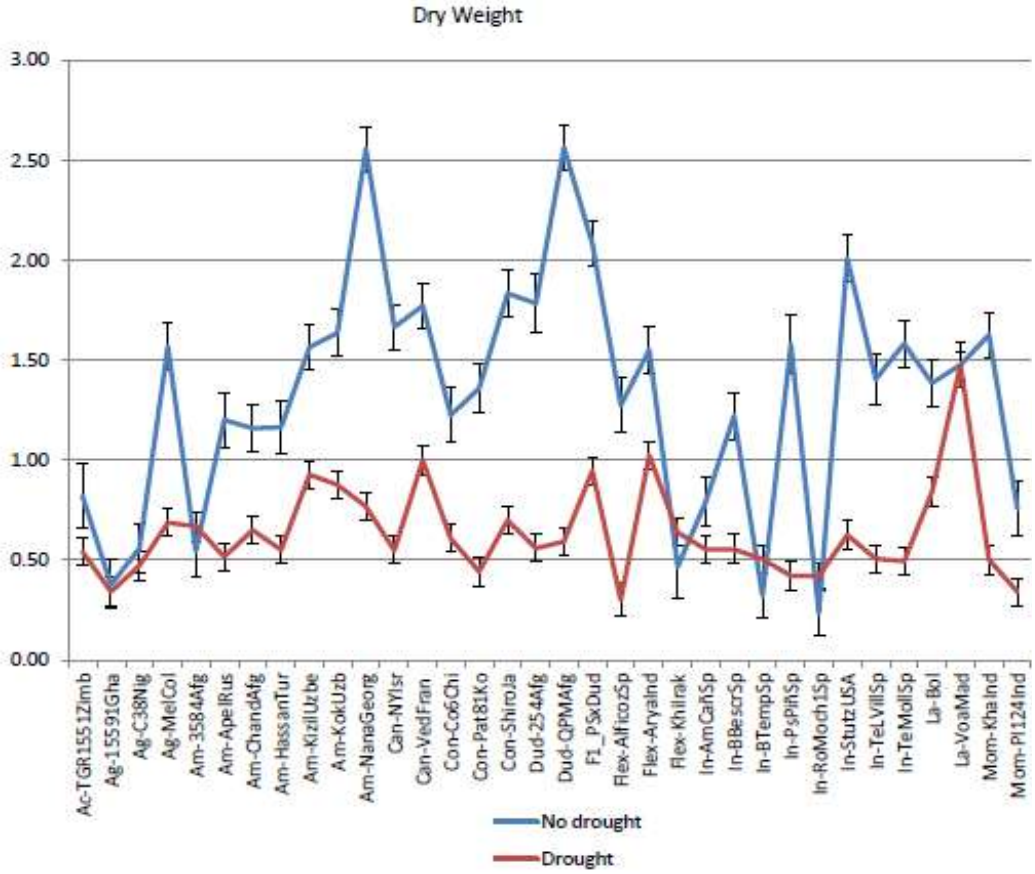

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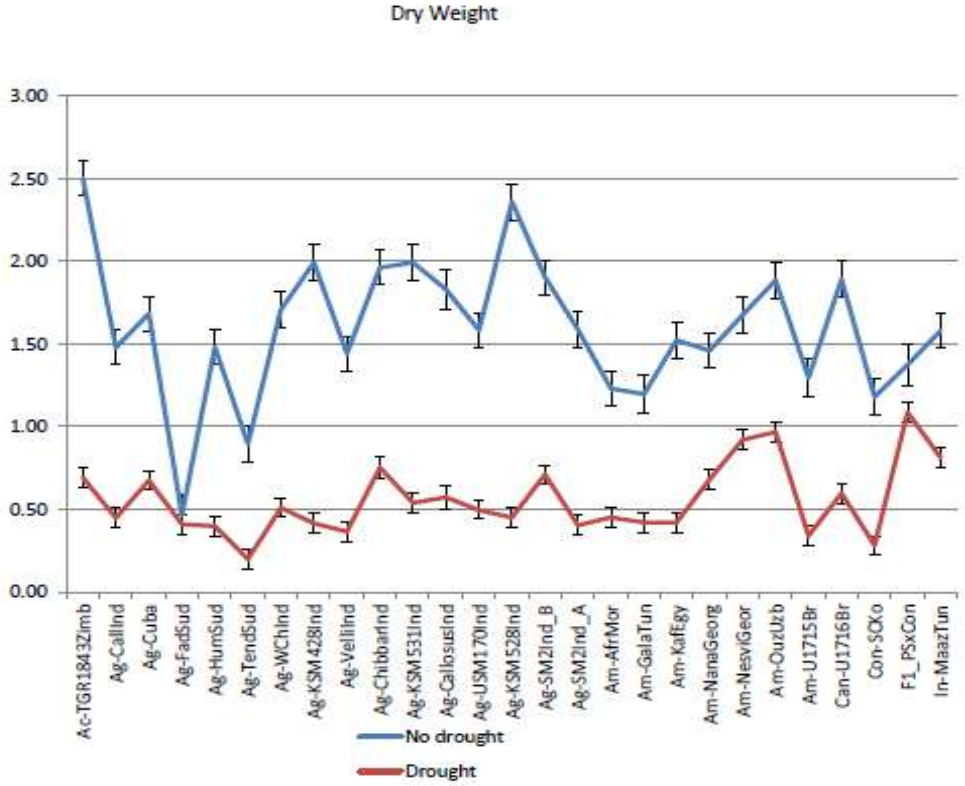

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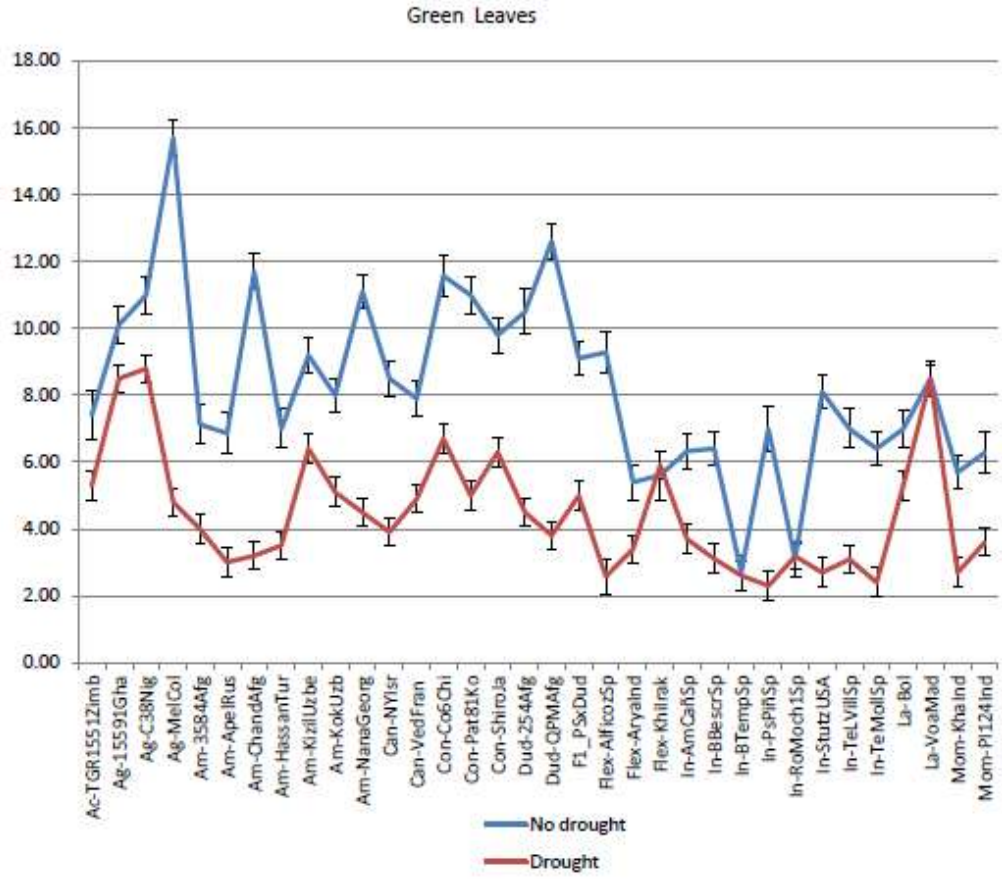

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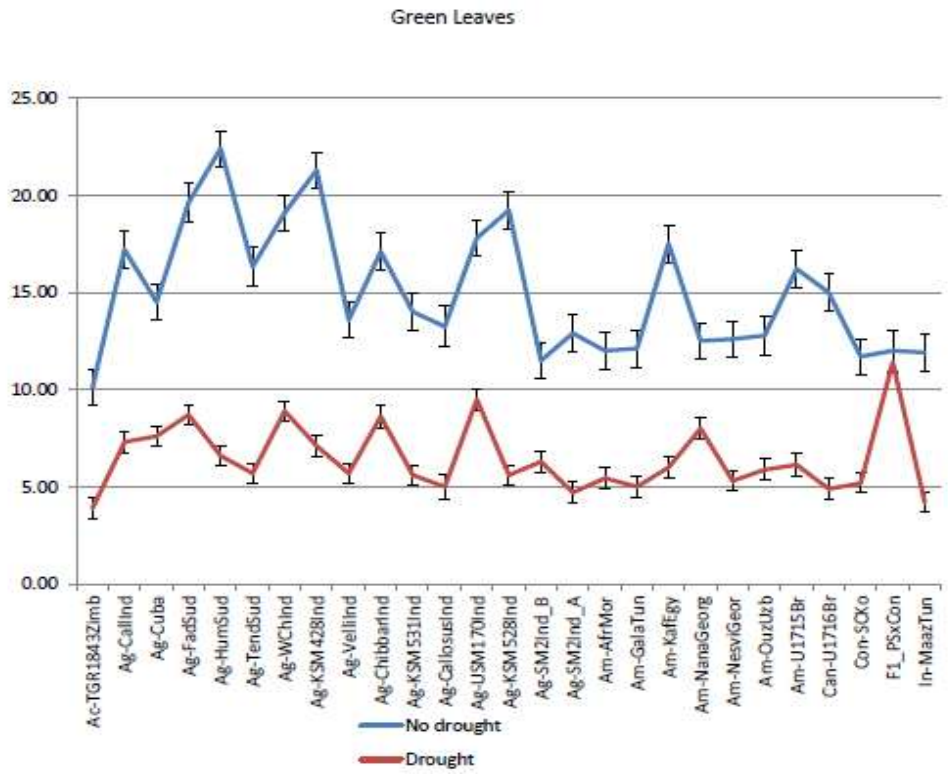

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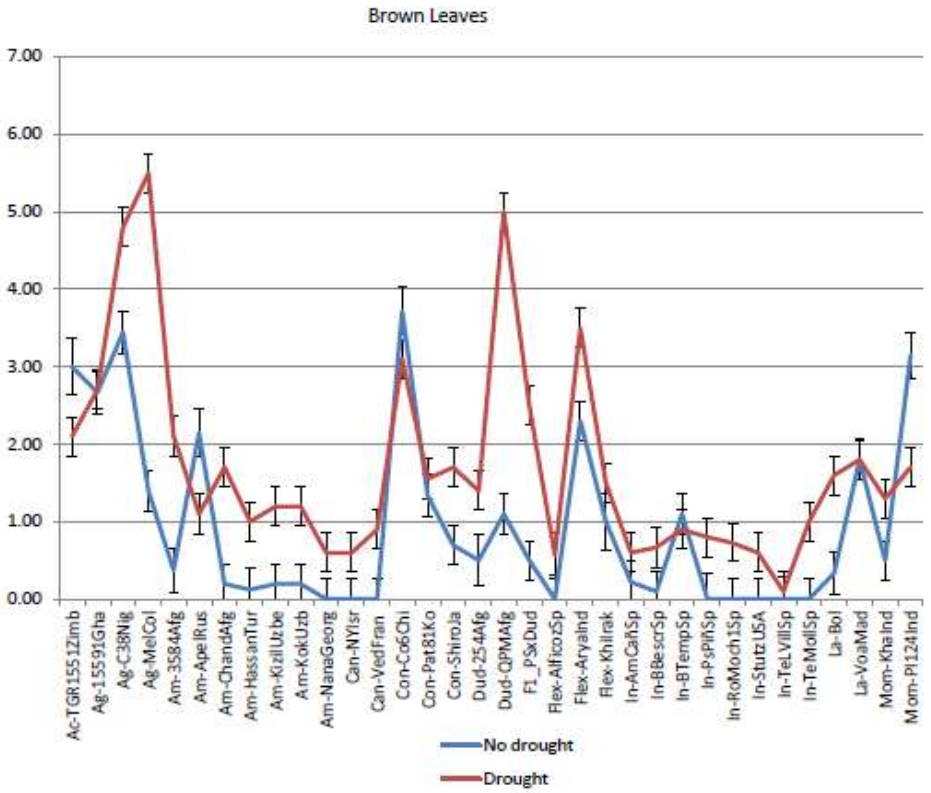

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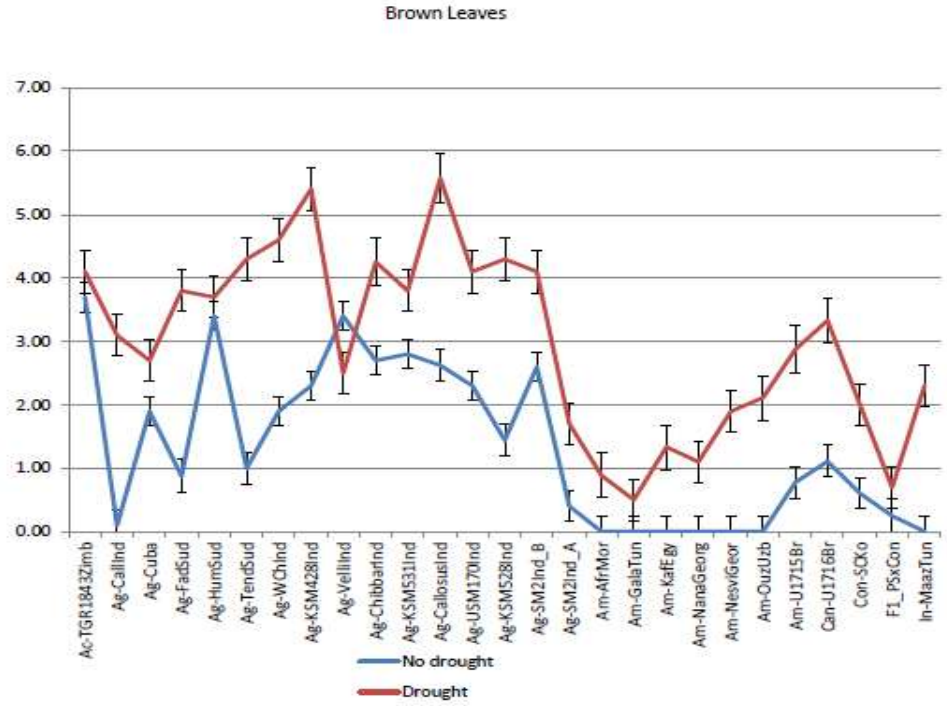

Supplementary file 1. Plots showing means and standard errors per genotype for each trait assessed in both assays under drought/no drought conditions.