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Assessment of flag leaf water status as drought tolerance discriminating trait in durum wheat (*Triticum turgidum var durum L.*)

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Abstract

Drought is a prominent limiting factor that impacts negatively durum wheat grain yield. Ten durum wheat breeding lines were evaluated under rainfall conditions at the Field Crop Institute Agricultural Experimental Station of Setif, Algeria, during the 2016/2017 cropping season. The investigation aimed to study the ability of flag leaf water status to discriminate among varieties for drought tolerance trait. Significant variability was observed among the tested varieties for leaf dry, wilted and turgid weights, leaf relative water content, water saturation deficit and excised water loss, after three wilting periods of 30, 60 and 90 minutes dehydration at 40°C. The assessed breeding lines were differentially categorized as drought tolerant and drought sensitive based on either relative water content or water saturation deficit or excised leaf water loss genotypic mean values. Correlation, principal components and cluster analyses indicated an unwanted significant association between excised leaf water loss and relative water content and water saturation deficit and classified the assessed entries into three clusters (CI, C2 and C3). Cluster C1 had high relative water content, low water saturation deficit but high excised water loss, while C3 had low relative water content, low excised leaf water but high-water saturation deficit, C2 being intermediate. Crosses between distant clusters (C1 vs C3) are proposed to generate more variability of the targeted traits in progeny population and to break undesirable linkage between alleles controlling leaf water status, allowing to select efficiently drought tolerant genotypes.

Keywords: *Triticum durum* Desf. Drought tolerance; Leaf water status; Cluster; Excised leaf water loss

1. Introduction

Drought is the most important abiotic stress factor affecting crop production worldwide, causing large economic losses. Induced by erratic and low rainfall, this stress causes severe yield reduction [1]. With a projected increase in drought events due to climate change, yield improvement under drought conditions is becoming a major goal of many plant breeding programs [2]. To develop drought-tolerant genotypes, it is essential to primarily understand mechanisms underlying this stress, and to identify their trait-markers. Three basic mechanisms are involved in drought stress resistance: escape, avoidance or tolerance, and resistance [2]. Plants, showing escaping mechanism, complete their life cycle before the onset of drought, during the brief period of favorable conditions. In the drought-resistance mechanism, plants adapt themselves to survive drought in two distinct strategies leading to drought avoidance and to drought tolerance. In the drought avoidance strategy plants minimize tissue dehydration, maintaining high cell water potential under limited water supply; while in the drought tolerance or dehydration tolerance strategy plants decrease cell water potential in order to reach a balance between water uptake by roots and water release by leaves [3]. Several physiological traits have been advocated as indicators of drought resistance and proposed to be used as tools for the development of drought resistant genotypes [4]. Efficient screening techniques applicable in early growth stage are

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desirable to eliminate unwanted plant material and to focus on promising one [5, 6]. Several tests based on leaf plant water status have been proposed as screening techniques for drought resistance. Relative water content (RWC) is closely related to cell volume and reflects the balance between water supply and transpiration [7]. Genotypic variation for this trait has been reported in wheat, making selection for RWC easier [5, 6, 8]. Hakimi et al., [9] mentioned that, under drought conditions, minimization of RWC decline was associated with drought resistance. Investigating excised leaf water loss (ELWL) variation in wheat, Clarke and McCaig [10] reported that this trait contributed positively to plant drought resistance, suggesting it as screening criterion. Randhawa *et al.,* [11] mentioned that leaf water retention ability was associated with drought tolerance in wheat. Datta et al. [12] reported that best performing genotypes under drought exhibited high RWC. Bilal et al., [13] mentioned that RWC discriminated efficiently between drought tolerant and drought sensitive genotypes. Dedio [14] found that Pelissier and Pitic 62, durum and bread wheat cultivars, respectively, were best water retainers under drought stress conditions. F3 selection for this characteristic, among progenies of crosses involving these genotypes as parents, was effective. Identifying varieties differing genetically in leaf water status, provides useful information about drought tolerance which could be used in breeding programs to develop high yielding and drought tolerant genotypes. This study aimed to investigate variation in leaf water status of a set of durum wheat (*Triticum durum* Desf.) advanced breeding lines under field conditions.

2. Material and methods

2.1. Site, plant material and experimental design

The experiment was conducted at the Agricultural Experimental Station, Field Crop Institute of Setif (AES- ITGC, Setif, Algeria, 36°15'N, 5°37'E, 1081 m altitude) during the 2016/17 cropping season. Ten durum wheat varieties, namely Canzone, Icarsha$_2$, Trouve, Habab, Chicca, Zeina, Waha, Hessept$_2$, Mammachan and Cucaraja were arranged randomly within each of four blocks. The tested varieties originated from the durum wheat nursery sent by Icarda-Morocco to its cooperators among which the AES- ITGC of Setif (Algeria). The experiment, seeded in mid-November 2016, was conducted under rainfed conditions. Plot dimensions were 6 rows, 5 m long with an inter rows spacing of 0.2 m. The experiment was fertilized with 80 kg/ha mono-ammonium phosphate (52% P_2O_5 + 12% N) just before sowing and 80 kg/ha urea (46% N) were applied at tillering growth stage. Weed control was performed chemically by application of 150 g/ha of Zoom herbicide. From September to June, monthly rainfall varied from almost zero in March, April and May 2017 to 48.3 mm in January 2017, with a total of 187.9 mm for the crop cycle. This was the driest cropping season when compared with the 354.9 mm average of the 1993-2013-period (Figure 1).

2.2. Leaf water status measurements

At the heading stage, flag leaves were sampled from the evaluated varieties for the determination of leaf water status. One set of five flag leaves was inserted in pre-weighed test tubes containing 10 ml of distilled water, according to the procedure described in [8]. The test-tubes were sealed and led to stand overnight in darkness to reach full turgidity, then leaf turgid weight (TW) was measured. The second set was placed in paper bags and transported to the laboratory for initial fresh weight (IFW) determination. Leaf samples were then placed in a ventilated oven, Memmert type, at 40°C to speed up leaf desiccation. Fresh weights (FW30, FW60 and FW90) were determined after 30, 60 and 90 minutes desiccation periods. Leaf samples were then oven dried at 70°C for 24 h for dry weight (DW) determination. Leaf relative water content (RWC) was calculated as follow: RWC (%) = 100*[(IFW-DW)/(TW–DW)], where IFW = initial fresh weight measured just after leaf excision [15]. Excised leaf water loss (ELWL), expressed in mg of water loss/g of dry weight [16] and as mg of water loss/min [17] was measured after 30, 60 and 90 min wilting periods: ELWL (mg/g) = (FW_t – FW_{t+30}) / DW, and ELWL (mg/min) = (FW_t - FW_{t+30}) / 30 min, where t= 0 or initial fresh weight, t+30 = fresh weight after 30 min wilting period [17]. Water saturated deficit (WSD) was determined according to [18]: WSD (%) = 100*[(TW - IFW) / (TW – DW).

2.3. Data Analyses

Recorded data were subjected to an analysis of variance using balanced anova subroutine implemented in Cropstat software [19]. Mean comparisons were made using the F-protected least significant difference test (F-protected LSD). The LSD was calculated according to [20] as follow: $LSD_{5\%} = t_{5\%}(\sqrt{2\sigma^2 e}) / r$, where $t_{5\%}$ is the tabulated t value at 5% probability level, $\sigma^2 e$ = mean square error and r = number of replications. Variables showing statistical significance were further explored through correlation, principal components and cluster analyses to determine useful associations between traits and genotypes for drought tolerance classification. Correlation, principal components and cluster analyses were performed using Past software version 3 [21]. Correlation coefficients significance was checked versus r table values at the 5% and 1% probability levels [20]. To reduce from the effect of multicollinearity, highly correlated variables were removed from the principal components and cluster analyses which were run using Euclidean distances of normalized variables and Ward's method as linkage criterion. Principal components showing Eigenvalue greater than unity were deemed significant [22] and discussed.

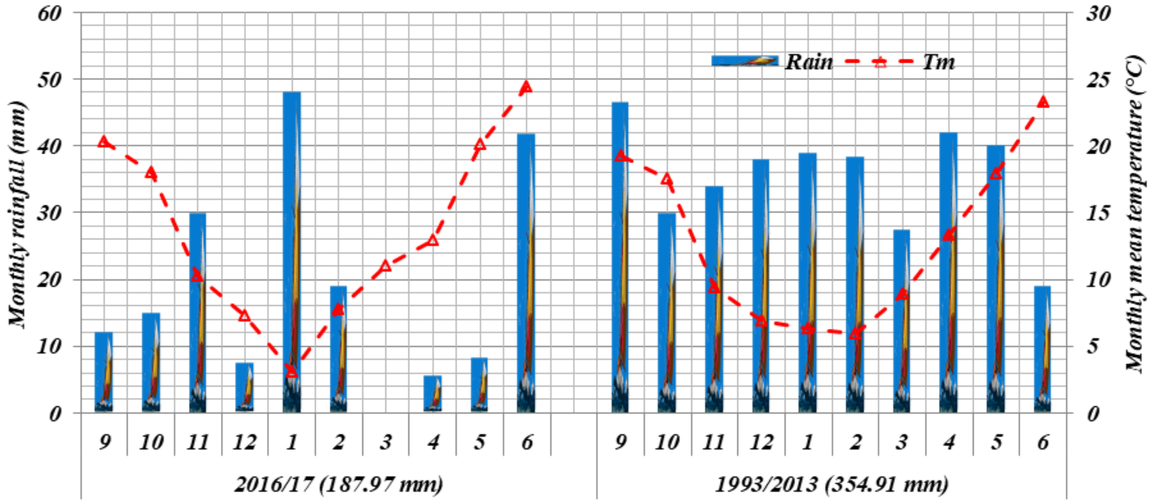

Figure 1 2016/17 cropping season and 20-year period (1993-2013) monthly mean rainfall and monthly mean temperature recorded at the experimental site [22].

3. Results

3.1. Traits variability

3.1.1. Flag leaf weights

Analysis of variance of fresh and dry weights of flag leaf samples indicated a significant variety effect for all measured variables (Table 1). This suggested the existence of inherent genetic variability among the assessed varieties which could be useful for drought tolerance selection. These differences originate from variation for both leaf water content and leaf accumulated dry matter. Mean values of the measured variables are reported in table 1. Habab exhibited significantly higher values, than the grand mean, (mean > $\bar{Y}$ + 1 LSD) for all the measured traits; while Mammachan and Cucaraja had significantly lower values (mean < $\bar{Y}$ - 1 LSD) for turgid weight (TW), initial fresh weight (IFW) and dry weight (DW). The remaining varieties had mean values not significantly different from the grand mean (mean > $\bar{Y}$ – 1 LSD and < $\bar{Y}$ + LSD).

3.1.2. Relative water content (RWC) and water saturation deficit (WSD)

RWC and WSD analysis of variance indicated significant variety effect (Table 2). This suggested that the observed inherent genetic variability for leaf sample weight between assessed varieties was partially caused by significant difference in water content. RWC mean values varied from 77.92%, mean of Mammachan, to 87.76%, mean of Canzone, with an overall mean of 79.97% (Table 2). Compared to the results of [18, 23, 24] who reported RWC mean values above 90.0% for varieties tested under irrigation conditions and below 85.0% for varieties tested under stressed conditions, the mean values reported in the present study are representative of drought stress conditions. Canzone and Icarsha2 exhibited significantly higher RWC than the overall mean (>$\bar{Y}$bar+1LSD) while Mammachan showed significantly lower mean (<$\bar{Y}$ -1LSD). The remaining varieties had mean values not significantly different from the grand mean (≈ $\bar{Y}$). Since plant water content is under genetic control [10], these results suggested that the tested varieties carry different alleles. Therefore, selection for this trait discriminates easily between drought tolerant and drought sensitive varieties.

Table 1 Analysis of variance mean squares and mean weights of flag leaf samples of the assessed varieties.

	Analysis of variance mean square					
Sources (DF)	TW	IFW	FW30	FW60	FW90	DW
Blocks (3)	2482.5	3397.5	3293.3	4290.8	3723.3	1655.8
Varieties (9)	95130.50*	89809.40*	70607.50*	58746.80*	49996.80*	6007.87*
Residual (27)	31934.4	25760.5	22373.9	16566.8	14857.6	1720.7
	Leaf weights					
Varieties	TW	IFW	FW_{30}	FW_{60}	FW_{90}	DW
Canzone	1306.67	1193.33	820.00	716.67	666.67	386.67
$Icarsha_2$	1275.00	1135.00	825.00	725.00	665.00	385.00
Trouve	1165.00	1020.00	800.00	670.00	625.00	320.00
Habab	1495.00	1310.00	1130.00	1015.00	940.00	460.00
Chicca	1375.00	1240.00	1035.00	900.00	820.00	400.00
Zeina	1280.00	985.00	925.00	885.00	850.00	385.00
Waha	1113.33	956.67	746.67	646.67	596.67	356.67
$Hessept_2$	1175.00	1040.00	780.00	695.00	660.00	380.00
Mammachan	950.00	805.00	665.00	595.00	555.00	320.00
Cucaraja	930.00	820.00	685.00	610.00	580.00	315.00
$\bar{Y}$	1206.00	1050.50	841.17	745.83	695.83	370.83
LSD5%	218.61	196.34	182.98	157.45	149.11	50.74

*= Variety effect significant at 5% probability level. TW= turgid weigh, IFW= Initial fresh weight, FW30 = fresh weight after 30 minutes desiccation, FW60 = fresh weight after 60 minutes desiccation, FW90 = fresh weight after 90 minutes desiccation, DW= dry weigh. Values are expressed in mg/sample of 5 flag leaves. $\bar{Y}$ = grand mean

Based on RWC observed means, Canzone and Icarsha2 are categorized as drought tolerant (water maintainers) and Mammachan as drought sensitive (water spender) varieties, with 6.84% difference. The remaining entries exhibited average drought tolerance. In this context, Hurd [25] mentioned that above average RWC expressed in the drought tolerant cultivars Pitic 62 and Pelissier is thought to be associated with an extensive root system in the deeper soil layers. Monica [26] reported a tendency for cultivars adapted to drought to have a lower leaf water content and water loss; and they lose 32.2% from their initial water content in the few hours after excision and 49.8% during the next 20 hours. Teulat et al. [27] noticed that RWC difference between drought resistant and sensitive genotypes varied from 18.6 to 21.8%. WSD mean values varied from 11.40% mean of Canzone to 33.32%, mean of Zeina, the overall mean of this trait being 18.84% (Table 2). These values compared well with those observed by [18] who reported means varying from 12.60% for the drought tolerant cultivar Pitic 62 to 28.90% for the drought sensitive cultivar Stewart. Among the assessed varieties, in the present study, Zeina, Waha and Mammachan, showed significantly above average WSD means, and therefore are categorized as drought sensitive; while Canzone and Chicca expressed significantly below average WSD mean and were classified as drought tolerant genotypes. Such classification based on WSD is not always straightforward since [25] mentioned that Pelissier (alias Hedba 3), a well-known drought tolerant cultivar, exhibited above average WSD.

3.1.3. Rate of water loss

The analysis of variance of the rate of water loss from excised flag leaves indicated significant desiccation period, variety and interaction effects (Table 3). Water loss rate varied substantially between desiccation periods, varieties and desiccation periods x variety combinations. Averaged over varieties, water loss rate declined from the first to the third desiccation periods, from 567.0 to 134.2 mg H_2O/g dry weight, and from 7.3 to 1.6 mg H_2O/min.

Table 2 Mean squares of the analysis of variance and mean values of relative water content and water saturation deficit of the assessed varieties flag leaf.

Sources of variation (DF)	RWC (%)	WSD (%)
Replication (3)	10.2	4.9
Variety (9)	156*	158*
Residual (27)	44.1	6.4
Varieties		
Canzone	84.76	11.40
Icarsha2	83.77	15.96
Trouve	81.36	17.56
Habab	80.01	18.07
Chicca	81.50	13.47
Zeina	62.58	33.32
Waha	81.65	22.10
Hessept2	81.23	14.80
Mammachan	77.92	23.88
Cucaraja	80.90	17.85
$\bar{Y}$	79.57	18.84
Lsd5%	2.93	1.71

*= Variety effect significant at 5% probability level. RWC= Relative water content (%), WSD= Water saturation deficit (%)

This variation is expected since water available for evaporation is greater at the first dehydration period compared to the last wilting period. However, if we assume that the duration of desiccation periods (30, 60, 90 min) could be viewed as variation of drought stress intensities, the above, mentioned results corroborate what has been observed by [24] who reported that under severe drought stress water loss rate declined. In such cases, stomatal control of water loss is suspected [28]. Averaged over desiccation periods, ELWL rate varied from 125.5 mg H_2O/g dry weight (Zeina) to 448.20 mg H_2O/g dry weight (Canzone). Canzone, Icarsha 2, Trouve, Chicca, Waha and Hessept2 showed significant above average water loss rate (mean > $\bar{Y}$ + 1 LSD). Habab, Zeina, Mammachan and Cucaraja exhibited significantly below average ELWL rate (mean < $\bar{Y}$ - 1 LSD). Similar varietal ranking is observed when ELWL rate was expressed in terms of mg H_2O/min (Table 3). Genotypes losing water at a reduced rate (water retainer genotypes) are expected to be more drought stress tolerant than genotypes showing a relatively high-water loss rate (water spender genotypes). On this basis, the assessed varieties are clustered into drought tolerant (Habab, Zeina, Mammachan and Cucaraja) and drought sensitive (Canzone, Icarsha 2, Trouve, Chicca, Waha and Hessept 2). The desiccation periods x variety examination indicated that the assessed varieties categorization is easier after a short desiccation period than after a long one, because the expressed differences for ELWL rates are larger after 30 minutes desiccation period than after 60 or 90 minutes (Figure 2).

3.2. Traits relationship

Scrutinizing the correlation coefficients matrix indicated that the measured variables clustered into two groups: variables related to water content (RWC, WSD and ELWL) and variables related to leaf weight (TW, IFW, FW30, FW60, FW90 and DW). Within group variables were highly correlated to each other but no significantly correlated with variables from the other group, excepted IFW which presented significant correlations with variables from both groups (Table 4).

Table 3 Analysis of variance mean squares and water loss mean values of the assessed varieties.

Sources of variation (DF)	ELWL1	ELWL 2
Desiccations (D, 2)	2034980.0**	345.6**
Replications/D (9)	139.6	0.4
Varieties (V, 9)	101421.0**	14.0**
D x V (18)	61530.2**	6.2**
Pooled error (81)	75.0	0.3
Desiccation periods		
30 min	567.0	7.3
60 min	240.3	3.2
90 min	134.2	1.6
LSD5%	6.0	0.3
Varieties		
Canzone	448.2	5.6
Icarsha2	410.1	4.9
Trouve	355.9	4.7
Habab	270.2	4.1
Chicca	354.7	4.7
Zeina	125.5	2.3
Waha	337.9	4.3
Hessept2	327.5	4.4
Mammachan	256.9	2.9
Cucaraja	251.3	2.6
Ȳbar	313.8	4.0
Lsd5%	7.0	0.4

**= Desiccation period, variety and interaction effects significant at 1% probability level. ELWL1= excised leaf water loss expressed in mg H_2O/g dry weight, ELWL2= excised leaf water loss expressed in mg H_2O/min, respectively as regression coefficient b, Ȳ = Grand mean.

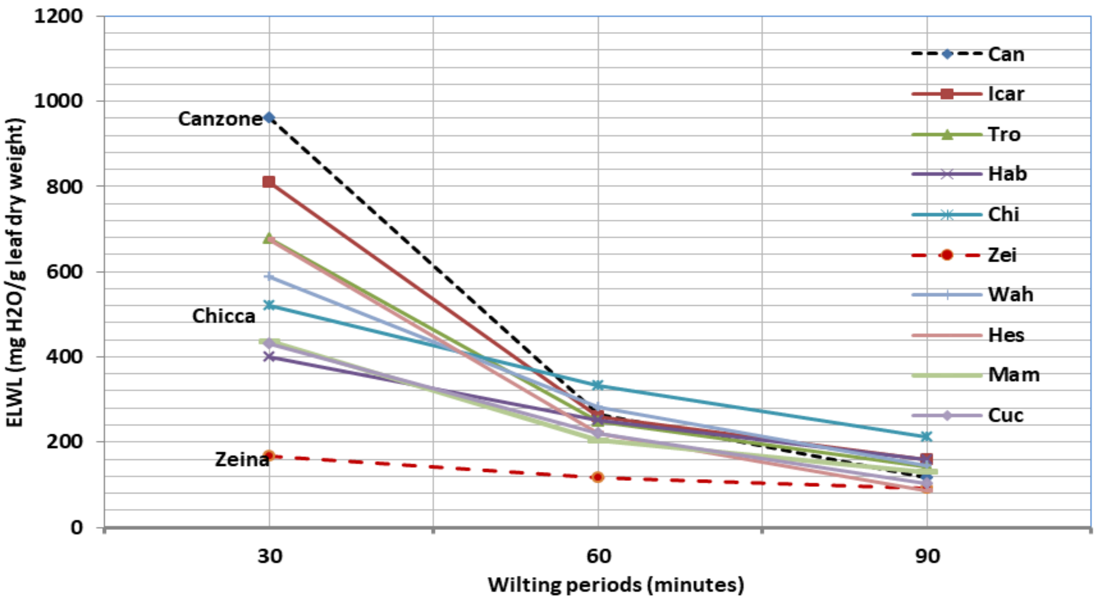

Figure 2 Variation of water loss rates among varieties and desiccation periods (interaction variety x wilting periods).

Table 4 Spearman's rank correlations coefficients among the measured variables of the assessed varieties (below diagonal correlation coefficients, above diagonal probability).

	RWC	WSD	ELWL1	ELWL2	TW	IFW	FW30	FW60	FW90	DW
RWC		0.000	0.003	0.000	0.162	0.019	0.310	0.293	0.405	0.177
WSD	-0.890**		0.003	0.000	0.162	0.019	0.310	0.293	0.405	0.177
ELWL1	0.830**	-0.830**		0.000	0.405	0.128	0.580	0.651	0.777	0.412
ELWL2	0.915**	-0.915**	0.976**		0.276	0.067	0.467	0.489	0.627	0.273
TW	0.479ns	-0.479ns	0.297ns	0.382ns		0.000	0.000	0.000	0.000	0.000
IFW	0.721*	-0.721*	0.515ns	0.600ns	0.915**		0.002	0.001	0.004	0.001
FW3	0.358ns	-0.358ns	0.200ns	0.261ns	0.939**	0.855**		0.000	0.000	0.001
FW6	0.370ns	-0.370ns	0.164ns	0.248ns	0.952**	0.867**	0.988**		0.000	0.000
FW9	0.297ns	-0.297ns	0.103ns	0.176ns	0.952**	0.818**	0.964**	0.976**		0.000
DW	0.463ns	-0.463ns	0.293ns	0.384ns	0.976**	0.890**	0.890**	0.921**	0.909**	

RWC = relative water content (%), WSD= water saturation deficit (%), ELWL1 = Excised leaf water loss (mg H2O/g dry weight), ELWL2= Excised leaf water loss (mg H_2O/min), TW= turgid weight (mg), IFW = initial fresh weight (mg), FW30= fresh weight after 30 min desiccation period, FW60 = fresh weight after 60 minutes desiccation period, FW90 = fresh weight after 90 minutes desiccation period, DW = dry weight (g), ns, *, ** = correlations coefficients non-significant and significant at 5 and 1% probability level, respectively.

RWC is negatively and significantly correlated with WSD and positively with ELWL (mgH_2O/g dry weight), ELWL (mg H_2O/min) and IFW. WSD is significantly and negatively correlated with ELWL and IFW; while ELWL (mgH_2O/g dry weight) is positively related to ELWL (mg H_2O/min). These relationships suggested that within the set of assessed varieties, genotypes exhibiting above average RWC are characterized by below WSD average and above IFW and ELWL averages. Traits related to leaf weight are positively and significantly related to each other, suggesting that variation in leaf fresh weight due to wilting is mostly induced by changes in water content rather than by changes in dry matter content. These findings contradict [27] who reported negative and significant correlation between RWC and ELWL and significant and negative correlation between ELWL and grain yield, under drought stressed conditions, proposing ELWL as an indirect selection criterion to improve grain yield under water-limited environment. In this context [29] reported no significant correlations coefficients between RWC, ELWL and grain yield, suspecting the absence of tight linkage between genes controlling these traits. However, [30] mentioned that the first canonical variables (RWC, WSD and ELWL), were highly associated with grain yield and harvest index, under drought stress conditions.

3.3. Varietal typology

To avoid using several highly correlated variables, in the PCA and cluster analyses, only RWC, WSD, TW, IFW and DW were retained to characterize the set of assessed varieties. Principal component analysis (PCA) allowed identifying traits which were decisive in varietal differentiation. Most of the variability existing within the data analyzed is absorbed by the first two principal components which had latent roots greater than one. These PC explained 95.47% of the total variance (Table 5). This percentage is appreciably high to discriminate among the assessed varieties for their drought tolerance ability based on flag leaf water content and weight. Major contributors to PC1 were WSD (-0.432), ELWL (0.404) and IFW (0.474). With 58.95% of the total variation explained, this PC is indicator IFW which the highest loading on this component. PC2 accounted for another 36.51% of variation with RWC (-0.419), TW (0.459) and DW (0.491) as the major loaded factors. PC2 is then indicator of water retention ability and leaf weights (Table 5). Canzone (2.187), Icarsha2 (1.344), Chicca (1.962), Waha (-0.665), Mammachan (-2.367) and Cucaraja (-1.782) had high scores on PC1. Based on the sign of their scores on PC1, Canzone, Icarsha2 and Chicca are classed as drought tolerant because of their ability to retain water (high IFW), minimizing WSD but having high ELWL. Waha, Mammachan and Cucaraja are classed as drought sensitive because of their low IFW, ELWL and large WSD (Figure 3). Trouve (-1.128), Habab (2.404), Zeina (3.009) and Heissept2 (-0.314) had high scores on PC2. Based on the sign of their scores, Trouve and Hessept2 had high RWC and low leaf turgid and dry weights,

Table 5 Eigenvalues, % variance, % cumulative variances and eigenvectors of the first two principal components for the excised leaf weight, water content and water loss of the tested varieties.

Parameters	**PC1**	**PC2**
Eigenvalue	3.53	2.19
% variance	58.95	36.51
% cumulative variances	58.95	95.47
Traits	Loading	
RWC	0.404	-0.419
WSD	-0.432	0.379
ELWL	0.404	-0.375
TW	0.382	0.459
IFW	0.474	0.299
DW	0.342	0.491

RWC = relative water content (%), WSD= water saturation deficit (%), ELWL = Excised leaf water loss (mg H2O/mg dry weight), TW= turgid weight (mg), IFW = initial fresh weight (mg), DW = dry weight (mg).

While Habab and Zeina had low RWC and high TW and DW (Figure 3). Cluster analysis classified the tested genotypes, based on their resemblance/dissemblance, into three groups. Habab, Chicca, Canzone and Icarsha2 formed cluster C1. Cluster C2 grouped Mammachan, Cucaraja, Trouve, Waha and $Hessept_2$, all together; while Zeina classified in a separate cluster C3 (Figure 3). Average values of the three clusters are reported in table 6, and between clusters differences, expressed as percent of the maximum mean values, are depicted in figure 4. Inspecting both tab 6 and figure 4, Cluster C3 (variety Zeina) is desirable for its low excised water loss, while cluster C1 is selectable for its high RWC, cluster C2 being intermediate for trait markers of drought tolerance. Since the targeted cultivar must exhibit high RWC, low WSD and low ELWL, the drawback of these selections is that C3 exhibited undesirable low RWC and high WSD; while cluster C1 exhibits high ELWL, making selection for drought tolerance ineffective. These relationships among the targeted traits originate from genetic linkage which may be broken through inter clusters crossing. The resulting crosses bring altogether the favorable alleles controlling the desired traits offering the opportunity to broaden genetic variability and to develop drought tolerant varieties.

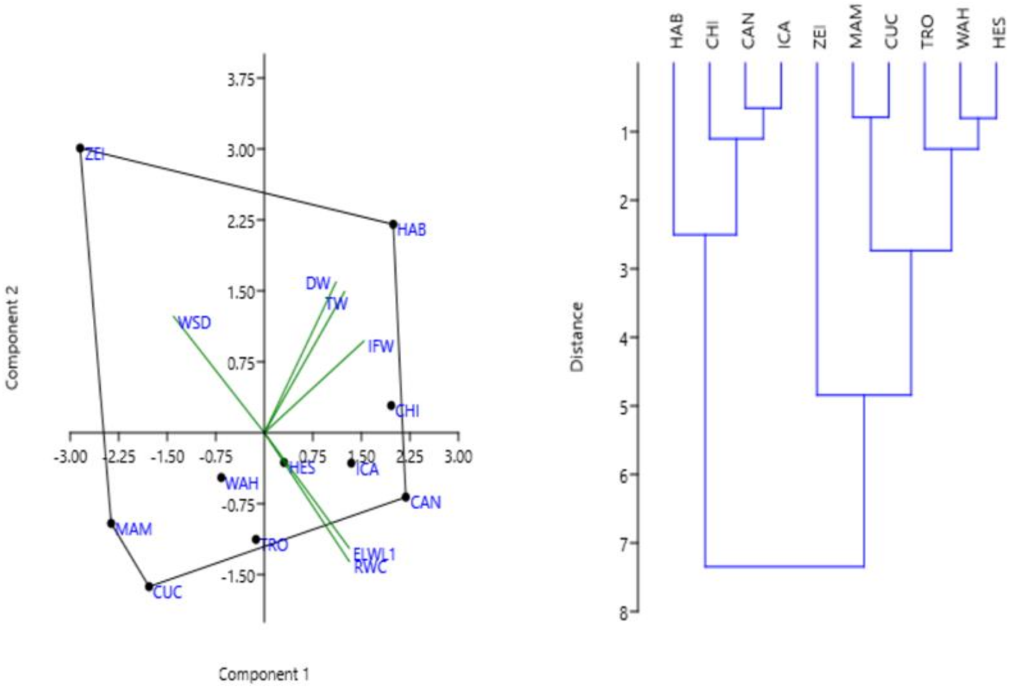

Figure 3 PC1-PC2 biplot and cluster dendogram of the assessed varieties based on flag leaf weight and water content**.**

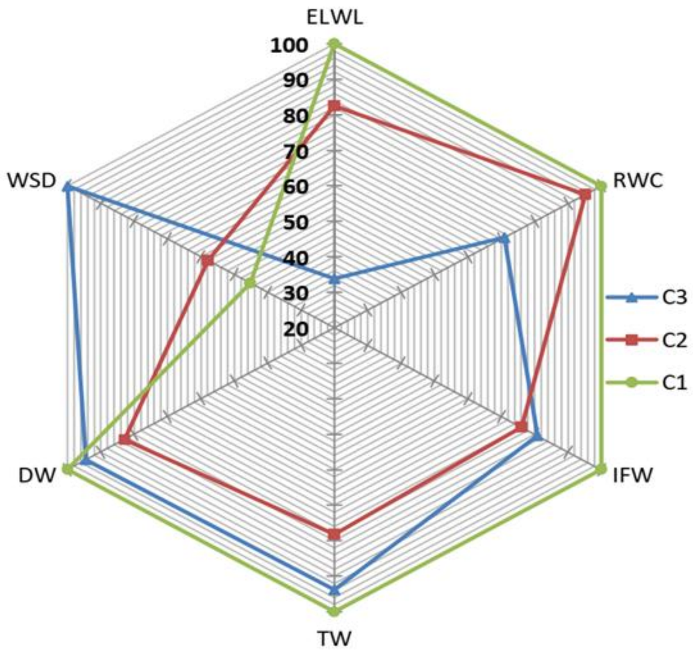

Figure 4 Cluster mean values expressed as % of maximum value of flag leaf weight and water content of the assessed varieties.

Table 6 Clusters average values of the measured traits of the assessed varieties.

Clusters	RWC	WSD	ELWL	TW	IFW	DW
C3	60.3	33.0	125.5	1280.0	985.0	385.0
C2	80.9	19.1	305.9	1066.7	928.3	338.3
C1	85.1	14.9	370.8	1362.9	1219.6	407.9

RWC = relative water content (%), WSD= water saturation deficit (%), ELWL = Excised leaf water loss (mg H2O/g dry weight), TW= turgid weight (mg), IFW = initial fresh weight (mg), DW = dry weight (mg).

4. Discussion

Water constitutes more than 80% of the fresh weight of growing plant, forming a continuous liquid phase from the root hairs to the leaf mesophyll cells. Plant cells require a high degree of internal water saturation to function efficiently [2]. Plant tissue water content fluctuates only within narrow limits if growth and development are to continue unimpaired. A change in water content of 15% to 20% of the value at full hydration will, generally result in cessation of growth. Even small changes of tissue water content trigger marked physiological changes [2]. Variation of plant tissue water content is generally monitored through leaf water status [10]. Leaf water status is extensively used to study the plant response to environmental stresses [31]. In this context water loss reduction from leaf surface under drought stress is a useful indicator of drought tolerance. Low cuticle transpiration rate reduces leaf dehydration, leading to leaf survival and maintain of photosynthesis [16]. Reducing leaf water loss under water stress is important to sustain plant production under harsh environmental conditions. Osmotic adjustment, water potential, ABA accumulation and stomata resistance are, among others, important physiological processes involved in leaf water loss regulation [27]. Identification of drought tolerant plant materials is of paramount importance to develop high yielding and stress tolerant genotypes. In this context assessment of leaf water content and leaf water loss seems to be promising [31]. Differences among genotypes for water content and/or rate of water loss, could be used to screen for drought resistance. According to Lugonan et Ciulca, [32], drought tolerant varieties showed less reduction in RWC, this trait exhibited appreciable variability, is moderately heritable and easily measured on a large number of progenies, with acceptable precision. Quantitative trait loci (QTL's) were reported for RWC, WSD and ELWL [33]. Ali et al. [34] mentioned that genotypes showing low water loss rate yielded significantly more than genotypes having high water loss rate in the driest environments, suggesting that selection based on low water loss rate is desirable. Varieties having high RWC postponed leaf senescence and recovered easily after stress than sensitive one. Literature review showed that flag leaf area, specific leaf weight, leaf dry matter, excised leaf weight loss, relative dry weight, relative water content and residual transpiration had been widely exploited as reliable morph-physiological markers contributing towards drought tolerance for various crop plants [16]. The results of the present investigation indicated that the assessed breeding lines were differentially categorized as drought tolerant and drought sensitive based on either relative water content or water saturation deficit or excised leaf water loss genotypic mean values. Classification based on more than one trait was less effective because of an unwanted significant association between excised leaf water loss and relative water content and water saturation deficit. Cluster analysis classified the assessed entries into three groups. Cluster C1 had high relative water content, low water saturation deficit but high excised water loss, while C3 had low relative water content, low excised leaf water but high-water saturation deficit, C2 being intermediate. Crosses between distant clusters (C1 vs C3) are proposed to generate more variability of the targeted traits in progeny populations and to break undesirable linkage between alleles controlling leaf water status, allowing to select efficiently drought tolerant genotypes. An alternative method would be to construct and use a selection index based on a combination of relative water content, excised leaf water loss, water saturation deficit and initial leaf fresh weight to identify the most drought tolerant breeding lines among those herein tested.

5. Conclusion

Findings indicated the presence of appreciable variability for leaf water status among the tested breeding lines. Selection, based on any of the various leaf water content traits, classified effectively the tested varieties into drought tolerant and drought sensitive. However, selection based on more than one trait was less effective because of undesirable association between relative water content, water saturation deficit and excised leaf water loss. Crosses between distant clusters are proposed to increase variability of the target traits and to break undesired genetic linkage, allowing efficient selection within progeny populations.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no interest conflict.

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