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Investigation of water retention capacity (WRC) as a new physiological indicator related to plant water status for screening drought tolerant genotypes in wheat

Hojjat Hasheminasab*, Ezatollah Farshadfar, Anita Yaghotipoor

Department of Agronomy and Plant Breeding, College of Agriculture, Razi University, Kermanshah, Iran

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Key words: Water retention capacity, wheat, water stress, plant water status.

Abstract

In developing a breeding program to improve the drought tolerance of a crop plant it is necessary to gain knowledge about the physiological mechanisms of tolerance. In order to introduce and evaluate water retention capacity (WRC) as a new physiological indicator related to plant water status for screening drought tolerant genotypes, fifteen bread wheat (*Triticum aestivum* L.) genotypes with wide range of sensitivity to drought were used in a randomized complete block design with three replications under two different environments (irrigated and rainfed) in 2012-2013 at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran. The results of the present study showed that considerable variations among genotypes for WRC were observed when grown under water stress and non-stress conditions. The highest WRC were observed in tolerant genotypes Pishtaz, Azar2, Rijaw and Chamran, and the lowest in susceptible genotypes Alamut, Zarin, Flat, Shiraz and Bahar under stress condition. The intermediate ratios were observed in Tabasi, Roshan, Niknejad and Darab2 (intermediate genotypes). The results of different statistical methods used in this study showed that WRC had a close relationship with relative water content (RWC). The visualizing graphic of scatter plot and biplot of principal component analysis identified WRC, RWC, relative water protection (RWP) and Canopy temperature depression (CTD) as the best indicators for screening drought tolerant genotypes. Discriminant and canonical discriminant functions analysis provided strong statistical evidence of significant differences among the genotypic groups for WRC, RWP, RWC, yield stability index (YSI) and relative water loss (RWL) with producing low Wilks' lambda. Our results suggested that WRC was a reliable index for classification and separation of drought tolerant genotypes.

***Corresponding Author:** Hojjat Hasheminasab ✉ hojathashemi@gmail.com

Introduction

Drought is one of the most widespread environmental stresses when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration and evaporation (Kramer, 1980; Razi and Assad, 1999). Although this situation has been more serious due to global climate change, in certain tolerant crop plants physiological and metabolic changes occur in response to water stress, which prevent the water loss from the leaf and contribute towards adaptation to such unfavorable constraints (Blum, 1985). Among crop plant, wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and it is also the first grain crop in Iran. This crop is widely adapted from temperate irrigated to dry and high rainfall areas, and from warm humid to dry cold environments. However, in arid and semi-arid regions, its yield is severely limited by water stress (Mohammadi and Amri, 2008; Hasheminasab *et al.*, 2012a).

Water plays a key role in the life of plant. It is the most abundant constituents of most organisms. Water is very essential for plant growth and makes up 75 to 95 percent of plant tissue. A vast amount of water moves throughout the plant daily. Plants use water and carbon dioxide to form sugars and complex carbohydrates. Water acts as a carrier of nutrients and also a cooling agent. It also provides an element of support through turgor and as an intercellular reaction medium. Many of the biochemical reactions occur in water and water is itself either a reactant or a product in a large number of those reactions (Ashraf and Harris, 2005; Vince and Zoltán, 2011).

Almost all of the water lost from leaves is lost by diffusion of water vapour through the tiny stomatal pores. The stomatal transpiration accounts for 90 to 95% of water loss from leaves. The remaining 5 to 10% is accounted for by cuticular transpiration. The important factor governing water loss from the leaf is the diffusional resistance of the transpiration pathway, which consists of two varying components: 1) the resistance associated with diffusion through the stomatal pore and 2) the resistance due to the layer of unstirred air next to the leaf surface through which water vapor must diffuse to reach the turbulent air of the atmosphere (Canny, 1998; Vince and Zoltán, 2011).

Physiologists have often suggested that the detection and selection of physiological traits related to plant water status are reliable methods to breeding for higher yield, and could be a valuable strategy for use in conjunction with normal methods of plant breeding (El Jaafari *et al.*, 1993; Blum, 2005). Relative water content (RWC), stomata resistance (SR), Leaf temperature (LT) and transpiration rate (E) are among the main physiological criteria that influence plant water relations and have been using for assessing drought tolerance (Siddique *et al.*, 2000; Anjum *et al.*, 2011). Relative water protection (RWP) is another important physiological index in assessing the degree of water stress. RWP is indicating plant water status related to water stress, as well as reflecting the metabolic activity in tissues (Hasheminasab *et al.*, 2012a). The canopy temperature (T_c) measured with infrared thermometers (IRTs) provides a reliable method for rapid, non-destructive monitoring of plant response to drought stress (Siddique *et al.*, 2000; Yuan *et al.*, 2004). They also stated that the behavior of T_c both under stress and non-stress conditions provided clues for crop water status and yield performance during drought. The main objective of the present study was to introduce and evaluate of water retention capacity (WRC) as a new physiological traits related to plant water status for screening drought tolerant genotypes.

Material and methods

Plant material and experimental conditions

Fifteen bread wheat (*Triticum aestivum* L.) genotypes with wide range of sensitivity to drought stress listed in Table 1 were used in a randomized complete block design with three replications under two different environments (irrigated and rainfed) at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran (47° 20′ N latitude, 34° 20′ E longitude and 1351m altitude) during 2012-2013. Climate in this region is classified as semi-arid with mean annual rainfall of 478mm and mean annual temperature of 13.8°C. Soil of the Experimental station was of clay-loam texture with EC = 0.550 dS/m and pH = 7.1. The plots consisted of 2m rows and at 15×30 cm inter-plant and inter-row distances, respectively. For measurement of measured attributes, flag leaves of all wheat cultivars at the flowering stage were harvested and weighed.

Relative water content (RWC).

RWC was measured using the method of Barrs (1968). A sample of 10 flag leaves was taken randomly from different plants of the same cultivar and their fresh weight (F_W) measured. The leaf samples were placed in distilled water for 24 h and reweighed to obtain turgid weight (T_W). After that, the leaf samples were oven-dried at 70°C for 72 h and dry weight (D_W) measured. However, RWC was calculated using the following formula:

$$RWC = \frac{F_W - D_W}{T_W - D_W}$$

Relative water protection (RWP)

RWP was determined according to Hasheminasab *et al.* (2012a). Ten randomly selected flag leaves were taken and weighed for fresh weight (F_W). The leaves were then allowed to wilt at 25°C for 8 h and weighed again (Withering weight, W_W). Then the samples were oven-dried at 70°C for 72 h and reweighed (Dry weight, D_W). RWP was calculated using the following equation:

$$RWP = \frac{W_W - D_W}{F_W - D_W}$$

Water retention capacity (WRC)

WRC is a combination of two value indexes, including RWC and RWP, therefore this index is calculated as the ratio of water out of the leaf and the water entering the leaf. WRC is indeed the proportion of actual water that is protected and not evaporated from the leaves after drying, because turgid leaf weight (maximum leaf water capacity) is located in the denominator of the formula. To measure WRC, ten randomly selected flag leaves were taken and placed in distilled water for 24 h and reweighed to obtain turgid weight (T_W). The leaves were then allowed to wilt at 25°C for 8 h and weighed again (Withering weight, W_W). Finally, the leaf samples were oven-dried at 70°C for 72 h and dry weight (D_W) measured. However, WRC was calculated using the following formula:

$$WRC = \frac{W_W - D_W}{T_W - D_W}$$

Leaf water content (LWC), relative water loss (RWL) and excised leaf water loss (ELWL)

Randomly selected leaves were weighed spontaneously after their harvesting (W_1). The leaves were then wilted at 25°C and weighed again over 2, 4 and 6 h (W_2, W_3 and W_4). Then the samples were oven-dried at 70°C for 72 h and reweighed (W_D). LWC, RWL and ELWL was worked out using the following formula devised by Clarke and Caig (1982), Yang *et al.* (1991) and Manette *et al.* (1988):

$$LWC = \frac{W_1 - W_D}{W_1}$$

$$RWL = \frac{(W_1 - W_2) + (W_2 - W_3) + (W_3 - W_4)}{3 \times W_D (T_1 - T_2)}$$

$$ELWL = \frac{W_1 - W_3}{W_1 - W_D}$$

Canopy temperature depression (CTD)

The crop canopy temperature was measured with a portable infrared thermometer (IRT). Four measurements were taken per plot at approximately 0.5 m from the edge of the plot with an approximately 30-60° from the horizontal position. Two to seven days after irrigation in each experiment, canopy temperatures were measured between 12:00 to 14:00 hours on cloudless, bright days. Ambient temperatures (AT) were measured with a common thermometer held at plant height. CTD was worked out according to Dong and Yu (1995):

CTD = AT – CT

Stomatal resistance (SR) and leaf temperature (LT)

Stomatal resistance (mmol m^{-2} s^{-1}) and leaf temperature (°C) was measured by Porometer-AP4 (Delta Devices, Cambridge, UK). Three random plants were selected in each plot for determining gas exchange parameters. All measurements were made on the portion of the flag leaf exposed to full sunlight, at about halfway along its length. The measurements were also made over the same time period as for the canopy temperature depression.

Evapotranspiration efficiency (ETE)

According to total consumed water through wheat life circle, ETE were calculated by referring to Ehdaie and Waines (1993). The ETE are defined as the ratio of total dry matter (TDM) production to total water use (TWU), respectively. TDM was recorded under normal and stress conditions at physiological maturity stage. The physiological maturity stage was considered when 90% of seed changed color from green to yellowish and stopped photosynthetic activity. The ETE were calculated using the following formulae:

$$ETE = \frac{TDM}{TWU}$$

Grain Yield and Yield Stability Index (YSI)

Grain yield was recorded at physiological maturity stage. The physiological maturity stage was considered when 90% of seed changed color from green to yellowish and stopped photosynthetic activity. Yield stability index (YSI) was calculated according to Bouslama and Schapaugh (1984) using the following formula:

$$YSI = \frac{Ys}{Yp}$$

Where, Ys and Yp represent yield under stress and non-stress conditions, respectively.

Statistical analysis of data

The measurement data of the studied traits across two environment conditions were analyzed by the statistical methods including descriptive statistics, principal component analysis (PCA), biplot analysis, scatter plot, discriminant analysis, canonical discriminant functions analysis and cluster analysis using SPSS software packages 16.0 (SPSS, 2007), Minitab version 14 and Microsoft Office Excel (2007).

Results and discussion

The results of the present study showed that considerable variations among genotypes for water retention capacity (WRC) were observed when grown under irrigated and rainfed conditions (Fig. 1). The genetic variability of these genotypes in response to water deficit was indicated by the results could help in identifying possible drought tolerant genotypes and also suitable indicators for screening these genotypes (Razi and Assad, 1999; Farshadfar *et al.*, 2013). The highest WRC were observed in tolerant genotypes Pishtaz, Azar2, Rijaw and Chamran, and the lowest in susceptible genotypes Alamut, Zarin, Flat, Shiraz and Bahar under stress condition. The intermediate ratios were also measured in Tabasi, Roshan, Niknejad and Darab2 (intermediate genotypes). Several reports underlined the significant relationship between the ability to maintain leaf water content and drought tolerance in various plants (Turkan *et al.*, 2005; Renu and Devarshi, 2007; Hasheminasab *et al.*, 2012a). Dong *et al.* (2008) in wheat and Yousfi *et al.* (2010) in alfalfa reported that under stress conditions, higher leaf water retention was a resistant mechanism to drought which the result was a reduction in stomatal conductance and transpiration rate. Loveys (1984) and Gowing *et al.* (1993) reported that under drought condition, abscisic acid (ABA) is increased in plant tissue and this causes a variety of physiological effects, including stomata closure in leaves. By opening and closing stomata, the guard cells control transpiration to regulate water loss or retention. They also stated that tolerant plant had higher rates of ABA as compared with susceptible. As seen in Fig. 1, dryland wheat genotypes Azar2 and Rijaw had the highest WRC under both environmental conditions. Thus, it can be concluded that WRC is a reliable indicator for screening drought tolerant genotypes. Farshadfar and Hasheminasab (2012) reported that genetic gain in developing tolerance in bread wheat could be achieved through indirect selection of physiological indicators related to leaf water status, because the additive genes mainly controlled these traits.

Table 1. Characteristics of investigated wheat genotypes.

Genotype	Code	Pedigree	Reaction to drought
Bahar	1	ICW84-0008-013AP-300L-3AP-300L-0AP	Susceptible
Shiraz	2	Gv/D630//Ald"s"/3/Azd	Susceptible
Falat	3	Kvz/Buho"s"//Kal/Bb=Seri82	Susceptible
Zarin	4	PK15841	Susceptible
Alamut	5	Kavz/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/3/Pi/Ndr//Hys	Susceptible
Darab2	6	Maya"s"/Nac	Intermediate
Niknejad	7	"F13471/Crow"s	Intermediate
Roshan	8	Roshan	Intermediate
Tabasi	9	Tabasi	Intermediate
Alvand	10	CF1770/1-27-6275	Intermediate
Chamran	11	(Attila.(CM85836-50Y-OM-OY-3M-OY	Tolerant
Kavir	12	Stm/3/Kal//V534/Jit716	Tolerant
Rijaw	13	PATO/CAL/3/7C//Bb/CNO/5/CAL//CNO/Sn64/4/CNO//Bad/DAR/3/ KL/6/Sabalan	Tolerant
Azar2	14	Azar2	Tolerant
Pishtaz	15	Alvand//Aldan/Ias58	Tolerant

Table 2. Principle component analysis of measured traits in wheat under drought stress condition.

Variable	Dimension			
	1	2	3	4
WRC	-0.606	0.771	0.182	-0.052
RWP	-0.974	0.135	-0.127	0.079
RWL	0.982	-0.03	-0.034	-0.031
RWC	-0.455	0.872	0.11	-0.106
LWC	0.478	0.604	-0.132	0.594
ELWL	0.94	-0.036	-0.01	-0.313
CTD	-0.819	0.083	-0.31	-0.022
SR	-0.827	-0.482	-0.223	0.119
LT	-0.215	-0.412	0.811	0.308
ETE	-0.84	-0.476	-0.192	0.091
YSI	-0.827	0.061	0.342	-0.315
Eigenvalue	6.387	2.381	1.038	0.688
Proportion (%)	58.059	21.645	9.436	6.259
Cumulative (%)	58.059	79.704	89.14	95.399

Principal component analysis (PCA)

PCA of the data in Table 2 showed that four main components together explained 95.39% of the total variation, which, in conventional analyses. PCA is a multivariate statistical method which transforms a number of possibly correlated variables into a smaller number of variables called principal components (Gabriel, 1971; Dong *et al.*, 2008). From Fig. 2, it was observed that an increase in the number of the components was associated with a decrease in eigenvalues, which is an important indicator in general genetics and very valuable for evaluating drought tolerant genotypes and also efficient indicators for screening these genotypes. The trend reached its maximum for four components. Thus, it is reasonable to assume that the PCA divided total estimated variables into four main components. Data presented in Table 2 showed that the first component (PC1) explained 58.059% of the total data variation and had a highly positive correlation with RWL and negative correlation with RWP under stress condition. Therefore the PC1 can be named as water loss dimension and it separates the drought susceptible genotypes from tolerant ones

The second component (PC2) explained 21.645% of the total variability and correlated positively with RWC and WRC. Therefore, the second component can be named as a component of plant water status with high leaf water retention in a stressful environment. In other words, this component was able to separate the genotypes with high tolerance. Thus, selection of genotypes that have low PC1 and high PC2 are suitable for rainfed condition. The third and fourth dimensions (PC3 and PC4) included LT and LWC, respectively, which accounted for 9.436 and 6.259% of the total variability in the dependent structure and they were named LT and LWC factors. Therefore PC3 and PC4 can be screening the genotypes with low evapotranspiration and high LWC under stress condition, respectively. Similar results were obtained by Naroui Rad *et al.* (2012) who stated that factor analysis had classified the eight physio-biochemical variables into three main groups which accounted for 77.093% of the total variability in the dependence structure. Also, Dong *et al.* (2008) by factor analysis in wheat explained four main components together accounted for 83.27% of total variation in some physiological variables.

Table 3. Discriminant analysis of measured traits in wheat under drought stress condition.

Variable	Wilks' Lambda	F	df1	df2	Sig.
WRC	0.203	23.539	2	12	0
RWP	0.278	15.547	2	12	0
RWL	0.405	8.815	2	12	0.004
RWC	0.429	7.996	2	12	0.006
LWC	0.978	0.138	2	12	0.873
ELWL	0.632	3.492	2	12	0.064
CTD	0.802	1.485	2	12	0.265
RS	0.567	4.583	2	12	0.033
LTS	0.598	4.035	2	12	0.046
ETE	0.601	3.983	2	12	0.047
YSI	0.266	16.53	2	12	0

Table 4. Canonical discriminant function coefficients of measured variables.

Variable	Function	
	1	2
WRC	-0.257*	-0.066
YSI	-0.215*	0.072
RWP	-0.207*	0.115
RWL	0.158*	0.032
RWC	-0.146*	-0.124
SR	-0.113*	-0.041
ELWL	0.099*	0.001
CTD	-0.065*	0.017
ETE	-0.075	0.291*
LT	-0.072	-0.283*
LWC	0.01	0.061*
Eigenvalue	59.01	4.58
Proportion (%)	92.8	7.2
Cumulative (%)	92.8	100

*: Significant at the 0.05 probability levels.

Data obtained of PCA were graphed in a biplot analysis, so that the eigenvalues of PC1 were plotted against those for PC2 for both the genotypes and for the physiological traits (Fig. 3). The biplot is a helpful tool for revealing clustering, multicollinearity, and multivariate outliers of a dataset and it can be also used to display Euclidean distances, variances and correlations of variables of large datasets (Gabriel, 1971; Kohler and Luniak, 2005). In the biplot, the length of the lines approximates the variances of the physio-biochemical traits. The longer the line, the higher is the variance. According to Fig. 3, ELWL had the highest variance among the variables in the biplot and LT the lowest. The correlation coefficient between any two traits is approximated by the cosine of the angle between vectors drawn from the origin to the trait. An angle of 0° or 180° degrees reflects a correlation of 1 or – 1, respectively, and an angle of 90° represents a correlation coefficient of 0 (Gower and Hand, 1996). The biplot indicated that the angles between RWC and WRC were acute (Fig. 3). Therefore, these traits had significant and positive correlation and could be classified in a group. YSI, RWP and CTD were placed in the same group with an acute angle between them. The biplot in Fig. 3 showed a strong and positive relationship between SR with ETE and ELWR with RWL, and a strong and negative relationship (180°) between LWC with LT and ELWL with YSI. According to PC-axis, the ranking of efficiency indices for screening drought tolerant genotypes were: RWP > YSI ≈ ETE ≈ SR ≈ CTD > WRC > RWC ≈ LT > LWC > RWL > ELWL.

The distance from a genotype to a trait name is an indication of the rank of that trait for that genotype. Genotype can be compared by determining their position relative to each other and to a trait name (Yan and Rajcan, 2002). Therefore, genotypes Rijaw (13), Kavir (12), Azar2 (14), Roshan (8) and Chamran (11) with low PC1 and high PC2 had the highest relationship with WRC, RWC, RWP and CTD traits and confirmed these genotypes are superior for stress environment. Biplot presentation depicted genotypes Zarin (4), Bahar (1) and Shiraz (2) were close to ELWL and RWL traits and away from the other drought tolerance indicators, and were identified as susceptible genotypes. According to Fig. 3, genotype Zarin (4) was selected as the most susceptible genotype with high PC1 and lowest PC2. Previous studies have demonstrated that the drought tolerant genotypes acclimated better than susceptible genotype by maintaining higher water relations, low membrane injury, pigment photo-oxidation and chlorophyll degradation by inducing well-coordinated antioxidant defense, which results high photosynthesis and YSI under stress environment (Yousfi *et al.*, 2010; Anjum *et al.*, 2011). Thus, it can be concluded that selected traits are suitable for screening drought tolerant genotypes and quantifying water stress response. As seen in the biplot, a large number of genotypes were close to WRC, RWC, RWP and CTD; it shows that these indicators are the best for evaluating tolerant rates of genotypes. Several reports demonstrated that RWC and CTD were the most often reliable index to quantify crop water stress based on plant water status (Golestani and Assad, 1999; Siddique *et al.*, 2000; Hasheminasab *et al.*, 2012a).

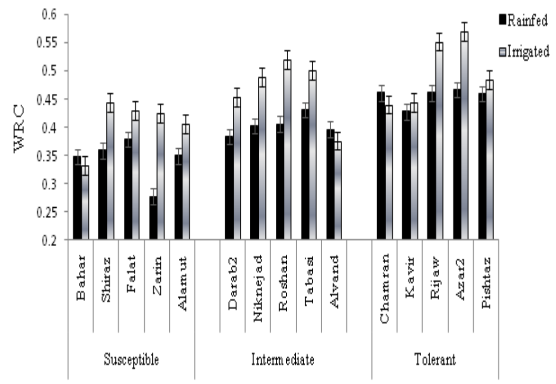

Fig. 1. Water retention capacity (WRC) in wheat genotypes grown under irrigated and rainfed conditions, vertical bars stand for the mean ± S.E. (n = 3).

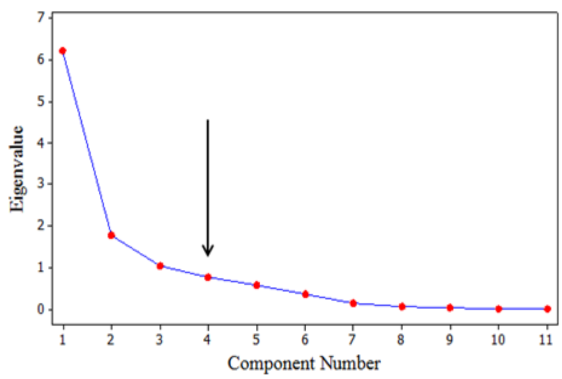

Fig. 2. Eigenvalues in response to number of components for the estimated variables of wheat genotypes.

Scatter plot

A scatter plot is a tool for analyzing relationships between two variables. One variable is plotted on the horizontal axis and the other is plotted on the vertical axis. The pattern of their intersecting points can graphically show relationship patterns. Most often a scatter plot is used to prove or disprove cause-and-effect relationships, while a scatter diagram can be used for screening datasets (Weisberg, 1985). Fig. 4 showed a scatter plot of the three genotypic groups including tolerant, intermediate and susceptible to drought based on the measured traits under drought stress condition. As seen in Fig. 4, scatter diagrams of WRC (x-axis) on the other traits (y-axis) were efficient for screening drought tolerant genotypes. These diagrams clearly revealed that the three groups can be separated from each other by this new index, but there is a large amount of overlap among these groups when used of LT, ELWL and LWC as drought tolerance indicators. The results indicated that WRC, RWP, RWC and YSI were the most reliable traits for screening all the three groups of genotypes, while LT, ELWL and LWC were the most unreliable. Renu and Devarshi (2007), Amjad *et al.* (2011) and Hasheminasab *et al.* (2012b) reported that drought stress tolerant and intermediate tolerant genotypes were superior to susceptible ones in maintaining membrane stability, leaf water content and yield stability under stress condition.

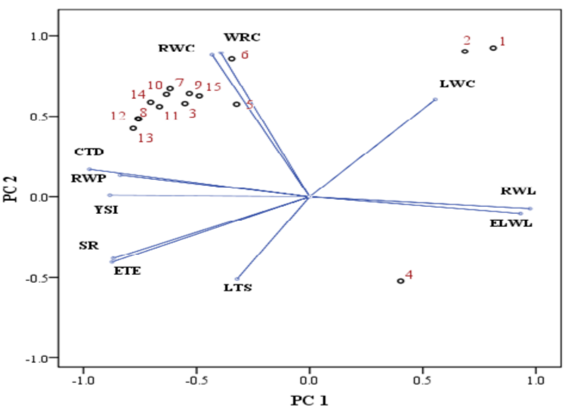

Fig. 3. Biplot analysis of the measured traits and fifteen wheat genotypes.

Discriminant analysis

Discriminate Analysis is a powerful statistical method to investigate differences between groups on the basis of the attributes of the cases, indicating which attributes contribute most to group separation (Agresti, 2002). Table 3 provides strong statistical evidence of significant differences (P < 0.01) among the three groups of genotypes for WRC, RWP, RWL, RWC and YSI with producing very high value F's. While SR, LT, and ETE were significant at the 5% probability, and also the other variables were not significant. Wilks' lambda in the Table 3 indicated the significance of the discriminant function and provided the proportion of total variability not explained (Agresti, 2002). The result showed that LWC, ELWL and CTD unexplained 97.8, 63.2% and 80.2 of variability among groups and WRC, RWP, RWL, RWC and YSI unexplained 20.3, 27.8, 40.5, 42.9 and 26.6% of variability, respectively. The descriptive technique successively identifies the linear combination of attributes known as canonical discriminant functions (equations) which contribute maximally to group separation (Agresti, 2002). The results of canonical discriminant functions analysis showed that the first two functions explain 100% (function 1 = 92.8%, function 2 = 7.2%) of the total variation (Table 4). The first function explained 92.8% of the total data variation and had the highest relationship with WRC (-0.257*), YSI (-0.215*) and RWP (-0.207*) under stress condition. Therefore, these traits were the best for classification the genotypes.

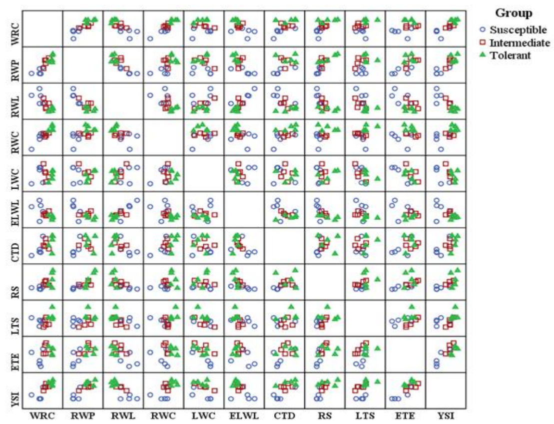

Fig. 4. Scatter plot of three genotypic groups including tolerant, intermediate and susceptible to drought based on the measured traits under drought stress condition.

Cluster analysis

The objective of cluster analysis is to assign observations to groups/clusters so that observations within each group are similar to one another with respect to variables or attributes of interest, and the groups themselves stand apart from one another. In other words, the objective is to divide the observations into homogeneous and distinct groups (Tryfos, 1997; Saed-moucheshi *et al.*, 2013). The groupings of wheat genotypes (Fig. 5) and physiological traits (Fig. 6) were shown in the tree diagram (dendrogram). Cluster analysis showed that the genotypes based on physiological traits divided into four groups with 4, 6, 3 and 2 genotypes under drought stress condition, respectively (Fig. 5). The results from the dendrogram showed that a many intermediate genotypes were located in the first group. Also all susceptible genotypes were in the second group. Drought tolerant genotypes with except Roshan were placed in the third and fourth groups. The lowest distance or similarity between genotypes was observed for genotypes Azar2 and Pishtaz. Cluster analysis indicated that these physiological traits related to water status could be useful for classification of genotypes for drought tolerance (Fig. 5). The cluster analysis of variables also separated physiological traits into five groups with 6, 1, 1, 2 and 1 variables (Fig. 6). WRC, RWC, RWP, YSI, ETE and SR were located in the similar cluster (group1), and Also RWL and ELWL were placed in the group 4. The highest similarity between studied traits was observed between WRC and RWC. Variables WRC, RWC, RWP and YSI were placed in a similar subset of group 1. In the present study, different statistical methods were identified this subset as a superior group for screening drought tolerant genotypes.

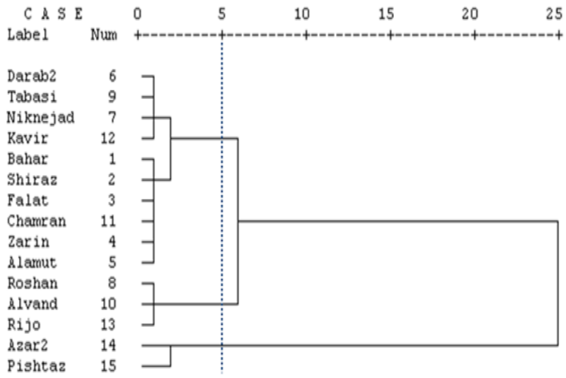

Fig. 5. Tree diagram of cluster analysis of fifteen wheat genotypes based on measured traits.

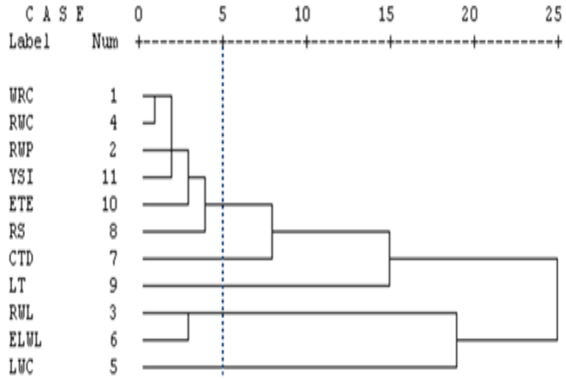

Fig. 6. Tree diagram of cluster analysis of the measured traits based on fifteen wheat genotypes.

Conclusion

The results of this study showed that genotypes respond differentially to drought stress as a result of variations in their WRC. The highest WRC were observed in tolerant genotypes Pishtaz, Azar2, Rijaw and Chamran, and the lowest in susceptible genotypes Alamut, Zarin, Flat, Shiraz and Bahar under stress condition. The intermediate ratios were observed in Tabasi, Roshan, Niknejad and Darab2 (intermediate genotypes). The results of different statistical methods used in this study showed that WRC had significant relationship with RWC. The visualizing graphic of scatter plot and biplot of principal component analysis detected WRC, RWP, RWC and YSI as the best indicators for screening drought tolerant genotypes. Discriminant and canonical discriminant functions analysis provided strong statistical evidence of significant differences among the genotypic groups for WRC, RWP, RWL, RWC and YSI with producing low Wilks' lambda. Our results suggested that WRC was reliable for classification and separation of drought tolerant genotypes.

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