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Full Length Research Paper

Physiological and biochemical relationship under drought stress in wheat (*Triticum aestivum*)

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Some statistical procedures like correlation, stepwise regression, factor analysis and cluster analysis were used to study the relationship between wheat grain yield and some physiological parameters under drought conditions. Results reveal that the ratio fv/fm of chlorophyll fluorescence is the most effective parameter to select for tolerant genotype with more grain yield in water limited condition. Based on the results, it is reasonable to assume that high chlorophyll capacity of wheat plants under drought conditions could be identified by selecting breeding materials with high chlorophyll capacity and less proline content.

Key words: Wheat grain, drought, chlorophyll, tolerant genotype.

INTRODUCTION

Plant improvement for drought resistance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions where a large amount of genotypes can be evaluated efficiently (Ramirez and Kelly, 1998). In addition, drought stress is the most widespread environmental stress that affects growth and productivity, and it induces many physiological and biochemical responses in plant. Understanding of physiological mechanisms that enable plants to adapt to water deficit and maintain growth and production during stress period could help in screening and selection of tolerant genotypes and the use of this trait in breeding programs (Zaharieva et al., 2001).

Developing high yielding wheat cultivars under water deficit conditions in arid and semi-arid regions is an important key for breeding programs and understanding the factors that limit crop yields is essential for regional yield forecasting and improvement of crop management techniques. Moreover, such factors must be known for the development of adaptation strategies to climate change, for example, effective irrigations at key crop growth stages under a warmer and dryer climate scenario. Moisture stress is recognized as the dominant limiting factor to spring wheat yields in the world (Hay and Walker, 1989; Raddatz, 1993; Campbell and Close, 1997). Accordingly, the increased attention on the production of resistant plant species for prolonged food production under different conditions indicates the necessity of performing breeding experiments (Martin et al., 2008; Van de Wouw et al., 2010). Some appropriate methods, cluster analysis, principal component analysis (PCA) and factor analysis, for genetic diversity identification, parental selection, tracing the pathway to evolution of crops, centre of origin and diversity, and study interaction between the environment are currently available (Bhatt, 1970; Carves et al., 1987; Mohammadi and Prasanna, 2003; Eivazi et al., 2007). The knowledge of genetic association between grain yield and its components under water deficit conditions would improve the efficiency of breeding programs by identifying appropriate indices for selecting wheat varieties (Evans and Fischer, 1999). Naroui Rad et al. (2006) determined the six factors which explained all the characteristic

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Table 1. Basic statistics {minimum and maximum values, arithmetic mean and standard deviation (SD)} for the estimated variables of wheat.

Trait	Minimum	Maximum	Mean	SD
Chl*a* (mg/g Fresh weight)	0.54	4.61	2.4154	.88013
Chl*b* (mg/g Fresh weight)	0.59	2.77	1.2025	0.478
Ch(*ab*) (mg/g Fresh weight)	1.13	7.15	3.6179	1.33
Plant yield (gr/plant)	11.97	20.70	14.8879	1.97
Proline content (mg/g Fresh weight)	0.12	0.63	0.2647	0.12
Chlorophyll content (SPAD)	24.93	41.03	34.8552	3.74
Stomatal conductance (mol.m-2 s-1)	100.33	484.00	290.3048	94.03
FV/FM fv/fm ratio	0.63	0.83	0.7311	0.04

variation among wheat landraces in southern east of Iran, using factor analysis. Some appropriate methods like cluster analysis, principal component analysis and factor analysis are used for genetic diversity identification, parental selection, tracing the pathway to evolution of crops, centre of origin and diversity, and study of interaction between the environment (Bhatt, 1970; Carves et al., 1987; Mohammadi and Prasanna, 2003; Eivazi et al., 2007). Factor analysis suggested by Walton (1972) has been widely used to identify growth and plant characters related to wheat (Moghaddam et al., 1998; Mohamed, 1999). It has been also used in soybeans (Leilah et al., 1988), sugar beet (Naser and Leilah, 1993) and sesame (El-Deeb and Mohamed, 1999). The degree of association between plant traits and judged by correlation coefficient and the rate of change in one trait due to changes in the other are measured by regression coefficient and this information is important in selection practice for the prediction of correlated response (Lane, 1958). The aim of this study was to evaluate the relationship among some physio-biochemical traits, to obtain information about their behaviour in drought stress condition and to get better selection for tolerant genotypes.

MATERIALS AND METHODS

This experiment was carried out in the agricultural experimental field under shelter at the University Putra Malaysia Department of Agrotechnology in 2010. Seeds were obtained from the Agriculture and Natural Resources Research Center of Sistan-Iran. All genotypes (eight parents with twenty eight crosses that were obtained among their parent) were sown in pots with a soil mixture of soil/sand/organic matter in a ratio of 1:1:1. Four seeds were sown in each pot. The pots were irrigated after 75% depletion of the soil water and genotypes were arranged as a completely randomised block design. The chlorophyll content was measured 3 times. Measurements were made on the flag leaf on two seedlings per pot, with a chlorophyll meter, (SPAD-502, Soil Plant Analysis Development (SPAD) Section, Minolta Camera Co, Osaka, Japan). Three readings were taken along the middle section of the leaf, and mean used for analysis and values were expressed as SPAD units. Chlorophyll "Chla and Chlb" were estimated by extracting the leaf material in 80% acetone. Absorbances were recorded at 645 and 665 nm, Chla and Chlb and total chlorophyll (a+b) were calculated (Arnon, 1949). Proline was quantified by using ninhydrin reagent and measured according to Bates et al. (1973).

Proline was extracted from 0.5 g of leaf in 10 ml 3% sulfosalicylic acid. Two millilitres of extract was reacted with 2 ml acid- ninhydrin and 2 ml of glacial acetic acid for 75 min at 100°C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and vortexed. The absorbance of toluene layer was spectrophotometrically determined at a wavelength of 520 nm. Concentration was determined from a standard curve and calculated on a fresh weight basis (mg /g fw^{-1}). Chlorophyll meter readings were taken from two flag leaf samples in each pot, chlorophyll fluorescence was measured using Handy Pea (Hansatech Instrument) and the ratio of **fv/fm** was calculated. Plant yield was obtained by mean of grain weight (g) in three plants. Stomatal conductance of leaves was determined using a portable porometer (Delta-T AP4, Delta-T Devices, Cambridge, UK). The measurements were taken on the surface of the leaf at the heading stage. Analysis of data was done by using SAS package (SAS Institute 1997).

RESULTS AND DISCUSSION

Table 1 shows the minimum and maximum values, arithmetic mean and standard deviation for all estimated variables of wheat. One criterion for character to be an index of drought tolerance is having positive significant correlation coefficient with grain yield under drought stress. Correlation (Table 2) between traits under study showed that there is a just positive correlation between ratio fv/fm and grain yield (r = 0.556**) in other report which shows a high degree of correlation with the grain yield (Björkman and Demmig, 1987). On the other hand, there is a high and significant correlation between chlorophyll a and chlorophyll b (r = 0.936**), total chlorophyll (a + b) (r = 0.992**) but this relation with proline was negative and significant (r = -0.340*) and these results are in accordance with other researches (Schonfeld et al., 1988; Bayoumi et al., 2008). Results of stepwise regression (Table 3) analysis of mean grain yield (dependent variable) on selected yield components (independent variable) indicated that ratio fv/fm was the

Table 2. Matrix of simple correlation coefficients

Esitmated varible	Cha	Chb	Ch(a+b)	Grain yield	Proline content	Chlorophyll content	Stomatal conductance	fv/fm
Cha	1							
Chb	0.936**	1						
Ch(a+b)	0.992**	0.973**	1					
Grain Yield	-0.133	-0.130	-0.134	1				
Proline content	-0.340*	-0.363*	-0.353*	0.050	1			
Chlorophyll content	-0.020	-0.018	-0.019	0.291	0.011	1		
Stomatal conductance	-0.040	-0.096	-0.061	-0.065	0.138	0.312	1	
fv/fm	-0.271	-0.265	-0.273	0.556**	-0.072	0.190	-0.061	1

*and** Significant at 5% and 1% levels, respectively

Table 3. Relative contribution (model R2) in predicting wheat grain yield (the stepwise procedure analysis).

Trait	Model R^2	b	SE
FV/FM	56.7	0.556	3.4

The predicted equation for grain yield ($Y^{\wedge}$) was: Y = -3.164 + 0.556x

Table 4. Rotated (varimax rotation) factor loadings and communalities for the estimated variables of wheat.

Variable	Factor 1	Factor 2	Factor 3	Communality
Chla	0.960	-0.160	0.038	0.950
Chlb	0.961	-0.141	-0.004	0.944
Ch(a+b)	0.975	-0.155	0.024	0.976
Grain Yield	-0.036	0.846	0.090	0.724
Prolin content	-0.517	-0.203	0.251	0.371
Chlorophyll content	0.063	0.405	0.737	0.710
Stomatal conductance	-0.104	-0.176	0.838	0.745
fv/fm	-0.170	0.846	-0.053	0.748
Variance	40.778	20.211	16.104	
Cumulative variance	40.778	60.989	77.093	

single most important yield component which accounted for 56.7% of the variation in grain yield. Table 4 shows that three main factors (based on Eigen values higher than one) accounted for 77.09% of the total variability in the dependent structure. The first factor (group) included chlorophyll a, chlorophyll b and proline which accounted for 40.778% of the total variability in the dependent structure. The suggested name for this factor is bio-chemical capacity. The second factor included grain yield and ratio fv/fm which accounted for 20.211% of the total variability in the dependent structure and it was named the yield factor. The third factor included chlorophyll content and the stomatal conductance which accounted for 16.104% of the total variability in the dependence structure. The suggested name for this factor is stomatal conductance. Similar results were obtained by Mohamed (1999) who stated that factor analysis had classified the ten wheat variables into two main groups which accounted for 80.79% of the total variability in the dependence structure. Also, Rajiv et al. (2010) reported similar results according to factor analysis. Naroui Rad et al. (2010) by factor analysis in wheat explained 6 factors which accounted for 74.26% of total variation in the studied variables.

Toker and Cagirgan (2004) reported three factors that explained 92.9% of the total variance seen in the characters. Factors 1, 2 and 3 explained 51.3, 24.8 and 16.8% of total variance explained. Cluster analysis can be used to identify variables which can be classified into main groups and subgroups based on similarity and

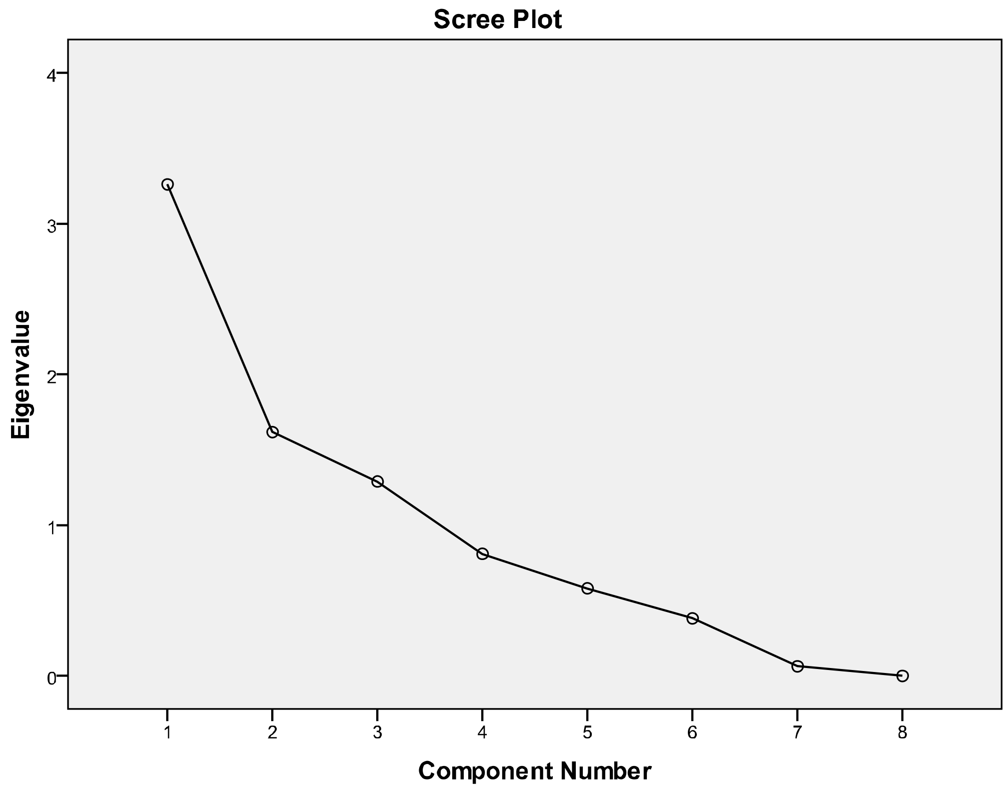

Figure 1. Eigen values in response to number of components for the estimated variables.

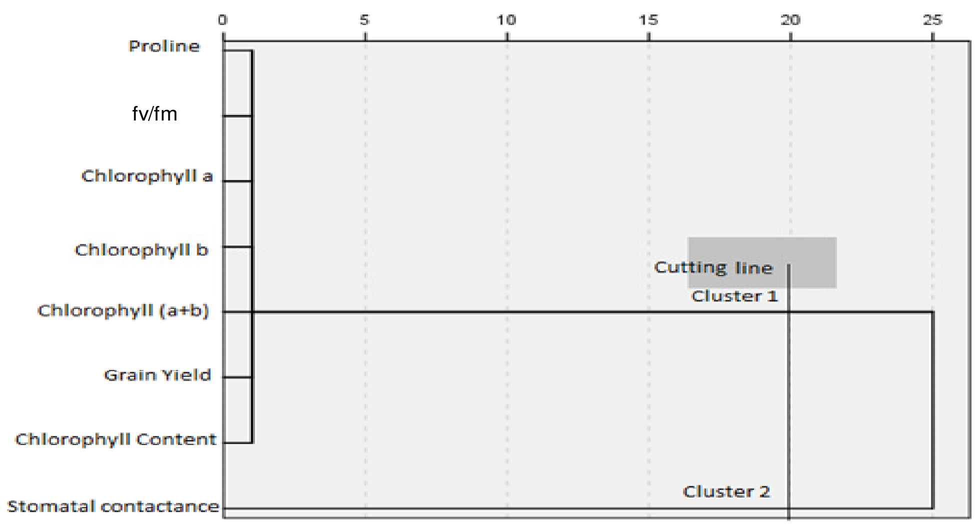

Figure 2. Dendrogram from cluster analysis of wheat genotypes under drought stress based on some physiological traits.

dissimilarity. This technique is useful for parental selection in breeding programs (El-Deeb and Mohamed, 1999) and crop modeling (Jaynes et al., 2003). Many algorithmic methods have been proposed for cluster analysis in this study. Eigen values in response to number of components for the estimated variables are shown in Figure 1. Hierarchical cluster analysis with wheat variables was used and is shown in a dendrogram (Figure 2). The results show all traits except stomatal conductance are placed in cluster one and stomatal conductance is placed separately in second cluster.

Conclusion

The multiple statistical procedures used in this study showed that chlorophyll fluorescence parameter, especially ratio fv/fm is the most important trait for tolerant genotype selection and also based on factor analysis, there is a positive relationship between chlorophyll content and stomatal conductance, and maybe high induction of proline content is the cause of more stomatal conductance.

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