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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (1): 118-122, 2008
ISSN 1028-8880

Molecular Aspects of Drought Tolerance in Bread Wheat *(T. aestivum)*

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Abstract: In order to evaluate molecular indices of drought tolerance and selection of drought tolerance genotypes in bread wheat a randomized complete block design was carried out with three replications in the field of research station of Sararood, Kermanshah, Iran. Positive significant correlation coefficient was observed between grain yield and proline, soluble sugar and total protein under rainfed condition. Based on the grain yield, proline content, soluble sugar and total protein the genotypes were classified into four clusters using cluster analysis and UPGMA method. Genotypes numbers 4, 9, 10, 11, 18 and 19 in the first clusters indicated the highest performance.

Key words: Drought tolerance, SDS-PAGE, electrophoresis, total protein, proline content, soluble sugar

INTRODUCTION

Water deficit is the most important factor in yield reduction in the semiarid regions (Kristin *et al*., 1997; Kirigwi *et al*., 2004; Farshadfar and Sutka, 2003).

Positive correlation with relative yield performance of genotypes in rainfed condition is a start point for identification of characters related to drought tolerance (Farshadfar and Sutka, 2003; Ghasempour and Kianian, 2007).

Drought tolerance is not easily quantifiable plant attribute, rather it is the outcome of interaction among complex morphological, physiological and molecular character associated with low molecular weight biochemical materials acting as osmoticum, these include proline, betaine, choline, betain aldeid, 3 etil butarat and dietil glycerin (Khazaei, 2001; Ghasempour *et al*., 2007; Ildiko and Galiba, 2000).

Under drought stress, high molecular weight (>100kd) soluble protein will decrease in wheat leaves, while low molecular weight will increase (Kochaki, 1997; Sujin and RayWu, 2004).

Drought stress decompose starch and fade it from the plant. Reduction of starch is the result of amylase activity that increase soluble sugar (Vaezi, 2005; Ghasempour *et al*., 1998). Genotypes with high soluble sugar show drought resistance.

Decreases phospholipids in the cell membrane (Zarei, 2006) suggesting biochemical attributes such as: free proline content, soluble sugar, total protein and chlorophyll stability can be used as drought tolerance indicators for selecting drought resistant genotypes (Khazaei, 2001; Sujin and RayWu, 2004).

The objectives of the present investigation were to screening molecular indicators of drought tolerance and selection of drought tolerant genotypes.

MATERIALS AND METHODS

In order to screen drought tolerant genotypes twenty fall bread wheat cultivars were incubated at 4°C for one week for vernalization and evaluated in a randomized complete block design with three replications under drought and irrigated conditions in the field. Each plot consisted of 3 rows with row to row distance of 50 cm.

In the rainfed condition besides Grain Yield (GY), from each experimental unit 5 plants were randomly selected and the following metabolic traits were measured from the flag leaves:

Soluble sugar extraction: Soluble sugar content was determined by the modified phenol sulphoric acid method (Dubois *et al*., 1956; Kennedy, 1987). Data were measured at 485 nm by Bausch and Lomb spectrophotometer 70. A standard curve; 0, 5, 10, 15, 20, 25, 30 and 40 mg of glucose were prepared. Glucose content of treated extracts was calculated using the standard curve and recorded.

Assessment of proline in leaves: Proline of leaves was determined by Bates *et al*. (1973) method. Data were measured at 520 nm by Bausch and Lomb spectrophotometer 70. A standard curve; 1.9, 7.8, 15.62, 31.25 and 125 μg of proline were prepared. Proline content of treated extracts was calculated using the standard curve and recorded.

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Measuring the total protein (T. protein): In order to quantify the total protein of leaves in each sample, a 0.05 g of dry weight of leaves was evaluated by Lowry *et al.* (1951) method. The total protein was determined with Folin reagent and the color compared with Bovine Serum Albumin (BSA), serving as the standard for determining protein content, read at A660 (OD) and recorded.

Protein SDS-PAGE and gel electrophoresis analysis: Peterson (1977) method was used to determine the protein concentration of fresh leaves of treated plants.

For one-dimensional SDS-PAGE the supernatant of samples was diluted with UKS-buffer (9.5 Urea, 5 mM K_2CO_3, 1.25% (w/v) SDS) (1:1).

For each well 20 μL was applied. A Hoeffer SE 600 vertical unit was employed and coomassie blue used for staining.

In order to analyze molecular weight and mobility of protein bands the UV. Doc program was used and molecular weight of marker bands entered to the program. The program gives the molecular weight of the bands on the stained gel.

Chlorophyll content: Using Ashraf *et al.* (1994) method spectrophotometer with 663 and 645 nm and the following formulas, chlorophyll content was determined:

Chlorophyll a (Ch.a) (mg mL^{-1}): $0.0202a_{645}+0.008a_{663}$
Chlorophyll b (Ch.b)(mg mL^{-1}): $0.0127a_{663}- 0.00269a_{645}$
Total chlorophyll (T.Ch.) (mg mL^{-1}): $0.0229a_{645}- 0.00468a_{663}$

Statistical analysis: Analysis of variance, mean comparison, correlation and cluster analysis were done with MSTAT-C and SPSS statistical softwares.

RESULTS AND DISCUSSION

The results of analysis of variance under water stress condition (Table 1) revealed high significant differences between genotypes for proline content, soluble protein, chlorophyll a, b and total chlorophyll and grain yield indicating the presence of genetic variation and possibility of selection for drought tolerant genotypes.

Mean comparison (Table 2) for total chlorophyll ranged from 7.62 mg mL^{-1} for genotype 4 to 17.78 mg mL^{-1} for genotype 8, chlorophyll a ranged from 3.22 for genotype 6 to 7.63 for genotype 8 and chlorophyll b ranged from 3.70 for cultivar 4 to 10.15 for cultivar 8. Maximum total protein (137.8) and grain yield (367.3 g) was related to genotype 20. Genotypes number 9 and 11 exhibited maximum amount of proline (93.01) and soluble sugar (44.41), respectively.

Proline content and soluble sugar were larger during the grain filling period than preanthesis, hence the best

Table 1: Analysis of variance for various characters investigated under water stress condition

		Mean squares						
SOV	df	Prolin	Sugars	T. protein	Ch.a	Ch.b	T.Ch.	GY
Replication	2	862.70**	15.00ns	165.500**	33.63**	338.25**	580.57**	334.517ns
Genotype	19	3916.54**	771.30**	20.580**	11.07**	33.75**	77.94**	937.220**
Error	118	54.72	5.66	246.378	0.53	1.58	1.32	1346.552+

ns: non significant; **: Significant at 0.01 probability level; +: Error = 38

Table 2: Mean comparisons of characters studied under water stress condition +

Genotypes	T.Ch.	Ch.b	Ch.a	GY	T. protein	Sugars	Prolin
1	10.68^{fghi}	5.21^{efg}	5.46^{bcdefg}	199.7^{c}	108.60^{abcde}	35.58^{cd}	70.33^{ef}
2	10.04^{fghi}	5.74^{efg}	4.29^{fghi}	259.7^{abc}	57.43^{hi}	34.06^{cd}	45.21^{def}
3	10.75^{fghi}	5.83^{defg}	4.92^{defgh}	238.0^{abc}	77.38^{cdefgh}	32.84^{cde}	50.08^{def}
4	7.62^{i}	3.70^{g}	3.91^{ghi}	300.0^{abc}	96.67^{bcdefg}	41.81^{ab}	78.50^{abc}
5	15.05^{abcd}	8.60^{abc}	6.44^{abcd}	246.3^{abc}	64.89^{ghi}	30.80^{def}	47.59^{def}
6	8.05^{i}	4.82^{efg}	3.22^{abcdef}	262.0^{abc}	72.22^{ghi}	25.35^{fgh}	39.51^{def}
7	7.70^{i}	3.93^{g}	3.76^{a}	234.3^{bc}	59.12^{hi}	27.62^{efg}	33.37^{ef}
8	17.78^{a}	10.15^{a}	7.63^{ab}	244.3^{abc}	73.10^{ghi}	31.46^{cde}	49.12^{def}
9	16.40^{ab}	9.60^{ab}	6.79^{ab}	342.3^{ab}	120.30^{abc}	44.35^{a}	93.01^{a}
10	10.33^{ghi}	4.74^{efg}	5.58^{bcdef}	3467.7^{ab}	106.70^{ab}	43.96^{a}	90.14^{ab}
11	15.63^{abc}	8.99^{abc}	6.63^{abc}	342.7^{ab}	119.80^{i}	44.41^{a}	92.51
12	11.89^{efgh}	5.99^{defg}	5.89^{bcdef}	243.0^{abc}	129.00^{ab}	33.13^{cde}	77.74^{abc}
13	14.15^{bcde}	7.93^{bcd}	6.21^{abcde}	222.0^{bc}	47.04^{i}	19.96^{hi}	48.09^{def}
14	12.46^{defg}	6.38^{def}	6.07^{abcde}	197.0^{c}	83.76^{defgh}	18.03^{i}	57.63^{cde}
15	12.96^{cdef}	6.97^{cde}	5.98^{bcde}	237.0^{abc}	88.81^{cdefgh}	19.84^{hi}	40.19^{def}
16	9.12^{hi}	4.25^{fg}	4.87^{defgh}	229.3^{bc}	78.17^{efghi}	21.86^{ghi}	48.44^{def}
17	10.19^{fghi}	5.06^{efg}	5.12^{cdefgh}	200.0^{c}	104.10^{abcdef}	17.01^{i}	27.88^{f}
18	9.53^{ghi}	4.55^{fg}	4.97^{defgh}	320.3^{abc}	94.15^{cdefg}	37.24^{bc}	64.03^{bcd}
19	9051.00^{ghi}	4.73^{efg}	4.77^{efgh}	343.7^{ab}	116.00^{abcd}	37.39^{bc}	77.74^{abc}
20	11.43^{efgh}	5.71^{efg}	5.70^{bcdef}	367.3^{a}	137.80^{a}	43.19^{ab}	80.43^{abc}

+: Genotypes with common letter(s) have no significant differences

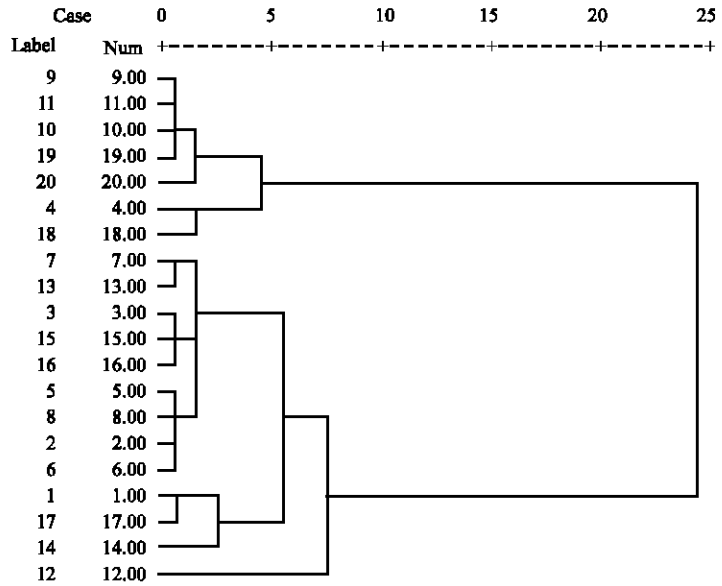

Fig. 1: Cluster analysis based on the characters with positive correlation with grain yield

Table 3: Correlation coefficients between characters studied

	Proline	Sugar	Protein	Ch.a	Ch.b	T.Ch.	GY
Proline	1.00						
Sugar	0.75**	1.00					
Protein	0.66**	0.57**	1.00				
Ch.a	0.30	0.09	0.211	1.00			
Ch.b	0.56	0.07	-0.03	0.85**	1.00		
T.Ch.	0.24	0.08	0.08	0.94**	0.98**	1.00	
GY	0.83**	0.82**	0.82**	0.049	0.041	0.046	1.00

*, **: Significant at 5 and 1% level of probability, respectively

stage for selection of drought tolerant genotypes will be postanthesis and grain filling period (Hien *et al*., 2003).

With regard to this fact cultivars number 9, 10, 11, 12 and 20 have the highest amount of proline content, soluble sugar and total protein.

As chlorophyll has an important role in photosynthesis rate, therefore, genotypes with high amount of photosynthesis in grain filling period will have higher grain yield and resistant to water stress. With this regard and what mentioned before, genotype number 11 will be outstanding regarding grain yield and all metabolite and physiological traits.

Correlation analysis: One criterion for a character to be an index of drought tolerance is having positive significant correlation coefficient with grain yield under water stress (Sujin and Ray Wu, 2004), as proline, soluble sugar and protein showed high significant correlation coefficients with grain yield under drought condition (Table 3), therefore they can be considered as drought tolerance indicators (Ghasempour *et al*., 2001).

Soluble sugar revealed significant positive correlation coefficient with proline (r = 0.750**) and protein (r = 0.66**). Which is in accordance with Siegien and Leszczynska (2004) and Zarei (2006). Proline and soluble protein also exhibit positive high significant correlation coefficient (r = 0.663**).

It can be concluded that effect of water stress on genotypes with higher soluble protein causes protein degradation and release more free proline (Vaezi, 2005). Chlorophyll a and b and total chlorophyll did not show any significant correlation with grain yield and other characters, hence according to the results of this investigation they can not be considered as drought tolerance indicators.

Based on the characters having significant variation and significant positive correlation with grain yield (proline, soluble sugar and protein) cluster analysis using UPMGA showed four clusters so that high yielding and drought tolerant genotypes 4, 9, 10, 11, 18, 19, 20 with regard to biochemical traits were grouped in cluster one (Fig. 1). Genotypes number 1, 14, 17 and 12 were grouped in clusters 3 and 4. As genotype 1, 12, 14 and 17 (cluster 3 and 4) and genotypes 4, 9, 10, 11, 18, 19 and 20 (cluster one) have the most genetic distance, hence their crossing will contribute to heterosis and transgressive segregating in the segregation populations (Altinkut *et al*., 2001; Vaezi, 2005).

Evaluation of width and number of protein bands in SDS-PAGE indicates that after stress 12 protein bands appeared.

With regard to this fact that each protein band is an indicator of one character, therefore different between protein bands exhibited genetic variation between wheat genotypes.

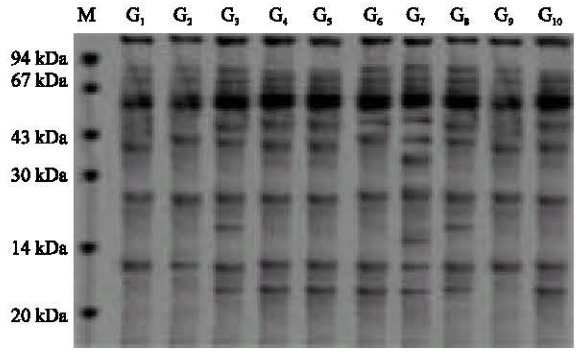

Fig. 2: Protein profile (1-10) genotypes (before stress)

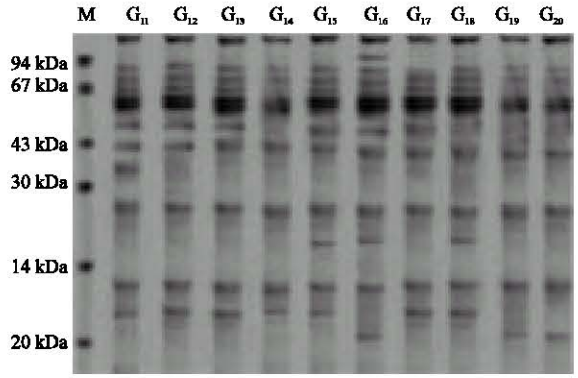

Fig. 3: Protein profile (11-20) genotypes (before stress)

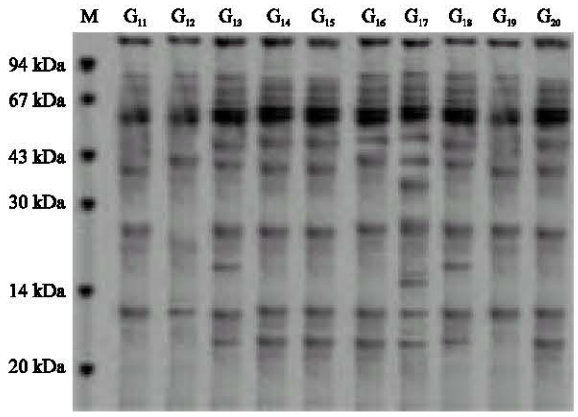

Fig. 4: Protein profile (1-10) genotypes (next stress)

Figure 2-5 also revealed that in some genotypes some new bands appeared and some disappeared. For example in genotype 1 the first band at the end appeared after water stress, while in genotype 2 the first band at the end disappeared. In genotype 3 a new band appeared, in genotype 7 third band disappeared, in genotype 19 and 1 appeared. In genotypes No. 1, 2, 3 an 4 the intensity of

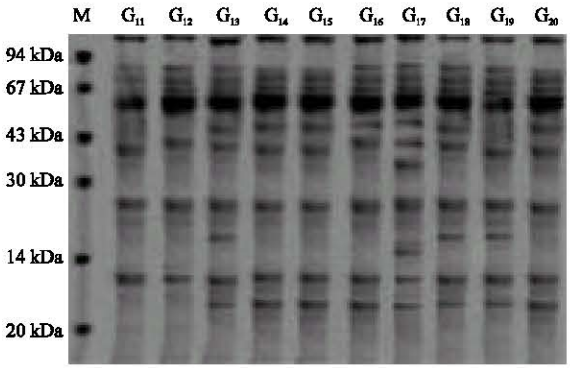

Fig. 5: Protein profile (11-20) genotypes (next stress)

band No. 2 and in genotypes 13, 14, 15 and 16 the intensity of band No. 3 increased while in genotype 12 the intensity of band No. 3 decreased. From the above mentioned results it is concluded that the increase of the intensity of bands is the results of the increase of proteins with low molecular weight (Zimmermann, 1998) reported that water stress in wheat leaves causes soluble proteins with low molecular weight increase while, soluble proteins with high molecular weight decrease (Vaezi, 2005; Ghasempour *et al*., 2007).

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