Zagazig J. Agric. Res., Vol. 35 No.(3) 2008 517-529

BIOCHEMICAL GENETIC MARKERS ASSOCIATED WITH DROUGHT AND SALINITY STRESSES IN WHEAT

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Accepted 7/4/2008

ABSTRACT: This study was aimed to find out molecular genetic markers associated with drought and salt tolerance in Egyptian wheat. Three varieties, Sids 1 (drought tolerant), Gemmiza 7 (salt tolerant) and Sakha 69 (drought and salt sensitive), and their corresponding F1's and F2 grains were grown under either drought or salt stresses. F2 seedlings were classified in groups according to their performance under either stresses. Samples of the parents, F1's and the two extreme groups of F2 individuals (most drought or salt tolerant and most drought or salt sensitive), as bulked F2 segregants, were taken and subjected to molecular SDS-protein analysis. The drought tolerant parent and the most tolerant F2 segregants exhibited approximately the same numbers of bands, having a common band of 46 KD which may be considered as a molecular marker associated with drought stress.

On the other hand, the salt tolerant parent and the most salt tolerant F2 segregants exhibited higher number of bands compared with their corresponding salt sensitives. Two common bands, having MW of 42 and 44 KD, were detected among the tolerant parent and its tolerant F2 progeny, suggesting that either one or both bands could be considered as molecular markers associated with salt tolerance.

The SDS-PAGE protein banding patterns were used to construct similarity matrix and to generate dendrograms to determine the genetic relationships between wheat genotypes under drought or salt stress conditions.

Key words: Drought, salinity, tolerance, sensitive, molecular markers, SDS-PAGE, wheat.

INTRODUTION

Bread wheat (*Triticum aestivum, L.*) is one of the most important cereal crop grown in the world based on area under cultivation and total production. In Egypt, there is a wide gap between crop production and wheat consumption. The Abiotic stresses are major causes of limited production. Water and nutrient stresses are responsible for about 50% reduction of the potential yield of crops. However, drought and salt stresses are the most important factors in selecting new genotypes.

One of the strategies available for crops with drought or salinity stresses is to select tolerant genotypes to such stresses (Bohnert *et al.*, 1995; Bohnert and Jensen, 1996).

Molecular markers developed by protein analysis, isozymes, random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) have shown excellent potential to select quantitative trait loci associated with such stresses (Stuber, 1992). In this connection, Bahieldin *et al.* (1994) detected RAPD-PCR markers for salt tolerance in wheat, and Abdel -Tawab *et al.* (2003) developed markers, using SSR-PCR technique, associated with drought tolerance in Egyptian wheat.

Molecular markers offer specific advantages to asses genetic diversity and studying phylogenic relationships and consequently in trait-specific crop improvement (Demek *et al.*, 1997).

The objective of this investigation was to develop some molecular markers, using SDS-protein bands, associated with drought or salt tolerance in wheat employing bulked segregant analysis technieque. The relationships between genotypes was also studied.

MATERIALS AND METHODS

This study was carried out in the Farm and Molecular Genetics Laboratory, Genetics Department, Faculty of Agriculture, Zagazig University, during the period from 2003 to 2006.

Three Egyptian wheat varieties namely, Sids1 (drought tolerant), Gemmiza7 (salt tolerant) and Sakha 69 (drought and salt sensitive) were chosen after screening for both drought or salt tolerance of ten varieties. The three

parents were grown in the field and crossed to obtain F1 grains of two crosses using the sensitive parent as male one, some of F_1 grains were sown in the next season to obtain F_2 grains.

The parental grains, their F1 and F2 grains were sown on spongy sheets in dishs and two completely randomized experiments, one for drought stress and the other for salt stress besides control one with three replications for each were applied.

Each replicate comprised ten grains of each parent or F1 and sixty grains of F2. The grains on sheets were irrigated with treated solutions as well as with tap water for control.

Salinity treatment was conducted using 8000 ppm of NaCl, while in drought treatment, mannitol was used to induce drought stress at 7.5 bars.

The treated seedlings, 21 day old of F_2 were classified into groups according to their behaviour under drought or salt stresses. Samples of the parents, their F1's and the two extreme groups of F2 individuals (most drought or salt tolerant and most drought or salt sensitive) were taken for SDS protein analysis.

Protein analysis was performed using sodium dodecyle sulphate polyacrlamide gel electrophoresis (SDS- PAGE), according to Laemmli (1970) and modified by Studier (1973).

The presence or absence of protein bands were recorded for the studied genotypes under both stresses and converted into Euclidean similarity distance aranged in a genetic matrix (Johnson and Wichern, 1988) A cluster diagram was also constructed based on these similarities. The similarity matrix and dendrogram analyses were computed using SPSS (1995) procedure.

RESULTS AND DISCUSSION

Data in Figures 1 and 2 and Tables 1 and 2 illustrate SDS-PAGE protein profiles of the studied wheat parents, their F1 and F2 under drought and salinity stresses respectively.

The electrophoretic profiles of drought treatment revealed that the total number of protein bands was twenty eight, destributed widely among wheat entries, and having arange of molecular weights of 44 to 62 KD. The drought sensitive parent Sakha 69 exhibited higher

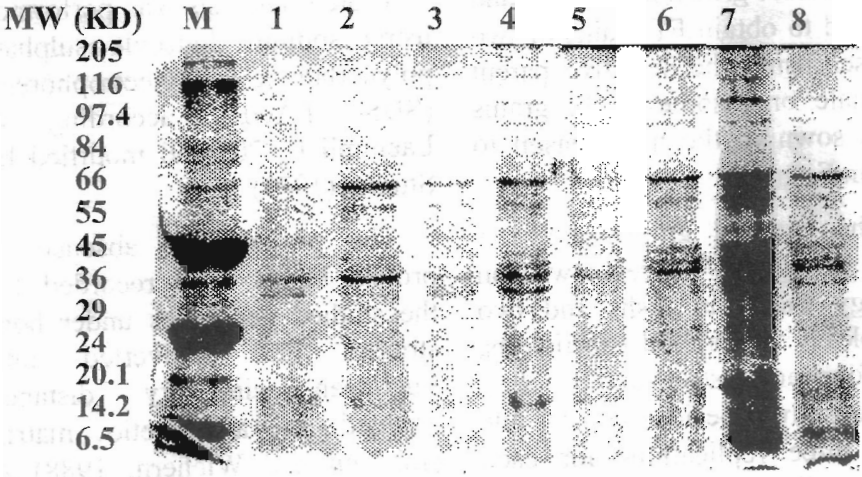

Figure 1. SDS. PAGE profiles of wheat genotypes under drought stress condition

1. Sids 1 control
2. Sids 1 treatment
3. Sakha 69 control
4. Sakha 69 treatment
5. F1 control
6. F1 treatment
7. Bulk F2 sensitive
8. Bulk F2 tolerant

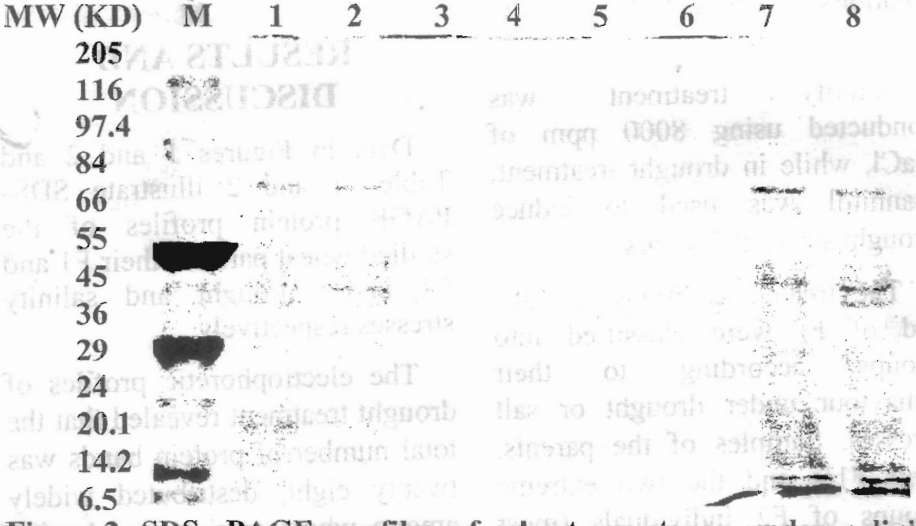

Figure 2. SDS. PAGE profiles of wheat genotypes under salinity stress condition.

1. Gemmiza 7 control
2. Gemmiza 7 treatment
3. Sakha 69 control
4. Sakha 69 treatment
5. F1 control
6. F1 treatment
7. Bulk F2 sensitive
8. Bulk F2 tolerant

Table 1. SDS-PAGE protein bands of wheat genotypes under drought stress conditions

No. of bands	RF	MW (KD)	Sids 1		Sakha 69		F1		F2 bulk	
			con.	treat.	con.	treat.	con.	treat.	sensitive	tolerant
1	0.06	62	-	-	+	-	-	-	+	-
2	0.07	62	-	-	-	+	-	-	-	-
3	0.13	59	-	+	-	-	-	+	+	-
4	0.14	59	-	-	-	+	-	-	-	-
5	0.17	58	-	-	-	-	-	+	-	-
6	0.18	58	-	-	-	-	-	-	+	-
7	0.19	57	-	-	-	+	-	-	-	-
8	0.30	53	-	-	-	-	-	-	+	+
9	0.31	53	-	-	-	-	-	+	-	-
10	0.32	53	-	-	+	+	-	-	-	-
11	0.33	52	+	+	-	-	+	-	-	-
12	0.34	52	-	-	-	+	-	-	+	-
13	0.35	52	-	-	-	-	-	+	-	+
14	0.36	52	-	-	-	+	-	-	-	-
15	0.37	51	-	+	-	-	-	-	+	-
16	0.46	48	-	-	-	-	-	-	+	-
17	0.48	48	-	-	-	-	-	+	-	-
18	0.49	48	-	-	-	+	-	+	-	-
19	0.50	47	-	+	-	-	-	-	+	-
20	0.51	47	-	-	-	-	-	-	-	+
21	0.52	47	-	-	-	-	-	+	-	-
22	0.53	46	-	-	-	-	+	-	-	-
23	0.54	46	-	-	-	+	-	-	+	-
24	0.55	46	+	+	-	-	-	-	-	+
25	0.56	46	-	-	-	-	+	-	-	-
26	0.57	45	-	-	-	+	-	-	-	-
27	0.58	45	-	-	+	-	-	-	-	-
28	0.61	44	+	-	-	-	-	-	-	-
	Total bands		3	5	3	9	3	7	9	4

+ = band present, - = band absent

Table 2. SDS-PAGE protein bands of wheat genotypes under salt conditions

No. of bands	RF	MW (KD)	Gemmiza 7		Sakha 69		F1		F2 bulk	
			con.	treat.	con.	treat.	con.	treat.	sensitive	tolerant
1	0.13	50	+	-	-	-	-	-	-	-
2	0.16	49	+	-	-	-	-	-	-	-
3	0.17	49	-	+	-	-	-	-	-	-
4	0.18	48	-	-	+	-	-	-	-	+
5	0.19	48	-	-	-	+	-	-	-	-
6	0.30	45	+	+	+	-	-	+	-	-
7	0.31	45	-	-	-	+	-	-	-	-
8	0.32	45	-	-	-	-	+	+	-	+
9	0.34	44	+	-	-	-	-	-	+	-
10	0.35	44	-	+	-	-	-	-	-	+
11	0.43	42	+	+	-	-	-	-	-	+
12	0.46	41	-	-	-	-	-	-	+	-
13	0.48	41	-	-	-	-	+	-	-	+
14	0.50	41	+	+	-	-	-	+	+	-
15	0.52	40	-	-	-	+	-	+	-	+
16	0.53	40	-	-	-	-	+	-	-	-
17	0.54	40	-	-	-	-	-	+	-	-
18	0.55	39	-	-	+	-	-	-	-	+
19	0.56	39	+	-	-	-	+	-	-	-
20	0.82	34	+	+	-	+	-	-	-	-
21	0.83	34	-	-	-	-	-	-	+	-
22	0.84	34	-	-	+	-	-	-	-	-
	Total bands		8	6	4	4	4	5	4	8

+ = band present, - = band absent

number of bands (9 bands) under drought treatment. This behaviour was also observed in its F1 progeny and sensitive bulk F2 segregants (7 and 9 bands, respectively). These results could be explained that the high bands number might be drought inducible which may be involved in plant adaptation for growth under drought stress. While, the drought tolerant parent Sids1 revealed a total of three and five bands in both control and drought treatment, respectively. The most tolerant F2 individuals showed approximately the same number of bands (4 bands), (Table 1).

It is interesting to note that the tolerant parent Sids 1 showed two bands having the molecular weights of 46 and 52 KD under both control and drought stress, and the first one of 46 KD was also observed among the most tolerant F2 individuals. This conclusion might suggest that this band of 46 KD could be considered as a molecular marker associated with drought tolerance.

In this regard, Kicheva *et al.* (1993) reported that SDS-PAGE analysis showed qualitative differences in *de novo* protein bands between drought tolerant and sensitive wheat varieties. They found three bands with MW of 58, 51 and 47KD in the tolerant variety under drought stress and unstress conditions and suggested that these bands might form a part of protective system of this tolerant one. However, Lopez *et al.* (2001) mentioned that the accumulation of dehydrin, a protein of 24 KD, in wheat varieties was associated with drought. Tolerance theysuggested that the expression of this 24-KD dehydrin might serve as a rapid and non-destructive screening technique for drought tolerance or seedling stage. Also, Abdel-Tawab *et al.* (2003) found four molecular markers associated with drought tolerance among eight Egyptian wheat cultivars using bulked segregants.

Concerning salt stress, SDS – PAGE protein profiles exhibited a total number of twenty two bands, destributed among wheat entries under saline treatment, having the molecular weights rangeding from 34 to 50 KD (Table 2). The salt tolerant wheat parent Gemmiza 7 exhibited higher number of bands under control or salt stress conditions showing eight and six bands respectively. This trend was also observed among most salt tolerant F2 segregants showing eight bands (Table 2). The higher

number of bands detected among salt tolerant wheat parent might be salt inducible and were involved in plant adaptation for growth under salt stress. These results agreed with those reported by Alamgiv (1995) who found (7-9) bands among wheat and rice cultivars under salt stress. However, the salt sensitive wheat parent Sakha 69 appeared by the presence or absence of some bands under both control and salt treatment, showing only four bands. The same number of bands was also detected in F1 and among most sensitive F2 individuals. These results indicated that salt stress might suppress gene (s) action, reflecting the absence of these bands.

Interestingly, the salt tolerant parent Gemmiza 7 exhibited four bands having the molecular weights of 34 , 41 and 42 and 45 KD under both salt stress and unstressed conditions, besides a bands with MW of 44 and 49 kd was observed only under salt stress. The two bands of molecular weights of 42 and 44 KD also appeard among most salt tolerant F2 individuals. This might suggest that either one or both bands could be served as molecular markers associated with salt tolerance.

In this connection, serveral investigators studied the molecular genetic characterization of cereals under salt stress conditions among them, Abdel-Tawab *et al.*, (2002 and 2003), Rashed *et al.* (2004 and 2006), and Abdel Bary *et al.* (2005).

The SDS-PAGE protein profiles of the studied wheat genotypes under drought and salt stress conditions were subjected to further analysis to construct a similarity matrix based on Euclidean distance (Tables 3 and 4). Also, two dendrograms were generated to determine the genetic relationships between the studied wheat genotypes under drought and salt conditions (Figures 3 and 4).

It is clear that the largest similarity was detected between the drought sensitive variety Sakha 69 and most sensitive F_2 individuals, and both entries were widely divergent than the other studied genotypes (Table 3 and Figure 3). The tolerant parent Sids1 under drought and unstressed condition was grouped in one cluster. The same trend was observed between the drought sensitive parent Sakha 69 and its F_1 under treated condition, forming one cluster. Both two clusters showed wide distance than the others.

Table 3. Similarity matrix of wheat genotypes under drought stress condition based on protein bands using Euclidean distance

Genotypes	Sids 1 control	Sids 1 treatment	Sakha 69 control	Sakha 69 treatment	F1 control	F1 treatment	Bulk F2 sensitive	Bulk F2 tolerant
1	0.000							
2	2.449							
3	2.828	2.828						
4	3.742	3.742	3.162					
5	2.449	2.449	2.449	3.464				
6	3.464	3.162	3.162	3.742	3.162			
7	3.742	2.828	3.464	4.234	3.742	4.000		
8	2.828	3.162	3.162	4.000	3.162	3.464	3.742	0.000

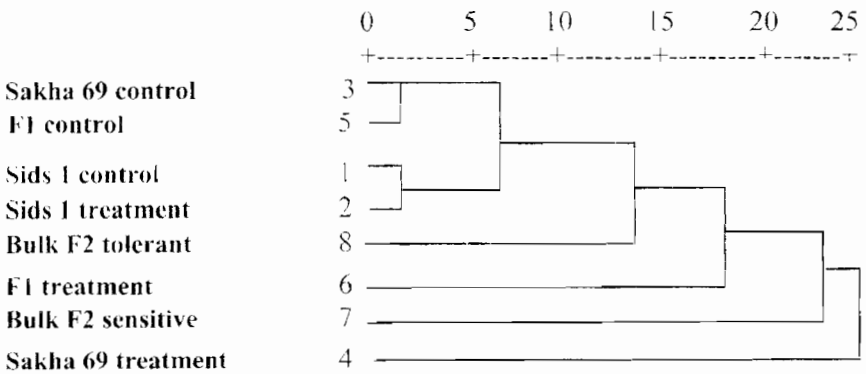

Figure 3. Dendrogram presentation of wheat genotypes under drought stress conditions based on protein bands

Table 4. Similarity matrix of wheat genotypes under salt stress condition based on protein bands using Euclidean distance

Genotypes	Gemmiza7 control	Gemmiza7 treatment	Sakha 69 control	Sakha 69 treatment	F_1 control	F_1 treatment	Bulk F_2 sensitive	Bulk F_2 tolerant
1	0.00							
2	2.83							
3	3.32	3.00						
4	3.16	2.83	3.00					
5	3.16	3.16	3.00	2.83				
6	2.83	2.45	2.65	2.83	2.45			
7	3.16	2.83	3.32	3.16	2.83	2.45		
8	3.61	3.32	2.45	3.32	2.65	3.00	3.32	0.00

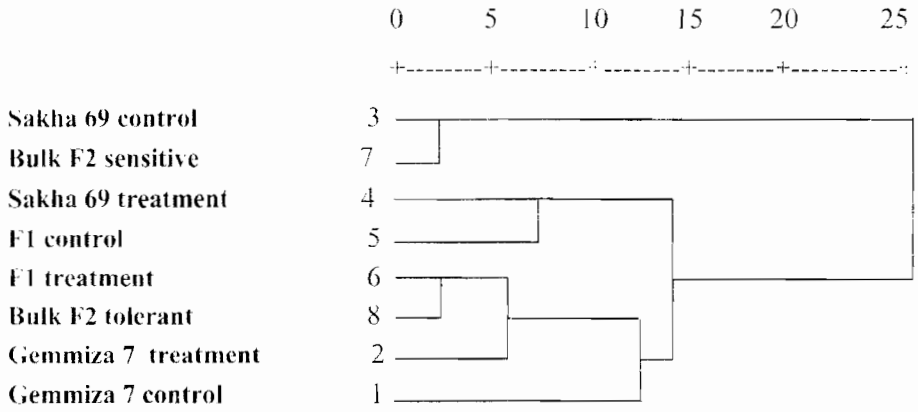

Figure 4. Dendragram presentation of wheat genotypes under salinity stress conditions based on protein bands

However, data in Table 4 and Figure 4 revealed that the salt tolerant parent Gemmiza 7, either under stress or unstress conditions, it's treated F_1 and most tolerant F_2 individuals exhibited a sort of similarity and grouped together in one cluster as shown in the dendrogram presentation. Likewise, the salt sensitive parent Sakha 69 and its most sensitive F_2 individuals were grouped together in another cluster. The two clusters were widely divergent. Generally, the two dendrograms of the wheat genotypes under drought or salt stresses showed minor differences in their clustering pattern, based on their relative similaries. In this regard, minor differences in clustering banding patterns generated by molecular markers were also reported on rice (Virk *et al.*, 2000) and on barley (Saker *et al.*, 2005).

Finally, these results might indicate that SDS-PAGE protein bands can be applied to differentiate between closely related genotypes under stress conditions.

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الأدلة البيوكيماوية الوراثية المرتبطة بإجهاد الجفاف والملوحة فى القمح

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هذه الدراسة تهدف إلى الحصول على بعض الواسمات الجزينية المرتبطة بتحمل الجفاف والملوحة فى القمح المصرى. وذلك باستخدام حبوب ثلاثة أصناف هى سدس١ (متحمل للجفاف)، جميزة٧ (متحمل للملوحة)، سخا ٦٩ (حساس للجفاف والملوحة)، والجيل الأول والجيل الثانى الناتج منها حيث زرعت تحت إجهادات الجفاف أوالملوحة. وقد قسمت بادرات الجيل الثانى إلى مجموعات تبعا لسلوكها تحت ظروف الإجهاد البيني وقد أخذت عينات من الآباء والجيل الأول وأكثر مجموعات الجيل الثانى المتحملة للجفاف أو الملوحة وأكثر مجموعات الجيل الثانى الحساسة للجفاف أو الملوحة بطريقة الإنعزالات المتفارقة وحللت بواسطة التفريد الكهربى فى وجود دوديسيل سلفات الصوديوم SDS-PAGE. وقد أظهر الأب المتحمل للجفاف ومعظم انعزالات الجيل الثانى تحملا للجفاف تقريبا نفس العدد من الحزم كما أنها تحتوى على حزمة مشتركة وهى ٤٦ كيلو دالتون والتى يمكن اعتبارها كواسم جزيئى مرتبط بتحمل الجفاف.

وعلى الجانب الآخر فقد أظهر الأب المتحمل للملوحة وكذلك معظم انعزالات الجيل الثانى المتحملة للملوحة عددا أعلى من الحزم بالمقارنة بمثيلاتها الحساسة للملوحة. كما تم الحصول على حزمتين ذات وزن جزيئى ٤٢ . ٤٤ كيلو دالتون مما يقترح معه أنه يمكن استخدام أى أو كل من الحزمتين كواسمات جزيئية لتحمل الملوحة.