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Molecular markers for drought tolerance in bread wheat

Tharwat El Ameen

Department of Genetics, South Valley University, Qena, 83523, Egypt.

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Random amplified polymorphic DNA (RAPD) primers associated with drought tolerance was used in this study to characterize drought tolerance in six wheat genotypes with developed marker assisted drought tolerance. Four of them were tolerant and two were drought sensitive genotypes. The results indicate that tolerant genotypes harbored seven positive RAPD markers, while sensitive genotypes had only one negative RAPD markers. In tolerant genotypes, seven positive PCR-RAPD markers with molecular sizes of 1050, 390, 200, 230, 850, 430 and 800 bp was exhibited by A-12, B-05, C-12, E-10 and B-02 primers. This study indicates that the seven positive markers can be used as indicators to discard drought tolerance in wheat marker -assisted breeding programmes.

Key words: Drought tolerance, PCR analysis, RAPD primer, wheat genotypes.

INTRODUCTION

Drought is the stress that has adverse effects on the growth of plants and crop yield. Physiological response to this stress arises from the changes in cellular gene expression profile, and a number of genes induced by exposure to such conditions (Shino and Yamaguchi, 2000). The constraints with the conventional breeding approaches are complexity of drought traits (Zhang, 2004) with low genetic variance of yield components under stress conditions, which make it very difficult for proper screening procedure (Alan, 2007).

Hence breeders are extremely interested in new technologies that could make this procedure more efficient. Traditional, the varietal selection is based on morphological feature hence, polygenic characters were very difficult to analyze, thus, such constraints can be overcome by using molecular marker assisted selection for trait of interest. As markers are currently available for relatively few traits, we believe that MAS must be integrated with ongoing conventional breeding to maximize its impact. When used in tandem with phenotypic selection, MAS will improve response to selection for certain trait, thereby increasing rate of genetic progress (William et al., 2007). Techniques which are particularly promising in assisting selection for desirable characters involve the use of molecular markers such as random amplified polymorphic DNA (RAPD) (Yang and Dong, 2003).

Most of the genetic diversity studied in wheat was concerned with the characteristics. Nowadays, PCR based molecular markers are used to analyze genetic relationships and genetic diversity using random amplified polymorphic DNA (RAPD) (Williams et al., 1990).

However, limited success has been achieved due to inadequate screening techniques and lack of genotypes that show clear differences in response to various environmental stresses (Bruckner and Frohberg, 1987). Stress tolerant genotypes of major food crops could be developed through breeding for wide or specific adaptation (Fisher et al., 1989), as well as, through the incorporation of certain morphological and/or physiological traits that confer tolerance under stress situation (Blum, 1979). Thus, the timely expression of stress responsive genes is crucial for the plants ability to survive under different environmental stress conditions (Chinnusamy et al., 2007). Many advances molecular mechanisms of antidrought and corresponding molecular breeding have taken place (Ramino et al., 2006; Wei et al., 2009; Ashraf, 2010). Randomly amplified polymorphic DNA primers (RAPD) associated with drought tolerance was used initially to search genetic diversity in wheat plants. It was found out that primer P_6 [TGGGGGGTTC] produced respectively a

E-mail: Thrawat_Ameen@yahoo.com.

Table 1. Wheat genotypes and their drought stress tolerant status.

Genotype	Origin	Drought susceptibility index	Reaction	Drought yield
No.1	Long spike -35 x Sakha-69	0.90	Tolerant	0.87
No.2	Long spike-35x Sakhra-69(F_6)	0.89	Tolerant	1.12
No.3	Long spike-35x Sakhra-69(F_6)	0.95	Tolerant	0.98
No.4	Long spike-35x Sakhra-69(F_6)	0.64	Tolerant	0.96
No.5	Long spike-35x Sakhra-69(F_6)	1.14	Sensitive	0.65
Giza-168	MRL/AUC/SERI	1.21	Sensitive	0.46

Table 2. Primer nucleotide sequence used to amplify DNA.

Primer designation	Sequence 5 → 3
A - 12	TCGGCGATAG
B - 05	TGCGCCCTTC
C - 12	TGTCATCCCC
A - 02	TGCCGAGCTG
E - 10	CACCAGGTGA
B - 02	TGATCCCTGG

920-bp band present mainly in drought tolerant and semi tolerant and absent in sensitive genotypes. P_7 primer [CTGCATCGTG] produced a 750- bp band that is not absolutely universal for all genotypes (Irada and Samira, 2010).

In the present research, RAPD molecular markers associated with drought tolerance in six bread wheat genotypes were analyzed.

MATERIALS AND METHODS

Plant materials

Six wheat genotypes were used in this study. These included one recommended cultivar (Giza-168), as well as, five advanced lines in the F_6 generation selected from the cross (long spike -35x Sakha-69).

They evaluated phenotypically for drought stress tolerance and were planted under two sowing dates [normal (25^{th} November) and late sowing date (10^{th} January)] over two seasons (2010 and 2011) to expose genotypes to different levels of drought stress. Four genotypes of which are known as drought stress tolerant, namely No. 1, No.2, No.3 and No.4, and genotype No. 5 and Giza-168 known as non drought tolerant were used. Relative drought sensitivity of these genotypes were determined over two years in two sowing dates (favorable and drought stress) based on grain yield through drought susceptibility index. Molecular marker assay (RAPD) was employed to determine the genetic diversity of six wheat genotypes and to determine drought tolerant genotypes associated with DNA marker. The six wheat genotypes were classified as drought tolerant or sensitive on the basis of field performance (Table 1) using drought susceptibility index as outlined by Fisher and Maurer (1978).

DNA extraction

For DNA extraction from leaves using the organs DNeasy, (Qiagen santa clara, CA) in the growth room, five to seven cut long piece of fresh leaf material was cut from the plants and the leaf tissues were ground. Total genomic DNA was isolated using protocol for plants (Murray and Thompson, 1980; Saghai et al., 1984; Kumar et al., 2003).

Estimation of DNA concentration

DNA concentration was determined by diluting the DNA in dH2O. The DNA samples were electrophoresed in 1% agarose gel against 10 ug of a DNA size marker. This marker covers a range of concentration between 95 and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker William and Betty (1985).

RAPD reactions

PCR reactions were performed according to Williams et al. (1990) method using six primers RAPD (Table 2).

RESULTS AND DISCUSSION

RAPD markers for drought tolerance

DNA isolated from the six wheat genotypes, which comprised four drought tolerant genotypes no.1 to 4 and two sensitive genotypes (no. 5 and Giza -168) is shown in Figures 1 and 2. They were tested against six pre-selected primers as shown in Table 2. Three primers only gave high polymorphism with the studied six genotypes (Table 4); five primers exhibited molecular markers for drought tolerance as summarized in Table 3. A-12, B-05, C-12, E-10 and B-02 primers exhibited seven positive molecular markers with molecular size of 1050 bp for A-12 primer, 390 bp for B-05 primer, 200 and 230 bp for C-12 primer, 850 bp for E-10 primer and 430 and 800 bp for B-02 primer, which were found only in tolerant genotypes and absent in sensitive ones. B-02 primer exhibited one negative molecular marker with molecular size of 300 bp for B-02 primer, which was found only in sensitive genotypes and absent in the tolerant ones as shown in Figures 1 and 2. These positive and one negative RAPD markers could be considered as reliable markers for drought tolerant in wheat. These results agree with those of many reports that detected RAPD markers for abiotic stresses tolerance. Abdel –Tawab et al. (2003) detected five positive and negative RAPD markers for drought tole-

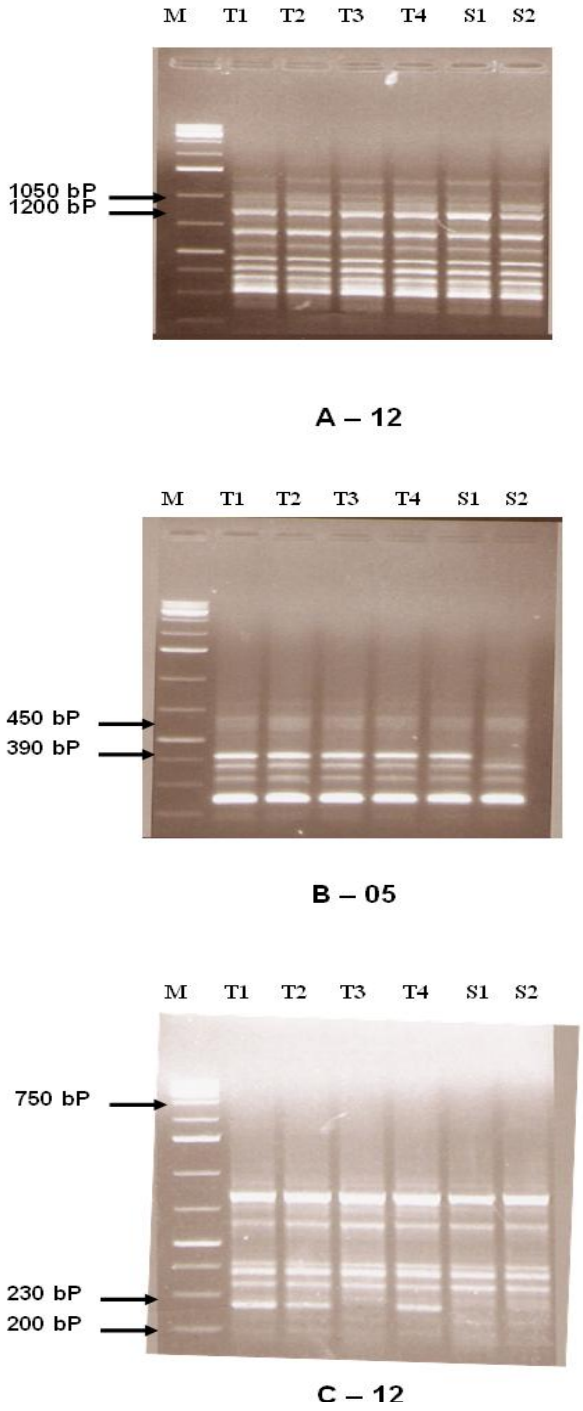

Figure 1. RAPD-PCR fragments of three primers (A-12, B-05 and C-12) for tolerant and sensitive genotypes.

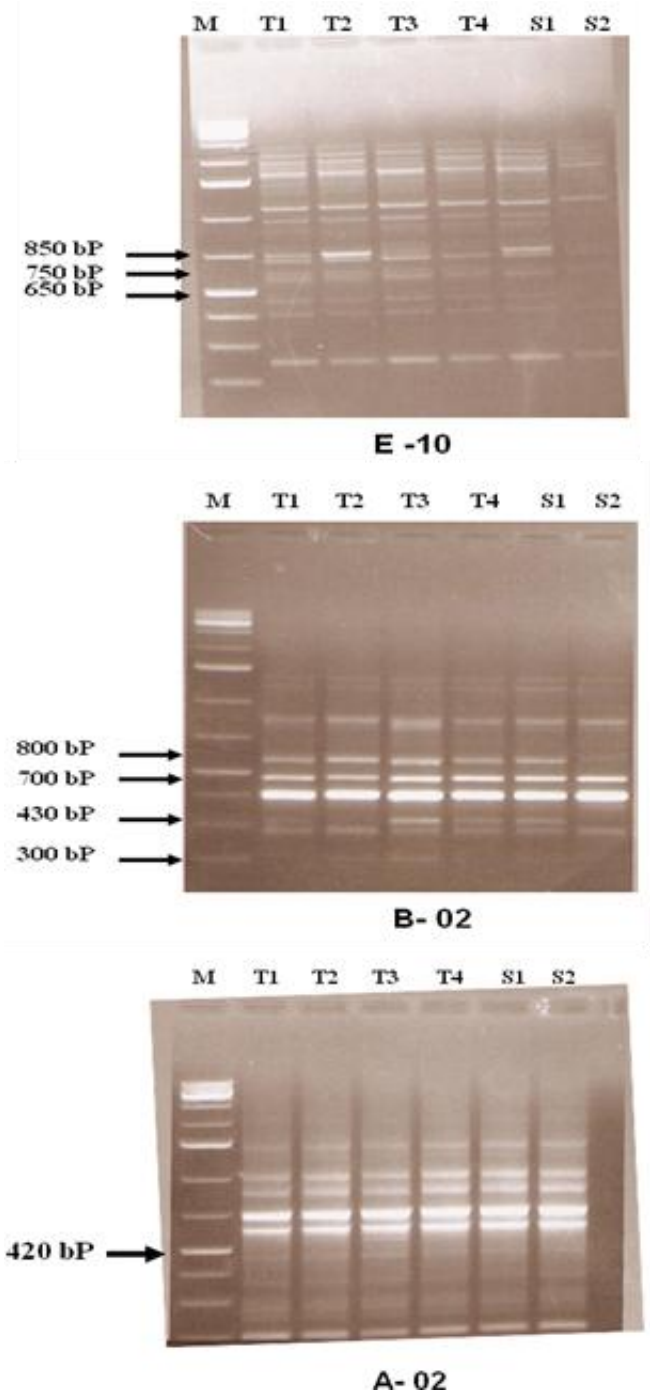

Figure 2. RAPD-PCR fragments of three primers (E-10, B-02 and A-02) for tolerant and sensitive genotypes.

rant in Egyptian bread wheat. In this context, Abdel-Bary et al. (2005) detected eight positive and negative RAPD markers for salinity tolerance in maize. Moreover, the results are in agreement with those reported by Bruckner and Forberg (1987), Rampino et al. (2006) and Alan (2007), who assigned RAPD markers to drought stress tolerance in wheat genotypes using molecular markers. The present results also agrees with those of Rashed et al. (2010), who studied the relation between yield related traits as grain yield, and yield components with some molecular markers in Egyptian bread wheat, and found several markers in these relationships with grain yield, yield components under drought stress. This indicates that there are potential markers to be used as marker assisted selection to improve drought stress tolerance by molecular breeding.

Table 3. Asurvey of the six tested primers with four tolerant and two sensitive genotypes.

Primer	Ms (bp)	T1	T2	T3	T4	S1	S2	MT
A -12	1050	1	1	1	1	1	0	P
	1200	1	1	1	1	1	1	-
B -05	390	1	1	1	1	1	0	P
	450	1	1	1	1	1	1	-
C -12	200	1	1	1	1	1	0	P
	230	1	1	0	1	0	0	P
	750	0	1	0	1	0	0	-
E -10	650	1	1	1	0	1	1	-
	850	1	1	1	1	1	0	P
B -02	430	1	0	1	1	1	0	P
	800	1	1	1	1	1	0	P
	700	0	0	1	1	0	0	-
A -02	300	0	1	1	0	1	1	N
	420	0	0	1	0	0	0	-

T, Tolerant genotypes; S, sensitive genotypes; Ms, molecular size; Mt, molecular type; P, positive; N, negative.

Table 4. Polymorphism percentage for the six wheat genotypes as generated by six primers.

Primer	Monomorphic	Polymorphic		Total band	Polymorphic
		Unique	Non unique		
A-12	11	0	1	12	0.09
B-05	6	0	1	7	0.14
C-12	8	0	3	11	0.27
A-02	10	1	0	11	0.09
E-10	11	0	3	14	0.21
B-02	6	1	3	16	0.30

Their results indicated the presence of four positive and two negative RAPD markers associated to drought tolerance in bread wheat. Ashraf (2010) developed different markers for different traits using RAPD analysis. Malik et al. (2000) used RAPD marker to detect DNA polymerphism between drought resistant and drought susceptible genotypes. Traditional varietal selection is based on morphological features hence polygenic traits which are very difficult to analyze; such constraints can be overcome by using molecular marker assisted selection for trait of interest (Zhang, 2004). Marker-assisted selection based on genotype mean performance will greatly increase breeding efficiency (Irada and Samira, 2010; Manavalan et al., 2009). Kamal et al. (2011) had a wide range of genetic variation in inbred lines of wheat with expected marker tag (EST). Nachit et al. (2000) associated grain yield and yield components with some molecular markers in durum wheat. Several markers showed strong relationships with grain yield and yield components under drought stress conditions. Sajida et al. (2010) used same primers to study molecular markers assisted selection for drought tolerance in wheat genotypes namely, A-02, A-17 and B-02. Their results indicate that two positive molecular markers with molecular size of 560 and 930 bp were exhibited only in KMP3 and SMPL genotypes. Seven bands were amplified by primer A-02 which was polymorphic with primers B-05 and B-07 and B-17 produced 75% polymorphic alleles. RAPD banding patterns for the six wheat genotypes by using six primers (A14, A18, B09, UBc30, UBc75 and UBc78) scored three negative and one positive molecular markers correlated to the relatively sensitive wheat genotypes and three positive molecular markers which appeared in the tolerant genotypes (Mar-5 and Gem-7). Also, UBc78 operon primer differentiates the highest salt tolerant genotype (Mar-5) by the positive unique band of (110 bp) Samy et al. (2007).

Conclusions

In conclusion, drought tolerant genotypes in bread wheat

were harbored in seven positive RAPD markers, while sensitive ones appeared only in one negative RAPD marker. These molecular markers could be used for characterizing bread wheat genotypes for drought tolerant to be used in molecular breeding, as well as, for early discovering of drought tolerant genotypes to be cultivated in suitable area of lower water supply and temperature increases.

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