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MOLECULAR MARKERS FOR DROUGHT TOLERANCE IN BREAD WHEAT

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ABSTRACT

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 indicated that tolerant genotypes were harboring seven positive RAPD markers ,while sensitive genotypes were having only one negative RAPD markers..In tolerant genotypes, seven positive PCR-RAPD markers with molecular sizes of 1050bp, 390 bp, 200bp.230bp ,850bp, 430bp and 800bp were exhibited by A-12 ,B-05,C-12 ,E-10 and B-02 primers. This study indicated that the seven positive markers can be used as indicators to discard drought tolerance in wheat marker -assisted breeding programes.
Keywords: 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 different to lake of the 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. 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 genetic diversity studied in wheat were 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 (Chinnusany *et al.*, 2007).

Many advances molecular mechanisms of antidrought and corresponding molecular breeding have taken place (Ramino *et al.* 2006; Wei *et al.*, 2009 and Ashraf 2010).

Randomly amplified polymorphic DNA primers (RAPD) associated with drought tolerance were used initially to search genetic diversity in wheat plants. It was found out that primer P_6 [TGGGGGGTTC] produced respectively a 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).

This study aimed to analyze 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 Ṣakha-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 ,while genotype No. 5 and Giza-168 are non drought tolerant..Relative drought sensitivity of these genotypes have been determined over two years in two sowing dates (favorable and drought stress) based on grain yield through drought sussceptability index , Molecular marker assay (RAPD) was employed to determine the genetic diversity of six wheat genotypes and to determine drought tolerant genotypes associated DNA marker.The six wheat genotypes were classified as drought tolerant or sensitive on the basis of field performance(Table 1) using drought susceptibility idex as outlined by Fisher and Maurer (1978).

DNA extraction

DNA extraction from leaves using the organs DNeasy (Qiagen santa clara, CA) in the growth room 5-7 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 Maroof et al., 1984 and Kumar et al., 2003).

Estimation of DNA Concentration :

DNA concentration was determined by diluting the DNA 1.5 in dH2O. The DNA samples were electrophoresed in 1% agarose gel against 10ug of a DNA size marker. This marker covers a range of concentration between 95 ng 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 et al., 1985) .

RAPD Reactions

PCR reactions was performed according to Williams et al.,(1990) using six primers RAPD (Table2).

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

Genotypes	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
Gp A – 12	TCGGCGATAG
B – 05	TGCGCCCTTC
C – 12	TGTCATCCCC
A – 02	TGCCGAGCTG
E – 10	CACCAGGTGA
B – 02	TGATCCCTGG

Table 3 . A survey 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	٠	١	٠	١	٠	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	-
	300	0	1	1	0	1	1	N
A -02	420	0	0	1	0	0	0	-

T = Tolerant genotypes **S = Sensitive genotypes**
Ms = Molecular size **Mt = Molecular type**
P= Positive **N = Negative**

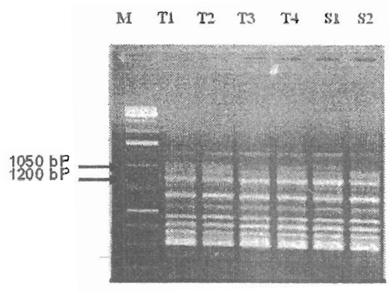
Table 4. Polymorphism percentage for the six wheat genotypes as generated by six primers.

Primer	Monomorphic	Polymorphic		Total bands	Poly morphic
		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

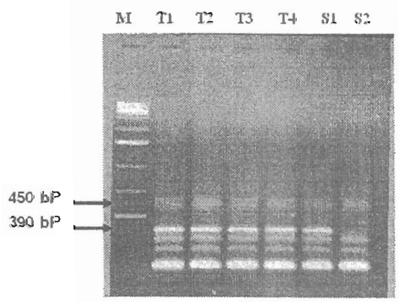
RESULTS AND DISCUSSION

RAPD markers for drought tolerance

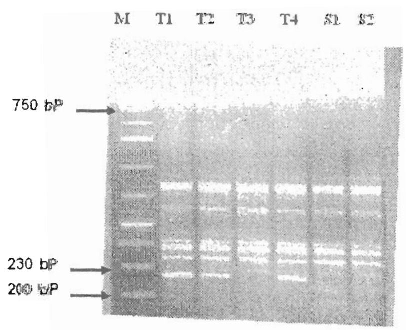
DNA isolated from the six wheat genotypes, which comprised four drought tolerant genotypes No1 to No 4 and two sensitive genotypes (No 5 and Giza -168) was seen in Figure (1and 2). They were tested against six preselected primers as shown in Table(2).Three primers only gave high polymorphism with studied six genotypes , five primers out of which 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 300 and 800bp for B-02 primer, which were found only in tolerant genotypes and absent in sensitive ones.As to 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 agreed with many reports detected RAPD markers for abiotic stresses tolerance. Abdel – Tawab *et al.,* (2003) detected five positive and negative RAPD markers for drought tolerant in Egyptian bread wheat.In this context, Abdel-Bary *et al.*, (2005) detected eight positive and negative RAPD markers for salinity tolerance in mize.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 are also agreed with those of Rashed *et al* ., (2010) ,who studied the relation between yield related traits as grain yield ,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 indicated that there are potential markers to be used as a marker assisted selection to improve drought stress tolerance by molecular breeding.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 detected DNA polymorphism between drought resistant and drought susceptible genotypes. Traditional varietal selection is based on morphological features hence polgenic traits which are very difficult to analyze, such constraints can be overcome by using molecular marker assisted selection for trait of interest (Zhang 2004).



A – 12



B – 05



C – 12

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

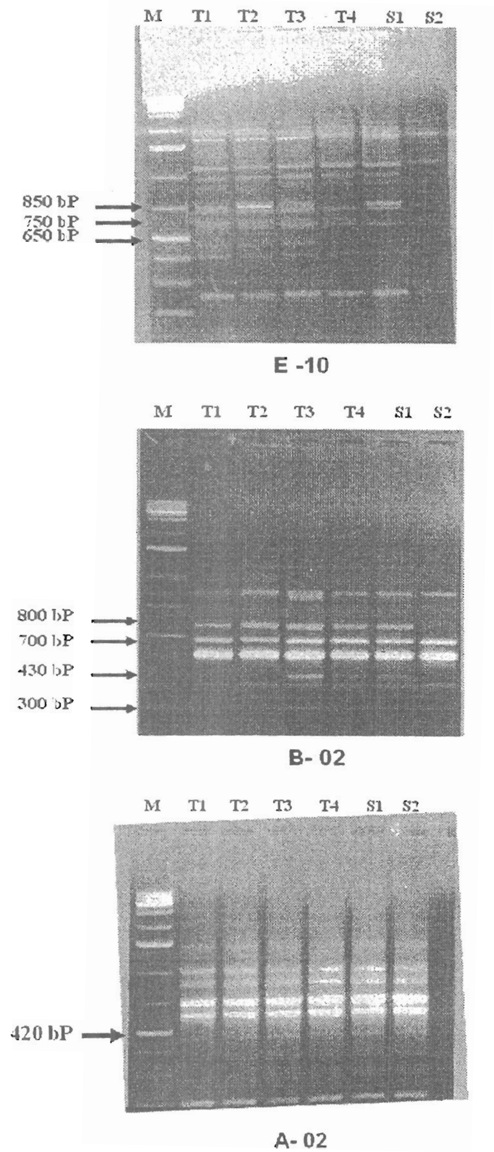


Fig 2. RAPD-PCR fragments of three primers (E-10, B-02 and A-02) for tolerant and sensitive genotypes.

Marker-assisted selection based on genotype meanperformance will greatly increase breeding efficiency (Irada and Samira 2010 and 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).Nachi *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 Bibi *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 indicated that two positive molecular markers with molecular size of 560 bp 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 ,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 harboring seven positive RAPD markers , while sensitive ones were appeared only 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 drought tolerant genotypes to be cultivating in suitable area of lowering water supply and temperature increases.

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الواسمات الجزيئية المرتبطة بتحمل الجفاف فى قمح الخبز

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تم فى هذة الدراسة إستخدام تكنيك ال RAPD بواسطة ست بادئات لدراسة الواسمات الوراثية الجزيئية المرتبطة بتحمل الجفاف فى ست طرز وراثية من قمح الخبز،أربعة من هذة الطرز الوراثية تتحمل الجفاف ،وإثنين منهم حساسة للجفاف. أظهرت نتائج الدراسة وجود سبع واسمات وراثية جزيئية موجبة لتحمل الجفاف فى الطرز الوراثية التى تتحمل الجفاف هى الطرز الوراثية رقم ١،٢،٣،٤ كما أظهرت نتائج الدراسة وجود واسمة جزيئية واحدة سالبة والتى ظهرت فى الطرز الوراثية الحساسة للجفاف. أسفرت نتائج هذة الدراسة عن إمكانية الاستفادة من الطرز الوراثية التى تتحمل الجفاف والتى ظهرت بها الواسمات الوراثية الجزيئية السبع فى برامج التربية لتحمل الجفاف ،والانتخاب للصفات التى يتحكم فى وراثتها عدد كبير من الجينات مثل صفة محصول الحبوب قيد الدراسة.

قام بتحكيم البحث

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