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RAPD Markers Associated with Drought Tolerance in Bread Wheat (*Triticum aestivum* L.)

H. Pakniyat and E. Tavakol
Department of Crop Production and Plant Breeding, Shiraz University,
Shiraz, Islamic Republic of Iran

Abstract: Randomly Amplified Polymorphic DNAs (RAPDs) were used to search genetic diversity and markers associated with drought tolerance in 20 bread wheat cultivars. These cultivars are extensively being used by farmers in Iran, 6 of them are known as drought tolerant. Initial screens involved growing 10 cultivars at seedling stage under drought conditions (-5 and -8 bar) exerted by PEG 6000 in a hydroponic experiment. These tests confirmed the tolerance of the 6 above mentioned cultivars. Thirty 10-mer RAPD primers were used for fingerprinting of the cultivars of which primers P6 (TCGGCGGTTC) and P7 (CTGCATCGTG) produced respectively a 920 and a 750 bp band present in drought tolerant (absent in others) cultivars. These bands may be associated with drought tolerance in bread wheat.

Key words: Drought tolerance, bread wheat, RAPD markers

INTRODUCTION

Water deficit stress is a major limiting factor for plant growth and productivity resulting large economic losses in many regions of the world (Ashraf and Harris, 2005). The exploitation of tolerant crops is one approach to the problem. Wheat is widely grown as a rain-fed crop in semi-arid areas, where large fluctuations occur in the amount and frequency of rainfall events. The development of tolerant cultivars, however, is hampered by low heritability for drought tolerance and a lack of effective strategies (Kirigwi *et al*., 2004; Bajji *et al*., 2001).

To differentiate drought tolerant genotypes, several selection indices have been suggested on the basis of a mathematical relationships between favorable and stress conditions (Clark *et al*., 1984; Huang, 2000). Tolerance (TOL) (McCaig and Clark, 1982; Clarke *et al*., 1992), Mean Productivity (MP) (McCaig and Clarke, 1982), Stress Susceptibility Index (SSI) (Fischer and Maurer, 1978), Geometric Mean Productivity (GMP) and Stress Tolerance Index (STI) (Fernandez, 1992) have all been employed under various conditions. Measuring these indices are tedious and time consuming, therefore, selection by aids of genetic markers may be very helpful for this purpose. A wide range of genetic markers is available for study in wheat. Earlier work with barley indicated that RAPD and AFLP markers are associated with salt tolerance (Pakniyat *et al*., 2004; Pakniyat *et al*. 1997). Here we use RAPDs as a quick and easy method to search for DNA markers associated with drought tolerance in selected lines of bread wheat contrasting for tolerance.

MATERIALS AND METHODS

DNA extraction: A total of 20 bread wheat cultivars including tolerant, semi-tolerant and non-tolerant were used (Table 1). Six of the cultivars were drought tolerant and extensively are planted by local farmers under drought conditions.

DNA was extracted from 10 days old seedlings using CTAB method (Murry and Thompson, 1980) with some modifications.

Polymerase chain reaction conditions: PCR was carried out essentially as described by Williams *et al*., 1990. Thirty 10-mer oligonucleotide primers were used for DNA amplification (Table 2). Amplifications were performed in a Technogene Co. thermocycler first 4 min at 94°C followed by 10 cycles of: 1 min at 94°C, 1 min at 36°C and 1 min at 72°C. After that, for next 35 cycles, 0.2°C was added to annealing temperature. After the final cycle, samples were incubated at 72°C for 15 min and then held at 4°C prior to analysis. Amplification products were

Corresponding Author: H. Pakniyat, Department of Crop Production and Plant Breeding, Shiraz University, Shiraz, Islamic Republic of Iran Tel/Fax: 0711-2286134

Table 1: Bread wheat genotypes and their drought tolerance status, used in the experiment

Cultivar No.	Cultivar name	Collected center	Reaction to drought*	Cultivar No.	Cultivar name	Collected center	Reaction to drought*
1	Azar 2	Yasooj	Tolerant	11	Shiraz	Zarghan	Sensitive
2	Gahar	Yasooj	Tolerant	12	Sardari	Zarghan	Tolerant
3	Koohdasht	Gachsaran	Tolerant	13	Falat	Zarghan	Sensitive
4	Bow	Gachsaran	Tolerant	14	Tajan	Zarghan	Sensitive
5	Zagros	Zarghan	Tolerant	15	Pishtaz	Zarghan	Sensitive
6	Cham	Gachsaran	Semi-tolerant	16	Marvdasht	Zarghan	Sensitive
7	Niknejad	Zarghan	Semi-tolerant	17	Chamran	Zarghan	Sensitive
8	El Neilairi	Gachsaran	Semi-tolerant	18	Estar	Zarghan	Sensitive
9	Bohoih	Gachsaran	Sensitive	19	Mahdavi	Zarghan	Semi-tolerant
10	Giza 164	Gachsaran	Sensitive	20	Darab 2	Zarghan	Semi-tolerant

*Reaction to drought reported by research centers which seeds collected

Table 2: Primer nucleotide sequence used to amplify DNA

Primer designation	Sequence 5'-3'	Primer designation	Sequence 5'-3'	Primer designation	Sequence 5'-3'
P1	ACACAGAGGG	P11	CCATTCCCCA	P21	TTCCCCGACC
P2	CCTCTCGACA	P12	GGTGAACGCT	P22	GAGGGCGGGA
P3	TCTCAGCTGG	P13	CTCCCTGAGC	P23	AGGGGCGGGA
P4	GTGTGCCCCA	P14	TTCCGGGTGA	P24	GAGGTCCAGA
P5	CCACGGGAAG	P15	GAGCTCGCGA	P25	GGGGGTTAGG
P6	TCGGCGGTTC	P16	CCTGGGCTTC	P26	ATCGGGTCCG
P7	CTGCATCGTG	P17	CCTGGGCTTG	P27	CCGTGCAGTA
P8	TGAGCCTCAC	P18	CCTGGGCCTA	P28	TAGCCGTGGC
P9	TCGGCACGCA	P19	CCTGGGCCTC	P29	GGCTAGGGGG
P10	CTGCGCTGGA	P20	TGCCCCGAGC	P30	TACGTGCCCG

separated by electrophoresis in 1.5% agarose gels and detected using ethidium bromide and UV light. Each amplification was performed using a single primer and gels scored for the presence and absence of products.

RESULTS AND DISCUSSION

Preliminary hydroponic experiment performed with a subset (10 cultivars) of bread wheat cultivars including the resistant ones under water deficit stress (-5 and -8 bars) exerted by PEG 6000, confirmed drought tolerance of the 6 tolerant cultivars shown in Table 1 (Tavakol, 2005). Twenty eight primers produced scorable amplification products. Two primers (P15 and P26) produced vague un-scorable bands. From 378 amplified band 156 (41%) were polymorphic and primers P3, P11 and P25 produced more polymorphic bands. Band sizes were between 300 to 1900 bp. Primers P6 (TCGGCGGTTC) and P7 (CTGCATCGTG) produced a 920 and 750 bp bands present in drought tolerant cultivars (Azar 2, Gahar, Koohdasht, Bow, Zagros and Sardari). These bands may be associated with drought tolerance in bread wheat (Fig. 1 and 2) and they may be used in selection of tolerant genotypes in breeding plans.

The results indicate that RAPD technology is a powerful tool in quickly identifying markers related to drought tolerance in wheat. It is interesting that in this

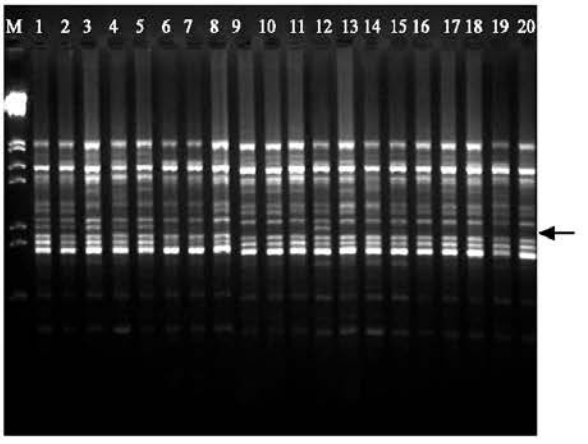

Fig. 1: Electrophoretic pattern generated by RAPD primer P7 (CTGCATCGTG). Arrow shows a 750 bp band present in drought tolerant (absent in others) cultivars

research, two out of 28 primers detected associations to drought tolerance in tolerant cultivars which they may be related to involved QTLs.

It is hoped that the discovery of markers associated with drought tolerance with the aid of the genes involved. One strategy for P6 and P7 products is to sequence the 920 and 750 bp bands and search against stress-related ESTs (expressed sequence tags) in public databases (Boguski *et al*., 1993) to identify the gene involved.

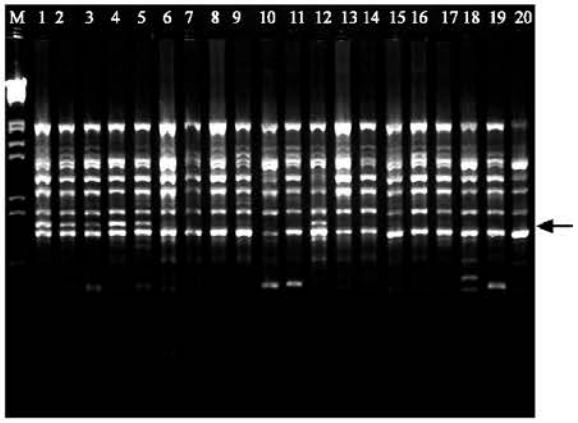

Fig. 2: Electrophoretic pattern generated by RAPD primer P6 (TCGGCGGTTC). Arrow shows a 920 bp band present in drought tolerant (absent in others) cultivars

Sequence data can also be used to develop more robust PCR primers as diagnostics for drought tolerance. Another strategy is to map the two products to confirm or otherwise their genetic co-location.

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