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Total Wheat DNA Variation in to Varieties Using Known Primers of the Genes Induced in Dehydration and Salinity Stress

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Abstract: Ten genotypes of wheat used characterised with their total DNA variation with special reference to known primers of the genes induced in dehydration and salinity stress. Amplification profiles of DRE primers in identification of polymorphic bands that can be used for finger printing the varieties. Four main groups were observed based on 138 polymorphic bands and variance was related to drought, pedigree or geographic origin. Similar pattern of genetic diversity was also observed when 200 markers were recorded from DRE combinations. Pakistani varieties showed higher degree of 70% dissimilarities, While exotic showed low genetic distance.

Key words: Biochemical analysis, molecular markers, RAPD, *Triticum aestivum*, DNA fingerprinting

Introduction

Wheat is the most important cereal crop and staple food of the world. It covers about one fifth of the Punjab rainfed tract, Where water deficiency most often severely effects the productivity and thus became one of the important limiting factor. The effects of drought on wheat have generally been studied in field and based on data recorded for agronomic, morphological and physiological plant traits. Such data characterize the varieties, but may not necessarily provide an accurate identification of genes for drought tolerance because of environmental influence (Aspinall, 1984). Bio-chemical markers have also been used; most recently DNA makers are being used for this purpose for assessment of genetic variability for drought/stress tolerance. Among the DNA markers, Random Amplified Polymorphic DNA (RAPD) has been reported as a useful tool for evaluating intra-specific variation (Dos Santos *et al*., 1994; Thormann *et al*., 1994) and for varietal characterization (Farooq *et al*., 1994). RAPD markers are generated by Polymerase Chain Reaction (PCR) with a single short oligo-nocleotides of arbitrary sequences and provide numerous markers that give genetic information at DNA level. RAPD are simple to produce, easy to handle and are rapidly detect variability used for varietal characterization (Farooq *et al*., 1994 and Linc *et al*., 1996).

Drought specific primers acts as a motif and called Dehydration responsive elements (DRE) identified by Shinozaki and Shinozaki (1994) are also being used these elements are conserved DNA sequence of about 9 base pair (TACCGACAT), present in the 5 ' promoter regions of genes that response to dehydration at transcriptional level in many plant species which are related to drought (Skriver and Mundy, 1990).

Materials and Methods

Ten pure-lines/varieties of wheat from diverse origin were used in the present study (Table 1). RAPD analysis was conducted in the Biological Resources Division Lab., (JIRCAS) at Tsukuba, Japan. Twenty seeds of each variety were sown in pots. After 30 days, young leaves were harvested and DNA was extracted using 20% SDS with extraction buffer [1 M Tris HCl (pH 8.0), 0.5 M EDTA (pH 8.0), 5 M NaCl and 10 m M of 2 mercaptoethanol]. The quantity was measured with spectra-photo-meter. Quantify of DNA used for amplification was 10 $ng^{-1}\mu l$ and also re-checked by mini gel using λ DNA as standard.

The PCR was performed in 0.5 ml reaction tube with 25 μl of DNA solution containing 3.0 μl genomic DNA (10 $ng^{-1}\mu l$), 2.5 μl of primer (10 μM), 2.0 μl dNTP (2.5 μM), Gene Taq $5.0l^{-1}$ μl (Nippon Gen. Co.) 0.3 μl, buffer (X 10) 2.5 μl and glycerol (7.5%) 14.7 μl. The amplification reaction was carried out in the Mj research PTC 100 machine with 94^0C for one min, 40^0C for 2 min, and 72^0C for 2 min completed 49 cycles. After that 72^0C for 5 min was applied for complete annealing prior to storage at 4 ^{0}C of the amplification reactor 15 random primers, and another 15 random primers with combination of Micro Satellite and 6 were with DRE primers (Table 2).

The data of polymorphic fragments were recorded. After electrophoresis, the content were loaded on to 13% Poly Acrylamide Gel, [13 ml H_2O, 5 ml Acrylamide (40%), 2.0 ml of 10 X TBE, 30% fresh APS and 10 μl of TEMED]. The solution was mixed thoroughly and poured in to the glass plates to let the gels polymerised. Before applying the sample, the wells of the gel were thoroughly washed and 7.0 μl from individual sample was applied to each value. Electrophoresis was conducted at 300 V for 120 min. Lambda (λ) DNA was used as molecular marker. After

Table 1: Varieties used for DNA fingerprinting with special reference to drought resistance

Variety	Varieties recommended for the area of (Drought status)	Origin/parentage
C 591	low rainfall	T-9 / 8B (Pakistan)
Blue Silver	short duration /low to medium rainfall areas	53 388/AN//Pit64/3/LR64 (India/Pakistan)
Rawal 87	Low to medium rainfall	Maya/Mon`s`//Kvz/Trm (Pakistan)
V 8203	Medium to high rainfall	4777(2)*{Cno-8150 x Tob-cno (No/12300 x LR64-8156}xTrf`S` (Pakistan)
V 90R34	low/med. Rainfall	CIMMYT (Mexico)
CB 51	Susceptible	(USA). HYS/T2484-35t-2t-1t-CB75-270. OWW76033-04P-1H-2S-0H (Mexico)
Chenab 70	Susceptible	C271/W1 (E)//Son64 (Pakistan)
Fukuho Kumugi	-	Japan
Oligo-Culm # 380	-	Israel
Janz	-	Australia

Table 2: Primers used for amplification in wheat

No	Primer Name	Sequence
1	P1-DIC	CCG TAT CAA CAG CGT G
2	P1-DICKA	GCC TTT ATG GTT CGA ATC
3	P2-DIC	TAC CGA CAT AAA GGT
4	P2-24F	GCC ACG TAC AGG CAT
5	P1-JOINT	CCT CAG GCG GTG ATT ACA
6	P2-JOINT	TTA CCA TTT ACA TTC G

electrophoresis, the gels were stained with silver nitrate. The banding profile appearing on the gel photodocumented and data was recorded and took photographs. The data were recorded as presence (1) or absent (0) and analyzed by UPGMA method (Sokal and Mitchener, 1958, Lance and Williams, 1966; Sneath and Sokal, 1973). Cluster analysis was formed on the basis of total 118 polymorphic fragments and fragments 36, 26, 4, 13, 16, 11 and 12 obtained by P1-DIC, P1-DICKA, P2-DIC, P2-24F, P1-joint and P2-joint primers separately.

Results and Discussion

Fig. 1 shows the amplification profile for overall primers P1-DIC, P1-DICKA, P2-DIC, P2-24F, P1-joint and P2-joint. The variety C 591 exhibited maximum genetic distance when compared with all other varieties (Table 3). Similarly Rawal 87 and F. Kumugi revealed maximum distance. Genetic distance based on 200 polymorphic bands obtained by DRE primers ranged from 4.36 (Janz Vs O. Culm # 380) to 10.54 (F. Kumugi Vs C 591). Genetic distance varied from 9.38 (O. Culm # 380 Vs. F.K.) to 14.53 (Rawal 87 Vs. F. K. and Rawal 87 Vs. O. Culm # 380) as presented in Table 4. The varieties from Pakistan exhibited high range of genetic diversity, which might be due to diverse parentage as most of wheat varieties have been bred from CIMMYT material. Genetic distance for 10 varieties of wheat based on 200 RAPD bands revealed four major groups. Group A represents one variety V 8203 that was a CIMMYT line. Group B represents three varieties viz., Oligo-Culm # 380 (O. Culm # 380), Janz and Fukuho-kumugi (F.K.) that were all exotic and the status for drought were not known. The group C represents five varieties viz., CB 51, Ch 70, V 90R34, Blue Silver and Rawal 87. Out of this group, Blue Silver was short duration and

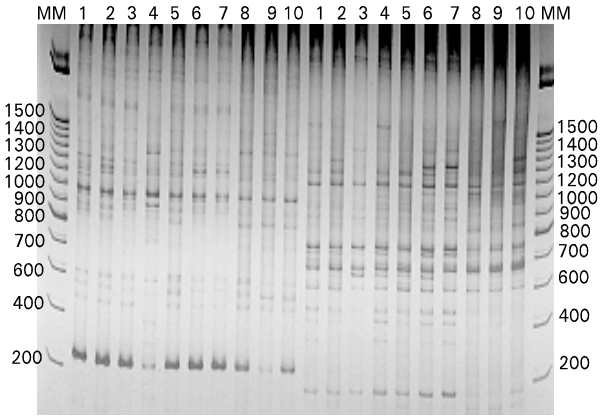

Fig. 1: Banding pattern of primers SK-7+OPF 08 (left) and SK-7+OPU 16 in 10 wheat varieties

escapes terminal drought; Rawal 87 and V 90R34 were bred for low to medium rainfall, whereas CB 51 and Chenab 70 were susceptible to drought. Group D represents the variety C 591 that is a drought resistant variety and well known for high bread making quality.

Table 6 gave the results of cluster analysis based on 422 RAPD fragments. Cluster IV comprising of two cultivars Rawal 87 and C 591 were drought tolerant. In relation with drought resistance, clustering on the basis of 422 fragments revealed more clear separation for drought stress. It might be due to more number of polymorphic fragments. Group D represents variety C 591 and Rawal 87, which were drought tolerant and these exhibited maximum genetic distance from other groups. Group B comprising of susceptible cultivars except Blue Silver and V 90R34 which bred for low to medium rainfall. Blue Silver has grouped with susceptible group although it is considered drought tolerant. In fact this variety is short duration and due to this feature it escapes drought. Overall analysis based on 422 polymorphic bands showed almost similar pattern except Rawal 87, which was grouped in cluster IV. According to the genetic distance, variety C 591 and Rawal 87 stand apart from all the other varieties and these both varieties were considered drought tolerant

Table 3: Fragment profile and cluster of each variety on the basis of dendrogram in cluster analysis for each primer and overall

Primer	P1-DIC	P1-DICKA	P2-DIC	P2-24F	P1-JOINT	P2-JOINT	P2-JOINT	Overall
Total Fragment	37	32	9	18	16	14	14	140
Polymorphism	36	26	4	13	16	11	12	118
Varieties	Fragment profile							
C 591	13A	21A	6A	13A	4A	9A	7A	73A
Blue Silver	22B	14B	6B	15D	1B	10A	6A	74B
Rawal 87	18C	16D	6C	16D	2B	11A	8B	77B
V 8203	12E	16D	6D	13B	6E	10A	11C	74D
V 90R34	22C	20D	6E	13C	4D	10A	9B	84B
CB 51	21C	18D	6H	12C	1C	8B	10B	76B
CH 70	13D	17E	6F	8E	1C	7B	8A	60C
F. Kumugi	13C	14C	7E	11B	0C	10C	8B	63C
O.Culm # 380	9D	15C	7G	13C	0C	8C	6A	58C
Janz	17B	14C	7H	13F	2B	8C	6B	67C
Total	150	105	13	77	21	61	59	486

Table 4: Genetic distance for 200 RAPD bands in wheat

	B.Silver	Rawal 87	V 8203	V 90R34	CB 51	Ch 70	F.Kumugi	O.C # 380	JANZ
C 591	9.43	8.48	10.1	9.05	8.54	9.85	10.54	10.15	10.1
Blue Silver		9.74	9.11	8.31	7.74	8.12	9.05	8.83	8.66
Rawal 87			9.8	7.87	7.68	7.94	10.25	9.75	9.59
V 8203				9.05	8.54	8.66	9.43	8.77	9.27
V 90R34					7.68	7.00	9.11	8.43	8.6
CB 51						6.63	9.38	8.94	8.54
CH 70							9.16	8.00	8.06
F.Kumugi								6.32	5.57
O.C # 380									4.36

Table 5: Genetic distance for 422 RAPD bands in wheat

	B.Silver	Rawal 87	V 8203	V 90R34	CB 51	Ch 70	F.Kumugi	O.C # 380	JANZ
C 591	12.5	12.04	13.34	12.88	12.17	13.49	14.49	14.42	13.86
Blue Silver		13.53	12.65	11.75	11.49	11.92	13.19	12.89	13.42
Rawal 87			13.38	11.87	11.53	12.12	14.53	14.53	14.46
V 8203				12.65	11.92	12.08	13.93	13.42	14.35
V 90R34					11.58	12.08	12.96	12.57	13.19
CB 51						9.7	13.64	13.42	13.42
CH 70							13.57	12.72	13.86
F.Kumugi								9.38	10.1
O.C # 380									9.38

Table 6: Clusters based on 422 fragments in wheat

Cluster	Variety	Group	Origin
I	F.K.,O.C # 380 and Janz	A	Japan, Isreal & Australia
II	V 8203	B	CIMMYT
III	Chenab 70, CB 51, B.Silver and 90R34	C	Pak., HRF area CIMMYT
IV	R-87, C 591	D	Pak, low Rainfall areas

(Table 5). The RAPD results conquered to the morphological, traits and groups could be placed in to groups based on drought and geographic region. Drought and geographic (rainfall) relationship of the crop plants as discussed by many earlier researchers (Rao *et al*., 1992; Sharma *et al.,* 1995).

It is evident from the results that RAPD can be used as a tool for the investigation of morphological and geographical relationship of wheat crop. Drought resistance can be identified by the use of RAPD analysis. This technique in combination with plant physiological analysis can efficiently be used to develop drought resistance cultivars to overcome wheat shortage in the country.

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