Identification of RAPD and ISSR markers for drought tolerance in wheat (*Triticum aestivum* L.)

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Abstract Drought tolerance is the essential trait that needs to be incorporated in cereal crops, particularly those grown under the rainfed cultivation. Drought tolerance being contributed by several regions of the genome requires identification of these regions, using suitable molecular markers. Therefore, present investigation was aimed at analyzing the genetic diversity present among the cultivars of rainfed and the irrigated areas with respect to the drought tolerant trait. In all, 14 RAPD and 90 ISSR markers were used to identify these genomic regions. Out of 14 RAPD markers, one RAPD primer exhibited polymorphic banding pattern with 18.6 % polymorphism, clearly separating drought tolerant and drought susceptible genotypes. Out of 90 ISSR primers, only 3 ISSR primers revealed polymorphism in relation to the drought tolerance trait exhibiting 21.38 % polymorphism.

Keywords Drought · Polygenic · ISSR · RAPD

Abbreviations

ISSR Inter Simple Sequence Repeats
SSR Simple Sequence Repeat
RAPD Random Amplified Polymorphic DNA
DNA Deoxyribonucleic Acid
PCR Polymerase Chain Reaction
CTAB Cetyl Trimethyl Ammonium Bromide
cv. Cultivar (s)

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Introduction

Wheat production is adversely affected by drought in 50 % of the area under cultivation in the developing countries (Trethowan and Pfeiffer 2000). Genetic gain for yield under these environments is estimated to be half of the yield compared to well-watered environments (Rajaram et al. 1996). It is difficult to make progress for grain yield and yield components under drought as they are complex characters influenced by many environmental factors and are characterized by low heritabilities and large genotype environment interactions under drought conditions (Smith et al. 1990). Breeding for drought tolerance of wheat cultivars is a major objective of wheat breeding program in arid and semi-arid regions of the world due to inadequate precipitation, shortage of water for irrigation and high water demand due to crop evapotranspiration in such climates. Development of drought resistant cultivars will considerably improve the rainfed wheat production.

Systematic evaluation of molecular diversity with respect to drought tolerance swathed in wheat genetic resource is essential in optimizing both conservation and utilization strategies. Morphological characteristics are used to ascertain proper selection of stress tolerant crops. While the sensitivity of morphological observations to environmental and developmental variations limits their applicability, molecular markers can be efficiently utilized to screen and identify proper tolerant plants even at an early stage. Since, drought tolerance is contributed by several regions of the genome; it requires identification which can be accomplished by the use of DNA markers as they offer a faster way to identify drought tolerance related regions. During the present investigation, RAPD and ISSR markers were used to fingerprint 12 cultivars of wheat grown in India especially to analyze the variations among these cultivars for drought tolerance and susceptibility.

In other words, efforts have been made to identify RAPD and ISSR markers to distinguish the drought tolerant from susceptible ones for breeding programmes.

Materials and methods

Drought tolerant and susceptible wheat genotypes were selected on the basis of the recommendations of field trials conducted for 5 years at different locations under Wheat Improvement Project, Department of Plant Breeding and Genetics, College of Agriculture, Jabalpur, India. The cultivars selected were classified on morphological basis suitable for rainfed (water stressed) and irrigated areas respectively. Five distinguished drought susceptible and seven tolerant cultivars were selected for molecular analysis (Table 1).

Leaf samples from 25 to 40 days old randomly selected plants were collected and stored at -20°C until use. Genomic DNA was isolated using CTAB method (Saghai-Maroo et al 1984). This method provided good quality as well as good quantity of DNA. The genomic DNA extracted was subjected to PCR amplification using RAPD and ISSR markers. PCR amplification of genomic DNA was performed with 14 RAPD (OPAW-6, OPAW-9, OPAZ-14, OPBA-14, OPBB-9, OPBE-10, OPBF-16, OPBG-19, OPZ-7, OPZ-13, OPB-5, OPO-5, OPZ-1 and OPBH-1) primers and 90 ISSR primers from UBC primers set # 9. 801–890. For RAPD primers, the reaction mixture was standardized to 20 μl (PCR buffer 1X, MgCl_2 2.5 mM, dNTPs 1 mM, Primer 50 ng, *Taq* Polymerase 1 unit, genomic DNA 25 ng. PCR programme was set as 45 cycles of 36°C : 1 min annealing, 2 min extension at 72°C and 7 min final extension at 72°C). The products of RAPD–PCR were analyzed on 1.5 % (w/v) agarose gel.

Table 1 The drought tolerant and susceptible lines used in the study

Cultivars	Code	Pedigree
GW273	Susceptible (S ₁)	CPAN2084/VW205
GW322	Susceptible (S ₂)	PBW173/GW196
HI1077	Susceptible (S ₃)	GALL/AUSTII61-151/CND NO66/3*KAL
GW190	Susceptible (S ₄)	VEE/3/BB*S*/SKA//ARJUN
DL803-3	Susceptible (S ₅)	K7537/HD2160MUT/HD2278/DL896-2
JW17	Tolerant (T ₁)	Selection from HHW 334
MP3020	Tolerant (T ₂)	C306/CB Spring (BW) 84
MP3173	Tolerant (T ₃)	HI1011/WH965-1
HI1500	Tolerant (T ₄)	HW2002*2/STREMOALLI/PWC 5
HI1531	Tolerant (T ₅)	HI1182/CPAN1990
HW2004	Tolerant (T ₆)	C306*7//TR380-14#7/3AG14
Sujata	Tolerant (T ₇)	Selection from C306

Likewise, ISSRs were detected by the use of repeat - anchored primer amplifying between simple sequence repeats (SSRs). The reaction mixture was standardized to 20 μl (PCR buffer 1X, MgCl_2 2.5 mM, dNTPs 1 mM, Primer 10 pmol, *Taq* Polymerase 1 unit, genomic DNA 50 ng; 40 cycles of 56°C : 1 min annealing, 2 min extension and 10 min final extension at 72°C). The products of ISSR–PCR were analyzed on 1.4 % (w/v) agarose gel. Gels were photographed under gel documentation system (Syngene™) and size of amplicons was detected using 1Kb DNA ladder (Fermentas Life Sciences).

Results and discussion

RAPD analysis

Out of 14 RAPD primers, only one primer OPO-05 (5' CCCAGTCACT 3') was found to be polymorphic across drought susceptible and drought tolerant cultivars. It amplified specific allele of 2,400 bp in all tolerant cultivars, which was completely absent in susceptible cultivars. Another polymorphic band of 550 bp was observed in cultivars JW 17, MP 3173, HW 2004 and Sujata (Fig. 1a) and was absent in other drought tolerant cultivars and all susceptible cultivars, revealing that these cultivars could even be more polymorphic or diverse involving further complexity as compared to other drought tolerant cultivars. The polymorphic loci which were developed through primer OPO-05 were restricted to drought tolerant cultivars only, indicating that this primer could be used as a putative marker for screening drought tolerant cultivars. These results showed similarity with the results obtained by Taghian and Abo-Elwafa (2003) and Pakniyat and Tavakol (2007). However, presence of more than one polymorphic locus exemplify that drought tolerant genotypes differed from susceptible ones in more than one respect. The results also suggest that RAPD technique has a great potential to find DNA based polymorphism between the genotypes of same species. These identified polymorphic bands can be considered as potential markers to identify drought tolerant cultivars for marker assisted selection (MAS) in wheat breeding programs.

Though drought tolerance is a polygenic character, there are examples (Ivandiç et al 2002; Liviero et al 2002) where specific alleles at some loci have been found to be associated with ecotypes better adapted to drought environments. RAPD and ISSR markers were specifically selected as the amplified segments are random, using short (decamer) primers. These primers bind somewhere in the sequence, eventually helping us at least in gathering information of trait related regions. Sequence selection is the important part of it, as different sequences produce many times entirely different banding pattern that allows more specific recognition of individual strains. Drought tolerance in wheat is a quantitatively inherited trait controlled by several genetic loci which their

Fig. 1 Electrophoretic banding pattern amplified by (a) RAPD primer OPO-05, (b) ISSR primer UBC 812, (c) UBC 834 and (d) UBC 881 lane M-1 Kb DNA ladder, lane S₁–S₅ susceptible varieties, lane T₁–T₇ tolerant varieties

genetic components are difficult to measure (Forster et al. 2000). RAPD is a PCR-based technique and extremely efficient for DNA analysis in complex genomes as it is relatively inexpensive and yields information on a large number of loci without having to obtain sequence data for primer design.

ISSR analysis

In present investigation, out of 90 ISSR primers only three primers developed precise polymorphism distinguishing tolerant and susceptible cultivars. The size of the amplified DNA bands ranged between 250 and 1,500 bp for primers 812 and 834 while for primer 881, it ranged between 250 and 2,000 bp. Primer 812 (5' GAGAGGAGAGAGAGAA- 3') developed a

specific marker band of 700 bp in all the drought tolerant cultivars (Fig. 1b). Trait specific band of 375 bp was also located in cv. GW273. In present study, three of the tolerant varieties MP 3020, HW 2004 and Sujata were derived from C306 (Table 1). The amplification of specific bands of similar size (400 bp) in HW 2004 and Sujata by the primer 812 indicates that both the derivatives share some similar ancestral sequences in part of their genome. Furthermore, primer 812 amplified two specific alleles (500 bp and 1,350 bp) in HI 1077 and one specific allele (540 bp) in cv. GW 190. Similarly, primer 834 (5' AGAGAGAGAGAGAGAGYT 3', Y = C/T) amplified polymorphic bands of 1,700 bp only in drought tolerant genotypes, a specific band of 2,500 bp in HI 1500 along with band of 1,000 bp in MP 3173 variety (Fig. 1c). On the other hand, primer 881 (5' GGGTGGGGTGGGGTG 3')

Table 2 RAPD and ISSR primers amplified specific alleles (bp) for drought tolerant cultivars and cultivar identification

Primer	Bands (bp) amplified by susceptible cultivars					Bands (bp) amplified by cultivars (bands specific for drought tolerance are mentioned in bold)							
	S ₁	S ₂	S ₃	S ₄	S ₅	T ₁	T ₂	T ₃	T ₄	T ₅	T ₅	T ₆	T ₇
RAPD													
OPO-5	–	–	–	–	–	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400
ISSR													
UBC812	375		500, 1,350	540	–	700, 385	700	700	700	700	700	700, 400	700, 400
UBC834	–	–	–	–	–	1,700	1,700	1,700, 1,000	1,700, 2,500	1,700	1,700	1,700	1,700
UBC881	–	–	–	350	350	1,500, 2,000	1,500, 2,000	1,500, 2,000	1,500, 2,000, 450	1,500, 2,000, 700	1,500, 2,000	1,500, 2,000	1,500, 2,000

amplified bands of 1,500 bp and 2,000 bp only in all the drought tolerant cultivars (Fig. 1d) and specific bands in two cultivars, one of 700 bp in HI 1531 and second 450 bp in HI 1500 (Table 2) which explains their identity among all the tolerant cultivars under analysis. Primer 881 also amplified two specific bands of 350 bp in susceptible cv. GW-190 and DL-803-3.

Among all RAPD and ISSR markers used to identify drought tolerant and susceptible wheat cultivars, one RAPD primer exhibited polymorphic banding pattern with 18.6 % polymorphism clearly separating drought tolerant and drought susceptible cultivars and 3 ISSR primers revealed polymorphism in relation to the drought tolerance trait, showing 21.38 % polymorphism. The specific bands, though varying between drought tolerant genotypes, were present only in drought tolerant cultivars, which are either directly or indirectly contributing to the drought tolerant loci. Even the ISSR markers revealed various bands that were either absent or present within tolerant cultivars which were altogether absent in susceptible cultivars.

Conclusions

Present study not only revealed the means for identification of RAPD and ISSR markers associated with drought tolerant loci but also defined polymorphism between susceptible and drought tolerant wheat cultivars. Though some more sophisticated methodologies are required to generate greater specificity and tight associations with drought tolerant loci, the RAPD and ISSR markers have nevertheless proved to be an easy, fast, simple and efficient means to identify such regions. Present effort have provided a base to critically judge these characters further by utilizing more advanced techniques wherein the marker termini will be sequenced to design longer primers such as SCAR for specific amplification of a particular locus. The identification of such loci

would consequently help breeders to introgress such traits in wheat.

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