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Validation of molecular markers linked with QTLs for heat and drought stress tolerance in wheat

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Abstract

Global climatic changes affect the production and productivity of wheat around the world. Increasing heat and drought are the most important abiotic stress factors that hinder productivity. To produce sufficient wheat there is urgent need to develop heat and drought tolerant wheat cultivars; therefore, identification of genetic diversity present among available wheat genotypes using SSRs markers is of prime importance. The allelic variation present among wheat genotypes can be deployed in breeding heat and drought tolerant cultivars. A set of 36 wheat genotypes was evaluated for evaluating genetic diversity for heat and drought tolerance among wheat genotypes using already reported SSR markers. A total of 24 SSR markers were analysed and out of them 19 were found polymorphic. Using SSR markers data cluster analysis was carried out to classify the wheat cultivars for genetic variation in heat tolerance. The dendrogram constructed using SSR markers data assigned the 36 wheat cultivars into three broad clusters. The polymorphic SSRs thus identified could be used to evaluate genetic diversity present among wheat genotypes and the wheat cultivars thus identified could be exploited in wheat breeding programs.

Keywords: Heat, drought, wheat, SSR markers, polymorphism, cluster analysis

1. Introduction

Wheat belonging to the *Poaceae* family is the most cultivated cereal crop and is the largest contributor in world grain production. FAO (2018, 2019) predicts an additional requirement of 198 million tonnes of wheat by 2050. In order to achieve the required levels, wheat production needs to be increased sufficiently in developing countries (Kumar *et al.* 2019).However, there are certain environmental factors that affect plants growth and development and impact wheat yields. Increasing global temperature intensifies drought stress and both are the most important limiting factor in agriculture affecting productivity. Parts of West and South Asia, North Africa and sub Saharan Africa faces drought and high temperatures during anthesis and early grain filling stage (Izanloo *et al*., 2008; Ortiz et al., 2008; Schillinger *et al*., 2008; Talukder *et al.,* 2014; Gbegbelegbe *et al*., 2016; Toreti *et al*., 2019). Drought and heat stress at reproductive stage significantly reduces wheat yields by reducing spikelet fertility, grain number, and single grain weight (Senapati *et al*., 2019). Increased temperature causes reduction in developmental stage duration thus resulting in lower biomass accumulation (Lobell *et al*., 2012; Chand *et al*., 2014).

Wheat is very susceptible to heat and drought at reproductive and grain filling stage (Alexander *et al*., 2006; Gaffen and Ross 1998; Hennessy *et al*., 2008). Heat and drought stress during the reproductive phase is more pronounced than during the vegetative phase due to the direct effect on grain number and dry weight (Wollenweber *et al*., 2013).Asseng *et al.* (2015) estimated that every 1°C rise in temperature may affect global

wheat production by 6%. Under abiotic stress conditions cellular structures are more prone to damage affecting various metabolic pathways, especially those relating to membrane thermo stability, photosynthesis and starch synthesis(Cossani and Reynolds, 2012; Ristic *et al*., 2008). Starch synthesis is highly sensitive to high temperature and drought stress due to the susceptibility of the soluble starch synthase in developing wheat kernels (Keeling *et al*., 1993; Jenner *et al*., 1994). Starch accumulation in wheat grains is significantly reduced by over 30% at temperatures between 30°C and 40°C (Stone *et al*., 1995). Identification and deployement of favourable alleles imparting tolerance to heat and drought are crucial to restrict yield losses (Furbank and Tester, 2011). Considerable genetic diversity in wheat germplasm for thermo and drought tolerance has been observed (Lopes *et al*., 2013, 2014; Sun *et al*., 2013; Chand *et al*., 2014; Tadesse *et al*., 2015). Owing to their genetic diversity, multi-allelic nature, high reproducibility and co-dominant inheritance SSRs are the most preferred markers for genetic diversity, linkage mapping and MAS assisted selection (Jaiswal *et al*., 2017, Sajjad *et al*., 2018). These markers have been harnessed extensively for assessment of genetic diversity and molecular genetic mapping of wheat (Abbasov, 2018; Henkrar *et al*., 2016). Sadat *et al.* (2013) affirmed use of various SSR markers linked with heat and drought tolerant traits in MAS for screening heat tolerant wheat genotypes. The SSRs used by him were linked with various heat and drought tolerant traits like Heat Susceptibility Index/ single kernel weight of main spike, HSI/grain filling duration and HSI/kernel weight under heat stress in MAS for screening wheat genotypes to heat stress.

Marker assisted selection provides better understanding of the underlying genetic basis of stress tolerance in crops (Liu *et al*., 2006; Momcilovic and Ristic, 2007). Responsiveness of different heat tolerant genotypes for enzymes like NRA (Nitrate reductase) and Peroxidase along with heat shock proteins is an indicative of thermo-tolerance. Due to difficulty in phenotype selection and general complexicity of stress tolerance MAS is the most suitable approach to impart stress tolerance.Validating QTLs linked to heat and drought stress in wheat would help to improve breeding efficiency and increase genetic gains (Tadesse *et al*., 2015). Efforts have been made to improve heat and drought tolerance of wheat through traditional breeding programmes, and studies have utilized molecular characterization and physiological traits for the characterization of genetic diversity (Semenov and Halford, 2009). Conventional wheat lacks the genetic diversity for heat and drought tolerance. Therefore, identification of allelic diversity in wheat germplasm has been a major breeding objective.Identification and characterization of alleles conferring tolerance to combined effect of heat and drought would be beneficial for wheat breeding programmes for the development of high-yielding, stable and heat-drought tolerant genotypes.

2. Material and methods

2.1 Plant material

The experimental material consisted of 36 diverse wheat cultivars procured from ICAR- Indian Institute of Wheat and Barley Research, Karnal. Seeds were surface sterilized by 0.1% $HgCl_2$ for 2 min and were germinated on filter papers in dark room at room temperature. After germination the seedlings were transferred to growth chamber under controlled light and humidity conditions (16 h light, 500 µmol fluorescent light, 22.15°C day/ night temperature with 60% humidity). After 8-9 days sufficiently grown leaf samples were harvested for DNA extraction.

2.2 DNA extraction and PCR amplification

DNA extraction was performed using CTAB method devised by Doyle and Doyle (1990) with little modifications (Sharma *et al*., 2014). Approximately 100 mg of leaf samples were crushed in 1.33 × CTAB buffer and incubated at 65°C for 30 min. The quality of DNA was evaluated on 0.8% agarose gel electrophoresis and quantification was done using nanodrop.

PCR profiling of all the 36 wheat genotypes was done using already reported 24 markers associated with QTL markers for heat and drought (Kumar *et al*., 2012; Gupta *et al*., 2017; Jaiswal *et al*., 2017). PCR reaction was performed in a volume of 20 µl containing 1 µl of DNA (25 ng), 10 µl of PCR master mix (Thermofisher Green taq), 10 µl of both primers (0.5 µl each of R and F primer) and 0.025 µl of DNA Polymerase and DNAse free water is added to make up a volume of 20 µl. PCR programme was run on Gradient thermal cycler S 1000 TM (BioRad). PCR amplification was performed with initial denaturation at 94°C for 5 min, followed by 35 cycle of 30 s at 94°C, 1min

at annealing temperature 55–60°C, 1 min elongation at 72°C and final extension at 72°C for 10 min. The amplified PCR products were electrophoresis in 0.5 × TAE buffer at 70 V on a 2.0% agarose gel. Gels were stained with ethidium bromide and DNA bands were visualized under UV light using Gel Documentation System (Alpha Imager, Systems and Control). The size of amplification bands was estimated by comparison with a 100-bp standard molecular weight (Merck Chemicals).

2.3. Data analysis

Dendrogram was performed using DARWIN (SAS 9.3 NC).The DNA fingerprint patterns obtained were converted into binary data matrices containing arrays of 0s and 1s. The SSR bands were scored visually for the presence (1) or absence (0) of bands of various mol. wt. sizes. Only polymorphic and reproducible bands were considered for further analysis. The allele number and polymorphic information content (PIC) (Botstein *et al.* 1980) were calculated using Power marker software (Liu and Muse, 2005).

3. Results and discussion

3.1. Heat tolerance patterns of SSR polymorphism and genetic diversity

In this study 24 already reported heat and drought (Table 1) were validated on a set of wheat cultivars to characterize the allelic diversity for heat and drought stress. The physiological traits associated with heat tolerance of these wheat cultivars are already reported (Sharma *et al.*, 2016). The total number of alleles and genetic diversity for heat and drought tolerance were determined for these genotypes and are presented in Table 1. Out of the 24 SSR markers 19 were found polymorphic and rest 5 were found monomorphic. The polymorphic markers were found to be distributed across different wheat chromosomes. The no of alleles for polymorphic SSR markers varied from 2 to 5 with *cfd43* and *Xbarc76* showing maximum no. of alleles. The PIC values that estimate the discriminating ability of any locus by considering the number of alleles per locus and their relative frequency varied according to markers (Anderson *et al.*, 1993).The mean value of PIC was 0.39 and ranged between 0.02 to 0.65 for the SSR markers *Xgwm293* and *Xgwm111* respectively. The SSR markers *Xgwm471, Xgwm389, Xgwm264, Xgwm146, cfd19, cfd233, Xgwm111, Xgwm148,Xbarc76* and *Xbarc128* were found to possess high polymorphism. These markers revealed the allelic diversity of 36 wheat genotypes for heat and drought stress tolerance. The banding pattern for SSR marker *Xgwm111* decipher considerable genetic variability as shown in Fig 1A. *Xgwm111* yielded 1- 3 alleles and the variation in molecular weight of the bands represent significant allelic diversity for heat and drought stress among these genotypes. *Xgwm389* amplified as a single band for most of the genotypes and the amplicon molecular weight varied significantly in certain genotypes suggesting considerable genetic diversity among the tested wheat genotypes (Fig. 1B). Considering polymorphism, the chosen markers are very informative. The marker *Xgwm63*-7A (PIC = 0.364) and *Xgwm133*-6B (PIC = 0.618) have been previously reported to be linked with the QTL for heat tolerance in wheat (Mohammadi *et al.*,2008). Our results corroborate findings of (Sharma *et al.*, 2016; Sharma *et al.*, 2014; Bousba *et al.*, 2012; Khanjari *et al.*, 2007). Rai *et al.*(2017) also validated SSR markers linked with drought and heat QTLs in bread wheat and found that six SSR markers were appropriate for selection of drought and heat tolerant lines for use in marker assisted backcross breeding programs.Therefore, these markers could be exploited in wheat breeding programme for heat and drought tolerance and the wheat genotypes thus identified to be genetically diverse at allelic level can be exploited in breeding programs.

3.2. Cluster analysis of marker based data

Cluster analysis was carried out to classify the 36 wheat cultivars for genetic variation in heat tolerance. The dendrogram constructed using SSR markers data assigned the wheat cultivars into three clusters (Fig.2). Cluster I consisted of twelve genotypes (GW322, MACS2496, NIAW34, VL616, PBW550, UP2338, HD2687, WH1080, RAJ4083, WH730, PBW343, DBW17). Cluster II consisted 17 cultivars (RAJ4079, RAJ4037, RAJ4210, WR544, MP4010, K7903, NI5439, MACS6145, HI1500, HUW510, HD2808, C306, WH147, UP2382, DBW14, DBW90, CBW38), while Cluster III had seven genotypes (PBW590, HD2967, HUW468, HD2932, HS240, NW1014, RAJ3765). Paudel *et al.* (2019) in a similar study determined genetic diversity of wheat genotypes for heat and drought tolerance using SSR markers and clustered 20 wheat genotypes into 5 major clusters. In present study the heat tolerant genotypes clustered in different groups

Table 1: SSR markers that were found polymorphic for heat and drought tolerance

Sl. No.	Marker	Primer sequence	Chromosomal location	No of allele	PIC
1	*Xgwm66*	F 5'CCAAAGACTGCCATCTTTCA 3' R 5'CATGACTAGCTAGGGTGTGACA 3'	4B	2	0.46875
2	*Xgwm95*	F 5'GATCAAACACACACCCCTCC 3' R 5'AATGCAAAGTGAAAAACCCG 3'	2A	2	0.496327
3	*Xgwm99*	5'AAGATGGACGTATGCATCACA 3' 5' GCCATATTTGATGACGCATA 3'	-	2	0.498457
4	*Xgwm337*	5'CCTCTTCCTCCCTCACTTAGC 3' 5' TGCTAACTGGCCTTTGCC 3'	1B, 1D	2	0.499314
5	*Xgwm493*	F 5'TTCCCATAACTAAAACCGCG 3' R 5'GGAACATCATTTCTGGACTTTG 3'	3B	3	0.634259
6	*Xgwm495*	F 5'GAGAGCCTCGCGAAATATAGG 3' R 5'TGCTTCTGGTGTTCCTTCG 3'	4B	3	0.368827
7	*Xgwm389*	F 5' ATCATGTCGATCTCCTTGACG 3' R 5' TGCCATGCACATTAGCAGAT 3'	3B	2	0.165123
8	*Xgwm264*	F 5'GAGAAACATGCCGAACAACA 3' R 5'GCATGCATGAGAATAGGAACTG 3'	3B	2	0.413194
9	*Xgwm471*	F 5'CGGCCCTATCATGGCTG 3' R 5'GCTTGCAAGTTCCATTTTGC 3'	7A	3	0.373016
10	*Xgwm146*	F 5'CCAAAAAAAACTGCCTGCATG 3' R 5'CTCTGGCATTGCTCCTTGG 3'	7B	2	0.202449
11	*Xgwm148*	F 5'GTGAGGCAGCAAGAGAGAAA 3' R 5'CAAAGCTTGACTCAGACCAAA 3'	2B	3	0.414201
12	*Xgwm293*	F 5'TACTGGTTCACATTGGTGCG 3' R 5'TCGCCATCACTCGTTCAAG 3'	5A	2	0.057093
13	*Xgwm111*	F 5' TCTGTAGGCTCTCTCCGACTG 3' R 5' ACCTGATCAGATCCCACTCG 3'	7B, 7D	4	0.716441
14	*Xbarc76*	F 5' ATTCGTTGCTGCCACTTGCTG 3' R 5' GCGCGACACGGAGTAAGGACACC 3'	2A, 6B, 7D	5	0.670976
15	*Xbarc186*	F 5'CGCTTCCCATAACGCCGATAGTAA R 3' 5'CGCCCGCATCATGAGCAATTCTATCC 3'	5A	4	0.599184
16	*cfd43*	F 5' AACAAAAGTCGGTGCAGTCC 3' R 5' CCAAAAACATGGTTAAAGGGG 3'	2D	5	0.768833
17	*wmc273*	F 5'AGTTATGTATTCTCTCGAGCCTG 3' R 5'GGTAACCACTAGAGTATGTCCTT 3'	7A	4	0.6218
18	*wmc455*	F 5'GCGTCATTTCCTCAAACACATC 3' R 5'AGAAGGAGAAGTGCCTCACCAA 3'	2A	2	0.3698
19	*wmc473*	F 5'TCTGTTGCGCGAAACAGAATAG 3' R 5'CCCATTGGACAACACTTTCACC 3'	4D	2	0.3415
20	*wmc11*	F 5'TTGTGATCCTGGTTGTGTTGTGA 3' R 5'CACCCAGCCGTTATATATGTTGA 3'	3A	4	0.6445
21	*wmc181*	F 5'TCCTTGACCCCTTGCACTAACT 3' R 5'ATGGTTGGGAGCACTAGCTTGG 3'	2A	4	0.5752
22	*wmc364*	F 5'ATCACAATGCTGGCCCTAAAAC 3' R 5'CAGTGCCAAAATGTCGAAAGTC 3'	7B	3	0.4768
23	*cfd19*	F 5' TACGCAGGTTTGCTGCTTCT 3' R 5' GGAGTTCACAAGCATGGGTT 3'	1D	4	0.3877
24	*cfd233*	F 5' GAATTTTTGGTGGCCTGTGT 3' R 5' ATCACTGCACCGACTTTTGG 3'	2D	2	0.2642

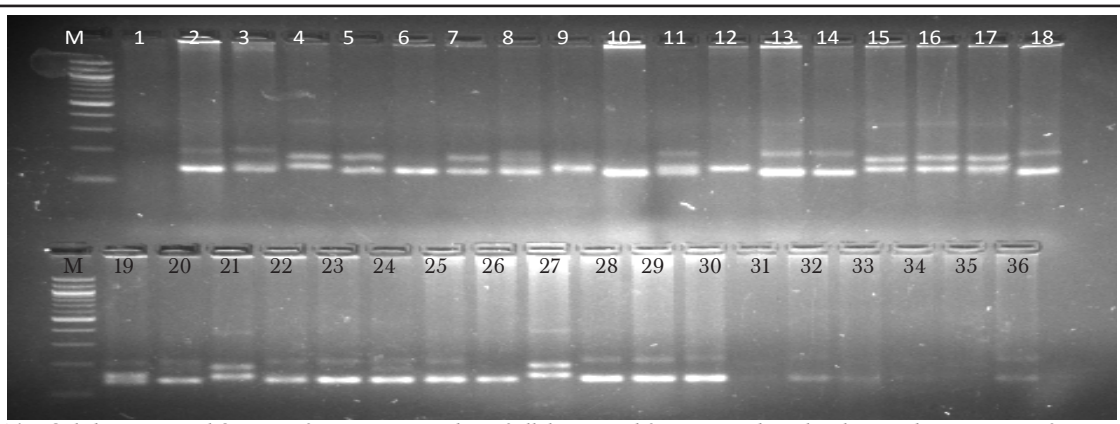

Fig1A: Gel showing amplification of *Xgwm111*, Number of alleles ranged from 1-3 with molecular weight in a range of 140-200

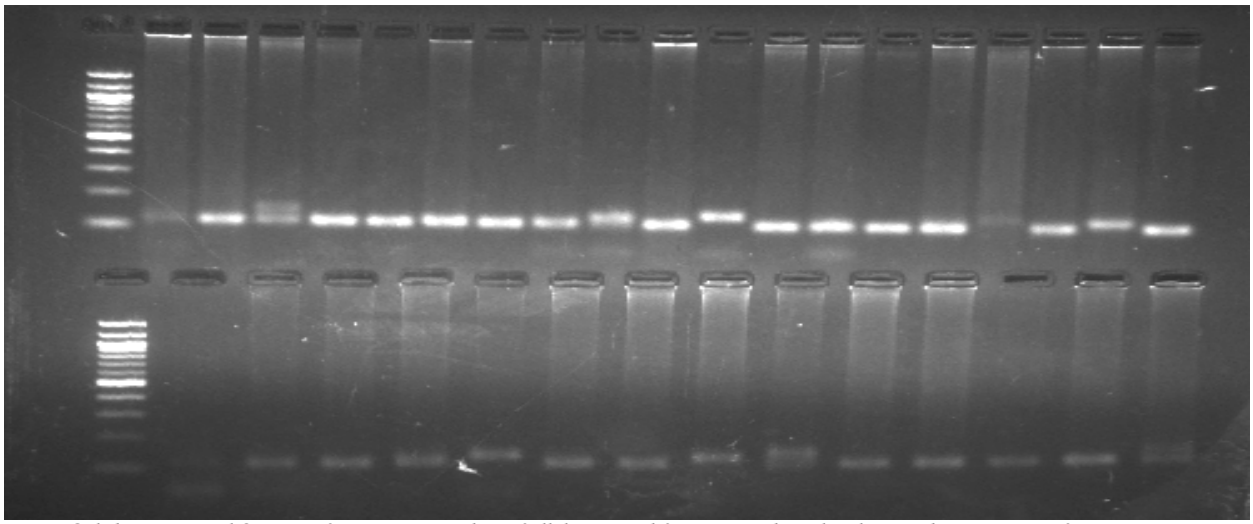

Fig1B: Gel showing amplification of *Xgwm389*,number of alleles ranged from 1-2 with molecular weight in a range of 110-130

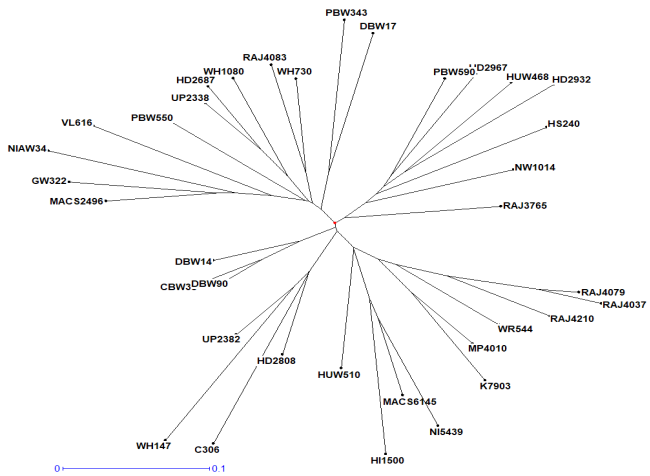

Fig. 2: UPGMA dendrogram based on genetic variation for heat and drought stress tolerance obtained through STRUCTURE analysis

highlighting different genetic basis of tolerance in various genotypes. Bhusal *et al.* (2017) also mapped 24 QTLs for grain yield components in wheat under heat stress in cultivar HD2808.

4. Conclusion

Genetic diversity among thirty-six wheat genotypes was assessed using already reported SSR markers. Genetically diverse heat and drought tolerant wheat genotypes thus identified provides the great potential to breed heat and drought tolerant wheat varieties. This information will be useful to identify new source of tolerance for breeding programme for improved wheat varieties capable of facing the environmental challenges.

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