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MOLECULAR CHARACTERIZATION AND VALIDATION OF MICRO-SATELLITE MARKERS LINKED WITH GRAIN YIELD COMPONENTS FOR DROUGHT TOLERANCE IN WHEAT (*Triticum aestivum* L.)

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ABSTRACT

The present study was aimed to enhance breeding efficiency, parental genetic distance and conferring drought tolerance in wheat. Eight, diverse wheat genotypes were selected as parents, crosses attempted in a diallel fashion (excluding reciprocals) and 28 F_1s developed. One hundred six F_2 plants taken from cross between DBW 17 (drought susceptible) and WH 1080 (drought tolerant) were evaluated. Grain yield showed significant positive correlation with effective tillers plant^{-1}, grains spike^{-1}, biomass plant^{-1} and harvest index and significant negative correlation with days to heading and days to anthesis in F_2 populations indicating the feasibility of improving grain yield. A total of 42 amplicons were detected in 106 F_2 plants and the number of amplicons locus^{-1} ranged from 1-3 with an average of 2.10 amplicons locus^{-1}. In cross DBW 17 × WH 1080 dendrogram was constructed and genotypes got clustered into eight major groups at the similarity coefficient value of 0.56. Cluster I included drought susceptible parent DBW 17 and cluster II had tolerant drought parent WH 1080. The markers showed 3.7 and 18.0% phenotypic variation in drought condition traits viz., effective tillers plant^{-1} and coleoptile length, respectively. Marker *Xgwm573* was found associated with days to heading and days to anthesis and *Xgwm369* with 1000-grain weight, coleoptile length and chlorophyll content.

Key words: Drought tolerance, microsatellites (SSRs), single marker analysis, variability, wheat

INTRODUCTION

Wheat (*Triticum aestivum* L. em. Thell) is the world's leading cereal grain, and worldwide India is the 2nd largest producer of wheat. In India, wheat is grown on an area of 31.34 million ha with a productivity of 30.61 q ha^{-1} (Anonymous, 2014). In Haryana, wheat is grown on an area of 2.50 million ha with a productivity of 47.22 q ha^{-1} (Anonymous, 2014). Drought stress tolerance has been seen in almost all crop plants but its extent varies from species to species and even within species. Improving drought tolerance and productivity is one of the most difficult tasks for cereal breeders (Devi *et al.*, 2011). In most of the breeding programmes, genetic improvement in drought tolerance is done through selection for yield. Wheat grain yield are highly affected by biotic and abiotic stress or environmental stresses (Shamsi *et al.*, 2010; Khavarinejad and Karimov, 2012). Drought is one of the most common environmental stresses that affect growth and development of plants (Nezhad-ahmadi *et al.*, 2013). Molecular markers are best tools used to determine the level of genetic

diversity among the plants and provide detailed characterization of genetic resources. SSR markers play important role in cultivar identification and genetic diversity assessment. These techniques provide better understanding of genetic basis of drought tolerance as well as provide an opportunity to dissect and understand the key processes involved in plant growth development (Devi *et al.*, 2011). Therefore, selection through molecular markers shall result in more precise targeting of factors limiting yield and also in faster rate of yield improvement in abiotic stress. Selection for high grain yield is an important focus in wheat breeding programs. Grain yield is a complex trait and usually controlled by a number of quantitative trait loci (QTL) with minor effects. The genetic variability of plants has been assessed more efficiently after the introduction of the method that reveals polymorphism directly at DNA level. Morphological markers with their complex and undeciphered genetic control are used in identification and diversity studies but they often may be affected by environmental factors and cultivation practices (Khan *et al.*, 2000).

The molecular markers are important tools for improving the efficiency of traditional plant breeding by facilitating indirect selection through molecular markers linked to the genes of the traits of interest. These markers are uninfluenced by environment and can be scored at all stages of plant growth. Microsatellites or simple sequence repeats (SSRs) serve as efficient tool in diversity studies to identify the degree of genetic similarity. SSRs are interspersed ubiquitously in the DNA of hexaploid wheat (Roder *et al.*, 1998). The chromosome-specific feature of SSR markers was also valuable for localizing linked amplicons and detecting the QTLs of interest. The quantification of drought tolerance often poses serious difficulties. Direct selection under field conditions is generally difficult as uncontrollable environmental factors adversely affect the precision and repeatability of such traits. Molecular and genetic approaches to identify and confer tolerance to abiotic stress shall not only facilitate marker-assisted breeding for drought tolerance but also shall pave the way for cloning and characterization of underlying genetic factors which could be useful in engineering plants with improved drought tolerance. The present study was aimed to enhance breeding efficiency, parental genetic distance and conferring tolerance to abiotic stress based on morphological and molecular markers (SSR).

MATERIALS AND METHODS

Yield and yield attributes of crosses

In present study eight diverse wheat genotypes (WH 730, WH 283, PBW 343, Raj 3765, DBW 17, WH 1080, HD 2967 and KRL 210) were selected as parents on the basis of their origin, adaptability, diversity, yield potential and drought tolerance characters. Crosses were attempted during *rabi* 2010-11 in diallel fashion (excluding reciprocals) at the research area of Wheat Section, Department of Genetics & Plant Breeding, CCSHAU, Hisar (India). Each genotype was crossed with all other selected parent and 28 F_1 hybrids developed. Further, F_1s were grown during off-season at IARI Regional Research Station, Wellington, Tamil Nadu (India) to obtain F_2 generation.

Two hundred six F_2 plants taken from the cross between DBW 17 (drought susceptible) and WH 1080 (drought tolerant) were evaluated during *rabi* 2011-12 for various morphological, yield components (days to heading, days to anthesis, days to maturity, plant height, effective tillers plant^{-1}, grains spike^{-1}, 1000-grain weight, grain yield plant^{-1}, biomass plant^{-1} and harvest index) and drought related traits (coleoptile length, chlorophyll content and chlorophyll fluorescence) under drought condition at research area of Wheat Section, Department of Genetics & Plant Breeding, CCSHAU, Hisar (India). The F_2 population from cross DBW 17 x WH 1080 along with parents (drought susceptible and drought tolerant) were grown in 10 rows of 2 m length under drought environment with date of sowing as 15 December, 2011 (late sown). No irrigation was applied. The data was recorded from 200 single plants of the cross, DBW 17 x WH 1080 along with their parents (average 5 plants) in stress environment only. For days to heading, the numbers of days were counted from

the date of sowing to the date of complete emergence of 1st spike, for days to anthesis from the date of sowing to date of 1st opening of flower and for days to maturity, date on which whole plant become yellowish in colour was recorded. The plant height was measured for each plant from ground level to the top of upper spikelets of main spike (excluding awns) at maturity. Effective tillers and grains plant^{-1} were counted for each plant. Thousand grains were counted from bulk of grain samples from each plant and weighed using electronic single pan balance. Grain yield plant^{-1} was also noted. Harvest index and the ratio of economic yield (grain) to biological yield were calculated as per Donald (1962):

$$\text{Harvest index (\%)} = \frac{\text{Grain yield/ plant (g)}}{\text{Biological yield/ plant (g)}} \times 100$$

Flag leaf chlorophyll content (Chl) was measured by a SPAD- chlorophyllmeter (Konika Minolta) and for chlorophyll fluorescence measurements, original fluorescence (F_O), maximum fluorescence (Fm), variable fluorescence (Fv) and maximum quantum yield of photosystem-II were recorded from flag leaves using a portable handy chlorophyll fluoremeter (model OS-30 p, Opti sciences, USA) at anthesis stage.

Molecular characterization

Fresh leaves from 2-3 week old seedlings of 106 selected F_2 plants of cross, DBW 17 × WH 1080 along with parents (drought susceptible and drought tolerant) were taken for DNA extraction of subsequent genotyping during 2011-12 winter season. The chemicals used for DNA extraction and PCR amplification were procured from Sigma Chemicals (USA) and Life Technologies (India).

Molecular markers and genomic DNA isolation: Eighty five simple sequence repeat (SSR) markers widely distributed on different wheat chromosomes were used in this study (*http://wheat.pw.usda.gov*). The molecular markers and their sources were taken from *www.graingenes.marker.com* and *http://wheat.pw.usda.gov* websites. Genomic DNA was isolated from each parental genotype and 106 F_2 population using CTAB method (Saghai-Maroof *et al*., 1984). A small amount of fresh leaf tissue (5.0 g) was taken from 21-23 day old plants, and finely ground using liquid nitrogen in a sterilized pestle and mortar. The leaf tissue powder was transferred into sterilized polypropylene centrifuge tubes to which 15 mL preheated (65ºC) CTAB buffer was added and the samples thoroughly mixed by inverting the tubes several times. The tubes were incubated at 65°C in water-bath for 90 min. and inverted a few times during incubation so as to mix the contents. The samples were then cooled to room temperature and 15 mL chloroform and isoamyl alcohol (24:1) solution added to it. The samples were again mixed thoroughly by gently inverting the tubes several times. Tubes were centrifuged for 10 min. at 10,000 rpm in a REMI C24 centrifuge and the upper aqueous layer transferred to fresh pre-sterilized, labeled centrifuge tubes and again centrifuged with chloroform: isoamyl alcohol (24:1) mixture. The extracted layer was transferred to centrifuge tube and equal volume of ice-cold isopropanol subsequently added to precipitate DNA and the tubes kept undisturbed for 15 min. The DNA was spooled out and put into 1.5 mL eppendorf centrifuge tubes. The DNA was washed with 70% ethanol and DNA pellet air-dried overnight at room temperature and subsequently dissolved in appropriate volume of TE buffer. Samples were stored at -20°C till further use.

Quality and quantity estimation of genomic DNA: Agarose gel (0.8%) electrophoresis was used to check the quality and quantity of genomic DNA by running DNA sample along with standard marker. The gel was visualized in a UV trans-illuminator. The DNA concentrations were estimated by visual assessment of band intensity in comparison with Lambda (λ) DNA of known concentration. The quality of DNA samples was estimated as good (if sharp and discrete band appeared), sheared (if smear was there), improper dissolution (if thin lane appeared).

Polymerase chain reaction (PCR) amplification: Out of 85 primers, 20 primers resulted in polymorphic bands between parents and were used for genotyping of F_2 populations.

Standardization of PCR condition was done by using DNA from parental genotypes. Different options for genomic DNA concentrations (25, 50 and 100 ng), Taq DNA (1, 1.5 and 2 units) and annealing temperature (55 and 60°C) were tried and PCR conducted in a reaction volume of 20 µL containing 2 µL 1X PCR buffer, 0.5 µL dNTPs, 0.5 µL of each primer, 1.5 unit Taq DNA polymerase and 25 ng genomic DNA. PCR amplification was carried out in MJ Research PTC – 0150 machine for parents and for 106 F_2 population amplification was carried out in PCR machine 'Benchtop Lab Systems-BT-B960' using conditions; initial denaturation (95°C for 5 min.), denaturation (94°C for 1 min.), annealing (50°C-65°C for 1 min.), extension (72°C for 1 min.) and final extension (72°C for 10 min.). Amplified products were stored at -20°C till further use.

Agarose gel electrophoresis: PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels. Agarose was melted in 1.0 X TBE buffer and ethidium bromide (5µg mL^{-1}) added. Gel solution was then poured into gel casting plate and appropriate comb inserted. After gel setting the comb was removed gently and sealing tapes removed from both the ends. Gel plate was placed in electrophoresis chamber and submerged using 1X TBE buffer. DNA samples were prepared by adding 2X loading dye and pulse centrifuged for proper mixing. The samples were loaded in the wells and electrophoresis carried out at constant voltage (3 v/cm of gel) until dye migrated to the other end of gel. PCR amplified products were viewed under UV light (350 nm) fluorescence and photographs taken by gel documentation.

Amplicon scoring and data analysis: SSR amplification profiles were scored visually based on the presence (taken as 1) or absence (taken as 0) of bands for each wheat genotype and with A and B codes for presence of specific band in susceptible and tolerant parent, respectively, as: A – homozygous susceptible, B - homozygous tolerant and H - heterozygous. Only clear and unambiguous bands were scored. The size (in nucleotide base pairs) of amplified bands was determined based on its migration relative to standard molecular size marker. The heterozygosity of a locus is defined as the probability that an individual is heterozygous for the locus in a population (Liu, 1998) and is calculated as:

$$H = 1 - \sum_{i=1}^{l} P_i^2$$

where P_i is the frequency for the i^{th} allele among a total of l alleles. PIC refers to the value of a marker for detecting polymorphism within a population, depending upon the number of detectable alleles and the distribution of their frequency. Thus it provides an estimate of discriminating the power of marker. The PIC value of an l-allele locus were calculated as (Nagy *et al.*, 2012)

$$\mathbf{PIC} = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2 P_i^2 P_j^2$$

where P_i and P_j are the population frequency of the i^{th} and j^{th} allele.

Molecular markers used for comparison and their sources, accession number and references are mentioned on *www.graingenes.marker.com*. The frequency of polymorphism between different F_2 plants of wheat for each type of SSR marker was calculated based on presence or absence of band (Ghosh *et al.*, 1997). The 0/1 matrix was used to calculate the genetic similarity distance using 'SIMQUAL' sub-programme of NTSYS-pc version 1.6 exeter software (Rohlf, 1990). Dendrogram was constructed by using distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc.

Statistical analysis

The genotypic (A, B and H code) and phenotypic data obtained from 106 F_2 population produced from the cross DBW 17 × WH 1080 was subjected to single marker analysis. The association between SSRs and phenotypic trait was assessed with simple regression analysis approach using one-factor ANOVA method as implemented in Microsoft Excel (MS Office 2007) programme. Magnitude of the marker associated with phenotypic effect was described by the coefficient of determination (%R^2) which represented the fraction of variance explained by the polymorphism of marker.

RESULTS AND DISCUSSION

Field evaluation of DBW 17 × WH 1080 F_2 plants

The plants from F_2 population of cross DBW 17 × WH 1080 exhibited wide range of variation for all the characters studied especially with respect to the yield, yield components and drought-related traits (Table 1). Grain yield plant^{-1} varied from 7.53 - 32.81 g. In parents DBW and WH 1080, the grain yield plant^{-1} was 12.84 and 13.47 g, respectively. Two hundred F_2 plants were taken for the measurement of morphological and drought-related traits and analysis of correlation coefficient analysis; while 106 F_2 plants were taken for molecular characterization. Out of 200 F_2 plants, grain yield plant^{-1} above the mean value of 18.14 g was observed in 83 plants. The segregating 200 F_2 populations displayed large variation for various quantitative and drought related traits.

Correlation coefficient analysis

A positive correlation of grain yield was observed with plant height (0.31**), tillers plant^{-1} (0.78**), grains spike^{-1} (0.46**), biomass plant^{-1} (0.96**), harvest index (0.36**) and chlorophyll content (0.17*), and a negative correlation with days to heading (-0.41**) and days to anthesis (-0.34**) [Table 2]. These findings are in conformity with Guendouz *et al*. (2012). Tomar *et al*. (2012) have reported that plant height, tillers plant^{-1}, grains spike^{-1}, biological yield and 1000-kernel weight depict significant positive correlation with grain yield in recombinant inbred lines.

Molecular characterization of F_2 population of cross DBW 17 x WH 1080

In present investigation SSR markers were used to study polymorphism among two wheat genotypes DBW 17 and WH 1080; and after screening 20 polymorphic SSR primers were selected (Table 3). Database of parental genotypes was generated visually using 20 SSRs based on presence or absence of the band (Fig. 1 and 2). The microsatellite or SSR primers generated 42 amplicons with the number of amplicons primer^{-1} varying from 1 to 3 with an average 2.1 amplicons primer^{-1} (Table 4). Similar results have been reported by Schuster *et al*. (2009) in a study on 36 wheat genotypes using 23 SSR markers and reported that a total number of amplified amplicons varied between 2 and 5 with an average of 3.2 amplicons locus^{-1}. The polymorphism ranged from as low as 20% to as high as 100% with average polymorphism of 23.0%. Ijaz and Khan (2009) also have reported high level of polymorphism ranging from 10.52 to98.42%. Bousba *et al*. (2012) found that polymorphism information content (PIC) values ranged from 0.38 to 0.94 with an average of 0.74. In our study the size of amplified DNA fragments varied from approximately 100 to 300 bp.

Table 1: Range, mean, CV and SD for various yield, yield components and drought related traits in DBW 17 × WH 1080 F_2 population of wheat

Traits	DBW 17	WH 1080	DBW 17 × WH 1080 F_2 population			
			Mean	Range	CV	SD
Days to heading (No.)	91.07 ± 0.98	89.80 ± 0.76	94.72 ± 0.18	90.00-102.00	2.70	2.54
Days to anthesis (No.)	93.00 ± 0.83	92.60 ± 0.58	95.85 ± 0.17	91.00-104.00	2.58	2.47
Days to maturity (No.)	120.00 ± 1.00	120.33 ± 0.88	125.20 ± 0.15	121.00-135.00	1.73	2.17
Plant height (cm)	81.67 ± 1.92	91.37 ± 2.59	72.77 ± 0.51	43.00-87.00	9.86	7.18
Effective tillers plant^{-1} (No.)	10.10 ± 0.67	10.27 ± 0.85	10.24 ± 0.36	4.00-20.00	49.04	5.02
Grains spike^{-1} (No.)	42.87 ± 1.31	40.13 ± 0.74	40.50 ± 1.27	10.00-87.00	44.27	17.93
1000-grain weight (g)	36.27 ± 0.78	39.40 ± 0.64	35.14 ± 0.31	26.09-47.50	12.59	4.42
Grain yield plant^{-1} (g)	12.84 ± 0.88	13.47 ± 0.30	18.14 ± 0.50	7.53-32.81	38.19	6.93
Biomass plant^{-1} (g)	34.87 ± 1.55	35.77 ± 1.97	47.69 ± 1.20	20.50-89.00	35.53	6.95
Harvest index (%)	36.76 ± 0.94	32.08 ± 0.39	37.90 ± 0.28	30.22-50.82	10.26	3.89
Coleoptile length (cm)	3.47 ± 0.09	4.10 ± 0.15	3.29 ± 0.04	2.40-4.60	15.32	0.50
Chlorophyll content (%)	43.30 ± 0.88	45.08 ± 2.03	47.96 ± 0.38	32.96-61.85	11.06	5.31
Chlorophyll fluorescence (Fv/Fm)	0.65 ± 0.02	0.73 ± 0.01	0.67 ± 0.01	0.54-0.80	7.42	0.05

Table 2: Correlation coefficients among yield, yield components and drought related traits in DBW 17 × WH 1080 F_2 population of wheat

Traits	Days to heading	Days to anthesis	Days to maturity	Plant height	Effective tillers plant^{-1}	Grains spike^{-1}	1000-grain weight	Biomass plant^{-1}	Harvest index	Coleoptile length	*Chl. content	Chl. fluore-scence
Days to anthesis	0.96**											
Days to maturity	0.35**	0.28**										
Plant height	-0.37**	-0.32**	-0.23**									
Effective tillers/plant	-0.47**	-0.39**	-0.26**	0.40**								
Grains spike^{-1}	-0.39**	-0.33**	-0.23**	0.39**	0.59**							
1000-grain wt.	-0.12	-0.12	0.28**	0.33**	0.09	0.24**						
Biomass plant^{-1}	-0.42**	-0.34**	-0.12	0.35**	0.80**	0.47**	0.11					
Harvest index	-0.10	-0.08	0.01	-0.08	0.11	0.06	-0.02	0.11				
Coleoptile length	0.01	0.06	-0.14	0.21**	0.06	0.17*	0.01	0.15*	-0.08			
*Chl. content	-0.14*	-0.13	0.12	0.08	0.28**	0.36**	0.35**	0.20**	-0.08	-0.05		
Chl. fluorescence	-0.27**	-0.29**	-0.19**	-0.06	0.06	-0.01	-0.18*	0.12	0.07	-0.11	-0.02	
Grain yield	-0.41**	-0.34**	-0.11	0.31**	0.78**	0.46**	0.10	0.96**	0.36**	0.12	0.17*	0.13

*Chl – chlorophyll; *Significant at 5%, **Significant at 1% level

Genetic similarity and cluster tree analysis

Dendrogram constructed from SSR showed that F_2 individuals alongwith parents were clustered in two main clusters-I and II at similarity coefficient of 0.56 (Fig. 3). Polymorphism

Table 3: DNA fingerprinting of parental genotypes (DBW 17 & WH 1080) as assessed by 20 SSRs

SSRs	Amplicons/ lane (No.)	Band size (bp)	Sequence	Annealing temp. (^{o}C)
Xgwm99-1A	Two	110, 150	5' AAGATGGACGTATGATCACA 3' 5' GCCATATTTGATGACGCATA 3'	60
Xgwm135-1A	Two	115, 140	5' TGTCAACATCGTTTTGAAAAGG 3' 5' ACACTGTCAACCTGGCAATG 3'	60
Xgwm369-3A	Two	190, 160	5' CTGCAGGCCATGATGATG 3' 5' ACCGTGGGTGTTGTGAGC 3'	60
Xgwm160-4A	Two	160, 200	5' TTCAATTCAGTCTTGGCTTGG 3' 5' CTGCAGGAAAAAAAGTACACCC 3'	60
Xgwm156-5A	Three	170, 190, 215	5' CCAACCGTGCTATTAGTCATTC 3' 5' CAATGCAGGCCCTCCTAAC 3'	60
Xgwm573-7A	One	190	5' AAGAGATAACATGCAAGAAA 3' 5' TTCAAATATGTGGGAACTAC 3'	50
Xgwm130-7A	Two	140, 120	5' AGCTCTGCTTCACGAGGAAG 3' 5' CTCCTCTTTATATCGCGTCCC 3'	60
Xwmc695-7A	Two	200, 270	5' GAGGGCACCTCGTAAGTTGG 3' 5' GGCAGGAGCCCCTACAAGAT 3'	61
Xgwm374-2B	Two	190, 200	5' ATAGTGTGTTGCATGCTGTGTG 3' 5' TCTAATTAGCGTTGGCTGCC 3'	60
Xgwm156-3B	Three	290, 270, 325	5' CCAACCGTGCTATTAGTCATTC 3' 5' CAATGCAGGCCCTCCTAAC 3'	60
Xgwm213-5B	Two	140, 160	5' TGCCTGGCTCGTTCTATCTC 3' 5' CTAGCTTAGCACTGTCGCCC 3'	60
Xgwm234-5B	Two	130, 150	5' GAGTCCTGATGTGAAGCTGTT G 3' 5' CTCATTGGGGTGTGTACGTG 3'	55
Xgwm577-7B	Two	140, 165	5' ATGGCATAATTTGGTGAAATTG 3' 5' TGTTTCAAGCCCAACTTCTATT 3'	53
Xgwm335-5B	Two	140, 180	5' CGTACTCCACTCCACACGG 3' 5' CGGTCCAAGTGCTACCTTTC 3'	60
Xgwm337-1D	Two	200, 240	5' CCTCTTCCTCCCTCACTTAGC 3' 5' TGCTAACTGGCCTTTGCC 3'	55
Xgwm642-1D	Three	145, 155, 170	5' ACGGCGAGAAGGTGCTC 3' 5' CATGAAAGGCAAGTTCGTCA 3'	60
Xgwm320-2D	Two	220, 245	5' CGAGATACTATGGAAGGTGAGG 3' 5' ATCTTTGCAAGGATTGCCC 3'	60
Xgwm565-5D	Two	180, 155	5' GCGTCAGATATGCCTACCTAG G 3' 5' AGTGAGTTAGCCCTGAGCCA 3'	60
Xgwm469-6D	Two	150, 175	5' CAACTCAGTGCTCACACAACG 3' 5' CGATAACCACTCATCCACACC 3'	60
Xgwm350-7D	Two	160, 140	5' ACCTCATCCACATGTTCTACG 3' 5' GCATGGATAGGACGCCC 3'	60

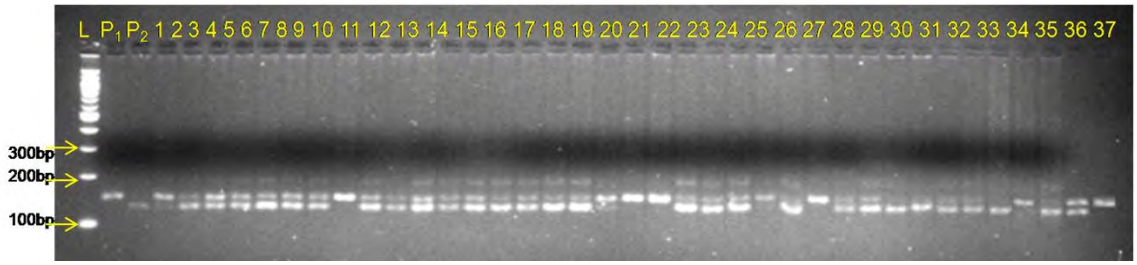

Fig. 1: Agarose gel showing allelic polymorphism among parental wheat genotypes and their F_2 (1-37) population used for molecular analysis using *Xgwm350-7D*, L = 100 bp P_1 = DBW 17, P_2 = WH 1080

Fig. 2: Agarose gel showing allelic polymorphism among parental wheat genotypes and their F_2 (1-37) population used for molecular analysis using *Xgwm156-3B*, L = 100 bp P_1 = DBW 17, P_2 = WH

Table 4: DNA amplification profile on parental genotypes DBW 17 and WH 1080 using 20 SSRs

Number of SSRs used	85
Number of markers that did not show amplification	15
Number of polymorphic markers	20
Number of amplicons detected using polymorphic markers	42
Range of amplicons	1-3
Average number of amplicons	2.10
Size of PCR products	100-300 bp

information content (PIC) values ranged from 0.56 to 0.95 and details of primers are given in Table 3. The parent WH 1080 was present in cluster-II and parent DBW 17 in cluster-I. Cluster-I comprised of drought susceptible parent-I (DBW 17) and one F_2 plant, whereas cluster-II comprised of drought tolerant parent-II (WH 1080). Thus, a wide range of genetic variability was revealed from the dendrogram. The NTSYS-pc UPGMA tree cluster analysis and two-dimensional PCA analysis revealed that two parental genotypes were quite distinct whereas 106 F_2plants interspersed between parental wheat geno-Types (Fig. 3 & 4). Genetic similarity coefficient analysis showed extensive genetic diversity (56 to 95%) among the parental genotypes, used for making cross. Similar findings have been reported by the use of microsatellites markers for the assessment of genetic diversity in wheat cultivars and their wild relatives (Hao *et al.*, 2008; Schuster *et al.*, 2009). Out of 85 microsatellite markers tested on parents, 20 were polymorphic which were used to genotype 106 individual F_2 plants. The number of polymorphic markers ranged from 1 (each on chromosome1A, 1D, 3B and 5D) to 4 (on chromosome 7A) [Table 5]. Using single marker analysis (SMA), 1 marker (*Xgwm573*) on 7A was identified to be associated with days to heading. It was common with days to anthesis. This marker explained 4.12% phenotypic variation for days to heading. SMA identified 1 SSR marker associated with days to heading and days to anthesis on chromosomes 7A. This marker explained upto 5.07% of phenotypic variation for days to anthesis.

Three markers *viz.*, *Xgwm156*, *Xgwm234* and *Xwmc695* in drought environment detected one each on 3B, 5B and 7A were validated to be associated with days to maturity explaining 7.32, 7.89 and 6.05% total phenotypic variance, respectively. Among these markers, *Xgwm234* and *Xwmc695* were common for chlorophyll fluorescence and tillers plant^{-1}, respectively. Using SMA, one marker *Xgwm213* on 5B was validated to be associated with plant height in drought condition and explained

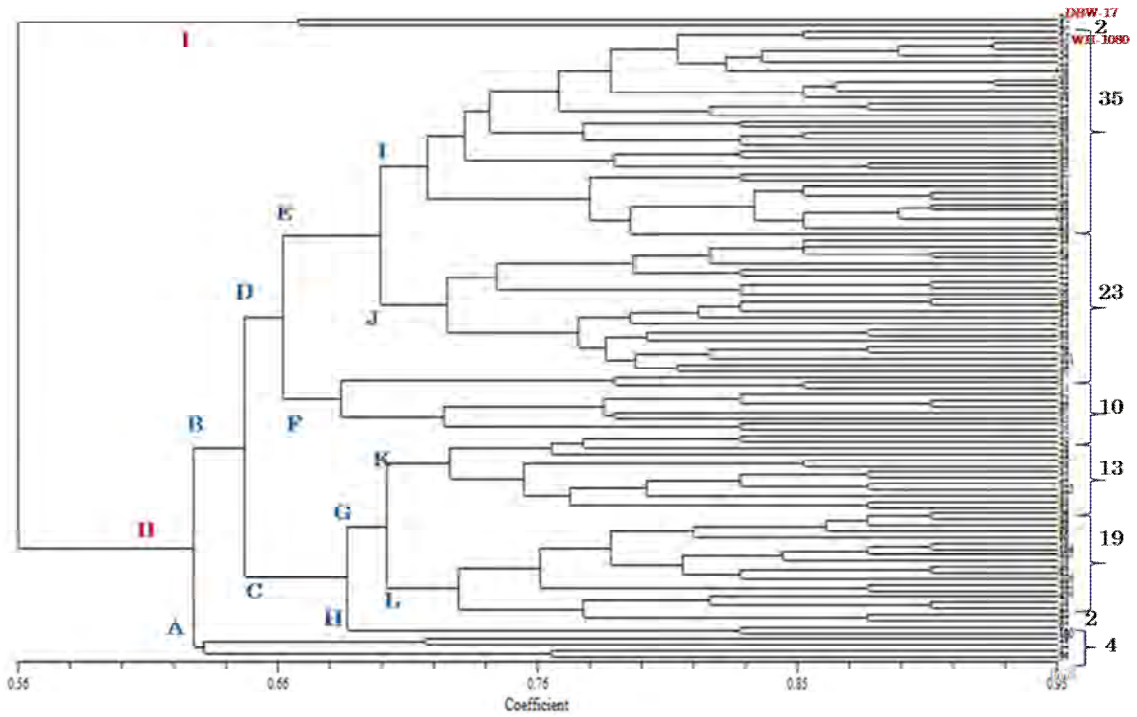

Fig. 3: Dendrogram 106 F_2 plants derived from wheat cross between DBW 17 × WH 1080 along with parental genotypes based on SSR data

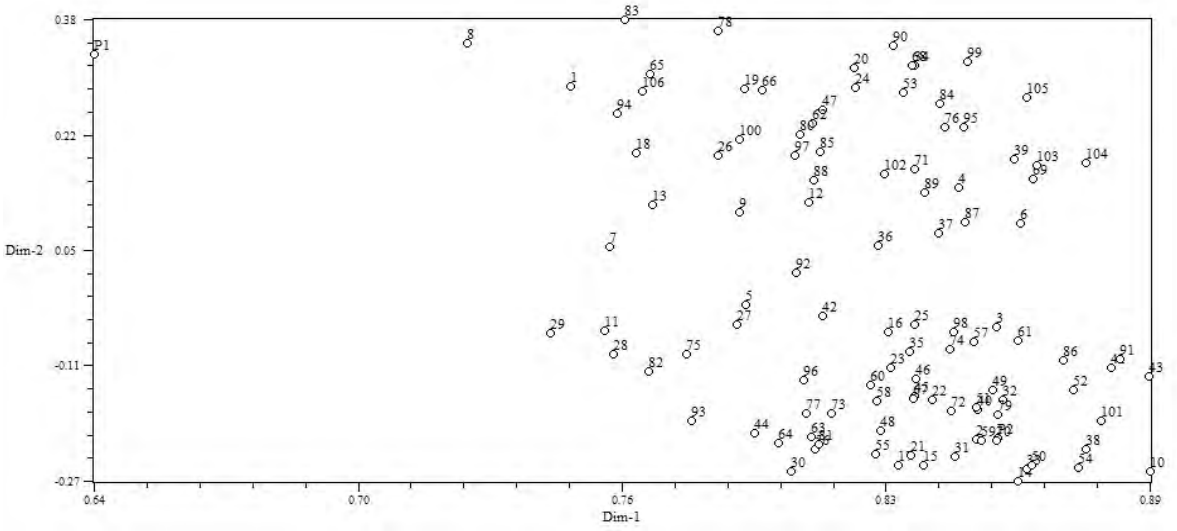

Fig. 4: 2-D picture of 106 F_2 plants derived from wheat cross between DBW 17 × WH 1080 along with parental genotypes based on SSR data

upto 5.70% phenotypic variance for plant height. Marker *Xwmc695* on 7A was detected under drought condition and explained about 3.70% phenotypic variance of tillers plant^{-1}.

Two markers (*Xgwm565-5D* and *Xgwm369-3A*) related to 1000-grain weight were validated in drought condition and explained 3.88 and 7.29% phenotypic variance. Marker *Xgwm369-3A* was also associated with coleoptile length and chlorophyll content.

Coleoptile length, chlorophyll content and chlorophyll fluorescence

The markers *Xgwm135-1A, Xgwm374-2B* and *Xgwm369-3A* were validated to be associated with coleoptile length explaining upto 18.0, 8.49 and 6.99% phenotypic variance, respectively. Using single marker analysis, two markers *Xgwm 642-1D* and *Xgwm369-3A* were validated to be associated

Table 5: Association of quantitative traits and markers detected by single marker analysis using F_2 population of cross DBW 17 × WH 1080

Trait	Markers	Location	R^2 (%)
Days to heading	*Xgwm573*	7A	4.12*
Days to anthesis	*Xgwm573*	7A	5.07*
Days to maturity	*Xgwm156*	3B	7.32*
	Xgwm234	5B	7.89*
	Xwmc695	7A	6.05*
Plant height	*Xgwm213*	5B	5.70*
Effective tillers/plant	*Xwmc695*	7A	3.70*
1000-grain weight	*Xgwm565*	5D	3.88*
	Xgwm369	3A	7.29*
Coleoptile length	*Xgwm135*	1A	18.00**
	Xgwm374	2B	8.49*
	Xgwm369	3A	6.99*
Chlorophyll content	*Xgwm642*	1D	3.75*
	Xgwm369	3A	8.11*
Chlorophyll fluorescence	*Xgwm234*	5B	5.50*

*Significant at 5%, **Significant at 1% level

with chlorophyll content in drought condition and explained upto 3.75 and 8.11% phenotypic variance for chlorophyll content. *Xgwm369-3A* was also associated with coleoptiles length and 1000-grain weight. SMA identified SSR marker *Xgwm234* associated with chlorophyll fluorescence and was present on chromosomes 5B. This marker explained upto 5.5% phenotypic variation for chlorophyll fluorescence.

Based on single marker analysis, 1, 1, 3, 1, 1, 2, 3, 2 and 1 markers were validated to be associated with days to heading, days to anthesis, days to maturity, plant height, effective tillers plant^{-1}, 1000-grain weight, coleoptile length, chlorophyll content and chlorophyll fluorescence, respectively. Some of these markers were validated to be associated with different traits. Marker *Xgwm573-7A* was associated with days to heading and days to anthesis, marker *Xgwm234-5B* was associated with days to maturity and chlorophyll fluorescence. Marker *Xwmc695-7A* was associated with days to maturity, effective tillers plant^{-1} and 1000-grain weight while *Xgwm369-3A* was associated with 1000-grain weight, coleoptile length and chlorophyll content. The markers explained between 3.70 and 18.0% of phenotypic variation in drought condition. A total of 15 putative SSRs were recorded to be linked to the traits by single marker analysis ranging from 1 for effective tillers plant^{-1}, plant height and chlorophyll fluorescence to 3 for coleoptile length.

The results of SMA revealed that about 18% phenotypic variation of coleoptile length could be explained by *Xgwm135-1A* marker in drought condition. This is in agreement with the reports of Huang *et al*. (2006) on marker-associated 1000-grain weight, Golabadi *et al*. (2011) on harvest index and Huang *et al*. (2003) on grain yield. Similarly, a minimum of 3-4% phenotypic variation for days to heading, tillers plant^{-1}, 1000-grain weight and chlorophyll content could be explained by *Xgwm573-7A*, *Xwmc695-7A*, *Xgwm565-5D* and *Xgwm642-1D* markers, respectively. Single marker analysis identified one SSR marker (*Xgwm234*) associated with chlorophyll fluorescence distributed on chromosomes 5B. This marker explained up to 5.50% phenotypic variation for chlorophyll fluorescence. Barakat *et al*. (2011) reported the single marker ANOVA analysis for heat tolerance in F_2 population which revealed that *Xgwm617*-linked QTL, *Xgwm132*-linked QTL and *Xgwm577*-linked QTL accounted for 3, 7 and 25% total phenotypic variation.

The present study suggested that SSR markers combined with single marker analysis could be used to identify molecular markers linked to coleoptile length, chlorophyll content and chlorophyll fluorescence gene as indicator for drought tolerance in wheat. Markers *viz.*, *Xgwm135*, *Xgwm374* and *Xgwm369* were associated with coleoptile length, markers *Xgwm642* and *Xgwm369* linked to chlorophyll content and marker *Xgwm234* linked to chlorophyll fluorescence. The results suggested that the selection for drought tolerance could become more efficient due to the availability of these markers linked to the drought tolerance components and these can be used in wheat breeding programs as a selection tool in early generations after validation using other mapping population.

Conclusions: The number of polymorphic markers ranged from 1 (each on chromosome 1A, 1D, 3B and 5D) to 4 (on chromosome 7A). The number of polymorphic markers on A genome was higher than on B and D genomes. The results of genetic similarity coefficient analysis showed that

extensive genetic diversity (from 56% to 95%) was present among parental genotypes which are used for making one cross. Markers *Xgwm135, Xgwm374* and *Xgwm369* were linked to coleoptile length, markers *Xgwm642* and *Xgwm369* linked to chlorophyll content and marker *Xgwm234* linked to chlorophyll fluorescence.

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