Development of mapping populations and their characterization for drought tolerance in wheat

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Abstract

Six sets of Recombinant Inbred Lines (RILs) *viz.*, HW2004/ HD2877, C306/MACS2496, NI5439/HD2012, C306/NI5439, C306/HD2851 and HD2888/HD2851 were produced by crossing drought tolerant and susceptible genotypes. A majority of traits differed between drought tolerant and the drought susceptible genotypes. The contrasting difference in mean indicated the suitability of parents for developing recombinant inbred lines. Positive correlation of plant height (r=0.531), tiller number (r=0.722), relative water content (r=0.729), thousand kernel weight (r=0.838), harvest index (r=0.733), biological yield (0.816) with yield were recorded in the genotypes. Similarly in RILs, plant height, tiller number, number of grains, biological yield and thousand kernel weight showed significant positive correlations with yield at Delhi and Indore. A total of 832 SSR markers were utilized to survey the parental polymorphism between drought tolerant (HW2004) and drought susceptible (HD2877) genotypes. Out of these 132 were polymorphic and only 91 markers covering all the 21 chromosomes were selected to characterize the parents involved in development of mapping populations. Amongst, 75.82 per cent were polymorphic between C306 and MACS2496, 58.43 between HD2888 and HD2851, 62.50 between C306 and HD2851, 56.91 percent between NI5439 and HD2012. Only 48.89 percent of SSR markers were polymorphic between drought tolerant genotypes, C306 and NI5439 which may be due to presence of similar genomic regions between the two. A higher per cent of polymorphic markers were located on B genome. Based on molecular analysis the eight genotypes involved in mapping populations were grouped into two clusters. Drought tolerant genotypes fell in one group while susceptible in second group. The mapping populations developed for drought tolerance are important resources for genetic dissection of drought tolerance in wheat.

Key words : **Wheat, drought tolerance, inbred lines, mapping population, recombinant, genetic diversity**

Introduction

Wheat is cultivated under wide range of environmental conditions in terms of temperature and moisture regimes. In the Indian sub-continent wheat production is often limited by sub optimal moisture conditions arising largely due to variations in rainfall from year to year and in different regions. Even under irrigated conditions, water for agronomic uses will increasingly become a limiting factor affecting wheat productivity. The availability of water is more important in dry and semi-arid regions, where it is a rising threat in the world [1-2]. Progress in developing drought tolerant cultivars has been slow due to difficulties in direct selection due to low heritability and significant genotype x environment interactions, lack of proper understanding of mechanisms of drought tolerance and poor understanding of inheritance of drought tolerance. Drought tolerance is a quantitative trait under complex genetic control [3]. It has been estimated that only 25 per cent of potential crop yield is realized under unfavourable environmental stresses [4]. Drought is a dynamic phenomenon and shows variations in frequency, duration and intensity thus making it difficult to select for drought tolerant genotypes. Selection for drought tolerance along with high productivity under favourable environments has been difficult [5]. Several physiological and biochemical traits have been proposed for enhanced drought tolerance but only a few have been demonstrated to be causally related with expression of tolerance to moisture stress under field conditions [6]. Genetic dissection of grain yield and its components are useful in elucidating the physiological route from gene-to phenotype for grain yield. Genetic associations of different agronomical traits with grain yield have been reported earlier [7-10].

In the last two decades, molecular markers have been increasingly used in crop improvement. However, success of marker assisted selection for a complex trait such as drought tolerance depends on identification of strong marker - trait (QTL) associations. Development of suitable mapping population is a pre-requisite for genetic dissection of complex traits using morpho-physiological and molecular markers. In this communication we describe the development of mapping populations, which can be useful in identifying QTLs associated with drought tolerance in wheat (*Triticum aestivum* L.).

Materials and methods

Plant material

The plant material comprised four each of drought tolerant (C306, HW2004, NI5439 and HD2888) and drought susceptible (HD2877, HD2012, MACS2496 and HD2851) genotypes and six sets of Recombinant Inbred Lines (RILs) derived from the crosses. The drought tolerant genotypes were selected on the basis of their cultivation under rain-fed situations over a long period of time in different ecological zones of India. The genotypes which are suitable in irrigated conditions were considered as drought susceptible. The pedigree and characteristics features of parents are given in Table 1. These mapping populations were derived from the crosses *viz.*, HW2004/HD2877 (F_8), C306/NI5439 (F_8),

NI5439/HD2012 (F_8), C306/MACS2496 (F_7), C306/HD2851 (F_7) and HD2888 /HD2851 (F_7) were developed following single spike descend method. From each F_2 plant a single spike was advanced to F_3 generation as progeny row. In F_3 and subsequent generations a single spike was taken randomly from each progeny row for advancement to next generation.

Phenotypic characterization of parents and RILs

The parents involved in six mapping populations were evaluated under moisture stress conditions for phenotypic characterization. Parental lines taken were highly contrasting phenotypically for the target trait (highly drought tolerant and susceptible lines). The genotypes were planted in two rows of 1.5m each, 30cm apart in two replications at normal seed rate (100 kg/ha) during 2008-09 and 2009-10. Only pre-sown irrigation was provided to ensure germination. Data were recorded on various morpho-physiological traits *viz.*, plant height, peduncle length, spike length, number of tillers/meter length, number of spikelets/spike, number of grains/spike, thousand kernel weight (TKW in g), biological yield/ meter length, relative water content (RWC in %) grain yield/ meter length and harvest index (%).

A mapping population comprising of 262 recombinant inbred lines derived from the cross HW2004 and HD2877 was phenotyped under moisture

Table 1.   Characteristic features of genotypes used in the development of Recombinant Inbred Lines (RILs)

S No.	Genotype	Characteristic Features	Pedigree
1.	C306	Tolerant to moisture stress (drought), high tillering and *Ne1*-carrier, a popular cultivar in rain-fed situations	Regent19473*CHZ//*2C591/3/P 19/C281
2.	HW2004 (Amar)	Backcross line from C306 with rust resistance genes *Sr*24/*Lr*24, tolerant to moisture stress, grown in rain-fed situations	C306*6//TR380-14*7/3Ag#14
3.	NI5439	Drought tolerant, short duration genotype for Peninsular India, non-carrier for hybrid necrosis genes	REMP 80/3*NP710
4.	HD2888	Drought tolerant, grown in rain-fed situation in North Eastern Plain Zone	C306/*T. sphaerococcum*//HW2004
5.	HD2012	Drought susceptible, *Ne2* -carrier	HD1467/HB208
6.	HD2877	Drought susceptible, non-carrier for hybrid necrosis genes	CDWR 9549/HD2347//HD2402
7.	HD2851	Drought susceptible, non-carrier for hybrid necrosis genes	CPAN3004/WR426/HW2007
8.	MACS2496	Drought susceptible, carrying 1BL.1RS translocation	Seri=Veery#5"5"=KVZ/BUHO"S"//KAL/BB

stress conditions at New Delhi and Indore. The parents and each RIL were planted in small plots consisting of two rows of 1.5m length in Augmented Block Design during *rabi* 2008-09 and 2009-10 under moisture stress conditions at New Delhi and Indore.

Measurement of physiological and agronomical traits

Relative water content (RWC) of flag leaf of main tiller was determined following standard procedure [11]. Leaves were cut and collected at mid-day to determine fresh weight (FW). The leaves were then kept in submerged water in petri dish. The turgid weight (TW) was then recorded after 4 hours rehydration at room temperature (25°C). To determine dry weight (DW), samples were oven-dried at 70°C for 24h. RWC was calculated according to the following equation

RWC% = (FW-DW/TW-DW) x 100

The harvest index was calculated from the total dry weight of biological yield of plant and their grain weight. Biological yield was calculated from the above-ground biomass including the number of tillers/meter and number of grains/spike. Correlation analysis was done using SPSS software (version 18).

Molecular analysis

A study on parental polymorphism was carried out in HW2004 and HD2877 using 832 SSR markers. Out of these 132 were polymorphic and 91 were used covering all 21 chromosomes to characterize remaining six parents. Out of these 30 markers were from A Genome, 34 markers from B Genome and 27 markers from D genome, respectively. The SSRs included public BARC, CFA, CFD, WMC and GWM sets [12-14].

DNA extraction, PCR amplification and electrophoresis

Plant DNA was isolated from fresh leaves as per the CTAB method [15]. The quality and quantity of DNA was determined using a UV spectrophotometer (Beckman, USA). The DNA samples were diluted to 25 ng/μl. DNA samples (50 ng) were amplified in 25-μL reaction volume containing 1x PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (v/v) gelatin] (Bangalore Genei, India), 0.2 mM of each dNTPs (Bangalore Genei, India), 10 pmol of each primer and 1 U of Taq polymerase (Bangalore Genei, India). PCR amplification was achieved in Thermal cycler (Eppendorf AG, Mastercycler Pro S, Hamburg, Germany) with the following thermal profile: one cycle of 4 min at 95°C (initial denaturation), followed by 40 cycles of 30 s. at 94°C (denaturation), 30 s. at 60°C (primer annealing) and 30 s at 72°C and 7 min at 72°C (final extension). PCR amplified products were resolved on 4% metaphor gels, stained with ethidium bromide and visualized under UV in a gel documentation system (Syngene G-Box, UK). The sizes of the amplified fragments were estimated using 50 bp and 100 bp DNA ladder (Bangalore Genei, India) as the standard size.

Statistical analysis

For each SSR marker-genotype combination, the presence or absence of band was given a score of 1 or 0. Pair wise GS was estimated using SIMQUAL of the software package NTSYS-pc version 2.0. The similarity matrices were used to construct a dendrogram for all the genotypes using NTSYS-pc based on UPGMA [16]. The correlation between similarity matrices based on molecular data was determined by MXCOMP module of the NTSYS-pc software.

Results and discussion

Diversity for agronomic traits in parents

A majority of traits differed between drought tolerant (HW2004, C306, HD2888 and NI5439) genotypes and the susceptible ones (HD2877, MACS2496, HD2012 and HD2851) (Table 2). The difference in mean indicated the suitability of parents for developing recombinant inbred lines. As most of the genotypes suitable for moisture stress conditions were tall ranging from 107cm to 117.5cm as compared to drought susceptible genotypes which were shorter in height (60cm to 95.8cm). Tall genotypes have the capacity to extract water from the deeper soils and also translocate the stored materials in the stem leading to higher grain yield under drought conditions in comparison with genotypes having shorter height [17]. Height of the plant directly or indirectly contributes to total biomass of the plant. Higher biomass is one of the selection criteria for drought tolerance [18]. A wide range (18.2-39.2cm) for peduncle length was observed among all the genotypes. The drought tolerant genotypes had comparatively longer peduncle (30.0 to 39.2 cm) to drought susceptible genotypes (18.2-29.7cm). HD2012 among the susceptible genotypes was an exception (34.2cm).

The higher peduncle length in drought tolerant genotypes may be due to higher plant height. Evans and Wardlaw [19] indicated that peduncle plays an important role in grain filling because it has higher green area which is likely to contribute to photosynthesis and

Table 2. Mean grain yield and its components of eight parents used in the development of RILs at New Delhi

S.No.	Genotypes	Relation to drought	RWC (in%)	Plant height (cm)	Tiller number	Peduncle length (cm)	Spike length (cm)	Spikelet number	Grain number	Thousand kernel wt (g)	Harvest index (in%)	Biological yield (g)	Grain yield (g)	Polymorphic markers (in%)
1	HW2004	tolerant	84.56	112.19	48.28	36.87	9.44	20.69	53.00	41.27	28.13	213.28	60.00	100.00
	HD2877	susceptible	58.77	73.73	28.00	23.27	11.63	21.80	49.00	33.70	18.22	172.00	31.34	
2	C 306	tolerant	82.11	117.53	50.50	39.25	9.42	20.09	51.60	39.45	28.93	206.00	59.60	75.82
	MACS2496	susceptible	60.23	82.00	36.00	29.70	11.30	21.00	40.00	20.70	17.65	136.00	24.00	
3	NI5439	tolerant	79.80	109.63	22.00	31.00	9.43	21.00	56.50	34.08	30.40	176.00	53.50	57.14
	HD2012	susceptible	68.24	95.88	32.00	34.25	12.10	21.50	43.00	22.35	16.48	126.00	20.76	
4	C 306	tolerant	82.11	117.53	50.50	39.25	9.42	20.09	51.60	39.45	28.93	206.00	59.60	48.35
	NI5439	tolerant	79.80	109.63	22.00	31.00	9.43	21.00	56.50	34.08	30.40	176.00	53.50	
5	C 306	tolerant	82.11	117.53	50.50	39.25	9.42	20.09	51.60	39.45	28.93	206.00	59.60	60.44
	HD2851	susceptible	62.00	60.00	24.00	18.20	8.20	19.00	44.00	22.80	26.57	120.00	31.88	
6	HD2888	tolerant	82.89	107.00	32.00	30.00	9.80	21.00	58.00	34.00	26.82	192.00	51.50	63.74
	HD2851	susceptible	62.00	60.00	24.00	18.20	8.20	19.00	44.00	22.80	26.57	120.00	31.88	

leads to increased grain yield under moisture stress [20]. Positive and significant correlation between peduncle length and grain yield of wheat is also reported [21]. In the present study also peduncle length exhibited positive correlation with grain yield (r = 0.364), thousand kernel weight (r=0.498), biological yield (r=0.546), relative water content (r = 0.686), plant height (r = 0.887) and tiller number (r = 0.708) under rain-fed conditions (Table 3).

Number of tillers/plant is considered to be a vital trait contributing towards grain yield in cereals particularly in wheat, rice barley and rye. The variation (range, 22-50.5) in tiller number/meter was recorded in the genotypes (Table 2). The drought tolerant genotypes had more tillers, C306 being the highest, than the drought susceptible genotypes. The ability of C306 and HW2004 to produce large numbers of tillers was evident in moisture stress conditions and tillering continued even at the later stage of maturity. The drought-resistant cultivars tolerate high internal stress by maintaining a large number of tillers [22]. Among the eight genotypes tiller number per meter showed positive and significant correlation with peduncle length, thousand kernel weight, biological yield and grain yield.

Number of spikelets/spike in drought tolerant and drought susceptible genotypes did not differ significantly. Drought tolerant genotypes did not show variation in spike length, whereas it varied (8.2cm to 11.6cm) in drought susceptible genotypes. The correlation recorded between spike length and spikelet number was not significant. However positively significant correlation between the two has been reported earlier under moisture stress conditions [23]. Drought tolerant genotypes produced higher grain number/spike (51.6-58.0) as compared to drought susceptible (40.0-49.0). HD2888 produced a maximum of 58 grains while MACS2496, a minimum of 40 grains/spike indicating the suitability of all the drought tolerant genotypes for developing RILs. Positive but non-significant correlation between grain number and number of tillers (r=0.225) was recorded (Table 3). Number of grains/spike is suggested as the most reliable selection criterion for drought tolerance [24].

Thousand kernel weight in drought tolerant genotypes was higher (34.0-41.27g) than the drought susceptible genotypes (20.7-33.7g). HW2004 had maximum thousand kernel weight whereas MACS2496 had lowest TKW. Plaut et al. [25] reported decrease in TKW and weight of kernels per spike under water deficit leading to reduced grain yield [26]. Thousand kernel weight showed significant positive correlation with RWC (r=0.746) and number of tillers (r=0.736).

The grain yield per meter length differed in drought tolerant and susceptible genotypes. The drought tolerant genotypes comparatively had more grain yield/m (51.5-60.0g) than the susceptible genotypes (21.7-31.3g). Apart from other yield contributing traits, number of tillers plays a major role in determining the grain yield. C306 produced highest number of tillers/m length. Minimum (120.0g) and maximum (213.28g) dry matter was recorded in HD2851 and HW2004 indicating that number of tillers and plant height enhanced biological yield under drought conditions. It is evident that moisture stress decreased the biological yield (BYD) in drought susceptible genotypes. Biomass (total dry matter) production is an important criterion to judge drought tolerance in crop breeding [27]. In different species including cereals such as wheat, rice, barely and sorghum, biological yield tend to increase due to efficient water use potential under moisture stress conditions [28]. In all the eight genotypes grain yield showed positive and significant correlation with relative water content (r=0.729), number of tillers/m (r=0.722), thousand kernel weight(r=0.838) and biological yield/m (r=0.816). Positive and significant associations between yield and other traits mentioned above have also been reported in other cereals [29-32]. The traits showing strong correlations with yield should be given more emphasis in breeding to improve yield in wheat under moisture stress conditions.

Measure of harvest index is a good indicator of plant efficiency in distributing photoysnthate towards development of grain under moisture stress. The harvest index in drought tolerant genotypes was higher as compared to drought susceptible genotypes. Harvest index and biological yield are the most important yield variables to be considered under drought conditions since both had the higher positive direct effect on yield [31]. RWC is an important indicator of water stress in leaves than other water potential parameters under drought stress [33]. RWC among drought tolerant genotypes was significantly higher (79.8-84.56%) as compared to drought susceptible (58.77-68.24%) (Table 2). HW2004 had the highest amount of RWC 84.56% followed by C306 indicated that these genotypes maintain aquatic relations under stress conditions whereas drought susceptible genotypes like HD2877 and MACS2496 are not able to retain the water level. Significant differences in RWC% in tolerant and susceptible genotypes of barley and wheat have been reported [34]. A high amount of RWC in leaf influences the ability of plants to recover from stress and consequently affect the yield and its stability [35-36].

High positive correlation was observed between RWC and other traits ranging from 0.714 to 0.911 (Table 3). The RWC exhibited significant and positive correlation with plant height, grain number, thousand kernel weight, biological yield and grain yield. It is evident from the results that RWC plays a significant role in producing more grains per unit area under moisture stress conditions.

Development of recombinant inbred lines

The drought tolerant genotypes namely, C306, HW2004 (improved backcross line from C306), NI5439 and HD2888 were found to be contrasting with susceptible genotypes (HD2012, HD2877, MACS2496 and HD2851) in most of the traits with respect to drought tolerance (Table 1). The six sets of RILs population in F_7-F_8 generation consisted of different number of lines from HW2004 x HD2877 in F_8 (262), C306 x NI5439 in F_8 (125), NI5439 x HD2012 in F_8 (86), HD2888 x HD2851 in F_7 (89), C306 X MACS2496 in F_7 (154) and C306 X HD2851 in F_7 (96). Only one set of RILs from the cross HW2004 x HD2877 was evaluated in moisture stress (rain-fed) conditions during *rabi* 2008-09 and 2009-10 at New Delhi and Indore. Significant differences with respect to plant height, number of tillers per plant, number of grains per spike, thousand kernel weight, biological yield, grain yield and harvest index were observed among the RILs derived from HW2004/HD2877.

Evaluation of RILs under moisture stress conditions

Transgressive segregants were observed for plant height, number of tillers, RWC, thousand kernel weight, biological yield and grain yield at New Delhi. The segregation in recombinant inbred lines followed a normal distribution (Fig. 1) and showed suitability of the data for QTL analysis. A histogram of the biological yield and grain yield showed a moderate degree of shift indicating the influence of genetic or genotype x environment interaction contributing to lowering the values of both the traits due to moisture stress. Positive and significant correlations of biological yield, grain number, thousand kernel weight and peduncle length with grain yield were observed among the RILs grown at New Delhi. The significant coefficient of correlation between yield and other traits ranged from 0.146 to 0.713 (Table 4). Positive correlation of plant height, tiller number, relative water content, spike length and spikelet number with yield were also recorded but the coefficient of correlation was of low magnitude at Delhi. However, plant height, tiller number, number of grains, biological yield, harvest index and thousand kernel weight had
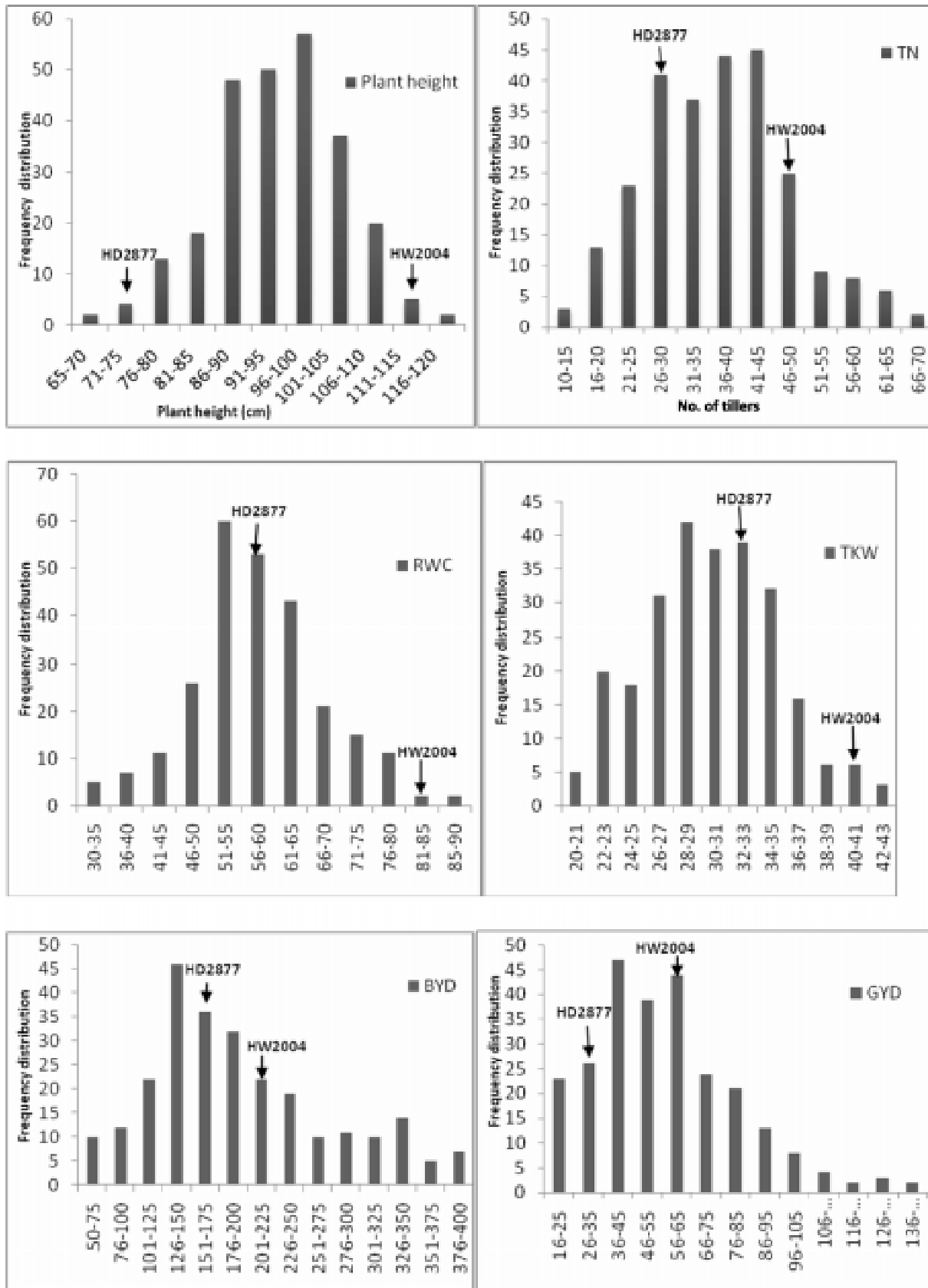
Fig. 1. Frequency distributions of five agronomic and physiological (RWC) traits in HW2004 x HD2877 RILs

Table 3. Correlations for yield and its related traits in parents at New Delhi

Trait	RWC %	Plant height (cm)	Tiller number	Peduncle length(cm)	Spike length(cm)	Spikelet number	Grain number	Thousand kernel wt(g)	Harvest index%	Biological yield (g)	Grain yield (g)
RWC%	1										
Plant height	0.911**	1									
Tiller number	0.645	0.683	1								
Peduncle length	0.686	0.887**	0.708*	1							
Spike length	-0.444	-0.101	-0.324	0.137	1						
Spikelet number	0.176	0.300	-0.165	0.135	0.557	1					
Grain number	0.714*	0.552	0.225	0.133	-0.356	0.475	1				
Thousand kernel weight	0.746*	0.682	0.736*	0.498	-0.327	0.142	0.686	1			
Harvest index	0.309	-0.024	-0.296	0.141	-0.856**	-0.643	0.243	0.321	1		
Biological yield	0.786*	0.746*	0.767*	0.546	-0.259	0.299	0.733*	0.966**	0.228	1	
Grain yield	0.729*	0.531	0.722*	0.364	-0.619	-0.166	0.611	0.838**	0.733*	0.816**	1

** and * significant at the 0.01 and 0.05 levels, respectively.

significant positive correlations with yield at Indore (Table 5). Carter *et al.* [37] reported that plant height was positively correlated to grain yield in RIL population derived from the cross Louise/ Penawawa, with taller plants having higher grain yield potential. However plant height showed negative correlation with harvest index at New Delhi (r = −0.071) and positive and low magnitude but non-significant at Indore (r = 0.089). Positive correlations at Indore may be due to sampling bias. Negative correlation between the above traits was also observed in pooled analysis (r = −0.031) (Table 6). On the other hand, plant height is reported to have direct relationship with yield related traits [38]. Correlations between all the traits mentioned above have been previously documented. The pooled analysis of data recorded at Delhi and Indore showed high correlations among the above listed traits at individual locations. The coefficient of significant positive correlation ranged from 0.154 (between plant height and grain yield) to 0.732 (between grain yield and biological yield) supporting the conclusions drawn based on the data recorded at individual locations (Table 6). Holland [39] observed that genetic correlations between traits are due to linkage and/ or pleiotropy indicating the magnitude and direction of correlated response to selection. He also emphasized the relative efficiency of correlations facilitating indirect selection. The present findings indicated that traits are highly correlated and it is suggested that plant breeders can exercise direct or indirect selection for the traits and thus maximize genetic gain in segregating populations. The selection based on correlations may be a useful breeding strategy for indirectly selecting for higher grain yield potential [40]. According to the fact that yield is a polygene trait, and it is difficult to improve it directly, traits which have high correlation with the yield might prove helpful and indirectly improve the yield [41].

Diversity analysis

In the last few years genetic information and molecular diversity in wheat is being assessed by molecular markers [42-43]. They are useful along with morphological and physiological characterization of genotypes because they are in abundance, independent of environmental effects and allow identification early in plant development [44]. SSR markers have the advantages of co-dominance and offer uniform genome coverage. The present study aimed at understanding the utility of molecular markers in attaining adequate levels of diversity in wheat genotypes for drought tolerance leading to development of highly segregating mapping population. A set of 91 markers were found

Table 4. Correlations for yield and its related traits in RILs derived from the cross HW2004/ HD2877 at New Delhi

	Plant height	Tiller number	Grain number	Biological yield	Grain yield	Harvest index	Thousand kernel weight	Relative water content	Peduncle length	Spike length	Spikelet number
Plant height	1										
tiller number	0.321**	1									
grain number	0.091	0.214**	1								
biological yield	0.264**	0.221**	0.242**	1							
grain yield	0.120	0.113	0.177**	0.713**	1						
harvest index	-0.071	-0.045	0.004	-0.043	0.570**	1					
thousand kernel weight	0.183**	0.038	0.212**	0.337**	0.454**	0.278**	1				
relative water content	0.295**	0.338**	0.325**	0.190**	0.114	-0.027	0.263**	1			
peduncle length	0.424**	0.088	0.122	0.178**	0.146*	0.038	0.364**	0.159*	1		
spike length	0.170**	0.059	0.264**	0.146*	0.004	-0.127	0.107	0.111	0.042	1	
spikelet number	0.190**	0.350**	0.396**	0.164**	0.005	-0.127	0.062	.379**	0.057	0.337**	1

** and * significant at the 0.01 and 0.05 levels, respectively.

polymorphic between HW2004 and HD2877. Since both these parents were contrast for drought tolerance, a polymorphism survey was conducted to study the genetic diversity at molecular level between other parents to know whether these parents, involved in the development of RILs, possess similar level of polymorphism (Fig. 2). Out of 91, 75.82 per cent SSR markers were polymorphic between C306 and MACS2496, 63.74 between HD2851 and HD2888, 60.44 between C306 and HD2851, 57.14 per cent between NI5439 and HD2012 (Table 2). Only 48.35 per cent of SSR markers were polymorphic between drought tolerant genotypes, C306 and NI5439. The existence of phenotypic variation in a crop depends on the product of underlying DNA diversity. Both the genotypes are grown under moistures stress conditions, the variations at nucleotide level observed in molecular analysis is low indicating that they carry similar set of loci for drought tolerance. HW2004 and HD2877 are contrasting parents for drought tolerance. Ninety one SSR markers polymorphic between these two were used to characterize other parental combinations.

A higher per cent of polymorphic markers were located on B genome among all the parents except C306 and HD2851 in which both B and D genome showed high percentage of polymorphic markers (Fig. 3). Liu and Tsunawaki [45] reported a higher genetic differentiation on B genome than on the A genome between *T. aestivum* and *T. spelta* using RFLP markers. Roder *et al.* 1998 [12] also reported similar results between *T. aestivum* and *T. durum* by use of microsatellites. A recent study revealed lower levels of polymorphism in the D genome than in the A and B genomes indicating that lateral gene flow from tetraploid to hexaploid species was frequent, while it was very limited between the diploid *Ae. tauschii* and hexaploid wheat [46]. Zhang *et al.* [47] using polymorphic DArT markers reported that these markers were fequently present on the B genome, but seldom occurred on the D genome, however, Akbari *et al.* [48] reported relatively lack of polymorphism by DArT markers. This agrees with the fact that the B genome presents the highest level of polymorphism, whereas the D genome shows the lowest level among the wheat ABD genomes [48-49]. This genome is the most recent addition to hexaploid wheat and therefore shows the lowest degree of polymorphism. Interestingly, in present study, the percent polymorphism on A genome between NI5439 and HD2012 was higher. It can be deduced, therefore, that NI5439 is a diverse genotype than the C306 and its derivatives HW2004 and HD2888 which are used in development of other sets of RILs.
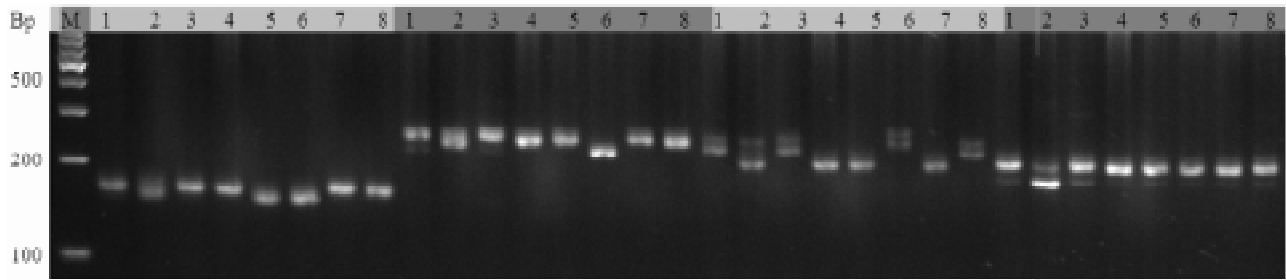
Fig. 2. Banding pattern of PCR amplified product of four microsatellite markers resolved in 4 % agarose gel showing allelic variation among parents used for the development of recombinant inbred lines. Base pairs (Bp), Marker (M), HW2004 (1), HD2877 (2), C306 (3), MACS2496 (4), HD2012 (5), NI5439 (6), HD2851 (7) and HD2888 (8)
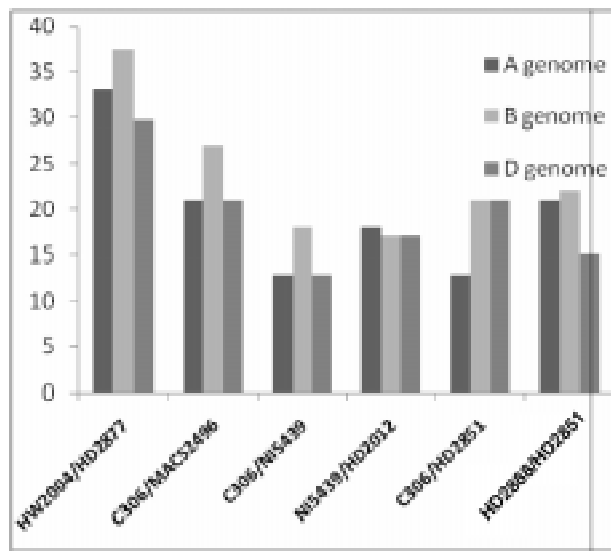

Fig. 3. Polymorphic SSR markers genome wise coverage in the homoeologous group between parents

Cluster analysis is a multivariate technique, which establishes the most suitable parents for useful combinations in breeding programme. Such multivariate analyses are used to ascertain genetic diversity among various parents [31]. Jaccard's similarity coefficients ranged from 0.43 to 0.89 with a mean genetic similarity of 0.65 at all the SSR loci. The highest similarity (0.89) was found between HW2004 and C306, while MACS2496 and HD2877 showed minimum (0.43-0.44) similarity (Table 7). The dendrogram generated from similarity coefficients indicated two main groups (Fig. 4) that were tested for statistical significance and confirmed at the 0.05 level of probability. One group was relatively homogeneous consisting well known drought tolerant genotypes *viz.*, C306, HW2004, HD2888 and NI5439 and a more heterogenous group including drought susceptible genotypes, namely, HD2877, MACS2496, HD2851 and HD2012. The clustering of genotypes proved the suitability of SSRs in detecting alleles characteristic of cultivars from distant genetic background. The closer association of HW2004,

Table 5. Correlations for yield and its related traits in RILs derived from the cross HW2004 and HD2877 at Indore

Trait	Plant height	Tiller number	Grain number	Biological yield	Grain yield	Harvest index	Thousand kernel weight
Plant height	1						
tiller number	0.173**	1					
grain number	0.114	-0.087	1				
biological yield	0.349**	0.405**	0.201**	1			
grain yield	0.312**	0.359**	0.195**	0.774**	1		
harvest index	0.089	0.058	0.095	0.027	0.588**	1	
thousand kernel weight	0.307**	-0.015	0.021	.339**	0.358**	0.161**	1

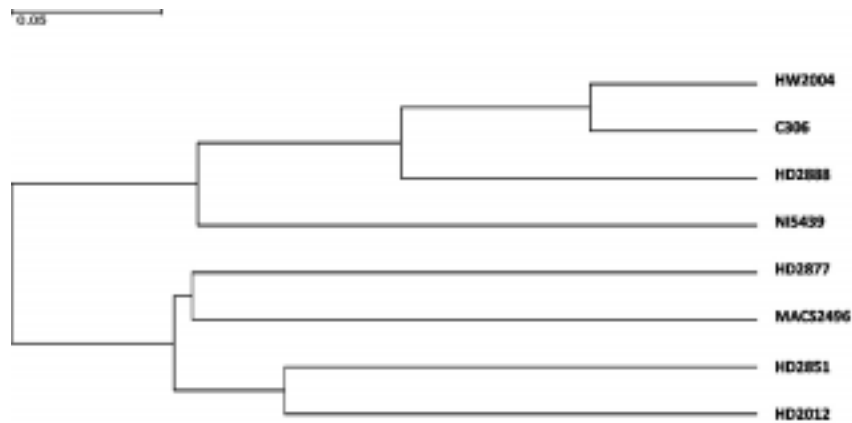** and * significant at the 0.01 and 0.05 levels, respectively.

Fig. 4. Dendrogram based on Jaccard's similarity coefficient generated using SSR markers in wheat genotypes

Table 6. Correlations for yield and its related traits in pooled data of RILs from HW2004/HD2877 at New Delhi and Indore

Trait	Plant height	Tiller number	Grain number	Biological yield	Grain yield	Harvest index	Thousand kernel weight
plant height	1						
tiller number	0.161[**]	1					
grain number	0.047	0.078	1				
biological yield	0.284[**]	0.339[**]	0.189[**]	1			
grain yield	0.154[*]	0.238[**]	0.180[**]	0.732[**]	1		
harvest index	-0.031	0.020	0.088	-0.009	0.570[**]	1	
thousand kernel weight	0.196[**]	0.006	0.051	0.319[**]	0.432[**]	0.296[**]	1

** and * significant at the 0.01 and 0.05 levels, respectively.

Table 7. Jaccard's similarity coefficients in drought tolerant and susceptible genotypes based on SSR markers

	HW2004	HD2877	C306	MACS2496	HD2012	NI5439	HD2851	HD2888
HW2004	1							
HD2877	0.44	1						
C306	0.89	0.46	1					
MACS2496	0.43	0.63	0.44	1				
HD2012	0.53	0.62	0.55	0.62	1			
NI5439	0.61	0.51	0.64	0.50	0.59	1		
HD2851	0.53	0.59	0.51	0.64	0.69	0.55	1	
HD2888	0.78	0.50	0.75	0.50	0.59	0.65	0.54	1

with the drought tolerant wheats HD2888 and NI5439 to a large extent reflects selection for genomic regions present in the drought tolerant genotypes. The mapping populations developed are important resource for genetic dissection of drought tolerance in wheat.

The eight cultivars with different responses to water stress were selected in order to develop mapping populations for genetic studies and to identify the genomic regions controlling drought tolerance in wheat. The study of correlation among yield and yield contributing traits suggests that relative water content,

number of tillers/meter, number of grains/spike and thousand kernel weight and biological yield were the most important characters, which possessed positive association with grain yield/meter under moisture stress conditions. Overall, an intensive selection for these traits is likely to improve grain yield in wheat under moisture stress conditions. Since correlations among the traits were significantly positive, selection in one of the traits will implicitly result in the improvement of the other traits. The integration of information accrued from molecular and morphological analysis leads to the identification of the most relevant loci controlling drought tolerance. Hence, in the near future, an integrated approach for drought tolerance could improve the yield under moisture stress conditions. With this knowledge, the plant breeder can make attempts to breed more widely adapted genotypes particularly for drought conditions.

Acknowledgement

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