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Evaluation of marker assisted backcross breeding derived lines for morpho-physiological characters under late sown heat stress condition in bread wheat

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Abstract

An experiment was conducted to study the performance of BC_2F_3 and BC_1F_4 lines derived in marker assisted backcross breeding (MABB) program for different morphological and physiological traits under late sown conditions. Two QTLs were introgresed into HD2733, one for days to anthesis and other for yield under stress conditions from an identified heat tolerant variety WH730 (donor). The lines were subjected to heat stress by sowing late in the season and the data was recorded on several characters at different phenological stages. A significant difference (P<0.05) between BC_2F_3 and BC_1F_4 lines for many traits at phenotypic level was observed but a few traits did not show significant difference and the lines were more and less similar with recurrent parent HD2733. Backcross derived lines showed improvement over recurrent parent for some important morpho-physiological traits including yield under high temperature stress condition. However, overall performance of BC_2F_3 lines was slightly better than the BC_1F_4 lines for morpho-physiologically important characters. For most of the DUS traits, both BC_2F_3 and BC_1F_4 lines were having closer values to the recurrent parent attributing to the recovery of the recipient parent genome. Seventeen BC_2F_3 and 10 BC_1F_4 lines were selected for their superior performance which will be further evaluated in multi-location trials.

Key words: Molecular marker assisted breeding, backcross, NDVI, CT, genome recovery

Introduction

Heat stress is becoming the most important constraint in achieving higher wheat productivity; which in conjunction with drought and radiation, affects different phenological stages at varying degrees. Exposure of wheat to sub-optimal temperatures at vegetative stages and supra-optimal temperature at reproductive stages results in reduced spikelet fertility, grain number per spike and eventually poor grain filling due to reduced period and rate of grain filling, which leads to low test weight and productivity of crop (Nagarajan and Rane 2002; Sphiler and Blum 1991). Popular cropping systems and proximity of South-Asian countries including India to the equator involves late sowing, which exposes wheat to high temperature (exceeding 35^{o}C) during reproductive stages (Rane et al. 2000). Bread and durum wheat genotypes show significant interaction with high temperature and hence the heat stress reduces the grain filling duration (Dias and Lidon 2009) and grain filling rate (Girish et al. 2013). Tolerance to heat stress is a quantitative trait and different morpho-physiological component traits, governed by different sets of genes/QTLs are essential to provide tolerance at different growth phases of wheat (Howarth 2005).

Heat stress affects wheat crop mainly at two stages, one during pre-anthesis and the other at post-anthesis. High temperature at post-anthesis stage (reproductive stages) affects number of spikelets/ spike, number of grains/spike, photosynthate translocation and starch granule deposition within kernels; the key traits involved in governing the

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productivity and quality of wheat (Mohammadi et al. 2004; Bhullar and Jenner 1985). Several morpho-physiological and yield traits significantly contribute to high temperature stress tolerance which includes canopy temperature, Normalised Difference Vegetation Index (NDVI), chlorophyll content, stomatal conductance, 1000 kernel weight, spike length, number of grains per spike etc. (Ramya et al. 2015; SaintPierre et al. 2010; Reynolds et al. 2007; Kamla et al. 2013). Since, most of the morpho-physiological traits are quantitative in nature their expression is controlled by more than one gene and interaction of genotypes with environmental conditions. Many researchers have identified chromosomal regions that control the expression of morpho-physiological traits like canopy temperature (Kumar et al. 2012), chlorophyll content (Kadam et al. 2012), stay green (Pinto et al. 2010), days to anthesis (Pinto et al. 2010), chlorophyll fluorescence (Kumar et al. 2012) and 1000 kernel weight (Pinto et al. 2010). The different physiological processes and morphological characters having high correlation with yield under stress conditions, significantly affect the tolerance to high temperature. Worldwide wheat breeders are searching for stress tolerance genes/QTLs to introgress into suitable genetic background of wheat to make high temperature tolerant lines which can escape the terminal heat stress in late sown condition (Punia et al. 2011). Dissection of genetic base of inheritance and mapping the positions of QTLs governing the stress tolerant traits with linked markers provided great advantage and opportunities to the wheat breeders to exploit emerging and efficient methodology like marker assisted breeding. MABB is one of the components of MAS, advantageous in transfer of genetic regions controlling the expression of quantitative traits (QTLs) (Gupta et al. 2010) and of one to two backcross generation selection relative to random or phenotypic selection helps to recover maximum recipient parent genome (Peter et al. 1996). The present study was therefore, undertaken to evaluate the lines derived from MABB program for different morpho-physiological traits.

Materials and methods

Marker assisted BC_2F_3 and BC_1F_4 lines were generated from the cross involving HD2733 and WH730. The wheat variety HD2733 (used as recipient) is suitable for normal sown irrigated and late sown high temperature stress conditions it gives reduced yield, whereas, WH730, an identified heat stress tolerant germplasm line was used as donor. This variety has been characterized as heat tolerant based on the low heat susceptibility index, thermo-tolerance of membrane, high kernel weight and grain number (Dhanda and Munjhal 2012). Variety HD2733 was improved by introgression of two Qtls controlling days to anthesis (Pinto et al. 2010) and grain yield under stress (Mohammadi et al. 2004).

Experimental design and layout

The experiment was laid out in an augmented design with 4 replications of parental checks in BC_2F_3 lines and 3 replications in BC_1F_4 lines. Two rows of each genotype were planted manually with gross plot size of 0.46 x 2.5 m, with rows at 23 cm apart. The standard cultivation practices prescribed for wheat under late sown conditions were followed precisely to expose them to heat stress. Parents were planted both at normal sowing date (mid of November) and also at late sowing date (first week of January) for accurate comparison of derived lines with parents. Materials for the present study were comprised of 39 BC_2F_3 lines and 21 BC_1F_4 lines containing either one or both the QTLs (*barc* 186 and *gwm* 190) along with replicated parents.

The field observations were recorded during crop season on 5 randomly selected plants from each plot for most of the traits. The observations were made on a total of 19 morpho- physiological and yield traits like days to flag leaf emergence (FLE), days to heading (DH), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), number of productive tillers per plant (tillers/pl), number of spikelets per spike (spk/sp), number of grains per 5 spike, 1000 kernel weight (TKW), grain yield per 5 plants, biomass, harvest index (HI), membrane stability index (MSI), normalised difference vegetation index (NDVI), chlorophyll content, stomatal conductance (SC), canopy temperature (CT), and early ground cover (GC). Observations on days to flag leaf emergence, days to heading, and days to maturity were measured by counting days from date of sowing to the respective stages of crop. Plant height, spike length, peduncle length, number of productive tillers per plant, number of spikelets per spike and number of grains per 5 spikes were recorded on 5 randomly selected plants and their means were used for statistical analysis. Biomass was recorded as above ground weight of five selected plants. Physiological traits like normalised difference vegetation index were measured with Greenseeker, a field portable NDVI sensor which works on the principle of absorbance of red light by healthy green canopy and reflectance of near infrared light

(Cossani and Reynolds 2012). Chlorophyll content was measured using Minolta SPAD-502 chlorophyll meter (a hand held battery portable optical meter) which works via light transmittance by absorbing red light at 650nm and infrared light at 940nm. Stomatal conductance was measured using Decagon: SC-1 hand held porometer. Early ground cover was measured as per the method of Mullan and Reynolds (2010). With the use of compact digital camera images were acquired without using zoom function. At 25 days after germination one image per plot was taken from a distance of constant 1m height and digital photographs were processed using 'Adobe Photoshop CS5 Extended' program. Canopy temperature was measured using hand held infrared thermometer (Kane May Model Infratrace 8000, USA). Two measurements per plot approximately 0.5m from the edge of the plot and approximately 1m above from the canopy with approximately 30^o-60^o angle from the horizontal were recorded. Membrane stability index (MSI) was determined according to the method of Sairam et al. (1997). Leaf material (100 mg) was taken in test tubes containing 10 ml of double distilled water. Initial (C1) (40^oC) and final (C2) (100^oC) conductivity of the solution was recorded on a conductivity bridge (Century, Water soil analysis kit, CMK 751). MSI was calculated as: MSI = [1 – (C1/C2)] × 100. Traits like stomatal conductance and canopy temperature were measured on clear sunshine days at 11AM to 12 PM hours. CT and NDVI were measured two times a day 11.00 to 11.30AM and 1.00 to 1.30 PM. All physiological characters were measured at three developmental stages, late boot stage, early milk stage and late milk stage which were considered as important and sensitive stages to heat stress.

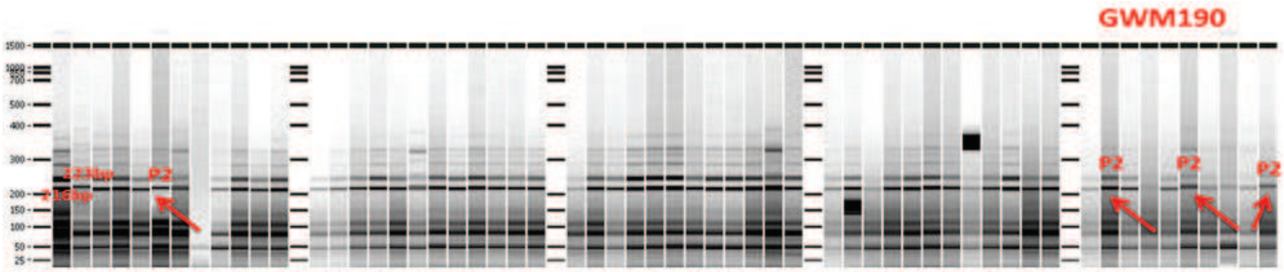
Improvement for the targeted traits and contribution of other morpho-physiological characters to yield under high temperature stress in the backcross derived lines were tested for significance at (P<0.05) and by using Critical Difference at 5 per cent level of significance (CD_5%). Comparison was made between BC_2F_3 and BC_1F_4 families for different characters on the basis of calculated (P<0.05) values and Anderson Darling test was studied to know the distribution pattern of lines in each population.

Results and discussion

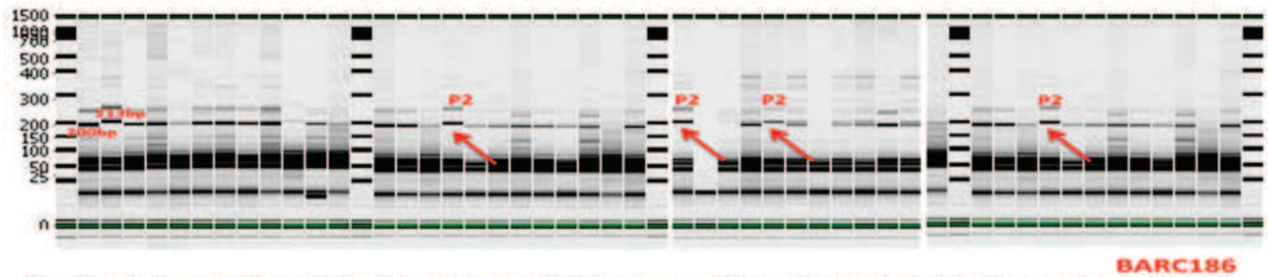
Evaluation of MABB derived lines for morphological traits

Wheat cultivar HD2733 was improved upon by introgression of two QTLs governing heat stress tolerance from tolerant variety WH730 through markers assisted backcross breeding method. WH730 was phenotypically evaluated for days to anthesis and stress tolerance index at the beginning of the experiment. The donor was selected for having positive allele (Babax) for barc186 marker linked to DA (Pinto et al. 2010) and positive allele (Kauz) for gwm190 marker (Mohammadi et al., 2008) linked to grain yield under stress (Unpublished data) which supplemented the phenotypic performance of the donor for the selected traits. In BC_1F_2 segregating population, the QTL linked to days to anthesis showed a phenotypic variance of 8.9% (R^2 = 0.089) and QTL linked to grain yield under stress showed 24.5% (R^2=0.245) phenotypic variance under high temperature stress. The BC_2F_3 and BC_1F_4 lines were evaluated for their improvement over recipient parent for morphological and physiological traits under heat stress conditions. The homozygous lines for *barc*186 and *gwm*190 markers linked QTLs governing days to anthesis and grain yield under stress conditions respectively, were identified in BC_2F_2 and BC_1F_2 generations (Figs. 1 and 2 respectively). These homozygous lines were forwarded to BC_2F_3 and BC_1F_4 generations by selfing. The recovery of the recurrent parent genome was calculated in each generation of both types of populations (Table 1) through background selection with SSR markers. The recovery per cent of selected lines increased from BC_1F_1 generation to BC_2F_3 and BC_1F_4 generations. However, the recovery was less in BC_1F_4 lines than in BC_2F_3 lines. These results clearly showed the expected results of backcross breeding where backcrossing is effective in recovering the recipient parent genome by replacing the donor parent alleles and also by making heterozygous loci into homozygous loci at more number of positions in the genome, thereby the two rounds of backcrossing resulted in more background recovery. Continuous selfing for three generations was effective in making the heterozygous loci into homozygous loci but not in replacing the donor parent alleles at non targeted regions, hence less recovery in selfed lines with one backcross. Based on the phenotypic similarity to recipient parent and higher recovery of recurrent parent genome, total of 39 BC_2F_3 and 21 BC_1F_4 selected lines in MABB program were subjected to late sown terminal heat stress conditions for recording data on different morpho-physiological characters.

In BC_2F_3 traits like spikelets/spike, tillers/spike, grains/5 spikes and in BC_1F_4 lines days to heading, spikelets/spike, tillers/spike, grains/5 spikes and



[Note: 1[st] lane-Ladder, repeated after every 12 lines, 2[nd] lane-recipient parent (P1), 3[rd] lane-donor parent (P2), Virtual image produced in Caliper life sciences electrophoresis system]



[Note: 1[st] lane-Ladder, repeated after every 12 lines, 2[nd] lane-recipient parent (P1), 3[rd] lane-donor parent (P2), virtual image produced in Caliper life sciences electrophoresis system]

Figs. 1 and 2. Homozygosity pattern of MABB selected lines in BC_2F_2 and BC_1F_2 generations for linked markers

Table 1. Recovery per cent of recurrent parent genome in selected BC_2F_3 and BC_1F_4 lines

Population	BC_1F_4 lines (21)			BC_2F_3 lines (39)		
Generation	BC_1F_1	BC_1F_2	BC_1F_4	BC_1F_1	BC_2F_1	BC_2F_3
Recovery %	73.6- 76.1	78.9-84.9	84.2-88.6	73.6- 76.1	85.09-94.7	90.5-97.9

harvest index were showing normal distribution (Anderson Darling test P>0.05). Days to flag leaf emergence, plant height, days to maturity and peduncle length were showing skewedness towards donor parent in both types of lines. However, traits like biomass/5 plant, 1000 kernel weight and grain yield under stress condition were showing significant improvement over both the parents in both generation lines.

The CD (Critical Difference) value at 5% level of significance was calculated for accurate comparison of the lines for their improvement over recipient parent HD2733 which has been introgresed with two targeted QTLs. There was a difference of 10-12 days for flag leaf emergence, days to heading and days to maturity under the normal sown conditions and of 9-10 days under late sown heat stress conditions between parents (Table 2), the mean values of BC_2F_3 and BC_1F_4 lines showed that they are behaving similar to donor parent for these traits, this may be because of introgresed QTL for days to anthesis which governs early anthesis and thereby early maturity and thus avoids exposure of crop to terminal heat stress. Improved BC_2F_3 and BC_1F_4 lines did not show any significant difference in days to maturity but they matured 8-10 days earlier than recipient parent HD2733 under stress condition to overcome the effect of terminal heat stress and perform better than the parent.

Plant height, spike length, spikelets/spike and tillers/plant were showing little improvement over HD2733 under stress condition but they were similar to HD2733 grown under non-stress condition. There was a significant difference in the number of grains/5 spikes in HD2733 grown under normal and stressed condition. We observed a 32% reduction in number of grains/5 spikes however; the donor parent was not affected for this trait under late sown stress condition. The selected BC_2F_3 and BC_1F_4 lines showed ~6% improvement for this trait over HD2733 under heat stress condition. BC_2F_3 lines were showing more biomass accumulation than BC_1F_4 lines and parents but selected BC_1F_4 lines performed better for thousand kernel weight and harvest index than BC_2F_3 lines and parents. Grain yield, the most important trait for which

Table 2. Mean values of parents and backcross derived lines under stress conditions (morphological and yield traits)

Traits	HD2733	WH730	HD2733	WH 730	BC_2F_3	BC_1F_4	t test	P value	$CD_{5\%}$
FLE	82.0	70.0	80.0	70.0	73.0	71.0	-3.83	0.000	1.43
DH	90.0	80.0	87.0	76.0	83.0	80.0	-3.04	0.004	4.3
DM	131.0	121.0	122.0	110.0	116.0	113.0	-3.44	0.001	2.86
PH	82.0	90.0	77.5	85.0	77.8	83.9	2.68	0.009	2.95
SL	10.1	12.8	9.3	11.3	10.6	10.6	0.01	0.994	1.76
PL	29.8	32.7	33.6	38.5	37.7	38.6	0.94	0.351	5.44
Spk/sp	19.4	19.6	18.4	19.6	19.2	18.6	-2.41	0.019	0.49
Tillers/pl	14.8	10.2	13.2	10.3	13.6	11.2	-3.80	0.000	1.35
Seeds/5sp	286.0	226.0	191.5	217.0	204.4	203.0	-0.10	0.922	27.94
BM/5pl	160.0	150.0	150.0	140.0	226.0	176.0	-3.60	0.001	0.34
TKW	44.51	41.84	42.46	45.46	40.9	46.04	2.70	0.009	3.86
HI	35.52	32.23	31.62	37.61	29.9	32.55	1.45	0.153	3.31
Yield/5pl	56.83	48.34	47.43	52.65	64.3	56.35	-2.45	0.017	3.71

N = Normal; S = Stress; M = Mean

a QTL has been introgresed into the lines in MABB programme were also evaluated, it was found that, there was a reduction of 16.5% in yield of HD2733 when subjected to stress condition, but the improved lines showed better performance than the recipient parent. BC_2F_3 lines showed an increase of 26% and BC_1F_4 lines showed 15.8% increase in yield under stress condition over the recurrent parent.

Large and significant differences were observed in molecular assisted backcross lines with 2 backcrosses and one backcross for days to flag leaf emergence, days to heading, days to maturity, plant height, tillers/plant, biomass/5 plants, 1000 kernel weight ($P<0.01$), number of spikelets/ spikes and grain yield/5 plants ($P<0.05$). However, for spike length, peduncle length and grains/5 spikes difference was non-significant between the two populations under terminal heat stress conditions (Table 2). Based on the CD values at 5% level of significance for individual traits (Table 2), it was found that all 39 lines were performing better than or similar to HD2733 with respect to days to flag leaf emergence, days to heading, days to maturity and spike length; 27 lines in plant height and harvest index, 28 lines in tillers/plant, 31 lines in peduncle length and grains/5 plant, 36 lines in biomass/5 plant and 37 lines in spikelets/spike and yield under late sown high temperature stress condition and rest of the lines had values either like recipient parent or in between two parents in BC_2F_3 lines. Similarly in BC_1F_4 lines it was found that all 21 lines were performing better than or similar to HD2733 in days to flag leaf emergence, days to heading, days to maturity, spike length, spikelets/spike and yield under stress condition, sixteen lines in peduncle length, biomass and harvest index, nineteen lines in seeds/5 spikes and 1000 kernel weight were similar to HD2733 under high temperature stress conditions. These values indicate that most of the lines were improved for their morphological characters to perform and yield better under heat stress conditions.

Evaluation of MABB derived lines for physiological traits

The MABB derived lines were also evaluated for physiological traits namely, CT, NDVI, GC, MSI, SC and chlorophyll content at different stages of crop period. Late boot (LB), early milk (EM) and late milky (LM) stages were selected for evaluation of the lines for physiological traits. In BC_2F_3 CT-LB, CT-LM, NDVI-LM, %GC, MSI, SC-LB, SC-EM, CHL-LB, CHL-EM CHL-LM were normally distributed (Anderson Darling test $P>0.05$). In BC_1F_4 CT-EM, NDVI-LM, %GC, MSI, SC-LB, SC-EM, CHL-LB, CHL-EM were found to be normally distributed (Anderson Darling test $P>0.05$).

BC_2F_3 and BC_1F_4 lines maintained cooler canopy at late boot (LB) and late milk (LM) stages but at early milk (EM) stage BC_2F_3 lines were with much cooler temperature (19.5^{o}C) than BC_1F_4 lines (20.55^{o}C), there was an observed difference of 1^{o}C temperature between parents.

Under stress condition per cent ground cover was measured to know the vigour of the derived lines. Both BC_2F_3 and BC_1F_4 lines had close values as that of recurrent parent. WH730 is a known source of membrane stability index (Dhanda and Munjhal 2012), the BC_2F_3 lines were found to be significantly improved for this trait as compared to recipient parent but BC_1F_4 lines were most similar in performance to recurrent parent. Stomatal conductance values measured at two stages were found to be in accordance with recurrent parent. Chlorophyll content was almost equal in BC_2F_3 lines and recipient parent but in BC_1F_4 lines it showed values similar to donor parent under heat stress condition.

The physiological traits, which are important in contributing to stress tolerance mechanisms in wheat under high temperature (Cossani and Reynolds 2012) were found to exhibit highly significant differences ($P<0.01$) for canopy temperature at early milk stage, NDVI at late boot, early milk and late milk stage, per cent ground cover, MSI, stomatal conductance at early milk stage and chlorophyll content at early milk and late milk stages (Table 3). On the basis of calculated $CD_{5\%}$, BC_2F_3 lines showing improvement or similarity with HD2733 were identified and it was observed that 38 lines were performing like recipient parent for canopy temperature in all the three stages (late boot, early milk and late milk), for NDVI 34 lines at late boot stage, 36 lines at early milk stage and 39 lines at late milk stage were showing similar values like HD2733. Membrane stability index which measures the lipid unsaturation was showing highly significant improvement (36 lines showed absolutely better performance than HD2733) over the recipient parent under the heat stress conditions indicating its strong association with introgresed QTLs or with other physiological traits. For per cent ground cover (%GC) which measures vigour of the lines, there were 31 lines exhibiting similar performance as that of recipient parent. Thirty six lines at late boot stage and 26 lines at early milk stage were performing similar to recipient parent in respect of stomatal conductance. For chlorophyll content, 37 lines at late boot stage, 32 lines at early milk stage and 34 lines at late milk stage showed improvement or were at bar with that of HD2733. In BC_1F_4 generation it was found that all 21 lines for CT (at all stages LB, EM and LM), NDVI (at LB and EM stage), per cent ground cover, SC (at LB stage) and chlorophyll content at late boot stage reacted like HD2733. Anderson darling test for physiological traits showed that traits like canopy temperature at early milk stage (BC_1F_4), late boot and late milk stage (BC_2F_3), NDVI at late milk stage (BC_1F_4 and BC_2F_3), per cent ground cover, MSI, SC at late boot and early milk stage, chlorophyll content at early milk stage (BC_1F_4), late boot, early milk and late milk stage (BC_2F_3) were distributed normally. CT at late boot and late milk (BC_1F_4) and late milk stage (BC_2F_3), NDVI at late boot and early milk stage (BC_1F_4 and BC_2F_3) and

Table 3. Mean values of parents and backcross derived lines under stress conditions (physiological traits)

Traits	Growth stages	HD2733	WH730	HD2733	WH 730	BC_2F_3	BC_1F_4	t test	P value	$CD_{5\%}$
Canopy temperature	LB	17.65	18.35	17.76	18.23	17.76	17.74	-0.32	0.749	0.42
	EM	19.6	20.35	19.84	19.89	19.5	20.55	3.95	0.000	1.47
	LM	27.55	29.75	23.53	25.85	23.3	23.46	0.98	0.331	1.95
NDVI	LB	0.83	0.81	0.82	0.81	0.81	0.82	2.44	0.018	0.028
	EM	0.81	0.79	0.8	0.76	0.78	0.79	6.06	0.000	0.036
	LM	0.54	0.46	0.63	0.53	0.63	0.57	-6.56	0.000	0.059
Per cent GC		36.97	29.87	21.85	15.67	20.93	23.72	7.48	0.000	1.66
MSI		163.85	310.15	172.44	318.12	287.95	199.91	-4.43	0.000	14.21
Stomatal conductance	LB	291.6	262.8	557.83	492.58	560.73	624.09	1.58	0.119	156.63
	EM	533.4	476.8	355.5	272.58	324.33	387.02	2.89	0.005	39.95
Chlorophyll content	LB	40.8	48.68	42.52	46.01	45.67	44.53	-1.71	0.093	1.92
	EM	48.12	50.88	49.51	49.98	51.03	48.71	-4.13	0.000	0.84
	LM	42.54	40.12	40.15	37.13	40.36	37.62	-3.53	0.001	2.26

N = Normal; S = Stress; M = Mean

chlorophyll content at late boot and late milk stage (BC_1F_4) were showing skewedness towards recipient parent. Improvement of derived lines for some physiological traits and similarity with recurrent parent for other physiological traits may be attributed to correlation among the traits under late sown heat stress condition.

Evaluation of marker assisted backcross derived BC_2F_3 and BC_1F_4 lines in this study is of wider application as it will help to know the performance of these lines for different morphological and physiological characters under heat stress condition. Contribution of different characters to the tolerance mechanism to reduce the effect of high temperature on grain yield can also be studied by measuring these independent traits. The present study revealed that grain yield under stress is governed by several morpho-physiological characters (Richards et al. 1999); the MABB derived lines which were introgresed with the QTLs for days to anthesis and grain yield under stress and improved for the respective linked traits performed better than recipient parent with respect to some morphological as well as physiological traits, however, some traits of these lines behaved similar to recipient parent indicating that derived lines are more tolerant to withstand the heat stress under late sown conditions.

This study has shown that the performance of the backcross derived lines was improved and more similar to recipient parent at phenotypic and physiological levels. The results can be attributed to the higher recovery of the recurrent parent genome in BC_2F_3 lines than in the BC_1F_4 lines indicating the success of backcross breeding program. The present study implemented the characteristic use of WH730 as an excellent source of heat stress tolerance for many physiological and morphological characters under heat stress, which can be further utilised in different breeding programs to generate mapping populations to map heat tolerant genes/QTLs and to develop high temperature stress tolerant varieties to combat the changing climatic scenarios. Based on the performance of the lines for morpho-physiological traits and yield under stress conditions, 17 BC_2F_3 and 10 BC_1F_4 promising lines showing improvement over recipient parent HD2733 were selected in the present study and the top five lines will be selected for their testing in multi locations. After their evaluation under multi-location testing they can be advanced to All India Coordinated Wheat Improvement Project (AICWIP) trials as improved lines over HD2733 and may be released as varieties.

Authors' contribution

Conceptualization of research (BA, GPS, KVP); Designing of the experiments (BA, GPS, KVP); Contribution of experimental materials (GPS, PKS); Execution of field/lab experiments and data collection (BA, KTR, PR, NJ, RN, KPS, TL); Analysis of data and interpretation (KTR, CPK, BA, HK); Preparation of the manuscript (BA, PKS, GPS).

Declaration

The authors declare no conflict of interest.

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