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Marker assisted stacking of drought tolerance QTL and rust resistance genes in an elite Indian wheat genotype

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Abstract

Backcross population derived from a cross between *Triticum aestivum* advance breeding line PBW703 (PBW343+*Lr24*+*Lr28*+*Yr10*+*Yr15*) and Babax, a drought tolerant line from CIMMYT was used for introgression of drought tolerance QTL (quantitative trait loci) regions in a leaf and stripe rust resistant background. Out of 24 polymorphic SSR markers reported to be linked to drought tolerance in earlier studies and 15 markers showed introgression on different chromosomes with minimum of two and maximum of nine introgressed regions in different BC$_2$F$_1$ plants. For phenotypic analysis, 364 BC$_2$F$_4$ progenies from best performing selected BC$_2$F$_1$ plants were evaluated for rust reaction, chlorophyll content, canopy temperature, stay green, plant height, number of tillers per meter, number of spikelets per spike, number of grains per spike, 100 grain weight under restricted irrigated conditions. Correlation analysis showed that there was significant and positive correlation between these morphological traits. Transgressive segregants were observed for all the traits which were out performing the recipient parent PBW703. A total of 42 plants carrying the Babax specific alleles for drought tolerance QTL and all the four rust resistance genes *Lr24*, *Lr28*, *Yr10* and *Yr15* were finally selected for agronomic evaluation. Selected improved lines for drought tolerance are being evalauted in the wheat breeding programme.

Key words. *Triticum aestivum*, Babax, drought tolerance, rust resistance genes, introgressed regions, yield related traits

Introduction

Bread wheat (*Triticum aestivum* L. em. Thell) is one of the most important cereal grains worldwide. India is the third largest producer of wheat in the world with an annual production of around 95.19 million tonnes in 2015 (FAO, 2015; Agriculture at Glance, 2014/15). Wheat has a wide range of climatic adaptability, but many biotic factors (diseases, insect pests and weeds) and abiotic factors (drought, high temperature, salinity, floods, freezing, high irradiation and nutrient deficiency or toxicity) limits its yield. Of all the abiotic stresses, drought is an important constraint to wheat productivity affecting different growth stages (Chavas and Oliveira 2004). Crops demonstrate various morphological, physiological, biochemical and molecular responses to tackle drought stress (Araus et al. 2002). Plants' vegetative and reproductive stages are intensively influenced by drought stress affecting the plant growth, tiller number, photosynthesis, number and size of the grains (Talebi 2011). In India, large areas under wheat in central region are rainfed which faces drought like conditions regularly. In the northern region, non-judicious use of ground water resources has led to depletion of ground water table. The most productive wheat growing areas (Punjab, Haryana and Western Uttar Pradesh) face imminent future problems due to water scarcity.

Rust diseases (stripe rust, leaf rust and stem rust) have historically been one of the major biotic production constraints worldwide. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* and leaf rust caused by *Puccinia triticina* continue to pose a significant threat to wheat crop production worldwide. The continuous emergence of new races through mutation or through the introduction of new pathotypes from outside regions, results in breakdown of resistance of wheat cultivars (Curtis 2002). Genetic resistance is the most economical and preferred method of reducing yield losses incurred due to rusts. Deployment of single

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resistance genes may result in significant shifts in the virulence pattern of the pathogen population leading to breakdown of resistance. Gene pyramiding is a breeding strategy where two or more genes are combined together within one genotype. A number of rust resistance genes have been introgressed in Indian wheat backgrounds using conventional as well as marker assisted selection (Sivasamy et al. 2009; Tiwari et al. 2014; Tomar et al. 2014).

Drought tolerance is a complicated trait which is controlled by polygenes and their expressions are influenced by various environmental elements. The complexity of stress tolerance traits, low genetic variance of yield components under stress conditions and the lack of efficient selection techniques impose limitations on traditional breeding approach (Gupta et al. 2012, 2017) for developing drought tolerant crops. This means that conventional breeding for this trait is difficult and marker assisted breeding, quantitative trait loci (QTL) mapping strategies and expression patterns of genes should be applied to produce drought tolerant genotypes (Bahari 2014). With the development of dense linkage maps and the development of powerful statistical tools, a large number of DT (drought tolerance) QTL have been identified in wheat in different studies making feasible the marker assisted introgression of drought related genes and QTLs in modern wheat cultivars (Budak et al. 2013). A RIL population derived from drought tolerant line Babax and Seri M82 has been extensively used for identifying QTLs for various drought related traits by screening under water deficit environments globally (Mathews et al. 2008; McIntyre-Lynne et al. 2010; Pinto et al. 2010; Lopes and Reynolds 2012). In the present study Babax was used as a donor for marker assisted transfer of drought tolerance QTLs to an Indian wheat genotype carrying two leaf rust (*Lr24*, *Lr28*) and two stripe rust (*Yr10*, *Yr15*) resistance genes in the PBW343 background.

Materials and methods

Plant material and experimental design

The experiment was carried out at Punjab Agricultural University, Ludhiana, India. *Triticum aestivum* advance breeding line (ABL) 'PBW703' which carried four rust resistance genes *viz*., *Yr10, Yr15, Lr24* and *Lr28* pyramided in the background of PBW343 (Chhuneja et al. 2011) was used as recipient parent and Babax a CIMMYT line (BOW/NAC//VEE/3/BJY/COC) highly tolerant to drought (Pinto et al. 2010), but susceptible to current races of rusts (unpublished data) was used as a donor parent. F_1 was backcrossed twice with the recipient parent PBW703 to develop BC_1F_1 and BC_2F_1 population. BC_2F_2 progeny of 74 selected BC_2F_1 plants was grown in the year 2013 and single plant selections were made on the basis of grain yield. A total of 364 $BC_2F_{2.3s}$ were planted in off-season at Keylong, Himachal Pradesh, India for the generation advancement. The 364 BC_2F_4 progenies along with the checks were sown under restricted irrigated conditions in augmented design and screened for various yield related traits. Two recently released cultivars WH1105, HD2967 and a sister line of recipient parent, PBW698, were used as experimental checks. Each genotype was sown in 2 m long pair rows with row to row distance of 20 cm and plant-to-plant distance of 10 cm. One pre-sowing irrigation was applied and then the trial was maintained under rainfed conditions using routine cultural agronomic practices. The mean rainfall for the months of December, January, February, March and April was 10.55, 6.45, 9.75, 17.3 and 8.96 mm and mean temperature was 12.1, 11.4, 15.9, 17.9 and 25.62^{o}C, respectively.

Molecular analysis

Molecular analysis was performed on BC_2F_1 plants for rust resistance genes and donor allele introgressions for DT QTL. Genomic DNA was isolated from leaves collected from backcross population and parents using CTAB method (Murray and Thompson 1980) with some modifications. The backcross plants/ progenies were analysed with SCAR/SSR markers reported to be linked with leaf rust and stripe rust resistance genes using linked markers *Lr24_Xgwm 114*, *Lr28_S421*$_{570}$, *Yr15_ Xgwm498* and *Yr10_E1* (Supplementary Table S1). For tracking donor drought tolerance QTL, the SSR markers linked to the reported DT QTL or the genomic regions harboring drought tolerance QTL (Pinto et al. 2010; Kirigwi et al. 2007; Wang et al. 2009; Kadam et al. 2012) were utilized. A total of 24 polymorphic SSR markers were used for introgression profiling (Supplementary Table S2).

Morphological traits evaluation

Phenotypic data for BC_2F_2 plants and BC_2F_4 progenies were recorded for various yield related morphological and physiological traits along with stripe and leaf rust reaction under restricted irrigated conditions. At adult plant stage, the stripe rust and leaf rust data was recorded as per cent of leaf area covered by the rust and scored according to modified Cobb's scale given by (Peterson et al. 1948). The observations were recorded for canopy temperature in ^{o}C (CT), stay green

(SG), chlorophyll content as SPAD values (CH), plant height (PH) (cm), number of tillers per meter (TL), number of spikelets per spike (SS), number of grains per spike (GPS), hundred grain weight (HGW) (g). The CT was recorded using infrared thermometer. Data was taken at late morning to early afternoon during cloudless period (10 AM to 2 PM) at Zadoks60 (Zadoks et al. 1974). Data were taken twice to minimize the influence of exposed soil. Leaf spectrometer was used to measure the stay green values from flag leaf at anthesis stage (Zadoks69). The chlorophyll content measurements were also made on the flag leaf with a chlorophyll meter SPAD at same stage. Ten plants were considered for recording the no. of tillers per meter, no. of spikelets/spike and thousand grain weight.

Statistical analysis

All data collected on BC_2F_4 progenies was subjected to Analysis of Variance (ANOVA) by online analysis from IASRI website (http://www.iasri.res.in/spadweb/default.aspx) according to augmented design. Pearson correlations were performed to understand the associations between recorded traits and the frequency distribution of traits among the BC_2F_4 progenies were studied using sigma plot software 13.0. The significant difference between the genotypic classes were calculated by using Z-test for two samples means.

Results

A total of 82 SSR markers reported to be linked with drought tolerance QTL were analyzed in PBW703 and Babax for parental polymorphism and 24 markers were observed to be polymorphic between the donor and recipient parents (Table 1). Molecular characterization for the drought tolerance QTL regions from the donor parent and four rust resistance genes from the recipient parent was conducted in BC_2F_1 generation.

Background selection for rust resistance genes

BC_2F_1 plants were analyzed for the presence of stripe rust resistance genes, *Yr10* and *Yr15* and leaf rust resistance genes *Lr24* and *Lr28*. Out of 95 BC_2F_1 plants 81, 91, 55 and 87 plants showed the presence of *Yr10*, *Yr15*, *Lr28* and *Lr24*, respectively. SCAR markers for *Yr10 and Lr28* were dominant, so it was not possible to distinguish heterozygous and homozygous plants. Number of plants carrying different combination of leaf and stripe rust resistance genes has been summarized in Table 2. A total of 44 BC_2F_1 plants were identified which carried all the four rust resistance genes.

Foreground selection for drought tolerance QTLs

A total of 95 BC_2F_1 plants were characterized using 24 polymorphic markers to identify the introgression of Babax specific allele for the drought tolerance QTL. Out of these, only 15 markers showed Babax specific introgression in the BC_2F_1 plants. These 15 markers showed introgression on eight wheat chromosomes i.e. 1B, 1D, 2D, 3B, 4A, 5A, 5B and 6D. A total of 94 BC_2F_1 plants showed introgression for one or the other chromosomal region carrying DT QTL. The introgression profile of the 43 BC_2F_1 plants with all the four rust resistance genes and introgression for drought tolerance QTL is summarized in Supplementary Table S3.

Evaluation of BC_2F_2 plants and BC_2F_4 progenies for agronomic traits

BC_2F_2 plants and BC_2F_4 progenies of the BC_2F_1 plants having leaf and stripe rust resistance genes and DT QTL along with the parents were evaluated for various agronomic traits under restricted irrigation conditions. A total of 1365 BC_2F_2 plants were sown in 2013-2014 cropping season and data were recorded for stripe and leaf rust reaction, tiller number/plant, grain weight and grain yield (data not given). On the basis of all these characters 364 best plants were selected for the advancement to BC_2F_3. Data were not recorded for any trait for the BC_2F_3 progenies. In main season of 2014-2015, 364 BC_2F_4 progenies derived from BC_2F_3 single plants were sown under restricted irrigation in augmented design and data were recorded on different yield related traits including stay green, chlorophyll content, canopy temperature, number of tillers per meter, number of spikelets per spike, number of grains per spike and 1000 grain weight under water stress conditions.

From the analysis of variance for various traits it was apparent that there were significant differences ($p<0.05$) among the genotypes for these traits (Table 3). Presence of block effect was also observed. The R^2 value of various traits varied from 0.96-0.97. The value of coefficient of variation did not exceed from 15 except for the traits chlorophyll content and tiller number per meter.

In the present study, the chlorophyll content of BC_2F_4 progenies ranged from 10.1 to 50.2 (Fig. 1a). We found that about 20 progenies showed statistically significantly superior chlorophyll content value than the recipient parent PBW703 indicating recurrent parent background was recovered well and some introgression

Table 1. SSR markers reported to be linked with drought tolerance QTLs and used in the present study

Chromosome	Total markers analyzed	Markers polymorphic between PBW703 and Babax	Associated traits	Reference
1A	5	*Xgwm497*	GFD	Wang et al. 2009
1B	14	*Xwmc269, Xwmc31, Xgwm498, Xgwm268, Xcfa2147*	DTA, CT	Pinto et al. 2010, Wang et al. 2009
1D	6	*Xcfd63*	DTM, CH	Pinto et al. 2010
2A	3	*Xgwm382*	NDVI	Olivares et al. 2007
2B	2	*Xcfd70*	CT, NDVI	Pinto et al. 2010
2D	2	*Xgwm484*	HI, GY	Kadam et al. 2012
3A	1	-	CT, CH	Kumar et al. 2012
3B	8	*Xbarc68, Xwmc533, Xbarc147*	CT, CH, KWI, GFD	Pinto et al. 2010, Wang et al. 2009, Kadam et al. 2012
3D	1	-	GY	Kadam et al. 2012
4A	12	*Xwmc48, Xwmc420, Xgwm468, Xwmc313, Xcfa2026, Xwmc429, Xwmc517*	GY, CT, PH, NDVI	Pinto et al. 2010 Kirigwi et al.2007
4B	16	*Xgwm165*	PH, HI, DSI, DTH,DTA,GY	Kadam et al. 2012
4D	4	-	DTM, DTA	Pinto et al. 2010
5A	1	*Xwmc327*	GY, HI, NDVI, CH, DTA, CT	Kadam et al. 2012, Pinto et al. 2010
5B	2	*Xwmc75*	DTM	Kadam et al. 2012
6D	2	*Xgdm132*	GFD	Wang et al. 2009
7A	2	-	DTA, DTM, TGW	Kadam et al. 2012
7B	1	-	NDVI	Kumar et al. 2010
TOTAL	82	24		

CH=Chlorophyll content, CT=Canopy temperature, DSI=Drought susceptibility index, DTA=Days to anthesis, DTH=Days to heading, DTM=Days to maturity, GFD=Grain filling days, GY=Grain yield, HI=Harvest index, KWI=Kernel weight index, PH=Plant height, NDVI=Normalized difference vegetative index, TGW=Thousand grain weight

Table 2. Summary of the marker analysis of BC_2F_1 plants for selection of pyramided rust resistance genes *Yr10, Yr15, Lr24* and *Lr28*

Gene/Gene combination	*Yr10*	*Yr15*	*Lr28*	*Lr24*	*Lr24+Lr28*
Yr15	81				
Lr28	91	55			
Lr24	78	80	56		
Lr24+Lr28	42	46	-		
Yr10+Yr15	-	-	43	64	44

from donor is also contributing towards it. Stay green trait value varied from 0.19-0.73 with a mean value of 0.5 which is lower than both the parents PBW703 and Babax having 0.6 and 0.59 stay green, respectively (Fig. 1b). About 101 progenies showed the superior value than the recipient parent PBW703. The range for canopy temperature varied from 18.80-31.75 with the mean value of 24.4. About 335 progenies showed superior value for canopy temperature than the recipient parent PBW703 which means these progenies had lower canopy temperature than PBW703 (Fig. 1c). Number of tillers per meter ranged from 24-170 with a mean of 88. Only nine progenies had more tillers than the recipient parent (Fig. 1e). No. of spikelets per spike varied from 11 to 20 with the mean of 15 and about 40 progenies had higher number of spikelets/spike than PBW703 (Fig. 1f). The number of grains per spike ranged from 13-58 with mean of 41 grains per spike (Fig. 1g). In the present study, 54 progenies showed increase in no. of grains per spike under restricted

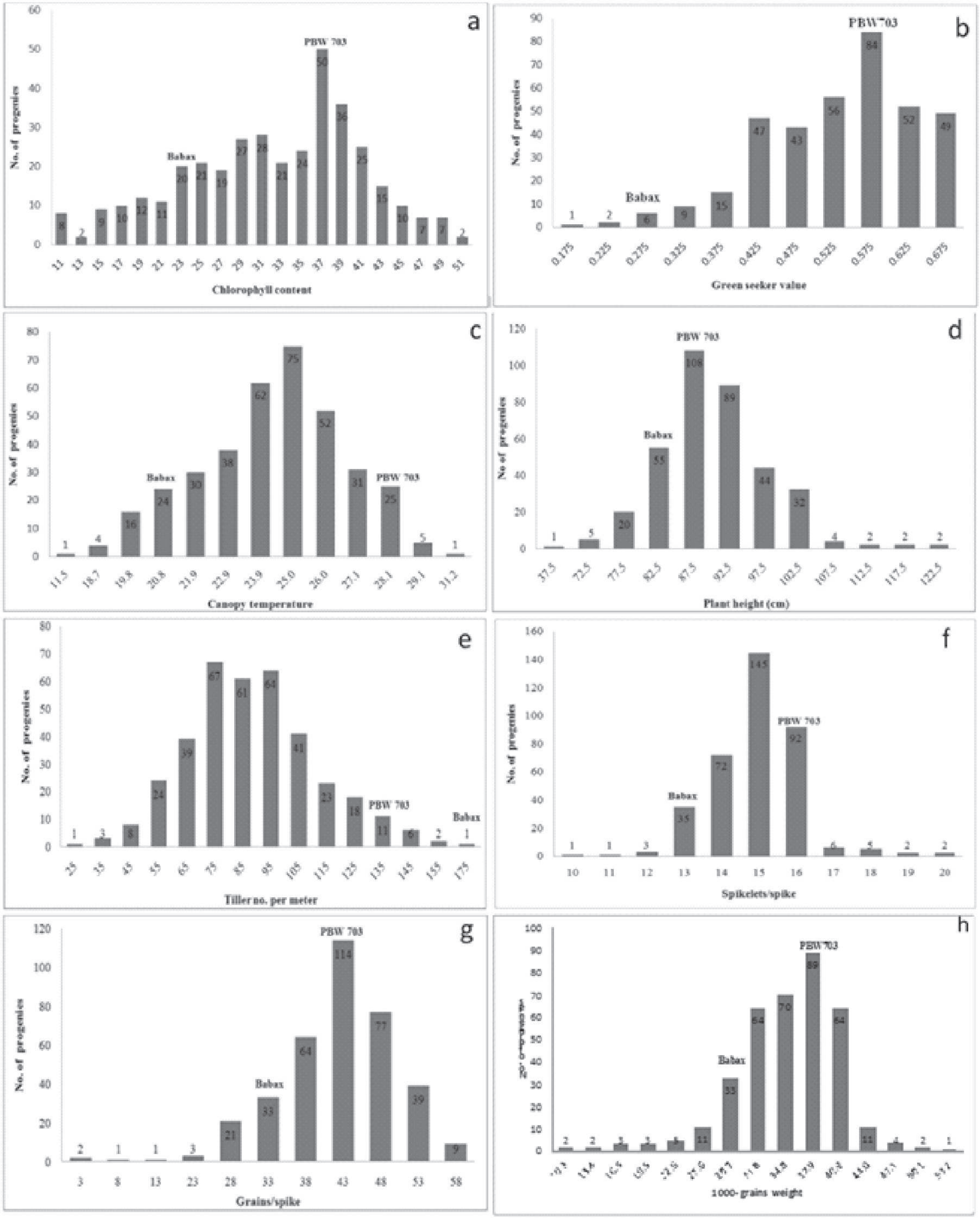

Fig. 1. **Frequency distribution of different yield related morphological traits of BC_2F_4 progenies under restricted irrigated conditions, (a) Chlorophyll content, (b) Stay green, (c) Canopy temperature, (d) Plant height, (e) Tiller number per meter, (f) No. of spikelets per spike, (g) No. of grains per spike, (h) Hundred grain weight**

irrigated conditions. Thousand grain weight ranged 8.7-54.7g with the mean value of 35.0g (Fig. 1h). Eighty two progenies have higher 1000-grain weight than PBW703, indicated that these progenies have some introgression from drought tolerant parent Babax, due to which they performed better under water limiting conditions.

Table 3. Analysis of variance of 364 BC_2F_4 progenies for various morphological traits

	df	CH	SG	CT	PH	TL	SS	GPS	HGW
MSS (genotypes)	363	62.82*	0.010*	4.75*	85.74*	551.30*	1.73*	60.20*	0.35*
MSS(block)	9	104.62	0.005	11.7	22.01	482.16	2.71	13.83	0.17
MSE(error)	27	40.4	0.004	2.14	29.98	271.3	1.23	24.32	0.17
R^2	-	0.97	0.98	0.98	0.98	0.97	0.96	0.97	0.97
CV	-	19.93	11.53	5.99	6.14	18.59	7.46	12.05	11.82
GM	-	31.9	0.53	24.42	89.25	88.59	14.85	40.93	3.48
CD(5)	-	18.44	0.17	4.24	15.89	47.79	3.21	14.31	1.19

* Significant at 5% level of significance; df- degree of freedom; CH=Chlorophyll content, SG=Stay green, CT=Canopy temperature, PH= Plant height, TL=Number of tillers per meter, SS=Spikelets per spike, GPS=Grains per spike, HGW=100 grain weight

Table 4. Phenotypic correlation coefficients among various morphological traits of BC_2F_4 progenies sown in cropping season, 2014-2015

	CH	SG	PH	TL	SS	GPS	HGW
CT	0.00286	0.0288	-0.0527	0.0171	0.135*	-0.00817	0.00612
CH		0.0161	0.117*	0.067	0.0612	0.0205	0.0324
SG			0.382	0.182*	0.218*	0.13*	0.0801
PH				0.194*	0.227*	0.112*	0.144*
TL					-0.0314	0.0277	0.0714
SS						0.132*	0.0162
GPS							-0.0291

* = significant at 5% level of significance; CH (SPAD meter)=Chlorophyll content, SG=Stay green, CT=Canopy temperature, PH (cm)= Plant height, TL=Number of tillers per meter, SS=Number of spikelets per spike, GPS=Number of grains per spike, HGW (g)=Thousand grain weight

Correlation analysis

Canopy temperature (CT) showed positive correlation with spikelets/spike (r=0.135; Table 4). Stay green showed positive correlation with tiller number (r=0.182), spikelets/spike (r=0.218) and grains/spike (r=0.12). Plant height showed positive correlation with number of tillers (r=0.194), spikelets/spike (r=0.227), grains/spike (r=0.112) and 1000 grain weight (r=0.144). No. of spikelets/spike showed positive correlation with grains/spike (r=0.132).

Selection of rust resistant and drought tolerant progenies

Based on extensive phenotyping we identified 42 progenies, which were significantly superior for one or other yield related traits under restricted irrigation conditions and were resistant to both stripe rust and leaf rust. The introgression for DT in selected 42 BC_2F_4 progenies was confirmed by amplifying only those SSR markers, which showed Babax specific introgression in BC_2F_1 plants. These selected progenies showed the presence of Babax specific introgression for various markers with maximum introgression observed for markers *Xcfa2041*, *Xcfd63, Xbarc147* and *Xgdm132*. Introgression profile and phenotypic data of the selected BC_2F_4 progenies is given in Table 5.

Overall, PBW703 contributed towards increase in different yield related traits, whereas Babax specific introgression contributed more towards improvement in drought tolerance under water limited conditions by improving the canopy temperature trait.

Discussion

To cope with the adverse effects of unpredictable climate change and rainfall pattern on plant yield, the development of drought tolerant wheat varieties is an alternative. In recent years, large numbers of QTLs have been identified in various wheat genotypes for

Table 5. Introgression profile and Phenotypic data of the selected BC_2F_4 progenies derived from the cross Babax/3*PBW703

S.No.	BC_2F_4 plant ID/ marker	Chromosome														Canopy temp.	Chloro- phyll content	Stay green	Plant ht	Tiller no.	Spkl/ spike	Grains/ spike	1000- GW
		1B Xgwm 498	1B Xwmc 269	1B Xgwm 268	1B Xcfa 2147	1D Xcfd 63	2D Xgwm 484	3B Xbarc 147	3B Xgwm 533	4A Xwmc 420	4A Xwmc 48	4A Xwmc 313	5A Xwmc 327	5B Xwmc 75	6D Xgdm 132								
1	GCPB-37	A	A	A	B	B	A	H	A	A	A	A	A	A	A	19.1	39.5	0.7	94.0	89.0	16.2	51.0	38.9
2	GCPB-39	A	A	A	A	B	A	-	A	A	A	A	A	A	A	22.1	38.4	0.5	88.0	59.0	15.4	48.0	41.9
3	GCPB-44	A	A	A	B	B	A	H	A	A	A	A	A	A	H	24.1	39.9	0.5	99.0	102.0	13.0	45.0	40.2
4	GCPB-54	A	A	A	B	B	B	-	A	A	A	A	A	A	A	21.1	40.0	0.6	100.0	94.0	16.2	45.0	40.0
5	GCPB-90	A	H	A	B	A	A	H	A	A	A	A	A	A	B	25.8	47.9	0.5	86.0	87.0	14.2	49.0	37.0
6	GCPB-112	A	H	A	B	B	A	H	A	A	A	A	A	H	A	24.5	38.1	0.5	87.0	89.0	14.8	52.0	38.2
7	GCPB-116	A	H	A	B	B	B	B	A	A	A	A	A	A	B	24.8	41.1	0.6	107.0	118.0	16.2	45.0	40.2
8	GCPB-124	A	A	A	B	B	B	H	A	A	A	A	A	B	A	24.1	44.5	0.6	90.0	61.0	15.2	51.0	39.6
9	GCPB-132	A	A	A	A	B	B	B	A	A	A	-	A	A	A	25.3	45.5	0.4	86.0	50.0	14.8	45.0	40.1
10	GCPB-143	A	A	A	A	A	A	B	A	A	A	B	A	A	B	27.1	43.4	0.5	74.0	69.0	15.6	46.0	39.5
11	GCPB-147	A	A	A	B	B	A	B	B	A	A	B	B	A	B	23.3	31.2	0.5	85.0	67.0	16.0	55.0	43.5
12	GCPB-161	A	A	A	B	B	B	B	A	A	A	A	A	H	B	26.1	41.7	0.5	75.0	95.0	14.8	54.0	37.3
13	GCPB-162	A	A	A	H	B	A	B	A	A	A	A	A	H	B	26.4	24.1	0.4	85.0	84.0	15.8	47.0	38.6
14	GCPB-175	A	A	A	H	B	A	A	A	A	A	A	A	A	A	27.6	32.3	0.6	89.0	106.0	14.8	46.0	39.2
15	GCPB-177	A	A	A	B	B	A	H	A	A	A	A	A	A	B	28.1	36.1	0.5	89.0	75.0	16.4	50.0	40.0
16	GCPB-178	A	A	A	A	B	A	H	A	A	A	A	B	A	B	26.1	18.2	0.4	87.0	69.0	15.0	53.0	37.7
17	GCPB-181	A	A	A	B	B	A	B	A	A	A	A	B	A	B	24.2	30.5	0.7	89.0	72.0	14.4	45.0	39.6
18	GCPB-187	A	A	A	B	B	A	H	B	A	A	A	A	A	B	27.6	22.6	0.6	79.0	88.0	14.8	47.0	38.7
19	GCPB-194	A	A	A	B	B	A	B	A	A	A	A	B	A	A	24.7	41.1	0.6	94.0	76.0	16.2	48.0	37.3
20	GCPB-199	A	A	H	B	B	A	H	A	A	A	A	B	A	A	24.9	24.4	0.6	89.0	89.0	15.6	48.0	37.5
21	GCPB-200	B	H	A	B	B	A	B	A	A	A	A	A	A	A	23.6	32.4	0.6	102.0	98.0	15.4	47.0	38.1
22	GCPB-207	A	A	H	B	B	A	A	A	B	B	A	B	A	A	25.2	42.9	0.6	93.0	51.0	16.6	48.0	41.3
23	GCPB-216	A	A	A	A	-	A	H	A	A	A	A	A	A	A	26.2	25.1	0.6	93.0	76.0	16.2	49.0	36.8
24	GCPB-226	A	A	A	A	B	A	H	A	A	A	A	A	A	A	25.6	34.2	0.4	93.0	148.0	14.0	47.0	42.4
25	GCPB-229	A	A	A	H	B	A	H	A	A	A	A	A	A	A	28.2	22.1	0.5	84.0	118.0	15.2	49.0	36.7
26	GCPB-230	A	A	A	H	B	A	H	A	A	A	A	A	A	H	28.2	32.1	0.5	86.0	81.0	15.0	46.0	37.9
27	GCPB-238	A	A	A	B	A	A	A	A	A	A	A	A	A	A	25.1	29.3	0.4	36.0	104.0	14.2	52.0	38.8

28	GCPB-246	A	A	H	B	B	A	H	A	A	A	B	B	A	A	22.1	22.6	0.4	94.0	99.0	14.8	49.0	36.8
29	GCPB-249	A	A	A	A	B	A	B	A	A	A	A	A	H	A	22.8	25.1	0.6	94.0	96.0	15.6	46.0	39.6
30	GCPB-251	A	A	A	B	B	A	H	A	A	A	A	A	A	A	24.6	35.1	0.5	96.0	94.0	15.6	48.0	41.8
31	GCPB-252	A	A	A	B	B	A	H	A	A	A	A	A	H	A	20.2	30.2	0.5	97.0	95.0	15.4	52.0	38.8
32	GCPB-258	A	A	-	B	B	A	B	B	A	A	A	A	B	A	20.2	31.2	0.6	93.0	94.0	14.6	54.0	36.8
33	GCPB-265	A	A	H	B	B	A	B	A	A	A	B	A	B	A	24.1	38.5	0.4	88.0	89.0	14.2	45.0	40.4
34	GCPB-270	A	A	A	B	A	A	B	A	A	A	A	A	B	H	23.6	22.2	0.5	96.0	87.0	15.8	45.0	39.7
35	GCPB-275	A	A	A	B	-	A	H	A	A	A	A	A	A	B	21.4	27.7	0.4	93.0	77.0	13.6	46.0	38.8
36	GCPB-290	A	A	H	B	A	A	H	A	A	A	A	B	A	B	23.8	37.7	0.4	86.0	103.0	13.6	49.0	40.5
37	GCPB-292	A	A	H	B	A	B	A	A	A	B	A	A	A	B	23.9	17.1	0.5	89.0	91.0	13.2	53.0	37.8
38	GCPB-294	A		A	B	B	A	B	A	A	B	A	A	A	A	22.1	18.0	0.6	100.0	97.0	13.0	47.0	38.5
39	GCPB-297	A		A	H	B	A	B	A	A	A	A	A	B	A	23.1	36.4	0.6	97.0	75.0	15.4	53.0	41.3
40	GCPB-305	A		A	B	B	A	B	A	A	A	A	A	B	H	22.9	41.9	0.6	104.0	79.0	16.2	51.0	39.1
41	GCPB-325	A		A	-	-	A	B	A	A	A	-	A	B	A	26.1	30.8	0.7	86.0	147.0	15.2	53.0	41.4
42	GCPB-326	A		A	-	-	B	B	A	A	A	A	A	B	A	27.1	19.6	0.5	93.0	94.0	14.6	49.0	40.7
43	PBW703	A	A	A	A	A	A	A	A	A	A	A	A	A	A	20.7	38.7	0.6	87.0	130.0	16.2	44.0	36.4
44	Babax	B	B	B	B	B	B	B	B	B	B	B	B	B	B	28.3	22.6	0.6	83.0	170.0	13.4	29.0	29.0

drought tolerance. Babax, an elite CIMMYT line was globally identified as drought tolerant genotype in such as Australia, Mexico and India in various studies (Mathews et al. 2008; McIntyre-Lynne et al. 2010; Pinto et al. 2010; Lopes and Reynolds 2012; Olivares-Villegas et al. 2007). A number of drought tolerant QTL have been mapped using Seri/Babax RILs population on chromosomes, 1B, 1D, 2B, 3B, 4A, 4D, 5A, 6A, 6B and 7A which are related to large number of yield related traits under drought stress conditions. The present study was conducted to transfer these identified QTLs from Babax to a stripe rust and leaf rust resistant version of mega variety PBW343 *viz*., PBW703. The backcross progenies of PBW703 and Babax were developed to combine good agronomic performance with disease resistance and drought tolerance QTLs from Babax. Markers/genomic regions linked to QTL reported in above studies were used to track drought tolerance QTL during backcrossing.

A number of morphological and physiological traits have been reported to be associated with the genetic variation in wheat under drought stress environment (Olivares-Villegas et al. 2007; Reynolds and Tuberosa 2008; Gupta et al. 2012; Gupta et al. 2017). In the present study, an improvement in the canopy temperature, chlorophyll content, stay green, tiller number, plant height, spikelets/spike, grains/spike and grain weight was observed under limited water conditions in the introgressed progenies. Babax specific introgression at chromosomes 1B might be contributing to the low canopy temperature, stay green and high chlorophyll content as has also been observed by Pinto et al. (2010). NDVI related QTL showed the most favorable increasing effect in 1B region controlled by Babax alleles. Most significant QTL for yield under drought was found on 4A by Pinto et al. (2010) and Kirigwi (2007) linked with SSR marker *Xwmc48*. In the present study introgression of *Xwmc48* and a closely linked marker *Xwmc420* was observed in BC_2F_1 but in the finally selected BC_2F_4 progenies only three progenies showed Babax specific introgression for these markers. Another QTL for grain yield was reported on chromosome 5B by Kuchel et al. (2007) and in our study we found that for this region, Babax allele increased the grains per no. of spike which ultimately will affect the yield.

From the molecular and phenotypic analysis of backcross progenies, we found that markers *Xcfa2041*, *Xcfd63* and *Xbarc147* showed introgression in most of the progenies. Under drought, CT was better associated with yield both phenotypically and genotypically (Olivares-Villegas et al. 2007). A number of significant associations of CT with other physiological and agronomic parameters supported that this parameter can be used for the selection of drought tolerant genotypes. These results indicate that Babax contained alleles that can improve agronomically important traits in elite cultivar and these QTLs may serve as potential targets for marker assisted selection to improve drought stress adaption. We can further use these progenies in breeding program for the development of rust resistance and drought tolerant lines by evaluating across the different locations and years.

QTL interval mapping and genome wide association studies (GWAS) have been conducted in wheat in a number of studies and a large number of QTLs have been reported for several traits related to drought tolerance. Besides the traits evaluated in the present investigation, other traits reported are coleoptile length, water-soluble carbohydrates (WSC) and root system (for a review, see Gupta et al. 2012; Gupta et al. 2017). Recent developments in genomics and phenomics will lead to precise and comprehensive characterization of the QTL regulating drought tolerance component traits. The introgressed progenies developed in the present investigation will serve a base for further studies.

Authors' contribution

Conceptualization of research (PC, KVP); Designing of the experiments (GSM, SK, PC); Contribution of experimental materials (PC, KVP); Execution of field/ lab experiments and data collection (GSM, SKB); Analysis of data and interpretation (PC, SK); Preparation of manuscript (GSM, SKB, SK, PC).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. List of the markers, expected amplicons for the rust resistance genes

Rust resistance gene	Marker	Type of marker	Diagnostic allele size (bp)	Resolving media	Reference
Lr24	*Xgwm114*	Co-dominant	120	6% PAGE	Pallavi et al. 2015
Lr28	*S421*$_{570}$	Dominant	670	1.5% Agarose	Gupta et al. 2008
Yr15	*Xgwm498*	Co-dominant	165	6% PAGE	Somers et al. 2004
Yr10	*E1*	Dominant	754	2.5% Agarose	Temel et al. 2008

Supplementary Table S2. SSR markers linked to drought tolerance allele reported in different studies

S.No.	Marker	Location	Sequence
1	*Xgwm498*	1B	5’GGTGGTATGGACTATGGACACT 3’ 5’ TTTGCATGGAGGCACATACT 3’
2	*Xwmc269*	1B	5’GCACCTTCTAACCTTCCCCAGC 3’5’ CCCTAATCCAGGACTCCCTCAG 3’
3	*Xgwm268*	1B	5’AGGGGATATGTTGTCACTCCA 3’5’ TTATGTGATTGCGTACGTACCC 3’
4	*Xcfa2147*	1B	5’TCATCCCCTACATAACCCGA 3’5’ATCGTGCACCAAGCAATACA 3’
5	*Xcfd63*	1D	5’ TCCTGAGGATGTTGAGGACC 3’5’ GAGAGAGGCGAAACATGGAC 3’
6	*Xgwm484*	2D	5’ ACATCGCTCTTCACAAACCC 3’5’ AGTTCCGGTCATGGCTAGG 3
7	*Xwmc533*	3B	5’ AATTGGATCGGCAGTTGGAG 35’ AGCAAGCAGAGCATTGCGTT 3’
8	*Xbarc147*	3B	5’ GCGCCATTTATTCATGTTCCTCAT 3’5’ CCGCTTCACATGCAATCCGTTGAT 3’
9	*Xwmc48*	4A	5’ GAGGGTTCTGAAATGTTTTGCC 3’5’ ACGTGCTAGGGAGGTATCTTGC 3’
10	*Xwmc420*	4A	5’ ATCGTCAACAAAATCTGAAGTG 3’5’ TTACTTTTGCTGAGAAAACCC 3’
11	*Xwmc313*	4A	5’ GCAGTCTAATTATCTGCTGGCG 3’5’ GGGTCCTTGTCTACTCATGTCT 3’
12	*Xwmc468*	4A	5’ AGCTGGGTTAATAACAGAGGAT 3’5’ CACATAACTGTCCACTCCTTTC 3’
13	*Xwmc327*	5A	5’ TGCGGTACAGGCAAGGCT 3’5’ TAGAACGCCCTCGTCGGA 3’
14	*Xwmc75*	5A/5B	5’ GTCCGCCGCACACATCTTACTA 3’5’ GTTTGATCCTGCGACTCCCTTG 3’
15	*Xgdm132*	6D	5’ ACCGCTCGGAGAAAATCC 3’5’ AGGGGGGCAGAGGTAGG 3’

Suplementary Table S1. Introgression profile of 43 BC_2F_1 plants derived from the cross PBW703/Babax//2*PBW703 using markers for chromosomal regions reported to carry drought tolerance QTLs

S.No.	BC_2F_1 plant ID/marker*	Chromosome														
		1B *Xgwm 498*	1B *Xwmc 269*	1B *Xgwm 268*	1B *Xcfa 2147*	1D *Xcfd 63*	2D *Xgwm 484*	3B *Xbarc 147*	3B *Xwmc 533*	4A *Xwmc 420*	4A *Xwmc 48*	4A *Xwmc 468*	4A *Xwmc 313*	5A *Xwmc 327*	5B *Xwmc 75*	6D *Xgdm 132*
1	357-7	H	A	A	H	A	A	H	A	A	H	A	A	A	H	A
2	357-9	A	-	H	A	-	-	A	A	A	A	A	A	A	A	H
3	359-1	H	-	H	A	A	H	H	A	H	H	A	A	A	A	H
4	359-3	A	A	H	A	A	H	A	H	H	H	A	A	A	A	A
5	359-5	A	A	H	A	A	H	H	A	A	A	A	A	A	A	A
6	359-9	H	H	H	A	A	H	A	A	A	A	A	A	A	A	A
7	363-1	H	H	H	A	A	A	A	A	H	A	A	H	A	A	A
8	363-10	A	H	-	H	A	H	A	A	-	A	A	H	A	H	A
9	363-4	H	H	A	H	A	A	A	A	H	A	A	A	A	A	A
10	364-2	A	A	A	A	H	A	A	A	H	A	A	A	A	A	H
11	364-6	A	A	A	H	H	H	A	-	H	A	A	H	A	A	A
12	365-8	A	A	A	H	H	A	A	H	H	A	A	H	A	H	A
13	366-2	A	A	A	H	A	A	A	H	A	A	A	H	A	H	A
14	368-1	A	A	A	A	A	A	A	H	A	A	A	H	H	A	A
15	368-2	A	A	A	A	H	A	H	-	A	H	A	-	H	A	A
16	368-6	A	A	A	A	H	A	H	H	A	A	A	H	A	A	A
17	369-1	H	H	H	H	A	A	H	H	A	A	A	A	A	H	H
18	369-3	H	H	A	A	A	A	A	A	A	A	A	A	A	A	H
19	370-7	H	H	H	A	H	A	A	A	A	A	A	A	A	A	H
20	371-2	A	A	H	A	H	H	-	A	H	H	A	A	A	A	H
21	372-1	A	A	A	H	H	H	A	A	H	H	A	A	A	A	H
22	372-7	A	A	A	H	H	A	A	A	-	H	A	H	A	A	-
23	373-1	H	H	A	H	A	A	A	A	H	H	A	H	A	A	H
24	374-1	H	H	A	H	A	H	H	H	H	H	A	A	H	A	A
25	375-1	H	H	H	A	H	H	A	A	A	A	A	A	H	A	A
26	384-3	A	H	H	A	A	H	H	H	-	H	A	A	H	H	H
27	385-1	H	A	A	H	A	A	H	A	A	H	A	A	A	A	H
28	385-2	A	A	A	A	A	A	A	H	A	A	A	A	A	H	H
29	385-3	H	A	H	A	A	A	A	H	A	A	A	H	H	H	-
30	386-10	H	A	H	A	A	A	A	A	A	A	A	A	A	H	A
31	386-2	A	H	H	A	A	A	A	A	A	A	A	-	-	A	A
32	386-5	H	H	A	A	A	A	H	A	A	A	A	A	H	H	A
33	387-2	A	A	A	A	A	H	A	H	-	H	A	H	H	H	A
34	389-2	H	H	H	A	A	A	A	A	A	H	A	A	A	H	A
35	402-4	A	A	A	A	H	A	-	H	A	A	A	A	A	H	H
36	402-8	H	A	A	A	H	H	-	A	A	H	A	H	A	A	H
37	413-1	H	H	A	A	A	A	A	H	A	A	A	A	H	H	A
38	416-1	A	A	A	A	A	A	A	H	-	H	H	A	H	A	A
39	417-1	H	H	H	H	A	A	A	A	A	H	H	H	A	A	A
40	421-2	A	H	A	H	A	A	A	A	-	H	H	A	A	A	A
41	421-4	A	H	H	H	H	A	H	A	H	H	H	A	A	H	-
42	427-4	A	A	A	H	H	H	A	H	H	H	H	A	A	A	A
43	430-2	A	A	H	A	-	H	A	A	-	H	A	A	A	A	A

***** A indicates PBW703 specific allele and H indicates Babax specific allele in heterozygous condition