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Short Communication

Introgression of drought tolerance QTLs through marker assisted backcross breeding in wheat (*Triticum aestivum* L.)

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Abstract

The present study reports the introgression of the genomic regions linked with drought tolerance traits *viz*., NDVI, staygreen, chlorophyll content/chlorophyll fluorescence and yield from a drought tolerant parent HI1500 in to a popular high yielding but drought susceptible wheat variety GW322 following the marker assisted backcross breeding. Background selection with 109 polymorphic SSR markers accelerated genome recovery of recurrent parent which ranged from 72.14 to 86.9% in BC_1F_1, 90.33 to 92.02% in BC_2F_1 and 91.6 to 94.95% with an average of 93.5% in BC_2F_2 generation. Eighteen homozygous BC_2F_3 progenies were found to be phenotypically superior for morpho-physiological and agronomic traits over the recurrent parent GW322.

Key words: QTLs, MABB, foreground selection, background selection, drought

Wheat is a crop of global significance in food security and the primary source of calories for millions of people worldwide. Abiotic factors are considered to be the main constraints (71%) in crop yield reductions (Boyer, 1982). Among the abiotic stresses, drought is the most difficult to deal with and breeding for drought tolerance is, therefore, a big challenge in wheat. Development of improved wheat cultivars with drought tolerance is critical for sustainable wheat production.

With the advent of molecular and genomic techniques, large number of QTLs for drought tolerance have been identified and mapped in crops (Tricker et al. 2018). Recently, there were attempts to validate and transfer the identified QTLs for drought tolerance traits in high yielding but susceptible wheat backgrounds (Rai et al. 2018a, 2018b; Jain et al. 2014). Marker Assisted Backcross Breeding (MABB) has been successfully utilised in many crops including rice, wheat, maize etc. for improvement of both qualitative as well as quantitative traits without substantial change in genetic background (Choudhary et al. 2019). In the present study, introgression of QTLs for drought tolerance related traits using MABB in wheat cv. GW322 was performed. GW322, a widely grown cultivar in central and peninsular zone of India. The donor parent HI1500 is drought tolerant and a stable yield performer under rainfed condition. F_1 was backcrossed to recurrent parent GW322 to produce BC_1F_1 seeds. Foreground selection was performed with DNA markers linked with targeted QTL region; Xgdm93 on 2D/2A (Oliveras et al. 2007) for NDVI, Xbarc68-Xbarc101 on 3B (Kumar et al. 2012) for chlorophyll content/chlorophyll fluorescence, Xwmc89 on 4A (Kiriwigi et al. 2007) for yield under stress and Xgwm111 on 7D (Kumar et al. 2010) for yield. For background selection, a set of 590 SSR markers spanning 21 chromosomes with minimum of ten loci per arm of chromosome were selected for polymorphism survey between the parents. For microsatellite assay, total genomic DNA was extracted from 3 week old seedling using standard protocol. The polymerase chain reaction was performed and the PCR products were analyzed by electrophoresis on 3% Metaphor gel. The progeny plants carrying targeted QTLs, high recovery of recurrent parent genome (RPG) and showing phenotypic similarity with recurrent parent were again backcrossed to generate BC_2F_1.

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For phenotypic evaluation, progenies selected with foreground and background markers in BC_1F_1, BC_2F_1 and BC_2F_2 generation were grown in rainfed condition (water stressed) in augmented design. The parents were grown in both rainfed and irrigated condition. In homozygous BC_2F_3 progenies, observations were recorded at grain-filling stage on five plants for traits like canopy temperature (CT), SPAD, NDVI, stomatal conductance (Gs) and delayed flag leaf senescence. Yield parameters like thousand kernel weight (TKW), number of tillers, number of grains per spike and plant height were measured on individual plants. Days to maturity and grain yield/m^2 were recorded on per plot basis. The mean difference for morpho-physiological traits between the parental lines and MABB derived lines were analyzed by an independent t-test. The chi square analysis for QTL positive and QTL negative plants was performed for goodness of fit to test the deviation of the observed segregants. The recovery of RPG was analyzed using Graphical Genotyping (GGT 2.0) software.

Marker-assisted foreground selection was used for the confirmation of the target alleles in the progeny of 540 BC_1F_1 plants. Tightly linked markers with trait/gene tend to be transmitted together in each generation. Among 540 BC_1F_1 plants, 252 were found heterozygous for the four target trait loci (Xgwm111, Xgdm93, Xbarc68-Xbarc101 and Xwmc98). The j”^{2} result fit to 1:1 revealing expected segregation ratio of BC_1F_1; j”^{2} = 0.1427 which is non significant at p= 0.05 accepting the null hypothesis. Finally, 66 plants were selected for further analysis based on foreground markers, background markers and phenotypic similarity to the recurrent parent.

Reduction of the donor genome segments other than the target gene requires evenly distributed polymorphic markers throughout the genome (Hospital 2001; Visscher 1996; Frisch et al. 1999). Out of the selected 590 SSR markers, 109 markers (18.47 %) were polymorphic between the parents. A total number of 87 polymorphic SSR (out of 109) markers with 4 to 5 markers/chromosome were used for RPG recovery in BC_1F_1. The overall RPG recovery ranged from 71.02% to 86.2 % in the 66 BC_1F_1 plants (Table 2). The selected BC_1F_1 plants were again back crossed with GW322 to develop BC_2F_1 population. Out of 484 plants in BC_2F_1 generation, which were obtained from selected BC_1F_1 plants, 223 plants were found QTL positive, each carrying 1-4 QTL. In this study, the expected 1:1 ratio of BC_2F_1 generation (j”^{2} = 0.084), non-significant at p=0.05 was observed. The background selection was performed in BC_2F_1 generation with the markers found heterozygous in BC_1F_1 along with additional set of 22 polymorphic markers (remaining 22 of the 109 markers). The extent of background recovery of GW322 ranged from 79.54 to 92.04% in BC_2F_1 generation with an average of 86.8%. Eighteen best plants were selected in BC_2F_1 on the basis of foreground selection, phenotypic resemblance with recurrent parent and background selection. GW322-40-86 with all four QTLs and 92.04 % background of GW322 was the best plant (Fig. 1). In this generation, the QTL positive plants were selfed to produce BC_2F_2 population.

In 760 plants of BC_2F_2 generation, plants with homozygous alleles of donor parent for foreground SSR markers were selected. For background selection of the homozygous lines, 46 SSR markers which were found heterozygous in BC_2F_1 generation were used. Eighteen BC_2F_2 plants with high RPG content ranging from 90.3% to 94.95% with different QTL combinations were selected (Table 1). The residual segments from the donor genome were distributed on chromosome 1D, 2A, 3B, 4A, 5B, 5D and 7A whereas perfect recovery of the recurrent parent's chromosomes was observed on chromosome 3A and 6B.

From BC_1F_1 to BC_2F_1 progressive increase in background recovery per cent was observed as a result of second backcrossing and additional marker data points used in background selection as compared to 85% in conventional back cross approach and further in BC_2F_2 generation increase in RPG was up to 94.95%. The percentage of substituted chromosome segments derived from HI1500 was merely ranging from 1.83% to 3.66%. This additional recovery is attributed to fixation of recipient allele from heterozygous allele which may be theoretically gained after 3-4 backcrossings in conventional approach. The homozygous lines had >90% genome of recipient parent GW322 except for segments carrying targeted regions. Also, selecting more number of plants for generation advancement and simultaneously practising phenotypic selection to remove undesirable plant in each generation enhanced the efficiency of MABB. The population size handled in each generation of this study was 540, 484 and 760 plants in BC_1F_1, BC_2F_1 and BC_2F_2, respectively. This increased the probability of getting desired individual with targeted QTLs governing favourable traits. Phenotypic performance of 18 MABB derived BC_2F_2 lines revealed

Table 1. Percent contribution of recurrent parent genome (RPG) in BC_1F_1, BC_2F_1 and BC_2F_2 generation of selected backcross derived lines introgressed with QTLs for drought tolerance

MABB derived lines MABB progenies	RPG (%)			No. of QTLs	LT, chlorophyll	Yield	NDVI	Staygreen
	BC_1F_1	BC_2F_1	BC_2F_2					
GW322-40-86-21	85.63	92.02	94.95	4	√	√	√	√
GW322-40-86-24	85.63	88.53	91.28	4	√	√	√	√
GW322-40-91-48	85.63	91.18	92.66	4	√	√	√	√
GW322-89-122-89	85.05	89.50	91.7	4	√	√	√	√
GW322-89-133-104	85.05	90.34	92.2	4	√	√	√	√
GW322-267-168-187	84.48	90.34	90.82	3		√	√	√
GW322-267-173-234	84.48	86.97	90.3	3		√	√	√
GW322-381-74-36	84.48	91.18	91.7	3		√	√	√
GW322-14-22-34	85.05	88.66	90.8	2			√	√
GW322-14-22-46	85.05	91.18	92.4	2			√	√
GW322-414-79-12	84.48	87.82	90.3	2			√	√
GW322-130-143-164	86.21	88.24	90.3	2			√	√
GW322-130-143-169	86.21	90.34	91.7	2			√	√
GW322-29-78-19	80.45	88.24	91.28	1				√
GW322-29-78-31	80.45	88.24	91.28	1				√
GW322-400-14-21	85.05	89.08	91.17	1				√
GW322-22-68-48	85.05	88.66	91.7	1				√
GW322-22-68-53	85.05	89.08	91.28	1				

Table 2. Phenotypic evaluation of backcross derived lines in BC_2F_3 for physiological and agronomic traits under stress

Traits	GW 322	Improved lines mean ±S.E
CT –Anthesis	38.4	27.14 ± 0.09
CT-Grain filling	34.5	31.50±0.26
SPAD-Anthesis	42	46.49±0.27
SPAD-Grain filling	36	42.12±0.20
NDVI-Anthesis	0.55	0.63±0.05
NDVI-Grain filling	0.36	0.48±0.03
Gs-Anthesis	286.5	269.06±1.98
Gs-Grainfilling	182.2	180.63±1.64
Staygreen	2	5.72±0.31
Plant height	91.29	93.71±0.93
No. of tillers	11	14±0.42
No. of grains per spike	48	56.22±0.30
Days to maturity	114	114.67±0.08
Grain yield/sq.m	480	548.61±1.12
TKW	40.5	43.54±0.25

majority of the derived lines were better than GW322 (Table 2). The lines GW322-40-86-21 and GW322-40-86-24 outperformed recurrent parent under non stress condition also. From the experimental results, it was evident that phenotypic performance of morpho-physiological and agronomic traits of the BC_2F_3 progenies was comparable with the performance of recurrent parent GW322.

Conventional breeding methods are time consuming, laborious and influenced by environment as compared to MAS based breeding methods which is simpler, efficient, robust and accurate. In this study, the recovery of the recipient parent genome was greatly accelerated emphasizing the increased efficiency of using markers to assist selection of backcross lines. The size of the donor chromosomal segment containing the target locus was reduced to ensure that there were minimal changes to the genetic composition of the recipient variety. The MABB derived lines will be a potential source of QTLs contributing drought tolerance and can be effectively utilised in wheat breeding programme under water stressed conditions.

Authors' contribution

Conceptualization of research (H, NJ); Designing of the experiments (H, NJ, LT); Contribution of experimental materials (PKS, NJ, H, GPS); Execution of field/lab experiments and data collection (LT, H, NJ); Analysis of data and interpretation (LT, H, NJ, GPS, KVP); Preparation of manuscript (H, NJ, LT).

Declaration

The authors declare no conflict of interest.

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