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Validation of SSR Markers Linked with Drought and Heat Tolerant QTLs in Bread Wheat (*Triticum aestivum* L. em.Thell.)

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ABSTRACT

Global warming caused by enhanced CO_2 in the atmosphere could disturb agriculture in future and lead to water deficit. Drought and heat stress is presently a major limitation to wheat productivity in arid, semiarid, tropical, and subtropical areas of the world. Marker-assisted selection which holds promise in improving selection efficiency and expediting the development of new cultivars with higher grain yield potential is used as a selection criterion for the development of stress tolerant varieties. Validation and use of markers linked with drought and heat tolerance traits that are previously reported across different populations would enhance the selection efficiency of MAS. In the present study, crosses were made between recipient parent HD2733 with two different donor parents HI1500 and WH730. In the BC_1F_2 population developed from the two crosses, different SSR markers viz., barc68-barc101, gdm93, gwm165 linked with drought tolerance QTLs, (chlorophyll content and canopy temperature), (normalized difference vegetation index), (grain yield) respectively and barc186, gwm190 linked with heat tolerance QTLs,(days to anthesis), (grain yield) respectively, were validated for their use in marker assisted selection (MAS) in BC_1F_2 segregating generation derived from cross HD2733X HI1500 (drought tolerance), HD2733X WH730 (heat tolerance). Both the markers gwm165 and gwm190 were linked with grain yield per plant in derived backcross populations. The six SSR markers were found appropriate for selection of drought and heat tolerant segregating lines in marker assisted backcross breeding (MAB) programs.

***Key words:** Drought and heat stress, Marker –assisted selection, SSR markers, grain yield*

INTRODUCTION

Wheat (Triticum spp.) is cereal grain, originally from the Fertile Crescent region of the Near East, but now cultivated worldwide. In 2009, world production of wheat was 682 million tons, making it the second most-produced cereal. Wheat is grown on more land area than any other commercial crop and is the most important staple food for humans. World trade in wheat is larger than for all other crops combined.

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According to FAO statistics[7] 729.5million metric tons harvested from 225.5 million ha area in the world (http://faostat.fao.org).The main wheat-growing areas of the world, predominantly with a Mediterranean climate, mean pan evaporation often exceeds average precipitation specifically during grain filling, leading to drought during reproductive and grain-filling phases, which is also known as 'terminal drought'[13] . Contact of higher than optimum temperature decreases yield and reductions in the quality of wheat[6,19]. Among all phenological stages in wheat reproductive and grain filling stages are most sensitive[16] and results in considerable yield losses. The severity and period of this drought stress determine the range of the yield losses.

Drought tolerance, an eminent complex trait is linked with a number of physiological and biochemical phenomena .Traits linked with such phenomena are commonly considered as secondary traits. Secondary traits used for selection purpose are mainly the root traits, osmotic adjustment and traits governing maintenance of plant water potential like relative water content[3,6] . Responses of plants to cope up drought situation are drought escape, drought recovery, drought tolerance, drought recovery and drought avoidance[1].

Heat stress in conjunction with drought, affects every aspect of plant growth by limiting the expression of full genetic potential of plant to yield better. But plants ability to yield well under different stress conditions is governed by several physio-morphic characters. Grain yield is reduced about 4 per cent, if temperature rises to 1^0C above the ambient temperature at the end of tillering and the beginning of grain filling, decreased number of spikes and number of grains per spike showed significant association with this yield reduction[6]. High temperature is a limiting factor as terminal heat stress in temperate environments, during anthesis and grain filling stage.

Quantitative trait loci (QTLs) are an unescapable crossroad for the molecular tailoring of wheat because most drought adaptive traits are polygenic. Various studies aim towards identification and mapping of QTLs related to several standardized drought adaptive physiological parameters in structured wheat populations (mapping populations). The knowledge of the number and effects of QTLs can benefit breeders to understand the genetic control of these traits and to project more efficient selection strategies for crop improvement[4].

The use of yield as a selection criterion for the development of stress tolerant varieties is prohibitive in early generations. As well, drought stress does not happen predictably and evenly in the field. Therefore, plant breeders look for alternative methods such as marker assisted selection (MAS). Rapid advances in genome research and molecular technology have led to the use of DNA marker-assisted selection which holds promise in improving selection efficiency and expediting the development of new cultivars with higher yield potential[17].

MATERIAL AND METHODS

The present study was carried out at the molecular lab, Division of Genetics, Indian Agricultural Research Institute, New Delhi during Rabi season, 2011 and 2012 under rainfed and irrigated condition.

Plant materials:

The study of validating SSR markers linked with drought and heat tolerance was conducted on BC_1F_2 segregating generation derived from cross HD2733X HI1500, HD2733X WH730. HD2733 is a high yielding variety released for North Eastern Plains Zone (NEPZ) of India under irrigated timely sown conditions. It is double dwarf (82cm), medium early maturing (130-135 days), resistant to leaf rust and leaf blight with average yield of 5.0 t/ha under timely sown irrigated condition[20]. HI1500 is released for central zone (CZ) under limited irrigations with average yield of 2.2 t/ha. It takes 126-134 days to mature, is 120-130 cm tall, resistant to stem rust and leaf rust, known to perform well under limited irrigation conditions (Annual report of AICW&BP) and hence was used as donor to transfer drought tolerant QTLs in the recurrent parent 'HD2733'.WH730 was selected under heat stress conditions, the variety recorded higher grain yield than other check varieties, this is because of low heat susceptibility index, thermo-tolerance of membrane, high kernel weight and grain number[5,12]. All these favorable traits led to characterization of WH730 into a heat tolerant variety (Annual report of AICW&BP). Specifications of the parental materials are given in Table 1.

Phenotypic differences were confirmed in parents for the targeted traits like chlorophyll content, canopy temperature, normalized difference vegetation index, days to anthesis and grain yield under drought and heat stress condition. The level of phenotypic differences were also confirmed at genotypic level by using the trait linked *barc68-barc101*[8], *gdm93*[13], *barc186*[14] *gwm165*[12] and *gwm190*[11] for chlorophyll content, canopy temperature, normalized difference vegetation index, days to anthesis and grain yield under stress condition respectively. The *barc68-barc101* marker linked allele was reported in RILs by intervarietal mapping of C306/HUW206 and favourable allele contributed by C306 parent with phenotypic variance of 35-40% [8], *gdm93* linked allele was reported in segregated population RILs of seri/babax[13] and the favourable allele contributed by Babax parent with a phenotypic variance of 20%,*barc186* linked allele was reported in the RILs population derived from the cross Seri/Babax and the favourable allele coming from Babax parent with a phenotypic variation of 6.4%[14],*gwm165* linked allele was reported in hexaploid wheat cross of Chinese spring/SQ1 to develop doubled haploid lines and favourable allele contributed by Chinese spring parent with phenotypic variance of 14.4% and *gwm190* was reported in RILs population of Kauz/ MTRWA116 and the favourable allele contributed by Kauz parent with a phenotypic variation of 44.3%[11] .

Table 1: Specification of the parents used in MABB program

Parents	Year of release	Institute	Parentage/Pedigree
HD2733	2001	IARI, New Delhi	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA
HI1500	2003	Regional Station IARI, Indore	HW2002*2/STREMPALLI/PNC5
WH730	2001-02	CCS HAU, Haryana	CPAN2092/Improved Lok1

DNA Extraction and SSR marker assay:
Total genomic DNA was extracted by micro-extraction protocol[15]. Polymerase chain reaction was performed in a thermal cycler (Applied Bio systems Inc., USA) using 10 µl total reaction volume. The reaction mixture contained 2-3µl (60-70ng/ µl) DNA, 2.0µl 10 x buffer with 25mM $MgCl_2$, 0.5µl dNTPs (10 mM) (Bangalore Genei, Bangalore, Karnataka, India), 1.0µl each forward and reverse SSR primers (20 mM) (Sigma Inc., St. Louis, MO, USA), 0.3µl *Taq polymerase* (3U/µl) (Bangalore Genei, Bangalore, Karnataka, India) and 5.2µl distilled water (sterile). Amplification of the template DNA was performed as per the annealing conditions of respective SSR markers used[8,11,12,13,14]. Amplified products so obtained were resolved on 3.2% metaphore agarose gel (Lonza, Rockland, ME, USA) stained with 0.1 µg/ml ethidium bromide (Amresco, Solon, OH, USA) along with a DNA size standard ladder (MBI, Fermentas) and documented in a Gel Documentation System (Biorad, Hercules, CA, USA). The markers difficult to score on metaphore agarose gels were run on automated chip electrophoresis system (Caliper life sciences, USA) for better resolution.

RESULTS

In the present study it was established that the targeted QTLs for canopy temperature, chlorophyll content, normalized differential vegetative index (NDVI),days to anthesis and grain yield in drought and heat stress condition were having better phenotypic expression in the segregating generation under drought and heat stress. Normalized differential vegetative index (NDVI) linked QTL located on 2A chromosome explained phenotypic variance of 9.3% (R^2 =0.093) in vegetative stage and phenotypic variance of 6.5% (R^2 = 0.065) in grain filling stage as against reported 20% with *gdm93* marker in segregated population. QFv/Fm ksu-3B, QChl ksu-3B, QLt ksu-3B controlling quantum efficiency of PS II, showed phenotypic variance of 13.5% (R^2 = 0.135) in grain maturity for chlorophyll content and phenotypic variance of 7.3% (R^2 = 0.073) in vegetative stage for canopy temperature in segregated population as against phenotypic variance of 35-40 % for canopy temperature and chlorophyll content flanked by markers *barc68-barc101* with 33cM interval. The days to anthesis linked QTL located on 5A had phenotypic variance of 8.9% (R^2 = 0.089) as against reported 6.4% with *barc186* marker in segregated population[14] grain yield under heat stress condition linked QTL located on 5D chromosome region with phenotypic variance 24.5% (R^2=0.245) as against reported 44.3% with *gwm190* marker in segregating population[11] under high temperature stress and grain yield under drought stress condition linked QTL region (segregated with *gwm165* marker) located on 4BS/L and 4DL chromosome showed 8.6% (R^2 = 0.086) for grain yieldthan the earlier report of 14.4% phenotypic variance[12] . Student's *t-test* for genotypic and phenotypic mean data was significantly different (P=0.01) for the targeted traits in segregating BC_1F_2 generation.

Table 3: Particulars of QTLs selected for introgression into HD2733 through MAB

S. No	QTL/Trait	Primer	Chromo	Sequence of Marker	Position	Annealing temp. (^{0}C)	R^2 %	Reference
1	Chlorophyll content	*barc68*	3B	F -5' CGATGCCAACACACTGAGGT 3' R-5' GCCGCATGAAGAGATAGGTAGAGAT 3'	66cM	55	35-40	Kumar *et al.*, (2012)
2	Canopy temperature	*barc101*	3B	F-5' GCTCCTCTCACGATCACGCAAAG 3' R-5' GCGAGTCGATCACACTATGAGCCAATG 3'	99cM	52	35-40	Kumar *et al.*, (2012)
3	NDVI	*gdm93*	2A	F-5'AAAAGCTGCTGGAGCATACA3' R-5' GGAGCATGGCTACATCCTTC3'	96cM	55	20	Oilvares *et al.*, (2008)
4	Days to anthesis	*barc186*	5A	F-5'GTGCTTGCTGAGCTATGAGTC3' R-5' GTGCCACGTGGTACCTTTG 3'	57cM	58	44.3	Pinto *et al.*, (2010)
5	Grain yield under heat stress condition	*Gwm190*	5D	F-5'GGAGTGTCGAGATGATGTGGAAAC3' R-5' CGCAGACGTCAGCAGCTCGAGAGG 3'	9cM	60	6.8	Mohammadi *et al.*, (2008)
6	Grain yield under drought stress condition	*gwm165*	4B	F-5' TGCAGTGGTCAGATGTTTCC 3' R- 5' CTTTTCTTTCAGATTGCGCC 3'	32cM	60	14.4	Quarrie *et al.*, (2005)

Note: NDVI: Normalized difference vegetation index

Table 4: Validation of QTLs directed for introgression.

Traits	Marker	R square	P value
NDVI (Vegetative stage) (Grain filling stage)	*gdm93*	0.093 0.065	0.0071 0.025
Canopy temperature (Vegetative stage)	*barc68-barc101*	0.073	0.017
Chlorophyll content (Grain maturity stage)	*barc68-barc101*	0.135	0.001
Days to anthesis	*barc186*	0.089	0.001
Grain yield (drought stress)	*gwm165*	0.086	0.009
Grain yield (heat stress)	*gwm190*	0.245	1.13E-08

Note: NDVI: normalized difference vegetation index

DISCUSSION

Yumbi Xu and Jonathan crouch 2007, suggested that published markers need to be validated in representative of breeding materials with appropriate population size. To rule out the possible statistical anomalies or errors the validation process tests whether the identified QTL show sufficient expression in the material generated by crossing parental lines and in other locations and or years and also to test its phenotypic expression when introduced into different genetic background. Because of allelic diversity, the genetic variation observed in one mapping population may not be similar in other population (at least recombination events in the target QTL regions). Thus, Nicholas, (2006) suggested that QTL or gene marker association identified in a single mapping population may not be directly used in unrelated population without validation. Similar suggestions were reported by Yumbi Xu Jonathan crouch 2007 for marker trait associations in representative parental lines and segregating breeding populations generated by crossing those lines. In segregating population BC_1F_2produced by crossing recipient parent HD2733 and donor parents HI1500 (drought tolerant), WH730 (heat tolerant). Our outcomes validate the existence of introgresed QTLs in the wheat chromosomes 2D, 3B, 5A, 5D and 4B inducing the phenotypic expression of quantitative traits. It was detected in drought tolerance cross, the association of *barc68-barc101* marker with quantum efficiency of PSII and chlorophyll content (*Qchl/ct*) QTL showed phenotypic variance of 13.5% at grain maturity stage, *gdm93* marker associated with normalized difference vegetation index (*Qndvi*) QTL showed phenotypic variance of 9.3% at vegetative stage and 6.5% at grain filling stage and gwm165 marker associated with grain yield (*Qyield*) under drought stress condition showed phenotypic variance of 8.6%.In heat tolerance cross, it was found that the targeted QTLs for days to anthesis and grain yield under stress condition were having better phenotypic expression in the segregating generation under heat stress,barc186 marker associated with days to anthesis (*Qanth)* QTL showed phenotypic variance of 8.9% and gwm190 marker associated with grain yield *(Qyield)* under heat stress condition showed 24.5%. Melchinger *et al*[9] found alike outcomes of QTL expression while studying complex traits with cross validation in $F_{4:5}$ populations of two elite cultivars in maize under 16 different environmental conditions. Stephanie and Kevin[18] also create similar results, where they have validated deoxynivalenol (DON) QTL in NILs population of barley which has been primarily identified through association mapping in elite breeding germplasm lines of barley. Mengistu *et al*[10] reported same kind of conclusions for QTL validation; they took previously identified QTLs in recombinant inbred chromosome lines (RICLs) developed from wheat cultivar.

CONCLUSIONS

Maker *barc68-bar101* linked with chlorophyll content and canopy temperature (*Qchl/ct*), *gdm93* linked with normalized difference vegetation index (*Qndvi*),*barc186* linked with days to anthesis (*Qanth*) and *gwm165* and *gwm190* linked with grain yield under drought and heat stress condition respectively (*Q grain yield*) showed polymorphism between selected parents. In the segregating BC_1F_2 population created in the marker assisted backcross breeding program under stress condition validation was done by a known polymorphic SSR markers connected with stress tolerant QTLs. Planned application of marker assisted foreground and background selection in individual generation was done for validated QTLs with high phenotypic variance for high temperature and drought stress responsive traits were transferred and stable in HD2733 background.

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