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Article in *Indian Journal of Genetics and Plant Breeding* · November 2014

DOI: 10.5958/0975-6906.2014.00893.1

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Molecular approaches for wheat improvement under drought and heat stress

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(Received : September 2014; Revised : November 2014; Accepted : November 2014)

Abstract

Novel molecular breeding selection strategies were implemented in breeding programs in India for wheat improvement under drought and heat stress. Elite Indian varieties HD2733 and GW322 were targeted for improvement to abiotic stresses through marker assisted backcross breeding approach. Backcross populations were advanced to BC₁/BC₂ F₂ after tracking QTLs for foreground selection covering traits such as canopy temperature (CT), chlorophyll content, normalized difference vegetation index (NDVI), grain filling duration (GFD), thousand kernel weight, grain yield and a range of polymorphic microsatellite markers covering entire genome (4-5 SSR markers per chromosome) being used for tracking the recurrent parent genome. Marker assisted recurrent selection (MARS) involved estimation of marker effects for several small effect/major QTLs followed by two recombination cycles. Designed intermating among selected F₅ families were carried out after conducting ANOVA and AMMI analysis on multi-location yield trials and polymorphic markers among the parents in two biparental base populations (F₅). Inter-family intermating among the best identified families carried out in different combinations to accumulate and recombine 4-8 QTLs per intermated progeny and confirmed in selfed homozygous lines.

Key words: Molecular breeding, wheat, MARS, QTL, molecular markers

Introduction

Wheat is an important staple crop worldwide and represents about 30% of the world's cereal area. In India, wheat occupies 29.9 million hectares area with annual production of 94.8 million tonnes. India along with China accounts for 48% of the total wheat consumed in the

developing countries [1]. In India, abiotic stresses are one of the major constraints towards realizing the yield potential and wheat occupies about 5-30 per cent of total cultivation under moisture and temperature stress with varying extent in different states. Drought and heat stress impairs wheat growth at different growth stages beginning from seedling to anthesis to the grain-filling stage. No remarkable progress has been made in the past due to quantitative inheritance of these complex traits and lack of understanding regarding the mechanisms underlying the genes. In the last few years, much emphasis is being laid on the fine-dissection of morpho-physiological traits for drought and heat tolerance [2, 3]. Earlier, the physiological parameters were not very explicitly used in breeding programmes, however recent advances in crop physiology has offered the breeders novel opportunity to combine adaptive traits. As a result, focused and integrated breeding programmes based on physiological traits based selection coupled with various genetics and genomics approaches are now being practiced to enhance stress tolerance in wheat. This is further complemented with the availability of a number of handy and portable instruments allowing precise and easy large scale field screenings under stress conditions. Thus a combination of precise phenotyping and high through-put genotyping has helped the breeders in exploiting the genetic gains for these complex traits under stress and enabled the improvement of genotypes in their breeding programmes. A brief overview of the physiological trait based selections and incorporation of MAS strategies for improvement of wheat under drought and heat stress is presented here.

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Physiological trait based germplasm characterization and selection

Precise phenotyping can lead to identification of genes associated with important stress adaptive traits [3]. Phenotypic trait variability can translate in better drought and heat tolerance and outline the genetic architecture including complex QTLs harboring physiological characters. A variety of morpho-physiological and agronomic parameters have been known for drought and heat tolerance, of which a few can be used to measure both. Certain physiological traits are known to be stress adaptive and may lead to variation in drought and heat stress responses. Increase in the rate of yield improvement can be achieved by targeting such traits that affects yield in dry [3] and warm [4] environments. Genetic gains associated with these traits have been measured in a range of environments and a core set has been compiled by CIMMYT representing variability for various morpho-physiological traits. We explored the variability among the core set under Indian conditions alongwith an Indian germplasm set and selected some promising lines as donors for breeding programmes.

The genetically diverse core set and Indian set was analysed under rainfed/restricted irrigation conditions to screen for drought stress and under delayed planting for heat stress. Drought stress was generated using a combination of restricted irrigation schedule (three instead of six) and no irrigation (rainfed). The performance of individual genotypes was assessed in timely, late and very late sown time schedules for heat stress. The major focus was on characterization of physiological and agronomic traits such as germination rate, days to heading (DH), days to anthesis (DA), days to maturity (DM), canopy temperature (CT), canopy temperature depression (CTD), stomatal conductance (Gs), flag leaf area (FLA), normalized difference vegetation index (NDVI), chlorophyll fluorescence (CFL) and SPAD chlorophyll meter reading chlorophyll content (CC), thousand kernel weight (TKW), biomass, yield and contributing traits. All physiological measurements were taken by portable instruments in field plots. Canopy temperature [CT] ($^{\circ}$ C) was measured during early boot (Z39) and 10 days after anthesis using infrared thermometer. Fv/Fm was measured during late boot stage through chlorophyll fluorescence meter. Green seeker was used to measure Normalized difference vegetation index (NDVI) at six different growth stages from early boot (Z 39) to dough development (Z 85-87) stage. Chlorophyll content was measured using SPAD meter. Morphological traits such as leaf area (square cms) was measured using leaf area meter. Plant

height (cms), spike length (cms) and grain yield (grams) per plot were measured under drought and heat stress.

Statistically significant correlation coefficients were observed between DH and DM, yield and DH (negative), TKW with DH, DM (positive), CT with TGW, yield (negative) and CTD with DH and yield (positive) (Table 1). Genotypes with higher stomatal conductance (Gs) and cooler canopies under warmer climate were identified. Genotypes Kauz/AA/Kauz, Sokoll, Tepoca/Rabe and RAC 875 had higher Gs. Indian lines HD 2932, HD 2987, FLW-18, HW 2004 and Raj 3765 were having equal potential. Chlorophyll fluorescence was also found to be an effective indicator of heat stress during grain-filling duration and superior genotypes correlated well with the Fv/Fm during reproductive stages.

Table 1. Correlation coefficients of various traits with yield and TGW among the core set under drought/heat stress

	DH	DM	CT	CTD	SPAD	NDVIgf	Fv/Fm
Yield	-0.22**	-0.11	-0.25**	0.38**	0.29**	0.32**	0.21**
TGW	0.20*	0.19**	-0.15*	0.04	0.273**	0.46**	0.12

Significance: *p=0.05, ** p=0.01

Stay-green in the post anthesis phase has been associated with drought and heat tolerance in several crops [5]. We observed significant variation across the genotypes for NDVI measured at different growth stages in wheat (Table 2). Genotypes have been selected from Indian and international core set for various physiological traits pertaining to drought and heat tolerance and are being used as donors in breeding programmes to introgress heat and drought tolerant traits. Table 2 represents the promising genotypes for their consistent performance in multi-year multi-location yield trials under restricted irrigation/late sown conditions.

Molecular breeding selection strategies

Recent developments in molecular genetics have strengthened the breeders with powerful tools to identify and select complex traits. Association between markers and traits reduce the influence of environment which is a major hindrance in conventional selection of complex quantitative traits [6]. Marker aided selection significantly increases the efficiency of selection by including approaches like marker assisted backcross breeding (MABB), and marker assisted recurrent selection (MARS).

Table 2. Genotype performance (best five) trait-wise under drought/heat stress among the core set based on multi-environment analysis

Canopy temperature	Stomatal conductance	F _v /F _m	NDVI boot stage	NDVI at dough stage	Grain yield	TKW
Sokoll	HGO94.9.1.52	Pastor//HX L7573/2*BAU/ 3/WBLL1	Babax	VJ01	Kukri/Excalibur	WBLL4//OAX 93.24.35/WBLL1
PASTOR//HX L7573/2*BAU/ 3/CMH82.575/ CMH82.801	FRTL//ATTILA /3*BCN	HGO94.9.1.52	QH_74-3	WBLL4/ Nursit	Bav92/Seri	PUB94.15.1.12/ FRTL
BAV92/Seri	Babax	Gladius	Kukri/ Excalibur	Pastor//HXL 7573/2*BAU/ 3/WBLL1	Granero inta	HGO94.9.1.3
SB025	Krichauff		Granero inta	Kukri/Excalibur	Kukri/Rac875	Sokoll/WBLL1
Janz	SSRW35		Excalibur	WH730	Babax	PARUS/3/CHEN/ AE.SQ//2*OPATA

Marker assisted backcross breeding

MABB approach produces a cultivar carrying the major gene/QTL from the donor background while the rest of the genomic constitution is from the recurrent parent [7]. MABB has extensively been used for improvement against biotic stresses but under explored for drought and heat tolerance owing to the complexity of traits and QTLs undergoing epistatic interactions with little or no phenotypic variance in different backgrounds. Elite Indian varieties HD2733 and GW322 from North-western plains zone and Central zone, and still covering a major share of breeder's seed indent, were targeted for improvement to abiotic stresses through marker assisted backcross breeding approach. Exotic wheat lines as Kauz, Excalibur, Rac875, Babax and indigenous lines viz. C306, HD2987, HI1500, WH730 and HD2888 were used as donor parents for transferring QTLs pertaining to drought and heat tolerance traits.

Stress linked QTLs [8,9] were validated in Indian set and international core set. The validated QTLs harboring SSRs for stress adaptive and agronomic traits were utilized for introgression of target loci in marker-assisted foreground selection (Table 3). High-yielding recurrent parent background selection was carried out using SSR markers covering all the chromosomes. Marker-assisted foreground selection was carried out in backcross populations by tracking QTLs linked to physiological traits such as canopy temperature (CT), chlorophyll content (SPAD values), stay-green habit, NDVI values, days to anthesis and agronomic traits as yield and its contributing traits for drought and heat

tolerance. Backcross populations were advanced to BC₁/BC₂ F₂ after tracking QTLs for fore-ground selection and a range of 70-90 polymorphic microsatellite markers covering the entire genome (4-5 SSR markers per chromosome) being used for tracking the recurrent parent genome. The lines with more than 90% recurrent parent background recovered in BC₁F₁ and BC₂ F₁ progenies (Table 4) were selfed and carried forward for development of homozygous lines. The selfed BC₁F₂ and BC₂F₂ homozygous lines were advanced for seed multiplication and homogeneity phenotyping.

Marker assisted recurrent selection

Abundance and availability of molecular markers have enabled the plant breeders to select on the basis of genotypic values derived from markers [10]. These include approaches like marker assisted recurrent selection that involves random intermating among the individuals selected on the basis of their marker genotypes [7]. MARS involves estimation of marker effects for several small effect QTLs or major QTLs followed by two or three recombination cycles [11]. MARS was exercised for drought and heat tolerance by conducting multi-location phenotyping at four target locations (IARI New Delhi, ARI Pune, JNKVV Powarkheda and PAU, Ludhiana) under rainfed and irrigated conditions in two F₄ base biparental populations comprising of 160-180 lines (Fig. 1). The polymorphic markers identified between parents of the two crosses were screened on the MARS populations to identify progenies carrying desirable QTLs as putatively detected. QTLs previously identified from marker-trait

Table 3. Stress linked QTLs for introgression into backcross populations

Backcross populations	Population size in BC ₁ F ₁	Traits targeted for foreground selection	Chromosomal location	References (foreground QTLs)
HD 2733/C306	645	Yield, DSI, root biomass CT	4B,1D	Kadam S. <i>et al.</i> , 2012 <i>Funct. Integr. Genomics</i> , 12 : 447-464.
HD2733/HD2888	454	Yield, above-ground biomass	1D,4A	Pinto R.S. <i>et al.</i> , 2010. <i>Theor. Appl. Genet.</i> , 121 : 1001-1021.
GW322/HI 1500	540	Stay-green, Chl content, CT	7D,4B	Kumar. <i>et al.</i> , 2010. <i>Euphytica</i> , 174 : 437-445.
GW322/HD2987	780	Stay-green, NDVI, Yield, HI, Biomass, grain filling rate, shoot biomass	3B,4A,4B	Kirigwi F. M. <i>et al.</i> , 2007. <i>Mol. Breed.</i> , 20 : 401-413.
HI1500/HD2733	516	Chl, Fv/Fm, CT, NDVI, Yield	3B,2B,4B	Kumar. <i>et al.</i> , 2012. <i>Euphytica</i> , 174 : 437-445.
Kauz/HD2733	136	Yield under thermal stress, Grain no. 5D, 7B		Pinto RS. <i>et al.</i> , 2010 <i>Theor Appl. Genet.</i> , 121 : 1001-1021

Table 4. Percent recurrent parent genome in BC₁ and BC₂ generations

Backcross populations	Progenies selected (foreground selection)	Background genome recovery in BC ₁ F ₁	Background genome recovery in BC ₂ F ₁
HD 2733/C306	62	75	94
HD2733/HD2888	20	76	94
GW322/HI 1500	28	90	91
GW322/HD2987	38	78	-
HI1500/HD2733	38	76	90
Kauz/HD2733	15	-	-

associations in biparental F₂ or F₃ populations for drought and heat tolerance and validated in our backgrounds were included for calculating marker effects. New QTLs were also identified among the F₄ base populations by employing marker-trait associations. The best progenies were selected on the basis of multi-location data and genotyping with SSR markers linked to stress adaptive traits [8,9].

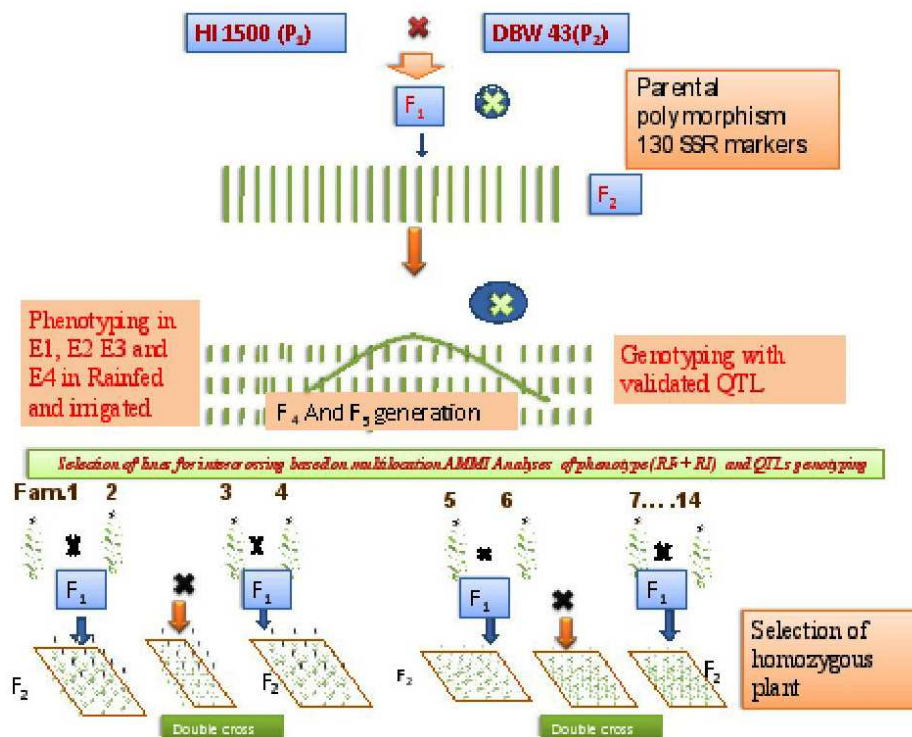
For combining favorable QTLs, stress adaptive traits like early vigour, SPAD values at vegetative and reproductive stage, canopy Temperature at vegetative and reproductive stage, NDVI, chlorophyll fluorescence, flag leaf area etc. were assessed in two base populations. ANOVA and AMMI analyses were conducted for yield using data from all locations and stable genotypes were identified across the locations. First round of designed intermating among selected F₅ families was carried out. Inter-family intermatings among the best identified families were carried out in F₅ generations in different combinations to accumulate and recombine 4-8 QTLs per intermated progeny (Table 5). The progeny of F_{5:2} and F_{5:1} X F_{5:1} hybrids were screened for genetic homogeneity and enhanced WUE and/or heat tolerance. Finally the homozygous intermated lines were screened phenotypically in field trials to identify best lines to be released as varieties. MARS thus implicates multi-trait improvement by combining favorable alleles pertaining to minor as well as major QTLs [12,13]. The F_{5:1} X F_{5:1} progenies were superior in performance to the parents (Table 6) and latest check variety HD 3043 and these promising lines have already been entered into station trials.

Table 5. A brief summary of two MARS populations intermatings

MARS population	Best identified progenies	SSR markers for genotyping	Single crosses for first round of recurrent selection	Double crosses for second round of recurrent selection	Entries made into station trials and CVT
DBW43XHI1500	10	80	100	27	17
HUW510XHI1500	12	20	40	25	2

Table 6. Performance of best five promising $F_{5:1} \times F_{5:1}$ MARS intermated progenies

Pedigree	Spike length	GW/10Spikes	TGW	NDVI gf	Biomass	CT	Yld
104-2/55-2	11.375	30.045	50.04	0.30	1795	23.85	797.5
1-6/93-1	10.75	35.695	52.20	0.29	1665	23.5	805.0
43-2/105-1	11.75	36.395	49.8	0.44	2037.5	24.6	850.0
55-16/104-3	11.375	30.725	49.1	0.31	1807.5	24.65	782.5
63-2/55-11	12.25	35.87	50.0	0.34	1890	24.75	785.0
HI1500	9.875	27.76	50.0	0.35	1875	24.00	742.5
DBW43	12.00	33.755	49.7	0.35	1649	23.25	667.5
HD 3043	10.125	30.31	45.5	0.32	1500	25.25	750.0

**Fig. 1.** Scheme representing inter-family intermatings in MARS progenies

Breeding wheat varieties for abiotic stress tolerance was dependent on conventional screening approaches. However, in the present day, abundant genomic approaches are available to breeders to adapt to the demands of specific target species and breeding objectives. A judicious combination of high-throughput phenotyping with modern genomics will lead to the development of stress tolerant cultivars that will enhance the total food security. The novel molecular breeding selection strategies such as marker assisted backcross breeding and marker assisted recurrent selection has been successfully implemented in wheat crop and has

enabled the development of stress tolerant varieties for future.

Acknowledgements

The financial support received from Generation Challenge Programme funded by CIMMYT, Mexico and National Initiatives on Climate Resilient Agriculture project supported by ICAR is gratefully acknowledged.

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