Indian J. Genet., 75(2): 256-259 (2015)
DOI: 10.5958/0975-6906.2015.00040.1

Short Communication

Improvement of leaf rust resistance in bread wheat variety DWR162 (*Triticum aestivum* L.) through marker assisted selection

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(Received: November 2014; Revised: February 2015; Accepted: March 2015)

Abstract

SCAR markers SCS1302 and SCS421 linked to leaf rust resistance genes *Lr24* and *Lr28* used in the present study genetically improved a bread wheat variety DWR162 using marker assisted selection. Evaluation of BC_1F_2 generation under field conditions indicated that plants with both *Lr24* and *Lr28* were superior in yield and its components and leaf rust resistance. Twenty six DUS characters used for identification of plants showed close resemblance with recipient variety DWR 162. The availability of a combination of the two major leaf rust resistance genes in desirable background would encourage the genetically enhanced DWR 162 with leaf rust resistance for its cultivation in a large area.

Key words: *Triticum aestivum*, leaf rust, marker assisted selection, resistance genes, pyramiding, DWR162

Leaf rust of wheat (*Triticum aestivum* L.) caused by *Puccinia triticina* Eriks, is one of the most important wheat foliar diseases worldwide (Kolmer 1996). Host resistance is the most economical, effective, and ecologically sustainable method of controlling the disease.

About 71 genes for resistance to leaf rust (*Lr*) have been cataloged in wheat (Singh et al. 2012). Most *Lr* genes confer race-specific seedling resistance and are vulnerable to defeat by new virulent races. Hence, the use of combinations of genes has been suggested as the best method for genetic control of leaf rust (Roelfs 1988). This activity of combining resistance genes can be achieved by pyramiding effective resistance genes. However, gene pyramiding is difficult using conventional breeding methods, but the availability of molecular markers closely linked with the target genes makes the identification of plants with two and three genes possible (Gupta et al. 2009). A set of alien leaf rust resistance genes *viz*., *Lr19*, *Lr24* and *Lr28* effective against prevalent and virulent leaf rust races in India were initially mobilized from the winter wheat stocks to improved genetic backgrounds (Tomar and Menon 1998). These near isogenic lines (NILs) constitute the basic parental material for the transfer and pyramiding of the above mentioned genes into other cultivated backgrounds.

The present investigation was carried out at Dr. Sanjay Rajaram Wheat Laboratory, All India Coordinated Wheat Improvement Project (AICWIP), Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad (UAS). The commercially cultivated but leaf rust susceptible bread wheat genotype DWR162 evolved from a cross Kavakaz/Buho//Kalyanasona/Bobwhite was selected for pyramiding of leaf rust resistance genes, *Lr24* and *Lr28*. Near isogenic lines of PBW343 with *Lr24* and *Lr28* developed through marker assisted backcross breeding were used as donor for introgression.

The crossing was attempted in polyhouse and in fields of UAS, Dharwad and in off-season at IARI, Regional Station, Wellington, Tamil Nadu. DWR162 plants were crossed with NIL's of PBW 343 with *Lr24* and *Lr28* and F_1 seeds were backcrossed with DWR162

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using it as female parent. BC_1F_1 seeds were generated during *rabi* 2011-2012 and space planted in *kharif* 2011. Individual plants of BC_1F_1's were confirmed for the presence of *Lr24* and *Lr28* using SCAR markers linked to target genes for resistance to leaf rust. Molecularly confirmed BC_1F_1 plants with both *Lr24* and *Lr28* genes were selfed to get BC_1F_2. The BC_1F_2 plants were evaluated for various agronomic parameters to assess the yield potential during *rabi* 2012-13.

When the plants were at four leaf stage, genomic DNA was extracted from leaf tissue using the modified CTAB (Dellaporta et al. 1983). DNA was quantified with a nano drop 3300 Fluorospectrometer and diluted to a final concentration of 40ng/µl. SCAR markers, SCS1302 and SCS421 were employed to follow leaf rust resistance genes *Lr*24 and *Lr*28, respectively during marker-assisted pyramiding of these genes (Gupta et al. 2006; Cherkuri et al. 2005). PCR reactions were performed in a total volume of 20 µl, containing 1× PCR buffer, 2.5mM of each dNTP, 20 ng of each primer, 1 U of Taq DNA polymerase (Banglore Genei Pvt. Ltd., India) and 100 ng of genomic DNA in a PTC-200 thermal cycler (MJ Research). Amplified PCR products were resolved in 2% agarose gel, stained with 2% ethidium bromide.

Each plant of BC_1F_2 was observed for 26 DUS (Distinctiveness Uniformity Stability) characters and also different morpho-physiological characters *viz*., days to 50 per cent flowering, plant height, number of productive tillers per plant, spike length, spikelets per spike, thousand grain weights and grain yield per plant. Data on leaf rust incidence under field conditions were recorded as percentage of leaf area covered with pustules by modified cobb's scale (Petersoon et al. 1998). The final disease severity data was converted into Coefficient of Infection (CI) by multiplying severity with a constant value for field response (Stubbs et al. 1998; Roelfs et al. 1992).

The recipient genotype DWR162 and near isogenic lines of PBW343 were confirmed for the presence/absence of *Lr24* and *Lr28* genes with the help of SCAR markers. These markers were consistent with amplification 719bp fragment with the SCAR marker S73719 of *Lr24* and 570bp fragment with SCAR marker S421570 of *Lr28* in PBW343. This confirmed the presence of resistance genes in PBW 343 and their absence in recipient parent DWR162. Individual plants of BC_1F_2 segregating generation characterized for the presence of leaf rust resistance genes *Lr24* and *Lr28* were also evaluated for agronomic traits such as days to 50% flowering, number of tillers/plant, spike length, spikelets/spike grain yield/plant, thousand kernel weight (TKW) and leaf rust resistance for studying the role of these genes in the background of DWR162 (Table 1).

Plants carrying only *Lr24* gene were superior in performance for all the traits except for grain yield per plant and thousand grain weight along with high leaf rust resistance. The alien segment carrying linked genes *Lr24/Sr24* does not impose any deleterious effect on yield as several cultivars carrying *Lr24* have been released for cultivation in India (Tomar et al. 2014). Plants carrying *Lr28* gene were superior for all agronomic characters such as spike length, spikelets/spike, grain yield/plant and TKW with moderate leaf rust resistance. Similar findings of increased grain yield, TKW and number of effective tillers/plant due to presence of *Lr28* gene with no association of any deleterious characters have been reported (Kumar and Raghavaiah 2004). *Lr28* is effective against currently

Table 1. Evaluation of individual plants of BC_1F_2 with different gene combinations for yield parameters and leaf rust resistance

Character	*Lr24*	*Lr28*	*Lr24* and *Lr28*	Nil	DWR 162	PBW 343
	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Days to 50% flowering	76.33 ± 4.91	72.43 ± 1.80	73.00 ± 3.29	72.21 ± 0.94	65.70 ± 1.61	84.9 ± 1.2
Plant height (cm)	61.67 ± 4.41	67.86 ± 4.61	72.25 ± 5.56	66.50 ± 1.60	80.10 ± 1.59	66.4 ± 1.37
Tillers Number of plant	20.67 ± 0.88	18.43 ± 3.28	19.00 ± 4.02	14.93 ± 1.55	19.50 ± 0.96	11.7 ± 0.83
Spike length (cm)	8.67 ± 0.33	10.00 ± 0.44	9.75 ± 0.63	10.11 ± 0.11	10.60 ± 0.31	9.4 ± 0.24
Number of spikelets/spike	16.33 ± 0.67	18.71 ± 0.92	20.50 ± 0.50	18.57 ± 0.27	18.90 ± 0.66	17.9 ± 0.43
Grain yield per plant (g)	17.40 ± 2.67	21.16 ± 1.61	25.50 ± 1.23	15.14 ± 1.34	23.42 ± 1.29	12.3 ± 1.17
Thousand Kernel weight (g)	37.05 ± 1.58	41.53 ± 1.97	40.85 ± 1.76	38.04 ± 1.78	38.67 ± 0.72	31.1 ± 1.26
Leaf rust incidence (CI)	0.07± 0.07	0.6± 0.57	0.00± 0.00	2.29± 1.67	65.00 ± 1.58	5.33 ± 1.32

prevailing leaf rust pathotypes except 121R60-1 (Bhardwaj et al. 2010) and therefore, represents a good candidate for gene pyramiding. Plants carrying *Lr24* and *Lr28* leaf rust resistance genes in combination were superior for all the agronomic traits as compared to plants with individual leaf rust resistantce genes *Lr28* and *Lr24* with high degree of leaf rust resistance. Similar reports of superior performance of back cross lines of PBW 343 pyramided with newly introduced leaf rust resistance genes *Lr24* and *Lr28* are available (Chhuneja et al. 2011; Tiwari et al. 2012). This kind of interaction between the combination of genes give higher level of resistance has been reported earlier (Kloppers and Betorius 1997) and often forms the basis of long term resistance.

Phenotypic evaluation of BC_1F_2 population resulted in the isolation of superior and rust free segregants with *Lr24* and *Lr28* donor genes under transfer. To see whether the plants of BC_1F_2 have recovered the genetic background of recurrent parent DWR162 to the satisfactory level, only phenotypic association is not effective, hence DUS characterization was followed. Twenty Six DUS characters of individual plants that mostly resembled DWR162 (Table 2) indicated 20 to 93% recovery of the characters of DWR162 in BC_1F_2. High resemblance recorded for the characters *viz*., grain (93%), glume (86%), spike (67%) and awns (74%). This indicated the possibility of getting superior rust free segregants resembling DWR162 with the help of backcrossing. Use of single backcross approach to capture genetic variation in achieving gene combination has been suggested (Bariana et al. 2004).

Four plants in BC_1F_2 generation were observed to have better yield potential than the recipient parent DWR162 showing enhanced resistance and without much deviation in the traits of adaptation. This would lead to early identification of potential cultivars and the resistance sources for future breeding programmes and these improved plants can be used as base genotype for developing genetically enhanced genotypes similar to DWR162 with leaf rust resistance.

Table 2. Number of individual plants in BC_1F_2 with rust resistance and DWR162 background based on DUS characters

Characters	No. of characters studied	No. of plants resembling DWR162	% recovery of DWR162 type background
Plant growth	3	13	45
Flag leaf	6	6	20
Spike	6	20	67
Aws	4	22	74
Glume	4	26	86
Grain	3	28	93

Acknowledgements

The first author expresses her gratitude to the Indian Council of Agricultural Research for the grant of fellowship to conduct the research for Ph.D. degree. The help provided by the Head, IARI Regional Station, Wellington, Nilgiris is also gratefully acknowledged.

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