



Short Communication

Transfer of leaf rust and stripe rust resistance genes *Lr19* and *Yr15* in to a susceptible wheat cultivar HS295

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Abstract

The present study was conducted to transfer multiple rust resistance in a popular but rust susceptible wheat cultivar HS295. Selected derivatives WBM3632 and WBM3635 have been developed from a cross, HS295*2/FLW20//HS295*2/FLW13 using bulk-pedigree method of breeding. Advance line WBM3697 selected from a breeding line WBM3532 was named as HS661. This line was evaluated for seedling resistance to a wide array of rust pathotypes and found to possess resistance to all the three rusts. HS661 was also tested under field conditions and showed adult plant resistance to leaf rust (AC1=0.6), stem rust (ACI=2.7) and stripe rust (AC1=3.8). Among 34 F₃ lines, 28 were tested positive for SSR marker *Xwmc221* indicating the presence of *Lr19/Sr25*. Out of 14 selected F₄ lines from F₃, nine were homozygous positive for *Lr19/Sr25*. The advanced breeding lines viz., WBM3632 (WBM3697) and WBM3635 were also positive for *Lr19/Sr25* with SCAR marker *SCS265*₅₁₂. SSR marker *Xgwm1* producing 215 bp band in *Avst-15*, *FLW13* and *HS661* confirmed the presence of *Yr15*. Agronomically, *HS661* was comparable with recipient variety *HS295* and superior to a standard check *HS490* under late sown restricted irrigation production conditions of NHZ. *HS661* may serve as a potential donor for creating new usable variability against all the three rusts.

Key words: Wheat, SSR marker, *Lr19/Sr25*, *Yr15*

Introduction

All the three rusts of wheat viz., leaf/brown (*Puccinia triticina*), stripe/yellow (*Puccinia striiformis* f.sp. *tritici*) and stem/black (*Puccinia graminis* f.sp. *tritici*) occur in India. Molecular marker assisted selection (MAS)

has become increasingly important in breeding programs because it permits breeders to combine genes for important agronomic traits and disease resistance including rusts. Due to continuous evolution of rust races, the resistance genes deployed in the cultivars are rendered ineffective in due course of time of their release (Chen 2013; Tomar et al. 2014). Recently five new virulent pathotypes of stripe rust viz., 46S117, 110S119, 238S119, 110S247 and 110S84 have overcome the resistance conferred by the genes, *Yr11*, *Yr12* and *Yr14* (Gangwar et al. 2015). A few genes such as *Yr5*, *Yr10* and *Yr15* are providing broad spectrum resistance to stripe rust races in India (Vinod et al. 2006). To combat stripe rust infection in wheat crop, a cultivar PBW757 carrying *Yr15* has recently been released for cultivation under very late sown irrigated conditions in North Western Plain Zone. During the past few years, a leaf rust pathotype 77 has mutated to about 13 new pathotypes prevailing in India (Bhardwaj 2012). However, the gene *Lr19* linked to *Sr25* still provides effective resistance to all the pathotypes of leaf rust prevalent world over including India (Tomar and Menon 2001; Huerta-Espino et al. 2011; Bhardwaj et al. 2016). The presence of *Lr19* in Indian wheat varieties PBN142 and WH533 is indicated from their pedigree as one of the parents involved is *Agatha* (Tomar et al. 2014).

Wheat variety *HS295*, released in 1992 has been popular among farmers under late sown rainfed

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conditions of Northern Hills Zone. This variety due to its wider adaptability, drought tolerance and good chapatti making qualities, remained choice of hill farmers for more than 18 years. However, it has now become susceptible to three wheat rusts. The objective of present study was therefore, to improve HS295 with multiple resistance to rusts.

The rust susceptible wheat cultivar HS295 (CQT/AZ//IAS55/ALD/3/ALD/NAFN/4/PJN/PEL SL 1276.69) was crossed to a genetic stock FLW20 (INGR07001 (PBW343/Agatha=T4=Tc+ *Lr19/Sr25*//FLW6) and the F₁ hybrid was twice back crossed to HS295 to obtain BC₂F₁. Similarly, another cross was made between HS295 and FLW13 (INGR05005 (WH542/Avocet-*Yr15*) to develop BC₂F₁ (HS295*2//FLW20, HS295*2//FLW13). Selected individual plants from the BC₂F_{1s} resistant to leaf and stripe rusts were inter crossed to develop F₁ (HS295*2//FLW20//HS295*2//FLW13) and subsequent segregating generations were handled through Bulk-Pedigree Method of breeding till the development of homozygous advanced bulk.

A total of 114 F₃ progenies derived from the above cross between two BC₂F₁ were screened to leaf rust pathotype 77-5 and to a mixture of stripe rust pathotypes (46S119 and 78S84) during 2013-14. Subsequently 47 F₄ lines were also screened against above mentioned leaf rust and stripe rust pathotypes separately under epiphytotic conditions in green house. The advanced breeding line WBM3697, selected from WBM3632 was screened in Initial Plant Pathological Screening Nursery (IPPSN) during 2016-17. The line WBM3697 was named as HS661 and evaluated for agronomic traits in Preliminary Yield Trial (PYT) along with other selected lines along with a check variety HS490 at Research Farm, IARI RS, Shimla during *rabi* 2016-17 in 5 rows (2.75m x 0.18m plot size) in RBD with three replications under late sown restricted

irrigated conditions. Data was recorded on days to 50% flowering, plant height (cm), 1000-grain weight (g) and grain yield (q/ha) in each of the three replications. Data were analysed using standard statistical procedures (Panse and Sukhatme 1995). Thereafter, HS661 was nominated to AICRP on wheat for testing under late sown, restricted irrigated production conditions of Northern Hill Zone during 2017-18.

Host-pathogen interaction (HPI) test for rust resistance

The seedling tests were conducted in green house with sets of differentials. Seven days old seedlings were inoculated with pure culture (5 mg uredospores per ml in light weight, non-phytotoxic isoparaffinic oil-Soltrol) of aggressive/predominant pathotypes of stem leaf and stripe rusts under artificial inoculated conditions following standard procedure. The seedling reaction for infection type (IT) of rusts was recorded after a fortnight according to Stakman et al. (1962). HS661 and check variety HS490 were also tested at adult plant stage against a mixture of leaf rust and stripe rust races. Average coefficient of infection was calculated according to a set procedure.

Standard procedure was followed for DNA extraction. Markers closely linked to rust resistance genes, their primer sequences, expected amplified PCR products and PCR conditions are presented in Table 1. Microsatellite co-dominant marker *Xwmc221* (Somers et al. 2004) and SCAR marker *SCS265*₅₁₂ (Gupta et al. 2006) were used to tag *Lr19/Sr25* in segregating and fixed populations of wheat. SSR marker *Xgwm11* was used to tag *Yr15* (Röder et al. 1998; Bariana et al. 2007; Yaniv et al. 2015) in fixed populations of wheat. Allele scoring was performed using Gene Mapper v 4.0 software (Applied

Table 1. Markers closely linked to rust resistance genes, their primer sequences, expected amplicon size (bp) of PCR products and PCR conditions

Gene	Marker	Type	Sequence	Amplicon size (bp)	AT °C	Reference
<i>Lr19/Sr25</i>	<i>SCS265</i>	SCAR	F5'GGCGGATAAGCAGAGCAGAG3'R5' GGCGGATAAGTGGGTTATGG3'	512	60	Gupta et al. 2006
<i>Lr19/Sr25</i>	<i>Xwmc221</i>	STS	F5' ACGATAATGCAGCGGGGAAT3'R5' GCTGGGATCAAGGGATCAAT3'	190	61	Prins et al. 2001
<i>Yr15</i>	<i>Xgwm11</i>	SSR	F5'GGATAGTCAGACAATTCTTGTG3'F5' GTGAATTGTGTCTTGTATGCTTCC3'	215	50TD	Röder et al. 1998 Bariana et al. 2007

AT-annealing temperature; TD-touch down

Biosystems). Proper negative and positive control DNAs were included in each of the marker analysis.

HS661 (WBM3697) was tested in IPPSN for leaf rust resistance at adult plant stage and found to be resistant to brown rust with Average Coefficient of Infection (ACI) ranging from 0.0 to 1.6 across all the locations of India. Similarly, it has shown ACI 2.7 and 3.8 for stem rust and stripe rust, respectively (Progress Report, ICAR-IIWBR 2017). The average coefficient of infection for all the rusts was much higher in recipient variety HS295 and standard control HS490 indicating that HS661 is an improved genotype with respect to adult plant resistance. HS661 were alongwith FLW28, FLW13, HS295 and Agra local (control) tested for seedling resistance to a wide array of rust pathotypes of all the three rusts at Regional Station, IIWBR, Shimla during 2017-18. The line HS661 displayed resistant reaction to 24 leaf rust pathotypes. Presence of leaf rust resistance in HS661 owing to *Lr19/Sr25* inherited from FLW20 has expressed resistance to pathotype 77-8 even though it has virulence to *Lr19/Sr25*. The resistance to 77-8 may be due to the presence of *Lr23* in recipient parent HS295 and donor stock FLW13 developed in the background of WH542, which carries *Secale cereal* derived resistance gene *Lr26*. The pathotype 77-8 is avirulent to *Lr9*, *Lr23*, *Lr24* and *Lr26* and is not commonly occurring in India, hence *Lr19* may still provide effective resistance (Tomar et al. 2014). HS661 is also resistant to 26 stem rust pathotypes because of the presence of *Sr25* linked to gene *Lr19*. Although the gene *Sr25* is ineffective to a virulent pathotype 40-2 even though it has virulence to *Sr25* but resistance against this pathotype may be ascribed to the presence of *Sr11* or some unknown resistance gene(s). HS661 has also been postulated to have *Sr2/Yr30*. Both these genes confer adult plant resistance to stem rust and stripe rust, respectively (Singh et al. 2000). HS661 is resistant to most prevalent and virulent stripe rust pathotypes, 46S119, 110S119, 110S84, 238S119 and 110S247. The seedling resistance provided by HS661 to 18 stripe rust pathotypes including the above mentioned pathotypes due to the presence of *Yr15* introduced from FLW13. *Yr15* has been reported to confer broad spectrum resistance against a large collection of pathotypes of stripe rusts from all over the world (Bariana et al. 2007; Murphy et al. 2009; Yaniv et al. 2015) including India (Prasad et al. 2018). HS661 carrying resistance to all the three rusts may be a very useful source of resistance genes.

Twenty eight lines identified from 114 derivatives in F_3 were tested positive for *Xwmc221* SSR marker indicating the presence of *Lr19/Sr25* (Fig. 1). Further,

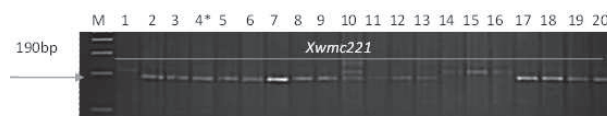


Fig. 1. M-100bp ladder; 1: HS295; 2: FLW20; 3: Positive control: COOK* 6/C-80-1-Lr19; *4-20: Representative F_3 progenies

nine F_4 lines, were homozygous positive for *Lr19/Sr25* were advanced to next generation. Microsatellite marker *Xwmc221* amplified 190bp band in donor parent FLW20 carrying *Lr19/Sr25*, positive control (Cook*6/C80-1+*Lr19/Sr25*) and same base-pair band was present in HS661, confirming the presence of *Lr19/Sr25*. The advanced breeding lines viz., WBM3632 and WBM3635 were found positive for *Lr19/Sr25* with SCAR marker *SCS265*₅₁₂. (Fig. 2). SSR marker



Fig. 2. M-100bp ladder, 1: HS295; 2: FLW20; 3: Positive control: COOK* 6/C-80-1-Lr19) 4: WBM3632, 5: WBM3635, 6: F_4 -63+*Lr19*

Xgwm11 produced 215bp band in Avst-*Yr15*, FLW13 and HS661 confirming the presence of *Yr15* (Fig. 3).

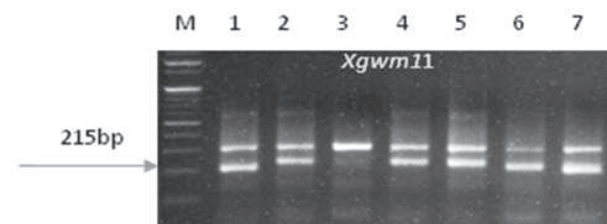


Fig. 3. M-100bp ladder, 1: HS295; 2: HS661; 3: FLW20+*Lr19*; 4: FLW13+*Yr15*; 5: Positive control (Avocet *Yr15*), 6: Cook*6/C80-1+*Lr19*; 7: Negative control (Agra Local)

Agronomic performance of HS661 was assessed under preliminary yield trial (PYT) consisting of 12 genotypes for grain yield and its contributing traits during crop season 2016-17. It recorded average grain yield of 28.8 q/ha which was higher (CD=3.0) over a standard check variety HS490 (25.7q/ha, 11.9%) under restricted irrigation situation at Shimla. HS661 was

also evaluated at seven locations in Northern Hills Zone under late sown, restricted irrigated conditions during 2017-18 and recorded average grain yield of 23.3 q/ha. The improved line (HS661) possess semi-erect growth habit, with 88cm of plant height maturing in 138 days and the grains are semi-hard ovate shaped amber in colour with thousand grain weight of 39g which is comparable to HS295.

Authors' contribution

Conceptualization of research (DP, SCB, KVP), designing of experiments (DP, MP), seedling resistance tests (SCB, SK, OPG), data generation, analysis and data interpretation (DP, SCB, PS), manuscript writing (DP, SCB).

Declaration

Authors declare no conflict of interest.

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