

HS628 : A potential genetic stock for resistance to new virulent pathotypes of black, brown and yellow rusts of wheat (*Triticum aestivum* L.)

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Three rusts of wheat *viz.*, leaf / brown (*Puccinia recondita* sp. *tritici*), stripe / yellow (*Puccinia striiformis* sp. *tritici*), stem / black (*Puccinia graminis* sp. *tritici*) are very devastating diseases causing huge losses to the wheat crop worldwide with India no exception. The 1BL.1RS translocation derived wheat varieties carrying *Yr9/Lr26/Sr31/Pm8* gene complex provided protection against losses due to leaf and stripe rusts in India till 1995. However, the evolution of pathotype (pt) 46S119 virulent to *Yr9* and *Yr2* resistance genes has changed the whole scenario (Nayar *et al.*, 1996). After breakdown of *Yr9* based resistance, some protection against stripe rust was rendered by “*Attila*” germplasm derived wheat varieties that had *Yr27* effective against pt. 46S119, was succumbed with new variant 78S84 (Prashar *et al.*, 2007). Recently five new highly virulent pathotypes of stripe rust *viz.*, 46S117, 110S119, 238S119, 110S247 and 110S84 have been identified in India (Gangwar *et al.*, 2015). Due to emergence of these pathotypes, *Yr12* became susceptible to pts. 110S84, 110S119, 110S247 and 238S119. Joss-Cambier carrying *Yr11* gene, also became susceptible to the new pts. 110S84 and 238S119 in addition to 46S119 pathotype (Bhardwaj *et al.* Pers. Comm). Another gene *Yr14* is also defeated by the new virulent pt. 110S119.

In the past few years, pathotype group 77 of leaf rust have evolved very rapidly and at present 13 pathotypes of this group exist in India (Bhardwaj, 2012).

The 7D.7Ag translocation (*Lr19/Sr25*) carrying effective resistance to leaf and stem rusts was effectively utilized in wheat to develop several rust resistant recombinant lines, of which many were released for cultivation world-wide. It provides effective resistance to all the pathotypes of leaf rust prevalent in Northern India (Bhardwaj *et al.*, 2016). Black rust had become the disease of past but the evolution of new race *Ug99* and its variants, virulent to many of the *Sr* genes including *Sr31* which used to protect 40% of wheat from black rust has alarmed the breeders to adopt proactive approach world-wide. The 7D.7Ag translocation carrying *Lr19/Sr25* not only provides leaf rust resistance but also effective against *Ug99* and its variants (Singh *et al.*, 2011). The emergence of new pathotypes and shift in virulence patterns rendered most of the resistant wheat varieties susceptible. Therefore, it is imperative to develop rust resistant plant genetic resources effective against newly evolved virulent pathotypes of rust. It not only provides opportunities to the plant breeders for incorporating viable genes into germplasm pools but also permits the system to release cultivars carrying diverse rust resistance genes. In this direction, HS628, a rust resistant genetic stock was developed from a double cross (HS240*2/FLW20 (*Lr19*)/HS240*2/FLW13 (*Yr15*)) using Bulk-Pedigree method of breeding at Indian Agricultural Research Institute, Regional Station, Tutikandi Facility, Shimla.

Host-pathogen interaction (HPI) test for seedling resistance: HS628, along with sets of differentials was raised in a mixture of loam soil and farm yard manure using aluminium trays. Seven day old seedlings were inoculated with pure culture (suspended in light weight, non-phytotoxic isoparaffinic oil-Soltrol) of virulent pathotypes of black, brown and yellow rusts. The inoculated seedlings were atomized with fine mist of water and placed in dew chambers for 48 hrs at $15\pm 2^{\circ}\text{C}$ for stripe rust, $20\pm 2^{\circ}\text{C}$ for leaf and stem rusts with 12-16 hours daylight for initiation of infection. The seedlings were then shifted on to the green house benches till recording of infection types (IT's). The seedling reaction for IT's of rusts was recorded after a fortnight according to Stakman *et al.* (1962). HS628 was evaluated for resistance to black rust and found to possess resistance to all the pathotypes including newly evolved pathotype 40-3 (Table1). The 7D.7Ag translocation derived *Sr25* tightly linked to *Lr19*, was postulated for its presence in HS628 through host-pathogen interaction test. It had shown seedling resistance to all the pathotypes except 77-8 (Table1) validating the presence of *Lr19/Sr25* in HS628 transferred from genetic stock FLW20 carrying *Lr19/Sr25*. The occurrence of 77-8 pathotype of brown rust has not been reported so far from Northern India. Therefore, it can be used as useful source of resistance

to brown rust by wheat breeders in Northern India. Besides resistant to black and brown rusts, HS628 also carries resistance to all the newly evolved pathotypes of stripe rust (Table1). It is pertinent to mention that recording of HS628 as susceptible to pathotype 110S84 was an inadvertent error during 2015-16. To confirm its resistance to stripe rust, HS628 was re-evaluated for resistance to yellow rust pathotypes in the year 2017-18 and found to carry resistance against all the pathotypes including newly evolved virulent pathotypes *viz.*, 110S84, 110S247, 110S119 238S119 and 111S68 (Table 2). These results indicated that though breakdown of resistance in 'Attila' lines is of grave concern but resistance genes are available in good agronomic backgrounds to compete with the newly evolved virulent pathotypes of rusts. The presence of *Yr9* and *Yr18* was postulated by (Nayar *et al.*, 2001) either singly or in various combinations in 95 among 136 released varieties of wheat in India but they emphasized the need for diversification of resistance against yellow rust as *Yr9* and *Yr27* had become ineffective due to virulence of 46S119 and 78S84, respectively. Moreover, all of the *Triticum aestivum* wheat or *Triticum durum* wheat check varieties being cultivated across the zones in the country were found susceptible to fast emerging highly virulent pathotype 110S119 during 2015-16 (Singh *et al.*, 2016).

Table1. Seedling Resistance Test (SRT) of HS628 against black, brown and yellow rust pathotypes during 2015-16.

Genotype	Black rust																				Postulated genes							
	11	11A	15-1	21	21-1	21A-2	24A	34-1	40A	40-2	40-3	42B	117A-1	117-1	117-3	117-4	117-5	117-6	122	295		Sr 25+ R						
R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R								
HS628	Brown rust																				Lr19+							
	11	12	12-2	12-3	12-5	12-7	12-8	77	77-1	77-2	77-5	77-7	77-8	77-10	77-12	77A-1	104-2	104-3	104B	106		104-4	107-1	108-1	162	162-2	162A	
R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
	Yellow rust																											
	46S119	110S119	38S102	238S119	K (47S102)	78S84	110S84	7S0	110S247	P (46S103)	20A (70S64)	238S119	6S0	T (47S103)	N (46S102)	46S68	111S68											
R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R											

R=Resistant, S=Susceptible

Table 2. SRT of HS628 against yellow and brown rust pathotypes during 2017-18.

HS628	Yellow rust											
	110S119	46S119	110S84	78S84	T (47S103)	238S119	P (46S103)	N (46S102)	L (70S69)	110S247	111S68	7SO
	0;	0;	0;	0;	;1	0;	;	0;	0;	0;	0;	0;
	Brown rust											
	77-5	77-9	77-7	77-10	104-2	12-5	77-8	16-1	77-2	12-2	12-7	11
	0;	0;	0;	0;	0;	0;	3+	;	0;	0;	0;	0;

0; (naught fleck) / ; (fleck) / ;1 (fleck1)=Resistant, 3+=Susceptible

Adult plant resistance: HS628 was also tested in Initial Plant Pathological Screening Nursery (IPPSN) for leaf rust resistance at adult plant stage and found to be resistant to brown rust across all the ten different locations of India. HS628 has shown adult plant resistance under epiphytotic conditions at hot spot locations with highest score of TR (ACI: 0.1) in South India and 5S (ACI: 0.6) in North India to leaf rust (Singh *et al.*, 2016).

Molecular marker assisted validation: The presence of *Sr25/Lr19* in HS628 was counter validated using STS markers PSY1-E1 (Zhang *et al.*, 2008) and Gb (Liu *et al.*, 2010). HS628 was tested positive for PSY1-E1 and Gb molecular markers indicating the presence of *Sr25/Lr19* (Table 3). The SCAR marker SCS265₅₁₂ considered as locus specific marker for single band amplification (Gupta *et al.*, 2006) was also used by (Pal *et al.*, 2015) to characterize backcross generations of wheat for the presence of *Lr19* gene.

Agronomic performance: HS628 was also evaluated at five locations under early sown, rainfed production conditions of Northern Hill Zone during 2015-16 under AICRP on wheat and barley improvement programme. The multi-location data on grain yield, ancillary and diseases were recorded, compiled and analyzed at IIWBR, Karnal. HS628 possesses good agronomic background with an average grain yield 20.1 q/ha under early sown and rainfed conditions of Northern Hill Zone (Tiwari *et al.*, 2016). HS628 possess semi-erect growth, non-waxy flag leaf and ear, medium ear length (11.0 cm), plant height (89.5cm), peduncle length (38.2cm), takes 189 days to mature. It has lustrous semi-hard ovate shaped amber grains with 38g thousand grain weight.

The rust resistance gene pool present in HS628 would prove an useful source for developing potential rust resistant genotypes and/ or serve as potent donor for creating new usable variability against new virulent pathotypes of rust diseases in wheat improvement programme of India.

Table 3. Response of molecular markers for detection of *Lr19/Sr25* in HS628 and its parental genotypes.

Genotype	Response to STS molecular marker	
	Gb	PSY1-E1
HS240	-ve	-ve
FLW20+ <i>Lr19/Sr25</i>	+ve	+ve
HS628	+ve	+ve
FLW13	-ve	-ve

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