CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Stacking effective ASR and APR rust genes for multiple disease resistance in bread wheat cultivars

Crop Breeding and Applied Biotechnology
23(1): e44082317, 2023
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332023v23n1a7

Rebekah Nisha P[1], Shajitha Panneer[1], Murugasamy Sivasamy[1*], Jayaprakash P[1], Venu Kumaran Vikas[1], SC Bhardwaj[2], O. P. Gangwar[2], Balaji V[1], Gokulakrishna M[1], John Peter[1] and Vijaishree Sivasamy[3]

Abstract: *Rusts and powdery mildews pose serious threats to wheat and have caused substantial yield losses worldwide. Host resistance is the most economical and sustainable approach for managing such diseases. In this study, an effective leaf rust resistance gene Lr45-derived from Secale cereale L. and a linked stem rust and powdery mildew resistance gene Sr36/Pm6-derived from Triticum timopheevii were successfully pyramided. They were validated into well-adapted Indian wheat cultivars that were already carrying the APR stem rust gene Sr2/Lr27/Yr30 through marker-assisted backcross selection (MABC) following two parallel backcrossing schemes. Three efficiently linked microsatellite markers, $G372_{185}$ (Lr45), Stm773-2(Sr36), and Xgwm533 (Sr2), were used to confirm introgression of these genes. Lines with resistance genes in each background showed improved agronomic traits in comparison to their recurrent parents. These lines could be used in wheat improvement programs as potentially resistant stocks for leaf, stem rusts and powdery mildew to develop new wheat cultivars.*

Keywords: T. aestivum*, Lr45, Sr36/Pm6, Sr2, pink awns*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely grown crop worldwide, covering approximately 222.14 million hectares (USDA 2023). Rusts caused by *Puccinia* sp. and powdery mildew (PM) caused by *Blumeria graminis* f. sp. *tritici* (Bgt) are key constraints on global wheat production. Of the three types of rust, leaf or brown rust were caused by *Puccinia triticina* Eriks. (*Pt*) is one of the most disruptive diseases affecting wheat crops worldwide because of its widespread occurrence (Bhardwaj et al. 2021) causing yield losses of up to 65% (Chhuneja et al. 2011). The stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) has caused yield losses of between 10% and 50% in recent years (Roelfs et al. 1992). However, the recent emergence and spread of the *Ug99* race and its variants have been reported to cause upto100% damage (Amulaka et al. 2013). In addition to rust, PM is an emerging disease that causes considerable yield losses of up to 35% (Sharma et al. 1996). Recently, the incidence of PM has increased in several wheat-growing regions worldwide, including India (Vikas et al. 2020).

***Corresponding author:**
E-mail: iariwheatsiva@gmail.com
ORCID: 0000-0003-4413-7570

Received: 22 November 2022
Accepted: 07 March 2023
Published: 20 March 2023

[1] 1 Indian Agricultural Research Institute (IARI), Regional Station, Wellington- 643 231, Tamil Nadu, India
[2] Indian Institute of Wheat and Barley Research (IIWBR), Regional Station, Flowerdale, Shimla- 171 001, Himachal Pradesh, India
[3] College of Horticulture, Tamil Nadu Agricultural University (TNAU), Coimbatore – 641 003, Tamil Nadu, India

The development of disease-resistant varieties by stacking effective genes is one of the most effective and eco-friendly methods for combating rust and PM (Singh et al. 2020). Currently, over 80, 60, and 100 genes for resistance to leaf, stem rust, and PM, respectively, have been formally cataloged in wheat (McIntosh et al. 2020) and many genes offer all-stage resistance (ASR). ASR/seedling resistance is governed by a single major gene that is often race-specific (Rosewarne et al. 2013). The effectiveness of such genes can be detected in the early stages of plant growth (Bariana et al. 2022). Generally, ASR fails within a few years of deployment, owing to the emergence of virulent variants (Chen 2005). Conversely, adult plant resistance (APR) is effective either at the post-seedling or adult plant stage and is usually race-specific, governed by minor genes with additive effects. A few also have pleiotropic effects in conferring resistance to multiple diseases (Herrera-Foessel et al. 2014). However, the number of APR genes identified and reported to date is limited. As the pathogen continues to evolve, stacking resistance genes is being pursued by wheat breeders to extend the resistance offered by these genes.

The translocation of T2AS-2RS.2 RL from Petkus rye (*Secale cereale L.*) postulated to carry the ASR gene*Lr45* (Naik et al. 2015) is reported to be effective in the seedling and adult plant stages against leaf rust pathotypes worldwide (Zhang et al. 2006) and has not been widely used. A distinct morphological trait known as "pink awns or glumes" is tightly linked to this gene (Sivasamy et al. 2010). It appears during the early anthesis and then gradually disappears as maturity progresses. It was observed that its expression differs across varied environments. *Triticum timopheevii* derived stem rust ASR gene *Sr36* originally transferred to wheat chromosome 2B and is effective in all the prevalent stem rust races, including the lineages of the Ug99 (Chemayek et al. 2017) except TTTSK (Jin et al. 2009). Although virulence has been reported for this gene, it is still effective on the Indian subcontinent (Sai Prasad et al. 2014) and continues to be widely exploited in combination with other minor or major genes (Jin et al. 2009). *Sr36* is also closely linked to the effective powdery mildew resistance gene, *Pm6* (Jorgensen and Jensen 1973). In India, the linked gene *Sr36/Pm6* confers effective resistance to stem and powdery mildew (Sivasamy et al. 2017).

The stem rust APR gene *Sr2* located on the short arm of wheat chromosome 3B (Hare and McIntosh 1979), provides broad–spectrum protection against stem rust and is associated with pseudo-black chaff (PBC), a dark pigmentation around the stem internodes and glumes post-anthesis.

Marker-assisted backcross (MABC) breeding is a recent approach for effectively transferring multiple genes for resistance, given that conventional breeding is time-consuming (Singh et al. 2018). The availability of specific microsatellite markers linked to resistance genes makes MABC easy and relatively rapid. The recurrent parents chosen in this study were released during the post-green revolution period, which had the highest level of adaptability to the changing environmental scenario and easy combining ability. However, the advancement of new virulent races renders these varieties more susceptible. Therefore, combining ASR and APR in such cultivars is desirable for the development of resistant stocks with higher levels of adaptability. With this objective, stacking multiple resistance genes for rust and PM in adapted wheat cultivars was initiated by pyramiding the ASR leaf rust gene *Lr45* and stem rust gene *Sr36/Pm6* with the APR stem rust gene *Sr2/Lr27/Yr30* through MABC in the background of eight well-adapted Indian bread wheat varieties.

MATERIAL AND METHODS

Plant material and breeding method

Leaf rust resistance gene *Lr45* was introgressed from a near-isogenic line (NIL) of Thatcher (Thatcher*7/ST−1 = RL6144) (Naik et al. 2015) into the background of eight well-adapted Indian bread wheat varieties viz., HD 2329, HD 2402, LOK-1, MACS 2496, NIAW 34, PBW 343, PBW 502, and RAJ 3077 that already carried race non-specific stem rust APR gene *Sr2/Lr27/Yr30/Pbc* (Bhardwaj 2011). *Sr2*+ alone is ineffective against stem rust. The selected recurrent parents were susceptible to leaf rust and PM. Likewise, the Australian line Cook (Cook*6/C80-1) carrying the *Triticum timopheevi*-derived gene *Sr36/Pm6* (Chemayek et al. 2017) was used as a donor to transfer the *Sr36* gene into the same background. These genes were introgressed into wheat varieties by adapting a backcross breeding approach assisted by linked molecular markers at the ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Wellington, Nilgiris, through two parallel backcrossing schemes. Appropriate agronomic practices were followed to raise crops in each generation.

The F_1s carrying individual genes were intercrossed to form a pyramid with *Lr45* and *Sr36*. The resulting F_1s was then backcrossed with the respective recurrent parents for up to three generations to raise the BC_3F_1 populations and recover the parental genome. The backcross progeny with the target genes were selected based on phenotype and MABC in each generation. From BC_3 onwards, the marker-selected homozygous plants were selfed for six generations to obtain homozygosity of the alleles received from the donor. The stable pyramided lines constituting BC_3F_6 were named HW 3641, HW 3642, HW 3654, HW 3655, HW 3657, HW 3660, HW 3661, and HW 3662.

Marker-assisted backcross breeding

Gene-specific microsatellite markers viz. $G372_{185}$ linked to *Lr45* (Naik et al. 2015), *Stm773-2* linked to *Sr36* (Tsilo et al. 2008), and *Xgwm533* linked to *Sr2* (Spielmeyer et al. 2003) were used for the selection of the genes in this study. DNA was extracted from 14-20 day old seedlings following a modified CTAB method (Doyle and Doyle 1990). PCR reactions were conducted in 20 µL reaction containing 25-50 ng of template DNA, 0.2 µM of each forward and reverse primer, Dream Taq Hot Start Green PCR mix (Thermo Fischer Scientific), and nuclease-free water in an Applied Biosystem thermocycler (Veriti). The details of the primers and their PCR conditions are given as Supplementary data (Table 1A). PCR products were resolved on a 3% agarose gel and visualized using a gel documentation unit (Syngene, Gene Genius Bioimaging System, UK). PCR markers were used to select heterozygous plants from the BC_1 to BC_3 generations and homozygous resistant plants from the BC_3F_6 generation.

Phenotypic selection

Phenotypic selection was performed for each generation to select plants resistant to leaf rust, stem rust, and powdery mildew under natural epiphytotic conditions. Wellington, being a natural hotspot for rusts and powdery mildew allows a natural selection of resistant lines. In 2021 in the summer and winter seasons, gene-pyramided plants in the BC_3F5 and BC_3F_6 generations were planted in one meter row of five lines, each with a line spacing of 23 cm between the rows. To ensure early disease onset and adequate disease pressure, infector rows were sown around the population. An aqueous suspension of viable mixed uredospores of prevalent *Pt* (*77-1, 77-5,77-9, etc.*) and *Pgt* (40A and 40-1) pathotypes in Wellington was sprayed at regular intervals of 15 d with a drop of Tween 20 (0.75 µL mL^{-1}).The field response to leaf and stem rust was recorded at the adult plant stage (Z80) (Zadoks scale) (Zadoks et al. 1974) according to the modified Cobb scale (Peterson et al. 1948) as the percentage of leaf and stem area covered with uredospores combined with the type of infection response. PM severity was scored on a 0–9 scale (Sheng and Duan 1991) where 0-3 was considered resistant.

Seedlings of the stable pyramided lines were evaluated for leaf and stem rust resistance under artificial glasshouse conditions at ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Regional Station, Flowerdale, Shimla in 2021. Seedlings that were approximately 10 d old sown in trays were inoculated with six pathotypes of *Pt* viz., 12-5 (29R45), 77-1 (109R63), 77-5 (121R63-1),77-8 (253R31), 77-9 (121R61-1) and 104-2 (21R55) and four pathotypes of *Pgt* viz., 11(79G31), 40A (62G29), 40-2 (58G13-3) and 117-6 (37G19). The infection type (IT) was recorded on a 0–4 scale as proposed by Stakman et al. (1962). The Infection types (ITs) 3, 3+ were considered susceptible, whereas lower ITs ("0", "1," "2," and "X") were considered resistant.

Agronomic evaluation of the advanced lines

Phenotypically and molecularly confirmed individual plants in the BC_3F_6 generation were planted with their respective recurrent parents in non-replicated plot trials in 2021–22. Ten uniformly resistant plants were selected from each background and data on plant height (PH) (cm), the number of productive tillers per plant (NPT), spike length (SPL) (cm), grain number per spike (GNS), and thousand grain weight (TGW) (g) were recorded.

RESULTS AND DISCUSSION

Genotypic Evaluation of gene stacked lines

Three microsatellite markers viz., $G372_{185}$, *Stm773-2,* and *Xgwm533* linked to *Lr45, Sr36/Pm6,* and *Sr2* were used in MABC. The low availability of molecular markers limits the use of *Lr45* in marker-assisted selection (Naik et al. 2015). Zhang et al. (2006) developed an amplified fragment length polymorphism (AFLP) marker for the detection of the *Lr45*

gene and Fein et al. (2009) developed a sequence-characterized amplified region (SCAR) marker. Naik et al. (2015) reported a highly polymorphic, co-dominant microsatellite marker ($G372_{185}$) that supported the efficient use of the *Lr45* gene in the wheat improvement programs. Microsatellite marker $G372_{185}$ linked to *Lr45* showed a single 185 bp allele in the homozygous resistant lines and a 127 bp allele or null allele in the lines devoid of the gene. Both alleles were present in the heterozygous lines (Figure 1A). Naik et al. (2015) validated the efficiency and specificity of this marker for *Lr45* in Thatcher-based NILs.

The STM (sequence-tagged microsatellite) marker *Stm773-2* linked to *Sr36/Pm6* developed a 155 bp amplicon in the pyramided lines homozygous for the presence of the gene and a 195 bp allele in the homozygous negative lines (Figure 1B). The codominant nature of this marker facilitates identification of heterozygotes (Tsilo et al. 2008). A microsatellite marker (*Xgwm533*) linked to *Sr2* amplified a 120 bp allele in the lines positive for the gene and a 150 bp allele or null allele in the lines without the gene (Figure 1C). Similar screening of *Sr2* in large populations using the microsatellite marker *Xgwm533* was reported by Vishwakarma et al. (2019).

Pyramiding multiple effective ASR genes with APR rust genes in the background of adapted cultivars is considered an effective strategy for enhancing resistance and durability (Jin et al. 2022). Stacking several disease-resistance genes in a single varietal background through phenotyping is difficult. Therefore, MABC is one of the most promising approaches

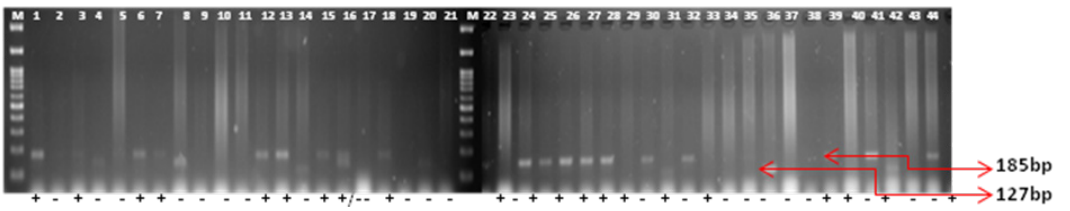

Figure 1A. Molecular confirmation of leaf rust resistance gene *Lr45* using marker $G372_{185}$ in the donor,recurrent parent, and pyramided lines (BC3F6). M: Marker; 1: RL 6144 (Positive control); 2: HD2329; 3-7: HW 3641, 8-HD 2402; 9-13: HW 3642, 14- Lok-1; 15-18: HW 3654; 19: MACS 2496; 20-22: HW 3655; 23: NIAW34; 24-28: HW 2657; 29: RAJ 3077; 30-33: HW 3662; 34: PBW343; 35-39: HW 3660; 40: PBW 502; 41-44: HW3661.

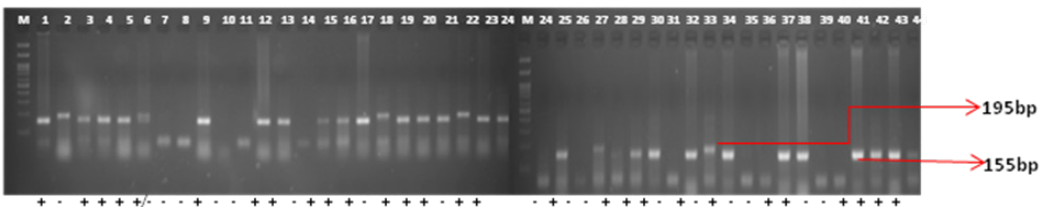

Figure 1B. Molecular confirmation of stem rust resistance gene *Sr36/Pm6* using marker *stm773* in the donor, recurrent parent, and pyramided lines (BC3F6). M-Marker (100bp); 1: Cook(Positive control); 2: HD2329; 3-6: HW 3641; 7: HD 2402; 8-13: HW 3642; 14: LOK-1; 15-17: HW 3654; 18: MACS 2496; 19-24: HW 3655; 25: NIAW 34; 26-30: HW 3657; 31: Raj 3077; 32-35: HW 3662; 36: PBW343; 37-39: HW3660; 40: PBW 502; 41-44: HW 3661.

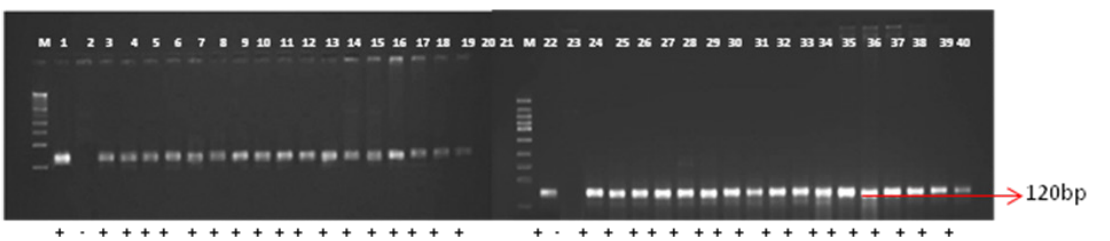

Figure 1C. Molecular confirmation of stem rust APR gene *Sr2* using marker *Xgwm533*in the donor, recurrent parents, and pyramided lines (BC3F6). M: Marker; 1: Kingbird (Positive control); 2: Agralocal (Negative control); 3: HD2329; 4-7: HW 3641; 8: HD 2402; 9-14: HW 3642; 15: LOK-1; 16-10: HW 3654; 11: MACS 2496; 12-17: HW 3655; 18: NIAW34; 19-23: HW 3657; 24: RAJ 3077; 25-29: HW 3662, 30: PBW 343; 31-33: HW 3660; 34: PBW 502, 35-40: HW 3661.

for the incorporation and stacking of resistance genes while retaining the essential characteristics of the recurrent parent (Collard and Mackill 2008).

Phenotypic evaluation and screening of backcross population

Advanced lines stacked with *Lr45, Sr36/Pm6,* and *Sr2+* were phenotypically evaluated in the field and were found to be completely resistant to leaf and stem rust. A susceptibility score of 20S-80S to both stem rust and leaf rust was recorded in recurrent parents (HD 2329, HD 2402, Lok-1, RAJ 3077, and NIAW 34). The recurrent parents, PBW 343, PBW 502, and MACS 2496, were resistant to stem rust because they carried the stem rust resistance gene *Sr31+* and were only susceptible to leaf rust (20S-40S). The well-known 1BL/1RS translocation carrying the gene *Sr31+* has contributed substantially to world wheat production, and several hundred cultivars possessing this gene locus were released during the mid-1990s and are continuing to be effective in the Indian subcontinent (Prasad et al. 2022). However, virulence has been reported extensively worldwide.

Table 1. Disease response in the seedling and adult plant stage and marker analysis data of the donor, recurrent parent and the pyramided lines

Parent/ Cross	Details	Seedling response leaf rust pathotypes						Seedling response stem rust pathotype				Adult plant response			Marker confirmation		
		12–5	77–1	77–5	77–8	77–9	104–2	11	40A	40–2	117–6	Leaf rust	Stem rust	PM	*Lr45 ($G372_{185}$)*	*Sr36/ Pm6 (stm773)*	*Sr2 (Xgwm533)*
HD 2329	RP		-	3+	3+	3+	1	2=	2C	-	-	80S	60S	6	-	-	+
HW 3641	HD2329 (*Lr45, Sr36/ Pm6, Sr2+*)	-	0	-	0	-		0	0		-	F	F	0	+	+	+
HD2402	RP	-	1	33+	-	3+	3+	12	12	0	-	40S	20S	6	-	-	+
HW 3642	HD2402 (*Lr45, Sr36/ Pm6, Sr2+*)	-	0	0	-	-		0	-	0		F	F	0	+	+	+
LOK-1	RP	33+	3+	3+	3+	3+	3+	3+	3+	0	3+	60S	60S	7	-	-	+
HW 3654	LOK-1 (*Lr45, Sr36/Pm6, Sr2+*)		0	1		-	1	12	0	-	0	F	F	0	+	+	+
MACS2496	RP	22+	12	3+	0	-	3+	2=	-	0	2=	20S	F	6	-	-	+
HW 3655	MACS2496 (*Lr45, Sr36/ Pm6, Sr2+*)	0	-	0	0	-	-	-	-	0	0	F	F	0	+	+	+
NIAW 34	RP	23	3+	X+3	-	3+	3+	-	12	2=	-	40S	20S	6	-	-	+
HW 3657	NIAW34 (*Lr45, Sr36/ Pm6, Sr2+*)	-	-	0	-	-		0	0	-	0	F	F	0	+	+	+
RAJ 3077	RP	-	1	23	-	3+	33+	-	-		-	80S	20S	7	-	-	+
HW 3662	RAJ3077 (*Lr45, Sr36/ Pm6, Sr2+*)	0	-	0	-	-	-	2+	12	0	-	F	F	0	+	+	+
PBW 343	RP	-	3+	3+	0	3+	3+	2=	0			40S	F	7	-	-	+
HW 3660	PBW343 (*Lr45, Sr36/ Pm6, Sr2+*)	-	-	0	-	-		0	0	0	0	F	F	0	+	+	+
PBW 502	RP	33+	3+	3+	0	3	3+	-	-	-		40S	F	7	-	-	+
HW 3661	PBW502 (*Lr45, Sr36/ Pm6, Sr2+*)	-	-	0	0	-	-	-	2=	0	-	F	F	0	+	+	+
RL6144 (*Lr45*)	Donor	0	-	0		-	-	0	3+	0	0	F	60S	4	+	-	+
COOK (*Sr36/ Pm6*)	Donor	3+	-	0	3+	3+	3+	-	2–	1	-	80S	F	0	-	+	-

* F - Free; S - Susceptible; RP - Recurrent Parent

The APR gene *Sr2+* in combination with other resistance genes is effective against the *Ug99* lineage (Alabushev et al. 2019). However, phenotyping the resistance offered by *Sr2* is difficult under field conditions because it confers partial resistance (Spielmeyer et al. 2003). Therefore, the microsatellite marker, *Xgwm533* linked to *Sr2* was used to accelerate the selection of lines carrying this gene.

Juvenile plants pyramided with the resistance genes showed a resistant infection response of 0–1 for the tested leaf and stem rust pathotypes whereas a susceptible infection type, 'IT' 33+ was recorded in the seedlings of the susceptible recurrent parents for leaf and stem rust races under controlled conditions. The rust and PM responses of the pyramided lines, donors, and recurrent parents are listed in Table 1.

In addition to phenotypic screening, morphological traits such as pink awns/glume linked to *Lr45* (Sivasamy et al. 2010) which appear during the early flowering period (anthesis) and then gradually disappear as maturity progresses and PBC linked to *Sr2+* further aided in the visual confirmation of the presence of the respective genes. However, direct selection based on these phenotypic traits is difficult because their expression varies across different environments.

Agronomic performance of the pyramided lines

Based on resistance to leaf and stem rust at the phenotypic and molecular level, ten plants from each background were selected and evaluated agronomically. The mean agronomic trait values are shown in Table 2. The gene-pyramided lines had agronomically superior traits, such as PH, NPT, SPL, GNS, and TGW, compared with their respective recurrent parents. There was a slight increase in spike length in the lax ear, which significantly increased TGW. From the data, it was observed that all gene-stacked lines carrying *Lr45, Sr36/Pm6,* and *Sr2* showed a better TGW than the recurrent parent. The main components contributing to wheat yield are SPL, GNS, and TGW (Zheng et al. 2020). In certain backgrounds, such as Raj 3077, PBW 343, and PBW 502, there was an increase in GNS and SPL. In wheat varieties PBW 343 and PBW 502, the lowermost 2–3 spikelets remain sterile depending on the environmental conditions. However, the fertility of the lowermost spikelet also recovered after introgression of the *Lr45* gene, which contributed to more GNS.

Suppression of other diseases

The MABC-derived lines were resistant to leaf and stem rust and were also resistant to PM because of the presence of the *Pm6* gene that inherited *Sr36*. Sivasamy et al. (2017) reported the effectiveness of *Pm6* on Indian wheat. The recurrent parents in the study also carried other resistance genes, such as *Lr13+, Lr10+, Lr23+, Lr1+, Sr8b+, Sr9b+,*

Table 2. Mean and Standard Deviation (SD) of agronomically significant traits of the recurrent parents and gene stacked lines

Parent/Cross	**PH** (cm)#	**NPT**	**SPL** (cm)	**GNS**	**TGW** (g)
HD 2329	70 ±2.07	8 ±0.54	9 ±0.25	51 ±3.42	26 ±0.83
HW 3641	80 ±1.65*	10 ±2.60*	9 ±0.13	48 ±2.12*	40 ±0.54*
HD2402	82 ±1.14	11 ±0.54	9 ±0.26	55 ±2.68	34 ±1.14
HW 3642	84 ±1.51*	12 ±1.67	12 ±0.54	56 ±1.64*	39 ±0.89*
LOK-1	89 ±0.65	7 ±1.09	9 ±0.2	45 ±2.12	32 ±1.22
HW 3654	75 ±2.75*	10 ±1.47*	10 ±1.03*	51 ±3.99*	34 ±2.45
MACS2496	89 ±0.68	8 ±0.89	11.2 ±0.22	65 ±3.87	32 ±0.54
HW 3655	90 ±1.03*	9 ±0.83	11.2 ±0.56	64 ±2.50	42 ±0.45**
NIAW 34	87 ±0.83	8 ±0.54	8.5 ±0.08	57 ±2.12	34 ±1.14
HW 3657	76 ±2.96*	12 ±1.64*	9 ±0.08	55 ±1.64	31 ±1.09**
RAJ 3077	83 ±2.12	9 ±0.89	7.7 ±0.17	45 ±2.12	24 ±1.14
HW 3662	82 ±1.51	14 ±1.30*	10.2 ±0.79*	49 ±1.64*	32 ±0.70*
PBW 343	83 ±2.38	12 ±1.09	8.6 ±0.11	54 ±2.68	28 ±2.07
HW 3660	80 ±2.02	14 ±0.70*	9.9 ±0.37*	61 ±2.68*	30 ±0.54*
PBW 502	74 ±1.14	9 ±0.54	9.8 ±0.20	51 ±2.12	26 ±1.92
HW 3661	82 ±1.17*	13 ±2.12*	10.6 ±0.65*	56 ±1.64*	32 ±0.70*

***** Recurrent parent and gene stacked line have significant differences ($P < 0.05$). ** Recurrent parent and gene stacked line have an extremely significant difference. **#** PH - Plant height, NPT - Number of productive tillers per plant, SPL - spike length, GNS - Grain number per spike, and TGW - Thousand grain weight.

Sr11+, Yr2+, Yr18+, Yr2KS+ and *Yr27+* (Bhardwaj 2011). Most of these genes were ineffective in the past. However, the accumulated residual effect of the defeated genes generally termed as the ghost effect (Martin and Ellingboe 1976) has also added to resistance when stacked with other effective ASR and APR resistance genes.

In conclusion, enhancing the genetic resistance of adapted or elite wheat cultivars to rust and PM is a highly decisive approach for tackling the rapid momentum of pathogen evolution. Resistance offered by a single ASR or major gene has an inherent risk of being overcome by newly evolving rust pathotypes. Therefore, the combination of the ASR and APR genes was found to have a more significant effect. In the process of stacking resistance genes, the synergistic effect of stacked genes has been reported to increase the lifespan of each gene (Klymiuk et al. 2018, Mundt 2018). Rapid stacking of genes could be conducted by taking advantage of the favorable environmental conditions prevalent in Wellington, which allows growing of two crop cycles per year. The gene-stacked lines developed with *Lr45*, *Sr36* and *Sr2* could be a potential source of new resistant varieties and could be suggested as potential germplasm resistant to leaf rust, stem rust, and PM. The co-dominant markers $G372_{185,}$ *Stm773-2,* and *Xgwm533* allow the efficient detection of genes in the homozygous state and would serve as an important tool in the rapid transfer of these genes into adapted wheat cultivars.

REFERENCES

Alabushev AV, Vozhzhova NN, Kupreyshvili NT, Shishkin NV, Marchenko DM and Ionova EV (2019) Identification of stem rust resistance genes in the winter wheat collection from Southern Russia. **Plants (Basel) 8**: 559.

Amulaka F, Maling'a J, Pathak R, Cakir M and Mulwa R (2013) Yield evaluation of a wheat line with combined resistance to Russian wheat aphid and stem rust race "Ug99" in Kenya. **American Journal of Plant Sciences 4**: 1494-1499.

Bariana HS, Babu P, Forrest KL, Park RF and Bansal UK (2022) Discovery of the new leaf rust resistance gene *Lr82* in wheat: molecular mapping and marker development. **Genes 13**: 964.

Bhardwaj SC (2011) Resistance genes and adult plant resistance of released wheat varieties of India. Regional Station, Directorate of Wheat Research, Flowerdale, Shimla -171002 (India), 32p. (**Technical Bulletin, 5**).

Bhardwaj SC, Kumar S, Gangwar OP, Prasad P, Kashyap PL, Khan H, Savadi S, Singh GP, Gupta N and Thakur R (2021) Physiologic specialization and genetic differentiation of *Puccinia triticina* causing leaf rust of wheat in the Indian subcontinent during 2016-2019. **Plant Diseases 105**: 1992-2000.

Chemayek B, Bansal UK, Quresh N, Zhang P, Wagoire WW and Bariana HS (2017) Tight repulsion linkage between *Sr36* and *Sr39* was revealed by genetic, cytogenetic and molecular analyses. **Theoretical Applied Genetics 130**: 587-595.

Chen XM (2005) Epidemiology and control of stripe rust (*Puccinia striiformis*f. sp. *tritici*) on wheat. **Canadian Journal of Plant Pathology 27**: 314-337.

Chhuneja P, Vikal Y, Kaur S, Singh R, Juneja S, Bains N, Berry O, Sharma A, Gupta S, Charpe A and Dhaliwal K (2011) Marker-assisted pyramiding of leaf rust resistance genes *Lr24* and *Lr28* in wheat (*Triticum aestivum*). **Indian Journal of Agricultural Sciences 81**: 214-218.

Collard BCY and Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. **Philosophical Transactions of the Royal Society B: Biological Sciences 363**: 557-572.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. **Focus 12**: 13-15.

Fein YH, Yan L, Gang GS, Xinag YW and Qun LD (2009) A SCAR marker for leaf rust resistance gene *Lr45*. **Scientia Agricultura Sinica 42**: 124-129.

Hare RA and McIntosh RA (1979) Genetic and cytogenetic studies of durable adult-plant resistances in 'Hope' and related cultivars to wheat rusts. **Z. Pflanzenzücht 83**: 350-367.

Herrera-Foessel SA, Singh RP, Lillemo M, Huerta-Espino J, Bhavani S, Singh S, Lan C, Calvo-Salazar V and Lagudah ES (2014) *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. **Theoretical and Applied Genetics 127**: 781-789.

Jin Y, Szabo LJ, Rouse MN, Fetch Jr T, Pretorius ZA, Wanyera R and Njau P (2009) Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. **Plant Diseases 93**: 367-370.

Jin X, Chi D, Wolfe D, Hiebert C, Fetch T, Cao W, Xue A, Humphreys G and Fedak G (2022) Wheat germplasm development by gene pyramiding for resistance to race TTKSK of stem rust. **Canadian Journal of Plant Science 102**: 760-763.

Jorgensen JH and Jensen CJ (1973) Gene *Pm6* for resistance to powdery mildew in wheat. **Euphytica 22**: 4-23.

Klymiuk V, Yaniv E, Huang L, Raats D, Fatiukha A, Chen S, Feng L, Frenkel Z, Krugman T, Lidzbarsky G, Chang W, Jaaskelainen MJ, Schudoma C, Paulin L, Laine P, Bariana H, Sela H, Saleem K, Sorensen CK, Hovmoller MS, Distelfeld A, Chalhoub B, Dubcovsky J, Korol AB, Schulman AH and Fahima T (2018) Cloning of the wheat *Yr15* resistance gene sheds light on the plant and emkinase-pseudokinase family. **Nature Communication 9**: 3735.

Martin TJ and Ellingboe AH (1976) Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding genes in *Erysiphe graminis* f. sp. *tritici*. **Phytopathology 66**: 1435-1438.

McIntosh RA, Dubcovsky J, Rogers WJ, Xia X C and Raupp WJ (2020) Catalogue of gene symbols for wheat: supplement. **Annual Wheat Newsletter 66**: 109-128.

Mundt CC (2018) Pyramiding for resistance durability: theory and practice. **Phytopathology 108**: 792-802.

Naik B, Vinod, Sharma J, Sivasamy M, Prabhu K, Tomar RS and Tomar S (2015) Molecular mapping and validation of the microsatellite markers linked to the *Secale cereale L*. derived leaf rust resistance gene *Lr45* in wheat. **Molecular Breeding 35**: 61.

Peterson RF, Campbell AB and Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. **Canadian Journal of Research 26**: 496-500.

Prasad P, Thakur RK, Savadi S, Bhardwaj SC, Gangwar OP, Lata C, Adhikari S and Kumar S (2022) Genetic diversity and population structure reveal cryptic genetic variation and long-distance migration of *Puccinia graminis* f. sp. *tritici* in the Indian subcontinent. **Frontiers in Microbiology 13**: 842106.

Roelfs AP, Singh RP and Saari EE (1992) **Rust diseases of wheat: Concepts and methods of disease management**. CIMMYT, Mexico, 81p.

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX and He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. **Theoretical and Applied Genetics 126**: 2427-2449.

Sai Prasad SV, Singh SK, Kumar V, Kantwa SL, Dubey VG, Ambati D, Prakasha TL and Mishra AN (2014) Pyramiding of resistance genes *Sr36* and *Sr2* in durum wheat background (HI 8498) through marker assisted selection for resistance to stem rust race 117- group pathotypes. In Porceddu E, Damania AB and Qualset CO (eds) **Proceedings of the international symposium on genetics and breeding of durum wheat**. CIHEAM, Bari, p. 419-429.

Sharma BK, Basandrai AK and Verma BR (1996) Disease resistance status of commonly grown wheat varieties in Himachal Pradesh. **Plant Disease Research 11**: 66-68.

Sheng BQ and Duan XY (1991) Improvement of scale 0-9 method for scoring adult plant resistance to powdery mildew of wheat. **Beijing Agricultural Sciences 9**: 38-39.

Singh A, Jaiswal JP and Badoni S (2018) Enhancing rust resistance in wheat through marker assisted backcross breeding. **Indian Journal of Genetics and Plant Breeding 78**: 19-25.

Singh H, Kaur J, Bala R, Srivastava P and Bains NS (2020) Virulence and genetic diversity of *Puccinia striiformis*f. sp. *tritici*i isolates in sub-mountainous area of Punjab, India. **Phytoparasitica 48**: 383-395.

Sivasamy M, Kumar J, Menon MK and Tomar SMS (2010) Developing elite, durable disease resistant wheat cultivars combining high grain yield and end-use quality by introgressing effective genes employing conventional and modern breeding approaches. **Annual Wheat Newsletter 56**: 87-94.

Sivasamy M, Vikas VK, Jayaprakash P, Kumar J, Saharan MS and Sharma I (2017) Gene pyramiding for developing high yielding disease resistant wheat varieties. In Singh DP (ed.) **Management of wheat and barley diseases**. Apple Academic Press, New York, p. 361-409.

Spielmeyer W, Sharp PJ and Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum L.*). **Crop Science 43**: 36.

Stakman EC, Stewart DM and Loegring WQ (1962) Identification of physiologic races of *Puccinia graminis*var *tritici*. United States Department of Agriculture, Washington (**Agricultural Research Service Bulletin, E617**).

Tsilo TJ, Jin Y and Anderson JA (2008) Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. **Crop Science 48**: 253-261.

USDA – United States Department of Agriculture (2023) World agriculture production. Available at <https://apps.fas.usda.gov/psdonline/circulars/production.pdf>. Accessed on January 17, 2023.

Vikas K, Sundeep K, Sunil A, Tyagi RK, Kumar J, Sherry J, Sivasamy M, Jayaprakash P, Saharan M, Basandrai AK, BasandraiD, Srinivasan K, Radhamani J, Parimalan R, Tyagi S, Kumari J, Singh AK, Peter J, Nisha R, Yadav M, Kumari J, Dhillon HK, Chauhan D, Sharma S, Chaurasia S, Sharma RK, Dutta M, Singh GP and Bansal KC (2020) Screening of 19,460 genotypes of wheat species for resistance to powdery mildew and identification of potential candidate using FIGS approach. **Crop Science 60**: 2857-2866.

Vishwakarma G, Sanyal RP, Shitre A, Gadekar DA, Saini A and Das BK (2019) Validation and marker-assisted selection of stem rust resistance gene *Sr2* in Indian wheat using gel-based and gel-free methods. **Journal of Crop Science and Biotechnology 22**: 309-315.

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. **Weed Research 14**: 415-421.

Zhang N, Yang WX, Yan H, Liu D, Chu D, Meng Q and Zhang T (2006) Molecular markers for leaf rust resistance gene *Lr45* in wheat based on AFLP analysis. **Agricultural Sciences in China 5**: 938-943.

Zheng W, Li S, Liu Z, Zhou Q, Feng Y and Chai S (2020) Molecular marker assisted gene stacking for disease resistance and quality genes in the dwarf mutant of an elite common wheat cultivar Xiaoyan22. **BMC Genetics 21**: 45.