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RESEARCH ARTICLE

Marker assisted improvement of stripe rust resistance in hybrid wheat

Neethu Mohan*, Shailendra Kumar Jha, Niharika Mallick, M. Niranjana, K. Raghunandan, Abhimanyu, Mahender Singh Saharan, R. Thribhuvan[1], Sanjay Kumar Singh, V.G. Dhanya[2] and Vinod

Abstract

Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (Pst), is one of the major rust fungi that causes severe reduction in yield across the world. Gene pyramiding offers to stack resistant genes in hybrids by incorporating them into their parents. An attempt has been made to introgress the stripe rust resistance genes *Yr10* and *Yr15* into maintainer line 365B and restorer line 1752 R to optimize the yield potential of hybrids developing at IARI, New Delhi. B (maintainer) and R (Restorer) lines were crossed with their respective donors for *Yr10* (Avocet*Yr10*) and *Yr15* (HD2967 + *Yr15*) genes. F_1 and backcross generations were screened with linked and validated SSR markers X*psp3000* for *Yr10* in B line and *Xgwm*273 for *Yr15* in R line. Based on marker association, plants carrying *Yr10* in B line and *Yr15* in R line in each backcross generation were further selected for background recovery. BC_2F_1 was selfed to obtain BC_2F_2. Marker assisted breeding helped to reduce the time and effort to improve the parental lines. These improved B lines and R lines will be used for hybrid development after doing background selections. The resistance in B and R lines will ensure the maximum harnessing of heterosis which could be declined due to disease occurrence.

Keywords: Hybrid wheat, Stripe rust, Restorer lines (R), Maintainer lines (B), Foreground selection, Marker-assisted breeding

Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple food grain crops preferred for consumption by people across the world. To meet the growing food demand of burgeoning population, the productivity of the crop has to be improved multiple folds in the future. Various biotic and abiotic stresses hamper the productivity of the wheat crop. Heterosis breeding has proven its worth in the form of the development of high-yielding hybrids that are resistant to biotic and abiotic stresses in many crops, including cereals. In addition to resistance against biotic and abiotic stress factors, hybrid offers many advantages over line varieties which results in higher productivity (Longin et al. 2014; Beukert et al. 2020).

Wheat is also receiving a lot of interest in hybrid development in India and other nations. In self-pollinated crop like wheat, in consideration with any other methods, hybrid development using ABR (A-male sterile lines, B-maintainer lines, R-restorer lines) lines ensures an efficient seed production system (Wurschum et al. 2017). Scientists at the Indian Agricultural Research Institute are making several attempts to develop and improve A, B and R lines in wheat. At the same time, hybrid wheat production is also hampered by several biotic and abiotic stresses, of which rusts are of major concern as some of the male sterile and restorer lines become susceptible to one or the other rust diseases.

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India.

[1]ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore 700 121, Kolkata, West Bengal, India.

[2]ICAR-Sugarcane Breeding Institute, Coimbatore 641 007, Tamil Nadu, India.

***Corresponding Author:** Neethu Mohan, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India, E-Mail: neethumohangenetics@gmail.com

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Fig. 1. Scheme of marker-assisted backcross breeding for yellow rust

Rusts are the most devastating fungal diseases that cause significant damage to wheat crop all around the world, leading to a decline in their productivity. Of the three rust diseases, yellow rust (Stripe rust) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) results in significant yield reduction (Zheng et al. 2017). A few of the male sterile lines and restorer lines that are developed at IARI, New Delhi, and utilized to create high-yielding hybrid wheat are susceptible to stripe rust, which causes an extreme reduction in yield. The best way to prevent wheat crops from being damaged by rust is by using wheat varieties that have a genetic ability to resist rust. Gene pyramiding is one of the promising methods of combining multiple resistance genes into one preferred genetic background. It is a practical and cost-effective way to ensure that a plant variety can survive in the field without being harmed by disease. When two resistance genes that protect against the pathogen are put together in a plant's genetic code, it would be unlikely for two simultaneous mutations to happen at the same time that can overcome the plant's resistance (Tiwari et al. 2014). Hybrid breeding is a good way to stack resistance genes together. The success of this method depends on how dominant certain genes are at specific locations. So, using hybrid breeding could be a good way to help develop resistance in plants (Longin et al. 2012; Beukert et al. 2020).

Marker-assisted introgression of rust resistance genes in A/B lines (female parent) and R lines (male parent) will ensure resistance in hybrids developed using these improved lines. Through the utilization of MAS technology, resistance breeding has effectively averted the development of artificial rust epidemics, enabling the selection of genes that bestow the resistance against rust.86 permanently recognized stripe rust resistance genes, 100 temporarily designated genes, and 363 quantitative trait loci (QTLs) with various designations have been identified in wheat to date (Klymiuk et al. 2022; Wang and Chen 2017). The resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17, Yr18, Yr19, Yr21, Yr22, Yr23, Yr25, Yr27* and *YrA* have lost their effectiveness against the prevalent and recently evolved pathotypes of *P. striiformis tritici* in India while all of the common pathotypes of *Pst* in India are still avirulent to the resistance genes *Yr5, Yr10, Yr15, Yr24/Yr26, Yr32*, and *YrSp* (Haider et al. 2023). An attempt has been made to incorporate *Yr-10* into the genetic background of the B line (The fertile counterpart of the A line) and *Yr-15* into the genetic background of R line to ensure the presence of both genes into a single hybrid developed using these improved lines.

Materials and methods

Plant material

The backcross breeding program was planned to transfer yellow rust resistance genes *Yr-10* and *Yr-15* into the genetic background of B line (maintainer line) and R line (Fig.1). Plant material included B line (365 B) and R line (1752R), both were developed and maintained at IARI, New Delhi as a part of hybrid breeding program. Details of the donor parents, recurrent parents and their stages are given in Table 1. Recommended agronomic practices were followed for raising the crop in the field.

Field test

Recurrent and donor parents were crossed to generate F_1 generation in 2017, *rabi* at IARI, New Delhi. Details of Scheme of marker-assisted backcross breeding for transfer of yellow rust resistance genes *Yr10* and *Yr15* in the parental background of hybrid wheat are given in Fig.1. Phenotypic observations and data recording for stripe rust were done by using the modified Cobb's Scale (Peterson et al. 1948) under natural infection in field conditions at weekly intervals between the beginning of January's second week and the end of March's first week during *rabi*. PBW 343 and Agra Local were used as susceptible checks. F_1 progenies were raised at IARI regional station, Wellington Nilgiris, during *kharif* 2018. The whole breeding program had shuttled between Nilgiri and New Delhi. F_1 plants and subsequent backcross generations were subjected to phenotypic and foreground selection to identify plants carrying resistance genes. BC_2F_1 generations were raised at IARI, Regional Station Wellington

Table 1. Plant material used to develop backcross generations

S. No.	Donor parent	Recurrent parent		Parentage	Stage
1.	Avocet *Yr10*	B365	B line (Maintainer line)	HS207/SONALIKA	BC_2F_2
2.	HD2967 + *Yr 15*	1752R	R line (Restorer line)	HW 2045/4099R	BC_2F_2

RP: Recurrent parent, Marker Assisted Backcross Breeding shuttled between Delhi and Nilgiris (Wellington)

Table 2. Details of marker associated with stripe rust resistance genes *Yr10* and *Yr15*.

S. No.	Gene	Marker name	Primer sequence	Product size	AT (C°)	Distance (cM)	Reference
1	*Yr10*	*Xpsp3000*	F:GCA GAC CTG TGT CAT TGG TC R:GAT ATA GTG GCA GCA GGTACG	240, 260	55	1.5	Bariana et al. 2002
2	*Yr15*	*Xgwm273*	F:ATT GGA CGG ACA GAT GCT TT R: AGC AGT GAG GAA GGG GAT C	165, 180	55	2.1	Tiwari et al. 2014

AT= Annealing Temperature; cM=Centimorgan

and those plants identified as resistant in accordance with phenotypic and foreground selection were subjected to selfing. BC_2F_2 seeds were obtained and BC_2F_2 was raised at IARI, New Delhi and was subjected to phenotypic and foreground selection.

Molecular markers for stripe rust resistance genes

Peculiar characteristics of molecular markers associated with stripe rust resistance genes *Yr10* and *Yr15*are presented in Table 2. These markers were used for the foreground selection of plants in F_1 and subsequent backcross generations. Genomic DNA was isolated from 22 to 27 days-old seedlings using the cetyl trimethyl ammonium bromide method (Murray and Thompson1980). DNA samples were quantified on 0.8% (W/V) agarose gel using lambda uncut DNA (100 ng) as a standard check. The final concentration of DNA was made up to 25 ng/µL of TE buffer before Polymerase Chain Reaction (PCR) amplification. The PCR reaction was performed using a reaction volume of 10 µL. About 10 µL Eppendorf tube contains a reaction volume of 2 µL of template DNA (25 ng), 1-µL each of forward and reverse primers, and 3 µL each of Taq DNA Polymerase RED 2× master mix (AMPLIQON A/S, Denmark) and nuclease-free water (THERMO FISHER SCIENTIFIC INC., USA). The PCR reactions were carried out in 96-well PCR plates in an APPLIED BIOSYSTEMS VERITI thermal cycler. PCR amplification was done by using an initial denaturation step of 94℃ for 4 minutes, 35 cycles, annealing temperature at 55℃, primer extension at 72°C for 1-minute and final extension at 72℃ for 10 minutes. PCR amplified products were resolved on 3.5% (w/v) Agarose (LONZA, Rockland, USA) gel stained with ethidium bromide. Final product visualization was done with the help of a UV-transilluminator gel documentation system (SYNGENE G-BOX, Cambridge, UK).

Results

The recipient genotypes 365 B and 1752 R, donor genotypes Avocet *Yr10* and HD 2967+*Yr15*, along with their F_1s and respective backcross generations (Fig.1), were subjected to natural infection of stripe rust disease under field conditions in both locations. The phenotypic selection was done at each backcrossing and selfing generation to avoid the association of plants with unfavorable traits (linkage drag). Plants showing brown glumes (morphological marker) (Rg1) were positively correlated with *Yr10* gene (Metzger and Silbaugh 1970). Hence, additional confirmation was done before going for the selection of plants for crossing. Recipient parental lines 365 B and 1752R have shown infection type IT(4), indicating the susceptibility to yellow rust similar to susceptible checks, while donor parents (Avocet *Yr10* and HD2967+*Yr 15*) and resistant plants carrying *Yr* genes identified through molecular tagging were shown infection type 0 (IT 0), *i.e*., resistance to yellow rust.

Molecular tagging of Yr10 gene

Microsatellite marker *Xpsp 3000* is co-dominant in inheritance. Irrespective of the growth stage, this marker helps to identify individuals (Clemence et al. 2018). B line (365B) with their respective donors (Avocet *Yr-10*) showed polymorphism for microsatellite marker *Xpsp* 3000 with 240 and 260 bp alleles. The fragment 260 bp was amplified in true hybrids of 365B/ Avocet *Yr10* (Fig. 2), which shows the presence of *Yr10* gene.

Molecular tagging of Yr15 gene

Co-dominant multiallelic microsatellite marker *Xgwm* 273 consists of 5 different types of alleles *viz*., 156, 165, 200, and 220 bp. With the help of these co-dominant markers, plants carrying *Yr15* were identified in the backcross populations of 1752R/HD 2967+*Yr15* (Fig. 3). This combination of 1752R and HD 2967+*Yr15* showed multi-allelic polymorphism for microsatellite marker locus *Xgwm 273*.

Out of 35 BC_2F_2 plants from 365B*Avocet *Yr10*, three plants were identified as positive for the presence of *Yr10* gene. Of 40 BC_2F_2 plants from 1752R*HD 2967+*Yr-15* yielded five plants positive for *Yr15* gene. The resistant and improved B and R lines obtained from BC_2F_2 generation can be used for developing hybrids with pyramided genes *Yr10* and *Yr15* that confer resistance against stripe rust. The stripe rust resistant and susceptible plants under field conditions are given in Fig. 4.

Discussion

The apprehension within the plant breeding community revolves around the occurrence of unfamiliar, new races of pathogens that are capable of overpowering the resistance offered by race-specific genes incorporated in plant cultivars. Developing cultivars with long-lasting resistance to pathogens is important in agriculture. Wheat rusts are a major problem for wheat plants and can be found in many places where wheat is grown (Khan et al. 2013). To prevent or

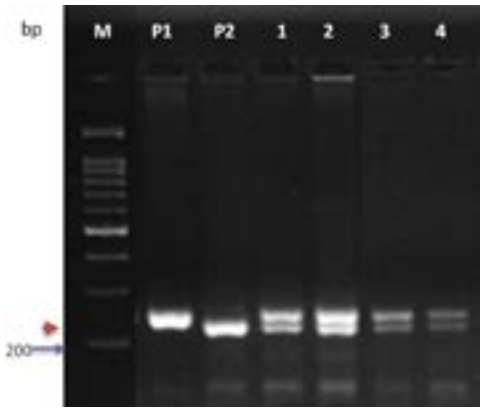

Fig. 2. Foreground selection for *Yr10* gene in B line (365B): P1 – Avocet, P2 – B line, 1-4: F_1 Samples, marker Xpsp3000.bp:base pair

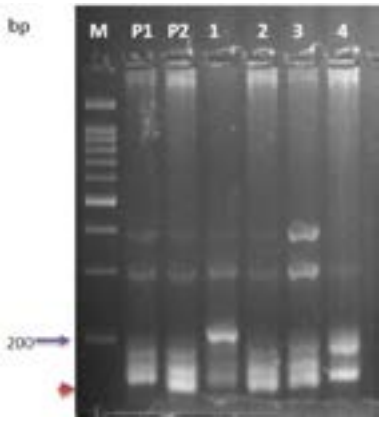

Fig. 3. Foreground selection for *Yr15* gene transfer in R line (1752R): P1, P2, 1-4: Population samples, Marker *Xgwm273*

mitigate damage to crop yield and quality, a lasting solution is to breed and cultivate wheat plants with robust genes that can combat rust diseases (Beukert et al. 2020). Choosing rust-resistant plants becomes more convenient with the use of molecular resistance breeding (Ordon et al. 1998). Marker-assisted selection (MAS), a frequently employed technique, enables the introduction of multiple genes to confer resistance to rust disease into a single genotype of wheat. The pyramiding of different resistance genes in the same genotype is important to verify that the current defenses maintain their effectiveness (Singh et al. 2005). Many stripe rust-resistant genes have been discovered in wheat. At the same time, new types of *Pst* with negative implications have also emerged in the past few years. However, employing a diverse combination of resistance genes in a single genotype ensures a proper defense mechanism to counter powerful genetic modifications or mutations in the stripe rust pathogen (Zheng et al. 2017). Using Marker-Assisted Backcross Breeding (MABB), scientists can combine multiple resistance genes into a single cultivar that provides durable resistance against evolving *Pst* races (Sobia et al. 2010). Many studies have also proven that combining genes is a good way to make wheat more resistant to stripe rust (Rosewarne et

Fig. 4. Stripe rust resistant (A) and susceptible plants (B) in the field

al. 2013; Zheng et al. 2017; Lowe et al. 2011; Yang et al. 2013; Aktar-Uz-Zaman et al. 2017). Liu et al. 2020 observed that breeders may rationally combine some effective *Yr* genes to achieve durable resistance and multigene pyramiding will not affect various agronomically important traits. An effective technique to combine multiple resistant genes into a single cultivar is hybrid breeding (Lognin et al. 2012), while its efficiency depends on the nature of the dominance of the gene. Therefore, hybrid breeding complements resistance breeding (Beukart et al. 2020). A marker-assisted breeding program separately conducted for incorporating different resistant genes into parental genetic background ensures durable resistance in hybrids.

The *Yr10* gene proved effective against almost all races of *Pst* reported so far in India, Iran, China, Pakistan, and the United States (Chatrath et al. 2007). The marker *Xpsp3000* is positioned at a distance of 1.2 cM from the gene *Yr-10* on chromosome 1BS. Due to its co-dominant inheritance it helps to identify genotypes irrespective of the growth stage (Elkot et al. 2016). In a similar fashion, *Yr-15* was also found to be effective against all prevalent races of *Pst* pathogen (Chen and Kang 2017). The brown glume color gene Rg1 helps identify *Yr10,* but it can only be used when the plant is fully grown (Metzger and Silbaugh 1970). The microsatellite markers *Xpsp* 3000 and *Xgwm* 273, which are located 1.5 cM and 2.1 cM away from *Yr10* and *Yr15* genes, respectively (Revathy et al. 2010; Marchal et al. 2018) show co-dominant inheritance. It can be used to determine the genetic makeup of individuals at any stage of growth. So, it would be a great choice for using this as a guide in markers-assisted selection. (Mallick et al. 2021). The present investigation shows that *Yr10* and *Yr15* genes show complete resistance in B and R lines and marker-assisted selection for *Yr10* and *Yr15* genes in parental lines of hybrids has accelerated the pace of the breeding program with reduced time and effort.

The marker-assisted backcross program provided an opportunity to transfer *Yr10* and *Yr15* genes into the maintainer and restorer lines within the stipulated time with reduced effort. The breeding material is in the BC_2F_3 seed stage. This needs to be grown and further evaluated

for background selection to ensure the complete recovery of the recipient genome.

Authors' contribution

Conceptualization of research (V, SKJ, MSS, NM); Designing of the experiments (NM, NM, V); Contribution of experimental materials (V, SKJ); Execution of field/lab experiments and data collection (NM, A, TR, RK, DVG); Analysis of data and interpretation (NM, NM); Preparation of the manuscript (NM)

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