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# Marker assisted stacking/pyramiding of stem rust, leaf rust and powdery mildew disease resistance genes (*Sr2/Lr27/Yr30, Sr24/Lr24* and *Sr36/Pm6*) for durable resistance in wheat (*Triticum aestivum* L.)

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**Abstract**
Wheat being a staple food crop is essential for food security. Rust diseases like stem and leaf rust continues to be a serious threat to wheat production at national and international level. In recent years, powdery mildew disease is emerging as a threat to wheat production due to the climate change and intensive crop cultivation practices. Development of resistant cultivar is the most economical, reliable and sustainable way to control rusts and powdery mildew diseases. Stem rust, leaf rust and powdery mildew resistance genes *viz*., *Sr2/Lr27/Yr30, Sr24/Lr24* and *Sr36/Pm6* were pyramided using marker assisted backcross breeding approach. Based on phenotypic observations, five populations were selected at $BC_4F_6$ with respect to yield traits, leaf and stem rust and powdery mildew reactions. These populations were subjected to molecular marker analyses. Out of the five populations, *Sr2*+ were present in all the populations, while *Sr24/Lr24* and *Sr36/Pm6* was present in the fourth and fifth population. The identified population carrying *Sr24/Lr24, Sr36/Pm6* and *Sr2*+ genes were stabilized at $BC_4F_7$ generation. The use of gene stacks/pyramids of major (*Sr24/Lr24* and *Sr36/Pm6*) and minor gene (*Sr2*+) that confer resistance to most of the pathotypes of stem rust, leaf rust and powdery mildew could impart durability and sustainability to the cultivars than single gene deployment.

**Key words**
rust diseases, powdery mildew, molecular markers, resistance

## INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops of the world, which is cultivated over a wide range of climatic conditions. Being the staple food crop, it needs constant progressive production to meet out the increasing human population. It faces many abiotic and biotic constraints of which diseases such as stem rust, leaf rust and powdery mildew are of major concern.

Rust diseases *viz*., leaf rust caused by *Puccinia triticina*, stem rust caused by *Puccinia graminis* f. sp. *tritici* have been managed, mainly by judicious deployment of effective rust resistance genes in different wheat growing zones in India. Leaf rust exists in all the wheat growing regions, while stem rust are common in warmer areas of Central and Peninsular India. Susceptible wheat cultivars to leaf rust suffer from yield reductions between 5 to 60% (Smith and Lauren, 2008) whereas to stem rust nearly 100% yield loss particularly after the emergence of Ug99 (Hawkesford *et al.,* 2013).

Another important foliar disease of wheat is powdery mildew which is gaining attention in the recent days due to the changing climatic conditions and modern cultivation

practices. Powdery mildew caused by *Blumeria graminis* f.sp. *tritici* can cause yield reductions upto 40% under the humid conditions (Bennett, 1984). Development and deployment of rust and powdery mildew resistant cultivars is the most economical, effective and environment friendly approach to prevent the damage caused by rust and powdery mildew diseases. In general, cultivars carrying single race specific resistance gene was found to be short lived due to rapid evolution of new races of pathogen. Therefore, pyramiding of minor and major resistance genes is considered as an effective strategy for enhancing the durability of resistance genes.

Wheat variety, HD2833 is a high-yielding variety released for cultivation under timely sown irrigated conditions of Peninsular zone in India. HD 2833 is reported to carry *Sr2* and *Sr24/Lr24* genes. A virulent pathotype, 40-1 (62G29-1), isolated from the Nilgiris in 1990 (Bhardwaj *et al.*, 1990), overcame the stem rust resistance gene, *Sr24.* To provide an enhanced resistance to stem rust disease, HD2833 was introgressed with *Sr36/Pm6*. *Sr2* is an adult plant resistance (APR) gene having minor effect against stem rust which is also reported to be linked with leaf rust and stripe rust resistance genes such as *Lr27* and *Yr30* respectively. *Sr24* is one of the major stem rust resistance gene linked together with the leaf rust resistance gene *Lr24*, which had been providing resistance to leaf and stem rust for quite long time. In this study, *Sr36/Pm6* was the target gene transferred into HD2833 from donor line, "COOK". *Sr36*, originally derived from *Triticum timopheevii* located in the chromosome arm of 2BS is an effective stem rust resistance gene in India so far. *Sr36* is also reported to be tightly linked to powdery mildew resistance gene *Pm6* (Jorgensen and Jensen, 1973) that is highly effective in India. Thus providing cross resistance to *Sr24*-virulent stem rust pathotype,40-1.

Selection of two or more genes in a single genotype can be difficult using conventional selection system. In such a scenario, phenotype neutral selection based on the marker-trait association becomes inevitable. 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). In this paper, we report the pyramiding of rust and powdery mildew resistance genes viz.,*Sr2/Lr27/Yr30, Sr24/Lr24*and *Sr36/Pm6* using marker assisted foreground selections in the background of wheat variety, HD2833.

## MATERIALS AND METHODS

Initially, HD2833 was crossed with the donor parent, Cook(*Sr36*/*Pm6*) to develop $F_1$ generation. The $F_1$ plants were backcrossed with HD2833 to produce the $BC_1F_1$ generation. Backcross was repeated for four consecutive generations to produce $BC_4F_1$ generation followed by selfing up to $BC_4F_7$ generation. Up to $BC_4F_5$ generations, genotypes with high yield, leaf and stem rust and powdery mildew resistance were selected based on phenotypic observation in the field. In $BC_4F_6$ and $BC_4F_7$ generation, genotypic screening using molecular markers was also performed along with phenotypic evaluation. Stabilized population with all the three rust resistance genes were grown for two more seasons to study the effect of resistance genes on the yield attributing traits. Crossing program and screening of the parents, $BC_1F_1$s, $F_2$s, $F_3$s, etc. for rusts and powdery mildew were carried out at the ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Wellington, The Nilgiris, Tamil Nadu. Being a hot spot for rust and powdery mildew diseases, inoculum is prevalent throughout the year at Wellington.

Stem and leaf rust scoring take into consideration the response and severity of infection. Severity of rust was recorded based on the modified Cobb's scale (Peterson *et al*., 1948). In this scale, the disease severity was assessed from 1 to 100 and was scored as percentage infestation of the disease on the plant (total area of stem and leaves covered by the disease). The host plant response to infection was scored as Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS) and Susceptible (S) as described by Logering (1959). Rust score of upto 20S is considered as resistant and >20S as susceptible.

For powdery mildew evaluation, scale (0-9) devised by Saari and Prescott (1975) for appraising the foliar disease intensity of wheat diseases was followed. Disease score of up to 5 is considered as resistant and > 5 as susceptible.
Genotypic selection - Marker assisted selection

Leaf tissues were collected from fresh twenty day old seedlings. Genomic DNA was isolated by using CTAB method of Doyle and Doyle (1990). The purity of the DNA samples were assessed by the ratio between the readings at 260 nm and 280 nm. The diluted DNA samples were then used for PCR analysis. The foreground selection of the targeted genes were carried out using sequence-tagged sites (STS) marker *Sr24#12* linked to *Sr24* (Mago *et al.* 2005) and sequence tagged microsatellite (STM) markers viz., *gwm533* linked to *Sr2* (Spielmeyer *et al.,* 2003), and *stm773-2,* linked to *Sr36* (Hayden *et al.*, 2001).

Polymerase chain reaction (PCR) was carried out in 10 µL reaction volumes with 25ng of genomic DNA, 1.0 unit *Taq* DNA polymerase (Bangalore Genei, Bengaluru, India), 200 µM of each dNTP (MBI Fermentas, Germany), 0.2 µM of both forward and reverse primers, 4mM Tris-HCl (pH 8.0), 20mM KCl and 0.8mM MgCl2. Polymerase chain reaction was performed in a Eppendorf (model Mastercycler pro S, Hamburg, Germany) thermal cycler using the conditions given in **Table 1**.The amplified products were resolved on 1.5% (*Sr24#12*) and 3.5% (*gwm533* and *stm733-2*) Metaphor (Lonza) agarose gel and visualized using ethidium bromide staining.

**Table 1. Details of the molecular markers and PCR conditions.**

| S.No. | Marker | Linked Genes | Amplicon size | PCR Conditions |
|---|---|---|---|---|
| | *Sr24#12* | *Sr24/Lr24* | 500bp | Initial denaturation: 94°C for 4 min,<br>Denaturation: 94°C for 30s,<br>Annealing :55°C for 30s (35 cycles)<br>Extension; 72°C for 30 s,<br>Final extension: 72°C for 10 min. |
| | *Xgwm533* | *Sr2/Lr27/Yr30* | 120bp | Initial denaturation : 95°C for 2 min, Denaturation: 95°C for 30 s,<br>Annealing: 60°C for 40s (35 cycles) Extension: 72°C for 50s<br>Final extension :72°C for 5 min. |
| | *Stm773-2* | *Sr36/Pm6* | 155bp | Initial denaturation : 95°C for 2 min, Denaturation: 95°C for 30 s,<br>Annealing: 55°C for 30s (35 cycles)<br>Extension: 72°C for 50s<br>Final extension :72°C for 5 min. |

## RESULTS & DISCUSSION

Rusts are devastating pathogens of wheat worldwide (Bhardwaj, 2013). Among the various foliar diseases of wheat, leaf and stem rusts cause significant yield loss, whereas powdery mildew is emerging as a threat in many wheat growing regions. Developing resistance varieties using combination of resistance genes is a vital strategy to combat the threat posed by rusts and powdery mildew. However, over a period of time, resistant cultivars became susceptible to rusts and powdery mildew due to the rapid evolution of new virulent races (Singh *et al*., 2015). To achieve durable resistance, pyramiding of multiple disease resistance genes into a single genotype is commonly attempted (Pietrusinska *et al.,* 2011). Keeping this in view, an effort was made to combine the major stem rust resistance genes *Sr36/Pm6* in the background of HD 2833, which carries minor and major stem rust resistance genes, *Sr2/Lr27/Yr30* and *Sr24/Lr24* respectively using marker assisted selection.

After initial crossing with the donor parent (Cook), $F_1$s were backcrossed with HD2833 ($BC_1F_1$, $BC_2F_1$, $BC_3F_1$ and $BC_4F_1$) for four times to recover the recurrent parent background (>95%) followed by selfing. Up to $BC_4F_5$ generation, high yielding, disease resistant lines were selected based on field phenotyping and forwarded to next generation. Five populations with relatively high yielding and disease resistant were selected. In $BC_4F_6$ generation, these five populations were subjected to molecular marker analyses for the confirmation of presence/absence of rust and powdery mildew resistance genes in addition to field phenotyping which showed resistance reaction against these diseases (**Table 2**). In the phenotypic scoring of $BC_4F_6$ generation, recurrent parent HD2833 had responded as moderately susceptible with severity of 20 for stem rust, whereas score for powdery mildew showed the susceptible reaction of 6. Population 1, 2 and 3 displayed resistant reaction of 10MR, 5MR and 5R respectively for stem rust and powdery mildew (5), while population 4 and 5 displayed immune response to both stem rust and powdery mildew. Susceptible check provided highly susceptible response to rusts and powdery mildew. Ten plants from each population were subjected to molecular marker analyses.

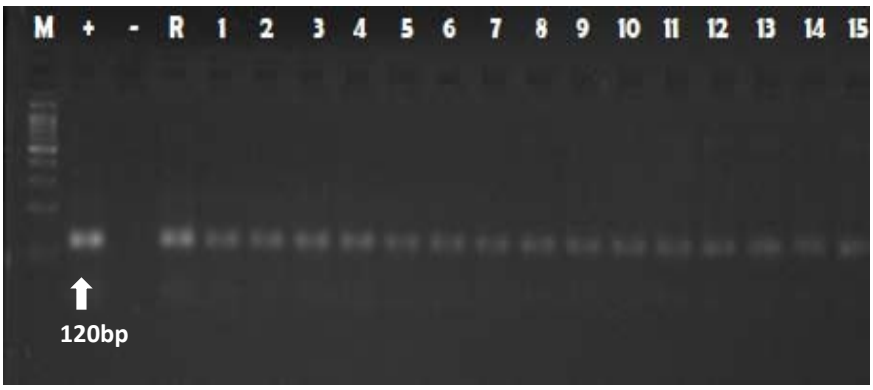
Of the five populations subjected to molecular marker analyses, all the populations carried stem rust resistance gene, *Sr2*+ (**Fig. 1**.), while *Sr24/Lr24* (**Fig. 2)** and *Sr36/Pm6* (**Fig. 3.**), were present only in fourth and fifth population. Adult plant reaction of population 4 and 5 confirmed the immune response to both rust and powdery mildew as observed in $BC_4F_6$ generation (**Table 2**). Thus,

**Table 2. Adult plant response of recurrent parent and introgressed lines to stem rust, leaf rust and powdery mildew diseases**

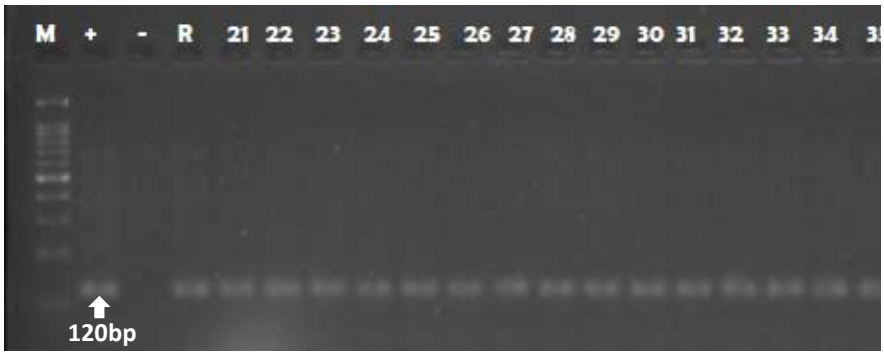
| Sl. No. | Genotype | Stem rust | | Leaf rust | | Powdery mildew | |
|---|---|---|---|---|---|---|---|
| | | $BC_4F_6$ | $BC_4F_7$ | $BC_4F_6$ | $BC_4F_7$ | $BC_4F_6$ | $BC_4F_7$ |
| 1 | Recurrent Parent | 20MS* | | 0 | | 6 | |
| 2 | Susceptible check | 60S | | 80S | | 8 | |
| 3 | Population 1 | 10MR | - | 5MR | - | 5 | - |
| 4 | Population 2 | 5MR | - | 10MR | - | 5 | - |
| 5 | Population 3 | 5R | - | 0 | - | 5 | - |
| 6 | Population 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | Population 5 | 0 | 0 | 0 | 0 | 0 | 0 |

*R:Resistance; MR: Moderately Resistant; MS: Moderately Susceptible; S:Susceptible; 0: Immune

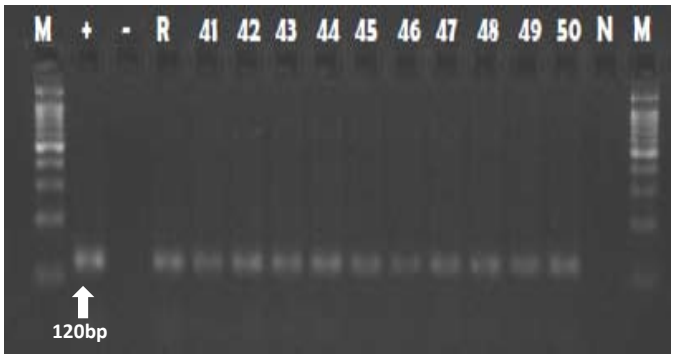
three genes were present in two populations and rest had only one gene. However, the field reaction showed resistance reaction in all the five populations irrespective of number of genes. Resistance in population with one stem rust resistance gene (*Sr2*+) may be due to the synergistic effect of *Sr2*+ with unknown resistance gene in the background, as *Sr2*+ alone cannot provide complete resistance. *Sr2/Lr27/Yr30* gene complex is an adult plant resistance gene which is tightly linked to a morphological marker, pseudo-black chaff (PBC) which involves melanin pigmentation of the glumes and stem, particularly below the uppermost node (Singh and McIntosh, 1984). This morphological marker aids in the identification of the lines carrying *Sr2* with visual observation. PBC was observed in all the five populations.




**(a)Population 1 and 2**

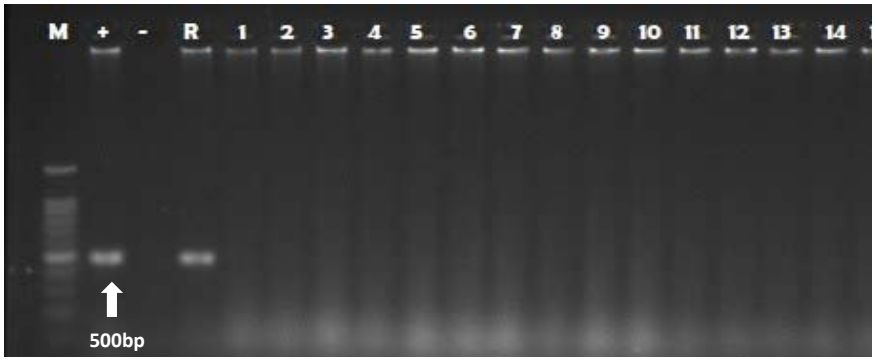



**(b)Population 3 and 4**

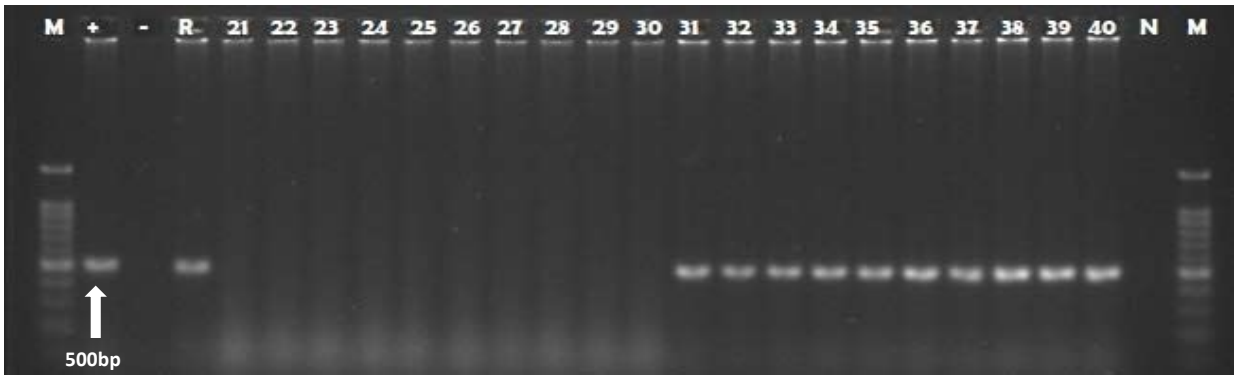



**(c)Population 5**

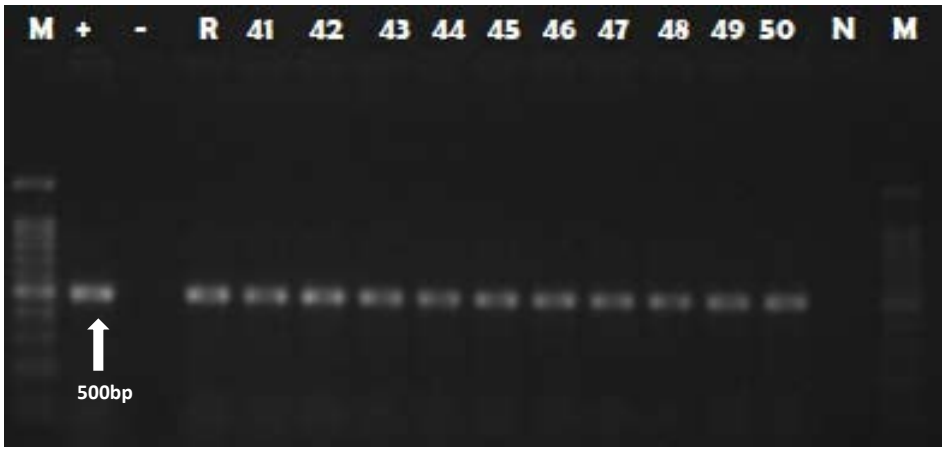
**Fig 1:Foreground selection for stem rust resistance gene, *Sr2/Lr27/Yr30* in$BC_4F_6$ generation(a: Population 1 and 2; b:Population 3 and 4; c:Population 5) (Lane: M: Marker; +: Positive control; -: Negative control; R: Recurrent parent; 1 to 50: Introgressed line; N: Distilled water)**




**(a)Population 1 and 2**

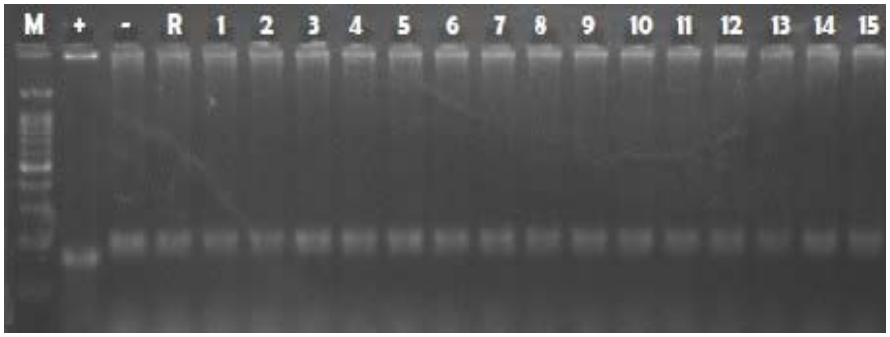



**(b)Population 3 and 4**

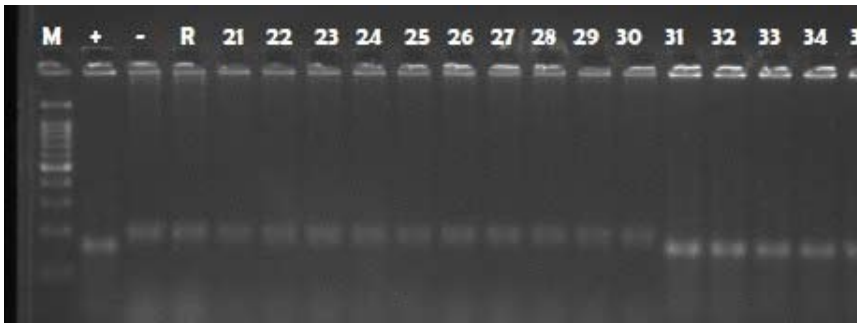



**(c)Population 5**

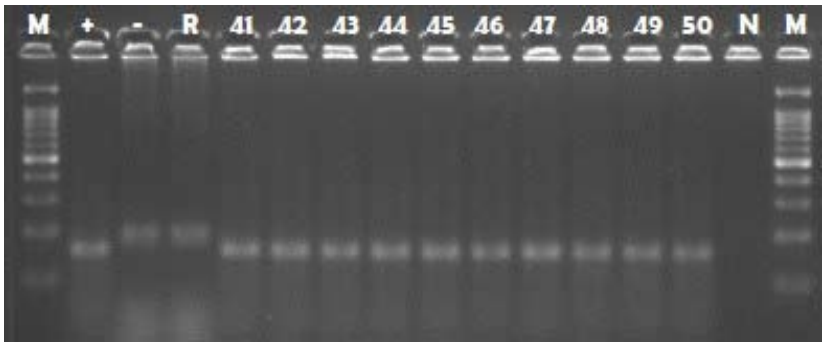
**Fig 2:Foreground selection for stem and leaf rust resistance gene, *Sr24/Lr24* in$BC_4F_6$ generation (a: Population 1 and 2; b:Population 3 and 4; c:Population 5) (Lane: M: Marker; +: Positive control; -: Negative control; R: Recurrent parent; 1 to 50: Introgressed line; N: Distilled water)**




**(a)Population 1 and 2**

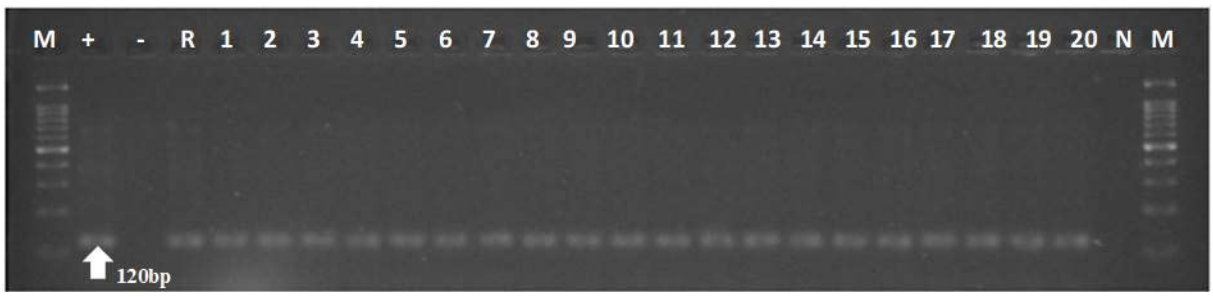



**(b)Population 3 and 4**

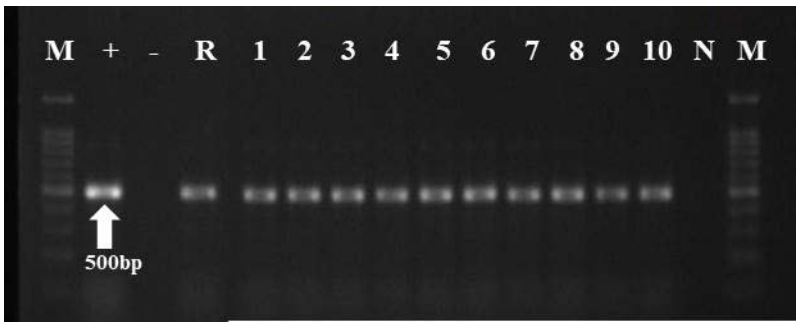



**(c)Population 5**

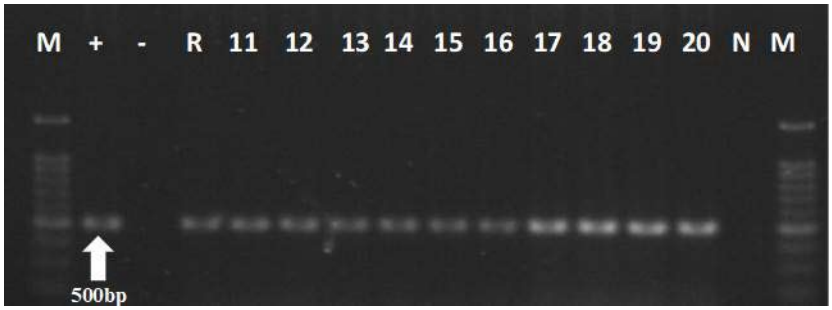
**Fig 3:Foreground selection for stemand powdery mildew resistance gene, *Sr36/Pm6* in$BC_4F_6$ generation (a: Population 1 and 2; b:Population 3 and 4; c:Population 5) (Lane: M: Marker; +: Positive control; -: Negative control; R: Recurrent parent; 1 to 50: Introgressed line; N: Distilled water)**



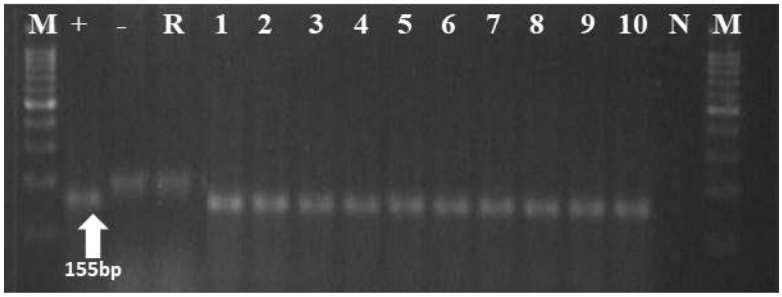

(a) Sr2/Lr27/Yr30



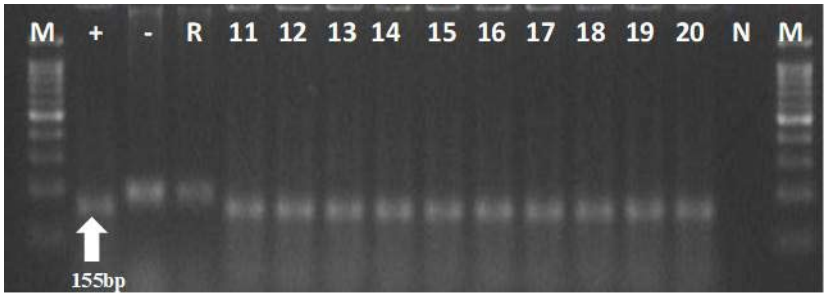

(b) Sr24/Lr24




(c) Sr24/Lr24




(d) Sr36/Pm6




(e)Sr36/Pm6

Fig 4:Fig 3:Foreground selection for stem, leaf rust and powdery mildew resistance geneinBC4F7generation (a:Sr2/Lr27/Yr30; b:Sr24/Lr24; c:Sr24/Lr24; d: Sr36/Pm6; e:Sr36/Pm6)(Lane: M: Marker; +: Positive control; -: Negative control; R: Recurrent parent; 1 to 20: Introgressed line; N: Distilled water)

Two populations carrying all the three rust resistance genes were forwarded to $BC_4F_7$ generation to stabilise the population. These were again subjected to molecular marker analyses confirming the presence of rust resistance genes, *Sr2*+, while *Sr24/Lr24* and *Sr36/Pm6* (**Fig. 4)**. Pyramided population were grown for two more seasons to study the yield attributing traits with respect to the recurrent parent. Two populations showed higher yield than the recurrent parent (data not shown). Yield reduction due to linkage drag is a common phenomenon while transferring one or more resistance genes in wheat. Growing in large population of introgressed lines and precise selection of genotypes could overcome such problem which was reflected in our study.

Interaction of two or more than two genes in a single genetic background may or may not work. However, stem rust resistance gene, *Sr36/Pm6* combined well with the *Sr2*+ and *Sr24/Lr24* in the background and didn't have a negative effect on yield and resistance mechanism. Close association between *Sr36* and *Pm6* rendered HD 2833 introgressed lines to be effectively resistant to powdery mildew also. In earlier studies, it was observed that the association of *Sr36* and *Pm6* is not very strong and therefore, they poorly recombine in some of the genotypes. However, during the present investigation, selections made in $BC_4F_6$ and $BC_4F_7$ generations resulted in successful recombination of stem rust and powdery mildew resistance. Marker assisted pyramiding of two or more disease resistance genes were reported for *Yr70/Lr76* & *Lr37/Yr17/Sr38* (Gautam *et al*. 2020), *QYr.nafu-2BL* & *QYr.nafu-3BS* (Hu et al. 2020), *Sr25, SrWeb* & *Sr50* (Yadav *et al.* 2015), *Lr19, Sr26* & *Yr10* (Mallick *et al.,* 2015), *Lr19* & *Lr24* (Singh *et al*., 2017), *Lr19* & *Lr28* (Bhawar *et al*., 2011) Marker-Assisted Selection (MAS) improves the efficiency of selection because the environment does not affect the expression of molecular markers. These markers can also be detected at all stages of plant growth, whereas phenotypic markers/expression often can only be identified at specific growth stage.

Marker Assisted Selection (MAS) provide the opportunity to select desirable lines on the basis of genotype in case of combining different genes in a single genotype. Combination of minor (*Sr2/Lr27/Yr30*) and major rust (*Sr24/Lr24* and *Sr36/Pm6*) resistance genes in the same background confer durable resistance than single gene deployment. Results of our study also showed the usefulness of the molecular markers for the precise identification of rust and powdery mildew resistance genes, *Sr2/Lr27/Yr30, Lr24/Sr24* and *Sr36/Pm6* which can be used in the wheat improvement programmes.

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