Indian J. Genet., 78(1): 19-25 (2018)
DOI: 10.5958/0975-6906.2018.00003.2

Enhancing rust resistance in wheat through marker assisted backcross breeding

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(Received: January 2017; Revised: November 2017; Accepted: December 2017)

Abstract

Rusts are among the most devastating fungal diseases of wheat worldwide causing major yield losses. The most effective way to control yield losses caused by rusts is by incorporating genetic resistance in wheat cultivars. In view of this, marker assisted backcrossing (MABC) was performed to transfer three highly effective genes, two for leaf rust resistance (*Lr19/Sr25* and *Lr24/Sr24*) and one for stripe rust resistance (*Yr15*) into a wheat cultivar UP 2338. The Avocet/ *Yr15* (*Yr15*) was used as donor for stripe rust and FLW 8 and FLW 21 were the donors for *Lr19/Sr25* and *Lr24/Sr24,* respectively. Two linked SSR markers, *Xgwm273* for *Yr15* and *Xwmc221* for *Lr19/Sr25* and a SCAR marker, *SCS1302* for *Lr24/Sr24* were used for foreground selection to select plants carrying respective gene(s). Foreground selection coupled with background selection identified plants homozygous for stripe rust and leaf rust resistance and materials have been advanced up to BC_3F_3 stage. Findings of this investigation show the usefulness of marker assisted selection in precise introgression of the genes for stripe rust and leaf rust resistance.

Key words: Wheat, rust, marker assisted backcrossing, foreground selection, background selection

Introduction

Wheat is one of the most important staple food crops of the world, occupying 17% (one sixth) of crop acreage worldwide, feeding about 40% (nearly half) of the world population and providing 20% (one fifth) of total food calories and protein in human nutrition (Gupta et al. 2008). India contributes about 16% to the total world wheat production. It is one of the major producers of wheat and occupies second place after China in terms of area and production among wheat growing countries of the world. Wheat is grown in India over an area of about 29.65 m ha with a production of 93.51 m t and productivity of 3153.8 kg/ha (Anon. 2015).

In recent years, wheat has achieved relatively higher production stability as compared to other cereal crops by adopting strategic gene deployment. Only marginal increase in wheat area is recorded but the strategic deployment of rust resistance genes has been crucial in sustaining the production levels (Tomar et al. 2014). However, recent break down of *Yr9* resistance in mega wheat variety PBW 343 and resurgence of five new pathotypes of *Puccinia striiformis* f. sp. *tritici*, namely, 110S119, 238S119, 110S84, 110S247 and 46S117 demands serious attention towards preparedness to fight against potential threat of this devastating pathogen.

Stripe rust, commonly referred as yellow rust, is caused by *Puccinia striiformis* West. f. sp. *tritici* Eriks. E. Henn. This disease is highly destructive and can cause up to 100% crop loss, though commonly in the range of 10 to 70% (Chen, 2005). Likewise, leaf rust, also known as brown rust, is caused by *Puccinia triticina* Eriks. The yield losses are mainly caused due to grain shrivelling and decreased floret set (Huerta-Espino et al. 2011). This is the most common rust in North Western Plains Zone (NWPZ), North Eastern Plains Zone (NEPZ) and Central Zone (CZ) of India.

The most effective, economic and ecofriendly strategy of controlling disease severity caused by rusts, is by deploying genetic resistance. This reduces the need for fungicide usage and thus offers an environment friendly alternative.

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
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Conventionally, breeding plants with resistance against a specific disease requires the identification of resistant parents, which are then crossed with agronomically acceptable but susceptible parents. A program of backcrossing to the susceptible parent and selection of resistant phenotypes leads to the production of plants that are similar to the susceptible parent but have the required resistance. Typically, this process takes 10 or more years, and by this time, the pathogen has evolved a variant that is not recognized by the improved cultivar, leading to susceptibility. To curtail the release time, the use of molecular markers to improve the efficiency and precision of conventional plant breeding *via* marker-assisted selection (MAS) is an important strategy. In majority of the MAS programmes, marker assisted back crossing (MABC) has been deployed which includes marker assisted foreground selection along with phenotypic selection to identify plants possessing desirable genes in segregating populations and background selection, utilizing the polymorphic markers to ensure the sufficient recurrent parent genome (RPG) recovery. The present study was carried out to improve stripe rust and leaf rust resistance of wheat variety UP 2338, a popular cultivar in NWPZ, through MABC along with stringent phenotypic selection.

Materials and methods

Plant material and molecular markers used

A widely adapted wheat variety UP 2338 (UP368/VL421//UP262) was used as recipient parent during the present study. Wheat genotypes, Avocet/*Yr15* (*Yr15*/6*Avocet S) for stripe rust resistance having *Yr15* gene and for leaf rust resistance, FLW 8 (HI1077/*Lr19*/*Sr25*) having *Lr19/Sr25* and FLW 21 (UP 2338/Centurk//UP 2338/*Yr15*) having *Lr24/Sr24* were taken as donors.

The linked markers, *Xgwm273* for *Yr15* (Peng et al. 2000), *Xwmc221* for *Lr19/Sr25* (Gupta et al. 2006b) and *SCS1302* for *Lr24/Sr24* (Gupta et al. 2006a) were used for foreground selection to select plants carrying respective gene(s). A total of 400 SSR (simple sequence repeats) markers covering all the 21 chromosomes of wheat were selected to detect polymorphism between recipient and donor parents. Markers showing parental polymorphisms were used for background selection to analyze the recurrent parent genome (RPG) recovery. The primer sequences were obtained from Grain-Genes 2.0: A database for *Triticeae* and *Avena*.

Marker assisted backcross breeding

DNA isolation and PCR analysis : DNA from one-month-old plants was isolated using a modified CTAB method (Doyle and Doyle, 1990) from parents and backcross progenies. The PCR amplification was carried out in a reaction mixture of 12µl containing 6.4µL ddH_2O, 1.5µL 10X PCR buffer containing 15mM $Mgcl_2$, 0.4µL of 10 mM dNTPs, 0.75µL of each forward and reverse primer, 0.2µL of 5U/µL Taq DNA polymerase (All reagents obtained from Genei), and 2µL of genomic DNA. PCR cycle consisted of an initial denaturation for 5 min at 94^oC, followed by 35 cycles each with 1 min at 94^oC, 1 min at 60ÚC annealing temperature (differs for different primers), 1 min at 72^oC with a final extension of 8 min at 72^oC. The amplified products were resolved on 2.5% agarose gel for the foreground as well as for the background selection.

Breeding scheme

Marker assisted backcrossing (MABC) was followed to transfer two leaf rust resistance genes, *Lr19/Sr25* and *Lr24/Sr24* and a stripe rust resistance gene *Yr15* into the genetic background of UP 2338. Recurrent parent UP 2338 was used as female and crossed with different donors as male to generate F_1 seeds in three separate breeding programmes. It was followed by three backcrosses (with UP 2338) events to effectively recover the genome of recurrent genotype with simultaneous foreground and background selection. The foreground selection was done up to BC_3F_3. Three cycles of self-fertilization was performed for homozygosity of the alleles received from the donor and simultaneous background selection was done. Detailed flowchart of MABC approach to transfer the above described genes is given in Fig. 1.

Phenotypic evaluation of backcross progenies for stripe and leaf rusts

Visual/phenotypic selection for stripe rust and leaf rust resistance was carried out before each round of backcrossing to select the resistant and moderately resistant plants for both the rusts in each backcross generation. Epiphytotic condition was created by inoculating the research materials at boot stage with the inoculum of important races (prevalent races in NWPZ) of stripe rust (78S84 and 46S119) and leaf rust (21R55 and 121R63-1). Besides this, infector rows were planted in the surrounding of segregating materials to create sufficient disease pressure. The observation was recorded after the first emergence of symptoms of the disease. The leaf and stripe rusts

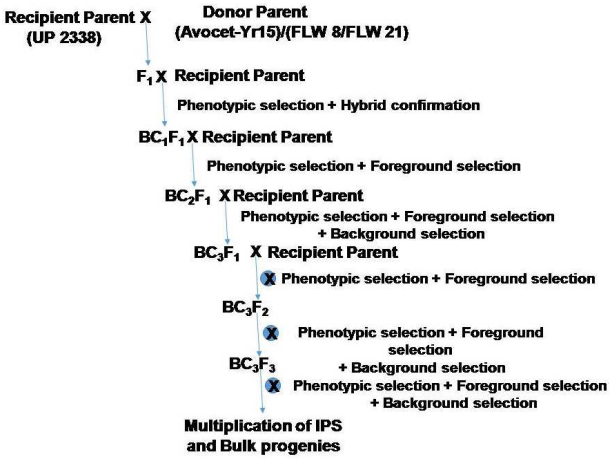

Fig. 1. Flow diagram of the marker assisted breeding scheme for introgression of stripe rust/leaf rust resistance genes (*Yr15/Lr19/Sr25/Lr24/Sr24*)

severity was recorded as per cent of infection from the individual plant according to modified Cobb's scale by Peterson et al. (1948).

Evaluation of advanced lines for agronomic performance

Field trials to evaluate advanced lines were conducted during *rabi* 2014-15 at Norman E. Borlaug Crop Research Centre (NEBCRC), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The selected individual plants (IPS) and bulks of BC_3F_3 generation for each introgressed genes were sown with recurrent parent as check to evaluate these lines for agronomic performance. Data on phenotypic traits was recorded on five selected plants for each rust resistant line both under field as well as at molecular level. Agronomic traits recorded were plant height (cm), spike length (cm), number of spikelets per spike, length of flag leaf (cm), width of flag leaf (cm) and 1000-grain weight (TGW in gram).

Estimation of the recovery of the recipient genome (RPG)

The recovery of RPG which is an important aspect in MAS was estimated in the derived progenies. It is measured by manual scoring of the gels for foreground positive samples amplified by the polymorphic markers, detected through parental polymorphism survey, using the following formula:

$$RPG = \frac{X+1/2Y}{N} \times 0$$

where X is designated as the number of markers showing homozygosity for recurrent parent allele; Y as the number of markers showing heterozygous state for the parental alleles and N as the total number of parental polymorphic markers screened.

Results

Screening of backcross population for foreground selection of Yr15 gene

It was observed that donor parent for *Yr15* produces band of 165 bp (presence of *Yr15*). In BC_1F_1 population, out of 182 plants, 42 showed the presence of 165 bp band indicating the presence of gene *Yr15* (Fig. 2).

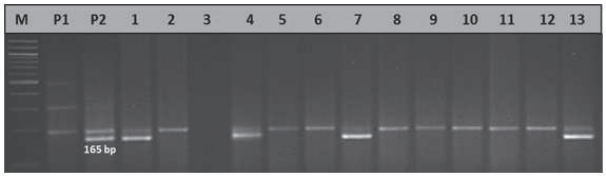

Fig. 2: Representative gel image of foreground selection for *Yr15* in BC_1F_1 generation: M- 100 bp step up ladder, P_1- UP 2338, P_2- Avocet-*Yr15*, 1-13: progeny of BC_1F_1 generation, genotype no. 1, 4, 7 and 13 shows the presence of donor allele of 165 bp

Plants positive for *Yr15* showed stripe rust resistance in the field also and were used to develop the BC_2F_1 population by crossing with the recurrent parent UP 2338. In BC_2F_1 population, 42 plants and in BC_3F_1, 40 plants were found to be foreground positive. Similarly, in BC_3F_2 population, 36 plants out of 72 were found to be foreground positive with the presence of 165 bp bands. Due to high level of stripe rust infection in the BC_3F_3 progenies, 56 single plants were selected and designated as individual plant (IPS).

Evaluation of selected IPS (Yr15) for agronomic performance

On the basis of resistance to stripe rust at phenotypic as well as at molecular level, eleven lines were selected from 56 IPS. These selected lines showed variable expression in respect of agronomic traits as well as for disease severity. All the traits *viz*., plant height, spike length, number of spikelets per spike, length of flag leaf and width of flag leaf were comparable with recurrent parent UP 2338, while disease severity was comparable with the donor Avocet-*Yr15*. Data on thousand grain weight of ten lines, out of eleven selected lines produced better TGW than that of recurrent parent under high disease severity. The date

on agronomic traits are presented in Table 1. Data on disease severity and response of the ten selected lines is given in Table 2.

Table 1. Agronomic data of selected improved lines in the background of UP 2338

Cross combination	Agronomic traits					
	Plant height (cm)	Spike length	No. of spikelets/ spike	Length of flag leaf	Width of flag leaf	TGW
UP 2338 x Avocet-*Yr15*						
1	91.20	20.2	12.0	27.60	2.00	35.07
2	90.35	20.2	11.2	29.50	1.83	36.00
3	89.50	19.4	11.5	27.10	1.93	34.50
4	92.00	19.6	11.3	28.67	1.83	31.47
5	90.50	20.0	11.2	27.37	2.10	33.40
6	90.00	20.2	11.4	27.93	1.83	37.87
7	89.25	20.4	11.5	29.17	1.73	36.90
8	88.80	18.4	11.8	28.10	1.93	33.67
9	90.25	19.0	11.4	27.90	2.53	32.00
10	87.00	18.6	10.5	28.67	1.77	41.82
UP 2338 x FLW 8						
1	91.50	19.8	10.8	28.43	2.23	42.64
2	90.25	18.6	11.4	26.67	2.37	39.40
3	89.50	19.4	11.6	27.90	2.33	39.57
4	92.20	19.6	12.6	29.00	2.23	32.30
5	88.60	18.8	11.2	28.67	1.97	34.82
UP 2338 x FLW 21						
1	89.75	19.2	11.5	29.30	2.13	33.80
2	90.00	19.4	11.2	26.30	1.90	32.62
3	91.25	19.0	10.8	28.50	1.78	34.75
4	89.50	18.6	12.0	29.67	1.87	35.22
5	88.90	18.8	10.9	28.67	1.73	34.20
6	91.60	19.2	12.1	29.00	1.77	34.42
UP 2338	90.00	19.4	11.4	27.33	1.93	26.80

Table 2. Disease severity and disease response of selected improved lines in the background of UP 2338

Cross combination	Disease severity (%)	Disease response
UP 2338 x Avocet-*Yr15*		
1	Trace	Resistant (tR)
2	Trace	Resistant (tR)
3	Trace	Resistant (tR)
4	10	Moderately resistant (10MR)
5	5	Resistant (5R)
6	Trace	Resistant (tR)
7	Trace	Resistant (tR)
8	5	Resistant (5R)
9	5	Moderately resistant (5MR)
10	Trace	Resistant (tR)
UP 2338 x FLW 8		
1	Trace	Resistant (tR)
2	Trace	Resistant (tR)
3	Trace	Resistant (tR)
4	5	Moderately resistant (5MR)
5	5	Resistant (5R)
UP 2338 x FLW 21		
1	5	Resistant (5R)
2	5	Moderately resistant (5MR)
3	Trace	Resistant (tR)
4	Trace	Resistant (tR)
5	Trace	Resistant (tR)
6	Trace	Resistant (tR)
UP 2338 (Recipient)	80	Susceptible (S)
Avocet-Yr15 (Donor)	0	No sporulation
FLW 8 (Donor)	0	No sporulation
FLW 21 (Donor)	0	No sporulation

Background selection

Background selection was performed using 47 polymorphic SSR markers distributed over all the 21 chromosomes. The recovery of the genome of recipient parent was evaluated in all foreground positive plants available in backcrossed generation, namely, BC_2F_1, BC_3F_2 and BC_3F_3 except for BC_3F_1 generation (Fig. 3). Background selections for recipient parent UP 2338 provided the RPG recovery with the average of 94.00% in a range of 86.60% to 95.35%.

MABC for Lr19/Sr25 and Lr24/Sr24 genes

The amplification using the marker *Xwmc221* had shown the desired allele of 200 bp (presence of *Lr19/Sr25*) with the donor genotype, whereas a band of 220

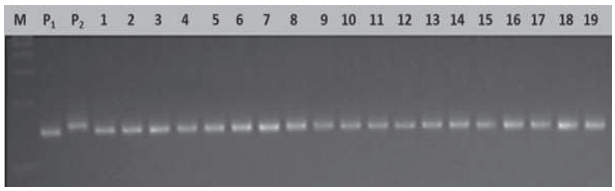

Fig. 3. Background selection for UP 2338 in BC_3F_3 generation: M- 100 bp step up ladder, P_1 = UP 2338, P_2 = Avocet-*Yr15*, Lanes 1-19=Genotypes show the presence (band) of allele corresponding to recurrent parent

bp (absence of *Lr19/Sr25*) in recipient parent. In case of marker *SCS1302*, a band of 609 bp (presence of *Lr24/Sr24*) was obtained in the donor genotype whereas in recipient parent no band was present.

Screening of backcross population for foreground selection of Lr19/Sr25 and Lr24/Sr24 genes

In BC_1F_1 population, out of 162 plants, 37 showed the presence of gene *Lr19/Sr25*. While, 29 out of 133 plants were found to be foreground positive for *Lr24/Sr24* gene. Plants positive for *Lr19/Sr25* and *Lr24/Sr24* were used to develop the BC_2F_1 population by crossing with the recurrent parent UP 2338. In BC_2F_1 population, out of 148 plants, 32 showed the presence of desirable band linked to *Lr19/Sr25,* and 21, out of 119 plants showed the presence of desirable band for *Lr24/Sr24*. Similarly, in BC_3F_1 population, out of 124 plants, 37 were found to be foreground positive for *Lr19/Sr25* gene, whereas in case of *Lr24/Sr24*, out of 115 plants, 35 were foreground positive. In BC_3F_2 population, 42 plants out of 75 were found to be foreground positive for *Lr19/Sr25* gene and 24 out of 54 showed the presence of desirable band for *Lr24/Sr24* gene (Figs. 4 and 5). The progenies were also found carrying *Yr9* and *Yr15* genes from the donor FLW 21, which was confirmed with the gene linked markers *Xgwm582* and Xgwm273, respectively (Figs. 6 and 7). Considering

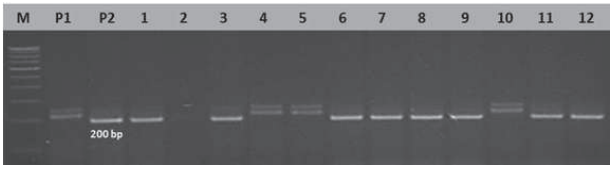

Fig. 4. Foreground selection for *Lr19/Sr25* in BC_3F_3 generation: M- 100 bp step up ladder, P_1=UP 2338, P_2=FLW 8, Lanes 1-12=progeny 1, 3, 6, 7. 8, 9, 11 and 12 shows the presence of donor allele of 200 bp

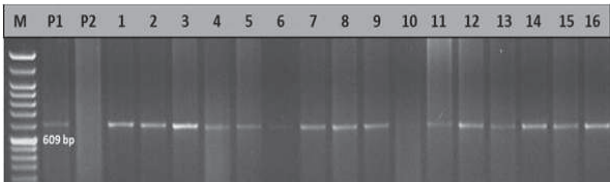

Fig. 5. Foreground selection in BC_1F_1 generation: M-100 bp step up ladder, P_1=FLW 21, P_2=UP 2338, Lanes 1-16=Genotype show the presence (band) of donor allele of 609 bp

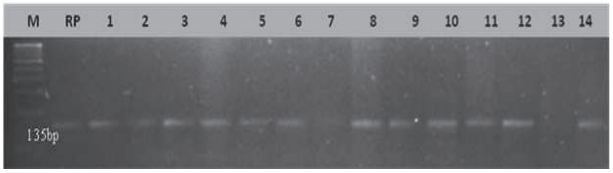

Fig. 6. Foreground selection of *Yr9* in BC_3F_3 generation: M-100bp step up ladder, RP=UP 2338, Lanes 1-14=progenies except lane 13 show the presence of *Yr9* with marker *Xgwm512* (allele size 135bp)

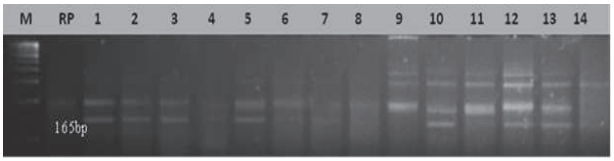

Fig. 7. Foreground selection of *Yr15* in BC_3F_3 generation: M=100bp step up ladder, RP=UP 2338, Lanes 1-14=The progenies 1,2, 3,5, 10, 12 and 13 have shown the presence of *Yr15* with marker *Xgwm273* (allele size 165bp)

the level of leaf rust infection and phenotypic uniformity for plant type, spike colour, shape and size in the BC_3F_3 progenies, some of them were selected as bulk (*i.e*. whole plot was harvested) and some were selected as IPS in both *Lr19/Sr25* (10 IPS and 8 bulks) as well as in *Lr24/Sr24* (10 IPS and 5 bulks).

Evaluation of selected IPS and bulks (Lr19/Sr25 and Lr24/Sr24) for agronomic performance

On the basis of resistance to leaf rust at phenotypic level as well as at molecular level, five lines from 10 IPS and 8 bulks of *Lr19/Sr25* gene and seven lines from 10 IPS and 5 bulks containing *Lr24/Sr24* gene were selected. These selected lines showed variable expression for agronomic traits disease severity. The traits, plant height, spike length, number of spikelets per spike, length of flag leaf and width of flag leaf were comparable with recurrent parent UP 2338 and so was the disease severity was comparable with the

respective donor parents FLW 8 (*Lr19/Sr25*) and FLW 21 (*Lr24/Sr24*) (Tables 1 and 2). Data on thousand grain weight of all the five lines carrying *Lr19/Sr25* gene and six, out of seven selected lines possesing *Lr24/Sr24* gene produced better TGW than that of recurrent parent under high disease pressure.

Background selection

Background selection was performed using 57 polymorphic SSR markers in progenies of *Lr19/Sr25* gene and 48 SSR markers for progenies of *Lr24/Sr24* gene which were distributed across the 21 chromosomes. The recovery of the genome of recipient parent was evaluated in all foreground positive plants available in backcrossed generation namely, BC_2F_1, BC_3F_2 and BC_3F_3 except for BC_3F_1 generation. Background selections for recipient parent UP 2338 provided the RPG recovery with the average of 94.30% in a range of 85.25% to 96.45% for *Lr19/Sr25* and for *Lr24/Sr24* the RPG recovery was found with an average of 94.65% ranging from 84.25 % to 96.45%.

Discussion

Rusts are the most devastating fungal diseases in wheat worldwide, which can primarily be managed through resistance breeding as chemical control is costly and not eco-friendly. However, cyclic breakdown of the resistance genes due to evolution of new pathogenic races demands for constant identification of new genes for resistance. Pyramiding of two or more genes can help to improve the durability of rust resistance but it is possible only if closely linked DNA markers are available (Vinod et al. 2010). With the advent of DNA markers it has now become possible to precisely transfer the desirable genes from unadapted germplasm to elite lines with minimum or no linkage drag (Young and Tanksley 1989).

In the present study, we successfully introgressed three rust resistance genes *viz*., *Yr15*, *Lr19/Sr25* and *Lr24/Sr24* into a popular wheat variety UP 2338 through marker assisted backcross breeding. Crosses were attempted in the lines of BC_2F_2 generations for the pyramiding of stripe rust (*Yr15*) and leaf rust (*Lr19* and *Lr24*) resistance genes in the background of the recurrent parent UP 2338. The cross seeds (F_1s) were raised and positive plants for both the genes were selected, harvested and forwarded for further evaluation. Pyramided genotypes carrying *Lr19/Sr25* and *Yr15* are shown in Figs. 8 and 9. Similarly, introgression of stem rust resistance gene (*Sr36*) into durum wheat was done by Prasad et al. (2014). MAS

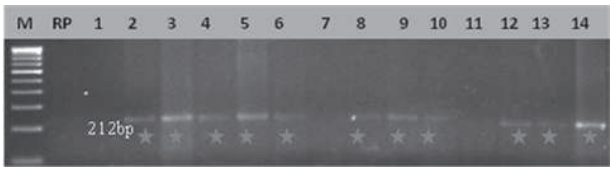

Fig. 8. Gel image showing the presence of *Yr15* in pyramided lines : M- 100bp step up ladder, RP-UP 2338, Lanes 1-14=Presence of gene in different genotypes have been marked with *. Product size for Yr15 gene with marker *Xgwm11* is 212bp

Fig. 9. Gel image showing the presence of *Lr19/Sr25* in pyramided lines: M=100bp step up ladder, RP= UP 2338, Lanes 1-14=Presence of gene in lines is been marked with *. Product size for *Lr19/Sr25* gene with marker *Xwmc221* is 230bp

also has been successfully implemented to introgress the gene through marker assisted backcrossing (MAB) and pyramid major genes/QTL through marker assisted pyramiding (MAP) for different traits in wheat (Vinod et al. 2006; Sivasamy et al. 2009; Revathi et al. 2010; Chhuneja et al. 2011 and Kumar et al. 2011). Agronomic performance of selected introgressed plants was comparable with recipient parent which shows that phenotypic selection along with markers assisted background selection led to maximum genome recovery of recipient parent in the present study. Background analysis exercised through phenotypic evaluation is reported to be useful in efficient recovery of the RPG (Singh et al. 2013).

Authors' contribution

Conceptualization of research (JPJ, SB, AS); Designing of the experiments (JPJ, SB, AS); Contribution of experimental materials (JPJ); Execution of field/lab experiments and data collection (AS, SB, JPJ); Analysis of data and interpretation (AS, SB, JPJ); Preparation of manuscript (AS, JPJ, SB).

Declaration

The authors declare no conflict of interest.

Acknowledgments

Authors are thankful to Department of Biotechnology, GOI, New Delhi for financial support under Accelerated Crop Improvement Programme (ACIP) (grant No. BT/PR11695) and to Dr. K.V. Prabhu, IARI, New Delhi for technical guidance to perform the study.

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