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Enhanced resistance in wheat against stem rust achieved by marker assisted backcrossing involving three independent *Sr* genes

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ABSTRACT

Marker assisted backcrossing (MABC) was used to transfer the three stem rust (*Puccinia graminis tritici*) resistant genes *Sr25*, *SrWeb* and *Sr50* into the popular Indian wheat cv. HUW234. The donor was the CIMMYT breeding line PMBWIR4, and each of the target genes was marked by a simple PCR assay. A three step screening strategy was adopted: the first screen comprised foreground selection, the second used markers lying close to the introgression borders to reduce the extent of linkage drag, and the last was background selection based on 149 informative microsatellite assays. Based on the background selection outcomes, at the BC_1F_1 generation the proportion of the genome fixed for cv. HUW234 alleles ranged from 55% to 67%. Three BC_1F_1 individuals were crossed a second time with cv. HUW234, resulting in the identification of six BC_2F_3 families carrying all three target *Sr* genes; the proportion of their genome inherited from cv. HUW234 ranged from 86% to 92%. BC_2F_4 and BC_2F_5 material was planted at three different locations under both disease-free and artificially induced epiphytotic conditions. Compared to recurrent parent, the improved versions of cv. HUW234 displayed significantly superior resistance to stem rust, and their agronomic performance was as good as, or even marginally better than that of the source cultivar.

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1. Introduction

Wheat, like all crop plants, is affected by numerous pathogens, amongst which the rust fungi are perhaps the most damaging [1]. All the three (leaf, stripe and stem) rusts frequently occur in India with stem rust (caused by *Puccinia graminis tritici*) being generally more important in Peninsular India [2]. The risk of a stem (black) rust epiphytotic has increased in recent years following the evolution in East Africa of race Ug99 [3,4], which was able to overcome the widely used host resistance gene *Sr31* [5]. Ug99 race was later on designated as race TTKSK [6] using the North American Nomenclature System [7]. Ug99 race also exhibited virulence toward a wide range of resistance genes of *Triticum* origin and also of alien origin. The pathogen evolved rapidly resulting in eight variants within the Ug99 lineage of wheat stem rust. Races virulent against carriers of *Sr24* (second highly utilized stem rust resistance gene after *Sr31* throughout the world) were detected in both Kenya and Ethiopia [6,8]. It caused severe epidemic in 2007 in Kenya [9], and it was predicted that Ug99 race will move to North Africa, Middle East and Asia [3]. Since then the race has been recorded in 12 neighboring countries including South Africa and Zimbabwe (rust-tracker.cimmyt.org; [10,11]).

In the Indian context, the first epidemic of stem rust occurred in 1786 in central India. The other epidemic of stem rust occurred in 1929 when wheat crop was destroyed severely in the state of Madhya Pradesh, India. The predominant pathotypes of stem rust in India are 40A (*62G29*), 40-1 (*62G29-1*) and 117-6 (*37G19*) while the resistance genes commonly used in Indian varieties have been *Sr2*, *Sr5*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9e*, *Sr24* and *Sr31* [9,12]. Both *Sr24* and *Sr31* have succumbed to Ug99. There are few genes (including *Sr2*) that provide good resistance to this race. However, there are several genes such as *Sr26*, *Sr36* and *Sr38* that remained effective against Indian stem rust races. Owing to global concern caused by vulnerability of *Sr31* gene, which happen to be the most commonly used gene in wheat varieties, South Asia was reported to be considered

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highly vulnerable to Ug99 with about 12.5 mha of wheat of India under threat of this race [3].

Nearly 60 stem rust resistant genes have been cataloged to date in wheat [13]. Those which give immunity at the seedling stage tend to be pathogen race-specific, while those which slow the development of the disease at the adult plant stage tend to be non-race-specific. The race-specific gene *Sr25* is effective against Ug99 [14], and is carried, together with *Lr19*, on a segment of a chromosome introgressed from the wild relative *Thinopyrum elongatum*. This resistance has been extensively used by the CIMMYT wheat breeding program. The gene *SrWeb*, first identified in cv. Webster, also confers resistance against the Ug99 race [15]; unlike *Sr25*, which is located on chromosome arm 7DL, this gene is present on chromosome arm 2BL. On its own, it is ineffective against several North American stem rust races [16]. The gene *Sr50*, derived from rye chromosome 1R, is either allelic or identical to *Sr31*. We found that it gives better resistance to stem rust in presence of other stem rust genes such as *Sr2*. Important disease resistance genes have been contributed by 1RS that confer resistance to rusts (*Puccinia spp.*) and powdery mildew. The 'Veery' lines developed by CIMMYT [17], derived from three way crosses between the Mexican spring semi dwarf and winter wheat variety 'Kavkaz' carry 1B/1R translocation from 'Petkus' rye [18,19], also contain 1RS translocations.

Developing genetically resistant cultivars has always been a core activity of crop breeding programs, but in recent years the way in which this can be achieved has been revolutionized by the advent of DNA marker technology [20]. Thus to provide durable resistance, diverse resistance genes to the pathogen have been identified and introgressed into the popular high yielding cultivars [9]. A susceptible cultivar is most easily converted to a resistant form by backcrossing, a process which can be accelerated by the introduction of marker assisted selection. The popularity in the NEPZ of cv. HUW234, along with its susceptibility to stem rust, has provided an ideal opportunity to perform a marker assisted backcrossing program, focusing on the resistance genes *Sr25*, *Sr50* and *SrWeb*. Our objective was to develop a stem rust resistant form of cv. HUW234 by simultaneously introgressing all three of these genes with the aid of markers.

2. Materials and methods

2.1. Plant materials and crossing program

The cultivar HUW234 was the recurrent parent, while the donor was the CIMMYT line PMBWIR4 (PRL/2*PASTOR//PBW343*2/KUKUNA/3/TACUPETO F2001*2/KUKUNA) which harbors *Sr25*, *Sr50* and *SrWeb*. HUW234 shows susceptible reaction to predominant Indian races 40A and 40A-1 [21] while donor parent is resistant to Indian as well as races from Kenya. The scheme of gene transfer using MABC approach is illustrated in Fig. 1. Two backcross generations were raised, the first in 2009 during the off-season at Wellington, Tamil Nadu and the second in the 2009 main season at Varanasi, Uttar Pradesh. The BC_2F_1 and BC_2F_3 generations were grown during the off-season in Wellington during 2010 and 2011, and were subjected to phenotypic selection for resistance against natural inoculum which was multiplied on the susceptible host cv. Agra Local. Selection in the backcross generations was based on foreground selection (the three *Sr* genes), recombinant selection to minimize linkage drag and background selection using SSR markers to recover as much as possible the recurrent parent's genotype. Selected BC_2F_4 families were planted in a randomized complete block design during the main 2011–12 season. At the BC_2F_5 generation, six families were planted at three locations in India (Varanasi, Uttar Pradesh, Indore, Madhya Pradesh and Dharwad, Karnataka), and data were recorded for stem rust severity along with key traits associated with yield.

2.2. Screens for stem rust reaction

Seven to ten day old seedlings were inoculated under green house at Shimla (Himachal Pradesh) using the three predominant Indian stem rust races, *62G29* (40A), *62G29-1* (40A-1) and *58G13-3* (40–2), following the protocol described by McIntosh et al. [22]. The plant response was recorded 10–14 days later, using the Stakman et al. [23] scale. Adult plants were monitored in the field at Varanasi and Indore, where the dominant races were, respectively, 21A-2 and 62G29. Disease severity was recorded using a modified Cobb scale [24] and the host response was classified as either 0 (immune), R (resistant), MR (moderately resistant), M (Intermediate), MS (moderately susceptible) or S (susceptible) [25]. The host response and disease severity scores were combined to obtain a coefficient of infection (CI) following Roelfs et al. [25].

2.3. Foreground and background selection

The foreground selection markers were STSLr19-130 [26] for *Sr25*, *Xgwm47* [15] for *SrWeb* and IB-267 [27] for *Sr50*. In the presence of target *Sr* gene, the three assays generated amplicons, respectively, 130 bp, 207 bp and 200–300 bp. All three markers were dominant, so were unable to distinguish between the heterozygous and the homozygous state. In BC_2F_1, a set of 12 informative SSRs (Table 1), mapping 4–6 cM from the ends of each of the introgression segments, was used as a pre-background screen to reduce the extent of linkage drag around the target loci. To assemble a set of informative SSR assays suitable for the background selection procedure, 880 established primer pairs were tested on the donor and recurrent parents, which allowed for the identification of 149 informative markers (between five and ten per chromosome) (Fig. 2). The total percent polymorphism between HUW234 and PMBWIR4 is given in Table 2. SSR map locations and primer sequences were obtained from the Gramene (www.gramene.org) and Graingenes 2.0 (wheat.pw.usda.gov) databases.

2.4. Genotyping

Genomic DNA was isolated from seedling leaves following Saghai-Maroof et al. [28] and used as template for 15 μL PCRs each containing 5–25 ng genomic DNA, 1.5 μL 10 mM Tris HCl (pH 8.8), 0.2 μL of 1 mM dNTP (MBI Fermentas), 0.2 μL 2.5 mM $MgCl_2$ (MBI Fermentas, Bangalore, India), 0.2 μL 5 U/μL Taq polymerase (MBI Fermentas) and 0.6 μL of 5 μM forward and reverse primers. Primer sequences of all the 3 linked markers with target Sr genes are given in Table 3. The amplicons were electrophoretically separated through 2.5% agarose gels and visualized by EtBr staining. An estimate of the contribution of the recurrent and donor parent to each of the progeny's genetic background was based on the SSR genotypic data, and was generated by GGT 2.0 software [29].

2.5. Agronomic performance of backcross progenies

The recurrent and donor parents, along with the six selected BC_2F_4 families were planted in a randomized complete block design with two replications at Varanasi. The interplant within row distance was 5 cm, and the inter-row distance was 22.5 cm. Data were recorded per entry for the number of days required for 50% of the primary florets on the leading tiller to have reached anthesis, plant height, the number of tillers per plant, the length of the spike on the leading tiller, the number of days to physiological maturity, the 1000 grain weight and the weight of grain set in 50 tillers. BC_2F_5

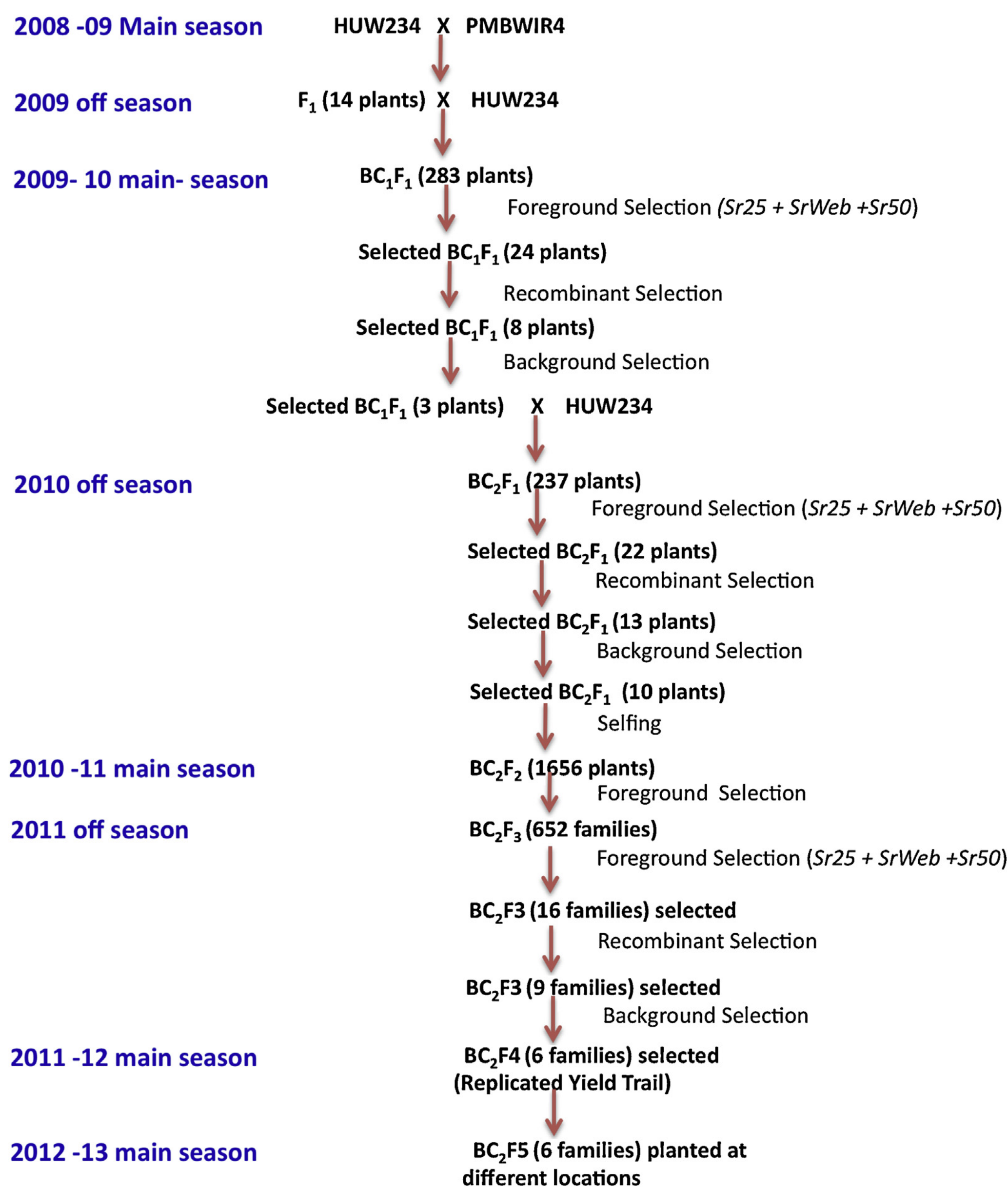

Fig. 1. Marker assisted backcrossing scheme used for the introgression of *Sr25*, *SrWeb* and *Sr50* into cv. HUW234.

Table 1
List of 12 SSR markers used for recombinant selection.

Gene	Marker	Chromosome
Sr25/Lr19	gwm302	7D
	wmc322	7D
	gwm344	7D
	barc340	7D
	gwm146	7D
	wmc232	7D
SrWeb	gwm319	2B
	wmc245	2B
	wmc272	2B
	wmc332	2B
	wmc361	2B
	gwm382	2B

selections were planted in a replicated trial at Varanasi, Indore and Dharwad. Each plot consisted of six 4 m rows, with an inter-row spacing of 22.5 cm. Observations were recorded for plot yield and yield traits.

2.6. Phenotyping for stem rust resistance in BC_2F_4 and BC_2F_5

The BC_2F_4 and BC_2F_5 selections were planted at Varanasi as pairs of 2 m rows with an infector (Agra Local) keeping row to row distance of 22.5 cm. Plants at the maximum tillering stage (GS 29) were sprayed with a suspension of 10^9 spores per mL with races 40A, 40A-1 and 40A-2. At Indore, a similar test was carried out using race 40A. To ensure effective infection, the inoculations were supplemented by an injection of the spore suspension into the plant stem. Disease severity was scored after anthesis.

3. Results

3.1. Foreground selection

The foreground genotyping of the 283 BC_1F_1 segregants identified 24 heterozygous plants for each of the three *Sr* markers. Following the application of the linkage drag screen, this number was reduced to eight. Of the 237 BC_2F_1 individuals generated, 22

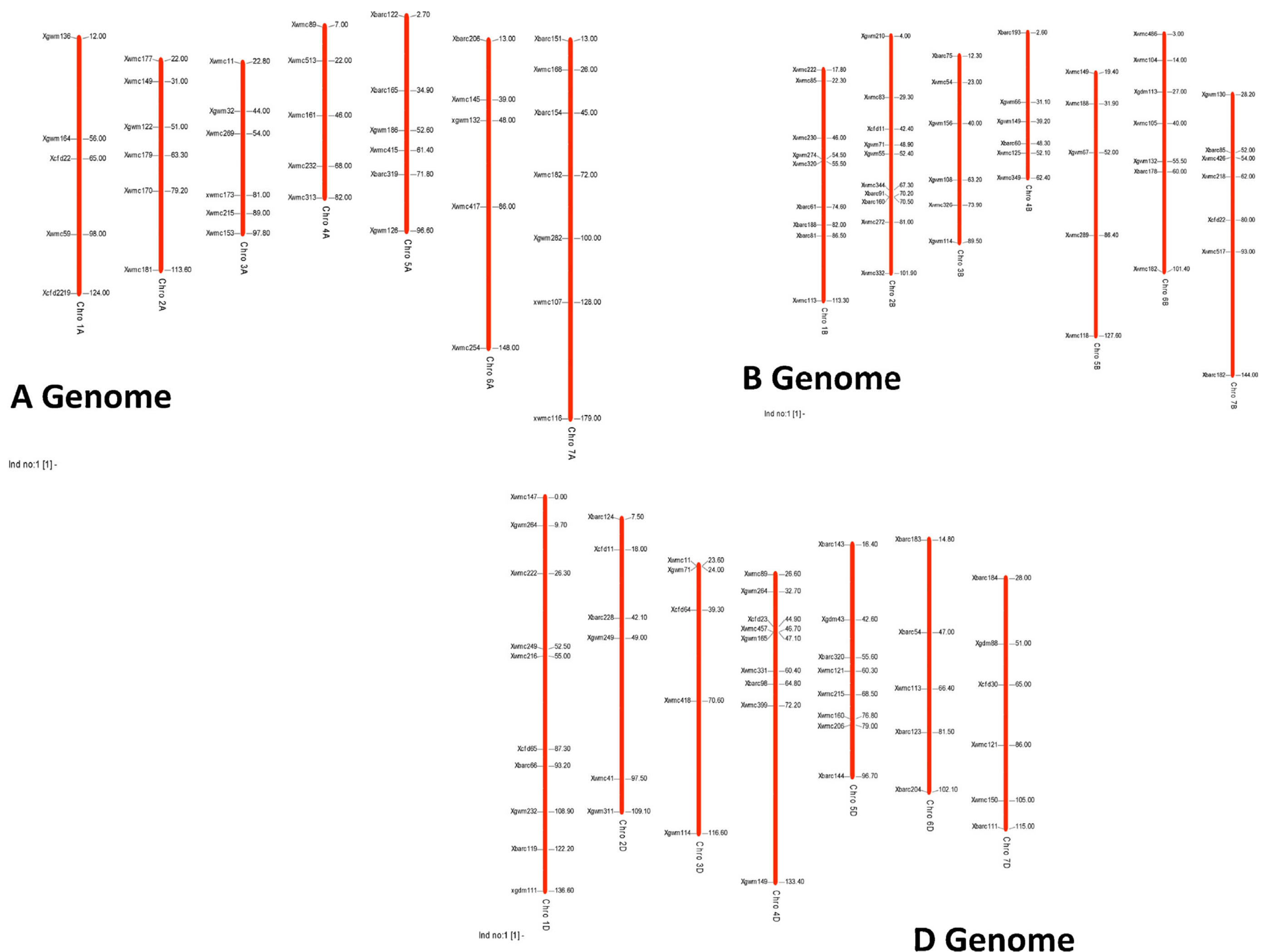

Fig. 2. The map location of informative SSR markers used for background selection.

Table 2
Total percent polymorphism observed in the cross HUW234 × PMBWIR4.

Chromosome	No. of polymorphic markers	No. of marker used	% polymorphic
1A	5	39	12.8
2A	6	42	14.3
3A	6	37	16.2
4A	8	46	17.4
5A	7	43	16.3
6A	8	46	17.4
7A	6	41	14.6
1B	9	43	20.9
2B	10	49	20.4
3B	6	38	15.8
4B	8	45	17.8
5B	5	35	14.3
6B	7	41	17.1
7B	8	45	17.8
1D	10	46	21.7
2D	7	42	16.7
3D	5	37	13.5
4D	9	40	22.5
5D	8	43	18.6
6D	5	38	13.2
7D	6	44	13.6
Total	149	880	16.9

retained all three markers for the *Sr* genes (Figs. 3–5), which was reduced to 13 following the application of the linkage drag screen. Ten BC_2F_1 selections produced 1656 BC_2F_2 progeny, of which 652 carried at least one copy of each of the three markers. In BC_2F_2, foreground selected plants were harvested separately and the seeds of individual plant constituted its family in BC_2F_3. A set of 16 BC_2F_3 families carrying all three markers was selected but was reduced to nine following the application of the linkage drag screen.

3.2. Recombinant selection to reduced linkage drag on carrier chromosome

The improved lines of HUW234 contain the segment of donor parent carrying genes; *Sr25*, *SrWeb* and *Sr50*. The selected lines may also contain certain amount of linkage drag along with the target genes. Therefore section for recombinants carrying the minimum size of donor fragment was performed with the help of 12 SSR markers, six each for *Sr25* and *SrWeb* (Table 1). The whole chromosome was recovered for the gene *Sr50* due to unavailability of SSR markers within 6-10cM distance. SSR markers; *gwm302*, *wmc322*, *gwm344*, b*arc340*, *gwm146* and *wmc232* were used for recombinant selection for the gene *Sr25* while *gwm319*, *wmc245*, *wmc272*, *wmc332*, *wmc361* and *gwm382* for the gene *SrWeb*. Based on selection on carrier chromosome (recombinant selection) six lines were selected which carried minimum segment of donor parent.

Table 3
Foreground selection: primer sequences and amplicon size for the three stem rust resistance gene markers used.

Gene	Marker	Sequence of primers 5′–3′	Size (bp)	References
SrWeb	gwm47	5′ TTGCTACCATGCATGACCAT 3′ 5′ TTCACCTCGATTGAGGTCCT 3′	207	[15]
Sr25/Lr19	STSLr19-130	5′ CATCCTTGGGGACCT C 3′ 5′ CCAGCTCGCATACATCCA 3′	130	[26]
Sr50	IB-267	5′ GCAAGTAAGCAGCTTGATTTAGC 3′ 5′ AATGGATGTCCCGGTGAPTGG 3′	200–300	[33]

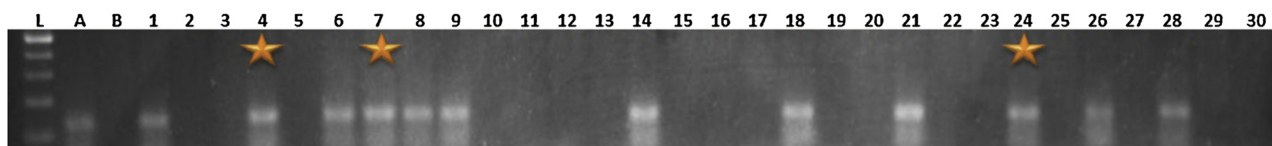

Fig. 3. BC_1F_1 segregants genotyped with the *Sr25* marker STSLr19-130. The presence of *Sr25* is predicted when the 130 bp amplicon is produced. A: PMBWIR4 (*Sr25* donor), B: cv. HUW234, 1–30: BC_1F_1 segregants. Segregants carrying *Sr25*, *Sr50* and *SrWeb* (individuals #4, #7 and #24) are marked by a star.

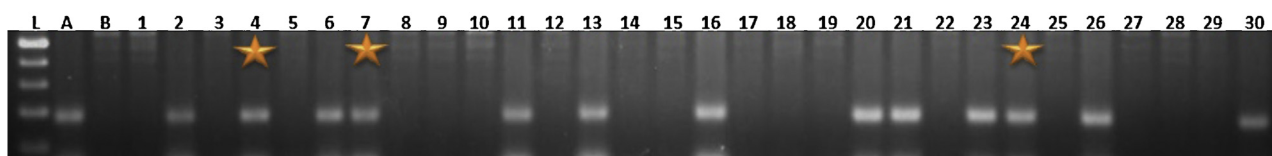

Fig. 4. BC_1F_1 segregants genotyped with the *SrWeb* marker *gwm47*. The presence of *SrWeb* is predicted when the 207 bp amplicon is produced. A: PMBWIR4 (*SrWeb* donor), B: cv. HUW234, 1–30: BC_1F_1 segregants. Segregants carrying *Sr25*, *Sr50* and *SrWeb* (individuals #4, #7 and #24) are marked by a star.

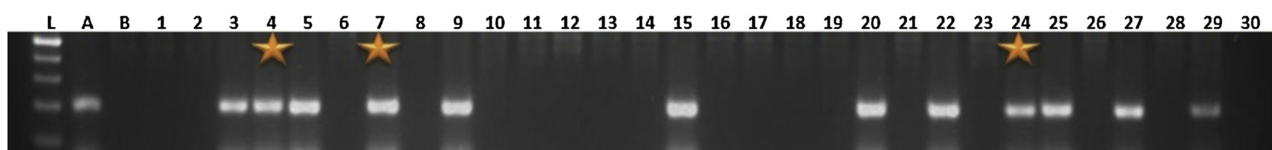

Fig. 5. BC_1F_1 segregants genotyped with the *Sr50* marker IB-267. The presence of *Sr50* is predicted when the 200–300 bp amplicon is produced. A: PMBWIR4 (*Sr50* donor), B: cv. HUW234, 1–30: BC_1F_1 segregants. Segregants carrying *Sr25*, *Sr50* and *SrWeb* (individuals #4, #7 and #24) are marked by a star.

3.3. Background selection

SSR analysis of the BC_1F_1 segregants showed that the proportion of loci which were fixed for the cv. HUW234 allele ranged from 61.6% to 72.6%. The three progenies which had inherited the highest proportion of cv. HUW234 alleles were chosen as the parent for the second backcross. The range of polymorphic SSR markers per chromosome ranged from 5 (chromosome 1A, 5B, 3D, 6D) to 10 (chromosome 1D, 2B) while in this polymorphic range 6 and 8 numbers were more frequent than others. The percent polymorphism of SSR markers ranged from 12.8% (chromosome 1A) to 22.5% (chromosome 4D). The overall proportion of the cv. HUW234 genome present in the six finally selected BC_2F_4 families was 93.7% in HUW234-09-183-71-04, 93.2% in HUW234-09-183-76-23, 94.6% in HUW234-09-183-76-74, 96.2% in HUW234-09-217-03-86, 94.2% in HUW234-09-217-56-73 and 93.8% in HUW234-09-217-79-64 (Table 6). These six HUW234 derived lines were temporarily named as HUW234-1, HUW234-2, HUW234-3, HUW234-4, HUW234-5 and HUW234-6, respectively.

3.4. Resistance and agronomic performance

The level of stem rust resistance was higher in BC_2F_4 as well as BC_2F_5 generations than in the parent cv. HUW234 (Tables 4 and 5). Likewise, there was evidence for enhanced performance of derived lines with respect to yield and yield traits (Tables 6 and 7). All the six HUW234 derived lines exhibited shorter stature in comparison to original recurrent parent HUW234 in both the generations; BC_2F_4 and BC_2F_5. Days to maturity is one of the important parameter as early maturity is associated with disease escape. MABC selected lines were early maturing compared to original HUW234. Thousand grain weight (TGW) in BC_2F_4 varied from 39.8 g to 40.7 g in HUW234 derived lines while HUW234 had 38.6 g TGW. The grain yield per plant of HUW234 was 14.86 g while in its derived lines it ranged from 16.23 g to 17.73 g in BC_2F_4 (Table 6). Likewise in BC_2F_5 the grain yield observed in case of HUW234 was 14.91 g while in derived lines it ranged from 16.48 g to 17.86 g (Table 7).

Table 4
Coefficient of infection for the six selected BC_2F_4 families grown in two locations and BC_2F_5 families grown in three locations, following infection with stem rust races 40A, 40A-1 and 40-2.

Genetic material	BC_2F_4		BC_2F_5		
	Varanasi	Indore	Varanasi	Indore	Dharwad
HUW234 (Recipient)	64	48	64	48	64
PMBWIR4 (Donor)	2	1	2	1	2
HUW234-09-183-71-4	8	4	8	2	8
HUW234-09-183-76-23	4	1	4	8	12
HUW234-09-183-76-74	4	4	2	4	4
HUW234-09-217-3-86	2	8	2	4	8
HUW234-09-217-56-73	8	4	8	8	2
HUW234-09-217-79-64	1	0	0	1	2

The level of stem rust resistance was higher in both the BC_2F_4 and the BC_2F_5 materials than in cv. HUW234 (Figs. 6 and 7). All the six HUW234 derived lines along with both parents were screened with stem rust races; 40A, 40A-1 and 40A-2. The coefficient of infection of improved lines that ranged from 0 to 12 exhibited much

Table 5
Disease reaction of parents and improved lines of wheat variety HUW 234 against stem rust pathogen (*Puccinia graminis tritici*).

Varieties/improved lines	Pathotypes used and resistance reaction			Resistance
	40A	40A-1	40-2	
HUW234	70SS	80S	40S	Susceptible
PMBWIR4 (*Sr25* + *SrWeb* + *Sr50*)	10RMR	5R	10R	Resistant
HUW234-09-14 (*Sr25*)	60MR	30RMR	40MR	Moderately resistant
HUW234-09-50 (*SrWeb*)	30MR	40RMR	40MR	Moderately resistant
HUW234-09-58 (*Sr50*)	40RMR	30RMR	30MR	Moderately resistant
HUW234-09-06 (*Sr25* + *SrWeb*)	40MR	20MS	30MR	Moderately resistant
HUW234-09-47 (*SrWeb* + *Sr50*)	20RMR	30MR	30RMR	Moderately resistant
HUW234-09-39 (*Sr25* + *Sr50*)	30MS	40RMR	20MSS	Moderately resistant
HUW234-09-24 (*Sr25* + *SrWeb* + *Sr50*)	20RMR	10MR	10RMR	Resistant

R = Resistant; MR = Moderately resistant; S = Susceptible; MS = Moderately susceptible.

Table 6
Agronomic performance and the recovery of the recurrent parent genotype in BC_2F_4 selections of the cross HUW234/PMBWIR4.

HUW 234 derived lines	PH	DH	DM	SL	TPP	TGW	50 TGW	YPP	RPG (%)
HUW234-09-183-71-4	98	74.3	111.7	10.6	10.7	40.7	100.8	17.29	93.7
HUW234-09-183-76-23	98.3	74.3	110.3	10.4	11.3	40.1	98.9	17.73	93.2
HUW234-09-183-76-74	98.4	74.7	110	10.5	10.7	39.8	100	16.23	94.6
HUW234-09-217-3-86	98.5	75	111.3	10.4	11.3	39.9	99.1	16.84	96.2
HUW234-09-217-56-73	98.3	74.7	110.7	10.2	10.7	40.2	99.8	17.16	94.2
HUW234-09-217-79-64	98.6	74.7	110.3	11.4	11	40.1	100.6	17.50	93.8
HUW234 (Recipient)	99.8	76	112	9.8	10	38.6	97.8	14.86	
PMBWIR4 (Donor)	110.8	79	119	13.4	13	41.7	103.2	20.18	
SE	1.54	0.56	1.04	0.39	0.31	0.31	0.57	0.53	
CD (0.05)	3.96	1.44	2.67	1.01	0.79	0.79	1.47	1.36	
CD (0.01)	6.21	2.26	4.19	1.57	1.25	1.25	2.29	2.14	

PH = plant height (cm); DH = days to heading; DM = days to maturity; SL = spike length (cm); TPP = tillers per plant; TGW = test grain weight; 50 TGW = 50 tillers grains weight; YPP = yield per plant; RPG = recurrent parent genome; PH = plant height (cm); DH = days to heading; DM = days to maturity; SL = splike length (cm); TPP = tillers per plant; CI = coefficient of infection.

Table 7
Agronomic performance of BC_2F_5 selections of the cross HUW234/PMBWIR4.

HUW 234 improved lines	PH (cm)	DH	DM	SL (cm)	TPP	TGW (g)	50 TGW (g)	YPP (g)
HUW234-09-183-71-4	98.2	74	110.7	10.6	10.3	39.8	99.7	17.34
HUW234-09-183-76-23	98	73.3	111	10.4	11	40.8	100.4	17.86
HUW234-09-183-76-74	98	74.6	112	10.7	11	40.6	100.7	17.56
HUW234-09-217-3-86	98.4	75.3	110.3	10.5	10.7	39.8	99.7	16.71
HUW234-09-217-56-73	98.8	73.7	110.7	10.5	9.7	40.7	99.6	16.48
HUW234-09-217-79-64	98.3	75	111.3	10.8	11.7	40.4	100.8	17.76
HUW234 (Recipient)	99.8	76	112	9.8	10	38.6	97.8	14.91
PMBWIR4 (Donor)	110.8	79	119	13.4	13	41.7	103.2	19.88
SE	1.54	0.64	1.01	0.38	0.37	0.32	0.54	0.50
CD(0.05)	3.96	1.65	2.59	0.98	0.95	0.82	1.39	1.29
CD(0.01)	6.21	2.58	4.07	1.53	1.49	1.29	2.18	2.02

PH = Plant height (cm); DH = Days to heading; DM = Days to maturity; SL = Spike length (cm); TPP = Tillers per plant; TGW = Thousand grain weight; 50 TGW = 50 tillers grain weight; YPP = Yield per plant.

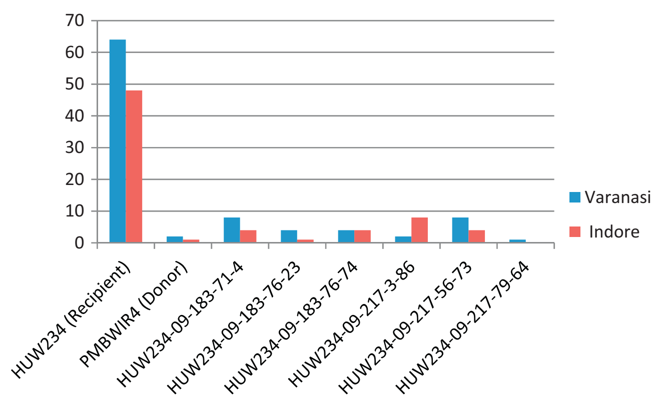

Fig. 6. Coefficient of infection for six HUW234 derived BC_2F_4 families along with parents grown in two locations; Varanasi and Indore (India), following infection with stem rust races 40A, 40A-1 and 40-2.

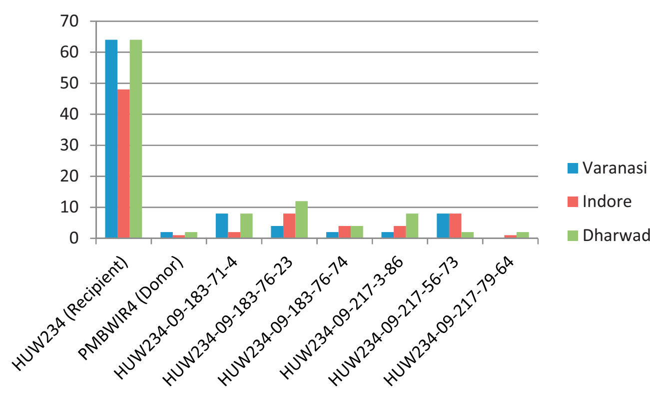

Fig. 7. Coefficient of infection for six selected BC_2F_5 families grown in three different locations; Varanasi, Indore and Dharwad (India), following infection with stem rust races 40A, 40A-1 and 40-2.

higher level of resistance in comparison to original HUW234 having coefficient of infection 64.

4. Discussion

Marker assisted backcrossing has been successfully employed in a number of wheat programs [26,30–38], as well as in other crops, such as rice [39–42], *Capsicum* [43] and tomato [44], among others. The objective of the present study was to use the MABC method to simultaneously introduce the three diverse genes *Sr25*, *SrWeb* and *Sr50* into cv. HUW234 as a means of preventing damage due to stem rust in this widely grown cultivar. This was readily achievable, since the appropriate foreground selection markers [15,26,27,45], and a large number of SSR assays are available in wheat (www.gramene.org and wheat.pw.usda.gov) for the purpose of background selection [46]. The marker strategy followed was a three stage one (foreground, linkage drag and background selection), rather than the more conventional two stage one (foreground and background selection) [47,48]. The additional step was designed to reduce the extent of linkage drag, thereby enhancing the recovery of recipient alleles in the vicinity of the three target genes prior to the genome-wide background selection step. Compared to cv. HUW234 itself, the improved versions of the cultivar exhibited a significantly better resistance against stem rust, while their agronomic performance was at least as good as, if not better than that of the original cultivar. Combining even two separate resistance genes for the same disease by conventional breeding is more time consuming than using the marker aided method, because the dominant nature of resistance means that it is difficult to distinguish between single and multiple gene carriers; this problem is avoided when the genes are selected for by independent marker assays [20,49]. A further advantage of markers is that they are unaffected by the growing environment, and thus the real risk of suffering a failed pathology test is no longer relevant [50].

DNA markers are well suited for assessing the relative contribution of each parent among the progeny of a biparental cross, and so are highly informative for estimating the extent to which the recurrent parent's genome (RPG) has been recovered during the course of a backcrossing exercise [51–54]. In a conventional backcrossing program, the general practice is to advance the material to BC_4, based on the genome-wide calculation that at this generation 97% of the genome will have been inherited from the recurrent parent. A feature of using markers for background selection is that recovery can be significantly accelerated. Phenotypic evaluation in conjunction with background analysis is also useful for efficient recovery of the recurrent parent genome [55]. In this exercise, phenotypic selection was performed for background traits among the progenies selected on the basis of foreground markers followed by background analysis. Stringent phenotypic selection for recovery of recurrent parent phenotype is the good substitute for the background selection in terms of judicious and maximum resource utility [41,55,56]. Here, background selection allowed for the identification of a BC_1F_1 individual with a genetic background comprising about 89% (compared to the unselected mean at this generation of 75%) inherited from cv. HUW234, and a BC_2F_1 individual with a recovery of nearly 92% (compared to 88%). Randhawa et al. [57] have reported 97% recurrent parent genome recovery after just two backcrosses in wheat, while Tanksley et al. [58], working with tomato, achieved 99% recovery by the third backcross generation, and Neeraja et al. [39] in rice managed 95% recovery.

A regular interval (about 20 cM) between two adjacent markers and at least four SSR markers for non-carrier chromosome appears to hasten maximum recovery of RPG in MABC [59,60]. Recently, by using computer simulations several new software's like PLABSIM and POPMIN were developed which optimized efficient, accurate and rapid introgression of target loci into the background of recurrent parent. The four variable factors of MABC, affecting its efficiency to a great context were the number of backcrosses, size of population, number and distance between adjacent markers and most revolving number of marker data points (MDPs). Surprisingly, RPG recovery was higher in two stage selection than the three or four stage selection [57]. In two stage selection the Qmin and Qmax value were 77% and 91%, respectively with 110 markers in comparison to three and four stage having Qmin 60% and 62.5%, respectively while Qmax for the latter two stages was 89.5% [57]. In our study, we get 89.2% genome wide recovery in BC_1F_1 and 92.8% in BC_2F_3 generation by using 149 polymorphic SSR markers. In BC_2F_4 generation, the maximum recovery of RPG was 96.2%. However, number of markers play a significant role in RPG recovery. Randhawa et al. [57] reported that genome wide selection with 110 markers in 100 plants/BC generation, recovery was 98% in BC_2 generation while same proportion was also obtained if 208 or 320 markers used instead of 110.

In backcross population, recovery of carrier chromosome is a great dispute. In 1999, Frisch et al. [47,48] reported that for efficient recombinant selection comparatively smaller population is better than the big one. Vishwakarma et al. [61] reported that one BC_2F_3 line had single small donor segment in the carrier chromosome 6B for the gene *Gpc-B1*. In our study, in BC_1F_1 and BC_2F_1, optimum size population was used but in BC_2F_2 large numbers of plants were screened for isolation of target gene due to dominant nature of tightly linked marker. The recombinant selection in BC_2F_2 was omitted because of the risk of losing homozygous plant carrying three genes. In addition it was time taking and tedious due to large size of population. Thus recombinant selection provide a unique opportunity for the selection of desirable plants carrying target gene(s) with minimum amount of linkage drag.

The converted forms cv. HUW234 performed agronomically as well as, if not better than the original cultivar. An improvement in performance may reflect the presence of favorable alleles (in addition to the *Sr* genes) inherited from the donor parent, either linked to the segments selected by foreground selection, or elsewhere in the genome. It appeared that the MABC technique along with the use of proper donor parent help in the rapid isolation of desired plants in segregating progenies of the cross in comparison to conventional backcross gene transfer method. Under conditions of little or no disease pressure, some of the improved versions of cv. HUW234 were marginally superior to cv. HUW234 in terms of yield, thousand grain weight, spike length and 50 tiller grain yield, while under high disease pressure, their advantage was more tangible. With respect to stem rust, field-grown cv. HUW234 plants exhibited an MS-S reaction, while the improved lines exhibited an R-MR reaction. This superior level of resistance is presumed to reflect the action of one or more of the *Sr* genes successfully introgressed by marker assisted backcrossing.

Author contribution statement

All authors contributed significantly in different ways. In the planning, coordination, conduct of the field experiments, data collection, tabulation, field and molecular analysis, interpretation and writing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

All experiments complied with the current laws of the India, the country in which they were performed.

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