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Marker Assisted Transfer of Stripe Rust and Stem Rust Resistance Genes into Four Wheat Cultivars

Mandeep S. Randhawa [1,2,*], Navtej S. Bains [3], Virinder S. Sohu [3], Parveen Chhuneja [4], Richard M. Trethowan [1], Harbans S. Bariana [1] and Urmil Bansal [1]

1 The University of Sydney Plant Breeding Institute, School of Life and Environmental Sciences, 107 Cobbitty Road, Cobbitty, NSW 2570, Australia
2 International Maize and Wheat Improvement Center (CIMMYT), ICRAF Campus, United Nations Avenue, Gigiri, P.O. Box 1041-00621, Nairobi, Kenya
3 Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141027, India
4 School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141027, India
* Correspondence: m.randhawa@cgiar.org; Tel.: +254-2072-24661

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Abstract: Three rust diseases namely; stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt), leaf rust caused by *Puccinia triticina* (Pt), and stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst), are the most common fungal diseases of wheat (*Triticum aestivum* L.) and cause significant yield losses worldwide including Australia. Recently characterized stripe rust resistance genes *Yr51* and *Yr57* are effective against pre- and post-2002 Pst pathotypes in Australia. Similarly, stem rust resistance genes *Sr22, Sr26,* and *Sr50* are effective against the Pgt pathotype TTKSK (Ug99) and its derivatives in addition to commercially important Australian pathotypes. Effectiveness of these genes make them good candidates for combining with known pleiotropic adult plant resistance (PAPR) genes to achieve durable resistance against three rust pathogens. This study was planned to transfer rust resistance genes *Yr51, Yr57, Sr22, Sr26,* and *Sr50* into two Australian (Gladius and Livingston) and two Indian (PBW550 and DBW17) wheat cultivars through marker assisted selection (MAS). These cultivars also carry other rust resistance genes: Gladius carries *Lr37/Yr17/Sr38* and *Sr24/Lr24*; Livingston carries *Lr34/Yr18/Sr57*, *Lr37/Yr17/Sr38,* and *Sr2*; PBW550 and DBW17 carry *Lr34/Yr18/Sr57* and *Lr26/Yr9/Sr31*. Donor sources of *Yr51* (AUS91456), *Yr57* (AUS91463), *Sr22* (*Sr22*/3*K441), *Sr26* (*Sr26* WA1), and *Sr50* (Dra-1/Chinese Spring ph1b/2/3* Gabo) were crossed with each of the recurrent parents to produce backcross progenies. Markers linked to *Yr51* (*sun104*), *Yr57* (*gwm389* and *BS00062676*), *Sr22* (*cssu22*), *Sr26* (*Sr26#43*), and *Sr50* (*Sr50-5p-F3, R2*) were used for their MAS and markers *csLV34* (*Lr34/Yr18/Sr57*), VENTRIUP-LN2 (*Lr37/Yr17/Sr38*), *Sr24#12* (*Sr24/Lr24*), and *csSr2* (*Sr2*) were used to select genes present in recurrent parents. Progenies of selected individuals were grown and selected under field conditions for plant type and adult plant rust responses. Final selections were genotyped with the relevant markers. Backcross derivatives of these genes were distributed to breeding companies for use as resistance donors.

Keywords: MAS; markers; stem rust; stripe rust; wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major food crops in the world and it is continuously threatened by several biotic stresses. Among the significant biotic stresses, rust diseases namely: Stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt), leaf rust caused by *Puccinia triticina* (Pt), and stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst), are the most common diseases of wheat worldwide. Rust diseases have been causing significant economic losses to wheat production in Australia. Stem

rust epidemic in the 1973–74 season caused economic losses of A$200 to 300 million [1]. Epidemics of stem rust and leaf rust in wheat crop in Western Australia in 1999 were estimated to result in A$50 million in losses [2]. Introduction of a new Pst pathotype in Western Australia has been continuously causing yield losses since its detection in 2002 [3,4].

The importance of genetic resistance to control rust diseases was first demonstrated by Biffen [5]. Since then, breeding for rust resistance is considered as the most preferred management strategy for rust control. This approach has been successful in reducing losses in Australian wheat growing regions, where rust resistant-wheat cultivars protected grain industry from epidemics since 1960s. The Australian grain industry was estimated to save A$289 million annually from stem rust, stripe rust, and leaf rust infections through resistance breeding efforts [6].

Long lasting control of rust diseases has been achieved through pyramiding resistance genes in a single genotype [7]. It is however difficult to combine more than two genes in conventional breeding programs, especially when genes to be deployed exhibit resistance against all available rust isolates and produce similar infection types. The detection of marker-gene associations has made marker-assisted gene pyramiding an achievable target in breeding programs [8].

Marker assisted selection (MAS) allows enrichment of positive alleles that condition economic traits at early generations in breeding programs and it is underpinned by the availability of tight marker-trait associations that are highly reproducible, cost-effective and user friendly [9,10]. The various approaches presently being used for MAS include marker-assisted backcrossing (MABC), forward selection, MAS-aided doubled haploid (DH) production and enrichment of positive alleles among F_2 selections.

Molecular markers linked with *Yr51* [11], *Yr57* [12], *Sr22* [13], *Sr26* [14], and *Sr50* [15] are available. Genes *Yr51* and *Yr57* are effective against all currently detected Pst pathotypes in Australia. Although a greenhouse isolate with virulence for *Sr50* was detected in 2014 [15], all three genes (*Sr22*, *Sr26,* and *Sr50*) are effective against the Pgt pathotype TTKSK (Ug99) and its derivatives [16]. This study was planned to transfer rust resistance genes *Yr51*, *Yr57*, *Sr22*, *Sr26,* and *Sr50* into four wheat cultivars through MAS.

2. Materials and Methods

2.1. Plant Materials

Wheat cultivars Gladius and Livingston (Australian), and PBW550 and DBW17 (Indian) were chosen as recurrent parents for transfer of stripe rust and stem rust resistance genes. Gladius is a spring wheat cultivar that was cultivated in South Australia and is moderately resistant (MR) to stem rust and moderately resistant to moderately susceptible (MR-MS) to stripe rust. Gladius carries all stage resistance (ASR) genes *Lr37/Yr17/Sr38* and *Sr24/Lr24* introgressions from *Aegilops ventricosa* and *Thinopyrum elongatum*, respectively. Livingston is cultivated in northern New South Wales (NSW) and Queensland and shows MR-MS level of resistance against both stripe rust and stem rust under field conditions. It carries adult plant resistance (APR) genes *Lr34/Yr18/Sr57* and *Sr2* in addition to ASR gene *Lr37/Yr17/Sr38*. PBW550 and DBW17 are high yielding cultivars widely cultivated in North Western Plain Zone (NWPZ) in India and are susceptible to stripe rust. Both PBW550 and DBW17 carry *Lr34/Yr18/Sr57* and *Lr26/Yr9/Sr31*. Seed of Indian cultivars was obtained as a part of Australia-India collaborative project funded by the Australian Centre for International Agricultural Research (ACIAR). Donor sources carrying *Yr51*, *Yr57*, *Sr22*, *Sr26,* and *Sr50* were obtained from the Australian Cereal Rust Control Program (ACRCP) staff (Table 1).

Table 1. List of plant material used to generate backcross derivatives.

Recurrent Parents	Donor Parents	Target Gene	Backcross Progenies
Gladius, Livingston, PBW550, DBW17	AUS91456	*Yr51*	BC_2F_2
	AUS91463	*Yr57*	BC_2F_2
	Sr22/3*K441	*Sr22*	BC_1F_2
	Sr26 WA1	*Sr26*	BC_1F_2
	Dra-1/Chinese Spring ph1b/2/3* Gabo	*Sr50*	BC_1F_2

2.2. Pathogen Materials

The Pst pathotype, 134 E16 A+Yr17+Yr27+, Plant Breeding Institute (PBI) culture number 617 and Pgt pathotype 34-1,2,3,4,5,6,7 (PBI culture number 103) were used to screen respective backcross derivatives along with recurrent parents at seedling stage in the greenhouse. The Pst pathotype 134 E16 A+Yr17+Yr27+ was virulent on *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27,* and *YrA*. The Pgt pathotype 34-1,2,3,4,5,6,7 carried virulence for *Sr6*, *Sr8a*, *Sr9b*, *Sr9g*, *Sr11*, *Sr15*, *Sr17,* and *Sr36*.

2.3. Greenhouse Tests

Four F_1 seeds and 10 to 20 seeds from each backcross (BC_1F_1, BC_2F_1) were sown in 9 cm pots filled with a potting mixture (pine bark and river sand in the ratio of 2:1). Recurrent parents (Gladius, Livingston, PBW550, and DBW17) and donor parents AUS91456 (*Yr51*), AUS91463 (*Yr57*), *Sr22*/3*K441 (*Sr22*), *Sr26* WA1 (*Sr26*), and Dra-1/Chinese Spring ph1b/2/3* Gabo (*Sr50*) were included as controls in respective experiments. Cultivar Morocco was used as the susceptible control. After sowing, pots were maintained in rust-free microclimate rooms at 20 °C. Twenty grams of water-soluble fertilizer Aquasol® was dissolved in 10 liters of tap water and applied to 100 pots. A single application of nitrogenous fertilizer urea was applied at the same rate as Aquasol® to the seven-day old seedlings. Inoculations were performed at the two-leaf growth stage by suspending urediniospores in light mineral oil (Isopar L, 5 mg spores per 10 mL oil) using a hydrocarbon propellant pressure pack. For stripe rust, the inoculated seedlings were placed under polythene hoods in water filled steel trays and incubated in a dark room at 9–12 °C for 24 h. Stem rust-inoculated seedlings were incubated under polythene hoods in water filled steel trays in a greenhouse microclimate room at 18–20 °C for 48 h. Following incubation, seedlings were moved to temperature- and irrigation- controlled greenhouse rooms. Post-inoculation temperatures of 27 °C and 17 °C were used for stem rust and stripe rust, respectively. Rust response assessments were made 14 days after inoculation according to scales described in Bariana and McIntosh [17]. Rust resistant selections were made by comparing infection types produced by backcross derivatives with donor sources.

2.4. Field Tests

The BC_2F_2 progenies of *Yr51* and *Yr57*, and BC_1F_2 progenies of *Sr22*, *Sr26* and *Sr50* were sown at Karalee (KRL) field site of the University of Sydney Plant Breeding Institute, Cobbitty. Thirty-five seeds from each cross were space (10 cm) planted at KRL for marker assisted selection. In order to create artificial epidemics, urediniospores of Pgt pathotypes (98-1,2,(3),(5),6 and 34-1,2,7+*Sr38*), Pt pathotypes (104-1,(2),3,(6),(7),11,13; 10-1,3,9,10,11,12 and 76-3,5,9,10+*Lr37*) and Pst pathotypes (134 E16A+*Yr17*+*Yr27*+ and 110 E143A+) were suspended in the light mineral oil (Isopar L) and atomized on experimental rows and susceptible infector rows using an ultra-low-volume applicator (Herbi 4, Microfit®, Micron Sprayer Ltd.) during three successive clear evenings.

2.5. DNA Isolation and Quantification

Total genomic DNA was isolated from seedlings of all test material following the procedure described in Bansal et al. [18]. Quality and quantity of DNA was estimated using agarose (1%)

gel and Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies Inc. Wilmington, NC, USA), respectively. Final dilutions of 30 ng/µl concentration were prepared with double distilled autoclaved water.

2.6. PCR Amplification and Gel Electrophoresis

For PCR amplification of SSR and STS markers, assays were performed in 10 µL reaction mixture containing 0.2 mM dNTPs, 1xImmolase PCR buffer (Bioline), 0.2 mM each of forward and reverse primer, 30 ng of genomic DNA and 0.2 U of Immolase DNA polymerase (Bioline). PCR amplifications were performed according to published protocols for respective markers (Table 2).

Table 2. List of molecular markers linked with rust resistance genes used in the present study.

Gene	Chromosome	Marker	Dominant/Co-Dominant	Base Pairs/Allele		Source
				Donor Parent	Recurrent Parent	
Lr34	7DS	*csLV34*	Co-dominant	150	229	Lagudah et al. [19]
Lr37	2AS	*VENTRIUP-LN2*	Dominant	252	Null	Helguera et al. [20]
Yr51	4AL	*sun104*	Dominant	225	Null	Randhawa et al. [11]
Yr57	3BS	*gwm389*	Co-dominant	150	145 or 147	Randhawa et al. [12]
Yr57	3BS	*BS00062676*	Co-dominant	A:A	G:G	Randhawa et al. [12]
Sr2	3BS	*csSr2*	Co-dominant	172, 112, 53	225,112	Mago et al. [21]
Sr22	7AL	*cssu22*	Co-dominant	237	335	Periyannan et al. [13]
Sr24	3DL	*Sr24#12*	Dominant	500	Null	Mago et al. [14]
Sr26	6AL	*Sr26#43*	Dominant	270	Null	Mago et al. [14]
Sr50	1DL.1RS	*Sr50-5p-F3, R2*	Dominant	470	Null	Mago et al. [15]

2.7. Marker Assisted Selection

Molecular markers linked with *Yr51*, *Yr57*, *Sr22*, *Sr26*, and *Sr50* were tested on respective backcross material to select seedlings positive for the target gene. Marker assisted selection was performed at each backcross generation. BC_2F_2s (*Yr51* and *Yr57*) and BC_1F_2s (*Sr22*, *Sr26*, and *Sr50*) were tested with markers linked with *Lr34/Yr18/Sr57*, *Sr24/Lr24*, *Sr2*, and *Lr37/Yr17/Sr38* to select resistance genes carried by recurrent parents. Markers details are listed in Table 2.

2.8. Chi-Squared Analyses

Goodness-of-fit of the observed segregation ratios with the expected genetic ratios of phenotypic and genotypic data of BCF_2 derivatives was tested using chi-squared (χ^2) analysis.

3. Results

3.1. Marker Assisted Transfer

Wheat cultivars Gladius, Livingston, PBW550, and DBW17 were crossed with each of the five donors *Yr51*, *Yr57*, *Sr22*, *Sr26*, and *Sr50* to produce F_1 hybrids which were in turn backcrossed with the respective recurrent parent. Marker assisted selection was performed at each generation. Figure 1 illustrates the crossing scheme used for the transfer of *Yr51* into Gladius as an example. Similar strategy was applied to the transfer of *Yr57*, whereas single back cross was used for transfer of *Sr22*, *Sr26*, and *Sr50* into each of the four wheat cultivars. Infection types produced by donor and recurrent parents are presented in Table 3. Details of transfer of each gene into four cultivars are discussed below:

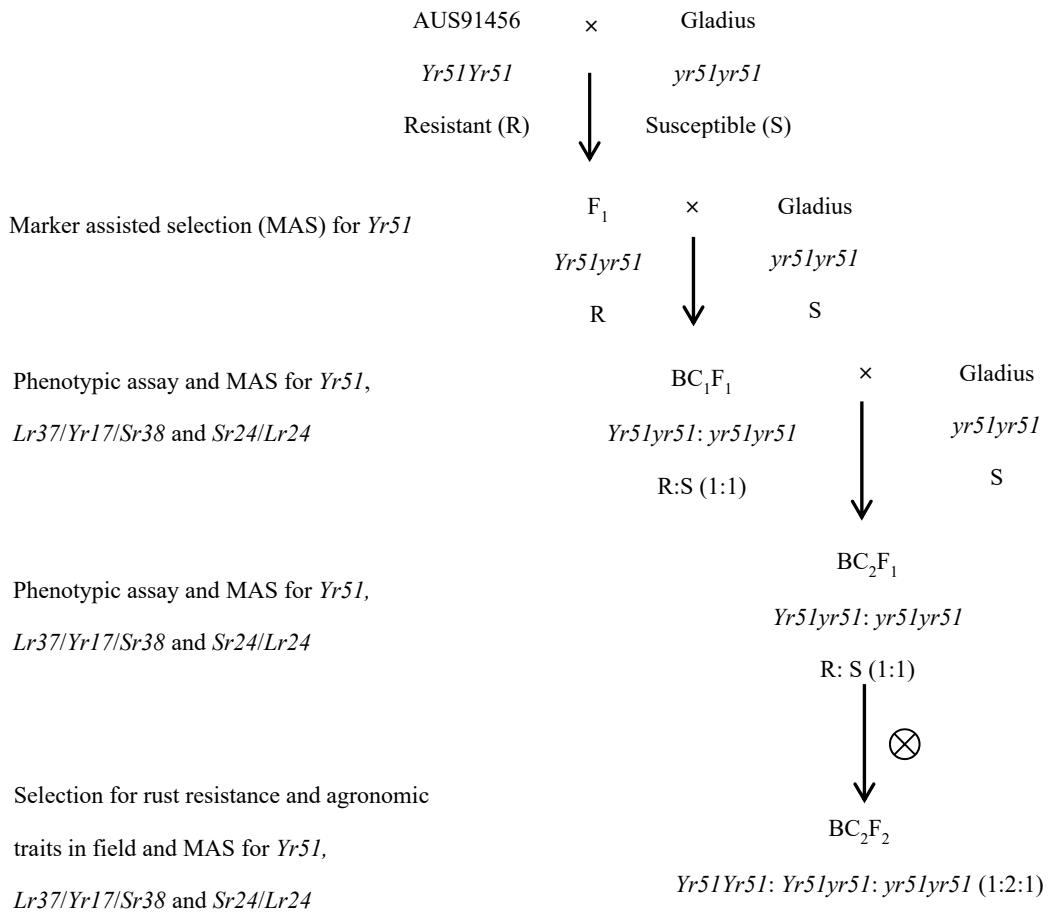

Figure 1. Schematic diagram depicting marker assisted transfer of stripe rust resistance gene *Yr51* to Gladius through phenotypic and marker assisted selection at each generation.

Table 3. Pedigree details and infection types produced by donor and recurrent parents, when tested against *Puccinia striiformis* f. sp. *tritici* (Pst) pathotype 134 E16A+*Yr17*+*Yr27*+ and *Puccinia graminis* f. sp. *tritici* (Pgt) pathotype 34-1,2,3,4,5,6,7.

Genotype	Pedigree	Infection type	
		134 E 16A+*Yr17*+*Yr27*+	**34-1,2,3,4,5,6,7**
Donor parent			
AUS91456	AUS27858/Westonia RIL #5515-1	;*n*-1*n*	-
AUS91463	AUS27858/Westonia RIL #5474-6	0;	-
Sr22/3*K441	-	-	;1+
Sr26 WA1	-	-	;1+c
Dra-1/Chinese Spring ph1b/2/3* Gabo	-	-	;1++c
Recurrent parent			
Gladius	(DH)Rac-875/Krichauff//Excalibur /Kukri/3/RAC-875/Krichauff/4/RAC-875//Excalibur/Kukri	3+	;1
Livingston	SUN-129-A/Sunvale	3+	23c
PBW550	WH-594/RAJ-3856//W-485	3+	;1−
DBW17	CMH-79-A-95/3*Ciano79//RAJ-3777	3+	;1

3.2. *Yr51*

Yr51-linked dominant marker *sun104* was genotyped on F_1 hybrids of *Yr51* and recurrent parents Gladius, Livingston, PBW550 and DBW17 to ensure identity of the cross. Genotyping of BC_1F_1 seedlings enabled identification of plants carrying the *Yr51*-linked allele $sun104_{225}$. Similarly, BC_2F_1

seedlings were also selected through MAS and those carrying the linked marker allele were advanced to BC_2F_2 stage. BC_1F_1 and BC_2F_1 plants showed 1:1 segregation ($sun104_{225}$: $sun104_{null}$) at the *sun104* locus (Table 4).

Table 4. Chi-squared analysis of marker segregation among BCF_1 and BCF_2 populations.

Cross	BC_1F_1				BC_2F_1			
	Yr51yr51	***yr51yr51***	χ^2 (1:1)	***p*-value**	***Yr51yr51***	***yr51yr51***	χ^2 (1:1)	***p*-value**
Yr51/Gladius	11	8	0.47	0.49	22	14	1.78	0.18
Yr51/Livingston	9	8	0.06	0.81	14	11	0.36	0.55
Yr51/PBW550	11	7	0.89	0.35	9	4	1.92	0.16
Yr51/DBW17	11	9	0.20	0.65	6	14	3.20	0.07
	Yr57yr57	*yr57yr57*			*Yr57yr57*	*yr57yr57*		
Yr57/Gladius	10	10	0.00	1.00	11	11	0.00	1.00
Yr57/Livingston	14	6	3.20	0.07	14	6	3.20	0.07
Yr57/PBW550	6	5	0.09	0.76	8	9	0.06	0.80
Yr57/DBW17	10	9	0.05	0.82	7	4	0.82	0.37
	BC_1F_1				**BC_1F_2**			
	Sr22sr22	***sr22sr22***			***Sr22sr22***	***sr22sr22***	χ^2 (3:1)	
Sr22/Gladius	4	6	0.40	0.53	9	7	3.00	0.08
Sr22/Livingston	5	4	0.11	0.74	13	3	0.33	0.56
Sr22/PBW550	3	5	0.50	0.48	10	6	1.33	0.25
Sr22/DBW17	7	5	0.33	0.56	13	3	0.33	0.56
	Sr26sr26	*Sr26sr26*			*Sr26sr26*	*sr26sr26*		
Sr26/Gladius	5	5	0.00	1.00	13	3	0.33	0.56
Sr26/Livingston	5	5	0.00	1.00	10	6	1.33	0.25
Sr26/PBW550	4	5	0.11	0.74	14	2	1.33	0.25
Sr26/DBW17	3	9	3.00	0.08	12	4	0.00	1.00
	Sr50sr50	*sr50sr50*			*Sr50sr50*	*sr50sr50*		
Sr50/Gladius	7	3	1.60	0.20	9	7	3.00	0.08
Sr50/Livingston	4	6	0.40	0.53	10	6	1.33	0.25
Sr50/PBW550	4	6	0.40	0.53	11	5	0.33	0.56
Sr50/DBW17	6	6	0.00	1.00	15	1	3.00	0.08

3.3. *Yr57*

Yr57-linked markers *gwm389* and *BS00062676* were co-dominant, and the *Yr57* source carried $gwm389_{150}$ and *BS00062676* 'A:A' alleles. The F_1 hybrids involving *Yr57* (AUS91463) and recurrent parents Gladius, Livingston, PBW550, and DBW17 were genotyped to ascertain the presence of *Yr57* and to exclude the possibility of selfing. DNA from 11 to 20 BC_1F_1 seedlings was isolated and tested using markers *gwm389* and *BS00062676*. BC_1F_1 seedlings carrying the *Yr57*-linked alleles were selected and backcrossed to respective recurrent parent to produce BC_2F_1 plants. Again *Yr57*-carrying BC_2F_1 seedlings were selected using markers and advanced to produce BC_2F_2 generation. Segregation of *Yr57*-linked markers *gwm389* and *BS00062676* on BC_1F_1 and BC_2F_1 plants fitted to 1:1 ratio (Table 4).

3.4. *Sr22*

The *Sr22*-linked marker *cssu22* is co-dominant and the F_1 plants from all four crosses carried *Sr22*-linked $cssu22_{237}$ allele together with recurrent parent alleles. The BC_1F_1 seedlings were either heterozygous at the *cssu22* locus or carried the respective recurrent parent allele (Table 2). Heterozygous BC_1F_1 plants were grown to produce BC_1F_2 generation. Marker *cssu22* showed monogenic segregation when tested on backcross derivatives (Table 4).

3.5. *Sr26*

The *Sr26*-linked marker *Sr26#43* follows dominant inheritance and amplifies 270 bp in genotypes carrying this gene. The F_1 plants were genotyped to ascertain the presence of *Sr26* and were crossed to the respective recurrent parent. The BC_1F_1 seedlings carrying the *Sr26*-linked allele in all crosses were selected and advanced to BC_1F_2 generation. Monogenic segregation at the *Sr26#43* locus was observed among BC_1F_1 and BC_1F_2 plants (Table 4).

3.6. *Sr50*

The *Sr50*-linked marker *Sr50-5p-F3, R2* amplified 470 bp product in genotypes carrying *Sr50* and no product was observed among recurrent parents indicating its dominant nature. Crosses of Dra-1/Chinese Spring ph1b/2/3* Gabo with all four recurrent parents were genotyped to ensure the presence of *Sr50* in F_1 plants and crossed with the respective recurrent parent. BC_1F_1 seedlings carrying the *Sr50*-linked allele were selected and advanced to produce BC_1F_2 families. The marker *Sr50-5p-F3, R2* showed monogenic segregation (Table 4).

3.7. Marker Assisted Selection of Gene Combinations Among BCF_2 Families

Bulked seed from marker positive BCF_2 families (BC_2F_2 for *Yr51* and *Yr57*, and BC_1F_2 for *Sr22*, *Sr26*, and *Sr50*) were space planted in the field during 2014 season and inoculated artificially with Pst, Pt, and Pgt pathotypes. While selection for triple rust resistance and agronomic traits were attempted, DNA from 35 plants from each cross was isolated to confirm presence of the target gene. Additional resistance genes carried by recurrent parents are listed in Table 5. These included *Lr37/Yr17/Sr38*, *Lr34/Yr18/Sr57*, *Sr24/Lr24*, and *Sr2*. Rust resistant and agronomically better backcross progenies of Gladius and Livingston were tested with *Lr37*-linked marker VENTRIUP-LN2 to select combination of *Lr37/Yr17/Sr38* with the gene targeted for backcrossing. The *Sr24/Lr24*-linked marker *Sr24#12* was used to select plants carrying *Sr24/Lr24* in combination with the target gene in crosses of Gladius. The *Sr2*-linked marker was monomorphic among parents and therefore progenies carrying *Sr2* were selected using seedling chlorosis linked with this gene. Backcross progenies of Livingston, PBW550, and DBW17 were tested with *Lr34*-linked marker *csLV34* to identify genotypes carrying *Lr34/Yr18/Sr57* and the target gene. Table 5 lists different combinations of rust resistance genes identified in backcross progenies of four cultivars.

Table 5. Resistance gene combinations observed in crosses with different wheat cultivars.

Recurrent Parents	Background Gene (s)	Gene Combinations	Number of Selected Plants
Gladius	*Lr37/Yr17/Sr38, Lr24/Sr24*	*Yr51 + Lr37/Yr17/Sr38 + Lr24/Sr24*	4
		Yr57 + Lr37/Yr17/Sr38 + Lr24/Sr24	7
		Sr22 + Lr37/Yr17/Sr38 + Lr24/Sr24	7
		Sr26 + Lr37/Yr17/Sr38 + Lr24/Sr24	13
		Sr50 + Lr37/Yr17/Sr38 + Lr24/Sr24	8
Livingston	*Lr34/Yr18/Sr57, Lr37/Yr17/Sr38, Sr2*	*Yr51 + Lr34/Yr18/Sr57 + Lr37/Yr17/Sr38*	4
		Yr57 + Lr34/Yr18/Sr57 + Lr37/Yr17/Sr38	11
		Sr22 + Lr34/Yr18/Sr57 + Lr37/Yr17/Sr38	12
		Sr26 + Lr34/Yr18/Sr57 + Lr37/Yr17/Sr38	2
		Sr50 + Lr34/Yr18/Sr57 + Lr37/Yr17/Sr38	4
PBW550	*Lr34/Yr18/Sr57*	*Yr51 + Lr34/Yr18/Sr57*	3
		Yr57 + Lr34/Yr18/Sr57	3
		Sr22 + Lr34/Yr18/Sr57	10
		Sr26 + Lr34/Yr18/Sr57	4
		Sr50 + Lr34/Yr18/Sr57	4
DBW17	*Lr34/Yr18/Sr57*	*Yr51 + Lr34/Yr18/Sr57*	3
		Yr57 + Lr34/Yr18/Sr57	2
		Sr22 + Lr34/Yr18/Sr57	9
		Sr26 + Lr34/Yr18/Sr57	3
		Sr50 + Lr34/Yr18/Sr57	5

4. Discussion

Diverse breeding methodologies are being used for selection of rust resistance in breeding programs. Backcrossing has been considered as an effective method for transferring resistance genes to agronomically superior genotypes as it allows capturing of recurrent parental background. Single backcross approach was used to achieve combinations of APR genes for resistance to rust diseases in wheat [22–24].

Gene-linked markers can be used to select and confirm gene combinations in segregating populations. Numerous reports about useful marker-trait associations for wheat are available. However, the number of publications detailing the practical application of MAS in wheat breeding is limited [25]. Molecular markers can assist in phenotype-neutral selection of traits that are difficult to select phenotypically, environmentally unstable, or are not cost-effective to assess [26]. Being a non-invasive and seed quantity independent alternative to phenotypic based selection, MAS can be applied at any point in a breeding program.

Close marker-trait associations increase the efficiency of MAS. This study demonstrated the usefulness of markers linked with rust resistance genes *Yr51*, *Yr57*, *Sr22*, *Sr26,* and *Sr50*. MAS was successfully employed to select combination of these genes with all-stage resistance and/or adult plant resistance genes present in recurrent parents. The MAS approach is even more useful in the case of genes that show recessive inheritance. In this study, backcrossing of the recessive gene *Yr51* was successfully achieved.

DNA isolation is the most expensive component of MAS and once DNA of a sample is available then markers linked with a range of traits can be genotyped to select a total genotypic package. However, larger population sizes are required to successfully select desirable number of genotypes carrying positive alleles for multiple trait loci. Bonnet et al. [27] estimated size of population for MAS of different number of loci using different breeding methodologies. A much lower number is needed in the case of doubled haploid/recombinant inbred line and single backcross methods.

Discovery of genetically diverse rust resistance loci and their close association with markers has exponentially increased in the last decade through advancement in genotyping technologies [28]. Validation of marker-trait linkages as demonstrated in this study will ensure assembly of the best training populations for genomic selection in wheat to beat environmental influences to increase selection efficiencies in breeding programs. The improved versions of four cultivars have been distributed to Australian and Indian wheat breeders involved in the Australia-India bilateral project funded jointly by the ACIAR and the Indian Council of Agricultural Research (ICAR) for use in respective breeding programs. This study resulted in improvement of stripe rust and stem rust resistance in wheat cultivars when evaluated in Australia and India. These results covered foreground and background selection to deliver triple rust resistant cultivars to farmers.

Author Contributions: H.S.B., U.B. and R.M.T. designed the experiments. M.S.R. performed all experiments and wrote the manuscript. H.S.B. supervised greenhouse and field evaluation of backcross progenies. U.B. supervised marker assays. N.S.B., V.S.S., and P.C. supplied seed of Indian wheat germplasm through collaborative research project and promoted material for releases as cultivars in India. All authors read the manuscript.

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