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Dissection of genetic factors underlying rust resistance of a widely-grown bread wheat (*Triticum aestivum*) cultivar 'PBW343'

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ABSTRACT

Genetic basis of rust resistance was investigated in a bread wheat (*Triticum aestivum* L. emend. Fiori and Paol) cultivar 'PBW343' which is grown in more than 8 million ha in north-western plains zone and adjoining areas of India. The study showed that 'PBW343' is resistant to leaf rust pathotypes except those virulent on *Lr26*. Single dominant gene imparted resistance against pathotype '109R31-1'. 'PBW343' was resistant to all stem rust pathotypes including those virulent on *Sr24*. At least 2 stem rust resistance genes were detected in 'PBW343'. Single dominant gene was responsible for resistance to stem rust pathotype '62 G29' and 2 independent dominant genes imparted resistance against pathotype '75 G5'. 'PBW343' was resistant to stripe rust pathotypes except a new race '78S84'. Two seedling resistance genes governed resistance against stripe rust in 'PBW343'. New pathotypes virulent on 'PBW343', identified in India (leaf and stripe rusts) or Central Asia (stripe rust) or Uganda (stem rust), are potentially capable of incurring economic loss to this cultivar which may adversely affect wheat production in India.

Key words: 'PBW343', *Sr31* virulence, Wheat rust resistance, *Yr27* virulence

Wheat (*Triticum aestivum* L. emend. Fiori and Paol) is grown throughout India in 6 diverse agro-ecological zones. Rusts are the most important diseases of wheat in India. Leaf rust *(Puccinia triticina* Eriks.) occurs throughout the country, stem rust *(Puccinia graminis* Pers. f.sp. *tritici* Eriks. & Henn.) assumes more importance in central and peninsular states and stripe rust *(Puccinia striiformis* Westend. f. sp. *tritici*) pose threat mainly in the north-western plains zone (NWPZ) as it proliferates in cool and moist climate. All present day high-yielding varieties are moderately susceptible to highly susceptible to either leaf rust or stripe rust or both. With the evolution of new stem rust pathotype virulent on *Sr31* in Uganda (Pretorius *et al.* 2000), resistance based on *Sr31*, the most frequent *Sr* gene in recent Indian wheat varieties (Bhardwaj *et al.* 2003), can no longer be safe in future. 'PBW343', which occupies more than 8 million ha, is the most important wheat cultivar in the NWPZ. Under present seed replacement situation, production of bread wheat in India is very much dependent on the fate of 'PBW343' against biotic and abiotic stresses. Very few samples of stripe rust were detected on 'PBW343' (Prashar *et al.* 2007), leaf rust appears on it at later stages of crop growth which enables it to evade economic loss and it is resistant to stem rust till date. Though this leading cultivar has successfully survived a decade but it may be rendered susceptible, especially after the detection of new virulent pathotypes of stripe and leaf rusts (Prashar *et al.* 2007). Genetic analyses were carried out to determine the number and nature of resistance genes in 'PBW343' (ND/VG9144//KAL/BB/3/YACO/4/VEE#5).

MATERIALS AND METHODS

The experimental material comprised F_1's, F_2 population and F_3 families of 'Agra Local'/'PBW343' and 'PBW343'/ Thatcher. 'PBW343'/Agra Local populations were tested against pathotypes of *Puccinia triticina*, *P. graminis tritici* and *P. striiformis*. Allelism test was conducted on 'PBW343'/ Tc*Lr10*, 'PBW343'/Tc*Lr26*, 'PBW343'/'Webster', 'PBW343'/Reliance and 'PBW343'/'Agra Local' F_2 populations.

Fully expanded primary leaves (7–9 days old) were inoculated with uredospores suspended in non-phytotoxic isoparaffinic oil (soltrol 170, Philips Chevron). Inoculations of stripe, leaf and stem rusts were carried out in temperature controlled glass house at 16°C, 22°C and 24°C, respectively. However, for testing the low temperature induced resistance of 'PBW343' against leaf rust pathotype '121R63-1', inoculation and incubation was done at 14°C. The inoculated seedlings were kept in a humid glass chamber for 48 hr.

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Infection types (IT's) recorded 14 days after inoculation. Seedlings were classified as per standard procedure on a scale of 0; to 3+. The F_1's, F_2 population, F_3 families were tested with pathotypes '109R31-1' and '45R35' of leaf rust, '62 G29' and '75 G5' of stem rust, '46S103' and '46S119' of stripe rust. Same set of F_3 families were evaluated wherever progeny testing was done simultaneously with two or more pathotypes and about 25 seedlings were analyzed per family. Chi square tests were applied to check the validity of postulated and observed genetic ratios.

Adult plants were inoculated after the appearance of flag leaf with uredospores suspended in light weight, non-phytotoxic isoparaffinic oil (soltrol 170). Adult plant resistance (APR) tests for leaf, stem and stripe rusts were done in separate green houses which were kept humid for 48 hr with the help of humidifiers. Temperature was maintained at 18–22°C with the help of sensor attached exhaust fans. Terminal disease severity scores were taken and rust severity was recorded as per the modified Cobb's scale.

Molecular marker (Fransis *et al.* 1995) was used to detect the presence of rye chromatin in 'PBW343'. Amplifications were performed in PTC200 thermal cycler (MJ Research). PCR products were analyzed in 1.5% agarose gel in 0.5X TAE buffer. PCR conditions were same as described by Fransis *et al.* (1995).

RESULTS AND DISCUSSION

The seedling infection types and adult plant resistance of the parents and checks with known resistance genes (Nayar *et al.* 2001, McDonald *et al.* 2004) are presented in Table 1.

Leaf rust resistance

The F_2 segregation patterns of 'Agra Local'/'PBW343' were in compliance with single dominant gene against pt. *109R31–1* and *45R35* (Table 2). In the allelism test, susceptible seedlings were not observed in the F_2 except in

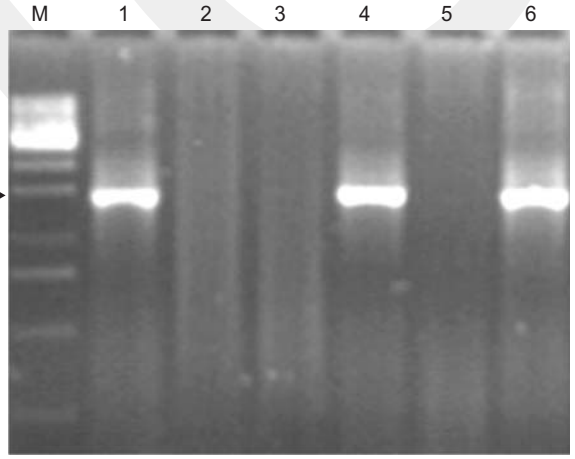

Fig 1 M- Marker, 1- Tc*Lr26*, 2- Tc*Lr23*, 3- Thatcher, 4- PBW343, 5- Agra Local, 6-Benno (*Lr26*)

'PBW343'/'*TcLr10'* and 'PBW343'/'Agra Local' (Table 2). Genetic analysis of F_2 and F_3 generations confirmed that 'PBW343' possess at least 2 major genes for leaf rust resistance. The differential host pathogen interaction of 'Tc*Lr26'* against pt.'109R63' and pt.'109R31-1' were same as 'PBW343' (Table 1) which indicated that the gene governing resistance to '109R31-1' in 'PBW343' can be *Lr26* as postulated earlier (Nayar *et al.* 2001). Further evidence of *Lr26* in 'PBW343' came from molecular marker of rye chromatin (Fig 1). Finally, the F_2 of 'PBW343'/'Tc*Lr26'* did not segregate when inoculated with pt. '109R31-1' which confirmed that 'PBW343' has *Lr26*. 'PBW343' was also resistant to pt. '45R35' which is virulent on *Lr26*. Therefore, this resistance must be due to a gene other than *Lr26*. Resistance to pt. '45R35' was imparted by a single dominant gene *'LrPB1'* (Datta *et al.* 2009). 'PBW343' was resistant to a highly virulent and predominant leaf rust pathotype '121R63-1' at low temperatures. An earlier genetic study from our lab has indicated involvement of two complementary genes (one dominant and one recessive) for low temperature resistance. 'PBW343' also showed resistance to pt. '121R127' at high temperatures (Table 2). High temperature dependent resistance was also observed in Agra Local and 'IWP94' (Datta *et al.* 2008). An earlier report on high temperature leaf rust resistance in 'PBW343' was indicative of partially dominant genes (Datta *et al.* 2009). In the areas of north western plains zone (NWPZ), where 'PBW343' is grown, high temperature resistance will only be effective during late crop growth stages and hence, this kind of resistance has little importance for varieties of 'NWPZ' including 'PBW343'. The flag leaves of 'PBW343' showed up to 80S response when inoculated with pt. '121R63-1' at 22°C, whereas at low temperature (14°C) the plants were highly resistant (Table 1). When tested with another highly virulent leaf rust pathotype '21R55', it neither showed low temperature dependent seedling resistance (IT's 3+) nor effective level of adult plant resistance (80S response). In the farmers' field leaf rust severity of 'PBW343' reaches up to 80S at the later stages of crop growth when temperature starts rising. During rust sample analyses it was observed that pt.'121R63-1' was declining, whereas pt. '21R55' was sowing upward trend in NWPZ (Jain *et al.* 2004). This observation is important because 'PBW343' is susceptible to the later pathotype even at low temperatures. Under present situation of leaf rust pathotype distribution in the NWPZ, 'PBW343' is able to escape the economic loss from leaf rust because it possess low temperature resistance factors against pt.'121R63-1'. In addition, some unidentified slow rusting gene/s or modifiers may also be present in 'PBW343' because the adult plants showed 80S terminal disease severity, whereas 'Agra Local' was fully susceptible (100S) (Table 1). Adult plant leaf rust resistance gene *Lr34* is documented in 'PBW343' (Nayar *et al.* 2001). However, such low level of adult plant resistance will not suffice to

Table 1 Rust resistance of 'PBW343', susceptible check and known gene lines against selected pathotypes

Pathotype	'PBW343'		'Webster' (*Yr27*)		'HS240' (*Yr9*)		'Agra Local' (AL)		'Reliance' (*Sr5*)
	SRT	APR	SRT	APR	SRT	APR	SRT	APR	SRT
tr '29R45'[b]	3^+	60S	12	10MR	0	a	3^+	100S	a
tr '109R63' [b]	3^+	60S	3^+	100S	3+	a	3^+	100S	a
tr '109R31-1' [b]	0	R	3^+	100S	3+	a	3^+	100S	a
tr '121R63-1' [b]	3^+	80S	3^+	100S	3^+	100S	3^+	100S	a
tr '121R63-1'[c]	1	R	3+	20MR	3^+	100S	3^+	100S	a
tr '21R55'[b]	3^+	80S	2	20MR	3	a	3^+	100S	a
tr '45R35'[b]		R	3^+	60S	0	a	3+	100S	a
tr '121R127'[d]		R	3+	100S	3+	a	1	R	a
st '46S103'[e]	0	R	;2	a	0	R	3^+	100S	a
st '46S119'[e]	1 to 2	R	;23	R	3^+	80S	3^+	100S	a
st '78S84'[e]	3^+	60S	3^+	80S		R	3^+	100S	a
gr '62 G29'[f]	2^-	10MR	2	10MR	2	a	3^+	100S	3+
gr '37 G19'[f]	0	R	2	10MR	0	a	3^+	100S	0
gr '37 G3'[f]	0	R	2	R	0	a	3^+	100S	0
gr '75 G5'[f]	0	R	3	R	0	a	3^+	100S	0
Ug99[g] (TTKS)	a	80–100S	a	a	a	a	a	a	a

SRT, Seedling resistance test; APR, adult plant resistance conducted under temperature controlled condition in the polythelene house; R, resistant; MR, moderately resistant; [a]not tested; [b]tested at 22°C; [c]tested at 14°C; [d]tested at 27°C; [e]tested at 16°C; [f]tested at 24°C; [g] tested in Kenya under field condition

Table 2 F_2 and F_3 segregation for rust response and tests of allelism in crosses of 'PBW343'

Cross	Pathotype	Number of seedlings/families			Expected ratio	χ^2value	P[a]
		Resistant	Segregating	Susceptible			
Leaf rust							
'Agra Local'/'PBW343'							
F_2	pt. '109R31-1'	226		93	3R: 1S	2.82	0.09
F_2	pt. '45R35'	211		85	3R: 1S	2.08	0.14
Stem rust							
'Agra Local'/'PBW343'							
F_2	pt. '62 G29'	239		87	3R: 1 S	0.45	0.50
F_3	pt. '62 G29'	35	80	39	1R: 2seg: 1S	0.44	0.80
F_2	pt. '75 G5'	217		18	15R: 1S	0.60	0.43
F_3	pt. '75 G5'	60	83	11	7R: 8seg: 1S	1.47	0.47
Stripe rust							
'Agra Local'/'PBW343'							
F_2	pt. '46S103'	424		33	15R: 1S	0.60	0.43
F_3	pt. '46S103'	49	62	6	7R: 8seg: 1S	0.53	0.76
Test of Allelism							
PBW343'/'Tc*Lr26* F_2	pt. '121R163-1'	279		0	1R: 0S		>0.99
PBW343/Tc*Lr10* F_2	pt. '45R35'	347		29	1R: 0S		<0.001
PBW343/Agra Local F_2	pt. '121R127'	328	46[b]	33	1R: 0S		<0.001
PBW343/IWP94 F_2	pt. '121R127'	245		0	1R: 0S		1R: 0S
PBW343/Webster F_2	pt. '46S119'	316		0	1R: 0S		1R: 0S
PBW343/Reliance F_2	pt. '75 G5'	331		0	1R: 0S		>0.99

Seg, Segregating; S, susceptible; R, resistant; [a]Values for significance at P = 0.05: at 1d.f. 3.84; at 2d.f.=5.9; [b]Intermediate response (IT 22^+)

Table 3 Co-segregation of F_3 families of 'Agra Local'/'PBW343' tested with pathotypes 62 G29 and 75 G5

		Reaction to pt. '62 G29'			
		HR	Seg	HS	Total
Reaction to pt. '75 G5'	HR	35	16	9	60
	Seg	0	64	19	83
	HS	0	0	11	11
Total		35	80	39	154
χ^2 1: 2: 1 pt. '62 G29'[a]	0.44				
χ^2 7: 8: 1 pt. '75 G5'	1.47				
χ^2 7: 14: 7: 8: 16: 8: 1: 2: 1 (independence)	106.68				

HR, Homozygous resistant; HS, homozygous susceptible; Seg, segregating; [a]values for significance at $P = 0.05$: at 2d.f. 5.9; at 8 d.f. 15.5

evade economic loss had the plants infected with leaf rust at early growth stages.

Stem rust resistance

Single dominant was involved in the resistance against pt. '62 G29' (Table 2). Two independent dominant genes imparted resistance to pt. 75 G5. Among 148 F_3 families, 60 were homozygous resistant (Table 2). The homozygous resistant families were further categorized as those which were uniformly 0; or uniformly 2^- or mixture of 0; and 2^- seedlings. Thirtyone progenies were homozygous for 0; IT's, whereas 6 families were homozygous for 2^- IT's. Preponderance of 0; families indicated that the gene responsible for 0; IT's was epistatic over the other gene that conferred 2^- IT's. The F_2 seedlings of 'PBW343'/Reliance *(Sr5*) was tested with pt. 75 G5 for detecting presence of *Sr5* in 'PBW343'. Absence of susceptible seedlings confirmed presence of *Sr5* in 'PBW343'.

The genetic analysis of F_2 and F_3 generations confirmed that 'PBW343' has 2 major genes for stem rust resistance. In the co-segregation analysis (Table 3), resistance against pathotype '75 G5' showed association with resistance to pt. '62 G29'. It was observed that all F_3 lines resistant to pt. '62 G29' were also resistant to pt.'75 G5' but many of the lines that were resistant to pt. '75 G5' were susceptible to pt. '62 G29'. Therefore, it was concluded that the gene imparting resistance against pt. '62 G29' was also effective against pt. '75 G5' but the second gene that is effective against pt. '75 G5' was susceptible to pt. '62 G29'. One of the stem rust resistance gene in 'PBW343' must be *Sr31* as it is completely linked with *Lr26*. Since *Sr31* is resistant to both pt. '62 G29' and '75 G5' (Table 1), it is the gene that provided resistance against both the pathotypes. The differential host pathogen interaction of *Sr5* and *Sr31* against pt. '62 G29', '37 G3', '37 G19' and '75 G5' indicated presence of *Sr5* in 'PBW343' (Table 1). The stem rust resistance genes *Sr5* and *Sr31* were previously postulated in 'PBW343' (Nayar *et al*. 2001). Based on genetic analysis and gene matching technique it was concluded that 'PBW343' carries at least two major stem rust resistance genes, namely *Sr5* and *Sr31*. Though the adult plants of 'PBW343' were resistant to stem rust pathotypes of India but it is important to note that it was heavily infected with stem rust at Kenya (Table 1) and, therefore, this variety is definitely vulnerable to stem rust pt. 'Ug99' which is prevalent in Kenya and other adjoining nations. This new stem rust pathotype may reach Indian sub-continent through either of the 3 possible pathways (Singh *et al.* 2006). Out of the three possibilities, natural migration of 'Ug99' in India is unlikely as per the *Puccinia* path (Nagarajan and Joshi 1985). Although this hypothesis sounds good but considerable risk is associated with the cultivation of 'PB343' in a large area either due to accidental introduction of 'Ug99' or evolution of similar isolate in peninsular India.

Resistance to stripe rust

The seedlings of 'PBW343' was resistant to pt. '46S119' and susceptible to pt. '78S84' whereas adult plants of 'PBW343' showed terminal disease severity to 60S against pt.'78S84' as compared to 100S response of 'Agra Local' (Table 1). Two dominant genes governed resistance against pt. '46S103' (Table 2).

An earlier genetic study indicated that single dominant gene governed resistance against pt. '46S119' (Datta *et al.* 2009). Co-segregation of resistance in the F_3 against pt. '46S103' and '46S119' showed association (Table 4). It was observed that few F_3 lines were either resistant or susceptible to both pathotypes, whereas some were resistant to only '46S103'. It was concluded that the gene imparting resistance against pt. '46S119' was also effective against pt. '46S103' but the second gene that is effective against pt. '46S103' was susceptible to pt. '46S119'. One of the dominant genes that gave resistance to pt. '46S103' must be *Yr9* which is postulated in 'PBW343' because it is completely linked with *Lr26*. The second stripe rust resistance gene of 'PBW343' must be *Yr27* because the characteristic infection types of

Table 4 Cosegregation of F_3 families of 'Agra Local'/'PBW343' tested with pathotypes '46S103' and '46S119'

		Reaction to pt. '46 S103'			
		HR	Seg	HS	Total
Reaction to pt. '46S119'	HR	23	0	0	23
	Seg	21	43	0	64
	HS	5	19	6	30
Total		49	62	6	117
χ^2 1: 2: 1 pt. '46S119'[a]	1.87				
χ^2 7: 8: 1 pt. '46S103'	0.53				
χ^2 7: 8: 1: 14: 16: 2: 7: 8: 1 (independence)	51.18				

HR, Homozygous resistant; HS, homozygous susceptible; Seg, segregating; [a]values for significance at P=0.05: at 2d.f. 5.9; at 8 d.f.=15.5

Webster (a line carrying *Yr27*) was similar to 'PBW343' (Table 1). The F_2 seedlings derived from 'PBW343'/ 'Webster' were tested with pt. 46S103. Absence of susceptible (3+) seedlings authenticated presence of *Yr27* in 'PBW343'. This conforms to the postulation of *Yr27* in 'PBW343' by McDonald *et al.* 2004. Therefore, based on genetic linkage, inheritance and characteristic infection types, *Yr9* (resistant to pt. '46S103') and *Yr27* (resistant to pt. '46S103' and '46S119') are confirmed in 'PBW343'. In an independent study (Nayar *et al. 2001*) stripe rust resistance gene *Yr18* was also postulated in this variety. An earlier report from our lab has indicated presence of at least one adult plant stripe rust resistance genes in 'PBW343' (Datta *et al.* 2009). In spite of adult plant stripe rust resistance genes, 'PBW343' has shown susceptibility to pt.'78S84' which was detected in 2001 but its population is negligible (Prashar *et al.* 2007). Therefore, under present circumstances 'PBW343' is practically free from stripe rust and it appears that it is safe from stripe rust in India until increase in frequency of pt. '78S84' or migration of more virulent pathotype or evolution of new pathotype. Though 'PBW343' possess unknown adult plant resistance genes in addition to *Yr9* and *Yr27* but it may not provide adequate protection against new virulent pathotypes and hence, if frequency of pt. '78S84' increases to an alarming level it may be devastating. The level of adult plant resistance in 'PBW343' was low when tested with Mexican stripe rust pathotypes having combined virulence for *Yr9* and *Yr27* (Singh and Huerta-Espino 2000).

'PBW343' possess *Lr26*, *Sr5*, *Sr31*, *Yr9*, *Yr27*. 'PBW343' also possessed at least 3 unknown leaf rust resistance genes and an unknown stripe rust APR gene (Datta *et al.* 2009). There is not only constant threat of new stripe rust pathotypes with combined virulence for *Yr9* and *Yr27* along the Punjab border but also there is considerable threat of 'Ug99' type stem rust pathotype either through introduction or due to evolution of existing pathotypes. This warrants to breed substitutes for 'PBW343' which have effective, multiple and diversified seedling and durable adult plant rust resistance genes.

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