Genetic basis of stripe rust resistance in transgressive derivatives of a cross between susceptible bread wheat parents

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Abstract

Parental lines, F_1s, F_2 populations as well as F_3 and F_4 families and F_6, F_7 RILs from bread wheat cross PBW 621 x HD 2967 were evaluated for stripe rust resistance against a highly virulent pathotype in experiments spanning six crop seasons. HD2967, the widely cultivated wheat variety in North Western Plains of India and PBW 621 have shown successively lowered levels of stripe rust resistance over the years. F_1 of these parents showed lack of dominance. The segregation pattern for stripe rust resistance in F_2, F_3 and F_4 showed transgressiveness for resistance. Chi-square analysis indicated that resistant segregants possess two genes, one contributed by PBW 621 and the other by HD 2967. These results were confirmed over two years using F_6 and F_7 RILs. Possible genetic mechanisms for this residual resistance and implications for breeding programme are discussed.

Key words: *Puccinia striiformis* f. sp*. tritici, Triticum aestivum,* genetic analysis, residual resistance, transgression.

Introduction

Wheat (*Triticum aestivum*) is the leading cereal grown and consumed globally. Stripe rust caused by *Puccinia striiformis* f. sp*. tritici* is presently the most important disease of wheat (Wellings, 2011). The wide distribution and frequent occurrence of stripe rust epidemics in wheat production zones demand continuous efforts for identification of new sources of resistance (Virdi et al. 2016) and developing and deploying control strategies (Chen et al. 2014). In India, stripe rust has assumed a regular and significant presence in an extensive belt adjoining the Himalayan foothills in the North Western Plains Zone since last one decade. This scenario has unfolded in the aftermath of breakdown of stripe rust resistance in mega cultivar PBW 343, followed by its high incidence since 2007 (Prashar et al. 2007). In face of the rapid evolution of *Pst*, the varietal replacement has lagged, making chemical control an important short term measure. Rapid and successive lowering of resistance was seen in an entire set of varieties including DBW 17, PBW 550, PBW 621 and HD 2967. The *Yr27* virulence which overcame resistance of PBW 343 has progressively widened its varietal host range. The pattern of evolution is typical of escalating aggressiveness rather than virulence. Large pathogen population sustained by presence of a susceptible host over a significant area, an environmentally robust race, multiple growth cycles per year, adaptive selection imposed by inoculum movement and over summering serves to automatically select for aggressiveness (Pariaud et al. 2009). The aggressiveness evolves to overcome quantitative resistance which is often governed by minor genes. These genes are however considered desirable for thwarting evolution of virulence. Thus, the evolution of aggressiveness and virulence represent two distinct modes of pathogen evolution. In the present study, an aggressive stripe rust strain carrying *Yr27* virulence and capacity for causing moderate to high levels of disease on PBW 621 and HD 2967 was used to evaluate the derivatives of PBW 621 x HD 2967 cross. As the two parents were initially resistant, the material developed from the cross was aimed at exploring and combining resistance in a highly productive genetic background. With the evolution of strains capable of causing significant levels of disease on the parental lines, the analysis of derived material has provided useful insights into phenomenon of residual, interactive resistance.

Materials and methods

The experimental materials comprised of parental lines, F_1 and F_2 populations, F_3, F_4 progenies, F_6 and F_7 recombinant inbred lines (RILs) of cross PBW 621/ HD 2967. Genotype PBW 343 was used as highly susceptible check. PBW 343 (ND/VG9144//KAL/BB/ 3/YACO/4/VEE#5) has been till recently most widely grown bread wheat variety in India, whereas PBW 621 (KAUZ//ALTAR84/AOS/3/ MILAN/KAUZ/4/HUITES) and HD 2967 (ALD/COC//URES/3/HD2160M/HD2278) are commercial cultivars, released in 2011 for timely sown irrigated conditions of North Western Plain Zone (NWPZ) of India. The parental lines were screened for four seasons (2010-11 to 2013-14) using stripe rust inoculum collected afresh from a single infection focus as it appeared in December or early January in the Anandpur Sahib area (31.23°N, 76.51°E) of Ropar district, Punjab, India. The screening for genetic analysis over three years (2013-14 to 2015-16) was performed by using inoculum from a single source, collected in 2013-14 and is hereafter referred to as *Pst* pt. AS. The inoculum was multiplied on PBW 343 grown in isolated chambers. The pathotype showed avirulence on PBW 343 isogenic materials carrying *Yr5, Yr10, Yr15* genes and moderate virulence on *Yr17, Yr40* and *Yr70* genes*. The F_{1s} along with F_2 and F_6 populations were screened in 2013-14 crop season, F_3 families and F_7 lines in 2014-15 and resistant and susceptible F_4 progenies bulks in 2015-16.

Pathotypes *Pst* pt. AS was used for seedling response tests of parental lines. Fully expanded primary leaves (7-9 days old) were inoculated with urediniospores suspended in a mixture of 30:70 mineral oil and petroleum ether. Plants were then transferred to a dew chamber set at 10°C with 16h light and 8h dark photoperiod for 48 hrs. The plants were then transferred to the green house set at 14-16°C temperature. Stripe rust assessment at seedling stage was performed 14 days after inoculation (Nayar et al. 1997). Infection types (ITs) for seedling response were recorded on a 0-4 scale, where 0 represents immune and 4 equates to highly susceptible (Roelfs et al. 1984). For adult plant reaction, parental lines (PBW 621 and HD 2967) were space planted during 2013-14 crop season in rows of 2m length with row to row spacing of 30 cm and were inoculated with *Pst* pt. AS in the field. Also, single plants of F_2 populations were space planted with rows of parental checks at regular intervals during this season. During 2014-15 crop season, about 40-50 plants of each F_3 families of the cross were screened in paired rows. The

parental lines were planted after every twenty five test entries. The F_4 progenies derived from single plants of non-segregating resistant and susceptible F_3 families along with parents were screened during 2015-16 crop season.

For field inoculations, urediniospores of pathotype *Pst* pt. AS suspended in 10L water added with two drops of Tween 20 were sprayed during end December to early January using an ultra-low volume applicator on clear evenings with good expectations of dew to ensure good disease development. In this study, field assessment of adult plant stripe rust responses was based on a continuous 1-9 scale (Bariana et al. 2007). Three successive disease observations were recorded on parental lines at 10 day intervals starting in first week of February. As observations were recorded on successive leaves, there is a possibility of increase as well as decrease in the level of disease. The disease score on other test entries was recorded when the susceptible check (PBW 343) had reached score of 8 on 1-9 scale, corresponding to 80-90% leaf area affected (as observations were recorded on successive leaves, there was a possibility of increase as well as decrease in the level of disease).

Statistical Analysis

Chi-square tests were used to determine the goodness of fit of the observed number of plants or lines to the predicted segregation ratios of F_2 population; F_3 and F_4 progenies to establish the number of stripe rust-resistance genes and mode of inheritance. The Chi-square value was calculated using standard procedure.

Results and discussion

The initial screening of parental lines was performed against new versions of stripe rust inoculum collected during initiation of epidemic in each crop season from the first foci that formed in the farmers' fields. The average scores on PBW 621, HD2967 and PBW 343 obtained from this screening performed over four seasons (2010-11 to 2013-14) is given in Table 1. The disease levels rose across the seasons, indicating rapid pathogen evolution, primarily for aggressiveness as rise was similar for both parental lines. Although environment was sequentially conducive over the years for pathogen sporulation, yet the same inoculum source was used for genetic analysis in each crop season and successively there was escalation in the pathogenicity of the pathotype, thus every time providing an enhanced susceptibility status of the

Table 1. Average disease score of parental lines against naturally available inoculum of *Pst* pt. AS of stripe rust at adult plant stage during different crop seasons

Parental lines	2010-11	2011-12	2012-13	2013-14
PBW 621	2.5	2.9	3.4	5.05
HD 2967	1.8	2.0	3.4	5.40
PBW 343 (Susceptible check)	8.40	8.50	9.00	9.00

parentals used in the study. This genotypic non-specific trend was also evident for several other lines (data not given here). The genetic analysis of related material was subjected to screening for the first season in 2013-14.

To obtain consistent and comparable inferences, this inoculum (pathotype strain representing aggressive *Yr27* gene virulence collected in the 2010-11 season from a farmer's field in the foothill area of Anandpur Sahib (31.23°N, 76.51°E) in Punjab, India and multiplied on PBW 343 for future inoculations) was preserved for use in screening in the subsequent two seasons as well. In the seedling tests of parental lines both PBW 621 and HD 2967 had a susceptible IT score of 3 as compared to highly susceptible infection type of 4 recorded on PBW 343. The adult plant stripe rust scores of parental lines and their F_1 is given in Table 2. The two parents showed moderate

dominance of two genes coming together, although the resultant disease development on F_1 was slightly less than the parents).

The segregation pattern for stripe rust resistance in F_2, F_3 and F_4 generations of the cross PBW 621 x HD 2967 is presented in Table 3. A small number of

Table 3. Segregation of F_2, F_3 and F_4 generations of cross PBW 621 x HD 2967 against *Pst* pt. AS

Phenotypic classes	Disease score	F_2 plants	F_3 lines	F_4 progeny	
				Homo-zygous	Segre-gating
Resistant	1-4	44	43	40	3
Intermediate or sgregating	5-7	515	505	-	-
Susceptible	8-9	41	52	50	2

χ^2 calculated for two additive genes (d.f. =2); 1.65(p=0.438); 3.10(p=0.212); 1.066(p=0.568)

qualitatively distinct classes with wide separations were not evident. However, extreme categories could be demarcated. For instance, there were plants in F_2 which showed greater resistance than the resistant parents in the crosses. These were clubbed in one transgressive category. This category was represented by seedling scores of 1 to 4 (up to 20S in the modified Cobb's scale). The parents PBW 621 and HD 2967 had shown an average score range of 5.05 to 5.65 and 5.40 to 6.20, respectively over the three years of testing (Table 2). Several F_2 plants displayed

Table 2. Average Disease Reaction of parental lines and their F_1s against *Pst* pt. AS of stripe rust at adult plant stage during different crop seasons

Parents and F_1 hybrid	2013-14 season			2014-15 season			2015-16 season		
	Score I	Score II	Final score	Score I	Score II	Final score	Score I	Score II	Final score
PBW 621	3.75	4.20	5.05	4.15	6.05	5.60	4.45	6.10	5.45
HD 2967	3.70	4.55	5.40	4.55	5.65	6.15	4.80	5.75	6.20
PBW 343 (control)	8.60	8.90	9.00	8.80	9.00	9.00	8.80	9.00	9.00
PBW 621/HD 2967	3.87	4.95	5.19	-	-	-	-	-	-

susceptibility with scores of 5.05 to 5.60 in case of PBW 621 and 5.40 to 6.20 for HD 2967. PBW 343 scores invariably touched highest level of 9. Stripe rust reaction was recorded on the available 87 F_1 plants of cross PBW 621 x HD 2967 and an average final score of 5.19 was obtained which was almost at par with the two parents (it was a resultant of lack of

susceptibility beyond these parental values and had rust scores of 8 to 9 (corresponding to 80S-100S on the modified Cobb's scale). Demarcating extreme categories and clubbing intermediate disease categories, the F_2 in the cross tended to have a symmetric distribution. Out of the 600 F_2 plants screened, 44 showed resistant scores of 1-4, 515

plants occupied intermediate category of 5-7 and 41 were highly susceptible (scores 8-9). This trend was largely preserved in the F_3 lines derived from the 600 F_2 plants. Presence of a strong genetic foundation at the base of the observed classes was evident from largely true breeding nature of both resistant and susceptible progenies. In the F_4 derived from randomly taken five plants from each seemingly homozygous F_3, it was observed that, only 3 of the 43 resistant progenies and 2 of the 50 susceptible progenies showed segregation. An F_4 family was declared segregating if at least one of the five single plant progenies from an F_3 line showed segregation. On the whole, F_4 further confirmed and fine-tuned the segregation pattern. In all these generations the resistance over and above the better parent is in all probability contributed by a combination of genes from both the parents. The resistant derivatives are thus hypothesized to harbour two genes, one each from PBW 621 and HD 2967. Conducting a chi-square analysis based on this hypothesis, on the observed categories over F_2, F_3 and F_4 generations gave highly acceptable chi-square values of 1.65 (p=0.438), 3.10 (p=0.212) and 1.066 (p=0.568), respectively (Table 3).

F_6 and F_7 RILs of cross PBW 621 and HD 2967 were also screened and analysed on the same pattern as employed for F_2 and F_3 generations to further substantiate the inferences drawn above. This set of RILs was derived independently from the above used F_2, F_3 and F_4 populations. The screening of F_6 RILs was done in 2013-14 and F_7 RILs were evaluated in 2014-15 using the *Pst* pt. AS inoculum. The large number of lines at homozygous stage, screened over two years, allowed for a highly reliable analysis. The F_6 RIL distribution showed that the highly resistant class comprised of 84 plants, intermediate class contained 1031 plants and highly susceptible class had 65 plants. A highly acceptable value of chi-square ($\chi^2_{1:14:1}$ = 2.46, p=0.292) in F_6 supported the inference of each parent contributing one resistance gene. The homozygous resistant class in F_7 contained 63 progenies and 88 progenies were present in homozygous susceptible class. The intermediate class contained 1029 progenies. The categories in F_7 were subjected to chi square analysis and an acceptable value of chi-square ($\chi^2_{1:14:1}$= 4.32, p=0.115) was obtained (Table 4).

The observed trend of increasing susceptibility of parental lines as observed for four years (2010-11 to 2013-14) in this study is well acknowledged among current workers in the region though scientific

Table 4. Segregation of F_6 and F_7 RILs of cross PBW 621 x HD 2967 against *Pst* pt.AS

Phenotypic classes	Disease score	F_6 RILs	F_7 RILs
Resistant	1-4	84	63
Intermediate or segregating	5-7	1031	1029
Susceptible	8-9	65	88
χ^2 for two additive genes (d.f. =2)		2.46 (p=0.292)	4.32 (p=0.115)

χ^2 1:141 table (d.f. =2) = 5.99 and 9.21 (p=0.01 and 0.05, respectively)

explanations may vary. Modern stripe rust lineages have shown a propensity for increased aggressiveness as reflected in their enhanced spore production potential (Milus et al. 2006). As a result, stripe rust has become severe in eastern United States, Australia and elsewhere since (Milus et al. 2009). The new isolates show improved adaptation to the warm temperature regimes for all parameters studied. A similar transition seems to be happening in stripe rust prone area of North Western India in the post PBW 343 breakdown era (Prashar et al. 2007). As a result, the inoculum collected from a specific geographic region over four seasons showed successively raised disease scores on the parental lines PBW 621 and HD 2967.

With respect to genetic analysis of resistance in PBW 621 x HD 2967 derivatives against the evolved *Pst* pt. AS, a clear indication of residual, cumulative, non-major gene resistance is available. Milus and Line (1986) had reported that the durable resistance can be partially recessive with no maternal inheritance and most of the gene actions among loci are additive. In wheat, once the genetic basis and diversity of slow rusting resistances became clearer, high-yielding lines that combined four or five additive, minor genes for both leaf rust and stripe rust resistances showing near-immune levels of resistance were developed through 3- and 4-way crosses involving lines carrying different minor genes (Singh et al. 2000) The present study had to contend with parents whose resistance had got partially dented though not fully defeated. In the absence of major resistance genes, the residual resistance is known to work in interaction with other minor genes, which originally couldn't provide adequate resistance (Singh et al. 2011). For example, German and Kolmer (1992) showed that *Lr34* gene enhanced the expression of several moderately effective race-

specific resistance genes by lowering the seedling infection types to races avirulent to the race-speciûc genes. Singh and Huerta-Espino (1995) reported that although *Lr16* gene only conferred moderate levels of leaf rust resistance in field trials in Mexico, the near-immune level of resistance in wheat varieties 'Ciano 79' and 'Papago 86' was based on the interaction of *Lr16* with two additional slow rusting genes. Similarly, immunity to stripe rust in wheat variety 'Pastor' involved the moderately effective race specific resistance gene *Yr31* and slow rusting genes *Yr29*, *Yr30* and possibly one additional minor gene (Singh et al. 2003). The alternate can be using these defeated genes in association with other minor genes. In a study by Silva et al. (2015) it has been shown that interactions of APR genes *Lr34*, *Lr68* and *Sr2* are very effective in achieving durable leaf rust resistance in wheat which is quantitatively inherited.

The results are thus in consonance with widely reported additivity of minor gene conferred rust resistance as well as of susceptible parents contributing to resistance in a complementary manner. It may be hypothesized that even when an aggressive race runs down a variety to almost complete susceptibility, it seems to overwhelm only a subset of diverse quantitatively determined resistance mechanisms. As a result, such susceptible lines have the potential for resistance when recombination provides the opportunity for a new assemblage of resistance components. The materials from the present study are being investigated further to assess if the surviving resistance components are more durable than the ones which were overwhelmed by the pathogen. Another moot question is whether the observed resistance is governed by the defeated major genes, the so called ghost resistance hypothesis. This is being addressed by monitoring presence of *Yr17* (from PBW 621) in resistant derivatives. Since, the source of resistance in HD 2967 is not known till now, so monitoring those gene(s) would be inappropriate in present frame of time.

Authors' contribution

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

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