



Generation mean analysis of resistance to white rust in Indian mustard

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Abstract

White rust is an economically important disease of oilseed *Brassicas* and generation mean analysis of three crosses was done to study the nature and magnitude of gene action governing resistance to this disease. Simple additive(d)-dominance(h) model could not explain the genetics of white rust resistance suggesting the presence of non-allelic gene interaction. The estimates of additive \times additive (i), additive \times dominance (j) and dominance \times dominance (l) gene effects were significant for the crosses Varuna \times BioYSR and Kranti \times Heera, however barring additive \times dominance (j) gene effect, all other gene effects were significant for the cross RH 749 \times BioYSR. The magnitude of non-fixable gene effect (h+l) was greater than fixable gene effect (d+i) for all the crosses. The opposite sign of h and l suggested presence of duplicate gene action for white rust resistance in all the crosses. The magnitude of broad sense heritability was high in all the crosses with desirable mid-parent heterosis.

Keywords: Epistasis, gene interaction, GMA, Indian mustard, white rust

Introduction

India is among the largest vegetable oil economies in the world next to USA, China and Brazil (Choudhary *et al.*, 2023). The diverse agro-ecological conditions in the country are favorable for growing nine annual oilseed crops which include seven edible and two non-edible types. However, India is among the largest importers of edible oils and oilseeds. Rapeseed-mustard comprise a group of important oilseed crop that stands next to soybean in terms of area and production however, contributes maximum (about 36%) towards edible oil in India (Rai, 2023). The major mustard producing states are: Rajasthan, Madhya Pradesh, Haryana, Uttar Pradesh, West Bengal, Gujarat, Jharkhand, Assam, Bihar and Orissa. Mustard seed contains about 38 to 43 per cent oil which is golden-yellow in colour, fragrant and is considered to be one of the healthiest and nutritious cooking medium.

Indian mustard (*Brassica juncea* L.) is affected by several biotic stresses of which, white rust caused by *Albugo candida* is an important disease leading to more than 40% yield decline in years with severe disease intensity (Lakra and Saharan, 1989). *Albugo candida* is an obligate oomycete biotroph that infects all the major oilseed brassica across continents. The disease symptom appears as white-blister like pustules on underside of foliage with systemic infection leading to hypertrophy and

hyperplasia of floral parts, termed as staghead. The microbe has a wide host range infecting about 63 genera and 241 plant species (Gupta and Saharan, 2002). Pound and Williams (1963) identified 6 races of *Albugo candida* and reported race 2 to infect *B. juncea*. The pathotypes of *Albugo candida* in Indian mustard has not yet been identified. The genome size of *Albugo candida* is 45.3 Mb. Oomycete fungus like *Albugo candida* possess a repertoire of several genes encoding pathogenicity related factors such as CRN effectors, R X LR effectors and Elicitins (Meijer *et al.*, 2014). The resistance against this pathogen has been observed to be governed by NB-LRR (Nucleotide Binding Leucine Rich Repeats) effector proteins in *Brassica juncea* (Arora *et al.*, 2019). Protective and systemic fungicides are being used against white rust however, resistant genotypes provide cost-effective, sustainable and eco-friendly approach for disease management. The most critical factor in developing white rust resistant cultivar through resistance breeding approach is the availability of genes for resistance against white rust in the gene pool of Indian mustard.

There are several known resistant sources available against white rust disease. It has been reported that white rust resistance in *B. juncea* is governed by a single dominant gene in several studies (Panjabi-Massand *et al.*, 2010; Behera *et al.*, 2016; Arora *et al.*, 2019). Furthermore, recent studies have also reported the white rust resistance to be governed by a pair of genes (Chand

et al., 2022). The genetics of inheritance determines the suitability of a particular breeding method in achieving desired objective.

The choice of appropriate breeding method depends largely on nature of gene action and number of genes governing the trait. Generation mean analysis is a suitable statistical procedure that detects nature of gene action involved in inheritance of traits including disease resistance, so as to select the most appropriate breeding programme to be followed. Therefore, a generation mean analysis study was undertaken to determine the nature and magnitude of gene action and, estimate heritability along with heterosis for resistance to white rust under field conditions.

Materials and Methods

Six generations (P_1 , P_2 , F_1 , F_2 , B_1 and B_2) of 3 crosses: Varuna \times BioYSR, RH 749 \times BioYSR and Kranti \times Heera, were grown in the *rabi* season of 2020-2021 at agricultural research farm, Banaras Hindu University, Varanasi in compact family block design. Previously, the parents were crossed in the year 2019 during *kharif* at IARI regional research station, Wellington and F_1 was obtained. The parents were again crossed in the *rabi* of 2019-20 to obtain fresh F_1 's and F_1 's obtained from Wellington were backcrossed with parents and simultaneously selfed to obtain backcross and F_2 generations, respectively. The alluvial sandy loam soil at experimental site was low in available N at the time of sowing. The content of available P, K and Zn in the soil was 23.6-34.2 kg/ha, 185-252 kg/ha and 6.4 ppm, respectively. The electrical conductivity value of the soil was 0.15 to 0.33 dS/m at 25°C and bulk density was 1.35 to 1.75 g/cm³. The weather data for *rabi* 2020-21 has been given in Figure 1.

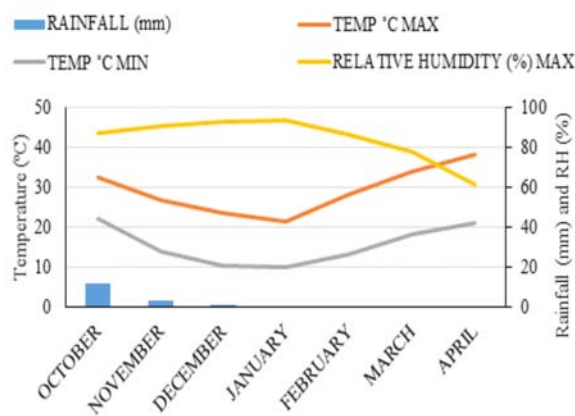


Fig. 1: Weather conditions during the crop growing period in 2020-21

Disease screening

Artificial epiphytotic condition was achieved in the experimental field by initial mixing of seeds and soil with stagheads containing oospores at the time of sowing and further inoculation with sporangial suspension at 35, 50 and 65 days after sowing. Frequent irrigations were done to maintain high moisture condition for disease development and all other recommended package of practices were followed. White rust sporangial inoculum was prepared by collecting fresh sporangia from naturally infected leaves and suspending collected zoosporengia at 4 °C for 2 hours in petri plate to facilitate its germination. The sporangial load in final sporangial suspension was adjusted to 2×10^4 sporangia/ml for spray inoculation. Percent Disease Index (PDI) at 75 days after sowing was recorded following 0-9 scale (Williams, 1985). The observations were recorded on 15 plants of P_1 , P_2 and F_1 ; 150 plants of B_1 and B_2 and 210 plants of F_2 .

Statistical Analysis

Estimates of PDI were transformed to arc sine values for generation mean analysis. The joint scaling test was done as per Cavalli (1952) and estimated chi-square values were tested for significance at 3 degrees of freedom. The presence of epistasis was detected using A, B and C scaling test proposed by Hayman (1958). The scales were tested for significance using t test. Generation mean analysis was done as per Hayman (1958). The notations used as per the model were: m = mean of the F_2 generation, d = additive gene effect, h = dominance gene effect, i = additive \times additive gene effect, j = additive \times dominance gene effect and l = dominance \times dominance gene effect. The significance of estimated gene effects was tested using t-test. The non-significant interaction effects were removed and estimation of gene effects was further done using weighted least-square method of Cavalli (1952) as per Hayman (1958) model. The re-estimated values were tested for significance using chi square values. The nature of epistasis was determined by the signs of estimates of h and l.

Results and Discussion

The mean and standard errors for parents (P_1 and P_2), F_1 , F_2 and backcross generations (B_1 and B_2) have been presented in Table 1. Varuna, RH 749 and Kranti had high values for PDI and thus, were susceptible to white rust. Varuna, Kranti and RH 749 has been reported to be susceptible (Uhukral and Singh, 1986; Yadav *et al.*, 1996; Sachan *et al.*, 2000; Panjabi-Massand *et al.*, 2010; Singh *et al.*, 2020a; Devi *et al.*, 2022). Varuna, Kranti and RH 749 are high yielding varieties of Indian mustard. Genotypes

BioYSR and Heera having low PDI values were observed to be resistant to white rust. BioYSR and Heera have been reported to be resistant against white rust (Yadav *et al.*, 1996; Varshney *et al.*, 2004; Panjabi-Massand *et al.*, 2010; Vignesh *et al.*, 2010; Singh *et al.*, 2020b; Chand *et al.*, 2022; Devi *et al.*, 2022). Thus, parents had contrasting values for the trait which is a prerequisite for generation mean analysis (Mather and Jinks, 1971). The PDI values for F_1 was skewed towards resistant parent for all the crosses with value of F_1 -Varuna \times BioYSR lower than its resistant parent BioYSR. Thus, resistance was observed to be dominant to susceptibility in this study, as has been previously reported (Vignesh *et al.*, 2010; Singh *et al.*, 2016; Chand *et al.*, 2022). The lower values of F_1 -Varuna \times BioYSR could be due to dispersion of genes

between parents or overdominance at one or more loci governing resistance. The F_2 mean values for PDI were lower than the susceptible parent and higher than the resistant parent for all the crosses due to segregation and recombination at loci governing resistance. The backcrosses of F_1 with susceptible high yielding parent, yielded progeny with PDI values greater than F_1 showing that the alleles contributed by susceptible parent leads to increased susceptibility. The backcross of F_1 with resistant parent led to progeny with lower values of PDI compared to resistant parent and F_1 , for the cross RH749 \times BioYSR indicating non-allelic interaction leading to increased resistance in B_2 generation. The B_2 of the crosses Varuna \times BioYSR and Kranti \times Heera had higher values of PDI than F_1 and resistant parent suggesting epistasis resulting in increased susceptibility.

Table 1: Means and standard error of all 6 generations of the three crosses studied

Generations	Crosses		
	Varuna \times BioYSR	RH749 \times BioYSR	Kranti \times Heera
P_1	44.5** \pm 0.6	42.4** \pm 1.7	37.9** \pm 1.0
P_2	3.7** \pm 0.9	14.2** \pm 0.5	1.8** \pm 0.5
F_1	3.2** \pm 0.9	15.3** \pm 0.9	2.7** \pm 0.8
F_2	16.1** \pm 1.2	33.0** \pm 0.9	33.9** \pm 1.0
B_1	32.9** \pm 1.1	27.0** \pm 1.2	26.8** \pm 1.2
B_2	22.2** \pm 0.4	13.3** \pm 0.3	14.0** \pm 0.3

The estimates of m, d and h as per joint scaling test of Cavalli (1949) has been given in Table 2. The significance of chi square value in joint scaling test suggested that simple additive-dominance model could not satisfactorily explain the inheritance of white rust resistance, in all the crosses. The significance of one or more scales in scaling test also reveals the inadequacy of simple additive-dominance model in explaining resistance. The estimates of A, B and C scales has been given in Table 3.

Furthermore, all three scales *i.e.* A, B and C were significantly different from zero for Kranti \times Heera and Varuna \times BioYSR. Scales B and C were significant for RH 749 \times BioYSR. Thus, interaction effects played an important role in genetics of white rust resistance. The presence of significant non-allelic interaction for white rust resistance has also been reported by Uhukral and Singh (1986) and Chaurasia *et al.* (2014).

Table 2: Joint scaling test for the three crosses

Gene effects and chi square value	Crosses		
	Varuna \times BioYSR	RH749 \times BioYSR	Kranti \times Heera
M	30.9** \pm 0.5	30.4** \pm 0.7	23.7** \pm 0.5
D	13.7** \pm 0.5	16.8** \pm 0.7	17.8** \pm 0.5
H	-15.8** \pm 1.0	-15.8** \pm 1.0	-8.3** \pm 0.9
Chi square value	656.0**	155.3**	642.9**

Table 3: Scaling test for three studied crosses

Scaling Test	Crosses		
	Varuna \times BioYSR	RH749 \times BioYSR	Kranti \times Heera
A	18.1** \pm 2.4	-3.7 \pm 3.1	13.0** \pm 2.7
B	37.6** \pm 1.5	-2.9** \pm 1.1	21.7** \pm 1.1
C	9.9** \pm 5.1	44.6** \pm 4.5	90.3** \pm 4.3

The estimated values of gene effects has been presented in Table 4. The estimated additive, dominance and non-allelic interaction effects were significant for crosses Varuna \times BioYSR and Kranti \times Heera. The cross RH 749 \times BioYSR showed non-significant additive \times dominance effect and therefore, this component of gene action was removed and re-estimation of gene effects was done using weighted least square method. The chi square value at 1 degree of freedom was non-significant indicating adequacy of estimated values. The significance of non-allelic interaction effects for white rust resistance has been reported by Uhukral and Singh (1986) and Chaurasia *et al.* (2014).

The magnitude of additive \times dominance (j) gene effect was low for Varuna \times BioYSR and Kranti \times Heera. The fixable components of gene effects viz. additive (d) and additive \times additive gene effects (i) can be harnessed by selection of superior homozygotes in later generations through conventional breeding. The non-fixable gene effects viz., dominance (h), additive \times dominance (j) and dominance \times dominance (l) can be utilised by heterosis breeding to obtain superior hybrids or; diallel selective mating or bi-parental mating with recurrent selection to obtain superior purelines. Dominance \times dominance (l) interaction effect was observed to be the most important factor for white rust resistance in the cross Varuna \times BioYSR whereas, dominance was the major contributor

in case of RH 749 \times BioYSR and Kranti \times Heera. The signs of dominance effects (h) and dominance \times dominance effects (l) were observed to be opposite in all the crosses, suggesting the presence of duplicate type of non-allelic gene interaction in trait inheritance. The gene-interaction is of complementary type when h and l have same sign whereas, opposite sign indicates duplicate epistasis (Kearsey and Pooni, 1996).

Although fixable gene effects viz. additive (d) and additive \times additive gene effects (i) were observed to be significant for white rust resistance, non-fixable gene effects viz. dominance (h) and dominance \times dominance (l) had larger magnitude having greater influence on rust resistance. Thus, mating design such as diallel selective mating or biparental mating coupled with recurrent selection can be employed to generate more heritable variation to exploit both additive and non-additive gene effects, for obtaining superior purelines (Shashikumar *et al.*, 2010). The predominance of non-fixable gene effects can also be exploited using hybrid breeding programme.

The estimates of broad sense heritability, mid parent heterosis and heterobeltiosis has been presented in Table 5. The estimates of broad sense heritability were high indicating greater contribution of genotype towards trait vis-à-vis environment in the study. The estimates of mid-parent heterosis was significant and considerably

Table 4: Gene effects, chi square values, nature of epistasis for all the crosses

Gene effects	Crosses			
	Varuna \times BioYSR	RH749 \times BioYSR	Kranti \times Heera	
	Hayman	Hayman	Cavalli	Hayman
m	16.1** \pm 1.2	33.0** \pm 0.9	78.9** \pm 4.0	33.9** \pm 1.0
d	10.7** \pm 1.2	13.7** \pm 1.2	14.0** \pm 0.7	13.7** \pm 1.2
h	24.9** \pm 5.4	-64.1** \pm 4.6	-120.3** \pm 8.5	-72.7** \pm 4.6
i	45.8** \pm 5.3	-51.2** \pm 4.5	-50.8** \pm 4.1	-55.6** \pm 4.5
j	-9.7** \pm 1.3	-0.4 \pm 1.5		-4.3** \pm 1.4
l	-101.4** \pm 7.0	57.8** \pm 6.6	56.7** \pm 4.9	21.0** \pm 6.6
$\div 2$ value	-	-	0.1(ns)	-
Epistasis	D	D	D	D

D: Duplicate epistasis, ns: non-significant

** Significant at 5%

Table 5: Broad sense heritability, mid-parent heterosis and heterobeltioses for the three studied crosses

Crosses	Broad sense heritability	Mid-Parent heterosis (%)	Better parent heterosis (%)
Varuna \times BioYSR	1.0	-86.6**	-13.1**
RH749 \times BioYSR	0.9	-45.8**	8.2**
Kranti \times Heera	1.0	-86.2**	55.0**

**significant at 5%

skewed in favour of resistance, further indicating the presence of dominance gene effect. The negative value of better parent heterosis for the cross Varuna × BioYSR showed that the hybrid was even better for disease resistance compared to its parent BioYSR for disease resistance. Thus, hybrids can be a suitable option for deployment in farmer's field to combat the disease.

Conclusion

The scaling tests and joint scaling test showed the inadequacy of additive-dominance model in explaining inheritance of white rust resistance in all the crosses. The estimates of non-allelic interaction effects were significant in all the crosses except additive × dominance component for the cross Varuna × BioYSR. Duplicate gene action for white rust resistance was observed in all the crosses suggesting the suitability of diallel selective mating or biparental mating coupled with recurrent selection to obtain desirable white rust resistant purelines. The estimates of broad sense heritability indicated that genotypic component of variance was high in the study. Among the crosses, Varuna × BioYSR showed significant desirable better parent heterosis while, all the crosses exhibited desirable significant mid-parent heterosis for white-rust resistance. Thus, hybrids can be deployed for white rust resistance, in farmer's field for resistance against white rust.

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