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CONTENTS

Identification of new source of white rust resistance in Indian mustard [*B. juncea* (L.) Czern & Coss] from germplasm collected from Uttarakhand hills

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ABSTRACT: Indian mustard (*Brassica juncea* L. Czern & Coss) is a major oilseed crop with more than 90% share in the acreage of oilseed brassicas grown in India. White rust is one of the major diseases that hamper the productivity and production to a considerable level. One collection (GP-11-222) from village-Van, Block-Dewal of district Almora (Altitude 7612 msl, Longitude 790 37.454; Latitude 300 11.45) grown in the field during 2011-12 showed segregation for several traits including reaction against white rust disease. The crop season during 2011-12 at Pantnagar was highly congenial for development of white rust disease therefore, helped in screening of germplasm for the diseases under natural conditions in the field. As a result of which 22 plants showing resistance to white rust were selected. Screening of descendants of selections from 2011-12 to 2016-17 in the field under artificially inoculated condition resulted into 14 promising lines. During 2015-16 and 2016-17 these 14 selected lines were screened in the field as well as in the glasshouse under controlled condition by the pathologist. All the 14 lines were found completely free from white rust infection in field condition while at cotyledonary stage four progenies have shown immune reaction and six progenies have shown resistance reaction. At true leaf stage only four progenies have shown resistance reaction. Out of these four progenies, only one progeny had also shown at par performance in terms of morphological traits. Genetic study indicated presence of single dominant gene conferring resistance against white rust. Allelic study showed presence of a different source of resistance gene in the identified line. Further studies on its molecular verification need to be done.

Key words: Germplasm, genetic study, Indian mustard, white rust resistance

Oilseed Brassica is the second most important edible oil crop of the world annually producing more than 26 million tonnes (mt) oil from almost 37.6 million hectare (mha) cultivated area (FAOSTAT, 2020). In India, it is the most important oilseed crop contributing 35% production (2.58 mt) to primary sources and almost one-fourth to total domestic edible oil production of 11.0 mt (DAC&FW, 2019), although it is second in normal acreage (6.13 mha) after soybean (11.25 mha). Indian mustard (*Brassica juncea* L.) is a major brassica oilseed crop with about 90 per cent of its share in area and production of rapeseed-mustard in India but still insufficient to fill the gap between demand and supply due to susceptibility to many diseases. Foliar diseases, particularly *Alternaria* blight, white rust and downy mildew are major contributors limiting crop productivity.

The white rust (WR) disease is one of the destructive diseases of mustard in India and Canada. Most of the released Indian cultivars are highly susceptible to white rust. The fungus causes local and systematic infection. White rust caused by *Albugo candida* is widely prevalent and most destructive disease of mustard causing upto 59 to 89.8% loss in seed yield. *Albugo candida* has a wide host range and about 300 hosts are known. Local infection produces white to cream-colored pustules on the undersides of leaves and on stems, while systemic infection causes inflorescence malformation known as staghead. The staghead is the major cause of the yield loss. Thirteen races of this pathogen have been reported from different *Brassica* species (Verma *et al.*, 1999), the predominant race of white rust infecting *B. juncea* being identified as race-2 (Petrie 1988, Rimmer *et al.,* 2000). Genetic analysis in *B. juncea* revealed that resistance to race-2 is controlled by a single dominant gene (Tiwari *et al.,* 1988 and Sachan *et al.*, 1995). Management of diseases had been suggested by scientists (Bhatt *et al*., 2009) to minimize the losses though development of

resistance cultivar is most inexpensive and effective approach. Studies on identification and cloning of plant defense resistance genes are in progress. Research towards development of resistant varieties is vigorously pursued for the past more than four decades in different crops (Singh and Pandey, 2007; Saxena *et al*., 2009) against major diseases involving conventional, Biochemical (Bhatt and Khanna, 2006 and Limbu *et al.,* 2013) and molecular techniques. Resistance sources, both exotic and Indian, have been identified and utilized to improve the inherent ability of new varieties. The germplasm have been collected and screened for unique traits like quality, fitness against different stresses (Tewari and Pandey, 2014; Sah and Khanna, 2010 and Reddy and Khanna, 2010). Indigenous sources developed particularly from adapted germplasm are potentially more useful for their direct use in breeding by conventional breeding methods especially if they possess agronomically desirable traits related to yield. Further the dynamic changes in race composition of the pathogen often result in short lived efficiency of host plant resistance in the improved cultivars. The exotic sources of resistance are of not much use as they are having very poor combining ability with the Indian varieties. The germplasm available as land races, wild relatives and farmers collections always serve as great reservoir of fitness traits that are otherwise not available in the improved varieties. Introgression of fitness trait in improved cultivar has been practiced since long back. The wild relatives and related generas have also been utilized as pre-breeding activities in almost all major crops through intersepecific and/or intergeneric hybridization (Sahoo *et al.*, 2021 and Bhatt and Khanna, 2010). The *Tarai* belt especially Pantnagar has been considered as '*hot-spot'* for many diseases thereby chances of prevalence of resistance sources are innumerable. Many areas in the hills of Uttarakhand are unexplored and untouched by the improved technologies hence the variability in the form land races is still abundant. These germplasm are rich sources of fitness trait but with poor yield potential. Identification and development of new source of resistance in mustard germplasm collected from Uttarakhand hills is reported in this paper.

MATERIALS AND METHODS

Exploration visit have been made in the hilly region of Uttarakhand during 2009-2010 and 2010-11. Germplasm accession (GP-11-222) was collected from village-Van, Block-Dewal of district Almora (Altitude 7612 amsl, Longitude 790 37.454; Latitude 300 11.45) during 2010-11. Germplasm was collected in the form of bulk sample. The collected sample was sown during 2011-12 in the oilseeds breeding block, Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar. Preliminary data were collected on morphological traits and disease reaction. The material was initially screened under natural condition in the field. Individual plants were selected from the bulk sample on the basis of disease reaction. Plant progenies were grown in multiple rows. Different lines (PWR-13-1 to PWR-13-14) were selected on the basis of their disease reaction. Bagging (selfing) is done to maintain purity of these lines. During 2015-16 and 2016-17 the selected lines (Coded as PWR-14-1 to PWR14-14) were also screened by pathologist under both natural and artificial conditions. Later on the PWR-13-8 has been evaluated for the agronomic features and utilized in the hybridization programme. Study on the inheritance and allelic relationship of white rust resistance was also done. EC-399301, Donskaja and PWR-13-8 were used as resistance sources and Varuna and PRB-2006-5 were used to susceptible parent. Crosses were made between resistant and susceptible parents. Further the F_2 and back crosses were also developed. For inheritance study P_1, P_2, F_1, F_2, BC_1 and BC_2 were used for study of inheritance. For allelic study crosses were made between resistance parents. F_2 were developed. F_1, F_2 along with parents were evaluated. χ^2 test was done test goodness of fit of ratios.

RESULTS AND DISCUSSION

The collected germplasm were grown and observed for the unique traits. A huge variation for leaf colour, plant type and white rust reaction was found. The pigmentation in the sample varied from complete

green to complete purple. The purple pigmented plants also showed immune reaction against the white rust under natural condition. Year-wise activity and its outcome have been presented in the Table 1.

The identified plants with immune reaction were also screened under controlled condition (Gairola *et al*., 2017). Out of the 14 progenies, all have shown the immune reaction under field condition while under controlled condition at cotyledonary stage four progenies have shown immune reaction and six progenies have shown resistance reaction. At true leaf stage only four progenies have shown resistance reaction. Out of these four progenies, only one progeny *i.e.,* PWR-13-8 (Fig.1) had also shown at par performance in terms of morphological traits.

GENETIC STUDY

Inheritance of white rust and its alleleic relationship with other resistance sources has also been worked out (Vigneash *et al*., 2009 and Purnima, 2020). It was found that the white rust resistance was controlled by single dominant gene. The genetics of white rust resistance have been reviewed and characterized in earlier reports (Li *et al*., 2008; Panjabi-Massand *et al*., 2010; Awasthi *et al*., 2012 and Arora *et al*., 2019). Three different sources have been used to find out the alleleic relationship. From the results it was found that the gene for resistance present in the new source is different from the other two sources. The source used in the present investigation belongs to different origins.

Table1: Year-wise activity for development of white rust resistance source

Year	Activity	Remarks
2010-11	• Collected as bulk from famers field	
2011-12	• Sown in 6 row plot, Segregating for leaf colour, plant type and white rust symptoms • Two plants with purple leaf showed less disease (<2 rating) selected, selfed seed harvested from one plant.	Screened under natural condition in the field
2012-13	• Progeny of selected plant grown in multiple rows. • Five plants with <2 disease score selected as per the guidelines prescribed in AICRP pathology.	Screened under natural condition inoculum sprayed
2013-14	• Plant progenies raised • One progeny with <1 disease rating selected as line	Screened under natural condition inoculum sprayed
2014-15	• Selected progeny raised in 10 rows of 5m. • 22 plants with zero white rust infection on leaves were selected.	Screened under natural condition inoculum sprayed
2015-16	• Selected progeny raised in 10 rows of 5m. • 14 progenies with zero white rust infection under natural condition on leaves were selected.	Screened under natural condition inoculum sprayed
2016-17	• Selected progenies (14) were grown in multiple row for collection of data	Field screening under natural condition
2017-18	• All progenies were free from disease under natural screening in the field condition. • Under artificial screening at cotyledonary stage, 4 progenies have shown immune where as 6 progenies showed resistant reaction • At true leaf stage four progenies have shown resistant reaction while rest all showed moderately resistant reaction under artificial inoculation condition.	Screened under field condition (inoculum spayed) as well as in glass house (artificial inoculum) condition.
2018-19	• Four line identified were PWR-13-8, PWR-13-9, PWR-13-10 and PWR-13-11	These lines were further evaluated for their agronomic performance. Used in hybridization.
2019-20	• One line PWR-13-8 was found completely free from white rust infection under field condition and found at par for agronomic features.	

Inheritance of white rust

Reaction of different generations of six crosses for white rust disease has been presented in Table 2. The study revealed that all the plants in all F_1s in all the crosses were resistant and resembled the resistant line used as P_1, suggesting that resistance in these line is a dominant trait. Since, the distribution of resistant and susceptible plants in F_2 generation gave a segregation of 3:1 (Resistant: susceptible), this thereby indicating that each resistant cultivar carries a single dominant gene. The monogenic dominant nature of the resistance gene to white rust was also confirmed from the results of backcross populations. In the backcross (BC_1) with the resistant parents, all the plants were resistant, whereas in the backcross (BC_2) with susceptible parents, test cross progenies segregated in 1:1 (Resistant: susceptible) ratio.

Table 2: Inheritance pattern of white rust resistance in Indian mustard

Cross	Generations	Total plants	Observed		Expected ratio (R:S)	Expected		χ^2 value	Probability
			Resistant (R)	Susceptible (S)		Resistant (R)	Susceptible (S)		
Donskaja x Varuna	P1	93	93	0	-	93	0		
	P2	100	9	91	-	0	100		
	F1	51	51	0	-	51	0		
	F2	241	170	71	3:1	181	60	2.69	0.10-0.20
	BC1	179	179	0	1:0	179	0		
	BC2	122	67	55	1:1	61	61	1.18	0.20.-0.30
PWR-13-8-8 x Varuna	P1	90	90	0	-	90	0		
	P2	89	16	73	-	0	89		
	F1	88	88	0	-	88	0		
	F2	257	202	55	3:1	193	64	1.68	0.10-0.20
	BC1	193	193	0	1:0	193	0		
	BC2	169	90	79	1:1	84	85	0.85	0.30-0.50
PWR-13-8-8 x PRB-06-5	P1	58	58	0	-	58	0		
	P2	65	0	65	-	0	65		
	F1	86	86	0	-	86	0		
	F2	196	155	41	3:1	147	49	1.74	0.10-0.20
	BC1	71	71	0	1:0	71	0		
	BC2	107	60	47	1:1	54	53	1.35	0.95-0.90
EC399301 x Varuna	P1	78	78	0	-	78	0		
	P2	61	0	61	-	0	61		
	F1	80	78	0	-	80	0		
	F2	220	159	61	3:1	165	55	0.87	0.30-0.50
	BC1	108	108	0	1:0	108	0		
	BC2	73	33	40	1:1	36	37	0.49	0.30-0.50

Table 3: Allelic relationship between different sources of resistance

Cross	Generations	Total plants	Observed data		Expected ratio (R:S)	Expected data		χ^2 value	Probability
			Resistant (R)	Susceptible (S)		Resistant (R)	Susceptible (S)		
Donskaja x PWR-13-8	P1	11	11	0	-	11	0		
	P2	10	10	0	-	0	10		
	F1	13	13	0	-	13	0		
	F2	180	164	16	15:1	169	11	2.27	0.10-0.20
Donskaja x EC399301	P1	20	20	0	-	20	0		
	P2	16	16	0	-	0	16		
	F1	12	12	0	-	12	0		
	F2	150	136	14	15:1	141	09	2.96	0.05-0.10

Genetic control of white rust resistance governed by single dominant gene was also reported by Paladhi *et al*. (1993), Sridhar and Raut (1998), Chauhan and Sharma (2001), Vignesh *et al*. (2009), and Dahiya *et al*. (2019).

Allelic relationship between various sources for white rust resistance

For ascertaining the allelic diversity in resistance sources, F_2 of the crosses Donskaja x PWR-13-8-8 and Donskaja x EC399301 were also screened for white rust reaction. A segregation ratio of 15:1 (R:S) was observed in the F_2 populations with a non-significant chi square value (2.42 and 2.96) in both the families (Table 3) demonstrated a goodness of fit to the presence of different single dominant genes in the three sources each and confirmed that the gene segregating in the population for resistance were non-allelic. Hence, the major gene governing white rust resistance could be easily transferred to the well adapted, high yielding but susceptible genotypes like Varuna and PRB-2006-5 through backcross breeding (Vignesh *et al*., 2009). The presence of resistance gene (s) in the three different resistant sources will be very useful in breeding for durable resistance by the diversification of resistant sources and gene pyramiding. However, more detailed studies would have to be conducted to analyze the virulence spectra and diversity in the pathogen population through controlled experiments with each of these genes.

Fig1: Field view of PWR-13-8

CONCLUSION

Gene introgression, its inheritance and screening of different traits with the help of molecular marker tools have been employed in different crops as speedy plant breeding technique which considerable enhances the efficiency of crop improvement (Singh and Pandey, 2007; Saxena *et al*., 2009 and Meena and Sachan, 2010). Dynamic changes in race composition of the pathogen have often resulted in short-lived efficiency of host resistance in the improved varieties. Therefore, a necessity to identify new sources of white rust resistance is imperative in breeding for durable resistance for this disease. The results of present study indicated that a new source of resistance from germplasm collection was developed that is different from already available sources in Donskaja and can be effectively used in future breeding programme. Further a detailed study to verify its resistance response, genetic control and allelic relationship with other known sources for resistance to white rust are needed under controlled condition. Molecular marker identification and its validation for new sources also help the breeder to speed up the improvement programme.

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