Pyramiding white rust resistance and Alternaria blight tolerance in low erucic *Brassica juncea* using *Brassica carinata*

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Abstract

The present study was undertaken to incorporate resistance to fungal diseases white rust (WR) and Alternaria blight (AB) in low erucic acid *Brassica juncea* from *B. carinata* through interspecific hybridization aided by ovule culture. Hybrids characterized through ISSR markers and morphological traits, indicated intermediate nature with strong influence of pollen donor. The hybrids showed high variation in morphology (6% *B. juncea* type plants in F_2 to 100% in BC_2) and exhibited high tolerance to both WR and AB in subsequent backcross generations under epiphytotic conditions. *B. juncea* type plants were identified from BC_2 and BC_1 self progeny with no detectable erucic and high oleic acid (44.7%) content along with high tolerance to both WR and AB.

Key words : A. candida, A. brassicae, interspecific hybridization, embryo rescue

Introduction

White rust (WR) and Alternaria blight (AB) are economically important fungal diseases of oilseed *Brassica* with worldwide prevalence that can reduce yields up to 60% (Kolte, 2002). *B. carinata* has resistance to *Albugo candida* races found in India and high tolerance to AB (Kolte, 1996). It has been utilized to incorporate resistance/ high tolerance to WR (Kumar et al., 2002, Singh and Singh, 1988) and AB (Katiyar and Chamola, 1995) in *B. juncea*. Nevertheless, with the ever-increasing changes in the genetic profile of the fungus due to evolution/ mutation, it is imperative to pyramid genes for multiple disease resistance in nutritionally superior genotypes. In this direction, appreciable efforts have been made for developing low erucic acid *B. juncea* (Agnihotri and Kaushik, 2003, Chauhan et al., 2002) and incorporation of WR resistance (Franke et al., 1999). This study was undertaken to incorporate WR and AB resistance/ high tolerance from *B. carinata* in the genetic background of nutritionally improved low erucic Indian mustard TERI (OE) M21-1.

Materials and Methods

The seeds of all the plant progenies were grown at TERI experimental field station following standard agricultural practices (Reddi and Reddy, 1980). Pollinations were performed under field conditions utilizing *B. juncea* line TERI (OE) M21-1 as the female parent and *B. carinata* cv. Kiran as the male donor. Pollinated ovaries were used for both *in vivo* seed set and *in vitro* ovule culture (Agnihotri, 1993) to obtain hybrids. The seeds obtained from *in vivo* left pods were germinated on half strength MS medium containing 3.0 % sucrose and 0.7% agar at 22 ± 2 °C under 16 h photoperiod with a photon flux of 170 mmol m^{-2} s^{-1}. *In vitro* developed plantlets were multiplied through axillary bud proliferation/ apical meristem culture on MS medium supplemented with 1.0 mg/ L Kn, 3.0 % sucrose and 0.7% agar at above mentioned temperature and light conditions. At 2- 3 leaf stage, plantlets were rooted on MS medium supplemented with 0.1 mg/L IBA, 2% sucrose and 0.65% agar for 3-4 weeks. The plantlets were hardened and transplanted in field at 4- 5 leaf stage, after 3- 4 weeks of sowing of parent line seeds for uniformity of the growth stages

and appropriate comparison. The putative hybrids were characterized using DNA based ISSRs marker and comparative morphological traits such as leaf and floral morphology. The percent pollen fertility was studied by staining pollen grains from freshly dehisced anthers using Fluorescein di-acetate (Shivanna and Rangaswamy, 1992).

The multiplied plantlets of individual hybrids were raised in single plant progeny (SPP) rows with their parents; *B. juncea* TERI (OE) M21-1, *B. carinata* cv Kiran and susceptible check var. Varuna. The plants in each generation were evaluated for morphological characteristics, WR and AB resistance, and oil quality. The resistant/ tolerant [disease index (DI) < 2] plants were selected, selfed/ backcrossed to their female parent, and F_2/ F_3/ BC_1/ BC_1self / BC_2 progenies of selected plants were forwarded as SPP rows alongwith their parents and *B. juncea* var. Varuna after every 10 lines as infector row/ susceptible controls (SC). Heavily infected leaves of *B. juncea* var. Varuna, collected from the Indian Agricultural Research Institute (IARI), New Delhi and GB Pant University of Agriculture and Technology (GBPUAT), Pantnagar (35 and 250 Km away from TERI field station, respectively), were utilized to prepare spore suspensions. For WR, 5 x10⁴ zoosporangia/ ml was prepared by the method of Singh *et al*. (1999). The density of conidial suspension for AB was adjusted to 1.5 x 10⁴ conidia/ ml. The plants were sprayed in the evening, when the temperature was low (12 ± 2 °C) and humidity was high (> 90%) to ensure optimum disease severity.

In order to create high disease pressure in field conditions, the plants were sprayed with AB inoculum at 40 days after sowing (DAS) and WR inoculum at 50 DAS. The third, fourth and fifth leaf from the base of the test plants were screened at 75, 90 and 110 DAS from 15 randomly selected parents and SC plants. The disease reaction was assessed on a 0-5 scale (Conn et al., 1990), where 0 = no visible disease symptoms, 1 = 1-5%, 2 = 6-10%, 3 = 11-25%, 4 = 26-50% and 5 = > 50% leaf area covered with disease symptoms. Plants with DI $\leq$ 1.0 (WR) / DI $\leq$ 2.0 (AB) were regarded as resistant/ highly tolerant.

All plant progenies were characterized for morphological traits viz., the leaf and floral morphology (shape, colour, and texture), inflorescence, branching pattern and seed colour. Five randomly selected BC_1self /BC_2 plants were also evaluated for yield attributing agronomic traits; days to 50% flowering/ maturity, number of primary/ secondary branches, pods on main shoot and 1000 seed weight. Pod length and seeds/pod was calculated as mean of ten randomly collected pods from the main branch and represented as mean $\pm$ standard error of mean (SEm). The fatty acid (FA) composition was determined in two replicates by Gas Liquid Chromatography (GLC) by the method of Kaushik and Agnihotri (1997).

The molecular data were analysed using statistical software package NTSYS version 2.0 (Rohlf et al., 1971). The DI of an individual plant was calculated as average DI on 3rd to 5th leaves, and overall mean of all plants was taken to get the DI for the progeny. Single Factor ANOVA was performed (Gomez and Gomez, 1984) using the mean DI of an individual plant to assess the statistical differences among the SPP rows. As most of the comparisons involved an unequal number of plants, the Least Significant Difference (LSD) was calculated at p= 0.05 as LSD= [Error mean square $(1/ni^{th} +1/mj^{th})^{0.5}$] $t_{0.05}$, where n and m is the number of plants in i^{th} and j^{th} progenies, respectively and $t_{0.05}$ denotes the tabulated value at p= 0.05.

Results of FA analysis were represented as pooled average of two replicates of different progenies along with Standard error of mean (SEm). The FA composition was compared with the parents using paired t-test assuming unequal variances. The data for agronomic traits of different progenies were compared with both female and male parent using LSD values calculated through Single factor analysis of variance (Gomez and Gomez, 1984).

Results and discussion

Detail of the experimental method with a synopsis of results is presented schematically (figure 1).
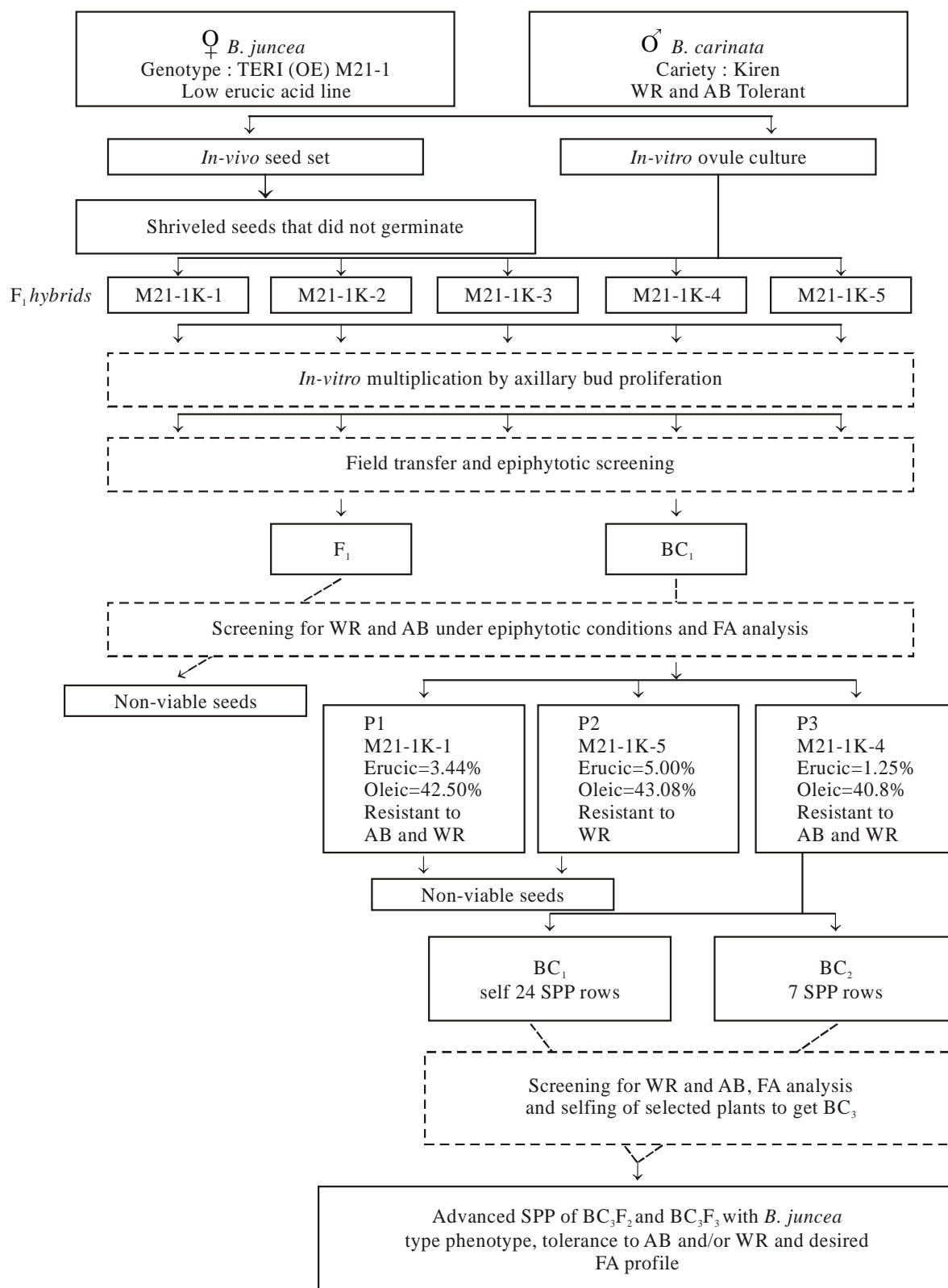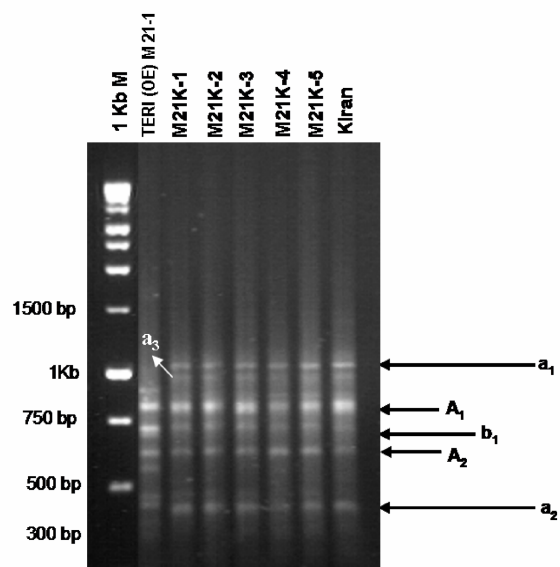
Figure 1: Schematic representation of experimental design and salient findings for introgression of disease resistance into low erucic *B. juncea* genotype using interspecific hybridization *(B. juncea* x *B. carinata)*

Molecular and morphological characterization of hybrids and disease evaluation:

Very few shrivelled seeds were obtained under *in-vivo* conditions. These seeds did not germinate under *in vitro* conditions. However, five hybrids (M21-1K-1 to M21-1K-5) obtained through *in vitro* ovule culture were characterized for hybridity through ISSRs and morphologically under field conditions. Figure 2 (a and b) details the molecular analysis of hybrids vis-a-vis parent genotypes. Two male parent specific bands amplified at 450 bp and 1000 bp. Based on the UPGMA analysis hybrids resembled more to the male parent at a GS value of 0.65 in comparison to the female parent (GS value 0.52). All hybrids except one (M21-1K- 1) resembled each other at a GS value of 1.0 (figure 2b).
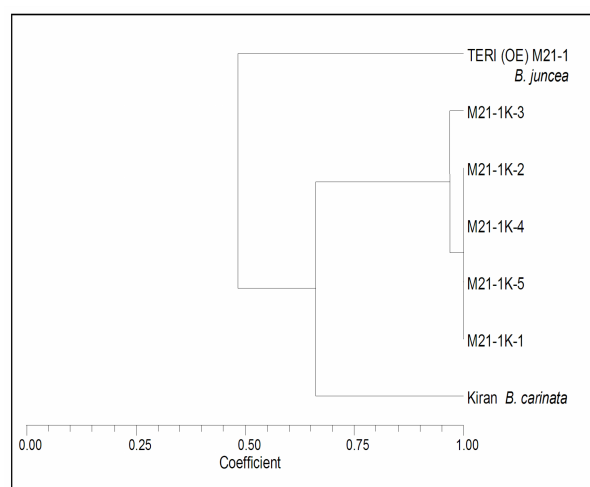

Figure 2 : Molecular confirmation of *B. juncea* x *B. carinata* hybrids produced from *in-vitro* ovule culture. (a) Banding profile of hybrids and their parents using ISSR primer UBC- 812. M is 1Kb ladder. Bands 'A1' and 'A2' were monomorphic to all genotypes; bands a1, a2 in all the hybrids were male specific bands, while 'a3' another male parent specific band showed polymorphism among different hybrids; whereas b1 was female specific band. (b) UPGMA based dendrogram showing clustering pattern of hybrids along with their parents.

Similar to the molecular data, all hybrids resembled each other being intermediate in morphological traits. The characteristic purple colour of the male donor *B. carinata* was observed on the stem, junction of branches, base of petioles, along the mid rib and on the green pods of all the hybrid plants. Corolla was light yellow like the male parent with presence of prominent red dot at the tip of anthers. Failure of interspecific cross to set seed *in vivo* could be due to genetic distance between the two parent species calculated through similarity matrix constructed using Jaccard's coefficient. Similar to our findings, hybrid seeds were difficult to obtain with *in vivo* approach in a *B. juncea* x *B. carinata* cross by Roy (1980). The morphological variations in most

inter-specific/ inter-generic hybridization are believed to arise from recombination of chromosomes (Stebbins, 1963) and in this direction taxonomic and cytogenetic studies in the past have elucidated that hybrids through tetraploid/ digenomic *Brassica* species possess high amount of pairing and thus reflect greater variation in morphological traits (Nishiyama *et al.,* 1991).

The anthers of hybrids ranged from rudimentary to well formed but the percent pollen fertility ranged from 6.7 to 14.79 % (average of eight replicates per hybrid) with an overall average of 8.92 ± 1.48 in comparison to more than 90 % in both parents. Seed set per siliqua was also poor with a pooled average of

1.86 ± 0.26 (calculated as number of seeds harvested from 10 randomly selected pods obtained through open pollination from main branch of each of the five hybrid). This low seed set and pollen fertility results from meiotic imbalance resulting in abortion of recombinant zygotes (Subudhi and Raut, 1995).

After epiphytotic inoculation, F_1's were completely resistant to WR up to 110 DAS with no visible disease symptoms. While for AB one or two dot like disease symptoms were recorded on the abaxial surface of the leaves with DI = 1.0 (average DI 0.28 ± 0.08). This observation confirmed that the genes conferring resistance to both the diseases were functional in the hybrid genetic background. Based on the resistance response of F_1's all the five hybrids were progeny forwarded, the harvested F_2 and BC_1 seeds were brown in colour and were evaluated for FA profile.

Evaluation of F_2 and BC progenies for morphology, disease response and oil quality

F_2 Progeny: High variability was observed in morphological traits in the F_2 plant progenies; *B. carinata* type, intermediate and *B. juncea* type with an overall ratio of 6:66:8 (total plants= 80). Hybrid plants having *B. carinata* specific red dot on anthers segregated to a ratio of 3:1, and presence of purple colour were reduced to 50% plants (36 out of 80 plants). Floral morphology of these F_2 plants revealed that they were partially sterile with very poor seed set. Similar to our findings, high frequency of intermediate plants in F_2 progeny have also been reported by Subudhi and Raut (1994) from interspecific cross of *B. juncea* x *B. napus*. However, wide phenotypic variation coupled with poor fertility may have resulted from meiotic irregularities caused by incompatible pairing of chromosomes during meiosis and/ or eventual segregation of aneuploid forms that generally occurs in interspecific crosses (Choudhary *et al.*, 2002).

The F_2 plants showed high resistance to WR ranging from no visible disease symptoms in progeny advanced from hybrid M21-1K-4 (n= 21) to a maximum of 0.23 ± 0.16 in M21-1K-2 (n= 10)

in comparison to susceptible female parent (figure 3), and was at par to the resistant male donor. However, the DI for AB in this progeny was comparatively high with an average of 3.14 ± 0.10 (ranging from 2.60 ± 0.18 in progeny derived from hybrid M21-1K-5, n=17 to a maximum of 3.57 ± 0.19 from M21-1K-1, n=15), showing high tolerance to WR as compared to the female parent (DI= 4.55± 0.11) and susceptible check var. Varuna (4.83±0.07; LSD $_{0.05}$= 0.27; figure 3). Six out of 80 F_2 plants (i.e. 7.5% plants) were identified with high tolerance to AB (DI $\leq$ 2.0).

Out of the total 77 F_2 plants that showed resistance to either AB or WR, seeds of only 28 plants were analysed for FA profile due to restricted seeds availability. A high variation was observed in FA profile content; the average FA profile was comparable to the male donor except for intermediate erucic content (t'= 9.31, p<0.05, table 1). Based on the disease response and FA profile, four selected plants were forwarded in the next generation but none germinated. Similar to these findings, production of non-viable seeds in interspecific crosses of *B. juncea* × *B. rapa* has reported earlier (Gupta *et al.*, 2007).

BC progenies: The plants in BC_1 progeny segregated into 66.6% *B. juncea* type and 33.4% intermediates (n=27). Red dot on the anthers was absent from all plants except one and the presence of purple colour was also reduced to 33% plants. This increase in frequency of female donor pheno-type in the BC generation has been reported earlier in interspecific crosses of brassicas (Gupta *et al.*, 2007, 2004). However, segregation of species specific morphological markers, like the red dot on anthers and purple pigmentation reported in the present study, possibly arrises from the partial homology that exists between the A, B and C genomes (Truco *et al.*, 1996). Chaudhary and Joshi (1999) have suggested that such segregation result from interspecific gene transfer during meiotic crossing over.

WR resistance response of F_1's was carried over to BC_1 plants; from no disease symptom to a maximum of 1.66 DI on individual plants and was

Table 1: Fatty acid profile of white rust/ Alternaria blight resistant plants in F_2, BC_1, BC_2 and BC_1 self progenies

Plant Progeny*	Number of plants analysed	Average Percent Fatty acid content (%) ± SEm[1,2,3,4] (Range)					
		16:0	18:1	18:2	18:3	22:0	22:1
(M21-1×K) self [F_2]	28[80]	4.95 ± 0.29 (3.3-11.95)	15.90± 0.74 (12.15-26.99)	21.83±0.93 (22.77-32.12)	8.63± 0.42 (4.93-16.0)	7.28±0.31 (6.31-11.4)	22.83± 1.12 (17.55-34.31)
(M21-1×K) × M21-1 [BC_1]	19[27]	5.34 ±0.46 (3.2-17.48)	25.03 ±1.35 (13.67-40.80)	25.35 ±0.61 (18.60-34.51)	10.84 ±0.41 (5.85-14.53)	8.43 ±0.45 (1.36-12.68)	21.971±1.58 (1.25-40.54)
[(M21-1×K) ×M21-1] self [BC_1self]	5[24]	4.69± 0.14 (4.12-5.18)	41.29± 0.85 (38.45-44.74)	35.32± 1.10 (31.93-37.89)	14.96±0.50 (13.66-17.26)	0.162±0.10 (ND-0.46)	ND
[(M21-1×K) ×M21-1 × M21-1 [BC_2]	7[7]	4.10 ±0.46 (4.32-6.23)	29.94± 3.53 (27.7-43.35)	27.56 ±3.30 (25.83-41.12)	10.31±1.14 (9.71-14.9)	2.32± 0.98 (ND-8.31)	4.59± 2.05 (ND-18.76)
B. juncea (♀)		5.305±0.02	41.435±0.04	34.83± 0.03	14.385± 0.16	0.11± 0.02	ND
B. carinata (♂)		3.65±0.02	15.355±0.08	17.77±0.52	11.05±0.01	8.54±0.04	42.13±0.16

[1] Figures in the square brackets are the total number of plants available

[2] 16:0=Palmitic acid, 18:1=Oleic acid, 18:2=Linoleic acid, 18:3=Linolenic acid, 22:0=Eicosenoic acid, 22:1 =Erucic acid

[3] Average is based on the total number of plants analysed in a progeny. The analysis for each individual plant was done in two replicates

[4] Values in bold letter indicates desirable quantum for nutritional quality

[5] ND= Not Detectable

* *B. juncea* parent TERI (OE) M21-1 is represented as M21-1 and *B. carinata* parent Kiran is represented by K

comparable to the male donor ($LSD_{0.05}$= 0.21; figure 3). Out of a total of 27 BC_1 plants, 22 plants (85.18% plants) had DI ≤ 1.0, seven plants showed no disease symptoms, but four did not bear pods or seed, hence 18 plants were identified with resistance to WR and normal seed set. The DI for AB in BC_1 plants ranged from 2.0-4.33 compared to the female parent (DI= 4.55 ± 0.11) and susceptible check (DI=4.83 ± 0.07; figure 1). Four plants were identified with DI ≤ 2.0 and three of these were also resistant to WR. Therefore, overall three plants (13.04% plants) were identified with resistance/ tolerance to both WR and AB.

High variability was also observed for major FA in all 19 resistant plants (table 1). The average oleic acid content was significantly higher than the male donor *B. carinata* (t'=3.01, p<0.05) but lower than the female parent *B. juncea* (t'=7.36, p<0.05); in contrast the erucic acid was significantly lower than the male donor (t'=9.38, p<0.05) and higher than

the female parent (t'=7.83, p<0.05). Three plants designated as P1 to P3 were identified with low erucic and high oleic acid; P1 (from hybrid M21-1K-1; 3.44% erucic, 42.50% oleic) and P3 (from hybrid M21-1K-4; 1.25% erucic, 40.80% oleic) were resistant/ tolerant to both WR and AB, while P2 (from hybrid M21-1K-5; 5.0% erucic, 43.08% oleic was resistant to WR only. These plants were selfed to obtain BC_1 self seeds or back crossed to obtain the BC_2 seeds.

Seeds of P1 and P2 did not germinate. Seven BC_2 plants and 24 BC_1self plants were raised till maturity in SPP rows forwarded from P3. All plants were *B. juncea* type but with profuse secondary branching in BC_2 and BC_1self progeny rows. In the BC progenies the resistance response for WR was higher but comparable to the male donor ($LSD_{0.05}$= 0.34; figure 3). Similarly resistance/ high tolerance was also observed for AB as compared to the female parent (figure 3). *B. juncea* type plants were

identified in the BC$_2$ progeny with either WR resistance (DI<1.0) or AB resistance/ tolerance (DI<2.0) or combined resistance to both. Although average disease response was varying for WR in the BC$_1$self and BC$_2$, but for AB, the average response of BC$_1$self (1.60 ± 0.12) and BC$_2$ (1.19 ± 0.11) was at par (LSD$_{0.05}$= 0.55; figure 3).

Overall the frequency of plants resistant to WR was higher and less varying (79.16- 100%) in all the progenies as compared to plants resistant/ tolerant to AB with frequency as low as 7.50% in F$_2$ to 100% in BC$_2$. These results indicate that the genes for disease resistance were successfully transferred to *B. juncea* background by the successive BC approach. Earlier reports suggest that the genes for WR resistance are localized on the C genome (Attia and Robbelen, 1986) and in the light of our findings, these were transferred to A genome of *B. juncea* since the A genome shares considerable sequence homology to the C genome (Howell *et al.*, 2008). The resistance/tolerance to AB on the other hand is

controlled by a number of minor genes and thus it is difficult to obtain high resistance in homozygous condition. In the present study hybrids showed DI ranging from 0.61 to 1.19 suggesting that the response is influenced by certain minor genes along with dominance of resistant gene. Subudhi and Raut (1994) and Krishnia *et al.* (2000) reported dominant nature of AB resistance in F$_1$ generation of *B. juncea* x *B. carinata* interspecific cross, with involvement of certain modifiers or minor genes. The significantly lower average DI of plants to AB in BC$_2$ progeny than their respective female parent indicates the introgression of resistance factors/ genes and intergenomic interaction that might play an important role in manifestation of resistance/ tolerance. The FAs analysis of all disease resistant BC$_2$ and five randomly selected resistant BC$_1$self plants was comparable to the BC$_1$ parent plant from which these SPP rows were derived. Four out of seven disease resistant BC$_2$ plants and all plants of BC$_1$self progeny showed non-detectable erucic acid.
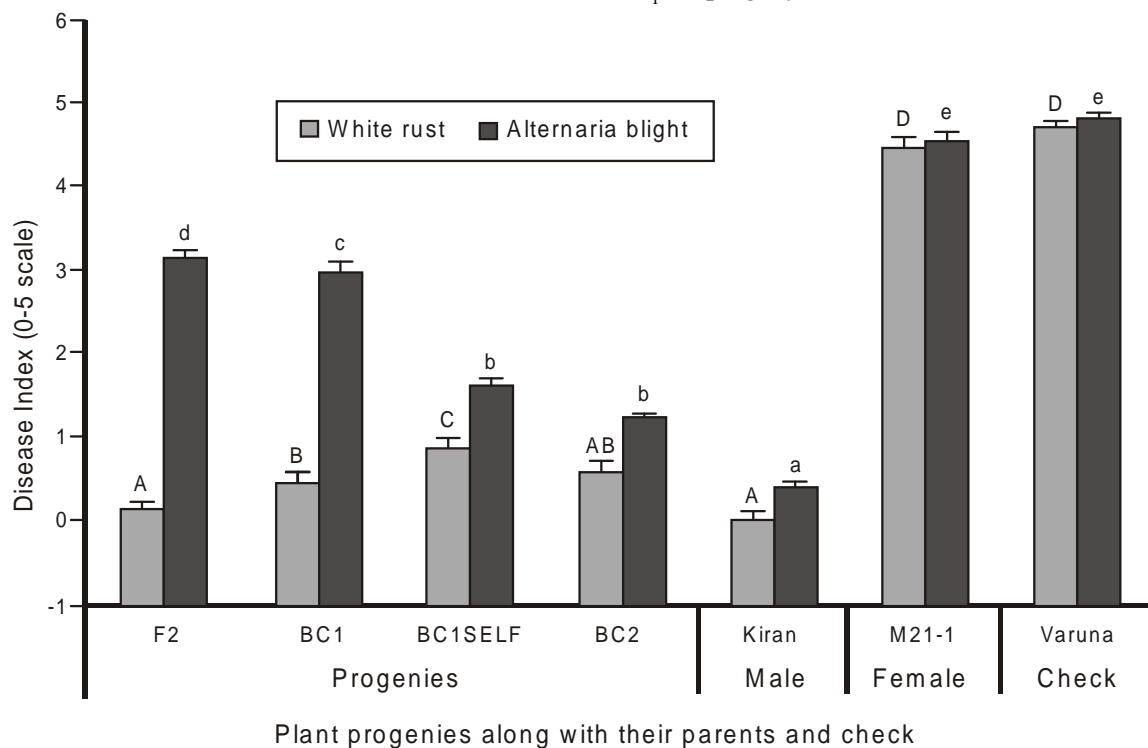

Figure 3 : Average DI of selfed and BC progenies of cross *B. juncea* TERI (OE) M21-1 x *B. carinata* cv Kiran along with parents and check to white rust and *Alternaria* blight at 110 DAS. Histograms represent pooled average DI and the bars represent standard error of mean. Absence of bar on white rust histogram of male parent indicates no disease symptoms. The progenies represented with same alphabet letter in a series do not differ significantly as compared by LSD using Single factor ANOVA at p=0.05.

The remaining three plants of BC_2 progeny showed moderately low erucic acid content ranging from 13.38 to 18.76%. There was no significant difference between the female parent and both BC_1 self and BC_2 progenies for average content of all important FAs as compared using paired t-test. The complete FA profile of the five analysed BC_1 self plants and all plants of BC_2 progeny resistant to both WR and AB along with the parents is depicted in figure 4.

For both WR and AB, segregation of plants into resistant and susceptible category could not be obtained in Mendelian ratios as meiosis rarely functions with complete normality in segregating interspecifc crosses and this is also applicable for the FA profile. Although the FA composition in F_2 progeny was skewed towards male donor, average erucic acid content was intermediate between parents. It represented a continuous variation and class overlap that did not allow formation of discrete classes of low and high erucic acid content. A similar trend was also observed by Banga and Banga (2002) in a *B. juncea* low erucic x high erucic cross and was attributed to additive gene effect. Erucic

acid content is controlled by two genes with additive gene effect both in *B. juncea* (Kirk and Hurlstone, 1983) and *B. carinata* (Fernandez *et al.*, 1988). This is further supported by reduced average content of erucic acid in BC_1 and BC_2 progeny. The palmitic acid varies from 0.71-11.6% and stearic acid from 0.1-3.6% in *B. juncea* (Chauhan *et al.*, 2002). Two transgressive segregants were identified in BC_2 plant progeny containing high palmitic (17.0%) and high stearic (6.8%) acid (figure 4). Such high levels of palmitic and stearic acid have not been reported in Indian mustard so far and can have diverse uses in replacing animal fats and tropical oils in margarines and confectionery products. High stearic acid and palmitic lines have been identified through anti sense technology in *B. napus* and are under field trials (Murphy, 1999). Nevertheless, further segregation of these *B. juncea* type plants in the present study needs to be studied for any substantial outcome.

Yield potential of selected progenies

BC_1 self and BC_2 *B. juncea* type plants selected for WR and AB resistance/ tolerance with
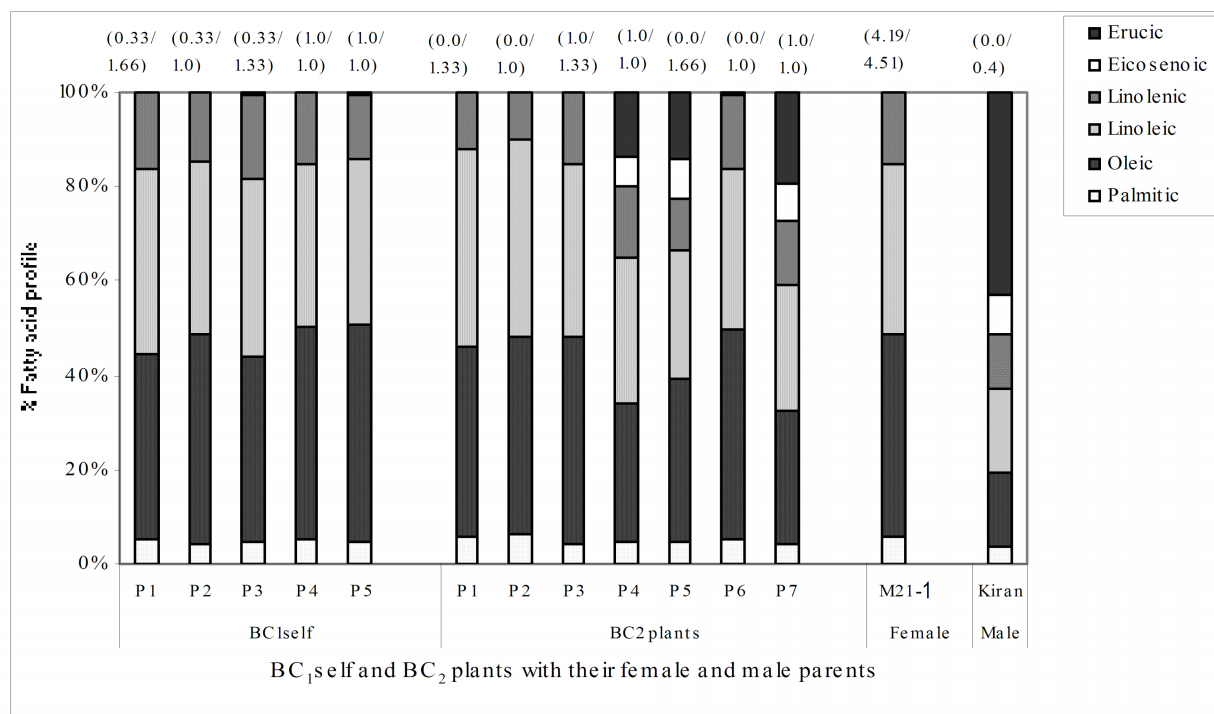

Figure 4: Fatty acid profile of selected low erucic *B. juncea* type plants from BC_1 self and BC_2 progeny resistant to both white rust and Alternaria blight along with parents. Each histogram represents the proportion of different fatty acids, and figures in the bracket represent the DI for white rust and Alternaria blight for individual plants.

desirable FA profile were evaluated for agronomic traits. A highly significant variation was recorded for five of the eleven traits evaluated in the BC_2 and BC_1 self progeny in comparison to the parents but the 1000 seed weight of both BC_2 and BC_1 self progenies was at par to both the female and the male donor (table 2). The selected plants were progeny forwarded on the basis of FA analysis and disease evaluation in each generation. The elite

genotypes, resembling *B. juncea* phenotype derived from advanced backcross progenies $BC_3 F_2$ and $BC_3 F_3$ have been selected for low erucic /high oleic acid and good tolerance to fungal diseases WR and AB (DI < 2).

To the best of our knowledge, this is the first report for incorporation of both WR and AB resistance in the genetic background of low erucic acid Indian

Table 2: Comparative agro morphological data of white rust and Alternaria blight resistant / tolerant BC_2 and BC_1 self plant progenies

Agronomic traits	Average ± SEm[1,2]				
	B. juncea TERI (OE) M21-1 (♀)	BC_2 progeny	BC_1 self progeny	*B. carinata* Kiran (♂)	$LSD_{0.05}$ and Significance level
Days to 50 % Flowering	52.0±0.0[a]	79.0±2.21[b]	91.0±0.63[c]	114.0±0.0[d]	7.43***
Days to Maturity	132.0±0.31[a]	140.0±4.11[a]	139.0±2.21[a]	161.0±0.32[b]	12.37*
Plant Height (cm)	153.6±3.24[c]	173.2±7.02[b]	180±6.89[b]	246±5.84[a]	18.40***
Distance from base to First branch	27.0±1.61[b]	8.6±1.74[c]	13.0±0.29[c]	94.8±2.47[a]	7.85***
Number of Primary Branches	5.0±0.24[c]	11.0±1.51[b]	8.0±0.94[a]	22.0±1.07[a]	3.38***
Number of Secondary branches	12.0±0.74[b]	20.0±3.12[ab]	29.0±5.36[a]	24.0±1.49[a]	10.80*
Pods per main Shoot	47.4±1.80[b]	49.0±3.30[b]	63.0±4.17[a]	16.0±0.75[c]	8.89***
Total pods	257±7.22[b]	882±78.0[a]	809±146.61[a]	930±48.88[a]	285.85***
Pod Length (cm)	4.49±0.09[a]	4.30±0.27[a]	4.36±0.172[a]	3.6±0.07[b]	0.50**
Seeds per Pod	15.8±0.58[a]	15.0±0.20[ab]	14.0±0.54[b]	13.4±0.24[b]	1.39*
1000 Seed weight (gm)	3.29±0.04[a]	3.68±0.31[a]	3.67±0.108[a]	3.50±0.10[a]	0.57[ns]
Yield per plant (gm)	9.88±0.21[b]	8.42±0.57[bc]	8.02±0.71[c]	15.36±0.29[a]	1.55***

[1] Average is based on data collected from five randomly selected resistant plants per genotype

[2] Means with same superscript letter for a agronomic trait in a row do not differ significantly compared by Least Significant difference (LSD) at p=0.05 using Single factor Analysis of variance (ANOVA); significance for the respective source of variation marked as: ns= non significant (P>0.05), *significant ($0.05 \leq P \geq 0.01$), **highly significant ($0.01 \leq P \geq 0.001$) and ***highly significant (P <0.001)

mustard. Apart from this, comparable agronomic yield attributing traits of the selected plants of BC_2 progenies to the female parent is a positive step in the direction of developing elite *B. juncea* germplasm. These elite genotypes have been advanced to $BC_3 F_2$ and $BC_3 F_3$ with strict selection of nutritional quality and disease resistance.

Acknowledgement

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