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Breeding for Alternaria Blight (*Alternaria brassicae*) Resistance in Mustard

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Introduction

In India, rapeseed-mustard *(Brassica species*) are placed at second position in total acreage (23.91 per cent) and production (27.19 per cent) after soybean among oilseeds crops. There are three ecotypes of *Brassica* (2n = 20) *i.e. Brassica campestris* ssp. Oleifera *viz.*, yellow sarson, brown sarson and toria, collectively called rape (syn. *B. rapa* L.), *B. juncea* or brown mustard (2n = 36) and *Eruca sativa* or Taramira (2n = 22) are commercially cultivated. Among these, Indian-mustard *(Brassica juncea)* accounts for > 80 per cent of the area under rapeseed-mustard crops in the country. Breeding system for a crop species mainly depends upon it's mode of pollination. Therefore, in plant breeding, breeders need to be aware of the existing genetic variability in terms of nature and magnitude, and wild relatives of a crop species, its reproductive behavior, adaptation to environments and cropping systems, and usage for the objective and methods chosen for its genetic improvement. The effectiveness and efficiency of selection is greatly advanced when the magnitude and nature of genetic variation is understood, and rapid and reliable screening techniques are available. Here, we will emphasize primarily on the breeding of Indian mustard *(Brassica juncea)* for Alternaria Blight *(Alternaria brassicae)* disease resistance.

Breeding Objectives

Increased seed yield with acceptable seed quality and stability in yield performance across environments is the major breeding objective in all crop improvement programmes. Stability in production is sought by incorporating resistance or tolerance to major biotic stresses, such as diseases like Alternaria blight *[Alternaria brassicae* (Berk.) Sacc.], white rust *[Albugo candida* (Pers. Ex Lev.) Kuntze], powdery mildew *[Erysiphe cruciferarum]* and Sclerotinia rot *[Sclerotinia sclerotiorum* (Lib.) de Bary]

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and the insect pests like mustard aphid *[Lipaphis erysimi]*, painted bug *(Bagrada cruciferarum [Bagrada hilaris]*), sawfly *[Athalia lugens proximo*], pea leaf miner [*Chromatomyia horticola]* and flea beetle *[Phyllotreta cruciferae])*, parasitic weeds (broomrape *[Orobanche])* and such abiotic stresses as frost, early or terminal heat, drought, acid, and saline toxicity.

Alternaria Blight *(Alternaria brassicae)* disease has been reported from all the continents of the world and is one of the important diseases of Indian mustard causing up to 47 per cent yield losses. In India, three distinct isolates of *A. brassicae* namely A (highly virulent), C (moderately virulent) and D (avirulent) has been reported (Vishwanath and Kolte, 1997). In Indian mustard, no proven source of resistance against *Alternaria* blight reported yet, but some sources of tolerance against this disease have been identified (Gupta *et al.* , 2001). Moreover, some resistance sources have been identified in related and wild species of *Brassica* like *Sinapis alba* L. (Hansen and Earle, 1997), *Camelina sativa, Capsella bursa-pastoris, Neslia paniculata* and taramira (Tewari and Conn, 1993), *Doplotaxis berthautii,* D. *catholica,* D. *cretacea,* D. *erucoides* and *Erucastrum gallicum* (Sharma *et al.*, 2002). Inheritance studies suggested that tolerance to *Alternaria* blight is governed by multigenes or cluster of genes with additive or partial dominance effects (Krishna *et al.,* 2000).

Inter-specific Variation

Weedy and wild taxa may become rich reservoir of genes for disease resistance. Lesion type, number and size has been used as selection criterion in breeding for Alternaria blight resistance. Among cultivated species, *B. carinata* and *B. napus* reported to be more resistant followed by *B. nigra, B. juncea* and *B. campestris* in that order (Bhowmik and Muncie, 1987). Sharma and Singh (1992) reported *B. hirta* as most resistant among *B. juncea, B. carinata, B. napus*. *B. rapa* and *B. hirta*. Contrary to these observations, Tewari (1991) reported that there is hardly any variability between resistant and susceptible host at the early stage of infection. Among the related species, *Camelina sativa,* Capsella *bursa-pastoris* and *Neslia paniculata* showed no disease symptoms mainly because of their inhibition capacity of fungal growth on leaf surfaces by producing phytoalexin shortly after infection. *Eruca sativa* (Miller) Thell showed localized flacks and *Armoracia rusticana* Gaertn., show slow necrosis and chlorosis. Pre and post fertilization barriers restrict the transfer of resistant genes from these

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wild species into *B. juncea* and *B. campestris*. Embryo rescue technique can help in transfer of resistance from these species.

Resistance against *Alternaria*

Host resistance against *Alternaria* species has various components and it is multilayered. Inheritance of resistance in inter and intra-specific crosses of *B. juncea* and *B. carinata* to *A. brassicae* is governed by additive genes, dominant genes, additive x additive type epistatic genes, additive x dominance and dominance x dominance type of non-allelic interaction genes. Inter-mating between tolerant plants helps in increasing the level of resistance against *A. brassicae* by pyramiding of resistant genes. High level of horizontal resistance in genotypes of oilseed *Brassicas* has been recorded (Saharan and Krishnia, 2001). Genotypes PR-8988, PR-9024, PAB-9511, PAB-9534, EC-399296, EC-399299, and PHR-2 show higher degree of tolerance/ partial resistance or slow blighting. Epicuticular wax (Candle, Tobin, and Tower), low number and narrow stomatal aperture (Tower, RC-781) provide resistance to *Alternaria* infection in *Brassica* species (Saharan and Kadian, 1983; Tewari, 1991). The concentration of phenolic compounds, activation of polyphenol oxidase and catalase is higher in tolerant genotypes. Chitinase modifying proteins (cmps) are secreted by fungal pathogens of crucifers, which interfere with fungalysin cmp activity to improve plant resistance against multiple fungal diseases (Naumann and Wicklow, 2013). GLIPl in association with ethylene signaling may be a critical component in plant resistance *(A. thaliana)* to *A. brassicicola* (Oh *et al.,* 2005). *B. juncea* plants transformed with chitinase gene tagged with an over expressing promoters, 35S CaMV give defense response by degrading the cell walls of invading fungi (Mondal *et al.,* 2003). -aminobutyric acid treatment leads to proper balance of oxidant and antioxidants suitable for expression of resistance in *B. carinata* against *A. brassicae* by curtailing pathogens entrance during early stages of colonization (Chavan *et al.,* 2013). Zeatin a cytokinin up regulates plant immunity via an elevation of MAPK-4 and antagonizes the effects of *A. brassicae* (Marmath *et al.,* 2013).

Transgenic expression of hevein, the rubber tree lectin, in *B. juncea* cv. RLM-198 confers protection against *A. brassicae* (Kanrar *et al.,* 2002), -amino-butyric acid pretreatment of *B. juncea* plants induces *A. brassicae* resistance mediated through an enhanced expression of pathogenesis related protein genes, independent of SA and

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JA accumulation (Kamble and Bhargava, 2007). The cDNA encoding Pm AMP 1 has been successfully incorporated into the genome of *B. napus*, and it is in planta expression confers greater protection against *A. brassicae*. Pm AMPl is a cysteine rich antimicrobial peptide isolated from *Pinus monticola* (Verma *et al.*, 2012). Combined expression of a barley class II chitinase and Type I ribosome inactivating protein in transgenic *B. juncea* provides resistance against *A. brassicae* (Chhikara *et al.*, 2012). Transcriptional responses to exposure to the brassicaceous defense metabolites camalexin and allyl-isothiocyanate in *A. brassicicola* have been recorded (Sellam *et al.*, 2007).

Elicitation and accumulation of phytoalexins in crucifers after exposure to Alternaria and their role in disease resistance have been demonstrated (Verma and Saharan, 1994). Calcium sequestration property of *A. brassicae* can be used in enhancing resistance to this pathogen in rapeseed by soil or foliar application of calcium compounds (Tewari, 1991). In-spite of several bottlenecks in the development of resistant cultivars, various methods/techniques including conventional as well as biotechnological approaches are being utilized to incorporate desired traits in cruciferous crops against Alternaria blight.

Breeding Approaches for Resistance

Genetics of Alternaria blight resistance in inter and intra-specific crosses of *Brassica juncea* and *Brassica carinata* have been determined for the inheritance of resistance to *Alternaria brassicae*. There is preponderance of additive gene effects compared to dominance gene effects. Therefore, reciprocal recurrent selection or diallel selective matings can be successfully used to develop tolerant/ resistant genotypes against Alternaria blight (Saharan and Krishnia, 2001). Inter-mating between resistant plants helps in increasing the level of resistance. The frequency of favourable genes for disease resistance increases in the population thus enhancing the probability of obtaining multiple disease resistance (Saharan and Krishnia, 2001). The selection for resistance against Alternaria blight should be done in inter-mated population rather than in F_2 and F_3 population, since the generations of inter-varietal hybrids would prevent the harmful effects of linkages and linkage disequilibrium,

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and shuffle the desirable genes in one recombinant.

Genetic Engineering

Non-availability of resistance sources within cross able germplasm of Brassica needs the use of genetic engineering approaches to develop genetic resistance against Alternaria blight. A number of genes for imparting resistance this fungus has been transferred to *B. juncea* via genetic transformation technique. *Osmotin* protein introgressed into Indian mustard delayed the appearance of symptoms of Alternaria blight disease (Taj *et al.*, 2004). Class I basic *glucanase* gene from tomato have been transformed by Mondal *et al.* (2007) into *B. juncea* v ar. RLM 198 and found that the transgenic plants expressing *glucanase* exhibited restricted number, size and spread of lesions caused by *A. brassicae* under pathogen-challenged conditions and the onset of disease was also delayed as compared to the non-transformed plants. Rustagi *et al.* (2014) reported a significant inhibition in hyphal growth of both A. *brassicae* and *Sclerotinia sclerotiorum* in transgenic Indian mustard plants developed with *msrAI* gene coding for antimicrobial peptides, potential for resistance against a broad spectrum of phytopathogens. Hada *et al.* (2015) developed transgenic *B. juncea* cv. Varuna plants with *thaumatin* gene *through Agrobacterium tumefaciens-mediated* genetic transformation technique. T_1 transgenic lines expressing the *thaumatin* gene showed an enhanced resistance against *Alternaria brassicae* by inhibiting the fungal growth up to 54 per cent as compared non-transformed plants.

Screening Techniques

Various screening techniques have been used by various workers for particular disease. However standard methods adopted in All India Coordinated Research Project (rapeseed and mustard) for screening of resistant/ tolerant genotypes against white rust, Alternaria blight, Sclerotinia stem rot and downey mildew is detaled below: (Proceedings, 24th AGM of AICRP-RM, ICAR-DRMR Bharatpur).

Method of Artificial Inoculation for Alternaria Blight

Test plants (including checks) should be inoculated twice i.e. at initiation of flowering and pod formation in the afternoon (after 1500 hrs) with conidial suspension (10^5 du/ ml) of pure culture of *Alternaria brassicae* using distilled water. Disease severity

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should be recorded 90 DAS/at maximum disease pressure on leaves and 15 days before harvest on pods.

Observations to be Recorded

1. Date of first appearance of each disease including bacterial rot
2. Data as percent disease severity /percent disease incidence for WR (75 DAS/at maximum disease pressure), AB/PM/BR (90 DAS/at maximum disease pressure) on leaves and pods and number of staghead (15 days before harvest) should be recorded on 10 randomly selected plants from each plot using 0-9 scale. Date of observation and date of sowing should be indicated in data sheet itself.
3. Cotyledonary infection due to downy mildew and pod infection due to Alternaria blight should be recorded separately. Staghead formation should be recorded as percent incidence and percent twigs infected. Staghead (per cent twigs affected) = (number of twigs infected/total number of twigs) x 100.
4. Data for all major diseases may be recorded as percent disease severity (AB, WR and PM) on leaves/pods or as percent disease incidence (SR, DM, CR, BR).
5. Date of each observation should be provided in the data sheet.
6. Data should be statistically analysed as per the design using ANOVA after arc sin transformation. Actual and transformed (in parenthesis) values along with mean, CD ($P< 0.05$) and CV (per cent) are to be submitted for report preparation.
7. Scale (0-9) for rating of entries for reaction to Alternaria blight, white rust and powdery mildew should be used.

(Immune for WR) = No lesion

(HR) = Non-sporulating pinpoint size or small brown necrotic spots, less than 5 per cent leaf area covered by lesion

3 (R) = Small roundish slightly sporulating larger brown necrotic spots, about 1-2 mm in diameter with a distinct margin or yellow halo, 5-10 per cent leaf area covered by lesions.

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5 **(MR)** = Moderately sporulating, non-coalescing larger brown spots, about 2-4 mm in diameter with a distinct margin or yellow halo, 11-25 per cent leaf area covered by the spots

7 (S) = Moderately sporulating, coalescing larger brown spots about 4-5 mm in diameter, 26-50 per cent leaf area covered by the lesions.

9 **(HS)** = Profusely sporulating, rapidly coalescing brown to black spots measuring more than 6mm diameter without margins covering more than 50 per cent leaf area

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