European Journal of Plant Pathology **105:** 217–220, 1999.

Short communication

Induction of resistance in mustard (*Brassica juncea*) against Alternaria black spot with an avirulent *Alternaria brassicae* isolate-D

Vishwanath, S.J. Kolte, M.P. Singh and R.P. Awasthi
Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar – 263 145, India (Fax: 091-05944-33473)

Accepted 4 January 1999

Key words: Alternaria brassicae, biological control, cross infection, disease management

Abstract

Resistance in susceptible mustard cv. PR-15 against the highly virulent *A. brassicae* isolate A (AbA) and moderately virulent isolate C (AbC) was induced using an avirulent *Alternaria brassicae* isolate D (AbD). The induction of resistance due to AbD against AbA or AbC resulted in significant reduction in disease severity. The *A. alternata* (Aa) failed to induce resistance against AbA and AbC, on the contrary it induced susceptibility against them.

Alternaria black spot (ABS) of rapeseed-mustard caused by *Alternaria brassicae* (Berk.) Sacc. is the most important disease in India, where it causes an average yield loss of 35–40% (Kolte et al., 1987), 5% in Western Canada (Tewari, 1991) and 10% in the United Kingdom (NIAB, 1985). The disease is also of major importance in Australia (Stovold et al., 1987), France (Brun et al., 1987) and Poland (Madej, 1986).

Three distinct *A. brassicae* isolates' A (highly virulent), C (moderately virulent) and D (avirulent) are prevalent in India (Kolte et al., 1991). More recent work done using several monoconidial cultures and different sets of differential host varieties confirms such variations in *A. brassicae* (Vishwanath and Kolte, 1997).

The present study was undertaken to explore the possibility of utilizing induced host resistance as a realistic alternative to classical fungicides in ABS management. It is well established that resistance can be induced in plants by biotic as well as by abiotic agents (Kessman et al., 1994a,b; Kombrink and Sommsich, 1995; Sticher et al., 1997). Induced resistance means enhancement of resistance in plants being otherwise susceptible to diseases without changing their genetic constitution, for example, by breeding or by genetic engineering. Thus, genes in the susceptible plants can be activated to show resistance by inoculation with avirulent forms of the pathogen, hypovirulent forms of the pathogen and non-pathogens or by restricted inoculation with pathogens (Deverall, 1995).

Such studies in the case of ABS disease of mustard using avirulent forms of pathogen have not been reported. Therefore, this research communication deals with induced resistance in mustard using an avirulent isolate of *A. brassicae* against virulent isolates of the same pathogen.

Single conidial cultures of *A. brassicae* isolates A, C and D from *Brassica carinata cv. PPCS-1* (*Ethiopian mustard*) *and A. alternata* from *B. juncea cv*. PR-15 were maintained for inoculum production on radish dextrose agar (RDA) medium supplemented with Rose Bengal 50 mg L^{-1} at $(20 \pm 2)^{\circ}C$ (Thakur and Kolte, 1985). Conidial suspensions of these cultures were prepared from 15-day-old cultures in sterilized distilled water. After passing through four layers of cheese cloth, the conidial suspensions were centrifuged at 3000 rpm for 5 min and unwanted material was removed. The conidial concentration of the inoculum was adjusted to 1.5×10^4 conidia mL^{-1} with the help of a haemocytometer. The 4th fully expanded true leaf of 30-day-old pot-grown *B. juncea cv*. PR-15 plants was inoculated both when attached on to the plant and detached from the plant. In the case of inoculation of attached leaves, in one

set of experiments one full leaf was inoculated while in another set only half the leaf was inoculated with the avirulent *A. brassicae* isolate D (AbD) and *A. alternata* (Aa) separately. After 12 and 24 h, the above leaves were challenged inoculated with the virulent *A. brassicae* isolate A (AbA) and *A. brassicae* isolate C (AbC) keeping appropriate check treatments, as per the details given below:

Inducer inoculation	Challenge inoculation	Treatment abbreviated as
Alternaria alternata (Aa)	*Alternaria brassicae* isolate A(AbA)	Aa–AbA
A. alternata (Aa)	*A. brassicae* isolate C(AbC)	Aa–AbC
A. brassicae isolate D (AbD)	*A. brassicae* isolate A(AbA)	AbD–AbA
A. brassicae isolate D (AbD)	*A. brassicae* isolate C(AbC)	AbD–AbC
A. alternata (Aa)	Distilled sterilized water only (w)	Aa–w (Check)
A. brassicae isolate A (AbA)	Distilled sterilized water (w)	AbA–w (Check)
A. brassicae isolate C (AbC)	Distilled sterilized water (w)	AbC–w (Check)
A. brassicae isolate D (AbD)	Distilled sterilized water (w)	AbD–w (Check)

After inoculation, the pots were kept at room temperature $20 \pm 2^{\circ}$C in a moist chamber with 90–95% RH for 3 days and later removed for symptoms to develop. Each treatment was repeated 3 times.

In the case of the detached leaf inoculation, the 4th fully expanded leaves were detached with a sterilized razor blade. The leaves, after washing gently under tap water, were surface-sterilized with 0.5% NaOCl solution for 3 min and rinsed 3 times with sterilized distilled water for removal of excess amounts of NaOCl. These leaves were also dipped in streptomycin solution (1000 ppm) to eliminate bacterial contamination and dried on sterilized blotting paper. Inoculation of these leaves was also done as mentioned above. The inoculated leaves for each treatment were kept separately inside Petri-dish moist chambers at $20 \pm 2^{\circ}$C up to 10 days. The experiment was repeated twice.

Observations were recorded 10 days after inoculation. The average number of spots (ANS) per 10 cm^2 leaf area, average size of spots (ASS) and average infection score (AIS) on individual leaf were recorded for each treatment using a 0–5 disease rating scale, where 0 = no symptoms; 1 = 1–10%; 2 = 11–25%; 3 = 26–50%; 4 = 51–75% and 5 = more then 75% leaf area covered with the spots. The treatment differences were statistically analyzed at 5% level of significance using the *F*-test.

The results revealed that when the avirulent isolate AbD was pre-inoculated either on full leaves or half leaves 24 h prior to challenge inoculation with virulent isolates AbA and AbC of the same pathogen, more than 60% reduction in disease severity in terms of ANS, ASS and AIS was obtained in comparison to their control(s) without pre-inoculation both in attached (Table 1) and detached (Table 2) leaves. The full expression of induction of resistance (IR) occurred after 24 h of induction period and remained consistent up to 72 h. It could thus be quite possible that a set of plant genes, known as resistance response or defense-related genes, in mustard are activated by biotic inducers like avirulent isolate AbD. Many such genes could encode products which are believed to participate in the inhibition of pathogen development through production of pathogenesis-related proteins (PR-proteins) like chitinase, β-1,3-glucanase, hydroxy-rich-glycoproteins, and by stimulating the activity of enzymes for lignin and phytoalexin synthesis as it has been well studied in many other cases of systemic acquired resistance (SAR) in plants (Ryals et al., 1994; Schneider et al., 1996; Uknes et al., 1996). Further studies in this direction in *Brassica–A. brassicae* system should yield some useful information.

Most of the earlier work suggested that necrotization is a key factor in SAR-induction (Kuc, 1983). In our study AbD showed a very weak disease reaction on the host with a few very small necrotic spots on the leaves, which may fulfill the requirement of triggering host defence response. Considering the overall impact of IR, the *Brassica–A. brassicae* (isolate D) system could be evaluated on field-grown plants with regard to

Table 1. Effect of pre-inoculation with *A. alternata* and avirulent *A. brassicae* isolate D on induced resistance in *B. juncea cv*. PR-15 on full attached leaf

Treatments[a]	Average no. of spots/10 cm^2 (ANS)	Average size of spots (mm) (ASS)	Average infection score (AIS)
Aa–AbA	15.0^{a}(+4.8)^{a}	4.13^{a}(+1.0)*	2.66^{a}(+33.0)
Aa–AbC	14.33^{ab}(+13.2)	1.86^{b}(−3.6)	1.83^{b}(+10.2)
AbD–AbA	2.66^{d}(−81.4)*	1.00^{c}(−75.6)*	0.66^{c}(−67.0)*
AbD–AbC	4.00^{cd}(−68.6)*	1.00^{c}(−48.2)	1.00^{c}(−39.8)*
Aa–w (Check)	0.00^{a}	0.00^{d}	0.00^{d}
AbA–w (Check)	14.33^{a}	4.09^{a}	2.00^{b}
AbC–w (Check)	12.66^{b}	1.93^{b}	1.66^{b}
AbD–w (Check)	5.66^{c}	1.00^{c}	0.83^{c}

[a]The figures given in the parentheses are the percent increase(+)/ decrease(−) over their respective checks. Values in a column with the same letter do not differ significantly at the 5% level using the *F*-test.

Table 2. Effect of pre-inoculation with *A. alternata* and avirulent *A. brassicae* isolate D on induced resistance in *B. juncea cv*. PR-15 on full detached leaf

Treatments[a]	Average no. of spots/10 cm^2 (ANS)	Average size of spots (mm) (ASS)	Average infection score (AIS)
Aa–AbA	14.08^{a}(+4.3)	4.00^{a}(+29.0)	2.33^{a}(+21.4)
Aa–AbC	12.16^{bc}(+2.8)	2.68^{bc}(+75.2)	1.00^{c}(−42.9)
AbD–AbA	0.96^{e}(−92.9)	0.46^{de}(−85.2)	0.46^{de}(−76.0)
AbD–AbC	4.67^{d}(−60.52)	1.26^{de}(−17.65)	0.67^{cd}(−61.7)
Aa–w (Check)	0.00^{a}	0.00^{e}	0.00^{e}
AbA–w (Check)	13.50^{ab}	3.10^{ab}	1.92^{ab}
AbC–w (Check)	11.83^{c}	1.53^{cd}	1.75^{b}
AbD–w (Check)	1.13^{c}	1.30^{de}	0.50^{d}

[a]The figures given in the parentheses are the percent increase (+)/ decrease (−) over their respective checks. Values in a column with the same letter do not differ significantly at the 5% level using the *F*-test.

whether it might be enough to be used as a component in integrated disease management.

In the present study, when Aa was pre-inoculated, it did not induce resistance against infection caused by AbA or AbC (Tables 1 and 2). Rather Aa failed to produce any disease reaction on the host and hence it was unable to recognize the host at all *vis-à-vis* being unable to trigger any induction of resistance in the host. These results are in contrast with the observations made by Mercer et al. (1993) and Coldwell et al. (1995), who reported that disease caused by *A. brassicae* could be reduced by prior inoculation of plants with *A. alternata*.

References

Awasthi RP and Kolte SJ (1989) Variability in *Alternaria brassicae* affecting rapeseed and mustard. Indian Phytopath 42: 275 (Abstr)

Brun H, Plessis J and Renard M (1987) Resistance of some crucifers to *Alternaria brassicae* (Berk). Sacc Proc 7th Int Rapeseed Congr Poznan, Poland. pp. 1222–1227

Coldwell MAH, Mercer PC, Bennett RN and Doughty KJ (1995) The interaction between *Alternaria alternata* (Fr.) keissler and oilseed rape (*Brassica napus* L.). Proc. 9th. Int. Rapeseed Congr. 4–7 July. Cambridge, U.K. Vol. 4: 1246–1247

Deverall BJ (1995) Plant protection using natural defense systems of plants. Adv Plant Pathol 11: 211–228

Kessman H et al. (1994a) Induction of systemic acquired disease resistance in plants by chemicals. Annu Rev Phytopathol 30: 439–459

Kessman H, Staub T, Ligon J, Oostendorp M and Ryals J (1994b) Activation of systemic acquired disease resistance in plants. European J Plant Pathol 100: 359–369

Kombrink E and Sommsich IE (1995) Defence responses of plants to pathogens. Adv Bot Res 21: 1–34

Kolte SJ, Awasthi RP and Vishwanath (1987) Assessment of yield losses due to Alternaria blight in rapeseed and mustard. Indian Phytopath 40: 209–211

Kolte SJ, Bardoloi DK and Awasthi RP (1991) The search for resistance to major diseases of rapeseed mustard in India. Proc 8th Int Rapeseed Congr 9–11 July, Saskatoon, Canada, Vol. 1: 219–225

Kuc J (1983) Induced systemic resistance in plants to disease caused by fungi and bacteria. In: Bailey JA and Deverall BJ eds. The Dynamics of Host Defence. Academic Press, New York. pp. 191–221

Madej T (1986) Incidence of disease affecting-cruciferous plants in Poland. Wiss Wilhelm-Pieck-Univ Rostock, Naturwiss Reihe 35: 50–51

Mercer PC, Ruddock A, Mee E and Papadopolous S (1993) Biological control of *Alternaria brassicae* of linseed and oilseed rape. Bull Int Org Biol Control 16: 89–99

National Institute of Agricultural Botany (NIAB) (1985) Diseases of Oilseed Rape and Fodder Brassicas. Cambridge, United Kingdom

Ryals J, Uknes S and Ward E (1994) Systemic acquired resistance. Plant Physiology 104: 1109–1112

Stovold GE, Maiter RL and Francis A (1987) Seed-borne levels, chemical seed treatment and effects on seed quality following a severe outbreak of *Alternaria brassicae* on rapeseed in New South Wales. Plant Prot Quart 2: 128–131

Schneider M, Schweizer P, Meuwly P and Metraux JP (1996) Systemic acquired resistance in plants. Int Rev Cytology 168: 303–340

Sticher L, Mauch-Mani B and Metraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35: 235–270

Tewari JP (1991) Current understanding of resistance to *Alternaria brassicae* in crucifers. Proc 8th Int Rapeseed Congr 9–11 July, Saskatoon, Canada Vol 2: 471–476

Thakur R and Kolte SJ (1985) Radish root extract agar, a suitable medium for the growth and sporulation of *Alternaria brassicae* Cruciferae News Letter 10: 117–118

Uknes S et al. (1996) Reduction of risk for growers: Methods for the development of disease resistant crops. The New Phytologist 133: 3–10

Vishwanath and Kolte SJ (1997) Variability in *Alternaria brassicae*: response to host genotypes, toxin production and fungicides. Indian Phytopath 50(3): 373–381