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Alternaria blight: a chronic disease in rapeseed-mustard

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Abstract

Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world affects most cruciferous crops and is one among the important diseases of rapeseed-mustard causing severe yield losses with no proven source of transferable resistance in any of the hosts. The pathogen is greatly influenced by weather with the highest disease incidence reported in wet seasons and in areas with relatively high rainfall. *A. brassicae* can affect host species at all stages of growth, including seed. Symptoms of the disease are characterized by formation of spots on leaves, stem and siliquae. Identification of signal molecules for induced resistance, development of bioformulations and disease forecasting module will enable trigger newer strategies for eco-friendly disease management for providing safer Alternaria blight free production of rapeseed-mustard with improved crop health.

Key words: *Alternaria blight, rapeseed-mustard, symptoms, variability, pathogen biology, survival*

Introduction

Rapeseed-mustard is group of crops contributes 32% of the total oilseed production in India, and it is the second largest indigenous oilseed crop. Out of 75.55 m tones of estimated rapeseed-mustard produced over 30.51 m ha in the world, India produces 7.36 m tones from 6.18 m ha with 1190 kg/ha productivity (GOI, 2009). Despite considerable increase in the productivity and production under Technology Mission, huge amount of money is spent on the import of edible oil. A wide gap exists between the potential yield and the yield realized at the farmer's field, which is largely because of number of biotic and abiotic stresses to which the rapeseed-mustard crop is exposed. Among the biotic stresses, Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world and is one among the important diseases of Indian mustard causing up to 47% yield losses (Kolte, 1985) with no proven source of transferable resistance in any of the hosts. Average yield losses in the range of 32-57 per cent due to Alternaria blight have been reported from Nepal (Shrestha *et al.*, 2005). *Alternaria* affects most cruciferous crops, including broccoli and cauliflower *(Brassica oleracea L.* var. *botrytis L.)*, field mustard and turnip *(B. rapa L.* (synonym: *B. campestris L.)*, leaf or Chinese mustard *(B. juncea)*, Chinese or celery cabbage *(B. pekinensis)*, cabbage *(B. oleracea* var. *capitata)*, rape *(B. campestris)*, and radish *(Raphanus sativus)*. *A. brassicae* and *A. brassicicola* are cosmopolitan in their distribution. *A. raphani* and *A. alternata* is widespread in the Northern hemisphere (Jasalavich *et al*., 1995). Different species of *Alternaria* on *Brassica* spp. vary in host specificity. *Alternaria brassicae* also produces a host-selective pathotoxin 'dextruxin B' (Pedras and Smith, 1997). The pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons and in areas with relatively high rainfall (Humpherson-Jones and Phelps, 1989). Black spot of oil seed rape, cabbage, cauliflower and mustard crops have been reported from many countries, *viz*., India (Kadian and Saharan, 1983), Italy (Tosi and Zazzerini, 1985), USA, UK and several other European countries (Gladders, 1987), Canada (Berkeramp and

Kirkham, 1989; Conn and Tewari, 1990), Iran (Nourani *et al*., 2008) etc.

Diagnostic symptoms

Symptoms of the disease are characterized by formation of spots on leaves, stem and siliquae. *A. brassicae* and *A. brassicicola* can affect host species at all stages of growth, including seed. On seedlings, symptoms include dark stem lesions immediately after germination that can result in damping-off, or stunted seedlings. *A. raphani* produces black stripes or dark brown, sharp-edged lesions on the hypocotyl of the seedling. It grows in the vascular system and rapidly infects the entire seedling (Valkonen and Koponen, 1990). Spots produced by *A. brassicae* appear to be usually grey in colour compared with black sooty velvety spots produced by *A. brassicicola*. Spots produced by *A. raphani* show distinct yellow halos around them. However, the symptoms may vary with the host and environment. Symptoms are first visible on lower leaves with appearance of black points, which later enlarge to develop into prominent, round, concentric spots of various sizes. With progress, the disease appears on middle and upper leaves with smaller sized spots, when defoliation of lower leaves occurs. Later, round black conspicuous spots appear on siliquae and stem. These spots may coalesce leading to complete blackening of siliquae or weakening of the stem with formation of elongated lesions. Rotting of the seed may be seen just beneath the black spot on siliqua of toria and yellow or brown sarson. Spots on mustard siliqua are brownish black with a distinct grey centre. When older plants become infected, symptoms often occur on the older leaves, since they are closer to the soil and are more readily infected as a consequence of rain splash or wind blown rain. Late infection or infection of older leaves does not characteristically reduce yields and can be controlled through intensive removal of infected leaves (Chupp and Sherf, 1960). Fruit-bearing branches and pods show dark or blackened spots that result in yield loss due to premature pod ripening and shedding of the seed. The infection of Alternaria blight on leaves and silique reduces the photosynthetic area drastically. The phase of infection on silique adversely affects the normal seed development, seed weight, colour of seed and percent oil content in seed and the quality of seed.

Identity and Biology of the Pathogen

Genus *Alternaria* was described by Nees in 1816 with *A. tenuis* as the type and only member of the genus, which later was renamed as *A. alternata* as type species. No clear consensus on *Alternaria* taxonomy emerged for over 100 years. Elliot (1917) suggested that the genus could be organized into six groups based upon common characteristics of conidia length, width and septation, with each group designated by a typical species. The need for additional groups encompassing taxa not covered in his work was clearly recognized. Neergaard (1945) proposed three sections for the genus based upon the formation of conidia in long chains (Longicatenatae), short chains (Brevicatenatae), or singly (Noncatenatae). According to Ellis (1971), it contains 44 species. *Alternaria* species are either parasites on living plants or saprophytes on organic substrate. The host range of pathogenic *Alternaria* is very broad. It is easy to recognize *Alternaria* sp. by the morphology of their large conidia. They are catenate, formed in chains or solitary, typically ovoid to obclavate, often beaked, pale brown to brown, multi-celled and muriform (Ellis, 1971). Modern concepts of these genera emerged from the work of Simmons (1967) in his paper "Typification of *Alternaria, Stemphylium*, and *Ulocladium"*. The historical controversy surrounding the taxonomy of these fungi is best illustrated by the fact that most "atypical" species have been placed into more than one of these genera since their initial identification. Simmons (1995) expanded concepts from both Elliot and Neergaard in loosely organizing the genus into 14 species-groups based upon characteristics of conidia and catenulation. Additional species-groups discussed in other work include the *A. arborescens, A. brassicicola, A. porri,* and *A. radicina* groups (Roberts *et aI*, 2000; Simmons, 1995; Pryor and Gilbertson, 2000, 2002). Although the use of species-group designation does not resolve definitive species boundaries within *Alternaria,* advantages of its use are that it organizes at the sub-generic level the morphologically diverse assemblage of *Alternaria* species and permits the

generalized discussion of morphologically similar species without becoming overly restricted due to nomenclatural uncertainty. In addition, the species-group concept has provided an important framework for hypothesis testing in advanced studies on *Alternaria* phylogeny.

Survival

Survival of the pathogen on diseased seed or affected plant debris in tropical or sub-tropical India has been ruled out (Mehta *et al*., 2002), unlike the situation in temperate conditions (Humpherson-Jones and Maude, 1982). In India, oilseed Brassicas are sown from late August to November, depending on the crop, prevailing temperature and availability of soil moisture for seed germination. Harvest occurs from February to May. Off-season crops are grown in non-traditional areas from May to September and this, coupled with harbouring of the fungal pathogen by vegetable *Brassica* crops and alternative hosts (*Anagallis arvensis, Convolvulus arvensis*), could be reason for carryover of the *A. brassicae* from one crop-season to another (Tripathi and Kaushik, 1984; Verma and Saharan, 1994; Mehta *et al*., 2002). Thus, air-borne spores of *A. brassicae* form the primary source of inoculum of this polycyclic disease (Kolte, 1985).

Variability in Pathogen

Variation in isolates of *A. brassicae* is indicated (Mridha, 1983, Vishwanath *et al*., 1999). Studies on pathogenic variability have to be the foundation for development of pre-breeding populations as strategic defence mechanism. Three distinct *A. brassicae* isolates, A (highly virulent), C (moderately virulent) and D (avirulent) are prevalent in India (Vishwanath and Kolte, 1997). Formation of chlamydospores is reported in *A. brassicae* and *A. raphani* while microsclerotia are found to be produced by the former. Some details of comparison of fungal structures among the three species of *Alternaria* encountered on rapeseed-mustard are given table 1. Variation among the Indian isolates of *Alternaria brassicae* has also been noted (Meena *et al*., 2005). Recently, 541 isolates of *Alternaria brassicae* were collected from 18 states of India, purified and catalogued (NRCRM, 2008).

Table 1: Comparison of fungal structures among the three species of *Alternaria* encountered on rapeseed-mustard

Fungal structures	*A. brassicae*	*A. brassicicola*	*A. raphani*
Mycelium	Septate, brownish grey	Septate, olive grey to greyish black	Cottony whitish to greenish grey or dark olive
Conidiophore	Dark, septate, arise in fascicles, 14-74µ x 4-8µ	Olivaceous, septate, branched, 35-45µ x 5-8µ	Septate, olive-brown, single or branched, 29-160µ x 4-8µ
Conidia	Brownish black, obclavate, muriform, produced singly or in chains or 2-3	Dark cylindrical to oblong, muriform, produced in chains of 8-10 spores	Olive-brown to dark, obclavate, muriform, more-or-less pin-pointed at each end, appear singly or in chains of up to 6 spores
Spore body (µ)	96-114 x 17-24	45-55 x 11-16	45-58 x 13-21
Spore beak length (µ)	45-65	none	1-25
Spore			
Transverse septation	10-11	5-8	6-9
Longitudinal septation	0-6	0-4	3-6
Rate of growth and sporulation on media	Rudimentary slow growth	Black sooty colony with distinct zonations, fast growing with abundant sporulation	Cottony mycelial colony with less abundant sporulation
Infection	Penetrates leaf only through stomata	Penetrates leaf directly or through stomata	Direct penetration

Pathogenic Variability at Molecular level

Variation at DNA level among *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein) Wiltshire, *A. raphani* J.W. Groves & Skolko and *A. alternata* (Fr.) Keissl have been established (Jasalavich *et al*., 1995) by restriction fragment length polymorphism (RFLP) (Botstin *et al*., 1980),

random amplified polymorphic DNA (RAPD) Hong *et al*. (1996) studied 53 strains of *A. brassicae* collected from regions in China for differences in virulence on 4 groups of Chinese cabbage with varying levels of resistance. Insight has recently been gained into genes being expressed during Alternaria infection of Brassica (Cramer and Lawrence, 2004). The authors used suppression subtractive hybridisation between RNA isolated from spores of *A. brassicicola*incubated in water and on the leaf surface of an ecotype of *A. thaliana* followed by cloning and sequencing of cDNA clones that were differentially expressed. One gene (P3F2), only expressed during infection, was identified. Its function remains to be determined. Labuda *et al*. (2008) clearly separated a new species, *A. jesenskae* from the other related large-spored and filament-beaked *Alternaria* species on the basis of sequences of the ITS1, 5.8S and ITS2 region as well as by its distinctive morphology.

Host- pathogen interaction

Activities of some compounds related to Camalexin ($C_{11}H_8N_2S$) and 6-methoxycamalex ($C_{12}H_{10}N_2SO$) were found to be toxic to *A. brassicae* (Dzurilla *et al*., 1998). Phytotoxin dextruxin B elicits phytotoxin response in *S. alba* (Pedras and Smith, 1997). Multi-layered resistance, multi-component–sensitivity to host-specific toxin dextruxin B (fig.1), quantitative and qualitative elicitation of phytoalexins, hypersensitive reaction, Ca sequestration, etc. determines fate of host-pathogen interaction (Tewari, 1991). *Alternaria*-

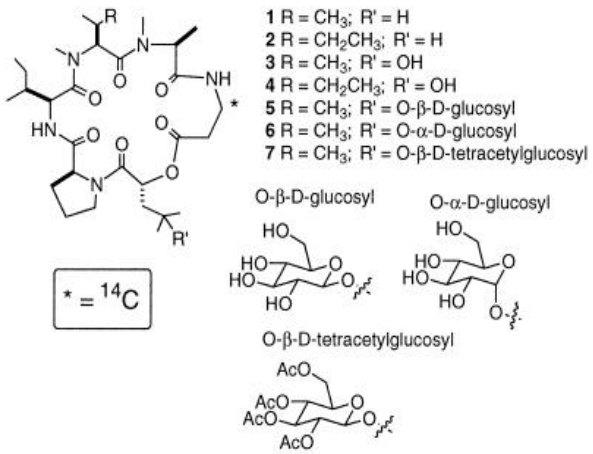

Figure 1: Chemical structure of dextruxin B

tolerant *Sinapis alba* has been found to detoxify dextruxin B (Pedras *et al*., 2001). Attempts to transfer resistance from wild crucifers to cultivated oilseed Brassicas were made (Shivanna and Sawhney, 1993). Cellulase enzymes (Nehemiah and Deshpande, 1977) and toxins (Hussain and Thakur, 1966; Durbin and Uchytil, 1977) are known to be produced by *A. brassicae*. However, their exact role in pathogenesis is not known. *Alternaria longipes* and *A. napiformae* are also reported on rapeseed-mustard from India (Purkayastha and Mallik, 1976; Rao, 1977). Signal transduction and expression analysis have been studied in *Alternaria – Brassica* interaction (Kalia *et al*., 2006).

Host-pathogen-environment- interaction

Efficient, economical and environment friendly control of the blight may be obtained through knowledge of its timing of attack in relation to weather factors, which may enable prediction of its occurrence so as to allow growers to take timely action in an efficient manner for crop management. Weather is an exceptionally important factor in the severity of Alternaria blight of oilseed Brassicas. Preliminary work indicates effects of temperatures, relative humidity (RH) and sunshine hours on occurrence of the blight on the oil Brassicas (Saharan and Kadian, 1984; Sinha *et al.*, 1992; Awasthi and Kolte, 1994; Dang *et al*., 1995). These reports indicate relationships between different weather factors and Alternaria blight occurrence through empirical models. However, there was a need to forecast accurately the crop age at first appearance of the disease and the risk that epidemic of the blight will occur on the crop to enable decisions to be taken regarding optimum time for spray of fungicide by farmers, and to avoid unnecessary pesticide application. Severity of Alternaria blight on leaves (Meena *et al*., 2002a) and pods (Sandhu *et al*., 1985) were higher in late sown crops. A delayed sowing results in coincidence of the vulnerable growth stage of plants as indicated earlier with warm (maximum temperature: 18-26^{o}C; minimum temperature: 8-12^{o}C) and humid (mean RH >70%) weather. Severity of Alternaria blight disease on leaves was favoured by a maximum temperature of 18-27^{o}C in

the preceding week, minimum temperature of 8-12°C, mean temperature >10°C, >92% morning relative humidity (RH), >40% afternoon RH and mean RH of >70%. Disease severity on pods was positively influenced by 20-30°C maximum temperature, >14°C mean temperature, >90% morning RH, >70% mean RH, >9 h sunshine and >10 h of leaf wetness. The regional and crop-specific models devised thereby could predict the crop age at which Alternaria blight first appears on the leaves, pods, the peak blight severity on leaves, pods and the crop age at peak blight severity on leaves, pods at least one week ahead of first appearance of the disease on the crop, thus allowing growers to take necessary action (Chattopadhyay *et al*., 2005).

In both 2002-03 and 2003-04, the *A. brassicae* spores started getting trapped on 23 Oct and in 2002-03 remained in the atmosphere till early April or the time when the crop got harvested from fields surrounding the trap and the daily mean temperatures rose beyond 35°C. During October-April, there was no major variation in daily total count of trapped spores noted after it reached a level following appearance on trap. However, a uniform diurnal variation in spore trap count was noted throughout the crop period viz., the variation in Feb 2003. The spore count on the trap was noted to rise gradually in the initial (0-6) hours of the day, which reached its peak between 2 PM – 3 PM before climbing down (Meena *et al*., 2005). This seemed to vary directly with the diurnal variation of temperatures and inversely with that of RH. This could be due to the production of spores during the night, which remained adhered to plant surfaces or spore producing lesions due to high RH or leaf wetness then. With rise in temperature and fall in RH (reduction of leaf wetness), the spores become free from the spore producing surfaces and are found to get trapped from the atmosphere (Humpherson-Jones, 1992). Sporulation of *A. brassicae* has been reported to be favoured by darkness (Humpherson Jones and Phelps, 1989).

Host resistance

Host resistance is one of the important components of integrated disease management. The regular appearance of this disease with no apparent variability among the released varieties of Indian mustard warrants immediate attention for the control of this disease. Among the oilseed *Brassica* species, *B. juncea* and *B. rapa* are more susceptible to Alternaria blight than *B. carinata* and *B. napus* (Skoropad and Tewari, 1977). Several sources of tolerance against Alternaria blight have been reported (Gupta *et al*, 2001), including dwarf *B. juncea* cv. Divya (Kolte *et al*., 2000). Sources of resistance to *A. brassicae* have also been identified in related and wild species, e.g., *Sinapis alba* L. (white mustard) (Hansen and Earle, 1997), *Camelina sativa* (L.) Crantz (false flax), *Capsella bursa-pastoris*, *Neslia paniculata* and taramira (Tewari and Conn 1993), *B. maurorum* (Chrungu *et al*, 1999), *B. desnottesii*, *Coincya pseuderucastrum*, *Diplotaxis berthautii*, *D. catholica*, *D. cretacea*, *D. erucoides* and *Erucastrum gallicum* (Sharma *et al*., 2002). Resistance to Alternaria blight in mustard has been found to be associated with leaf enzymes associated with the phenolic pathway, e.g., polyphenol oxidase, peroxidase and catalase, higher leaf sugar contents (Singh *et al*, 1999) and high deposits of leaf epicuticular wax forming a hydrophobic coating to reduce the adherence of water-borne inoculum, conidia germination and germtube formation (Saharan, 1992). The species *B. napus*, *B. carinata* and *S. alba* have relatively more epicuticular wax than *B. rapa* and *B. juncea* and tend to be less sensitive to Alternaria blight (Conn *et al*., 1984; Tewari, 1986). Older leaves are more susceptible than younger ones to *A. brassicae*. Systemic acquired resistance (SAR) induced by inoculation with avirulent race of *A. brassicae* has been reported (Vishwanath *et al*., 1999). Higher concentration of phenolics (polyphenol oxidase, peroxidase, catalase) in leaves, lower N (Singh *et al*., 1999), etc. have been related with resistance to Alternaria blight. Resistance to Alternaria blight of rapeseed-mustard is also found to be associated with factors like discouragement to conidial retention on plant surface like high deposits of epicuticular wax that form a physical barrier as a hydrophobic coating to reduce deposition of water-borne inoculum, reduce rate of conidia germination, germ

tube formation, etc. (Skoropad and Tewari, 1977; Saharan, 1992). *Brassica napus* (Tower, HNS-3), *B. carinata* (HC-2), *S. alba* have more wax on plant/ leaf surface compared to *B. rapa* (BSH-1, YSPB-24), *B. juncea* (RH-30) (Tewari, 1986). While studies on mechanism of tolerance to Alternaria blight have indicated effect of additive genes or polygene or cluster gene (Krishnia *et al*., 2000) with resistance being controlled by nuclear genes of partial dominance (Zhang FengLan *et al*., 1997), there has also been indication of components of resistance being significantly correlated to each other regarding slow blighting (Kumar and Kolte, 2001) and dominance (h) having a predominant role in genetic control of time of appearance, additive x dominance predominant for other disease progression factors viz., AUDPC, etc. (Kant and Gulati, 2002). Wild crucifers are found to elicit phytoalexins on challenge inoculation (Conn *et al*., 1988). Attempts to breed for resistance for these factors have yielded limited success.

Since resistance to Alternaria blight and leaf spots are found to be governed by additive or polygenes, breeding for resistance to these diseases could involve pyramiding of minor genes affecting the disease to provide additive/ polygene resistance against the disease, introgression of genes from material found resistant, reciprocal recurrent selection or diallel selective mating (Krishnia *et al*., 2000), wide hybridization (*B. alba*: Sigareva *et al*., 1999), molecular breeding (viz., from *Camelina sativa* by somatic hybridization: Sigareva and Earle, 1999; transgenic expressing *Trichoderma harzianum* endo-chitinase gene: Mora and Earle, 2001), pollen culture and sensitivity test to dextruxin B (Shivanna and Shawney, 1993), etc.

Rapid advances in techniques of tissue culture, protoplast fusion, embryo rescue, genetic engineering has made transfer of disease resistance traits across wide crossability barriers possible. Transgenic disease resistant plants which over-express different antifungal compounds like pathogenesis-related (PR) proteins (chitinase, glucanase, osmotin, etc.) and ribosome-inhibiting proteins (RIPs) viz., thionins, defensins and phytoalexins (Zhou *et al*., 2002) to inhibit growth of the pathogen seem less efficacious. Other transgenic disease resistant plants exploit defence mechanisms like hypersensitive response (HR) and systemic acquired resistance (SAR) through use of resistance (R) genes, signalling genes in SAR (like NPR) and two-component systems of R-Avr, Barnase-Barstar, etc. To exploit R genes for inducing HR, one requires better understanding of regulatory mechanisms and undesirable side effects from probable role of signalling genes in other pathways have to be appreciated.

A cDNA encoding hevein (chitin-binding lectin from *Hevea brasiliensis*) was transferred into *Brassica juncea* cv. RLM-198. Southern analysis of the putative transgenics showed integration of the transgene. Northern and Western analyses proved that the integrated transgene is expressed in the transgenics. In whole plant bioassay under glasshouse conditions, transgenics were found to possess parameters that are associated with resistance such as longer incubation and latent period, smaller necrotic lesion size, lower disease intensity and delayed senescence (Kanrar *et al*., 2002). Microarray studies have indicated that different defence signalling pathways converge to form substantial networks that control and coordinate regulatory interactions. Schenk *et al*. (2000) inoculated an ecotype of *Arabidopsis thaliana* with *A. brassicicola* (an incompatible interaction), or treated *A. thaliana* with various defence-related signalling molecules, such as salicylic acid (SA), methyl jasmonate (MJ) and ethylene. A study of 2,375 Expressed Sequence Tags (ESTs) representing a biased population of putative defence-associated and regulatory genes, identified genes that were differentially expressed in response to each of the treatments (pathogen, SA, MJ or ethylene), as well as a large group of genes that were coordinately expressed in the SA, MJ and ethylene treatments (Schenk *et al*., 2000). Progress in sequencing pathogens or beneficial microflora and the combination of bioinformatics and functional genomics are likely to provide a better understanding of plant-pathogen networks and lead to increase resistance to crop pathogens (Koltai and Volpen, 2003). In a breakthrough towards study of functional analysis of genes involved in *Alternaria-Brassica* interaction, use of linear minimal element is reported to provide high

throughput targeted gene disruption (Cho *et al*., 2006). A few expressed sequence tags have been identified in compatible *Alternaria-Brassica* interaction (Cramer *et al*., 2006). ²-aminobutyric acid has been reported to induce resistance in *B. juncea* against *A. brassicae* (Kamble and Bhargava, 2007).

Biological control

Till date chemical management was the only option against the problem. In many European countries, organic agriculture has rapidly been transformed from a farmers' movement to an institutionalised part of agricultural policy. In certification, compliance with published organic standards is verified through annual inspections on farms (Laura and Juha, 2004). However, some reports indicate possibility of biological management of the disease. Phyllosphere residents *Aureobasidium pullulans* and *Epicoccum nigrum* reduced the infection by *A. brassicicola*, especially when they were inoculated 14 h before the pathogen (Pace and Campbell, 1974). Spray of soil isolates of *Trichoderma viride* at 45 and 75 days after sowing could manage Alternaria blight of Indian mustard (*Brassica juncea*) as effectively as mancozeb (Meena *et al*., 2004), which have been confirmed later in multi-location trials (AICRP-RM, 2007). Botanicals viz., bulb extract of *Allium sativum* has been reported to effectively manage Alternaria blight of Indian mustard (Meena *et al*., 2004; Patni and Kolte, 2006).

Other methods of Alternaria blight management

Early sowing (Meena *et al*., 2002a) of well-stored clean certified seed after deep ploughing, clean cultivation, timely weeding and maintenance of optimum plant population, avoidance of irrigation at flowering and pod formation stages may help to manage the disease. Mancozeb was the best among all the treatments, resulting in the lowest disease severity on leaves of mustard (Meena *et al*., 2004). Iprodione (Rovral) spray has been found effective in checking silique infection due to *A. brassicae* (Cox *et al*., 1983). Soil application of K as basal has been found to check Alternaria blight disease in mustard (Sharma and Kolte, 1994).

Complete resistance against Alternaria blight is not available in the *Brassica* gene pool. Population improvement programme using the selected lines as base population is very likely to genetically enhance the level of resistance. In the absence of clearly defined isolates, the utilization of multi-location in the breeding programme can effectively address pathogenic variability besides efforts on biocontrol of the disease and epidemiological studies at different locations. Studies on variability at pathogenic and genetic level in *A. brassicae* could enable easier development of disease resistant material. Identification of signal molecules for induced resistance, development of bioformulations and disease forecasting techniques based on epidemiological findings will enable trigger newer strategies for environment-friendly disease management for providing safer Alternaria blight free production of Indian mustard with improved crop health.

Future Thrusts

The following issues need to be addressed to deal effectively against Alternaria blight in rapeseed-mustard:

- Relationship between pathogenic and molecular (sequence) variability among *Alternaria* isolates apart from relation with morphological and cultural data for the same.
- Molecular diagnostics of *Alternaria* taxonomy.
- Based on sequence data of type cultures, host differentials need to be fixed.
- Cross infectivity of *Alternaria* spp. (sub species) with other hosts (apart from designated host) need investigation.
- Establishment of relationship between *Alternaria* and *Stemphyllium* phylogeny.
- Complete picture of *Alternaria* spp. complex affecting each vegetable / *Brassica* crop (viz., which species of *Alternaria* infects what host plant).
- Maintenance / management of *Alternaria* cultures in repository by cryo-preservation / lyophilisation.

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