Journal of Applied and Natural Science 8 (2): 1110 - 1125 (2016)

JANS

Alternaria blight of oilseed brassicas: A review on management strategies through conventional, non-conventional and biotechnological approaches

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Received: September 15, 2015; Revised received: February 11, 2016; Accepted: May 11, 2016

Abstract: Oilseed Brassicas are contributing approximately 28 percent of the India's total oilseed production. This crop is gaining wide acceptance among the farmers because of adaptability for both irrigated as well as rainfed areas and suitability for sole as well as mixed cropping. Besides, it offers higher return with low cost of production and low water requirement. The production and productivity of oilseed brassicas are comparatively lower as compared to the world average due to the biotic and abiotic constraints. Among the biotic constraints, alternaria blight disease caused by *Alternaria spp.* has been reported from all the continents of the world, causing up to 70% yield losses in India. This disease was found on leaves, stems and siliquae and dark spots on the leaves and siliquae reduce the photosynthetic capacity and induce immature ripening, which causes reduced amount of quality seed and oil content. The severity of this disease depends upon weather conditions, varieties, age of host plants and virulence of the pathogens. Efforts are being done throughout the world for the management of alternaria blight of rapeseed-mustard. This paper comprehensively reviews the research of alternaria blight of rapeseed-mustard with special reference to management strategies through conventional, non-conventional and biotechnological approaches that leads to planning the future research. The present scenario demands the traditional and modern biotechnological techniques bringing together for integrated disease management according to the need and availability at farmers level for sustainable management of alternaria blight disease of oilseed brassicas.

Keywords: Alternaria blight, Management, Oilseed Brassicas, Pathogens, Symptoms

INTRODUCTION

Oilseed Brassicas are the third most important oil seed crop in terms of acreage and production after Soyabean and Palm in the world and occupy second position after groundnut in India (Kumar, 2012; Kumar and Chopra, 2014). Among the oilseed crops, India produced 8.03 mt of rapeseed-mustard from 6.36 mha of land with average productivity of 1262 q/ha during the 2012-13 season (Agricultural statistics at a Glance, 2014). The per capita consumption of edible oils in India has increased from 6.5 Kg. during the 1990-91 to 13.8 Kg during 2011-2012 (Economic survey, 2013-14). Brassicas have been cultivated in India for a very long time. The two species under cultivation are *B. rapa* and *B. juncea.* Among these two *B. juncea* is cultivated in about six million hectares in the northern states of India, Punjab, Haryana, Rajasthan, Gujarat, Uttar Pradesh, West Bengal and northeastern states during winter season. Three ecotypes of *B. campestris* ssp oleifera Brown sarson, Yellow sarson and toria are cultivated as catch crop in the northern India. In India, *B. juncea*, commonly known as Indian mustard, is a major cultivated brassica crop in north-west India, followed by limited cultivation of *B. napus* and *B. rapa* for vegetable oil production. To meet the growing demand and make India self-sufficient for edible oils, productivity of the oilseed crops should be increased since the possibility of increasing land under oilseed crops is very limited (Economic survey, 2013-14).

The development of high yielding varieties along with modern production technology leads to increase in production and productivity of rapeseed-mustard but the gap between potential yield and actual yields are widen due to the different biotic and abiotic factors. Amongst the major fungal diseases of oilseed brassicas prevalent in India, Alternaria blight disease is the most important and destructive disease causing heavy losses

all over the world attacking all *Brassica* species. *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 70% yield losses with no proven source of resistance against the disease reported till date in any of the hosts (Meena *et al.*, 2010; Meena *et al.*, 2012). This disease appears on leaves and stems of seedlings and adult plants and also in siliquae during the ripening stage. Dark spots on the leaves and siliquae reduce the photosynthetic capacity and induce immature ripening, which causes reduced amount of quality seed production in both vegetable and oleiferous brassicas (Kumar *et al.*, 2014).

The present review deals in brief about the fungal pathogens causing alternaria blight disease, host range, symptoms, epidemiology, disease cycle, Morphological and cultural characteristics, disease management strategies *viz.* conventional, non-conventional and biotechnological approaches.

Historical perspectives: The historical controversy surrounding the taxonomy of these fungi is because of their high variability in conidial morphology. There are more than 500 taxon of Alternaria in the literature. In addition to that about 400 species names in the old literature on *Macrosporium* Fries as well as 200 names published in other genera have been considered as basionyms for the names of *Alternaria species*. Angell (1929) considered Alternaria and Macrosporium synonymous and used the epithet Macrosporium to designate both the genera. Wiltshire (1933) agreed that Alternaria and Macrosporium are congeneric and stated that macrosporium in the original description was used to designate Alternaria type fungi having non-filiform spores and one spore per conidiophores and Alternaria used to designate both the genera. Neergaard (1945) suggested three sections for this genus on the basis of the formation of chains of conidia. The single, short (about 3-5 spores) and long chains (10 or more spores) conidia were known as Noncatenatae, Brevicatenatae and Longicatenatae respectively. He particularly considered the difference between the true beak (eurostrum), as an integral part of the conidium and the false beak (pseudorostrum), that is secondary conidiophores which are essential to generation of conidial chains Simmons laid the foundation of modern concepts for this genus through his research paper entitled "Typification of *Alternaria, Stemphylium,* and *Ulocladium"* published in Mycologia in 1967. He examined an authenticated specimen of the type species and redescribed the fungus and named *A. alternata* (Fries) keissler according to the International rules of Nomenclature. This specimen was assumed to represent Nee's *A. tenius* Nees (1816) and later compiled by Fries as *A. tenius* Fries (1832) with *A. tenius* Nees as a synonym. . According to Ellis (1971), genus Alternaria contains 44 species. *Alternaria* sp. is easily recognized 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, multicelled and muriform. Simmons (1992) redescribed numerous species and differentiated the genus into numerous species and also developed species-group system within the genus. Fourteen species groups were defined on the basis of conidial characteristics, the pattern of chain formation and the nature of the apical or sometimes lateral extensions of conidial cells.

Geographical distribution: Alternaria blight of rapeseed-mustard, the most common and destructive disease of oilseed brassicas is principally caused by an imperfect fungus, *Alternaria brassicae* (Berk.) Sacc. Three other species viz. *A. brassicicola* (Schw.) Wilts., *A. raphani* Groves and Skolko, *A. alternata* (Fr.) Keissler, are also associated with the affected plants under natural conditions in India (Kolte *et al.*, 1987).

In temperate climate countries such as Canada, United Kingdom, Poland, Sweden, France, Germany, Holland, Spain, and Cooler parts of India, the disease caused by *A. brassicae* is much more prevalent than other three species of Alternaria (Chahal and Kang, 1979; Tripathi *et al.*, 1980; Kadian and Saharan, 1983; Kolte, 1985b; Clear, 1992). As a result of survey of forty cruciferous crops, done in the year 1983 in Edinburgh area of United Kingdom, 73 per cent of total crops examined were infected with *A. brassicae* and only 16 per cent of the crops was infected by *A. brassicicola* (Anonymous, 1984). In the Alberta area of Canada, the disease caused by *A. alternata* is dominant on Polish type of rape and the same is more prevalent on the phylloplane of *Brassica campestris* and *B. napus* (Tsuneda *et al.*, 1978; Vaartnou and Tiwari, 1972; Visconti *et al.*, 1992). In contrast to the above, West Bengal state of India Alternaria blight is reported to be predominantly caused by *A. brassicicola* (Dasgupta *et al*., 1985; Sarkar and Sengupta, 1978; Satyabrata Maiti, 1989).

Economic importance: Alternaria blight of rapeseed-mustard has been found to be a constraint in the production of these crops in countries such as Germany (Stoll, 1948), Sweden (Borg, 1951), England (Loof, 1959), Canada (McDonald, 1959; Degenhardt *et al.*, 1974), and in India (Chahal and Kang, 1979; Kolte, 1982, 1985a, b; Kadian and Saharan, 1983; Kolte *et al.*, 1985, 1987). In India, the rapeseed-mustard crops are regularly affected by Alternaria blight which causes more than 46.57 per cent yield losses in yellow sarson and 35.38 per cent in mustard (Kolte, 1985a). Kolte *et al.* (1987) and Ram and Chauhan (1998) further reported that Alternaria blight may be responsible for 10-70 per cent average yield losses in rapeseed-mustard depending upon prevailing weather and disease situations. Alternaria infections have also been reported to affect the quantitative and qualitative changes in oil content. The loss in oil content of seed from heavily infected rapeseed plants by *A. brassicae* ranged from 14.58 to 39.57 per cent depending upon cultivars whereas in *B. juncea* it ranged from 14.12 to 29.07 per cent (Ansari *et al.*, 1988).

Host range: *Alternaria* species are either parasites on

living plants or saprophytes on organic substrate. The host range of pathogenic *Alternaria* is very broad. *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*).

Symptoms: All the three species of Alternaria are reported to infect seedling stage on cotyledons and in the adult stage on leaves, petioles, stem, inflorescence, siliquae and seeds. The variation in shape, size, colour and intensity of lesions are found on different host plants under different environmental condition. The initial infection on the lower leaves starts as minute brown to blackish lesions which multiply rapidly and later spread to the upper leaves, stem and siliquae. On the leaves, formation of concentric rings in the lesions and a zone of yellow halo around the lesion is very prominent (Kadian and Saharan, 1983). Several lesions on leaves unite to cause blighting and defoliation under humid weather. With progress, the disease appears on middle and upper leaves with smaller sized spots, when defoliation of lower leaves occurs. The lesions on leaves due to *A. brassicicola* are sooty black, velvety and copiously covered with black conidiophores and conidia, whereas those caused by *A. brassicae* are gray, dense and sparsely covered with brown conidiophores and conidia (Changsri, 1961). Spots produced by *A. raphani* show distinct yellow halos around them. However, the symptoms may vary with the host and environment. Later, round black conspicuous spots appear on siliquae and stem.The lesions on the stem are at first linear and then expand but remain usually elongated with pointed ends. Deep lesions on the siliquae cause infection in the seed. *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 hypocotyls of the seedling. It grows in the vascular system and rapidly infects the entire seedling (Valkonen and Koponen, 1990). The diseased seeds just beneath the black spot are small, shriveled, gray to brown in colour (Verma and Saharan, 1994). The blight also reduces seed size and impairs seed color and oil content (Kaushik *et al*., 1984).

Epidemiology: The optimum temperature for growth of *A. brassicae* and *A. brassicicola* is 22.5˚C and 25-27˚C respectively. The optimum temperature for conidial germination of *A. brassicae* is at 17-19˚ C and *A. brassicicola* is at 30˚C. Conidia germinate in both light and dark conditions but the maximum growth and sporulation of *A. brassicae* occurs in alternating cycles of light and dark. Dissemination of disease is favoured when the maximum temperature is in the range of 20-23˚C and minimum in the range 7-10˚C with an average relative humidity between 67 and 73% with frequent rains (70 mm) and the maximum frequency of rainy days *i.e.* 6-13 days during rosette to flowering stage of the crop (Awasthi and Kolte, 1989).

Disease cycle: Seeds have no effective role in initiation of the primary infection of *A. brassicae* to mustard crop in the subsequent crop season unless the seeds are stored in cold storage or in a region of low temperature. The primary infection can arise from either diseased stubbles or neighboring volunteer crucifers. The secondary infection takes place through conidia formed on infected leaves or pods and the infection cycle and spore production repeated throughout the growing season under favorable weather condition.

Causal fungi: Alternaria species: *A. brassicae* and *A. raphani* do not sporulate well as compared to *A. brassicicola* and *A. alternata* on PDA (Neergaard, 1945; Changsri, 1961; Prasad *et al.*, 1970; Goyal, 1977; Thind, 1977). *A. brassicae* has brown to brownish gray mycelium dark septate conidiospore with brownish black, obclavate, muriform long beak conidia. Maximum growth of *A. brassicae* has been observed at 20-25°C temperature and 5-8 pH. However, the sporulation of *A. brassicae* is reported to be increased on 10 per cent lucern seed decoction agar (McDonald, 1959) and PDA supplemented with mannitol (Thakur, 1985; Thakur and Kolte, 1985).

Alternaria spp. characterization

Morphological and cultural characterization: Fungi are among the most diverse organism groups on Earth (Hammond, 1995). Broad spectrum disease management strategy depends on understanding of pathogen population structure and mechanisms by which variation arises within populations. The variability of the pathogen is the foundation for development of prebreeding populations which leads to the development of resistant variety. Variation in pathogen populations can generally be detected with standard sets of differential hosts (Flor, 1971). The individual genotypes of a fungal pathogen may differ in many inherited characteristics, for example Morphology, physiology and pathogenecity (Person and Ebba, 1975). Most of the characteristics are governed by genes. New races of fungal pathogens are produced by mutation, recombination of nuclear genes during sexual reproduction and by re-assortment or exchange of genetic material in somatic cells.

The taxonomy of Alternaria is based primarily on the morphology and development of conidia and conidiophores and lesser degree on host plant association and colony morphology (Elliott, 1917; Wiltshire, 1933; Simmons, 1967). Classification of many Alternaria species is particularly difficult due to the variation and plasticity of colony and morphological characteristics. *A. brassicae, A. brassicicola A. raphani* and *A. alternata* exhibit some cultural, morphological, pathogenic and genetic variation (Verma and Saharan, 1994; Sharma and Tewari, 1995, 1998). The mycelium and

conidiophores of the *A. brassicae* are septate and the colour of mycelium and conidiophores are yellowish brown and chestnut brown to grayish brown respectively. Conidia of *A. brassicae* found single or in chains (upto four) and originated from the small pore of the conidiophore wall, straight or slightly curved, obclavate, 75-350 µ in overall length, 20-30 µ thick in broadest part, the beak about 1/3 to 1/2 the length of conidium and 5-9 µ thick. 10-11 transverse and 0-6 longitudinal septation are found in conidia. The growth of *A. brassicae* is slow and less sporulation. Conidia of *A. brassicicola* are mostly in chains of 20 or more, sometimes branched arising through small pore of conidiophore wall, straight or curved, more or less cylindrical but often slightly swollen at the base, septate, pale to mild olivaceous brown, 18-130 µ long, 8-30 µ thick in broadest part, the beak usually non-existent or 1/6 the length of conidium and 6-8 µ thick with less than 6 transverse septa and few upto 6 longitudinal septa. The growth pattern of A. *brassicicola* is fast, well developed black sooty colony and abundant sporulation. Conidia of *A. raphani* are commonly in chains of 2-3, straight or slightly curved, generally with short beak, dark golden brown or olivaceous brown, 50-130 µ long, 14-30 µ thick in the broadest part with 3-7 transverse and often a number of longitudinal septa. The cottony growth of mycelium and abundant sporulation are found in *A. raphani*. The colonies of *A. alternata* are usually black or olivaceous black, conidiophores arising solitary or in small groups, simple or branched, pale to mild olivaceous or golden brown. Conidia of *A. alternata* are branched, ellipsoidal, often with a short conical or cylindrical beak, overall length 20-63 µ, 9-18 µ thick in broadest part, beak pale, 2-5 µ thick with upto 8 transverse and usually several longitudinal septa. The study on cultural variability in *Alternaria* species in respect of mycelial growth and sporulation on different temperature, relative humidity, hydrogen ion concentration (Ansari *et al.*, 1989), media (Patni *et al*., 2005) and light (Ansari *et al*., 1989) have been done. Variability on the basis of morphology, sporulation, growth and other cultural characteristics also have been reported earlier. Goyal *et al.* (2011) showed high level of variability *in vitro* in respect of conidia length, width, beak length and number of septa. Kolte *et al.* (1989) distinguished three isolates of *A. brassicae*, on the basis of their morphology, sporulation, growth and cultural characteristics, which are prevalent in India. Three distinct *A.brassicae* isolates, A, C and D are highly virulent, moderately virulent and avirulent respectively (Vishwanath and Kolte, 1997). Pantnagar isolates A, C and D resembled the Bihar isolate BH1 and BH2 and Kanpur isolate K respectively (Kolte e*t al.*, 1991). Different temperatures were found optimum for growth and sporulation of *A. brassicae* in a range of 20–25^{o}C (Singh *et al.*, 2007) and 20–30^{o}C (Meena *et al.*, 2005), respectively. Mycelial growth, sporulation of *A. brassicae* is also affected by relative humidity and showed variation in its requirement (Meena *et al.*, 2005).

Molecular characterization: The amorphous physical characteristics, toxin production and colony characters of *Alternaria* spp. have made them difficult to classify satisfactorily (Rotem, 1994). Molecular methods have been used not only in differentiating between species (Kusaba and Tsuge, 1994; Jasalavich *et al*., 1995; Cooke *et al*., 1998; Roberts *et al.*, 2000), but also for assessing intra-specific variation (Adachi *et al*., 1993; Kusaba and Tsuge, 1994; Morris *et al*., 2000). Hong *et al*. (1996) studied 53 strains of *A. brassicae* collected from regions in China for differences in virulence on four differnt groups of Chinese cabbage with varying levels of resistance. Ribosomal DNA sequences, in particular the 5.8s ribosomal DNA (rDNA) and flanking internal transcribed spacer regions ITS1 and ITS2, have been used to study the phylogenetic relationships for one isolate each of *Alternaria brassicae, A. brassicicola, A. raphani, A. alternata* and *Pleospora herbarum*.. The 5.8 rDNA sequences from the *A. brassicae, A. brassicola, A. raphani* and *A. alternata* were identical but differed at only one base pair from *Pleospora herbarum*. The internal transcribed spacer sequences, especially ITS1, were very variable in both base composition and length. The 18s rDNA sequences were highly conserved, but enough variability was present to distinguish genera clearly (Jasalavich *et al*., 1995). Phylogenetic analysis of ITS and mitochondrial small subunit (SSU) rDNA sequences revealed that the *Stemphylium spp.* were distinct from Alternaria and *Ulocladium spp*. (Pryor and Gilbertson, 2000). 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. RAPD and RFLP markers were used for genetic variation in *Alternaria brassicae*, *A. brassicicola*, and *A. raphani* collected from geographically diverse regions of the world. UPGMA analysis of RAPD data of isolates of three *Alternaria species* showed four groups in which intra-regional variation between isolates was less apparent Variation was, however, higher in *A. brassicicola*, as based on RAPD analysis. Two isolates (from Canada and France) of *A. raphani* also showed variability with different RAPD profiles generated by all five primers tested. Five distinct polymorphic RAPD products were used as hybridization probes for RFLP analysis to detect inter- and intra-specific variation. Variation among *A. brassicae*, *A. brassicicola*, and *A. raphani* was evident. Non-radioactive probes were also used to hybridize with Southern blots of *A. brassicae*, *A. brassicicola*, *Leptosphaeria maculans*, *Rhynchosporium secalis* and *Brassica juncea* for the selection of *A. brassicae*-specific probe(s). Probe AbP3 specifically hybridized with restriction digests of *A. brassicae* but not with those of *A. brassicicola* or other tested species (Sharma and Tiwari, 1998). Morris *et al*. (2000) were able to identify a high level of genetic diversity within

A. alternata populations on tomato using RAPD within and between regions in California. Bock *et al*. (2002) studied the genetic variation within and between eighteen isolates of *Alternaria brassicicola*, five isolates of *A. alternata*, and a single isolate of *Rhynchosporium secalis* using AFLP. The AFLP analysis distinguished consistently within and between *A. brassicicola, A. alternata* and *Rhynchosporium secalis*, however multiple isolates from a particular location tended to cluster together. Despite the absence of an identified sexual stage, *A. brassicicola* would appear to have a means for generating and maintaining significant variation.

Disease management strategies: There are no single method are available currently which are effective and economical for alternaria blight disease management. Different methods are being integrated including cultural, biological, host resistance and biotechnological approach for the efficient management of alternaria blight diseases of cruciferous plants.

Conventional approach

Cultural Management: Crop management practices are the oldest and broadly applicable approach to minimize or control the plant diseases. Cultural management may reduce the activity of the pathogen by the use of disease free seeds, crop rotation, green manuring sanitation, rouging, time of planting and use of balanced nutrients.

Sanitation: Sanitation measures may be taken for alternaria blight management by eliminating or reducing the infected seed, debris and weed to prevent the spread the pathogen. Alternaria blight pathogen may survive on weeds and perennial crops viz. *Chenopodium album, Convolvulus arvensis, Crambe maritime, Crambe abyssinica and Camelina sativa* as chlamydospores/microsclerotia at low temperatures (Tsuneda and Skoropad, 1977) and these host assist this pathogen to propagate and serve as source of inoculums (Chupp and Sherf, 1960; Maude and Humpherson-Jones, 1980) and also in the infected seeds for at least a year at room temperature (Shrestha *et al*., 2003, Ahmad and Sinha, 2002).

Planting times: The eco-friendly and economical management of alternaria blight may be obtained through knowledge of its timing of incidence in relation to weather factors *viz.* temperatures, relative humidity (RH) and sunshine hours (Sinha *et al.*, 1992;Awasthi and Kolte, 1994; Dang *et al*., 1995). The extent of alternaria blight severity on leaves (Meena *et al*., 2002) and pods (Sandhu *et al*., 1985) were higher in late sown crops. A delayed planting results in coincidence of the vulnerable growth stage of plants as with warm (maximum temperature: 18-26oC; minimum temperature: 8-12oC) and humid (mean RH >70%) weather condition. Highest frequency of occurrence of *A. brassicae* on leaves and pods was found between 67-84 and 67-142 days after sowing respectively (Chattopadhyay *et al*., 2005). The minimum disease severity was reported in early sown at 45 cm row spacing as compared to broadcast method (Kumar and Kumar, 2006).

Biological management: Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system (Monte, 2001). *Trichoderma* species have been known as biological agents for control of plant diseases. They produce many components, which induce local or systemic plant resistance to abiotic stress. *T. harzianum* is a biocontrol agent in control of *A. alternata* (Roco and Perez, 2001; Monte, 2001; Sempere and Santamarina, 2007). 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). *Aspergillus flavus* produce fungistatic substance capable of preventing disease development of black leaf spot of mustard cabbage caused by *Alternaria brassicicola.* The fungistatic substances were inhibiting the germination of *A. brassicicola* conidia and stable under high temperature and high or low pH value (Chen *et al.*, 2011). The endophytic fungus *Heteroconium chaetospira* from roots of Chinese cabbage penetrates through the outer epidermal cells passes into the inner cortex of its host without causing pathogenic symptoms. This fungus suppressed the disease caused by *Pseudomonas syringae pv. macricola* and *Alternaria brassicae* on leaves (Hashiba and Narisawa, 2005).

The protective effects observed for certain Bacillus strains make them highly interesting for biocontrol agents in Brassica cultivation. Four Bacillus strains were tested for effects on plant fitness and disease protection of oilseed rape (*Brassica napus*). The strains belonged to plant-associated *Bacillus amyloliquefaciens* and a recently proposed species, *Bacillus endophyticus*. The fungal pathogens tested represented different infection strategies and included *Alternaria brassicae*, *Botrytis cinerea*, *Leptosphaeria maculans*, and *Verticillium longisporum*. The *B. amyloliquefaciens* strains showed no or a weak plant growth promoting activity, whereas the *B. endophyticus* strain had negative effects on the plant as revealed by phenological analysis. On the other hand, two of the *B. amyloliquefaciens* strains conferred protection of oilseed rape toward all pathogens tested. *In vitro* experiments studying the effects of Bacillus exudates on fungal growth showed clear growth inhibition in several but not all cases. The protective effects of Bacillus can therefore, at least in part, be explained by production of antibiotic substances, but other mechanisms must also be involved probably as a result of intricate plant-bacteria interaction (Danielsson *et al.*, 2007). *Bacillus subtilis* strain UK-9, an isolate from reclaimed soils, was studied for its biological control activity against Alternaria leaf spot disease of mustard. In dual culture, production of antifungal metabolites by the bacteria caused morphological alterations of vegetative cells and spores, disruption and lysis of their cell wall.

The antagonist reduced spore germination on leaves and disease incidence of the pathogen in plant trial as well as it also demonstrated plant-growth-promoting ability (Sharma and Sharma, 2008).The microbial metabolite fistupyrone isolated from the culture broth of a plant-associated *Streptomyces sp.* TP-A0569 inhibited infection of the seedlings of Chinese cabbage by *Alternaria brassicicola* in vivo (Igarashi *et al.*, 2000).

The botanical viz., Neem, Eucalyptus, Datura, Pudina, Tulsi,Lantana were evaluated under crude and boiled forms against *A. brassicae* under *in vitro* condition (Sasode *et al.*, 2012). The botanical *viz.*, Neem, Eucalyptus, Datura, Pudina, Tulsi,Lantana were evaluated under crude and boiled forms against *Alternaria brassicae* under in vitro condition 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) reported that *Eucalyptus globulus*, leaf extract showed significant reduction in the radial growth, sporulation and spore germination of *A. brassicae.* Brinjal (*Solanum nigrum*) extract component were identified which involved in controlling cabbage black leaf spot disease. An ethanol extract of *Solanum nigrum* inhibits spore germination of *Alternaria brassicicola*, the causative agent of cabbage black leaf spot disease. At a concentration of 500 mg/L, this ethanol extract also cause the germ tubes to become completely swollen. Extract-induced swellings of *A. brassicicola* germ-tube spores do not cause the symptoms of black spot disease on cabbage leaves. An n-butanol fraction of the ethanol extract exhibit strong antifungal activity; at a concentration of 25 mg/L, a derived sub fraction (Bu-11-13) show complete inhibition of spore germination (Lin *et al.*,2011). In the early stages of host-parasite interactions between cauliflower and *Alternaria brassicicola* lipase interacts closely with epicuticular leaf waxes for adhesion and/ or penetration of the fungal propagules. The lipase produced by ungerminated spores of *Alternaria brassicicola* plays a crucial role in the infection of cauliflower leaves (Berto *et al.*, 1999).The anti-lipase antibodies containing conidial suspension of *A. brassicicola* reduced the blackspot lesions on intact cauliflower leaves as compared to the surface wax removed cauliflower leaves.

Chemical management: Alternaria blight of rapeseed -mustard can be significantly managed with several fungicides. Kolte and Awasthi (1980) observed that to get effective control of Alternaria blight, the fungicide should be sprayed at appropriate time and at appropriate intervals. Kolte *et al.* (1989) suggested the iprodione (Rovral) is superior to mancozeb for control of pod infection of Alternaria. Four sprays of Rovaral at 10 days interval reduced the Alternaria blight incidence and increase the seed yield of mustard (Hussain, 1993). Prasad and Lallu (2006) revealed that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early date of sowing was the best combination in reducing the disease severity on leaves (18.7%) and pods (10.4%) higher realization yield (1295.8 kg/ha), 1000 seed weight (5.12 g) and oil content (42.6%). Ayub *et al.* (1996) reported that iprodione (Rovral) reduced disease severity and increased seed yield when applied on 40 days old plants. 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). Field evaluation of fungicides for the control of Alternaria blight of Indian mustard was done by DasGupta *et al.* (1985) and found that difolatan (captafol 0.2%) was highly effective when 4 sprays were given at 10 days intervals. Anwar *et al.* (2001) reported that Benlate showed best performance and reduced the leaf blight disease incidence of rapeseed-mustard by 76.6%. Godika *et al.* (2002) studied the efficacy of some fungicides against Alternaria blight disease of mustard and observed that Antracol sprayed plants showed lowest Alternaria blight severity. Ridomil MZ was most effective to reduce the disease severity of Alternaria blight followed by the combination Carbendazim and Captaf. Topsin-M at a concentration of 500 ppm was the most effective in reducing radial growth of the pathogenic fungi (Khan *et al.*, 2007). Iprovalicarb has an excellent toxicological and ecotoxicological profile. It has excellent fungicidal activity against *Plasmopara viticola*, *Peronospora vicia*, *Phytophthora sp*, *Alternaria sp* in grapes, potatoes, tomatoes, tobacco and vegetables (Maity and Mukherjee, 2009). Signum fungicide containing 6.7% pyraclostrobine + 26.7 % boscalid has been evaluated in comparison with the fungicides mancozeb, mancozeb + metalaxyl-M and azoxystrobin. It gave comparable or even better results than reference products for disease control against *Mycosphaerella spp*., *Albugo candida* and *Alternaria spp*., *Botrytis cinerea* and *Sclerotinia sclerotiorum* in vegetables (Callens *et al.*, 2005).

Alternaria blight resistance source: All cultivated Brassicas are essentially susceptible to *A. brassicae* and *A. brassicicola* but there are some differences in their degree of susceptibility (Jasalavich *et al*., 1993). *B. napus* and *B. carinata* are less susceptible to *A. brassicae* than *B. rapa* and *B. juncea* (Skoropod and Tewari, 1977; Bhoumik and Munde, 1987; Conn and Tewari, 1989; Katiyar and Chamola, 1994). Hydrophobic surface of epicuticular wax are primarily responsible for the difference of degree of susceptibility (Skoropad and Tewari, 1977; Conn and Tewari, 1989). Some field resistance to *A. brassicae* has been reported in *Brassica juncea* but under artificial inoculation conditions, most sources failed to withstand pathogen attack (Saharan and Kadian, 1983; Katiyar and Chopra, 1990). Several sources of tolerance against Alternaria blight have been reported (Gupta *et al*., 2001), including dwarf *B. juncea* cv. Divya (Kolte *et al*., 2000). Different components of horizontal resistance were analyzed in Brassica genotypes against *A. brassicae*

and found differences in the number of lesions, size of lesions, latent period, sporulation and infection rate (Saharan and Kadian, 1983). Size of lesions and amount of sporulation may be considered as important factors in determining the degree of resistance in Brassica to alternaria blight (Kolte, 1987). Some somaclones of *B. juncea* have high degree of field resistance to *A. brassicae* but not proved worthwhile in later generations (Katiyar and Chopra, 1990; Sharma and Singh, 1992). Inter specific hybridization between *Brassica carinata* and *B. rapa* revealed that F_1 hybrid were free from Alternaria blight but the meiotic studies showed major irregularities, leading to very less pollen stainabilty (Choudhary *et al*., 2000).

Primitive cultigens of mustard are more susceptible than the recent ones, indicating some degree of response to selection for resistance during cultivation. A great amount of genetic variation exists within the tribe brassicaceae for various traits of agronomic importance including disease resistance (Warwick, 1993). *Eruca sativa* was found to be highly resistant to a Canadian isolate (Conn and Tewari, 1986; Tewari, 1991b). *Sinapsis alba* was also found resistant to *A. brassicae* attack (Kolte, 1985a; Brun *et al*., 1987; Ripley *et al*., 1992; Sharma and Singh, 1992, Hansen and Earle, 1995, 1997). *B. desnottesii, Coincya pseuderucastrum, Diplotaxis berthautii, D. catholica, D. cretacea, D. eruciides and Erucastrum gallicum* were found completely resistant *in vivo* and *in vitro* against *A. brassicae* (Sharma *et al*., 2002). The high degree of resistance to *A. brassicae* was found in the wild relatives of *Brassica* outside the tribe *brassicaceae*. These are false flux (*Camelina sativa*), Shepherd's purse (*Capsella bursa-pastoris*) (Conn *et al*., 1988) and *Neselia paniculata* (Tewari and Conn, 1993). Inter-tribe gene transfer by conventional hybridization is very difficult. Inter-tribe hybrids were obtained with cultivated species but establishment of rooted shoots in soil was either unsuccessful (Narasimhulu *et al*., 1994; Hansen, 1998) or difficult (Sigareva and Earle, 1999). Under field conditions, inter generic hybridization between *Erucastrum canariense* and *Brassica rapa* appeared to be moderately resistant to Alternaria blight and also harboured a significantly lower population of mustard aphid than the cultivated *B. rapa* (Bhaskar *et al.,* 2002). Two inter generic hybrids involving wild species *Erucastrum cardaminoides* (2 n=18, E(cd) E (cd)) and two crop brassica species, *Brassica rapa* (2 n=20, AA) and *B. nigra* (2 n=16, BB), were synthesized. The *E. cardaminoides* x *B. nigra* hybrid and amphiploid appeared to be tolerant to alternaria blight under field conditions (Chandra *et al.,* 2004).

Resistance to *A. brassicae* in crucifers is layered and multicomponent (Tewari, 1991b; Tewari and Conn, 1993). The structure of wax in Brassica has important role in host resistance and acts as physical barrier without a direct chemical effect. The epicuticular wax forms a hydrophobic coating and reduces the rate of conidium germination and number of germ tubes formed by conidium. High deposition of epicuticular wax in some of the *B. napus* genotypes were responsible for resistance to *A. brassicae*, which acts as a water repellent resulting reduced conidial retention on the surface of leaves. The leaves of cultivars Midas and Tower have appreciable amounts of epicuticular wax (Skoropod and Tewari, 1977). The epicuticular wax of leaves is organized into two layers i.e. proximal and distal. The proximal layer has plate like crystals, while the distal layer is fluffy, consisting of wax crystals. In resistant genotypes, the distal layer is thicker (Skoropad and Tewari, 1977; Tewari and Skoropad, 1976). The alternaria blight resistant cultivars are characterized by relatively high concentrations of phenols and low concentration of sugars and nitrogen as compared to susceptible cultivars. Higher amount of phenols and lower amount of sugars and nitrogen were found in tolerant genotype RC 781 in comparison to susceptible genotype like Prakash. (Gupta *et al*., 1984). Yadav *et al.*, (2014) reported that out of 31 genotypes, NPN-1 was found to be resistant with 9.2% incidence of disease.

The phytoalexins plays significant role in host resistance against alternaria blight in Brassica. Phytoalexins are low molecular weight antimicrobial compounds that are synthesized and accumulated in plants after the interaction between the host and pathogen which results the inhibition of fungal growth on the leaf surface. Some wild crucifers are highly resistant to *A. brassicae* and elicit phytoalexins when challenged by this pathogen (Conn *et al*., 1988). Phytoalexins elicited in *Camelina sativa* (L.) crantz consist of camelexin ($C_{11}H_8N_2S$) and 6-methoxycamelexin ($C_{12}H_{10}N_2SO$) (Browne *et al*., 1991) and these appeared as a first report of naturally occurring thiazoyl-substituted indole phytoalexins which contain a 2- substituted thiazole ring. Thiabendazole, a well known synthetic fungicide, is a 4-substituted thiazole and closely related to camelexin. However, unlike camelexin, thiabenda zole is not toxic to *A. brassicae* (Maude et al., 1984; Conn *et al*., 1988). *Capsella bursa-pastoris* (L.) Medic. elicits camelexin, 6-methoxycamelexin and another phytoalexin, N-methylcamelexin ($C_{12}H_{10}N_2S$) upon challenge by *A. brassicae* (Jimenez *et al*., 1997). *A. brassicae* has a multitoxin system and produces three phytotoxins i.e. destruxin B, destruxin B_2 and homodestuxin B. Destruxin B is the major phytotoxin produced by *A. brassicae* which causes necrosis and chlorosis on host and non-host plants.

Destruxin B is also produced by *Metarhizium anisopliae* and some other fungi and it has some insecticidal properties also. The symptoms produced by destruxin B is light dependent and contributed in virulence. The phytotoxin, destruxin B also elicits phytoalexin response in *Sinapis alba* (Padres and Smith, 1997) and may be involved in resistance to crucifers to *A. brassicae.* Crucifers transform destruxin B to hydroxydestruxin B. The five cruciferous species *Arabidopsis thaliana*, *Thellungiella salsuginea*, *Erucastrum*

gallicum, *Brassica rapa* and *Brassica napus* are likely to produce a destruxin B detoxifying enzyme destruxin B hydroxylase similar to other cruciferous species (Pedras *et al.*, 2012)

Non-conventional approach: Brassica plant species has a relatively high nutrient requirement and most soils on which the crop is grown are deficient in one or more nutrients for optimum seed yield and oil and protein content (Grant and Bailey, 1993).

The effect of nutrients on disease resistance may be attributed to (i) effects on plant growth that can influence the microclimate in a crop and thereby affect infection and sporulation of the pathogen, (ii) effects on cell walls and tissues, as well as biochemical composition of the host, (iii) influence the rate of growth of the host, which may enable plants to escape infection in their susceptible stages and (iv) effects on the pathogen through alternations in the soil environments (Colhoun, 1973).

Effect of nitrogen: Nitrogen is the most abundant element in plants, and it has been intensively studied in relation to host nutrition and disease severity. It promotes vigorous growth, delays maturity and is essential for the production of amino acids, proteins, growth hormones, new protoplasm, chlorophyll, phytoalexins and phenols.

The evidence that excessive N fertilization increases susceptibility to obligate pathogens (Mildews, Club roots) seems to be conclusive, although the form of nitrogen available to the plants may also be significant (Huber and Watson, 1974; Kiraly, 1976). However, high levels of N usually increase resistance to facultative pathogen in fresh, green, young plant tissue (Kiraly, 1976). These differences in response result from differences in the nutritional requirements of the obligate and facultative type of pathogens. As a rule, all factors which support the metabolic and synthetic activities of host cells and delay senescence of the host plant also increase resistance to facultative pathogens (Marschner, 1986).

Mackenzie (1981) reported that higher doses of nitrogenous fertilizer decreased the disease of Alternaria blight of tomato. Higher dose of nitrogen (140 and 160 kg N/ha) increased the disease incidence of *Alternaria* leaf blight of mustard (Khatun *et al.*, 2011). Saharan *et al.* (1982) observed that the application of N fertilizer to tomato increased the incidence of Fusarium wilt of tomato plants. Enrichment of foliage with nitrogen may enhance host resistance to Alternaria (Blachinski *et al.*, 1996).

Effect of potassium: Potassium plays a major role in physiological activities of plants and is required in large amounts for adequate crop production. Potassium is a mobile regulator of enzyme activity, is involved in essentially all cellular functions, including photosynthesis, phosphorylation, protein synthesis, translocation, water maintenance, reduction of nitrates and reproduction. A balanced level of K induces thickened cell walls, accumulation of amino acids and production of new tissues (Mengel and Kirkby, 1978; Perrenoud, 1990; Grant and Bailey, 1993).The mode of action of K is primarily through plant metabolism and morphology. The K-deficient plants have impaired protein synthesis and accumulate simple N compounds, like amides, which are good nutrient source for invading pathogens. Tissue hardening and stomatal opening patterns which are regulated by K are closely related to infection intensity (Huber, 1980; Fageria *et al.*, 1991).

Sharma and Kolte (1994) reported that under natural conditions based on the number and size of spots, percent leaf and pod infection, average disease index on leaf and pods and number of spots on stem, K-fertilized plants reduced 30-45 per cent severity of Alternaria blight over N, P and NP fertilized plants in Toria. Higher dose of K (90 kg/ha) decreased the incidence of Alternaria leaf blight (Khatun *et al.*, 2011). Chung and Huang (1993) observed that application of KCl to soil increased the resistance of Chinese kale plants to black spot disease. Singh (1996) observed that under field conditions application of $KMnO_4$ and $KAl(SO_4)$ showed reduction in disease index of Alternaria blight in toria. Srinivas *et al.* (1997) reported that soil application of KCl reduced the disease intensity and increased the yield over control against Alternaria blight of sunflower.

Effect of sulphur (S): Sulphur is required in protein synthesis and it is an important constituent in the biologically active compounds like biotin, glutathione, thiamine and coenzyme A and is also important in energy transfer and protein structure (Bidwell, 1979). Sulphur is involved in synthesis of chlorophyll and is also required in Cruciferae for the synthesis of the volatile oils which accumulate as glucosinolates (Marschner, 1986). Its negligible toxicity to animals and low toxicity to plants have made sulphur attractive as a chemical control agent; it is also a common component of integrated pest management programmes because of its low toxicity to beneficial insects (Emmett, 2003).

Sulphur deficiency of oilseed rape negatively affects disease resistance caused by the reduction of sulphur dependent phytoanticipins (Dubuis *et al.*, 2005). Soil applied sulphur was found to increase resistance against a variety of fungal pathogens on different crops (Klikocka *et al.*, 2005). Vishwanath (1987) observed that application of S (through soil application @ 5 g/ m^2) showed significant increase of Alternaria blight severity on pods of toria, though it had resulted increase in yield as compared to check. In field trials 50 kg S/ha showed 40 per cent more disease severity of Alternaria blight over check (no sulphur). However, increasing the application of sulphur above 50 kg/ha showed inhibitory effect over disease development. It is interesting that yield in terms of both quality and quantity were highest 50 kg S/ha (Sharma, 1992). Singh (1996) reported that out of 15 micronutrients tested *in vitro*, $CuSO_4$ was found to be fungicidal and the remaining micro-nutrients were found to be fung-

istatic against AB, white rust, and downy mildew of rapeseed. *In vitro* growth studies also indicate that sulphur gave maximum fungal growth and sporulation to *A. brassicae* and *A. alternata* (Hasija, 1969; Vishwanath, 1987).

Effect of calcium (Ca): Although generally applied as fertilizer to neutralize soil pH, Calcium has critical role in cell division, cell development, carbohydrate movement, neutralization of cell acids, cell wall deposition and formation of pectate salts in the middle lamella (Huber, 1980). Calcium is known to be a factor in disease resistance (Agrios, 2005). The insoluble calcium polypectates are resistant to hydrolysis by pectolytic enzymes produced by the pathogens (Vidhyasekaran, 1988).

Examination of black spot lesions on rapeseed leaves by scanning electron microscopy in conjunction with energy dispersive x-ray microanalysis has revealed sequestration of calcium by *A. brassicae*. Therefore, there are possibilities of control of Alternaria blight of rapeseed by soil or foliar application of calcium compounds (Tewari, 1991a,b; Kumar *et al.*, 2014). Foliar spray of calcium compounds sequester the organic acids at the site of infection, and soil application has the potential of boosting calcium content of the plant (Verma and Saharan, 1994; Kumar *et al*., 2015). The foliar application of $CaSO_4$ at 0.5% concentration induced resistance to alternaria blight significantly in comparison to different concentration of KCl, K_2SO_4, $ZnSO_4$, and $Na_2B_4O_7$(Kumar *et al.*, 2014; Kumar *et al.*, 2015).

Effect of boron (B): Boron has been used as a fertilizer more than 400 years and it seems possible that its earlier use was associated with disease control (Mengel and Kirkby, 1978). Boron is functional in translocation, cellular differentiation and development, carbohydrate metabolism, pollen germination and the uptake and translocation of Ca.

Brandenburg (1938) pointed that the species of Brassica are more sensitive to boron deficiency and show symptom of infection much earlier and Powen's (1939) recommended boron application to soil around broccoli and cabbage plants and found promising results. *In vitro* effect of Borax, Boric acid, Sodium bicarbonate and TBZ against fruit rotting fungi of citrus showed that only TBZ was effective in inhibiting the mycelial growth (Javed *et al.*, 1995). Boric acid as foliar spray (0.53%) gave 20-64 per cent control of Alternaria blight (Vishwanath, 1987). Sharma (1992) observed positive correlation between the application of Boron in combination of micronutrients and fungicide and reduction in severity of Alternaria blight of toria.

Effect of zinc (Zn): Zinc is a metal component and serves as a functional, structural or regulatory co-factor of a large number of enzymes (Marschner, 1986). The most prominent physiological role of Zn is the interrelationship with auxin. It is thereby essential for cell elongation and growth, a well as being functional in respiration and enzyme regulation (Huber, 1980). Zinc application often increases host resistance to mildew and leaf spot and has suppressive effects on soil-borne diseases and bacterial and virus diseases (Graham, 1983). With Zn deficiency, a leakage of sugars inhibition of protein synthesis occurs with large increase in free amino acids which in turn favour disease development.

Application of $ZnSO_4$ with NPK in mustard give more biomass and seed yield per plant with an increase in number of branches and siliqua per plant by 28.5% and 21.8%, respectively as compared to NPK applied alone (Lallu and Shanker, 1995). Kaur (2000) reported that $ZnSO_4$ shows inhibitory effect on mycelial growth of *A. brassicae*. Zinc did not give any consistent effect on the severity of Alternaria blight of mustard (Vishwanath, 1987; Mian and Akanda, 1989).

Biotechnological approach: Non availability of resistance genes within crossable germplasms of Brassica compel to use the biotechnological strategies to develop genetic resistance against this pathogen. The pathogenesis related (PR) proteins are group of plant proteins that are toxic to invading fungal pathogens, but are present in plant in trace amount. Thus, overexpression of PR proteins leads to increased resistance to pathogenic fungi in several crops. Chitinase, capable of degrading the cell walls of invading phytopathogenic fungi, plays an important role in plant defense response. Chitinase gene tagged with an over expressing promoter 35 S CaMV inhibited the *Alternaria brassicae* colony size by 12-56%, lesion number and delay in the onset of disease over the non-trangenic contro (Mondal *et al.*, 2003). Glucanase hydrolyzes a major cell-wall component, glucan, of pathogenic fungi and acts as a plant defense barrier. Class I basic glucanase gene, under the control of CaMV 35S promoter, confirmed stable integration and expression of the glucanase gene in mustard transgenics and arrested hyphal growth of *Alternaria brassicae* by 15-54% and showed restricted number, size and spread of lesions (Mondal *et al.*, 2007).

Cysteine-rich antimicrobial peptides isolated from plants have emerged as a potential resource for protection of plants against phytopathogens. The cDNA encoding PmAMP1 isolated from western white pine (*Pinus monticola*) was successfully incorporated into the genome of *B. napus*, and it's in planta expression conferred greater protection against *Alternaria brassicae* (Verma *et al.*, 2012).

The fungal secondary metabolites derived from non-ribosomal peptide synthetases (NPSs) are phytotoxic virulence factors for niche competition with other micro-organisms in many plant pathosystems. However, many of the functions of NPS genes and their products are unknown. The predicted amino acid sequence of *A. brassicicola* NPS genes AbNPS2 showed high sequence similarity with *A. brassicae*, AbrePsy1, *Cochliobolus heterostrophus*, NPS4 and a *Stagonospora nodorum* NPS (Kim *et al.*, 2007). The hyphal filaments interconnected by bridges through anastomoses

and formed the fungal mycelium. These bridges facilitate the nutrients, water, and signaling molecules throughout the colony. Anastomosis is required for virulence of the fungal necrotroph *Alternaria brassicicola* Disruption of the anastomosis gene homolog (Aso1) in *A. brassicicola* resulted in both the loss of self-anastomosis and pathogenicity on cabbage (Craven *et al.*, 2008).

The expression levels of Mitogen-activated protein (MAP) kinase in Arabidopsis or Brassica could be a possible strategy for engineering defense against Alternaria blight disease. MAP kinases have been shown to be required for virulence in diverse phytopathogenic fungi. The amk1 MAP disruption mutants, a homolog of the Fus3/Kss1 MAP kinases in *Saccharomyces cerevisiae*, showed null pathogenicity on intact host plants. The mutants expressed extremely low amounts of several hydrolytic enzyme genes that were induced over 10-fold in the wild-type during infection. However, amk1 mutants were able to colonize host plants when they were inoculated on a physically damaged host surface, or when they were inoculated along with nutrient supplements (Cho *et al.*, 2007). The involvement of MAP kinase machinery in the pathogenesis of Alternaria blight was investigated in *Arabidopsis thaliana*. The MAP2K9/MAPK6 module is influenced during pathogenesis of Alternaria blight in *A. thaliana* ecotype Columbia (Kannan *et al.*, 2012). The expression of both MAP2K9 and MAPK6 simultaneously increased up to middle stage of disease progression. At late stage of disease progression the expression of MAP2K9 decreased and that of MAPK6 increased. The increased levels of MAP2K9 and MAPK6, seem to be necessary for plant to defend the pathogen up to middle stage of infection. Camalexin, the characteristic phytoalexin of *Arabidopsis thaliana*, inhibits growth of the fungal necrotroph *Alternaria brassicicola*. Camalexin has been found to activate both AbHog1 and AbSlt2 MAP kinases. Mutant strains lacking functional MAP kinases showed hypersensitivity to camalexin and brassinin. Enhanced susceptibility to the membrane permeabilization activity of camalexin has been observed for MAP kinase AbHog1 and AbSlt2 deficient mutants (Joubert *et al.*, 2011).

The members of plant WRKY transcription factor families are widely implicated in defense-related genes in response to fungal pathogens and hormone stimuli. A set of 13 BnWRKY genes were identified that are responsive to both fungal pathogens and hormone treatments genes in canola (Yang *et al.*, 2009). The novel defence genes in canola by using a cDNA microarray from Arabidopsis were identified. The abundance of transcripts corresponding to 2375 Arabidopsis expressed sequence tags (selected for defence gene identification) following inoculation of canola plants with the fungal necrotrophic leaf pathogen, *Alternaria brassicicola* were studied. Homology searches using a canola expressed sequence tag database with approximately 6000 unique clones led to identification of canola defence genes (Schenk *et al.*, 2008).

Of the other non-host-resistant/tolerant plants, *Sinapis alba*, white mustard, is considered to be the most important apart from Arabidopsis. The suppression of 46% of the genes in the compatible background indicates the possibility of effective and specific recognition of Alternaria *in S. alba.* Analysis of the 118 genes up-regulated specifically in infected *S. alba* compared with *B. juncea* showed that 98 genes have similarity to proteins such as receptor-like protein kinase genes, genes involved with calcium-mediated signalling and salicylic acid-dependent genes as well as other genes of known function in Arabidopsis (Ghose *et al.*, 2008). Cramer and Lawrence (2004) identified *Alternaria brassicicola* genes expressed in planta during pathogenesis of *Arabidopsis thaliana*. 47 cDNA clones differentially expressed between Alternaria infected Arabidopsis leaves and spore germination in water was selected for sequencing. Seventy-seven percent (36) of the cDNAs had significant homology to fungal sequences from databases examined, including available fungal genomes, while 13% (11) had no homology to sequences in the databases. All 36 genes had significant matches with genes of fungal origin, while 11 genes did not have significant hits in the databases examined. Five sequences were expressed on the plant leaf surface but not during spore germination in water. These five cDNAs were predicted to encode a cyanide hydratase, arsenic ATPase, formate dehydrogenase, major Alternaria allergen, and one unknown. In order to identify candidate fungal pathogenicity genes and characterize a compatible host response, a suppression subtractive hybridization (SSH) cDNA library enriched for *A. brassicicola* and *Brassica oleracea* genes expressed during the interaction was created, along with a fungal cDNA library representing genes expressed during nitrogen starvation (NS). A total of 3749 and 2352 expressed sequence tags (ESTs) were assembled into 2834 and 1264 uni sequence sets for the suppression subtractive hybridization (SSH) and nitrogen starvation (NS) libraries, respectively. BLASTX analyses of the 2834 uni sequence set using the Gen Bank non-redundant database identified 114 fungal genes. Further BLASTN analyses of the genes with unidentifiable origin using a database consisting of the 1264 fungal unisequence set from the nitrogen-starved library identified 94 additional fungal genes (Cramer *et. al.*, 2006).

Conclusion

Oilseed brassicas crop is gaining importance globally due to its advantage over other oilseeds *viz.*, higher yield potential, low moisture requirement, higher return at low cost of production, wider adaptability for various farming conditions, etc., But, the area, production and productivity of the crop is declining due to various biotic stresses. Alternaria blight of rapeseed-mustard is the most common and destructive disease of oilseed brassicas and that causes reduction in quantity as well

as quality of seed and oil contents. Due to unavailability of proven source of resistance in cultivated varieties of oilseed brassicas, other strategies of management could be used. The traditional *viz.* cultural, chemical, biological, nutritional and modern *viz.* biotechnological approaches may be employed together according to the needs/demands to develop integrated disease management strategies for sustainable, environment friendly and effective management of alternaria blight disease of oilseed brassicas to narrow down the gap between potential and actual yield.

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