Eco-friendly Management of Alternaria Leaf Spot and Blight Disease through SAR Activation in Plants using Abiotic elicitors

Dr. Anindita Nan

Department of Botany, Mrinalini Datta Mahavidyapith, Kolkata, India

1. Introduction

Immobile plants have evolved various sophisticated and effective mechanisms to recognize and combat pathogenic microorganisms when attacked and have thrived over millions of years. They are equipped with genetic information which allows them to resist infection by a wide range of pathogenic organisms including viruses, bacteria, oomycetes and fungi. These defense responses may be short or long term and are either constitutive or inducible. Thus plants when attacked by pathogens and use several defense mechanisms such as strengthening of tissue at the site of infection, production of different types of anti-microbial compounds and synchronous stimulation of defense genes and their protein products (Agrios, 1997).

As pre-existing mechanisms do not always protect plants against pathogen attack sufficiently, plants reinforce further protection by developing active defense mechanisms. Several events take place when inducible defenses are activated. Reinforcement of the cell wall takes place by cross-linking its components with polyphenolic compounds and by callose (β-1, 3 glucan) deposition. This makes the plants resistant to hydrolytic enzymes of the pathogens (Heath, 2000; Richter and Ronald, 2000). In addition, the plants produce phytoalexins and antimicrobial pathogenesis related (PR) proteins immediately after pathogen recognition (Sticher *et al.*, 1997).

Inducible plant defense is also activated by the plant growth regulators, viz. salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Ton *et al.*, 2002; Beckers & Spoel, 2006). It has been reported that signal transduction pathways required for inducing resistance are different for different pathogens. While SA-dependent signal transduction pathways are essential for resistance to biotrophic pathogens (Glazebrook, 2005), JA and ET signalling are generally required for resistance to necrotrophic pathogens. Feys and Parker (2000) further reported that SA- and JA/ET dependent signalling pathways interaction was of primarily mutual antagonism. Inter-pathway communication has been speculated to help plants adjust and design defense responses on detecting multiple signals (Beckers & Spoel, 2006).

Insight of the intrinsic mechanisms involved in such defense responses helps in the basic understanding of plant-pathogen interactions and can be exploited to produce improved disease resistance in crops.

Alternaria leaf spot and blight, reported in the early 20th Century (Fawcett, 1909), is one of the most widespread fungal diseases of vegetable crops, cereals, ornamentals etc.belonging to a wide range of families throughout the world (Sharma and Kolte, 1994; Saharan, 1991). A widely diverse pathogen *Alternaria* infects in many crop plants which causes huge yield losses and reduce the economic value of the crop plants in conventional production system which are very difficult to manage. One of the common methods of management of Alternaria disease is using of fungicides like dithane M-45, antrocol, captan, difolaton, blitox-50 gave satisfactory control but are dangerous to the ecosystem. Because these fungicides are chemicals which have direct toxic effect on the plant in addition to being noxious to the environment. They have a narrow spectrum of defense with short lasting protection and are economically costly also. Management to control of plant disease is a high pressing need for agriculture in India given the growing human population and reducing land availability. The increasing requirement for sustainable food supply is met through higher inputs which includes chemical applications in the form of fertilizers and pesticides. However, the continuous use of these chemicals result in the environmental pollution and may also lead to development of resistance in the target organism. As India is now doing natural farming and for organic farming, it is very important to address crop plant disease management also using eco-friendly approaches, for maintaining ecosystem, human health and human nutrition through balanced food chain in agro-ecosystems. Another safest way is to use resistant or tolerant varieties against the disease. Currently in India, resistance among the cultivable varieties of radish are ranges only from very susceptible to moderately resistant. Under these circumstances, not only is there a serious threat to control by chemical means but there is also a great concern since management of the disease through the development of resistant varieties is not always successful.

The hazardous and adverse effects of toxic chemicals or their degradation products on environment, beneficial microflora and human health strongly necessitates the search for a new economic, harmless and viable alternative means of disease control for Alternaria diseases. Some eco-friendly methods like using biocontrol agents and many plant products to suppress plant disease, offers a powerful alternative tool to synthetic chemicals with similar targets as well as induced protection of plants against various pathogens by biotic or abiotic agents has been reported since 1930s. Since the late 1950s, an increasing body of evidence on the natural phenomenon of induced resistance has been accumulated, culminating in its successful practical application (Kuc, 2001) as an alternative, less hazardous and economic methods for plant disease management. Disease containment through eco-friendly noble compounds as abiotic elicitors are now becoming an inevitable component in the integrated management strategy of the disease. When induced resistance is activated, a normally compatible plant-pathogen interaction can be converted into an incompatible one (Mauch-Mani and Slusarenko, 1996; Mauch-Mani *et al*., 1998). For planning long-term sustainable disease management strategies for Alternaria leaf spot and blight disease, exploration of induction of systemic resistance through the use of biotic or abiotic elicitors can be done.

2. Occurrence of *Alternaria* and Symptomatology of Alternaria disease

Alternaria species are widespread and can be found in various climates and on a wide range of host plants. They are commonly found on agricultural crops such as tomatoes, potatoes, cucumbers, mustard, cabbage, radish and various other fruits and vegetables. They can also affect ornamental plants and trees. Alternaria disease in plants typically manifests as leaf spots, blight, stem cankers, and fruit rot. The symptoms can vary depending on the host and the specific *Alternaria* species involved (Conn and Tewari, 1990). Common symptoms include dark, necrotic lesions on leaves, which may have concentric rings, and cankers or lesions on stems and fruit that often appear dark brown or black.

Alternaria infection on different crops is causing world-wide economic loss. Alternaria blight and leaf spot diseases are the most dominant among the different diseases caused by the genus *Alternaria* that causes average yield loss in the range of 32-57 per cent (Conn and Tewari, 1990). According to Sharma *et al.* (2005) , the pathogen *Alternaria alternata* causing leaf spot on apple has shown the appearance of reddish brown necrotic spots with 1.3 mm diameter on young apple leaves, later many spots became coalesce to form larger blotches and given blighted appearance to the leaves which followed by defoliation.

Kamalakannan *et al.* (2008) reported that the leaf spot diseases of *Aloe vera* which caused by *Alternaria alternata* (Fr.) Keissler agg. In 2010, Vikas *et al*. has worked on the symptoms of Alternaria leaf blight in sunflower and reported that in sunflower, the symptoms appeared in the form of characteristic small circular, brown patches on the surface of leaves. Later on, as the disease progressed, these brownish patches expanded in size and finally coalesced to cover the entire surface of leaves developing blight symptoms. Finally, the blighted leaves got curled and became dark black in colour. In 2011, Zhang *et al.* reported that the symptoms of *A. alternata* in *Euphorbia lathyris* in early summer on stems were characterized by circular or irregularly shaped, small, brown to black spots or/and on shrivelled leaf apices. The lesions rapidly grew around the stems or along the leaf blades in the rainy season. According to Shokooh Farhood and Shervin Hadian (2012), at the initial stage of the infection, the symptoms of *A. alternata* on gerbera leaves were brown, small, scattered spots that gradually became characterized by round or irregular spots and later spots coalesced to affect large areas of foliage causing defoliation.

Many *Alternaria* species also produce toxins that diffuse into host tissues ahead of the fungus. Therefore, it is not uncommon to see a yellow halo or chlorotic halo (Humpherson-Jones, 1992) that fades into the healthy host tissues that surround the target spot. Dark, sunken lesions are produced on roots, tubers, stems, and fruits. The fungus may sporulate in these cankers, causing a fine, black, velvety growth of fungus and spores to cover the affected area (Laemmlen, 2001).The pathogens also occur on many weed species, which may serve as inoculum reservoirs (Farr *et al*., 1989; Cucuzza *et al*., 1994; Maringoni, 1997).

3. The genus *Alternaria*

Alternaria has more than 50 species (Ellis, 1971; Anuj Mamgain *et al.*2013). *Alternaria* species are either parasites or saprophytes. Pathogenic *Alternaria* has a very broad range of host. *Alternaria* species can be recognized very easily by the morphology of their large polymorphous conidia. They are catenate, formed in chains or solitary, typically ovoid to obclavate, often beaked, pale brown to brown, multicelled and muriform (Ellis, 1971).

4. Nature of Systemic acquired resistance (SAR):

Induced resistance to pathogens can be classified into two broad categories viz., (1) systemic acquired resistance (SAR) and (2) induced systemic resistance (ISR). Systemic acquired resistance (SAR) is a mechanism of induced defense that plays an important role in the ability of plants to give long lasting protection themselves against a broad spectrum of pathogens (Ryals *et al*., 1996; Van Loon, 1997). SAR has been recognized as a plant response to pathogen infection for almost 100 years (Chester, 1933).

After the development of necrotic lesions in host plant, either as a part of the hypersensitive response (HR) or localized acquired resistance (LAR) or as a symptom of disease (Van Loon, 1997), SAR is activated via signalling molecule salicylic acid depended process (Hammerschmidt and Becker, 1997; Hammerschmidt, 1999) and is associated with accumulation of pathogenesis-related proteins and enhanced activity of various defense related enzymes, which develop enhanced resistance in the distal, uninfected plant parts against secondary pathogen attack (Kuc, 1995; Ryals *et al*., 1996). Evidence has shown that faster and stronger activation of defense responses at the sites of secondary infection results in a decrease in disease symptoms (Durrant and Dong, 2004) reflecting the SAR state (Ross, 1961). The establishment of SAR involves the generation and transport of signals via phloem to the uninfected distal tissues (Guedes *et al.*, 1980; Tuzun and Kuc, 2001) and confers a long-lasting protection that can last for weeks to month, and sometimes throughout an entire season (Kuc *et al.*, 1987).

Exogenous application of SA also induces SAR in several plant species (van Loon *et al*., 2006; Pieterse *et al*., 1998; Kessmann *et al*., 1994). The classic form of SAR can be triggered biologically by challenging a plant to virulent, avirulent, and non-pathogenic microbes, as activators of plant defense responses. But the utility of this form of plant inoculation is limited in the field because of the likelihood of pathogen spread following application. Although a range of organisms is commercially available for use as biocontrol agents, nearly all are based on a direct antibiotic principle. In addition, exposing a plant to natural or synthetic chemical elicitors such as salicylic acid, 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), β-Aminobutyric acid (BABA) etc., induces SAR in plants (van Loon, 1998; Laura Mejía-Teniente *et al*., 2010).

5. Elicitors and Their Mode of Action

The use of Systemic Acquired Resistance (SAR) for controlling Alternaria infection in plants in an eco-friendly manner is a promising approach. To enhance the plant resistance against pathogens, SAR involves inducing a plant's natural defense mechanisms. This method can reduce the need for chemical pesticides, fungicides etc., minimizing the environmental impact. According to research, it is suggested that SAR can effectively suppress Alternaria blight and leaf spot disease by priming the plant's immune system. However, the success of SAR depends on various factors like plant species, timing of induction, and environmental conditions. It's a sustainable strategy worth exploring further, as it aligns with eco-friendly agricultural practices.

The eco-friendly management of Alternaria leaf spot and blight disease through Systemic Acquired Resistance (SAR) and its elicitors presents a comprehensive approach to control plant diseases. Systemic Acquired Resistance (SAR) involves activating a plant's innate defense responses, enhancing its resistance to pathogens like *Alternaria* sp. Elicitors are compounds that trigger SAR, amplifying the plant's defense response. This SAR inducing strategy minimizes reliance on chemical treatment which is benefiting the environment in a sustainable manner. Research also suggests that SAR, coupled with well-chosen elicitors, can effectively mitigate Alternaria leaf spot and blight. The combined approach promotes sustainable agricultural practices, reducing hazardous negative ecological impacts. However, practical implementation of inducing SAR in plants against pathogens requires consideration of various factors, including plant specificity, elicitor application methods, and local conditions. Overall, the integration of Systemic Acquired Resistance (SAR) and elicitors holds promise for eco-friendly disease management of crop plants.

The application of abiotic elicitors for eco-friendly management of Alternaria leaf spot and blight is a noteworthy approach. Abiotic elicitors are non-living compounds that can stimulate a plant's defense mechanisms, enhancing resistance against various and detrimental pathogens like *Alternaria* sp. These elicitors can include substances like salicylic acid, 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), β-Aminobutyric acid (BABA) etc., (van Loon, 1998; Laura Mejía-Teniente, 2010). or even nanoparticles, which have shown promising results in inducing systemic acquired resistance. However, the success of this approach depends on factors such as the choice of elicitors, application methods, and specific crop requirements. While abiotic elicitors offer an environmentally friendly alternative, further studies and field trials are essential for optimizing their efficacy in real-world scenarios.

In past, originally the elicitor was used for inducing the production of phytoalexins, but at present elicitors are commonly used for compounds stimulating any type of plant defense (Sinha P.P.and Prasad RK.,1989 Boller T.,1992). Normally, the induction of defense responses may lead to enhanced disease resistance against plant pathogens. Elicitors are substances of pathogen origin (exogenous elicitors) and also the compounds which released from plants after pathogen infection due to pathogen action (endogenous elicitors) (Ebel J. and Cosio E. G.,1994; Boller T.,1995) . Elicitors are classified depending on their origin and molecular structure as physical or chemical, biotic or abiotic and complex or defined .

Elicitors may also be divided into two types, “general elicitors and “race specific elicitors. General elicitors are capable to trigger defense against pathogens both in host and non-host plants and in case of race specific elicitors, it induces defense responses which leads to disease resistance only in specific host cultivars. A complementary pair of genes in a particular pathogen race and a host cultivar determines the cultivar specific (gene-for-gene) disease resistance. An avirulence gene (avr gene) which present in a particular race of a pathogen encodes a race specific elicitor and this can elicit resistance only in a host plant variety carrying the corresponding resistance gene. This has been reported that in the absence of either gene product will often result in disease (Hammond-Kosack K. E. and Jones J. D. G.,1996; Cohn J., Sessa G., and Martin G. B., 2001). According to Nürnberger (1999), general elicitors give signal the presence of potential pathogens to both host and nonhost plants (Nürnberger, T., 1999). Shibuya, N. and Minami, E. (2001) reported that the nonspecific nature of general elicitors is relative, however some of these are only recognized by a restricted number of plants. At low concentrations, elicitors play a role in signal transduction mechanism in host plant as signal compounds providing information for the plant to trigger primary immune response.

6. Induction of SAR in plants with abiotic elicitors:

In a field experiments, cotton (*Gossypium hirsutum, G. barbadense*) plants were exposed to foliar applications of the synthetic activators like INA or BTH and the severity of natural infection with *Alternaria macrospora*, *Xanthomonas campestris* pv. *malvacearum* and *Verticillium dahliae* was evaluated (Colson-Hankse *et al*., 2000). Percentage leaf area infected with *A macrospora* was significantly (P=0.05) lower in cotton plants following one application of INA or BTH respectively over control and significantly (P=0.05) less defoliation also reported in the treated plants. The reduction in susceptibility of the cotton (*Gossypium hirsutum*) plants to *Alternaria* leaf spot, bacterial blight and *Verticillium* wilt is attributed to systemic acquired resistance following application of INA or BTH.

Induction of defense responses was observed against *Alternaria* rot by different elicitors such as salicylic acid (SA), oxalic acid, calcium chloride, and antagonistic yeast *Cryptococcus laurentii* in harvested pear fruit (*Pyrus pyrifolia* L. cv. Yali) (Tian *et al*., 2005).The possible mechanism of induced resistance was also determined by elicitors treated pear fruit against postharvest disease. The results showed that all the elicitors could significantly enhance defense related enzyme activities, such as β-1,3-glucanase, phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase activity and significantly (P=0.05) reduce the disease incidence caused by *A. alternata* in pear fruit. Among these different elicitors, SA treatment was known to be the best abiotic elicitor for inducing the defense responses and reducing the decay in pear fruit.

Chitra and co-workers (2006) investigated the effect of SA in inducing resistance in groundnut plants against *A. alternata*. Foliar application of SA at the concentration of 1 mM showed significant reduction in the leaf blight disease intensity and increased the pod yield under glasshouse conditions. They reported the changes in the activities of PAL, chitinase, β-1, 3-glucanase and in phenolic content on groundnut after SA application and *A. alternata* inoculation. It was observed that SA-treated plant leaves showed an increase in phenolic contents, five days after challenge inoculation with *A. alternata* in groundnut plants pre-treated with SA. There was a marked increase in chitinase activity in SA-treated pathogen inoculated leaves. Increased activities of chitinase, β-1,3-glucanase were observed in SA treated groundnut leaves. Even foliar applications of SA, induced the peroxidase and polyphenol oxidase activities upon challenge inoculation with pathogen in groundnut.

Flors and co-workers (2008) conducted an experimental set up to understand the role of the callose synthase PMR4 in basal resistance and β-Aminobutyric acid-induced resistance (BABA-IR) in *Arabidopsis thaliana* against the hemibiotrophic pathogen *Pseudomonas syringae* and the necrotrophic pathogen *A. brassicicola*. Treating the host plants with BABA boosted the already elevated levels of PR-1 gene expression and further increased the level of resistance in pmr4-1 mutant. Conversely, pmr4-1 plants showed enhanced susceptibility to *A. brassicicola*, and failed to show BABA-IR. Wild-type plants showed BABA-IR against *A. brassicicola* and also produced increased levels of JA. The pmr4-1 mutant produced less JA upon *A. brassicicola* infection than the wild-type. It was revealed that blocking SA accumulation in pmr4-1 mutants restored basal resistance, but not BABA-IR against *A. brassicicola*. This suggests that the mutant's enhanced susceptibility to *A. brassicicola* is caused by SA-mediated suppression of JA, whereas the lack of BABA-IR is caused by its inability to produce callose. *A. brassicicola* infection also suppressed ABA accumulation. The effect of five antioxidants (citric acid, salicylic acid, benzoic acid, ascorbic acid, and sodium citrate) on the resistance of tomato plants (*Lycopersicon esculentum* Mill) to early blight disease caused by *A. solani* was studied by Awadalla, (2008) *in vitro* and *in vivo*. The results showed that all the antioxidants tested at the highest concentration (10.0 mM) significantly inhibited the mycelial growth of *A. solani in vitro* and the degree of inhibition was directly proportional to the antioxidant concentrations. Salicylic acid, ascorbic acid, and citric acid were found to be more effective throughout the concentration ranges in inhibiting the mycelial growth than sodium citrate and benzoic acid respectively. During in *vivo* study tomato seeds soaked with all antioxidants prior to sowing showed increased levels of resistance to early blight pathogen, *A. solani*. With varied concentration all antioxidants markedly reduced the appearance of early blight disease on tomato plants and the disease incidence was completely inhibited at the highest concentrations of every antioxidants tested. Salicylic acid, ascorbic acid, and citric acid were found to be the most effective in controlling the disease and then followed by sodium citrate, and benzoic acid. Phytoalexin (tomatine) production was also greatly increased in antioxidant-treated inoculated tomato plants.

To study the efficacy of seven SAR activators, 2,6-dichloroisonicotinic acid (INA), benzo-thiadiazole S-methyl ester (BTH), β-aminobutyric acid (BABA), K_2HPO_4, K_3PO_4, $Ca(OH)_2$ and $CaCO_3$ to induce SAR for the management of *Alternaria* leaf blotch of apple, Sofi *et al*. (2013) applied all these SAR activators on two year old grafted seedlings of Red Delicious apple cultivar 48h before and after *Alternaria mali* spore inoculation. The SAR activators along with a conventional synthetic fungicide (penconazole) were evaluated against most virulent isolate Am-1. All the SAR activators significantly lowered the disease intensity as compared to control (only dist.water). BABA was most effective with least disease intensity before and after pathogenic inoculation which followed by penconazole. Applying SAR activators before pathogen inoculation showed significantly lower disease intensity (12.71%) in comparison to SAR application after pathogen inoculation (14.77%). This induced resistance exploiting natural defense mechanisms of plants could be proposed as a non-conventional and eco-friendly approach for plant protection from various pathogens.

Chavan and Kamble (2013) tested exogenous foliar application of β-aminobutyric acid (BABA) to evaluate the reduction in disease severity on *Brassica carinata* caused by *A. brassicae*. Changes in defense-related enzymes like phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO), isoform analysis of superoxide dismutase (SOD) and peroxidase (POX) were studied to understand the induction of SAR in treated plants. BABA-treated plants showed a significantly increased level of PAL, PPO enzyme activities and total phenolic content in response to pathogen inoculation. However, isoform analysis of SOD and POX revealed no change in number of isozymes but resulted in a quantitative change in activity in response to pathogen.

Thakur (2014) studied the role of elicitors such as SA and BTH for inducing resistance to combat *Alternaria* blight in *Brassica juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) *in vitro*. The effect of different concentrations of elicitors on antioxidative enzymes *viz.*, peroxidase (PO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD); pathogenesis related (PR) proteins *viz*., chitinase, β-1,3-glucanase; phenolics; pigments; ascorbic acid and sugars was investigated in both *Brassica* species. Elicitor treatments enhanced the activities of defense related enzymes and phenolic contents as compared to control plants (only water applied). Similarly, maximum contents of photosynthetic pigments, ascorbic acid, total soluble protein, free amino acids, total sugars and reducing sugars were recorded in elicitor treated plants than control, in both the species. Among all the elicitor treatments, combinations of elicitors like BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) were found most effective. Disease severity was less in plants treated with the combinations of elicitors. The decrease in disease severity and increase in seed yield might be attributed to the role played by defense related enzymes and phenolics. It is a well-known fact that elicitors *viz*., BTH and SA play an important role in enhancing the defense mechanism against wide range of pathogen in various plants species. In addition, this study suggested that elicitors when applied in combinations show markedly effect on elicitation of defense response in *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) against *Alternaria* blight. Although both biotic and abiotic agents have been reported to induce resistance in plants, chemicals are better as elicitors or inducers, because they are easy to formulate and handle and less sensitive to the environment than biological inducers (Kuc, 1995). Chemical inducers may provide better means of application possibilities for induction of resistance, provided that they are easily accessible and not harmful. The induced resistance following treatments with abiotic factors has been considered to be a great potential approach for the control of plant diseases (Latha *et al.*, 2009). There are certain biochemical changes that occur after the application of resistance inducing agents, and can act as markers for induced systemic resistance (Schönbeck *et al.*, 1980). These biochemical changes include the accumulation of certain enzymes and phenolic compounds (He *et al.*, 2002).

7. Conclusion

The pathogen *Alternaria* species are either parasites or saprophyte and destructive pathogen causing widespread destruction in vegetables and economically important crops. But with the utilization of advanced knowledge, tools and techniques in plant disease management it becomes easier to control this detrimental fungus. and has a very broad range of host. Due to this there is a wide level of variability in the pathogen and it exhibits low sensitivity against fungicide(s) or shows resistance/ tolerance to fungicides. A broad host range further helps it to acquire greater survival ability. These abilities not only pose serious threat in controlling the disease by chemical means but also cause great concern for its management by development of resistant varieties. Further, as a part on the integrated disease management approach exploration by induction of systemic acquired resistance through the use of abiotic elicitors becomes a necessity. The application of biotic and abiotic elicitors in crop protection and pest management is still in the very early stages of use as a new control method to control diseases in crop plants and thus the current experiences come from several experimental trials and not yet from large scale agricultural use. Keeping health hazards in view, alternate and eco-friendly method of disease control of crop plants using elicitor treatments with some advantages are like (i) reduced damage from fungi, insects, pests, and herbivores, (ii) reduced environmental hazards and human health hazards as elicitors affect directly on the crop plant, and their acute toxicity to other organisms which is lower than that of pesticides, (iii) elicitors can be used with the current spraying technology as protective agrochemicals, (iv) treatments using elicitors could be an alternative to genetically modified (GM) crop plants for better attraction of natural enemies of pest organisms on cultivated plants (Kappers *et al.*,2005) and (v) crop plants which were treated with biotic and abiotic elicitor bear lower ecological risks than genetically modified GM plants (Poppy and Wilkinson, 2005).

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