CHANGES IN ANTI OXIDANT ENZYME ACTIVITIES IN *Pseudomonas syringae* pv *syringae* (BACTERIAL BROWN SPOT) INFECTED SEEDLINGS OF *Vigna radiata* L.

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ABSTRACT
Bacterial brown spot disease caused by *Pseudomonas syringae* is a great menace to a wide variety of crops including pulses. The bacteria is commonly found in the infected seeds and hence transferred from plant to plant and nearby fields by wind, splashing rains, sprinkler irrigation, surface-drainage water, insects, birds, large animals, humans, farm machinery, tools and other agencies. The bacteria survive for 6 to 18 months in plant refuse. *Pseudomonas syringae* has been proved to be pathogenic to *Dolichos*, *Macrophilium*, *Pueraria* and *Vigna* spp. In the present study, *P.syringae* pure culture was injected using a sterile needle in the main stem at its junction with the stipules at the youngest node in 15 d-old *Vigna* seedlings. The disease symptoms appeared on the stem and the leaves positively after four days of infection. The severity of the disease was monitored at the antioxidant enzyme level namely *viz*, peroxidase, polyphenoloxidase, catalase and SOD in infected seedlings after 5 and 10 days of growth. There was a hike in the activity of all the above enzymes under pathogen treatment with maximum changes in SOD activity. The rise in the antioxidant enzymes level upon bacterial infection is correlated to the resistance phenomenon exhibited by the plants.

Keywords *- Pathogen, Pathogenicity, P.syringae, Brown spot disease, Antioxidant enzymes,*

1. INTRODUCTION

Plant pathogenic bacteria can multiply rapidly inside plant tissue under favourable conditions, causing many serious diseases of crops, with major economic impacts. Disease symptoms caused by bacterial pathogens include wilts, galls, specks, spots, cankers and chlorosis (yellowing).The most studied plant pathogenic bacteria belong to the genera *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Ralstonia* and *Agrobacterium*. Among these pathogenic bacterias *Pseudomonas* sp gains more importance as it is a great menace to food legumes like mung bean, cowpea, cluster bean and so on.

Mungbean (*Vigna radiata*) is a food legume that is very rich in protein and essential aminoacids with the exception of the sulphur aminoacids, methionine and cysteine which may be nutritional limited. It is a good source of soluble carbohydrate, and contains very high amount of crude fiber [1][2]. However, this all important food crop is beset by a number of bacterial diseases namely: bacterial blight, halo blight, bacterial wilt and bacterial spot which result in yield loss.In mung bean bacterial brown spot disease is caused by *P.syringae* and result in losses of yield[3].

Plants have developed strategies to defend themselves against pathogen attacks. One of the earliest defense responses is the production of reactive oxygen species (ROS) after pathogen recognition[4]. Reactive oxygen species includes the superoxide anion radicals and the hydroxyl radicals produced as by-products of oxidation/reduction reactions as a consequence of aerobic metabolism[5]. The interaction between pathogen and plant, leads to production of ROS at early time points of the interaction. This oxidative burst seems to be effective in controlling pathogen infection in incompatible interactions [6].

Plants have a variety of mechanisms for ROS detoxification. Superoxide dismutase (SOD) catalyses the dismutation of O_2^- to H_2O_2, catalase (CAT) scavenges H_2O_2 to oxygen and water. Superoxide dismutases are classified into three groups based on the cofactor utilize: those which consist of iron SOD (Fe-SOD), manganese SOD (Mn-SOD), and copper-zinc SOD (Cu/Zn-SOD)[7]. PPO has also been suggested to function as a defense against pests and pathogens. Thus, considering the above mentioned informations the study is aimed at observing the changes in the level of antioxidant enzymes mung bean plants upon *P.syringae* infection

2. MATERIAL AND METHODS

2.1 Procurement of Seeds

Certified seeds of *Vigna radiata*(L.) were procured from Tamilnadu Agricultural Research Station, Kovilpatti. The seeds obtained were checked for the viability by conventional method. Nearly 80% germination was noticed in seed sample.

2.2 Cultivation of Seedlings

The viable seeds were soaked in distilled water for overnight and allowed to germinate. Seedlings were raised in earthen pots (125 x 25 cm) filled with a mixture of red soil, black soil and sand (in the ratio of 2:2:1). Twenty seeds were sown at equal distances at a depth of 2cm in each pot. The pots were categorized into two sets i.e., control and treated.

2.3 Procurement of Microbes

Pure cultures of *P.syringae* pv *syringae* were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. They were cultured in Nutrient Agar Medium

2.4 Subculture of Bacteria

The bacterium was sub-cultured in Nutrient broth (NBA). The broth was sterilized in an autoclave at 120°C for 15 minutes. The bacterial inoculum was isolated from NA medium and inoculated in 250 ml Erlenmeyer flask containing 100 ml of NBA broth incubated 24 h in an incubator cum shaker (Orbitek, India) at 250 rpm at 36°C. The subculture was maintained at -20°C.

2.5 Estimation of Activities

In vivo nitrate reductase activity was assayed by Jaworski's[8] method. The catalase activity was quantified by the method of Kar and Mishra[9]. Peroxidase activity was quantified by the method of Addy and Goodman[10]. The Polyphenoloxidase activity was analyzed by colorimetric method of Mukherjee and Ghosh[11]. SOD activity was analyzed by the method of Bowler[12].

2.6 Statistical Analysis

The results were expressed as Arithmetic Mean ± Standard deviation. Group difference was tested by one way analysis of variances (ANOVA).All statistical calculations were performed using online statistical tool 'http;// vassarstats.Net/anova/u.html; the level of significance was expressed as $p<0.05$.

3. RESULTS AND DISCUSSION

The present investigation was aimed at studying the changes in the antioxidant enzyme activities in *Vigna radiata* plants infected with the pathogen *Pseudomonas syringae*. When a plant recognizes an attacking pathogen, one of the first induced reactions is to rapidly produce superoxide (O^{-2}) or hydrogen peroxide (H_2O_2) to strengthen the cell wall. This prevents the spread of the pathogen to other parts of the plant, essentially forming a net around the pathogen to restrict movement and reproduction. Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide.

As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Similarly catalase, which is concentrated in peroxisomes located next to mitochondria, reacts with the hydrogen peroxide to catalyze the formation of water and oxygen. Glutathione peroxidase reduces hydrogen peroxide by transferring the energy of the reactive peroxides to a very small sulfur-containing protein called glutathione.

3.1 Infection of Plant Using Pathogen

To assess survival, twenty plants were inoculated by dipping a sterile 25 gauge needle into a 24-hr old-culture of *Pseudomonas syringae* grown on Nutrient agar medium and inserting the needle through the crown region of a 2-week-old seedling, thus ensuring transfer of a similar inoculum quantity to each crown. Control plants were wounded with a sterile needle previously dipped in sterile distilled water (SDW). The inoculated plants and the control plants were kept in a greenhouse and checked regularly.

3.2 Symptoms and Signs

Lesion size can vary, but generally lesions are small, circular, and brown, often surrounded by a yellow zone. As the disease progresses, lesions begin combining to form linear, necrotic streaks bound by leaf veins (Fig 1).Old lesion centers fall out, leaving tattered strips or "shot holes" on affected leaves and evidence of water soaking may be visible in the edge of tissue next to the shot holes.

3.3 Changes in Catalase Activity

In the results obtained by our investigation, there was an increase in the catalase activity in the pathogen treated plants to about 14% and 17% in both 20 and 30 days old *Vigna* plants respectively, over their corresponding control plants (Fig. 2).

Fig.1: Typical symptoms of "Brown spot" disease induced by *P. syringae* pv *syringae* in the leaves of *V. radiata*

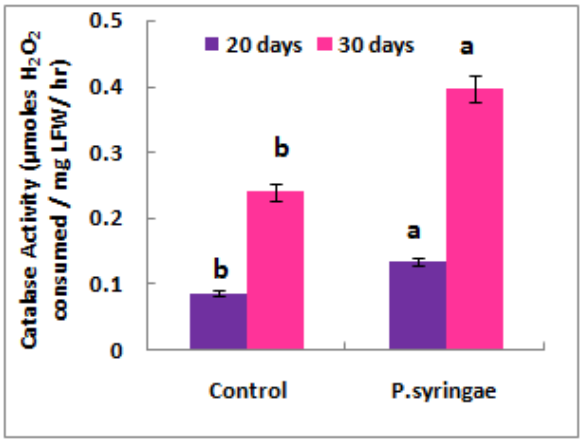

Fig.2: Changes in catalase activity in 20 and 30 days old *Vigna* plants infected with *Pseudomonas syringae*

3.4 Changes in Peroxidase Activity

PO is a key enzyme in the biosynthesis of lignin [13]. Increased activity of cell wall bound peroxidases has been elicited in different plant such as cucumber[14], rice[15], tomato[16] and tobacco[17] due to pathogen infection. In bean, rhizosphere colonization of various bacteria induced the peroxidase activity[18]. Increased peroxidase activity has been recorded in *P.fluorescens* isolate Pf1-treated plants challenged with the pathogen [19]. Chen[14] reported the higher PO activity in cucumber roots treated with *P. corrugata* challenged with *P.aphanidermatum*. The present study also indicates the same increase in peroxidase activity in the *Pseudomonas* treated plants. The hike was about 31% and 48% in 20 and 30 days old *Vigna* plants over their respective control plants (Fig.3)

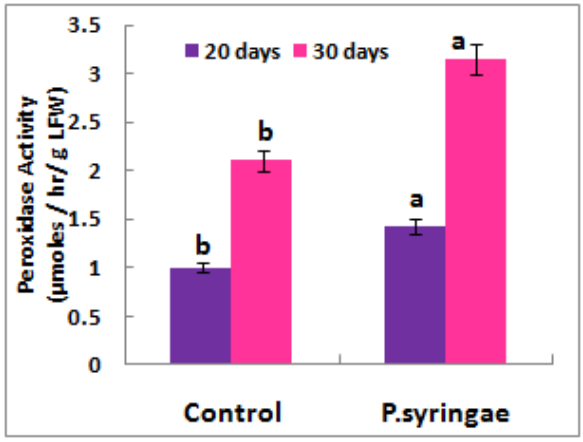

Fig.3: Changes in Peroxidase activity in 20 and 30 days old *Vigna* plants infected with *Pseudomonas syringae*

3.5 Changes in Polyphenoloxidase Activity

Pathogen-induced PPO activity continues to be reported for a variety of plant taxa, including monocots and dicots[14][20]. Similarly, studies describing correlations of high PPO levels in cultivars or lines with high pathogen resistance continue to provide support for a pathogen defense role of PPO[21]. In par with the above said earlier inferences our findings also has shown a significant increase in PPO activities in *Pseudomonas* treated plants. The increase was about 14% in both 20 and 30 days old *Vigna* plants over their corresponding control plants (Fig. 4).

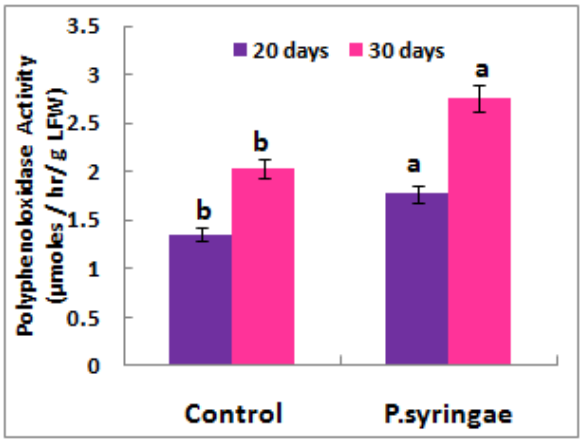

Fig.4 Changes in polyphenoloxidase activity in 20 and 30 days old *Vigna* plants infected with *Pseudomonas syringae*

3.6 Changes in SOD Activity

Superoxide Dismutase, whose changes in activity may indicate an increased concentration of intracellular O_2 [22], showed greater activity in the leaves of inoculated plants, which is in accordance with the greater O_2concentration. SOD catalyzes the dismutation of O_2 into H_2O_2 and O_2, thereby converting one ROS to another, which should also be balanced to keep cells at steady-state [23][24][12]. In this way, the increased SOD activity may have contributed to an increase in H_2O_2 concentration at advanced stages of *X. gardneri* infection. Our results also predicts a gradual increase of SOD to about 8% in *Pseudomonas* infected 20 days old *Vigna* plants and in contrary there was a sudden increase to about 68% in the later stage of infection in 30 days old *Vigna* plants over their respective control plants (Fig. 5)

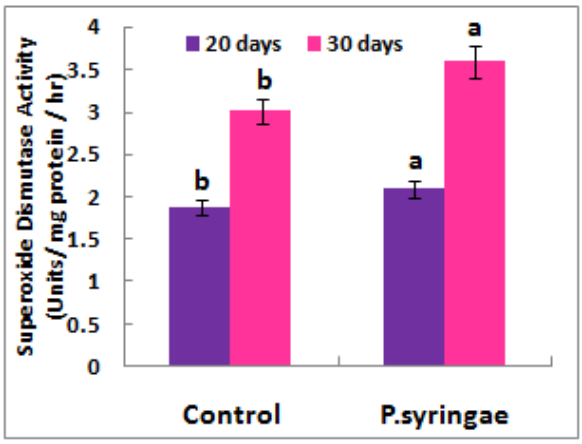

Fig.5: Changes in SOD activity in 20 and 30 days old *Vigna* plants infected with *Pseudomonas syringae*

4. CONCLUSION

Different enzyme activities such as CAT, POX, PPO and SOD which are indicative of cell metabolism when there is an interaction between the host plant and pathogen were determined. Our results have exhibited that there is a significant correlation between the induction of systemic resistance and the activities of these enzymes. The results obtained so far have shown a wide variety of reactions triggered by *Pseudomonas syringae*. Such induction of all available defense mechanisms seems to be the optimal defense tactic against different pathogens[25]. This impact of the bacteria encourages further studies of new strains in experimental systems with different plants to induce defense response and resistance in plants in a variety of biotic and abiotic conditions.

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