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Assessment of Jasmonate in *Pseudomonas syringae*, Induced Brown Spot Disease in *Lablab purpureus* L. (Sweet)

G. Mahalakshmi[1], K. Lingakumar[2*]
[1]Research Scholar, Ayya Nadar Janaki Ammal College, Srivilliiputhur, State Highway 42, Sivakasi, Tamil Nadu, India
[2]Head and Associate Professor, Centre for Research and PG studies in Botany, Ayya Nadar Janaki Ammal College, Srivilliiputhur, State Highway 42, Sivakasi, Tamil Nadu, India

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***Corresponding author**
K. Lingakumar

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Abstract: The effect of *Pseudomonas syringae* on morphological and antioxidant enzyme of *Lablab purpureus* L. (Sweet) was studied. Jasmonic acid are important endogenous chemical signals that play a key role in enhancing plant defense response *Pseudomonas syringae* cultures were injected into the stem region of 15 days old *Lablab purpureus* seedlings. Manual injection was found to be successful in inducing brown spot of *Pseudomonas syringae*. Post treatment of JA given as foliar spray resulted in enhance in the plant defense response. It was found that the level of Peroxidase, Polyphenol oxidase, Catalase and SOD was affected to a level of 85%, 35%, 44% and 60% respectively. Post treatment of JA at 0.5 µM, 1 µM, 2 µM and 5 µM each for 3 days resulted in enhanced resistance to brown spot disease. JA caused alleviation of responses induced by the pathogen in all aspects. Thus, JA treatment was found out to be considerably beneficial in reducing symptoms protecting the *Lablab* against the pathogen.

Keywords: Jasmonate, *Pseudomonas syringae, Lablab purpureus* L. (Sweet), Antioxidant enzymes.

INTRODUCTION

Lablab purpureus belongs to the family Fabaceae cultivated thoughout the tropic world. The species of *Lablab purpureus* (L.) Sweet is known as a bean and is considered to be one of the most important economically valued plants in the world. Brown spots are the one of the most frequent diseases in pulses.

The complete decreasing leaves size. Symptoms of brown spot initially appear as small (1/8 to 3/8 inch), circular, necrotic (brown) spots on the leaves, often surrounded by a narrow yellow halo. The spots sometimes fall out, giving the leaf a "shot-hole" appearance. Water-soaking and bacterial ooze are not usually seen with this disease. Wasternack [1] and Gfeller *et al.*, [2] studied that the derivatives of a linolenic acid viz. jasmonic acid and jasmonoyl-L-isoleucine (JAIle) are accumulated in response to biotic and abiotic stress. Several plant-growth promoting bacteria have been shown to enhance plant's resistance against biotrophic and necrotrophic pathogens by increasing SA and JA levels, respectively [3, 4]. Jasmonates are one of the newest plant growth regulators which cause decrease in damages due to environmental stresses on plant system [5]. The objective of the present study was to identify/characterize the JA induced defence related antioxidant activities from *Lablab purpureus* plants upon different treatments viz., *Pseudomonas syringae* treatment, JA + *Pseudomonas syringae* treatment along with untreated controls. The present investigation was carried out to study the effects of different concentrations (0.5µM, 1µM, 2µM, 5µM) of Jasmonic acid on activity and antioxidative enzymes such as Peroxidase, Polyphenol oxidase, Catalase and SOD.

MATERIALS AND METHODS

Procurement of seeds

Certified seeds of *Lablab purpureus (L.)*Sweet was procured from Tamilnadu Agricultural Research Station, Kovilpatti.

Cultivation of seedlings

The viable seeds were soaked in distilled water for overnight and allowed to germinate. The percentage of germination was nearly 85%. Seedlings were raised in earthen pots (125 x 25 cm) filled with a mixer 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.

Inoculation of seedling with *P.syringae*

Pure bacterial culture of *Pseudomonas syringae* was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India and sub-cultured in Nutrient Agar Medium. *P.syringae* was sub-

cultured in Nutrient broth (NBA). The bacterial inoculum isolated from NA medium was inoculated in 100 ml of NBA broth and incubated for one day in an incubator cum shaker (Orbitek, India) under shaking condition at 250 rpm at 36°C. The subculture was maintained at -20°C. Bacterial cells were harvested in sterile dilution method concentration of 10^5 and bacterial suspension was adjusted to OD600= 0.01 using UV–visible spectrophotometer (ELICO SL 171 model) to obtain the concentration of 1×10^5 cfu ml^{-1}. Pathogenicity test was performed on 2 week old seedlings.

Jasmonate Treatment

Jasmonic acid was obtained from Sigma chemical Co., (St. Louis, U.S.A). JA was initially dissolved in 100 µl of acetone and concentrations of 0.5 µM to 5 µM were made up with distilled water containing 0.02% Tween 20. JA foliar application of brown spot disease infected plants *Lablab purpureus* seedlings at the fully developed trifoliate stage (3 week old seedlings) Control plants were sprayed with equal volume of sterilized distilled water and inoculated similarly as the *Pseudomonas syringae* treated plants. The experiment had 3 treatments. 2 week old *Lablab* plants of similar size were selected and divided into three groups. Group 1: healthy 1 control, plants sprayed with water; Group 2: infected control, plants inoculated with *Pseudomonas syringae*; Group 3: Plants treated with 0.5 µM to 5 µM JA + challenge inoculation with *Pseudomonas syringae*.

Estimation of Enzyme Activities

To assay the catalase activity, 1 ml of H_2O_2 and 1 ml of enzyme extract was added to 3ml phosphate buffer. The reaction mixture was incubated at 25°C for a minute. The reaction was terminated by the addition of 1ml H_2SO_4. The reaction mixture was titrated against 0.01 N $KMNO_4$. The end point was the persistence of pink colour for at least 15 seconds. The catalase activity was expressed in µmoles H_2O_2 catalyzed per unit time per mg protein [6]. To assay peroxidase activity, the enzyme extract was added to pyrogallol which gets oxidized to colored derivative in the presence of H_2O_2 (1% v/v). The amount of purpurogallin formed during the reaction was assayed spectrophotometrically [7]. Polyphenol oxidase activity was analysed by colorimetric [8]. To 2ml of enzyme extract 3ml of 0.1m phosphate buffer (pH 6.0) was added and mixed thoroughly by inverting the cuvette and placed in calorimeter. Super oxide Dismutase (SOD) activity was analyzed by Bowler *et al*., [9] method. The absorbance was measured at 560nm.

RESULTS AND DISCUSSION

Lablab purpureus (L.)Sweet seedlings were post-treated with treatment of Jasmonic acid. The concentration of hormones used in µM concentration. Antioxidant enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase activity and catalase were investigated in hormone treated *Lablab purpureus (L.) Sweet* seedlings. JA significantly enhanced the disease resistance in brown spot susceptible cultivar plants.

Morphology

The intensity of disease alleviation was tested by measuring the leaf symptoms, leaf parameters, enzyme activities**.** The necrotic lesions were angular with brown centers and ranged from 0.2 to 5.0 mm in diameter. It was found that was *Pseudomonas syringae infection* caused 15% reduction in leaf area as compared to control (Fig-1a). Post-treatment of JA application to the leaves of 15 days old seedlings caused 18% increase in leaf area (Fig-1b).

Peroxidase activity

In the present investigation, the peroxidase activity was found to increase to 85% in *P.syringae* infected *Lablab* leaves (Fig-2a). Peroxidase activity in *Lablab* seedlings infected with *P.syringae* which was latter supplemented with jasmonate is shown in (Fig-3b).

Poly phenol

Pathogen infection caused 35% increase in Poly phenol activity in *Lablab* (Fig-2c). Supplementation of JA to *P.syringae* infected leaves was found to cause 15% decrease in the Poly phenol activity (Fig-3c).

SOD activity

P.syringae treatment caused an increase in SOD activity to about 44% in *Lablab* (Fig-2a). Supplementation of *P.syringae* treated seedlings with JA at 2 µM caused an increase in superoxide dismutase activity to about 19% (Fig-3b).

Catalase activity

Pathogen infection caused 60% increase in catalase activity in *Lablab* (Fig-2a). Supplementation of JA to *P.syringae* infected leaves was found to cause 17% decrease in the catalase activity (Fig-3b).

In the present experiment, the plants were post-treated with Jasmonate. Leading *P.syringae* disease the leaf area was compact in *Lablab* seedlings as compared to the control plants. Sayyari *et al*., [10] reported a reduction in leaf area under severe stress condition. The present results show that the leaf area was found to be increased under jasmonate treatment alone. In our findings a considerable increases in the rate of catalase activity was observed in *Lablab* seedlings. Mary and Subramanian [11] reported highest catalase activity on the fifth day in control whereas *Fusarium oxysporum* inhibited the activity significantly. Andreev and Shaw [12] reported the peroxidase activity increases in response to infection of plants by pathogens and higher rate of increase has been related with resistance of the plants. The increase in total phenol

observed in the present study was also reported by others using different plant pathogen interactions. In the present study most of the enzyme activities are increased under JA in *Lablab purpurieus* than control plants. To counteract the toxicity of reactive oxygen species, plants have developed a highly efficient antioxidant enzymes defense system, mainly including SOD, CAT and POD, increasing tolerance to different stress factors [13]. This includes enzymes like SOD, CAT, APX, GPX and non enzymes like ascorbate and glutathione [14]. Tariq *et al.,* [15] reported that the activities of CAT, POX and SOD were increased in the leaves on account of MeJA treatment. They also mentioned that the application of MeJA further enhanced the activities of all antioxidant enzymes, both in nonstressed and stressed plants, by supplementing the ROS scavenging mechanism. MeJA could induce antioxidant defense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant. Mitigation of ROS effects by MeJA was reported in the case of strawberry under drought stress and in maize seedlings subjected to paraquat [16].

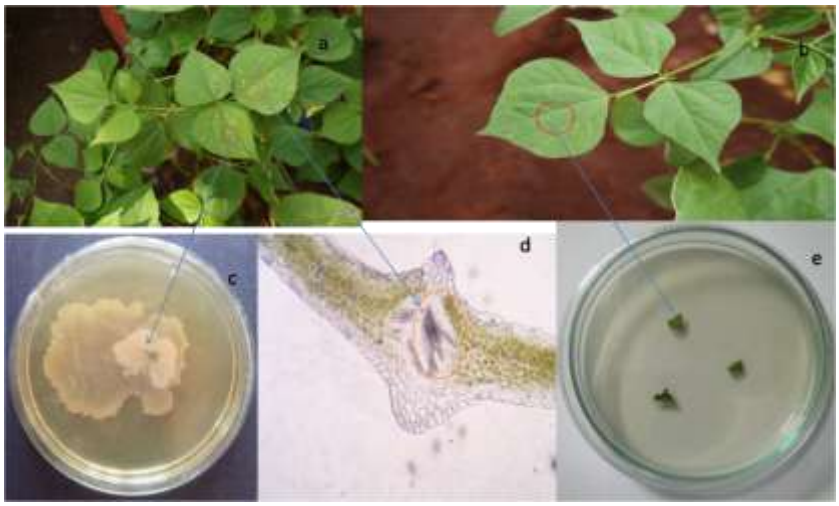

Fig-1: Symptoms (a) and identification (c,d) of *Pseudomonas syringae* infection in *Lablab purpurieus (L.) Sweet.* after 11 days of inoculation

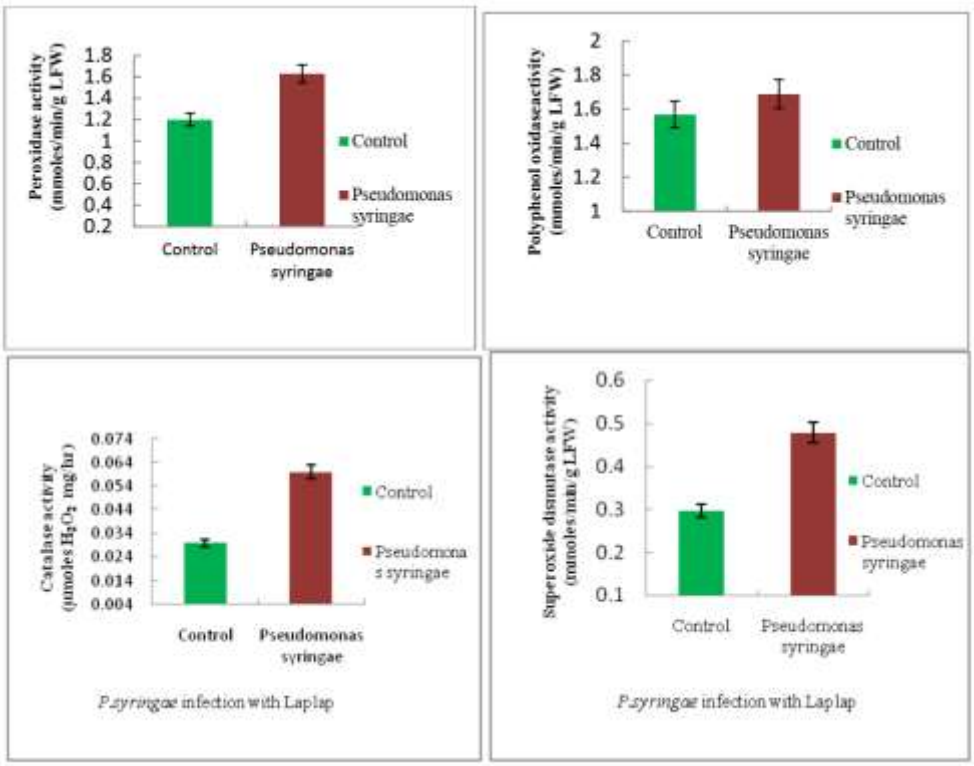

Fig-2 (a, b, c and d): Changes in biochemical constituents and No. of brown spots of *Lablab purpurieus (L.)* Sweet seedlings after infection with *Pseudomonas syringae*

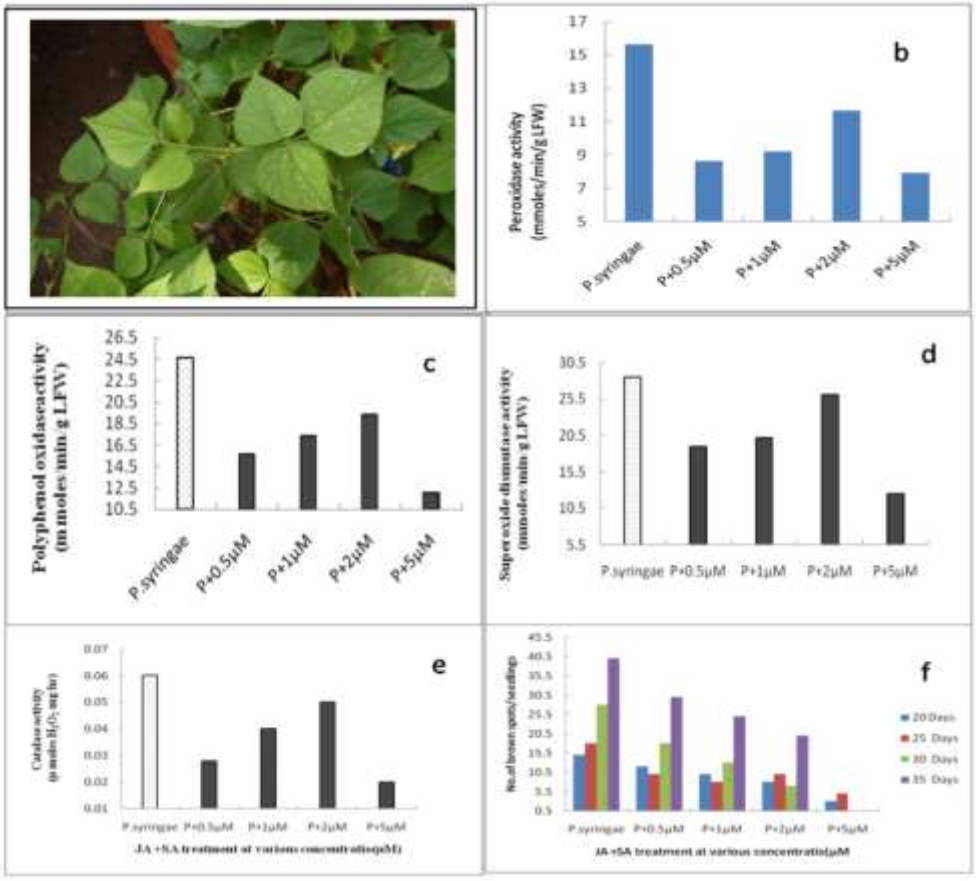

Fig-3: (a, b, c, d, e and f) Changes in biochemical constituents and No. of brown spots of *Lablab purpurieus (L.)* Sweet seedlings after infection with *Pseudomonas syringae* Post infection treatment with Jasmonate at various concentrations

CONCLUSION

Pseudomonas syringae culture at log phase was selected to infect *Lablab purpureus*. Tiny yellow spots appeared on the leaves of *Lablab purpureus* 5 days after *Pseudomonas* inoculation. The spots became visibly necrotic after 8days of pathogen inoculation. The necrotic lesions were angular with brown centers and ranged from 0.2 to 5.0 mm in diameter. Enzyme activities like catalase, peroxidase, polyphenol oxidase, superoxide dismutase activity increased with *Pseudomonas syringae* infection. Foliar spray of JA of various concentrations 0.5µM to 5.0µM were extensively enhanced the leaf area. Among 5 µM of JA concentration was most favorable concentration after 30 days of growth seedling of *Lablab purpureus*. The enzymes activities such as peroxidase, ploy phenol oxidase, superoxide dismutase activity were increased with JA treatment. Various concentrations of JA were proved to be effective in relieving the *P.syringae* induced inhibition. Jasmonate solutions (0.5, 1.0, 2.0 and 5.0µM) were foliar sprayed on the leaf surface of *Lablab purpureus* with *Pseudomonas syringae* pre-treatment. The results obtained conform the Pathogenic nature of *Pseudomonas syringae* and *Lablab purpureus*, the symptoms of "Wildfire" disease were noticed on the young and old leaves of *Lablab*. In order to alleviate the severity of disease JA seedlings were formed to beneficial at 5 µM.

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