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ON THE POSSIBLE EFFECTS OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* IN *VIGNA RADIATA* [L.] SPECIAL REFERENCE TO PHOTOSYNTHETIC PIGMENTS AND BIOCHEMICAL CONSTITUENTS

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Abstract

Pseudomonas syringae* pv. *Syringae is one of the mainly intensively consider bacterial plant pathogens. An attempt was made to induce pathogenesis by injecting Pseudomonas syringae in to the stem region of 15-d-old green gram seedlings. Manual infection was found to be successful in inducing symptoms on the leaves. The infected leaves were subjected to photosynthetic pigments and biochemical constituents. It was found that the photosynthetic pigments were highly affected especially chl and carotenoids. Nearly 75% reduction in total chl was noticed. On the contrary, the non-photosynthetic pigments viz., anthocyanin and flavonoids got increased upon infection. Biochemical constituents such as soluble protein, NR activity, phenol content, catalase, peroxidase activity were also increased in infected leaves. From the inoculation site, lysis occurred on the epidermal cells, palisade and spongy parenchyma cells, with strong staining of the cellular contents with abnormal intercellular spaces in the palisade and spongy parenchyma, hypertrophy and hyperplasia of mesophyll cells and partial destruction of chloroplasts. The results are useful to understand the plant-pathogen interaction which causes a serious damage to plant growth and yield.

Key words: Anthocyanin, Catalase, Flavonoids, NR activity, Peroxidase, Pseudomonas syringae, Chlorophyll, Vigna.

I. INTRODUCTION

Green gram (*Vigna 106adiate*) is one of the most important vegetable crops grown in south India know for its rich protein content essential for human nutrition. India is the world's largest producer as well as consumer of green gram. Gopalan *et al*. 2007 have reported that 100 g of green gram produces 334kCal of energy. Most of the bacterial pathogen of plants includes Gram-negative bacteria such as *Pseudomonas, Erwinia, Ralstonia, Xanthomonas* and a few Gram-positive bacteria also causes disease in plants (Buonaurio, R 2008). *Pseudomonas syringae* is a rod-shaped, gram negative bacterium with polar flagella. Bacterial brown spot disease is caused by *Pseudomonas syringae pv. Syringae* in vigna radiate seedlings. Pathogenic bacteria incite disease in plants by penetrating into host tissues through natural openings, such as hydathodes, stomata lenticels wound and bacteria (Melotto, M and Kunkel, B.N. 2013).

II. MATERIALS AND METHODS

Seeds of *Vigna radiata* (L.) Wilczek were procured from Agricultural research Station, Kovilpatti, Tamilnadu, India and surface sterilized with 0.1% $HgCl_2$ for a minute and washed repeatedly with distilled water. Healthy seeds were elected and sown in pots containing assortment

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of red soil, black soil and sand assorted in the ratio of 2:2:1. The seeds were tolerable to germinate in dark for 48 hours. The percentage of seed germination was more than 80%. Soon after emergence, the seedling were shifted to daylight conditions and covered with 40% cut off mesh filter to avoid hot temperature for an initial period of 2-3 days. Those plants which served as control received only distilled water foliar spray.

Pure bacterial culture of *Pseudomonas syringae pv. Syringae* was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India and sub-cultured in Nutrient Agar Medium. The broth was autoclaved at 120°C ffor 15 minutes. The bacterial inoculation isolated from NA medium was inoculated in 100ml of NBA broth and incubated for one day in an incubator cum shaker (Orbitek, India) under shaking condition at 250 rpm at 36 ^{o}C. the subculture was maintained at -20°C.

To induce pathogenicity, fifty seedlings of 15 days old were inoculated by dipping a sterile 25 gauge needle into a 24-h-oldl-culture of *Pseudomonas syringae* developed on a Nutrient agar medium and inserting 0.1 ml of the needle in the crown of a 15 days old *Vigna* seedling. Control plants were wounded with a sterile needle previously dipped in sterile distilled water. Both the plants maintained in the green house of the department.

The procedure of extracting of photosynthetic pigments from freshly harvested leaves was the same as that of (Lingakumar, K. and Kulandaivelu, G. 1996). The amount of Chl a, Chl b, total chlorophyll and carotenoids was measured at 662, 645, 470 nm respectively using a Hitachi U-200 double beam spectrophotometer and estimate using the formulae of Welburn, A.R. and Lichtenthaler, H.1994.

The leaves were homogenized in ice-cold 0.1 Mm phosphate buffer (P^{H} 6.8) and filtered through 4 layer cheese cloth. The homogenate was centrifuged at 10, 000 rpm for 10min at 40°C and the supernatant as used to determine soluble protein content. Protein content was determined following the method of Bradford, M. 1976. Having BSA as standard The proline content in fresh leaf samples was determine following the method of Bates *et al.* 1973.

In- vitroNR activity was measured following the method of Jaworski, E.G. 1971.To assay the catalase activity, 3ml, phosphate buffer was to 1 ml of H_2O_2. And 1 ml of enzyme extract (Kar, M and Mishra, D. 1976). The reaction mixture was incubated at 25Oc for a minute. The reaction was terminated by the addition of 1ml H_2SO_4. The reaction mixture was titrated against 0.01 $NKMNO_4$. The end point was the persistence of pink colour for at least for 15 seconds. The catalase activity was expressed in µmoles H_2O_2 catalyzed per unit time per mg protein. To assay peroxidase activity, the enzyme extracted was added to pyrogallol which gets oxidized to coloured derivative in the presence of H_2O_2 (1% v/v). the amount of purpurogallin formed during the reaction was assayed spectrophotometrically (Addy, S.K. and Goodman, R.N. 1972).total phenol content of the fresh leaf sample was estimated by Folin Ciocalteau method (Bray, M.G. and Thorpe, M.V. 1954).the amount of total phenols present in the sample was calculated from a standard curve prepared using phenol.Known amount of leaf bits were incubated in 80% acidified methanol (methanol:water:HCl;80:20:1) for 12 h at 4°C in dark with intermittent shaking to extract the flavonoids. The absorbance of the acidified methanol extract at 315 nm was used to quantify the flavonoids content (Mancinelli. *et al* 1975). Anthocyanins were extracted by grinding the leaves in 80% acidified methanol and the clear extract was used to estimate the anthocyanin concentration by measuring the absorbance at 530 and 657 nm (Mirecki, R.M. and Teramura, A.H. 1984).

III. RESULTS

The experiment was designed to study the pathogenicity of *Pseudomonas syringae* in *Vigna radiata* (L.) Wilczek. The intensity of disease in terms of Photosynthesis, non- Photosynthesis and biochemical parameters was tested by analyzing the Photosynthesis, non- Photosynthesis enzyme actions and biochemical constituents. In the current study, the chl a, chl b, chla+b, total chlorophyll content was established to decrease to a level of 68% after 18 days of infection with *P.syringae* pv. *syringae* Figure 1 (A,B,C,D). Treatment of *P.syringae* considerably increased the anthocyanin

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content in leaf to about 75% in *Vigna radiata*. Over the uninoculated control Figure 1 (E). *P.syringae* treated plants having maximum content of flavanoids in leaf to about 84 % in *Vigna radiata*. Figure 1(F). Flavonoid content was found to be increased under *Pseudomonas* infection in *Vigna radiata***.** The infection of *P.syringae* response was also witnessed in the intensity of soluble protein and NR activity content *P.syringae* stressed seedlings have shown +12% amount of decrease in NR content as compared to the control Figure 1 (G). The study exposed that there was a decline in protein content in pathogen infected plants. *P.syringae* infection caused 72% lessening in the leaf soluble protein content than the uninfected control Figure 1 (H). The phenol content was found to be increased to about +35% in *P.syringae* treated plants of *Vigna radiata*. Over the control plants Figure 1 (I). The changes in catalase and proline activity under *Pseudomonas syringae* treatment is shown in Figure 1(J, K). Pathogen infection caused +10% and +12 increases in catalase and proline activity respectively in *Vigna*. In the present analysis, the peroxidase activity was starting to increase to above 10% in *P.syringae* infected *Vigna* leaves Figure 1 (L). Peroxidase constitutes an important group of resistance enzymes that defend plants against various stresses.

IV. DISCUSSION

In our results a significant reduction in the proportion of chlorophyll a and b activity was observed in *Pseudomonas syringae pv. syringae* infected seedlings. Scarpari *et al*. 2005 reported reductions in chlorophyll a and b in the *Theobroma cacao* plants infected by the pathogen *Crinipellis perniciosa*. In our observation a significant difference in the frequency of Carotenoid activity was observed in *Pseudomonas syringae pv. syringae* infected seedlings. Carotenoid levels presented significant reductions on the 8th and 12th days as a result of the infection expansion and consequent cell death produced by the pathogen in leaf tissue (Tomankova I *et al*. 2006). The changes in the protein content in the *P.syringae* treated plants could be due to the change in the metabolic activity due to the plant pathogen interaction. Similarly Farkas and Kiraalg 1962 stated that there was a significant decrease in the protein content as a result of pathogen infection which may be due to some activities related to a hypersensitive response. Results of the present study reveal that, a higher amount of proline was observed in the *P.syringae* infected *V. radiata* than the control. Similarly, Lubaina and Murugan,(2013) reported that the maximum amount of proline was observed in the infected same compared to the respective control. In our findings a considerable reduction in the rate of NR activity was observed in *Pseudomonas syringae pv. syringae* infected seedlings. Rajeswari, (2015) reported that in *Arachis hypogea* the activity of NR enzyme was found to be highest in the control (1250 nmol/mg). The least activity was recorded in the infected plants (900 n mol/ mg). Significant reduction in NR activity was observed in the *Fusarium oxysporum* infected plants. Since the contemporary outcomes, hereby located intensification in catalase activity was originated in *Vigna radiata* plants followed by subsequent infection of *Pseudomonas syringae*. Mary and Subramanian,() reported catalase activity on the fifth day of control showing highest activity (0.15 unit/min/g) and the *Fusarium oxysporum* inhibiting the activity (0.2 unit/min/g). In this regard, peroxidase activity was maximal in *P.syringae* treated plants. Andreev and Shaw ,(1965)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 phenolics observed in the present study have been reported by others using different plant pathogen interactions. Girdhari *et al.* 2008 also reported that increased total phenol content was found in rice leaves after treatment with biotic inducers. Lo and Nicholson 1998 reported that Chinese cabbage leaves responded to feeding damage and fungal infection, respectively, by raising the levels of total anthocyanins. In the present investigation it was found to be increased in *Pseudomonas syringae* treated plants when compared to the control plants. Lo and Nicholson anthocyanin and flavonoid content (Lo and Nicholson 1998) reported that Chinese cabbage leaves responded to feeding damage and fungal infection, respectively, by raising the levels of total anthocyanins. Larson 1988 reported that drought often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids in willow leaves.

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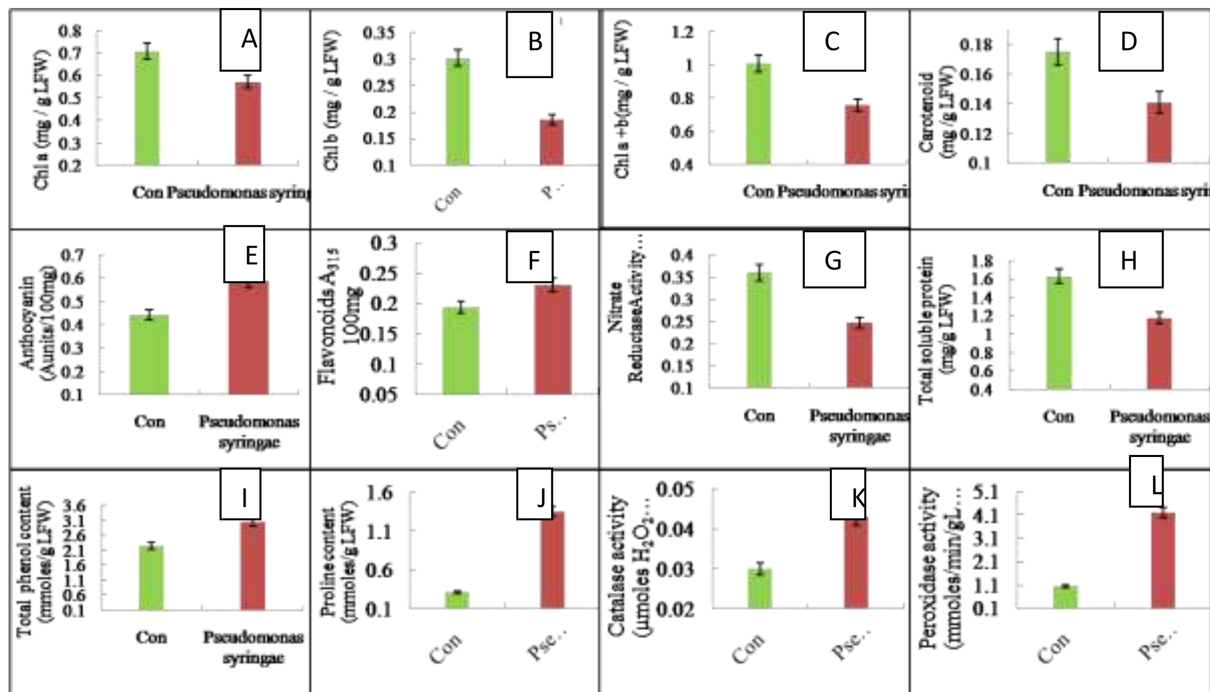

BIBILOGRAPHY

[1] Gopalan, C., Sastri, B.V.R., Balasubramanian, S.C. 2007. Nutritive Value of Indian Foods. National Institute of Nutrition, Hyderabad: ICMR; p.161.

[2] Addy, S.K. and Goodman, R.N. 1972. Polyphenol oxidase and Peroxidase activity in apple leaves inoculated with a virulent or an avirulent strain of Erwinia amylovoro. Indian Phytopath. 25: 575-579.

[3] Bray, M.G. and Thorpe, M.V. 1954. Analysis of phenol compound of interest in metabolism. Methods. Biochem. Anal. 1: 27-52.

[4] Mancinelli, A.L., Yang, C.P.H., Lindguist, P., Anderson, O.R. and Rabino, I. 1975. Photoregulation of anthycyanin synthesis III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiol. 55: 251-254.

[5] Mirecki, R.M. and Teramura, A.H. 1984. Effects of ultraviolet-B irradiance on soybean: V. The dependence of plant sen-sitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol. 74:475–480.

[6] Scarpari, L.M,, Meinhardt, L.W., Mazzafera, P., Pomella, A.W.V., Schiavinato, M.A., Cascardo, J.M.C. 2005. Biochemical changes during the development of witches' broom: the most important disease of cocoa in Brazil caused by Crinipellis perniciosa. J. Experimental. Bot.56:865–877.

[7] Santos, N., Israelian, G., Mayor, M. 2005. A&A, 437, 1127 Astronomy and Astrophysics, Volume **437**:1127-1133.

[8] Tománková, K., Jeřábková, P., Zmeškal, O., Veselá, M., Haderka, J. 2006. Use of image analysis to study growth and division of yeast cells. J Imaging Sci Techn.;50:1–8. J.ImagingSci.Technol.50:6(583).

[9] Farkas, G.L. and Kiraaly, Z. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. J. Phytopathol. 44: 105-150.

[10] Lubaina, A.S., Murugan, A.K. 2013. Ultrastructural changes and oxidative stress markers in wild and cultivar Sesamum orientale L. following Alternaria sesami (Kawamura) Mohanty and Behera inoculation. Ind. J. Exp. Biol. 51: 670-680.

[11] Rajeswari, P. 2015. Control of Fusarium Oxysporum Causing Fusarium Wilt by Trichoderma Spp and Pseudomonas fluorescens on Arachis hypogaea L. Intl.J.Adv.Biotechnol.Res. 6(1): 57-65.

[12] Buonaurio, R. 2008. Infection and plant defence responses during plant-bacterial interaction, In: plant-Microbe Interactions, E Ait Barka and Clement (eds.), Research signpost, India: 169-197.

[13] Mary, S. and Subramanian, R.B. 2014. Biochemical and Enzymatic Response of Cajanus cajan Plant Towards the Biotic Stress Induced by Fusarium oxysporum Afr. J. Basic Appl. Sci. 6(3): 65-75.

[14] Andreev, L. A.N. and Shaw, D.M. 1965. A note on the effect of rust infection on peroxidase isoezymes in flax.Can. J. Bot. 43: 1479-1483.

[15] Girdhari, L.K., Biswas, S.K., Srivastava, S.S.L. 2008. Biochemical evidence of defense response in paddy induced by bio-agents against brown leaf spot pathogen. Indian Phytopath.61(2):197-203.

[16] Lo, S.C. and Nicholson, R.L. 1998. Reduction of light induced anthocyanin accumulation in inoculated sorghum mesocotyls. Implications for a compensatory role in the defense response. Plant Physiol. 116(3): 979-89.

[17] Larson, R.A. 1988. The antioxidants of higher plants.Phytochem. 27: 969–978.

[18] elotto, M. and Kunkel, B. N. 2013. “Virulence strategies of plant pathogenic bacteria,” in The Prokaryotes – Prokaryotic Physiology and Biochemistry 4th Edn eds Rosenberg E.,

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[19] Lingakumar, K. and Kulandaivelu, G. 1996. Reversal of ultraviolet-B (280-320nm) radiation induced changes in Vigna unguiculata L. Chloroplasts by supplemental "white light". Photosynthetica 32(3): 467-469.
[20] Wellburn, A.R and Lichtenthaler, H. 1984. Formulae and program to determine total carotenoids and chlorophyll a and b of leaf extracts in different solvents. In:Advances in Photosynthesis Research(Sybesma, C. ed.,) Martinus Nijhoff/ Dr. W. Junk, The Hague. 2 : 9-12.
[21] Bradford, M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding . Analytical Biochemistry, 72:248-254.
[22] Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of the proline in water stress studies. Plant and Soil. 39: 205-208.
[23] Jaworski, E.G. 1971. Nitrate Reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43: 1274-1279.
[24] Kar, M. and Mishra, D. 1976. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 57: 315-319.

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