Journal of Global Biosciences
ISSN 2320-1355
Volume 6, Number 3, 2017, pp. 4863-4870
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

LOCAL AND SYSTEMIC PROTECTION AGAINST *Pseudomonas syringae* pv.*phaseolica* IN *Vigna unguiculata* L. BY FOLIAR APPLICATION OF JASMONATE AND SALICYLATE

Pandiselvi, T., G. Mahalakshmi, R. Kuralarasi, M. Veeralakshmi and K. Lingakumar

Centre for Research and Postgraduate Studies in Botany,
Ayya Nadar Janaki Ammal College,
(Autonomous Re-accredited (3 rd cycle) with A' Grade (with CGPA of 3.67 out of 4) by the NAAC & College,
of Excellence by UGC), Sivakasi- 626124, Tamil Nadu, India.

Abstract

Pseudomonas syringae is a gram negative bacterium infecting crop species with more than 50 pathovars poses a serious menace. Phytohormones like jasmonate and salicylate are known to induce defensive responses in plants against abiotic stresses. In the present study foliar application of JA and SA at critical concentrations offered resistance to *P.syringae* infection. The parameters tested include morphological symptoms and biochemical constituents. Infection of *P.syringae* into the crown of 15days-old- *Vigna* seedlings resulted in typical foliar symptoms. Biochemical constituents such as NR activity, catalase, peroxidase, SOD activity were analyzed in control, infected and SA and JA treated seedlings. Differential response was noticed in all these parameters. The results were useful to understand the plant-pathogen interaction causing a serious damage to plant growth and yield. The pathogenic nature of *P.syringae* on *Vigna* was assayed in terms of foliar symptoms and biochemical constituents. The hormonal treatment was found out to be considerably beneficial in inducing symptoms agent in protecting the cowpea against the pathogen.

Key words: Catalase, Jasmonate, NR activity, peroxidase, Salicylate, SOD, *Vigna unguiculata*.

INTRODUCTION

Pseudomonas syringae pv. phaseolica is a rod-shaped, Gram negative bacterium with polar flagella. As a plant pathogen, it can infect a wide range of species and exists as 50 different pathovars, all of which are available from NCPPB and ICMP. Salicylic acid is a class of phytohormone which has an affinity to bind with the enzymes like CAT, APX, aconitase and carbonic anhydrase [1] and some of the enzymes are involved in ROS metabolism and in redox homeostasis. Anderson *et al*. [2] reported that SA affects the lipid peroxidation playing a key role in initiating defense response and induction of SAR

in plants when challenged with pathogens [3]. Similarly, Jasmonates and their derivatives play a key role in activating genes involved in defense responses against abiotic and biotic stresses [4]. The discovery of wound-induced proteinases expressed in tomato [5] and potato [6] along with the findings that expression of the corresponding genes was induced by exogenous JA treatment were crucial for the establishment of a first study model for signaling triggered by wounds in plants [7]. Thus, it was demonstrated that treatment with MeJA is an alternative strategy for stimulating natural defense mechanisms in *V. vinifera*, reducing the need for fungicides against powdery mildew.

MATERIAL AND METHODS

CULTIVATION OF SEEDLINGS: Certified seeds of *Vigna unguiculata* (L.) Walp were procured from Tamilnadu Agricultural Research Station, Kovilpatti. 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 mixture of red soil, black soil and sand (2:2:1). Twenty seeds were sown in equal distances at a depth of 2cm in each pot. The pots were categorized into six sets. First set of plants served as control which received only distilled water spray. First set of plants served as control that received only distilled water spray. The second set of plants was infected with *Pseudomonas syringae*. The third set of plants was sprayed with different concentrations (0.5 µM, 1.0 µM, 1.5 µM, 2.0 µM, 5.0 µM) of Jamonate and Salicylate only. Fourth set of plants treated with *Pseudomonas syringae* and further sprayed with Jamonate and Salicylate at different concentrations (0.5 µM, 1.0 µM, 1.5 µM, 2.0 µM, 5.0 µM).

PROCUREMENT OF BACTERIAL CULTURE: Pure bacterial culture of *Pseudomonas syringae* pv.phaseolica 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 broth was autoclaved at 120°C for 15 minutes. 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.

INOCULATION OF SEEDLING WITH *P.syringae*: To induce pathogenicity, fifty seedlings of 15 days old were inoculated by dipping a sterile 25 gauge needle into a 24-h-old- 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 in stem region. Control plants were wounded with a sterile needle previously dipped in sterile distilled water. Both the plants maintained in the greenhouse of the department.

ESTIMATION OF ENZYME ACTIVITIES: *In vivo* nitrate Reductase (NR) activity was assayed following the procedure to Jaworski [8]. The Nitrate was estimated with the help of nitrite standard curve. To assay catalase activity, 3ml, phosphate buffer was added to 1ml of H_2O_2 and 1ml of enzyme extract [9]. The reaction mixture was incubated at 25^{o}C for 1 minute. The reaction was terminated by the addition of 1ml of H_2SO_4. The reaction mixture was titrated against 0.01N $KMN0_4$. The end point was the persistence of pink colour at least for 15 seconds. The catalase activity was expressed in micro moles H_2O_2catalyzed per unit time per mg protein. To assay peroxidase activity, the enzyme extract was added to pyrogallol which gets oxidized to coloured derivative in the presence of hydrogen peroxide (1% V). The amount of purpurogallin formed during the reaction was assayed spectrophotometrically [10]. Polyphenol oxidase activity was analysed by colorimetric [11]. 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* [12] method. The absorbance was measured at 560nm.

RESULTS

Pseudomonas syringae pv. phaseolica culture at log phase was selected to infect the selected crop viz., *Vigna unguiculata* (cowpea). Tiny yellow spots appeared on the leaves of *Vigna unguiculata* after 8days of *Pseudomonas* infection (Fig. a). After 15 days of infection the necrotic lesions were angular with brown centers and ranged from 0.5 to 5.0 mm in diameter (Fig. b). Fig. (c) shows the anatomical details of the infected leaves. The pathogen was found to enter in to the sub-epidermal region of the leaves. With increase in age of the leaf, the nature of the lesion appeared highly necrotic (Fig. d). The infected seedlings were given foliar spray of various concentrations of Jasmonate (Fig. e) and Salicylate (Fig. f). The intensity of disease alleviation was tested by measuring the leaf symptoms, leaf parameters, enzyme activities.

LEAF AREA: 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 0.5 to 5.0 mm in diameter. It was found that was *Pseudomonas syringae* pv. phaseolica infection caused 18.5% reduction in leaf area as compared to control (Fig. g). JA and SA application alone to the leaves of 15 days old seedlings caused 11.6% and 16% increase in leaf area (Fig. h). JA and SA at 1.5µM caused 13% and 17% respectively increase in leaf area over pathogen infected control (Fig. i).

C**ATALASE ACTIVITY:** Pathogen infection caused 60% increase in catalase activity in *Vigna* (Fig. j). Among the five concentrations of JA and SA tested, 1.5 µM of JA and SA was found to cause a decrease in catalase activity to a tune of 3-19% and 9% to 31% respectively (Fig. k). Supplementation of JA and SA to *P.syringae* infected leaves was found to cause 3-17% and 11-41% decrease in the catalase activity (Fig. l).

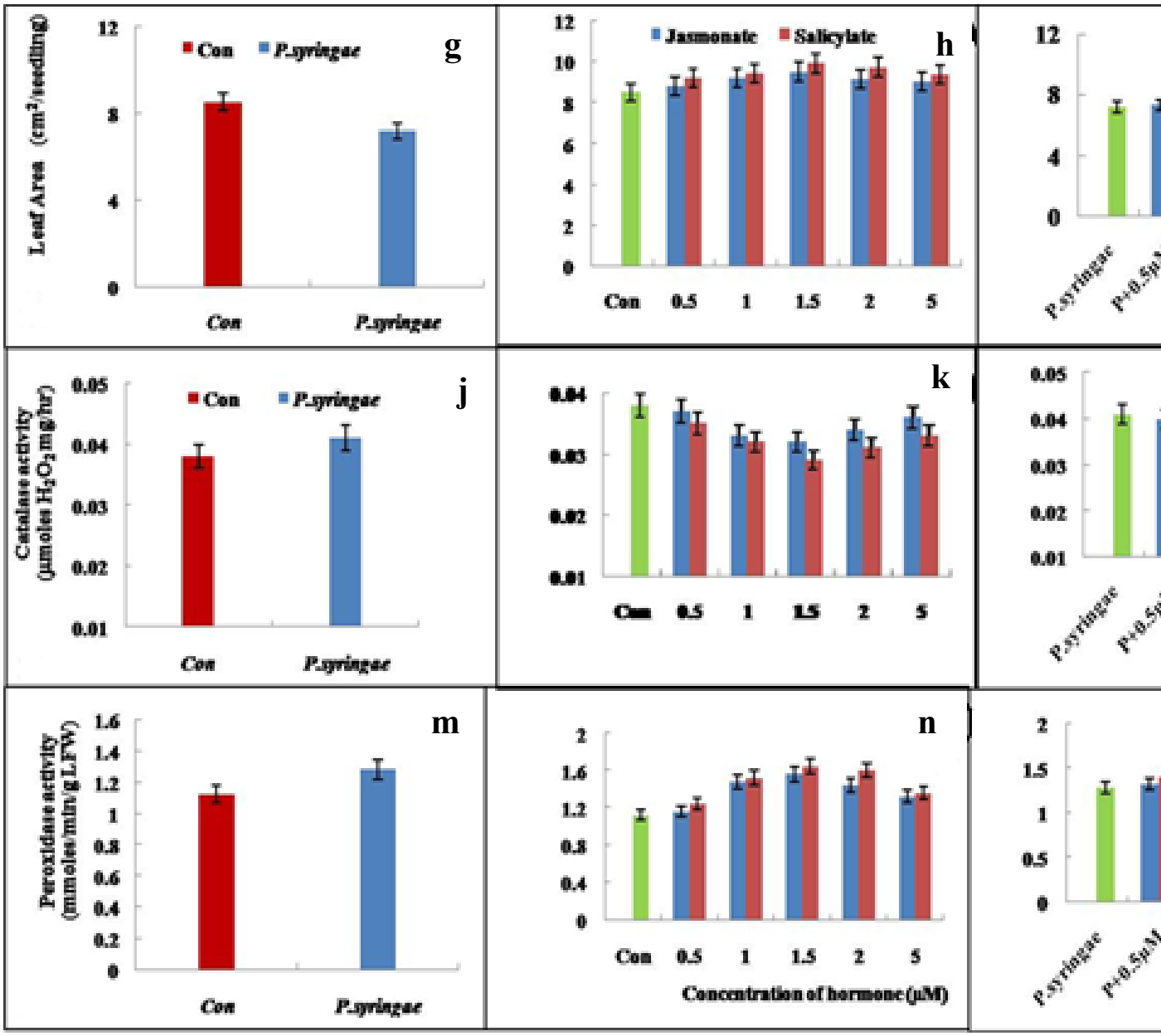
P**EROXIDASE ACTIVITY:** In the present investigation, the peroxidase activity was found to increase to 44% in *P.syringae* infected *Vigna* leaves (Fig. m). Among the various concentrations tested, 1.5 µM of JA and SA caused significant increased level was found to be 4- 39% under JA treatment and 11- 46% under SA treatment (Fig. n). Peroxidase activity in *V.unguiculata* seedlings infected with *P.syringae* which was latter supplemented with jasmonate and salicylates are shown in (Fig. o).

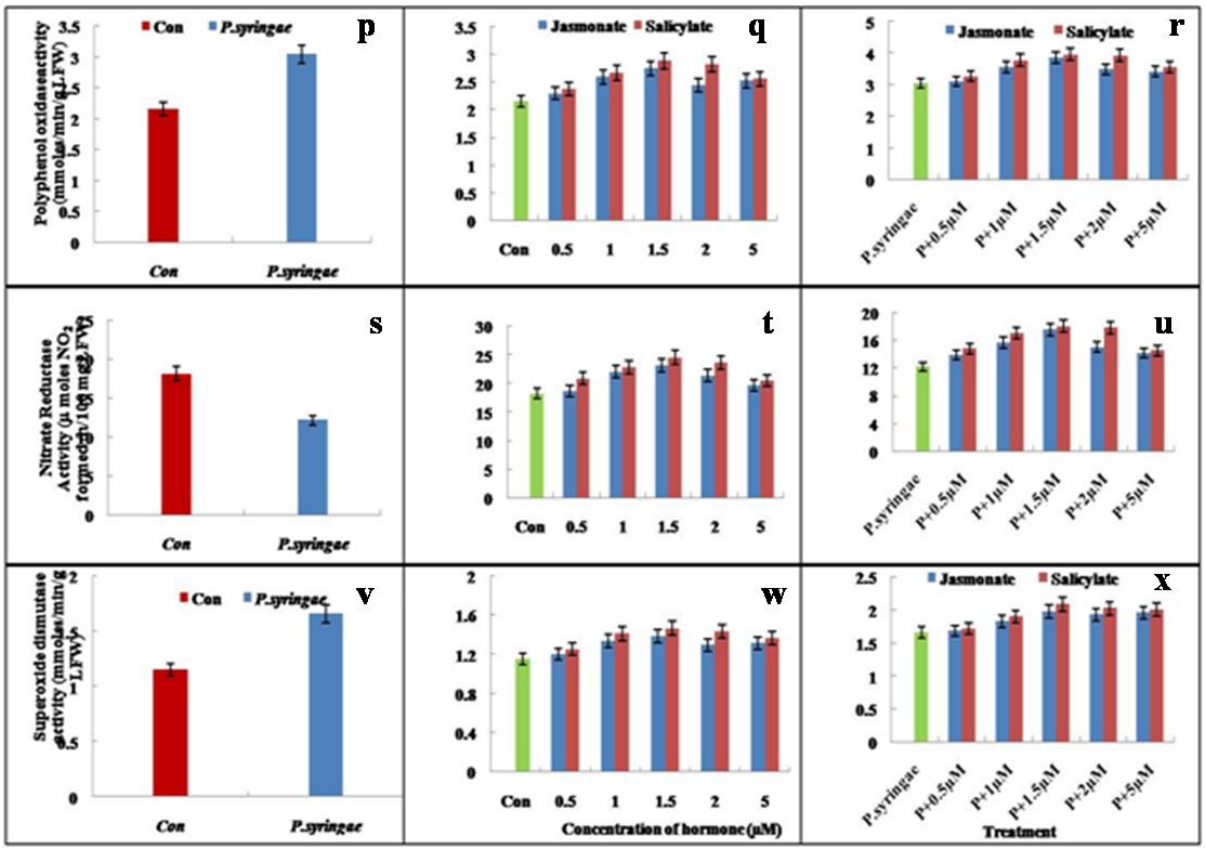
POLYPHENOL OXIDASE ACTIVITY: The present result showed an increase in the polyphenol oxidase activity to about 41% in *Vigna unguiculata* (Fig. p). PPO activity was found to be increased to about 7-28% in JA treatment and 10-34% in SA treatment (Fig. q). In *V.unguiculata* both jasmonate and salicylate at 1.5 µM concentration was found to cause a slight increase to a level of 2-27% and 7-30% respectively (Fig. r).

NR ACTIVITY: In the present study, the NR activity was found to decrease to a level of 66% after 18 days of infection with *P.syringae* pv. phaseolica (Fig. s). Hormones such as JA and SA given as foliar spray at 1.5µM caused 3-28% increase in NR activity whereas SA caused 15-35% increase (Fig. t). In the case of pathogen infection and subsequent hormone treatment, a stimulation of NR to 13-44% and 19-48% was observed after JA and SA treatment to diseased leaves (Fig. u). In *V.unguiculata* both jasmonate and salicylate at 1.5 µM concentration was found to cause a slight increase to a level of 2-27% and 7-30% respectively.

SOD ACTIVITY*: P.syringae* treatment caused an increase in SOD activity to about 44% in *Vigna* (Fig. v). The results showed that the SOD activity in *V.unguiculata* showed

changes in response to jasmonate and salicylate. At 1.5 µM concentration of JA, the SOD activity was found increased to about 4-20% followed by 1 µM concentration. Similarly, SA at 1.5 µM concentration also showed an increase in the activity of superoxide dismutase to a tune of 9-27% followed by 2 µM concentration (Fig. w). Supplementation of *P.syringae* treated seedlings with JA at 1.5µM and SA at 1.5µM caused an increase in superoxide dismutase activity to about 1-19% and 3-25% respectively (Fig. x).


g
Leaf Area (cm²/seedling)
Con
P.syringae
h
Jasmonate
Salicylate
Con
0.5
1
1.5
2
5
j
Catalase activity (μmoles H2O2 mg/hr)
k
m
Peroxidase activity (mmoles/min/g LFW)
n
Concentration of hormone (μM)



DISCUSSION

Upon *P.syringae* pv. phaseolica infection the leaf area was reduced in *Vigna* seedlings as compared to the control plants. Sayyari *et al*. [13] reported a reduction in leaf area under severe stress condition. The present results shows that the leaf area was found to be increased under jasmonate treatment alone and in plants pretreated with pathogen and later supplemented with jasmonate and salicylate. In our findings a considerable reduction in the rate of NR activity was observed in *Pseudomonas syringae pv.* phaseolica infected seedlings. According to Padma Singh [14] decreased nitrogen content in the *Alternaria* sp. infected onion leaves was due to the disruption of cell structure coupled with enhanced proteolytic enzyme activity when enhanced disease development. From the present findings, there was an increase in catalase activity was found in *Vigna unguiculata* plants after infection of *Pseudomonas syringae*. Chamnongpol *et al*. [15] reported catalase as a mediator of defense response, because the reduction in its activity was related to the accumulation of H_2O_2 in tobacco.

In this regard, peroxidase activity was maximal in *P. syringae* treated plants. 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 [16]. 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 [17].The superoxide dismutase activity was high in *Pseudomonas syringae* treated plants over the control plants. Similar to our results, increased levels of SOD are reported from *Nicotiana benthamiana* to infection with two strains of pepper mild mottle virus. In the present study most of the enzyme activities are increased under JA treatment alone and in plants pretreated with *Pseudomonas syringae* and later supplemented with JA in *Vigna unguiculata* than control plants. Vranova *et al*. [18] and Neill *et al*. [19] reported that the compounds that are able

to reduce the damaging effects of certain stresses may be of great importance from both theoretical and practical points of view. ROS are dangerous cytotoxic molecules, but also act as intermediate signaling molecules to regulate the expression of genes associated with antioxidant defense mechanisms. 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 [20]. In our findings polyphenol oxidase activity was found to be increased in plant supplemented with JA alone and also the same result was obtained in *Vigna* plants pretreated with *P.syringae* and which was later supplemented with JA over the corresponding control plants. Queiroz *et al*. [21]; Tran and Constabel [22] stated that the PPO is a copper-binding oxidative enzyme, it catalyzes the conversion of monophenols and *o*-diphenols to *o*-quinones in the presence of molecular oxygen, causing browning and cross-linking in wounded tissues. The current study shows that the Superoxide dismutase activity was maximum in plants treated with JA alone and in plants pretreated with *P.syringae* and later supplemented with JA over the control plants. Similarly, Jung [23] reported that in *Arabidopsis thaliana*, the total SOD activity decreased three days after Jasmonate treatment and increased two fold after seven days of treatment.

ACKNOWLEDGEMENT
The authors thank the Management and Principal for providing all facilities to carry out this work.

REFERENCES

1. Slaymaker, D.H., Navarre, D.A., Clark, D., Del-Pozo, O., Martin, G.B. and Klessig, D.F. 2002. The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proc.Natl. Acad.Sci.,* U.S.A. 99: 11640–11645.
2. Anderson, M.D., Chen, Z. and Klessig, D.F. 1998. Possible involvement of lipid peroxidation in salicylic acid-mediated induction of PR1 gene expression. *Phytochem.* 47: 555–566.
3. Shah, J. 2005. Lipids, lipases and lipid modifying enzymes in plant disease resistance. *Annu.Rev.Phytopathol.,* 43: 229–260.
4. Rosahl, S. and Feussner, I. 2005.Oxylipins.*In*: Murphy, D.J. (ed.). *Plant Lipids: Biology, Utilization and Manipulation*. Blackwell Publishing Ltd. CRC Press, Oxford and Boca Raton. Pages: 329-354.
5. Green, T.R. and Ryan, C.A. 1972. Wound inducible proteinase inhibitors in plant leaves: A possible defense mechanism against insects. *Science.,* 175: 776–777.
6. Sanchez-Serrano, J., Schmidt, R., Schell, J. and Willmitzer, L. 1986. Nucleotide sequence of proteinase inhibitor II encoding cDNA of potato (*Solanum tuberosum*) and its mode of expression. *Mol.Gen.Genet.,* 203: 15-20.
7. Belhadi, A., Saigne, C., Telef, N. and Cluzet, S. 2006. Methyl jasmonate induces defense responses in grapevine and triggers protection against Erysiphe necator. *J.Agric.Food.Chem*., 54: 9119-9125.
8. Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissues. *Biochem.Biophys. Res. Commun.,* 43: 1274-1279.
9. Kar, M. and Mishra, D. 1976. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol.,* 57: 315-319.

10. 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.
11. Mukherjee, P.K., Gosh, J.J., Sci, K. and Cult, J. 1975.Phenol oxidase activity in reaction to resistance of rice to infection by *Helminthosporium oryzae*.*Sci. culture.,* 41: 433-435.
12. Bowler, C., Van Montagu, M. and Inze, D. 1992.Superoxide dismutase and stress tolerance. *Annu.Rev.PlantPhysiol.Plant Mol.Biol.,* 43: 83-116.
13.Sayyari, M., Ghavami, M., Ghanbari, F. and Kordi, S. 2013.Assessment of salicylic acid impacts on growth rate and some physiological parameters of lettuce plants under drought stress conditions. *Intl.J. Agri. Crop Sci.,* 5(17): 1951-1957.
14. Padma Singh, S. 2000. Permeability changes andmineral metabolism in the leaves of onion infected with *Alternaria porri*. *Ad.Plant Sci*., 13: 323-325.
15. Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann, H.J.R., Van Montagu, M., Inze, D. and Van Camp, W. 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. *Proc.Natl.Acad.Sci.,USA.,* 95: 5818–5823.
16. 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.
17. Hakmaoui, A., Perez-Bueno, M.L., Garcia-Fontana, B., Camejo, D., Jimenez, A., Sevilla, F. and Baron, M. (2012). Analysis of the antioxidant response of *Nicotiana benthamiana* to infection with twostrains of Pepper mild mottle virus. *J. Exp. Bot.,* 63: 5487–5496.
18. Vranova, E., Inze, D. and Van Breusegen, F. 2002. Signal transduction during oxidative stress. *J.Exp.Bot.,* 53:1227 1236.
19. Neill, S.J., Desikan, R., Clarke, A., Hurst, R.D. and Hencock, J.T. 2002. Hydrogen Peroxide and nitric oxide as signaling molecules in plants. *J.Exp.Bot.,* 53:1237–1247.
20. Jiang, M.Y. and Zhang, J.H. 2002. Water stress-induced abscisic accumulation triggers the increased generation of reactive oxygen species and upregulates the activities of antioxidant enzymes in maize leaves. *J.Exp.Bot.,* 379: 2401–2410.
21. Queiroz, C., Lopes, M.L., Fialho, E. and Valente Mesquita, V.L. 2008. Polyphenol oxidase: Characteristics and mechanisms of browning control. *Food Rev. Int.,* 24(4): 361-375.
22. Tran, L.T. and Constabel, C.P. 2011. The polyphenol oxidase gene family in poplar: phylogeny, differential expression and identification of a novel, vacuolar isoform. *Planta.,* 234(4): 799-813
23. Jung, S. 2004. Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. *Plant Physiol.Biochem.,* 42: 225–231.