Research Paper :

Induced Systemic Resistance by Plant Products and Nutrients in Green Gram Challenged with Powdery Mildew Pathogen (*Erysiphe polygoni* DC.)

P. MAHALAKSHMI AND D. ALICE

International Journal of Plant Protection, Vol. 2 No. 2 : 157-160 (October, 2009 to March, 2010)

See end of the article for authors' affiliations

Correspondence to :
P. MAHALAKSHMI
Department of Plant Pathology, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

SUMMARY

Induced systemic resistance in green gram by foliar spray of plant products and mineral nutrients were studied for the management of powdery mildew pathogen. Foliar application of plant extracts *viz.*, *Allium sativum*, *Prosopis julifora*, *Trianthemum portulacastrum* and Zinc sulphate (0.1%) induced the enzyme activity *viz.*, peroxidase (P0), polyphenol oxidase (PPO) and phenyl alanine ammonia lyase (PAL) in green gram leaves challenged with the powdery mildew pathogen.

Key words :
Green gram, Powdery mildew, Botanicals, Induced systemic, *Erysiphe graminis*

Accepted :
June, 2009

Plant pathogens play an important role and pose challenges on the increased production of pulses, among which the fungi form the most important group of pathogens affecting pulse crops. In green gram, considerable losses in the production occur as a result of powdery mildew (*Eryisphe polygoni*.DC) in all areas having rice based cropping systems of the country (Abbaiah, 1993). Disease reduction by mineral nutrients is most often attributed to improved nutrition that boosts host defense or directly inhibits fungal growth and its activity. In few cases, nutrition indirectly stimulates the indigenous population of microorganisms that are beneficial to plant growth and act as antagonists to pathogen.Induction of plant defense genes by prior application of inducing agents is called induced systemic resistance (ISR) (Hammerschmidt and Kuc, 1982)and is thought to be a novel plant protection strategy.

MATERIALS AND METHODS

Physiology and biochemical aspects of induction of resistance by nutrients

For assaying induced resistance by botanicals and mineral nutrients, the plants were sprayed with appropriate concentration of plant extracts and mineral nutrients on 30 days old crop. After 48 hours the treated plants were challenge inoculated with the conidial suspension of *E. polygoni* (10^6/ml) Then the plant samples were collected on 0,1,3,5 and 7 days after inoculation.

Enzyme extraction:

Using pre chilled pestle and mortar, 1 g of leaf sample was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant was used as enzyme source for the assay of peroxidase activity (PO).

Assay of peroxidase:

Peroxidase activity was assayed spectrophotometrically. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of one per cent H_2O_2 which was incubated at room temperature. At start of enzyme reaction, the absorbance of the mixture was set to zero at 420 nm in a spectrophotometer and the change in absorbency was recorded at 30 seconds intervals for 3 minutes. Boiled enzyme extract served as control. PO activity was expressed as change in the absorbance of the reaction mixture per min per g on fresh weight basis (Hammerschmidt and Kuc, 1982).

Assay of polyphenol oxidase:

The enzyme source for the assay of PPO was prepared as that of PO. The polyphenol oxidase was determined as per the procedure given by Mayer *et al*. (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium

phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm per minute per g on fresh weight basis (Table 2).

Estimation of phenol:

Total phenol (mg per g of leaf) was estimated at 0, 3, 6, 9 days after pathogen inoculation. An uninoculated control was maintained as check.

Preliminary extract of green gram leaf in alcohol:

The leaf samples were chopped into small bits of one cm length and plunged into 80 per cent ethanol kept in water bath for 10 minutes and cooled in running tap water. The tissues were crushed thoroughly in a pestle and mortar and the extract was centrifuged at 5000 rpm for 10 min. The supernatant was collected and extract was diluted with 25 ml of distilled water and was used for estimation.

Total phenols:

From the ethanol extract, 0.1 ml was taken in test tubes and evaporated in a boiling water bath. To this, 6 ml of water was added and shaken well. Then 0.5 ml of Folin ciocalteau reagent was added to each test tube and after 8 minutes 2 ml of 20 per cent sodium carbonate was added. After 30 minutes blue colour developed which was measured at 660 nm wavelength in a spectrophotometer (Malik and Singh, 1980). From the standard graph, the amount of phenol present was calculated and expressed as mg per g of leaf (Table 3).

Phenylalanine ammonia lyase (PAL):

Leaf sample (1gm) was homogenised in 5 ml of 0.1M sodium borate buffer (pH 8.8) using chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used for the assay of PAL activity (Dickerson *et al.*, 1984).The reaction mixture contained 3.1 ml of 0.1m sodium borate buffer (pH 8.8), 0.2 ml of enzyme extract and 0.1 ml of 12 mM L – phenyl alanine prepared in the same buffer. The reaction was stopped by adding 0.2ml of 3 N HCl. The absorbance was read at 290 nm. PAL activity was expressed in micro moles of cinnamic acid produced per min per g on fresh weight basis (Table 4).

RESULTS AND DISCUSSION

Foliar application of zinc sulphate (1%) significantly induced peroxidase activity of 14.13 unit/ min/g of fresh weight, polyphenol activity of 3.66 unit/min/g of fresh weight and phenylalanine ammonia lyase of 0.35 n/mol/ transcinnamicacid/mg of leaf tissue as against the control (Table 1). This was followed by urea 1% and bulb extracts of *Allium sativum*. The phenol content of the plant treated with a foliar application of zinc sulphate (0.1%) was individually significantly superior and recorded 3.48 mg/g of fresh weight on the fifth day after inoculation against the control, which has recorded 0.22 mg/g of fresh weight. The next best treatments was foliar spray of urea (1%) followed by bulb extracts of *Allium sativum* (Table 3). The following chemical compounds *viz.*, K_2HPO_4, K_3PO_4, Na_3PO_4 and Na_2PO_4 sprayed on the under surface of leaves of cucumber also induced systemic resistance against *C. langenarium* (Helen *et al.*, 1990). Application of silicon significantly stimulated the activity of peroxidase and polyphenol oxidase after infection with *Pythium* spp. infecting cucumber (Cherif *et al.*, 1994).

Peroxidases (PO), poly phenol oxidase (PPO) are reported to be capable of oxidizing phenols to quinones and they are associated with disease resistance in plants. Increased phenyl alanine ammonia lyase (PAL) activity levels leads to the *denova* synthesis of phenolics (Glazzener, 1982). PAL is an enzyme of phenyl propanoid

Table 1 : Changes in peroxidase activity in *E. polygoni* challenged green gram treated plants under pot culture condition

Treatments	*Peroxidase activity (changes in absorbance / min / g fresh weight at 420 nm)				
	Days after inoculation				
	0	1	3	5	7
Urea (1%)	6.21^{ab}	8.71^{b}	10.41^{bc}	12.88^{b}	9.61^{b}
Zinc sulphate (1%)	6.30^{a}	9.70^{a}	12.71^{a}	14.13^{a}	11.08^{a}
A. sativum (10%)	6.05^{ab}	7.80^{c}	9.81^{b}	11.02^{c}	8.78^{c}
P. juliflora (10%)	5.94^{b}	7.40^{d}	9.87^{c}	10.57^{d}	8.07^{d}
T. portulacastrum (10%)	5.45^{c}	6.58^{e}	7.81^{d}	9.04^{e}	7.64^{e}
Control (healthy)	3.90^{e}	4.02^{f}	4.12^{e}	4.34^{g}	4.30^{g}
Control (inoculated)	4.50^{d}	4.58^{f}	4.61^{e}	4.63^{f}	4.62^{f}

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different at 5% level by DMRT

Table 2 : Changes in polyphenol oxidase activity in *E. polygoni* challenged green gram treated plants under pot culture condition

Treatments	*Polyphenol oxidase activity (change in absorbance/min/g fresh weight at 470 nm)				
	Days after inoculation				
	0	1	3	5	7
Urea (1%)	1.00[a]	1.40[a]	2.09[b]	3.32[a]	2.52[a]
Zinc sulphate (1%)	1.02[a]	1.40[a]	2.10[b]	3.66[a]	2.67[a]
A. sativum (10%)	0.92[b]	1.13[bc]	1.97[c]	2.79[ab]	2.18[b]
P. juliflora (10%)	0.89[b]	1.20[b]	2.01[c]	2.68[b]	2.14[b]
T. portulacastrum (10%)	0.64[c]	0.98[c]	0.80[d]	1.01[c]	1.77[c]
Control (healthy)	0.13[d]	0.15[d]	0.18[d]	0.19[d]	0.14[e]
Control (inoculated)	0.30[d]	0.34[d]	0.36[d]	0.38[d]	0.37[d]

* Mean of three replications
In a column, means followed by a common letter (s) are not significantly different at 5% level by DMRT

Table 3 : Changes in phenol content in *E. polygoni* challenged green gram treated plants under pot culture condition

Treatments	*Phenol content (mg/g of fresh weight of leaf tissue)				
	Days after inoculation				
	0	1	3	5	7
Urea (1%)	2.32[a]	2.11[c]	2.22[c]	2.83[b]	2.66[b]
Zinc sulphate (1%)	2.48[a]	2.54[a]	3.27[a]	3.48[a]	3.26[a]
A. sativum (10%)	2.02[b]	2.32[b]	2.44[b]	2.53[c]	2.28[c]
P. juliflora (10%)	1.80[c]	1.98[d]	2.13[c]	2.45[c]	2.16[c]
T. portulacastrum (10%)	1.72[c]	1.81[d]	1.97[c]	2.07[d]	1.77[d]
Control (healthy)	0.71[d]	0.70[e]	0.43[e]	0.22[f]	0.19[f]
Control (inoculated)	1.02[e]	1.05[f]	1.16[d]	1.28[e]	1.16[e]

* Mean of three replications
In a column, means followed by a common letter (s) are not significantly different at 5% level by DMRT

Table 4 : Changes in phenylalanine amonia lyase activity in *E. polygoni* challenged green gram treated plants under pot culture condition

Treatments	*Phenyl alanine ammonia lyase activity ($nmol^{-1}$ transcinnamic acid /mg of leaf tissue)				
	Days after inoculation				
	0	1	3	5	7
Urea (1%)	0.34[ab]	0.35[b]	0.37[a]	0.35[a]	0.32[a]
Zinc sulphate (1%)	0.36[a]	0.37[a]	0.38[a]	0.35[a]	0.33[a]
A. sativum (10%)	0.34[ab]	0.35[b]	0.36[a]	0.35[a]	0.32[a]
P. juliflora (10%)	0.33[b]	0.35[b]	0.36[a]	0.35[a]	0.31[a]
T. portulacastrum (10%)	0.27[c]	0.31[c]	0.32[b]	0.28[b]	0.27[b]
Control (healthy)	0.12[e]	0.12[e]	0.13[c]	0.13[d]	0.13[d]
Control (inoculated)	0.19[d]	0.19[d]	0.21[c]	0.20[c]	0.17[c]

* Mean of three replications
In a column, means followed by a common letter (s) are not significantly different at 5% level by DMRT

pathway and may have a definite role in the defense mechanism of plants (Friend and Threshfall, 1976). PAL activity is also believed to be associated with the production of specific phenolic compounds including some antifungal isoflavonoids, phytolaxine in legumes (Friend, 1981). The phenolic compounds, apart from enhancing the mechanical strength of host cell wall, may also inhibit the fungal growth. Hence they are also fungitoxic in nature.

Earlier investigations revealed the increased activity of PO, PPO and PAL due to the application of neem seed kernel extract (NSKE) on *Colletotricum capsici* infected chilli plants (Renukadevi, 1995), *Sarocladuim oryzae* infected rice plants (Boopathikannan, 1995), and

the leaf extracts from *P. juliflora*, *Ziziphus jujuba* and *A. sativum* on *Pyricularia oryza*e infected rice plants (Kamalakannan, 1994).Thus, the results clearly revealed that foliar spray of *A. sativum* (10%) and zinc sulphate (1%) caused bio chemical changes like increasing the activity of enzymes, accumulation of phenols and increasing protein content which led to resistant reaction against the powdery mildew pathogen in green gram apart from their direct action by creating an unfavorable situation for the conidia to establish and grow. The studies have also shown the possibility of management of powdery mildew disease of green gram by using plant extracts and mineral nutrients which are non hazardous to environment eco friendly and economical.

Authors' affiliations:
D. ALICE, Department of Plant Pathology, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

REFERENCES

Abbaiah, K. (1993). Development of powdery mildew epidemics in urd bean in relation to weather factors. *Indian J. Pulses Res.,* **6**: 186-188.

Boopathikannan, R. (1995). Studies on sheath blight of rice – III. Management through non-chemical methods. M.Sc. (Ag.). Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 149 pp.

Cherif, M., Asselin, A. and Belanger, R.R. (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Phythium* spp. *Phytopathol.,* B **4:** 236-242.

Dickerson, D.P., Pascholati, S.F. Hagerman, A.E., Butler, L.G. and Nicholson, R.L. (1984). Phenylalanine ammonia lyase and hydroxy cinnamate COA ligase in maize mesocotyls inoculated with *Helminthosporium maydis***.** *Physiol. & Mol. Pl. Pathol.,* **25:** 111-123.

Friend, J. (1981). Plant phenols lignification and plant disease progress**.** *Phytochem.,* **7:** 197-267.

Friend, J. and Threshfall, D. (1976). *Biochemical Aspects of Plant Parasite Relationship*. Academic Press, London, 354pp.

Glazzener, J.A. (1982). Accumulation of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopathology,* **72**: 105-150.

Hammerschmidt, R. and Kuc, J.A. (1982). Lignification as a mechanism for induced systemic resistance in cucumber. *Physiol. & Mol. Pl. Pathol.,* **20**: 61-71.

Helen, R., Joseph. I. and Kuc, A. (1990). Total and systemic induction of peroxidase chitinase and resistance in cucumber plants by KH_2PO_4. *Physiol. & Mol. Pl. Pathol.,* **37**: 355-366.

Kamalakannan, A. (1994). Management of rice blast disease by using chemicals and plant extracts. M.Sc. (Ag.) Thesis Tamil Nadu Agricultural University, Coimbatore, India.

Malik, C.P. and Singh, M.B. (1980). In: *Plant Enzymology and Histo enzymology***.** A text Manual Kalyani Pub., New Delhi, 286 pp.

Renukadevi, P. (1995). Management of chilli die-back and fruit rot disease with plant extracts and fungicides. M.Sc.(Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 118 pp.

****