Research Paper :

Effect of foliar application of *Pseudomonas fluorescens* on the activity of lytic enzymes in response to *Cercosporidium personatum* in groundnut

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SUMMARY

Late leaf spot incited by *Cercosporidium personatum* is the serious disease in groundnut. *Pseudomonas fluorescens* Pf1 formulation induced chitinase activity in groundnut leaves in the present study. When lower most leaves of groundnut plants were treated with Pf1 formulation, an increase in chitinase activity was seen in the upper most leaves. Pf1 application on lower leaves resulted in an increase in β-1,3-glucanase activity on upper leaves. Further, steep increase in β -1,3-glucanase activity was observed when the Pf1 treated plants were inoculated with pathogen. The induction of both chitinase and β-1,3-glucanase activity was observed due to Pf1 treatment.

Key words : *Pseudomonas fluorescens*, Lytic enzymes, Groundnut, *Cercosporidium personatum*

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In groundnut, late leaf spot caused by *Cercosporidium personatum* is the destructive foliar disease. Numerous reports on the control of foliar diseases with *P. fluorescens* applied to foliage are available (Mew and Rosales, 1986). Systemic acquired resistance (SAR) is a vital plant defense system that confers broad spectrum resistance accompanied by coordinate expression of genes. Fluorescent pseudomonads are known to induce disease resistance against foliar diseases (Liu *et al.*, 1995). Besides the capacity to colonize roots intensively for an extended period of time, other mechanisms are involved that makes the fluorescent pseudomonads as an effective biocontrol agent. Chitinases and β-1,3-glucanases (laminarinase) are two important enzymes which degrade the fungus cell wall components, chitin and β-1,3-glucan, respectively (Henis and Chet, 1975). Bacterial strains differ in their ability to induce resistance in different plant species and plants show variation in the expression of ISR upon induction by specific bacterial strains.

MATERIALS AND METHODS

The lower most leaves of 45 day old groundnut plants (variety, TMV7) were sprayed with Pf1 formulation (1 kg ha^{-1}). The upper most leaves of each plant were inoculated with *C. personatum* at 2 or 5 or 30 days after application of *P. fluorescens*. Plants mock-inoculated with talc formulation without *P. fluorescens* on the lower leaves followed by mock inoculation with sterile water without *C. personatum* on the upper leaves were kept as control. At various times after inoculation of pathogen, leaf samples were collected and analysed for the changes in the activity of enzymes *viz*., chitinase and β-1,3-glucanase.

The colorimetric assay of chitinase was carried out according to the procedure developed by Boller and Mauch (1988). The assay mixture consisted of 10 µl of 1 M sodium acetate buffer (pH 4.0), 0.4 ml of enzyme solution and 0.1 ml of colloidal chitin (1 mg). N-acetyl glucosamine (GlcNAc) was used as standard and absorbance value at 585 nm was measured using a Hitachi 200-20 Spectrophotometer. The enzyme activity was expressed as nmol GlcNAc min^{-1} mg^{-1} fresh tissue. Total β-1,3-glucanase activity was colorimetrically assayed by the laminarin-dinitrosalicyclate method (Pan *et al.,* 1991). The crude enzyme extract of 62.5 µl was added to 62.5 µl of laminarin (4%) and then incubated at 40^0C for 10 minutes. The blank was the crude enzyme preparation mixed with laminarin with zero time incubation. The enzyme activity was expressed as nmol min^{-1} mg^{-1} fresh tissue.

RESULTS AND DISCUSSION

Plants respond to pathogen infection by producing a number of proteins believed to be important in protecting them from the deleterious effects of the pathogen (Lamb *et*