*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 $40^0$C 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*

*al.,* 1989). These include enzymes capable of hydrolyzing structural components of the pathogen. In the present study Pf1 formulation induced chitinase activity in groundnut leaves. When lower most leaves of groundnut plants were treated with Pf1 formulation, an increase in chitinase activity was observed in the upper most leaves (Table 1). Further, steep increase in chitinase activity in upper leaves was seen at five days after inoculation with pathogen. The increase in chitinase activity was similar in the mock inoculation with *P. fluorescens* or mock inoculation with *C. personatum*. When the upper leaves were inoculated with *C. personatum* at 30 days after

**Table 1 : Effect of foliar application of lower leaves with *P. fluorescens* Pf1 formulation on chitinase activity of upper leaves inoculated with *C. personatum***

| Time of inoculation of *C. personatum* on upper leaves after spraying *P. fluorescens* on lower leaves (days) | Spraying with *P. fluorescens* on lower leaves | Inoculation with *C. personatum* on upper leaves | Chitinase activity (nmol GlcNAc $min^{-1}$ $mg^{-1}$ fresh weight) Days after inoculation with *C. personatum* | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 3 | 5 | 7 |
| 2 | + | + | $4.8^{a}$ | $12.8^{a}$ | $15.2^{a}$ | $16.1^{a}$ | $13.5^{a}$ |
| | + | - | $2.6^{b}$ | $6.4^{b}$ | $9.2^{b}$ | $11.6^{ab}$ | $8.5^{ab}$ |
| | - | + | $3.4^{ab}$ | $7.4^{b}$ | $9.6^{b}$ | $8.2^{b}$ | $6.4^{b}$ |
| | - | - | $1.9^{c}$ | $2.2^{c}$ | $2.6^{d}$ | $2.2^{c}$ | $1.8^{c}$ |
| 5 | + | + | $4.4^{a}$ | $10.2^{a}$ | $12.4^{a}$ | $15.1^{a}$ | $11.6^{a}$ |
| | + | - | $2.3^{b}$ | $5.1^{b}$ | $7.8^{c}$ | $10.3^{b}$ | $6.4^{b}$ |
| | - | + | $3.1^{b}$ | $7.2^{b}$ | $8.4^{b}$ | $7.9^{b}$ | $6.2^{b}$ |
| | - | - | $2.1^{bc}$ | $2.6^{c}$ | $2.4^{d}$ | $2.4^{c}$ | $2.0^{c}$ |
| 30 | + | + | $4.2^{a}$ | $8.6^{ab}$ | $12.1^{ab}$ | $13.8^{a}$ | $9.5^{a}$ |
| | + | - | $2.1^{bc}$ | $5.0^{bc}$ | $6.9^{c}$ | $8.4^{b}$ | $6.2^{b}$ |
| | - | + | $2.8^{b}$ | $5.4^{b}$ | $8.1^{bc}$ | $7.6^{bc}$ | $6.1^{bc}$ |
| | - | - | $2.1^{bc}$ | $2.8^{c}$ | $2.4^{d}$ | $2.1^{c}$ | $1.9^{c}$ |

+ = Treatment with *P. fluorescens* (or) *C. personatum*
- = Mock inoculation
For mock inoculation with *P. fluorescens*, lower leaves were sprayed with talc powder formulation (1 kg $ha^{-1}$) without Pf1.
For mock inoculation with *C. personatum*, upper leaves were sprayed with sterile water.
Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT.

**Table 2 : Effect of foliar application of lower leaves with *P. fluorescens* Pf1 formulation on β-1,3-glucanase activity of upper leaves inoculated with *C. personatum***

| Time of inoculation of *C. personatum* on upper leaves after spraying *P. fluorescens* on lower leaves (days) | Spraying with *P. fluorescens* on lower leaves | Inoculation with *C. personatum* on upper leaves | Chitinase activity (nmol GlcNAc $min^{-1}$ $mg^{-1}$ fresh weight) Days after inoculation with *C. personatum* | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 3 | 5 | 7 |
| 2 | + | + | $28^{a}$ | $52^{a}$ | $58^{a}$ | $41^{a}$ | $32^{a}$ |
| | + | - | $26^{a}$ | $38^{ab}$ | $45^{a}$ | $32^{a}$ | $28^{a}$ |
| | - | + | $21^{b}$ | $24^{bc}$ | $31^{b}$ | $28^{b}$ | $24^{ab}$ |
| | - | - | $14^{c}$ | $16^{c}$ | $18^{c}$ | $15^{c}$ | $15^{c}$ |
| 5 | + | + | $27^{a}$ | $46^{a}$ | $54^{a}$ | $41^{a}$ | $30^{a}$ |
| | + | - | $24^{ab}$ | $34^{b}$ | $39^{b}$ | $28^{b}$ | $22^{b}$ |
| | - | + | $20^{b}$ | $22^{c}$ | $28^{bc}$ | $26^{b}$ | $21^{b}$ |
| | - | - | $16^{c}$ | $18^{c}$ | $20^{c}$ | $17^{c}$ | $16^{bc}$ |
| 30 | + | + | $24^{ab}$ | $34^{b}$ | $42^{ab}$ | $31^{ab}$ | $24^{ab}$ |
| | + | - | $24^{ab}$ | $28^{b}$ | $22^{c}$ | $22^{bc}$ | $18^{b}$ |
| | - | + | $18^{bc}$ | $19^{c}$ | $23^{c}$ | $22^{bc}$ | $19^{b}$ |
| | - | - | $15^{c}$ | $18^{c}$ | $19^{c}$ | $17^{c}$ | $14^{c}$ |

+ = Treatment with *P. fluorescens* (or) *C. personatum*
- = Mock inoculation
For mock inoculation with *P. fluorescens*, lower leaves were sprayed with talc powder formulation (1 kg $ha^{-1}$) without Pf1.
For mock inoculation with *C. personatum*, upper leaves were sprayed with sterile water.
Data followed by the same letter in a column are not significantly different (p=0.05) by DMRT.

treatment with Pf1 formulation, no appreciable increase in chitinase activity was observed when compared to inoculation of pathogen at upper leaves at two and five days after treatment with Pf1 formulation (Table 1). *P. stutzeri* VPL-1 has been found to reduce the disease caused by *Fusarium solani* mainly by laminarinase and chitinase activities (Lim *et al*., 1991).

Pf1 application on lower leaves resulted in an increase in β-1,3-glucanase activity on upper leaves. When these upper leaves were challenge inoculated with pathogen, a sharp increase in β-1,3-glucanase activity was observed. When groundnut leaves treated with Pf1 formulation were challenge inoculated with pathogen at 30 days after treatment, no significant difference in enzyme activity was observed compared to untreated groundnut leaves. The increase in activity was similar when upper leaves were challenge inoculated with pathogen at two and five days after treatment with Pf1 formulation (Table 2). Fridlender *et al.* (1993) documented the involvement of β-1,3-glucanase produced by *P. cepacia* in decreasing the incidence of disease caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium ultimum*.

The results indicated the possibility of involvement of β-1,3-glucanase in the defense mechanism against late leaf spot of groundnut. A direct role for β-1,3-glucanases in defense against pathogens has been proposed because the substrate of these enzymes is a major component of the cell walls of many fungi (Lim *et al*., 1991). The induction of both chitinase and glucanase was observed due to Pf1 treatment in the present study. The synergistic effect of these two defense related enzymes might have a possible role in defense mechanism.

From the above study, it could be concluded that Pf1 formulation was able to induce systemic resistance against this foliar disease. Studies conducted so far have thus reinforced the prospects of using this biocontrol agent on a commercial scale as a successful alternative for chemical control of foliar disease and have further implicated the role of *P. fluorescens* in the defense mechanism against the pathogen.

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