Role of chitinase and β-1,3 glucanase elicitation in the Trichoderma harzianum induced systemic resistance in Capsicum

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Members of the chitinase and β-1,3 glucanase gene families are found in all plants which express them inducibly as PR proteins and constitutively in tissues vulnerable to pathogen attack (9,10). Several lines of evidence indicate that chitinases play a distinct role in plant defense by attacking chitin, a β-1-4-linked polymer of N-acetyl-D-glucasamine and a major component of fungal cell wall, and β-1,3 glucanases by hydrolysing β-1,3 glucans- which form the matrix in which chitin is embedded. Synergistic antifungal relationship exists between chitinase and β-1-3 glucanase (2,8). The role of these enzymes in plant defense against pathogens is thus multi-faceted. The systemic induction of two PR proteins, chitinase and β -1-3 glucanase by Trichoderma harzianum is reported in this paper.

Culture of Trichoderma harzianum Jh2, maintained on 0.1% malt extract agar (MEA) slants and stored at 4°C were used. For pot studies, T. harzianum was multiplied on 2% MEA for 10 days at 28°C.

Seeds of four chilli cultivars with known differential reaction to Rhizoctonia solani causing root-rot were used. Of these, Shivani and S-77, were resistant, while G-4 and NP- 46A were susceptible. The seeds procured from the local market were surface sterilized with 0.1% sodium hypochlorite solution. Two experiments were conducted in pots, the first one for evaluation of biocontrol potential of T. harzianum against R. solani, and the other for assay of enzyme activity.

To evaluate the biocontrol potential of T. harzianum Jh2 against Rhizoctonia solani in chilli varieties, seeds were sown in autoclave sterilized soil-FYM mixture (3:1) in earthen pots (20 cm diam). Cultures of T. harzianum multiplied on maize cob- wood saw dust was placed on top layer of each pot at 50 g / pot, and gently mixed in soil. Pots without T. harzianum were maintained as control. Five replications for each cultivar with and without T. harzianum were maintained, and arranged in the completely randomized design. The pots were lightly irrigated on alternate days to provide adequate moisture. 10-days -old seedlings (with and without T. harzianum) were challenge inoculated with a sclerotial suspension (1X10^3 sclerotia ml^-1) of R. solani at 50 ml / pot. Observation for root-rot was recorded 15 days after the challenge inoculation, and the plants were then uprooted. Soil adhering to roots was gently collected with the help of a brush for estimating the population densities (c.f.u. /g soil) of T. harzianum and R. solani, using specific techniques and media (1).

To assay the enzyme activity, after ten days sowing of seeds as described earlier the soil was drenched with a spore -suspensions of T. harzianum ( 1 x 10^6 conidia ml^-1, 50 ml in each pot). The pots were irrigated on 0, 2, 4, 6, 8, and 10 days after drenching, and samples of roots and leaves were collected for analysis. Roots and leaves were washed in running tap water followed by glass distilled water and surface blotted with blotting paper.

The enzymes were extracted with citrate buffer (pH 5.0; 0.2M) containing 0.25 mM EDTA, and 15mM β-mercaptoethanol (E. Merck, Germany) using 2 ml buffer for each g of leaf or root tissue.
Since the root extracts contained less β-1, 3-glucanase activity, these were concentrated by ammonium sulphate precipitation. The cold (0-5 °C) extract was 80% saturated with ammonium sulphate (E. Merck, India) followed by centrifugation. The precipitate was dissolved in citrate buffer and dialyzed against the same buffer overnight with at least two changes of buffer. The reaction mixture containing 1 mg chitin azure or laminarin azure (Sigma chemical Co. USA) and 250 µl enzyme extract was shaken on an orbital shaker for one hour at 25°C and centrifuged at 4°C in a microfuge. The O.D. was measured at 570 nm with a Cary 50 UV-VIS spectrophotometer. The O.D. of blanks with substrate alone and enzyme alone incubated for same time were deducted from the O.D. of test samples. Enzyme activity of the samples drawn at 0 hr was considered as control.

Significant suppression of root-rot and \textit{R. solani} population density was observed in pots treated with \textit{T. harzianum}, compared to those in the untreated ones. Least root-rot was observed in Shivani, and maximum in NP 46A. Higher population densities of \textit{T. harzianum} and lower of \textit{R. solani} was observed in the resistant cultivars compared to that in the susceptible ones (Table 1).

Chitinase activity in the leaves was also increased following \textit{T. harzianum} inoculation (Fig. 2). The activity in the leaves of tolerant varieties (Shivani and S–77) on 10th day was nearly 2.5 times more than that of the susceptible cultivars (G-4 and NP 46-A).

β-1, 3-glucanase activity in roots (Fig. 3) and leaves (Fig. 4) also increased following drenching with \textit{T. harzianum} spore- suspension. The enzyme activity on tenth day was nearly 2 to 5 times higher in the roots, and 2.5 to 4.5 times in the leaves of tolerant cultivars compared to that in the susceptible ones.

Chitinases and β-1,3-glucanases besides being antifungal themselves on account of their hydrolytic action on the fungal wall, are also known to release fungal cell wall fragments which elicit other defense responses (9). The induction of these two enzymes in chilli plants in response to \textit{T. harzianum} may therefore trigger multi pronged defense responses contributing to both local and systemic resistance to the pathogen. This may considerably augment the defense capability of the BCA in addition to its other modes of action like release of alkyl- pyrone antibiotics, hyperparasitism, and competition (5-7).

De Meyer \textit{et al.} (4) reported SAR like responses from strain T-39 of \textit{T. harzianum}. We have earlier shown that \textit{T.harzianum} induced a reiterant micro oxidative burst and micro hypersensitive response concomitant with the development of systemic resistance to \textit{Colletotrichum capsici} and \textit{R. solani} in chilli plants (3). Systemic acquired resistance may therefore be an important component of \textit{T. harzianum} induced resistance.

### Table 1.
Root rot* and population densities (x 10³ c.f.u. /g soil) of \textit{Trichoderma harzianum} and \textit{Rhizoctonia solani} in rhizosphere of four chilli cultivars

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chilli cultivars</th>
<th>Root Rot (%)</th>
<th>Population density</th>
<th>Untreated T. harz. (%)</th>
<th>R. solani (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Shivani</td>
<td>9.8</td>
<td>146.4</td>
<td>15.8</td>
<td>23.4</td>
</tr>
<tr>
<td>2.</td>
<td>S-77</td>
<td>12.3</td>
<td>190.0</td>
<td>16.2</td>
<td>25.2</td>
</tr>
<tr>
<td>3.</td>
<td>G4</td>
<td>33.5</td>
<td>138.3</td>
<td>22.0</td>
<td>35.5</td>
</tr>
<tr>
<td>4.</td>
<td>NP 46 A</td>
<td>66.7</td>
<td>99.5</td>
<td>25.0</td>
<td>56.8</td>
</tr>
</tbody>
</table>

CD (5%) 8.3 26.3  6.2 12.8 15.3

* mean values of five replications
Fig. 1. *Trichoderma harzianum* induced chitinase activity in chilli roots following root drenching

Fig. 2. *Trichoderma harzianum* induced chitinase activity in chilli leaves following root drenching
Fig. 3. *Trichoderma harzianum* induced β-1,3 Glucanase activity in chilli roots following root drenching.

Fig. 4. *Trichoderma harzianum* induced β-1,3 Glucanase activity in chilli leaves following root drenching.
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REFERENCES


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