Effect of Heat Shock on Red Light-Induced Resistance of Broad Bean against *Botrytis cinerea*

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**Abstract**  Heat shock treatment at 55 °C for 5 to 10 sec before inoculation induced the disease susceptibility of broad bean to *B. cinerea*. Such heat-induced susceptibility was manifested by lesion development and hyphal growth of pathogenicity-lost strains of *B. cinerea* in heat-shocked broad bean leaflets. On the other hand, when virulent strain of *B. cinerea* was inoculated to the broad bean, appressorium formation and infection hyphal growth were also promoted in the dark. Under red light, however, infection behaviors were inhibited even in heat treated broad bean. Higher antifungal activity was observed in inoculation droplet recovered from heat-shocked broad bean leaflet kept under red light compared with that in the dark. These studies suggested that red light suppressed the heat-induced susceptibility in broad bean leaves inoculated with *B. cinerea* by accumulation of antifungal substance(s) in inoculation droplet.

Key words: *B. cinerea*, broad bean, red light, heat-shock, antifungal substance(s)

**Introduction**

It was reported that plants have some disease resistance mechanisms for their survival in nature against the wide variety of plant pathogenic agents. Understanding the mechanism of host-plant resistance to pathogens has attracted much attention, especially for the control of plant disease using new biotechnology to improve host resistance (Gasser and Fraley, 1989; Staskawicz et al., 1995). A new aspect for plant disease management, red light induced resistance has been reported in some plants (Islam et al., 1998, 2002; Rahman et al., 2003; Umezu et al., 1999). However, the specific mechanism of red light induced resistance in broad has not yet been elucidated clearly.

Heat shock can alter or negate the disease resistance even to non-pathogenic agents (Cruickshank and Perrin, 1965; Heath, 1979). Heat shock treatment is an established method which is commonly used to prevent or delay resistance response (Elliston et al., 1977; Jerome and Muller, 1958; Oka et al., 1975a & b; Yarwood, 1956). Lesion development and sporulation are useful criteria for measuring heat-induced susceptibility of rice plant against blast fungi (Arase et al., 1982a & b). Yoshida et al. (1999) reported that pre-inoculated heat treatment of cruciferous plant cotyledons suppress defense reactions against *Peronospora parasitica*. The effect of heat shock on red light-induced disease resistance in plant is yet to be revealed, though, the response of plants to heat-shock has recently received much attention. Thus, thermo-biological approaches on red light induced resistance in broad bean against *B. cinerea* seem to provide some valuable information on the mechanism of red light-induced disease resistance in plant. Therefore, the effect of heat treatment on the lesion development and infection behaviors in red light irradiated broad bean leaflet inoculated with *B. cinerea* was investigated.

**Materials and Methods**

**Plant**

Broad bean plants (*Vicia faba* L., cv. Taito) were grown in a glasshouse throughout the experiment. Seeds were sown in 16-cm-diameter pots containing 3 kg of commercial garden soil (Kureha Chemicals, Osaka, Japan) 1 seed/pot (Islam et al., 1998).

**Pathogen**

A virulent strain BC 304 of *B. cinerea* was isolated by single spore culture from infected strawberry fruit, and stocked on potato-strawberry agar (100-100-20g/l) in slants for maintaining its pathogenicity. An avirulent strain BC 1 which had lost the pathogenicity during the continuous subculture was also used in this study. Both strains of *B. cinerea* were cultured on potato sucrose agar medium at 22 5°C under continuous near ultraviolet irradiation provided by fluorescent lamp (FL 10BLB, 360 µW/cm², Toshiba, Japan). A lawn of uniform
sporulation was formed within 6 to 8 days. A spore suspension was prepared by flooding plates of 6-8-day-old cultures with sterilized, distilled water and dislodging spores with a glass rod. Spore densities were estimated with a hemacytometer and adjusted to $2 \cdot 10^6$ spores/ml.

**Heat treatment and inoculation**

Fully expanded unblemished leaflets were collected and dipped into hot water at 50 and 55 °C for 5 and 10 sec. Each leaflet was divided into two-half leaflets by its midrib, and placed on a frame of glass rod in a Petri dish lined with sterilized moist filter paper. Heated and unheated half leaflets were inoculated with 20 µl drops of a spore suspension of *B. cinerea* (2-3 $\cdot 10^6$ spores/ml) and then kept under red light (FL 20S · R-F, National: 600-700 nm, max. 650 nm, 258 µW/cm²) or in the dark for 48 h at 25°C.

**Light microscopic observation**

Leaf tissue under inoculation droplets were cut into a small pieces and boiled in a lactophenol-alcohol solution (1:2, v/v) until the chlorophyll was removed. The leaf pieces were immersed into saturated chloral hydrate solution for 1 day to make the leaf tissue transparent. The tissue was stained with 1% cotton blue in lactophenol on a glass slide and the fungal infection behavior was observed under a microscope. Three hundred appressoria from six pieces were observed for each replication. Each experiment was repeated at least twice with three replications.

**Bioassay of anti-fungal activity of infection droplets**

The heated and unheated broad bean leaflets were washed thoroughly with distilled water. Each leaflet was divided into two-half leaflets by its midrib and placed in plastic case lined with moist paper. The leaflets (upper surface) were inoculated with three to four drops (50 µl) of *B. cinerea* (2-3 $\cdot 10^6$ spores/ml) and then incubated under red light from a fluorescence lamp (FL 20S · R-F, National: 600-700 nm, max. 650 nm, 258 µW/cm²) For dark control, one half was inoculated with a spore suspension and incubated in plastic cases covered with aluminum foil. After 48 hr, inoculation droplets were recovered from the leaflets by micropipette. The droplets were centrifuged (2500 rpm for 10 min) to remove the spores. New spores of *B. cinerea* from 5-6 days old culture were suspended in supernatants from inoculation droplets giving the concentration of 2 $\cdot 10^6$ spores/ml. The drops (50 µl each) of spore suspension were incubated on glass slide at 25°C. After 24 h, spore germination was investigated. Approximately 400 spores were evaluated for each replication.

**Results**

**Effect of red light on heat-induced susceptibility in broad bean against *B. cinerea***

1) *Avirulent strain of B. cinerea*

When an avirulent strain BC1 of *B. cinerea* was inoculated to the unheated broad bean, small necrotic lesions appeared slightly in some inoculated sites of broad bean leaflets in the dark (Fig 1, A). In the heated leaflets, however, small necrotic lesions appeared at some inoculated sites 12 h after inoculation in the dark and then after 24 and 48 h, large necrotic lesions appeared at all inoculated sites irrespective of heat doses (Table 1) and developed around the healthy tissues (Fig. 1, B). Under red light, however, lesion formation was suppressed even in heated broad bean, as compared to that in the dark (Fig. 1, B).

**Table 1** Effect of heat shock before inoculation on lesion formation in broad bean inoculated with an avirulent strain of *B. cinerea* in the dark

<table>
<thead>
<tr>
<th>Strain</th>
<th>Heat dose</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC1</td>
<td>50 °C, 5 sec</td>
<td>$\Box^{+\times}$</td>
<td>$+^{1+}$</td>
<td>+</td>
</tr>
<tr>
<td>BC1</td>
<td>50 °C, 10 sec</td>
<td>$\Box$</td>
<td>$+$</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>$\Box^{-\times}$</td>
<td>$\Box$</td>
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</tbody>
</table>

- a) Small necrotic lesions were formed in some inoculated sites.
- b) Large necrotic lesions were formed in all inoculated sites.
- c) No lesion formation was observed.

2) *Virulent strain of B. cinerea*

When heated and unheated broad bean leaflets were inoculated with a virulent strain of *B. cinerea* and kept in the dark, respectively, large necrotic lesions were formed in the inoculated sites of both heated and unheated leaflets (Fig. 1, C and D). There was no difference in lesion formation between the heated and unheated broad bean leaflets. Under red light, however, lesion formation was significantly inhibited even in heated broad bean, as compared to that in the dark.

Inhibition of lesion development in heated broad bean un-
der red light was also recognized at the hyphal growth level by the microscopical observation. There was no difference in appressorium formation of *B. cinerea* between light treatments or heat treatments. Under red light, however, the infection hypha formation and also hyphal growth of *B. cinerea* were significantly inhibited even in heated broad bean tissues. More than 98% of appressoria produced infection hyphae in heated broad bean leaflets in the dark, whereas 75% of appressoria produced under red light. Number of infected cells per infection hypha in heated broad bean was 2 to 7 in the dark, whereas that under red light was 1 to 3 (Fig. 2).

**Effect of red light on production of antifungal substances in heated broad bean**

Antifungal activity in infection droplets on heated and unheated broad bean leaflets was investigated (Fig. 3). In the dark, spore germination in inoculation droplets was more than 92% in both heated and unheated broad bean leaflets. Under red light, however, antifungal activity was observed in droplets recovered from heated broad bean leaflets (41.1%), although its activity decreased, as compared to that in droplets from unheated broad bean leaflet (10.2%).

**Discussion**

Heat-induced susceptibility of beans to some viruses and fungi was demonstrated by Yarwood (1956). Temporary suppres-
Fig. 3. Antifungal activity of inoculation droplets collected from heated and unheated broad bean leaflets inoculated with *Botrytis cinerea*. *B. cinerea*-inoculated broad bean leaflets were kept under red light or in the dark. After 48 h, inoculation droplets were used for antifungal activity test. Antifungal activity was checked by spore germination of *B. cinerea* on a glass slide 24 h after incubation. Data are the means of three experiments with three replications. Bars represent ± SD. Mean followed by the same letters are not significantly different at 5% level according to New Duncan’s multiple range test.

In conclusion, present studies demonstrated that red light induced resistance was heat stable and red light-dependent production of antifungal substances were involved in resistance of heated broad bean leaflets against *B. cinerea*.

References


