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Effect of far-red light on red light-induced resistance of broad bean infected by *Alternaria tenuissima*

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Abstract : Effect of far-red light on lesion development in broad bean leaflets inoculated with *Alternaria tenuissima* was investigated. Lesion development by *A. tenuissima* was significantly inhibited under red light. Furthermore, red light-induced resistance was observed even in additional far-red light treatment. In the far-red light conditions, however, large lesions were induced and the lesion size was similar to that in broad bean leaflets in the dark. Our study showed that red light is required for the suppression of lesion developed in broad bean against *A. tenuissima*, whereas far-red light had no suppressive effect to the resistance of broad bean.

Keywords: Far-red light, induced resistance, broad bean, *A. tenuissima*

I Introduction

Disease resistance in some plants against many fungi is induced by exposure to light. Recently, effect of red light on plant disease development was reported (Islam *et al*., 1998, 2002; Rahman *et al*., 2003a, 2005; Schuerger and Brown, 1994, 1997). Furthermore, we demonstrated that red light-induced resistance against *Botrytis cinerea* was observed even in heated broad bean leaflets (Rahman *et al*., 2003b) and such resistance in broad bean was pathogen non-specific (Rahman *et al*., 2003a). Recent study also showed that photo-and protein-syntheses of broad bean was involved in the expression of red light-induced resistance (Rahman *et al*., 2002). It was demonstrated that production of antifungal substance(s) (Islam *et al*., 1998; Rahman *et al*., 2003b); papilla formation (Akashi *et al*., 1999) and activity of antioxidant enzyme catalase (Khanam *et al*., 2005) were significantly enhanced in broad bean by red light. However, receptors of red light in broad bean are not yet elucidated clearly.

It is well known that far-red and red lights are playing an important role in plant morphogenesis. Tibbitis and co-workers (1988, 1994) reported that the far-red light suppressed the red light enhanced non-pathogenic gall (oedema) formation in tomato. But the effect of far-red light on disease resistance in plant caused by biotic agent is still unknown. Therefore, in this study, an attempt was made to determine the effect of far-red light on lesion development in broad bean inoculated with *Alternaria tenuissima* for additional information of elucidation of red light-induce resistance in plant.

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Materials and Methods

Growing of plants

Broad bean plants (*Vicia faba* L. cv. Taito) were grown in a glasshouse throughout the experiment as described by Islam *et al*. (1998).

Culturing of fungi

A. tenuissima was isolated by single spore culture from the infected broad bean leaves collected from farmer's field in Shimane prefecture, and stocked on V-8 juice agar medium in slants. *A. tenuissima* was cultured on potato sugar agar (PSA) medium at 25℃ under continuous near ultraviolet (NUV) irradiation provided by fluorescent lamp (FL20S.BL-B, National, Japan). A lawn of uniform sporulation formed within 6 to 8 days.

Preparation of spore suspension

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 concentration of each suspension was adjusted using a hemacytometer.

Inoculation and light treatments

Fully expanded leaflets were cleaned by washing with distilled

water. Each leaflet was divided into two-half leaflets by cutting its midrib and placed on a frame of glass rod in a Petri dish lined with sterilized moist filter paper. Leaflets were slightly injured by scratching with a scalpel, inoculated at injured sites with 20μl spore suspension of *A. tenuissima*, and then kept under red light (600-700 nm, max. 650 nm, 287 μW/cm^2, FL 20S·R-F, National, Japan). For far-red treatment Petri dishes were kept in plastic cases, which were covered with the sharp cut filter (SC 70, Fuji Photo Film Co. Ltd., Tokyo, Japan). Then cases were kept under far-red light (700-800nm, 149.6μW/cm^2, FL20S. FR-74, 20 W, Toshiba).

To determine the effect of red or far-red light intensity on lesion development, light intensity was changed with neutral density filters (Fuji Photo Film Co. Ltd.) having attenuation rates of 95, 90 and 50% for the experiment on the light intensity effect. Petri dishes with inoculated leaflets were kept in plastic cases, which covered with different neutral density filters and then irradiated by red or far-red light at 25℃ for different durations. Four florescent tube lamps were suspended above filters. Light intensity of red and far-red lights which passed through the saran wrap without neutral density filter was 490μW/cm^2 and 576.9μW/cm^2, respectively.

Data collection

In all experiments, the diameter of each lesion was determined at different intervals after inoculation by measuring across two diameters through centre of the lesion. Six-half leaflets were used per replication. All experiments were repeated three times with three replications.

Results and Discussion

Red light treatment inhibited lesion development in broad bean leaflets by *A. tenuissima* and average lesion size in diameter was ca. 2mm (Fig. 1) which agreed with our previous report (Rahman *et al*., 2003a). Under far-red light, however, lesion development was not inhibited and average lesion size in diameters was ca. 6mm. No inhibition in lesion development was also observed in the dark. Lesion development was inhibited with the increase of duration of red light irradiation and its most significant inhibition was found 48h after red light irradiation, while lesion size increased slightly with increasing duration of far-red light irradiation (Fig. 2). It was found that lesion development decreased with increasing intensity of red light. As shown in Fig. 3, significant disease suppression was definitely dependent on the red light intensity, but not on the far-red light intensity. Red light-induced resistance in broad bean was not suppressed under combined red and far-red lights as demonstrated by the formation of small necrotic lesions in diameter, compared with that under far-red light or in the dark (Fig. 4). As shown in Fig. 5, red light inhibited lesion development in the broad bean leaflets, regardless of continuous or alternative treatment. In continuous far-red and dark treatment and their alternative treatments, however, large lesion formation was observed and there was no significant difference in lesion size among light treatments. The effect of red or far-red light with the other treatments on broad bean leaflets varied on duration and sequence of red or far-red light irradiation. But there was no significant effect when far-red replaced with

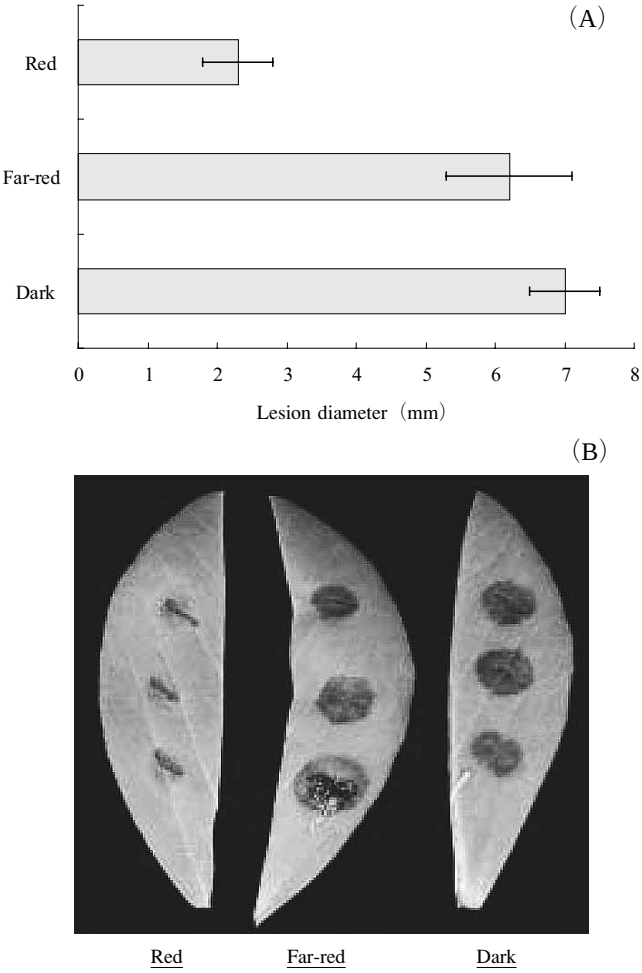

Fig. 1. Effect of red or far-red light on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept under the red light, far-red light or in the dark for 48h. (A): Data are the means of three experiments with three replications. Bars represent ±SD. (B): Lesion developed in broad bean leaflet.

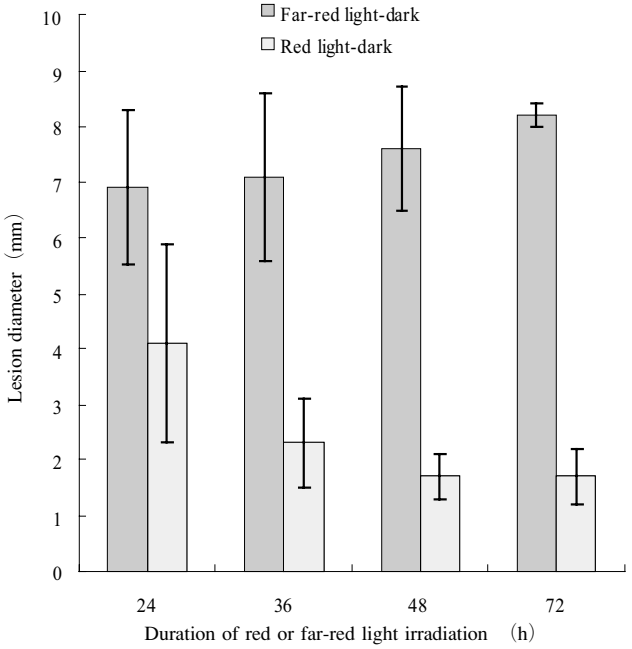

Fig. 2. Effect of duration of red or far-red light irradiation on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept under red and far-red lights, respectively. Red and far-red light durations were alternated with dark treatment at 24, 36, or 48 h after inoculation during 72h incubation period. Bars represent ± SD.

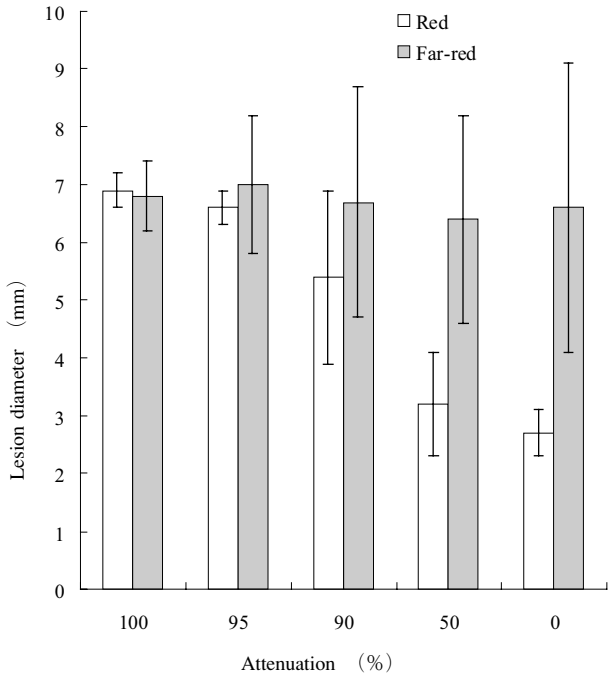

Fig. 3. Effect of red or far-red light intensity on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept in Petri dishes which were covered with saran wrap film (attenuation rate 0%), neutral density filters and aluminum foil (attenuation rate; 100%) under red or far-red light. Lesion diameter was measured 48h after inoculation. Bars represent±SD.

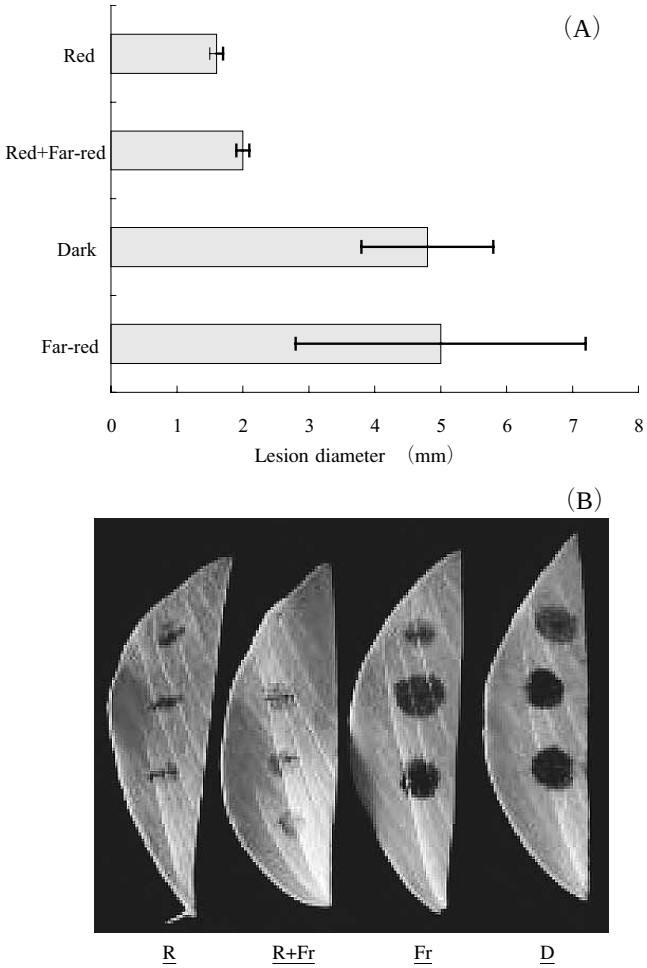

Fig. 4. Effect of red and far-red lights on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept under the four light conditions for 48h. (A): Data are the means of three experiments with three replications. Bars represent±SD. (B): Lesion developed in broad bean leaflet. R: Red light; Fr: Far-red light and D: Dark.

darkness alternatively 24h cycle for 96h incubation period (Fig. 6).

These results indicated that both light intensity and exposure time of red light were necessary for induction of resistance in broad bean against *A. tenuissima*, whereas far-red light had no suppressive effect to the resistance of broad bean. As per our knowledge, effect of far-red light on lesion development in broad bean leaflet, presented in this paper has not been reported previously. In fact, Morrow and Tibbitts (1988) reported that far-red light (700-800nm) prevented non-pathogenic tumor development on solanaceous spp. which was induced by red light (600-700nm) and they concluded that effectiveness of red and far-red light irradiation in the regulation of tumor development indicated phytochrome involvement in red light induced non-pathogenic tumor development in to-

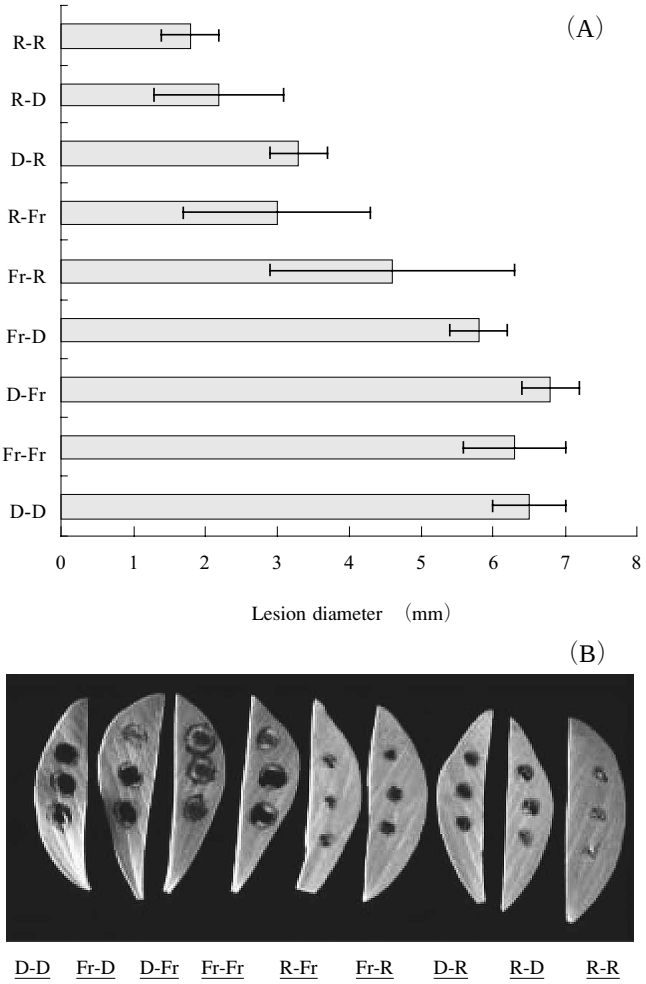

Fig. 5. Effect of alternative light treatments on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept under the red light (R), far-red light (Fr) and dark (D) conditions, respectively. After 24h each leaflet was kept under the several light conditions for 24h. (A) : Data are the means of three experiments with three replications. Bars represent±SD. (B) : Lesion developed in broad bean leaflet 48h after inoculation.

mato plant. These finding confirms earlier work implicating the red light-induced resistance in broad bean leaflets, as far as is known, and provide additional information that far-red light has no effect on red light induced resistance.

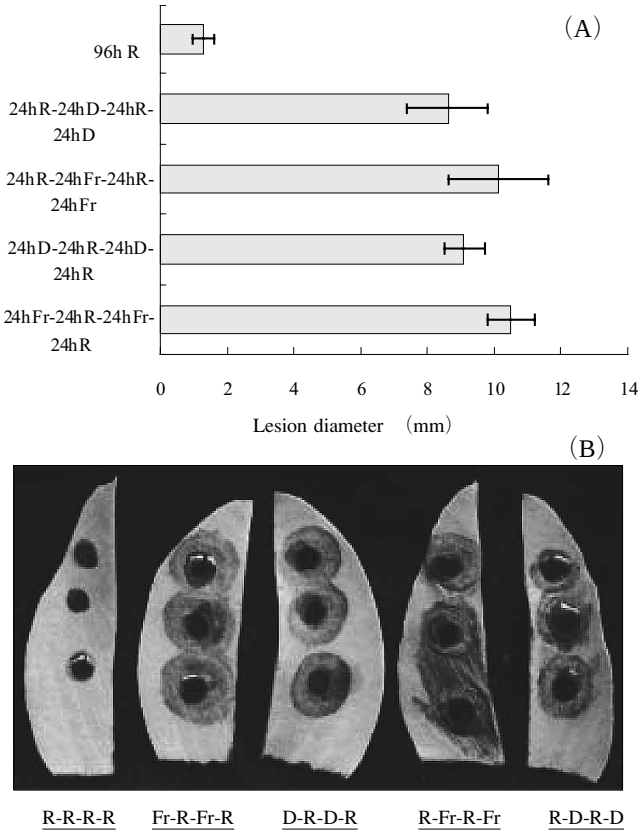

Fig. 6. Effect of successive 24h cycles of alternative light treatments on lesion development in broad bean leaflets inoculated with *A. tenuissima*. Inoculated broad bean leaflets were kept under red (R), far-red (Fr) lights or in the dark (D), respectively. After each 24h, each leaflet was kept under several light conditions for 96h incubation period. (A) : Data are the means of three experiments with three replications. Bars represent±SD. (B) : Lesion developed in broad bean leaflet 96h after inoculation.

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