

INVESTIGATIONS OF THE INDUCED RESISTANCE TO *PLASMOPARA HALSTEDII*

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Abstract

Although downy mildew of sunflower (*Helianthus annuus* L.) can be effectively controlled, protection can be hindered by several factors. The goal of our study was to test the effectiveness of an immune activator, Bion 50 WG against *Plasmopara halstedii* in sunflower examining various host-pathogen combinations. Bion treatment significantly reduced fungal sporulation as well as the number of plants damped off in compatible interactions. Cell necrosis, intensive fluorescence and secondary cell division found to be associated with infected and Bion-treated susceptible plants resembled that usually appearing in genetically resistant inoculated sunflowers. Bion treatment had no effect on plants with total resistance (HA 335), while host response (hypersensitive reaction, necrosis and active oxygen species) of RHA 340 characterised by HLI resistance markedly decreased after treatment.

Introduction

Downy mildew of sunflower caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni is one of the most destructive diseases worldwide. Although the pathogen can be effectively controlled by using resistant plant cultivars and fungicidal seed dressing, protection from the disease can be hindered by several factors. One of these is the pathogenic variability of *P. halstedii* (Gulya et al., 1996, Kormány and Virányi, 1997), while the appearance of tolerance (resistance) to the fungicide metalaxyl is another problem (Albourie et al., 1998).

In the present work, we examined an immune activator, Bion 50 WG (benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester) to test its effectiveness against the downy mildew disease of sunflower in various compatible and incompatible host-pathogen combinations. In addition, the histology of genetic and systemic acquired resistance was also compared.

Materials and Methods

The pathotype (race) of *Plasmopara halstedii* (virulence code 700) as well as two compatible (RHA 274 and HA 89) and two incompatible (HA 335 and RHA 340) sunflower inbred lines were used for examination. While HA 335 is characterised by total resistance (immunity), RHA 340 shows HLI (hypocotyl-limited) resistance type (Virányi and Gulya, 1996).

Pre-germinated seeds (25 for each treatment) were inoculated with sporangia of the downy mildew fungus (50,000 sporangia/ml) using the whole seedling inoculation technique (Cohen and Sackston, 1973) directly after treatment with Bion 50 WG at 160 mg/l. Seedlings were soaked in Bion solution for 3 hours. The seedlings were then planted into pots and grown in a glasshouse for a month. Ten days after planting, plants were covered with plastic bags to induce fungal sporulation and disease assessment was made by using a 0-4 scale (Oros and Virányi, 1987). The incidence of damping off as one of the most visible symptoms of downy mildew of sunflower was evaluated as well.

Histological examination of both Bion-treated and untreated host-pathogen combinations were undertaken by fluorescence microscopy (Olympus, Japan; filter block BX 50, excitation 460-490 nm, transmission >515 nm). A series of cross-sections of sunflower stems were examined to detect fungal elements (hyphae, haustoria) and host cell response (hypersensitive reaction /HR/, production of active oxygen species and necrosis). The production of active oxygen species was visualised by the DAB staining method keeping the sections in a 0.1 % diaminobenzidin solution for 2 hours. Infection (fungal elements), necrosis and the formation of active oxygen species were assessed on a 0-4 scale based on their appearance in one, two, three and four quarters of the cross-sections both in the cortical and pith parenchyma.

Experiments were repeated twice. Data were subjected to analysis of variance ($\alpha=0.05$) using Fisher's multiple comparison with the MINITAB 10.2 statistical package.

Results and Discussion

Bion treatment at 160 mg/l significantly reduced fungal sporulation on cotyledons in both compatible host-pathogen combinations examined. There was no infection found in the incompatible interactions. Furthermore, Bion 50 WG highly reduced the number of plants damped off in susceptible lines.

Fluorescence microscopy of cross-sections of sunflower stems revealed that in compatible combinations the fungus could readily colonize parenchyma tissues. Bion treatment, however, significantly decreased the development of fungal structures; i.e., the appearance of hyphae and haustoria. Furthermore, cell necrosis, intensive fluorescence and secondary cell division found to be associated with infected and Bion-treated plants resembled that usually appearing in genetically resistant inoculated sunflowers.

There was a marked difference in the host reaction between the two incompatible inbred lines. Investigation of cross sections of HA 335 (with total resistance) revealed a hypersensitive reaction on the third day and the occurrence of active oxygen species and necrosis on the fourteenth day after infection. In contrast, host response characterised by extent cell necrosis was relevant on the tenth day following infection in the RHA 340 resistant inbred line. Bion treatment had no effect on total immunity, while host response of RHA 340, i.e., HR, necrosis and active oxygen species markedly decreased after Bion treatment.

Conclusions

The immune activator Bion 50 WG effectively restricted downy mildew development in the host-pathogen combinations examined. Furthermore, induced resistance was very

similar to genetic resistance at host tissue level. Several workers have investigated the histopathology of sunflower downy mildew including compatible and incompatible interactions (Virányi and Mohamed, 1985; Mouzeyar et al., 1993, 1994, 1995; Mazeyrat et al., 1999; Tosi et al., 1999), but this is the first paper providing histological data on the systemic acquired resistance (SAR) in the case of *P. halstedii* and sunflower.

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