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INDUCTION OF SYSTEMIC RESISTANCE IN CUCUMBER AGAINST ANTHRACNOSE BY PLANT GROWTH PROMOTING FUNGI

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ABSTRACT

Plant growth promoting fungi(PGPF) obtained from zoysiagrass rhizosphere offer dual advantages - induse systemic disease resistance response in cucumber to C. *orbiculare* infection and cause enhancement of plant growth and increase yield. PGPF protected plants either by colonizing roots or by their metabolites. PGPF offer an advantage by protecting plants for more than 9 weeks and 6week in the greenhouse and field. PFPF-induced plants linited pathogen spore germination and decreased tne number of infection hyphae on the leaf, and increased lignification at places of attempted pathogen infection, thus reducing thepathogen spread. PGPF elicited increased activities of chitinascs, glucanases, peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase to C. orbiculare infection in cucumber plants. The role of PGPF in elevating cucumber defense response to pathogen infection suggests potential application of PGPF as biological control agents.

INTRODUCTION

Pathogens including viruses, bacteria, and fungi were shown to induce systemic resistance in a variety of plants (Ross, 1961; Yarwood, 1956; Kuc, 1982; Dean and Kuc, 1985). The resistance acquired by plants to pathogen infection has been very effective against further attack by pathogen(s) and lasted for the life of an annual plant (Dean and Kuc, 1987; Kuc and Tuzun, 1990; Madamanchi and Kuc, 1991). The results of experiments conducted by several researchers also revealed that nonpathogenic microorganisms (Elliston et al., 1971, 1976; Schonbeck et al., 1980; Strobel, 1989) and their metabolites induce resistance when treated to plants (Dehne et al., 1984; Trivedi and Sinha, 1976; Ozeretskovskaya et al., 1987). Other than

nonpathogens, certain beneficial rhizobacteria have been reported to induce systemic resistance in bean (Alstrom, 1991), carnation (van Peer et al., 1991), and cucumber (Liu et al., 1991; Wei et al., 1991). Lately, rhizosphere fungi that promoted plant growth were also reported to induce systemic resistance in cucumber (Meera et al., 1994; Meera et al., 1995 a, 1995 b).

Over the past few years, our research aims were to establish: 1) that certain saprophytic fungi having biological control activity could be used to induce systemic resistance to Colletotrichum orbiculare in cucumber, 2) the durability of acquired systemic resistance in the greenhouse as well as in the field, and 3) the molecular mechanism(s) of resistance systemically induced with PGPF. In this paper, we intend to give a comprehensive account of the work done, until now, on PGPF-induced systemic resistance in cucumber.

INDUCTION OF SYSTEMIC RESISTANCE

Rhizosphere fungi (Phoma sp. and nonsporulating fungi) from zoysiagrass that enhanced cucumber plant growth were selected and mass cultured axenically on autoclaved barley kernels at room temperature (Meera et al., 1994). The fungal colonized barley kernels were the main source of PGPF inocula for most of the experiments. The fungus colonized inoculum was added to autoclaved commercial potting medium at various concentrations.

Plants were grown for the period of 3 weeks and leaf 2 were challenge inoculated with an optimum spore concentration of 20 10 l drops of 105 spores ml-1 of the pathogen. Consistently significant protection was obtained when fungal colonized barley kernels were added to potting medium at the rate of 2% (w/w) and the challenge inoculum was limited to 105 spores ml-1 of the pathogen. Plant treatment with 2% PGPF inoculum, at a pathogen concentration of more than 106 spores ml-1 had less significant effect on plant protection (Meera et al., 1994).

Experiments were conducted to test whether the mycelial inoculum and cell free culture filtrate (CF) of PGPF also induced systemic resistance. PGPF was grown in potato dextrose broth for 8-10 days at 25 C and the mycelial mat was harvested and ground with distilled water (1 g in 4 ml) to prepare mycelial inoculum. Culture filtrate was separated and filter-sterilized. The mycelial inoculum or CF was diluted with sterile water (1:4) and treated to roots of 3-week-old plants (Meera et al., 1994). Treated plants were challenge inoculated as described. Comparison of results of barley kernel inoculum, mycelial inoculum, and CF indicated that certain PGPF induced systemic resistance when treated as barley kernel inoculum, mycelial inoculum or CF or in all forms (Meera et al., 1994). Based on these results we assumed that elicitors of induced systemic resistance might be present in the fungal inocula and or its metabolite. Experiments were also conducted to fractionate elicitors from the fungal cell wall or CF. The fungal cell wall

fractions such as cell wall that retained proteins and lipids, cell wall that lacked proteins and lipids, and cell wall lipid fraction were obtained from the mycelia of root colonizing PGPF or noncolonizing PGPF and treated to roots of plants. Results indicated that only lipid fraction of mycelial cell wall of noncolonizing PGPF was effective in eliciting resistance response, while the cell wall lipid fraction as well as sugars of root colonizing PGPF were effective. This may well suggest two types of elicitation mechanisms of defense response of cucumber plants. On the other hand, CF of PGPF were separated into three fractions based on the molecular weight by serial dialysis. Culture filtrate fractions with molecular weight less than 8000 and above 12000 consistently reduced disease (Shivanna et al., 1995 b) and these fractions were methanol soluble. A further characterization of the elicitors of PGPF origin to reveal the active chemical component(s) is necessary to understand the mechanism of elicitation of plant defense response.

PERSISTENCE OF INDUCED SYSTEMIC RESISTANCE

Experiments were conducted to test the duration of induced systemic resistance due to selected PGPF in the greenhouse and field. The barley kernel inoculum of PGPF was added to commercial potting medium (2%, w/w) and plants were grown for 9 weeks in the greenhouse. At an interval of 7 days up to 3 weeks and at an interval of 15 days from 3 to 9 weeks, the top most maximally expanded leaves of PGPF-treated plants were challenge inoculated with the pathogen. In the field, soil from the pit were removed and added with PGPF inoculum (2%, w/w) and replaced back to the pit and grown with a plant. Plants were challenged, as done in the greenhouse, at 6 weeks after planting. Most of the PGPF tested, that induced systemic resistance at 3 weeks in the greenhouse, also induced 40-60% protection up to 5 weeks against the pathogen. The protection decreased gradually after 5 weeks and at 9 weeks it was 35-45% (Meera et al., 1995 a). In the field, the PGPF induced an average of 30% protection when tested at 6 weeks (Meera, 1994). This suggested that PGPF-induced protection in cucumber plants against anthracnose persisted for at least 6-9 weeks.

PGPF induced a remarkable increase of plant growth over uninduced plants in a nutrient-deplete soil. PGPF treatment has been shown also to increase yield of soybean and wheat in the field (Shivanna et al., 1994, 1995 a). Separate experiments with PGPF also demonstrated that they survive in soil and can induce growth promotion of subsequent crops (Shivanna et al., 1996 b). The yield increase in plants treated with PGPF might be associated with the reduction of damage caused by pathogens in the field. Certain PGPF were shown to compete with soilborne pathogens for infection sites on roots thus reducing the disease (Shivanna et al., 1996 a).

Root colonization by certain PGPF seems to be necessary for inducing resistance to C.

orbiculare. There was a positive correlation between the root colonization and protection caused by PGPF at different intervals (Meera et al., 1995 a). When these PGPF were provided as barley kernel inocula, they effectively protected plants against the pathogen. However, when the barley kernel inoculum of same PGPF was autoclaved, they failed to induce systemic resistance response suggesting that they induced resistance by producing elicitors during the process of root colonization (Meera et al., 1995 a). The root colonizing isolates were found to colonize the epidermal and outer cortex layers of cucumber roots (Shivanna et al., 1995 b). On the other hand, certain other PGPF that failed to colonize roots, also induced resistance. Such of the PGPF when treated as autoclaved inoculum, protected plants to some extent suggesting that they might produce certain thermostable elicitors (Meera et al., 1995 a). Further, when their CF was treated to roots, they induced systemic resistance indicating the role of certain elicitors produced by noncolonizing PGPF during their growth in soil or around roots (Meera et al., 1994). The root colonization ability also depended on the cultivar. The roots of susceptible cultivar colonized by PGPF was more than in the roots of moderately susceptible or resistant cultivars (Meera et al., 1995 b). Since more roots were colonized by PGPF in a susceptible cultivar than in the resistant, increased protection in the susceptible cultivar might be related to the quantity of PGPF hyphae available to plant roots.

MECHANISMS OF PGPF-INDUCED SYSTEMIC RESISTANCE

The spore germinability of C. orbiculare on leaves of plants induced with PGPF decreased significantly and the number of infection hyphae produced from appressoria were considerably less when tested on nuclepore membrane (Shivanna et al., 1995 b). Our observation, as well as those of others, indicated that immunized plants produce certain antifungal substances that inhibited spore germination and production of infection hyphae. Further, lignification was also induced at points of attempted penetration by the pathogen in PGPF-induced plants (Shivanna et al., 1995 b). A rapid limitation of fungal growth to a few cells by the process of lignification is suggested to be an important step in the reduction of lesion area spread (Dean and Kuc, 1986; Hammerschmidt, 1984; Vance et al., 1980).

Plants systemically induced with PGPF were also tested for the increased activity of peroxidase (PO). Activities of PO have been directly implicated in induced systemic resistance. Our observation on the systemically increased activity of PO is one of the manifestations of the mechanism of PGPF-induced systemic resistance. In PGPF-induced cucumber system, PO activity was increased considerably by 3 days after challenge in plants of a susceptible cultivar, while in a resistant cultivar the increased systemic activity was noticed even before 3 days. However, at 6 days after challenge, the activity of PO decreased in both cultivars that were induced with

PGPF, but still the activity was higher than in uninduced challenged plants. On the other hand, in uninduced plants the activity remain unchanged. The PGPF-induced plants produced a slight increase in the accumulation of polyphenol oxidase (PPO) compared to uninduced plants. The activity of PPO reached a peak at 6 days in PGPF-induced plants and decreased afterwards in both susceptible and resistant cultivars. This indicated that systemically increased activities of PO and PPO peaked at 6 days when the lesion spread was also maximum, and when the lesion spread decreased by 9 days, the activities of PO and PPO were also decreased. This might be suggestive of the roles of PO and PPO in regulating the lesion spread in immunized plants.

Chitinases and -1,3-glucanases, among hydrolases, have received much attention since they are known to accumulate systemically upon fungal infection of many plants (Mauch et al., 1984; Netzer et al., 1979; Pearce and Ride, 1982). Chitinases and glucanases have endoglycolytic activities and they partially hydrolyse fungal cell walls releasing oligosaccharides from the substrates, chitin, -1,3-glucan or laminarin (Boller and Metraux, 1988; Keen and Yoshikawa, 1983; Mauch et al., 1988; Wessels and Sietsma, 1981). Chitinases and -1,3-glucanases were also shown to have synergistic antifungal activity in vitro (Mauch et al., 1988). Cucumbers immunized with C. lagenarium, Pseudomonas fluorescence, P. lachrymans, tobacco necrosis virus or other elicitors accumulated increased activities of chitinases and glucanases (Binder et al., 1989; Ji and Kuc, 1995; Metraux et al., 1988; Schneider and Ullrich, 1994). The PGPF-induced cucumbers also accumulated increased activities of endochitinase and -1,3-endoglucanase (Shivanna et al., 1995 b). Their accumulation started as early as 3 days after challenge with C. orbiculare in susceptible cultivars induced with PGPF. However, in case of resistant cultivar, PGPF induced a clear significant increase at 6 days after challenge and the activity increased considerably thereafter. The activities of exochitinase and -1,3-exoglucanase in PGPF-induced plants also increased consistently in susceptible as well as in resistant cultivars (Shivanna et al., 1995 b). This indicated that PGPF-induction caused increased activities of chitinases and -1,3-glucanases irrespective of the resistant situation of the cultivar.

PGPF-induced plants showed a marked increase in the activity of phenylalanin ammonia lyase (PAL) over uninduced control during the first 6 days after challenge, but the activity decreased by 9 days roughly equalling that of uninduced control. A rapid increase of PAL activity was observed just before the appearance of lesions and it declined when the lesion spread was almost on the decline. This might well suggest that the early increase in PAL activity might result in earlier lignification and hence could be one of the mechanisms of defense response of PGPF-induced plants. Since the activity was almost similar in PGPF-induced and uninduced plants at 9 days after challenge, PAL accumulation appears to be not needed to plants that is already in the state of induction.

Cucumber plants protected with C. orbiculare accumulated more exochitinase, endochitinase and peroxidase than those protected with PGPF. On the other hand, activities of

-1,3-endoglucanase, PPO, and PAL were higher in PGPF-induced plants. Resistant plants induced with certain PGPF accumulated more exochitinase and -1,3-exoglucanase activities than those induced with the pathogen. This suggests that PGPF-induced plants might slightly deviate from pathogen-induced plants in their accumulation of some of these defense-related components.

CONCLUSION

Plant growth promoting fungi (PGPF) have been implicated in the induction of systemic resistance to the foliar pathogen Colletotrichum orbiculare in cucumber. Barley grain inoculum, mycelial inoculum, culture filtrates and elicitors from fungal cell wall, and culture filtrates of PGPF induced systemic responses in cucumber plants. Elicitation mechanisms of defense response of cucumber plants by root colonizing and noncolonizing PGPF are different. The elicitor substances of PGPF origin are also diverse. The spore germinability of the pathogen on leaves of plants induced with PGPF decreased significantly and the number of infection hyphae produced from appressoria were considerably less. Lignification was induced at points of attempted penetration by the pathogen in PGPF-induced plants, thus limited the fungal growth to a few cells and reduced the lesion area spread. Time course activities of chitinases, β-1,3-glucanases, and peroxidase to pathogen infection increased significantly in PGPF-induced plants. Polyphenol oxidase and phenylalanine ammonia lyase that accumulated up to 6 days after challenge decreased thereafter. The results of our investigation as well as of others support the contention that induced systemic resistance in cucumber is multicomponent (Dixon and Harrison, 1990; Kuc, 1990). Increased activities of PO, chitinase, and glucanase in systemically induced plants seems to be coordinately regulated (Irving and Kuc, 1990; Ward et al., 1991). Based on the experiments conduced by several researchers, defense mechanism of an induced plant to pathogen is thought to depend on the host system and the type of inducer. Even at the gene level, structurally dissimilar genes that are present in different plants, however, express similar functions. Metraux and Boller (1986) showed that cucumber expressed genes are not structurally related to the abundant PR proteins of tobacco but are thought to share functional analogy. This suggests that different plants display different patterns of molecular mechanisms when they are induced systemically. Some examples of plants that differ from this trend to synthesize increased quantities of PR proteins suggest that there are still many undiscovered components of the plants' defense. This could be mainly attributed to the complexity of the inducer-host-pathogen interaction.

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