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Preliminary results on the evaluation of the effects of elicitors of plant resistance on chrysanthemum yellows phytoplasma infection

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Abstract

Delay of syndrome development and of plant death has been used to evaluate the activity of the arbuscular mycorrhizal fungi *Glomus mosseae* and *G. intraradices*, the rhizobacteria *Pseudomonas putida* S1PF1, *Pseudomonas aureofaciens* 30-84 and *Streptomyces* sp. SB20, chitosan, and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) as inducers of resistance against chrysanthemum yellows (CY) phytoplasma infection. While *G. mosseae* and *P. putida* S1PF1 slightly reduced the number of CY-infected plants and extended the life span of the affected plants, only 2.4 mM BTH provided some protection from the disease and a delay of the syndrome. Two concentration of chitosan dissolved in acetic or chloride acids were not effective, although some protection from the disease was present in plants sprayed only with acetic acid. Both acids induced phytotoxic effects on daisy plants.

Key words: *Glomus mosseae*, *Glomus intraradices*, *Pseudomonas* spp., *Streptomyces* spp., chitosan, BTH.

Introduction

Arbuscular mycorrhizal fungi (AM) are naturally present in the roots of most fruit trees. They establish a mutualistic association with the plant, which results in an improved resistance to abiotic (Leyval *et al.*, 2002) and biotic stresses (Berta *et al.*, 2005). It has been reported that the AM fungus *Glomus mosseae* may reduce Stolbur infection of tomato plants of about 70% (Lingua *et al.*, 2002). Also the presence of non-pathogenic rhizosphere bacteria may induce a systemic resistance (ISR) in plants. Efficient ISR has been described towards several pathogens, including fungi, viruses and bacteria (van Loon *et al.*, 1998).

Few natural or synthetic compounds, with no obvious deleterious effect on the pathogen, may also induce the activation of the plant defence machinery against several pathogens (Ryals *et al*., 1996). The effect of these chemicals influences the regulation of the multigenic plant defence system and therefore it avoids the risk of selecting resistant strains of the pathogen. Chitosan, widely present in nature and not very toxic to the plant, is known to induce an efficient control of fungal, bacterial and viral plant diseases (Chirkov, 2002). Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) has also a well-known ability to elicit host plant defence against different pathogens and to reduce phytoplasma infection of *Arabidopsis thaliana* plants inoculated with X-disease phytoplasma-infective vectors (Bressan and Purcell, 2005).

Some preliminary results on the activity of AM fungi, rhizobacteria, chitosan and BTH, potential resistance inducers, on chrysanthemum yellows (CY) disease of daisy are presented.

Materials and methods

The '*Candidatus* Phytoplasma asteris', strain CY, originally isolated from *Argyranthemum frutescens* (L.) Schultz-Bip plants in Liguria (Italy) (Conti *et al*., 1987), was maintained on daisy (*Chrysanthemum carinatum* Schousboe) by vector transmission.

Healthy colonies of *Macrosteles quadripunctulatus* (Kirschbaum) were reared on potted oat plants inside nylon cages in growth chambers at 25 °C, photoperiod L16: D8. For transmission experiments, nymphs were fed for 1 week on CY-infected plants, transferred on healthy oat for 2 weeks to complete latency and then transferred singly to 10 daisies for each elicitor treatment for an inoculation access period of 3 days. The plants were then treated with insecticides and maintained in the greenhouse until death.

C. carinatum plants were grown from seed in the greenhouse until 10 cm high, transferred to a growth chamber (25 °C, photoperiod L16:D8) and used for inoculation experiments.

Biotic treatments were conducted with 3 bacterial (B1-B3) strains and 2 AM fungi (F1, F2). Daisy seedlings were watered with 5 ml of 10^8 cfu/ml of *P. putida* S1PF1 (B1), *P. aureofaciens* 30-84 (B3) and *Streptomyces* sp. SB20 (B2) in Long Ashton solution 2 weeks after transplanting. Each inoculum was repeated after 3 weeks. For AM fungi treatments, daisy seedlings were added with 25% of *G. mosseae* (F1) or *G. intraradices* (F2) inocula (Biorhize, Dijon, France). Treated plants were exposed to infectious leafhoppers 6 weeks after transplanting.

Abiotic treatments were conducted with chitosan (Fluka, Low MW, 73 kDa, 80-85% acetylation) (C1,

C2) and BTH (Bion 50 WG; Syngenta) (BTH1 – 3). For chitosan, seedlings were sprayed at run off point with a) 0.1 or 0.2 % chitosan in 0.1 % acetic acid (C1), in a single application 2 weeks after transplanting or b) 0.1 or 0.2% chitosan in 0.1% acetic acid or in 0.035 M HCl, 2 weeks after transplanting, followed by a second application of the same chitosan solutions 3 days after the 1st treatment (C2). Treated plants were exposed to infectious leafhoppers 4 weeks after transplanting.

For BTH, daisy seedlings were sprayed at run off point with 1.2 mM, 2.4 mM and 4.8 mM in distilled water according to Bressan and Purcell (2005), in a single application 2 weeks after transplanting. Treated plants were exposed to vectors 3 weeks after transplanting. The experiment was repeated 4 times. Daisies treated with water and exposed to vectors were used as transmission controls. For each elicitor, treated plants not exposed to vectors were used as treatment controls. Presence and severity of CY symptoms were evaluated 3 times a week starting from 3 days after the beginning of inoculation and classified in 5 classes of severity (0 = no symptoms, 5 = dead plant).

Results

Two biotic treatments (B1 and F1) slightly reduced the number of CY-infected plants and also extended the life span of the affected plants, as compared to the controls.

In addition, F2, B1 and B3 overcame the stem branching induced by the phytoplasma. In plants treated with two bacterial strains (B1 and 2) serious phytoplasma symptoms appeared a week earlier than in the controls.

Plants sprayed with 0.2% chitosan in both acetic and chloride acids, in single and double applications, developed CY symptoms few days later than the control plants; these treatments also delayed the development of the disease and the death of the plants of 4-5 days. Single and double treatment with acetic acid (without chitosan) resulted in a lower number of symptomatic plants. Acetic acid induced the development of severe necrotic lesions on the treated plants, while control plants sprayed with HCl developed chlorotic lesions on the leaves.

Treatment with 2.4 mM BTH resulted in a lower number of symptomatic plants. Infected plants showed a delay in disease development. Two plants that were infected at 40 dpi became symptomless at 60 dpi and remained healthy until the end of the experiment. These plants were phytoplasma-free at the end of the experiment. Treatments with 1.2 and 4.8 mM BTH were less effective.

Discussion

Among the biotic elicitors, *G. mosseae* and *P. putida* S1PF1 were associated with a slower disease development and their combined application is under testing. Among the abiotic elicitors, BTH provided some protection from the disease and also a delay of the syndrome. Actually, BTH showed a strong protection activity against X-disease phytoplasma when applied to *A. thaliana* plants (Bressan and Purcell, 2005). Chitosan, in spite of its known activity against several pathogens, has not shown efficacy towards phytoplasma infection. The observed delay in symptom development is likely due to the solvents required to solubilise the polymer. Moreover, acetic acid alone reduced the number of infected plants. It is worth to note that both acetic and chloride acids induced phytotoxic effects on daisy plants. The most promising biotic and abiotic treatments identified in this preliminary screening will be further studied following their application under controlled conditions. Quantification of the pathogen titre and electron microscopy analysis of the plant phloem will be evaluated to monitor the effect of the different elicitors on the phytoplasma, while several morphological and physiological parameters as well as the expression of resistance-related proteins will be measured to evaluate the effects of the elicitors on the plants. The possible interactions between elicitors and insect vectors will also be investigated.

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