Induction of Resistance to Fire Blight in Apple (*Erwinia amylovora*)

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Abstract

Apple genotypes show different levels of resistance to fire blight caused by the bacterium *E. amylovora*. This resistance is considered to be polygenic, thus relying on a number of defense mechanisms. On the assumption that in susceptible genotypes these defenses may be present but not activated during the infection process, we attempted their induction by various abiotic and biotic elicitors. Two kinds of experiments were performed in the greenhouse and in the orchard: i) evaluation after controlled inoculation of the level of protection conferred to the plant by each elicitor and ii) investigation of various defense responses potentially involved in the protection. Results showed that some elicitors provided a level of overall protection of about 50% in the orchard while higher levels may be obtained on seedlings in the greenhouse. The comparison of some defense responses in protected and unprotected treated plants showed that i) elicitation of defense is not necessarily associated with control of fire blight and that ii) some responses at least may be associated with the observed protection.

Keywords: defense inducers; bacterial diseases; control; resistance mechanisms

Control of fire blight of apple and pear is difficult. Classical direct chemical control as well as utilization of resistant varieties present specific negative aspects. In this context, new approaches for control are needed. Among them, the activity of defense inducers in apple was worth to be tested. It has been recently shown that resistance can be induced against fungal diseases in japanese pear (ISHII *et al*. 1999) and against fire blight in apple (BRISSET *et al*. 2000; MAXSON-STEIN *et al*. 2002) by the plant activator Bion. It can be assumed that other activators may induce defense in apple. However except for a growth regulator (Regalis) known to have some beneficial side-effect on diseases of apple and pear, not many of them have been tested so far. The aim of our work was therefore to investigate biotic and abiotic candidate-inducers, for their role in defense against fire blight in apple.

MATERIALS AND METHODS

Elicitors. 9 elicitors were tested, at least in the first step (greenhouse): 6 abiotic (Aliette, β-1-3-glucan, Bion, Regalis, Messenger, Stimulase), 3 biotic (3 different avirulent *hrp* mutants of *E. amylovora*) (THARAUD *et al*. 1997).

Test for protection. A standard procedure of sprays (either 7 days, 4 days or 3 hours before inoculation) of apple seedlings was used with all elicitors. The two candidates selected for field experiments were sprayed before bloom and at the beginning of shoot growth, for blossom (spray) and shoot (injection) inoculations with *Erwinia amylovora* respectively.

Defense mechanisms. Leaves from seedlings were sampled at intervals after infiltration of elicitors under vacuum and extracts were prepared for enzymatic activities or, alternatively, for transcripts of specific genes, as described elsewhere (BRISSET *et al*. 2000; VENISSE *et al*. 2002).

RESULTS

Greenhouse tests. The mean results of at least three repeats per conditions showed that the elicitors could be placed in 3 categories: Stimulases, Glucan, Mes-

senger plaid no role in protection against fire blight; Aliette and the avirulent mutants of *E. amylovora* were not able to protect plants significantly except when sprayed just before (3 hrs) inoculation. Finally Bion and Regalis only provided a high level of protection (75%) even if sprayed 7 days before inoculation.

Orchard tests. Two inducers were selected for activity in the orchard: Bion and Regalis. Each produced a significant reduction of infection on blossoms and shoots: (55 and 60%, respectively for Bion, 40 and 75%, respectively for Regalis).

Elicitation of defenses. Induction of peroxidase and glucanase activities was detected after infiltration of Bion. In addition, transcripts of glucanase, PR5 or PAL were detected in tissues treated with Bion, Regalis, Messenger, Glucan or avirulent mutants (stimulase was not included in this test). The pattern of induction was different for each inducer.

DISCUSSION

Among 9 potential elicitors of defense in apple, 6 were shown to be active in plant tissue in conferring a certain level of resistance to apple. Nevertheless, 2 only of these 6 induced a level of protection high enough (75%) and for long enough (at least 7 days) to be potential candidates to be sprayed in the orchard for fire blight control: Bion and Regalis. Regalis is basically a plant regulator, and its effect on plant growth must be considered together with its protective effect. It is possibe that low dosages have a limited influence on shoot growth, but keep a role in fire blight protection. Transient symptoms of phytotoxicity were observed after Bion application (200 μg/ml), only in the glasshouse.

Most tested elicitors were able to induce defense mechanisms in apple leaf tissues after infiltration. Nevertheless no association between one elicited gene and efficacy of control was evidenced. This would show either that other (not tested) mechanisms were involved in protection for fire blight, or that spraying of elicitors on the plant surface did not allow elicitation within plant tissue, conversely to infiltration in the leaf.

Several questions remain to be adressed before the use of these (or others) inductors in practical control either alone, or in an integrated management approach. Among them the responses of diverse genotypes (or species: pear ...) should be determined, as well as the dose of application, the spray schedule, and the durability of the protection conferred by one spray. In addition, the level of protection is never a 100%, but is at best 50–75%: is this level economically valuable? This may depends again on the variety. Finally some side effects would be worth to be considered, such as toxicity (to plant and/or animal), and activity against other major diseases and pests of apple (and pear): scab, mildew and aphids.

Control of fire blight with the help of plant elicitors seems to be a feasible approach. But the number of candidates for use in practical conditions is at present very limited. It has been noted that different elicitors induce responses from different defense genes. Which of these are really active against *E. amylovora* in apple is not known. Therefore detection of markers associated in apple with resistance to *E. amylovora* would be a very useful breakthrough, particularly in providing a great help in the screening for new elicitors.

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