Induced resistance in plants against insects and diseases
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Induced systemic resistance against systemic viruses: a feasible approach?

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Abstract: Induction of resistance to plant viruses causing localized infections has been widely used to study HR and SAR mechanisms. However, in Nature true virus diseases are produced by viruses able to systemize in the plant and SAR is scarcely effective against them. Thus, a more successful strategy relays in the induction of resistance against both the virus and its vector. In this work, using the pathosystem bean common mosaic virus (BCMV)-*Phaseolus vulgaris* we made attempts of inducing resistance separately to both the pathogen and the aphid vector *Myzus persicae*, with the aim of dissecting the two resistance levels inducible with the most used chemical elicitors. Results showed that BTH and chitosan are able to reduce the infection degree in BCMV mechanically inoculated plants, however not preventing the infection. On the other hand, chitosan and 2-isobutyric acid (IBA), applied as root-drench, could reduce aphid population by half. Therefore, combining the two effects and using chitosan, partially effective against both the virus and the vector, it could be possible to raise an acceptable resistance level in the field, where BCMV is actively spread by aphids. To verify this hypothesis, experimental transmission with viruliferous aphids in chitosan and IBA treated plants are now in progress.

Key words: BABA, BTH, BCMV, chitosan, IBA, *Myzus persicae*

Introduction

Challenging a plant with an HR-inducing virus is one of the simplest experimental method to verify the effectiveness of a SAR inducer, as the acquired resistance can be quantified by counting the number and measuring the size of necrotic local lesions produced on treated and control leaf tissues. By using tobacco necrosis virus (TNV) and tomato bushy stunt virus (TBSV), two HR-inducing pathogens in bean, we have previously demonstrated the efficacy of BTH and chitosan in inducing SAR in this plant specie, also describing the different mechanisms operating with the two elicitors (Faoro *et al.*, 2001; Faoro & Iriti, 2006; Iriti & Faoro, 2008). However, in nature, virus diseases are produced mainly by compatible viruses that are able to systemize and, unfortunately, SAR does not seem to be so effective in controlling them (Pennazio & Roggero, 1998; Palukaitis & Carr, 2008).

Up to now, partial resistance against systemic viruses has been obtained in the lab by using, among others, BTH, BABA and chitosan (reviewed in Schreiber & Desveaux, 2008). However, the efficacy of these componds in open field was acceptable only when treatments were coupled with specific agricultural practices (i.e. UV-reflective plastic mulch, non-woven covers, insecticides). By this strategy the most successful results were obtained in controlling tomato spotted wilt virus (TSWV) and tomato yellow leaf curl sardinia virus (TYLCS) and their vectors (Momol *et al*., 2004; Fanigliulo *et al*., 2009)

In this work we have investigated the possibility of inducing resistance to bean common mosaic virus (BCMV, genus *Potyvirus*) and its vector *Myzus persicae* with chitosan, BTH, BABA and 2-aminobutyric acid (IBA). BCMV, is a wordwide diffuse virus, infecting

systemically several *Phaseolus* species and cultivars and transmitted in a non-persistent manner by numerous aphid species. Furthermore, it is efficiently transmitted by seed and pollen, constituting a dangerous inoculum source in the field (Agrios, 2005).

Material and methods

Plant material.
Phaseolus vulgaris plants, cv. Borlotto Nano Lingua di Fuoco (BLF), were sown in 12 cm pots and grown in greenhouse at temp. 24 ± 2 °C, RH 60 ± 5%, 16 h/8 h light/dark period. Ten-20 days after sowing, when the first pair of true leaves was almost completely expanded, plants were ready for treatments.

Insect rearing
Myzus persicae was cultured on bean plants in an insect growth facility where temperature was maintained at 20 ± 2 °C, relative humidity ranged between 50-80% and light/dark period was 16 h/8 h. Aphids were restricted to plants by enclosing them in perforated plastic bags. Before experiments, aphid population was synchronized and only aptera adults of the same age were used.

Induction of resistance to BCMV
Bean plants were sprayed with water solution of BTH [Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (trade name Bion®, Syngenta, CH) at the concentration of 0.3 mM, prepared from a wettable formulation containing 50% (w/w) active ingredient (a.i.). Control plants were sprayed with water containing the wettable powder alone. Chitosan (Sigma-Adrich, Low molecular weight) was dissolved in 0.05% acetic acid at the concentration of 0.1% and the pH adjusted at 5.6. In this case, control plants were sprayed with 0.05% acetic acid. A partial purified suspension of BCMV (non-necrotizing strain), was mechanically inoculated on primary leaves of bean plants, using a 600 mesh carborundum as an abrasive. Four groups (BTH-treated, chitosan- treated and respective controls) of 10 plants each, were tested in three repeated experiments. Inoculation was carried out either 1, 2 or 7 days after BTH treatment, or 1, 2 or 4 days after chitosan spraying. Infection development was monitored up to 30 days. Every 5 days symptoms severity was recorded and classified in 4 infection levels as follows: 0 = no symptoms; 1 = mild mosaic, 2 = leaf chlorosis; 3 = chlorosis and leaf malformation. Percent of infection (P) was calculated following Kremer & Unterstenhöfer (1967) formula:

$$P = [\Sigma (n \times v) / Z \times N] \times 100$$

where n = number of plants with the same infection level, v = infection level, Z = maximum infection level, N = total numbers of plants.

Induction of resistance to Myzus persicae
Resistance was assessed as the reduction of nymph production by adult aphids. At this purpose, apterous adult *M. persicae* that had been reared and synchronized on untreated plants were transferred to bean plants that had been treated as a root drench 2 days earlier with 50 ml of 1% chitosan in acetic acid 0.05 M or 50 mM β-aminobutyric acid (BABA) in water, or 50 mM 2-isobutyric acid (IBA) in water. All chemicals were from Sigma-Aldrich. Groups of three aphids were transferred in 3 leaves of 5 plants for each treatment in four repeated experiments. Plants were checked every 24 h, adult survival recorded and any nymphs present were counted up to 7 d.

Results and discussion

Induction of resistance to BCMV

Resistance induction experiments showed that both BTH and chitosan were not able to protect plants from BCM infection whatever the induction time (i.e. the time elapsed between treatment and inoculation) used in the different trials (Figure 1). However, both compounds could delay the appearance of symptoms and at the end of the observation period the percentage of infection was significantly lower in the treated plants in comparison with controls. At this regard, chitosan applied 2 days before inoculation was the most effective (Figure 1E). Nevertheless, its double application every other day did not ameliorate its efficacy in controlling BCMV (Figure 1F). BTH applied 1 or 2days before inoculation behaved similarly (Figure 1A, B) but it was almost ineffective when sprayed a week before virus challenging (Figure 1C). This result is surprising as a week of induction phase completely prevented infection when bean plants were challenge with an HR-inducing virus (Faoro *et al.*, 2001; Faoro & Iriti, 2006).

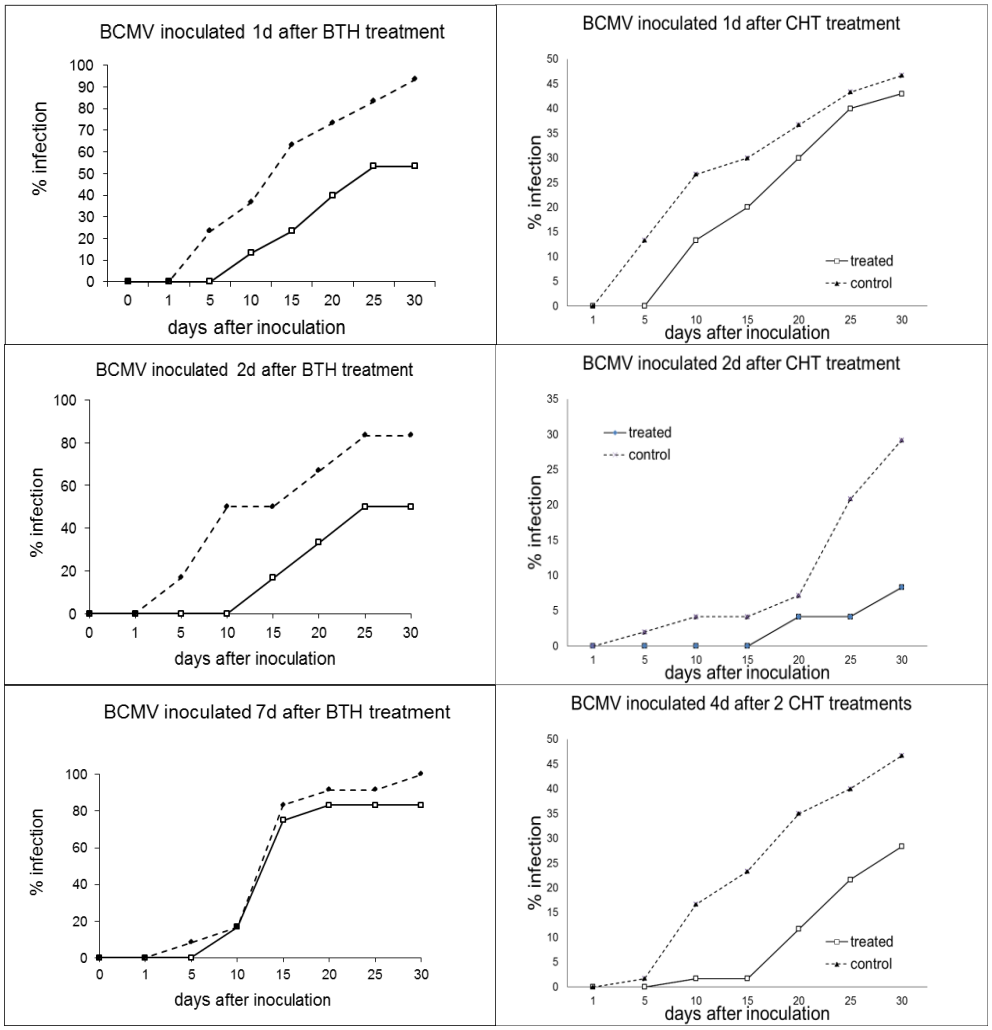

Figure 1. Effect of different elicitor treatments on the infection percentage calculated following Kremer & Unterstenhöfer formula in bean infected plants.

Induction of resistance to Myzus persicae

Among the different elicitors applied as root drench, IBA and chitosan were the most effective in reducing aphid population after a week of observation, while efficacy of BABA was much lower and not significantly different from the control (Figure 2A). Initially, the adults on treated beans had similar rates of reproduction as those on control plants, as shown in Figure 2A that is representative of the four experiments carried out. However, after 5d, CHT and IBA treated plants were producing about half of the nymphs compared to controls. A similar situation was found after a week, as reported in Figure 2B that compares the average number of aphids of all the four repeated experiments. Though we could not observe BABA effectiveness in reducing *M. persicae* population, as previously reported in *Vicia faba* (Hodge & Powel, 2012), its isomer IBA was more effective. This is possibly due to IBA inhibitory effect on ethylene biosynthesis (Malerba *et al*., 1996) as it is known that *M. persicae* fitness decreases significantly in plants impaired in the production of this hormone (Mantelin *et al*., 2009). Also chitosan was similarly effective in reducing aphid population, but in this case a possible reason resides in its capacity to elicit salicylate and jasmonate pathway (reviewed in Iriti & Faoro, 2009), both involved in resistance to aphids (Kaloshian & Walling, 2005).

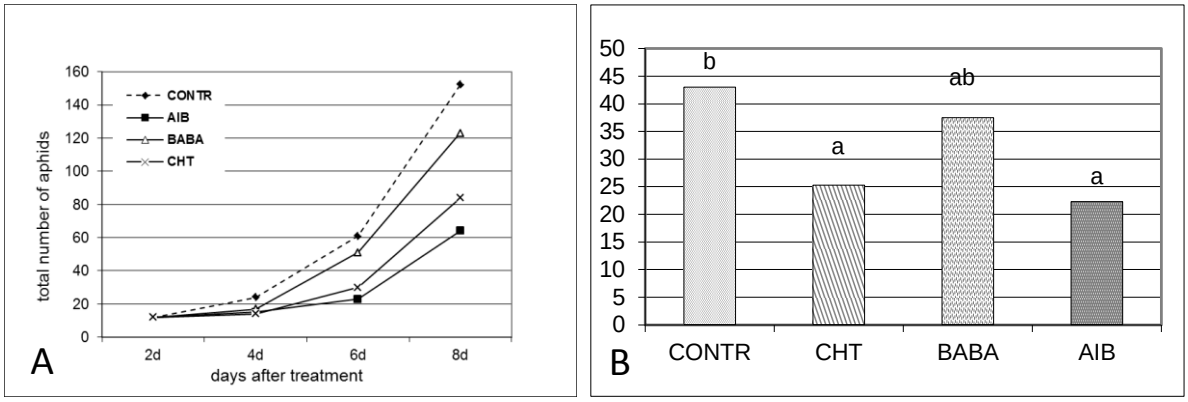

Figure 2. Effect of different elicitor treatments on *Myzus persicae* population in treated and control plants. A. Trend of aphid growth in a representative experiment. B. Average number of aphids after a week in four repeated experiments.

In conclusion, the above data besides confirming that a reasonable degree of induced resistance against a systemic virus is a very difficult task to be achieved, indicates once again that a possible successful strategy includes the induction of resistance to its vector. In the case of BCMV, as chitosan was partially effective against both the virus and the vector, it could be possible to raise an acceptable ”combined” resistance level in the field, where BCMV is actively spread by the aphid. To verify this hypothesis, experimental transmission with viruliferous aphids in chitosan and IBA treated plants are now in progress.

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