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Short communication

Acibenzolar-*S*-methyl induces resistance in oilseed rape (*Brassica napus* L.) against branched broomrape (*Orobanche ramosa* L.)

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ABSTRACT

Branched broomrape (*Orobanche ramosa*) has displayed a worrying expansion in winter oilseed rape crops over the last decade in France. The efficacy of both plant defence activators, acibenzolar-*S*-methyl (ASM, 0.05 g a.i. L^{-1}) and potassium phosphonate (1 g a.e. L^{-1}) in the induction of oilseed rape resistance was evaluated under controlled conditions through repeated bi-weekly applications as foliar sprays or soil drenches. These activator concentrations did not affect the growth of the non-infected oilseed rape. ASM slightly reduced broomrape seed germination while no direct effect was observed for potassium phosphonate. Regardless of the application method, potassium phosphonate did not induce resistance, as shown by the high number of broomrape attachments per treated plant and the near 50% reduction in biomass observed in the infected oilseed rape, whether treated or not. On the contrary, both foliar and soil applications of ASM reduced broomrape attachment by 70% and prevented the loss of crop biomass.

1. Introduction

Broomrapes (*Orobanche* spp.) are root parasitic plants devoid of chlorophyll that develop a haustorium serving as both an attachment organ to host roots and a bridge for water, mineral and organic nutrient uptake from host vascular tissues. Branched broomrape (*Orobanche ramosa* L.) is the most widespread broomrape in the world, causing severe damage to several crops in the Mediterranean region and southeast Europe, particularly to hemp (*Cannabis sativa* L., Gonsior et al., 2004), tobacco (*Nicotiana tabacum* L., Buschmann et al., 2005a), tomato (*Lycopersicum tuberosum* Mill., Cagan and Toth, 2003) and oilseed rape (*Brassica napus* L., Zehhar et al., 2003; Buschmann et al., 2005c). Oilseed rape is the primary European oilseed crop, and production areas have increased steadily since 2005, especially in France, Germany and the UK, as a consequence of a stepped-up demand for oilseed rape-derived biodiesel production. In addition to known virulent oilseed rape pathogens such as insects and fungi, branched broomrape has become a major phytosanitary problem in winter rape fields in France, causing heavy seed yield losses as high as 80% in highly infested fields (Delos et al., 2006). Thus, on 1 million hectares of oilseed rape fields in France 10% are estimated to be infested by *O. ramosa* (CETIOM: Centre Technique Interprofessionnel des Oléagineux Métropolitains, unpublished data). The infestation levels have also increased in hemp and tobacco crops in France (Gibot-Leclerc et al., 2003). By using molecular markers and cross infestations, several French populations of *O. ramosa* have been differentiated, including the pathotype C which displayed higher virulence on oilseed rape than on tobacco or hemp (Benharrat et al., 2005; Brault et al., 2007).

Several control strategies are employed against broomrapes but none has enjoyed unequivocal success. The methods are either uneconomic or hard to achieve, or result in incomplete protection. In addition, resistance to broomrape is scarce and complex, making resistance breeding difficult; this is particularly true for oilseed rape challenged with branched broomrape since most of the tested cultivars are susceptible (Buschmann et al., 2005c). Moreover, chemical treatments display a relatively high phytotoxicity for oilseed rape (CETIOM, unpublished data). Thus alternative or supplementary methods should be considered to prevent infection.

As such, chemical induced resistance (IR) is a suitable strategy that utilizes natural plant defences to control pathogens. Mediated by salicylic acid (SA) or jasmonate (JA), IR comprises plant response activated before pathogen infection (Rayals et al., 1994). IR can be activated by exogenous application of SA or its synthetic functional analogue acibenzolar-*S*-methyl (ASM; also known as benzo (1,2,3) thiadiazole-7-carboxylic acid, BTH) which is active as an inducer of the SA-mediated defence pathway (Lawton et al., 1996; Achuo et al., 2004). ASM is one of the most potent disease resistance activators used in crops to control pathogens including viruses (Šindelářová et al., 2002), bacteria (Oh et al., 2004) and fungi (Achuo et al., 2004). In France, Bion® 50WG (50% ASM, Syngenta) is registered for wheat

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protection against fungi, and Bion® MX (4% ASM, 40% metalaxyl m, Syngenta) is used for tomato protection against bacterial diseases and for the control of mildew in vegetable crops including spinach, radish, and lettuce. As underlined by several studies on different plant species including clover (Kusumoto et al., 2007), pea (Pérez-de-Luque et al., 2004), tobacco, hemp (Gonsior et al., 2004) and sunflower (Sauerborn et al., 2002; Buschmann et al., 2005b; Müller-Stöver et al., 2005), ASM application also triggers IR against broomrapes. ASM is efficient independently of the application method (seed treatment, soil drench or leaf spray), resulting in a strong reduction of broomrape attachments and in some cases a total inhibition of parasite emergence from soil. In sunflower, induction of IR against broomrape by seed treatment with ASM was associated with an activation of phytoalexin, H_2O_2 and chitinase protein production (Sauerborn et al., 2002). In clover, however, root application of ASM favoured broomrape (*O. minor* Sm.) avoidance by activating another resistance pathway through the inhibition of parasite radicle elongation in the vicinity of roots and the enhanced lignification of the root endodermis (Kusumoto et al., 2007). IR is activated in a typical way in oilseed rape by pre-treatment of leaves with ASM (Borges et al., 2003). Nevertheless, ASM efficacy against branched broomrape infection needs to be checked in oilseed rape.

Many other defence activators have been characterized including cell wall components of marine brown algae (Klarzynski et al., 2000; Aziz et al., 2003), harpin-producing bacteria extracts (Dong et al., 1999), prohexadione-calcium (PHDC, Rademacher, 2004), phosphate and phosphonate salts (Reuveni and Reuveni, 1998). Some of these activators were successfully assayed on plants challenged with broomrape including sunflower (PHDC, Fan et al., 2007), tobacco and hemp (Proradix®, Goemar Fruton Spezial®, Gonsior et al., 2004). Phosphonate salts protect plants against oomycete pathogens by acting as both fungicides and inducers of localized or systemic resistance (Bécot et al., 2000; Pajot et al., 2001). Plant responses to phosphonate include notably high phytoalexin accumulation, synthesis of phenolic compounds and lignin deposition (Nemesthoty and Guest, 1990; Jackson et al., 2000). Several studies reported similar responses in resistant cultivars of *Orobanche* susceptible species when challenged with the parasite (Dörr et al., 1994; Goldwasser et al., 1999; Serghini et al., 2001; Pérez-de-Luque et al., 2005; Echevarria-Zomeno et al., 2006; Mabrouk et al., 2007).

Consequently, the aim of this study was to evaluate the efficacy of ASM and phosphonate applications to induce resistance in oilseed rape challenged with branched broomrape (pathotype C). Leaf spray and soil drench were used as application methods on plants artificially infected by *O. ramosa*.

2. Materials and methods

2.1. Plant materials

Oilseed rape seeds (*B. napus* L. cultivar 'Yudal') were kindly provided by Dr. R. Delourme (INRA Le Rheu, France). *O. ramosa* seeds type C were collected from shoots harvested from an infested oilseed rape field in 2004 (Benet, France) and kept at 20 °C before use. Viability of pre-conditioned broomrape seeds was checked using Evans blue (1 g L^{-1}) that stains living cells (Véronési et al., 2005). Germination rates were then corrected by taking into account the rate of seed viability.

2.2. Broomrape seed germination

Broomrape seeds were surface sterilized in 12% sodium hypochlorite for 5 min and then rinsed with sterile distilled water. Twenty-five mg of seeds (around 5000 seeds) were spread on a sterilized moistened fiber glass paper filter in a Petri dish. After pre-conditioning in darkness for 7 days at 25 °C, broomrape seeds were spread on fiber glass paper (10 mm diam, Macherey-Nagel) and then transferred to small Petri dishes (25 mm diam) containing 500 µl of GR24 (1 µg L^{-1}, optimal concentration), a synthetic analogue of strigol that stimulates germination (Johnson et al., 1976), or 500 µl water (controls). ASM (Bion® 50WG, from Syngenta) and potassium phosphonate were applied at 0.05 g a.i. L^{-1} and 1 g e.a. L^{-1}, respectively, in both GR24 assays and water controls. Petri dishes were incubated at 25 °C for 7 days before germination was assessed. Data are means ± confidence intervals ($n = 15$, $\alpha = 0.05$, Student's t test).

2.3. Plant culture and treatments

Broomrape seeds (4 mg kg^{-1} of soil, about 1250 seeds) were mixed with a peat–sand–clay mixture in a 1.3 L pot (1:1:1 by vol). Seed pre-conditioning was managed by protecting watered pots from light for 1 week at 25 °C. Three oilseed rape seeds were then sown directly into each pot. Two weeks after oilseed rape emergence, seedlings were thinned to one per pot. Pots containing broomrape-free soil were used as controls. Plants were grown under greenhouse conditions at 23 ± 5 °C with 300 µmol m^{-2} s^{-1} photosynthetic active radiation and a 16 h photoperiod.

Two treatment procedures were assessed. For foliar application, treatment was started on 2-week-old oilseed rape plants by spraying ASM (0.05 g a.i. L^{-1}) or potassium phosphonate (1 g e.a. L^{-1}) solutions on both surfaces of the first true leaves until run-off. Excess of stimulator was collected at the soil surface on a 3 M Whatman paper. Treatments were repeated on all the leaves at 4, 6, 8 and 10 weeks after sowing. Tween 20 (0.1%, v/v) was added as a surfactant for leaf application of ASM. Controls were performed by spraying 0.1% (v/v) Tween 20 or water. For application as soil drench, pots were supplied with 20 mL of ASM (0.05 g a.i. L^{-1}) or potassium phosphonate (1 g e.a. L^{-1}) solutions 2, 4, 6, 8 and 10 weeks after sowing. Tween 20 was not added to ASM solution for soil application and plants only supplied with water corresponded to controls. In addition, non-infected plants were grown and treated in the same conditions in order to estimate the respective impact of stimulators and parasitism on oilseed rape biomass.

2.4. Data analysis

Twelve weeks after sowing, oilseed rape plants were uprooted. Total number and biomass (DW: dry weight) of broomrape attachments per host plant were measured as well as the total host plant DW. DW was determined following incubation of fresh plant material in an oven at 80 °C for 48 h. Controls and treatments were carried out on 12 oilseed rape plants. The data are means ± confidence intervals ($n = 12$, $\alpha = 0.05$). The data were subjected to analysis of variance using SigmaPlot 10.0 (Student–Newman–Keuls test, $n = 12$, $P \geq 0.001$).

3. Results

3.1. Potassium phosphonate treatments

Under *Orobanche* free-conditions, no significant impact of the potassium phosphonate treatment on oilseed rape biomass was observed when compared to non-treated plants (Fig. 1). Otherwise, phosphonate-treated plants were infected almost as strongly as the non-treated plants (Fig. 2). By soil drench treatment, potassium phosphonate reduced significantly the parasite emergence while the total number of attached parasites did not change. Similarly they displayed a near 50% loss of biomass on infection (Fig. 1). We also showed that potassium phosphonate did not affect broomrape