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Full Length Research Paper

Sucrose effect on broomrape (*Orobanche crenata*) development on narbon bean (*Vicia narbonensis* L.)

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The growth and development of broomrapes *(Orobanche* spp.) fully depends on the nutritional connection established between the parasitic plant and the root of the corresponding host plant. In the present study, narbon bean plants infected with *Orobanche crenata* were watered with different concentrations of sucrose (0.014, 0.044, 0.088 and 0.146 M) in order to evaluate its effect on the early growth stages of the parasite. The germination of *O. crenata* seeds decreased with increasing sucrose concentrations and the number of infection attachments of crenata broomrapes decreased significantly when sucrose was present. A parallel experiment was conducted with identical sorbitol concentrations in order to determinate the role of the osmotic potential in the inhibition of the parasite growth. Our results showed that while low sucrose concentrations significantly reduced seed germination in *O. crenata*, similar concentrations of sorbitol have no significant effect thus indicating that the effect of sucrose is not simply osmotic. Sucrose phytotoxicity was also studied by considering the dry weight of the host plants.

Key words: Sucrose*, Orobanche crenata, Vicia narbonensis* and broomrape control.

INTRODUCTION

Narbon bean (*Vicia narbonensis* L.) is an ancient grain and forage legume used for livestock feed in the Mediterranean region. It has a tap root system, erect habit, strong stems and it grows on neutral/alkaline soils with medium and low rainfall conditions, seed yielding up 1 t/ha under extremely arid conditions (104 mm) and up 2.5 t/ha on 400 mm rainfall (Berger et al., 2002). Although this species has a low incidence of disease and insect attack in the field, it is ddescribed as clearly susceptible to broomrape (*Orobanche crenata* Forsk.) (Linke et al., 1993; Nadal and Moreno, 2007). This parasitic plant attacks its seedlings to the root system of the host plant, establishes a connection with the vascular system of the host via a specialized organ (haustorium) and deprives the host of water, mineral nutrients and metabolites (Parker, 1991). In infested soils broomrape represents the major constraint for narbon bean crop and where this holoparasitic plant is endemic, it is necessary to use some control strategy.

The understanding of metabolic and developmental aspects of root parasite is of great importance because each developmental stage is crucial for the growth and dispersal of these root parasites being each step a potential target for their control. Considering this, the finding of compounds that could interfer with the parasite in these initial phases could help in controlling it. In this sense, different control means such as ammonium-containing nutrients or essential amino acids have been already proposed to control *Orobanche* (Nandula et al., 1996; Vurro et al., 2006).

Since sucrose is the main carbohydrate transferred from the host to the parasite (Aber 1984), this molecule can induce or repress a huge variety of genes which act as regulators of different processes such as germination, early seedling developments or tuberization (Rolland et al., 2002; Gibson, 2004). González-Verdejo et al. (2006) proposed the use of sucrose as a control method of *Orobanche ramosa* on tomato plants, but no studies are already available regarding its effects on *O. crenata*- legumes interaction. The objective of the present study was to

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Table 1. Effect of different sucrose and sorbitol concentrations on the germination of *O. crenata* seeds and effect of sucrose on nodes per root length of *O. crenata* seeds. Values within a column with not letters in common differ significantly at $p < 0.05$.

Sucrose concentration (M)	% Germination		Nodes/root lenght
	Sucrose	Sorbitol	Sucrose
0	27.8 ab	32.2	0.20 a
0.014	31.1 a	27.1	0.07 b
0.044	13.6 c	22.1	0.03 b
0.088	16.5 c	23.6	0.03 b
0.146	21.1 bc	25.7	0.02 b
CV	18.15	41.5	50.7

determinate the effects of different sucrose concentrations on the germination and nodulation of crenate broomrape on narbon bean growing under *in vitro* conditions, trying to find a differential situation where the target weed is sucrose inhibited but the crop is not.

MATERIALS AND METHODS

Seeds disinfection

Narbon bean seeds (sel 556 - 2376, broomrape susceptible entry from ICARDA) were surface-disinfected by immersing them first in 70% ethanol for 2 min, then in 3.5% sodium hypochlorite containing 0.1% Tween 20 for 10 min, and finally rinsing three times with sterile distilled water. *O. crenata* seeds were collected from plants parasitizing narbon bean plants in Córdoba (37º 51´ 42´´N and 4º 48´ W, Spain). *Orobanche* seeds were surface-sterilized by treating them for 2 h with a solution of 0.5% formaldehyde and 0.1 Tween 20, followed by 20 min incubation at 50ºC. Subsequently, seeds were rinsed three times with sterile distilled water as proposed by González-Verdejo et al. (2005).

Sucrose assays

Assays were performed following the methodology described by Pérez de Luque et al. (2004) with slight modifications. Narbon bean (*V. narbonensis*) seedlings, previously pre-germinated in vermiculite, and approximately 6 mg of *O. crenata* seeds were simultaneously sown in 12 x 12 cm squared Petri dishes containing perlite and a sheet of glass-fibre filter (Whatman International, Kent, UK). Afterwards, the plates were wrapped in aluminium foil in order to maintain *Orobanche* seeds in the dark, watered and vertically stored under growth chamber conditions (21ºC, 16 h light). After 10 days of *Orobanche* conditioning period, sucrose (Merk 141621) at different concentrations (0.014, 0.044, 0.088 and 0.146 M) was added to the watering water. Plates without any sucrose treatments were used as negative controls

Four independent plates were set per each of the four treatments and per sugar assays. Percentages of seed germination were determined using a stereoscopic microscope. In order to refer the germination data to root length (germination rate per root cm.) the host root length was estimated by the intercept method of Tennant (1975). The final number of attachments and dry weight per plant were determined per plate at the end of the experiment. In a separated experiment, the same protocol was used, but in a broomrape free environment. The data of both experiments were analyzed separately by analysis of variance according to randomized blocks design, and the means were compared using the Tukey comparisons test at 0.05 probability level.

RESULTS AND DISCUSSION

O. crenata seed germination in presence of sucrose

The mean percentage values of *O. crenata* germination for the tested sucrose concentrations (0.014, 0.044, 0.088, 0.146 M) were 31.1, 13.6, 16.5 and 21.1% respectively (Table 1). The germination rate became significantly lower with the 0.044 M sucrose concentration, thus, indicating that only the lowest sucrose concentration in the watering solution could not reduce significantly the number of germinating seeds. In the case of *O. ramosa* germinating seeds in tomato roots, González et al. (2006) studying similar sucrose concentrations reported that low and intermediate concentrations (0.044 and 0.088 M) did not reduce the number of germinating seeds and only the highest concentration of sucrose (0.18 M) caused a moderate reduction on germination.

Considering that in non-parasitic plants like *Arabidopsis,* 90 - 100% of the seeds germinated in the presence of high glucose or sucrose (Laby et al., 2000) and in the case of *O. ramosa* only high concentrations alter significantly the germination of the parasitic seeds in the presence of tomato roots, *O. crenata* seems to detect earlier the sucrose presence thus preventing seed germination at lower concentrations. In this sense, differential sensitivities to nitrogen containing nutrients have been observed regarding germination between two different *Orobanche* species: *Orobanche aegyptiaca* and *O. ramosa* (Nandula et al., 1996). The observation that *O. crenata* is more sensitive than *O. ramosa* to sucrose application indicates that there are inherent differences between these closely related species in response to this carbohydrate.

O. crenata tubercles formation in presence of sucrose

Sucrose reduced significantly ($p < 0.05$) the number of nodes per root plant at all studied concentrations when compared to the control treatment (Table 1). The mean number of nodes per root length cm was 0.07, 0.03, 0.03 and 0.02 at 0.014, 0.044, 0.088 and 0.146 M sucrose

Table 2. Effects of different sucrose concentrations on dry weight of narbon bean plant under broomrape conditions and free broomrape conditions. Values within a column with no letters in common differ significantly at $p < 0.05$.

Sucrose concentration (M)	*Orobanche* non inoculated	*Orobanche* inoculated
0	0.51 a	0.49
0.014	0.27 ab	0.45
0.044	0.35 ab	0.36
0.088	0.41 ab	0.40
0.146	0.16 b*	0.38
CV	43.2	28.4

*Dead plants.

concentrations, being that of the control plate of 0.20 nodes per root cm. Sucrose exposure at the lowest concentration caused an effect statistically comparable to that caused at the highest one indicating that even the lowest sucrose concentration was enough to prevent tubercles formation in vitro when compared to the concentrations needed to inhibit the germination stage. In this sense, all the treatments with sucrose avoided *Orobanche* attachments at the time that prevented a successful seedling establishment and tubercle formation.

The avoidance of tubercles formation in the presence of sucrose can be explained by the fact that in *Orobanche*, the exogenous supply of sucrose might mimic the later stages of infection when the parasite is connected to the host vascular system. With the exogenous application of sucrose the parasitic seedling receives a carbohydrate as it does when penetrating the host root, thus preventing the activation of genes involved in attachment.

Avoidance of successful attachment of *Orobanche* to the host plant is a valuable mean for its control. In this sense, reports of inhibitory effects of nitrogen on the growth of *Orobanche* has been common in the literature for many years (Sauerborn, 1991; Parker and Riches, 1993) and field experiments were able to reduce the number of emerging *Orobanche* shoots with the application of ammonium sulphate and to increase the yield of faba bean significantly (Kukula and Masri, 1984). Moreover, the exogenous application of appropriate amino acids to a root zone might result in control of *O. ramosa* as reported by Vurro et al. (2006). Just recently Gonzalez-Verdejo et al. (2006) has also proposed sucrose as a possible control strategy for *O. ramosa* control.

Effect of osmotic potential

Since an appropriate osmotic potential plays a key role for seed germination, to determine if sucrose itself or changes in the osmotic potential after the treatments are responsible for the changes detected in the germination rate of *O. ramosa*, identical sucrose concentrations were also tested with sorbitol. In this study, while low sucrose concentrations significantly reduced seed germination in *O. crenata*, similar concentrations of sorbitol have no significant effect (Table 1) thus indicating that the effect of sucrose is not simply osmotic. This evidence has been also found by other authors when determining the effect of glucose against sorbitol and manitol in *Arabidopsis thaliana* (Price et al., 2003; Dekkers et al., 2004). In the case of *Orobanche*, Nandula et al. (1996) when studying the effect of nitrogen-containing nutrients also considered the osmotic potential as a mechanism for inhibition of germination in different species of *Orobanche* and concluded that although osmotic stress reduces germination in three different *Orobanche* species, *O. crenata* germination percentage seemed not to be affected by the osmotic potential.

Sucrose effect on the dry weight of narbon plants

According to the host plant, the effect of sucrose was tested by determining the dry weight of no inoculated and inoculated narbon bean plants. The mean values of the control (no sucrose added) and the tested concentrations (0.014, 0.044, 0.088 and 0.146 M) in both situations (inoculated and non-inoculated plants), were those showed in Table 2. Although in the case of no inoculated plates, significant differences were found among treatments with decreasing values of host dry weight with increasing sucrose concentrations, no significant differences were found for the same treatments in the inoculated plants. In this sense, even dead plants were observed in the 0.146 M treatment under broomrape-free conditions, evidencing a poorer growth on very high concentrations compared with moderate concentrations of sucrose due to osmotic stress. In contrast, we did not observe statistical differences among host plants under inoculated conditions, since broomrape parasite acts as a strong carbohydrate sink that reduced the damage provoked by sucrose.

Although the feasibility, effectiveness and application sucrose as a control strategy need to be determined, this work can be considered as an opening step in the study of sucrose as a regulator of broomrape germination. In the future, the determination of the best timing and concentration of sucrose as well as the evaluation of its application considering its stability and movement into the soil, in order to consider the potential of its direct application and to evaluate their influence in the environment, will be highly desirable. Moreover, although no phytotoxicity is evident in inoculated plants, considering the future use of sucrose as a field control mean, the determination of the minimum concentrations needed to control *Orobanche* will be of great importance since the carbohydrate seems to clearly interfer with the normal root development of non infested host plants and phytotoxicity could compensate the benefit of the control provided. Finally, differences in sucrose sensitivity between *O. crenata* and *O. ramosa* suggested in this study and that reported by González-Verdejo et al. (2006) caution against generalizing about the efficacy of *Orobanche* control by sucrose application.

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