Chitinase Expression in Strawberry Root Colonized by *Azospirillum Brasilense* and V. A. Mycorrhiza

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Abstract

Spores of *Glomus intraradix* and *Azospirillum brasilense* were inoculated on 5 varieties of Strawberry. In all case, both microorganisms develop chitinase expression on plants, finding that the highest values of this enzyme are produced when the inoculation is altogether with both microorganisms. Associated to this process it was found increments in the number of bacteria and nitrogenase activity with the dual inoculation.

Introduction

The obligate symbionts V. A. mycorrhizal fungi are exceptionally common among terrestrial flowering plants (Genderman 1968, Newman et al 1987). On the other hand, several studies have pointed out that *Azospirillum* colonizes many species of root plants (Döbereiner et al 1980, Okon 1985, Okon et al 1994), and that the dual inoculation produces a beneficial effect on crops.

The fungi involved in this symbiosis have chitin in their wall cell (Bomfante-Fasolo et al 1982), and, the bacterias have N-acetyl glucosamine, a monomeric component of chitin which has been implicated as an inductor in the interactions of the chitinase, and phytoalexine production as a response of defence against fungi, bacterias and virus which commonly invade the plants (Bowles 1990, Roby et al 1987, Metraux et al 1986, Schulumbaum et al 1986, Boller et al 1983).

The mycorrhizal fungus increases the chitinase activity (Spanu et al 1989) besides some other enzymes such as cellulase and pectinase (Bellone 1983, Bellone et al 1993). Equally, *Azospirillum* increases the pectinolitic and cellulolitic activity in root tissues by itself or combined with mycorrhizal fungi (Bellone 1983, Bellone et al 1993) but there is

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not information about the chitinase production during the interaction of both microorganisms. The pectic fragments obtained by enzymatic action are considerated to be responsible for the elicit chitinase accumulation (Broekart et all 1988) and defensive response regulation (Benhamou et al 1990).

In this experiment we examine the combined effect of VAM *G. intraradix* and *A. brasilense* in strawberry root plants and the chitinase production.

Methods

Five different varieties of strawberry: Chandler, Pájaro, Oso grande, Selva and Fern, were cultivated "in vitro" using the Murashige and Skoog media (1962). We used plants from meristems just in order to eliminate pathogens of the tissues, therefore to avoid a probable interference in the chitinase expression. Seedlings from the 5 varieties (5 month old) were inoculated with sterile spores of *G. intraradix* and with *A. brasilense*, both isolated from the rhizosphere of commercial crops.

Each seedling has got 100 spores and 1 x 10^5 bacterias cultured in NFb media (Döbereiner et al 1980). They have been in greenhouse under a day/night grade of 14/10 hs, with temperature of 27° C during the day and 20° C during the night. They were watered with sterile tap water and with Hoagland type nutrient solution once a week. The plants inoculated with both microorganisms and the controls were tested 4 week after the inoculation.

The infection produced by *Glomus* was estimated by the Giovaneti and Mosse method (1980) and *Azospirillum* by its presence in NFb media (Döbereiner et al 1980). The results are given as a percentage of root infected by the fungus and by the presence of *Azospirillum* in roots cultivated in NFb media.

For the chitinase assay, it was extracted from the roots with a 50 mM Tris buffer, adjusted to pH 8,0 with HCl, 500 mM NaCl and 0,2% of Triton X 100. The chitinase was assayed as described by Boller et al (1983), and the proteins were determined according to Bradford (1976), using bovine serum albumin as standard. All experiments were repeated 5 times, using for each treatment 10 replication. Nitrogenase activity was measured using the acetylene reduction assay (ARA) 15 day after of the inoculation. The plants were sealed into 200 ml bottles stoppered with suba seals and 10% acetylene was injected into

the bottles. After 24 h the ethylene concentration in the bottles was measured on a Carle gas chromatograph fitted with a 50 cm porapak column and a hydrogen flame ionization detector.

Results

Table 1. Chitinase and nitrogenase activity. Presence of *Azospirillum* and % of infection by *Glomus* in strawberry roots.

Treatment		Chandler	Pajaro	Oso grande	Selva	Fern
Mycorrhiza	(1)	32±12	42±10	38±14	28±11	36±14
(M)	(3)	325±53	230±32	279±48	317±40	265±58
Azospirillum	(2)	+	+	+	+	+
(A)	(3)	98±18	112±28	69±12	76±19	96±14
	(4)	22±4	18±6	20±8	30±12	16±16
	(1)	69±30	78±28	86±31	75±25	92±32
M + A	(2)	+	+	+	+	+
	(3)	462±69	342±78	378±61	441±69	392±65
Control		-	-	-	-	-

1:% of mycorrhiza - 2: presence (+) or absence (-) of *Azospirillum*.

3: Chitinase activity (nkat/mgr protein ± SE).

4: Nitrogenase activity (nM the etylene/mgr protein ± SE).

The plants express chitinase activity only by the induction of both microorganisms, as it is shown in table I.

Glomus colonized about 32 to 42% of the roots during the period of cultivation in greenhouse.

In plants inoculated with *Glomus* and *Azospirillum* altogether around 69 to 92% of the root length was colonized by *Glomus*, at the same time there was also an increment of the Nitrogenase activity.

Discussion

The roots of strawberry plants were extensively colonized by *Glomus* and *Azospirillum*, and that ability does not differ to much between the varieties used, and it was not affected by the chitinase expression studied here. The microscopical analysis did not reveal any conspicuous difference in the structure of hyphae, vesicles and arbuscules in any of the varieties tested. The induction of chitinase in strawberry plants, as well as some other enzymes such as proteinases and glucanases inducted by the same microorganisms in other plants, suggest that the signalling system, which induce and regulate these enzymes, would belong to several genes associated to the same process.

The different amount of chitinase induced by both microorganisms is due to the fungi structures, as they make possible to find more target regions to induce chitinase activity than the bacteria.

We still do not know the role of the inducer in the root system, whether it is involved directly in the chitinase production just because of their presence or whether by the substances they produce during the infection into the roots. According to this supposition we suggest that both microorganisms associated to strawberry plants can be able to use host defensive compounds as recognition cues. Thus, it seems possible to use the antifungal properties of chitinase production induced by *Glomus* and *Azospirillum*, without reducing their symbiotic potential.

Inductive expression of chitinase, in combination with other enzymes may provide a better protection against a wide range of pathogens.

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