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NODULATION OF FRANKIA-DISCARIA SYMBIOSIS AFTER INOCULATED PRECROPS

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The effect of preculture of actinorhizal (*Discaria americana*) and non-actinorhizal plants (wheat and canola) on the *Frankia-Discaria* symbiosis was studied. Factorial design was performed with two levels for the *Frankia* factor (presence/absence) and four levels for the precrop factor (wheat-canola-*Discaria* and bare soil). The infectivity parameters evaluated in *Discaria* plants were shoot length, dry weight of roots , shoots and nodules, and number of nodules.The results indicated that all three precrops significantly increased the parameters evaluated. Values were higher for *D. americana* as precrop and no differences were obtained between canola and wheat. The data suggest that infective capacity was increased in substrata where previously actinorhizal and non-actinorhizal plants had been cultivated. The possible causes of this increment are discussed.

Key words: *Brassica- Discaria- Frankia*-Nodulation-Rhamnaceae- *Triticum*

INTRODUCTION

Actinomycetes of the genus *Frankia* are nitrogen-fixing microsymbionts living within root nodules of eight *Dicotyledoneous* families (actinorhizal plants). Although a considerable amount of information is available on the physiology of *Frankia* in pure cultures and within the nodules, the nature and extent of its free living existence is still unclear (Benson, Silvester 1993). During the colonization stage of host plants (actinorhizal plants) a profuse development of *Frankia* in the rhizosphere has been observed (Diem *et al.* 1982; Cusato, Tortosa 1993) although a similar colonization of *Frankia* has been reported also in non-actinorhizal plants (Smolander *et al*, 1990; Ronkko *et al* 1993). The last may be the explanation of the fact that *Frankia* can survive and retain infectivity in soils devoid of actinorhizal plants (Arveb, Huss Danell 1988; Huss Danell, Frej, 1986) The objective of this paper was to further investigate whether actinorhizal and non-actinorhizal plants have any effect on the development of *Frankia* in the soil.

MATERIAL AND METHODS

The soil was a Typic Udipsament which, main characteristics in the upper 20 cm were: pH 7.5 (2.5 ml H_2O: 1g soil); organic carbon 0.15; electrical conductivity:0.30 dS m^{-1}; C:N ratio 7.5. Plastic pots (5 dm^3) containing a mixture of soil and vermiculite (1.5:8.5 by vol) were used. The soil utilized was collected from the rhizosphere of *D. americana* nodulated plants, living in their natural habitat at La Lucila del Mar (Provincia de Buenos Aires, Argentina, 36°05'S, 56°02'W). Samples were carried to laboratory in plastic bags, air dried 2 weeks later and stored for 90 days. The species utilized were wheat (*Triticum aestivum* L. cultivar Pampa INTA), canola (*Brassica napus* L. cultivar Samurai) and quina (*Discaria americana* Gill. et Hook.). Seeds of the latter had been also collected in La Lucila del Mar. Seedlings were surface sterilized (5% sodium hypochlorite, 15 min) and germinated at 28° C in sterilized Petri dishes. When the radicle had emerged, the young seedlings were transplanted to the pots. Seedling of *D. americana* for the second experiment were cultivated for seven days from germination to transplatation in the pots, in sterile glass tubes filled with complete nutritive solution. Pots were capped with aluminium foil with five small holes for the plants. Pots were placed in a greenhouse with a temperature range of 15-25° C, with a light photosynthetic photon flux density *ca.* 350 μMol m^{-2} s^{-1} 10 hours a day. Pots were drenched 2 times a week with nitrogen free nutritive solution which contained: KH_2PO_4, 0.5 *mM*; $MgSO_4 7H_2O$, 0.5*mM*; K_2SO_4, 0.75 *mM*; $CaCl_2$, 1*mM*; micronutrients (H_3BO_3 11.5 μM; $MnCl_2$ $4H_2O$, 2.3 μM; $ZnSO_4 7H_2O$, 0.2 uM; $CuSO_4$ $5H_2O$, 0.08 μM $NaMoO_4 2H_2O$, 0.03 μM; $CoCl_2 6H_2O$, 0.03 μM) and chelated iron ($FeSO_4 7H_2O$, 0.03 μM; Na_2 EDTA, 0.03 μM; pH 7). A complete solution was prepared by adding a nitrogen supplement solution (NH_4NO_3, 0.09 *mM*) to the nitrogen -free solution, final concentration. Soil contained in the pots was the *Frankia* inoculum (SFI). The presence of *Frankia* in the soil was previously tested, using *Discaria americana* seedlings as traps. Soil used in controls (SE) was previously drenched with water and sterilized by autoclave (3 succesive days at 120 °C, 30 min). Four treatments were performed with their respective controls1) Bare substratum with *Frankia* inoculum (SFI), 2) Substratum with *Frankia* inoculum (SFI) and wheat as precrop, 3) Substratum with *Frankia* inoculum (SFI) and canola as precrop,4) Substratum with *Frankia* inoculum (SFI) and *D. americana* as precrop. For controls of 1, 2, 3, and 4, the substratum contained sterilized soil (SE). Each treatment included five pots with five replicated plants. For this purpose *Discaria americana* (actinorhizal), wheat and canola (non actinorhizal) -called here precrops- were cultivated in pots with infected (with *Frankia*) soil. After harvesting, all pots were used to cultivate *Discaria americana,* and in these plants the infectivity and effectivity of *Frankia* were evaluated. Pots without plants and pots with sterilized soil were conducted in parallel as controls. Pretreatments were conducted for 48 days, drenched with sterile nitrogen free nutritive solution after which the plants were removed and the pots remained without any watering.

Table 1: Effect of different precrops on growth and nodulation of *D. americana* seedlings. SFI: substratum with *Frankia* inoculum; SE: substratum without *Frankia*.

Substratum	Precrop	Height (cm)	Seedlings Nod Root dry weight ([mg plant^{-1}]$^{0.213}$)	Shoot dry weight ([mgplant^{-1}]$^{0.322}$)	Nodules number (nodules.plant^{-1})	Dry weight (mg.plant^{-1})
SFI	*Discaria*	63.00 a	4.22 a	10.87 a	6.92 a	182.40 a
SFI	wheat	52.65 b	2.57 b	6.90 b	3.60 b	55.17 b
SFI	canola	59.45 a	2.56 b	7.15 b	3.86 b	70.68 b
SFI	bare soil	38.94 c	2.34 b	4.96 c	2.76 c	29.92 c
SE	*Discaria*	24.40 d	2.08 c	4.03 d	0	0
SE	wheat	19.45 d	2.05 c	3.73 d	0	0
SE	canola	20.70 d	2.07 c	3.85 d	0	0
SE	bare soil	20.68 d	1.88 c	2.93 d	0	0

Mean values in the columns followed by same letters are not significantly different from each other at P>0.05

A hundred and thirty days after precrop plants were uprooted, five *D. americana* seedlings were transplanted to each pot of the previous treatments. The cultivation of these plants were the same as indicated for precrops. At the end of the experiment, 116 days after trasplanting the seedlings, plants were removed and washed thoroughly, nodules counted and individual plants measured and weighed. For dry weight plants were treated at 70° C for 18 h. The following growth parameters were measured: number and dry weight of nodules, plant height and dry weight of roots and shoots. All data were analyzed by a two-way ANOVA (*Frankia*, precrops). Because of the non-normality of the data, two of the original variables were transformed so that Z (shoot dry weight) became $Z^* = Z^{0.322}$ and Y (root dry weight), $Y^* = Y^{0.213}$, before they were analyzed by F test (Montgomery 1991), then Duncan test was performed. Finally non parametric correlation (Tau coefficient of Kendall) between dry weight (root and shoot) and length on one side, and number of nodules and dry weight of nodules on the other side, were calculated. We have employed a non parametric statistic for correlation both, because of the non normality of the data and the low value of n. Moreover, Kendall's T is an efficient and easily calculable measure of correlation (Kendall 1975).

RESULTS AND DISCUSSION

The average of shoot length, dry weight (root, shoot and nodules) and number of nodules and the results of statistical analysis are showed in Table 1,. Presence of *Frankia* inoculum (SFI) gave higher values ($P<0.05$) in all treatments for the five variables studied than sterile soils (SE).

When *Frankia* inoculum was present, the shoot lenght, dry weight (root, shoot and nodules) and number of nodules variables resulted significatively higher for precrops than for bare soil, and higher when the precrop was *D. americana* than in the case of non actinorhizal species. Number of nodules and dry weight of nodules showed a positive correlation with the rest of parameters (Table 2). Whereas sterilized sustratum was used no nodulation ocurred and no differences among treatment resulted (Table 1).

Our experiment showed that both host and non host-precrops increased the nodulation rate in the *Frankia - Discaria* symbiosis, although response was higher when precrop was an actinorhizal plant. The positive correlation between number of nodules and the rest of parameters demonstrated the efectivity of nodulation in *D. americana*. Root exudates, with a great variety of carbon sources (Smith 1976), could be responsible for an increment of *Frankia* biomass and/or for an increase of the physiological activity of *Frankia* in the rhizosphere (Mehgard, Killham 1995). In turn these seem to be the causes of the relative high rates of nodulation observed after precrops culture.

Table 2: Kendall Tau correlation coeficients between the traits

Variables	Number of nodules	Dry weight of nodules
Heigth	0.474	0.500
(1) Shoot dry weight	0.534	0.610
(1) Root dry weight	0.548	0.670

(1) data transformed, see Table 1
All coefficients resulted significative at 1% level

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