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PROSPECTS FOR EVALUATION OF *FRANKIA* –*CASUARINA* ASSOCIATION UNDER EGYPTIAN CONDITIONS II- INTERACTING EFFECTS OF *FRANKIA* WITH *CASUARINA* SPECIES, SOIL TYPES AND VA MYCORRHIZAL INOCULATION

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ABSTRACT

The interacting effects of four isolates (UF010,UF011,UF012 and UF013) or two reference strains (UF001 and UF002) of *Frankia* and VA mycorrhizas on growth, nodulation and N_2-fixation of *C. glauca* and *C. cunninghamiana* were evaluated in loamy sand and clay soil. In the two soils, seedlings representing the two species of *Casuarina* were either uninoculated or inoculated with *Frankia*, VAM or both where VAM inoculum preceded or followed that of *Frankia*. Data showed that all studied factors seemed to influence the performance of the 2 above mentioned hosts. Generally, the performance of inoculated *Casuarina* was greater than those of uninoculated control plants. This finding was more obvious in loamy sand soil compared with the clay one and with *Frankia* UF013 applied solely or when conjugated with either of inoculation schedule of VA mycorrhizas. On the other hand, application of *Frankia* UF010 or UF011 to *C. glauca* and UF001 to *C. cunninghamiana* prior to VA mycorrhizas developed higher number and dry weight of nodules than the opposite treatment. Athought it was also the case for N_2-fixation, significant amounts of acetylene were reduced with *Frankia* applied singly or followed with VAM. Mycorrhizal infection did not show significant difference due to soil type, single or dual inoculation treatment.

Key words: *Frankia* VA Mycorrhizas, *Casuarina*, Inoculation, Nodulation, N_2-fixation, Dual inoculation.

INTRODUCTION

Actinorhizal plants, originating from diverse geographical locations and habitats (**Chouglu, 1990; El-Lakany, 1990; Merwin, 1990; Midgley, 1990; Baker & Mullin, 1992 and Selim 1995**) have a wide range of potential use in forestry. The genus *Casuarina,* is by far the most intensively studied one and species of this genus are reported to be largely responsible for high levels of soil nitrogen

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worldwide **(Chonglu, 1990; El-Lakany, 1990; Merwin, 1990; Midgley, 1990; Diem & Dommergues, 1990; Baker & Mullin, 1992 and Selim & Schwencke 1995)**. Interestingly, *Casuarina* plants depend on symbiotic associations between their roots and beneficial soil microorganisms for successful establishment and growth. Two of these symbioses are crucial for tree growth in infertile soil, nitrogen-fixing nodules developed by *Frankia* **(Midgley, 1990; Baker & Mullin, 1992 and Selim 1995)** and vesicular arbuscular mycorrhizal root infection, **(Gardner, 1986; Russo, 1989 and Diem, 1996)** Both associations function to enhance growth by increasing the nutrient supply available to the tree **(Lumini *et al* 1994)**. However, the tripartite system of plant, nodules and mycorrhizas is one of the most complex symbiotic associations **(Russo 1989 and Diem, 1996)**. Each endophyte interacts with the other and the host plant with each microorganism **(Lumini *et al* 1994; Diem, 1996 and Hurd and Schwintzer 1997)**.

This paper comprise data on growth, nodulation and N_2-fixation of *C. glauca* and *C. cunninghamiana* as influenced by inoculation with four isolates and two strains of *Frankia* and/or VA mycorrhizas in clay and loamy sand soil.

MATERIAL AND METHODS

Soils and seeds

Two representative samples of loamy sand and clay soil were collected from El-Bostan location, Beheira Governorate and King Mariout city, Alexandria Governorate, respectively. The samples were air dried, ground to pass 2 mm sieve, mixed thoroughly and analyzed for chemical and physical properties (Table 1).

Seeds of *C. glauca* and *C. cunninghamiana* were kindly provided by Desert Development Center (DDC), American university in Cairo, Egypt, to be used in this study.

Raising of *Casuarina* seedlings

Seeds of the two species of *Casuarina* were surface sterilized by immersing for 2 min in concentrated H_2So_4, and then washed with sterile water until reaching to neutral pH **(Selim and Schwencke, 1995)**. Two month old seedlings were transferred into 20 cm diameter pot (two plants), irrigated for at least 2 weeks (twice a week) with ¼ Hoagland solution with (NH_4^+) and then 3 weeks with NH_4^- free ¼ Hoagland solution under greenhouse condition **(Selim & Schwencke, 1995)**.

Frankia and VA mycorrhizal inocula

Four *Frankia* isolates **(Eweda *et al* 2003)** and two reference strains identified by **Selim (1999)** were used for inoculating the 2 above-mentioned species of *Casuarina*. The designations and origins of those strains are given in Table (2). *Frankia* inocula were prepared by inoculating 1 μg of mycelial protein **(Bradford, 1976)** per ml of-BAP liquid medium **(Fontaine *et al* 1986)**. The inocula composed of exponentially growing *Frankia* cells which were disrupted 5-6 times by a 0.6 mm sterile needle. Cultures were incubated at 28±2°C for 5 days under stirred conditions, then washed and centrifuged (5000 rpm for 15 min) in NH_4^+ free BAP liquid medium and once in Hoagland solution without N-source

Table 1. Some mechanical and chemical analysis of soils used in the greenhouse experiments.

Mechanical analysis

	Sand %	Silt %	Clay %	Soil texture	PH 1:2.5	E.C mmhos cm^{-1}	$CaCO_3$ %
Type (1)	85.52	4	10.48	Loamy sand	8.3	0.16	3.2
Type (2)	35.52	16	48.48	Clay	8.0	0.85	40.8

Chemical analysis*

	Cations mg L^{-1}				Anions mg L^{-1}			Macroelements ppm				Microelements ppm		
	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	SO_4^{--}	Cl^{-}	CO_3^{--}	N	P	K	Fe	Cu	Zn	Mn
Type (1)	0.8	0.3	0.87	0.24	0.51	0.5	1.2	12	1	100	10.8	0.02	0.4	0.4
Type (2)	3.4	2	2.75	0.96	6.63	1.5	1	15	4	356	1.0	0.14	0.24	0.2

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Table 2. Origin of *Frankia* used in this study

Frankia isolates	Original host	Geographical origin	Reference
UF 010	*C. glauca*	Sadat City	**Eweda *et al* 2003**
UF 011	*C. glauca*	Nobaria City	**Eweda *et al* 2003**
UF 012	*C. cunninghamiana*	Cairo –El-Zagazig rood	**Eweda *et al* 2003**
UF 013	*C. cunninghamiana*	Kafr El-Sheikh	**Eweda *et al* 2003**
UF 001	*C. glauca*	Ismailia	**Selim 1999**
UF 002	*C. glauca*	Zagazig	**Selim 1999**

(**Hoagland and Arnon, 1938**). Cells were resuspended in the same solution and were homogenized by 0.6 mm sterile needle.

VA mycorrhizal spores were extracted by wet sieving and decanting technique (**Gerdemann and Nicolson, 1963**) from soil around the roots of wheat grown on the Expermental Field, Fac. Agric., Ain-Shams Univ., Cairo, Egypt. The spores used represented two main genera of VAM, i.e, *Glomus* and *Gigaspora*.

Experimental techniques

Interactions among *Frankia*, *Casuarina* and VA mycorrhizas in two soils

A green house pot experiment was conducted in the Unit of Biofertilizers, Fac. Agric., Ain-Shams University to study the effects of inoculation with *Frankia* alone or conjugated with VA mycorrhizas at 2 reversed application (before and after *Frankia* inoculation) on the performance of 2 species of *Casuarina* in 2 different soils. For this purpose, polyethylene bags (20x30cm) with 5kg capacity were packed with either of loamy sand or clay soil. Two month old seedlings of *C. glauca* or *C. cunninghamiana* were transplanted into polyethylene bags contained either of the 2 tested soils. Seedlings were fed with Hoagland solution containing NH_4+ for 2 weeks and for 3 weeks with the same solution without NH_4+ (**Selim and Schwencke, 1995**). *Casuarina* plants were then inoculated with either of the tested *Frankia* by adding 20 µg of the mycelial protein /plant into holes (3 cm depth) at soil around the stem (**Selim and Schwencke, 1995**). *Frankia* inocula were applied either singly or in conjugation with 2 ml of VAM spores (contained 50 spores/ml) in 2 variable timing to give the following treatments.

i) Inoculation with *Frankia* with planting (F)
ii) Inoculation with VAM with planting (M)
iii) Inoculation with VAM after 7 days from *Frankia* inoculation (FM)
iv) Inoculation with VAM 7 days before *Frankia* inoculation (MF)
v) Uninoculation (Control)

The above-mentioned treatments were arranged in complete randomized design with 10 replicates. Developed plants were fed with Hoagland solution without N source (**Selim and Schwencke, 1995**) for 6 months.

At the end of the experiment period *Casuarina* plants grown under different treatments were harvested to record: shoot height (cm/plant), total dry weight (g/plant), nodulation frequency (%), number of nodules/plant, dry weight of nodules (mg/plant), acetylene reduction assay (Hardy et al., 1968) expressed in n moles C_2H_4/h/plant and percentages of mycorrhizal root infection (**Phillips and Hayman, 1970**). The results were statistically analysed according to **Snedecor and cochran (1969)**.

RESULTS

The interacting effects of *Frankia* and / or VA mycorrhizas on performance of *C. glauca* and *C. cunninghamiana* in two soils

Growth

Results presented in Table (3) show that shoot length of the 2 species of *Casuarina* inoculated with *Frankia*

Table 3. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on shoot length (cm/plant) of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	44.0	57.3	34.0	52.7	39.0	26.8	76.8	66.8	65.8	60.3	72.0	71.0
UF011	50.0	52.3	70.8	65.8	67.0	38.0	64.5	63.0	78.3	53.8	43.0	62.0
UF012	56.0	71.8	41.5	60.5	71.0	62.5	62.0	58.3	59.8	65.3	71.3	59.0
UF013	52.5	58.8	66.3	57.0	67.3	58.3	97.5	71.0	82.3	91.0	70.3	75.5
UF001	70.3	40.5	52.5	36.3	61.3	50.8	53.0	60.5	65.5	56.3	61.3	65.3
UF002	73.8	55.0	47.3	67.0	75.3	82.0	62.8	70.5	62.5	57.3	71.5	71.5
Control		30.3			29.3			40.5			46.7	
M		64.5			57.5			66.0			54.5	

		1%	5%		1%	5%
LSD	Soil	2.9	2.05	Soil x Plant	4.1	2.91
	Plant	2.9	2.05	Soil x treatment	13.03	9.22
	Treatment	9.19	6.49	Plant x treatment	13.03	9.28
				Soil x plant x treatment	18.37	13.03

* Control without inoculation

F : inoculated with *Frankia*

FM : Inoculated with *Frankis* and after 7 days with mycorrhizae

M : inoculated with mycorrhizae

MF : Inoculated with mycorrhizae and after 7 days with *Frankia* and/or VAM and grown in loamy sand soil were significantly higher than those of plants grown in clay soil. Among *Frankia* isolates, the UF012 was particularly superior to other tested isolates when applied solely or proceeded with VAM to *Casuarina glauca* grown on the clay soil (56.0 and 71.8 cm/plant, respectively). This observation was only recorded with dually inoculated *Casuarina cunninghamiana* grown on the same soil. Application of VAM following to inoculation of *C. glauca* with the same *Frankia* isolate gave lower records of shoot lengths 41.5 cm/plant. However, the view was changed in the loamy sand soil where higher records of shoot lengths were obtained from *C. glauca* and C. *cunninghamiana* inoculated with *Frankia* UF013 solely. The recorded figures under the above mentioned conditions were 97.5 and 91.0 cm/plant respectively. The same finding seemed to be also true, but to lower extents, with the 2 other inoculation pattern, i.e. MF and FM being more pronounced with the latter treatment. The recorded figures were 71.0, 82.3 and 70.3, 75.5 cm /plant for *C. glauca* and *C. cunninghamiana* inoculated with *Frankia* UF013 preceded and flowed by VAM, respectively. Records of total dry weights of *Casuarina* inoculated with *Frankia* were generally greater than those of uninoculated control plants (Table 4) and also for plants grown on loamy sand soil compared with the clay one. Again, the dry matter contents of *C. glauca* grown on clay or loamy sand soil, being higher in the latter soil and inoculated with *Frankia* UF013 solely or conjugated with VAM were greater than other treatments. The recorded figures were 2.3, 5.07. and 3.22 g/plant against 10.71,7.51 and 8.55 g/plant for *C. glauca* inoculated with *Frankia* UF013 (F) and when preceded or followed by VAM in the clay and loamy sand soil, respectively. The pronounced performance of *C. cunninghamiana* with the same above-mentioned isolate of *Frankia* solely or conjugated with VAM was also true in the loamy sand soil. However, the UF012 appeared to be more effective in the clay soil.

Nodulation and N_2-fixation

It is obvious from data recorded in Table (5) that no significant differences were observed in number of nodules due to soil type or tested *Frankia*. The highest number of nodules were obtained from *C. glauca* and *C. cunninghamiana* plants grown on the clay soil and inoculated with VAM with planting followed with *Frankia*-UF010 or UF001, respectively. On the other hand, application of *Frankia* prior to VAM developed higher number of nodules on plants grown on clay soil, than the opposite inoculation treatment. This observation was shown in *C. glauca* inoculated with *Frankia* UF010 or UF011 and also in *C. cunninghamiana* inoculated with strain UF001. The percentages of seedling that formed root nodules (nodulation frequency) were generally higher in clay soil than in loamy sand except for *Frankia* UF002 (Table 6). The above mentioned strain gave higher nodulation frequencies on the roots of the 2 hosts in loamy sand soils in the presence or absence of VAM. The dry weights of nodules obtained from *C. glauca* and *C. cunninghamiana* grown on clay soil and inoculated with VAM followed with *Frankia* UF011 and UF001 were particularly distinguishable being 1485 and 1950 mg/plant, respectively. Also, *C. cunninghamiana* inoculated solely with *Frankia* UF012 and grown on

Table 4. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on the total plant dry weight g/plant of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	1.97	3.69	2.09	2.13	1.06	1.35	9.71	6.68	5.86	4.38	5.73	5.06
UF011	1.89	1.63	2.42	2.07	3.46	1.49	4.95	3.64	14.26	4.30	2.69	3.66
UF012	2.06	4.48	2.23	3.86	5.45	4.75	3.61	4.16	7.79	4.34	7.25	4.95
UF013	2.30	5.07	3.22	2.62	3.66	2.08	10.71	7.51	8.55	8.44	5.03	7.10
UF001	6.52	6.47	4.49	1.32	3.07	2.30	2.79	3.91	5.00	2.34	4.50	4.91
UF002	4.56	1.87	1.51	2.55	4.61	7.08	2.70	4.02	5.08	3.93	8.55	4.13
Control		1.41			2.41			2.00			2.11	
M		2.90			1.99			9.38			7.40	

		1%	5%		1%	5%
LSD	Soil	0.54	0.38	Soil x Plant	0.76	0.54
	Plant	0.54	0.38	Soil x treatment	2.39	1.69
	Treatment	1.69	1.197	Plant x treatment	2.39	1.64
				Soil x plant x treatment	3.39	2.39

* Control without inoculation
F : inoculated with *Frankia*
FM : Inoculated with *Frankis* and after 7 days with mycorrhizae
M : inoculated with mycorrhizae
MF : Inoculated with mycorrhizae and after 7 days with *Frankia*

Table 5. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on the number of nodules per plant of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	1.7	14.3	1.7	1.0	3.0	1.3	5.3	3.3	3.3	1.0	1.0	1.3
UF011	4.3	14.3	2.3	1.0	2.0	2.0	1.0	1.0	1.0	7.7	2.3	1.0
UF012	4.7	6.3	1.3	12.3	5.7	3.7	1.3	2.3	2.3	2.3	2.0	2.0
UF013	2.7	2.0	2.3	1.0	2.7	3.0	2.0	2.7	3.0	2.3	4.0	11.7
UF001	8.7	5.0	4.3	2.0	14.7	1.0	1.7	7.0	1.7	3.0	3.7	1.7
UF002	2.0	1.0	1.0	1.7	2.7	1.0	6.0	3.7	3.0	3.7	4.7	3.7
Control		0.0			0.0			0.0			0.0	
M		0.0			0.0			0.0			0.0	

		1%	5%		1%	5%
LSD	Soil	1.3	0.9	Soil x Plant	1.81	1.28
	Plant	1.3	0.9	Soil x treatment	5.44	3.85
	Treatment	3.85	2.72	Plant x treatment	5.44	3.85
				Soil x plant x treatment	7.69	5.44

* Control without inoculation
M : inoculated with mycorrhizae
F : inoculated with *Frankia*
MF : Inoculated with mycorrhizae and after 7 days with *Frankia*
FM : Inoculated with *Frankis* and after 7 days with mycorrhizae

Table 6. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on the nodulation frequency of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	40	100	50	30	60	30	60	80	30	10	20	30
UF011	80	100	60	30	20	30	20	20	20	80	30	20
UF012	100	100	30	60	60	60	30	30	60	40	60	60
UF013	50	30	30	20	30	40	30	30	30	30	30	80
UF001	80	60	60	60	60	20	30	80	30	50	60	30
UF002	50	30	30	60	50	50	100	60	100	60	50	100
Control		0			0			0			0	
M		0			0			0			0	

		1%	5%		1%	5%
LSD	Soil	0.54	0.38	Soil x Plant	0.76	0.54
	Plant	0.54	0.38	Soil x treatment	2.39	1.69
	Treatment	1.69	1.197	Plant x treatment	2.39	1.64
				Soil x plant x treatment	3.39	2.39

the clay soil gave high record of nodules dry weights being 1029.3 mg/plant Table (7). Lower amounts of dry matter were observed in the 2 hosts when grown on the loamy sand soil. Under that condition, the highest nodule dry weight was obtained from *C. cunninghamiana* inoculated with *Frankia* UF013 followed by VAM being 866.3 mg/plant. This superiority of FM inoculation pattern in enhancing nodule dry matter content of *C. cunninghamiana* was shown by the same above-mentioned isolate applied alone but to a lower extent being 640.0 mg/plant (Table 7).

Data presented in Table (8) show that acetylene reduction activity significantly differed according to soil type and plant species in favor of loamy sand soil and *C. glauca*. However, application of VAM with planting followed with *Frankia* induced higher levels of N_2 – ase activity than application of *Frankia* alone or *Frankia* followed with VAM .The highest activities of acetylene reduction were obtained from *C. glauca* plants grown on loamy sand soil inoculated with VAM followed with either *Frankia* UF010, UF011, UF012 or UF002 being 8482.9; 7776.5; 5145.0 and 4801.3 nmol C_2H_4 h plant respectively. The corresponding figures in clay soil were, 9267.9; 10692.6 and 4429.9; 746.9 nmol C_2H_4 /hr/ plant in the same above – mentioned respective order. Relatively pronounced acetylene reduction activities were observed in *C. cunninghamiana* when inoculated with *Frankia* UF012 solely or combined with VAM in the 2 inoculation pattern. The recorded figure were 7610.7, 4667.0 and 2406.6 nmolC_2H_4/h/plant for the UF012, F, MF and FM, respectively. Although, the latter inoculation treatment induced lower level of N_2-fixing activity in the clay soil, it was the most effective in the loamy sand soil as it gave 5671.9 nmol C_2H_4/h/plant.

Mycorrhizal infection

No significant differences were observed between VAM infection percentages due to soil type or single inoculation with VAM compared with dual inoculation of VAM plus *Frankia* (Table 9). However, distinguished percentages infection were shown in the roots of *C. glauca* inoculated with *Frankia* UF012 followed by VAM in the loamy sand soil being 61%. The same level was also recorded in the roots of *C. glauca* with the same above-mentioned inoculation pattern but under clay soil condition. Application of VAM prior to *Frankia* seemed also to give similar level of infection development. This finding was observed when VAM inoculation preceded *Frankia* UF010 or UF001 applied to *C. glauca* grown in clay and loamy sand soil, respectively. This inoculation pattern induced the lowest level of infection in *C. cunninghamiana* inoculated with the UF012 isolate of *Frankia* being 28 and 38 % in clay and loamy sand soil, respectively.

DISCUSSION

Success in introducing *Casuarina* in poor soil is often impeded by both nitrogen and phosphate deficiency. This deficiency can be overcome by adding fertilizers or ensuring the establishment of effective symbioses by inoculating the plants with compatible endophytes *Casuarina* is among one of the most important genus of actinorhizal plants that is capable of fixing N by virtue of root nodules

Table 7. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on the dry weight of nodules per plant (mg) of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	172.6	327.0	25.3	13.5	154.6	20.4	64.3	99.1	235.2	10.7	8.60	47.2
UF011	368.7	148.5	267.0	85.0	179.0	176.7	8.2	8.5	8.3	177.0	43.7	14.6
UF012	121.4	281.5	24.7	1029.3	148.2	180.0	44.7	59.7	201.7	192.7	269.7	206.0
UF013	94.0	234.3	200.7	58.7	209.7	199.5	325.7	162.3	190.5	640.0	502.0	866.3
UF001	125.7	143.7	549.7	44.8	1950	20.4	89.0	610.0	360.3	225.0	265.7	166.3
UF002	68.0	92.3	47.7	45.7	773.0	22.63	195.0	161.3	71.3	82.7	467.3	197.7
Control		0			0			0			0	
M		0			0			0			0	

		1%	5%		1%	5%
LSD	Soil	69	49	Soil x Plant	97	69
	Plant	69	49	Soil x treatment	119	84
	Treatment	84	59	Soil x strains	168	116
	Strains	119	84	Plant x treatment	119	84
	Plant x Strain	516.8	119	Soil x Plant x Treatment	504	357

* Control without inoculation
M : inoculated with mycorrhizae
F : inoculated with *Frankia*
MF : Inoculated with mycorrhizae and after 7 days with *Frankia*
FM : Inoculated with *Frankis* and after 7 days with mycorrhizae

Table 8. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on acetylene reduction activity (ARA) n molC_2H_4/h/plant root of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	713.5	9267.9	5437.7	651.3	1800.85	881.425	3137.0	8482.9	2610.35	790.6	790.45	970.28
UF011	2171.8	10692.6	1532.9	748.7	1327.95	1337.05	841.35	7776.5	3460.65	4222.4	1624.05	840.8
UF012	24090.7	4429.9	900.93	7610.7	4667.0	2406.6	1068.0	5145.0	1635.9	1398.1	1480.13	5671.9
UF013	1762.2	1291.5	1548.6	1055.3	1876.6	1717.5	1512.5	1425.3	1800.5	1471.0	2015.9	1487.1
UF001	3912.3	3630.0	2836.0	1382.7	1216.2	796.5	1006.1	1208.8	5273.8	2215.5	2371.6	2541.3
UF002	1509.5	746.9	698.8	1239.5	806.0	856.8	656.1	4801.3	3276.0	2914.9	2926.03	2555.5
Control		0			0			0			0	
M		0			0			0			0	

		1%	5%		1%	5%
LSD	Soil	134.47	95.1	Soil x Plant	190.17	134.49
	Plant	134.47	65.1	Soil x treatment	232.91	164.72
	Treatment	164.69	116.48	Soil x strain	329.39	232.95
	Strains	232.91	164.72	Plant x treatment	232.91	264.72
	Plant x strain	329.39	232.95	Soil x plant x treatment	806.84	570.61

Table 9. Effect of inoculation with different isolates and strains of *Frankia* and/or mycorrhizae on % infection by mycorrhzae of *C. glauca* and *C. cunninghamiana* grown on clay and loamy sand soils for 6 months.

	Clay						Loamy sand					
	C. glauca			*C. cunninghamiana*			*C. glauca*			*C. cunninghamiana*		
	F	MF	FM	F	MF	FM	F	MF	FM	F	MF	FM
UF010	0	47	48	0	62	56	0	47	43	0	30	50
UF011	0	52	50	0	44	48	0	52	58	0	47	53
UF012	0	48	44	0	28	54	0	53	61	0	38	46
UF013	0	48	38	0	54	61	0	48	42	0	48	56
UF001	0	38	50	0	58	47	0	61	47	0	50	42
UF002	0	52	38	0	48	49	0	42	45	0	46	42
Control		0			0			0			0	
M		47			60			38			50	

		1%	5%		1%	5%
LSD	Soil	1.21	0.86	Soil x Plant	4.396	3.11
	Plant	1.21	0.86	Soil x treatment	2.11	1.49
	Treatment	3.12	2.196	Plant x treatment	2.11	1.49
				Soil x plant x treatment	6.21	4.39

formed by the actinomycete *Frankia*. The roots of *Casuarina* species have been also found to support vesicular arbuscular mycorrhizas and ectomycorrhizas in addition to nitrogen fixing nodules (**Diem *et al* 1981**).

The symbiotic fixation of atmospheric N_2 by *Frankia* in root nodules forming on *Casuarina* species is one attribute which make these trees important for land reclamation in infertile soils of the tropics, agroforestry and fuelwood production (**Midgley *et al* 1983**). Studies conducted by **Dommergues (1963) and Gauthier *et al* (1985)** on plantings of *C. equisetifolia* under N-deficient conditions of coastal dunes had estimated fixation rates of 40-60 Kg N $ha^{-1}yr^{-1}$. Other studies had demonstrated that the amount of N_2 fixed in nodules of *Casuarina* species can vary considerably depending upon the strain of *Frankia* (**Reddell and Bowen, 1985**), the host tree genotype (**Sougoufara *et al* 1987 & 1989 and 1992**) and soil characteristics such as P status (**Reddell *et al* 1986 and Selim *et al* 2000**), moisture content (**Kant and Narayana, 1978**) and other root associated microorganisms such as mycorrhizal fungi (**Diem and Gauthier, 1982**) which also reflected on plant growth rates.

Differences between isolates of *Frankia* in their abilities to increase the growth of trees from one provenance of *Casuarina cunninghamiana* were reported by **Reddell *et al* (1988)**. In that study, *Frankia* ORS020607 was much more effective compared to the other strains of *Frankia* in stimulating tree growth and height of these plants being almost 3 times that of the uninoculated treatment. These results are in agreement with those reported in this study where *Frankia* isolates UF012 and UF013 were more effective than other tested *Frankia*. The overall data demonstrate the potential to increase growth of *Casuarina* by selection of effective strains of *Frankia* for use in nursery inoculation programs.

Vesicular arbuscular mycorrhizas are association that function to increase the efficiency of soil nutrient use by wide range of host plants including *Casuarina* trees. In an experiment conducted by **Diem and Gauthier (1981)** using seedlings of *C. equisetifolia* inoculated with *Frankia*, *G. mossease* or both and grown on a sterile soil containing only 10 ppm available P, the number of nodules and the total N of shoots were more than twice as great in dully inoculated plants as in plants inoculated with *Frankia* alone. It is well documented that the intimate physiological association existing between actinorhizal plants and their nodular endophyties could be enhanced by VA mycorrhizal infection due to increased phosphate uptake. This profound advantage could establish effective nodulation and N_2–fixation which result in overall enhancement of plant growth.

The pronounced increase in shoot height and dry matter in *Casuarina* plants inoculated with *Frankia* and/or mycorrhizas could be attributed to stimulation of the nodule development and N_2-fixing activity of those endophytes and their ability to modify the metabolism of auxin, gibberellins and cytokinens in actinorhizal root nodules (**Miguel *et al* 1978 and Diem, 1996**). In this study, the timing of inoculating *Casuarina* seedlings with *Frankia* and VA mycorrhizal fungi was investigated to verify the nature of their interaction at early stages of symbiotic association. Application of *Frankia* prior to VA mycorrhizal (FM) induced good responses on growth, nodulation, and N_2-fixation than opposite treatment (MF). However, mycorrhizal infection did not show significant differences due to studied factors, i.e., host species, soil type and *Frankia* / mycorrhizal inoculation. In this respect, the study of **Diem (1996)** showed no competition between *Frankia* and VA mycorrhizas for infection sites of tree roots.

Further studies are needed to achieve an improved understanding of other aspects associated with this symbiotic system.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(١) ، ١٢١ - ١٣٨ ، ٢٠٠٣

اتجاهات تقيم تكافل الفرانكيا و الكازوارينا تحت الظروف المصرية
٢- التأثيرات المتداخلة للفرانكيا مع نوع الكازوارينا , التربة والتلقيح بفطريات الميكورهيزا المكونة للحويصلات والتفرعات الشجرية

[١٠]

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تم فى هذا البحث دراسة التأثيرات المتداخلة للتلقيح بكل من أربعة عزلات من الفرانكيا هى UF010, UF011, UF012,UF013 وكذلك السلالاتين المرجعيتين UF001, UF002 منفردة أو جراثيم فطريات الميكورهيزا للحويصلات والتفرعات الشجرية أو خليطهما على النمو وتعقيد الجذور وتثبيت الأزوت الجوى فى نوعى الكازوارينا *C. cunninghamiana*, *C. glauca* النامبين في تربة رملية طميية وأخرى طينية وفى معاملة التلقيح المزدوج تم دراسة توقيت التلقيح من خلال فارق زمني يفصل بين التلقيح بالفرانكيا الميكورهيزا أو العكس وقد أظهرت النتائج أن كل العوامل مجال الدراسة لها تأثير على أداء نوعى الكازوارينا.

وبصفة عامة تفوقت الكازوارينا الملقحة عن تلك الغير ملقحة ولوحظ ذلك بصورة وأكثر وضوحاً فى التربة الرملية الطميية مقارنة بالتربة الطينية وكذلك باستخدام *Frankia* UF013 بصورة منفردة أو مع جراثيم الميكورهيزا بغض النظر عن أسبقية التلقيح ومن ناحية أخرى فان تلقيح بادرات *C. glauca* بواسطة *Frankia* U F010 , UF011 وبادرات *C. cunninghamiana* بواسطة *Frankia* UF001 سابقاً للقاح الميكورهيزا أدي إلى زيادة في الأعداد والأوزان الجافة للعقد الجذرية بالمقارنة بالمعاملة العكسية وعلي الرغم من هذا كان أيضا ملحوظاً بالنسبة لقياسات تثبيت الأزوت الجوي فأن كميات معنوية من ألاستلين قد اختزلت في معاملات التلقيح بالـ *Frankia* منفردة أو سابقة للقاح الميكورهيزا وعموما فان مستويات الإصابة بالميكورهيزا لم تظهر فروق معنوية نتيجة اختلاف نوع التربة أو ما إذا التلقيح منفرداً أو مزدوجا.

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