J. Agric. Sci. Mansoura Univ., 33(11): 8361 - 8383, 2008

ASSESSMENT OF CULTURAL, BIOCHEMICAL AND GENETIC CHARACTERISTICS OF *FRANKIA* ISOLATES, THEIR NODULATION AND SYMBIOTIC NITROGEN FIXATION WITH *CASUARINA* TREES

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ABSTRACT

The microsymbiont *Frankia* genus belongs to the order of filamentous bacteria, the actinomycetales, family *Frankiaceae*, is Gram-positive bacteria fixing atmospheric nitrogen which forms symbiotic association with actinorhizal *Casuarina glauca*. *C. glauca* is an important introduced tree species in Egypt, valued for windbreaks and shelterbelt; land stabilization and soil improvement.

The present study concerned with the phenotypic, ecological and genetic characters; symbiotic competence and host specificity of some local *Frankia* isolates with *Casuarina* trees.

- Sixteen native *Frankia* pure isolates were isolated from root nodules of *Casuarina* trees, collected from different ecological Egyptian soils and their characteristics assessed in comparison with foreign reference *Frankia* isolates.
- *Frankia* isolates were grown well in synthetic nutrient medium. Vegetative hyphae, vesicles; sporangia and reproductive torulose hyphae (RTH) were observed. According to their physiological properties and plasmids number it was found that the isolated *Frankia* were identified as fifteen different *Frankia* isolates belonging to *Frankia Casuarina*. The characterization of *Frankia* isolates at DNA level revealed that existence of a large diversity with the exception of two isolates that were completely identical in all parameters using RFLP analysis.
- Inoculation of *Casuarina* seedlings with the various local *Frankia* isolates induced nodulation (nodules number and dry weights) and enhanced their vegetative growth parameters (stem lengths, branches and shoots dry weight) over the foreign reference strain.
- Some environmental factors included resistance to different antibiotics, neutral salinity and various C-sources utilization were investigated well in comparison with imported foreign reference strain.
- Performance symbiotic N_2-fixation of various indigenous *Frankia* isolates were judged by the percent shoots and roots N-content and N-uptake (mg/seedling) of *Casuarina* seedlings cultivated in both calcareous and sandy soils. Efficacy of *Casuarina* seedlings to N_2-fixation was significantly increased upon inoculation with the most local *Frankia* isolates, which resulted in augmentation of N-content and uptake over the reference strain.
- Highly efficient, native indigenous *Frankia* isolated from Kafr El-Sheikh and those from Ismailia regions identified as salinity tolerant, characterized by well intrinsic antibiotics resistance, showed greatly response to different C-sources and displayed highly symbiotic competence and effectiveness on *Casuarina glauca*, which surpassed the imported foreign reference *Frankia* strain.

Therefore, such isolates were strongly recommended to be applied as inocula for *Casuarina* species grown in Egyptian soils.

The obtained data clearly emphasize the importance of isolation of proper *Frankia* strains for high compatible, resistant processed inocula for *Casuarina* cultivated under ecological conditions similar to initial from which they were isolated i.e. initial niches.

Keywords: *Frankia*, *Casuarina*, isolation, nodulation, actinorhizal plants, antibiotics, plasmids, microsymbionts; and symbiotic N_2-fixation.

INTRODUCTION

Efforts are made to increase and introduce N_2-fixing trees to Egyptian agriculture practices. Also, the erosion control and sustaining the urban and rural conditions needs efficient soil stabilization which mainly can be achieved by forestation (Diem & Dommergues 1990b). Therefore investigations are continued to provide informations on such trees concerning ecology, microbiology and inoculation with specific microsymbionts (endophytes).

The N_2-fixing partnership between *Frankia* and actinorhizal non-leguminous woody shrubs and trees such as *Casuarina* is very important to agricultural and silviculture practices. This symbiosis have the potential to free the host plant from dependence on nitrogenous fertilizers as well as to increase soil fertility. The full realization of this potential depends on maximizing the contribution of each partner, catering to specificity in the association and providing conditions for plant growth and nodules formation. El-Lakany (1983a, b; 1985 and 1986) reported that nitrogen fixing trees with special reference to *Casuarina* are being plants on a wide scale in tropics to provide fuel wood construction materials, fodder and nitrogen-rich biomass for improving soil fertility. The use of N_2-fixing trees in agroforestry systems is receiving considerable attention as a source of nitrogen and much needed organic matter. Furthermore, these trees are used in the rehabilitation of damaged soils and in the protection of these soils against erosion. *Casuarinaceae* is the most important non-leguminous, dicotyledonous actinorhizal plants from the economical and ecological points of views including 96 species of woody trees and shrubs (Johnson & Wilson, 1989), their roots are nodulated by a nitrogen-fixing actinomycetes of genus *Frankia* (Mariana *et al.*, 2003).

Casuarina trees are the most planted in Egypt under different ecological conditions. El-Lakany (1986) Egypt is one of the poorest countries in natural forests in the world. Most of these species symbiotically fix nitrogen with *Frankia* which was recently isolated from *Comptonia peregrina* by Callaham *et al.* (1978) and from nodules of *Casuarina equisetifolia* by Diem & Dommergues (1983). First report on isolation and culture of effective *Frankia* strains in Egypt was by Girgis *et al.* (1990). The first trial to isolate *Frankia* directly from soil was achieved by Baker & O'Keefe (1984) using sucrose-density fractionation method. The microsymbiont *Frankia* genus belongs to the order of flilamentous bacteria, the actinomycetales, family *Frankiaceae*. The species concept in *Frankia* was based on host specificity and ultra structure of endophytes (Becking, 1981). Recent developments in *Frankia*

classification and diversity are reviewed elsewhere (Benson & Silvester, 1993 and Mullin & Dobritsa, 1996).

Recently, *Casuarina* trees have proved to increasing annually the soil-N content by their biological N_2-fixation ability around their rhizosphere in higher rates comparable to those of legumes-Rhizobium symbiosis (Long, 1996 and Franche *et al.*, 1998).

In Egypt, a N_2-fixing potential of 288 kg N/ha/year has been reported for *Casuarina* (Gauthier *et al.*, 1984). In addition, different actinorhizal species have the ability to grow well under a rang of environmental stresses such as high salinity, heavy metal, extreme pH (Dawson, 1990).

The basic knowledge of the symbiotic association between *Frankia* and actinorhizal plants still poorly understood, although it offers striking differences with the *Rhizobium*-legume symbiosis (Pawlowski & Bisseling, 1996; Long, 1996 and Franche *et al.*, 1998).

Also, little is known about the two partners and the ecological, genetical conditions affecting the cultural and symbiotic characteristics of local isolates of *Frankia* compared with a foreign reference strains.

The present study concerned with the morphological; cultural and ecological characteristics of native, local and high effective pure isolates of *Frankia* obtained from *Casuarina* root nodules cultivated in different ecological locations of Egyptian soils. Symbiotic performance of isolated cultures of *Frankia* were assessed i.e. assessment their efficiency in renodulating capacity, growth and symbiotic N_2-fixation ability of *Casuarina glauca* seedlings under green house conditions.

In addition, the isolates were studied for their intrinsic antibiotic resistance (IAR); salt tolerance as well as carbon source utilization. The genetic diversity of isolated *Frankia* was determined using the restriction fragment length polymorphism (RFLP) of their DAN.

MATERIALS AND METHODS

Collection of *Casuarina* nodules:

Casuarina nodules were collected from different ecological locations of Egypt i.e. Ismailia; Kafr El-Sheikh and the New Valley Governorates (Table 1). The nodules were sampled with small parts of roots at the depth of 10-30 cm from soils surface. The viable nodules that had light brown color were selected, separated and transferred under controlled conditions.

Table (1): Locations and depths of *Casuarina* nodules samples and soil types.

Locations	Soil types	Host trees	Root nodules depth (cm)	Nodules diameter (cm)
Kafr El-Sheikh Fac. of Agric. Farm Kafr El-Sheikh Univ.	Clay loam	*C. cuinninghamiana*	20	3-5
New Valley. Mout city	Clay loam	*C. glauca*	36	2-4
Ismailia.Ismailai-Suez road (10 km) apart	Sandy clay loam	*C. glauca*	15	3-4

Characterizations of the studied soils:

Representative composite arable soil samples were collected from Ismailia, Kafr El-Sheikh and the New Valley, from which *Casuarina* nodules were collected. Another soil samples of virgin calcareous and sandy soils, represent the new reclaimed soils, were collected also from Mariout Research Station and El-Nobaria city for the greenhouse experiments. The two groups of the soil samples were air dried, ground to pass through 2 mm sieve and kept for analyses at room temp.

The selected soil properties and total-N of roots and shoots of *Casuarina glauca* seedlings were determined using classical methods which reported by (Page *et al.*, 1982; Klute, 1986; Carter, 1993 and Burt, 2004).

Table (2) represent some physico-chemical characteristics of the studied soils.

Table (2): Main analytical properties of the tested soils from different ecological regions..

Parameters / Soils	Particle size distribution				Water holding capacity WHC (%)	Chemical analysis											
	Sand	Silt	Clay	Texture class		pH 2.5: 1	EC dS/m	$CaCO_3$ %	Soluble cations meq/L				Soluble anions meq/L			O.M.%
	%								Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$ + HCO_3^{-}	Cl^{-}	$SO_4^{=}$	
Soils of collected nodules																
Kafr El-Sheikh	8.9	32.8	58.3	Clay loam	50	8.2	12.9	2.9	32.8	26.4	67.3	2.5	3.5	85.0	40.5	1.98
New Valley	27.9	36.4	35.7	Clay loam	40	7.9	0.9	3.5	3.0	1.8	3.9	0.3	0.2	5.5	3.3	1.13
Ismailia	29.5	25.7	44.8	Sandy clay loam	33	7.8	10.6	2.9	25.5	18.6	57.4	4.5	2.3	66.6	37.1	1.36
Soils of green house experiments																
Mariout Research Station (MRC)	5.3	30.7	64	Clay loam	40	8.3	0.51	30.2	1.4	1.0	2.2	0.5	0.1	3.1	1.9	0.79
El-Nobaria city	92.5	5	2.5	Sandy	26	7.7	1.7	1.4	4.8	3.1	8.7	0.4	0.3	11.3	5.4	0.51

Microbiological analyses of the tested soils:

Pour-plate method was employed using the technique described by Louw & Webley (1959). The serial dilution pipette method was used for the microbial counts on different selective media as following as listed in Table (3).Total microbial counts on nutrient agar medium (Difco, 1976). Total fungi counts on Martin's medium (Allen, 1953). Total actinomycetes counts on yeast extract-malt extract agar (Szabo 1974). Spore-formers counted on nutrient agar medium (Difco, 1976) after heating the dilution tubes for 10 minutes at 80-90°C. Total N_2-fixing microorganisms counts on combined carbon medium (Rennie, 1981).

Table (3): The microbiological analyses of the tested soils from which nodules were collected and of soils which used in the green house experiments.

Microorganism groups / Locations	Total microbial counts	Total fungal counts	Total Actinomycetes counts	Total N_2-fixing microorganisms counts	Spore-forming Bacilli
	CFU units/g soil				
Locations of collected nodules					
Kafr El-Sheikh	6.0×10^7	6.4×10^4	2.2×10^5	1.3×10^5	1.3×10^5
New Valley	4.8×10^5	4.6×10^4	1.7×10^5	1.0×10^5	1.4×10^5
Ismailia	11×10^7	4.5×10^4	2.1×10^5	2.1×10^5	1.2×10^5
Soil used in the green house experiments					
Mariout Research Station (MRC)	1.0×10^7	6.1×10^4	2.7×10^5	1.3×10^5	1.7×10^4
El-Nobria city	3.4×10^5	2.7×10^4	1.1×10^5	5.3×10^5	1.6×10^5

Isolation of *Frankia*:

A number of selected fresh active nodules belonging to various locations were used to isolate different typical phenotypic *Frankia* isolates from the developed colonies on slants of *Frankia* medium according to the method of Benson (1982). The modified BAP liquid medium (Fontaine *et al.*, 1986) was used for inocula preparation.

Reference strain and probes:

A *Frankia* foreign reference strain (Br) (ORS 020608) isolated from *Casuarina equisetifolia* grown in Brazil was kindly obtained from the Department of Plant Ecology, Faculty of Science. Chile (Muller *et al.*, 1991).

Source and germination bioassay of *Casuarina* seeds:

Seeds of *Casuarina glauca* were kindly obtained from Desert Development Center (DDC) of American University in Cairo, Egypt.

The seeds were surface sterilized by the immersing for 2 minutes in concentrated (H_2SO_4), then washed with sterile tap water until the wash reached pH 7 according to the method which reported by Selim & Schwencke (1995).

The prepared seeds were germinated in sterile growth medium composed of washed sand and peatmoss (1: 1) per volume for two weeks under room temperature.

Intrinsic antibiotic resistance (IAR) of isolated *Frankia*:

Antibiotics sensitivity of *Frankia* isolates were carried out by determining the growth inhibition plate assay according to Louis *et al.* (1999) using the following ten different types of antibiotics, rifampicin; kanmamycin; chloramphenicol; spectinomycin; tetracycline; ampiciline; erythromycin; neomycin sulfate; streptomycin sulfate and vancomycin hydrochloride. Different concentrations from each antibiotic, 15, 30 and 60 µg ml^{-1} medium were used. Sterile Petri dishes were inoculated with 0.1 ml of 10 days-old of the respective cultures. Qmod melted agar medium (Lalonde & Calvert, 1979) flaks were supplemented with the respective antibiotics concentrations, then poured into the inoculated Petri dishes and incubated at 28 $\pm$ 2°C. The

developed colonies on various antibiotic concentrations were recorded after 7 days of inoculation.

Response of *Frankia* isolates to different C-sources:

Five carbon sources i.e. sodium pyruvate; sodium acetate; glucose; sucrose and mannitol were tested as substitutions to sodium propionate that is considered the recommended carbon source for *Frankia* growth. BAP medium (Fontaine *et al.,* 1986), without carbon source was autoclaved at 121°C for 15 minutes, while the various tested carbon sources were sterilized separately by filtration through sterile membrane filter of 0.45 µm pore size supplemented to sterile Büchner set. After sterilization both BAP medium and various tested carbon were mixed together to give the required concentration (0.2 g carbon/L). Conical flasks of 250 ml volume containing 100 ml of BAP medium including the respective carbon source were prepared and each was inoculated with homogenized 0.1 ml of a freshly prepared *Frankia* isolate (10 days-old) as well as the reference strain. After incubation at 28 ± 2°C for 10 days on a reciprocal shaker, each culture was counted using the serial dilution method.

Tolerance of *Frankia* isolates to salinity:

The tolerance of isolated and reference *Frankia* to different NaCl concentrations was studied by the growth inhibition plate. A stock solution of sodium chloride (40%) and Qmod liquid medium (Lalonde & Calvert, 1979) were prepared and autoclaved separately. After sterilization, the medium flasks were supplemented with aliquots of NaCl solution to give the final NaCl concentrations: control, 2.0; 4.0; 6.0; 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10 and 10.5%, and inoculated with 0.1 ml of freshly prepared (10 days-old) homogenized liquid cultures of the tested *Frankia* isolates. After 10 days inoculation period at 28 ± 2°C in a reciprocal shaker, each culture was counted on solid Qmode medium, containing the same previously mentioned NaCl concentrations, using the serial dilutions plate count.

DNA and plasmid isolation of *Frankia* isolates:

Plasmid numbers, sizes as well as DAN isolation and analyses of *Frankia* isolates were determined using the miniscreen method described by Rodriguez & Tait (1983).

Nodulation assay:

Local *Frankia* isolates and the foreign reference (Br) strain were tested for potential infectiveness and nodulation of host plant *Casuarina glauca* plant seedlings in a pot experiments under green house conditions of National Research Centre (NRC). Sterile plastic pots of 15 cm diameter were packed with 350 g sterile mixture of washed sand and peatmoss (1: 1) per volume. The growth medium was autoclaved at 121°C for 2 hours for three successive days.

The content of each pot was moistened with 500 ml sterile N-free Evan's nutrient solution (Evans *et al.,* 1972), then mixed with 25 ml 10 days old culture of the respective *Frankia* isolate.

Sterilized seeds of *Casuarina glauca* were sown in pots, then covered with a thin layer of the same sterile growth medium. The pots were frequently irrigated with N-free Evan's nutrient solution. After four months, the

seedlings were uprooted, their roots were washed and the formed nodules were collected, counted, weighted and dried at 70°C for 48 hours for each seedlings. These fresh seedlings were subjected to estimate their vegetative growth parameters.

N_2-fixation assay:

Isolated *Frankia* and reference (Br) strain were tested for their efficiency to fix symbiotically the atmospheric nitrogen.

The virgin calcareous and sandy soils were sterilized and packed in sterile plastic pots (3 kg/pot) under green-house conditions of NRC. Pregerminated seeds of *Casuarina glauca* were transplanted (3 seedlings/pot), inoculated with 25 ml of 10 days-old culture of the respective. *Frankia* isolates and covered with a thin layer of the same sterile soil. Irrigation was done with N-free Evan's nutrient solution at intervals according plant needs (Huss-Danell, 1990) continuously for 10 months. At the end, shoots and roots N-content % and plant N-uptake (mg/seedling) were estimated.

RESULTS AND DISCUSSION

Isolation; purification and phenotypic characteristics of isolated *Frankia*:

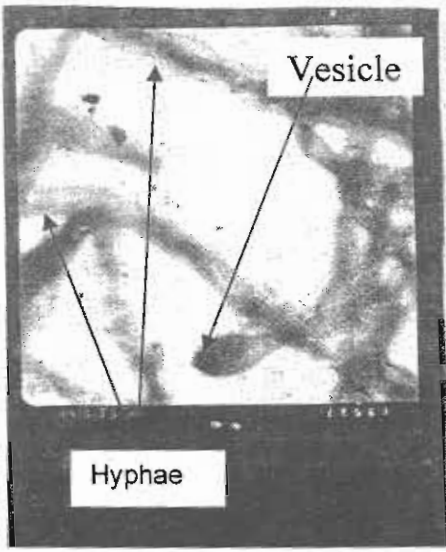
The selected active nodules, which were collected from different locations represent different ecological sites of Egypt, were used for isolation and identification of different local isolates of *Frankia* using the microdissection method. Sterilized crushed nodule exudates was cultured on *Frankia* medium (Benson, 1982), containing sodium pyruvate as sole source of carbon and incubated at 28 ± 2°C for one month.

Sixteen local isolates of *Frankia* were selected and subjected to more morphological studies using scanning electron microscopy SEM (10000x) for two isolates (Figs. 1 and 2).

Frankia differentiate into three cell type: Vegetative hyphae, sporangia and vesicles. These different cell types can be produced in pure culture, in plant and presumably in soils (Huss-Danell, 1990).

Fig (1) illustrates that the hyphae and vesicles of *Frankia* isolates were similar harmony of those findings by Lalonde *et al.* (1981) and Fontaine *et al.* (1984) who stated that *Frankia* sp. strain HFPAr 13 induced different formation of specialized structures, called vesicles, which are the proposed site of N_2-fixation (localization of nitrogenase), Meesters *et al.* (1985) and Schultz & Benson (1989).

Fig. (2) shows the specific fourth structure of *Frankia* that called reproductive torulose hyphase (RTH). Diem & Dommergues (1984 and 1990a) found that some *Frankia* strains (ORS 021001) isolated from *Casuarina junghuhniana* produced a fourth structure results from conversion of a vegetative hypha into a wide torulose hypha with a thick double-layerd wall. These hyphae may be disrupted into spore-like cells and serve as the surviving and regenerating structure in *Frankia* strains from *Casuarina* when all vegetative hyphae are lysed.



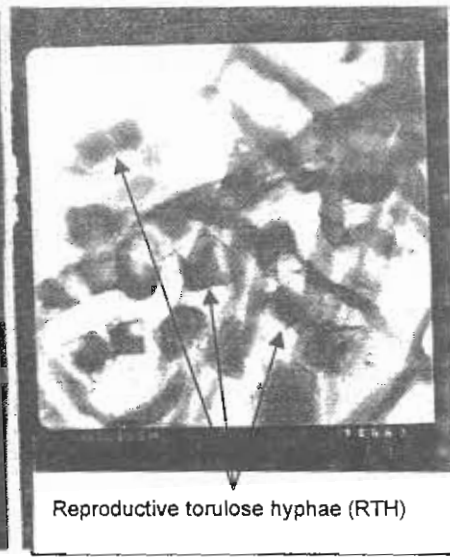


Fig (1): SEM of hyphae and vesicles **Fig (2): SEM of RTH**

During growing the selected local *Frankia* isolates in the BAP liquid medium, New Valley isolates N01; N03; N04 and N05 as well as IO1 were produced colored pigments ranged from yellow to pink. The general morphological cultural characteristics of the different local *Frankia* isolates grown in BAP liquid medium as follows: *Frankia* isolates of Kafr El-Sheikh K01; K02 and K03 were formed clusters, without producing colored pigments in the BAP medium.

These clusters collected together and precipitated at the bottom formed white lumps with diameter of colony ranged between 1.2-2 mm. Meanwhile, the other isolates were weakly grown only on the bottom $K0_4$ and dispersed in the whole liquid $K0_5$.

Isolates of New Valley were relatively slow grown; forming colonies (diameter 1-2 mm); producing soluble colored pigments in the BAP liquid medium with the exception of isolate N02. The isolate N01 formed small colonies firmly stickled on the wall and produced soluble red pigment in the medium. Isolates N02 and N03 were faster grown, formed larger colonies and clusters precipitated on the bottom, but isolate N03 secreted yellow soluble pigment.

The isolate N04 formed large colonies without producing clusters and secreted red pigment in the medium. Isolate N05 formed small colonies firmly attached to the wall, grew on the surface of growth liquid medium with grey color.

Frankia isolated from Ismailia displayed the lowest growth on the BAP medium, formed small colonies (diameter 1-2.5 mm) without forming soluble colored pigments in the medium, with the exception of isolate I01. The isolate I01 formed small colonies and clusters precipitated as granules

with pink pigment. Isolate I02 gave weak growth on the wall, formed clusters, deposited on the bottom. Isolate I03 showed weak gray growth dispersed in the liquid medium without forming film on the inner walls. Isolate I04 showed very weak growth on the inner walls without precipitated on the bottom. I05 showed heavy white growth on the surface forming pellicle without forming clusters or precipitates. I06 had a medium growth on the surface with grey colored mycelium.

The imported reference foreign *Frankia* strain Br was characterized by weak growth with small colonies 1-1.5 mm in diameter and formed clusters on the bottom. On solid medium it formed gray mycelium.

Effect of some ecological conditions on growth of isolated *Frankia*:

1. Intrinsic antibiotic resistance (IAR):

Antibiotic sensitivity of *Frankia* isolates have been used to differentiate and facilitate the identification of these isolates. Table (4) showed that the sixteen local isolates and the foreign reference (Br) strain differed markedly in their resistance towards the tested (10) antibiotics.

The tested *Frankia* isolates, either locally isolated from the studied soils or the foreign reference strain (Br) were incapable to grow at any of the tested concentration levels (15, 30 and 60 μg ml^{-1}) of kanamycin, tetracycline and neomycin sulfate antibiotics.

Frankia isolates N01, N02 and N03 failed to grow on any of the tested antibiotic at any concentrations. From Table (4) it was observed that just one *Frankia* isolate N04 was able to grow on the concentration levels of vancomycin hydrochloride, but all the remainder isolates completely failed to grow on any of the tested concentrations.

Indigenous *Frankia* isolates of Kafr El-Sheikh and Ismailia showed the greatest capability to grow on various concentration levels of the most different tested antibiotics. However, local *Frankia* isolates of the New Valley displayed the lowest one in the exception of isolate N04, which was the most efficient isolate.

These observations could be ascribed to the adaptation of the isolates to their locations from which they were isolated as given in Tables (3 and 4).

Foreign reference (Br) strain was more sensitive to all tested antibiotics at the three concentration levels and displayed the absolutely lowest resistance in the exception of erythromycin.

These results are in agreement with the findings of Carrasco *et al.* (1995) and Louis *et al.* (1999). They found that all the strains demonstrated sensitivity to some antibiotics.

Table (4): Resistance of *Frankia* isolates to certain concentrations of examined antibiotics.

Antibiotic	Conc. (µg/ml)	*Frankia* isolates																
		Br	K01	K02	K03	K04	K05	N01	N02	N03	N04	N05	I01	I02	I03	I04	I05	I06
Rifampicin	15	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-
	30	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kanamycin	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloramphenicol	15	-	+	+	-	+	+	-	-	-	+	+	+	-	+	-	-	-
	30	-	+	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-
	60	-	+	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-
Spectinomycin	15	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+
	30	-	+	+	+	+	+	-	-	-	+	+	-	+	+	-	+	-
	60	-	-	+	+	+	+	-	-	-	+	+	-	-	+	-	+	-
Tetracycline	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampicilline	15	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
	30	+	+	-	+	+	+	-	-	-	-	+	-	+	+	-	+	-
	60	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Erythromycin	15	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+
	30	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-
	60	+	+	+	-	+	+	-	-	-	+	+	+	-	+	-	+	-
Neomycin sulfate	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptomycin sulfate	15	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	30	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vancomycin hydrochloride	15	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

Notes: + Means growth, - means no growth.
Br. : The reference strain
K01-K05 : *Frankia* isolates of Kafr El-Sheikh
N01-N05 : *Frankia* isolates of New Valley
I01-I06 : *Frankia* isolates of Ismailia

2. Screening of the local and reference *Frankia* isolates under NaCl-Salinity stresses:

Neutral salinity stress was used for differentiating between the tested *Frankia* isolates. Tolerance of the indigenous, native *Frankia* isolates and the foreign reference strain, referred to as their growth of *Frankia* colony counts (CFU/ml) were assessed using Qmod liquid medium contained different NaCl concentration levels ranged from 2% to 10.5%. Data illustrated in Fig. (3) revealed that the *Frankia* isolates and the foreign strain exhibited great differences in their NaCl-stresses tolerance. Local *Frankia* isolates of New Valley region failed to grow above 4% NaCl, which showed the highest sensitivity to salinity stresses. The growth of the foreign reference *Frankia* strain was absent above 9% NaCl.

Kafr El-Sheikh *Frankia* isolates showed the greatest resistance against NaCl-salinity levels. These isolates were able to grow up to 9.5% with the exception of K04. Isolates K03 and K05 were the most tolerant *Frankia* to salinity stresses, since they were able to grow up to 10% NaCl.

Further, Ismailia *Frankia* isolates came second in salt tolerance. They tolerated NaCl concentration levels up to 7.5%. Two of six strains i.e. I06 and I06 had the ability to grow at 9.5% NaCl. These results are in the line with the findings obtained by Khalil (1999).

Indigenous *Frankia* isolates of Kafr El-Sheikh region surpassed the imported foreign reference *Frankia* strain and could be strongly recommended to be applied as inocula for inoculation shrubs and trees of *Casuarina* in different Egyptian salt-affected soils.

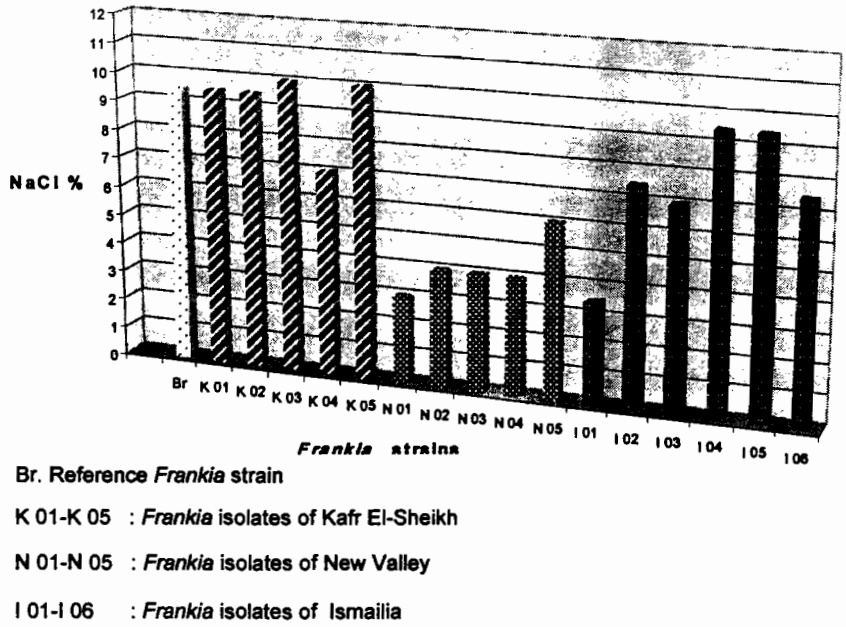


Fig. (3): Relation between *Frankia* isolates and NaCl-salinity stresses.

Utilization of various carbon sources:

Table (5) demonstrates the response of using different carbon sources (6) by differed *Frankia* isolates and Br reference strain in BAP medium. Different types of C-forms were utilized by the tested isolates in different degrees represented by their total *Frankia* colon counts units (CUF/ml).

Data revealed that local isolates were more efficient than the foreign one. Generally, Kafr El-Sheikh isolates showed the highest utilization of the used carbon. The obtained results are in agreement with findings of Benson & Schultz (1990) and Carrasco *et al.* (1995) who found that the utilization of carbon sources by *Frankia* strains was related to the genomic species that infect the members of the Elaeagnacease and *Casuarinaceae*, these strains grown on sugars and organic acids.

Generally the tested *Frankia* isolates differed in their carbon utilization from all different carbon sources. Most Fankia isolates from Kafr El-Sheikh displayed greater response to different C-sources and surpassed the foreign Br strain.

Table (5): Utilization of several carbon sources by *Frankia* isolates.

Frankia isolates	Carbon sources					
	Na-propionate	Na-pyruvate	Na-acetate	Sucrose	Glucose	Mannitol
	Frankia colony count units (CFU/ml)					
Br	3.8×10^3	3.9×10^3	2.4×10^3	2.3×10^3	5.9×10^4	> 30
K 01	7.1×10^4	3.6×10^4	7.0×10^4	5.9×10^5	5.1×10^5	3.7×10^5
K 02	8.4×10^5	4.2×10^4	1.7×10^5	6.0×10^5	1.6×10^8	1.1×10^8
K 03	1.9×10^4	1.6×10^7	4.1×10^4	5.7×10^3	1.7×10^7	9.0×10^4
K 04	1.5×10^5	1.8×10^4	4.9×10^4	3.1×10^4	1.3×10^5	2.5×10^4
K 05	1.9×10^4	5.0×10^4	4.8×10^4	1.2×10^5	9.0×10^5	< 30
N 01	2.7×10^2	6.1×10^3	< 30	87.5×10^1	1.0×10^1	< 30
N 02	1.4×10^3	5.4×10^3	1.3×10^3	4.0×10^4	2.8×10^3	2.0×10^4
N 03	3.2×10^1	3.6×10^5	< 30	6.2×10^4	3.3×10^2	4.4×10^3
N 04	< 30	2.2×10^4	< 30	4.2×10^3	< 30	8.4×10^2
N 05	2.4×10^2	3.7×10^2	2.0×10^2	2.5×10^3	1.5×10^3	2.6×10^4
I 01	3.5×10^1	4.5×10^3	1.6×10^3	1.5×10^4	1.3×10^3	8.5×10^3
I 02	1.5×10^4	4.7×10^3	1.2×10^4	2.1×10^2	5.0×10^2	5.2×10^3
I 03	9.6×10^1	1.0×10^5	3.2×10^2	8.9×10^2	7.2×10^2	5.2×10^3
I 04	8.5×10^2	9.7×10^3	5.3×10^3	1.1×10^3	1.4×10^3	1.6×10^3
I 05	6.5×10^4	4.1×10^3	1.2×10^3	8.6×10^3	3.2×10^3	1.3×10^3
I 06	6.1×10^1	1.7×10^2	7.9×10^1	1.3×10^2	2.3×10^2	2.7×10^2

Br. : **The reference strain**
K 01-K 05 : ***Frankia* isolates of Kafr El-Sheikh**
N01-N05 : ***Frankia* isolates of New Valley**
I01-I06 : ***Frankia* isolates of Ismailia**

Differentiation between *Frankia* isolates using DNA analysis:

To differentiate between the isolated *Frankia* as well as the reference Br strain, the DAN plasmid analysis was carried out using the method of alkaline lysis with some modification. This method is able to isolate high molecular weight plasmids of *Frankia* (Rodriguez & Tait, 1983). Harbor plasmid DNA as part of the genome could be used to differentiate between different *Frankia* isolates on genetic bases. The DNA plasmid patterns of *Frankia* isolates and the reference (Br) strain which had different molecular weights are present in Fig. (4). Reference strain Br showed three high molecular weight plasmids (Lane 17). No small size plasmids were found in

any of the tested isolates. Plasmids number of the tested *Frankia* isolates ranged from one to three plasmids per isolate. The *Frankia* isolates of Kafr El-Sheikh yielded one to three plasmid bands upon gel electrophoresis; one band was found in K 02 isolate (lane 2); three bands found in K 01 (lane 1) and two bounds in each of K 03; K 04 and K 05 (lanes 3, 4 and 5) respectively.

The *Frankia* isolated from New Valley harbored plasmids ranged from one to two plasmid bands. One band has been found in each of N 01, N 03 and N 05 (lanes 6, 8 and 10); two plasmid bands noted in N 04 (lane 9), but no plasmid bands found in N 02 (lane 7).

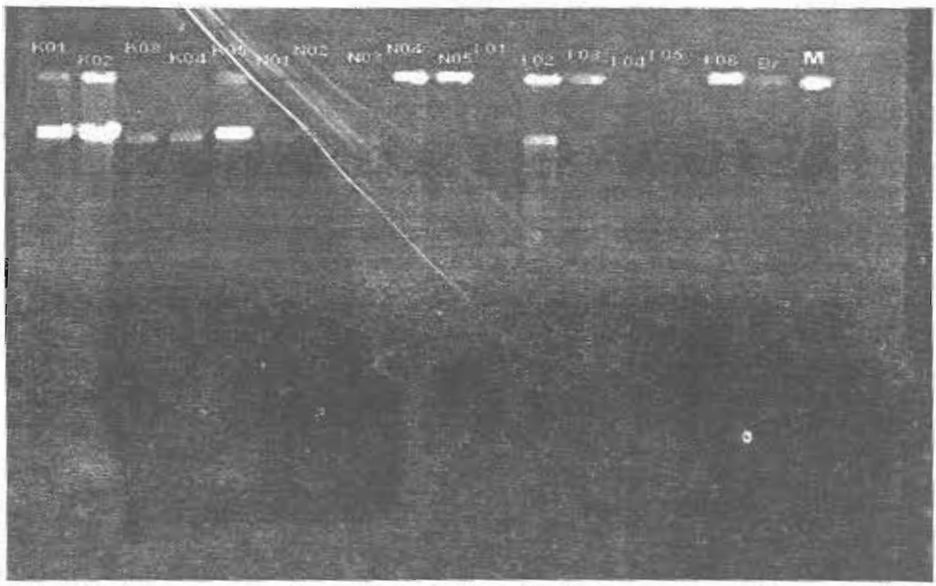


Fig. (4): The DNA plasmid patterns of isolated *Frankia* and their reference strain.

Br. : The reference strain
K01-K05 : *Frankia* isolates of Kafr El-Sheikh
N01-N05 : *Frankia* isolates of New Valley
I01-I06 : *Frankia* isolates of Ismailia

The *Frankia* isolates of Ismailia displayed that three of them I01, I04 and I05 (lanes 11, 14 and 15) have no plasmid bands, but isolate I 02 harbored two plasmids (lane 12), while isolates I 03 and I 06 (lanes 13 and 16) indicated only one plasmid band. In general, the highest number of plasmid bands was presented by Kafr El-Sheikh isolates and the lowest by Ismailia isolates. The different plasmid patterns among various isolates of *Frankia* are indication for their genetic diversity. The same results were reported among population of *Frankia*, where a wide variety of distinct plasmid profiles was found (Normand *et al.*, 1983).

As illustrated in Fig. (5), total genomic DNA of the 16 *Frankia* isolates were isolated and migrated by electorphoresis in 0.7% agarose gel to

differentiate between isolates. Results showed the differentiation between isolates in their migration through agarose gel electrophoresiss. These results represented that genetic diversity among *Frankia* isolates (An *et al.*, 1985).

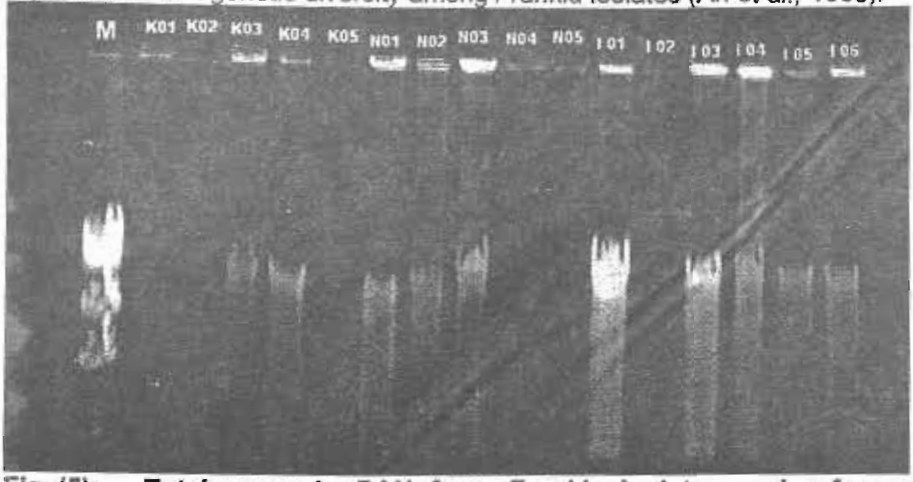


Fig. (5): Total genomic DAN from *Frankia* isolates and reference strain.

Br. : The reference strain
K01-K05 : *Frankia* isolates of Kafr El-Sheikh
N01-N05 : *Frankia* isolates of New Valley
I01-I06 : *Frankia* isolates of Ismailia

The characterization of *Frankia* isolates at the DNA level has revealed the existence of a large diversity (Rouvier *et al.*, 1996) using RFLP of their DNA.

According to the different identification parameters which used to differentiate between the various *Frankia* isolates i.e. application of certain concentrations of different antibiotics; NaCl concentrations; types and utilization of different C-sources as well as the genetic differentiation by plasmid DNA analysis; no similarity was recorded between the various tested *Frankia* isolates except the two isolates number N 01 and N 03 that were completely identical in all parameters.

Table (6) summares the obtained above mentioned results. So, it could be concluded that, the obtained *Frankia* isolates (15) are belonging to *Frankia Casuarina* strains.

Response of *Casuarina* seedlings to nodulation and growth:

Nodulation and vegetative growth of *Casuarina glauca* seedlings upon inoculation with local and Br foreign *Frankia* isolates were carried out under green-house conditions. Data listed in Table (7) revealed that no nodules were formed on Casuarina seedlings of the control treatment, i.e. sterilized growth medium and seeds. Nodules number ranged from 11 (I 03) to 18 (I 04) per seedling due to inoculation with *Frankia* isolates.

Most of local *Frankia* isolates induced nodulation and surpassed the foreign reference Br stain (12 nodules). Figs. (7 and 8) represent a nodulated *Casuarina* roots.

The nodule dry weights ranged from 140 for (K 05 and N 04) isolates and 820 mg/seedling with I 04 isolate. Also, the majority local *Frankia* isolates were significantly overcame the Br reference strain in nodule dry weights.

Table (6): Schematic diagram showing the summarized behavior of various tested *Frankia* isolates towards the tested parameters.

Carbohydrates		NaCl conc.	Antibiotics	Plasmids No.
Na-Propionate	I 02	– 7%	– 30 R& 30sp. 30Amp. & 30 Er.	2
	I 05	– 9.5%	– 60 Sp. & 60 Amp. & 60Er.	-
Na-Pyruvate	N 01	2%	Failed	1
	N 03	2%	Failed	1
	N 04	2%	– 30 R. & 60 Ch. 60 sp. 60Van. 60 Er.	2
	I 03	– 6.5%	– 30 R.&15 ch. & 60 sp. 60 Er 60 Er. &30 Amp.	2
	I 04	– 9.5%	– 15 Amp.	-
Sucrose	K 01	– 9.5%	– 60 Ch. & 30 Sp. & 60 Er. 30 Amp. & 30 St.	1
	K 04	– 7.5%	– 30 Ch & 60 Sp. & 60 Er. 30 Amp.	2
	K 05	– 10%	– 60 Ch & 60 Sp. & 60 Er. 30 Amp. & 30 St.	2
	N 02	2%	– Failed	-
	I 01	2%	– 60 Ch. & 15 Amp.& 15 Sp. & 60 Er.	-
Glucose	K 02	– 9.5%	– 30 Ch & 60 Sp. & 15 Amp. & 60 Er. 30 St.	1
	K 03	– 10%	– 60 Sp. & 60 Amp. & 30 Er.	2
Mannitol	N 05	– 6%	– 30 Ch. & 60 Sp.& 30 Amp & 60 Er.	1
	I 06	– 7.5%	– 15 Sp. & 15 Amp. & 15. Er.	1
	5 groups	13 groups	15 groups	15 groups

K01-K05 : *Frankia* isolates of Kafr El-Sheikh
N01-N5 : *Frankia* isolates of New Valley
I01-I06 : *Frankia* isolates of Ismailia

Most of *Frankia* isolates produced nodule numbers and nodule dry weights overmatched that obtained with Br reference strain being the highest with the strain I 04. These findings could be attributed to the high efficiency of active local *Frankia* isolates, which have more adaptation to Egyptian ecological conditions than the foreign Br strain. These obtained results were in line with those reported by Masuk & Makoni (1995) and Zayed (2000) who

found that inoculation with *Frankia* sp. increased nodules number and their dry weights.

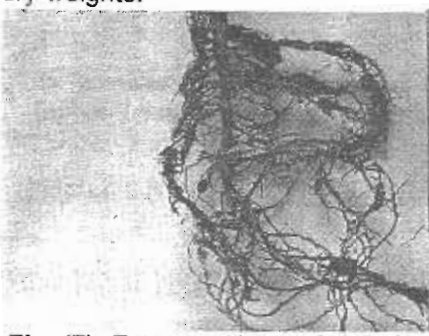


Fig. (7): Represent a nodulated *Casuarina* roots.

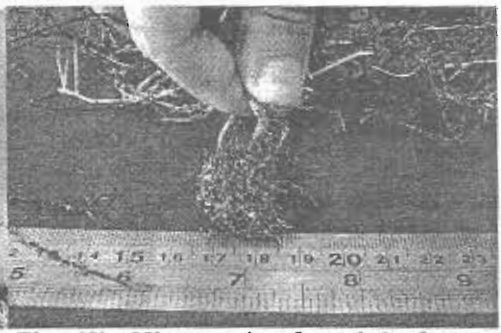


Fig. (8): Micrograh of nodule formed on *Casuarina* roots by K 01 isolate.

From Table (7) it could be concluded that inoculation with local *Frankia* isolates increased nodule numbers and their dry weights. Also, inoculation enhanced lengths and root dry weights, stem lengths and increased their branches numbers. Generally, *Frankia* isolate I 04 recorded the highest assessed nodulation and the vegetative growth parameters.

Table (7): Effect of inoculation with *Frankia* isolates and Br reference strain on the nodulation of *Casuarina glauca* and its vegetative parameters.

Parameters / Treatments	Nodules (No/ seedling)	Nodules D.Wt. (mg/ seedling)	Root length (cm)	Root D.wt. (g/ seedling)	Shoots D.Wt. (g/seedling)	Branches (no/ seedling)	Stem length (cm)
Control	0	0	10.33	0.20	0.96	4.0	15.33
Br	12	180	20.33	0.57	3.58	2.7	24.33
K 01	17	620	20.00	1.37	4.57	2.7	25.00
K 02	15	250	16.67	0.73	2.35	1.7	32.67
K 03	13	440	16.67	0.92	3.55	3.3	27.67
K 04	14	590	16.00	0.88	2.56	4.3	24.33
K 05	13	140	165.00	0.69	3.55	4.0	31.67
N 01	13	560	13.67	0.33	1.89	4.7	24.0
N 02	14	420	20.00	0.53	1.86	2.0	22.33
N 03	14	380	17.33	0.61	1.19	1.7	21.67
N 04	14	140	18.00	0.51	1.27	1.0	23.33
N 05	13	210	15.33	0.70	2.41	4.0	25.67
I 01	13	330	13.00	0.82	2.30	2.0	29.00
I 02	13	270	14.33	0.57	2.81	5.0	17.00
I 03	11	260	18.33	0.94	2.50	4.0	32.00
I 04	18	820	25.00	1.30	5.53	6.3	36.00
I 05	11	250	17.33	0.56	3.36	5.0	33.33
I 06	14	440	12.67	0.69	2.53	5.7	24.33

Each value is a mean of 3 replicates

Br. : The reference strain

K01-K05 : *Frankia* isolates of Kafr El-Sheikh

N01-N05 : *Frankia* isolates of New Valley

I01-I06 : *Frankia* isolates of Ismailia

Efficacy of *Casuarina* seedlings to symbiotic N_2-fixation:

Performance symbiotic N_2-fixation trials of local isolated *Frankia* from different ecological regions in comparison with the foreign *Frankia* reference strain from Chile (ORS020608) were judged by the N-content of roots and shoots as well as N-uptake (mg/seedling) of *Casuarina* seedlings cultivated in the studied soils. Data listed in Table (8) showed that these N-parameters of non-inoculated *Casuarina* seedlings with *Frankia* were recorded the lowest values in both calcareous and sandy soils (control) in comparison with the inoculated seedlings. Efficacy of *Casuarina* seedlings to N_2-fixation was significantly increased upon inoculation with *Frankia* strains. As shown in Table (8) N-parameters of inoculated *Casuarina* seedlings with foreign *Frankia* strain were significantly increased in comparison with the control.

In addition, inoculation of *Casuarina* seedlings with indigenous isolated *Frankia* led to highly significant increasing in N-parameters in comparison with the corresponding values of the (Br) reference *Frankia* strain.

This means that local *Frankia* isolates were significantly surpassed the foreign *Frankia* strain in N_2-fixation efficiency. Inoculation of cultivated *Casuarina* seedlings with *Frankia* isolates of Ismailia region (I.S 146.6 mmoles L^{-1}) gave significantly the highest N-values in Mariout calcareous soils (I.S7.25 mmoles L^{-1}, $CaCO_3$ 30.2% and clay content (64%). Grand mean values of N-content of roots; shoots and N-uptake were 2.73%, 1.84% and 1376.4 mg/seedling respectively.

Isolate I04 displayed the greatest values. However these isolates recorded the lowest values in El-Nobaria sandy soils (I.S 23.65 mmoles L^{-1}, $CaCO_3$ 1.4% and sand content 92.5%), which were 0.94%, 0.95% and 461.46 mg/seedling. It could be concluded that, N_2-fixation capability of *Frankia* isolates of Ismailia in calcareous soils was more higher than those in sandy soils. Inoculated *Casuarina* seedlings with *Frankia* isolates of Kafr El-Sheikh (I.S. 178.85 mmoles L^{-1}) cultivated in Mariout calcareous soils gave the lowest significant values which were 1.63%, 1.14% and 403.9 mg/seedling respectively. These isolates registered the highest values in El-Nobaria sandy soils, which were 2.06%, 1.99% and 1222.75 mg/seedling respectively. K01 isolate represented the greatest values.

N_2-fixation by *Frankia* isolates of Kafr El-Sheikh in sandy soils was more efficient than in calcareous soils. Inoculation of *Casuarina* seedling with *Frankia* isolates of New Valley (I.S. 13.05 mmoles L^{-1}) displayed moderately values.

This observations could be ascribed to the adaptation of the studied isolates to the areas from which they were isolated (Table 1 and 2), which are in harmony with those obtained by Zhang *et al.* (1990) and Khalil (1999) who reported that different *Casuarina* genotypes inoculated with pure *Frankia* strains showed significant N_2-fixation and N-uptake per plant.

From the above mentioned investigations it is clear that the most of local *Frankia* isolates specially Kafr El-Sheikh and Ismailia isolates could be recommended as highly efficient and specific biofertilizer for *Casuarina*

inoculation under different drought conditions in Egypt for rough agricultural, ecological stabilization.

Table (8): N-content of roots, shoots and N-uptake of *Casuarina glauca* seedlings in studied soils as affected with *Frankia* isolates.

Parameters / Treatments	Calcareous soil			Sandy soil		
	N-content (%)		N-uptake	N-content (%)		N-uptake
	Roots	Shoots	(mg/seedling)	Roots	Shoots	(mg/seedling)
Cont.	0.20	0.37	45.26	0.45	0.40	39.98
Br	1.14	1.45	258.22	1.50	1.50	612.90
K01	2.80	2.23	695.65	3.55	3.35	2782.77
K02	1.50	0.90	320.64	0.76	1.40	555.98
K03	0.15	0.32	93.72	1.63	1.40	1055.65
K04	2.05	0.30	575.75	1.90	1.70	639.61
K05	1.65	1.95	333.72	2.45	2.10	1079.72
Grand mean K	1.63	1.14	403.9	2.06	1.99	1222.75
N01	1.38	1.65	310.88	2.05	2.00	804.61
N02	1.42	1.00	734.95	2.45	1.65	1276.33
N03	2.37	0.90	710.57	1.75	1.78	883.60
N04	2.45	2.55	1383.3	1.05	1.78	557.60
N05	2.90	2.15	575.70	1.80	2.30	1306.26
Grand mean N	2.10	1.65	743.1	1.82	1.90	965.68
I01	2.70	1.05	1035.00	0.85	0.64	371.46
I02	2.30	1.75	963.90	0.79	0.85	463.63
I03	3.55	3.35	1538.70	1.40	1.10	683.43
I04	3.70	2.55	3031.25	0.55	1.25	352.13
I05	2.25	1.55	1113.40	0.85	0.95	525.01
I06	1.90	0.80	576.18	1.20	0.85	373.10
Grand mean I	2.73	1.84	1376.4	0.94	0.95	461.46
Total grand mean	2.16	1.54	841.13	1.60	1.61	883.29

BR : The reference strain
K01-K05 : *Frankia* isolates of Kafr El-Sheikh
N01-N-05 : *Frankia* isolates of New Valley
I01-I06 : *Frankia* isolates of Ismailia

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تقييم الخصائص المزرعية ، البيوكيميائية والوراثية للعزلات المختلفة من الفرانكيا ، وقدرتها على تكوين العقد الجذرية ، وتثبيت الأزوت الجوى تكافليا مع أشجار الكازورينا

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** تهدف هذه الدراسة إلى عزل وتنقية بعض عزلات الفرانكيا *Frankia* المحلية من العقد الجذرية النامية على جذور بعض أشجار الكازورينا المتأقلمة فى مناطق ايكولوجية مختلفة ومقارنتها بسلالة قياسية أجنبية للفرانكيا مستوردة من شيلى Foreign *Frankia* Reference Strain (ORS 020608).

** تم إجراء بعض التحليلات الكيميائية والميكروبيولوجية لعينات التربة التى جمعت من ريزوسفير الكازورينا التى عزلت من جذورها العقد الجذرية المحتوية على ميكروب الفرانكيا ، للتعرف على الظروف البيئية السائدة فى هذه الأراضى ومدى تأقلم هذه الميكروبات فى الظروف المختلفة.

** أجريت دراسات مورفولوجية مزرعية للتأكد من أن هذه العزلات المحلية نقية وتتبع جنس الفرانكيا عن طريق تنميتها على البيئات المتخصصة لنموها (بيئة BAP السائلة) ، ودراسة الأشكال المختلفة التى تتميز بها الفرانكيا وهى الخيوط الخضرية (الهيفات) والجراثيم والحويصلات ومقارنة ذلك بالسلالة الأجنبية القياسية بعمل قطاعات وفحصها بالميكروسكوب الإلكترونى Scanning Electron Microscopy (10000X).

** أجريت عدة دراسات بيوكيميائية لاختبار قدرة هذه السلالات المعزولة على تحمل بعض الظروف البيئية الصعبة ، بهدف التفريق بين هذه العزلات من الفرانكيا ، شملت مقاومتها ومدى تحملها لبعض المضادات الحيوية وتركيزاتها (IAR) ، مدى تحملها لتركيزات عالية من الاجهاد الملحى ، قدرتها على استخدام وتمثيل مصادر كربونية مختلفة كمصدر وحيد للطاقة ومقارنة ذلك بالسلالة الأجنبية القياسية.

** أجريت دراسات جينية وراثية لدراسة التنوع الجينى ، وذلك بعزل الحمض النووى الكروموسومى لهذه العزلات وتحديد وجود أو عدم وجود البلازميدات DNA-Plasmids باستخدام الهجرة الكهربائية Electrophoresis ومقارنة ذلك بالسلالة المرجعية.

** اختبار قدرة هذه العزلات المحلية من الفرانكيا على تكوين عقد جذرية فعالة (عدد العقد ووزنها الجاف) على جذور شتلات الكازورينا جلوكا *Casuarina glauca* النامية فى مخلوط رمل و Peatmoss فى الصوبة ، و دراسة بعض القياسات الخضرية (طول الساق والوزن الجاف للساق والجذر وعدد الأفرع) ومقارنتها بالسلالة القياسية.

** دراسة تأثير التلقيح بعزلات الفرانكيا المحلية والقياسية المستوردة على كفاءة هذه العزلات فى تثبيت النيتروجين الجوى تكافليا مع شجيرات الكازورينا فى الصوب وذلك بدراسة المحتوى النيتروجينى للمجموع الجذرى والخضرى والكمية الممتصة من النيتروجين (N-uptake) كدالة لكفاءة عملية تثبيت النيتروجين بشتلات الكازورينا المنزرعة تحت ظروف أراضى النوبارية الرملية وأراضى مربوط الجيرية التى تمثل أراضى الاستصلاح الجديدة.

عموما يمكننا تلخيص النتائج المتحصل عليها من هذه الدراسة فى الآتى:

١- تم عزل ١٦ من عزلات الفرانكيا المحلية من مختلف المناطق الإيكولوجية وأجريت عليها الدراسات السابقة حيث وجد أن منها ١٥ سلالة مختلفة الصفات عن بعضها.

٢- أظهرت عزلات الفرانكيا المحلية والمستوردة المقدرة على تكوين عقد جذرية فعالة على شتلات الكازورينا ، وقد أوضحت النتائج تفوق معظم السلالات المحلية للفرانكيا على السلالة القياسية المستوردة فى عدد العقد الجذرية ووزنها الجاف وكذلك الصفات الخضرية لشتلات الكازورينا.

٣- أظهرت النتائج أن تلقيح شتلات الكازورينا بالفرانكيا أدى إلى زيادة المحتوى النيتروجينى للمجموع الجذرى والخضرى وكذلك كمية النيتروجين الممتص لهذه الشتلات ، مما يعكس كفاءة عملية تثبيت النيتروجين الجوى ، وبصفة عامة فإن معظم عزلات الفرانكيا المعزولة تفوقت على السلالة القياسية الأجنبية فى قدرتها على تثبيت الأزوت الجوى فى العقد الجذرية لجذور شتلات الكازورينا المنزرعة فى الأراضى الجيرية والرملية تحت ظروف الصوبة.

على ضوء النتائج المتحصل عليها من هذه الدراسة يمكن التوصية باستخدام العزلات المحلية التى تم عزلها من مناطق إيكولوجية مختلفة من أراضى كفرالشيخ والإسماعيلية فى صورة لقاحات ميكروبية كمخصب حيوى فى تلقيح شتلات الكازورينا عند استصلاح الأراضى الرملية والجيرية تحت ظروف البيئة المصرية الجافة والمتأثرة بالأملاح وكذلك للثبات الإيكولوجى والزراعى ، حيث أن هذه العزلات تتميز بأنها ذات كفاءة عالية فى تثبيت النيتروجين الجوى ولها المقدرة على مقاومة تركيزات عالية من الملوحة وأنواع مختلفة من المضادات الحيوية بتركيزات مختلفة بالإضافة إلى قدرتها فى تكوين عقد جذرية فعالة وتمثيل واستخدام مصادر كربونية مختلفة كمصدر وحيد للطاقة.