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ROOTING OF JUJUBE (*Ziziphus jujuba* MILL) LI VARIETY CUTTINGS, USING SOME ROOT PROMOTING MICRO-ORGANISMS AND PLANT GROWTH REGULATORS

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ABSTRACT

This study was conducted at the experimental nursery of the Horticulture Research Institute at Giza, Egypt to study the effect of plant growth promoting rhizobacteria (PGPR) and plant growth regulators on rooting of jujube cuttings during 2008 and 2009 seasons. Sub-terminal cuttings were taken on mid April from mature 15- years old trees of jujube (*Ziziphus Jujuba* Mill) Li variety (difficult to root). Rooting treatments included inoculation with *B*acillus *polymyx, Bacillus circulance, Bacillus megaterium, Bacillus pasteruii, Pseudomans florescence* or mixed inoculants from previous PGPR strains or yeast *(Saccharomyces cerevisae)*. In addition, Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) were also tested at concentration of 1000 and 2000 ppm as compared with untreated (control). Data showed that the effect of *Bacillus megaterium* as PGPR resulted in the highest significant rooting percentage (60% and 50%). After eight months of transplanting, bacterial strain (*Bacillus megaterium)* followed by *Pseudomans* strain surpassed the other treatments in survival percentage, average number of roots/transplant, stem and root length, number of leaves, number of branches/transplant as well as leaves, stem and root fresh and dry weight (g). On the contrary, the lowest significant effect of treatments was found as a result of NAA at 1000 ppm and control during the two seasons of study. Histological studies revealed that, the callus originated from the cambial and phloem parenchyma cells below the cork cells, from these protrusions the adventitious roots were developed. The new roots established their connections with the vascular tissue of the cutting treated with *Bacillus megaterium*. Generally, it can be concluded that inoculation jujube (Li variety) sub-terminal cuttings with *Bacillus megaterium* or *Pseudomans florescence* can promote root formation as well as increase survival percentage and enhance vegetative growth of the produced transplants.

Keywords: PGPR, Rooting Jujube, Growth regulators, Anatomical studies.

INTRODUCTION

The Jujube (*Ziziphus Jujuba* Mill) belongs to the genus Zizuphus that belongs to Rhamnaceae family. The genus includes about 40 species of plants in tropical and subtropical regions of the northern hemisphere (Lyrene, 1979) of which the species *Zizuphus jujube* Mill is the most important in terms of distribution and economic significance. Li is an early ripening variety and is one of the most commonly available cultivar. Jujube fruit contains potassium, phosphorus, manganese and calcium as the major minerals. There are also high amounts of sodium, zinc, iron and copper. Jujube contains vitamin C about 20 times the amount of that of citrus fruits, riboflavin and thiamine *(*Jin-Wei *et al.,* 2007*)* and 18 of the 24 amino acids. The water extract of jujube

fruit inhibits tumor cells and may have useful compounds for medicinal use *(*Ki-Yeon *et al.,* 2010*).* The Jujube trees can withstand a wide range of temperatures, tolerance of marginal land thrive and grow well under semi-arid zone *(*Reddy *et al., 1998).* Few researches are available concerning propagation of jujube by cutting (Hatta *et al.,* 1996). Jujube propagation as stem cuttings is limited and very difficult to root even with the application of indole butyric acid (Yan-Ting & King, 2001). Stem cutting is the most simple and economical method particularly in horticulture, for mass production within a short time. Application of growth substances such as Naphthalene Acetic Acid (NAA) and Indole-3- Butyric Acid (IBA) as a growth regulators are typically the principal and commercially which can be applied. They have positive effect on rooting, specially cuttings which are considered hard to root (Ercili & Guleryz, 1999 and Delker *et al.,* 2008). Biofertilizers are considered group of organisms plays an important role in soil biofertility, because of their capability for producing hormones, amino acids and vitamins (Omar *et al.,* 1991). Biofertlizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). A promising trend for increasing the efficiency of biofertilizers is the use of different mixtures of bio preparations as nitrogen fixers, phosphate- and silicate-solubilizers (Wu *et al.,* 2005). Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers or seeds (Kloepper *et al.,* 2004). Some of the other reported benefits of using PGPR include the ability to control soil-borne fungi, enhance plant survival and induce systemic resistance to foliar pathogens (Liu *et al.,* 1995). PGPB use different mechanisms of action to improve plant growth and health, which could be active either simultaneously or sequentially at different stages of plant growth (Arshad & Frankenberger, 1998). Some bacteria have been found to stimulate plant growth through their 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activities (Shaharoona *et al.,* 2006). ACC-deaminase lowers ethylene levels in plants by converting ACC into α-ketobutyrate and ammonia, which is in contrast to activity of the enzyme ACC-synthase or ACC-oxidase, the latter are known to promote ethylene synthesis. Since higher concentrations of ethylene have been reported to inhibit root growth and nodulation (Arshad & Frankenberger, 2002), the bacteria containing ACC-deaminase may completely or partially eliminate potential inhibitory effects of higher ethylene concentrations in plants (Glick *et al.,* 1998). In addition, microbial activities can be made more efficient by maintaining high bacterial populations in the rhizosphere of a plant throughout the life cycle (Cheuk *et al.,* 2003). The direct growth promoting mechanisms by PGPR are as follows i) nitrogen fixation, ii) solubilization of phosphorus, iii) sequestering of iron by production of siderophores, iv) production of phytohormones such as auxins, cytokinins, gibberellins, and v) lowering of ethylene concentration (Banerjee, *et al.,* 2006). The use of PGPR offers an attractive way to replace chemical fertilizer and supplements most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants (Kloepper *et al.,* 2004).

Yeast (*Saccharomyces cervicisae*) is considered as a new promising biofertiltzer for many crops. The positive effects of applying yeast could be due to one or more of the merits. It contains some natural growth regulators, i.e. auxin (IAA) (Moor, 1979) and cytokinins (Cks) (Ferguson *et al.,* 1987) and it was also found to encourage the uptake of various nutrients (Vilsmeier and Amberger, 1988). In addition, it contains some important nutrients as N, P and K and some common amino acids (Abou-Zaid, 1984). This evaluation may be essential to examine some growth regulators and plant growth promoting rhizobactria on rooting ability of jujube (Li variety) semi hardwood cuttings.

MATERIALS AND METHODS

This investigation was conducted through two seasons of 2008 and 2009 on jujube trees (*Ziziphus Jujube* Mill) Li variety grown in the Experimental orchard station of Horticulture Research Institute at Giza, Egypt. Productive and nearly uniform trees similar in their vigour , disease free and subjected to the same horticulture practices were carefully selected and devoted as a source for the required cuttings under mist. This work was performed to study the effect of some root promoting micro-organisms as *Bacillus polymyxa (*BP), *Bacillus circulans* (BC), *Bacillus megaterium* (BM), *Bacillus pasteruii* BPa, *Pseudomonas florescence* (PF), or their mixture, Yeast (SV) and plant growth regulators (IBA or NAA) and control on rooting ability of jujube Li variety (hard rooting) cuttings. Both experimental seasons started at April 15th. Sub-terminal cuttings were prepared from one year old branches. Cuttings were about 10 cm in length and 1 – 1.5 cm in diameter and with 4 – 6 buds. The basal cut was made just below a nod and all leaves were removed, except two leaves left at the cutting top. The basal end of cuttings to about 2 cm was quickly dipped for 10 seconds just before planting in two different concentrations (1000 or 2000 ppm) of either indole butyric acid (IBA) or naphthalene acetic acid (NAA). In each PGPR strain and yeast treatments cuttings were inoculated for a period of one hour.

Origin and preparation of the microorganisms for inoculation:

Bacillus polymyx, *Bacillus circulance, Bacillus megaterium, Bacillus pasteruii, Pseudomans, Saccharomyces cerevisae*, yeast were provided from the Microbiology Dept. Soil & Water Environment Res. Instit. Agric. Res. Center, Giza, Egypt. Commercial preparation of the active dry yeast has the following composition as shown in Table (1).

Table (1): Composition of the dry matter of yeast

Constituents	Protein	Fats	Glycogen	Cellulose, gum, etc.	Ash
Percentage	52.41	1.72	30.25	6.88	8.74

Modified Ashby S' media (Abdel-Malek and Ishac, 1968) and semi-solid malate (Dobereiner, 1978) were incubated at 30 C^{o} and 32 C^{o} for 7 days. Also, Bunt and Rovira medium (1955) modified by Abdel-Hafez (1966)

was used. One liter from each of the previously prepared bacterial strain was diluted with one liter of water. Then all treated cuttings were planted in plastic flats containing vermiculite and perlite (1:1). Each treatment was replicated three times and each replicate represented by 25 cuttings. Planted flats were directly kept under intermittent mist for 16 weeks. Misting was applied according to seasonal and daily weather conditions, within a range of 5-15 seconds ON and 2, 5.5 min OFF. Inoculations with all the tested microorganisms were applied after planting every two weeks till the time of transplanting.

The tested treatments were arranged as follow:

T1. Untreated (dipping in water)
T2. Inoculated with *Bacillus polymyxa (*BP)
T3. Inoculated with *Bacillus circulans* (BC)
T4. Inoculated with *Bacillus megaterium* (BM)
T5. Inoculated with *Bacillus pasteruii* (BPa)
T6. Inoculated with *Pseudomonas florescence* (PF)
T7. Inoculated with Mixed inoculants (BP + BC + PF + BM + Bpa)
T8. Inoculated with Yeast (*Saccharomyces cerevisae*) (SV)
T9. Indole butyric acid (IBA) with 1000 ppm
T10. Indole butyric acid (IBA) with 2000 ppm
T11. Naphthalene acetic acid (NAA) with 1000 ppm
T12. Naphthalene acetic acid (NAA) with 2000 ppm

This experiment was terminated at mid September during both seasons of the study. The following measurements and determinations were recorded:

I. Rooting and vegetative growth of cuttings:

1-Rooting (%)
2-Average root length (cm)
3- Average number of roots/cutting
4- Stem length (cm)
5-Number of leaves/cutting
6-Number of sprouted shoots/cutting

Rooted cuttings were transplanted into black plastic bags 15 cm in diameter (one rooted cutting/bag) containing a mixture of peatmoss and sand (1:2).

II. Survival of transplants:

Eight months after transplanting the following measurements were recorded:

1. Survival percentage: It was estimated on the number of rooted cuttings that remained alive eight months later from recording the rooting measurements
2. Average root length (cm)
3. Average number of roots/ transplant
4. Stem length (cm)
5. Number of leaves / transplant
6. Number of branches/ transplant
7. Leaves, stem and root fresh weight (g)
8. Leaves, stem and root dry weight (g)

III. Histological studies

Histological studies were carried out on jujube sub-terminal cuttings in 2008 and 2009 seasons. Samples of two cuttings per each treatment were periodically taken at one week interval from planting date, till root formation, i.e. The basal portion (5cm) of jujube cutting was used for anatomical studies. Softening samples were soaked in tap water for two days before preparation of section. Sections of about 18-20 microns in thickness were prepared by using a sledage microtome. The sections were stained by the Safranine-Pecro-Amilye-Blue Method (Johansen, 1940). Sections were dehydrated, cleared xylol and mounted in canda balsam. Then sections were microscopically examined and photographed.

Statistical analysis

Experiments conducted in this study followed a Complete Randomized Design. The obtained data were subjected to Analysis of Variance (ANOVA) according to Snedecor and Cochran (1980). Differences between treatments were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

I. Rooting and vegetative growth of cuttings:

- **Rooting %**

Data presented in Table 1 and Figures 1-6 indicated that, all applications of (PGPR) except for mixed inoculants recorded the highest significant values of rooted cuttings percentage in both seasons compared with control and other treatments. Inoculated jujube cuttings with *Bacillus megaterium* had the highest significant increment (60% & 50%) followed by inoculation with *Pseudomonas florescence* (40% & 40%) in both seasons. On the other hand, NAA at 1000 or mixed inoculants and control didn't produce any roots (zero %), the rest treatments were in between in both seasons. These results are in accordance with observations of Strobel and Nachmias, (1985) on *Pruus amygdalus*, and Mcafee *et al.,* (1993) on Pinus spp. They found that A. *rhizogenes* is capable of improving propagation of jujube cuttings and quality of the subsequent root system. The ability of A. *rhizogenes* to infect and produce hairy roots in host plant tissue, however, is very specific depending on the strains of the bacterium and state of the host plants. Huang *et al.* (1991) suggested that compatibility between A. *rhizogenes* and host plants. T-DNA, phytohormona production and juvenility of the host tissues are important factors in inoculation success and hairy root production.

- **Average Root length (cm)**

Data in Table, 1 also show that the highest significantly values of root length (cm) was obtained when jujube cuttings inoculated with yeast following by *Pseudomonas florescence* and *Bacillus polymyxa* in the first season, in the second one the highest values were achieved when jujube cuttings inoculated with *Pseudomonas florescence* following by *Bacillus polymyxa* and *Bacillus megaterium*. However, not any roots were recorded with the untreated (control) cuttings or treated with NAA at 1000 or mixed

inoculants, where the other treatments were in-between, this was true in both seasons of study.

Fig (1)

Bacillus polymyxa

Fig (2)

Bacillus pasteruii

Fig (3)

Bacillus circulans

Fig (4)

Bacillus megaterium

Fig (5)

Yeast

Fig (6)

IBA

Figures (1-6): A comparison between rooting ability of treated cuttings with some plant growth promoting rhizobacteria and control (untreated) of jujube (Li variety).

These results were confirmed with those obtained by Cleyet-Marel *et al.* (2001), they found that the plant response to PGPR is obviously a very complex phenomenon resulting from the combination of mechanisms which affect several aspects of mineral nutrition and root development. Another feature that has been reported for PGPR is the production of phytohormones, which could affect root development directly. The nutritional capacity and developmental control are very much dependent on each other because they both affect growth rate of the plant. The positive effects of PGPR on plant growth are always correlated with remarkable changes in root morphology, namely increased lateral root length and root hair number and length (Bertrand *et al.,* 2000).

- **Average number of roots**

It could also be concluded from the obtained results in Table 1 that number of roots ranged from 4.25 & 5.00 in inoculated jujube cuttings with *Pseudomonas florescence* and *Bacillus pasteruii* and reached zero roots when the cuttings were treated with NAA at 1000 or mixed inoculants and control in both seasons. The enhancement of plant growth due to inculcation with asymbiotic N-fixers could be attributed to the capability of these organisms to produce growth regulators such as auxins, cytokinines and gibberillin which affect the production of root biomass and nutrients uptake (El-Ghinbihi &Fetouh, 2001 and Abou El-Khashab, 2002).

Table 1. Effect of some plant growth promoting rhizobacteria and growth regulators on rooting percentage, root length (cm) and number of roots after four months of planting jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Rooting (%)		Root length (cm)		Number of roots/cutting	
	2008	2009	2008	2009	2008	2009
Control	0.00 e	0.00 g	0.00 h	0.00 g	0.00 e	0.00 d
Bacillus polymyxa	40.00 b	30.00 c	27.4 b	28.00 b	3.00 c	4.00 b
Bacillus circulans	40.00 b	25.00 d	24.75 c	17.00 f	3.80 ab	1.00 c
Bacillus megaterium	60.00 a	50.00 a	24.75 c	28.00 b	3.20 c	5.00 a
Bacillus pasteruii	21.00 d	22.00 e	22.50 d	24.00 c	4.25 a	5.00 a
Pseudomonas florescence	40.00 b	40.00 b	27.70 b	32.00 a	4.10 a	5.00 a
Mixed inoculants	0.00 e	0.00 g	0.00 h	0.00 g	0.00 e	0.00 d
Yeast	30.00 c	18.00 f	28.75 a	20.00 e	3.25 bc	1.00 c
IBA (1000 ppm)	1.00 e	1.00 g	20.00 e	22.00 d	1.00 d	1.00 c
IBA (2000 ppm)	2.00 e	2.00 g	16.00 f	18.00 f	1.00 d	1.00 c
NAA (1000 ppm)	0.00 e	0.00 g	0.00 h	0.00 g	0.00 e	0.00 d
NAA (2000 ppm)	1.00 e	1.00 g	15.00 g	17.00 f	1.00 d	1.00 c

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

- **Stem length**

The overall trends in the effects of tested treatments on stem length are presented in Table 2. An increase in stem length was significantly observed in the jujube cuttings inoculated with (PGPR), the lowest values recorded in control, mixed inoculants and NAA at 1000 ppm in both seasons,

while in the second one the proper treatments were the inoculated cutting with *Bacillus megaterium* and *Bacillus pasteriuuu* followed by inoculated with *Pseudomonas florescence.* The other treatments were in between. The aforementioned results agree with those reported by Kloepper *et al*. (2004), they found that, inoculation with PGPR offers an attractive way to replace chemical fertilizers, pesticides, and supplements; most of the isolates result in a significant increase in plant height.

Table 2. Effect of some plant growth promoting rhizobacteria and growth regulators on stem length (cm), number of leaves/cutting and number of sprouted shoots/cutting of jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Stem length (cm)		Number of leaves/cutting		Number of sprouted shoots/cutting	
	2008	2009	2008	2009	2008	2009
Control	0.00 e	0.00 f	0.00 h	0.00 g	0.00 d	0.00 c
Bacillus polymyxa	16.6 c	15.0 d	3.33de	5.00 c	1.00 c	1.00 b
Bacillus circulans	18.0 b	14.0 e	3.30de	2.00 f	2.00 a	1.00 b
Bacillus megaterium	17.6bc	18.0 a	7.00a	9.00 a	1.00 c	3.00 a
Bacillus pasteruii	20.7 a	18.0 a	3.66 d	3.00 e	1.00 c	1.00 b
Pseudomonas florescence	19.8 a	17.0 b	5.62 b	8.00 b	1.00 c	3.00 a
Mixed inoculants	0.00e	0.00 f	0.00 h	0.00 g	0.00 d	0.00 c
Yeast	15.0 d	15.0 d	4.66 c	3.00 e	1.50 b	1.00 b
IBA (1000 ppm)	15.0 d	16.0 c	3.00ef	4.00 d	1.00 c	1.00 b
IBA (2000 ppm)	15.0 d	16.0 c	2.00 g	3.00 e	1.00 c	1.00 b
NAA (1000 ppm)	0.00 e	0.00 f	0.00 h	0.00 g	0.00 d	0.00 c
NAA (2000 ppm)	15.0 d	17.0 b	2.00 g	3.00 e	1.00 c	1.00 b

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

- **Number of leaves/cutting**

Data presented in Table 2 also showed that the greatest values of number of leaves/cutting (7.00 & 9.00) were obtained by inoculated jujube cuttings with *Bacillus megaterium* following by inoculated with *Pseudomonas florescence* (5.62 & 8.00) in both seasons, respectively. The lowest values were found as a result of control or mixed inoculants and treatment with NAA at 1000 ppm (0.00 & 0.00), the other treatments were in between.

- **Number of sprouted shoots/cutting**

In this respect, jujube cuttings inoculated with *Bacillus circulans* (1st season) and Bacillus megaterium & *Pseudomonas* (2nd season) were the proper treatments. While, the lowest values (0.00 & 0.00) were found as a result of control, mixed inoculants and treatment with NAA at 1000 ppm, the rest treatments were in-between. Similar results were reported by Arshad & Frankenberger (1998), they recorded that the soil bacteria influencing the plant growth positively by any metabolic process are referred to as plant growth-promoting bacteria (PGPB). PGPB use different mechanisms of action to improve plant growth and health, which could be active either simultaneously or sequentially at different stages of plant.

II. Survival of transplants:

Data concerning survival (%), vegetative growth of transplants and leaves, stem & roots fresh and dry weight after eight months of transplanting are presented in Tables (3, 4, 5 & 6).

- **Survival (%)**

Data in Table 3 showed that inoculated jujube cuttings with *Bacillus megaterium* treatment following by inoculated with *Pseudomonas florescence* treatment gave the highest values in this respect (70%, 71% & 65%, 64%), respectively, in both seasons. The lowest percentages were found as a result of control, mixed inoculants and treatment with NAA at 1000 ppm as they recorded 0.00%, the rest treatments were in between. Benefits of using PGPR include the ability to control soil-borne fungi, enhance plant survival, and induce systemic resistance to foliar pathogens (Liu *et al.,* 1995).

- **Average root length (cm)**

The highest values of root length after eight months from transplanting were recorded by inoculated cuttings with *Bacillus megaterium* (35.00, 37.00 cm) in both seasons as well as the inoculated cuttings with *Bacillus polymyxa* in the second season only (37.00 cm). The minimum values were recorded from control, mixed inoculants and NAA at 1000 ppm in both seasons where the cutting didn't have any roots. The rest treatments were in between.

- **Average number of roots /transplant**

Number of roots/transplant after eight months was affected significantly by the tested treatments. The most stimulus effect were recorded by inoculated jujube cuttings with *Bacillus megaterium* (12.00, 12.00) in both seasons following by *Pseudomonas florescence*, *Bacillus circulans* and, *Bacillus polymyxa,* they had the same value (10.00) in the first season. Control, mixed inoculants and NAA at 1000 ppm took the other way around as they didn't have the ability to produce any roots in both seasons. The rest treatments were in between.

- **Stem length (cm)**

Stem length (cm) was significantly increased in all inoculated cuttings with (PGPR) strains than control or mixed inoculants and plant growth regulators in both seasons Table, 4. The cuttings inoculated with *Bacillus megaterium* caused a significant increase in stem length after eight months in both seasons (29.00, 28.00 cm) as well as the inoculated with *Bacillus pasteruiiin* in the second one (28.00 cm). On the contrary, the mixed inoculation and growth regulators treatments as well as the untreated cuttings didn't show any increase in transplant stem length.

- **Number of leaves/transplant**

It is interesting to note in Table 4 that, all cuttings inoculated with (PGPR) strains significantly improved this parameter compared with control or mixed inoculants and plant growth regulators in both seasons. The highest values were achieved when cuttings were inoculated with *Bacillus megaterium* (41and 35) in both seasons, respectively.

- **Number of branches/transplant**

Table 4 indicated that, number of branches/transplant increased significantly as a result of inoculation with all PGPR strains when they were added solely. On the other hand, PGPR mix, control and NAA 1000 ppm treatments didn't significantly have any effect on number of branches. Where, the rest treatments were in-between in the two seasons of study. PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR (Joseph *et al.,* 2007). The stimulus effect of bio-fertilizer application may be attributed to the prompting effect on the parameters of plant growth which are enable to absorb more minerals by root system (Adam, 2002).

- **Leaves , stem and root fresh weight (g)**

Data presented in Table 5 indicated that leaves, stem and root fresh weight (g) increments were significantly more pronounced by inoculation with *Bacillus megaterium* following by *Bacillus polymyxa, Bacillus circulans* and *Pseudomonas florescence* in both seasons expect for stem fresh weight in second season, where the differences were in insignificant between them . Generally, there were insignificant differences between both IBA concentrations (1000 & 2000 ppm) and the higher concentration of NAA (2000 ppm).

Table 3. Effect of some plant growth promoting rhizobacteria and growth regulators on survival (%), root length (cm) and number of roots after eight months of transplanting of jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Survival (%)		Average root length (cm)		Average number of roots/transplant	
	2008	2009	2008	2009	2008	2009
Control	0.00 f	0.00 f	0.00 i	0.00d	0.00 g	0.00d
Bacillus polymyxa	60.00 c	59.00 c	33.00 b	37.00 a	10.00 b	11.00 a
Bacillus circulans	60.00 c	59.00c	32.00 bc	34.00 b	10.00 b	11.00 a
Bacillus megaterium	70.00 a	71.00 a	35.00 a	37.00 a	12.00 a	12.00a
Bacillus pasteruii	50.00 d	50.00 d	29.00 e	34.00 b	7.00 d	8.00 b
Pseudomonas florescence	65.00 b	64.00 b	31.00 cd	34.00 b	10.00 b	11.00 a
Mixed inoculants	0.00 f	0.00 f	0.00 i	0.00 d	0.00 g	0.00 d
Yeast	50.00 d	50.00 d	30.00 de	34.00 b	8.00 c	8.00 b
IBA (1000 ppm)	20.00 e	15.00 e	25.00 f	30.00 c	5.00 e	4.00 c
IBA (2000 ppm)	20.00 e	18.00 e	20.00 g	29.00 c	3.00 f	4.00 c
NAA (1000 ppm)	0.00 f	0.00 f	0.00 i	0.00 d	0.00 g	0.00 d
NAA (2000 ppm)	18.00 e	18.00 e	17.00 h	29.00 c	3.00 f	4.00 c

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 4. Effect of some plant growth promoting rhizobacteria and growth regulators on stem length (cm), number of leaves/ transplant and number of branches/ transplant after eight months from transplanting of jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Stem length (cm)		Number of leaves/transplant		Number of branches/ transplant	
	2008	2009	2008	2009	2008	2009
Control	0.00 e	0.00e	0.00 i	0.00 f	0.00 d	0.00 d
Bacillus polymyxa	24.60b	25.00b	28.00b	31.00b	3.00 a	3.00 a
Bacillus circulans	24.00b	25.00b	24.00cd	28.00c	3.00 a	2.00 b
Bacillus megaterium	29.00a	28.00a	41.00a	35.00a	3.00 a	3.00 a
Bacillus pasteruii	22.00c	28.00a	21.00 e	22.00d	2.00 b	2.00 b
Pseudomonas florescence	24.00b	25.00b	25.00 c	27.00c	3.00 a	3.00 a
Mixed inoculants	0.00 e	0.00e	0.00 i	0.00 f	0.00 d	0.00 d
Yeast	22.00c	23.00c	23.00d	22.00d	2.00b	2.00 b
IBA (1000 ppm)	19.00d	19.00d	18.00 f	17.00e	1.00 c	1.00 c
IBA (2000 ppm)	19.00d	20.00d	12.00 h	17.00e	1.00 c	1.00 c
NAA (1000 ppm)	0.00 e	0.00e	0.00 i	0.00f	0.00 d	0.00 d
NAA (2000 ppm)	19.00d	19.00d	15.00 g	17.00e	1.00 c	1.00 c

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 5. Effect of some plant growth promoting rhizobacteria and growth regulators on leaves, stem and root, fresh weight (g) after eight months from transplanting of jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Fresh weight (g)					
	2008			2009		
	Leaves	Stem	Root	Leaves	Stem	Root
Control	0.00 e	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d
Bacillus polymyxa	1.83 b	4.21 b	2.69 b	1.75 b	5.90 a	2.80 b
Bacillus circulans	1.80 b	4.18 b	2.50 b	1.70 b	5.92 a	2.75 b
Bacillus megaterium	2.30 a	6.11 a	3.17 a	2.15 a	5.95 a	3.00 a
Bacillus pasteruii	1.40 c	3.00 c	1.00 c	1.38 c	3.00 b	1.55 c
Pseudomonas florescence	1.82 b	4.17 b	2.41 b	1.70 b	5.89 a	2.70 b
Mixed inoculants	0.00 e	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d
Yeast	1.50 c	3.20 c	1.00 c	1.40 c	3.00 b	1.50 c
IBA (1000 ppm)	1.01 d	2.05 d	0.94 c	0.95 d	2.50 c	1.50 c
IBA (2000 ppm)	0.92 d	2.00 d	0.90 c	0.90 d	2.40 c	1.53 c
NAA (1000 ppm)	0.00 e	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d
NAA (2000 ppm)	1.00 d	2.00 d	0.69 c	0.95 d	2.40 c	1.45 c

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 6. Effect of some plant growth promoting rhizobacteria and growth regulators on leaves, stem and root, dry weight (g) after eight months from transplanting of jujube (Li variety) sub-terminal cuttings during 2008 and 2009 seasons.

Treatments	Dry weight (g)					
	2008			2009		
	Leaves	Stem	Root	Leaves	Stem	Root
Control	0.00 f	0.00 g	0.00 g	0.00 d	0.00 f	0.00 d
Bacillus polymyxa	0.40 b	2.59 c	1.17 b	0.35 b	2.50 b	1.00 b
Bacillus circulans	0.37 bc	2.60 c	1.15 b	0.33 b	2.25 c	1.00 b
Bacillus megaterium	0.67 a	3.46 a	1.44 a	0.50 a	3.30 a	1.30 a
Bacillus pasteruii	0.30 d	2.15 d	0.70 c	0.22 c	1.95 d	0.94 b
Pseudomonas florescence	0.35 c	2.65 b	1.13 b	0.31 b	2.55 b	1.00 b
Mixed inoculants	0.00 f	0.00 g	0.00 f	0.00 d	0.00 f	0.00 d
Yeast	0.33 cd	2.12 d	0.73 c	0.30 b	1.90 d	0.95 b
IBA (1000 ppm)	0.25 e	1.10 e	0.50 d	0.20 c	0.95 e	0.40 c
IBA (2000 ppm)	0.24 e	1.00 f	0.33 f	0.20 c	0.91 e	0.30 c
NAA (1000 ppm)	0.00 f	0.00 g	0.00 g	0.00 d	0.00 f	0.00 d
NAA (2000 ppm)	0.20 e	1.07 e	0.48 de	0.20 c	0.93 e	0.35 c

Values have the same letter(s) in the same column are not significantly different at 5% level using Duncan's Multiple Range Test.

- **Leaf, stem and root dry weight (g)**

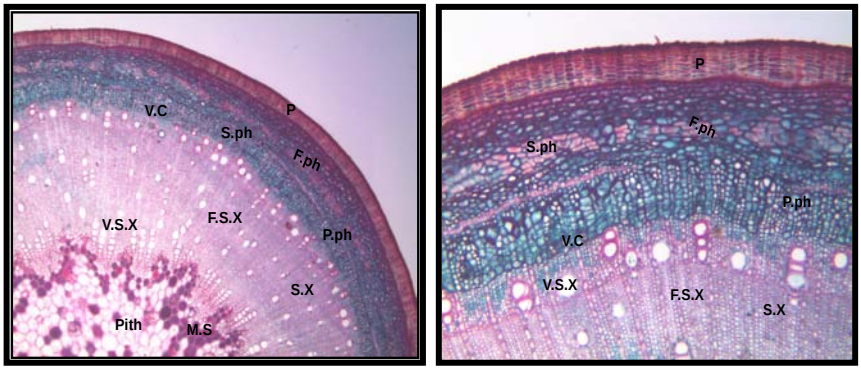
It could be concluded from the obtained results in Table 6 that cuttings inoculated with *Bacillus megaterium* had significantly appositive effect on leaf, stem and root dry weight (g) in both seasons as compared to other treatments. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers or seeds Weller (1988). The aforementioned results agree with Kloepper *et al.* (2004), they found that, the use of PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements; most of the isolates result in a significant increase in dry matter prod uction of shoot and root of plants. Generally, it can be concluded from the previous results that, inoculation jujube (Li variety) sub-terminal cuttings with *Bacillus megaterium* or *Pseudomans* can promote root formation as well as increase survival (%) and enhance vegetative growth of the produced transplants.

- **Histological studies**

The stem of jujube (Figs, 7 & 8) has a well differentiation periderm. The vascular tissues are continuous ring of secondary xylem and secondary phloem. The secondary xylem consists of solitary wide vessels, fibres and few pareuchynatous cells. The secondary phloem has elements, companion cells and parenchyma. Between the vascular tissues i.e, the xylem and phloem a narrow vascular embroil zone, which was bordered by remains of the primary phloem and cortex. The primary phloem has wide strands of fibers. That was separated with narrow parenchyma zones which prevents the emergence of adventitious roots. The primary xylem is observed lining the wide pith which is composed of large parenchyma cells. Some of these cells have dark contents, which observed in the outer part of the pith which called the (medullary sheath). The anatomical structure of jujube stem cutting is

nearly closed to jojoba *(Simmondsia chinensis)* as provably mentioned by Sayed *et al.,* (2010). In most plants adventitious roots are originated in the vicinity of differentiating vascular tissue. These places are root close to xylem, phloem and facilitates rapid establishment of vascular connection (Satoo, 1956). Biofertilizers are considered to be the alternate source of fertilizers to meet the nutrient requirements of crops and to bridge the future gaps. This group of organisms seems to play an important role in soil biofertility, because of their capability for producing hormones, amino acids and vitamins (Omar, *et al.,* 1991). The direct growth promoting mechanisms by PGPR are as follows i) nitrogen fixation ii) solubilization of phosphorus iii) sequestering of iron by production of siderophores iv) production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration (Glick *et al.,* 1999). There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for crops (Banerjee, *et al.,* 2006). The emergence of adventitious roots was observed about eight weeks after cuttings were grown. The transverse sections in some rooted cuttings revealed the development of few callus cells occurred on the destroyed parts of the cuttings (Fig, 9). The callus cells were proliferated and form small protrusions and originated from the cambial and phloem parenchyma cells below the cork cells from these protrusions the adventitious roots were developed. Sooner or later the new roots established their connections with the vascular tissue of the cutting treated by *Bacillus megaterium* (Fig, 10).

Fig (7) **Fig (8)**



S.ph: Secondary phloem. **S.X: Secondary xylem.**
F.X.: Fibers of xylem. **F.ph.: Fibers of primary phloem.**
P.ph.: Parenchyma of phloem. **V.C.: Vascular cambium.**
M.S.: Medullary sheath. **A.r.: Adventitious root.**

Figs (7 &8): A cross section in jujube plant stems cutting (control) showing the different tissues

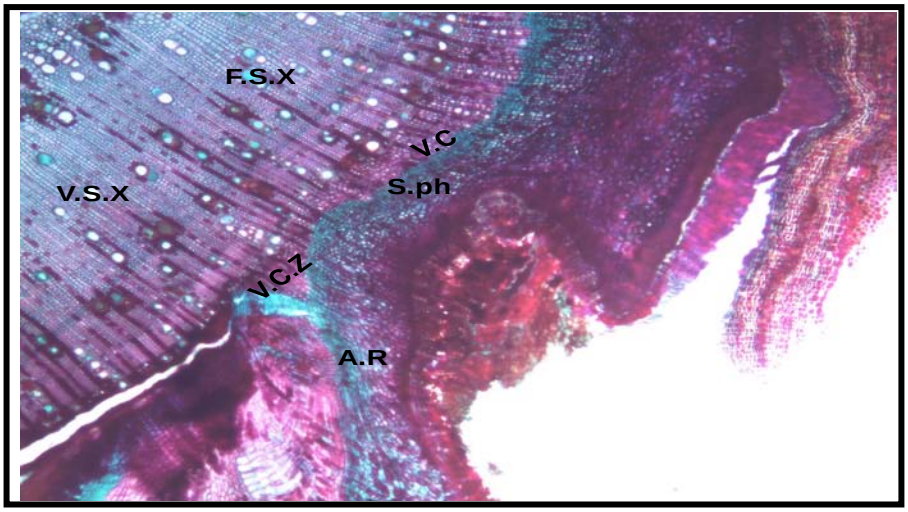


Fig (9): A cross section in jujube plant stem cutting showing the development of few callus cells.

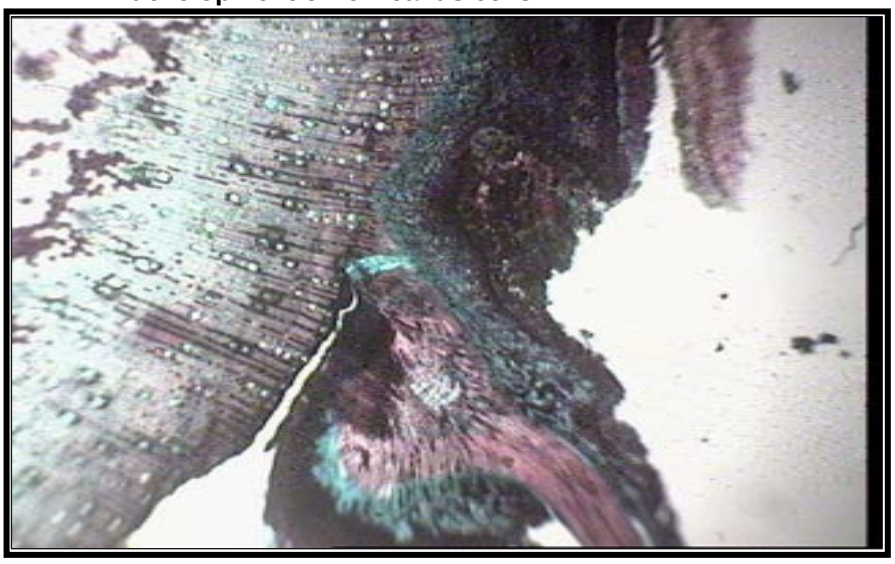


Fig (10): Across section in jujube plant stem cutting showing the well development root.

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تجذير عقل العناب (*Ziziphus Jujuba Mill*) صنف لى بأستخدام بعض الكائنات الدقيقة المشجعة للتجذير ومنظمات النمو النباتية

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اجريت هذة الدراسة فى معهد بحوث البساتين بالجيزة – مصر وذلك لدراسة تأثير بعض الكائنات الدقيقة المشجعة للتجذير ومنظمات النمو على تجذير العقل تحت الطرفية فى منتصف شهر ابريل والمأخوذة من افرع عمر سنة من أشجار العناب (عمرها 15 سنة) صنف لى الصعب التجذير بالعقلة خلال موسمى الدراسة 2008-2009. وقد تم نقع قواعد العقل بالمعاملات الآتيه:

بكتريا بأسلس بوليمكسما - بأسلس سركيولينز- بزيدومنس فلورسينس- بأسلس ميجتيريم- بكتريا بأسلس بيستيريو- مخلوط من انواع البكتريا السابقة وخميرة سلالة سكارواميسز بالاضافة الى المعاملة بمنظمات النمو مثل: اندول بيوتريك اسيد و نفثالين استيك اسيد بتركيز 1000 – 2000 جزء فى المليون مقارنة بالكنترول (عقل تم نقعها فى الماء). ومن أهم النتائج المتحصل عليها إن نقع قواعد العقلة ببكتريا باسلس ميجتيريم أعطى أعلى نسبة تجذير فى الموسمين على التوالى (50 %- 60%). بعد ثمانية اشهر من الزراعة بكتريا باسلس ميجتيريم يليها بزيدومنس فلورسينس اعطت اعلى نسبة بقاء وكذلك متوسط عدد الجذور/شتلة وطول الساق والجذر وعدد الاوراق/شتلة والوزن الطازج والجاف للجذر والساق والاوراق. بينما معاملة الكنترول وخليط البكتريا ومعاملة نفثالين اسيتك أسيد بتركيز 1000 جزء فى المليون كانت الاقل خلال موسمى الدراسة. وقد أوضحت الدراسة التشريحية لقواعد العقل أن الجذور العرضية تنشأ من الكامبيوم وبرانشيمه اللحاء وذلك عند خروج الجذر. عموما يمكن التوصية باستخدام النقع ببكتريا باسلس ميجتيريم او باسلس بزيدومنس فلورسينس لتجذير العقل الساقية تحت الطرفية للعناب صنف لى (فى منتصف شهر ابريل) وذلك للحصول على نسبة عالية من التجذير ونسبة البقاء وكذلك تشجيع تحسين نمو الشتلات الناتجة.

قام بتحكيم البحث

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