

INFLUENCE OF SOME PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON VEGETATIVE GROWTH, NITROGEN AND PHOSPHORUS CONTENTS AND ANATOMICAL CHARACTERISTICS OF *Taxodium distichum* Rich. TRANSPLANTS

(Received: 26. 9. 2010)

By

H. H. Hammad, A. A. Awad * and O.S. El-Kobisy**

*Timber Trees and Forestry Department, Horticultural Research Institute, Agricultural Research Center, Giza * Soils, Water and Environment Research Institute, Agricultural Research Center, Giza** Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza*

ABSTRACT

Growth of *Taxodium distichum* Rich. transplants was stimulated by inoculation with Plant Growth Promoting Rhizobacteria (PGPR). Results show that significant increases in the plant height, stem diameter, branch number and fresh and dry weights per transplant were observed in the plants inoculated with *Azospirillum brasilense*. While, higher total phosphorus percentage was found in the plants inoculated with *Bacillus polymixa* (0.78%) compared with the control (0.45%) in the stem of *Taxodium distichum* transplants. Such effects were confirmed by anatomical studies. These results suggest that the growth promoting substances provided by *Azospirillum barsilense* may enhance the growth of *Taxodium distichum* transplants and shortening the time of growth.

Key words: *anatomy, Azospirillum brasilense, Azotobacter chroococcum, Bacillus polymixa, plant growth promoting rhizobacteria (PGPR), and Serratia sp., Taxodium distichum, vegetative growth.*

1. INTRODUCTION

Taxodium distichum Rich. belongs to family Taxodiaceae which is an unusual and interesting tree often growing over 100 m in height and over 3m in diameter. This beautiful tree is used as a specimen or in small groves particularly in permanent wet areas of the landscape and along roadsides. The wood has a multitude uses and is well known for its ability to resist decay, it is fairly durable and has many uses in outdoor constructions (Bowers and Melhuish, 1988). Cypressene, is an oil extracted from the wood, is believed to give bald cypress high decay resistance. For this reason, cypress wood has long been favored in the building construction fences, posts, planking in boats, doors, shingles, blinds, flooring, caskets, interior trim, cabinetry and many other uses. (Walker 1990). Resins that can be obtained from the cones have been used as an analgesic in folk remedies for skin ailments. Horticultural, bald cypress can be grown on many upland soils and it is seen in cities as a shade tree or ornamental (Wilhite and Toliver,

1990). Gutierrez Manero *et al.* (1996) reported that the influence of native rhizobacteria on the growth of European alder (*Alnus glutinosa* Gaertn). PGPR has become a new class of biofertilizers and physiological stimulators in recent years. PGPR have been a renewed interest for inoculation of agricultural crops. PGPR hold great promise as potential agricultural and forestry inoculants and could reduce the use of agrochemicals including chemical fertilizers and pesticides(Zahir *et al.*, 2004). A number of (*Rhizobacteria*) strains with a positive effect on plant development [Plant Growth Promoting *Rhizobacteria* (PGPR)] have been reported (Belimov *et al.*, 2001).Many strains have been catalogued as (PGPR) due to their effect on plant pathogens (Mei *et al.*, 1984., Schippers *et al.*, 1991; Bashan and de Bashan , 2002 and Al-Kahal *et al.*, 2003) or to their ability to induce plant growth promoting (Bashan,1999 and Mekhamar ,2001). Most of these strains belong to *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Azospirillum* (Reddy and Rahe 1989). Phytohormones such as

indol-3-acetic acid (IAA) or cytokinins are among the plant growth promoting compounds often produced by bacteria (Hubble *et al.*, 1979 and Muller *et al.* 1989). However, other compounds, known as auxins- like IAA¹ are often responsible for the promoting effects (Oberhansli *et al.*, 1990 and Selvadurai *et al.*, 1991).

The aim of this work was to investigate the effect of different PGPR strains on growth, N, P content and the anatomical characteristics of *Taxodium distichum* transplants. Moreover to determine the best treatment that could be used to promote the growth of the seedlings in the nursery.

2. MATERIALS AND METHODS

This study was conducted at the nursery of the Timber Trees and Forestry Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt with the cooperation with Soils, Water and Environment Res. Institute, Agric. Research Center, Giza, Egypt, and the Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza during two successive seasons 2008/2009 and 2009/2010.

Seeds of *Taxodium distichum* Rich. were inoculated with *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus polymixa* and *Serratia* sp. that were planted in plastic cups 5 cm diameter filled with sterilized media on the first of January 2008, at the nursery of the Timber trees and Forestry Department. After three months, the seedlings were transplanted in plastic cups 10 cm diameter on the first of April 2008. On the 15th of June 2008 homogenous seedlings were transplanted into plastic pots 30 cm diameter filled by the sterilized container media and inoculated again by the same bacterial strains. The seedlings were placed in a shaded area and after two weeks from transplanting, seedlings were removed outdoors to a sunny area and common cultural practices including regular irrigation were followed (twice weekly in winter and daily in summer). Every transplant received starter nutrition consisted of NPK 0.5g from Kristalon 19:19:19.

2.1. Organisms and growth conditions

Azospirillum brasilense (SP 245), *Azotobacter chroococcum*, *Bacillus polymixa* and *Serratia* sp. (Local strain) were supplied by the Microbiology Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. *Azospirillum* was grown in Yeast

Extract Peptone (YEP) medium (Vanstocken *et al.*, 1987) *Azotobacter* was grown in Asheby medium (Hegazi and Neimela, 1976). *Serratia* and *Bacillus* were incubated at 25°C for three days until early log phase was developed of 10⁷ viable ml⁻¹. Container media. The container media used in this study were consisted of separated manure, Sphagnum peat and an organic component (vermiculite No-2) (1:1:1 v/v). Physical and chemical characteristics of the used media were analyzed before cultivation according to Abdel-Wahab and Ahmed (2003) and their properties are presented in Table (1).

Table(1): Physical and chemical characteristics of the used cultivation media.

Property	Value
pH	6.93
E.c.(ds/m)	4.23
Organic-C(%)	19.65
Total-N(%)	1.10
C/N ratio	17.86
Total -P(%)	0.78
Total -K(%)	1.23

2.2. Data concerned

In both seasons 2008/2009 and 2009/2010, the vegetative growth parameters were recorded including plant height (cm), stem diameter, out bark (mm) measured at 10 cm above soil surface, fresh and dry weights of the shoots and leaves (g/ plant).

2.3. Chemical composition

The chemical constituents in dried shoot samples were determined using the method described by Piper (1950). The nitrogen content were determined using the modified micro - Kjeldahl method as described by Pregl (1945), while the phosphorus content was estimated using the method recommended by King (1951).

2.4. Anatomical characteristics

The anatomical study was performed on the stem and lateral roots as a cross section at the age of 30 months from transplants planted in first of January 2008 till the first of July 2010. It was intended on plant materials taken from the median portion of the main stem and lateral root. Specimens were killed and fixed for at least 48 hrs in formalin acetic acid (F.A.A) 10 ml formalin, 5 ml glacial acetic acid, 50 ml ethanol alcohol 95% and containing 35 ml distilled water. The selected

materials were washed in 50 % ethyl alcohol , dehydrated in a normal butyl alcohol series embedded in paraffin wax of melting point 56°C , sectioned to a thickness of 20 microns and double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam, Willey (1971). Sections were analysed to detect histological manifestations of noticeable responses resulted from using Plant Growth Promoting *Rhizobacteria*.

2.5. Experimental design and statistical analysis

The pot experiment was carried in randomized complete blocks design. Each main plot contains 5 treatments each treatment represented 25 replicates. Each replicate consisted of 5 pots, with a single plant per pot. The data were subjected to statistical analysis of variance and the means were compared using the Least Significant Difference (L.S.D) test at 5% level, as described by Steel and Torrie,(1980).

3. RESULTS

3.1. Effects of some PGPR on *Taxodium distichum* transplants.

3.1.1. Vegetative growth

Data presented in Table (2) show that inoculation PGPR to container medium had generally favorable effect on the vegetative growth parameters in both seasons , compared to transplants un- inoculated . Among the different

PGPR strains, *Azospirillum brasilense* strain was clearly the most effective for promoting vegetative growth of *Taxodium distichum* transplants in both seasons. In this respect the highest values of plant height , stem diameter, branches number, fresh and dry weights per transplant were :91.33 cm, 13.4mm , 10.00 branches, 73.47 and 21.80 g, respectively, in the first season , and reached 100.00 cm,15mm, 8,00 branches,72.50 and 21.50 g ,respectively in the second season. In the first season, the percentages of increases as a result of using *Azospirillum barsilense*, compared to uninoculated medium, were 62.1%, 67.5%, 233.3%, 184.8% and 161.4% for plant height , stem diameter, branch number , fresh and dry weights /transplant over the control, respectively.

In addition,data in Table (2) indicate that no significant differences were observed between using the strains of *Bacillus ploymixa* and *Serratia* sp. While , *Azotobacter chroococcum*, gave the least vegetative growth values as compared to any other used strains.The results were confirmed in the second season, with the same applications.

Table (2) indicates that *Taxodium disitchum* seedlings responded favorably to inoculation with PGPR strains specially *Azospirillum brasilense*. A similar significant trend was noticed when the growth media was inoculated with PGPR. The growth parameters of the seedlings in the inoculated

Table (2):Influence of PGPR on vegetative growth of *Taxodium distichum* transplants in both studied seasons 2008 / 2009 and 2009/2010.

Characters Treatments	The First season 2008/2009					The Second season 2009/2010				
	Plant height (cm)	Stem diameter (mm)	Number of branches	F.W* (g)	D.W** (g)	Plant height (cm)	Stem diameter (mm)	Number of branches	F.W* (g)	D.W** (g)
Control	56.33	8.00	3.00	25.80	8.34	60.00	8.50	3.00	28.80	9.50
<i>Azospirillum brasilense</i> M1	91.33	13.40	10.00	73.47	21.80	100.00	15.00	8.00	72.50	21.50
<i>Azotobacter chroococcum</i> M2	68.67	9.70	4.00	32.60	10.11	72.33	11.00	4.00	35.80	12.00
<i>Bacillus polymixa</i> M3	83.50	10.80	4.00	51.67	14.90	88.50	13.00	5.00	53.00	16.00
<i>Serratia</i> sp. M4	84.00	9.60	7.00	54.67	14.93	88.50	13.00	5.00	54.67	16.00
L.S.D.at 5%	7.50	1.40	3.46	11.60	3.13	6.67	1.10	2.46	9.60	2.95

F.W* : fresh weight , D.W**: dry weight

media increased by 66.6%, 67.5%, 166.7%, 151.7% and 126.3% for plant height, stem diameter, branches number, fresh and dry weights per transplant, when compared to the uninoculated media. These results indicate that the *Rhizobacteria* exhibited positive effects on the growth parameters of the inoculated host plants as previously reported by Zahir *et al.* (2004) and Mekhamar *et al.* (2007).

3.1.2. Chemical analysis

Data presented in Table (3) indicate that significant increases in N were observed in shoots and roots of the seedlings inoculated with *Azospirillum* which showed higher N content being 2.14 and 0.81%, respectively than those recorded for transplants grown in other media.

readily available for plant uptake, and this should be considered as a mechanism for enhanced plant growth (Glick, 1995). Several reports suggested that PGPR can stimulate plant growth by increasing solubilization *via* releasing siderophores or organic acids and facilitate the uptake of mineral nutrients by the plant (Chabot *et al.* , 1996; Biswas *et al.* , 2000 a and b ; Dazzo *et al.* , 2000).

These results are in agreement concerning such effects, It was similarly demonstrated by several authors Abo El-Soud *et al.* (2007), and Mekhamar *et al.* (2007) who explained that improving effects arising from microbial inoculation are due to producing growth promoting substances such as auxins , gibbrillins and cytokinins.

Table (3): Nitrogen and Phosphorus contents (%) Shoots and roots of *Taxodium distichum* transplants as influenced by PGPR inoculation in both seasons

Characters Treatment	The First season 2008/2009				The Second season 2009/2010			
	Shoot N%	Root N%	Shoot P%	Root P%	Shoot N%	Root N%	Shoot P%	Root P%
Control	1.32	0.55	0.45	0.30	1.33	0.55	0.45	0.30
<i>Azospirillum</i> M1	2.14	0.80	0.55	0.53	2.11	0.81	0.54	0.50
<i>Azotobacter</i> sp. M2	1.48	0.52	0.47	0.32	1.45	0.50	0.45	0.33
<i>Bacillus</i> sp. M3	1.75	0.75	0.78	0.43	1.75	0.73	0.74	0.43
<i>Serratia</i> sp. M4	1.50	0.63	0.50	0.42	1.50	0.65	0.50	0.40
L.S.D. at 5%	0.24	0.18	0.15	0.10	0.23	0.18	0.15	0.10

Additionally , it is well demonstrated that in both seasons chemical analysis of dried shoot and root of the seedlings which were grown in different inoculum strains in the growing media, reported in (Table 3) that the P content was considerably increased by addition of *Bacillus polymixa* to the bio composite media. The P content varied from 0.45% in the transplants grown in an uninoculated control media to 0.78% in transplants grown in media inoculated by *Bacillus polymixa* strain. This means that the addition of (PGPR) strains to the media increased P content in the transplants shoots and roots. Solubilization of mineral nutrients such as phosphorus and iron by PGPR made them more

3.1.3. Anatomical study

3.1.3.1. The stem

Data presented in Table (4) and Fig. (1) illustrate that stem diameter of *Taxodium* transplants in all treatments was thicker when compared to the control and reached their maximum thickness with treatment (M1), as it reached 18.5% over the control. Predermal layer was thin in both (M1) and (M3), while in the other treatments, it was equal to the control. Cortex thickness decreased when applied all treatments and reached it's minimum decrease with treatments (M1) and (M3), where all treatments compensated the decrease in this trait by the highest increase in xylem thickness

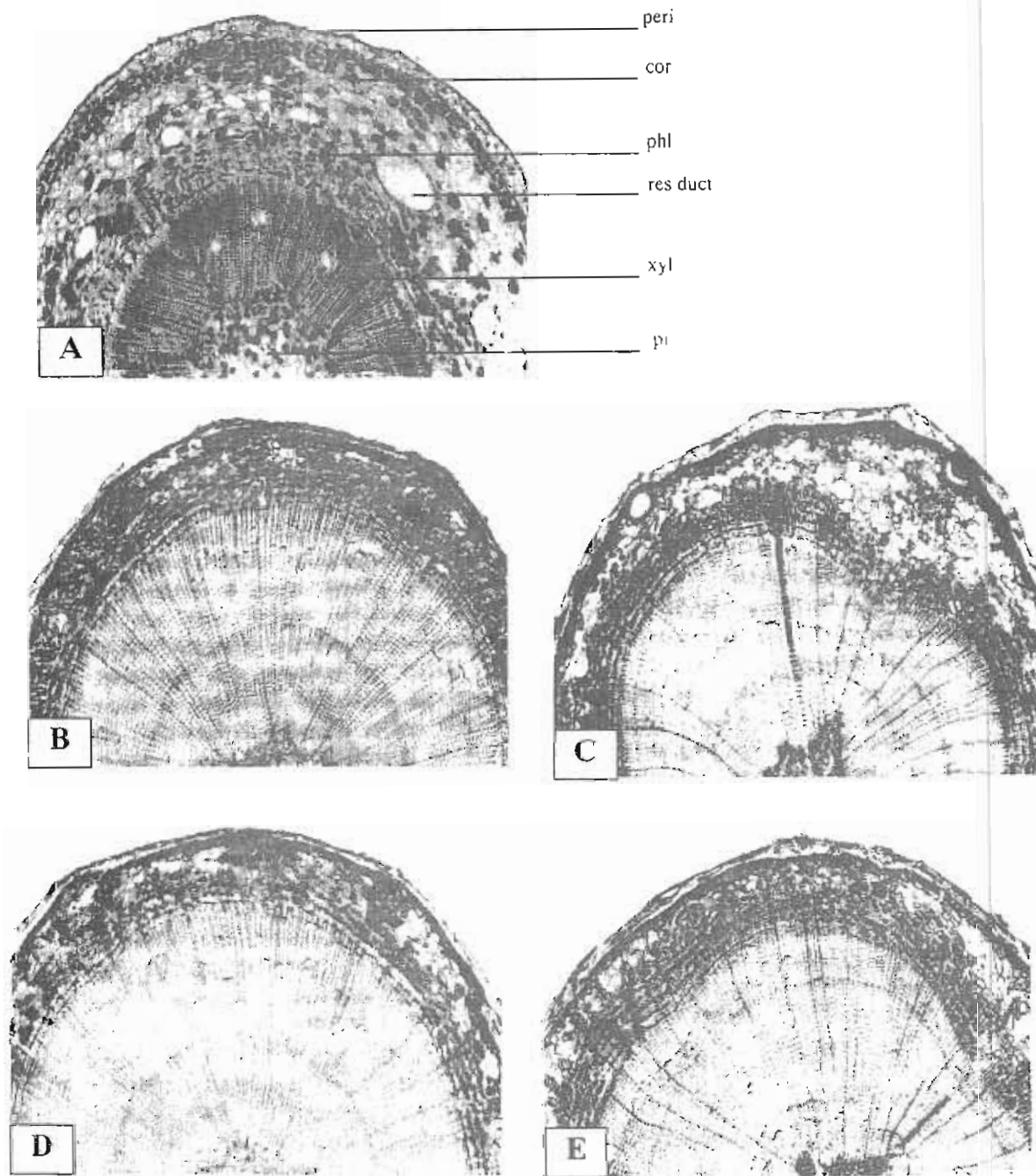


Fig. (1) : Transverse section through the middle part of the median internode of the main stem of *Taxodium* transplants (X=54)
A-Control, B- M1, C-M2, D-M3, E-M4

over the control. Fibrous thickness was more pronounced in the treatments (M1, M2 and M3). Thickness of phloem increased with treatments, (M1) and (M4) representing 33.3% and 50% increases over the control, respectively and that may play a role for increasing diameter and vigor. Thickness of xylem verified the importance for using treatments which exhibited the high increases than other traits in all treatments (M1, M2, M3 and

the cortex it is proved that, in all applied treatments, the number of glands decrease than control also the disappear of these ducts in xylem in all treatments under studies. On the other hand, annual rings were fuzzy in the control where other treatments exhibited a clear rings which were 2- 3 rings in all treatments. These results of vigour growth attributed to the role of PGPR on seedlings growth, while the control treatment gave the lowest values which

Table(4): Counts and measurements in (micron) of certain histological features in transverse sections through the middle part of the median internode of the main stem of bald cypress plants as affected by PGPR inoculation treatments (M1,M2,M3 and M4).

Characteristics	Cont.	M1	M2	M3	M4
Stem Thick	3090.5	3662.5	3597	3487.5	3560
Priderm Thick	150	112.5	150	112.5	150.00
Cortex Thick	750.00	225	330	225	300.00
Fiberous Thick	-	150	150.00	150.00	-
Phloem Thick	300.00	400	300.00	300.00	450.00
Xylem Thick	825	2025	1907	1950	1970.00
Pith Thick	1062.5	720	750.00	750	712.50
No.of rating gland in cortex	11.00	7.00	7.00	6.00	8.00
No.of rating gland in xylem	8.00	-	-	-	-
Annual ring	Not clear	3 rings	3 rings	3 rings	2 rings

M1: *Azospirillum brasilense*
M3: *Bicillus polymixa*

M2: *Azotobacter chroococcum*
M4: *Serratia* sp.

M4) which recorded increases Nitrogen and Phosphorus contents (%)in shoots and roots of *Taxodium distichum* as influenced by PGPR inoculation in both seasons up to 145.5, 131.2,136.4 and 138.8% over the control, respectively. So this increase in main value of this traits affected pith area which showed low values in all treatments than the control. According to the number of glands in

exhibited the importance of using PGPR to improve the growth of *Taxodium* transplants.

3.1.3.2. The root

Data presented in Table (5) and Fig. (2) indicate that anatomical features of the root did not show a considerable differences between the control and other treatments except the treatment (M1) which showed an increase in root diameter by 2.7 % over

Table(5) : Counts and measurements in micron of certain histological features in transverse sections through the middle part of the lateral root of bald cypress plants as affected by 4 treatments (M1-M2-M3 and M4).

Characteristics	Cont.	M1	M2	M3	M4
Root Thick	1840.00	1890.20	1557.00	1585.00	1835.00
Epi.Thick	18.75	18.75	18.75	18.75	18.75
Cortex Thick	963.00	1229.00	950.00	962.50	1195.00
Vs.Cylinder Thick	675.00	412.50	420.00	412.50	450.00
Pith Thick	168.75	168.75	131.25	131.25	150.00

M1: *Azospirillum brasilense*
M3: *Bacillus polymixa*

M2: *Azotobacter chroococcum*
M4: *Serratia* sp.

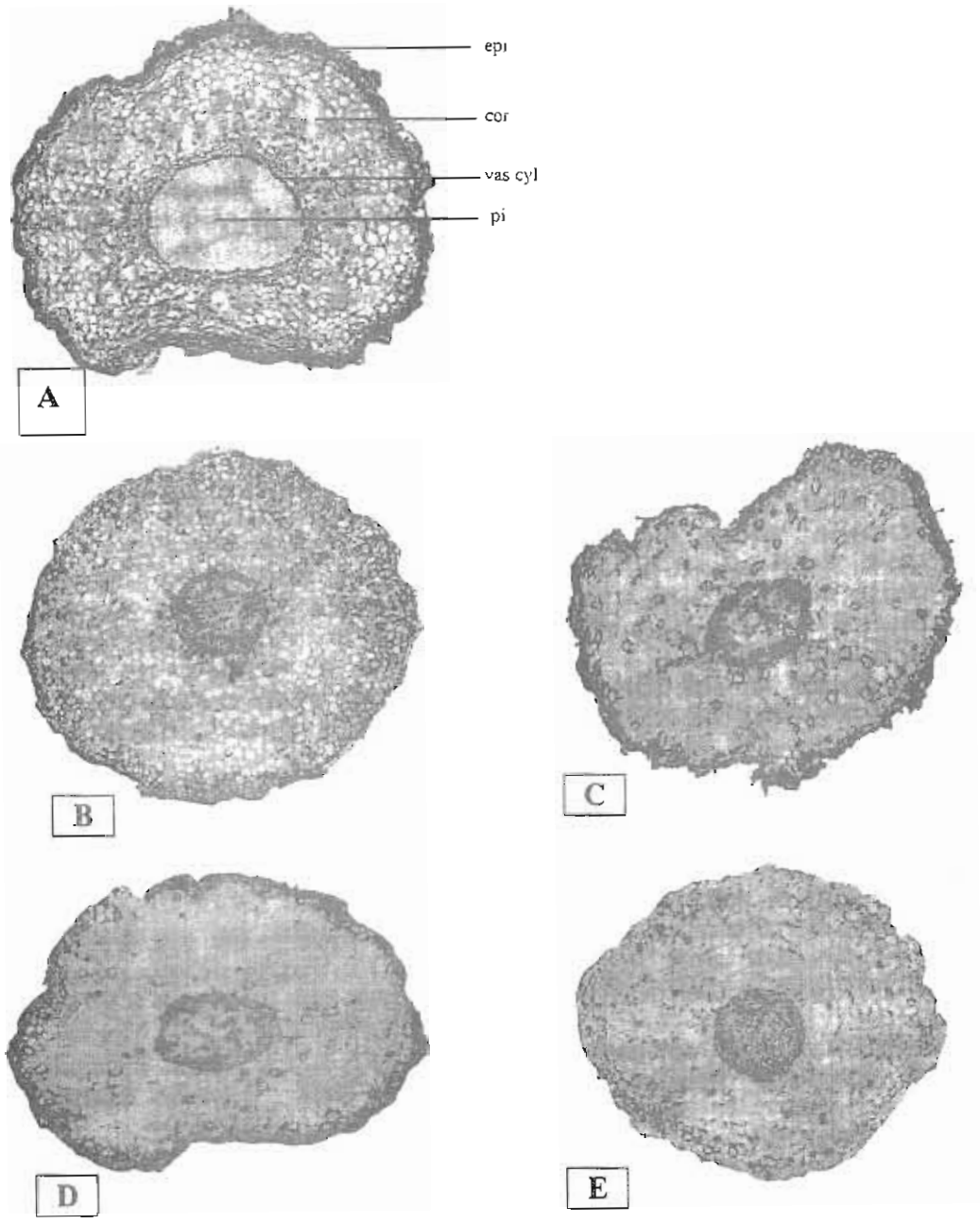


Fig. (2): Transverse section through the middle part of the lateral root of *Taxodium* transplants (X=50)
A-Control , B-M1. C-M2, D-M3, E-M4

showed an increase in root diameter by 2.7% over the control. Thickness of cortex recorded high value for both treatments (M1) and (M4) which recorded 27.6% and 24.09% increases respectively, over the control. While, other treatments caused a decrease below the control. Other traits were either equal or decreased than uninoculated one.

4. DISCUSSION

Many results are inconclusive, but encouraging enough to improve selection procedures and the production of quality inocula for practical application. As PGPR-mediated processes involved in nutrient cycling include those related to non-symbiotic nitrogen-fixation, and those responsible for increasing the availability of phosphate and other nutrients in the soil. Many asymbiotic diazotrophic bacteria have been described and tested as biofertilizers Kennedy *et al.*, (2004). The selection of effective PGPR diazotrophs is critical for further development of this technology. *Azospirillum* species are also considered to be PGPR Lucy *et al.* (2004) and Zahir *et al.* (2004). A significant activity of these bacteria is the production of auxin-type phytohormones that affect root morphology and, thereby, improve nutrient uptake from the soil. This may be more important than their N₂-fixing activity. *Azospirillum* species are being used as seed inoculants under field conditions Dobbelaere *et al.*, (2001); Lucy *et al.*, (2004); Zahir *et al.*, (2004). Despite many studies reporting the benefits of *Azospirillum* inoculation, some studies present inconsistent results. However, it can be assumed that, upon establishing appropriate management practices, the use of these inoculants will have a beneficial effect on plant nutrition. It has recently been postulated that an additional mechanism for plant growth promotion by PGPR could be their altering of microbial rhizosphere communities Ramos *et al.* (2003). Agreeing with such an indirect mechanism, it would be interesting to evaluate the actual impact of this activity in rhizosphere biology. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria PGPR. In recent years, the use of PGPR to promote plant growth has increased in various parts of the world. PGPR can affect plant growth by the production and release of secondary metabolites (plant growth regulators/phytohormones / biologically active substances), preventing deleterious effects of phytopathogenic

organisms in the rhizosphere and / or facilitating the availability and uptake of certain nutrients from the root environment. Selection of effective PGPR is the most critical aspect to have maximum benefits from this technology. (Glick 1995; Nguyen *et al.* 2002; Zahir *et al.*, 2004; Abo EL-Soud, *et al.*, 2007 and Mekhamar *et al.*, 2007).

Conclusion

Inoculation with Plant Growth Promoting Rhizobacteria (PGPR) comparatively enhance the growth of *Taxodium distichum* transplants and shortening the time of seedling growth.

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تأثير بعض البكتيريا المشجعة للنمو على النمو الخضري ومحتوي النيتروجين والفوسفور والصفات التشريحية لشتلات التاكسوديوم

حسام حسن حماد - عادل عبد العزيز عوض* - أسامة سليمان القبيصي**

قسم بحوث الأشجار الخشبية و الغابات بمعهد بحوث البساتين - مركز البحوث الزراعية . الجيزة
*قسم بحوث الميكروبيولوجي - معهد بحوث الأراضي و المياه و البيئة - مركز البحوث الزراعية . الجيزة
** قسم النبات الزراعي - كلية الزراعة - جامعة القاهرة الجيزة مصر .

ملخص

اجري هذا البحث على شتلات نبات التاكسوديوم خلال موسمين زراعيين متتاليين (٢٠٠٨/٢٠٠٩ و ٢٠٠٩/٢٠١٠) بهدف دراسة تأثير بعض سلالات البكتيريا المشجعة للنمو وهي :
(*Azospirillum brasilense* , *Azotobacter chroococcum* , *Bacillus polymixa* and *Serratia* sp.)
أوضحت النتائج المتحصل عليها ما يلي:
١. حدوث زيادة معنوية في نمو الشتلات وقيم التحليل الكيميائي عند استخدام اللقاحات البكتيرية مقارنة بالكنترول.

٢. استخدام لقاح *Azospirillum brasilense* في بيئة النمو كان له تأثيراً معنوياً على طول النبات و قطر الساق و عدد الأفرع و كذا الوزن الغض و الجاف للشتلات و كذلك قيم التحليل الكيميائي و أيضاً الزيادة في جميع القراءات و أعداد الأنسجة المكونة للساق و الجذر.
٣. أدى استخدام لقاح *Azospirillum brasilense* إلى حدوث زيادة معنوية في نسبة المحتوي النيتروجيني في المجموع الخضري للشتلات. بينما أدى استخدام لقاح *Bacillus polymixa* إلى زيادة نسبة محتوي الفوسفور في شتلات التاكسوديوم حيث تراوحت من ٠.٤٥% في الكنترول غير الملقح إلى ٠.٧٨% بالنسبة للشتلات الملقحة
٤. أكدت النتائج التشريحية جميع الاختلافات المورفولوجية حيث اشتملت النتائج على الزيادة في معظم القراءات و اعداد الأنسجة المكونة للساق بالإضافة إلى ظهور الاختلافات في التركيب التشريحي للجذر أيضاً.

بتوصية

وبناء على ما تم التوصل اليه من نتائج فانه يوصي بتلقيح شتلات التاكسوديوم بالبكتيريا المشجعة للنمو للحصول على شتلات قوية بالمثل في فترة زمنية اقل بكثير من المعتاد و هذا سوف يكون له مردود اقتصادي كبير على عملية الانتاج.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (٦٢) العدد الاول (يناير ٢٠١١): ٢٩-٣٩.