Influence of soaking date plam plantlets (*Phoenix dactylifera* L.) in different Cyanobacteria, *Azolla pinnate* and humic acid on adventitious roots induction and survival percentage during acclimatization stage

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ABSTRACT

A greenhouse expermint was carried out to study the effect of soaking date palm plantlets cv. Bertmoda in different fresh cyanobacterial cultures including, Spirulina platensis, Nostoc muscorum, Anabaena oryzae, Azolla pinnate either individually or a (mixture of all) and humic acid on adventitious roots per plantlet (pre treatment) before planting in soil and subsequent survival percntage in acclimatization stage. The number and length of adventitious roots formed on the basic root of plantlets significantly increased when plantlets were soaked in all inocula and humic acid respectively with the superiority of Spirulina Platensis. Also numbers of leaves, leaf length, leaf width, number of main roots, length of main root, length of adventitious roots after 6 months of adaptation significantly increases, the highest increases were observed when plantlets were treated by Spirulina *platensis. Besides survival percentage of plantlets and formed indol, chlorophyll a, b and carotenoids contents, in plantlet leaves after 6 months of adaptation significantly increased in response to the various treatments used with the superiority of Spirulina platensis. It is recommended to soak plantlets (pre treatment) received from tissue culture micropropagation technique of date palm in 20cm Spirulina platensis for 3-4 days before planting in soil and drench soil with Spirulina platensis after planting to obtain the best adventitious root and high survival percntage of plantlets at acclimatization stage.*

***Key words**: Phoenix dactylifera, Azolla pinnate, Cyanobacteria, Humic acid, Plantlets Acclimatization stage*

INTRODUCTION

Palms belong to the family Palmacea (Arecaceae) (Corner,1966) which contain several members, such as date palm (*Phoenix dactylifera L.*) coconut palm, Cocas muciferal and oil palm *Elasis guineesis* toca are widely cultivated for their fruit crop products in addition to numerous fenoum important products such as filer, fuel and furniture. Date palm is considered one of the most important commercial crops in the Arab world. Date palm fruits have a large percentage of sucrose up to 80% and some amounts of fiber, protein, fat, vitamins and minerals. Date palms have been proven to be among the most salt tolerant fruit crops, therefore, have the potential to help combat desertification processes (Bauchireb and Clark, 1997). Blue-green algel extract contains

a great number of substances that influence plant growth and development (Ordog, 1999). These microorganisms have been reported to benefit plants by producing growth promoting regulators (gibberellin and auxin) ,vitamins , amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen bioccontrol and polymers, especially exopolysaccharides, that improve plant growth and productivity (Zaccaro *et al.*,1999) . Also Ghallab and Salem (2001) studied the effect of some bioferilizer treatments, Cerealin (*Azospirillm pp*.)and Nemales (*Serratia* spp.) on wheat plant ,in field experiment and found that both bioferilizers increased growth characters and nutrients, sugar, amino acids , growth regulators (IAA,GA) as well as protein content in plant . Many, though not all, non-heterocystous cyanobacteria can fix N_2 and convert it into an available form of ammonia required for the plant growth. Nevertheless, these organisms may make a substantial contribution to the global nitrogen cycle (Bergman *et al*., 1997).

Cyanobacteria species are recommended to be used as biofertilizers instead of utilizing the expensive industrial chemical fertilizers. Liu-ShiMing and Liang-ShiZhong (1998) noticed that Nostoc extracts increased root, epicotyl and hypocotyl growth, number of roots and plant fresh and dry weights of mung bean.

Humic acid provides natural compounds that transport nutrients and vitamins into plants more efficiently. So, plants grow faster and have stronger cellular processes. Various kinds of media were studied by several investigators. Stevenson (1994) showed that humic substances isolated from different materials contained 45 – 65% carbon, 30 – 48% oxygen, 2 – 6% nitrogen and about 5% hydrogen. Humic substance (HS) are extremely important soil component because they constitute a stable fraction of carbon, thus regulating the carbon cycle and release nutrients, including nitrogen, phosphorus and sulphur. Additionally, the presence of HS improves water-holding capacity, pH buffering and thermal insulation. Liu and Liang (1998) on creeping bentgrass (*Garostis stolonifera*), reported that, humic acid at 400 mg/l significantly increased net photosynthesis on all four observation dates. Chlorophyll content was unaffected by HA rate at each observation date. HA increased tissue contents of Mg, Mn and S and decreased those of Ca, Cu and N. Cooper *et al*. (1998) also on creeping bentgrass indicated that, humate incorporated to depth of 10 cm in sand culture gave a 45 % increase in root mass at the 0 to 10 cm depth and 38 % increase in root mass at the 10 to 20 cm depth compared with the control Abdel-Galeil *et al.* (2010) who postulated that HA at 10 ml/L greatly improved rooting%, root length, no of roots and rooting efficiency index (REI%) of *Phoenix dactylifera* cv. Zaghloul offshoots. Morover Abdel - Galeil *et al* (2009) reported that plantlets of date palm cv. Bartamoda cultured on basal inorganic salts medium supplemented with different concentration of humic acid showed improved root system and adventitious roots formation and increased survival percentage of plantlets during acclimatization stage. *Azolla* has been used extensively and effectively as green manure in rice fields, instead of chemical fertilizers (Wagner, 1997 and Elzeky *et al*., 2005).Azolla is also used successfully as a biofertilizer to increase the yield of rice in many countries such as Vietnam and China (Lumpkin and Plucknett,1982). The establishment of root system *in vitro* is vital for subsequent success throughout acclimatization to autotrophic condition. As the high survival adventitious roots quality before acclimatization. Rooting is an important *in vitro* stage of a micropropagation protocol of date palm as the high survival percentage

of acclimatized plants is concerned ,the success of entire *in vitro* cycle of date palm depends mainly on the adventitious roots quality before acclimatization .Rooting stage can be divided into adventitious root initation and root development step**.** The well-rooted plantlets may be shifted into the greenhouse through an intervening *in vitro* hardening step (Abul-Sood *et al*., 1999).

The acclimatization phase is the most important stage in the protocol of date palm micropropagation because if not optimized, the whole process will be inefficient. This study aimd to investigate the effect of different fresh cyanobacterial cultures of *Spirulina platensis, Nostoc muscorum ,Anabaena oryzae, Azolla pinnate* either individually or a (mixture of all) and humic acid on adventitious roots per plantlet (pre-treatmet) and survival percentage of plantlets during acclimatization stage.

MATERIALS AND METHODS

A trial was conducted under greenhouse conditions at Central Laboratory of Research and Development of Date Palm, Giza to investigate the influence of soaking plantlets in different type of cyanobacteria, *Spirulina platensis, Nostoc muscorum ,Anabaena oryzae ,Azolla pinnate* either individually or a mixture of all and humic acid on adventitious roots per plantlet (pre-treatmet) before planting in soil and drench soil with the same cyanobacteria and humic acid after planting to obtain the best adventitous root in order high survival percentage of plantlets in acclimatization stage . Healthy and strong of date palm (cv.Bartamda) received successfuly from tissue culture technique used for this purpose with average 10-12 cm height,2-3 leaves/plantlet and 2-3 main roots/ plantlet with an average 5-7 cm in height . Plantlets were rinsed thoroughly with tap water to remove any medium residues then soaked in *Spirulina platensis ,Nostoc muscorum, Anabaena oryzae, Azolla pinnate* and the mixtures of *(Spirulina +Azolla + Nostoc + anabaena)* (20 cm for ech one) and humic acid (20cm) for 3- 4 days ,then plantlets were transferred individually into 5 x18 cm plastic pots filled with peat moss + sand (2:1 ratios by v/v). Plantlets were watered with 20 cm fresh cyanobacteria weekly for 6 months. All plants under various trestments were irrigated once every week with fresh water. Control treatment contained tap water without cyanobacteria .

Cyanobacteria strains were maintained in BG11 medium (Rippka *et al*., 1979) except *Spirulina platensis* which was cultured in Zarrouk medium (Zarrouk, 1966). Cultures were incubated in a growth chamber under continuous illumination (2000 lux) and temperature of 25 ± 2 ˚C for all strains except the mesophilic alga *Spirulina platensis* (32 ± 2 ˚C). *Azolla pinnata* was grown on modified Yoshida medium (Yoshida *et al*., 1976). Plantlets were planted with 3 replicates each one containd 3 plantlets. Data were tabulated and SAS program (1994) was used for statistical analysis whereas Duncan's multiple range test (1955) was employed to verify the differences among means of treatments. Cyanobacteria and Azolla extract were supplied from the Dept. Agric. Microbiol., Res. Inst., Soils, water and environ**.** Res. Inst., ARC, Giza,Egypt

Data recorded after 6 month

Plant height (cm), leaf width (cm), number of leaves per plantlet, number of main root/ plantlet ,length (cm) of main roots , length (cm) of adventitious root, number of adventitious roots/plantlet and survival percentage of date palm plantlets were determined. In fresh leaf samples, photosynthetic pigments (chlorophyl1 a, b and carotenoides) in mg/g f.w. were determined

according to Saric *et al*. (1976) and total indole content were assessed as described by Larsen *et al*. (1962).

RESULTS AND DISCUSSION

Effect of soaking date palm cv. Bertamoda plantlet in different cyanobactiral cultures

It is clear from data presented in Table (1) and Fig (1) that number of leaves / plantlet, number of main roots per plantlet length (cm) of main root number of adventioius roots/plantlet, length (cm) of adventioius roots/plantlet singnificantly increased after soaking in the different types cyanobacteria *Spirulina platensis*, *Nostoc of muscorum* , *Anabaena oryzae* , *Azolla pinnate* ,and the mixture of (*Spirulina* + *Nostoc* + *Anabaena*+ *Azolla*) at 20 cm for each one per plant and humic acid (20cm) for 3-4 days ,with the superiority of *Spirulina platensis* which gave the utmost high means in all vegetative growth parameters, number of main roots/plantlet (3.33), number of adventioius roots/ plantlet (10) .length of main roots (6.17cm) and length of adventioius roots were recorded (4.47cm). This may indicate the synergistic effect of cyanobacteria that fix atmospheric nitrogen and secrete more vitamins and growth promoting substances. Similar results were obtained by Liu-ShiMing and Liang-ShiZhong (1998) who noticed the effectiveness of cyanobactreia extracts. Mung bean *Phaseolus radiatus* seeds were soaked in extracts from Nostoc commune and placed in the dark for 3-4 days before being transferred to the light (1200 Lx). Nostoc extracts increased root, epicotyl and hypocotyl growth, number of roots and plant fresh and dry weights.

Table (1): Effect of soaking date palm plantlets cv. Bertamod in different cyanobactira during acclimatization stage.

Treatments	No. of leaves	No . main roots /plantlet	Length(cm) of main roots	No.of dventioius roots/plantlet	Length (cm) of adventioius roots/plantlet
Control (tap water)	2.00 b	2.33 c	5.13 d	0.00 f	0.00 d
Spirulina platensis	2.67 a	3.33 a	7.17 a	10.00a	4.47 a
Azolla pinnate	2.33 ab	2.33c	5.90 c	3.67 d	2.08 bc
Nostoc muscorum	2.00 b	2.67 b	5.33 d	2.00 e	1.67 c
Anabaena oryzae	2.33 ab	1.67 d	5.17 e	2.67 e	2.00 c
Spirulina+Azolla+ Nostoc+ Anabaena	2.33 ab	2.67 b	6.50 b	4.67 c	1.67 c
Humic acid	2.33ab	2.33c	6.37e	6.17b	2.50b

Means within a column having the same letters are not significantly different accoding to Duncan's Multiple Range Test at 5% level of probability .

Effect of different cyanobactira on vegetative growth of date palm plantlet during acclimatization stage

Cyanobacteria play a key role in improving growth when applied as biofertilizers. This evidence was clearly appeared in growth criteria of date palm plantlet represented in Tables (2 and 3) and Fig. (2). The perfrmance of date palm plantlets cv. Bertamoda, in terms of number of leaves/plant, number of main roots /plantlet , length(cm) of adventioius roots, number of adventioius roots/plantlet. was enhanced by cyanobacteria application with the superiority of *Spirulina platensis* treatment which gave the highest records of number of leaves /plant (4.00), leaf length (16.83cm), leaf width (0.73 cm), numder of adventions roots / plantlet (15.00), numder of main roots/ plantlets (14.67), length of main roots/ plantlet (17.33

cm), length of adventitious roots/ plantlet (10.83 cm). This may indicate the synergistic effect of cyanobacteria, which fix more atmospheric nitrogen and secrete more vitamins and growth promoting substances.

Fig. (1): Effect of soaking date palm plantlets in Spirulina platensis (pre- treatment) for 4 days before planting in soil.

Fig. (2): Survived plantlets of date palm after 6 months of cyanobacteria treatment during acclimitazation stage.

In this concern, Adam (1999) studied the effect of cyanobacteria as biofertilizer on seed germination and related processes of wheat, sorghum, maize and lentil. It was observed that growth parameters were significantly increased compared with controls. Also, Nanjappan- Karthikeyan *et al*. (2007) found that cyanobacteria have growth promoting activity as inoculants of wheat.In addition, Ordog (1999) reported that blue-green algel extract excretes a great number of substances that influence plant growth and development. Moreover, Zaccaro *et al.* (1999) stated that these microorganisms have been reported to benefit plants by producing growth promoting regulators (gibberellin and auxin), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve plant growth and productivity. Safinaz and Ragaa (2013) reported that, using marine algae as biofertilizers improved the vegetative characters of maize plants.

Table (2): Effect of Spirulina platensis, Nostoc muscorum, Anabaena oryzae, Azolla pinnate either individually or a mixture between of all and humic acid on vegetative growth of date palm plantlets cv. Bertamoda during acclimatization stage.

Treatments	No. of adventitious roots /plantlet	No. of leaves	Leaf length(cm)	Leaf width (cm)
Control (tap water)	5.33c	1.67d	11.00d	0.43c
Spirulina platensis	15.00a	4.00a	16.83a	0.73a
Azolla pinnate	9.67b	2.67bc	13.67c	0.50bc
Nostoc muscorum	10.33b	2.67bc	13.83c	0.50bc
Anabaena oryzae	10.00b	2.33c	14.00c	0.53bc
Spirulina+ Azolla+ Nostoc+ Anabaena	9.67b	3.00b	14.67b	0.60b
Humic acid	10.00b	2.67c	14.00c	0.56b

Means within a column having the same letters are not significantly different accoding to Duncan's Multiple Range Test at 5% level of probability .

Table (3): Effect of Spirulina platensis, Nostoc muscorum, Anabaena oryzae, Azolla pinnate either individually or a mixture of all and humic acid on vegetative growth of date palm plantlet cv.Bertamoda during acclimatization stage.

Treatments	No. of main roots/plantlet	Length of main roots/plantlet (cm)	Length of adventitious roots /plantlet (cm)
control(tap water)	*4.00e*	*6.83d*	*4.05d*
Spirulina platensis	*14.67 a*	*17.33a*	*10.83a*
Azolla pinnate	*7.67d*	*9.43c*	*8.23c*
Nostoc muscorum	*8.33 cd*	*10.58bc*	*10.18ab*
Anabaena oryzae	*8.67cd*	*11.26b*	*10.01ab*
Spirulina+ Azolla+ Nostoc+ Anabaena	*9.33bc*	*11.83b*	*9.43b*
Humic acid	*10.33b*	*10.55bc*	*10.18ab*

Means within a column having the same letters are not significantly different accoding to Duncan,s Multiple Range Test at 5% level of probability .

Effect of cyanobactira on survival percentage of date palm plantlet cv. Bertamoda during acclimatization stage

The acclimatization phase is the most important stage in the protocol of date palm micropropagation because if not optimized, the whole process will be inefficient. Factors affecting the successful production of free-living date palm, including length of plantlets, strength of root system, humidity conditions, and number of leaves and composition of the soil have been reviewed**.** The survival percentages of plantlets were recorded after 6 months from transplanting. Data in Table (4)

showed that survival percentages of plantlets was significantly increased due to the different treatments used in this study ,with the superiority of *Spirulina platensis* type of cyanbacteria which gave the highest survival percentage during acclimatization stage (73.33%), whereas the control treatment gave the lowest value (38.33%).

Table (4): Effect of cyanobactira, and humic acid on survival percentages of date palm plantlets cv. Bertamoda during acclimatization stage.

	Survival%
Treatments	
Control (tap water)	38.33d
Spirulina platensis	73.33a
Azolla pinnate	61.67c
Nostoc muscorum	67.33b
Anabaena oryzae	66.33b
Spirulina+ Azolla+ Nostoc+ Anabaena	67.33b
Humic acid	68.33b

Means within a column having the same letters are not significantly different accoding to Duncan's Multiple Range Test at 5% level of probability .

Chemical composition

Chlorophyll content of leaves (%)

Table (5) indicates that application of cyanbacteria, *Spirulina platensis*, *Nostoc muscorum, Anabaena oryzae,, Azolla pinnate* either individually or a mixture of all and humic acid caused a significant increment in the leaf content, chlorophyll a , b and carotenoids over control plants. The data indicated that the highest value of chlorophyll a (4.25 mg /g f.w) was recorded in the leaves of plants treated with *Spirulina platelets*, while control treatment gave the lowest values (2.03 mg /g f.w).

Chlorophyll b content

The highest value of chlorophyll b (3.86 mg /g f.w) was recorded in the leaves of plantlets treated with *Spirulina platensis*, while control treatment gave the lowest values (1.00 mg /g f.w) (Table5).

Carotenoids content

As for carotenoids content, the highest values of carotenoids (2.82 mg /g f.w) was recorded by the treatment with *Spirulina platensis* (Table 5). These results are in agreement with Ordog (1999) who found that , extracts of cyanobacteria and microalgae contain a special set of biologically active compounds including plant growth regulators, which can be used for treatment to decrease senescence, transpiration as well as to increase leaf chlorophyll a and b ,protein and root and shoot development. Haroun and Hussein (2003) stated that cyanobacteria extract enhanced chlorophyll formation. Moreover Prasanna *et al*. (2008) found that BGA and *Azotobacter* in different combinations with chemical fertilization gave the highest values of chlorophyll. Amal *et al.* (2010) found that total chlorophyll of plant leaves responded positively to BGA inoculation.

Table (5): Effect of cyanobactira types and humic acid on chlorophyll a,b and carotenoids (mg g .f.w) contents of date palm plantalet during stage .

Treatments	Chlorophyll a	Chlorophyll b	Carotenoids
control(tape water)	2.03 d	1.00e	0.61d
Spirulina platensis	4.25a	3.86a	2.82a
Azolla pinnate	3.72ab	3.37b	2.31c
Nostoc muscorum	3.64b	2.28d	2.37c
Anabaena oryzae	3.74ab	2.44d	2.37c
Spirulina+Azolla+Nostoc+ Anabaena	2.73c	2.83c	2.49b
Humic acid	3.77ab	2.30d	2.54b

Means within a column or row having the same letters are not significantly different accoding to Duncan's Multiple Range Test at 5% level of probability

Indoles content

Indoles are considered an important factor affecting plant growth characters. Data in Table (6) showed that the highest significant values of indoles in the leaves resulted from plants treated with *Spirulina platensis* (4.17mg/100gm f.w). On the other hand, control treatment gave the lowest value (2.03). These current results are in agreement with those of Tiwari (2009), who proved that *Azospirillum*, *Azotobacter*, *Klebsilla* and *Pseudomonas*sp produced highly significant indole contents.

Table (6): Effect of cyanobactira types and humic acid on indoles content (mg/ 100 gf.w) in the leaves of date palm plantalet during acclimatization stage.

Treatments	Indole content
Control (tap water)	2.03d
Spirulina platensis	4.17a
Azolla pinnate	2.67c
Nostoc muscorum	2.50c
Anabaena oryzae	2.50c
Spirulina+ Azolla+ Nostoc+ Anabaena	2.63c
Humic acid	3.10b

Means within a column having the same letters are not significantly different accoding to Duncan's Multiple Range Test at 5% level of probability .

From the previous results ,it could be recommended to soak date palm plantlets(pre-treatment) received from tissue culture micropropagation technique in 20cm *Spirulina platensis* for 3- 4 days before planting in soil and drench soil with the cyanobacteria *Spirulina platensis* after planting to obtain the best adventious root and to get high survival percentage in acclimatization stage.

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الملخص العربي

تأثير غمس نبيتات نخيل البلم فى السلالات المختلفة من الطحالب الخضراء المزرقة (السيانوبكتريا) – الازولا وحمض الهيومك على حث خروج الجذور العرضيه والنسبه المئويه لنجام النبيتات خلال مرحله الاقلمه

لبنى محمد عبد الجليل *, عزه أحمد محمد عبد العال**

*المعمل المركزى لابحاث وتطوير نخيل البلح – مركز البحوث الزراعيه -الجيزه – مصر

**قسم بحوث الميكروبيولوجيا –معهد بحوث الآراضى والمياه والبيئه – مركز البحوث الزراعيه -الجيزه – مصر

أجريت هذه التجربه فى الصوبه التابعه للمعمل المركزى لنخيل البلح لدراسة تأثير غمس نبيتات نخيل البلح والناتجه من زراعة الأنسجه فى السلالات المختلفة من الطحالب الخضراء المزرقة (السيانوبكتريا) (*Spirulina platensis, Nostoc muscorum ,Anabaena oryzae, Azolla pinnate*) كلا بمفردة) أو خلط نسب متساويه منهم وكذلك إستعمال الهيومك على تحسين خروج الجذور العرضيه قبل الزراعه فى التربه (كمرحله أوليه) بالتالى رفع النسبه المئويه لنجاح النبيتات فى مرحلة الأقلمه - سجلت القياسات زيادة معنويه فى عدد وطول الجذور العرضيه المنتجه على الجذور الأصليه عندما غمست النبيتات فى سلالات الطحالب الخضراء المذكورة سواء بمفردها أو الخليط منها وحمض الهيومك على التوالى مع تفوق المعامله بالغمس فى طحلب *Spirulina platensis* – وقد أمكن الحصول على أتجاه مشابه فيما يتعلق بزيادة نسبة نجاح االنبيتات ومحتوى الأوراق من الأندولات وكذلك كلوروفيللى a,b والكاروتينويدات بعد ٦ أشهر من الآقلمة وكانت أفضل الزيادات فى النبيتات التى عوملت بطحلب *Spirulina platensis* . من النتائج يمكن التوصيه بغمس النبيتات الناتجة من زراعة الأنسجه فى٢٠ سم من طحلب *Spirulina platensis* لمدة٣- ٤ يام قبل الزراعه بالتربه وريها بنفس الطحلب *Spirulina platensis* لتحسين وزياده خروج الجذورالعرضيه وبالتالى رفع النسبه المئويه للنجاح خلال مرحله الأقلمه .