In vitro hardening affects growth in the nursery of date palm c.v Medjool vitroplants

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ABSTRACT

In vitro hardening of date palm (Phoenix dactylifera L.) c.v Medjool shoots were accomplished as pre-acclimatization using ascorbic acid 0, 50,100,150 or 200 mg/L in the MS (Murashige and Skooge, 1962) rooting medium. Positive response occurred with ascorbic acid (150 mg/L) which scored the highest significant values of root length (11.66 cm) and root circumference (1.93 mm) after 8 weeks of incubation. Subsequently affects saplings cultured in growing mixture containing compost and perlite (1:1, v/v) which raised saplings survival from 80% to 93.33% after 12 weeks in acclimatization as well as reflected significant values of total chlorophyll (2.93 mg/g D.W), total soluble sugars (29.33%) and ascorbic peroxidase activity (1102.33 Units/g, F. W). Results revealed that plastic leggy bags [20 cm (w) X 40 cm (h)] filled with the growing mixture sand + coarse sawdust (2:1, v/v) and fertigated with Stimifol (0.5 g/L) once a week for 9 months under greenhouse conditions in the nursery, recorded the highest significant values of total leaves no. (7.3), pinnate leaves no. (5.5), plant height (57.0 cm), leaf width (5.3 cm) and stem circumference (2.7 cm). This was accompanied by the highest significant values of dry weight (15.79%), T.H. Carbohydrate (38.99%), lipids content (4.48%). As well as accompanied by a consequence higher value of total soluble phenol (0.85 g/g protein) and the highest significant values of crude protein (36.15%) and PAL activity (220 nkat/g protein). However, growing mixture containing sand + foam recorded the lowest values of all growth characters and chemical analysis under study. Results considered very helpful to the researchers and highly supportive to the nurseries at the commercial level. Since introduce effective and cheapest natural raw materials to fulfillment of the huge demand of insufficient big quantities required. In addition, it has national environment great beneficial effect through implement rice shell and coarse sawdust good practical recycling and environmental bioremediation and finally reduces production costs.

Key words: Phoenix dactylifera L., tissue culture, micropropagation, ex vitro, phenylalanine ammonialyase (PAL), Environmental bioremediation, waste recycling.
INTRODUCTION

Somatic embryogenesis is one of the most important technologies for plant regeneration. There are two morphogenetic pathways ensuring the production of somatic embryos. The first pathway is direct somatic embryogenesis, which is yet to be fully developed for massive plant regeneration in date palm (Sudhersan et al., 1993; Hegazy et al., 2006b; Hegazy and Aboshama, 2010). The second pathway is an indirect method which is based on the induction of embryogenic calli (Al-Khayri, 2005). Direct regeneration of vegetative buds minimizes the risk of somaclonal variation among regenerates. Moreover, the duration of culture period is limited by frequent renewal of the plant material. Actually, there are few laboratories that use this technique to produce date palm vitro-plants at the commercial level (Abahmane, 2011). In reviewing date palm (Phoenix dactylifera L.) plantlets nearly there is very few studies considers the period after acclimatization stage and subsequently plants growth and development (Hegazy et al., 2006b). The use of in vitro techniques such as somatic embryogenesis and organogenesis is highly suitable for large-scale plant multiplication of vegetative propagated crops. The success of these techniques is highly genotypic dependent. However, it have been successfully applied for plant propagation in wide ranging crops including date palm (Jain, 2007). The major obstacle to the practical application of date palm tissue culture to mass propagation is low rate of plantlets survival during acclimatization under greenhouse conditions (Hegazy et al., 2006a; Hegazy, 2014). Plantlets performance during acclimatization was determined to a large extent by the degree of autotrophy (Kozai, 1993). In addition, Transfers of plantlets to greenhouse are depending primarily upon the quality and type of materials produced in the previous stages (Hegazy et al., 2006a). Micropropagation via organogenesis or direct shoot formation is extensive labour-oriented. Somatic embryogenesis may reduce labour cost and also assist in developing automated somatic embryo production. However, genetic fidelity of micropropagated plants should be maintained with minimal somaclonal variation, otherwise there will be severe economic loses to the growers. On the other hand, Jain (2012) stated that molecular markers investigate only a small part of the genome. Hence, field performance analysis remain the most reliable strategy to assess genetic integrity in date palm. Micropropagated plants do not survive easily after transfer from in vitro conditions to greenhouse or field environments. Greenhouse or field environment have substantially lower relative humidity, higher light intensity, and septic conditions that are stressful to micropropagated plants compared to in vitro conditions (Debergh and Read 1993). Date palm plantlets in
acclimatization stage face many stresses. The water deficit was the main stress as a result of high evapotranspiration (Carvalho et al., 2001). The continuation process of these plants in growth and development depends on abundance of other factors, which affect growth such as growing mixture type and fertigation level (Hegazy et al. 2006a). However, plantlets in culture are grown in conditions that provide little physiological stress since a carbon source is reducing the need for photosynthesis as well as aseptic environment reduces the stress of pathogenic organisms. These conditions produced plants uniquely unsuited for survival directly in greenhouse and field conditions. So, the culture environment defines plantlets initiated in culture medium and they must have the same environments as they did in vitro after transfer to the greenhouse. Carvalho et al. (2001) mentioned that leaves formed during the acclimatization period might still have a lower photosynthetic capacity than leaves of greenhouse-grown plants. Moreover, Zaid and Hughes (1995) stated that none-acclimatized in vitro date-palm leaves have only 15% of the wax deposits found on greenhouse-grown seedlings, polyethylene glycol treatment of vitroplants increased the amount of wax deposition on leaf surfaces and as a consequence it was able to decrease water losses which were observed during acclimatization.

The objectives of this study was to follow the relation between rooting and acclimatization stages and evaluate the growth of the identical phenotypically vitroplants produced through direct embryogenesis pathway using four growing mixtures types.

MATERIALS AND METHODS

This work was carried out in Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City Univ., and Biochemistry Department, Faculty of Agric., Cairo Univ., Egypt, during the period 2010-2012.

In vitro hardening:

In this work, individual phenotypically shoots produced from direct somatic embryogenesis pathway were subjected to in vitro hardening by cultured on the MS basal medium (Murashige and Skooge, 1962) in addition to IBA (indole-3-butyric acid) at the concentration of 1.0 mg/L, sucrose (30 g/L) and supplemented with ascorbic acid 0, 50, 100, 150 or 200 (mg/L). medium was solidified with phyto- agar (6.0 g/L). The pH of the media was adjusted to 5.6 with 0.1 M KOH or 0.1 M HCl prior to gelling agent addition. Media were dispensed in a glass tubes (2.5 x 25 cm; Borosil) capped with Bellco plastic caps containing 15 ml and autoclaved at 121°C and 1.2 Kg/cm² for 20 min. Fifteen glass tubes (replicates) were used for each treatment. Cultures were incubated at 25±1°C and exposed to 16-h photoperiod using ordinary fluorescent tubes with a light
intensity of 3000 lux. After 8 weeks of incubation, roots length (cm) and root circumference (mm) were recorded.

**Acclimatization:**
Well-rooted Plantlets removed from rooting medium (Fig. 1- a), rinsed and soaked under tap water for 2 h., roots were immersed in fungicide (Benomyl) solution (0.5%, w/v) for 5 min. Then, planted in plastic pots (5 ×18 cm) filled with growing mixture of compost (call-val universal compost- England) [compost and perlite (1:1, v/v)]. Plantlets were covered with transparent polyethylene sheet and sub-irrigated in case of need. The potted plants were incubated for 4 weeks in phytotron at 25 ± 1°C, relative humidity (80-90 %) and 16 h photoperiod with a light intensity of 1500 lux. Acclimatization was achieved through gradually removing the plastic sheet each day till it totally removed after 4 weeks and transferred to plastic greenhouse in tunnel under shade condition (black saran 63%) and were left to grow for another 8 weeks. Plants were sub-fertigated once a week with commercial fertilizer of NPK (Sangral, 1.0 g/L) at a ratio of 20: 20: 20. After 12 weeks survival % was recorded as well as chemical analysis of total chlorophyll, total soluble sugars and ascorbic peroxidase activity were analyzed.

**Nursery transplantation:**
 Twelve weeks old well-acclimatized vitroplants were transferred to plastic greenhouse covered with black tent (Seran 63 %) and transplanted to black polypropylene plastic leggy bags [ 20 cm (W) X 40 cm (H) ] filled with different growing mixture types as follows: Sand + Foam (2:1, v/v), Sand + Perlite (2:1, v/v), Sand + Rice shell (2:1, v/v) and Sand + Coarse sawdust (2:1, v/v). Plants of each growing mixture type treatment (Fig. 1- c, d, e & f) were weekly fertigated with a nutrient solution (180 ml) containing commercial fertilizer of NPK (Stimifol, 1.0 g/L) at a ratio of 19:19:19 and monthly with microelements, Fe, Zn and Mn (0.25 g/L each) for 9 months. Plants were irrigated twice a week in summer and once a week in winter. The growing plants were maintained under plastic-greenhouse covered with black tent (Seran 63%) shade, at 25 ± 2 °C, relative humidity of (60-70 %) and expose to the normal daylight photoperiod and intensity. After 9 months, 12 plants for each treatment were taken and vitroplants growth characters; total leaves number, pinnate leaves number, plant height (cm), leaf width (cm) and stem circumference (cm) were recorded.

**Chemical analysis:**
Fresh sample of second emerged leaf were taken for determination of dry weight percentage as well as the concentrations (%) of the following: crude protein, total
phenols, lipids, total hydrolysable carbohydrate and total soluble sugars.

Total hydrolysable carbohydrates and total soluble sugars determined in the ethanolic extract using the phenol-sulfuric acid method according to Dubois et al., (1956).

Dry weight, lipids as well as total nitrogen and crude protein (usual micro kjeldahl methods) were determined according to the methods described by (A.O.A.C. 1990).

Determination of pigments, Chlorophyll a, b were determined in plantlets leaves according to the method of Arnon (1949).

**Assay of peroxidase activity:**

Two grams of fresh sample were homogenized either in cold phosphate buffer (0.05 M at pH 6.5). The homogenate was centrifuged at 1000 rpm for 10 min. The pigments were removed from the supernatant by adsorbing on activated charcoal and filtered. The filtrate was completed to a known volume and used to determine enzymes and total soluble protein. Peroxidase (EC 1.11.1.7) was assayed following the method of Kar and Mishra (1976) with slight modification. Five mL of the assay mixture contained 300 μM of phosphate buffer (pH 6.8), 50 μM catechol, 50 μM H2O2 and 1 mL of crude enzyme extract was prepared. After incubation at 25ºC for 5 minute, the reaction was stopped with the addition of 1 mL of 10% H2SO4. The colour could be detected at 430 nm and the enzyme activity was expressed as enzyme activity/g fresh weight/hour.

The colorimetric method of Folin-Denis as described by Swain and Hillis (1959) was employed for the determination of total soluble phenols in ethanolic extracts of leaf samples. Extraction and assay of phenylalanine ammonialyase (PAL) were done according to Lamb et al., (1979).

**Statistical analysis:**

Data were statistically analyzed by one factorial randomized complete block design using the SAS (1988) package. The Least Significant Differences among levels of each treatment were compared using L.S.D. test at 5%, according to Steel and Torrie (1980).

**RESULTS AND DISCUSSIONS**

**In vitro hardening**

The process between rooting and acclimatization is known *in vitro* hardening. It is a very important step to complete propagation process and raised survival percentage. In this stage plantlets are grown in optimum conditions (moisture, salts, sucrose and water). Therefore, plantlets leaves don't have enough cuticle layer and the transpiration is remained high.

Data presented in Table (1) and Fig. (1- a) demonstrated that *in vitro* hardening of date
palm (Phoenix dactylifera L.) c.v Medjool shoots were accomplished as pre-acclimatization with well-developed root system after 8 weeks in vitro, subsequently raised survival % of plantlets received in vitro hardening treatment after 12 weeks in acclimatization. Result indicated that ascorbic acid addition at the concentration of 150 mg/L to the rooting medium recorded the highest significant values of root length (11.66 cm) and root circumference (1.93 mm) after 8 weeks in vitro and subsequently

Table (1) Effect of different ascorbic acid concentrations as in vitro hardening treatment at rooting stage on root length (cm), root circumference (mm) and subsequently survival % of date palm c.v Medjool plantlets in acclimatization.

<table>
<thead>
<tr>
<th>Treatments of ascorbic acid (mg/L)</th>
<th>Rooting stage (8 weeks)</th>
<th>Acclimatization stage (12 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length (cm)</td>
<td>Root circumference (mm)</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>3.92e</td>
<td>1.05g</td>
</tr>
<tr>
<td>50</td>
<td>5.67d</td>
<td>1.13d</td>
</tr>
<tr>
<td>100</td>
<td>7.67c</td>
<td>1.5c</td>
</tr>
<tr>
<td>150</td>
<td>11.66a</td>
<td>1.93a</td>
</tr>
<tr>
<td>200</td>
<td>10.08h</td>
<td>1.83b</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at P=0.05 according to the L. S. D. test.

Affect saplings in acclimatization cultured in growing mixture (previously superior) containing compost and perlite (1:1, v/v) which raised saplings survival from 80 to 93.33 % after 12 weeks in acclimatization (Table 1) as well as reflected the highest significant values of total chlorophyll (93 mg/g D.W), total soluble sugars (29.33 %) and higher values of ascorbic peroxidase activity (1102.33 Units/g, F.W) as shown in Table (2). In this regard, the obtained data from Table (1) revealed that the application of different concentrations of ascorbic acid positively affected the root length and root circumference and this was accompanied by the highest total chlorophyll, total soluble sugars and peroxidase activity with the 150 ppm ascorbic acid treatment as compared with the control. That is may be due to the antioxidant activity of ascorbic acid that suppress the degradation of chlorophyll a and b under in vitro hardening as pre-acclimatization process when the highest accumulation of total soluble sugars have been observed also the antioxidant activity of antioxidant enzyme peroxidase have been reported in a positive correlation with highly ascorbic acid administration treatment. These data is in accordance with Flowers et al., (1977); Sacher and Staples (1985) have shown that true vascular
Halophytes often produce copious amounts of nitrogenous compounds (e.g., proline and betain), while salt sensitive vascular glycophytes generally produce carbohydrates (e.g. polyols and sucrose), to counteract the stress of acclimatization process. A number of roles have been proposed for proline in salinity tolerance. The first is that it acts as a store of energy that can be rapidly broken down and used when the plant is relieved of stress. The second is that it acts as an osmolyte and reduces the osmotic potential of the cell, thus reducing toxic ion uptake (Hare et al., 1998). In this case, the latter is more likely, with the stress tolerant plants not only producing more proline when stressed, but also having, in most cases, no significant drop in the chlorophyll content. This indicates that the increase in proline is reducing the physiologically detrimental effects of the salt (Delauney and Verma, 1993; Hare et al., 1998).

### Nursery

Data presented in Table (3) and Fig. (1-f) showed that date palm plantlets c.v Medjool transplanted to the growing mixture containing of sand + coarse sawdust (2:1, v/v) recorded the highest significant values of total leaves no. (7.3), pinnate leaves no. (5.5), plant height (57.0 cm), leaf width (5.3 cm) and stem circumference (2.7 cm) after 9 months in the nursery as compared to those produced under the other different growing mixture types. On the other hand, plantlets cultivated in growing mixture containing sand + foam recorded the lowest values of all growth characters Fig. (1-c) as well as chemical analysis under study.

### Table (2): Effect of different ascorbic acid concentrations on in vitro hardening of c.v Medjool plantlets chlorophyll and total soluble sugars and ascorbic peroxidase activity.

<table>
<thead>
<tr>
<th>Treatments of ascorbic acid (ppm)</th>
<th>Total chlorophyll (mg/g D.W)</th>
<th>Total soluble sugars (%)</th>
<th>Peroxidase (Units/g F. W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>684.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>1.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>810.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>981.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>2.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1102.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>2.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1076.33&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at P=0.05 according to the L. S. D. test.
Table (3): Effect of growing mixture type on some growth characters of date palm c.v Medjool vitroplants grown for 9 month in greenhouse in the nursery

<table>
<thead>
<tr>
<th>Treatments Growing mixture type</th>
<th>No. of Total leaves</th>
<th>Plant height (cm)</th>
<th>Leaf width (cm)</th>
<th>Stem circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand + Foam (2:1, v/v)</td>
<td>5.4^d</td>
<td>30.8^d</td>
<td>3.5^d</td>
<td>1.8^d</td>
</tr>
<tr>
<td>Sand + Perlite (2:1, v/v)</td>
<td>5.8^c</td>
<td>38.0^c</td>
<td>4.2^c</td>
<td>2.0^c</td>
</tr>
<tr>
<td>Sand + Rice shell (2:1, v/v)</td>
<td>6.7^b</td>
<td>41.1^b</td>
<td>4.4^b</td>
<td>2.2^b</td>
</tr>
<tr>
<td>Sand + Coarse sawdust (2:1, v/v)</td>
<td>7.3^a</td>
<td>57.0^a</td>
<td>5.3^a</td>
<td>2.7^a</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at P=0.05 according to the L.S.D. test.

Chemical analysis:

Results obtained from growth characters are in parallel with chemical analysis (Table 4 & 5) of the best growing mixture sand + coarse sawdust (2:1, v/v) and fertigated with 0.5 g/L Stimifol. Another evidence for the superiority of vitroplants growing in the growing mixture were recorded the highest significant values of dry weight (15.79 %), T.H. Carbohydrate (38.99 %), lipids content (4.48 %). As well as was accompanied by a consequence higher value of total soluble phenol (0.85 g/g protein) and the highest significant values of crude protein (36.15 %) and PAL activity (220 nkat/g protein).

Table (4): Effect of different growing mixture types on dry weight (%), T.H. Carbohydrate (%), lipids content (%) of date palm c.v Medjool vitroplants grown for 9 month in greenhouse after acclimatization stage.

<table>
<thead>
<tr>
<th>Treatments Growing mixture types</th>
<th>Chemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry weight (%)</td>
</tr>
<tr>
<td>Sand + Foam (2:1, v/v)</td>
<td>13.03^d</td>
</tr>
<tr>
<td>Sand + Perlite (2:1, v/v)</td>
<td>13.85^c</td>
</tr>
<tr>
<td>Sand + Rice shell (2:1, v/v)</td>
<td>14.14^b</td>
</tr>
<tr>
<td>Sand + Coarse sawdust (2:1, v/v)</td>
<td>15.79^a</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at P=0.05 according to the L.S.D. test.

That could be lead to the highly mineral availability offered from soil to plants and healthy growth of plants. Marschner, (1995) found that the uptake systems for nutrients are regulated by demand from the shoot. This feedback regulation also extends to enzymes.
involved in nutrient assimilation and the mobilization of nutrients from the rhizosphere (Raghothama, 2000).

Which in turn represents the main source of water and nutrients for growing roots. Therefore, it must retain enough moisture, has sufficient porous so that excess water drains away, permitting adequate aeration to the roots and finally remains the nutrients in available form for plants to uptake. Thus, it appears that growing mixture consisted of sand + coarse sawdust (2:1, v/v) could spare the aforementioned requirements, since sand could provide sufficient porous and permit adequate aeration while coarse sawdust may release represent permanent source of available nutrients for the growing plants, which was reflected on the growth characters in parallel with the chemical analysis recorded.

**Table (5): Effect of some growing mixture types on crude protein, total soluble phenol and phenylalanine ammonia-lyase (PAL) activity in leaves of date palm c.v Medjool vitroplants grown for 9 month in greenhouse after acclimatization stage.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Growing mixture types</th>
<th>Crude Protein (%)</th>
<th>Total soluble phenols (g / g protein)</th>
<th>PAL activity (nkat / g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand + Foam</td>
<td>(2:1, v/v)</td>
<td>32.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sand + Perlite</td>
<td>(2:1, v/v)</td>
<td>33.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>186&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sand + Rice shell</td>
<td>(2:1, v/v)</td>
<td>35.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>200&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sand + Coarse sawdust</td>
<td>(2:1, v/v)</td>
<td>36.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>220&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within each column followed by the same letter are not significantly different at P= 0.05 according to the L. S. D. test.

Of course, reduction of N<sup>+</sup> level could be directly related to their availability for the basic anabolic processes, which in turn could play an important role on metabolism and subsequently plant development. In this regard, Kevers et al., (1984) stated that an initiating stress factor, such as an excess of NH<sub>4</sub><sup>+</sup> ion, might enhance the activity of soluble and membrane-bound peroxidases through a rapid modification of phenolic level. On the other hand, in the first step of the phenylpropanoid metabolic pathway, phenylalanine ammonia-lyase catalyzed the conversion of L-phenylalanine to E-cinnamate, with the release of ammonium ion (NH<sub>4</sub><sup>+</sup>). Therefore, increase of NH<sub>4</sub>NO<sub>3</sub> in the plant could act as an inhibitor of PAL activity, a mechanism which has long been postulated to occur via feed-back inhibition by end-products.
Meanwhile, Gilbert and Tully (1982) found that ammonia reduced phenylalanine uptake and pool size and so may repress enzyme synthesis through inducer exclusion. In this regard, Ganapathi and Kargi (1990) reported that, phosphate and many other nutrients could affect the production of secondary products in cell cultures. Ahmed et al., (2000) found a very positive correlation between phosphorus content and secondary products production, ascribing that to the increment of mRNA enzymes system responsible for secondary products synthesis. On the other hand, data in Table (4) describe the concentrations of both total soluble phenols and tannins as well as the activity of phenylalanine ammonialyase (PAL) enzyme. All the three components are contributed to the formation of protective materials, i.e. lignin, suberin and flavonoids, which could reflect in somehow the speed of plant development. The results of crude protein, total soluble phenols well as PAL activity confirmed that growing mixture type containing Sand + coarse sawdust (2:1, v/v) and fertigated with 0.5 g/L Stimifol (0.5 g/L) once a week increased the concentrations of both crude protein and total soluble phenols as well as PAL activity in leaves of growing plants as compared with the other three growing mixture types. The obtained results are in conformity with those obtained from the leaf growth parameters (Table 3). van-Heerden et al., (1996) reported that the primary metabolic fate of phenylalanine, following its deamination by activity of PAL enzyme in plants, is conscription of its carbon skeleton for lignin, suberin, flavonoid, and related metabolite formation. Moreover, Kevers and Gaspar (1985) reported that, the higher lignin level of normal carnation tissues corresponded to a higher phenylalanine ammonialyase activity.

It could be concluded from the obtained data that in vitro hardening of date palm c.v Medjool shoots were accomplished as pre-acclimatization using ascorbic acid at different levels in the MS (Murashige and Skooge, 1962) rooting medium. Ascorbic acid at the concentration of 150 mg/L occurred positive response with produced plantlets. It scored the highest significant values of root length (11.66 cm) and root circumference (1.93 mm) after 8 weeks of in vitro incubation. Followed by raised saplings survival from 80% to 93.33% after 12 weeks in acclimatization on growing mixture containing compost and perlite (1:1, v/v) as well as reflected significant values of total chlorophyll (2.93 mg/g D.W), total soluble sugars (29.33%) and ascorbic peroxidase activity (1102.33 Units/g, F. W). Results revealed that sand + coarse sawdust (2:1, v/v) was the best growing mixture treatment for vitroplants to grow in black plastic leggy bags after 9 months in the nursery.
and fertigated with Stimifol (0.5 g/L) once a week. Which recorded the highest significant values of total leaves no. (7.3), pinnate leaves no. (5.5), plant height (57.0 cm), leaf width (5.3 cm) and stem circumference (2.7 cm). This was accompanied by the highest significant values of dry weight (15.79%), T.H. Carbohydrate (38.99%), lipids content (4.48%). As well as accompanied by a consequence higher value of total soluble phenol (0.85 g/g protein) and the highest significant values of crude protein (36.15%) and PAL activity (220 nkat/g protein). However, growing mixture containing sand + foam recorded the lowest values of all growth characters and chemical analysis under study.

Results considered very helpful to the researchers and highly supportive to the nurseries at the commercial level. Since introduce effective and cheapest natural raw materials to fulfillment of the huge demand of insufficient big quantities required. In addition, it has national environment great beneficial effect through implement rice shell and coarse sawdust good practical recycling and environmental bioremediation and finally reduces production costs.

ACKNOWLEDGEMENTS
The author is grateful to S. Y. Rania, Lecturer of Biochemistry, Faculty of Agriculture, Cairo University, Egypt for her valuable support through offered biochemistry lab.

Equipments and facilities for chemical analysis.
Fig. (1): In vitro hardening of date palm c.v Medjool vitroplants and grown in plastic leggy bags on different growing mixture types. Weekly fertigated with Stimifol (0.5 g/L).

a- Plantlets received in vitro hardening treatment using ascorbic acid (150 mg/L).
b- Saplings transplant to leggy plastic bags in nursery showed lanceolate leaf.
c- Saplings cultured on growing mixture contained Sand + Perlite (2:1, v/v).
d- Saplings cultured on growing mixture contained Sand + Compost (2:1, v/v).
e- Saplings cultured on growing mixture contained Sand + Rice shell (2:1, v/v).
f- Saplings cultured on growing mixture contained Sand + Coarse sawdust (2:1, v/v).
LITERATURE CITED


التقسيمة عملاً توثر في نمو نباتات نخيل تمر الأنسجة صنف مدجول في المشتل

عادل السيد حجازي
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أجريت هذه الدراسة في معامل زراعة الأنسجة وصويدة معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية جامعة
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استخدمت في الدراسة نموات خضرية لنبات النخيل صنف مدجول ناتجة من مرحلة التضاعف العددي من خلال تكوين
الجذور الجماعية المباشرة عملياً. تم عمل نسقية عملية في مرحلة التجذير للفروع الخضرية بإضافة حمض الأسكوريك
بتركيزات 0، 150، 200، 400، 600 ملم/لتر في بنية الأساس لمرشح وسجج مضاف إليها إضافة حمض
البيوتريك بتركيز 1 ملم/لتر. أظهرت النتائج أن حمض الأسكوريك بتركيز 150 ملم/لتر حقق توقع معنوي في
طفل النبات (10.08 سم) و قطرها (1.83 سم) معاً بالمقارنة بالنباتات السابقة تحت الدراسة. وقد أثر ذلك بشكل
موجب على رفع نسبة نجاح النباتات عند أقل منها 80% إلى 93.33% لزراعةها على مخلوط من الكروموزت
و البريليوم بنسبة متساوية لمدة 3 أسابيع في الصوبة البلاستيكية. كما صاحب ذلك زيادة معنوية في الكروتين في الكلي (2.49 ملم/جم وزن جاف) والسكرات الكلية الثنائية (27 جم/100 جم وزن طازج) زيادة في نشاط الأنزيم
الأسكوريك بروكسيداز (1102.33 وحدة/جرام وزن طازج). وقد نقلت نباتات الأنسجة بعد الأقلمة للنمو في أكياس
بلاستيك سوداء مخزنة تحت ظروف الصوبة في المشتل.

أظهرت النتائج أن أفضل معاملة خلطة في آلرسبا لنمو نباتات الأنسجة بعد 9 أسابيع في المشتل هي مخلوط من الرمل
مع نشارة الخشب الخشنة بنسبة (2 / 1 حجم/حجم) مع التسديد مرة في الأسبوع بحلول سمادي ستيميپول (19-19)19
تركيزه 0.5 جرام/لتر قد عكس استجابة موصلة في قياسات النمو مثل عدد الأوراق الكلية (7.3) وعدد الأوراق
الريشية (5.5) وارتفاع النبات (57 سم) وعرض الورقة (5.3 سم) و قطر قاعدة الساق (2.7 سم) وذلك بالمقارنة
بباقي المعاملات تحت الدراسة. كما أكدت نتائج التحليل الكيميائي نتائج قياسات النمو وحققت هذه المعاملة نتائج
معنوية في الوزن الم جم (15.79 %)، الليبيدات الكلية (4.48 %)، الكروتيديات الكلية الثنائية (38.99 %)
، الفئولات الكلية الثنائية (85.0 جم/جم بروتين) والبروتينات الخام (36.15 % علامة على زيادة نشاط إنزيم
الفيبين الأثيلين أمينتامين (220 نانو كتيل/جم بروتين) في الأوراق.

هذا النتائج يمكن للباحثين الاستفادة منها وعلى نطاق التجاري تساعدهم المستقبليين والمشتري لاحظ كبير في توفير
نفقات الإنتاج حيث أنها تقدم مواد خام طبيعية فعالة ورخصية يمكن استخدامها في المشتري وحلاً أن المشتري يمكن
أن تحتاج وتستجيب للبكتات كبيرة من هذه المواد الطبيعية في تدوير النباتات. علاوة على ذلك لها مرود ذو تأثير بيئي
عظيم في تدوير الخلفيات والاستفادة منها مثل سرسة الأرز ونشرات الخشب الخشنة بما يؤدي إلى تشجيع الإصحاح
البيئي وخفض تكاليف الإنتاج.