EFFECT OF AUXINS AND GROWING STAGES ON *IN VIVO* ACCLIMATIZATION OF DATE PALM (*Phoenix dactylifera* L) ZAGHLOUL CV.

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Abstract

This work was carried out in The Central Lab. of Date Palm Research and Development, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt during 2005-2006. This investigation was planned to study the effect of auxins (IAA, IBA and NAA), growing stages (plantlet root system) and stem diameter on acclimatization of date palm (*Phoenix dactylifera* L) Zaghloul cv derived from *in vitro* under the different temperature and humidity treatments. The viability of acclimatization was significantly differing depending on plantlet growth stage, auxin type and level; being optimum for plantlets which formed complete root system (primary + adventitious + side roots).

The best results when using 15 p.p.m of IBA for 45 seconds and plantlate stem diameter with >3 cm grown under 27 C and 90 % RH had the highest survival % during 4 months of the acclimatization.

Key words: Auxins – Acclimatization – date palm – humidity temperature – in vivo.

INTRODUCTION

Efforts in plant cell and tissue culture techniques have been directed to optimize the conditions for *in vitro* stages of micropropagation, but the process of acclimatization of micropropagated plants to the soil environment has not fully been studied. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants. Plantlets or shoots that have grown *in vitro* have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and optimum conditions for plant multiplication. Plantlets were developed within the culture vessels under low level of light, aseptic conditions, on a medium containing ample sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high level of humidity. These contribute a culture-induced phenotype that cannot survive the environmental conditions when directly placed in a greenhouse or field. The physiological and anatomical characteristics of

micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field. Although specific details of acclimatization may differ, certain generalizations can be noted (Hazarika, 2003).

After transfer from the *in vitro* to the *ex vitro* conditions the plantlets have to correct the above-mentioned abnormalities. In the greenhouse, and especially in the field, irradiance is much higher and air humidity much lower than in the vessels. Even if the water potential of the substrate is higher than the water potential of media with sucrose, the plantlets may quickly wilt as water loss of their leaves is not restricted. In addition, water supply can be limiting because of low hydraulic conductivity of roots and root-stem connections (Fila etal., 1998). Many plantlets die during this period. Therefore, after *ex vitro* transplantation plantlets usually need some weeks of acclimatization with gradual lowering in air humidity (Bolax et al . 1998, Kadlecek, 1997 and Preece and Sutter *et al.* 1991). Acclimatization units have been developed with temperature, humidity, irradiance, CO2 concentration and air flow rate controlled by computer (Hayashi, *et al.* 1988).

The aim of this study is to increase the percentage of acclamized tissue culture plantlets.

MATERIALS AND METHODS

This work was carried out in the Central Lab. of Date Palm Research and Development, Agricultural Research Center, Ministry of Agriculture, Cairo Egypt during 2005-2006. These investigations were planned to study:

1- The effect of auxins [indole acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA)] on in vivo acclimatization of date palm (*Phoenix dactylifera* L) Zaghloul cv derived from tissue culture. The root zone of the plantlets was dipped in the different auxin levels (0.0, 5, 10, 15 and 20ppm) for 15, 30, 45 and 60 seconds. Plantlets were cultured in polyethylene pots (5×18cm) containers containing a mixture of peat moss: vermiculite: sand (1:1:1) medium and covered with white transparent polyethylene bags during the 1st. tow months. The cultures were incubated in greenhouse under 30°C ± 2°C and 80-90% of relative humidity (RH) for 4 months.

2- The effect of plantlet diameter, relative humidity and temperature degrees on *in vivo* acclimatization of Zaghloul plantlets derived from tissue culture. Plantlets with the different bulbs diameters (>0.5, > 1.0, > 2.0 and > 3 cm.) were cultured in polyethylene pots (5×18cm) containers containing a mixture of peat moss:

vermiculite: sand (1:1:1) medium and covered with white transparent polyethylene bags during the 1st. tow months. The cultures were incubated in greenhouse under 60 or 90 %±10% of relative humidity and 27 or 32°C±2 °C for 4 months.

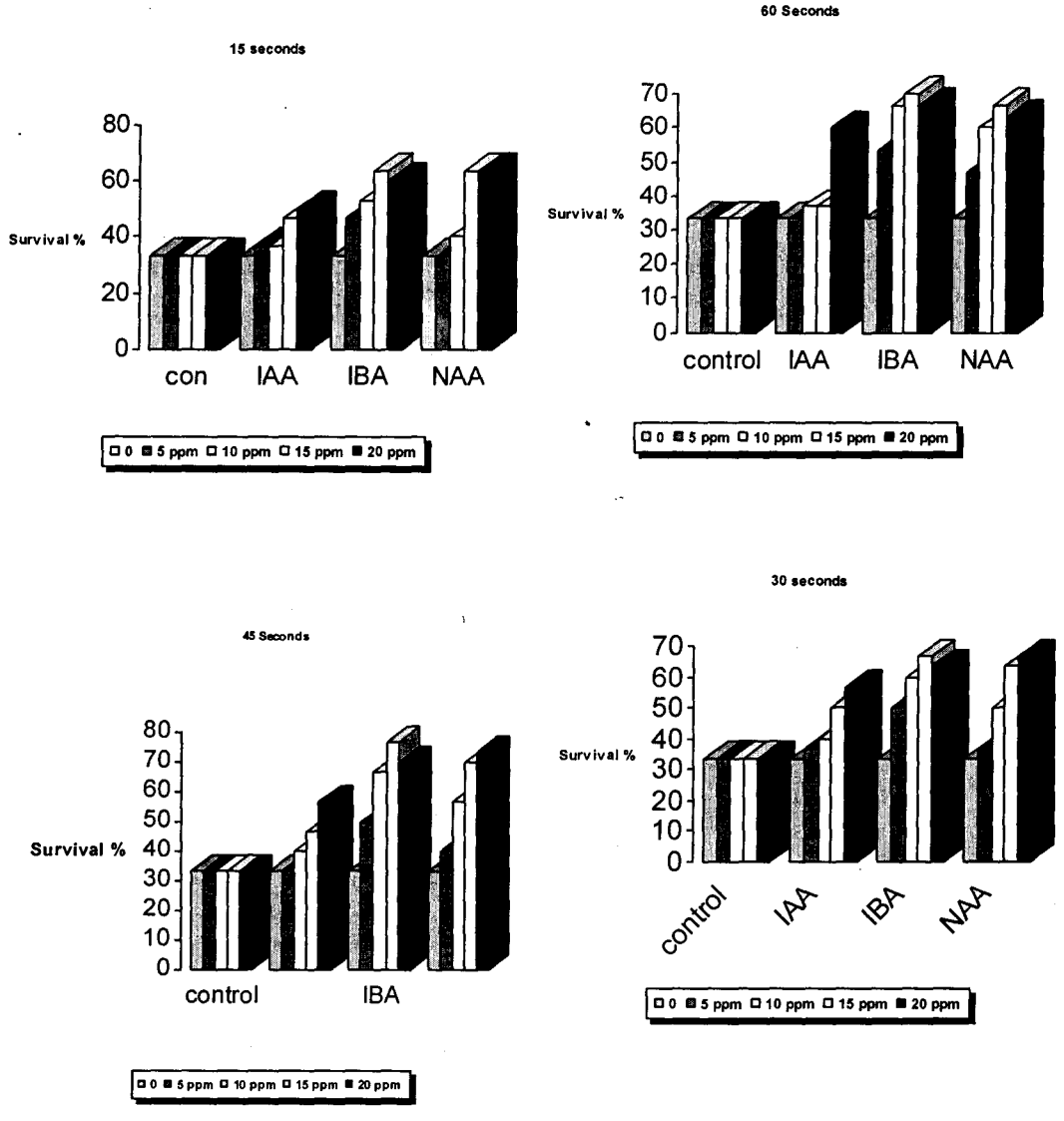
3- The effect of plantlet root system, relative humidity and temperature degrees on *in vivo* acclimatization of date palm Zaghloul cv derived from tissue culture. Plantlets with the different root system [primary roots (PR), primary roots with adventitious roots (PR+AR), primary roots with adventitious and side roots (PR+AR+SR)] were cultured in polyethylene pots (5×18cm) containers containing a mixture of peat moss: vermiculite: sand (1:1:1) medium and covered with white transparent polyethylene bags during the 1st. tow months. The cultures were incubated in greenhouse under 60 or 90 %±10% of relative humidity and 27 or 32°C±2 °C for 4 months. Survival percentage was recorded.

The experiments were carried out using completely randomized bloks design with three replicates. The results were analyzed using analysis of variance and the means were compared using new L.S.D at 5% level, All obtained data were subjected to analysis of variance according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of auxins(IAA, IBA and NAA) on *in vivo* acclimatization of date palm(Zaghloul cv.)

From the presentations of data in Fig. (1) it is evident that the different auxins have their stimulant effect on acclimatization of Zaghloul cv. through the different auxin level. In this concern, survival % significantly correlated with increasing the root dipping period of zone of the plantlets in the different auxin levels (0.0, 5, 10, 15 and 20ppm) from 15- 60 seconds 45 or 60 seconds being optimum with 15 p.p.m in the most cases, regardless of auxin type. However, using 15 p.p.m of IBA for 45 seconds seems to be the most suitable treatment in this order.



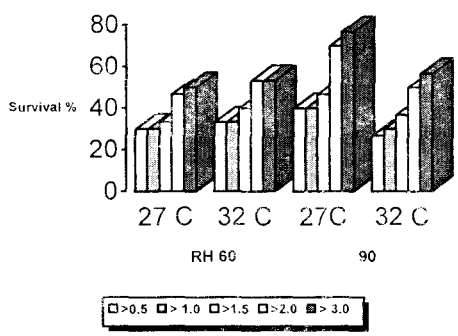
New LSD for: Auxins = 6.97, Dipping period = 3.87 and Interaction = 13.94

Fig. 1. Effect of auxin levels on survival % of *in vivo* acclimatization of date palm (*Phoenix dactylifera* L) Zaghloul cv.

2 * Effect of Relative Humidity, plantlet dimater and temperature degrees on *in vivo* acclimatization of date palm (Zaghloul cv.)

Data of Fig. 2 show clearly that, plantlet diameter, relative humidity and temperature degrees remarkably affected *in vivo* acclimatization of date palm Zaghloul cv derived from tissue culture. Through the different bulb diameter, plantlet with > 3 cm grown under 27 °C and 90% RH had the highest survival % during 4 months of the acclimatization.

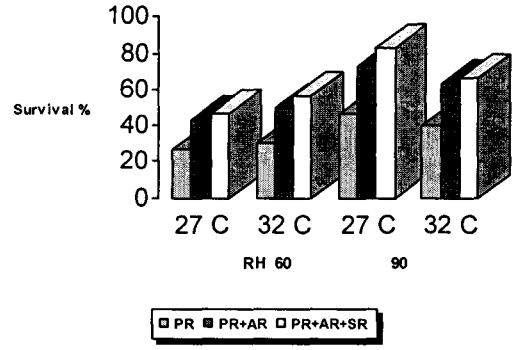
The plants that develop under lower relative humidity (60%) have fewer transpiration and translocation problems *ex vitro*, and persistent leaves that look like normal ones. The low deposition of surface wax, stomatal abnormalities and a non-continuous cuticle are typical anatomical features of herbaceous plants growing under conditions of abundant moisture. This typical *in vitro* anatomy can be prevented by increasing the vapour-pressure gradient between the leaf and the atmosphere. Lowering the relative humidity *in vitro* has been done experimentally with varying results. A relative humidity of 85% decreased the multiplication rate of carnation but increased the number of levels. Improved plant survival rates after transplantation have been promoted by the reduction of relative humidity (Smith, *et al.* 1991). Humidity inside the culture vessel has been reduced to improve the internal structure of plantlets and give a more successful establishment in the glasshouse (Ziv, *et al.* 1983). Short, *et al.* (1987) Reported that optimum growth and *in vitro* hardening of cultured cauliflower and chrysanthemum occurred when plantlets were cultured at 80% relative humidity.



New LSD for plantlet diameter = 6.13, RH = 3.88, Temperature (T) = 3.88,
Interaction between diameter and (T or RH) = 8.67
Interaction between diameter and (T and RH) = 12.27
Fig. (2): Effect of plantlet diameter, temperature and relative humidity on survival % of *in vivo* acclimatization of date palm Zaghloul CV

3- Effect of plantletroot system, temperature degrees and relative humidity on *in vivo* acclimatization of date palm (Zaghloul cv.)

It is evident from the data of Fig. 3 that survival % of *in vivo* acclimatized date palm Zaghloul cv significantly affected by root system, temperature and relative humidity; being optimum with the full root system plantlets grown under 27 °C and 90% RH.



New LSD for ROOT SYSTEM (RS) =5.54, RH=4.52, T4.52, RS ×T=7.84, RS×RH= 7.84, RS×T×RH=11.08

Fig. 3. Effect of root system, temperature and relative humidity on survival % of *in vivo* acclimatization of date palm Zaghloul cv.

Many commercial laboratories do not root micro cutting *in vitro*, because it is labour-intensive and expensive. The process of rooting *in vitro* has been estimated to account for approximately 35 to 75% of the total cost of micropropagation (Debergh and Mecene, 1981). An approach combining advantages of *in vitro* and *ex vitro* rooting had been successful for apples (Zemmerman and Fordhan, 1985). Sharma, et al (1999) reported that acclimatization and hardening in tea micropropagation could be accomplished as a one-step procedure within a short period of time before transplanting. Optimization of time of harvesting of micro shoots, shoot size, soil pH (4.0–6.4), plant growth regulator treatment (IBA 500 mg/L, 30 min), CO2 enrichment and light conditions in specially designed hardening chambers made a significant impact on the percent of success for hardening in tea micropropagation. However the *in vivo* conditions during root formation are not so important, provided optimal plant material is produced at the end of the *in vitro* cycle (Maene and Debergh, 1983). In contrast, the untreated control plantlets had a comparatively low survival of about 33.3%. This is due to the fact that after root colonization, they become empowered with extra molecular weapons to tackle the situation.

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تأثير الأوكسينات ومراحل النمو على الأقلمة داخل المعمل لنخيل البلح صنف زغلول

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أجرى هذا العمل في المعمل المركزي للأبحاث وتطوير نخيل البلح – مركز البحوث الزراعية – وزارة الزراعة – جيزة – مصر . أثناء موسم ٢٠٠٥ – ٢٠٠٦ . وهذه الدراسة خططت لدراسة تأثير الأوكسينات (أندول حمض بيوتريك ، أندول حمض الخليك ونفثالين حمض الخليك) ومراحل النمو ونظام التجذير في النبيتات وحجم الساق) لنخيل البلح صنف زغلول الناتج من الأنابيب تحت مختلف درجات الحرارة ومعاملات الرطوبة.

وكانت النبتات القادرة على الأقلمة مختلفة معنويا معتمدة على مرحلة النمو ونوع الأكسين وكانت أعلى مستوى عندماً تكون النظام الجذري بالكامل (الجذور الأولية ، الجذور العرضية ، الجذور الجانبية) . وكانت أفضل النتائج عندما استخدم ١٥ جزء في المليون من الأندول بيوتريك أسيد لمدة ٤٥ ثانية وعندما كانت ساق النبيتات بسمك أكبر من ٣ سم ونامية تحت ٥٢٧ م في ظل رطوبة ٩٠% وذلك بعد ٤ شهور من الأقلمة .