A comparative anatomical study of date palm vitroplants

(Received: 02.04.2003; Accepted: 14.04.2003)

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ABSTRACT

Because the rate of survival of tissue culture-derived date palm plantlets (vitroplants) in acclimatization stage is significantly low, this study was suggested in order to focus on the anatomical structures of roots and leaves of date palm vitroplants. The obtained data showed the complete absence of vascular elements in roots of some sorts (clones) of vitroplants; meanwhile in other clones, the vascular elements were found to be in early stages of development. Anatomical variations were also recorded in leaf topography and anatomy among different clones of vitroplants originated from the same explant and subjected to the same media regime. The recorded anatomical variations and the poor development of vascular elements and consequently poor connection and transportations of water, solute, mineral and gases within the vascular elements, may be involved in severe wilting of date palm vitroplants in acclimatization stage. Accordingly, it could be suggested that successful acclimatization of date palm vitroplants starts in rooting stage in vitro.

***Key words**: Date palm, vitroplants, acclimatization.*

INTRODUCTION

As a result of its multipurpose uses, tolerance to extremely adverse environmental conditions and low cultivation and harvesting costs, compared with other fruit crops, date palm is the most important fruit crop in Middle East, North of Africa and is the major plantation crop in some Arabic countries, such as Saudi Arabia (FAO, 1984). Unfortunately, insufficient number of offshoots resulting from the mother tree through the traditional vegetative propagation hinders the expansion of date palm-cultivated area. Moreover, the long life cycle of palm tree makes its improvement through traditional breeding programmes a tedious endeavour (Moursy and Saker, 1998). Accordingly, employment of recent advances in plant biotechnology, including plant tissue culture, marker assisted selection (MAS) and transformation, seem to be the appropriate solution for quick propagation, selection of new date palm breeds and production of pest auto-resistance varieties (Saker and Moursy, 2003).

Although, date palm is considered a recalcitrant monocot plant to respond to *in vitro* conditions, extremely high number of successful attempts were made on its *in vitro* propagation (for instance, Tisserat, 1982; Dass et al., 1989; Bhansali et al., 1988; Zaid and Hughes et al., 1995; Bekheet and Saker, 1998 and El-Bahr et al., 2003a&b). However, the applicability of the published results is rare due to low percentage of survival and development in the acclimatization stage.

Previously published data on acclimatization of tissue culture-derived date palm plantlets have been focused on optimization of factors such as composition of acclimatization soil, humidity, pests, temperature and other green house conditions (Tisserat, 1982, 1984 and Al-Salih et al., 1986). Although, such studies has led to significant enhancement in the percentage of survival of tissue culture-derived date palm plantlets in acclimatization stage up to 70% (Quraishi et al., 1997), low rate of success in acclimatization stage still represents a dilemma in commercial micropropagation of date palm. Accordingly, other factors may also be affecting this limiting stage of *in vitro* propagation of date palm for commercial purposes. In this context, Zaid and Hughes (1995) reported that water content and water potential of tissue culture-derived date palm plantlets has a direct impact on successful *ex-vitro* acclimatization. On the other hand, recently published reports indicated that the development of root system and vascular elements, as revealed by anatomical investigations, may significantly affect *ex-vitro* acclimatization of tissue culture-derived date palm plantlets (El-Bahr *et al.*, 2003a&b).

The present study focuses upon the anatomical studies of roots and leaves of tissue culture-derived date palm plantlets (vitroplant) as a first step towards deep understanding the low rate of success of date palm vitroplants in acclimatization stage.

MATERIALS AND METHODS

Establishment of date palm tissue culture

Date palm offshoots of the cultivar Zaghlool were secured from Edco, El-Beherah governorate during the fruiting season (September, 1998). Shoot tip explants were separated, sterilized and cultured on callus induction medium containing 100 mg/l 2,4-D + 3 mg/l 2ip. Embryogenic callus proliferated after six months of cultivation on callus induction medium, were subcultured on basal MS medium for further development and plantlets recovery. The recovered plantlets were divided into two groups according to the morphology of the root and shoot systems. A set of plantlets, showing the highest percentage of survival in acclimatization stage, was included in this study as the third group. Roots and leaves of the different individual plantlets represent the three-above mentioned groups were subjected to anatomical investigations.

Anatomical investigations

Transverse sections were taken from the middle parts of the leaves and main root (3-5 cm behind apex of the main root). Samples were killed and fixed in formalin, acetic acid and ethyl alcohol, at a ratio of 90:5:5, dehydrated in ascending concentrations of ethyl alcohol, then cleared by soaking in a series of absolute alcohol and xylene and imbedded in paraffin wax (M.P. 55-58 oC). Using a rotary microtome, serial cross-sections (15-20) were taken. Samples were then stained with crystal-violet-erythrosine combination and mounted in Canada balsam. Examination and observations were carried out by Nikon light Microscope and Photographed by Nikon Camera FX-35.

RESULTS AND DISCUSSION

Date palm tissue culture

Different tissue culture-derived date palm plantlets (Vitroplants) proliferated from shoot tip explants (CV. Zaghlool) via somatic embryogenesis were divided into three groups, based on morphological criteria. Data presented in Table (1) summarize the morphological description of the three different groups. The root system of the first group was a cluster of fine weak roots and it is

difficult to distinguish which one is the main root. The leaves of the planlets of this group were curled (twisted) and dark green in color. The root system of the second group consists of two to three main roots with numerous adventitious and hairy roots. The root system of this group, in general, appears as a network of roots. Meanwhile the leaves of the plantlets of this group are faint green leaves with visible veins. Finally, the root system of the third group of the plantlets consists of one main long root with few adventitious roots and the leaves were straight long green with visible veins (Table 1).

Anatomical investigations

1-Leaflets anatomical structure

Transverse sections through the middle parts of the leaflets of date palm plantlets belonging to the three groups (Table 1) indicated that the leaflets consist of one layer of epidermal cells which were vary in shape and size (Fig. 1). A thin layer of cuticle covering the upper epidermal cells was observed in leaflets of the three groups. The mesophyll tissue of the leaflets of the three groups consists of one type of mesophyll cells, which appeared, variously in shape and size with narrow intercellular spaces between cells. The narrow and thin leaflets blade observed in leaflets of the first group was mainly due to compactness and reduction occurred in the size and number of mesophyll cells and layers (Fig. 1). The mesophyll cells were compacted, ruptured darker and heavy stained in the central area of mesophyll tissue. On the contrast, the thick and wider leaflet blades observed at the leaflets of the second and third groups, were mainly due to the increasing number and size of mesophyll cells (Fig. 1), which was composed of seven to nine layers of mesophyll cells. Therefore, the differentiation and development of mesophyll tissue cells and layers have an essential role on the shape and size of leaflets blades in tissue culture-derived date palm plantlets. Such differences in the mesophyll tissue cells (shape and size) could be a result of some conditions during *in vitro* cultivation resulting in the formation of plantlets with abnormal physiology, anatomy and morphology and in some cases increased genetic variations (Saker *et al.*, 2000 and El-Bahr *et al.*, 2003b).

No clear differences of vascular tissues were observed among plantlets of the three groups (Fig. 1). However, small area occupied by undifferentiated vascular elements was observed in the three groups. This area appeared to be composed of undifferentiated parenchymatous cells, which were not developed to phloem or xylem vessels elements. On the other hand, the large vascular vein well developed to both phloem and xylem vessels element surrounded by sclerenchmatous elements were clearly developed to the pro and metaxylem vessels but they lost their normal arrangements. Such effect appeared to be due to the environmental conditions of culturing *in vitro* as reported by Rady and Ali (1999) on *Beta vulgaris*; Ali and Rady (2000) on *Lavandulla officinallis* and El-Bahr et al., (2003b) on date palm.

Table (1): Morphological description of different in vitro derived-plantlets subjected to anatomical investigation.

Description	Group I	Group II	Group III
Root system	A cluster of fine roots	Three main roots with numerous adventitious and hairy roots	One long main root with few adventitious roots
Shoot system	Curled dark green leaves	Faint green leaves with visible veins	Straight long faint green leaves with visible veins

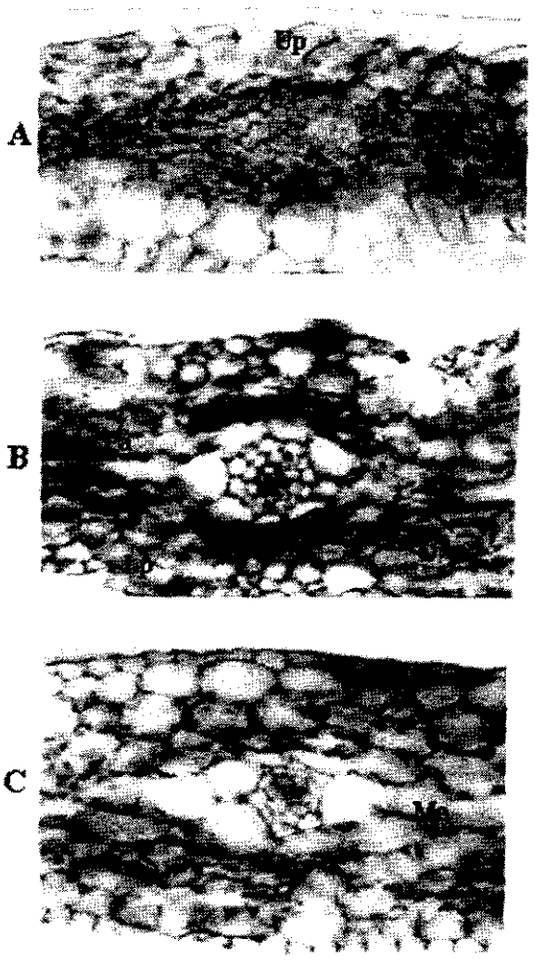

Fig. (1): Cross-sections of date palm vitroplants leaflets representing clone I (A), clone II (B) and clone III (C).Lp: lower epiderm, Up: upper epiderm, Me: mesophyll tissue, Pa: parenchyma cells and Vb: vascular bundles.

2-Leaf surface topography

Epidermal cells on both abaxial and adaxial of leaf surfaces of the three groups of leaflets were straight and of thick cell walls as presented in Fig. (2). The epidermal cells on the lower surface of the leaflets was slightly smaller than that found on the upper surfaces of the leaflets. Mostly, the epidermal cells were elongated in shape (with very extended length and short diameter) with few numbers of rounded, cubic and polygonal shapes). Stomata of the three groups of the leaflets were morphologically differing in number and large size of stomata was observed at the second and third groups of leaflets. Whereas, less number and size of stomata was observed at the first group of leaflets. Predominantly, each stoma was surrounded by four epidermal cells differing in shape and size from the other epidermal cells.

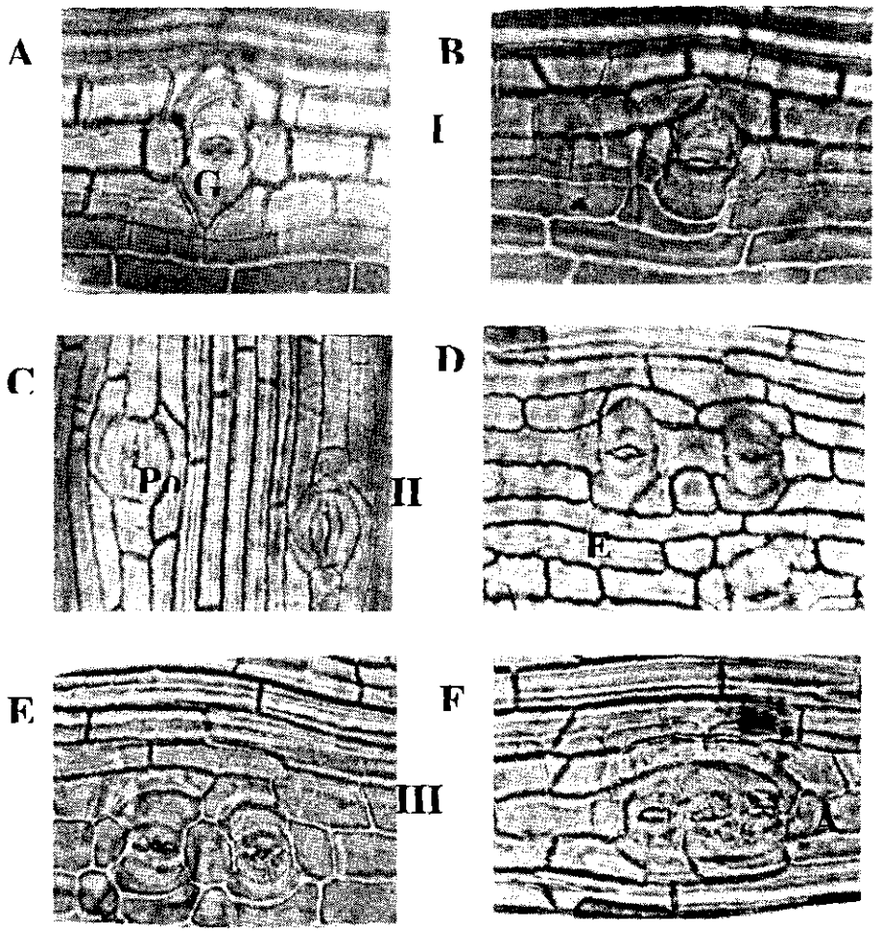

Fig. (2): Upper (A,C and E) and lower (B,D and F) surface leaflets topography of date palm vitroplants representing clone I, clone II and clone III. Ep: epidermal cells, Gr: guard cell, Po: stomatal pore and Ab: abnormal stomata. All Figs X=80.

3-Root anatomy

Transverse sections of primary rootlets of tissue culture derived date palm plantlets showed different levels of differentiation and development as presented in Fig. (3). The transverse sections of roots of the first group (a cluster of fine roots) were shown in Fig. (3). The roots appeared in an early primary stage of growth and differentiation. The epidermal and subepidermal layers could not be distinguished for their dark staining or disruption (Fig. 3). The cortical zone consists of 7-9 layers of cortical cells, which appear, variable in shape and size. The inner region of the cortical zone was degenerated and disrupted whereas the outer cortical zone (3-4 layers) was normal and compact in shape. The central zone of the vascular tissues (stele) was devoid from differentiation and development of vascular elements except a mass of small parenchymatous cells.

Transverse sections of the primary rootlets of tissue culture derived date palm plantlets of the second group (main root with numerous adventitious and hairy roots) were slightly more differentiated and developed than the first group. The outline shape of roots was extended and appeared oblong or oval in shape. The epidermal and subepidermal layers could not be clearly distinguished for their

compactness and dark staining. Both number and layers of root cortical cells were increased. The cortical cells appeared smaller in size and tightly packed with smaller intercellular spaces between cells. The changes occurred on the outline shape of roots in the transverse sections might be due to the increased multiplication of the root cortical cells and layers which increased pressure toward inner and outer zones of root cross sections. Small vascular elements were scattering on inner and outer layers of cortical zone (Fig. 3). The central zone of the vascular elements (stele) was slightly elongated in shape, the pericycle and endodermal layers were hardly to recognize for their compactness, whereas well differentiation and development of xylem vessels elements embedded in a mass of undifferentiated and smaller parenchyma cells were observed (Fig. 3).

Transverse sections of primary roots of tissue culture-derived date palm plantlets of the third group (one main root and a few adventitious roots) were more advanced in differentiation and development than the roots of the first and the second group (Fig.3). The outline shape of the main root appeared normal and rounded in shape. The epidermal and subepidermal layers were compacted and darkly stained or disrupted (Fig. 3). Most regions of the cortical zones were disrupted or degenerated (Fig. 3). Such effect may be due to the differentiation and development of vascular elements in the central zone of vascular tissues (stele) as observed with initiation and development of secondary root primordia (Fig.3) or to more differentiation and development of central zone vascular elements. The central zone of vascular tissues (stele) was round in shape and its size is proportional to the size of transverse section. The pericycle and endodermis were darkly stained as observed in Fig. (3) or clearly distinguished as in Fig.(3). Xylem vessel elements were arranged at the peripheral layer of the central zone of vascular elements. The xylem vessels elements were imbedded in a mass of smaller and differentiated parenchymatous cells.

It is well known and documented that a well developed root system on tissue culture derived date palm plantlets is the most important stage of any *in vitro* protocol aiming at mass micropropagation of date palm. In this regard, Tisserat (1982 and 1984) reported that date palm plantlets derived from tissue culture did not establish easily when transferred to free living conditions. Al-Salih *et al.*, (1986) mentioned that death or failure of date palm plantlets during acclimatization stage may be attributed to the lack of root cell differentiation caused by a deficiency in growth regulators, insufficient sugar or hormonal or sugar balance. In this context, El Bahr *et al.*, (2003b) pointed out that leaves, roots and stomatal morphology of date palm plantlets were different in structure and shape when compared with those produced from acclimatized plantlets.

Such disruption appeared to be a constitutive structure of date palm roots, to transfer root's cortex to aereanchyma, which enhance the diffusion of atmospheric or photosynthetic oxygen from the shoot to the roots (Naidioo and Naidioo 1992, Bruch, 1994 and Bruch and Merida, 1995). The compactness of the hypodermal and exodermal layers in roots produced *in vitro* may play a role in preventing the collapse of the cortex and may also be a necessary structural framework for aereanchyma formation (Seago and Marsh, 1989). The abnormalities in the structure of leaflets and root vascular tissues of the *in vitro* plantlets could be attributed to extreme environment in the cultivation vessels such as CO_2, water, potential, air humidity and photosynthetic ability.

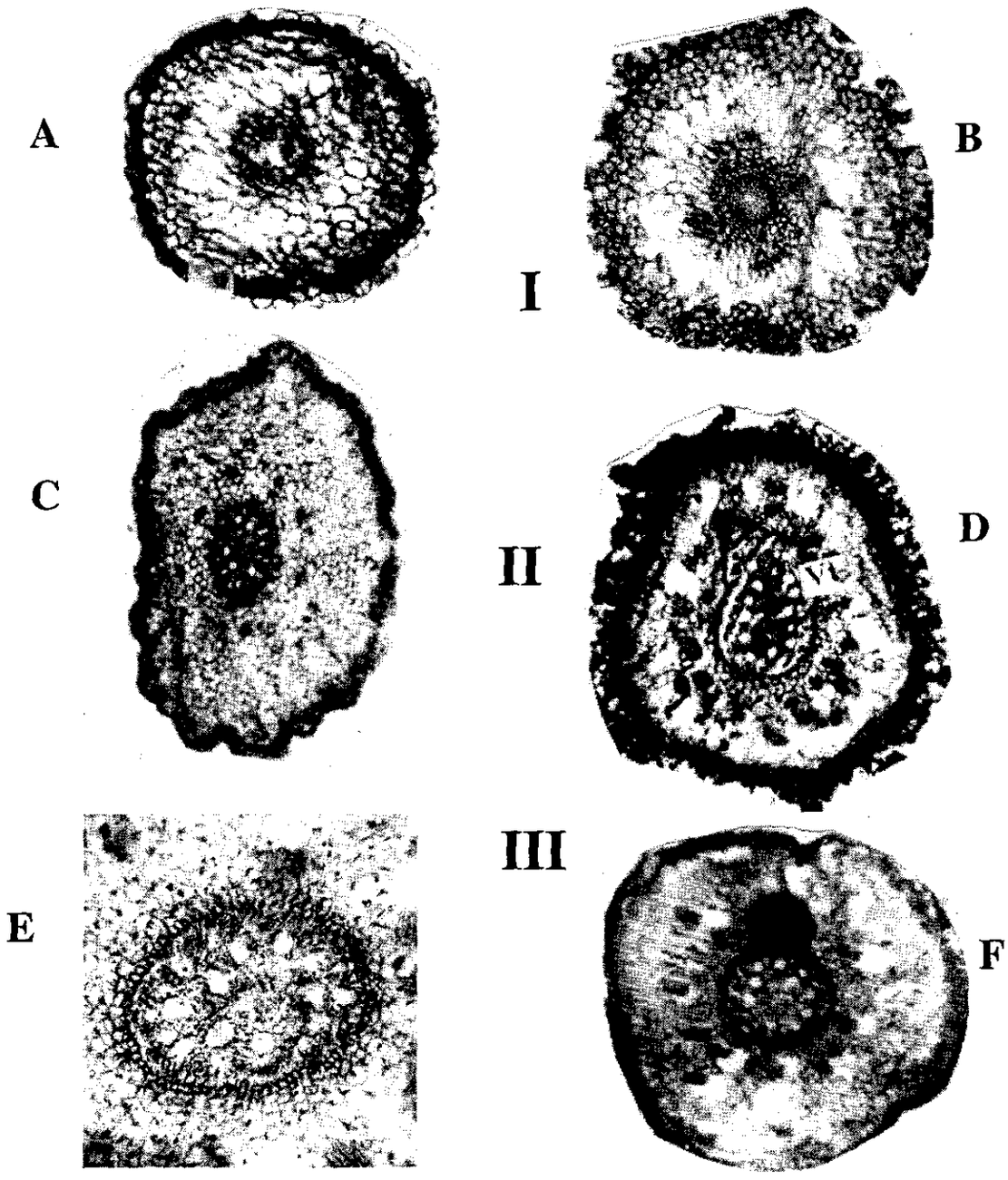

Fig. (3): Transverse sections of roots of date palm vitroplants representing clone I, clone II and clone III. Ep: epidermal layer, Co: cortical layer, Pa: parenchyma layer, Vb: Vascular bundle and Se: sclerenchymatous layer. All Figs X=80.

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الملخص العربي

دراسة تشريحية مقارنة لنخيل البلح الناتج من زراعة الأنسجه

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بالرغم من نجاح إكثار نخيل البلح معمليا إلا أن عملية أقلمة النبات في الصوبة تعتبر عقبة أمام تطبيق البروتوكول على المستوى الاقتصادي، و قد استهدفت هذه الدراسة التغلب على انخفاض نسبة بقاء النباتات الناتجة معمليا أثناء مرحلة الأقلمة، حيث تم التركيز على التركيب التشريحي لجذور و أوراق نباتات نخيل البلح الناتجة من زراعة الأنسجة،قد أظهرت النتائج الغياب الكامل للأوعية في جذور بعض النباتات الناتجة من زراعة الأنسجة، بينما كانت تلك الأوعية في المرحلة المبكرة الأولى من التطور في بعض النباتات، وقد سجلت أيضا بعض الاختلافات التشريحية في طبوغرافية و تشريح الورقة بين سلالات مختلفة لنباتات ناتجة من نفس المنفصل النباتي و ناميه على نفس بيئات الزراعة. الاختلافات التشريحية المسجلة و قلة تطور الأنسجة الوعائية و بالتالي قلة الاتصال و انتقال الماء و العناصر المغذية و التبادل الغازي ربما يكون سببا في ذبول نباتات نخيل البلح الناتج من زراعة الأنسجة خلال مرحلة الأقلمة، و بناءا على الدراسة يمكن القول ان نجاح عملية الأقلمة تبدأ في مرحلة التجذير في المعمل كعملية تهيئة أو أقلمة أولية.