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FACTORS AFFECTING *IN VITRO* ROOTING OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

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Date palm is a dioecious, perennial monocot plant species of the Arecaceae family cultivated in 56 countries including European and American countries. The success of date palm micropropagation is strongly linked to the *in vitro* root quality. The identical shoots of 8-10 cm long of three different varieties (vars.) were cultured in rooting experiment to study the combined impact of genotype, sucrose concentration, activated charcoal (AC), basal salts (MS or MS & B5) and subculture number. The adventitious roots were planned to be initiated on media included different combinations of sucrose and basal salts for a couple of subcultures (each a month) and after then were transferred onto media contained 1.5 and 3.0 g/l AC for additional two subcultures. Findings indicated that using 40 g/l sucrose in the rooting medium encouraged the adventitious roots production where 5 roots per plantlet of 7.8 cm long and 1.4 mm width were averagely induced. The average leaves number reached 3-4 leaves per plantlet, leaf length 18-28 cm and the leaf width 3.5-6.9 mm. Full strength of MS basal salts proved better than macro elements of B5 & micro elements of MS. The leaves and roots growth was affected by the genotype since the shoots of var. Gajar were initiated before other two varieties and in higher number. Incorporation of 3.0 g/l AC to the rooting medium found to be necessary to enhance the whole plantlet growth as the length of white roots and leaves increased. AC addition encouraged the secondary and tertiary roots formation. By adjusting factors affected rooting, the ideal *in vitro* roots have been achieved within a short period of time (4 months) and the survival percentage of *ex vitro* plants exceeded 90%. The *in vitro* roots development and proper handling of the plantlets during rooting stage was described in current study.
Keywords: Acclimatization, activated charcoal, basal salts, date palm, *in vitro* rooting, genotype, sucrose.

INTRODUCTION

Date palm is one of the oldest fruit crop mainly cultivated in North Africa, Middle East, Near East of Asia and some dispersed areas of Europe and America (Zaid, 2002; Hodel and Johnson, 2007; Haider *et al*., 2013). Micropropagation has been extensively used for the rapid and large scale multiplication of many plant species. However, its more widespread use is restricted by the often high percentage of plants lost or damaged (50 to 90%) when transferred to *ex vitro* conditions (greenhouse or field) (Wardle *et al.,* 1983; Ziv, 1986; Pospisilova *et al*., 1999; Kumar and Rao, 2012; Uzma *et al*., 2012). Even when gradual acclimatization has been used, poor survival and slow growth of plantlets have been commonly reported (Lee *et al*., 1985; Pierik, 1987; Crane and Hughes, 1990). Being monocot date palm trees produce fasciculate and mostly fibrous roots. Primary roots develop from seed and secondary roots develop from primary root. Differentiation of root cortex is a key factor in the transport of compounds inside and outside of the root, subsequently to other parts of the plant body (Fatima, 2011). 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). In this context, El Bahr *et al*. (2003b) pointed out that leaves, roots and stomata morphology of date palm plantlets were different in structure and shape when compared with those produced from acclimatized plantlets.

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. In order to reach ideal rooting in a short period of time, rooting stage can be divided into adventitious roots initiation and roots development steps. Detached individual shoots from the multiplication stage almost are short and may have a primary root. Consequently it may need to be subjected to an elongation stage before rooting (Abul-Soad *et al*., 2006). The well-rooted plantlets may be shifted into the greenhouse through an intervening *in vitro* hardening step (Abul-Soad *et al.,* 1999). Therefore, shortening the time of rooting stage could help to reduce the entire production cycle. However, this period may vary because of the small size of the multiplied shoots, the origin of these shoots and presence of the primary root. The type of the individual small shoot is either a direct somatic embryo with a primary root or a

detached shoot without any roots from a cluster during the multiplication stage (Abul-Soad *et al*., 2004a,b). The direct somatic embryos were found to be much more powerful to grow and develop roots compared to the detached shoots from the multiplication stage (Abul-Soad *et al.,* 2002a,b, 2004a, 2005; Sidky *et al*., 2007). For instance, addition of AC in the rooting medium from the first subculture produced thin and disbranched roots, and inhibited the adventitious roots formation (Abul-Soad *et al*., 1999). There seems to be other factors affecting rooting that may include AC, type of the basal salts, sugar, variety (var.) and number of subcultures. These factors need to be studied. However, concentrations of MS basal salts, sucrose, light, type and concentration of the plant growth regulators have been studied, but separately (Abul-Soad *et al*., 1999, 2006; Ibrahim *et al*., 1999a,b). However, the current study focused on the combined effect of some major factors at the appropriate subculture during rooting stage in order to get rapid and satisfied adventitious rooting and subsequently increase the survival percentage of the acclimatized plants in the greenhouse. Also, the study extended to describe proper handling of the *in vitro* plantlets during rooting stage to sustain excellent rooting.

MATERIALS AND METHODS

This work was carried out at Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan in 2011-12. The *in vitro* shoots of three different varieties Gajar, Kasho Wari and Gulistan used in the experiment were collected from the proliferated clusters of shoots in the multiplication stage at the subculture number 13. Typical 8-10 cm long shoots were separated individually and cultured onto the basal medium composed of 2.4 g/l Agar, 1.4 g/l Gel, vitamins of MS, 0.1 mg/l of GA_3 and NAA, and supplemented with 3 different concentrations of sucrose (30, 40 and 50 g/l) and 2 salts formula [Macro of B5 (Gamborg *et al*., 1968) & Micro of MS (Murashige and Skoog, 1962) which named as MMS and macro & micro of MS (Murashige and Skoog, 1962) which named as MS] for 2 subcultures each for a month. After then, two concentrations of AC (1.5 and 3.0 g/l) were added to the nutrient media for an additional couple of subcultures. All primary roots of the selected shoots were trimmed to the minimum length possible (1-2 mm) before starting the experiment (Fig. 1).

As long as the plantlets were growing through the different 4 subcultures, equal amounts of 20 ml medium were poured into three different size tubes covered with aluminum foil closures. These were small-size tubes (25 × 150 mm), medium-size tubes (25 × 200 mm) and long-size tubes (25 × 250 mm). After each subculture, the shoot (leaf) and root length (cm), leaf and root width (mm), and leaves and roots number (no.) per plantlet were measured. The *in vitro* rooting experiment was repeated twice to confirm the results.

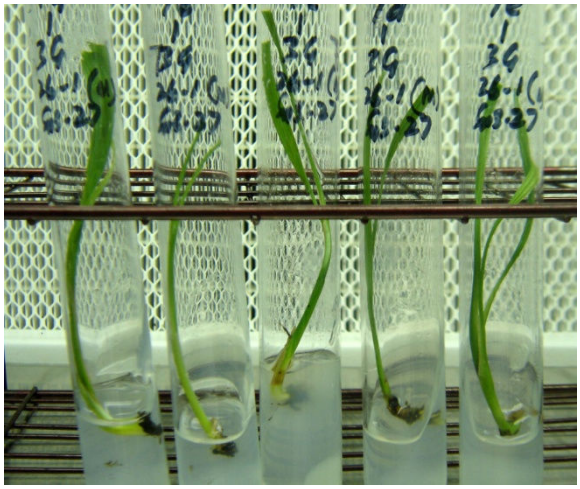

Figure 1. 8-10 cm initial shoots (explant) cultured on different treatments at the beginning of rooting experiment.

The rooted plantlets were transferred to a fiberglass house for acclimatization. Handling the *in vitro* plantlets into the greenhouse was according to the method described by Abul-Soad (2011) and the following procedure was followed:

1. In the greenhouse, the plants were picked from the tubes and roots were washed gently in lukewarm distilled water to remove any residual medium.
2. The plants were immersed in 0.5% (w/v) Topsin M fungicide solution for 5 minutes.
3. The plastic bags of 350 m^3 were filled with soil mixture of washed sand: peat moss (1:1 v/v) and 10% of total volume Perlite. Crushed stones were placed in the bottom of the bags to allow drainage of excess irrigation water.
4. The plants have been kept in the greenhouse under natural day light and high relative humidity (95-100%) using a low tunnel of white-transparent polyethylene sheet kept closed for one week.
5. Process of acclimatization of plants in greenhouse conditions (30±5ºC and 40-50% relative air humidity) was achieved by gradual reduction in humidity at the ambient conditions of plants by removing the plastic sheet gradually through first couple of months. After then plants were allowed to grow under greenhouse conditions.
6. The plants were watered once after a month without fertilization up to 3 months. The survival percentage was recorded after 3 months of transplanting for the 12 different treatments (3 concentrations sucrose × 2 basal salts × 2 AC treatments) of the three varieties.

Each treatment consisted of 45 shoots for each variety (3 replicates of 15 shoots each and each test tube contained an

individual shoot). Factorial Randomized Complete Block Design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using LSD test at 5% according to Steel *et al*. (1997).

RESULTS AND DISCUSSION

Successful transplanting of the *in vitro* date palm plantlets depends mainly on the *in vitro* adventitious roots. One of the main goals of this study was to initiate ideally the adventitious roots of the young shoots. Consequently the required time for rooting will be reduced simultaneously with enhancement overall growth and development. Ultimately, the whole production cycle of micropropagation will be shortened.

Prior to develop the current protocol for rooting, other researcher's groups reported in several publications on the successful rooting protocols of date palm (Abul-Soad *et al*., 1999, 2006; Ibrahim *et al*., 1999a, b). However, their experiments had some limitations.

***Variety dependent responses*:** The variety (genotype) affected adventitious roots formation. Data in Table 1 represent the best treatment of the integration impact of major root stimulators. These data showed the plantlets development of 3 different varieties through 4 subcultures on MS basal medium supplemented with 40 g/l sucrose and 3 g/l AC.

Shoot development in terms of number of leaves, leaf length and leaf width significantly varied among the 3 varieties (Table 1). The average number of leaves and leaf length of var. Gajar significantly was higher than other two varieties in S1. However, var. Gulistan was characterized with wider and shorter leaves than other two varieties.

Regarding the shoot growth during the different subcultures, the average of leaves number, leaf length and leaf width gradually increased from S1 through S4 but the major increment occurred after 2 months (S2). In S4, overall average number of leaves ranged from 3-4 but var. Gulistan appeared with lower leaf number 2.8 per plantlet but, had more healthier and wider leaves where the leaf width reached almost to 6.9 mm while var. Gajar 4.5 mm and var. Kashoo Wari 3.5 mm.

Roots development as expressed by number of roots, leaf number and root diameter was significantly different. Var. Gulistan was able to initiate the adventitious roots only after the S1 but other two varieties started to initiate their roots within a month. However, var. Gulistan produced more roots where the average roots number was 6.0 and 6.0 after S2 and S4, respectively. The average of root width was 0.9 mm and increased to reach 1.4 mm at S4. All parameters of roots development increased gradually from S1 through S4. Furthermore, similar results were obtained whether the shoots cultured onto MS basal salts or modified MS salts (MMS). The results indicated that the genotype could have impact on early root initiation of the young shoots. Therefore, the morphology of the *in vitro* date palm plantlets was variety independent response.

***The integration impact of the major root stimulators*:** Data in Table 2 indicated a significant difference between the MS and MMS media for var. Kashoo wari at the three used sucrose concentrations. The overall average shoots and root development significantly increased by increasing the sucrose concentration in the MS basal medium while the increment was not significant when MMS basal medium was used. After 2 months of culturing the maximum no. of leaves (2.73) & roots (3.78), the average leaf (14.06 cm) and root length (3.31 cm), and the average leaf (3.2 mm) & root (1.09 mm) thickness achieved using MS medium with 40 g/l sucrose concentration followed by 30 & 50 g/l sucrose concentration. The least response was observed on overall average growth development when MMS medium was used with 30 & 50 g/l sucrose. This result is in full accord with Ibrahim *et al.* (1999b) who reported that root and shoot

Table 1. Leaves and roots development of the *in vitro* date palm plantlets for three different varieties (Gajar, Kasho Wari and Gulistan) during 4 consecutive subcultures.

Growth parameters	Subcultures and Varieties															
	Gajar					Kasho wari					Gulistan					
	S1[z]	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	LSD
Leaf No.[w]	3	3.2	4.0	4.0	3.55	2.4	2.3	2.3	3.0	2.5	2.3	2.3	2.8	2.8	2.55	0.43
Leaf Length[v]	13.6	15.4	21.2	24.5	18.67	13.5	16.1	19.0	20.0	17.15	11.9	13.6	15.6	18.2	14.82	2.45
Leaf Width (mm)	1.8	2.8	3.8	4.5	3.22	1.6	3.3	3.5	3.5	2.97	4.3	6.0	6.3	6.9	5.87	0.55
Roots No.	3	4	4.2	4.2	3.85	2.4	3.8	4.3	4.5	3.75	0.0	6.0	6.0	6.5	4.62	2.37
Root Length	1.8	5.3	6.3	6.5	4.97	2.0	4.6	4.9	6.0	4.37	0.0	2.6	5.6	7.8	4.0	1.88
Root Diameter (mm)	1.1	1.2	1.3	1.3	1.22	0.9	1.1	1.3	1.3	1.15	0.0	0.9	1.3	1.4	0.9	0.50

LSD at 0.05; [z]Subculture Number (1-4), [y]KashoWari variety, [x]Gulistan variety, [w]Leaf number/plantlet, [v]Leaf length (cm).

Table 2. Effect of basal salts and sucrose concentrations on *in vitro* rooting of date palm plantlets after 2 subcultures in var. Kashoo wari.

Sucrose g/l	Basal Medium											
	No. of leaves		Leaf length cm		Leaf width mm		No. of roots		Root length cm		Root thickness mm	
	MS	MMS	MS	MMS	MS	MMS	MS	MMS	MS	MMS	MS	MMS
30	2.48	2.55	14.06	11.84	2.96	2.61	2.56	1.43	2.97	2.14	1.02	0.96
40	2.73	2.7	14	12.99	3.2	3.12	3.78	1.43	3.31	2.84	1.09	0.91
50	2.61	2.3	14.04	13.27	2.87	2.3	3.16	2.15	3.11	3.22	1.22	0.96
Mean	2.60	2.51	14.03	12.7	3.01	2.67	3.16	1.67	3.13	2.73	1.11	0.94
LSD at 0.05	0.30	0.40	0.23	0.55	0.55	0.31	0.30	0.39	0.30	0.55	0.22	0.01

Table 3. Combined impact of basal salts, sucrose concentration and activated charcoal (g/l) on roots and shoots formation of var. Kasho Wari plantlets after 4 subcultures.

Basal Salts	Sucrose conc. (g/l)	Activated Charcoal Conc. (g/l)							
		1.5				3.0			
		Root No.[x]	Root Length[w]	Leaf No.	Leaf Length	Root No.	Root Length	Leaf No.	Leaf Length
MS[z]	30	3.75	4.20	3.00	16.60	3.20	6.75	3.00	24.00
	40	5.25	4.50	3.90	21.25	8.00	9.12	4.00	28.16
	50	4.75	4.00	2.80	18.00	4.00	4.50	3.00	20.00
LSD at 0.05		0.47	0.16	0.24	0.68	0.19	0.05	0.19	0.40
MMS[y]	30	2.00	3.70	2.80	15.60	2.50	3.75	2.90	20.50
	40	3.66	3.80	3.00	20.00	4.00	4.30	3.20	21.20
	50	3.25	3.50	3.00	17.00	3.50	4.00	2.80	18.00
LSD at 0.05		0.05	0.39	0.65	0.69	0.36	0.04	0.60	0.61

[z]MS (macro & micro salts of MS); [y]MMS (Macro of B5 (Gamborg *et al*., *1968*) & Micro of MS (Murashige and Skoog, 1962); [x]Roots number/plantlet, [w]Roots length (cm).

formation affected by sucrose concentration in the rooting medium, since it increased by elevating the sucrose concentration from 30 g/l to 50 g/l, but the opposite effect was observed by using 60 g/l sucrose.

The interaction impact of sucrose concentration and basal salts reveled that using MS full strength as basal salts in the rooting medium was better than using the modified MS. The number of adventitious roots was higher as compared to the modified MS. Nevertheless, Abul-Soad *et al.* (2007) reported that ¾MS strength was better than the full strength of MS.

***The appropriate time to add AC in the rooting medium*:** Data in Table 3 showed a significant difference between the two doses of AC given after 2 subcultures along with three different sucrose concentrations in MS and MMS media for var. Kashoo wari. The overall average shoots and root development significantly increased by increasing the AC dosage and sucrose concentration in the MS basal medium. After 2 months of culturing the maximum no. of leaves (4) & roots (8) and the average leaf (28.16 cm) and root length (9.12 cm) observed on MS medium supplemented with 3 g/l AC at 40 g/l sucrose concentration followed by 30 & 50 g/l sucrose concentration. The least response was observed on overall average growth development when 1.5 g/l AC with MMS medium used with 30 & 50 g/l sucrose. The minimum number of leaves (2.8) & roots (2) and the average leaf (15.6 cm) and root length (3.7 cm) was noticed on MMS medium supplemented with 1.5 g/l AC at 30 g/l sucrose concentration. However, the minimum number of leaves/plantlet (2.8) was also observed using MS with 1.5 g/l AC at 50 g/l sucrose concentration and MMS medium with 3 g/l AC at 50 g/l sucrose concentration. The minimum root length (2.5 cm) was observed on MMS medium with 1.5 g/l AC at 50 g/l sucrose concentration.

AC is a familiar component to be used in the *in vitro* date palm rooting media but, time of implementation found vital. Although incorporation of the AC in the beginning of rooting stage strongly enhanced the root length and leaves growth; however, reduced the adventitious roots initiation. In current study, two doses of AC were added in the last 2 subcultures to enhance the already initiated adventitious roots and encourage their secondary and tertiary roots formation. Data in Table 3 showed the average of shoot and root length for the interaction between sucrose concentration and AC. In case of using 30 g/l sucrose in the rooting medium, no difference between the two doses of AC 1.5 and 3 g/l was observed. By increasing the sucrose concentration the average shoot and root length significantly increased.

Time of the addition was selected to be after the adventitious root initiation after S2 since the negative impact of AC on the adventitious root number was stated by Abul-Soad *et al*. (2006). However, the AC addition proved important to produce healthy plantlets (Fig. 2). The usual color of the primary root of young somatic embryos and shoots was white or creamy. Sometimes during the proliferation stage, newly formed shoots appeared with green, thin and long roots. Most of the time green roots formation was associated with culturing on a nutrient medium contained AC. The sharp trimming of such green primary roots stimulated the new white adventitious roots formation. The plantlets with green root were able to grow in the greenhouse; however, with no additional adventitious root formation.

Figure 2. Adventitious roots development on charcoal and free charcoal media and development of secondary roots.

***Behavior of the root growth and development*:** It was important to uniform the used explant in current study, i.e. individual shoots to reduce the variability and to reflect consequently the impact of the tested factors. In current study a lot of care was taken to select similar shoots before the experiment. Most of these shoots were with no primary roots. It was observed that removing the initial roots completely or trimming to 1-2 mm enhanced thicker-white adventitious root formation. Leaving the primary roots without trimming during rooting stage inhibited the adventitious roots formation which is important for the further growth in the acclimatization stage.

The root growth behavior showed a systematic development. The growth was started since the new somatic embryo differentiated with intact root tip. These primary roots increased as long as the leaf enlarged. Mostly a single or two roots emerged from the base of a shoot. Rarely, folded roots were observed which after a certain length branched into 2 separate roots. However, major root growth occurred when the primary root was removed or trimmed. Hence, at least an adventitious root has initiated. Also, trimming enhanced the formation process of secondary and tertiary roots on the remainder part of the trimmed roots.

After trimming, the new adventitious roots mostly formed at a point near the cut surface of original trimmed root. But rarely the new roots grew as extension of original trimmed root. Therefore, the remainder terminal part of original root was converted into a structure looked like a theca (Fig. 3). Mostly the process of new roots formation after trimming was similar to the growth and development of date palm roots in open field if faced any mechanical constraints like cut at any point of growing root. It was observed that the trimming stimulated the second and third level of roots formation compared to complete removal of original roots. Moreover, the newly formed secondary and tertiary roots were formed on the remaining part of trimmed root and newly formed roots as well within 1-2 months. On the other side, if the original roots were completely removed, the formation of secondary and tertiary roots (functional roots) took 2-4 months at least.

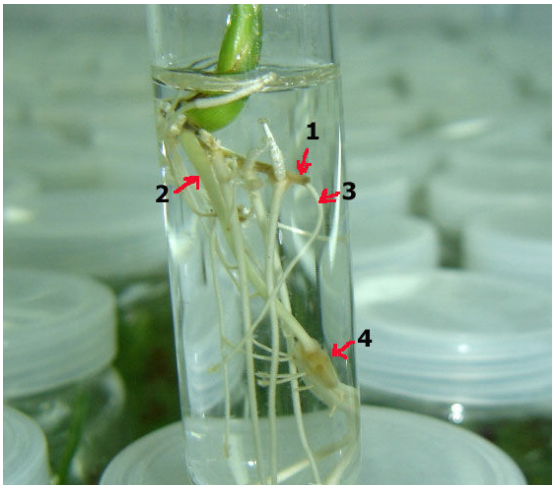

Figure 3. Development of new adventitious roots after trimming and protective theca formation, note the two main trimmed roots (1 and 2), the new root after trimming (3), and theca (4).

It was noticed that plantlets without sub-culturing for a long period of time (3-6 months) the agar medium turned to liquefy that unexpectedly showed a positive impact on the growth of both roots and shoots. It could be because of secreted like-acid substances that lower down the pH and liquefied the agar (Jatoi, 2013). The associated enhancement of the growth could be due to the effect of these substances (lactic acid) which help the plant to grow under anaerobic conditions of long incubation period or enhance mineral uptake by the enough number of produced roots. Also, it could be some exudates from the roots, directly benefited the plantlets. The thickness of the stem has notably increased

that gives firmness to the plantlets during transplanting in the greenhouse.

Emergence of rootless plantlets of var. Gulistan at S1 during rooting stage can be explained as inaccurate and inexperienced handling of those plantlets mostly due to damage of the shoot base. It might be occurred during the young shoot detachment from a cluster of shoots or by the trimming process. Consequently such shoots showed slow or no further development whether to initiate roots or pursue the growth of leaves. Much care should be paid during shoot detachment or root trimming processes to avoid any damage for the shoot base and trimming the root to 1-2 mm.

***Low rate of the ex vitro survived plants in the soil bed*:** Data presented in Table 4 showed significant differences concerning the interaction among type of Basal salts supplemented with 3 different sucrose concentrations and 2 different dosage of AC on survival percentage of tissue culture derived date palm plantlets in greenhouse conditions. Since the date palm plantlets which cultured on MS basal medium containing 40 g/l sucrose and 3 g/l AC during *in vitro* conditions resulted in highest survival percentage (90%). The least response was observed on MS and MMS basal salts supplemented with 30 and 40 g/l Sucrose at AC 1.5 g/l, respectively.

Table 4. The survival percentage (%) of *ex vitro* date palm plants after 3 months in transplanting for the plants coming from the 12 *in vitro* treatments during rooting stage.

Basal Salts	Sucrose Conc. (g/l)	Activated Charcoal Conc. (g/l)	
		1.5	3.0
MS[z]	30	40	70
	40	60	90
	50	50	80
MMS[y]	30	50	50
	40	40	80
	50	50	70

[z]MS (macro & micro salts of MS)
[y]MMS (Macro of B5 (Gamborg *et al*., 1968) & Micro of MS (Murashige and Skoog, 1962)

Rooting quality of the *ex vitro* plantlet of date palm was the vital factor which increased the survival percentage in the greenhouse. Most of the reports indicated low survival percentage (25-35%) during acclimatization stage rather than it used to be a big obstacle in the whole micropropagation protocol (Abul-Soad *et al*., 1999; Hegazy *et al*., 2006). But in current study and based on the utilization of high sugar concentration, AC after adventitious roots formation and proper handling of plantlet, the survival percentage reached more than 90% (Table 4, Fig. 4). The used soil bed was a simple mixture of washed sand and peat-moss (1:1 ratio). It was noticed that when the *in vitro* plantlets left without removal or trimming of original roots the survival percentage decreased to about 70% and those were not able to produce any new adventitious roots from the base of the plant. After 2 months of acclimatization of those plants, the overall growth of the plant was very slow; the thickness of original primary-root did not increase whereas the shoot color remained green. The follow up observation for a year in greenhouse showed a significant difference in growth rate between those plants having the primary root and the plants possessed a few adventitious roots.

Figure 4. Successfully acclimatized a month-old *ex vitro* date palm plants.

Roots formed in the test tubes can be beneficial for enhancing early growth following transfer from the laboratory to the greenhouse. The optimum growth rate of *ex vitro* plantlets frequently did not occur until new leaves and roots developed in the greenhouse environment (Hegazy *et al.,* 2006). Furthermore, the inferior quality of root-shoot system led to extend the required time for early growth of the *ex vitro* plants from 1 up to 3 months. During which the plants will be exposed to be attacked by fungal infection under humid conditions at early phase of the acclimatization stage.

During acclimatization the pulp of the plant (plant base) kept uncovered by the soil bed especially if this soil bed was not sterilized to secure enough aeration for this moisture-sensitive part of the plant. Uncovered base which existed above the soil bed surface has avoided to wide extent the fungal infection and the further plant growth was not affected i.e., higher survivability.

***CONCLUSION*:** The genotype in terms of variety had impact on early root initiation of the young shoots. Furthermore, the morphology of the whole plantlet (shoot and root) was variety dependent response. The difference in rooting among varieties has been confirmed as the root

induction in var. Gulistan occurred after a month of culturing on the rooting medium whereas Gajar and Kashoo Wari varieties were easier and rapid to initiate the new roots after 2-3 weeks. Also, the leaves measurements indicated a difference since var. Gulistan had the lowest number of short but wide leaves as compared to Gajar and Kashoo Wari varieties.

Adventitious roots formation was achieved using nutrient medium involved 40 g/l sucrose and full strength MS basal salts within 4 subcultures and in last 2 subcultures AC was added. The average number of leaves, leaf length and leaf width were improved and reached up to 3-4 leaves/plantlet, their length was 18-24 cm and leaf width 3.5-6.9 mm. Moreover, most of the plantlets were able to produce adventitious roots 4-6 roots/plantlet, which extended up to 6-8 cm with thickness 1.3-1.4 mm. Few plantlets failed to initiate roots. Thus much care should be paid during shoot detachment or root trimming processes to avoid any damage for the shoot base and trimming the root to 1-2 mm to encourage further adventitious root formation. Eventually, enhancing rooting in *in vitro* plantlets can increase the survival percentage of the *ex vitro* plantlets (90-95%) in the greenhouse.

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