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ESTABLISHMENT OF AN EFFICIENT PROTOCOL FOR ROOTING AND ACCLIMATIZATION OF TWO QATARI DATE PALM CULTIVARS SHISHI AND LULU

*Rehab Sidky; Muna AL-Salahi, Amel Al-Mahmoud
Agriculture Research Department, Ministry of Municipality and Environment. Qatar
*Corresponding author email: sidky1234rehab@hotmail.com

ABSTRACT
This study aimed to establish a new protocol for *in vitro* rooting and acclimatization of date palm (*Phoenix dactylifera* L.) in different cultivars "Shishi and Lulu" using natural substances compared with growth regulators. In order to increase growth, achieve a higher survival rate of plantlets through the acclimatization stage with reduce costs of production plants. The plantlets of the two cultivars were cultured in media in three successive steps (each of 6 weeks). In First and second steps kinetin and malt extract were tested to optimize the quality of plantlets in the presence of α-Naphthalene acetic acid. Third step were cultured plantlets onto liquid media with polyethylene glycol. The obtained results showed that, plantlets grown on malt extract at 750 mg/l had wider and greener leaves 0.81, 0.74 mm and encouraged the adventitious roots number per plantlet 6.88, 6.10 in Shishi and lulu cultivars. Also results demonstrated that the new established protocol is efficient in acclamate date palm. The highest survival rate was 98.66, 87.66 % of two cultivars at the acclimatization stage within a short period (3 month).

KEY WORD: Date palm, Malt extract**,** acclimatization, rooting, Kinetin, *in vitro.*

INTRODUCTION

Plant tissue and organs are grown in *vitro* on artificial media, which supply the nutrients necessary for growth .The success of plant tissue cultures as means of plant propagation is greatly turned in flounced by the nature of culture medium (Edwin *et al*., 2008). Rooting is an important *in vitro* stage of a micropropagation protocol of date palm. Therefore, transplanting of the *in vitro* date palm plantlets depends mainly on *in vitro* good roots and trunk thickness. The well-rooted plantlets may be shifted into the greenhouse through an intervening *in vitro* hardening step (Abul-Soad *et al*., 1999).

For produce a good root-shoot system some factors such as explants source, genotype, plant nutrition and tissue growth greatly modified the regulation of shoot formation. Since the discovery of auxin/ cytokinin responses in plant cell culture system, many additional compounds have been discovered that influence culture growth and morphogenesis. It has been reported that the success of many *in vitro* techniques in higher plants depend on the success of plant regeneration which usually control by auxin and cytokinen concentration (Flick *et al.*, 1983a).

Many undefined supplements were employed in early tissue culture media. Their use has slowly declined as the balance between inorganic salts has been improved, and as the effect of amino acids and growth substances has become better understood (Edwin *et al*., 2008).

Employing natural substances have beneficial effects on *in vitro* plant cell and tissue cultures (Molnár et *al.*, 2011). Malt extract, mainly a source of carbohydrates, was shown to initiate embryogenesis in nucellar explants (Rangan *et al*., 1968; Rangan, 1984). Some plant hormones, such as auxins and gibberellins have been identified in malt extract (Dix and Van Staden, 1982).

Micropropagated plants on being transferred to *ex vitro* conditions are exposed to (altered temperature, light intensity and water stress) conditions so need acclimatization for successful establishment and survival of plantlets (Chandra *et al*., 2010), also it is possible to acclimatize plantlets during *in vitro* as well as *in vivo* by various methods and thereby facilitating the successful transfer of *in vitro* cultured plantlets to soil (Kumar and Rao 2012).

The aims of this study is reducing the use of growth regulators in culture media as well as reduce costs of production plants, simultaneously with enhancement overall growth and development. Moreover to initiate ideally roots of the young plantlets in shorter time and subsequently obtain successful protocol for acclimatize plantlets with high rate of survival.

MATERIALS & METHODS

This work was carried out at Agriculture Research Department, Plant Tissue Culture Laboratory, Doha, Qatar. In this paper, three steps were taken to produce a good root-shoot system, the *in vitro* individual shoots without roots of two different varieties Shishi and Lulu were cultured on the basal medium composed:

First step: ¾MS (Murashige and Skoog, 1962) salt-strength medium supplemented with 60 g/l sucrose, 1 mg/l NAA. Also 100 mg/l myo-inositol, 170 mg/l potassium dihydrogen phosphate (KH_2PO_4), 3.0 mg/l Ca-panthothianate with two substance kinetin (kin) at 0.3 mg/l or malt extract at 750 mg/l and solidified with 0.6 % agar to enhance the root system and leaf formation.

Second step: ¾ MS salt-strength medium supplemented with 50 g /l sucrose, 1 mg/l NAA, 2mg/l IBA. Also 100 mg/l myo-inositol, 170 mg/l potassium dihydrogen phosphate (KH_2PO_4), 3.0 mg/l Ca-panthothianate, 0.5 g/l Activated Charcoal(A.C) with two substance kinetin (kin) at 0.3 mg/l or malt extract at 750 mg/l and solidified with 0.6 % agar, to improve root formation and adventitious root. In the first and second steps, individual shoots were cultured at small tubes (25 × 150) mm for 6 weeks.

Third step: ½MS salt-strength liquid medium supplemented with 30 g /l sucrose 8 g/l polyethylene glycol (PEG). Also 100 mg/l myo-inositol, 170 mg/l potassium dihydrogen phosphate (KH_2PO_4), 3.0 mg/l Ca-panthothianate, without agar. Plantlets were cultured at large tubes (28 × 250 mm) for 6 weeks.

The pH was adjusted to 5.8 for solid media and 5.2 for liquid media prior to autoclaving at 121 for 20 min and 103 kPa. The cultures were maintained at 25 ±2 ºC and 75 % relative humidity under cool fluorescent light at 6000 lux.

Plant acclimatization

Handling the *in vitro* plantlets into the greenhouse as procedure was followed:

Plantlets were removed from the culture medium; the root system was washed in running water to remove all adhering medium, and then soaked for15 min in a solution of 2g/L systemic fungicide (Ridomil). Plantlets were cultured is in pots (5 x 18 cm) filled with soil containing a mixture of peat-moss, vermiculite (2:1 v/v). During initial 90 days of hardening, high humidity was maintained by covering the plants with acclimatization hood of polyethene plastic into greenhouse and watered with mist every two hours 3 seconds. Acclimatized by slow and gradual exposure from high (90–95%) relative humidity (RH) and low (24–26 °C) temperature to low (40–50%) RH and high (30–32 °C) temperature by gradually opened the plastic hood of polyethene after four to six weeks. The plantlets were watered after a month with fertilization 1 g/l N-P-K (17-17-17). Remove plastic tunnel after the first leaf appeared, the survival percentage, plant length, number of roots and root length were recorded after 3 months. Each treatment consisted of 36 plants (3 replicates of 12 plants).

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

Effect of different media on rooting and plantlets growth

The morphogenetic responses of plantlets cultures to various media are shown in Table 1 and Fig. 1a, b. In terms of leaf width, increase in leaf size appears to be an important factor for plant's overall growth which develops strength in the plantlets to withstand the septic conditions of outside environment, in our result plantlets grown on malt extract at 750 mg/l had wider and greener leaves than those on medium containing kin at 0.3 mg/l after 18 weeks of culture, the optimum width of leaf was (0.81mm). Moreover the highest number of roots were obtained when they were cultured in the presence of 750 mg/l malt extract, the number of adventitious roots per plantlet was higher and thick (6.88) (Fig.1b).

TABLE 1. Effect of different treatments on *in vitro* rooting of date palm plantlets after 18 weeks of date palm cultivar Shishi in three steps.

	Average			
Treatments mg/l	Leaf width (mm)	No. of roots	Root length (cm)	Trunk thickness (mm)
0.3 kin	0.78	4.80	6.40	8.77
750 malt extract	0.81	6.88	5.88	6.38
L.S.D at 0.05	0.62	2.48	2.76	7.70

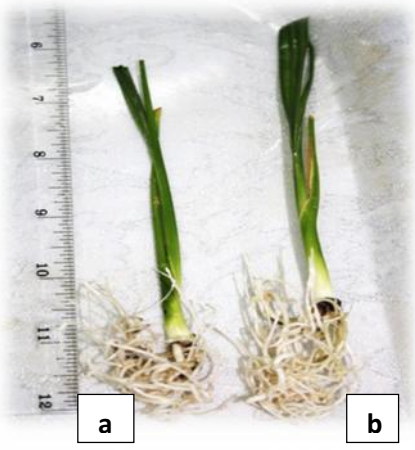

FIGURE 1: plantlets from different treatments showing differences in their development with new development of adventitious root a) 0.3 mg/l kin, b) 750 mg/l malt extract

Roots cultured on media supplemented with cytokinin showed faster growth in root length and trunk thickness. The heights of root length (6.40 cm) were observed on media contacting 0.3 mg/l kin. As well as thickness of trunk is strongly by adding 0.3 mg/l kin to rooting medium (8.77).

TABLE 2. Effect of different treatments on *in vitro* rooting of date palm plantlets after 18 weeks of date palm cultivar Lulu in three steps.

	Average			
Treatments mg/l	Leaf width (mm)	No. of roots	Root length (cm)	Trunk thickness (mm)
0.3 kin	0.68	5.44	6.16	7.57
750 malt extract	0.74	6.10	5.32	5.47
L.S.D at 0.05	0.54	4.61	2.89	7.32

The results of *in vitro* rooting are shown in Table 2. The statistical results indicated that addition of malt extract improved the general health and growth of the leaves; leaves were more increased size and width (0.74 mm) at 750 mg/l malt extract than medium containing kin at 0.3. The maximum numbers of roots per plantlet was 6.10 grown on MS medium supplemented with 750 mg/l malt extract. Generally, plantlets developed on malt extract media were greener than media with kin.

Furthermore, it was found that the root length occurred on a medium supplemented with 0.3 mg/l kin (6.16 cm), successful transplanting of the *in vitro* date palm plantlets depends mainly on the *in vitro* trunk thickness, the strongly of trunk thickness was obtained on media contacting 0.3 mg/l kin (7.57 mm). Generally, shoots developed on media supplemented with kin were thicker, and more vigorous.

TABLE 3. Plants after 3 months in greenhouse development in response to different treatments of date palm cultivar shishi

	Average			
Treatments mg/l	Survival percentage (%)	Plant length(cm)	No. of roots	Root length (cm)
0.3 kin	87.66	28.60	2.61	24.53
750 malt extract	87.33	29.26	4.81	21.53
L.S.D at 0.05	1.30	13.23	1.12	5.56

Plant acclimatization

The plantlets obtained from different treatments were transferred to the greenhouse for acclimatization, and their parameters were assessed after 3 months (Fig. 2a and b). The plantlets showed different sign, depending on which medium they were derived from. Data in Table 3. showed that, plantlets are developed on a medium containing 0.3 mg/l kin gave the highest rate of survival percentage (87.66%) without significant difference with medium containing 750 malt extract (87.33%) as well as increasing root length, the highest root length was 24.53cm at 0.3 mg/l kin while in media containing 750 mg/l malt extract was 21.53 cm.

In terms of Plant length, the highest Plant length (29.26 cm) was observed when medium was supplemented with750 mg/l malt extract; this value was not significantly different from those obtained with media containing kin (28.60 cm).

There was a significant difference among two treatments in number of roots; culturing plantlets in media containing750 mg/l malt extract significantly increased the number of formed roots with an average of 4.81per plantlets.

TABLE 4. Plants after 3 months in greenhouse development in response to different treatments of date palm cultivar Lulu.

	Average			
Treatments (mg/l)	Survival percentage (%)	Plant length(cm)	No. of roots	Root length (cm)
0.3 kin	98.66	23.30	4.86	23.94
750 malt extract	96.33	28.99	5.16	20.10
L.S.D at 0.05	2.06	1.96	1.00	9.17

Our findings showed that in Table 4. The survival rate was significant in plants that had been cultured on medium supplemented with 0.3 mg/l kin before acclimatization; the highest survival rate was 98.66 % in Table 4. As well as, root length, plantlets grown on media containing 0.3 mg/l kin before acclimatization had higher root length than those on media containing malt extract. The highest root length was (23.94 cm).

Excellent growth with larger leaves green and health with an average of 28.99 cm plant length was observed on media supplemented with 750 mg/l malt extract. Furthermore, the average number of roots was always greater in media containing malt extract compared with media containing kin. This number increased at 750 mg/l malt extract (5.16).

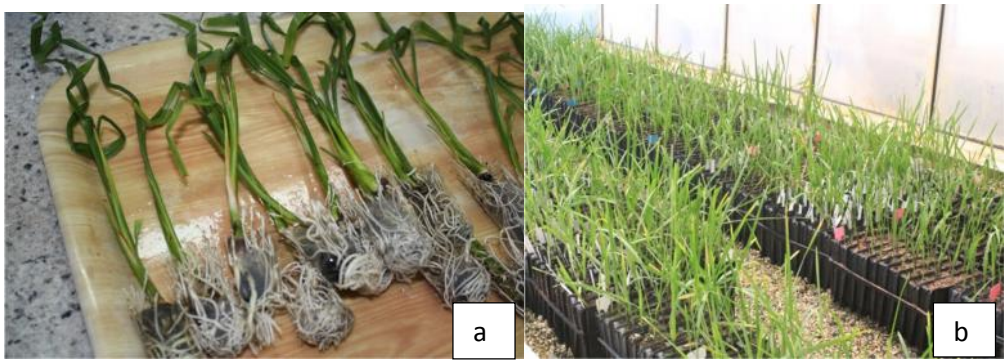

FIGURE 2a) Plantlets before acclimatization, sample of shoots from treatments b) Plantlets after 3 months in the greenhouse.

DISCUSSION

The present work showed that the use of natural substances such as malt extract or use cytokinins was beneficial *in vitro* rooting stage , malt extract played roles in increasing root formation and leaf width while cytokinin improve root length and trunk thickness. The addition of complex mixtures to the culture medium, such as coconut water, malt extract, and banana juice, has been successfully used for different species since the beginnings of tissue culturing (Loewenber and Skoog, 1952). As well as malt extract promoted germination of early cotyledonary stage embryos arising from *in vitro* rescue of zygotic embryos of sour orange (Carimi *et al*., 1998), in the promotion of plantlet formation from somatic embryos derived from styles of different Citrus cultivars (De Pasquale *et al*., 1994). In our study, Plantlets were cultured on media supplemented with750 mg/l malt extract greener and wider leaves.

In this study cytokines were used for improve rooting and acclimatization stage. Plantlets were affected by cytokine (kin), all Plantlets will culture on media supplemented with 0.3 mg/l kin in the presence of NAA had strongly, thicker and increase of root length. This was agree with (Gana, 2011) cytokines usually promote cell division, especially when they are added with an auxin. Shoot elongation requires transfer of shoots from the multiplication medium to another medium with a high auxin/cytokinin ratio (Beauchesne *et al*., 1986; Loutfi and Chlyah, 1998). A combination of NAA (1mg/l), BA (0.5 mg/l) and Kin (0.5 mg/l) enhances shoot growth and elongation. Some authors have suggested the use of media supplemented with PGRs for shoot elongation and rooting (Sidky *et al*. 2007; Othmani *et al*., 2009), while others recommended PGR-free media (Mazri and Meziani, 2013). The frequency of plantlets in the glasshouse was higher in all parameters that had been cultured on media containing the previous steps in materials. Consequently, It must be gradual steps from media containing hormone to media without and it turns out the importance of the pre-acclimatization in the laboratory with free-hormone (third step), the survival percentage reached to 98.66%. This confirm with Sane *et al.* (2006) reported that the transition of shoots in a PGR-free medium led to the formation of branched roots that showed poor survival after transfer 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 (Abul-Soad and Jatoi, 2014). The nature of the substrate plays an important role in the success of acclimatization. Hegazy and Aboshama (2010) tested the survival of the cultivar Medjool on four different substrates (1:1 composts and, peat, perlite, or vermiculite).

CONCLUSIONS

The results obtained in this study indicate that, plantlets were cultured in the presence of malt extract gave the best effect as well as it is lowest price, which leads to lower production costs. We recommended to use malt extract and kin together it will be became more effective to improve plantlets before acclimatization stage than either of these substances are used alone, also the protocol reported here is useful to obtain the highest survival rate in greenhouse.

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