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Thidiazuron-induced *in vitro* bud organogenesis of the date palm (*Phoenix dactylifera* L.) CV. Hillawi

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The objective of the present was to enhance the frequency of plant regeneration in date palm (*Phoenix dactylifera* L.) cv. Hillawi. Explants were incubated on Murashige and Skoog (MS) medium supplemented with 1 mgl^{-1} 6-benzyladenine (BA) and different concentrations (0.1 to 2.0 mgl^{-1}) of thidiazuran (TDZ), or free of BA and TDZ (control treatment). The results indicate that the Maximum response (66.67%) was observed on medium supplemented with 1.0 mgl^{-1} BA and 0.5 mgl^{-1} TDZ, producing an average of 4.2 and 18.2 buds per culture after 16 and 24 week from culture, respectively. TDZ at concentrations higher than 0.5 mgl^{-1} resulted in suppressed buds formation, where a decrease in the number of buds was noticed when the concentration of TDZ was increased from 0.5 to 2 mgl^{-1}. Regarding the activity of antioxidant enzyme peroxidase during budding of date palm cv. Hillawi, (The chemical analyses of peroxidase compounds were spectrophotometrically performed) the applied concentration of 0.5 mgl^{-1} TDZ with 1 mgl^{-1} BA enhanced peroxidase activity, where peroxidase activity was associated with increased number of buds formation. Histological studies revealed that adventitious buds were formed directly from epidermal cells without callus formation, and adventitious buds were developed from meristematic cells in shoot tip tissues. Shoots were elongated on 0.5 mgl^{-1} GA3+ 0.1 mgl^{-1} NAA MS media and rooted on MS media supplemented with 0.2 mgl^{-1} of α-naphthaleneacetic acid (NAA). Rooted shoots were successfully acclimatized and established in a mixture of peat moss and perlite (2:1) with 80% success.

Key words: Date palm, thidiazuran (TDZ), adventitious buds, peroxidase (POD), histology.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) the dioecious, monocotyledon species belonging to the family Arecaceae is a multipurpose tree having food, medicinal and ornamental importance. With the present uncertainty in the world food supply and the expected increase in demand, the date palm could be a good source of food of high nutriational value (Khan and Bi, 2012). It has long been one of the most important fruit crops in the arid regions of the Arabian Peninsula, North Africa, and the Middle East (Chao, 2007). Iraq was one of the top ten date producers

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producers in the world between 1991 and 2001, where it contributed a 7.5% of the total world dates production (FAO, 2011). The vegetative multiplication of date palm is traditionally achieved by offshoots. This offshoot propagation has limitations such as slow propagation rate, transmission of disease-causing pathogens and insects and production of offshoots in a limited number for a certain period in the lifetime of a young palm tree (Gueye et al., 2009). Date palm readily grows from seeds but half of the seedlings may turn out to be males and high proportion of inferior quality segregates (Al-Khalifah and Shanavaskhan, 2012; Mohammad, 2013). Furthermore, seedlings take 6 to 10 years to fruit, so male and female trees are not identifiable until flowering (Othmani et al., 2009). Hence, *in vitro* propagation is the only available alternative to produce disease free, uniform and good quality planting material to establish large scale cultivation within a short period of time (Zaid and De- Wet, 2002). *In vitro* production is applied through two main protocol, one of them is the somatic embryogenesis and the second is via meristim apexes or buds in the axil bottom of the leaves (Bekheet et al., 2001; Eke et al., 2005). Micropropagation through direct organogenesis lacking callus phase, has the advantage of producing highly identical plants to the mother plants in their vegetative characteristics (Khan and Bi, 2012). Shoot tips are most appropriate explant used for date palm *in vitro* multiplication (Al-Mayahi, 2013). Thidiazuron (TDZ) is a cytokinin-like substance that has often been used for shoot regeneration in recent years (Erisen et al., 2011). Also, it is a powerful regulator of *in vitro* plant regeneration and subsequent growth (Murthy et al., 1998). There are many reports showing that the application of thidiazuron (TDZ; N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) results in a better shoot regeneration capacity in comparison with other cytokinins (Srikandarajah et al., 2001; Zhang et al., 2001; Thomas, 2003; Husain et al., 2007). TDZ is used as a plant growth regulator to stimulate high rate of axillary shoot proliferation in many woody plant species, and its releases the lateral bud dormancy and stimulates shoot formation in wide variety of plant species (Malik and Saxena, 1992; Anandan et al., 2011). In date palm, N-phenyl N'-1,2,3-thidiazol-5-ylurea (TDZ) is used in the stimulation of direct somatic embryo regeneration from shoot tip explants (Sidky and Zaid, 2011). The plant cells possess highly efficient defence systems for elimination of the harmful effect of oxidative stress. Guaiacol- peroxidase (EC 1.11.1.7), catalase (EC 1.11.1.6) and ascorbate-peroxidase (EC 1.11.1.11) are among enzymes expressing antioxidative functions, where peroxidase (EC 1.11.1.7) is considered among enzymes expressing antioxidative functions (Kapchina-Toteva and Yakimova, 1997). There are still limited data concerning plant regeneration using thidiazuron (TDZ), and the peroxidase activity for date palm is through direct budding. The overall objectives of this study were to determine the optimal concentration of TDZ for adventitious buds regeneration from shoot tip and investigate the relationship between TDZ and peroxidase through budding in date palm tissues cultured cv. Hillawi *in vitro*. Cultivar Hillawi is a good soft date with good quality and famous in Iraq. It early ripens early and yields about 94.3 kg/palm and it is best for raw eating at rutab stage (Vij et al., 2005). But this cultivar is suffers from some problems; the ageing of the adult plants as well as study the histological events related to direct regeneration from shoot tips.

MATERIALS AND METHODS

Plant material

The experiments were conducted in the Laboratory of Plant Tissue Culture at the Palms and Dates Research Centre, University of Basra. The success of tissue culture largely relies on the selection of suitable explants for use as the starting material for the experiment. The selected offshoots cv. Hillawi were 3 to 4 years old.

Cleaning of explants

Cleaning of the explants was done according to Junaid and Khan (2009). In short, the offshoots were washed with tap water to remove the attached soil and other debris. The outer large leaves and fibers were carefully removed with a sharp knife until the shoot tip zone was exposed.

Sterilization efficient

The explants were kept in 1% sodium hypochlorite solution mixed with one drop/100 ml of Tween-20 for 20 min followed by 4 to 5 rinses in distilled water. It is recommended to give sterilization for 10 min with fresh sodium hypochlorite solution. Then, the explants were immersed in 0.1% mercuric chloride solution for 5 min, followed by 4 to 5 washes in distilled water. Sterilized explants were kept in a cold sterilized solution of ascorbic and citric acid (150 mgl^{-1}) to avoid browning (Al-Khalifah and Shanavaskhan, 2012).

Establishment of initial cultures

In this study, shoot tips were used for culture initiation (Figure 1). The shoot tip terminal, about 1 to 1.5 cm long, was sectioned longitudinally into four sections. Several media were tested. Murashige and Skoog (MS) (1962) basal medium supplemented with 1.0 mgl^{-1} NAA, 1.0 mgl^{-1} Naphthoxy acetic acid (NOA), 1.0 Indol butyric acid (IBA), 1.0 mgl^{-1} kinetin (K), 100 mgl^{-1} glutamine, 5 mgl^{-1} thiamine HCl, 1 mgl^{-1} biotin, 30 gl-1 sucrose, 2.0 gl^{-1} activated charcoal, and solidified with agar at 5.0 mgl^{-1} were used. All the media were adjusted to pH 5.8 with 0.1 N NaOH or HCl, before the addition of agar. Media were dispensed into culture jars. All jars with media were autoclaved at 121°C and 1.04 kg/cm² for 15 min. Cultures were kept under complete darkness at 27±2°C which provide the cultivation on initiation medium that enhanced the percentage of explant survival which was 60%, where explants started exhibiting signs expansion after 3 to 4 weeks, as well as reduced browning. Similar reports are given by (Al-Maarri and Al-Ghamdi, 1997; Al-Mayahi, 2014a).

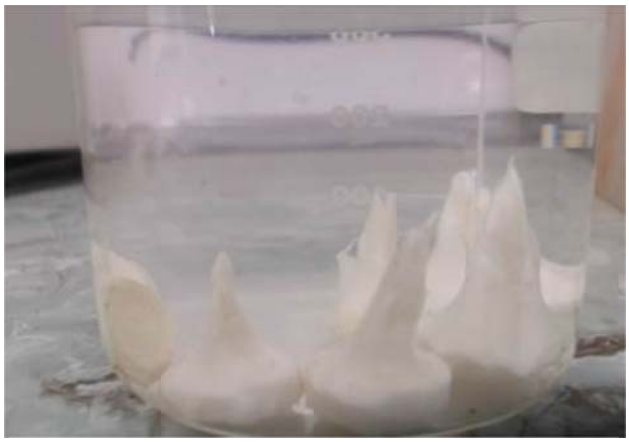

Figure 1. Apical buds used in this study.

Figure 3B. Rooted plantlets ready for transplanting to plastic pots.

Figure 2. Subcultured buds on to fresh media.

Figure 3A. Shoots formed on the elongation medium.

Buds initiation and multiplication

The developing explants were shifted on initiation and multiplication media (MS) containing 1.0 mgl^{-1} BA and Thidiazuron TDZ at different concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 mgl^{-1}), or used as free of 6-benzylaminopurine (BA) and TDZ (control treatment). All the cultures were incubated in a culture room maintained at 27 ± 2°C under 16/8 h, and light/dark with 55 to 60% relative humidity (RH). Every treatment in the experiment was replicated 6 times. Buds formed on this medium were divided and subcultured on to fresh media every 6 weeks until obtaining enough number from buds to complement this study (Figure 2). The data was recorded as follows: The percentage of response of cultures on direct bud formation after 16 week; number of direct buds/explant after 16 and 24 weeks.

Shoot elongation, rooting and acclimatization of date palm plantlets

Developed buds were isolated and transferred to the elongation medium supplemented with 0.5 mgl^{-1} Gibberellic Acid GA3+ 0.1 mgl^{-1} α-Naphthalene acetic acid (NAA). Well-developed shoots (5 cm long) (Figure 3A) were separated from each other, and transferred individually to rooting medium supplemented with NAA (0.2 mg^{-1}) (Al-Maarri and Al-Ghamdi, 1997). The rooted plants were gently removed from the vessels, washed initially to remove adhered agar and traces of the medium to avoid contamination (Figure 3B). Then, the plantlets were washed with distilled water and treated with fungicide (Benlet 500 mg^{-1}) for 20 min and transferred to plastic pots containing autoclaved a mixture of peat moss and perlite (2:1) (AL-Mayahi, 2014b). The plants were covered with glass bottles to maintain humidity (Figure 3C). The plants were initially irrigated with quarter-strength inorganic salts of MS medium for 2 week followed by tap water. Potted plantlets were grown in culture room (25±2°C, 55±5% RH, under 16 h of photoperiod with a light intensity of 40 µmol m^{-2} s^{-1}) for 45 to 60 days. The glass bottles were gradually removed upon emergence of new leaves and acclimatized plantlets were transferred to the greenhouse.

Estimation of peroxidase at a budding stage

To extract the enzyme, 1.0 g of homogenized buds tissues were ground in 20 ml. cold distilled water in a mortar at 0°C. The extract was obtained by filtering off the debris with a clean cloth and centrifuging at 3000 rpm for 15 min in a refrigerated centrifuge. The

Figure 3C. Coverage of plant by glass bottle.

Table 1. Effect of different concentrations of TDZ in combination with 1.0 BA on percentage of cultures responding and number of buds for date palm cv. Hillawi.

Treatment (mgl^{-1})	Percentage of explants responding (After 16 weeks)	Average number of Buds / explants (S.E)*	
		After 16 weeks	After 24 weeks
0	0.0±0.0^{e}	0.0±0.0^{e}**	0.0±0.0^{e}
0.1 TDZ 1 BA+	33.34±2.06^{c}	2.0±0.2^{c}	0.29 c± 4.0
0.5 TDZ 1 BA+	66.67±1.11^{a}	4.2±0.14^{a}	1.2a±18.2
1.0 TDZ 1 BA+	50.0±1.05^{b}	2.8 ±0.7^{b}	0.14b±7.2
1.5 TDZ 1 BA+	33.34±2.06^{c}	1.5±0.4^{cd}	0.75 d±2.6
2.0 TDZ 1 BA+	16.67 ±0.0^{d}	1.0 ±0.0^{d}	0.0 d±2.0

* ± Standard error (n = 6). ** Values followed by the same letter are not significantly different at P<0.05.

supernatants were recovered and kept in a tube in an ice bath until assayed. POD activity was assayed spectrophotometrically Model CECEIL CE-2021 at 470 nm using guaiacol as a phenolic substrate with hydrogen peroxide (Díaz et al., 2001). The reaction mixture contained 0.15 mL of 4% (v/v) guaiacol, 0.15 mL of 1% (v/v) H_2O_2, 2.66 mL of 0.1 M phosphate buffer pH 7 and 40 µL of the enzyme extract. The blank sample contained the same mixture solution without the enzyme extract.

Histological analysis

Histological examinations during bud formation were carried out using a freezing microtome. Microtome slide preparation and observation were made following the methods as described by Sarker and Awal (1999)

Experimental design and statistical analysis

Completely randomized design was used. The data was subjected to the analysis of variance and mean values were compared using revised LSD at 5% (Snedecor and Cochran, 1989).

RESULTS

Buds initiation and multiplication

The adventitious buds developed from shoot tips on MS medium supplemented with a combination of TDZ and BA after 4 to 6 months of culture without basal callusing (Table 1). The optimal response percentage of explants producing buds with the highest number of buds per explant was recorded on MS medium supplemented with 1 mgl^{-1} BA+ 0.5 mgl^{-1} TDZ. On this medium, 66.67% of the cultures responded (Figure 4A) with an average of 4.2±0.14 and 18.2±1.2 buds per explant after 16 and 24 week, respectively (Table 1, Figures 4A and B), which was statistically significant compared with the other treatments, followed by 1 mgl^{-1} BA + 1.0 mgl^{-1} TDZ (Figure 4C). The response percentage of explants producing buds and frequency of direct bud regeneration decreased significantly when the concentration of TDZ was increased over 0.5 mgl^{-1}, whereas TDZ at higher concentra-

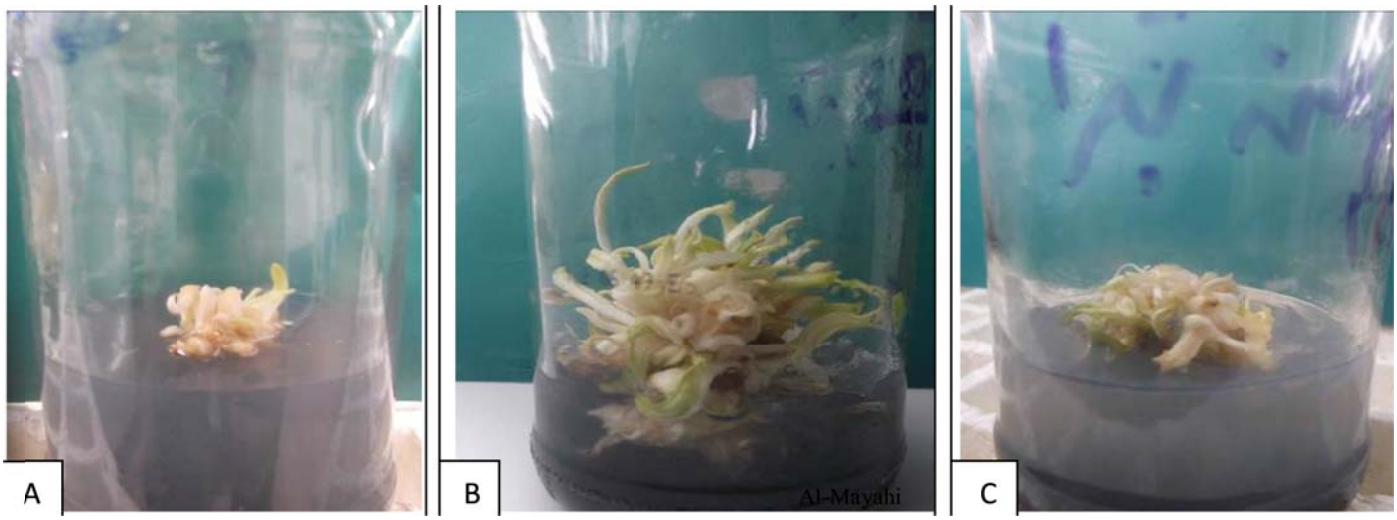

Figure 4. Bud proliferation and multiplication **A)** Bud induction from shoot tip on MS media supplemented with 0.5 mgl^{-1} TDZ + 1.0 mgl^{-1} BA after 16 weeks. **B** and **C)** Bud proliferation on 0.5 mgl^{-1} TDZ + 1.0 mgl^{-1} BA and 1.0 mgl^{-1} TDZ +1.0 mgl^{-1} BA media after 24 weeks, respectively.

Table 2. Effect of different concentrations of TDZ in combination with 1.0 BA on Activity of guaiacol-peroxidase through direct budding in *in vitro* cultured date palm cv. Hillawi.

Treatment (mgl^{-1})	POD activity (U/ml)
0	14.748[d]
0.1 TDZ 1 BA+	19.794[c]
0.5 TDZ 1 BA+	33.159[a]
1.0 TDZ 1 BA+	24.166[b]
1.5 TDZ 1 BA+	21.039[c]
2.0 TDZ 1 BA+	16.899[d]

* Values followed by the same letter are not significantly different at P<0.05.

tions, specially 1.5 and 2.0 mgl^{-1}, resulted in suppressed buds formation. In the absence of BA and TDZ (control treatment) there was not any response for direct buds formation, implying that these compounds are critical for bud regeneration in date palm cv. Hillawi.

Peroxidase "POD" activity

On the basis of the obtained results in the present study, Table 2, illustrates a measure of the activity of guaiacol-peroxidase "POD" regarding the effect of TDZ on bud development of *in vitro* cultured date palm cv. Hillawi. Peroxidase activity in date palm buds was stimulated by TDZ with BA. Thus, cytokinins increase peroxidase activity whereas, lowest activity was observed in control buds. In buds grown on medium containing 1 mgl^{-1} BA+ 2.0 mgl^{-1} TDZ, the activity of peroxidase did not differ significantly from control buds. More increase was exhibited in buds cultured on medium supplemented with 1 mgl^{-1} BA and 0.5 mgl^{-1} TDZ, where activity of peroxidase differed significantly compared with the other treatments.

Histological origin of adventitious budding

The anatomy of buds regenerated *in vitro* by direct organogenesis from shoot tips was investigated in date palm cv. Hillawi. The repeated cultivation on bud-forming medium in the presence of cytokinin, produced tissue masses which rapidly propagated and divided, and can always produce new buds which grow or appear at the surface of the masses or inside them. Histological sections showed that the epidermal cells were the source of organogenesis. The structure exhibited large cells not uniform in size and compactness. Also, there were scattered cell clusters near the epidermis. These cells were distinguished by their fineness and compactness, which was considered as the reason of the protrusions' formation, and consequently the formation of the promeristematic tissue. Simultaneously, with the differentiation of epidermal cells and the subsequent adventitious buds formation, meristematic cells (MC) became more and more abundant due to the continuing division in the shoot tip tissue (Figure 5A). Cell differentiation became evident through appearance of large nucleus undergoing division containing dense cytoplasm. The meristematic isolates emerged from large and strongly vacuolated parenchymatous cells. There meristematic zones could develop into meristems (Figure 5B). From the MC cells, apical meristem (AM) and leaf primordia (LP) differentiated under the same conditions in culture (Figure 5C). Several meristematic regions differentiated and these were

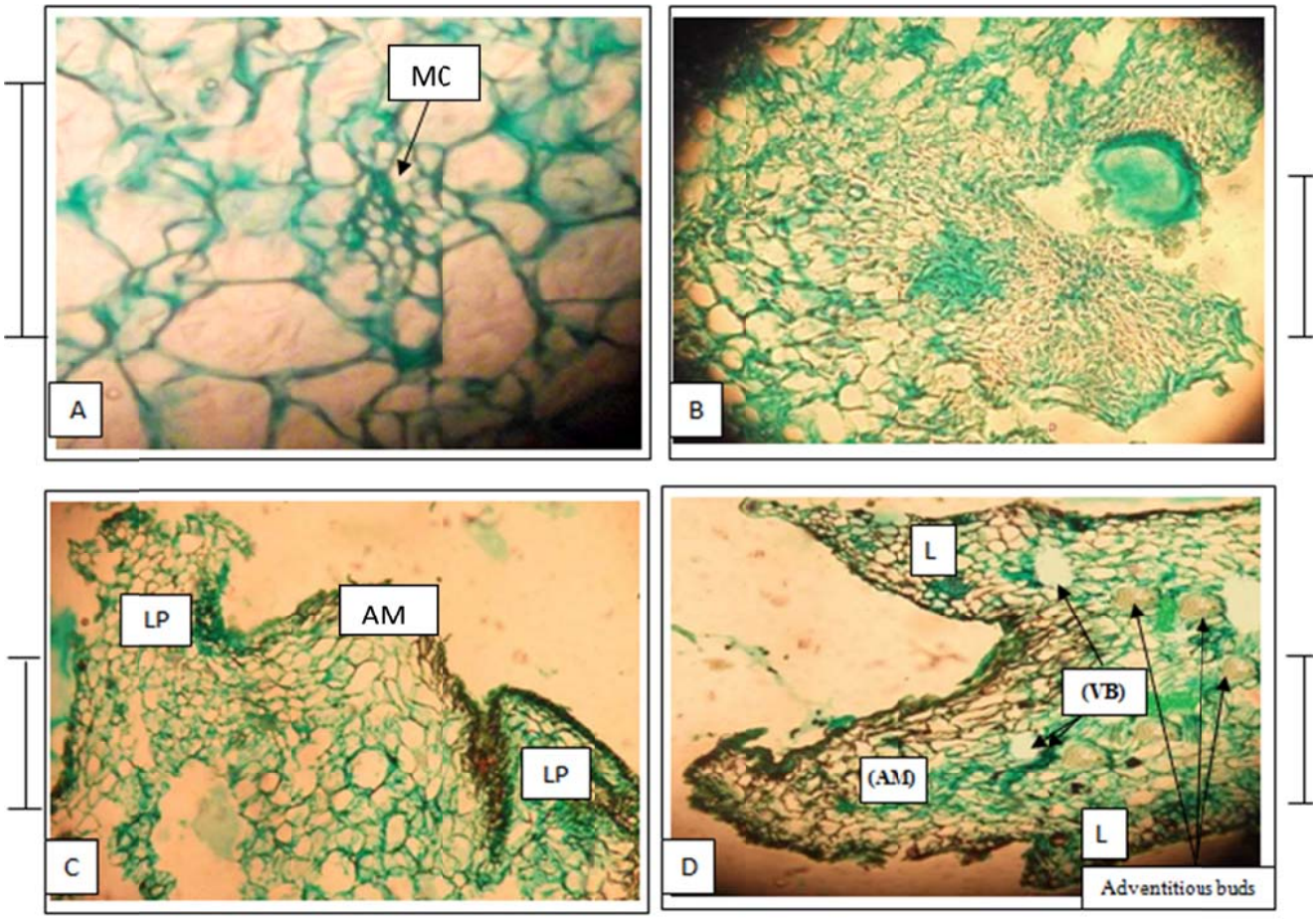

Figure 5. Transverse histological section of an adventitious bud developed from shoot tip of date palm cv. Hillawi cultured on MS + 0.5 mg/l TDZ + 1 mg/l BA. **A)** Mristematic center (MC) originating from the shoot tip, consists of very fine active cells, scale bar 100 µm. **B)** Formation of meristematic which constituted by small cells, scale bar 50 µm. **C)** Apical meristem (AM) and leaf primordia (LP), scale bar 50 µm. **D)** Adventitious buds with apical meristem (AM), leaves (L) and vascular bundles (VB), scale bar 50 µm.

responsible for the formation of adventitious buds. Continuity of cells division led to vascular bundles which can be either induced to form other independent nodules, or differentiating into a bud. It is worth mentioning the adventitious buds induction process showed that it emerged successively from basal superficial of node. Initially, small bulges raised from the epidermal cell of the node (Figure 5D). Also, the nodules developed into buds when moved to an auxin free medium. Such nodules developed into distinct buds or leaf primordium. Figure 5D shows well developed buds with apical meristem, leaves (L) and vascular bundles (VB).

Shoot elongation, rooting and acclimatization of date palm plantlets

The budding tissues formed in the second step, were transferred to the elongation medium supplemented with 0.5 mgl^{-1} GA3 + 0.1 mgl^{-1} NAA. After 8 to 10 weeks, the buds were found from shoots (Figure 6A). The regenerated shoots were transferred to rooting medium MS + 0.2 mgl-1 NAA (Figure 6B), and rooted successfully (80%) with rapid elongation, with an average of 4.4 roots per shoot and shoot of an average root length of 5.2 cm. After 6 weeks of culture (Figure 6C). Complete plants were obtained 6 to 8 weeks after the regenerated plantlets were transferred to this medium. The rooted plants were acclimatized successfully in a mixture of peat moss and perlite (2:1) with 80% after 10 weeks of transferred to plastic pots (Figure 7). All the micropropagated plants were free from external defects.

DISCUSSION

Direct regeneration is the useful means of production of plantlets with a lower risk of genetic instability than by other routes (Khan and Bi, 2012). The composition of induction media is important for adventitious buds

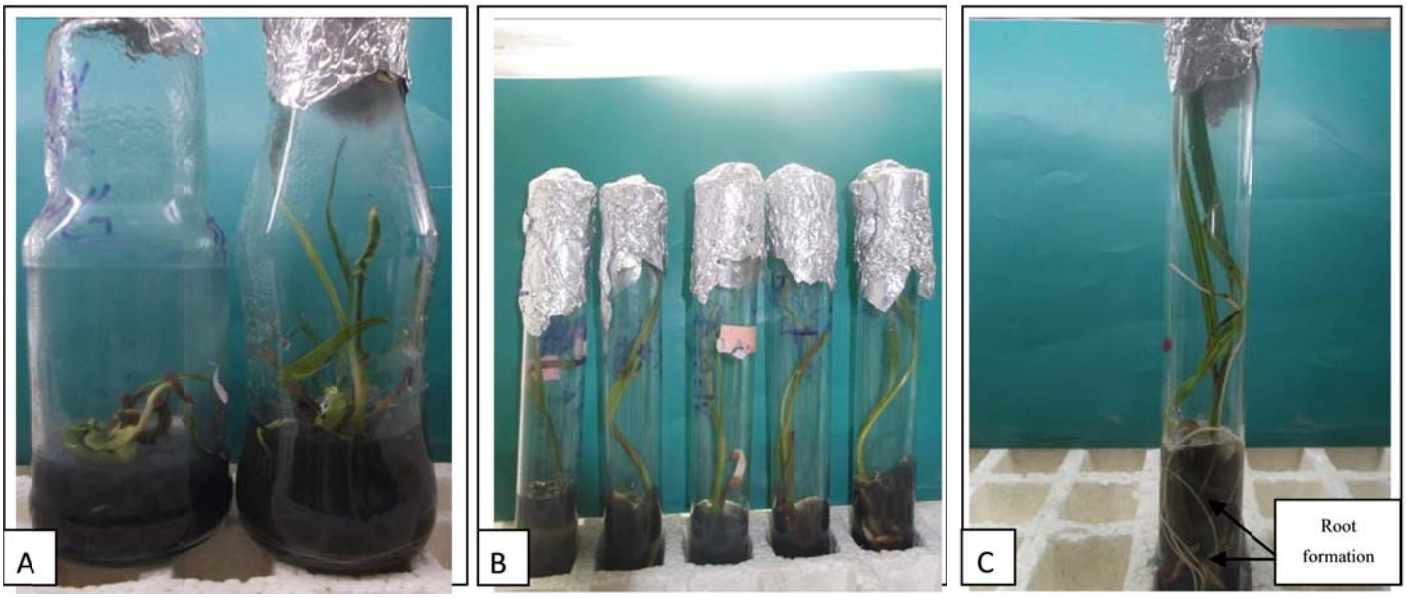

Figure 6. A) elongation of shoots on MS media supplemented with 0.5 mgl-1 GA3+ 0.1 mgl-1 NAA. **B)** Shoots on rooting medium supplemented with 0.2 mgl^{-1} NAA. **C)** Rooting of shoots on same media after 6.

Figure 7. Date palm plantlets transplanted in plastic pots filled with mixture of peat moss and perlite 2:1 ratio (v/v).

development, the presence of cytokinin is critical for buds induction and for differentiation from explants of date palm, where adventitious buds were not observed in cytokinin-free medium (control treatment). Cytokinin (BA) and cytokinin-like compound (TDZ) break apical dominance (Tawfik and Mohamed, 2006). Thidiazuron (TDZ) has gained a considerable attention during the past decades due to its efficient role in plant cell and tissue culture. The highest number of buds (18.2 per explant after 24 weeks) was induced from shoot tip explants on MS medium with 0.5 mgl^{-1} TDZ and 1.0 mgl^{-1} BA (Table 1 and Figure 5B). Such a response may perhaps be due to the increase in the levels of endogenous cytokinins by the effect of the TDZ used, which brings about an increase in the level of naturally occurring cytokinins, and it is likely to have a common site of action with the naturally occurring cytokinins (Ruzić and Vujović, 2008). Also, Casanoval et al. (2004) determined the effects of TDZ on endogenous plant growth regulators in organogenesis as low TDZ levels. Also, the TDZ mediated alteration in the cytokinin biosynthetic pathway might be responsible for the depletion of the endogenous 2iP pool and the elevated concentrations of the other purine metabolites (Zhang et al., 2005). TDZ can highly induce synthesizing, gathering and modifying other produced cytokinins (Visser et al., 1992). It is well known that cytokinins stimulate plant cell division and participate in the release of lateral bud dormancy, in the induction of adventitious bud formation, in the growth of lateral buds and in the cell cycle control (Gaspar et al., 2003). TDZ-treated plant tissues enhanced endogenous auxin metabolism and transport (Murch and Saxena, 2001). Also, Nabila et al. (2003) found that TDZ had been useful for the production of economically important secondary metabolites in some plant species. The other possibilities include the modification in cell membranes, energy levels, nutrient uptake, or nutrient assimilation (Murthy and Saxena, 1998; Murthy et al., 1998; Guo et al., 2011).

Moreover, Abbasi et al. (2011) reported that TDZ inhibits synthesis of abscisic acid according (Li and Yang, 1998), where the frequency of induction and growth of buds was found to vary significantly depending on the concentrations of TDZ. It might be due to the low concen-

trations of TDZ inhibiting synthesis of abscisic acid was more than high concentrations. These results agree with the reports of Lincy and Sasikumar (2010) which suggested that combinations of TDZ and other plant growth regulators could be more effective than TDZ used alone. Also, these results are similar to those reported by Husain et al. (2007) and Husaini and Abdin (2007) who confirmed that the frequency of shoot regeneration ability declined markedly at higher concentrations of TDZ. Also, higher concentrations of TDZ hindered further growth and development of the regenerates (Shirani et al., 2010). Generally, explants cultured on media supplemented with low levels of TDZ were positive for adventitious buds regeneration. TDZ has been shown to stimulate buds regeneration at low concentrations and was used mainly in combination with other plant growth regulators. These results suggest that the response of explants to buds formation ensure the development of budding, necessary to modify the hormonal balance in favour of the cytokinins. Moreover, it is reported that TDZ is the best choice compared with other phytohormones for protoplast proliferation (Murthy et al., 1998; Xiao et al., 2007).

The acceleration of peroxidase activity was associated with increased number of buds under TDZ effect, and had more stability and important role in POD synthesis. The stimulation of peroxidase in the present experiments in accordance to enhancement of bud growth and development at 0.5 mgl^{-1} TDZ, supports the view that TDZ might make this enzyme active, thus controlling the level of H_2O_2 and the rate of cell division (Kapchina-Toteva and Yakimova,1997). In addition, we suggest that the activity of peroxidase could be used as a biochemical marker of development in the plant object studied. Moreover, TDZ promotes the activities of POD, which may be one reason for budding. Sharifi and Ebrahimzadeh (2010) and Mamaghani et al. (2010) reported that antioxidant enzymes play an important role in the organogenesis of 20 plants as confirmed by analysis of POD which was also, reported by Ezaki et al. (1996). This enzyme has high activity as a marker during stressful conditions. In addition, such an enhancement of peroxidase activity can be caused by stress due to changes in media composition; where the TDZ is the induction of a stress response (Murthy et al., 1998; Abbasi et al., 2011), and stress has been considered to be a stimulus to developmental switch by reprogramming gene expression and reorganizing cellular state (Fehér et al., 2003). During this process, some defense-related genes may be induced to adapt the stress conditions. The ability of the explant tissue to survive the applied stresses of the culture process seems to be an integral part of the morphogenic phenomena and some studies provide indication of the factors involved in the regulation of plant regeneration, with various active forms of peroxidase involved in growth regulation, development and organogenesis. The acceleration of enzyme activity was associated with formation of more shoots (Kapchina-Toteva et al., 2005) since plant peroxidases are involved in many functions such as growth, vegetative development, resistance to biotic and abiotic stresses (Gonzalez-Verdejo et al., 2006). The results of the present study are in agreement with the results of other studies related to using cytokinins on peroxidase activity (Synková et al., 2006), and with Wang et al. (1991) who reported that many of the TDZ-stimulated enzymes were associated with cell walls membranes and membrane fluidity was modified.

Also, this result is in accordance with earlier reportof an enhancement of peroxidase activity in response to TDZ (Todor and Iordanka, 1995). As the overall result, it seems that changes in POD activity, is an index for regeneration. TDZ is resistant to oxidases, is stable, but biologically more active at low concentrations. These properties may enhance future use in tissue culture manipulations. These findings may promote further investigations of the physiological properties and selectivity of phenylurea cytokinins. The importance of the cytokinins in releasing the process of meristems and, consequently, formation, is well-known; these formations may be caused by the cytokinins that enhance the multiplication of the DNA, and the chromosomes separation which encourages the cell division (Auge, 1984). The formation of the organs in the moncotyledonae is generally, enhanced by addition of the cytokenines (Duhoux, 1988). Also, the nodules developed into buds when moved to an auxin free medium. Moreover, organogenesis, due to the influence of growth regulators present in the medium, is the result of dedifferentiation of certain cells showing a mitotic activity. This also showed that cell division is initiated in the epidermal layers and that from a multiple-layered epidermis occasional meristematic bulges are produced. Regeneration of adventitious bud meristems formed directly on explants *in vitro* is often initiated by cell divisions beginning in the epidermal. Adventitious buds primordial is initiated as a result of organized directional growth of cells from meristematic cells. Although mitotic activity was found throughout the explant, activity was concentrated in the epidermis regions that were in close contact or adjacent to the nutrient media. Clusters of cells began to appear in the subepidermal region of the explant. Regeneration in this manner is widespread, occurring in monocotyledons (Crinum macowanii) (Slabbert et al., 1995). The initial cell divisions result in a mass of small cells forming new meristematic primordia. Continuity of division cells led to vascular bundles, where development of the vascular bundles is essential to guarantee the transport between tissues and distant organs, assuring the growth.

Conclusion

In the present study, the *in vitro* protocol for regenerating plantlets of date palm cv. Hillawi using shoot tip explants was described. Since the plantlets were developed direct-

ly without intervening of callus phase, it can be concluded that results obtained in this study, permit the development of a mass propagation protocol with a good budding rate and a high regeneration percentage. Data suggest that TDZ and BA were indispensable for *in vitro* propagation of date palm since no excisable shoots were produced on MS-0 medium (control). 0.5 mgl^{-1} TDZ with 1 mgl^{-1} BA are recommended as a component of culture media. Stimulation of number of buds was accompanied by an enhancement of guaiacol peroxidase activity. Histological studies revealed the development of meristematic regions, which later developed into buds meristems.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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