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# Plant regeneration from immature female inflorescence explants of date palm (*Phoenix dactylifera* L.) via direct somatic embryogenesis

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This study was conducted to investigate the induction of direct somatic embryogenesis from immature female inflorescence explants of date palm cv. Amhat (soft cultivar). Explants cultured on MS medium containing 1 or 2 mg1$^{-}$ of thidiazuron (TDZ) or benzyladenine (BA) supplemented with 0.5 mg1$^{-1}$ naphthalene acetic acid (NAA). The results showed that the highest formation of direct somatic embryos performed on MS medium containing 0.5 mgl$^{-1}$ NAA and 1 or 2 mg1$^{-1}$ TDZ, which also induced the lowest value of callus formation and browning. However, cultured explants on medium free growth regulators (control) and that of adding 0.5 NAA mg1$^{-1}$ had no response to induce direct somatic embryos. Subsequently, development of the embryos was completed on MS medium with 0.1 mg1$^{-1}$ NAA. In conclusion, for plant regeneration from immature inflorescences of date palm via direct somatic embryos, TDZ with NAA was more efficient to initiate direct somatic embryos.

**Keywords:** ***Phoenix dactylifera*** **L., Immature inflorescence, Somatic embryogenesis.**

## INTRODUCTION

Micropropagation of date palm (*Phoenix dactylifera* L.) is hampered by the limited number offshoots needed for multiplication. Alternatively, immature female inflorescence tissue represents an abundant and successful source of explants for date palm micro propagation (Loutfi and Chlyah, 1998). Also, histological analysis revealed that the immature female flowers appear as small mass of meristematic cells subtending with small bracts (Zayed and Abdelbar, 2015). Direct somatic embryogenesis without a callus phase is an important and suitable method in development economic mass production of date palm (Sudhersan et al, 1993). This method was successful with the plant material contains meristematic cells which became crucial requirements for this process (Al-Khayri, 2003). Also, success floral tissues of date palm to reverse into vegetative tissue are depends on cultured in early stage in suitable medium that contain specific plant growth regulators. These tissues are available source instead of offshoots (Drira and Al-Shaary, 1993). The main factors control in the stimulation of embryogenesis and the kind (direct or indirect) of morphogenesis depend on the type, concentration, and exposure time of the plant growth regulators employed (Gaj, 2004). Direct somatic embryogenesis was obtained from inflorescences explants of areca nut which is ideal because it allows the production of plants without a callus phase leading to somaclonal variation and hence useful for efficient genetic transformation (Radha et al, 2006). Exogenous auxins and cytokinins are the main plant growth regulators (PGRs) which, controlled of cell division and differentiation (F´eher et al. 2003)

## MATERIALS AND METHODS

The present study was performed during the period from 2016 to 2017 in the Tissue Culture Lab. for Agricultural Systems Development Project (Egypt California project) and Faculty of Agriculture, Cairo University.

### Plant material and tissue culture protocol

Immature female inflorescences of date palm cv. Amhat were taken from adult female trees which grow in the special field in Abou-Homos area belong to Behera governorate, Egypt. The explants were collected at 15–30$^{th}$ January the average spathe length 6–7 cm. Explants sterilization is performed by soaking in 40% of commercial Clorox (5.25% sodium hypochlorite) for 20 min and then rinsed with sterilized distilled water three times, and then protective sheath and part of base were removed. Sterilized inflorescence explants were divided longitudinally into 2–3 segments (spikes with part of inflorescence base) for use as explants (Zayed, 2011). Explants were cultured horizontally with a good contact with the surface of the induction media. The basic cultured medium consists of MS salts (Murashige and Skoog, 1962) adding 0.5 NAA mg1$^{-1}$ supplemented with sucrose (30 g1$^{-1}$) and the media were gelled by agar (6 gl$^{-1}$). Different concentrations of TDZ and BA (0.0, 1.0 and 2.0 mgl$^{-1}$) were added individually with adding 0.5 NAA mg1$^{-1}$. The pH of the media was adjusted to 5.7± 0.1 prior to sterilization in the autoclave and the cultures also were incubated in total darkness at 27±2°C and transferred to fresh media every 6 weeks.

### Data were taken during three subcultures as follows:

The average length of elongation (cm), The average percentage of swelling /explants, The average percentage of browning/explants, The average percentage of callus formation/explants and the average percentage of direct somatic embryos.

### Statistical analysis.

The used designed was in a complete randomized design with three replicates, using L.S.D test at 5% according to Snedecor and Cochran (1989)**.**

## RESULTS AND DISCUSSION

Date in Table showed that different concentrations of TDZ and BA with NAA added to culture media had a significant effect on elongation of Amhat cv. Inflorescence explants, it was clearly observed that added MS+ 0.5 mgl$^{-1}$ NAA, control ,added 2 mgl$^{-1}$ TDZ + 0.5 mgl$^{-1}$ NAA , 1 mgl$^{-1}$ TDZ + 0.5 mgl$^{-1}$ NAA , 2 mgl$^{-1}$ BA+0.5 mgl$^{-1}$ NAA and 1 mgl$^{-1}$ BA+0.5 mgl$^{-1}$ NAA culture media recorded the highest significant values of elongation of inflorescence explants (4.3, 4, 3.8, 3.67, 3.11 and 3.6 respectively) without significant differences among them .

Date in Table revealed that the swelling percentage values of inflorescence explants affected significantly with different TDZ, BA and NAA concentrations added to culture medium, date indicated that the highest significant value of swelling was produced by cultured inflorescence explants on MS medium +0.5 mgl$^{-1}$ NAA (50.2),followed significantly by the same values of swelling of explants cultured on medium supplemented with 1 mgl$^{-1}$ BA + 0.5 mgl$^{-1}$ NAA (47.2%) while, the lowest significant value of swelling of inflorescence explants (40.6%) was produced by cultured on media without TDZ and BA.

Date about browning showed that different concentrations of TDZ, BA and NAA added to culture media don't affect significantly the browning percentage of inflorescence explants. The Highest percentage value of explants was produced by without TDZ or BA. and NAA. while the lowest significant value of browning percentage was produced by culture the explants on medium 1 mgl$^{-1}$TDZ + 0.5 mgl$^{-1}$NAA (21.7 %).

Date in Table (1) showed that the value of callus formation percentage initiation from inflorescence explants effected significantly by different TDZ and BA concentration added to culture media, The highest significant value of callus formation was achieved (22.0 % ) when TDZ added to culture media at 2.0 mgl$^{-1}$ + 0.5 mgl$^{-1}$ NAA followed significantly by addition TDZ at 1.0 mgl$^{-1}$with 0.5 mgl$^{-1}$NAA to culture media (21 %) while the addition of 1 mgl$^{-1}$ BA+0.5 mgl$^{-1}$ NAA and control to culture media the lowest significant value of callus formation percentage was achieved (0.0 %).

Date indicated that the percentage of direct somatic embryos which formed directly from initial meristems of the florets were affected significantly by adding different concentrations of TDZ and BA culture media.

The inflorescence explants cultured on medium supplemented with 2.0 mgl$^{-1}$ BA and 0.5 mgl$^{-1}$ NAA at showed significant reducing in the value of direct somatic embryo (20%) where the highest

significant value of direct somatic embryo percentage was achieved when explants cultured on medium supplemented with 1 $mgl^{-1}$ TDZ and 0.5 $mgl^{-1}$NAA (34%) followed significantly by the value of direct somatic embryo percentage formed from inflorescence explants cultured on medium supplemented with 2.0 $mgl^{-1}$ TDZ and 0.5 $mgl^{-1}$NAA (32.0 %) as shown in Table (1) and Fig. (1) Other treatments cannot able to form any direct somatic embryos. TDZ was effective to induce *in vitro* morphogenesis in several plants, such as shoot regeneration and proliferation and direct somatic embryogenesis (Chen and Chang, 2001 and 2002)

**Table 1: Effect of different concentrations of BA and TDZ added with NAA at 0.5 mg $l^{-1}$ on elongation (cm), swelling, browning, callus formation and direct somatic embryos of date palm inflorescence (Amhat) after 18 weeks in culture (throughout 3 subcultures).**

| Treatments (mg/l) | Elongation (cm) | Swelling % | Browning % | Callus formation% | Direct somatic embryos% |
|---|---|---|---|---|---|
| **Control** | 4 | 36.5 | 25.2 | 0.0 | 0.0 |
| **MS+0.5NAA** | 4.3 | 50.2 | 24.3 | 22 | 0.0 |
| **1BA+0.5NAA** | 3.6 | 47.2 | 22.2 | 0.0 | 22 |
| **2BA+ 0.5NAA** | 3.11 | 40.6 | 23.5 | 13.5 | 20 |
| **1TDZ+0.5NAA** | 3.67 | 43.1 | 21.7 | 11 | 34 |
| **2TDZ+0.5NAA** | 3.8 | 40.8 | 22.4 | 21 | 32 |
| **L.S.D** | 0.690 | 5.884 | 2.690 | 10.538 | 9.852 |

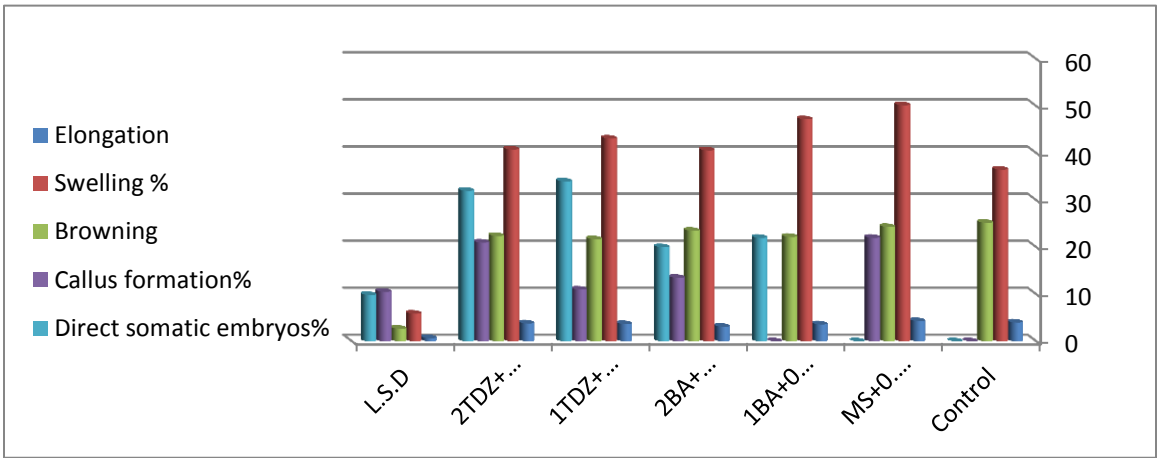


**Figure (1): Effect of different media on elongation, swelling, browning, callus formation, and direct somatic embryos % of female flowers of Amhat cultivar.**

Resulted data showed that, the highest formation of direct somatic embryos achieved on MS basal medium containing 0.5 NAA and 1 or 2 $mg1^{-1}$ TDZ. Beauchesne et al. (1986) found that, auxins at low concentration, enhanced date palm bud growth in vitro after four to six months gave some signs of budding which is indicates giving true-to-type plantlets. Date palm shoot tips cultured on medium containing low auxin concentrations initiated leaves and in some cases roots while, high auxin concentrations resulted in the formation of callus (Tisserat, 1979) culturing explants in vitro determine a continuous supply of auxins and cytokinins added either individual or in combination at diverse ratios, (Ziv, 1991). Moreover, Date palm shoot tips cultured on medium containing low auxin concentrations initiated leaves and in some cases roots while, high auxin concentrations resulted in the formation of callus (Tisserat, 1979).

Murthy et al. (1998) mentioned that TDZ is a substituted phenyl urea that has been reported to exert a high cytokinin activity and aid regeneration in many plant species, TDZ a urea-derived cytokinin, is a potent cytokinin for woody plant tissue culture. TDZ was effective to induce *in vitro* morphogenesis, such as shoot regeneration and proliferation (Chen and Chang, 2002). Velcheva et al. (2005) indicated that cultured young inflorescences explants of *Aloe arborescens* on MS medium supplemented with BA or TDZ produced shoot regeneration. Moreover, exogenous TDZ is responsible for increasing zeatin, which indicates the active extent of cell division and metabolism of the plant (Casanova et al, 2004). This greater effectiveness of TDZ As a result of its slow metabolism in tissue culture. It is also, TDZ is a urea-based cytokinin and, subsequently, is non-degradable by cytokinin oxidase enzymes in plant tissue. These reasons are distinctive TDZ to be persistent in tissue and to modify endogenous hormones either directly or indirectly and produce response in cells and tissue necessary for their division and regeneration (Guo et al, 2011).

## CONCLUSION

Plant regeneration from immature inflorescences of date palm via direct somatic embryos has been considered the protocol for distinguishing high genetic stability and true-totype vitro plants. TDZ with NAA was more efficient to initiate direct somatic embryos.

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## CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

## AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.



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