

The Influence of plant growth regulators and slicing methods inducing direct organogenesis of meristematic florets of date palm

Eman M.M. Zayed

The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Cairo, Egypt

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ABSTRACT

In date palm micropropagation protocols, the female inflorescences play an important role, being available tissue culture-explants, alternatively of the limited number of renewing offshoots explants of date palm that used for *in vitro* propagation. This study conducted to investigate the effect of different auxins types (2,4-D, NAA, NOA) at different concentrations, supplemented in induction culture medium of constant concentration of cytokinins, on inducing the direct organogenesis by adventitious shoots differentiation, of the date palm immature female inflorescences explants, of Barhe cultivar. Also, the interaction effect of slicing method (for the first time studying) of the starting spikelet explants was studied. The results showed that, the induction medium of the appropriate ratio of the NAA auxin type to cytokinins of induction medium, with applying the slicing method of fine chopping for the cultured spikelet, showed the most satisfying results, of direct adventitious shoots formation, direct somatic embryos, and less formation of direct adventitious roots. Developing the date palm micropropagation protocol by inflorescences explants considered a reliable and rapid opportunity to avoid the shortage of date palm offshoots and covering the commercial demands on the date palm plantlets.

Keywords: Date palm micropropagation, female inflorescences, direct organogenesis, chopping, plant growth regulators.

Introduction

Date palm (*Phoenix dactylifera* L.) tree, is growing as a monocotyledon plant, belonging to the Arecaceae family. It is a perennial dioecious 'tree' and well known as the most important economic crop in the Middle East and North Africa (Chao and Krueger 2007; Jain, 2012 and Gantait *et al.*, 2018). Date palm plantation widely expanded all over the world, because of the high nutritional value of date fruits, their pharmaceutical importance (ElHadrami, and Al-Khayri, 2012; Al-Mssallem *et al.*, 2019 and El-Dawayati *et al.*, 2020) and also, the important domestic industries products gaining from the tree parts (Gantait *et al.*, 2018). Commonly, this generous tree propagated by the vegetative method through offshoots, but, the slow growth, high costs with a limited number of renewing offshoots are the serious problems faced the vegetative propagation in date palm, which hampering the increasing demands in their plantation expansion (Johnson, 2011 and Abahmane, 2017). Since the sexual propagation of date palm by seeds is not an effective method at all, due to, the highest longevity of the growing period until reaching the bearing stage, moreover, genetic heterozygosity occurrence in the new seedlings progeny (Zayed, 2011). Therefore, the advanced propagation by tissue culture technique, using the meristematic cells of shoot tips (Othmani *et al.*, 2009; Al-Khayri 2011 and Mazri *et al.*, 2018) or immature (Zayed, 2011 and Fayek *et al.*, 2017) and mature (Kriaa *et al.*, 2012 and Zayed, 2017) female inflorescences, has an important role in adjusting the faster and higher production of new plantlets that are true-to-type to the mother plants, which ensuring the expansion of palm plantations. In general, date palm micropropagation can be accomplished through, indirect somatic embryogenesis, where somatic embryos differentiated from the induced embryogenic callus, which subsequently converted into complete plantlets (Fki *et al.*, 2003; Aslam *et al.*, 2011 and El-Dawayati *et al.*, 2018). Meanwhile, the other pathways are, the direct somatic embryogenesis or the direct organogenesis (Bekheet, 2013; Fayek *et al.*, 2017 and Ali and Abdulzghara, 2018), as the complete plantlets are directly produced, without the callus stage interfere. Anywhere, callus phase absence ensures avoidance of the genetic variation among the regenerated plantlets. Furthermore, plants regeneration via direct organogenesis, is the most

Corresponding Author: Eman M.M. Zayed, The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Cairo, Egypt

preferred method for most plants' micropropagation, even if the frequency of plantlets production decreased, but the somaclonal variation occurrence decrease (Asthana *et al.*, 2011; Amit *et al.*, 2014; Kazeroonian *et al.*, 2018 and Zhang *et al.* 2018).

Recently, there is a worldwide increasing demand for *in vitro* produced plantlets of date palm cultivars. Therefore, there is an urgent need to available source and develop a high-frequency of identical genetic plant regeneration protocols for date palm.

It is worth mention that, various studies in date palm micropropagation have demonstrated female inflorescences explants to be an available and alternative source for shoot tip explants, whenever deficiency of offshoots needed to start the *in vitro* propagation protocol (Zayed, 2011).

There are several studies on date palm micropropagation by female inflorescences explants using indirect somatic embryogenesis (Abul-Soad 2012 and Zayed and Abdelbar, 2015) but, a limited studies using the direct somatic embryogenesis (Zayed, 2011; Sidky and El-Dawyati, 2012 and Fayek *et al.*, 2017). On the other hand, a few studies conducted to induce the direct adventitious bud (Kriaa *et al.*, 2012; Zayed *et al.*, 2016 and Khierallah *et al.*, 2017). In general, many factors control the meristematic cells morphogenesis, as the plant growth regulators, the other components of culture media, the chronological age of explants, the developmental stage, and the surrounded environmental conditions (Tilkat and Onay, 2009 and Bar and Ori, 2014). It is well known that, plant growth regulators playing a critical role in the regulation of cell division tissue and organ differentiation Traas (2019). Research results on plant material from palm inflorescences showed that this type of tissue is very sensitive to growth regulators, especially auxins (Abahmane, 2013), also it was reported that, the plant hormone auxin presented as a central regulator of organ initiation (Bar and Ori, 2014). Moreover, with the other signaling pathways, in particular, cytokinin is an essential factor for the correct organ initiation (Bar and Ori, 2014; Traas, 2019 and Zhang *et al.*, 2018). The induction of direct organogenesis from female inflorescence have been achieved by adjusting the ratio of cytokinin to the auxin (Abahmane, 2005; Abul-Soad 2012; Zayed 2017 and Khierallah *et al.*, 2017), however (Zayed, 2011) succeeded in inducing the direct somatic embryogenesis from female inflorescence cultured on induction media rich of cytokinines only. Meanwhile, several studies demonstrated that, the source and size of explant affects the efficiency of regeneration (Plaza *et al.*, 2015). The explant size found to be an important factor in the date palm female inflorescence somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* (Sidkey and El-Dawayati, 2012), In addition the fine chopping of cultured explants of date palm shoot tip sections significantly improved the proliferation of somatic embryogenesis (Fki *et al.*, 2003; Sane' *et al.*, 2006 and Othmani *et al.*, 2009). On the other hand, Benkirane *et al.* (2000) reported that the smallest explants sizes presented a higher morphogenetic capacity in the inflorescences fragments of *Triticum durum*.

On the light for the above introduction, the aim of present study is to develop an efficient protocol for date palm micropropagation by using inflorescences explant source, through investigating the effect plant growth regulators of different auxins type with constant concentration of cytokinins, accompanied with (the first time studying) the slicing method for the starting spikelet explant by (the fine chopping or the intact small segments) of female inflorescences explants of Barhe cultivar, on inducing the direct adventitious bud formation, to ensure identical plantlets production by tissue culture process for date palm inflorescences explant source.

2. Materials and Methods

The present study was conducted in the Central Lab. for Date Palm Researches and Development, Agricultural Research Center (ARC), Giza, Egypt.

2.1. Plant material and explants preparation

Immature female inflorescences of date palm (*Phoenix dactylifera* L.) cv. Barhe collected from adult female trees that grow under Egypt climate. spathes collected in early spring (February). Spathes are cream in color, soft, and 10-15 cm in length. Soap the collected spathes for 1h, and wash under running tap water. Disinfect the spathes by soaking in 40% of commercial Clorox (5.25% sodium hypochlorite) while shaking for 20 min. Rinse the spathes with sterilized distilled water three times and

then the protective sheath was removed. Soak the sterilized spike explants in the antioxidant solution (100 mg/l⁻¹ ascorbic acid and 150 mg/l⁻¹ citric acid).

Experiment design

Two factors were studied to enhance the micropropagation protocol of date palm using immature female inflorescences explants: -

1- The slicing methods for the inflorescence explant: -

The sterilized female inflorescences spikes were sliced by two method

A- Spikes explants were cut into equal segments at 2-3 cm in length and contained 4–6 immature florets (the original slicing) (Fig 1a).

B- The same size of previous spikelet explant has completely chopped by sterile sharp scalpel, into small pieces (chopping slicing) (Fig. 1b).

All cultured explants have to be well contacted to the surface of the culture medium, as recommended by (Zayed, 2011).

2- The induction culture medium

The effect of different auxins types (2, 4-D, NAA, NOA) at different concentrations treatments (0.5, 1.0, and 2.0 mg/l⁻¹) on the proliferation of inflorescences explants cultured on the induction culture medium.

The basic components of the induction culture medium are, the nutrient salts and vitamins of Murashige and Skoog (1962), 30 g/l⁻¹ sucrose, 200 mg/l⁻¹ glutamine, 40 mg/l⁻¹ adenine sulfate, and TDZ at 0.6 with BA at 1.0 mg/l⁻¹ as described according to (Zayed, 2011). The pH was adjusted to. Media were dispensed 5.7 ± 0.1 before autoclaving into culture small jars (150 ml) at the 35 ml/jar and were capped with polypropylene closures. The medium was then autoclaved for 20 min at 121°C 15 lb/in². Each treatment contained 3 replicates and each replicate contained one explant. Cultures were incubated in a temperature-controlled room at $27 \pm 2^\circ\text{C}$ under darkness and transferred to fresh media every 6 weeks' interval.

Data were collected for different parameters during four subcultures as follows:

- 1-The average value of adventitious bud formation/explant.
- 2-The average value of direct somatic embryo formation/explant.
- 3-The average value of adventitious roots formation/explant.
- 4- The average degree value of browning /explant.

All data were evaluated by visual observation as degree Poor (1), Fair (2), Good (3), very good (4), and Excellent (5), as a followed recommendation by (El-Dawayati *et al.*, 2018).

Plant formation

Transferred all differentiated direct adventitious shoots and somatic embryos obtained from all treatments to MS medium supplemented with 0.1 mg/l⁻¹ NAA and 0.05 BA mg/l⁻¹ maintain cultures at $27 \pm 2^\circ\text{C}$ under a 16 h photoperiod of 40 $\mu\text{mol}/\text{m}^2/\text{s}$ (cool-white fluorescent lamps) to resume their development.

Plant acclimatization

Plantlets hardened by culture on 1/2 MS liquid medium supplemented with 10g/l⁻¹ sucrose and 0.1 mg/l⁻¹ NAA (Zayed, 2011) Incubate cultures for 6 weeks at $27 \pm 2^\circ\text{C}$ under 16-h photoperiod, and light intensity 80 $\mu\text{mol}/\text{m}^2/\text{s}$ provided by (cool-white fluorescent lamps). Transfer plantlets in the greenhouse and soaked in 0.5 % (w/v) Benlate fungicide solution for 1-2 min. Put plantlets in plastic pots 5 × 18 cm (torpedo) containing a mixture of peat moss, vermiculite, and sand (1:1:1, v/v/v).

Statistical analysis

Data were expressed as mean \pm standard error (SE) and analyzed by ANOVA. To examine the significant differences among the treatments, (Duncan, 1955) multiple range test at ($P < 0.05$), performed.

3. Results

3.1. Direct adventitious bud formation

The present data reveal the effect of the addition of different types of auxins at different concentrations to the induction medium of MS salts supplemented with cytokinins of 0.6 mg/l⁻¹ TDZ and 1.0 mg/l⁻¹ BA, and the slicing method for the cultured spikelet explants of the immature female inflorescence of date palm, to induce the direct adventitious bud differentiation). Direct somatic embryos, adventitious roots formation and browning phenomena were observed.

Data in Figure (2) illustrated that auxin type significantly effect on the formation of direct adventitious bud, where cultured spikelet explants of the immature female inflorescence on induction medium with cytokinins of 0.6 mg/l⁻¹ TDZ and 1.0 mg/l⁻¹ BA and NAA auxin gave the highest significant value of direct adventitious bud differentiation, followed significantly, by the value of direct adventitious bud differentiated from the spikelet explants on induction medium with 2, 4-D. On the other hand, spikelet explants cultured on induction medium with NOA completely failed to produce direct adventitious bud, also a necrosis appearance observed in the presence of NOA auxin.

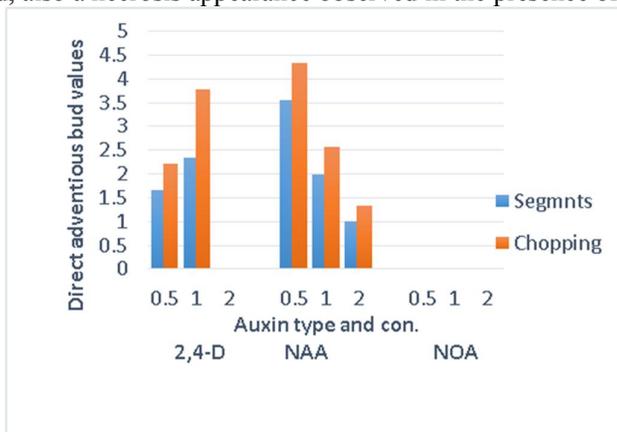


Fig. 2: Influence of auxin concentrations and slicing method on inducing direct adventitious bud from female inflorescences of date palm cv. Barhe.

The results also showed that, low level of auxin (0.5 mg/l⁻¹) supplemented in the induction medium with cytokinins of 0.6 mg/l TDZ and 1.0 mg/l⁻¹ BA recorded the highest significant value of adventitious bud developed directly on the spikelet explants Fig. (3 a,b,c). But increasing the level of auxin to (2.0 mg/l) in the induction medium showed a dramatic reduction in the value of adventitious bud differentiation from cultured spikelet explants. Regarding the effect of the slicing methods of spikelet (Segments or fine Chopping), the present data revealed that fine Chopped spikelet gave the higher significant value of formation of direct adventitious bud than spikelet segments Fig. (3 d, e).

From data it can be indicated that differentiation of the direct adventitious bud from inflorescence explants affected significantly by the type of auxin, concentration, and the slicing method, as fine chopped spikelet cultured on induction media containing NAA at 0.5 mg/l recorded significantly the highest value of differentiated direct adventitious bud Fig.3a, whereas the cultured segments or fine chopped explants on induction media containing NOA, did not develop any direct adventitious bud from inflorescence explants, at all the studied concentrations.

Direct somatic embryos

Data in Fig. (4) indicated that the value of direct somatic embryos that developed from the initial meristems of the florets explants affected significantly by the type of auxin that added to the induction medium with cytokinins of 0.6 mg/l⁻¹ TDZ and 1.0 mg/l⁻¹ BA. The cultured spikelet explants on induction medium containing NAA showed the highest value of direct somatic embryos formation, followed by, the value of direct somatic embryos formation of spikelet explants cultured on the induction medium containing 2,4-D. In contrast, the induction medium with cytokinins of 0.6 mg/l⁻¹ TDZ and 1.0 mg/l⁻¹ BA supplemented with NOA gave the lowest value of direct somatic embryos formation of spikelet explants.

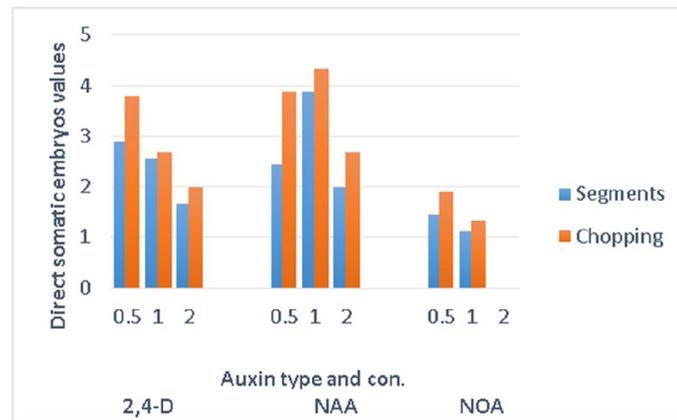


Fig. 4: Influence of auxin concentrations and slicing method on inducing direct somatic embryos from female inflorescences of date palm cv. Barhe.

The results showed that increasing the concentration of auxin in induction medium to (2 mg/l⁻¹) recorded the lowest significant value of direct somatic embryos formation of cultured spikelet explants. On the other hand, the highest values of somatic embryos developed directly obtained from spikelet explants cultured on induction media containing auxin concentration at 0.5 and 1.0 mg/l⁻¹ without significant difference in between. Regarding the slicing methods of the spikelet explants, it can be indicated from the results in Figure (2) that the slicing method for the spikelet explants highly affected the direct formation of somatic embryos, as the finely chopped spikelet explants had the highest significant value of direct somatic embryos frequency comparing to the segmented explants Fig. (5 a, b).

Direct adventitious roots

The results in Fig. (6), indicated that the highest proliferation of undesired formation of the abnormal direct adventitious roots from female inflorescences spikelet explants, observed clearly, with those spikelet explants, cultured on the induction medium, containing NOA. While, the spikelet explants, cultured on the induction medium, containing NAA, showed the lowest significant value of the formation of the direct adventitious roots.

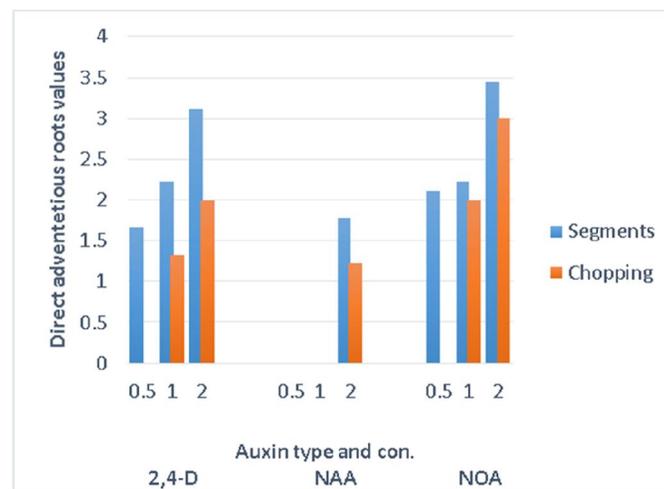


Fig. 6: Influence of auxin concentrations and slicing method on inducing direct adventitious roots from female inflorescences of date palm cv. Barhee.

Also, results showed that induction medium supplemented with a high concentration of auxin at (2.0 mg/l⁻¹), produced the highest value of direct adventitious roots formation. Whereas explants cultured on the induction medium supplemented with auxin, at 1.0 and 0.5 mg/l⁻¹, produced the lowest value of direct adventitious roots formation, without a significant difference in between. Also, finely

chopped explants showed less value of adventitious roots formation than those explants, sliced as segments (Fig. 7a).

Browning degree

The observed browning degree on cultured spikelet explants in Fig. (8) highly increased when NOA implemented in the induction medium. Methods of slicing spikelet explant were also significant.

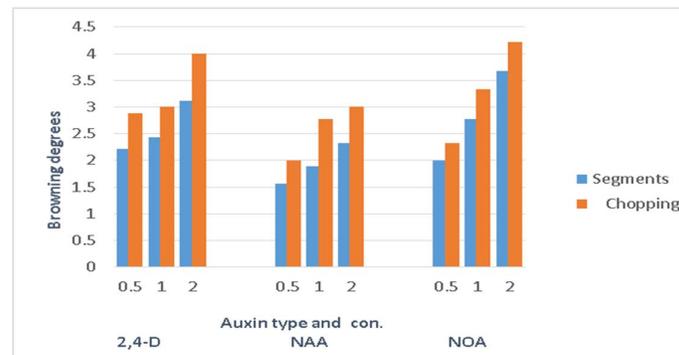


Fig. 8: Influence of auxin concentrations and slicing method on browning degree from female inflorescences of date palm cv. Barhee.

Also, spikelet explant presented the highest values of browning degree with increasing in the concentrations of auxins to (2.0 mg/l⁻¹) whereas the lowest concentration of auxins at (0.5 mg/l⁻¹) recorded the lowest significant degree of browning appearance. Data indicated that, fine chopping for cultured explants, highly increased the browning degree appearance Fig. (7b), comparing to those spikelet explants cultured on induction medium as sliced segments.

Table 1: Effect of slicing method on the accelerating of direct adventitious shoots and direct somatic embryos induction from cultured spikelet explants

| Slicing methods | Time period of morphogenesis response (week) | | | |
|-------------------|--|----------------|-------------|----------|
| | Direct shoot | Direct somatic | Direct root | Browning |
| Segment explant | 24 | 18 | 8 | 4 |
| Chopping explants | 18 | 12 | 12 | 1 |

In general, the initial visible observation for all cultured explants of all treatments appeared after 4-6 weeks as a slight enlargement Fig.9. accelerating of direct adventitious shoots and direct somatic embryos induction from cultured spikelet explants.

The direct adventitious bud formed mostly faster in fine chopping spikelet explants (18 weeks) comparing to the sliced segment spikelet explants (24 weeks). Also, the direct somatic embryos initiated by the chopping slicing method of spikelet explants after 12 weeks whereas initiated by segment slicing method of spikelet explants after 18 weeks without passing of callus phase for each. Browning phenomena observed within a week from chopped spikelet explants while observed after 4 weeks with segments spikelet explants.

Finally, all received plantlet via direct adventitious bud of date palm immature inflorescences, immediately transferred successfully to acclimatization stage with higher survival rate Fig. (10). Similarly, plantlets differentiated through direct somatic embryogenesis, also exhibited good growth and high survival rate.

4. Discussion

The immature female flowers histologically appear as a small mass of meristematic cells, subtending with small bracts (Zayed and Abdelbar, 2015), thus considered a successful source as regenerative explants of date palm micropropagation (Loutfi and Chlyah, 1998, Fayek *et al.*, 2017). Zayed, (2011), reported that using the culture medium of the MS salts nutrient, supplemented with cytokinins (0.6 mg/l⁻¹ TDZ+1.0 mg/l⁻¹ BA), effectively induced the direct somatic embryogenesis, from the immature female flowers spikelet. The present study showed that adding different types of auxins

at different concentrations to the previous induction medium could also induce the direct adventitious bud formation from the cultured spikelet explants, which sliced as small segments (2-3cm) or fine chopped into tiny sections. The obtained data revealed that spikelet explants cultured on medium containing low concentrations of NAA (0.5 mg/l^{-1}) was the best to initiate direct organogenesis followed by 2,4-D at 1 mg/l^{-1} , the same result found with date palm inflorescences that developed adventitious bud by using NAA (Khierallah *et al.*, 2017), also, adventitious shoot regeneration achieved in leaves of wild pear (*P. communis* var *pyraster* L.) by using the lowest NAA concentration ($1.0 \mu\text{M}$), together with $8.8 \mu\text{M}$ BA (Caboni *et al.*, 1999). Meanwhile, it was suggested that the proliferation of the young female inflorescences explants on a media characterized by a low auxin to cytokinin ratio, leading to bud formation either directly or after the multiplication of floral parts (Loutfi and Chlyah, 1998; Wang *et al.*, 2001 and Schaller *et al.*, 2015). Moreover, the combination of one auxin and two cytokinins was found to be more effective on tissue growth and bud formation in date palm (Abahmane, 2005; Khierallah *et al.*, 2017 and Mazri *et al.*, 2017), also it was reported that the induction of embryogenesis and organogenesis were accomplished on media supplemented with auxin (2,4-dichloroacetic acid or *a-naphthaleneacetic acid*) alone or in combination with cytokinin (Trieu and Harrison 1996 and Kouassi *et al.*, 2017). On the other hand, the presented study showed that using NOA auxin at different concentrations failed to induce proliferation of direct adventitious bud from inflorescences explants, also, the lowest value of direct somatic embryos recorded, moreover, the explants turned in necrotic appearance. Similar result was found by Abahmane (2005) that using mg/l^{-1} of NOA in culture media of date palm inflorescences explants resulted in 50% of culture necrosis during the first 3 months of culture.

Our result showed that the culture explants on high level of auxins, resulted in abnormal direct adventitious roots formation. Regarding the growth regulator balance on floral explants of date palm, it has been shown that high auxin/cytokinin ratios NOA (0.5 mg/l^{-1}), IBA (0.5 mg/l^{-1}), BA (0.1 mg/l^{-1}) allow a high ratio of tissue reactions, but root formation was the most frequent (Abahmane, 2013). Ikeuchi *et al.*, (2013) maintained that the balance between two plant hormones, auxin and cytokinin, determines the state of differentiation and dedifferentiation, since a high ratio of auxin-to-cytokinin induces root. Moreover, Canher *et al.*, (2020) illustrated that, the wound-induced signal, highly modulated auxin content by affecting the subsequently expression of auxin biosynthesis and polar auxin transport genes, thereby stimulating adventitious roots induction.

In general, this type of differentiated adventitious roots, was not functional and by the time they turned in brown and completely dried, but it inhibits any subsequent normal differentiation from the meristematic cells.

Concerning to the browning phenomena appearance in the present study, it is well known that oxidation and phenolic accumulation followed by rapid explants browning has common in date palm tissue culture (Meziani *et al.*, 2016). Meanwhile, Fayek *et al.*, (2017) reported that the lowest value of browning was produced from cultured immature of *Phoenix dactylefra* inflorescences explants on medium containing 1.0 mg/l^{-1} TDZ and 0.5 mg/l^{-1} NAA, and in the leaf and inflorescence slices, of *Areca catechu* L. cultured on medium at high level of auxin, recorded the highest value of browning degree (Karun *et al.*, 2004), which agreed with the obtained results in the presented study.

It was reported that, the source and size of explant affects the efficiency of regeneration (Plaza *et al.*, 2015). In the present study, slicing method by fine chopping for the initial spikelet explants of immature date palm inflorescences, significantly influenced on their response to produce direct organogenesis during short period of proliferation, The same finding agreed with Othman *et al.*, (2009) who noted that chopped explant segments at (5–10 mm long) of juvenile leaves of the date palm shoot tip, cultured on 5 mg/l^{-1} 2,4-D, firstly resulted in direct embryogenesis without a callus phase presence. Also, in date palm, dividing small cells had an embryogenic appearance, with a protein-rich cytoplasm, small vacuoles, and a large nucleus (Sane *et al.*, 2006 and AL-Mayahi 2015). Sane *et al.*, (2012) chopped the granular calli of date palm to initiate embryogenic cell suspensions. This is could be explained by the higher morphogenetic capacity of the small explants fragments, as mentioned by Benkirane *et al.*, (2000). Also, Gugsu *et al.*, (2011) reported that the in vitro growth of small and intermediate-sized tef explants successfully started to initiate direct somatic embryos, at the edges of the scutellum, before callus formation. Whereas, the large explants, tended to form initial callus before appearing of the somatic embryos. It was suggested that explants with reduced size, showed the synthesis of new cell wall components, such as oligosaccharides, that can act as signals, to the cell to

resume again the mitotic cycle (Tran Thanh Van and Bui, 2000). Moreover, the small explants, highly exposed to contact with the surface of the culture medium, and can be considered to be more stressed, which increasing the cell's metabolism (Feher *et al.*, 2003). On the opposite side, Nair *et al.*, (2018) found that in the male immature flower bud explants of banana, the optimum length at (3 cm), exhibited better survival, while smaller explants size, found to be not suitable for culture initiation, it was suggested that the larger explants possessing more nutrient reserves and endogenous plant hormones to sustain the culture El Boullani *et al.*, (2017).

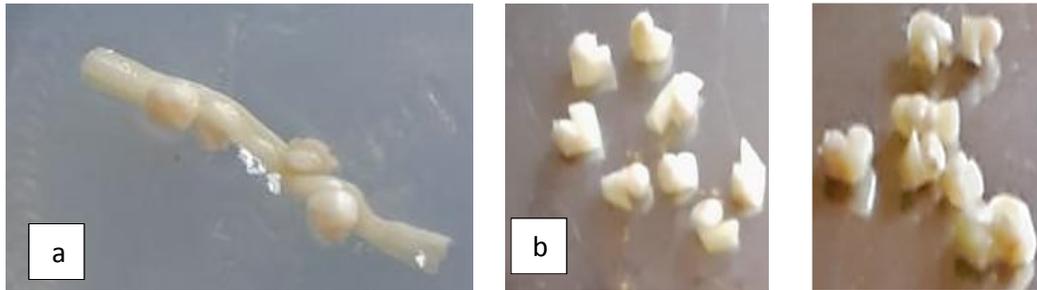


Fig. 1: Fine Chopped spikelet gave the higher significant value of formation of direct adventitious buds.

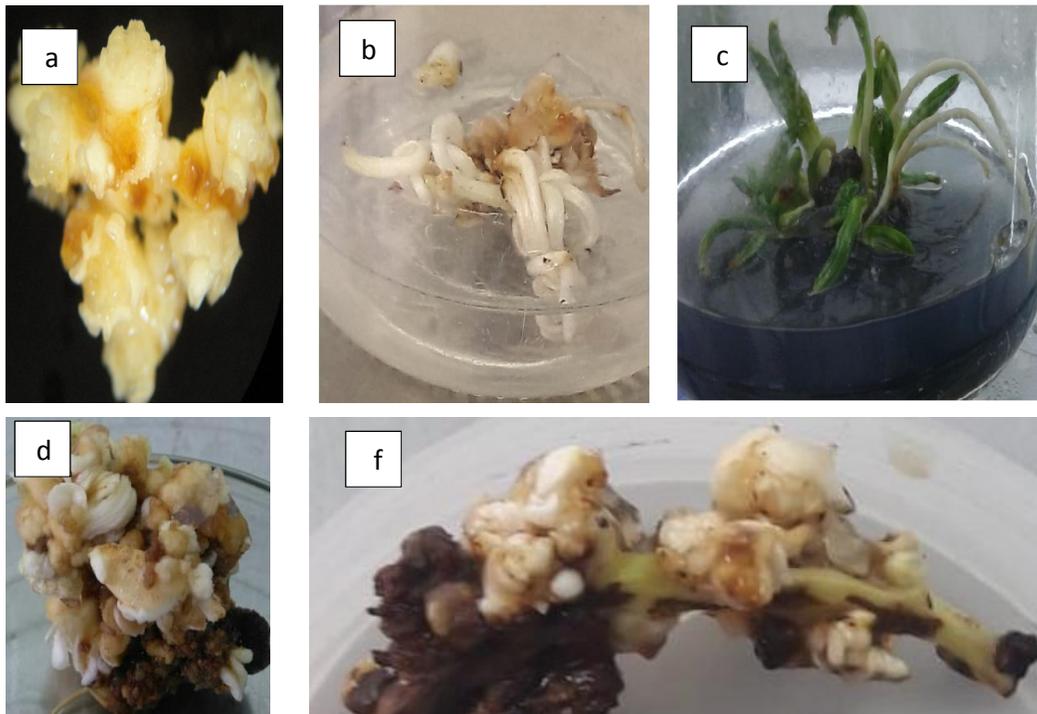


Fig. 3: Fine Chopped spikelet showed the higher ability for initial direct adventitious buds formation, especially cultured on induction medium, of.5mg/l NAA and cytokines (a). Differentiated and Developing direct adventitious shoots after 18 weeks from Chopped spikelet explants (b,c). High frequency of direct adventitious shoots occurring by chopping (d) than the intact segments (f) of slicing method for the spikelet explants.

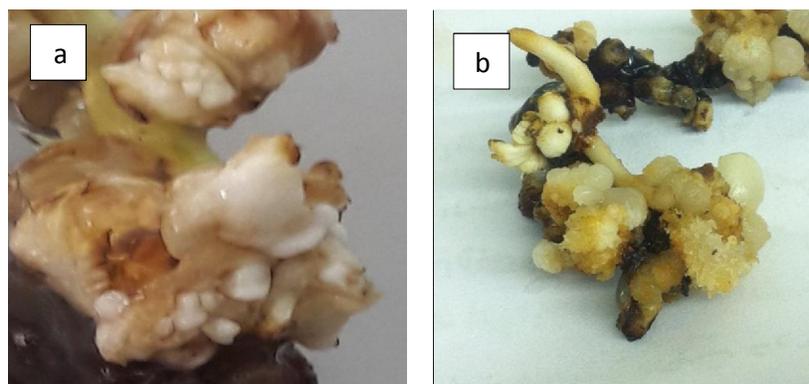


Fig. 5: High frequency of direct somatic embryogenesis occurring by chopping(a) than the intact segments (b) of slicing method for the spikelet explants.

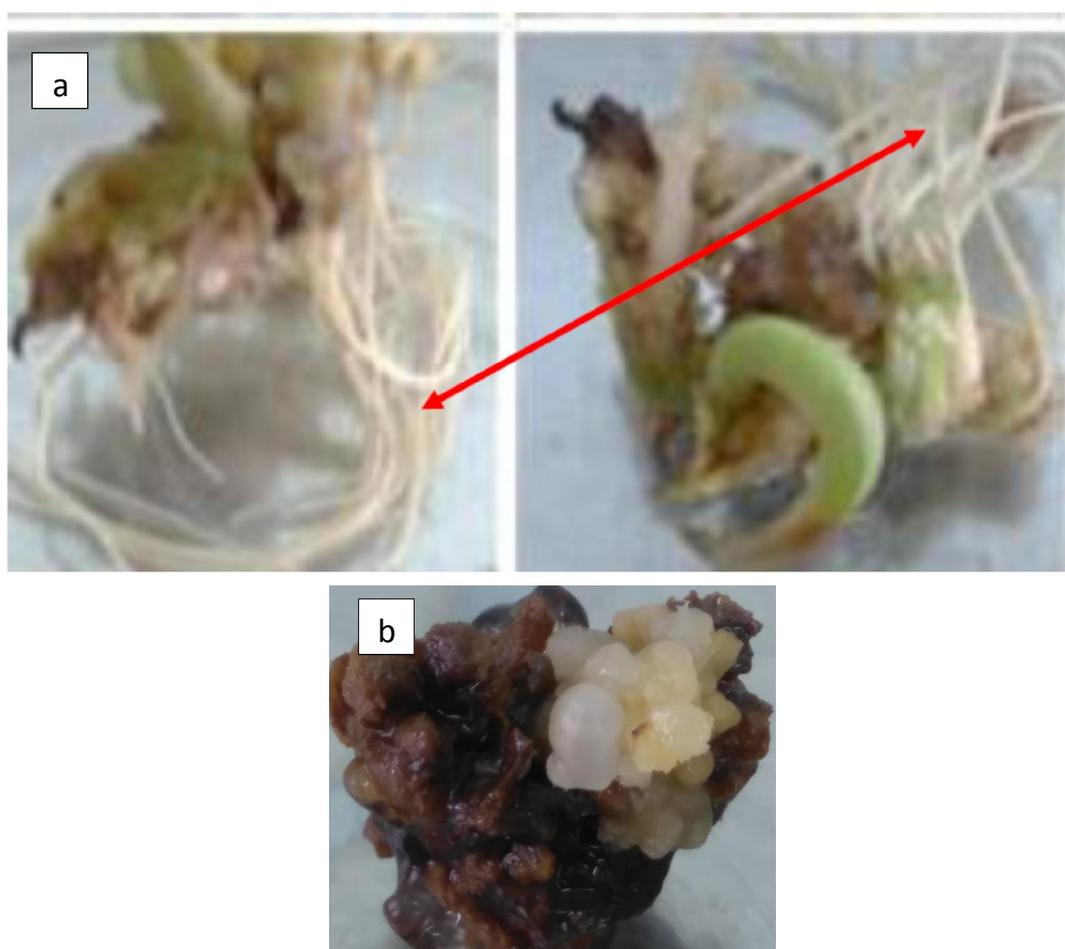


Fig. 7: Chopped explants showed less value of adventitious roots formation than those explants, sliced as segments(a), but fine chopping for cultured explants, the browning degree appearance highly increased in chopped cultured spikelet explants (b).



Fig. 9: Slight enlargement appeared after 4-6 weeks in chopped cultured spikelet explants in all treatments.



Fig. 10: Plantlet via direct adventitious bud of date palm immature inflorescences, immediately transferred successfully to acclimatization stage with higher survival rate.

5. Conclusion

This study demonstrated that, culturing immature inflorescences of date palm cv. Barhe on the induction medium of the appropriate ratio of the NAA auxin type at 0.5 mg/l, with two cytokinins of TDZ at 0.6 mg/l and BA 1.0 mg/l⁻¹, in addition to using the slicing method by fine chopping for the spikelet explants, showed the most satisfying results, to induce direct organogenesis by adventitious bud formation, where the hormonal balance and appropriate explants size, are important to induce the immature inflorescences proliferation in date palm. Developing the date palm micropropagation protocol by inflorescences explants considered a reliable and rapid opportunity to avoid the shortage of date palm offshoots and covering the commercial demands on the date palm plantlets.

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