In vitro Morphogenesis of Direct Organs in Date Palm (*Phoenix dactylifera* L.) cv. Siwy

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Abstract— *Direct regeneration of organs offers a suitable approach for in vitro plantlets production evading the risk of genetic instability (mutations) associated with other regeneration routes involving callus phase. To stimulate adventitious bud formation, shoot tip explants of date palm Siwy cv. were cultured on MS medium containing 1 mg l^{-1} α-naphthaleneacetic acid (NAA) and 1 mg l^{-1} of 6-benzylaminopurine (BA), 6-furfurylaminopurine (Kin) and 2-isopentenyladenine (2iP) added individually or in combinations. The highest percentage of responsive explants (36.36%) was observed on a medium containing 1 mg l^{-1} NAA combined with 1 mg l^{-1} each of BA, Kin, and 2iP where 9.20 shoots per explant formed after 32 weeks of culture (i.e. the end of the fourth subculture). In contrast, the medium containing 1 mg l^{-1} NAA combined with 1 mg l^{-1} of only one cytokinin enhanced root formation but completely inhibited shoot development. Thus, the combinations of NAA, BA, Kin and 2iP at 1 mg l^{-1} each resulted in the highest regeneration percentage of shoots.*

Key words— ***Phoenix dactylifera L, Tissue culture, Direct regeneration, Morphogenesis.***

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is propagated through either seeds or offshoots but both methods suffer inadequacies and limitations [1-3]. Micropropagation has created new opportunities for overcoming these problems and provides uniform and good quality planting material for establishing large-scale plantations [4].

In vitro production is applied through two main modes: somatic embryogenesis and organogenesis [5]. Both modes can occur directly from the explant or indirectly *via* callus phase. Somatic embryogenesis is one of the most successful methods widely used for mass propagation of date palm throughout the world [6]; however, the most disadvantage of this method is the possibility of mutation and abnormalities occurrence which may not be apparent until the fruiting stage [7-9].

In vitro organogenesis is the process by which cells and tissues are induced to undergo changes that lead to the production of a unipolar structure, namely, a shoot or root primordium, whose vascular system is often connected to the maternal tissue. [10, 11].

Micropropagation through direct organogenesis lacking callus phase has the advantage of production of plants that are genetically identical and true-to-type with the mother plant in their vegetative characteristics with no abnormalities or disorders, therefore this method represents an effective mean of large-scale vegetative propagation of date palm [7].

The technique of direct organogenesis is consisting of a multi-staged: initiation of meristematic buds, shoot development (multiplication and elongation), and rooting followed by acclimatization [12, 13]. The achievement of this technique depends on the accomplishment of the initial step which requires a well-trained staff [14]. Furthermore, most of the problems encountered in the succeeding stages (multiplication, elongation, rooting) may have their origin at initiation phase [15].

The direct induction of adventitious buds from explants is a morphogenetic process that is under the influence of exogenous plant growth regulators and its interactions with endogenous phytohormones [16]. The growth, differentiation, and organogenesis of tissues become feasible only with the addition of one or more plant growth regulators (PGRs) to a medium.

Studies on date palm organogenesis are scarce, and successful protocols were reported for some cultivars such as cv. Sukry [3], cv. Maktoom [7], cv. Deglet Nour [8], cv. Khlass [17], cv. Najda [12], cv. Hillawi [4], cvs. Medjool and Mazafati [18], and cv. Mejhoul [19].

Nevertheless, it is well known that developing a micropropagation protocol through organogenesis in date palm is highly dependent on genotype [14, 20] and no standard PGRs combination has been established for initiation and multiplication of organogenic [21]. Thus, it is important to develop an appropriate *in vitro* culture protocol for each desired genotype. Comparatively, many other date palm varieties, cvs. Siwy (semi-dry), Bartamuda, Malakaby, Shamia, and Gondila (dry) are the most recalcitrant to micropropagation in Aswan, Egypt.

Furthermore, regeneration through organogenesis in date palm encountered certain physiological disorders that decrease the proliferation capacity of shoot buds, namely hyperhydricity, early appearances of the roots (precocious

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rooting) and tissue culture browning [22, 23]. There have been few studies to reduce the incidence of these phenomenon, but research has indicated that their frequency is affected by factors such as mineral salt concentration, the type or concentration of PGRs, and the texture of the medium (liquid or semi-solid) [14].

The purpose of this investigation was to evaluate the effects of different composition of auxin (NAA) and cytokinins (Kin, BA, and 2iP) on adventitious bus initiation during *in vitro* organogenesis of Siwy cv. and attempts to understand the mechanism of exogenously applied plant growth regulators to induce organogenesis.

Also, the effects of these factors on the frequencies of swelling, fresh weight, tissue browning and precocious rooting (early rooting), besides describing morphological structures forming consecutive stages of development during the *in vitro* cultivation period.

II. MATERIAL AND METHODS

This work was carried out in the Biotechnology Department, Central Laboratory for Date Palm Research and Development, Agriculture Research Center (ARC) Giza, and Plant Physiology Section, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period of 2013-2016.

2.1. Establishment of initial culture

Effect of α-naphthaleneacetic acid (NAA) with different types of cytokinins 6-benzylaminopurine (BA), 6-furfurylaminopurine (Kin), and 2-isopentenyladenine (2iP) at 1 mg l^{-1} each one (alone or combined) on growth character and morphogenesis (root or shoot) of date palm Siwy cv. *via* direct organogenesis were conducted.

2.1.1 Plant material and Explant preparation

Young offshoots (3-4 years) of date palm Siwy cv. were detached from the mother palm plant for using them as starting material for the protocol. Isolation of the explant was carried out according to Aslam and Khan [24]**;** and Bekheet [9]. Briefly, the outer large leaves and fibers were carefully removed with a hacksaw (sprayed with 70 % ethanol before use) until the shoot tip zone was exposed.

The shoot tips were sterilized with 0.01% (w/v) mercuric chloride ($HgCl_2$) for 1 h. followed by three times rinsing with sterile distilled water [25]. To prevent browning, the sterilized explants were kept in a cold sterilized antioxidant solution consisting of 150 mg l^{-1} citric acid and 100 mg l^{-1} ascorbic acid until cultured [26]. The shoot tips terminal, about 1 to 1.5 cm long was sectioned longitudinally into four sections. Shoot tips explant were cultured individually on culture initiation medium.

2.1.2. Culture media

The culture medium consisted of MS [27] basal salts supplemented with 200 mg l^{-1} glutamine, 100 mg l^{-1} myoinositol, 170 mg l^{-1} $KH_2PO_4.2H_2O$, 40 g l^{-1} sucrose, 1 mg l^{-1} thiamine-hydrochloride, 0.5 mg l^{-1} nicotinic acid, 0.5 mg l^{-1} pyridoxine hydrochloride, 2 mg l^{-1} glycine, 10 mg l^{-1} Ca-pantothenate, 1 mg l^{-1} cysteine, 40 mg l^{-1} adenine sulfate, 100 mg l^{-1} biotin, 0.3 g l^{-1} activated charcoal [28] and 6 g l^{-1} agar. The pH of the medium was adjusted to 5.6 ± 0.1 with 0.1 N NaOH or HCl, prior to adding the agar.

Plant growth regulators were added to the basal medium to study the effect of NAA combined with BA, Kin, and 2iP on date palm direct organogenesis. The plant growth regulators are added as follows:

Control: free of plant growth regulators
Treatment 1: 1 mg l^{-1} NAA and 1 mg l^{-1} BA
Treatment 2: 1 mg l^{-1} NAA and 1 mg l^{-1} Kin
Treatment 3: 1 mg l^{-1} NAA and 1 mg l^{-1} 2iP
Treatment 4: 1 mg l^{-1} NAA+1 mg l^{-1} BA+1 mg l^{-1} Kin+1 mg l^{-1} 2iP

Culture medium was dispensing into 200-ml culture jars at the rate of 40 ml/jar and jars were capped with polypropylene closures and autoclaved at 121 ^{o}C with pressure of 1.5 kg/cm^2 for 20 min.

2.1.3. Culture conditions

The cultures were incubated in total darkness to reduce phenolic secretion from the explants at 25±2 ^{o}C and the explants were re-culturing every 8 weeks to the same previously mentioned freshly prepared media. Different morphological observations were recorded at each subculture for 4 subcultures (8 weeks each subculture).

2.2. Data recorded and statistical analysis

Data were taken during four subcultures as follows:

- The average of the browning degree and swelling degree were scored visually according to Pottino (1981) as follows:

1	(+)	Negative results
2	(++)	Slight results
3	(+++)	Average results
4	(++++)	Above average results
5	(+++++)	Good results

- The average value of fresh weight (g).
- The percentage of meristemoids formation.
- The percentage of adventitious roots (early rooting).
- The percentage of direct shoot formation.

The experimental design used in this study was a randomized complete design [29] the data were statistically analyzed using SAS Version 9.1 and mean average was carried out using T-test multiple range test at 5%, and wherever there was a significant effect, least significant difference (LSD) was used to compare means at 5 % level of probability.

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III. RESULTS

Auxin and cytokinin have been demonstrated to regulate *in vitro* shoot and root formation in many plant tissues [30]. These concentrations were used based on initial experiments were conducted using different concentrations of auxin and cytokinin widely. As an auxin, NAA was selected because of their stability during autoclaving of the medium and during the 8 weeks culture period. Shoot tip explants showed different morphological responses depending on the hormonal combination as well as the culture duration which is expressed in the number of subcultures.

3.1. Swelling degree

Data presented in Table 1 clearly showed that swelling degree was not significantly affected by the cytokinins added to the culture medium. However, culture medium devoid of growth regulators (control) gave the lowest value of tissue swelling (1.04) after the first subculture.

Swelling degree was enhanced significantly at the end of the second subculture Table 2 when shoot tip explants were placed on NAA+2iP (T4) and NAA+BA+Kin+2iP (T5) and recorded the values 3.33 and 3.28 respectively. Data presented in Table 3 revealed that the culture medium supplemented with PGRs gave significant values of swelling degree as compared to the control treatment.

Finally, at the end of the fourth subculture Table 4 all treatments significantly increased swelling degree as compared to the control treatment. The treatment NAA+Kin (T3) and NAA+2iP (T4) gave the highest significant values of swelling degree 3.72 and 3.67 respectively.

Table.1: Effect of NAA, different types of cytokinins (BA, Kin, and 2iP) added alone or combined at 1 mg l^{-1} on growth character and morphogenesis (root or shoot) of date palm via direct organogenesis (cultured in vitro for 8 weeks)

Treatment (1 mg l^{-1})	Growth character			Morphological structure	
	Swelling degree	**Fresh weight (g)**	**Browning degree**	**M root %**	**Shoot %**
Control (PGR-free)	1.04	1.37e	2.30a		
NAA+BA	1.32	5.83d	1.06b	-	-
NAA+Kin	1.12	6.71b	1.00b	-	-
NAA+2iP	1.33	7.90a	1.07b	-	-
NAA+BA+Kin+2iP	1.19	6.41c	1.11b	-	-
LSD (0.05)	**NS**	**0.10**	**0.26**		

M = Meristemoids

Table.2: Effect of NAA, different types of cytokinins (BA, Kin, and 2iP) added alone or combined at 1 mg l^{-1} on growth character and morphogenesis (root or shoot) of date palm via direct organogenesis (cultured in vitro for 16 weeks)

Treatment (1 mg l^{-1})	Growth character			Morphological structure	
	Swelling degree	**Fresh weight (g)**	**Browning degree**	**M root %**	**Shoot %**
Control (PGR-free)	0.97d	1.43d	3.33a		
NAA+BA	1.48c	8.52c	1.43bc	20	-
NAA+Kin	2.83b	9.22b	1.15c	30.77	-
NAA+2iP	3.33a	10.72a	1.42bc	30	-
NAA+BA+Kin+2iP	3.28a	10.70a	1.70b	-	-
LSD (0.05)	**0.43**	**0.13**	**0.45**		

M = Meristemoids

Tabel.3: Effect of NAA, different types of cytokinins (BA, Kin, and 2iP) added at 1 mg l^{-1} each on growth character and morphogenesis (root or shoot) of date palm via direct organogenesis (cultured in vitro for 24 weeks)

Treatment (1 mg l^{-1})	Growth character			Morphological structure	
	Swelling degree	**Fresh weight (g)**	**Browning degree**	**M root %**	**Shoot %**
Control (PGR-free)	1.00b	1.63e	3.30a		
NAA+BA	3.25a	10.90c	1.88c	50	-
NAA+Kin	3.50a	9.03d	2.50b	38.5	-
NAA+2iP	3.44a	12.12ab	2.10bc	44.44	-

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NAA+BA+Kin+2iP	3.31a	12.80a	3.11a	-	-
LSD (0.05)	**0.55**	**0.18**	**0.52**		

M = Meristemoids

Table.4: Effect of NAA, different types of cytokinins (BA, Kin, and 2iP) added Alone or combined at 1 mgl^{-1} on growth character and morphogenesis (root or shoot) of date palm via direct organogenesis (cultured in vitro for 32 weeks)

Treatment (1 mg l^{-1})	Growth character			Morphological structure	
	Swelling degree	Fresh weight (g)	Browning degree	True root %	Shoot %
Control (PGR-free)	1.13c	1.47e	3.73a		
NAA+BA	3.28b	11.24c	2.11c	50	-
NAA+Kin	3.72a	9,84d	2.67b	37.5	-
NAA+2iP	3.67a	12.52b	2.17c	42.86	-
NAA+BA+Kin+2iP	3.38ab	18.40a	3b	-	36.36
LSD (0.05)	**0.35**	**0.17**	**0.34**		

3.2. Fresh weight

When shoot tips explant were grown on culture medium containing different treatments Table 1, fresh weight values were increased significantly with all treatment as compared to the control treatment at the end of the first subculture. Moreover, treatment NAA+2iP (T4) resulted in the highest significant value.

At the end of the second subculture treatment NAA+2iP (T4) and treatment NAA+BA+Kin+2iP (T5) gave the highest significant values of fresh weight 10.72 and 10.70g respectively as compared to the control treatment Table 2.

Explant tissues grown more and reach to its highest significant values of fresh weight 12.80 and 12.12g respectively as shown in Table 3 when they were cultured on NAA+BA+Kin+2iP (T5) and NAA+2iP (T4). However, control medium reduced the fresh weight value at the end of the third subculture.

The medium supplemented with NAA+BA+Kin+2iP (T5) was superior at the end of fourth subculture that gave the highest significant value of fresh weight (18.40 g) as compared to the control treatment Table 4.

3.3. Browning degree

Data presented in Tables 1-4 clearly showed that all treatments except NAA+BA+Kin+2iP (T5) in Table 3 resulted the least significant values of browning degree during all four subcultures when compared with the control treatment. Treatment T5 in Table 3 is nearly equivalent in effectiveness and insignificant as compared to control treatment. They were presumably due to browning phenomena was occurred in the external cell layer while the internal cells don't suffer from browning and continued in its division and morphogenesis.

3.4. Morphological structures

3.4.1.Meristemoids percentage

Data presented in Table 1 indicated that during the first subculture there isn't any root formation in all tested treatments as well as control treatment. Root meristemoids were noticed when culture media were supplemented with NAA and one type of cytokinin (T2, T3 and T4) during the second subculture. Culture medium supplemented with NAA+ Kin (T2) enhanced root meristemoids formation (30.77%) followed by culture medium added with NAA+2iP (T3) (30%) and medium that supplemented with NAA+BA (T1) (20%) (Fig. 1 a, b, e and f).

No meristemoids formed in PGR–free culture medium. However, meristemoids were absent at the periphery surface of the explants that were cultured on 1 mg l^{-1} NAA and (BA+Kin+2iP) at 1 mg l^{-1} for each of them but when the later were cut longitudinally, the meristemoids were seen deeper in the internal tissue of the explants. These meristemoids developing further and giving rise to both shoots and roots in the fourth subculture (Fig. 3 a, b). The same trend in Fig. 1 c, d was observed during the third subculture and developing of root meristemoids. Root meristemoids developed from meristematic activity area [31].

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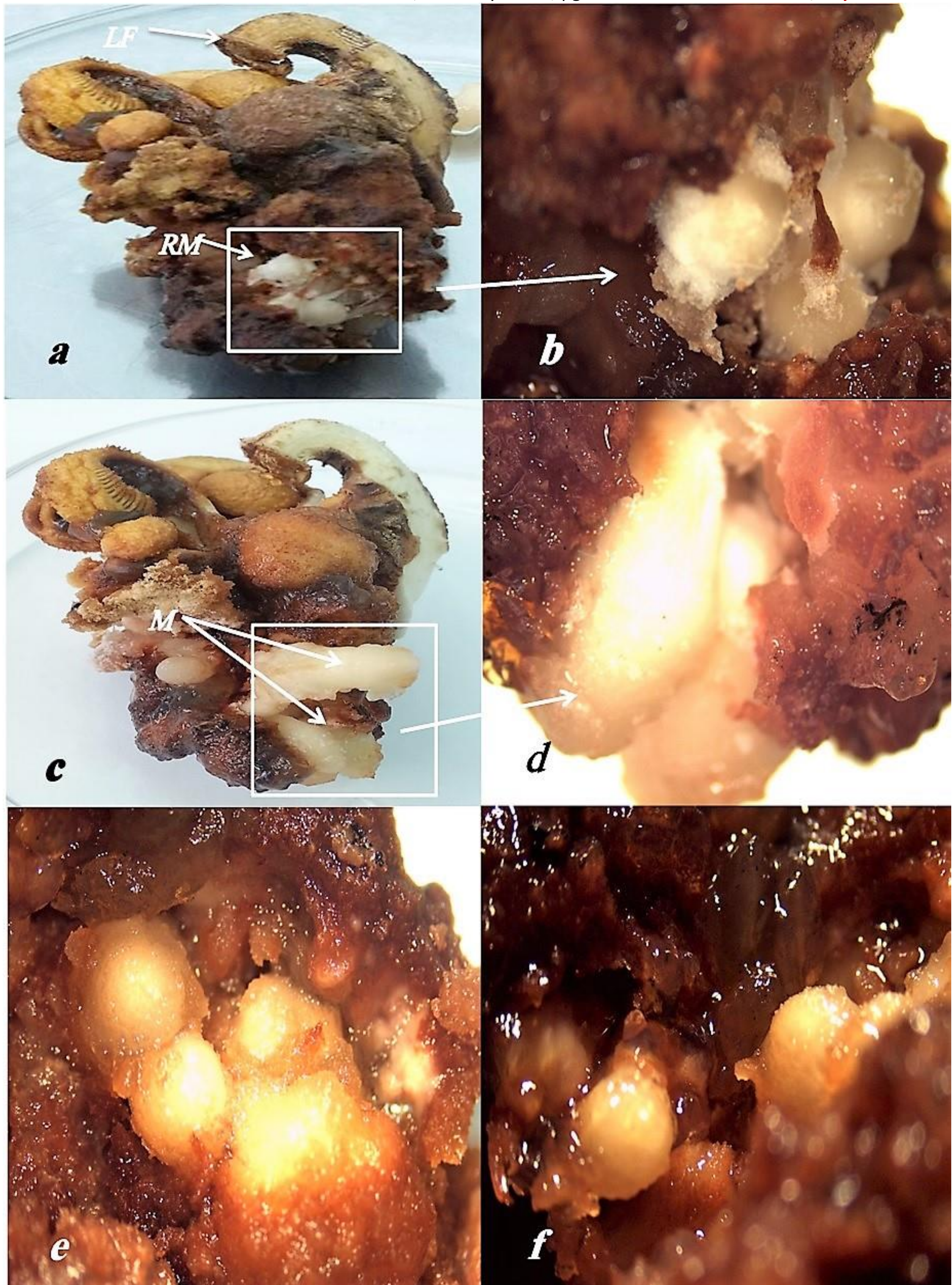

Fig. 1: Meristemoids formation of date palm Siwy cv. derived from shoot tips explant that were cultured on 1mg l^{-1} NAA plus 1mg l^{-1} of each BA or Kin or 2ip. (a): shoot tip explant at the end of second subculture note root meristemoids also notice undifferentiated leaf primordial, (b): An enlarged view from Fig. 1.a showed root meristemoids formation at the basal part of explants, (c): developing RM at the end of third subculture, (d): An enlarged view from Fig. c showed root meristemoids take an elongated appearance, (e): meristemoid root, (f): merstimoids root formation after the second subculture.

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3.4.2.Frequency of precocious rooting

The visually noticed true root formation was occurred during the fourth subculture (after 32 weeks). These formed true roots were histological examined (data not shown). Treatment 2 (NAA+BA) in Table 4 and Fig. 2 a and b was the superior which inducing roots formation on the cultured explants after the fourth subculture (50%).

Fig. 2: Direct rhizogenesis of date palm Siwy cv. derived from shoot tip explants that were cultured on 1 mg l^{-1} NAA and mg l^{-1} of each BA or Kin or 2iP. (a and b): shoot tip explant at the end of fourth subculture note root formation also notice undifferentiated leaf primordial.

3.4.3. Shoot formation percentage

One of the main goals of this study was to evaluate the effects of various combinations of PGRs on shoot bud proliferation in the date palm Siwy cv. The results showed no shoot formation noticed during the first, second, and third subcultures. After 32 weeks of culture, higher percentage of shoot bud proliferation (36.36%) was observed on medium supplemented with NAA+BA+Kin+2iP at 1 mg l^{-1} each. Treatment 5 produced 9.2 shoots per explant after 32 weeks Table 4 and Fig 3 c, e and Fig 4 a, b). Shoot formation did not occur in the other culture hormonal treatments. Regarding the precise origin of shoots and roots formation; it has been noted that basal parts of shoot tips were more responsive to direct plant regeneration. this result was consistent with those of Ahmed, Chokri [8], who reported that basal part of leaves was more responsive than its surface to direct and indirect plant regeneration.

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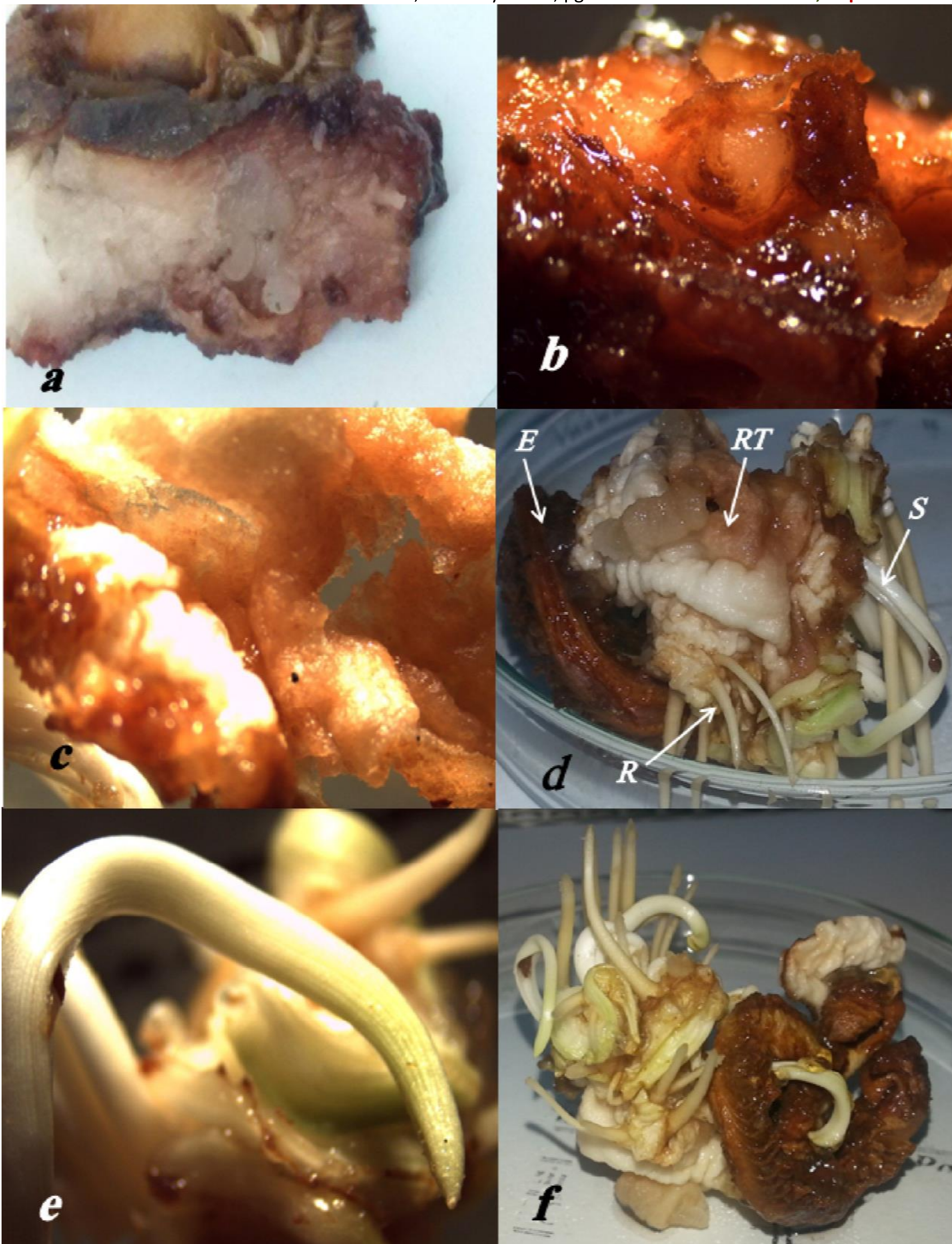

Fig.3: Direct regeneration of shoots derived from shoot tip explants Siwy cv. after the fourth subculture of culturing on 1 mg l^{-1} for each NAA+BA+Kin+2ip. (a): internal view of shoot tip explants at the end of the second subculture, note meristemoids (M) formed at the internal layer at the basal part of original explant (b): An enlarged view for meristemoids at the end of the second subculture, (c): note the regenerative tissue (RT) which seems to be an active dividing cells and these cells develop further to give meristemoids for both shoots and roots, (d): shoots (S), roots (R) and regenerative tissue(RT) formed in the original explants, (E) at the end of the fourth subculture, (e): shoots, (f): shoots and roots formed in the original explants..

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Fig. 4: Direct regeneration of shoots derived from shoot tip explants cv. Siwi after the fourth subculture of culturing on 1 mg l^{-1} for each NAA+BA+Kin+2ip.

IV. DISCUSSION

The increase fresh weight and swelling degree as the first indicator of growth and tissue response. The growth is the most complex physiological processes known so far, it is one of the salient features of the attributes of living matter (Srivastava, 2002). The current obtained results clearly showed that there was a gradual increase in swelling degree from the first subculture to the fourth subculture. The primary mode of action of the known natural growth regulating substances is probably their action on membrane systems, particularly their control of ion fluxes. This in turn could lead to many of the biochemical changes which they are known to induce response in specific ways [32].

Auxin/cytokinin is responsible for the initiation of cell division, i.e., re-entry of cells into the mitotic cell cycle [32, 33]. Cell division seems to be regulated by the joint action of auxin and cytokinins each of which appears to influence phases of cell cycle, auxin exerts an action on DNA replication, while cytokinins were required only for mitosis [34].

In contrast for date palm, the most commonly used cytokinin added to date palm micropropagation is 2iP. The exogenous auxin/2iP balance was used in several date palm investigations [9, 18, 35, 36]. It seems likely that, 2iP is presumably is a suitable cytokinin that triggers cell division. Kinetin and BA are nearly equivalent in effectiveness, perhaps BA being slightly more effective [30].

Tissue browning is a physiological disorder observed in many plant species during *in vitro* propagation. Browning of date palm tissues is a major problem affecting its micropropagation through either somatic embryogenesis or organogenesis [21]. This phenomenon, caused by the high levels of caffeoylshikimic acids contained in date palm tissues [37], hampers the multiplication efficiency of explants and leads to their death [38].

Physical characters and culture condition play a major role in the process of browning. High temperature during incubation increased browning. Abohatem, Zouine [39] illustrated that reducing the time between subcultures decreased the intensity of browning in somatic embryogenesis. The Culture was grown under dark also showed less browning than those incubated under light conditions [26]. Mazri [22] indicated that plant growth regulators and carbon sources might affect the intensity of tissue browning during organogenesis.

In order to reduce browning in date palm tissues cultured *in vitro*, researchers have incorporated compounds such as activated charcoal and polyvinylpyrrolidone (PVP) [37]. Activated charcoal prevented discoloration by adsorbing phenolic and rendered polyphenol oxidase and peroxidase inactive, thus increasing explant survival and organogenesis [40]. Whereas, it has been postulated that this browning could be minimized without addition of charcoal to the nutrient medium by presoaking of explants in an anti-oxidant solution of 150 mg l^{-1} of citric acid and 100 mg l^{-1} of ascorbic acid, employing small explants, i.e. apical meristem, and re-culturing them to fresh medium after a short period of incubation [41].

Concerning morphological observation, Prior morphogenesis and after active cell division, it appears that localized active cell division leading to *de novo* meristemoids and subsequent root organogenesis *in vitro*. The results also indicated that the compact large-size meristemoids usually formed roots (Fig. 1 a, b, c, d, e and f).

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Typically, overt organogenesis is preceded by the appearance of small precursor of cell population or meristemoids as described by Roubelakis-Angelakis and Van [11]. Regeneration process of plants *in vitro* firstly started with undergoing the pattern of cell division leading to meristematic center or meristemoids. This was consistent with Thrope (1970) who mentioned that meristemoids are considered as a key of the morphological feature of *de novo* organogenesis. The cells of meristemoid characteristically are small, isodiametric and strong walled with densely staining and prominent nuclei.

Precocious rooting (early rooting) is another major problem affecting of date palm micropropagation by organogenesis. During the starting stage, roots are formed on cultured explants instead of buds. The appearance of roots in this stage inhibits of bud formation and leads to culture elimination [14] because nutrients are diverted to root formation instead of to shoot bud proliferation [42].

These results suggest that the high NAA level in the medium enhancing root formed on cultured explants and instead of adventitious buds formation Table 4. This was consistent with the findings of Abahmane (2011) who mentioned that the auxin NAA proved to be an important plant hormone for induction of rhizogenesis in date palm plantlets. Some studies have shown that root initiation requires high auxin concentration, especially NAA 3 mg l^{-1}. In contrast, root elongation occurs on culture medium with low auxin concentration 0.1 mg l^{-1}.

Cytokinins can sometimes induce or promote root growth [43]. The interactions affect axillary shoot growth. Also, showed that NAA or BA at it's the highest concentration used suppressed shoot growth. This result indicates that NAA must be lowered than 1 mg l^{-1}. This confirms with Mater (1990) who indicated that NAA must be lowered to the range 0-0.01 mg l^{-1} if the objective is to attain maximum shoot growth within BA range of 0-1 mg l^{-1}. Barendse, Croes [44] reported that when supplied NAA and BA at equal concentrations for 24 h. the NAA uptake was up to 10-fold higher than BA uptake.

Whereas, it has become clear that the effects of a particular hormone are dependent on other hormones and that the applied hormone may trigger changes in the endogenous levels of the other hormones. There is also evidence that endogenous IAA levels are affected by the application of different exogenous plant growth regulators. Cytokinin may also alter the level of auxin in some tissue. Lau and Yang [45] reported that kinetin increases the free auxin content of *mung bean* hypocotyl segments exposed to IAA both by enhancing IAA uptake and suppressing the conversion of IAA to indoleacetylaspartic acid. Kinetin increases the auxin content of *coleus blumei*, though the major increase was in bound auxin [46].

Moreover, the time of year when explants are introduced *in vitro* seems to have an important effect on root formation. Rooting percentage of 16% and 40% are observed when explants were cultured respectively in July and December (Anjarne and Zaid 1993) cited after [14]. Furthermore, low concentrations of mineral nutrients in culture medium and incubation of culture in darkness for long periods also lead to early rooting of buds [19, 47].

One of the main goals of this study was to evaluate the effects of various combinations of PGRs on adventitious buds initiation in the date palm Siwy cv. Exogenous PGRs interact with endogenous plant hormone to induce or stimulate morphogenesis in cultured tissues. It is surely understood the control of organogenesis (organ formation) in the majority of cultures is largely a function of the quantitative interaction between the exogenous auxin/cytokinin ratio within a particular range of all type of growth including organ formation [24, 48]. This is completely dependent on the type of explants and the plant species [49].

The results showed that adventitious buds were produced on medium supplemented with NAA+Kin+BA+2iP 1 mg l^{-1} each after 4 subcultures. In date palm cultivar, Medjool and Mazafati Rad, Zarghami [18] reported that the best treatment was 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} NOA (naphthoxyacetic acid), 1 mg l^{-1} BA and 1 mg l^{-1} 2iP (4.51 buds). Khierallah and Bader [7] indicated that The medium supplemented with 2 mg l^{-1} 2iP and 1 mg l^{-1} BA, 1 mg l^{-1} NAA and 1 mg l^{-1} NOA gave a better result in the date palm cultivar, 'Maktoom'. In the cultivar Hillawi the maximum response (66.67%) was observed on medium supplemented with 1 mg l^{-1} BA and 0.5 mg l^{-1} TDZ [4]. Hegazy [17] recommended NOA 0.5 mg l^{-1} in combination with equal level of BA 1.0 mg l^{-1} , kin 1.0 mg l^{-1} and 2iP 1.0 mg l^{-1} were recorded the highest significant values of higher significant percentage values of axillary bud growth (33.33%) cv. Khlass. These results could simply reflect differences in the requirements for exogenous PGRs among date palm genotypes.

The results of this study showed that culturing organogenic clusters without any PGR, or with only an auxin or a cytokinin, was no effective than culturing such clusters on a combination of an auxin and a cytokinin so, the composition of induction medium is important for adventitious buds development. This was consistent with th findings of Gaspar *et al*. (1996) who illustrated that many aspects of cell growth and organogenesis in tissue and organ cultures are controlled by the interaction between cytokinins and auxins.

The presence of cytokinin is critical for buds induction and differentiation from explants of date palm [4]. It is

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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 cell cycle control [50]. Al-Mayahi, (2014) cited after Duhoux [51] illustrated that the formation of the organs in the monocotyledonous is generally enhanced by the addition of the cytokinins.

Cytokinins promote the unfolding of a complex gene expression program in tissue culture that results in the formation of shoots [52]. Rad, Zarghami [18] reported that the presence of cytokinins such as BAP and 2iP in culture medium, causing bud regeneration and by reducing them, initiation of vegetative buds was reduced. Khierallah and Bader [7] indicated that the superiority of BA over other cytokinins (Kin and 2iP) for the initiation and development of buds.

The results of this study confirmed that the date palm cultivar 'Siwy' required both exogenous auxin and cytokinin for efficient shoot bud initiation. Combined optimum of adventitious rooting and axillary shoot growth could be obtained at NAA concentrations between 0.01-1 mg l^{-1} and BA excluded from the media [53].

V. CONCLUSIONS

The *in vitro* protocol for direct organogenesis of date palm Siwy cv. using shoot tip explants was developed. Medium containing 1 mg l^{-1} NAA+1 mg l^{-1} 2iP+1 mg l^{-1} BA+1 mg l^{-1} Kin is recommended as a component of culture medium. The key morphological feature of *in vitro* organogenesis is the formation of the meristematic center or meristemoid. The cultures produced adventitious shoot buds directly at the basal part of shoot tips. Direct organogenesis methods undergo serious technical problems, such as browning and early rooting. Our results will be beneficial for the efficient micropropagation of this elite date palm cultivar.

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