Endogenous Hormones and Amino Acids Contents Influence Somatic Embryogenic Potential of *Phoenix dactylifera* L.

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Abstract

Somatic embryogenesis is a means by which plants can regenerate bipolar structures from somatic cells. Furthermore, a high frequency and synchronous embryogenic system is necessary to obtain the complete advantages of somatic embryogenesis. Additionally, examining the regenerability of embryogenic callus (EC) and its ability to develop further into advanced stages of somatic embryos (SEs) could therefore help in the mass production of date palm SEs. For that reason; the embryogenic callus (EC), non-embryogenic callus (NEC) and the other developing SEs phases [pro-embryos (PE) and fully developed SEs (FDSEs)] were undergone to determine the endogenous plant growth regulators (PGRs) as well as different amino acids levels. Indole-3-acetic acid (IAA), gibberellic acid (GA_3), zeatin (Z) and 2-isopentenyladenine (2iP) concentrations reached their lowest levels in EC in comparing with the other developing SEs phases (PE and FDSEs). However, NEC had the greatest level of ABA. Furthermore, FDSEs had high levels of GA_3 and total cytokinins (CKs). In contrast to FDSEs and NEC, which had high 2iP/zeatin ratios, EC and PE had elevated IAA/CKs ratio. IAA/ABA ratio increased significantly in PE. On either hand, EC had the highest significant quantities of aspartic, alanine, histidine and lysine than PE and FDSEs. Whereas, NEC had the highest significant levels of glycine, tyrosine, and phenylalanine, but no proline was detected in NEC or PE. Serine, glutamic, and arginine levels were high in FDSEs. In PE, the level of threonine increased considerably. These determined biochemical components are highly of importance since the discovery that the induction and expression of somatic embryogenesis are totally dependent on their endogenous levels from PGRs and free amino acids.

Key words: Date palm, somatic embryos, phytohormones, amino acids fractionation.

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Introduction

Phoenix dactylifera L. is one of the oldest cultivable crops and an important multipurpose tree. It is one of the most significant cash crops in the Middle East, accounting for over 90% of the global production (Aslam and Khan, 2009). Somatic embryogenesis procedure comprises induction, proliferation, histo-differentiation, maturation and germination phases. Furthermore, these stages are similar to zygotic embryogenesis and the development of

somatic embryos (SEs) is unconnected to the parent tissues (Von-Arnold *et al*., 2002). Although the basic mechanisms of plant regeneration (somatic embryogenesis and organogenesis) have been extensively studied, little is known about their influencing elements, benefits, limitations, and applications in crop development (Desai *et al*., 2022). Explants responding to endogenous cues during cell differentiation, triggering the development of signaling responses and, as a result, modifying the cell's gene program (Grzyb *et al*., 2017; Méndez-Hernández *et al*., 2019). Because endogenous phytohormone levels are closely related to explant differentiation and regeneration in tissue culture, optimizing the application of plant growth regulators throughout different stages of somatic embryogenesis may allow SEs to develop and mature successfully (Jiménez *et al*., 2005). It's easy to establish the embryogenic callus cultures in date palm, when the shoot tip explants are placed into a culture medium containing 2,4-dichlorophenoxy acetic acid (2,4-D) (Othmani *et al*., 2009; Sané *et al*., 2012; Zein Eldin and Ibrahim, 2015). However, use of 2,4-D in high concentrations resulted in formation of black and necrotic calli after 9th week of culturing date palm shoot tips, while low concentrations produced creamy, compact and globular calli (Aslam and Khan, 2009). Jiménez and Bangerth (2001) observed that two types of calli as a result of continuous 2,4-D treatment; the first one of calli developed into pre-globular and globular embryos, the other one was translucent, watery and lacking of any sign of organization. Additionally, it has been demonstrated that the EC of palms is highly heterogeneous in structure, with non-synchronously developing embryos and non-accessory dividing cells (Tisserat and De Meason, 1980). Furthermore, a recent study by Zein El Din *et al*., (2021) showed that the date palm calli of Barhee cv. might develop into EC tissues or degenerative embryogenic calli (DEC). The latter one had some anatomical and biochemical features which led to a stop cell division state and inhibit the further development of EC cells into embryos. In this regard, Sung and Okimoto (1983) found that carrot callus cultures initiated in high cell density in 2,4-D free medium generated a mixture of malformed early embryos and callus. Moreover, Jiménez and Bangerth (2001) found that wheat callus cultures lost their embryogenic competence as a result of long culture, having lower endogenous IAA levels nearly equaling those found in NEC. As a result, endogenous IAA concentrations may be a key indicator of embryogenic competence and determining the endogenous hormones during induction, development, maturation, and SEs conversion is highly of importance for a rapid embryogenic system (Ivanova *et al*., 1994). Ibrahim *et al*. (2005) found a positive correlation between high endogenous levels of IAA, GA_3 and the potential of date palm shoot tip explants cv. Bartamuda to respond to 2,4-D induction treatment. Exogenous auxin especially 2,4-D is usually necessary to initiate somatic embryogenesis, in order for the dynamic processes of IAA synthesis (Michalczuk *et al*., 1992), transport, conjugation, degradation, and distribution to happen, exogenous auxin must be lowered when cells begin to organize in tissues and at the beginning of embryo histo-differentiation (Márquez-López *et al*., 2018). While exogenously supplied cytokinins (CKs) are required for induction and maintenance of embryogenic calli cultures, endogenous CKs may hinder the expression of embryogenic competence in many plant species. High endogenous CKs content was found in non-embryogenic lines (Wenck *et al*., 1988; Rajasekaran *et al*., 1987). Moreover, Sáenz *et al*. (2010) reported that the embryogenic capacity of *Cocos nucifera* L. was correlated with the

endogenous cytokinins contents. Exogenously applied ABA is well known to regulate many physiological processes during embryo maturation, enhance the formation of high quality SEs, and inhibit precocious germination (Ammirato, 1985; Rai *et al*., 2011). Saeedpour *et al*. (2021) found that ABA levels in Barley Golden promise cv. (which had high regeneration ability) were almost seven times lower than those in Iranian cultivar (which had low regeneration ability). Similar findings were obtained by Tretyakova *et al*. (2021), who found that the embryogenic cultivar of L. sibrica explants had a lower ABA level than the non-embryogenic cultivar. A complex hormonal regulatory system influences the growth and development processes at every stage of somatic embryogenesis. As a result of this control, a number of metabolic processes are exhibited, including the metabolism of sugars and amino acids. It is well known that the latter metabolite act as receptors to transmit hormonal signals into the proper physiological responses (Bartos *et al*., 2018). Pinto *et al*. (2019); Méndez-Hernández, *et al*. (2021) on the other hand, confirmed that the auxin homeostasis mechanism carried out by Gretchen Hagen 3 (GH3) proteins through amino acid conjugation is a vital step in the induction of somatic embryogenesis. Generally, organic nitrogen (N) is a growth limiting factor in plant tissue culture, when glutamate was added, the lag phase was reduced. Addition of date palm tissue culture media with high concentrations of organic nitrogen, particularly glutamine, improved callus induction, stimulated embryogenesis, and increased SEs proliferation (Abo El-Nil, 1986; Zounie and El Hadrami, 2007). Moreover, during the embryogenic process, amino acids function as the pivotal components for protein synthesis and as a crucial form of nitrogen transportation (Bartos *et al*., 2018). Since amino acids are stored throughout embryonic development and are regarded as a source of nutrition in the early stages of plant development, this metabolite is often quite significant (Cangahuala-Inocente *et al*., 2013). Further, Sen *et al*. (2002) investigated the endogenously presence of several amino acids during different morphogenetic processes reflecting a probable requirement for special amino acids for specific events. Therefore, it's critical to distinguish some biochemical and physiological events associated with each stage of date palm somatic embryogenesis and understand the factors that contribute to calli's loss of embryogenic competence, such as the endogenous hormones and free amino acids. Furthermore, it is important to recognize obstacles that face date palm somatic embryogenesis in order to overcome them and enable the continuous production of mature SEs.

Materials and methods

1. Establishment of date palm somatic embryogenesis

This study took place at the tissue culture lab of the Central Laboratory for Date Palm Researches and Development, Agricultural Research Center (ARC), Giza, Egypt and the Departments of Agricultural Botany and Biochemistry, Faculty of Agriculture, Ain Shams University from 2019 to 2022. Offshoots of date palm Medjool cv. were selected, gathered from Bahariya Oasis, Giza governorate and used as starting material for somatic embryogenesis pathway. The callus cultures were then established by culturing shoot tip explants on 3/4 MS (Murashige and Skoog, 1962) medium supplemented with 10 mg L^{-1} 2,4-D and 3 mg L^{-1} 2-isopentenyladenine (2iP). Repetitive culture of explants was required

on the same freshly medium composition to callogenesis (every 6 weeks for 6-8 months). Friable embryogenic callus was formed when 2,4-D was replaced by a weaker auxin such as naphthalene acetic acid (NAA) at 0.1mg L^{-1} for another two subcultures. For somatic embryos (SEs) maturation, friable embryogenic callus was placing on medium included 0.5 mg L^{-1} abscisic acid (ABA), 5 g L^{-1} polyethylene glycoal (PEG- 4000) and 10 g L^{-1} phytagel as described by Zein Eldin and Ibrahim (2015). During the maturation period, while some friable calli grew in a proliferative manner without any SEs formation, the other portion of friable calli grew, became more nodular and globular in appearance as well as different developmental stages of somatic embryos were formed. On the other hand, all the cultures were incubated under total darkness at 27± 2°C until the appearance of fully developed somatic embryos (FDSEs). Morhophological observations and photomicrographs were carried out using Stereo Microscope with Integrated LED llumination and Digital 3 MP Camera Leica EZ4 D. Finally, the samples (EC, PE, FDSEs and NEC) were harvested for hormonal and amino acid analyses.

2. Biochemical analyses

2.1. Hormones separation by HPLC

2g fresh tissue was homogenized with cold 70% (v/v) aqueous methanol HPLC grade and stirred for 24hr at 4°C. The extract was filtered through a Whatman filter paper No. 42 and the methanol was removed by evaporation under vacuum. The remaining aqueous solution was pH-adjusted to 8.5 using 0.1 M phosphate buffer and then three times partitioned with ethyl acetate. After the ethyl acetate phase was eliminated, 1 N HCl was used to bring the pH of the aqueous phase to 2.5. The solution was three times partitioned with equal volumes of ethyl acetate, the combined ethyl acetate phase was evaporated under vacuum to dryness, and then the residue was dissolved in 2.0 mL of methanol HPLC-grade and kept in vials at 4°C until separation by HPLC. HPLC separation was carried out according to Kelen *et al*. (2004) method using Agilent 1260 series. The separation was conducted using an water 1525 binary HPLC pump, an Eclipse C18 column (1.3x300 mm) The mobile phase consisted of acetonitrile (HPLC grade 99.9%) water (26:74; 30:70% ; v/v). In these media, 30 mM phosphoric acid was adjusted to different pH values with sodium hydroxide. The separation was conducted by an isocratic elution system with a flow rate of 0.8 mL/min. An injection volume was 10µL used for each of the sample solutions. The column temperature was maintained at 25°C.water 2489 UV/ VIS detector was monitored at 254 for acids and 269 for cytokinin.

2.2. Free amino acids separation by HPLC

2 g tissue was macerated in 10mL Methanol 70% for 24hr at 50°C. After cooling, extract was filtered using Whatman filter paper No.1 , then the extract was evaporated under vacuum .The dried extract was dissolved in1 ml distilled deionized water. The precolumn derivatization with OPA reagent was carried out according to Wang *et al*. (2010) method as follows : 70 µL extract was derivatized with 10 µL of OPA reagent (O-phthalaldehyde and 3-mercaptopropionic acid) at 25°C and pH of 9.5 for 2 min. This mixture was immediately separated by HPLC. HPLC analysis was carried out according to Henderson

and Brooks (2010). Method using an Agilent 1260 series. The separation was carried out using Eclipse Plus C18 column (4.6 mm x 250 mm i.d., 5 µm).The mobile phase consisted of solvent A (buffer Sodium phosphate buffer pH 7.8) and solvent B (acetonitrile: Methanol: water 45:45:10 v/v) at a flow rate 1.5 ml/min. The mobile phase was programmed consecutively in a linear gradient as shown in Table (1):

Time (Min)	A %	B %
0.00	98	2
0.84	98	2
33.40	43	57
33.50	0	100
39.30	0	100
39.40	98	2
40.0	98	2

The flow rate was 0.8 mL/ min, injection volume was 10 µL, and the column temperature was maintained at 40 °C. The analysis was monitored by DAD at 338nm (Bandwidth 10 nm).The Fluoresence detector was adjusted as the following: - From 0 to 25 min at 340/450 nm (Excitation/Emission) and from 25 to 40 min at 266/305nm (Excitation/Emission).

2.3. Statistical analysis

The experiment had a completely random design; the mean and standard deviation of the three replicates for the biochemical data were introduced. According to Snedocor and Cochran (1980), results were statistically analyzed using Costat software (1998- 2005), and compared with Tukey's Studentized Range test by the honest significant difference (HSD) at $p \leq 0.05$ levels between treatments.

Results

3.1. Hormonal analysis

Embryogenic callus (EC) was triggered to be competent and differentiate into SEs when cultured on maturation medium supplemented with ABA and PEG at 0.5mg L^{-1} and 5 g L^{-1} respectively. However, during the sub-culture system a part of date palm embryogenic calli lose their regeneration ability, stop dividing and fail to develop further to form SEs and are recognized as degenerative embryogenic calli (DEC) as described by Zein El Din *et al.* (2021). During the current investigation, the analysis of endogenous hormones in the EC, pro-embryos (PE) and fully developed SEs (FDSEs) revealed that, there was a gradual increase in IAA, GA_3, 2iP and ABA concentrations from EC to FDSEs as shown in (Table 2) While zeatin level increased from 0.078 mg $100g^{-1}$ FW in EC to 0.109 mg $100g^{-1}$ FW in PE then, dropped again in FDSEs (0.094 mg $100g^{-1}$ FW). Concerning the endogenous plant hormonal ratios among the different developmental stages of date palm SEs cv. Medjool, data clearly showed that IAA/CKs ratio recorded high levels in EC and PE (0.489 and 0.484, respectively). However, FDSEs and NEC contained lower levels of IAA/CKs ratio (0.248, 0.252, respectively). While PE contained the highest IAA/ ABA ratio (0.804), NEC

had the lowest level of this ratio (0.162). Nevertheless, FDSEs contained the highest significant ratio of 2iP/zeatin (4.649) followed by NEC (3.56).

Table (2): Variation in endogenous plant hormones concentrations and ratios between embryogenic callus, Non- embryogenic callus afs well as in pro-embryos and fully developed SEs of date palm cv. Medjool.

Endogenous hormones mg 100g^{-1} FW	Developmental stages				HSD p≤0.05
	EC	PE	FDSEs	NEC	
IAA	0.083C±0.0086	0.107B±0.0116	0.128A±0.0115	0.125AB±0.0085	0.0191
GA_3	3.19C±0.251	9.11B±0.486	26.20A±0.629	25.22A±2.989	2.9201
Zeatin	0.078B±0.0155	0.109A±0.00901	0.094AB±0.0106	0.112A±0.0156	0.0244
2iP	0.095B±0.1167	0.113B±0.0241	0.431A±0.0871	0.387A±0.060	0.1029
Total CKs	0.173B±0.027	0.222B±0.036	0.526A±0.082	0.498A±0.045	0.0959
ABA	0.138C±0.003	0.133C±0.0033	0.231B±0.0016	0.768A±0.0073	0.0083
IAA/CKs	0.489A±0.086	0.484A4±0.093	0.248B±0.0585	0.252B±0.0344	0.1068
IAA/ ABA	0.603B±0.0487	0.804A±0.0773	0.553B±0.0481	0.162C±0.01195	0.0979
IAA/ GA_3	0.026±0.0042	0.0118±0.0013	0.0049±0.00056	0.0050±0.000267	NS
2iP/Zeatin	1.22B±0.094	1.025B±0.176	4.649A±1.2302	3.560A±1.545	1.5369

The bars show the average over three replicates ± standard deviation; according to the Tukey's Studentized Range (HSD) test, different letters (a-e) indicate significant differences ($p \leq 0.05$).

In comparison to EC, NEC had greater concentrations of endogenous IAA, zeatin and 2iP (0.125, 0.112 and 0.387 mg 100g^{-1} FW, respectively). Additionally, the levels of GA_3 and ABA were around 6 and 8 times higher in NEC than EC one (25.22 and 0.768 mg 100g^{-1} FW, respectively). However, EC had considerably higher ratios of IAA/ total CKs, IAA/ ABA and IAA/ GA_3 (0.489, 0.603 and 0.026, respectively) than NEC (0.252, 0.162 and 0.005, respectively). 2iP/ zeatin ratio on the other hand, increased significantly in NEC (3.56) than EC (1.22).

3.2. Amino acids

Roowi *et al*. (2010) reported that amino acids function as a basic component for the synthesis of protein and as an important form in nitrogen transportation during embryogenic process. Data presented in Table 3 show variation in free amino acids concentrations (mg 100 g^{-1} FW) in different developing stages of date palm somatic embryos (cv. Medjool) as well as in NEC. Cocerning the different developmental stages, EC contained the highest significant levels of aspartic (ASP), α-alanine (ALA), valine (VAL), isoleucine (ILE), leucine (LEU), histidine (HIS), lysine (LYS), arginine (ARG) and proline (PRO) (5.631, 48.598, 39.607, 17.45, 34.66, 5.248, 24.68, 66.939 and 16.496 mg 100 g^{-1} FW, respectively). The highest concentrations of serine (SER), glutamic (GLU), and arginine (ARG) were found in FDSEs (38.086, 73.289 and 70.268 mg 100 g^{-1} FW, respectively). On the other hand, PE had the highest concentration of threonine (THR) 16.351 mg 100 g^{-1} FW. When comparing NEC with EC, it was found that NEC included more glycine (GLY), tyrosine (TYR), and phenylalanine (PHE) (13.225, 30.341, and 29.465 mg 100 g^{-1} FW, respectively) than EC. While, EC had a higher concentrations of ASP, ALA, HIS, LYS and proline than NEC.

Table (3): Variations in the level of some endogenous amino acids in different developmental stages of date palm cv. Medjool (embryogenic callus, pro-embryos and somatic embryos) and within non- embryogenic callus.

Amino acids mg 100 g^{-1} FW.	Different developmental stages of somatic embryogenesis				HSD
	EC	PE	FDSEs	NEC	p≤0.05
Asparatic (ASP)	5.631A±0.46	0.000D±0.00	0.774C±0.03	2.407B±0.57	0.69
Threonine(THR)	13.087BC±1.23	16.351A±0.60	11.396C±1.58	13.521B±0.36	2.00
Serine(SER)	32.028B±1.80	19.085C±0.81	38.086A±1.17	17.947C±0.27	2.18
Glutamic (GLU)	6.890C±1.17	21.003B±1.42	73.289A±0.77	6.0910C±0.87	2.05
Glycine (GLY)	8.800B±1.89	6.896B±1.09	6.099 B±2.09	13.025A±2.15	3.49
Alanine(ALA)	48.598A±1.04	44.532B±2.19	24.486D±2.03	36.719C±1.69	3.38
Valine (VAL)	39.607A±3.05	23.235B±3.70	22.870B±1.99	39.967A±1.33	5.04
Isoleucine (ILE)	17.45A±0.45	8.01B±1.39	10.59B±1.43	17.32A±1.81	2.66
Leucine (LEU)	34.660A±2.76	17.193B±1.32	12.698B±1.54	31.231A±4.96	5.67
Tyrosine (TYR)	4.7220D±0.81	16.517B±2.52	13.271C±0.34	30.341A±1.43	2.87
Phenylalanine (PHE)	17.091B±2.36	4.200C±0.53	6.680C±0.09	29.465A±2.13	3.04
Histidine (HIS)	5.248A±0.17	2.794B±0.18	1.306C±0.28	2.769B±0.13	0.37
Lysine (LYS)	24.680A±0.49	17.472B±1.75	6.568C±1.43	5.460C±2.45	3.17
Arginine (ARG)	66.939A±4.94	51.634B±2.72	70.268A±5.63	8.515C±0.98	7.56
Proline (PRO)	16.496A±0.99	0.000C±0.00	2.711B±0.89	0.000C±0.00	1.25
Cysteine (CYS)	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	ND
Methionine	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	ND
Total	341.93A±1.36	248.92C±6.10	301.10B±6.81	254.78C±10.14	12.92

The bars show the average over The bars show the average over three replicates ± standard deviation; according to the Tukey's Studentized Range (HSD) test, different letters (a-e) indicate significant differences ($p \leq 0.05$).

Discussion

4.1. Phytohormones

It is well established that external auxins and CKs are the principal plant growth regulators (PGRs) involved in the control of cell division and differentiation (Feher *et al*., 2003). As a result of exogenously supplied PGRs, induction of signaling response was triggered which modifies the gene program of the cells. Upon, somatic cells are induced to generate cells with embryogenic potential; the new cells can form structures which have an ability to regenerate into complete plantlets (Méndez-Hernández *et al*., 2019). The current study's data supports Wu *et al*. (2021) 's finding that all estimated endogenous phytohormones increased in cotyledonary stage and decreased in EC. According to their research, EC was better at encouraging embryogenic development since it contained less CK, GA_3, and ABA.

4.1.1. Auxins

The results of the present study revealed that EC had the lowest level of IAA content, because auxin is necessary for pre-embryonic cell mass (PEMs) proliferation but inhibits their development into somatic embryos, endogenous IAA must be decreased to enable organized embryo growth (Li *et al*., 2022). However, PE contained higher level than EC. The later finding is in line with those of Loschiavo *et al*. (1991), who found that carrot pro-embryos don't synthesize auxin before they become polarized at post-globular stage.

Regarding gradual increase in IAA levels from PE to FDSEs, Márquez-López *et al*. (2018) found that endogenous IAA is localized in the cells that are generating the apical shoot meristem (SAM) and root meristem. Furthermore, the latter authors discovered that the cells that formed the cotyledon had a high content of IAA. Moreover, our data are in line with Tran *et al*. (2016) results which indicated that at day 21, when some globular embryos of banana began to form, the IAA concentration in somatic embryos arose. While, at day 35, when SAM formation started, it then decreased sharply. Furthermore, Pérez-Pastrana *et al*. (2021) discovered a positive association between an increase in IAA levels and the cell differentiation, elongation and maturation during both zygotic and somatic embryogenesis of *Capsicum chinese* Jacq. A loss of embryogenic potential of calli may be due to a long time of culture as described by Jiménez and Bangerth (2001). The hormonal analysis of date palm NEC showed that it was contained higher IAA than EC, this finding insured the previous results related the fact that IAA levels must be regulated during the early stages of embryonic differentiation and when cells begin to organize in tissues (Márquez-López *et al*., 2018). According to a recent paper by Zein El Din *et al*. (2021), the overabundance of naringenin and the presence of cinnamic acid in date palm (cv. Barhee) degenerative embryogenic callus (DEC) were the causes of the NEC's inability to develop into somatic embryos. Naringenin is considered as a precursor of most flavonoids as well as being a negative regulator of auxin transport (Steenackers *et al*., 2017). Also cinnamic acid inhibits the polar transport of IAA especially during globular phase (López Arnaldos *et al*., 2001). It is well documented that auxins are the prominent participants in the process of establishing cell polarity (apical-basal axis). It has been proposed that the formation of bilateral symmetry during plant embryogenesis depends on the polar transit of auxin in early globular embryos (Liu *et al*., 1993). These findings indicated that IAA transportation is essential for the embryos' proper growth.

4.1.2. Cytokinins

The common agreement is that low concentrations of cytokinins (CKs) encourage auxin-induced embryogenesis, while large quantities are thought to hinder this response. Our results show that, compared to EC, pro-embryos and SEs showed high levels of zeatin. This finding is consistent with that of Tran *et al*. (2016), who found that zeatin levels dramatically increased at day 35 when the development of banana somatic embryo organs has occurred. During this period somatic embryo apical meristem differentiation started at the late globular stage. Regarding the high level of 2iP and total CKs recorded in FDSEs among the other developing stages, it could be possible owing to the *de novo* synthesis of CKs. It is well known that natural cytokinin biosynthesis appears to take place mostly at the roots, although some production does occur in other actively growing tissues (Chen *et al*., 1985). 2iP on the other hand is the most widely distributed CK in nature and was the second CK to be isolated from natural sources. It occurs both naturally and as a constituent of tRNAs (Zhu *et al*., 1995). Additionally, an increment CKs during the maturity of hazelnut seeds may be an indication of preparation for eventual germination and seedling growth, in which these CKs play a significant role (Centeno *et al*., 1997). Data provided throughout the current investigation show that total endogenous CKs ratios and levels may serve as precise indicators of the embryogenic response. Total CKs levels fell more in EC

than NEC. These findings confirm earlier results acquired by Pintos *et al*. (2002), who found that the total CKs level increased in *Medicago arborea* L. NEC. Zeatin and GA_3 contents increased significantly in date palm NEC than in EC under the current investigation, in this respect (Ammirato, 1977) reported that zeatin and GA_3, especially when combined, both raise the frequency of aberrant forms. Zeatin induces the formation of multiple shoots, leafy and aberrant cotyledons, and in the dark, larger hypocotyls; GA_3 affects root elongation and polycotyledony development. Also data obtained here showed that the 2iP and total CKs contents decreased significantly in EC than NEC. The latter findings could be explained by the fact that 2iP serves as a biosynthetic precursor for the synthesis of another CK required for cell division but not for the later stages of SEs formation and maturation (Centeno *et al*., 1997; Fraga *et al*., 2016). Therefore, a low recorded level of 2iP in EC cells may be due to its consumption to synthesize those needed for triggering cell division, while 2iP being at high level and don't shift to zeatin like compounds in NEC. Moreover, Zhu *et al*. (1995) investigated that while inoculated rice calli grown on MS medium supplemented with a sufficiently high 2iP level did yield a considerable quantity of embryogenic callus, the majority of them failed after a few days. In this regard, Pintos *et al*. (2002) made the assumption that isopentenyl derivatives at high concentrations don't seem to favor the development of embryogenic response. Additionally, it was found that over production of endogenous CKs caused an over accumulation of phenolic compounds, increase in peroxidases (POD) activities and moderately high lignin content (Schnablová *et al*., 2006). In this respect, Zein El Din *et al*. (2021) reported that formation of DEC was related with accumulation of phenolic compounds, H_2O_2 and over accumulation of naringenin.

4.1.3. Abscisic acid (ABA)

Our observations, showing that endogenous ABA concentrations rise in parallel with somatic embryos (SEs) development, may help to explain other studies' findings which showed that ABA plays a crucial role in promoting somatic embryogenesis. ABA has an impact on various SEs features, especially during the somatic embryo maturation phase, when it controls the synthesis and deposition of storage compounds, improve the SEs quality, inhibit precocious germination, promotes desiccation tolerance and causes somatic embryo dormancy or quiescence (Rai *et al*., 2011; Fraga *et al*., 2016). The results of the current study indicate that date palm EC contained a moderate level of ABA, this result supports the earlier results obtained by (Ackerson, 1984) which showed that there was an accelerated accumulation of ABA during cell division and an extensive synthesis of RNA and DNA. However, ABA content decreased in PE then peaked in date palm FDSEs under the current investigation. The latter finding may assist in understanding why ABA was previously believed to be a dormancy inducer and was well known for its inhibitory effects on seed germination according to Rock and (Quatrano, 1995). ABA seems to selectively inhibit the synthesis and translation of mRNA's encoding germination enzymes such as isocitrate layse as described by (Ackerson, 1984). Data given in Table 2 regarding ABA content revealed that EC possessed a lower level than NEC. This result was consistent with that of Saeedpour *et al*. (2021), who discovered that the ABA level in the cultivar Golden Promise (which had a high regeneration rate) was approximately seven times lower than an

Iranian cultivar (which had lower regeneration rate). Also, Kępczyńska and Orłowska (2021) found that ABA level in EC was over six times lower than NEC of *Medicago truncatula* Gaertn. Furthermore, the fact that young embryos respond to low levels of ABA by promoting growth (at this stage, the embryonic tissue is primarily meristematic) may help to explain why NEC accumulates more ABA than EC. However, once cell division and differentiation stop, embryos primarily transform into storage organs. At this stage, ABA suppresses growth while having little effect on protein synthesis. It is well known that low quantities of ABA have been demonstrated to stimulate somatic embryogenesis in *in vitro* culture medium, whereas high concentrations, on the other hand, prevent it (Seldimirova *et al*., 2019).

4.1.4. Gibberellic acid (GA_3)

GA_3 level peaked in FDSEs, followed by PE. This noticeably levels of endogenous GA_3 may be related to expansion growth which occurred especially in the PE as shown in (Zein Eldin and Ibrahim, 2015) study. According to their findings, date palm pro-embryos (mid embryogenesis) grown *in vitro* were distinguished by their high level of dehydroascorbic acid (DHA), which stimulates the cell wall extensibility needed for this type of growth and causes cell enlargement. Thus, it could be a strong association between expansion growth during the middle stage of somatic embryogenesis and endogenous GA_3 and DHA. Additionally, the over-accumulation of GA_3 in FDSEs may bring support to the theory that it plays a vital role in the germination of both zygotic and somatic embryos. Our presented data are in accordance with (Rock and Quatrano, 1995) who reported that although GA_3 is found in seeds in relatively high concentrations, its main function appears to be related to the early stages of embryo development, followed by germination and seedling growth (GA catalyze α-amylase mRNA which requested for starch hydrolyzation and germination of embryos). Otherwise, GA might be crucial for the development of xylem and lignification in carrot roots (Wang *et al*., 2017). Besides, results obtained here also showed that NEC contained approximately eight fold increase of GA_3 than EC; this over-accumulation of it may inhibit the development of early stage SEs by inducing abnormal development of SEs. In this respect Cordewener *et al*., (1991) discovered that more expanding and vacuolation of carrot pro-embryo masses resulted in formation of un-normal SEs and the exogenously supplied of peroxidase enabled these pro-embryo masses to develop further by stopping of such undesirable processes. Further, Noma *et al*. (1982) found that a nonembryogenic strain and undifferentiated carrot cells had high polar gibberellin levels. Moreover, recently it was detected that date palm DEC which failed to develop further had a higher level of DHA than EC one (Zein El Din *et al*., 2021). Thus it could be assumed that a more accumulation of GA_3 as noted from the current study and DHA as detected in a recent report (Zein El Din *et al*., 2021) may use as negative biochemical markers for inducing the formation of such type of calli.

4.1.5. Hormonal ratios

The interaction between endogenous hormones can be expressed by their ratios, which can be used to evaluate the balance of endogenous hormones at different developmental stages of somatic embryos (Wu *et al*., 2021). Concerning IAA/ CKs ratio, EC and pro-embryos contained the high ratios of them. However, FDSEs and NEC contained low levels of this

estimated parameter (0.248 and 0.252 respectively). However, FDSEs had the highest significant 2iP/zeatin ratio. Our data are in agreement with Vondrakova *et al*. (2018) who found that the IAA/ CKs ratio peaked during maturation (3week after culturing the tissues on ABA containing medium). The authors suggested that a high IAA/CKs ratio is needed for proper formation of apical and root meristems. In this regard, Leljak-Levanic *et al*. (2016) noted that the auxin/cytokinin ratio is crucial for sustaining the embryogenic potential. The regeneration capacity of the habituated embryogenic line of pumpkin was not observed to be positively correlated with high cytokinin levels, which may be related to the involvement of other growth regulators. Also, their data reflected that mature SEs contained the lowest level of IAA/ CKs during desiccation stage. The results obtained here related to IAA/ABA and IAA/GA_3 were in agreement with those of Wu *et al*. (2021), who noted that IAA/ABA and IAA/GA_3 steadily reduced from the embryogenic callus to cotyledon stages of *Ormosia henryi* Prain. Also, the authors supposed that these low ratios were the cause of lost embryogenic ability of EC. Moreover, in the current study, date palm EC had endogenous hormonal ratios that were noticeably greater than NEC, despite having a significantly lower 2iP/zeatin ratio than the NEC. In this respect, Centeno *et al*. (1997) found that the cotyledons of embryogenic genotype of hazelnut (cv. Casina) contained nearly twice as much IAA/ABA as the non-embryogenic genotypes (cv. Negretta). Furthermore, the authors hypothesized that for the induction and expression of somatic embryogenesis in explants, the IAA/ABA ratio appeared to be more significant than their IAA or ABA contents. However, when compared to an EC, the NEC of wheat had a two-fold greater IAA/ABA ratio (Seldimirova *et al*., 2019). Furthermore, Wu *et al*. (2021) found that high IAA/ABA and IAA/GA_3 ratios favored EC induction in the early stages of SE. The somatic embryo's development and germination were enhanced by the low IAA/CKs ratio. While here, IAA/ABA in PE recorded the high level (0.804) among the other developmental stages and NEC. Concerning high ratio of 2iP/ zeatin in date palm NEC than EC, Our data support Pintos *et al*.(2002)'s report that the embryogenic potential of *Medicago arborea* L. calli arose as a result of a reduction in the 2iP/Z ratio.

4.2. Amino acids

It has been demonstrated that several endogenous amino acids play a role in differentiation and morphogenesis (Magnaval *et al*., 1995). As a result, figuring out the value of amino acids at each developmental stage of date palm somatic embryos may be a good marker for calli that well be oriented to embryogenic growth. Furthermore, it was shown earlier in study by Thom *et al*. (1981) that organic nitrogen (N) was selectively absorbed over inorganic nitrogen during early cell growth cycle. The rapidly dividing cells utilized glutamine and other amino acids as an energy source during this phase. Results related to determination of endogenous asparatic (ASP) revealed that EC contained the highest level, while it wasn't detected in pro-embryos (PE). Lack of free ASP in PE may be linked to its conjugation with free IAA, as IAA conjugation is essential for converting free IAA into an inactive form required for proper SEs polarity. In this respect, Méndez-Hernández *et al*. (2019) discovered preserved regions that might act as binding sites for nucleotides and certain amino acids for the conjugation of IAA during *C. canephora* embryogenesis. Furthermore, according to Abo EL-Nil (1986), peptidic-bound auxins (like indole

acetylglutamate and indole acetylaspartate) regulate free auxin levels to meet the needs of developing tissues and are also thought to be involved in the detoxification of applied auxins in date palm callus cultures. However, NEC contained a lower level of ASP than EC. In this regard, Maadon *et al*. (2016) showed that numerous amino acids found in embryogenic structures, including asparagine, glutamine and proline, are related to different metabolic processes. ASP is one of the essential elements of nitrogen metabolism, which involves frequent inter-conversion with arginine. The large increase in ASP content in date palm (cv. Medjool) EC may have been caused by its correlation with the high protein deposition that characterized this phase, as stated by Zein El Din *et al*. (2022). In this regard, Atkins *et al*. (1975) showed that asparagine metabolism occurred prior to the protein synthesis *via* asparaginase enzyme. According to the link between embryo growth and asparaginase activity, the enzyme's activity increases tenfold between five and seven weeks after anthesis and reaches a peak before the embryo exhibits its quickest rate of growth and protein deposition. Data concerning threonine (THR) content clearly revealed that pro-embryos had the highest amount of this kind of amino acid. However, NEC and EC had moderate amounts of THR. In this respect Yang *et al*. (2020) confirmed that besides the traditional function in the synthesis of intracellular proteins, essential amino acids (EAAs) also play other important roles in energy deficiency, including the direct entry of LYS and isoleucine (ILE) catabolism into the tricarboxylic acid (TCA) cycle and the conversion of THR and methionine into isoleucine. The latter suggestion may be a sign of a predominant requirement for protein synthesis and the highly metabolic activity which distinguish the EC. The existence of THR in NEC may also be explained by the histological analysis done by Zein El Din *et al*. (2021), since it was noted that cell division took place in both EC and NEC, and this process requires energy, which may lead to THR catabolism into ILE, then the latter enter TCA cycle upon its breakdown. However, NEC cells stop dividing state and degenerate (their cellular aggregates represent the initial ontogenesis of somatic embryos). Further, it is interesting to discover that the aspartate family pathway is also closely linked to GLY, and PRO as mentioned previously by Yang *et al*. (2020). GLY, and PRO are involved in stress-related amino acids. It is probably due to its consumption to detoxify the negative effects of oxidative stress, date palm NEC's lack of PRO throughout the conducted study may be understood. Our results indicated that serine (SER) content increased significantly in FDSEs among the other developmental stages (EC and PE). Serine is earlier described as being precursor of tryptophan for endogenous auxin synthesis. Since date palm FDSEs had a considerable level of endogenous IAA during the current study, it is likely that increasing the SER content of FDSEs will be utilized for the *de novo* synthesis of IAA. According to our results, FDSEs had the largest quantity of glutamic acid (GLU), which increased by nearly eleven and four fold, respectively, as compared to EC and PE. It was discovered that glutamine and glutamic acid were very stimulatory in inducing embryo differentiation (Kamada and Harada, 1984). Furthermore, the high accumulation of GLU in FDSEs may be explained by earlier finding that the cytosol and chloroplasts are the two compartments in the leaf cells of higher plants where glutamine is synthesized (Durzan, 1987). As reported earlier, date palm embryogenesis was greatly accelerated when glutamine was added together with 2,4-

D to the culture medium (Zouine and El Hadrami, 2007). According to their results glutamine induced rapid cell proliferation, increased SEs numbers and active protein synthesis of cvs. Jihel and Bousthami Noir. Moreover, exogenous application of glutamine greatly enhanced the quality of formed SEs in peanuts (Murch *et al*., 1999). The latter authors discovered that although fewer embryos were produced when proline or glutamine were added to the culture media, the resultant embryos were large, green, and developed more synchronously. Glutamine is regarded as the preferred endogenous amino acid involved in plant metabolism, providing nitrogen for the production of amino acids, nucleic acids, and acting as an amino group donor in the transamination reactions (Jeyaseelan and Rao, 2005). The amount of glycine (GLY) in the EC, PE, or FDSEs did not alter significantly, according to our data; however, the level of GLY in the NEC increased. GLY probably acts as protector for cell membrane from osmotic and temperature stresses during isolation of cucumber leaf mesophyll protoplast and plant regeneration (Orczyk and Malepszy, 1985). Recently, Zein El Din *et al*. (2021) data 's revealed that date palm calli that were losing their ability to produce embryos had elevated levels of malondialdehyde (MDA) and H_2O_2. In *Ficus deltoidea*, H_2O_2 also performs the role of an oxidative stressor, causing damage to cell structure, cell membrane, and cell loss their function (Nurnaeimah *et al*., 2020). Therefore, the rise in GLY level in oxidatively stressed date palm NEC may be explained by glycine's ability to protect cell membranes from the toxic effect of H_2O_2. Moreover, Glycine-rich LEA (late embryogenesis abundant) proteins are hypothesized to be crucial for membrane stabilization and protecting proteins from becoming denatured when the cytoplasm dehydrates (Janska *et al*., 2010). Date palm EC had the highest concentration of α-alanine (ALA), which, according to Kamada and Harada (1984), was incorporated into developing cells and embryos during the development of globular embryos and sped up cell division and proliferation while active protein synthesis was occurring. α-ALA seems to be rapidly converted to glutamic acid by alanine aminotransferase after being incorporated into cells and used as a nitrogen source. Concerning the Valine (VAL), ILE and LEU contents which were increased significantly in EC than PE and FDSEs, VAL has primarily been described as a reduced nitrogen source in popular culture, but its presence is often essential for callus or cell growth (Magnaval *et al*., 1995). Also, This results are in a harmony with Schmidt *et al*. (1997) who reported that, a leucine-rich repeat receptor-like kinase-encoding gene has been discovered to be specifically up regulated during the extremely early stages of the SE process, while the majority of the molecular SE markers discovered with the last phases of embryo development. Additionally, during the current investigation, the contents of VAL, ILE, and LEU were comparable in both EC and NEC. Both EC and NEC have extremely high metabolic and dividing rates, as we have already mentioned, thus their roles are critical during this phase. VAL is involved for callus development, (Magnaval *et al*., 1995), and direct entry of LYS and ILE catabolism into the tricarboxylic acid (TCA) cycle during the cases needed high energy (Yang *et al*., 2020). Moreover, Batista-Silva *et al*. (2019) reported that owing to its function as an osmo-regulation element, isoleucine is essential for plant stress resistance. The authors also investigated that abiotic stress in plants results in a variety of alterations to amino acid metabolism. Tyrosine and phenylalanine were

present here in higher concentrations in date palm NEC than EC. These findings are consistent with those of Polesi *et al*. (2022), who found that the bamboo *G. chacoensis* NEC contained higher levels of certain aromatic amino acids. According to Tzin and Galili (2010); Ng *et al*. (2016), who proved that; in addition to being necessary elements for protein synthesis, the aromatic amino acids phenylalanine, tyrosine, and tryptophan in plants act as precursors for a variety of secondary metabolites that are crucial for plant growth. Additionally, the aromatic amino acids act as precursors for a number of plant hormones, including auxin and salicylate. According to the latter finding and the data from this study, which showed that NEC had higher quantities of phenylalanine, tyrosine, and endogenous IAA than EC, this type of callus' capacity to develop into successive SEs was inhibited. Lysine (LYS) content peaked significantly in EC followed by PE. While FDSEs and NEC contained the lowest levels of this amino acid. Regarding pro-embryos (early stage of SE), Angelovici *et al*. (2011) found that although lysine is considered as an essential amino acid, a large increase in its concentration in Arabidopsis seeds inhibits germination. Several metabolic responses are regulated by the accumulation of lysine such as starch biosynthesis, unfolded protein responses, and maintaining the normal osmotic potential. The aspartate family pathway is complicated in plants and is influenced by lysine metabolism, which also has an impact on other metabolic processes (Yang *et al*., 2020). Also, the latter authors proposed that during stress responses, LYS metabolism activates the jasmonate signal pathway and tryptophan metabolism. In respect of arginine (ARG) content, date palm FDSEs and EC had significant amounts of this amino acid. This result may be explained by Allona *et al*. (1994) finding's, which showed that ARG is thought to be a storage globulin-specific amino acid because of its high nitrogen concentration. Moreover, ARG is the primary source of N for the formation of nitrogenous compounds in the developing seedling (Canovas *et al*., 2007). ARG plays a crucial role in the formation of polyamines *via* the arginine decarboxylase pathway which has a regulatory role in embryogenesis (Minocha *et al*., 2004). Moreover, Polesi *et al*. (2022) found that ARG content increased in EC of bamboo *G. chacoensis* than NEC. The authors reported that several amino acids, including ALA, GLU, ARG, SER, and PRO, asparagine, glutamine are also tightly linked to the metabolism of carbohydrates. These amino acids are directly catabolized, releasing tricarboxylic acid cycle precursors or intermediates (TCA). Additionally, Ng *et al*. (2016)'s findings that fingerroot ginger's EC contained higher amounts of ARG and LYS than NEC, which are consistent with those reported here.

Our research showed that the highest concentrations of ARG, SER, and glutamic acid were found in FDSEs. These observations are in line with those of Khan *et al*. (2014). Their findings suggested that ARG and SER were considerably more abundant in SEs of *Silybum marianum* L. than in NEC. It has been demonstrated that few endogenous amino acids contribute to morphogenesis and differentiation (Magnaval *et al*., 1995). The latter authors confirmed that the coconut calli which exhibited strong tendency towards embryogenesis were distinguished by rising proline, valine, and leucine contents. Proline is a stress-related amino acid, required for the induction of somatic embryogenesis, and provides organic nitrogen in addition to act as a stress buffer during *in vitro* culture and embryogenesis (Sen *et al*., 2002). Caligo *et al*. (1985) discovered that both serine and proline affect the growth

of carrot SEs. While, proline enabling growth and differentiation modifies the usual pattern of ontogeny, serine impacts growth by preventing cell division after a certain phase. By the other words, the addition of both of them at the time zero after hormonal removal from the carrot culture medium was important, serine arrested the development of SEs at the globular forms. While, proline induced globular structures lacking normal expression of polarity to develop further and triggered some severely malformed torpedoes to differentiate into normal plantlets. According to the finding of Zein El Din *et al*. (2021) which indicated that NEC of date palm was made up of cohesive cellular aggregates that represented the initial ontogenesis of somatic embryos (the majority of these aggregates were in the globular stage) and displayed a cessation of cell division, may be a good explanation for the lack of proline in NEC during the current investigation. Furthermore, our results indicated that serine content was around 1.78 times higher in EC than NEC. The latter discovery confirms the finding of Caligo *et al*. (1985) that serine affects the polarity of carrot SEs. Hisidine (HIS) content recorded high significant value in date palm EC, then decreased gradually in PE and FDSEs. In Valencia sweet orange, early somatic embryogenesis is characterized by the expression of a gene called CsHPt1, which Maul *et al*. (2005) have identified as encoding a histidine-containing phosphotransmitter protein that is also elevated in other tissues with embryogenic potential.

Conclusion

The findings given here may aid in the clarification of the biochemical and physiological changes that take place during date palm somatic embryogenesis as well as recognition the factors that contribute to calli's lack of embryogenic competence. it was shown that the induction and expression of somatic embryogenesis are entirely dependent on their endogenous levels from PGRs and free amino acids, these identified biochemical components have gained significant importance. While, EC had lowest concentrations of IAA, GA_3, Z, and 2iP, the high amounts of GA_3 and total cytokinins (CKs) were found in FDSEs. The most contents of GA_3 and ABA were found in NEC. Furthermore, IAA/ABA, and IAA/GA_3 ratios were lower in NEC than EC. The most significant concentrations of aspartic, alanine, histidine, lysine, and proline were found in EC than the other embryonic developing stages (PE and FDSEs). On the other hand, NEC did not contain any proline and had the greatest significant quantities of glycine, tyrosine, and phenylalanine. The amounts of serine, glutamic acid, and arginine were elevated in FDSEs. Threonine content significantly rose in PE.

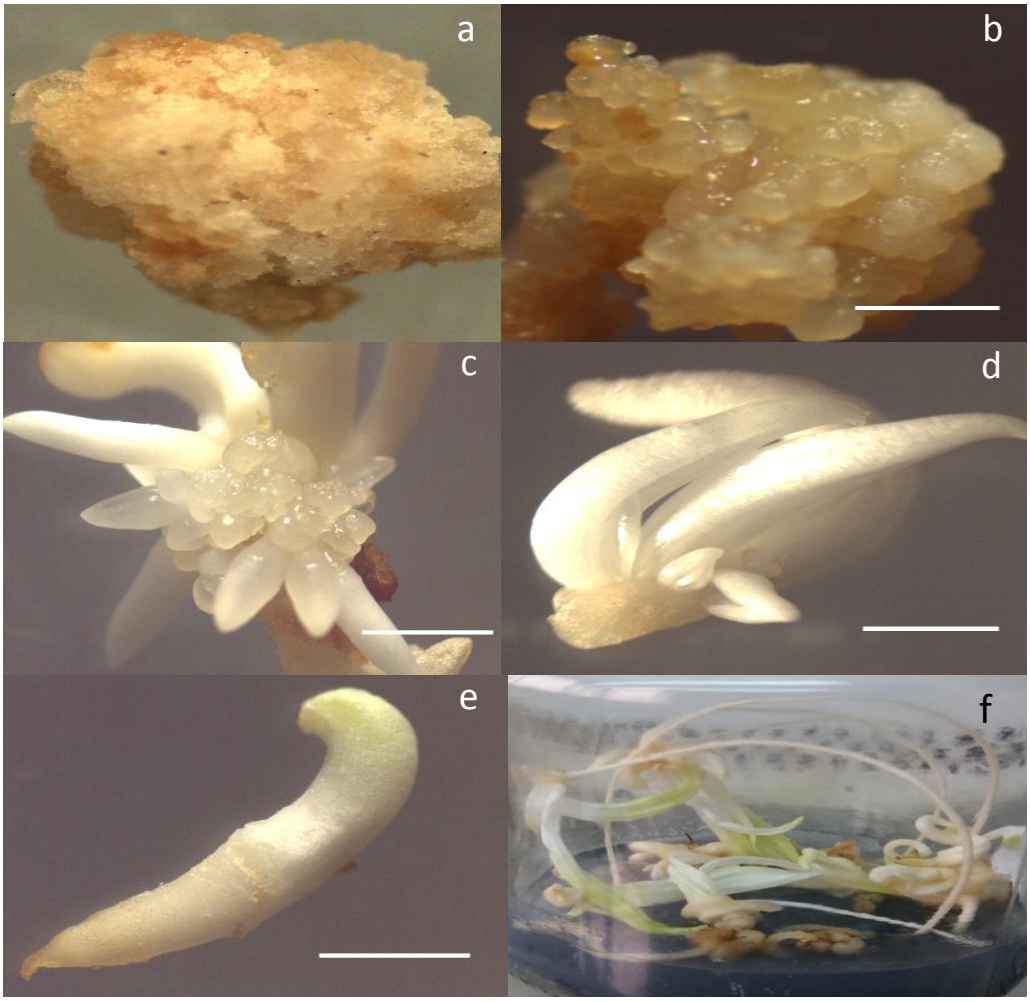

Fig. (1): Somatic embryos in date palm cv. Medjool at different developmental stages as well as non embryogenic callus NEC. (a): NEC; (b): embryogenic callus (EC); (c) early and late stages of somatic embryos; (d, e): cluster and individual of somatic embryos, (f): germination and conversion of somatic embryo into plantlets. Bar; a, b = 0.1 cm, c = 0.5 cm and j = 0.7 cm.

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تأثير الهرمونات النباتية الداخليه والأحماض الأمينية على القدرة الجنينية لنخيل البلح

Phoenix dactylifera L.

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الملخص العربي

توفر وسيلة إكثار النباتات خضريا داخل المعمل عن طريق أنتاج الاجنة الجسدية إنتاج عالى ومتزامن للأجنة الجسدية ثنائية الأقطاب. وكذلك يمكن أن يساعد إختبار مدى كفاءة وقدرة الكالس الجنينى (EC) على التطور لإنتاج أجنة جسدية وتحولها لنبيتات نخيل البلح معمليًا. ولهذا السبب تم تقدير الهرمونات النباتية الداخلية والاحماض الأمينية فى الكالس الجنيني (EC) ، والكالس الغير جنيني (NEC) والمراحل المتطورة الأخرى للأجنة الجسدية (مبادئ أجنة وأجنة كاملة التكوين) لما لهذه المكونات من دور فعال فى تطور الكالس الجنينى وتكشفه إلى أجنة جسدية وبالتالي إنبات الأجنة إلى نبيتات كاملة. وصلت تركيزات إندول-٣- حامض الخليك (IAA) وحمض الجبريليك (GA3) والزياتين (Z) و ٢-إيزوبنتنيل أدينين (2iP) إلى أدنى مستوياتها في الكالس الجنينى (EC) ومع ذلك، وصل حمض الأبسيسك (ABA) إلى أعلى مستوياته فى الكالس الغير جنينى (NEC).

علاوة على ذلك، إحتوت ألاجنة الكاملة التطور (Fully Developed Somatic Embryos) مستويات عالية من GA_3 و السيتوكينينات الكليه (زياتين وايزوبنتيل ادنين).

وعلى عكس الأجنة الجسدية (SEs) والكالس الغير جنينى (NEC)، المحتويان على نسب عالية من 2iP /zeatin، إحتوى الكالس الجنينى ومبادئ الأجنة على معدل مرتفع من IAA / CKs ، بينما زاد نسبة IAA / ABA فى مبادئ الأجنة.

بينما أشارت نتائج تحليل و تفريد الأحماض الأمينية على إحتواء الكالس الجنينى (EC) على أعلى كميات معنوية من الأسبارتيك، والألانين، والهيستيدين، والليسين، والبرولين، بينما أحتوى الكالس الغير جنينى (NEC) على أعلى مستويات معنوية من الجليسين، والتيروزين، والفينيل ألانين، ولكن لم يتم إكتشاف البرولين في هذا النوع من الكالس الغير متكشف (NEC) أو مبادئ الأجنة (Pro-Embryos). كانت مستويات السيرين والجلوتاميك والأرجينين عالية في الأجنة المكتملة التكوين (FDSEs)، بينما زاد مستوى الثريونين بشكل كبيرفى مبادئ الأجنة(Pro-Embryos).

تعتبر هذه المكونات البيوكيميائية ذات أهمية كبيرة منذ اكتشاف أن الحث والتعبير عن التطور الجنيني الجسدي (Induction and expression of somatic embryogenesis) يعتمدان كليًا على المستويات الداخلية من منظمات النمو (PGRs) والأحماض الأمينية الحرة، حيث تلعب الهرمونات النباتية والأحماض الأمينية دورا فعالًا فى تكشف و تطور الاجنة الجسدية.

الكلمات الدالة: نخيل البلح، الأجنة الجسدية، الهرمونات النباتية، تجزئة الأحماض الأمينية.