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ORIGINAL ARTICLE

THE EFFECT OF PUTRESCINE AND SPERMIDINE ON SOMATIC EMBRYOGENESIS AND REGENERATION OF DATE PALM (*PHOENIX DACTYLIFERA* L.) CV. BARHEE

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Abstract: The present study was conducted to determine the effect of different concentrations of putrescine and spermidine at all stages of regeneration (callogenesis, somatic embryos multiplication, germination and rooting)) of date palm cultivar Barhee. Shoot tips were eradicated from 2-3 years old offshoots, surface sterilized and inoculated onto Murashiege and Skoog, 1962 (MS) medium supplemented with 20 mg/L 2,4-D and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was obtained after 24 weeks on the nutrient medium. Calli were then transferred onto fresh MS medium containing 0.0, 50, 100 or 150 mg/L of putrescine or spermidine individually. Results were recorded after 12 weeks. A significant increase in embryonic callus fresh weights reached 4.093 g at the concentration 100mg/l of Spermidine and 3.817 g at 100 mg/L of putrescine. Embryogenic callus was developed on MS media using different concentration 0,50,100 or 150mg/L of putrescine or spermidine. Results indicated that the highest embryo number reached 28.67embryo at the concentration 100mg/L of spermidine. The highest significant root number 5.20 root/plant appeared with rooting medium supplemented 100mg/l of Spermidine. Addition of Putrescine as a supplement to the rooting medium at concentrations 100mg/l reached 2.60 root/plant. It is concluded that both putrescine and spermidine may play a positive role in increasing callus growth and regulation of somatic embryogenesis in *Phoenix dactylifera* var. Brahee tissue cultures.

***Key words*:** *Phoenix dactylifera* L., Embryos, Regeneration, Spermidine, Putrecine.

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1. Introduction

Rapid propagation of date palm through tissue culture is the most promising technique for production of sufficient plant materials with high quality. The benefit of using this technique is the production of true to type, disease free. Moreover, production is on a commercial scale [Sane *et al.* (2006)]. In reviewing date palm micropropagation, several reports have been published. The early attempts via zygotic embryos have been done by Schroeder (1970). However, various explants tissues have been examined *i.e*. shoot tip, leaf primordial, axillary bud, root and finally inflorescence. Organogenesis and somatic embryogenesis are the two techniques currently used in various laboratories in the world for *in vitro* mass propagation of date palm. However, other *in vitro* pathway involved the induction of embryogenic callus prior to somatic embryogenesis which reveals a number of advantages over other *in vitro* techniques. This indirect somatic embryogenesis technique allows the succession cultures of cell suspension or secondary embryos [AL- Khayri and Naik (2017)]. Amino acids such as L-Glutamine, Casein hydrolysate, L-Asparagine and Adenine are generally used as source of organic nitrogen in culture media growth of date palm callus tissue was significantly stimulated by the addition of amino acids specifically glutamine, which plays an important role in nitrogen assimilation as it is an intermediate in the trsansfer of

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ammonia into amino acids [Elmeer (2013)]. The most common types of polyamines are spermidine, spermine and their diamine precursor, putrescine. Beneficial effects of polyamines on *in vitro* regeneration and somatic embryogenesis were documented in several crops. Moreover, the promoter role of polyamines in the conversion of somatic embryos or shoot regeneration has been evidenced in several plant species [Aydin *et al.* (2016)]. Hegazy and Aboshama (2010) recorded the highest multiplication rate of date palm embryo by adding 100mg/L putrescine. They also suggested an efficient novel pathway in date palm tissue culture protocol by induction of direct somatic embryogenesis from bud tissues cultured in a medium containing through putrescine at 150 $mg.L^{-1}$ along with plant growth regulators. Ibrahim *et al.* (2014) reported that a significant increase in embryonic callus fresh and dry weights of date palm cv. Bream was recorded reached 2.3 and 0.3 g, respectively at 2.0 mM of putrescine and 5.0, 0.27 g at 3.0 mM of salicylic acid. Number of mature embryos increased up to 10.3 achieving 1.7 g fresh weight for ten embryos at the concentration of 3.0 mM putrescine.

Recently El-Dawayati *et al.* (2018) reported that date palm culture media with organic nitrogen, especially high concentrations of glutamine improved callus induction and rate of growth and stimulated embryogenesis. The best callogenetic response was obtained when 100 $mg.L^{-1}$ spermidine alone or in combination with higher concentration of glutamine (EA) in solid media was used. Higher concentration of glutamine (EA) combined with putrescine in the liquid media produced maximum number of excellent somatic embryos (32.33) with good callus induction. On the other hand, highest embryonic callus fresh weight (5.49 g) was produced by the explants contained spermidine alone. Therefore, the aim of this study is to examine the effect of two types of polyamines (putrescine and spermidine) on enhancing somatic embryos induction from embryonic callus during maturation stage of date palm cv. Barhee, in order to develop micropropagation cycle of date palm plantlet.

2. Materials and Methods

Offshoots of Barhee cultivar (2-3 years old) were chosen. Leaves were dissected acropetally. Shoot tips of 3 cm in length (apical meristem with soft inner leaves), were eradicated along with immature fiber of 2 cm in diameter. Explants were dipped in antioxidant solution consisted of 150 mg/L citric acid plus 100 mg/L ascorbic acid. Explants were surface sterilized with 20% sodium hypochlorite solution containing eight drops of Tween-20 as emulsifier for 15 minutes and rinsed three times with sterile distilled water. They transferred to Petri dishes where leaf primordia were removed except the two pairs surrounding the apical meristem which then divided longitudinally into four equal segments and cultured in jars aseptically. The medium used in the initiation stage was Murashig and Skoog(1962) (MS) salts plus the following (in mg/L): thiamine-HC1 1.0; pyridoxine-HCl 1.0; adenine sulfate.$2H_2O$ 40; myo-inositol 100; $NaH_2PO_4.2H_2O$ 170; sucrose 30000 activated charcoal 2000 and agar-agar 7000. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or HC1 [Ahmed (2020)], before the addition of agar. The medium was dispensed into culture jars with aliquots of 25 ml in each, then covered with polypropylene caps and autoclaved under 1.04 kg/cm^2 at 121°C for 15 minutes. Callus initiation medium was supplemented with 20 mg/L 2, 4- D and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was obtained after 24 weeks of growth in full darkness. Calli were then transferred onto fresh MS medium containing different concentrations 0.0, 50, 100 or 150 of putrescine or spermidine individually, with regular transfer to fresh medium of the same supplements every 4 weeks. Cultures were incubated in a growth room under low light intensity of 1000 lux for 16 hours daily at 27±1°C for four weeks. Results of callus fresh weights, number of germinated embryos were recorded after 12 weeks. And after that germinated embryos transfer to medium with different concentrations 0.0, 50, 100 or 150 of putrescine or spermidine individually, supplemented with 1 mg/l NAA plus 1 mg/l BA allowed asexual plantlets to develop within 8-16 weeks in culture, number of leaves/plant and root number per explant were recorded [Almemary *et al.* (2020)]. Experiments were conducted as factorial using Complete Randomized Design (CRD), with ten replicates. Least significant differences (LSD) were used to compare means at 5% level probability.

3. Results and Discussion

Callogenesis of date palm

Data in Table 1 showed a significant increase in fresh weight 4.093 g at the concentration 100mg/l of Spermidine followed by 3.280g at the concentration 50mg/l. Addition of Putrescine as supplement to the embryonic callus medium at concentrations 100mg/l led

Table 1: Callus fresh weight initiated on MS medium supplemented with 20 mg/L 2, 4-D and 3 g/L 2iP at different concentrations of putrescine and Spermidine for 24 weeks.

Concentration mg/l	Callus fresh weight g	
	Spermidine	Putrescine
0	1.375 d	1.357 c
50	3.280 b	2.650 a
100	4.093 a	3.817 a
150	2.243 c	2.717 b
LSD	0.339	0.293

to a significant increase in callus fresh weight reached 3.817g, while the lowest amount 1.375g was recorded in control treatment. Results are in accordance with those obtained by El-Dawayati *et al*. (2018) they reported that the highest embryonic callus fresh weight (5.49g) was produced by the explants contained 100 mg/L spermidine. Putrescine is a polyamine with low molecular weight. It has been implicated in many cellular processes such as cell division, protein synthesis and DNA replication. The recent work of Ravindra and Nataraja (2013) reported that putrescine enhances callus growth, somatic embryogenesis and plant regeneration in many plant species including Pinus gerardiana at a concentration of 2.0 mg/L, however, it is a genotype dependent.

Somatic embryos multiplication

Data in Table 2 shows that the number of formed embryos increased proportionally after the inclusion of Spermidine to the medium till reached to a significant level at the concentrations of 100 and 150 mg/l recording 28,67 embryos and 24.67 embryos respectively. Also the result shows that all levels of Putrescine resulted in a significant increase in number of formed embryos 25.33 embryos at the concentrations of 100 mg/l compared with the lowest number of embryos 16.33 embryos in control treatment. Similar results were obtained by Hegazy (2008) on date palm floral buds ''Selmy'' that embryos cultured on modified MS medium in addition to putrescine (100 mg/L) obtained significant values of multiplication rate and growth value as well as total soluble protein and PAL activity.

Table 2: Mean number of germinating embryos initiated from calli after supplementation with different concentrations of putrescine and Spermidine after 12 weeks.

Concentration mg/l	Embryo number	
	Spermidine	Putrescine
0	16.33 c	16.33 b
50	22.67 b	20.67 ab
100	28.67 a	25.33 a
150	24.67 b	21.33 a
LSD	3.686	4.922

leaf number

Regarding the effects of polyamine data presented in Table 3 indicated that embryos cultured on MS medium in the presence of 1 mg/L of each of NAA and BA in addition to Spermidine (100 mg/L) recorded the highest significant number of leaves 5.33 as compared with the other treatments. Also the result shows that the Putrescine at the concentrations 100 mg/l recorded the highest significant number of leaves 3.67 compared with the lowest number of leaves 2.33 in control treatment. Srivastava (2002) published that PAs metabolism is affected by auxins, cytokinins and gibberellins in several plant systems and that PAs are essential for many of the growth responses attributed to these hormones. Polyamines (PAs) are generally recognized as active regulators of plant growth. They are present in all cells and their millimolar titer is responsive to physiological effects caused by many

Table 3: Effect of different concentrations of Spermidine and Putrescine on the number of leave after 8 weeks of culture in the presence of 1 mg/L of each of NAA and BA.

Concentration mg/l	leaf number	
	Spermidine	Putrescine
0	2.33 c	2.33 b
50	4.33 b	2.67 ab
100	5.33 a	3.67 a
150	3.00 c	2.33 b
LSD	0.941	1.08

Table 4: Effect of different concentrations of Spermidine and Putrescine on the number of roots after 8 weeks of culture in the presence of 1 mg/L of each of NAA and BA.

Concentration mg/l	Root number	
	Spermidine	Putrescine
0	1.20 c	1.20 c
50	3.20 b	1.80 bc
100	5.20 a	2.60 a
150	3.80 b	2.40 ab
LSD	0.764	0.67

agents, such as hormones, light and stress, but their precise mode of action in plant growth and development is still unclear.

Root formation

Regarding the effects of polyamines types data presented in Table 4 indicated that individual shootlets cultured on basal MS medium supplemented with Spermidine (100 mg/L) in the presence of 1 mg/L of each of NAA and BA were recorded the highest significant values of growth characters' number of roots, compared with the control treatments. The highest significant root number 5.20 root/plant appeared with rooting medium supplemented with 100mg/l of Spermidine followed by 150 mg/l which gave 3.80 root/ plant, while the lowest root number 1.20 root/plant appeared at control treatment. Addition of Putrescine as a supplement to the rooting medium at concentrations 100mg/l reached 2.60 root/plant. Handa and Mattoo (2010) reported that Biogenic amines putrescine, spermidine and spermine are ubiquitous in nature and researchers because they are essential for cell division and viability and due to a large body of their pharmacological effects on growth and development in most living cell. Results are in accordance with those obtained by Srivastava (2002) who reported that because auxin application caused a large increase in PAs content, it was suggested that auxins act through PAs to promote growth in this tissue and the genetic analysis further indicated that high and low rooting responses were probably controlled by multiple genes.

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