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INFLUENCE OF SOME FACTOR ON SOMATIC EMBRYOS INDUCTION AND GERMINATION OF DATE PALM BARHI C.V BY USING CELL SUSPENSION CULTURE TECHNIQUE

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Abstruct

Various physical conditions of medium and different concentrations of Abscisic acid (ABA) and salicylic acid (SA) were examined for their effect on somatic embryo growth and on germination of somatic embryogenesis in *Phoenix dactylifera* Barhi cv. leaf primordial and shoot tips were excised from 2-3 years old offshoot. surface sterilized with 0.1% of mercuric chloride and inoculated onto Murashiege and Skoog, 1962 (MS) medium supplemented with 50 mg/L NAA and 3 mg/L N6-2-isopentyl adenine (2ip). callus was obtained after 24 weeks on the nutrient medium. Then established cell suspension culture after that studied physical condition for medium by transferred cells were resulted from cell suspension culture to four physically different media (semi solid media, fixed liquid media, movement liquid media by using shaker and movement liquid media by using plant form bioreactor) Results were recorded a highest value of fresh weight of somatic embryos and N.O of globular embryo by using liquid media especially movement liquid media by using plant form bioreactor was 0.210 g 162.0 embryo respectively while solid media gave highest value of Dry weight (g) and N.O of elongation embryo was 0.084 g, 62.0 embryo respectively. Different concentrations of ABA (0, 0.1, 0.5 and 1) % was effective on the germination and percentage of germination of embryos, were the 0.5 % of ABA gave highest number with significant different for number of germinating embryos and percentage of germination of embryos 42.58% and 35.28, while SA (0.1, 0.5 and 1) % not gave significant effect on same characters were control (0)% gave highest average 31.58 embryo and 26.16% respectively, the interaction treatment of ABA+SA(0.5, 0)% reached to(50.33 embryo and 41.7%).

Key words : *S*omatic Embryos, Germination, Palm Barhi.

Introduction

The propagation of date palm (*Phoenix dactylifera* L.) by Sexual method does not consider for commercial purposes due to the high degree of genetic heterozygous progeny. Traditional vegetative propagation through offshoots is taken into account, which is hindered by slow growth rate and give limited numbers of offshoots (Faki, 2017). While the production of date palm through in-vitro technique preferred widely for mass production of true-to-type plants (Aldaej *et al.,* 2014; Shehata *et al.,* 2014; Aleid *et al.,* 2015). This technique is successfully adopted using shoot tip, lateral buds, leaf primordial or inflorescences explants (Al-Khalifah and Shanavaskhan, 2012; Zayed and Abd El-Bar, 2015; Hussein *et al.,* 2016).In-vitro date palm plantlets are produced by direct organogenesis or somatic embryogenesis (Sidky and El-Dawayati, 2012; Abd El-Bar and El-Dawayati, 2014; Ali *et al.,* 2017). However, other in-vitro pathway involved the induction of embryogenic callus prior to somatic embryogenesis (Al-Samir, 2015), which detect a number of advantages over other in-vitro techniques. This indirect somatic embryogenesis technique allows the sequence cultures of cell suspension or secondary embryos. Several studies have been conducted to optimize somatic embryogenesis of date palm by culture in different medium ingredients and physical conditions (Al-Khairy and Al-Bahrany, 2012; Baharan *et al.,* 2015). Were The physical state of the medium plays an integral role in the growth and development of plant parts and is therefore a determinant of success for tissue culture. In addition the Ventilation Is important factor in the success of the parts cultured, the parts of plant in semi-submerged of liquid medium will reduce the important oxygen supply and thus

reduce the amount of liquid medium in the container or work more like bridges to make the vegetation float on the liquid surface and thus reduce the submerged volume, In other cases Submerge of the culture so shakers are used at the same time, it is necessary to take into account the damages caused by the shaking process resulting from the collision of the cells some of them and with the walls of the container planted in it. The liquid medium may provide good growth for the culture parts through the supply of nutrients in addition, the resulting secretions are diluted in the liquid medium (pierik, 1999). There is a lot of research confirmed the need to use the semi-soild medium for most stages of plant parts and is prepared by adding the material agar to the medium, Tisserate & Gabr (1985) emphasized the use of solid medium in all phases of cultivation because cultivation in liquid media performs what is known as tissue vitrification, in the same direction A study conducted by Othmani *et al*., (2009) showed that the semi solid medium stimulated the formation of somatic embryos and germination of embryos while the less germination of embryos in liquid media because occurrence vitrification, In another side some Researchers found that use of liquid medium gave positive results in increase fresh weight of callus and number of embryo, Were khieralla (2007) found the Agitated liquid medium by shaker gave highest adventitious bud 18 buds during of duration 54 days from inflorescences callus tissue of *phoenix dactyliferae* Barhi cv while the number of buds was 12.4 in semi solid media with duration 72 days. And recently Nayyef (2019) found best result by using liquid media in (Plant form Bioreactor) with change with different immersion duration, the result showed highest number of buds of phoenix dactyliferae 75.6 at 4 min / sec duration of immersion.

In general somatic embryogenesis protocol for date palm includes a series of consecutive stages beginning with callus induction, embryogenesis callus multiplication, somatic embryo maturation and somatic embryo germination (El Bellaj, 2000). In general, embryogenic calluses were induced on medium containing growth regulators, especially 2, 4-D and NAA (El Hadrami, 1995; ELBellaj, 2000; Fki *et al.,* 2003 and Gadalla, 2007). Maturity of somatic embryos may be induced via application of exogenous Abscisic acid (ABA) (El Bellaj, 2000). Bawis *et al*., (2015) found that ABA plays an important role in both zygotic embryo and somatic maturation. These same authors indicated that ABA promotes embryo maturation, supports the accumulation of storage proteins, starch and; lipids it prevent the formation of abnormal embryo structures and, finally prevents the mature embryo from germinating. Choi *et al*., (1999); Kim *et al.,* (1999) and Klimaszewska *et al.,* (2001) reported that the culture medium constituents particularly osmoticum, has a marked effect on somatic embryos. Also, the attempt to increase the quality of somatic embryos by using the high molecular mass osmoticum, PEG 4000, and ABA was accomplished by insertion of a maturation phase of culture between multiplication (maintenance) and regeneration phase. The combined application of ABA and PEG has become a routine method for stimulation of somatic embryo maturation in some gerera of coniferales (Bozhkov and Von Arnold 1998) and selected tree species such as *H. braziliensis* (Linossier *et al.,* 1997).

Salicylic Acid (SA) is One of the important phenolic compounds, containing an aromatic ring with a hydroxyl group or its derivatives, found in plants. (Aberg, 1981). Exogenously supplied SA was shown to affect a large variety of processes in plants, including stomatal closure, seed germination, fruit yield and glycolysis (Cutt *et al.,* 1992). Salicylic acid is widely used in organic synthesis and its function as a plant hormone. It appears to have a role in systemic acquired resistance to pathogens and is able to induce various pathogen resistance proteins (Goerge *et al.,* 2008).

Therefore the aim of this study is to examine various physical conditions of medium on somatic embryo growth and different concentrations of Abscisic acid (ABA) and salicylic acid on germination of somatic embryogenesis in *Phoenix dactylifera* Barhi cv.

Materials and Methods

Off shoots (2-3 years old) of Barhi cultivar were chosen and detached from mother palm. leaves were dissected acropetaly. Shoot tips of 2 cm in length with leaves primordial (apical meristem with soft inner leaves). Explants were dipped in antioxidant solution consisted of 150mg/ L ascorbic acid plus 100 mg/L ascorbic acid (Tisserat. 1991). Explants were surface sterilized via 0.1% chloride solution containing few drops of tween-20 for 15 minutes under vacuum, and rinsed three times with sterile distilled water, The medium of callus initiation stage was composed of Murashige and Skoog (1962) (MS) salts plus the following (in mg/L); thiamine-Hcl 1.0; pyridoxine-Hcl 1.0; adenine sulfate.$2H_2O$ 40; myo-inositol 100; $NaH_2PO_4.2H_2O$ 170; sucrose 3000 ; activated charcoal 2000 and agar-agar 7000. The pH of the medium was adjustes to 5.7 with 0.1 N NaOH or HCl, before addition of agar. The medium was dispensed into culture jars with aliquots of 25 ml and then covered with polypropylene caps and autoclaved under 1.04Kg/cm^2 at 121 C for 15 minutes callus initiation medium was

supplemented with mg/L of NAA and 3 mg/LN6-2-isopentyl adenine (2ip), primary callus was obtained after 20 weeks of growth in full darkness. The primary callus was later transferred in medium supplemented 50 mg NAA with same component as the medium above where it was obtained embryo callus, then established cell suspension culture by transferred 1 g of friable callus from semisolid media and inoculate into a 250 ml Erlenmeyer flask containing 50 ml MS liquid medium supplemented with 30 g /L sucrose, 1.5 mg / L 2ip, and 10 mg mg /L NAA without agar . incubated cultures at 16 h photoperiod of cool – white florescent light, 40 mmol/ m^2/ Second +- 23c and agitated at 150 rpm via shaker . maintain cultures by regular sub culturing at 2- week interval. the next stage was studied physical condition for medium by transferred cells were resulted from cell suspension culture to four physically different media (semi solid media, fixed liquid media, movement liquid media by using shaker and movement liquid media by using plant form bioreactor). We make a comparison among the medium as dry and fresh weight and number of globular and elongation embryos, the best physical condition of the four medium, it will be germination media in later stage by using different concentration of ABA (0, 0.1, 0.5, 1)% and SA (0, 0.1, 0.5, 1)% in order to study their effect on germinating embryos and percentage of germinating embryos.

Results and Discussion

Table 1: Effect of physical conditions of medium on the characters.

Treatment	Fresh weight (g)	Dry weight (g)	No. of globular embryo	No. of elongation embryo
Fixed liquid media	0.178	0.0577	156.7	30.0
Liquid agitated media	0.191	0.0627	159.3	36.0
Liquid media in PLB	0.210	0.067	162.0	40.0
Semi-solid media	0.169	0.084	120.7	62.0
LSD	0.017	0.0061	8.42	9.37

The liquid media in plant form bioreactor exhibited significant difference in fresh weight and number of globular embryo (0.21g,162.0 embryo) also Liquid agitated media and Fixed liquid media gave high value (0.191 , 0.178) g (159.3, 156.7) embryo for character respectively compared to Semi-solid media gave lowest value in fresh weight and number of globular embryo (0.169,120.7) respectively. But Semi-solid media exhibited a significant increase in both dry weight and number of elongation embryo (0.084 g, 62.0 embryo) respectively while Fixed liquid media, Liquid agitated media and Liquid media in PLB gave lowest value (0.0577, 0.0627 and 0.067)g (30.0,36.0 and 40.0) embryo for both characters respectively.

Table 2: Effect of different concentration of Abscisic acid and salicylic acid and their interaction on number of germinating embryos cultured in solid media.

ABA	SA				Mean of
	0	0.1	0.5	1	ABA
0	11	13	13.33	12.33	12.42
0.1	39	28.67	37.33	29	33.5
0.5	50.33	39	41.33	39.67	42.58
1	26	35	24	31	29
LSD	5.012				2.506
Mean of SA	31.58	28.92	29	28	
LSD	2.506				

The results of table 2 showed the concentrations of ABA at 0.5 % give highest value (42.58) embryo with significant increase compared with rest concentrations (0.1, 1)% gave (33.5,29) embryo, while the control (0)% gave lowest value (12.42) embryo.

The number of germinating embryos was decreased with increase concentrations (0.1, 0.5 and 1) % of SA while the control (0)% led to significant increase in number of germinating embryos (31.85)%. The result of same table showed the treatment of interaction ABA + SA (0.5 +0)% led to a significant increase (50.33) embryo beside that the treatment of interaction (0.1+0)% (0.1+0.5) %(0.5 +0.1) (0.5+0.5)% (0.5+ 1)% gave high value was (39, 37.33, 39, 41.33, 39.67) embryo respectively. While the treatments (0+0)% , (0 + 0.1)%, (0 + 0.5) % and (0 + 1)% gave lowest values (11, 13, 13.33 and 12.33) embryo respectively.

Table 3: Effect of different concentration of Abscisic acid and salicylic acid and their interaction on percentage of germinating embryos.

ABA	SA				Mean of
	0	0.1	0.5	1	ABA
0	9.11	10.77	11.04	10.22	10.28
0.1	32.31	23.74	30.93	24.02	27.75
0.5	41.7	32.31	34.24	32.86	35.28
1	21.54	28.99	19.88	25.5	23.98
LSD	4.107				2.054
Mean of SA	26.16	23.95	24.02	23.15	
LSD	2.054				

The addition of ABA table 3 as a supplement to the number of germinating embryos. at concentrations (0.5 and 0.1)% led to a significant increase in percentage of germinating embryos reached (35.28 and 27.75) respectively compared to control (0) gave 10.28%. also we found the concentration of SA 0% gave highest

percentage 26.16% with significant increase compared with the concentrations (0.1,0.5 and 1) gave lowest percentage (23.95, 24.02 and 23,15)%. The results of same table showed interaction between ABA and SA at (0.5+ 0)% achieved highest value of percentage (41.4)%, beside this the interaction treatments ABA+SA (0.1 +0), (0.1 +0.5), (0.5+ 0.1), (0.5+0.5), (0.5+1)% gave high percentage (32.31,30.93,32.31, 34.24,32.86)% respectively compared with treatments interaction (0+0), (0+0.1)(0+0.5) and (0+1)% gave lowest percentage of germinating embryos (9.11), (10.77), (11.04) and (10.22) % respectively .

Conclusion

The experiment of table1 showed that liquid media with difference physical conditions achieved increasing of fresh weight and Number of globular embryos , may be back to containment of the liquid medium for nutrient elements and high level of water that help cells to division and increased of fresh weight and number of globular embryos, but continuously stay of the cells for long period (from established cell suspension culture until culture in treatments of liquid medium) that may led to be a type of stress, this result decrease of its growth and next development stages assimilate of dry weight and number of the elongation embryos, in the other side we found the semi solid media give high average of dry weight and number of the elongation embryos may be back to need's of the cells to the desiccation after stay in long time in cell suspension culture and treatment of liquid medium, finally desiccation factor help to growth of cells with embryos maturation and development positively. Othmani *et al*., (2009) reported that semi solid media stimulated formation somatic embryogenesis and number of elongation embryos while liquid medium led to occurrence vitrification in date palm. Dawayati *et al.*, (2018) found the liquid media increase fresh weight of cells of *phoenix dactyliferae* sewi cultivar.

The result of (Table 2, 3) showed the concentrations of (ABA) was positively effect on the number of embryo germination and increasing percentage of germinating embryos compared with (SA) concentrations are not up to a significant level, that may back to the role of ABA in the regulation of many physiological processes, promote maturation of somatic embryogenesis and enhance somatic quality by increasing desiccation to tolerance. (George *et al.*, 2008 and Somaidai, 2017).

Sidky and Gadalla (2014) indicated use of ABA with different concentration positively effect on germination and maturation of embryos were the concentrations (0.3 and 0.5) % gave highest number of embryo while 0.1% of ABA achieved highest percentage of germinating of embryos in date palm. AL-khayri and AL-Bahrany (2012) reported that high concentration of ABA increase number and percentage of germination embryos in date palm phoenix dacty liferae.

References

Abd El-Bar, O.H. and M.M. El Dawayati (2014). Histological changes on regeneration in-vitro culture of date palm (*Phoenix dactylifera*) leaf explants. *Australian Journal of Crop Science*, **8:** 848-855.

Aberg, B. (1981). Plant growth regulators. XLI. Monosubstituted benzoic acids. *Swedish J. Agric. Res*., **11:** 93-105

Aldaej, M., S. Alturki, W. Shahata and H. Ghazzawy (2014). Effect of Potassium Nitrate on Antioxidants Production of Date Palm (*Phoenix dactylifera* L.) in vitro. *Pakistan Journal of Biological Sciences*, **12:** 1209-1218.

Aleid, S.M., J.M. Al-Khayri and A.M. Al-Bahrany (2015). Date palm status and perspective in Saudi Arabia. In: Al-Khayri JM, Jain SM, Johnson DV, Eds. Date palm genetic resources and utilization, Springer, Dordrecht, 49-95.

Ali, K.M., A.M. Sabbour, M.K. Khalil, A.S. Aly and A.F.Z. El-Din (2017). In-vitro morphogenesis of direct organs in date palm (*Phoenix dactylifera* L.) cv. Siwy. *International Journal of Advanced Agricultural Science and Technology*, **4:** 1-12.

Al-Khairy, J. and A.M. Al-Bahrany (2012). Effect of abscisic acid and polyethylene glycol on the synchronization of somatic embryo development in date palm (*Phoenix dactylifera* L). *Biotechnology*, **11:** 318-325.

Al-Khalifah, N.S. and A.E. Shanavaskhan (2012). Micropropagation of Date Palms. Asia-Pacific Consortium on Agricultural Biotechnology and Association of Agricultural Research Institutions in the Near East and North Africa, 54.

Al-Samir, E., S. Al-Utbi and M. Abass (2015). Phytotoxic effect of 2,4-D and dicamba on date palm (*Phoenix dactylifera* L.) tissue cultures at initiation stage. *Advances in Agriculture and Botanics*, **7:** 96-108.

Baharan, E., P. Pour, E. Mohammadi, S. Shahbaziand and Z. Hosseini (2015). Effects of some plant growth regulators and light on callus induction and explants browning in date palm (*Phoenix dactylifera* L.) *in-vitro* leaves culture. *Iranian Journal of Plant Physiology*, **5:** 1473-1481.

Bawis, H.A., K.A. Bujliah, M.A. Bu Ali, M. El Bellaj and A.B. Abdallah (2015). Effect of abscisic acid (ABA) on somatic embryogenesis in date palm (*Phoenix dactylifera* L)., **4(2):** 031-034.

Bozhkov, P.V. and S. Von Arnold (1998). Polyethylene glycol promotes maturation but inhibits further development of *Picea abies* somatic.

Choi, Y.E., D.C. Yang, E.S. Yoon and K.T. Choi (1999). High

efficiency plant production via direct somatic single embryogenesis from preplasmolysed cotyledon of Panax ginseng and possible dormancy of somatic embryos. *Plant Cell Rep*., **18:** 493-499.

Cutt, J.R. and D.F. Klessig (1992). Salicylic acid in plants a changing perspective. *Pharmaceut. Technol*., **16:** 26-34.

El Bellaj, M. (2000). Etude de quelques parame'tres biochimiques en relation avec l'acquisition des potentialite´s embryoge'nes et la maturation des embryons somatiques chez le Palmier dattier (*Phoenix dactylifera* L.). The'se de Doctorat. Universite´ Cadi Ayyad, Faculte´ des Sciences-Semlalia, Marrakech.

El-Dawayati, M.M., H.S. Ghazzawy and M. Munir (2018). Somatic embryogenesis enhancement of date palm cultivar Sewi using different types of polyamines and glutamine amino acid concentration under in-vitro solid and liquid media conditions. *International Journal of Biosciences*, **(12): N1**. 149-159.

Fki, L., W. Kriaa, A. Nasri, E. Baklouti, O. Chkir, R. Masmoudi, A. Rival and N. Drira (2017). Indirect somatic embryogenesis of date palm using juvenile leaf explants and low 2,4-D concentration. In: J.M. Al-Khayri, S.M. Jain and D.V. Johnson. Eds. Date Palm Biotechnology Protocols, Volume II: Germplasm Conservation and Molecular Breeding, Springer, New York, 99-106.

Fki, L., R. Masmoudi and A. Rival (2003). Anoptimised protocol for plant regeneration from embryogenic Suspension culture of date palm, (*Phoenix dactylifera* L.) cv. *Deglet Nour. Plant Cell Rep.*, **21(6):** 517-524.

Gabr, M.F. and B. Tisserat (1985). Propagating palms *in vitro* with special emphasis on the date palm (*Phoenix dactylifera* L.). *Scientia Hort.,* **25:** 255-262.

Gadalla, E.G. (2007). High frequency somatic embryo production and maturation into plantlets in date palm (*Phoenix dactylifera* L.) through suspension culture. *Egypt.J.Agric.Res*., **85(B):** 349-365.

George, E.F., A. Michael and G.D. Klerk (2008). Plant propagation by tissue culture. Third Edition. Springer, Netherland.

Hussein, J., A. Khaun and D. Abdulrahman (2016). Improving the germination of somatic embryos in date palm Berhi cultivar *in-vitro. International Journal of Agronomy and Agricultural Research*, **8:**17-23.

Khierallah, H.S. (2007). Micro propagation of Two Date Palm *Phoenix dactylifera* L. Cultivars Using Inflorescences and Study The Genetic Stability Using AFLP Markers, Dissertation, university of Baghdad- Iraq.

Kim, Y.W., J. Youn, E.R. Noh and J.C. Kim (1999). Somatic embryogenesis and plant regeneration from imature zygotic embryos of japanes larch (*Larix leptolepis*). *Plant Cell Tissue and Organ Culture*, **55:** 95-101.

Klimaszewska, K., Y.S. Park, C. Overton, N. Ian and M.J. Bonga (2001). Optimized somatic embryogencis in *Pinus strubus* L. *In vitro Cell Dev. Biol. Plant,* **37:** 392-399.

Linossier, L., P. Veisseire, F. Cailloux and A. Coudret (1997). Effect of abscisic acid and high concentration of PEG on *Hevea brasiliensis* somatic.

Nayyef, M.N. (2019). Impact of temporary immersion system (TIS) by using plantform bioreactor and light source on date palm micropropagation Thesis, university of Baghdad-Iraq.

Othmani, A., C. Bayoudh, N. Drira, M. Marrakchi and M. Trifi (2009). Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L. cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. *Plant Cell Tissue Organ Cult*., **97(1):**71-79.

Pierik, R.L. (1999). In Vitro Culture of Higher Plants. Third Edition. Martinus Nijn off Publishers. Nether Lands.

Shahata, W., M. Aldaej, S. Alturki and H. Ghazzawy (2014). Effect of Ammonium Nitrate on Antioxidants Production of Date Palm (*Phoenix dactylifera* L.) *In vitro*. *Biotechnology* **3:** 116-125.

Sidky, R.A. and E.G. Gadalla (2014). Somatic embryogenesis in *Phoenix dactylifera*: maturation, germination and reduction of hyperhydricity during embryogenic cell suspension culture Proceedings of the Fifth International Date Palm Conference.

Sidky, R. and M. El-Dawyati (2012). Proliferation of female inflorescence explants of dare palm. *Annals of Agricultural Sciences*, **57:** 161-165.

Tisserat, B. (1991). Clonal propagation of palms. *Plant tissue culture manual*, **C2 :** 1-14.

Zayed, E.M.M. and O.H. Abd Elbar (2015). Morphogenesis of immature female inflorescences of date palm in-vitro. *Annals of Agricultural Science*, **60:**113-120.