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THE EFFECT OF SOME HORMONES ON THE *IN VITRO* CULTURE OF DATE PALM (*PHOENIX DACTYLIFERA* L.) OF BOU-SAÂDA, ALGERIA

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Abstract

The Oasis of Bou-Saada is gradually deteriorating due to the attack of insect pests such as white scale *Parlatoria blanchardi* Targ., the palm worm *Myeloïs ceratoniae* Zell and some fungal pests. In addition, polluted irrigation water coming from the wadi Bou-Saada has seriously damaged the oasis palm. Consequently, 23 promising date palm varieties are facing extinction. The Rehabilitation of the oasis palms of Bou-Saâda requires mass propagation of the endangered date palm varieties to conserve the fauna and the genotypes. The Tissue Culture technique is the only method through which date palm oases Bou-Saâda can be restored in a short period as compared to growing the trees through seeds or offshoots. As the first phase of conservation of the oases, this research project was initiated whereas propagation of two highly promising varieties *Deglet-Nour* and *Mech-Degla* was carried out through tissue culture technique on MS medium using various concentrations of three different hormones (AIB, GA3, 2,4-D) to obtain organogenesis. Results revealed that the growth in length of explants of the variety *Deglet-Nour* and its overall development was better as compared to the variety *Mech-Degla* with the AIB (2mg/l). The GA3 hormone performed the best in relation to the development of organogenesis in the two varieties followed by AIB hormone than the 2,4-D with overall mean lengths of 1.36, 1,26 and 0.84 centimetres, respectively. The formation of the callus was noticed only in the case of the variety *Deglet-Nour*.

Key words: Bou-Saâda oasis, *In vitro* culture, Deglet-Nour, Mech-Degla, 2,4-D, AIB, GA3, Organogenesis.

Introduction

The date palm trees *Phoenix dactylifera* L. (Arecales: Arecaceae) are among the oldest known fruit crops grown in the Middle East and North African (MENA) region for at least five thousand years (Moussouni *et al.,* 2017; Mohammed, 2019). As far as Algeria is concerned, the date palm is considered as a major fruit crop in the country. The crop is cultivated in numerous oases located mainly in the southern part of Algeria where a dry and hot climate favours the cultivation and growth of the date palm crop. The statistical data reveal that about 18 million date palm trees have been grown in different regions of the country on an area of 169,380 hectares with average annual produce of 500,000 metric tonnes of dates. Hence, the date palm crop serves as a backbone for the economy of the country especially for southern provinces (wilayates), where the economy is largely dependent on the cultivation, production and sale of the dates and their by-products including date paste, date syrup, date flour, alcohol and vinegar (Bouguedoura *et al*., 2015; Anon., 2021: Harkat *et al.,* 2022). Unfortunately, various biotic and abiotic factors damage the crop and cause huge economic losses annually. A review of the literature reveals that the date palm crop in Algeria suffers from112 species of insect and mite pests belonging to 10 different orders and 42 families of agricultural pests (Roumani *et al*., 2018). The most important pests include Old World Mite *Oligonychus afrasiaticus*, the date palm scale insect *Parlatoria blanchardi*, the palm butterfly *Myeloïs Ceratoniae* and fungal diseases such as rot disease flowering (raw) caused by the fungus *Mauginiella scaeta* and Bayoudh diseases *Fusarium oxyusporum* f.sp. albedinis, which has spread to Morocco and Algeria, causing the destruction of 3 million palm trees in Algeria (Bounaga and Djerbi, 1990; El-Juhani, 2010). The protection of the date palm crop is vital both for the livelihood of the people as well as the economy of the country especially the residents of Bou-Saada where the crop is facing serious threats due to the attack of the above-mentioned insect pests as well as polluted irrigation water. Bou-Saada is considered as the door of the Sahara and is among the old oases, the date palm is the key plant of the oasis ecosystem of Bou-Saada. There have been identified 23 cultivars originating from Bou-Saada, which are distributed in four zones located on both sides of the Oued Bou-Saada: Djenane Nakhara, Djenane Btom, Djenane Hmaïd, and Djenane Khachbet-Mimoun (Guettouchi *et al*., 2015). The alarming situation and threats faced to data palm crop in the oases of Bou-Saada warrants immediate remedial actions including adopting appropriate plant protection measures, mass propagation and growing of the promising insect pest-resistant varieties to conserve the genotypes of date palm of Bou-Saada oases. Literature reveals date palm is generally propagated through three methods (Nixon & Carpenter, 1978; Zaid & deWet, 2002; Jatoi *et al*., 2015; Hassan *et al*., 2021) including offshoot propagation (traditional-technique), Seed propagation, and the recently developed is the tissue culture techniques. Unfortunately, the propagation through offshoot is very slow and requires a pretty long time to establish a new crop. On the other hand, there are several genotypes that either do not produce offshoots and are difficult to grow from seeds. Besides, the seed-propagated date palm trees are often not true to type owing to heterozygosity and require about seven years to reach the fruiting stage. (Othmani *et al*., 2009). Under the circumstances, Tissue Culture (TC) technique seems to be the only promising and feasible technique for mass propagation of date palm genotypes to protect the Oases of Bou-Saada.

Since the 1970s, rigorous work has been carried out for micro-propagation of date palm using organogenesis and somatic embryogenesis techniques (Ammar & Benbadis, 1977; Tisserat, 1979; Drira & Benbadis, 1985; El-Hadrami *et al*., 1995; El-Hadrami *et al*., 2009, Al-Khateeb, 2008; Othmani *et al*., 2009; Zayed *et al*., 2016). The studies have revealed that apical meristems and auxiliary buds may be used to achieve organogenesis, whereas plant parts such as young leaves, shoots, rachilla and such other meristematic tissues can be used to achieve embryogenesis. It has also been found that date palm propagation through tissue culture depends upon a lot of internal and external factors including the genotypes of cultivars, growth media, temperature, humidity and optimal growth conditions which vary from one cultivar to the other. Generally, a period of 6 years is required to reach production through the use of the Tissue Culture technique, whereas two more years are required to produce the cultivars on a commercial scale. Although progenies produced through the tissue culture techniques represent characteristics similar to those produced from offshoot propagation, yet the tissue culture progenies may exhibit somaclonal variation (off-types). The somaclonal variation represents numerous typical phenotypes, such as variations in the structure of leaves, variegation and diversity in colour of the leaves, as well as overall growth patterns of the plants. There may be trees that exhibit abnormal floral development or even do not form inflorescences. Likewise, there may be trees that produce seedless parthenocarpic fruits. Gurevich *et al*., (2005) stated that most of the somaclonal variations, as well as the variants, can be spotted at early stages in the laboratory, yet sometimes it takes several years to detect some variations and variants after planting in the field or after flowering, setting of fruits and at the time of maturation of the trees. The occurrence of somaclonal variation in Tissue culture propagated date palms trees maybe sometimes on the higher side significantly, yet the mechanisms responsible for such variations are not clear and are still under investigation. In short, all the above literature reveals the promise of the *In vitro* tissue culture technique for propagation of date palm as compared to the conventional methods (Quiroz-Figueroa *et al*., 2006; Hassan *et al*., 2017).

Factors of declining of palm oasis of Bou-Saada: The decline of the Bou-Saada oasis palms is attributed to several factors. The most important factor is the attack of a number of agricultural pests, wherein the white scale *Parlatoria blanchardi* Targis is one of the most common insect pests. Apart from insect attacks, irrigation water and environmental pollution are also significant factors responsible for the deterioration of the oasis of Bou-Saada (Guettouchi *et al*., 2016). The damage has posed a serious threat to the local date palm genotypes, which are important for the region in terms of their economic importance and genetic potentials. Today the oasis palm trees are in worse condition and some of its local varieties are facing extinction (Guettouchi *et al*., 2016).

The present research project was, therefore, carried out to conserve the 23 identified date palm genotypes of the oases of Bou-Saada by their mass propagation using TC technique. In the first phase, we have selected *Deglet-Nour* and *Mech-Delga* varieties, which are of high economic importance due to their high yield and high vulnerability to extinction.

Materials and Methods

Plant material and explants preparation: Young offshoots (2-3 years old) of date palm *Deglet-Nour* and *Mech-Degla* were detached from mother palm (Fig. 1). The explants are the fragments of the hearts of the rejections (white parts), the fragment of the rachis and small leaves. The ultimate size of the excised shoot tip was about 0.5-1 cm in width and 2-3 cm in length. Later on, we sterilized the shoot tips with sodium hypochlorite supplemented at 12°C for 20 min, rinsed thrice with distilled water under aseptic conditions.

Initiation of cultures: Shoot apical tips (about 3-cm length, 1-cm diameter) of offshoots were excised in small pieces (5mm length) and inoculated on MS (Murashige & Skoog, 1962) basal medium comprising of (per litre): 100 mg Myo-inositol, 30g sucrose, 3g activated charcoal and 10g agar incorporating different concentrations with 0.5, 2 and 50mg/l 2.4-D, AIB, GA3 (Table 1). The pH was adjusted at 5.7 prior to autoclaving (20min, 120°C). The cultures were kept at 25°C under dark conditions.

Data analysis: The measurement of the size of explants developed in the media (organogenesis) was made at the end of each week till the 10^{th} week. The results were tabulated and subjected to statistical analysis to determine the behaviour of each variety in response to different growth hormones and concentrations at different time intervals (1-10 weeks). The standard Analysis of Variance was worked out to determine the significance of independent variables on the growth of explants at a 95% level of probability using SPSS software version 23.

Results and Discussion

The results of Analysis of variance presented in (Table 2) reveal statically significant response of varieties F (1, 899) =130.82, p=0.000), hormones F (2, 899) =65.930, p=0.000), Concentrations F (2, 899) =6.795, p=0.001), Time period F (9, 899) =22.457, p=0.000) as well as the interaction of verities and hormones F (9, 899) =8.554, p=0.000) as well as Verities and time F (9, 899) =22.457, p=0.000). Likewise, there was found significant interaction between verities and concentrations F (2, 899) =21.880, p=0.000), verities and time F (9, 899) =4.333, p=0.000), hormone and Concentrations F (4, 899) =2.767, p=0.026), hormone and time F (18, 899) =2.098, p=0.005) as well as among verities, hormone and concentration F (4, 899) =5.911, p=0.000). The results further revealed that there was found no significant interaction between concentrations and Time as well as among Verities-Hormones-Time, Varity-Concentrations- Time, hormones-concentrations-time and verities-hormones-concentrations-times at 0.5% level of confidence.

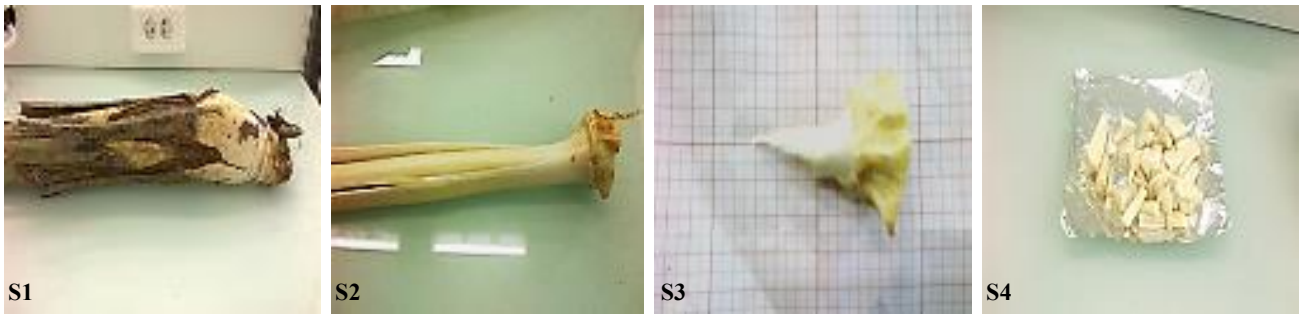

Fig. 1. Stages of harvesting date palm explants (S1-S4).

(Fig. 2a) Development of *Deglet-Nour* explant at concentration 2 mg/l of 2,4-D after six weeks, **(Fig. 2b)** Development of *Deglet-Nour* explant at concentration 2 mg/l of GA3 after seven weeks, **(Fig. 2c)** Development of *Deglet-Nour* explant at concentration 50 mg/l of GA3 after ten weeks. **(Fig. 2d)** Development of *Mech-Degla* at concentration 0.5 mg/l of GA3 after ten weeks.

A perusal of the mean values indicated that the maximum growth was recorded in the case of variety *Deglet-Nour* with an overall mean size of 1.38 cm followed by *Mech-Degla* with an overall mean length of 0.93 cm. As far hambones are concerned maximum growth was visible in the case of GA3 with a mean score of 1.36 cm followed by AIB and 2,4-D with overall mean lengths of 1.26 and 0.84 cm, respectively. As far as the impact of different concentrations of hormones on organogenesis or somatic embryogenesis of the two verities is concerned, maximum growth was recorded in media with 0.5mg/l concentrations of the hormone measuring an overall mean length of 1.15 cm followed by 2m/l and 50mg/l concentrations, respectively. The time factor proved a decisive factor in the organogenesis and somatic embryogenesis of the two verities. There was found a positive correlation between the length of the embryonic plant and the time period (Fig. 2a, Fig. 2b, Fig. 2c, Fig. 2d).

According to the results stated above, it has been observed that the low concentrations of hormones (0.5 and 2mg/l) resulted in organogenesis whereas the high concentration (50 mg/l) lead to embryogenesis. In general low concentration of the growth hormones and regulators leads to organogenesis and consequently, the callus phase is avoided. The findings are in line with the previous work accomplished by Rathore *et al*., 2011 and 2014. Direct regeneration of vegetative buds minimizes the risk of somaclonal variation among regenerants. (Abahmane, 2011). According to Bhan *et al*., (2013; Al-Khayri & Naik, 2017) *In-vitro* organogenesis is the promising technique to control the problem of somaclonal variations as well as to obtain true-to-type date palm plants. When morphogenesis occurs (organogenesis), it maintains the changes already present in the cultured tissue or in the callus stage, but it blocks the appearance of new changes. Cells divide and organize to form a bud (Demarly & Sibi, 1996) and well-visualized structures (Rathore *et al*., 2020). The morphological response is not the same in all the explants, there was a clear and visible growth since the second week for the two varieties. Previous studies conducted in Morocco used the technique of organogenesis from young leaves of hearts of offshoot, which allows, in principle, a certain varietal validity as compared to the somatic embryogenesis technique (Azeqour *et al*., 2002). However, callus passage can induce genetic variations in the quality of fruits, level of disease resistance as well as other significant morphological and horticultural characters. (D'Amato, 1977, 1978; Khierallah *et al*., 2015). As far as the effect of time on growth is concerned, there was found a positive correlation between the two factors. The overall average growth of both varieties gradually increased from week 1 to week 10 with a mean length of 0.73 and 1.15 cm, respectively (Table 3).

Table 1. Blueprint of the research material used.

Verities	***Deglet-Nour***									***Mech-Degla***								
Hormone	***2,4-D***			***AIB***			***GA3***			***2,4-D***			***AIB***			***GA3***		
Concentration (mg/l)	0.5	2	50	0.5	2	50	0.5	2	50	0.5	2	50	0.5	2	50	0.5	2	50
Time intervals for Taking measurements	Weekly upto 10thweek																	

Table 2. Analysis of variance showing the effect of various factors on *In vitro* growth of date palm varieties at $p<0.05$.

Source	df	Mean square	F	Sig.
Corrected model	**179**	**1.616**	**3.898**	**0.000**
Intercept	1	1430.991	3452.194	0.000
Varieties	1	54.228	130.823	0.000
hormone	2	27.329	65.930	0.000
Concentration	2	2.817	6.795	0.001
Time	9	9.309	22.457	0.000
Varity * hormone	2	0.213	0.514	0.598
Varity * Concentration	2	9.069	21.880	0.000
Varity * Time	9	1.796	4.333	0.000
hormone * Concentration	4	1.147	2.767	0.026
hormone * Time	18	0.870	2.098	0.005
Concentration * Time	18	0.210	0.507	0.956
Varity * hormone * Concentration	4	2.450	5.911	0.000
Varity * hormone * Time	18	0.175	0.422	0.984
Varity * Concentration * Time	18	0.333	0.804	0.698
hormone * Concentration * Time	36	0.158	0.381	1.000
Varity * hormone * Concentration * Time	36	0.211	0.510	0.993
Error	899	0.415		
Total	**1079**			
Corrected total	**1078**			

Table 3. Effect of Time period on the growth (mean lengths in cm) of *Deglet-Nour* and *Mech-Degla.*

Time in weeks	Mean lengths (cm)	N	Std. deviation
1.00	0.7315	108	0.29023
2.00	0.7991	108	0.28531
3.00	0.8916	107	0.41004
4.00	0.9954	108	0.49035
5.00	1.1324	108	0.62170
6.00	1.1861	108	0.66508
7.00	1.3065	108	0.89215
8.00	1.4157	108	1.01292
9.00	1.5352	108	1.03836
10.00	1.5259	108	1.05000
Total	**1.1522**	**1079**	**0.78359**

Conclusion

Date palm (*Phoenix dactylifera* L.) is one of the significant major fruit crops grown in arid regions of the Middle East and North Africa. The tree may be grown and propagated either sexually through seeds or vegetatively by using the offshoots. However, the tissue culture propagation technique has a great potential leading to rapid propagation of date palm through the two main routes somatic embryogenesis and the axillary branching technique known as organogenesis. In this research, we have multiplied *In vitro*, two varieties through their offshoot by organogenesis. Using MS medium with three concentrations of three hormones explants taken from the base of a young leaf. From our research we have the following results:

- *Deglet-Nour* gave the best result for organogenesis; Mech-degla shows better development only with AIB with a concentration equal to 2 mg/l.
- The GA3 hormone is the best for the development of organogenesis in both varieties followed by the AIB hormone then 2,4-D for all concentrations (0.5, 2, 50 mg/l);
- 0.5 and 2 mg/l of the hormones AIB and GA3 promote the development of organogenesis in both varieties.

In vitro culture especially organogenesis is the best method to save the date palm varieties of the oasis of Bou-Saada. It is therefore recommended that the project may be continued, and all endangered date palm varieties may be propagated using tissue culture technique to conserve the date palm flora of the oasis of Bou-Saada.

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