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In vitro effects of culture media on potential regeneration of date palm (*Phoenix dactylifera* L.) in Boussaâda oasis, Algeria

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Abstract

Date palm (*Phoenix dactylifera* L.) of Boussaâda has varietal diversity and a very important nutritional value in Algeria. Nevertheless, today the palm groves are threatened, not only climatically but also in terms of varietal diversity such as disappearance of varieties, the low production and even discharges serving mainly to vegetative propagation which can be used as a source of explants on *in vitro* somatic embryogenesis technique. The aim of this study is to use *in vitro* culture techniques to regenerate the phoenicicultural orchards at Boussaâda oasis. Two types of experimental protocol are used; firstly, to make an inventory in field in order to contain palm groves and then date palms existing; and the secondly is to applicant the somatic embryogenesis technique in the laboratory and then regenerate the good cultivar. Results indicate that plant growing is an important axis that needs to be developed in the oasis by introducing varieties to select the most suitable and profitable for a good diversification of cultivars and consequently the improvement of production.

1. INTRODUCTION

In Algeria, there are more than thirteen thousand date palm (*Phoenix dactylifera* L.) trees and in Boussaâda region only, there are seven thousand trees" (CRSTRA-Biskra, 2014). Date palm is a species of great socio-economic interest. It is the main axis of agriculture which provides the main food and financial resource of people in these regions. However, palm groves cited below are subject to severe genetic erosion and environmental threats which is manifested by the disappearance of many cultivars, leading to the depletion of date palm genetic resources (Bougerfaoui., 1998). Date palm diversity is facing crucial problems also such as different abiotic stresses like water efficiency and sodium chloride concentration; and biotic stress like Bayoud (*Fusarium oxysporium* f. albedinis), which varies in level and nature of the problems in addition to climate conditions, and cultural practices (Zaid and de Wet, 2002). These factors globally cause genetic erosion and uncontrolled commercial exploitations of cultivars (Abul-Soad et al., 2017). In order to limit the genetic erosion of date palm, it is very interesting to make an overall census of the feet of the date palm spread over several micro-oases in these regions on the one hand and to conserve the phoenicicultural diversity of the Saharan zone in South-Eastern of Algeria (Belguedji, 2002). Several studies have already been done using phenotypic markers (Zehdi et al., 2004). These molecular tools are currently used to study genetic diversity which they will make it possible to identify genetics resources of this species in order to organize their conservation and safeguarding. According

to Bouguedourra et al. (2015), the main objective is the preservation and sustainability of oasis ecosystem by maintaining date palm genetic diversity. The aim of this study is to choose the best technique used to regenerate and safe conservation of date palm grown on in vitro conditions (Engelmann, 2010).

2. MATERIAL AND METHODS

2. 1. Study area

Boussaâda region is located at 69 km south-west of M'sila city. It is located between the mountains of Jebel Kerdada and Jebel Azzedine; it is crossed by a perennial flow namely Valley of Bousaâda that irrigates the garden area of the city and its palm grove. The total area is 255 Km^2. It is limited to the North, by the Ouled Sidi Brahim region, in the North-East, by the Maarif region, in the East, by EI-Houamed region, in the West, by Temsa region, in the South-East, by the municipality of Oultem and in the South-West by El-Hamel region (Bennaceur & Benderradji, 2016).

Table 1: Average monthly rainfall (mm) and relative humidity (%) in Boussaâda region for the year 2018

Months	J	F	M	A	M	J	J	A	S	O	N	D	Year
Rainfall (mm)	34	22	29	25	25	14	14	14	24	24	32	35	Total 282
Relative Humidity (%)	70.7	65.8	62	52	52	32	30.9	29.6	31.5	50.8	66.7	71.7	Average 51.3

2. 2. Climatic conditions region

In this region, the climatic parameters such as the rainfall and relative humidity (Table 1) have acts on the density of the populations by causing a decrease in the number of individuals when the hygrometric conditions are unfavorable on the organisms (Dajoz, 1971). In the region of Boussaâda, it has been noted that the month of December is characterized by the highest relative humidity (71.7%), against the lowest monthly average that was recorded during July with 30.9%. The annual average is 51.3% (Table 1).

2. 3. Inventory of date palm cultivars

The survey was implemented at the 4 study sites made it possible to determine several cultivars with a different number of trees. The inventory method was carried by establishment this survey by asking farmers in the region and collected data.

2. 4. *In vitro* culture media

Date palm offshoots of date palm cultivar namely Mech-Degla, very appreciated by local population, were collected from the four palm groves of Boussaâda region and rinsed for many time periods with water and then were prepared to be used in laboratory as explants for regeneration of this cultivar. Three culture media, such as MS (Murashigue and Skoog, 1962); B5 (Gamborg et *al.,* 1968) and KNOP (Gautheret, 1949) were prepared (Table 2-4).

Explants were sterilized and incubated in growth chamber at average temperature (25 ± 2°C) with photoperiod of 16h/day using white light lamps.

2. 5. *In vitro* regeneration

Tissue culture method such as somatic embryogenesis technique was applied to improve and regenerate a very famous cultivar of date palm namely Mech-Degla which was the thumbprint in this region using her offshoot which appeared able to give entire plantlets according to cells division activation as described above.

Date palm offshoot of Mech-Degla variety was used in this study. After the removal of the outer leaves until the appearance of the white and soft part, explants are cut from 0.5 to 1cm in size for use on *in vitro* culture (Fig. 1).

Fig. 1. Date palm offshoot (left) and its heart (right) of Mech-Degla variety.

Table 2. Composition of MS culture medium (Murashigue & Skoog 1962)

Macro-elements	mg/l	Micro-elements	mg/l	Inorganic elements	mg/l	Organic elements	mg/l
NH_4NO_3	1650	KI	0.83	$FeSO_4.7H_2O$	27.8	Myo-Inositol	100
KNO_3	1900	H_3BO_3	6.2	$Na_2EDTA.2H_2O$	37.3	Nicotinic acid	0.5
$CaCl_2.2H_2O$	150	$MnSO_4.4H_2O$	22.3			Pyridoxine-HCl	0.5
$MgSO_4.7H_2O$	250	$ZnSO_4.7H_2O$	8.6			Thiamine-HCl	0.1
KH_2PO_4	170	$Na_2MoO_4.2H_2O$	0.25			Glycine	2
		$CuSO_4.5H_2O$	0.025				
		$CoCl_2.6H_2O$	0.025				

Table 3. Composition of B5 culture medium (Gamborg, 1968)

Macro-elements	mg/l	Micro-elements	mg/l	Inorganic elements	mg/l	Organic elements	mg/l
KNO_3	2500	$MnSO_4.H_2O$	10	$FeSO_4.7H_2O$	27.8	Myo-Inositol	100
$CaCl_2.2H_2O$	150	$ZnSO_4.7H_2O$	2	$Na_2EDTA.2H_2O$	37.3	Nicotinic acid	1
$(NH_4)_2.SO_4$	134	H_3BO_3	3			Pyridoxine-HCl	1
$MgSO_4.7H_2O$	250	KI	0.75			Thiamine-HCl	10
$NaH_2PO_4.H_2O$		$Na_2MoO_4.2H_2O$	0.25			Glycine	10
		$CuSO_4.5H_2O$	0.025				
		$CoCl_2.6H_2O$	0.0125				

Table 4. Composition of the 'KNOP' culture medium (Gautheret, 1957)

Elements	$CaNO_3$	KNO_3	$MgSO_4$	KH_2PO_4	$FeSO_4$
Quantity (mg/l)	1	0.25	0.25	0.25	0.05

2. 5. 1. Culture media and explants

Sterilization of the culture media is done by autoclave (120°C) for 20 minutes. Thermolabile substances (vitamins, hormones, and amino acids) are subjected to microfiltration (0.22 µm filter paper). The metal instruments and / or glassware are put in the oven at a temperature of 120°C for 15 minutes. The sterilization of the explants comes from the offshoot, was carried out by putting firstly in ethanol to eliminate the maximum of the contaminant and in Ascorbic acid to avoid browning of explants, and then in a solution of sodium hypochlorite (6%) for 20 minutes, then rinsed with sterile distilled water 3 times for 5 minutes each time under laminar flow hood (Benderradji et *al.,* 2007).

2. 5. 2. Seeding of the explants

Seeding and incubation of explants were made, and vitamins and filtered hormones were added to the autoclaved culture media and then poured into Petri dishes. Explants of the offshoot were cut from 0.3 to 0.6 cm in length in order to make small explants and then put them in different culture media MS, B5 and KNOP. Six explants per petri dishes are sown with three replicates (Table 5) and then Petri dishes well

Table 5. Repeats number and different concentrations of 2.4-D.

Culture media	**MS** (Murashigue and Skoog)			**B5** (Gamborg)			**KNOP** (Gautheret)		
[2.4-D (mg/l)]	5	10	20	5	10	20	5	10	20
Number of replicates	3	3	3	3	3	3	3	3	3
Seeded explants per replicate	6	6	6	6	6	6	6	6	6

closed and sealed by the parafilm. The addition of 2.4 dichlorophenoxyacetic acid at different concentrations for each medium was performed as indicated below. After this step, explants were incubated in a growth room at a temperature of 25°C ± 2, under a fluorescent light at a rate of 16 hours light / 8 hours dark.

3. RESULTS AND DISCUSSION

3. 1. Description of the Boussaâda's oases

Environmental factors are contributing to the deterioration of Boussaâda Oasis and contributing to its dispersion, these factors are real components of an environmental threat, such as the pollution of the Valley of Boussaâda, which is the only source of irrigation of palm trees, especially since the irrigation system in the oasis is a traditional flood irrigation through a network of canals developed to provide water to all farmland evenly, so the orientation from farmers to other fruit crops like Apricot and olive, vegetables and legumes rather than date palms which made competing in water uses. Date palm resources of this region is also exposed in a serious way to threats and biotic damage caused by multiple pests, diseases, and weeds (Idder et *al.,* 2015, Idder-Ighili et *al.,* 2015). The Valley of Boussaâda receives polluted water used for irrigation of all date palm groves cited below (Fig. 2).

3. 2. Genetic resources of date palm

The inventory should be noted that the Boussaadiya cultivar is abundant in all the study sites or palm groves. This can be interpreted by adapting this cultivar to environmental constraints on the one hand and secondly because this cultivar is specific to the region, which gives it some tolerance to different biotic and abiotic stresses existing in the region (Fig. 3)

3. 3. Biotechnological improvement of the date palm

Result should be noted that a development of the explants of offshoot heart is recorded indifferently in media MS, B5 and KNOP supplemented with 5 mg/L 2.4 Dichlorophenoxyacetic acid., Compared to the concentrations 10 mg/L and 20 mg/L. Nevertheless, it is reported that the browning of explants and contamination of some explants, are the two main causes recorded in this study.

The obtained results showed also that culture media are indifferent to contamination and successful rate of regeneration. Data show that all culture media could regenerate explants with 2, 4-D concentration of 5 mg/L (Fig. 4; Table 6).

Fig. 2. Polluted water of Boussaâda's valley used for irrigation of date palm groves

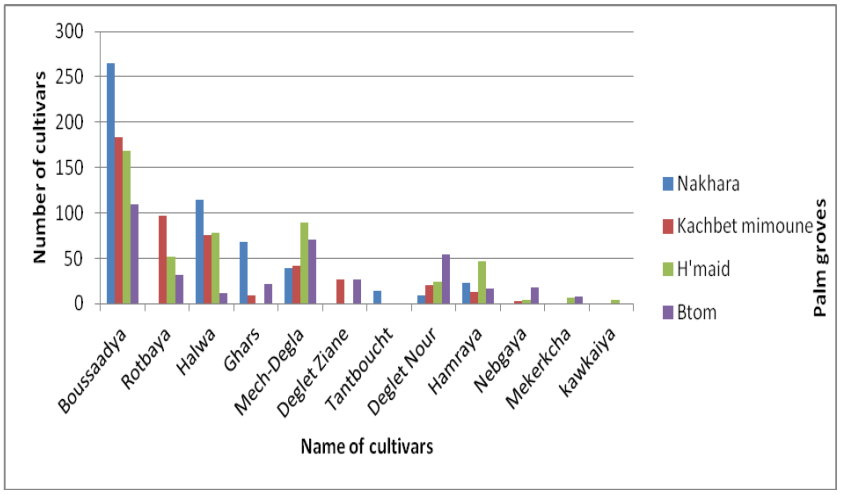

Fig. 3. Cultivars number in different palm groves (colored bars) in the region of Boussaâda (Algeria)

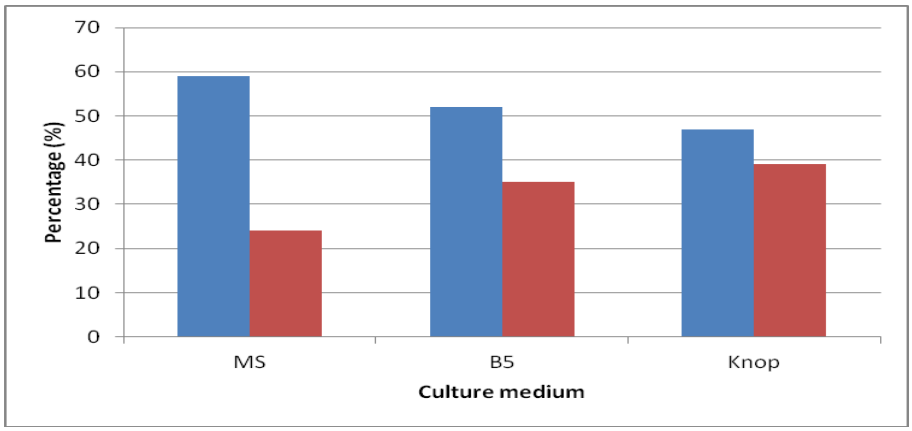

Fig. 4. Regeneration (blue bars) and contamination (red bars) of Mech-Degla cultivar in different culture media. (MS = Murashigue and Skoog; B5= Gamborg; Knop = Gautheret)

Table 6. Growth regulator and culture media used for *in vitro* regeneration of date palm, Mech-Degla variety

Growth Regulator	**Culture medium**		
2,4-D (mg/L)	**MS** (Murashigue and Skoog)	**B5** (Gamborg)	**KNOP** (Gautheret)
5	**+**	**+**	**+**
10	**-**	**-**	**-**
20	**-**	**-**	**-**

4. CONCLUSION

Date palm is a species with crucial socio-economic and ecological importance for arid and semi-arid regions. Its slow growth is well known to propagate by vegetative methods through offshoot using *in vitro* culture techniques. Date palm cultivars in the locality of Boussaâda, are exposed to many environmental threats which make a seriously factors of deterioration of phoenicicultural orchard such as biotic and abiotic constraints, so official authorities, scientist, and researchers are invited to protect and preserve this famous genetic patrimony. These traditional plantations contain all the cultivars that retain varietal diversity, although it is less important. In general, cultivars in this region are a real economic importance; so, it is recommended to improve a suitable multiplication method to conserve the date palm, which is more urgent.

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REFERENCES

Abul-Soad, A. A., Jain, S. M., Jatoi, M. A. (2017). Biodiversity and Conservation of Date Palm. In: M. R. Ahuja and S. M. Jain (eds.), Biodiversity and Conservation of Woody Plants, Sustainable Development and Biodiversity 17, Springer International Publishing AG.

Bougerfaoui M. (1998). Vitrification and its effect on tissue culture of date palm. Proceeding de la conférence sur le palmier dattier organisé par le ''Réseau de Recherche et Développement du Palmier dattier'' ACSAD, Marrakech, Maroc, 16 -18 février 1998, pp : 230-236.

Belguedji M. (2002). Les ressources génétiques du palmier dattier : Caractéristiques des cultivars dans les palmeraies du Sud-est Algérien. 3D – Documents – Débats - Dossiers.

Benderradji L., Bouzerzour H., Ykhlef N., Djkoun A., Kellou K. (2007). Réponse à la culture *in vitro* de trois variétés de l'Olivier (*Olea europaea* L.). Sciences & Technologie 26: 27-32.

Bennaceur M, Benderradji L. (2016). Phoeniciculture in the M'sila region: Current situation and methods of improvement, Master Thesis in Plant Biotechnology and Meta-genomics, Faculty of Sciences- M'sila University, 47p.

Bouguedoura, N., Bennaceur, M., Babahani, S., Benziouche, S. E. (2015). Date Palm Status and Perspective in Algeria. In: 125 Springer Science & Business Media Dordrecht 2015, J. M. Al-Khayri et *al.* (ed), Date Palm Genetic Resources and Utilization: Vol. 1: Africa and the Americas, DOI 10.1007/978-94-017-9694-1_4.

C. R. S. T. R. A. (2014). Centre de Recherche Scientifique et Technique sur les Régions Arides, Numéro Spéciale, ISSN : 1112-3273.

Dajoz, R. (1971). Précis d'écologie. Paris, Dunod (2ème édition), 434 p.

Engelmann, F. (2010). Use of biotechnologies for conserving plant diversity. Acta Hort., (ISHS), 812 : 63-82.

Gautheret, R. J. (1949). La culture des tissus végétaux et les phénomènes d'histogenèse. Colloque International du C. N. R. S. sur la morphogenèse. (Strasbourg, Juillet 1949), pp. 719-744.

Gamborg, O. L., Miller, R. A., Ojima, K. (1968). Nutrient requirements of suspension culture of soybean root cells. Ex. Cell. Res.; 50: 15-158.

Idder, M. A., Ighili, H., Mitiche, B., Chenchouni, H. (2015). Influence of date fruit biochemical characteristics on damage rates caused by the carob moth (*Ectomyelois ceratoniae*) in Saharan oases of Algeria. Scientia Horticulturae, 190, 57-63.

Idder-Ighili, H., Idder, M. A., Doumandji-Mitiche, B., Chenchouni, H. (2015). Modeling the effects of climate on date palm scale (*Parlatoria blanchardi*) population dynamics during different phenological stages of life history under hot arid conditions. International Journal of Biometeorology, 59(10), 1425-1436.

Murashigue, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with Tobacco tissue culture, Physiologia plantarum, 15: 473- 497

Zaid, A., De Wet, P. F. (2002). Pollination and bunch management. In: Zaid A (ed.) Date palm cultivation. F. A. O. Plant Prod. & Prot. Paper 156 Rev, Vol 1, pp 227-269.

Zehdi, S., Trifi, M., Billotte, N., Marakchi, M., Pintaud, J. C. (2004). Genetic diversity of Tunisian date palms (*Phoenix dactylifera* L.), revealed by nuclear microsatellite polymorphism. Hereditas 141: 278–287.