



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

Laid Benderradji^{1,2}, Mourad Bennaceur¹, Roumeissa Djerboua^{1,2}, Sara-Narimène Mazaoui^{1,2}, Samir Medjekal¹, Mouloud Ghadbane¹, Walid Saibi³ & Faical Brini³

¹Faculty of Sciences, University Mohamed BOUDIAF of M'sila, Algeria

²Biodiversity and biotechnological techniques for plant resources valorization laboratory, University Mohamed BOUDIAF of M'sila, Algeria

³Biotechnology and Plant Improvement Laboratory, Centre of Biotechnology of Sfax (CBS), University of Sfax, Tunisia

Article info

Abstract

History:

Received 09/02/2022

Accepted 26/04/2022

Keywords: *Phoenix dactylifera* L., Boussaâda oases, date palm cultivar Mech-Degla, *in vitro* culture, Somatic embryogenesis.



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* Corresponding author

laid.benderradji@univ-msila.dz

Conflict of Interest: The authors declare no conflict of interest

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 phoenicultural 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 phoenicultural 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 Km². It is limited to the North, by the Ouled Sidi Brahim region, in the North-East, by the Maarif region, in the East, by El-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 Total
Rainfall (mm)	34	22	29	25	25	14	14	14	24	24	32	35	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 ₄ NO ₃	1650	KI	0.83	FeSO ₄ .7H ₂ O	27.8	Myo-Inositol	100
KNO ₃	1900	H ₃ BO ₃	6.2	Na ₂ EDTA.2H ₂ O	37.3	Nicotinic acid	0.5
CaCl ₂ .2H ₂ O	150	MnSO ₄ .4H ₂ O	22.3			Pyridoxine-HCl	0.5
MgSO ₄ .7H ₂ O	250	ZnSO ₄ .7H ₂ O	8.6			Thiamine-HCl	0.1
KH ₂ PO ₄	170	Na ₂ MoO ₄ .2H ₂ O	0.25			Glycine	2
		CuSO ₄ .5H ₂ O	0.025				
		CoCl ₂ .6H ₂ O	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 ₂ O	10	FeSO ₄ .7H ₂ O	27.8	Myo-Inositol	100
CaCl ₂ .2H ₂ O	150	ZnSO ₄ .7H ₂ O	2	Na ₂ EDTA.2H ₂ O	37.3	Nicotinic acid	1
(NH ₄) ₂ .SO ₄	134	H ₃ BO ₃	3			Pyridoxine-HCl	1
MgSO ₄ .7H ₂ O	250	KI	0.75			Thiamine-HCl	10
NaH ₂ PO ₄ .H ₂ O		Na ₂ MoO ₄ .2H ₂ O	0.25			Glycine	10
		CuSO ₄ .5H ₂ O	0.025				
		CoCl ₂ .6H ₂ O	0.0125				

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

Elements	CaNO ₃	KNO ₃	MgSO ₄	KH ₂ PO 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			B5			KNOP		
	(Murashigue and Skoog)			(Gamborg)			(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

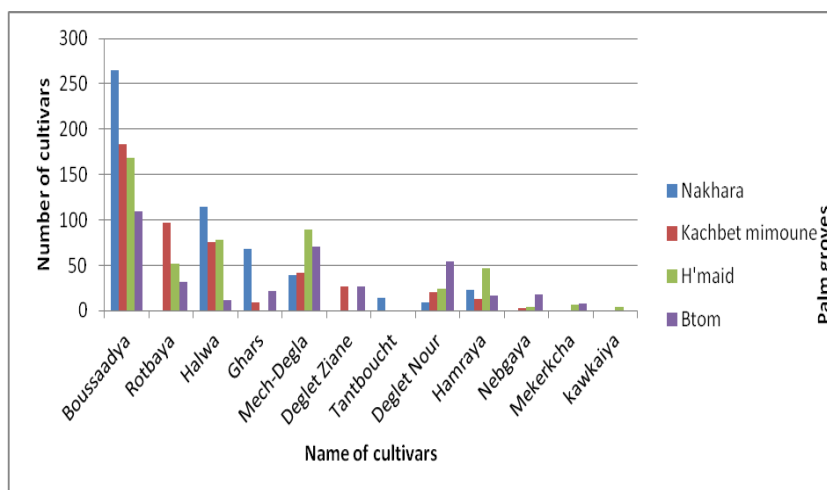


Fig. 3. Cultivars number in different palm groves (colored bars) in the region of Boussaâda (Algeria)

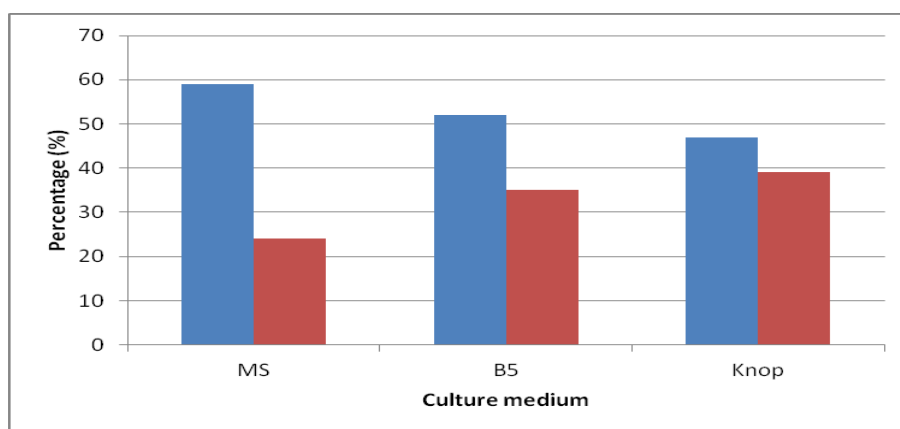


Fig. 4. Regeneration (blue bars) and contamination (red bars) of Mech-Degla cultivar in different culture media. (MS = Murashigue and Skoog; B5= Gamborg; Knop = Gautheret)

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 phoenicultural 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.

Acknowledgements

This research was supported by DGRSDT, Ministry of Higher Education and Scientific

Research (Algeria). The authors would like to thank, the chairman of Boussaâda municipality and his technical staff for their help and assistance.

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