

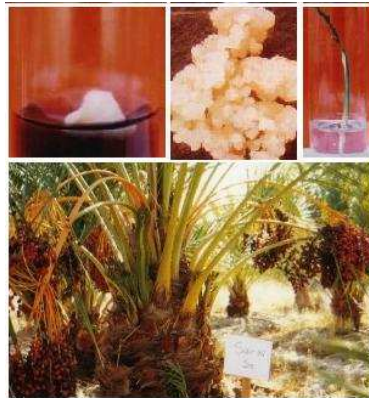
Links:

- <http://www.takween.com/biotechnologies/palmier-vitro-plants.html>
- <http://www.biotech-ecolo.net/date-palm-cultivars-morocco.html>
- <http://www.biotech-ecolo.net/date-palm-maghreb.html>
- <http://www.biotech-ecolo.net/palmier-vitro-culture.html>
- <http://www.biotech-ecolo.net/palmier-stress.html>
- <http://www.biotech-ecolo.net/palmier-embryogenese-mycorhization.html>
- <http://www.biotech-ecolo.net/palmier-phoenix-dactylifera.html>



Mohammed BAAZIZ

Biochimie et Biotechnologies des Plantes (BBP), Université Cadi Ayyad, Faculté des Sciences-Semlalia, B.P 2390, Marrakech, Maroc. E-mail : baaziz@ucam.ac.ma



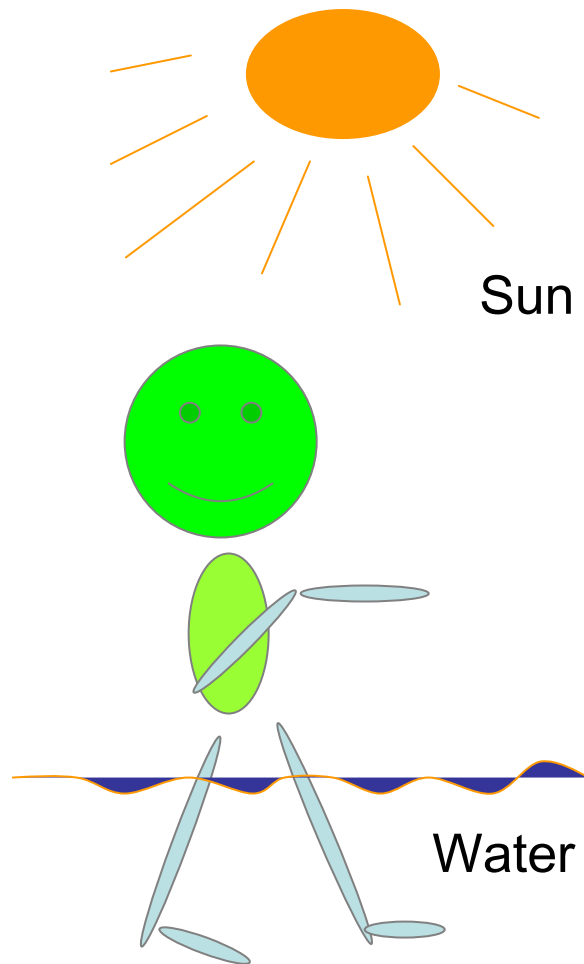
DATE PALM TISSUE CULTURE Takween

Variability in inflorescence and offshoot productions of date palm clones derived from tissue culture. Scientific research and outputs on education related to plant biotechnology

- **WHAT IS DATE PALM (*Phoenix dactylifera* L.) ?**
- **WHAT ARE THE WAYS FOR DATE PALM PROPAGATION ? ADVANTAGES AND DISADVANTAGES**
- **MICROPROPAGATION OF DATE PALM BY TISSUE CULTURE. THE MOROCCAN EXPERIENCE IN GREEN BIOTECHNOLOGIES.**
- **PROBLEMS RAISED FROM DATE PALM TISSUE CULTURE, IN PARTICULAR THOSE RELATED TO POLLINATION AND ALTERED GROWTH (OFFSHOOTS)**
- **OUTPUTS OF SCIENTIFIC RESEARCH ON EDUCATION RELATED TO BIOTECHNOLOGY AND GENETIC DIVERSITY**



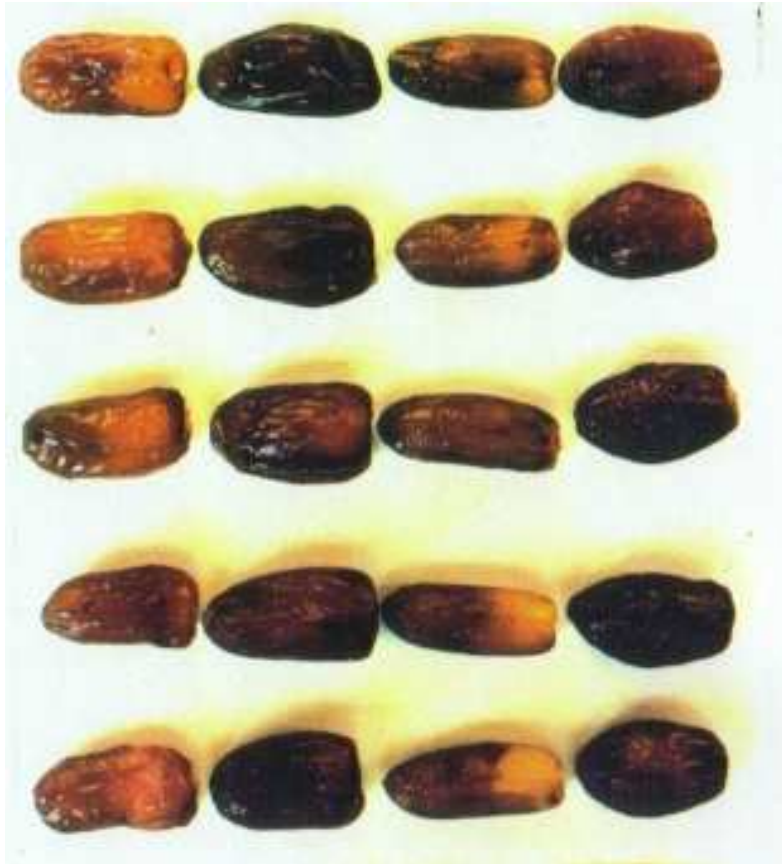
WHAT IS DATE PALM (*Phoenix dactylifera* L.) ?



- **LONG LIVEDE DIOECIOUS MONOCOTYLEDON WITH A LONG TRUNK ENDING IN A CLUMP OF LEAVES. OFFSHOOTS (SUCKERS) ARE FORMED AT THE BASE. HIGHLY DEVELOPED ROOT SYSTEM**
- **MOST FAVORABLE GROWING AREAS : 24°-34° LATITUDE NORTH. PRESENT IN THE NORTHERN (+ SOUTHERN) HEMISPHERE**
- **THERMOPHILOUS SPECIES. MAXIMAL VEGETATION AT 30-40°C.**
- **SPECIES ALWAYS SEEKING WATER BY A VERY DEVELOPED ROOT SYSTEM.**

أصناف عديدة لنخل التمر

DATE PALM SPECIES WITH MANY CULTIVARS



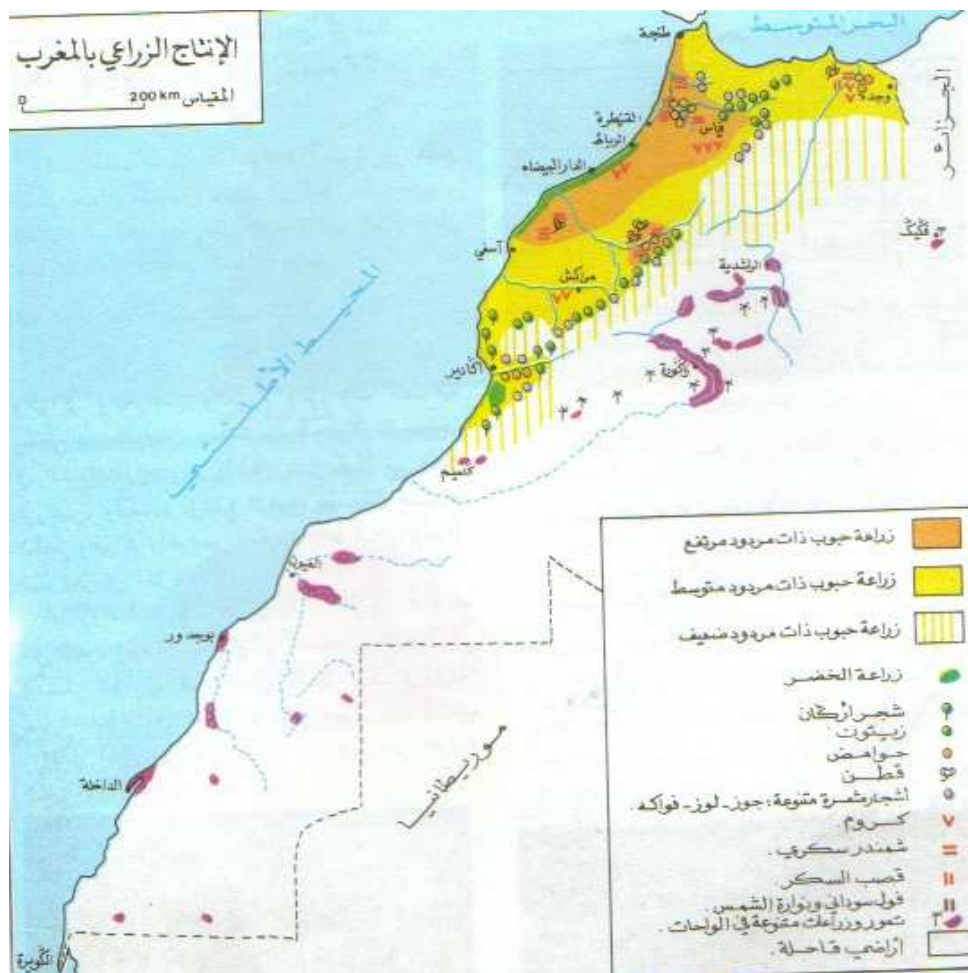
- لنخل التمر أصنافا عديدة قد تتعدى الآلف في بعض البلدان و تعتمد تسميتها في الغالب على نوعية التمور.

DATE PALM HAS MANY VARIETIES (CULTIVARS). THE NUMBER MAY EXCEED ONE THOUSAND IN SOME COUNTRIES. THE DETERMINATION IS BASED PRIMARILY ON THE QUALITY OF FRUIT (DATE)

EXAMPLES OF DATE PALM CULTIVARS IN MOROCCO: 'Mejhoul', 'Bou-Feggous' (good fruit quality), 'Bou-Stahammi Noire', 'Jihel' (Resistant to Bayoud disease)

EXAMPLES OF DATE PALM CULTIVARS IN TUNISIA AND ALGERIA: 'Deglet Nour', with good fruit quality, but low resistance to Bayoud

أنواع واحات النخيل بالمغرب. KINDS OF PALM GROVES IN MOROCCO.



’واحات نخيل منتجة’

‘DATE PRODUCING PALM GROVES’

OUARZAZAT:	1 800 000 TREES (42.4%)
ERRACHIDIA:	1 200 000 TREES (28.3%)
TATA:	850 000 TREES (20.0%)
TIZNIT:	141 600 TREES (3.3%)
GUELMIM:	135 500 TREES (3.2%)
FIGUIG:	120 000 TREES (2.8%)
AUTRES:	3 100 TREES

TOTAL 4250200 المجموع:

’واحات نخيل هامشية’

‘MARGINAL PALM GROVES’ (NON DATE PRODUCING)

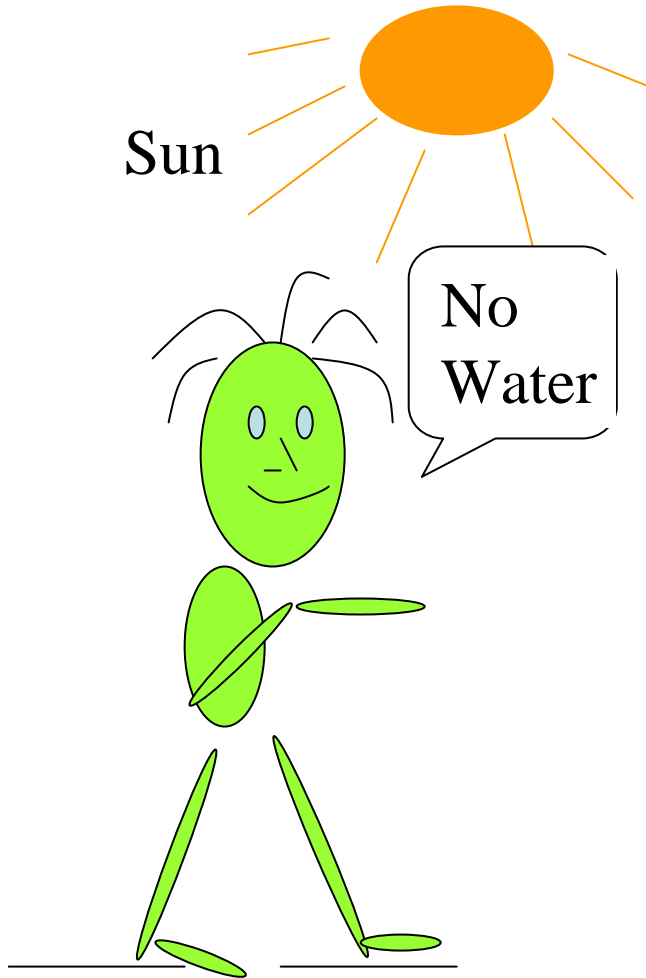
Example: MARRAKECH: 150 000 PALMS

الأصناف: حوالي 230 CULTIVARS:

مثل بوفقوص، مجهول، جيهل،

ما هي أهم مشاكل زراعة نخل التمر بالمغرب ؟

KEY CONSTRAINTS OF DATE PALM CULTURE IN THE MAGHREB ?



- جفاف، ملوحة، تصحر، ...
- **DROUGHT, SALINITY, DESERTIFICATION.**
- عدم الاهتمام من طرف السكان
- **LACK OF INTEREST FROM POPULATIONS**
- تعرية وراثية
- **GENETIC EROSION.**
- شيخوخة النخيل
- **AGING OF THE PALM GROVES.**
- ظهور أمراض فتاكة كمرض البيوض
- **APPEARANCE OF DAMAGING DISEASES LIKE BAYOUD.**

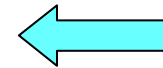
زراعة نخل التمر. تأثير مرض البيوض

DATE PALM CULTURE. IMPACT OF BAYOUD DISEASE



لاستدراك الخسائر و الإسراع في إعادة إعمار الواحات، يجب استعمال الطرق السريعة في إكثار النخيل

IN ORDER TO QUICKLY RESTORE DESTROYED PALM GROVES, WE MUST USE EFFICIENT (RAPID) METHODS IN PROPAGATING DATE PALMS



WHAT ARE THE WAYS FOR DATE PALM PROPAGATION ? ADVANTAGES AND DISADVANTAGES

✓ Seed-based propagation:

disadvantages are: Cultivar loss, heterozygosity

✓ Offshoot-based propagation:

This method maintains the genetic integrity of cultivars but does not increase the genetic diversity between individuals. Scarce cultivars and Bayoud-resistant varieties cannot be propagated quickly and distributed on a large scale, because of few offshoot production.

✓ Vitro-plant-based propagation:

Advantages: rapid

Disadvantages: variations occur when using embryogenesis



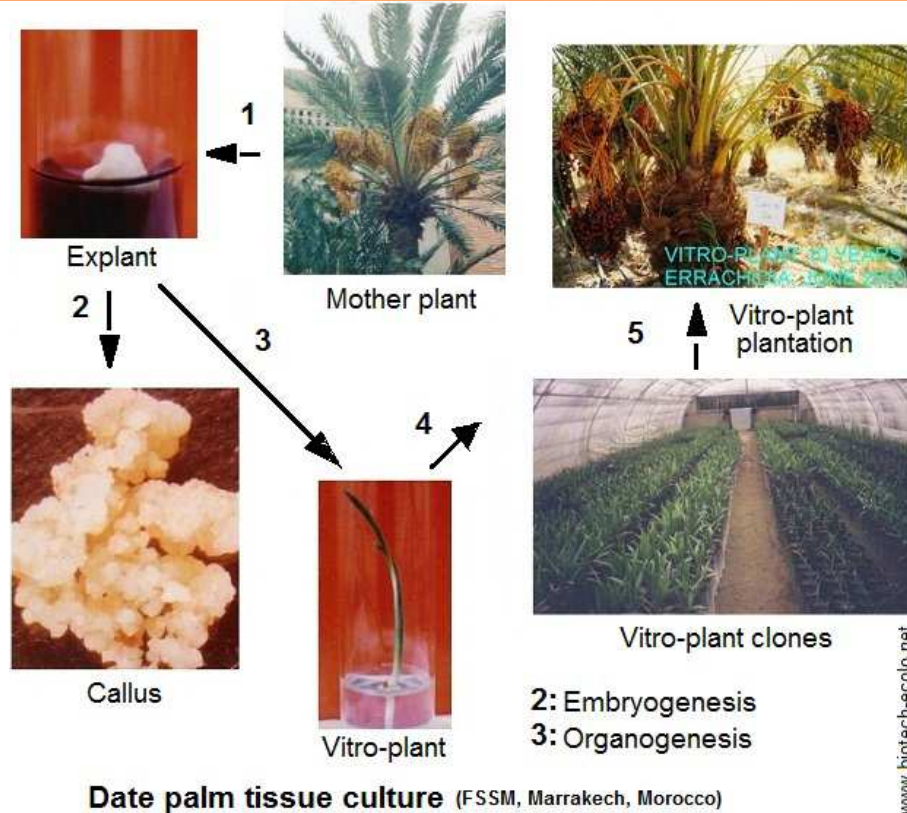
فسائل جانبية
OFFSHOOTS

إنتاج الفسائل الأنبوبية عند نخل التمر
Date palm vitro-plant production



Morocco has leading experience in date palm tissue culture. Moroccan researchers are developing this biotechnology in Africa (Namibia, as example) and the Middle East (Emirates, Saudi Arabia, as examples).

MICROPROPAGATION OF DATE PALM BY TISSUE CULTURE. THE MOROCCAN EXPERIENCE IN GREEN BIOTECHNOLOGIES



Considering its power of multiplication, the *in vitro* culture method is judged in Morocco as the unique suitable technique to be used in the restoration of date palm groves destroyed by Bayoud disease.

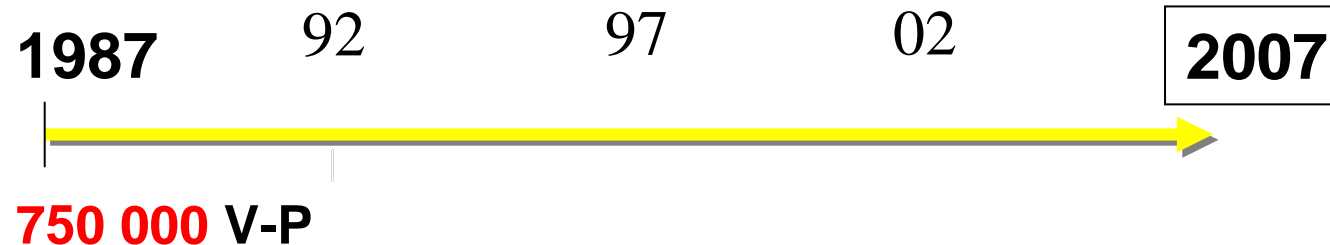
← Examples of tissue-culture-based projects for restoration of palm groves in Morocco

MICROPROPAGATION OF DATE PALM BY TISSUE CULTURE. THE MOROCCAN EXPERIENCE IN GREEN BIOTECHNOLOGIES

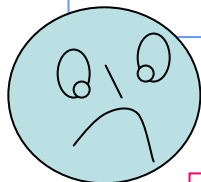
PROPOSED PROGRAMME FOR DATE PALM RESTORATION BY USING TISSUE CULTURE.

- **PROJECT OF PLANTING 3 MILLIONS VITRO-PLANTS IN MOROCCO DURING THE 20 YEARS PERIOD (1987 A 2007):**

→ **ESTIMATIONS** :



→ **REALIZATIONS** :



The goal was not achieved. A Lack of production was noticed

Another dream with Green Morocco Plan's strategy

THE GREEN MOROCCO PLAN'S STRATEGY FOR DATE PALM CULTURE

The Green Morocco Plan's strategy concerns Agriculture a sector which is directly responsible for the food security of 30 million consumers.

- Starting from the fact that domestic production of **dates** is limited at approximately 90 000 tonnes / year (normal year), about 3% of the world production and the fact that,
The fact that In 2007, Morocco imported nearly 50,000 tons of dates,
-
- The objective of the Green Morocco Plan's strategy is the production of **160,000 tons of dates / year** instead of 90,000 tonnes actually.
- In 2009. Project to plant **one million date palms** in the oases of Tafilalet in **2015**. **Much of them corresponds to vitro-plants**.



However, the project does not specify how the objectives will be achieved !!!!



What are problems raised from date palm tissue culture ?

MICROPROPAGATION OF DATE PALM BY TISSUE CULTURE. THE MOROCCAN EXPERIENCE IN GREEN BIOTECHNOLOGIES

Problems raised from date palm tissue culture:

استعصاء إكثار جميع الأصناف

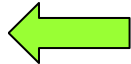
Inability to multiplication of all date palm cultivars. Recalcitrance of some cultivars to tissue culture. This leads to monovarietal palm groves

مشاكل تطابق صنف الفسائل الأنبوبية:

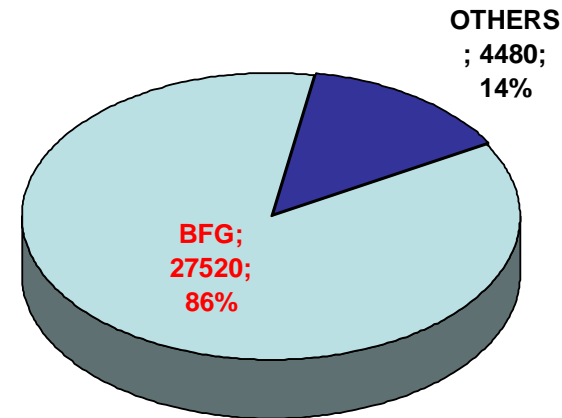
Problems of true-to-type for vitro-plants

مشاكل التلقيح والنمو

Problems related to pollination and growth alterations (offshoots)



RECOVERY PROGRAM OF DATE PALM IN TAFILALET DURING THE PERIOD 1988-1990 PROVIDED THE PLANTATION 150000-VITRO PLANTS. THE AVAILABLE WAS ONLY 32000 VITRO-PLANTS WITH 86% FROM BFG CULTIVAR.



DEFORMATIONS IN DATE PALM VITRO-PLANTS PRODUCED BY TISSUE CULTURE



VITRO-PLANT DE PALMIER DATTIER.
MALFORMATION DU RACHIS
ET DESSECHEMENT DES FOLIOLES



PALMIER DATTIER.
MALFORMATIONS



VITRO-PLANT DE PALMIER DATTIER. EMISSION EXCESSIVE DE REJET S

M. Azeqour et al., C. R. Biologies 325 (2002) 947-956

EXCESS PRODUCTION OF OFFSHOOTS

LEAF DEFORMATION



PALMIER DATTIER.
FRUITS PARTHENOCAPIQUES

**ALTERED POLLINATION
(Parthenocarpic fruits)**



Morphological variations and isoenzyme polymorphism of date palm clones from *in vitro* culture acclimatized and established on soil in South Morocco

M. Azeqour^{1,2,3}, K. Majourhat¹ & M. Baaziz^{1,*}

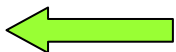
¹Laboratoire de Biochimie et Amélioration des Plantes, Université Cadi Ayyad, Faculté des Sciences-Semlalia, B.P. 2390, Marrakech, Morocco; ²Département de Biologie, Université My.Ismail, Faculté des Sciences et Technique, B.P. 509, Boutalamine, Errachidia, Morocco; ³Station Expérimentale, Office de Mise en Valeur Agricole de Tafilalet (ORMVAT), B.P. 17, Errachidia, Morocco; (*author for correspondence, e-mail: baaziz@ucam.ac.ma)

Received 5 June 2000; accepted 23 February 2001

Key words: cultivar, date palm, inflorescences, isoenzyme polymorphism, micropropagated plants, morphological variation, offshoots, *Phoenix dactylifera* L.

Summary

Five clones of date palm (*Phoenix dactylifera* L.) corresponding to three reputed Moroccan cultivars (BFG, JHL and BSK) and two selected seedling genotypes (S16 and S35), derived from *in vitro* culture, were acclimatized and planted in Errachidia soil (South Morocco) in 1989. After 10 years of culture, clones were evaluated on the presence/absence of the two principal morphological characters (offshoots, inflorescences). Then, their leaf extracts were subjected to electrophoresis on polyacrylamide gels in order to determine their isoenzyme polymorphism. Inflorescence formation is relatively more frequent in the clones S16 and S35 than in clones JHL and BSK. Clone BFG showed an intermediate situation. High isoenzyme variations were found for the oxidoreductases (peroxidases (POX) and polyphenoloxidases (PPO)), the transferase (glutamate oxaloacetate transaminase (GOT)) and the hydrolases (esterases (EST) and endopeptidases (ENP)) enzymes. The factorial component analysis exhibited a negative correlation between the two principal morphological characters (presence of inflorescences and offshoots formation). Date palm clones typified respectively by high inflorescences and high offshoots formed two separated groups showing each one some isoenzyme characteristics. All morphological and enzyme variations were discussed in respect to the genotype effect on the micropropagation process.



DEFORMATIONS IN DATE PALM VITRO-PLANTS PRODUCED BY TISSUE CULTURE

Five (5) clones of date palm developed from tissue culture at the INRA Centre (Marrakech) and Meknes Laboratory, were planted in Errachidia and Ouarzate since 1989. They are: Cultivars BFG, JHL, BSK and Sairs 'S16' and 'S35'

After 10 years, vitr-plants were evaluated on two morphological characters (inflorescence and offshoot productions) and four enzymes (ENP, EST, GOT, PPO, POX)

Four types of plants were obtained (Azeqour, Majourhat & Baaziz. 2002. Euphytica 123, 57-66):

- Vitro-plants with inflorescences (morphotype I)
- Vitro-plants with offshoots, only (morphotype O (offshoots))
- Vitro-plants with inflorescences and offshoots (morphotype M (medium))
- Vitro-plants without inflorescences and offshoots (morphotype N (none))



VITRO-PLANT DE PALMIER DATTIER. EMISSION EXCESSIVE DE REJET S

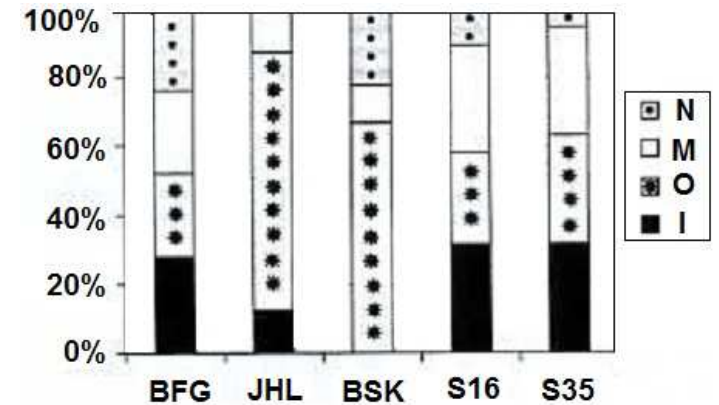
DEFORMATIONS IN DATE PALM VITRO-PLANTS PRODUCED BY TISSUE CULTURE

- Vitro-plants from JHL and BSK cultivars mainly produced offshoots reaching 80% and 60%, respectively.
- Clones S16 and S35 exhibited high numbers of inflorescences
- BFG clones showed an intermediate situation: I: 25%, O: 25%, M: 30% and N: 20%

→ ARE THESE CHARACTERS RELATED TO THE MATERIAL OF ORIGIN OR NEWLY APPEARED IN CLONES PRODUCED BY TISSUE CULTURE ?

Biochemical and molecular analysis:
Factorial Component analysis (FCA) exhibited:

- Morphotype O (Offshoots) is associated by a high peroxidase polymorphism
- Morphotype I is associated by a high polyphenoloxidase polymorphism



Parts (%) of types I, O, M and N, obtained in 5 date palm clones corresponding to the cultivars BFG, JHL and BSK and the genotypes S16 and S35 after 10 years of culture on soil



VITRO-PLANT DE PALMIER DATTIER, EMISSION EXCESSIVE DE REJET S

DEFORMATIONS IN DATE PALM VITRO-PLANTS PRODUCED BY TISSUE CULTURE

Two hypotheses could be proposed to explain deformations in date palm vitr-plants:

- VARIATIONS DUE TO THE TECHNIQUE OF MICROPROPAGATION (HERE, ORGANOGENESIS)
- VARIATIONS LINKED TO GENOTYPES OF THE MOTHER PLANTS

	BFG	JHL	BSK
Ability of the plant material of origin to form offshoots	64%	91%	64%

(Toutain & Rhiss, 1973)

Offshoot formation due to the in vitro technique (O + M) ...	48%	87%	78%
---	------------	------------	------------

(Azeqour, Majourhat & Baaziz. 2002. Euphytica 123, 57-66)

➡ **THE EFFECT OF DATE PALM GENOTYPE (ORIGIN) IS THE MOST PROBABLE FACTOR UNDER THE APPEARANCE OF EXCESS OFFSHOOTS**

➡ **AFTER MANY YEARS OF CULTURE, DEFORMATIONS DISAPPEARED. THEY MIGHT BE RELATED TO EPIGENIC VARIATIONS, ONLY.**



VITRO-PLANT DE PALMIER DATTIER. EMISSION EXCESSIVE DE REJETS

M. Azeqour et al., C. R. Biologies 325 (2002) 947-956

← This work was an example of our studies dealing with true-to-type analyses in date palm

Emir. J. Agric. Sci. 2003. 15 (1) : 01-16
<http://www.cfs.uacu.ac.ac/research/ejas.html>

Techniques for determination of true-to-type date palm (*Phoenix dactylifera L.*) plants: A literature review

K. J. Kunert¹, M. Baaziz² and C. A. Cullis³

¹ Botany Department, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 001, South Africa

² BAP Laboratory, Cadi Ayyad University, Marrakech 40000, Morocco

³ Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106, USA

Abstract: Production of date palm by *in vitro* techniques has been established in several commercial laboratories to produce large numbers of date palm plants at a competitive cost. *In vitro* propagation of plants requires that produced plants remain true-to-type as an important part of the quality assurance. With the advancement of biotechnology, several screening techniques for identification of true-to-typeness on the protein and the DNA levels have been developed, which might be applicable for identification of cultivars and detection of plant off-types. The advantages and disadvantages of these protein and DNA-based techniques are presented and strategies of their possible use as a quality assurance tool into commercial plant micropropagation laboratories are discussed.

Key Words: date palm, DNA markers, isozymes, somatic embryogenesis, *in vitro* propagation.

أساليب تحديد مدى تطابق أشجار النخيل مع نوعها الأصلي

ك. ج. كونرت¹، م. بعزیز²، أ. كوليس³

ملخص: تستخدم العديد من المختبرات التجارية أسلوب زراعة أنسجة نخيل التمر لإنتاج أعداد كبيرة من تلك الأشجار بأسعار تنافسية. ومن بين عناصر ضمان الجودة في هذه العملية أن تكون الأشجار المنتجة مطابقة لنوعها الأصلي. ونتيجة للتقدم في مجال التقنية الحيوية تم استحداث أساليب عديدة للتعرف على مدى مطابقة الأشجار الجديدة للنوع الأصلي من حيث مستويات البروتين والحمض النووي (DNA). ويمكن تطبيق هذه الأساليب في التعرف على الأنواع واكتشاف النباتات ذات الصفات المختلفة عن النوع الأصلي. وفي سياق هذا البحث نستعرض مزايا ومساوئ أساليب الكشف عن البروتين والحمض النووي، كما نناقش استراتيجيات استخدامها الممكنة كإداة لضمان الجودة في المختبرات التي تقوم بالإكثار الخضري على نطاق تجاري



What about Training and education through scientific research ?

OUTPUTS OF SCIENTIFIC RESEARCH ON EDUCATION RELATED TO BIOTECHNOLOGY AND GENETIC DIVERSITY



1. What is epigenetics ?

Epigenetics is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence

2. How could you check true-to-type in date palm cultivars propagated by tissue culture ?

3. What are the effects of biotechnology on plant genetic diversity ?



Development of biochemical and molecular markers