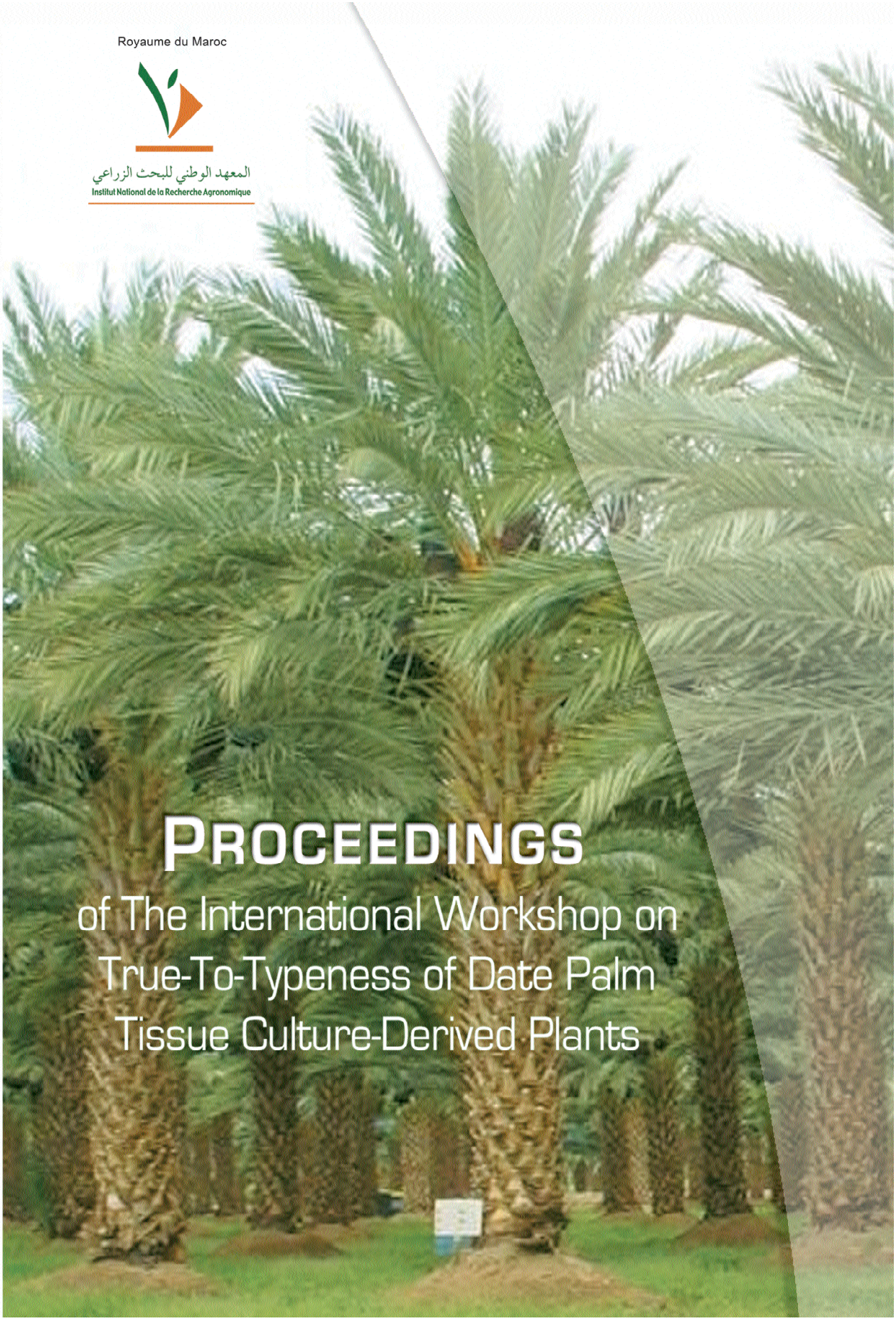
Royaume du Maroc

المعهد الوطني للبحث الزراعي

Institut National de la Recherche Agronomique



PROCEEDINGS

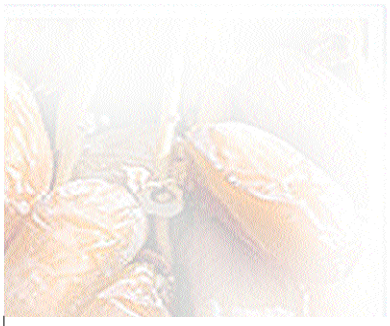
of The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants

Proceedings of The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants

2005

PROCEEDINGS

of The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants



The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the United Arab Emirates University, Food and Agriculture Organization of the United Nations, the United Nations Office for Project Services, nor National Institute of Agronomic Rsearch, concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

Réalisation

NADACOM Design - Rabat

Tél. : 037 68 25 50

Dépot légal : **2407/2005**

ISBN : 9981-1994-8-6

For inquiries on this publication, please contact:

Date Palm Research & Development Programme (UAE/2000/002)
Headquarters of Date Palm Global Network
Plant Tissue Culture Laboratory, UAE University,
United Nations Office for Project Services,
P.O.Box. 81908, Al Ain, UAE.

Edition INRA 2005

Information and Communication Division
Phone : 212 37 20 36 68
Fax : 212 37 20 36 60

PROCEEDINGS

OF THE INTERNATIONAL WORKSHOP

On
True-To-Typeness of Date Palm
Tissue Culure-Derived Plants

Morocco
23 – 25 May 2005

Organized by:

Date Palm Global Network;
Food and Agriculture Organization of the United Nations
(AGPC & RNE).

Hosted by:
National Institute of Agronomic Research / INRA - Morocco.

THE INTERNATIONAL WORKSHOP ON TRUE-TO-TYPENESS OF DATE PALM TISSUE CULTURE-DERIVED PLANTS

MOROCCO; 23 – 25 MAY, 2005.

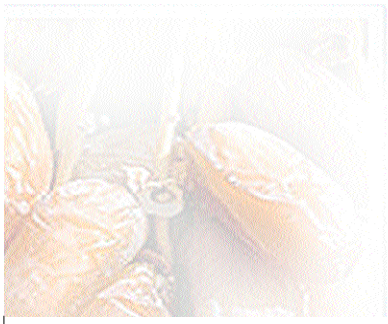
Chairpersons, Conveyor and Organizing committee

Chairpersons:

Session I : Dr. E.J. Arias.
Session II : Prof. A. Oihabi.
Session III : Dr. F. Taher.
Session IV : Prof. A. Zaid.

Conveyor:

Prof. Abdelouahhab Zaid
Chief Technical Advisor
Coordinator General of DPGN
P.O.Box. 81908, Al Ain, UAE.
Tel.: +971 3 78 32 334
Fax: +971 3 78 32 472
E-mail: zaid@uaeu.ac.ae



CONTENTS

THE INTERNATIONAL WORKSHOP ON TRUE-TO-TYPENESS OF DATE PALM TISSUE CULTURE-DERIVED PLANTS

MARRAKECH / MOROCCO, 23 – 25 MAY, 2005.

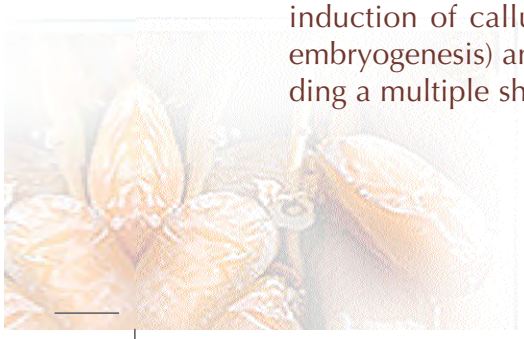
INTRODUCTION

The date palm, Phoenix dactylifera L., has been cultivated since antiquity. Dates provide a staple food crop for several countries located in the arid tropical and sub-tropical regions of the world.

Date palms are vegetatively propagated by offshoots which are usually produced by juvenile palms. The rapid vegetative propagation of a particular date palm or a mature specimen is limited by the number of offshoots produced during the palm's lifetime. Seed propagation of date clones and cultivars is impractical for several reasons. Half of the progeny will be males and half will be females and seedling females usually produce late maturing fruit of variable and generally inferior quality.

The development of tissue culture propagation method enabled date palm to be rapidly propagated at a large-scale from elite varieties already in existence, or from the F1 hybrids of previously select, and seed-only-originated palms, as well as from clones selected from high yield, disease resistant cultivars, or males having superior pollen with useful metaxenic characteristics.

In propagation by tissue culture, there are two approaches: one is through induction of callus which, in turn differentiates plantlets (called asexual embryogenesis) and the other method is called organogenesis which is yielding a multiple shoots formation.



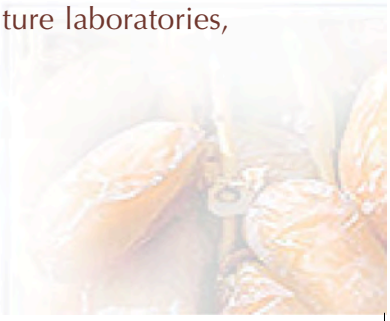
The production of genetically conform and stable vitro date palms is of a great and critical importance. The appearance of some physiological and morphological abnormalities is occurring with date palms of asexual embryogenesis origin. This situation already started to cause massive financial losses for date growers and embarassment for the concerned commercial laboratories.

Since the seventies, various techniques have been applied to study the true-to-typeness of tissue culture-derived plants. Such techniques include histo-cytological examination, iso-enzyme, and molecular biology / fingerprinting techniques. However, morphological field verification is the commonly used mean to confirm if tissue culture-derived plants are true-to-type to the mother tree.

Abnormalities so far recorded are several and can be listed as follow: broader leaves, leaf variegation, dwarfing, leaf whitening, delayed flowering and pollination failure.

WORKSHOP AIMS

- The workshop will be useful in updating the magnitude of the tissue culture abnormalities problem;
- The development of a program of future activities in this field;
- Strengthen communication between T.C. laboratories and specialists;
- Establish plan for continues communication among T.C. production staff that are facing abnormalities in the field;
- The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants is to provide an opportunity for updating scientific information on different aspects of date palm in vitro propagation. Date Palm scientists and experts from around the world will be able to exchange their know-how and experiences;
- Another important aim of the workshop is to present and compare the date producing countries experiences and to foster International technical cooperation on date palm. Several senior officials, scientists and technicians from date growing countries, public and private sector, commercial tissue culture laboratories, will be invited to attend the workshop.



CONTENT

The main topic of The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants can be summarized as follows:

- Asexual embryogenesis technique and its application to date palm;
- Organogenesis technique and its application to date palm;
- Other in vitro techniques to mass propagate date palm;
- True-to-typeness of derived date plants;
- Assessment of abnormalities so far observed and documented; and
- A round table discussions about the above.

WORKSHOP PERIOD & VENUE

The workshop was hosted by National Institute of Agronomic Researcg (I.N.R.A) / Morocco, during 23 – 25 May, 2005 at Marrakech, Morocco.

LANGUAGE OF THE WORKSHOP

English was the language of the workshop.

THE INTERNATIONAL WORKSHOP ON TRUE-TO-TYPENESS OF DATE PALM TISSUE CULTURE-DERIVED PLANTS

MARRAKECH / MOROCCO, 23 – 25 MAY, 2005

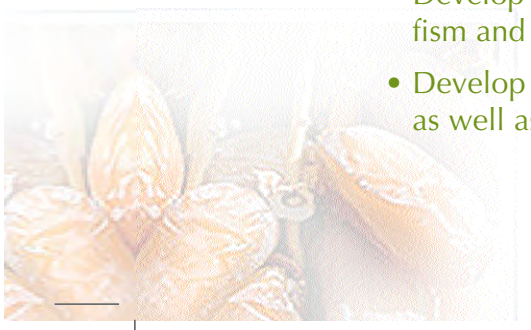
CONCLUSIONS AND RECOMMENDATIONS

- The workshop was useful in updating the magnitude of tissue culture abnormalities; Presentations from various date growing countries illustrated these variations which were summarized as follow: dwarfism, pollination failure and abnormal fruiting, abnormal morphology of the tree and the leaves, twisted inflorescences and offshoots, leaf whitening and delayed flowering time.

- At the exception of the two cases (one in the Kingdom of Saudi Arabia as well as in some additional countries, with Barhee trees originating from DPD / England) and (the other case related to plants produced by Palm Dat which are presently planted in Israel, Jordan, Namibia, Niger, and Yemen), which could be considered as accidents, the abnormalities of tissue culture-derived date palms are of a small incidence (less than 5 %).

- These abnormalities were classified by the participants in three categories: i) Dwarfism, ii) Parthenocarpic fruits and iii) Morphological problems (as leaf whitening, leaf size, and deformed offshoots). Off-types carrying symptoms similar to "Black Scorch" disease of Medjool cv. were also mentioned.

- An important aim of the workshop which was the development of a program of future activities in this field was also reached. Indeed the following initiatives were adopted:
  - Develop a reliable marker that can identify at early stage the dwarfism and another one for the parthenocarpy phenomenon.
  - Develop a data base for many markers of the date palm cultivars as well as the related abnormalities.



- Collaborative approach involving plant anatomists, tissue culturists and molecular geneticists will provide solutions to clonal fidelity issues. Hence, it is highly recommended to strengthen the on going research on all the above fields.
- Micro propagation protocols are to be refined to eliminate factors inducing variations. It is highly probable that these abnormalities resulted from improper handlings during the micro-propagation process.

❖ Both commercial laboratories and the research units dealing with date palm in vitro propagation were present in the workshop. They all agree on strengthening their collaboration and ensuring the continuous exchange of information in order to solve common problems.

❖ With the worldwide increase of potential countries coming into commercial cultivation of date palm, tissue culture is certainly the most appropriate tool to provide these countries with their needs of date palm plants. However it is highly recommended that all enterprises working with this commodity develop a safe tool that grantee a safe product to the end user.

❖ Tissue culture production staff that are facing abnormalities in the field did all agree to establish a continuous communication among them by e-mail. The participants of these workshop were invited to continue communication and informing each others and providing updates about the tissue culture abnormalities in the form of an electronic site. Dr. Ait Chitt Mustapha from Morocco was nominated to create and ensure the follow up of this web site which will be linked to the DPGN web site.

❖ The workshop did indeed provide an opportunity to present and compare the date producing countries experiences and to foster international technical cooperation on date palm. Scientists attending this workshop were coming from 14 countries (Austria, Canada, Egypt, France, Iraq, Israel, Italy, KSA, Morocco, Namibia, Spain, UAE, UK, and USA).

❖ An important recommendation was to continue the survey approach in order to well assess these abnormalities and also to carry out an international field evaluation.

- Some of the abnormalities could be due to environmental and / or management factors.
- The variability Seems to be strongly related to the conditions of regeneration of the vitro plants (Mother material, production protocol, personnel capacity, laboratory conditions, etc) and to a lesser degree to the technique (Somatic embryogenesis or organogenesis).
- Date palms from seeds like from tissue culture produce frequently inflorescences of juvenile type. This juvenile morphogenesis corresponds to a very classical rejuvenation phenomenon similar to the one affecting the leaves.
- So called abnormalities are often transitory juvenility traits (even dwarfness and "Thielaviopsis sensitivity") but prolonged juvenility induced by specific tissue culture process are detrimental. Their epigenetic origin makes them undetectable by molecular fingerprint techniques.
- It was quite probable that failure of normal fruiting in young tissue culture trees was due to many interrelated events that led to slow growth of pollen tube at early stages of fruit growth and which might possibly be accentuated by the relatively high ABA content during this period.

LIST OF PARTICIPANTS

Country Full Name / or Institution	Mailing Address	Tel. No.	Fax. No.	E-mail address
Dr. Fawzi Taher FAO/Egypt.	Regional Crop Production Officer FAO Regional Office for the Near East, Cairo.	+202 33 1 6000 +202 33 1 6134	+202 7495981	*fawzi.taher@fao.org*
Dr. E. Arias FAO/Italy.	Agricultural Officer, AGPC, FAO, Rome, Italy.	+39 348051 9088 (Mobile); +3906 5705 4581 (Office)	+39 06 5705 6347	*Enrique.Arias@fao.org*
Dr. A. Zaid UNDP/UAE.	Chief Technical Advisor / Director, UAE University, P.O.Box. 81908, Al Ain, UAE.	+971 3 7832 334 +971 50 6633 598 (Mobile)	+971 3 7832 472	*zaid@uaeu.ac.ae*
Dr. A. Oihabi FAO/KSA.	Senior Date Production Expert, Al Hassa, KSA.	+966 35 30 1660 +966 50 6929 412 (Mobile)	+966 35 30 1896	*oihabi@yahoo.fr*
Dr. C. Ainsworth UK.	Imperial College at Wye, United Kingdom.	+44 78157 31198	+44 20 759 42640	*c.ainsworth@imperial.ac.uk*
Dr. C.A. Cullis USA.	Dept. Biology, Case Western Reserve Univ. Cleveland, OH 44106-7080, USA.	+1 216 368 53 62	+1 216 368 46 72	*cac5@case.edu*
Dr. M. Aaouine Morocco.	Residence Jawhara, 4, Rue Nehrou, B.P. 7115, Meknes.	+212 61 07 6056 (Mobile)	+212 55 5235 19	*maaouine@menara.ma*
Dr. Hassan Ali Dinar KSA.	UTFN / SAU / 002 / SAU – Riyadh, KSA.	+966 50 710 1959 +966 50 6929412 (Mobile)	+966 1 4033 563 (Tel / fax)	*oihabi@agrwat.gov.sa*
Dr. Abdullatif Ali Al Khateeb KSA.	CHead of the Date Palm Centre King FaisalUniversity, P.O.Box. 400, Al Hassa 31982, KSA.	+966 50 4920 397	+966 581 6630	*aalkhateeb@kfu.edu.sa*

Country Full Name / or Institution	Mailing Address	Tel. No.	Fax. No.	E-mail address
Dr. Yuval Cohen Israel.	Dept. of Fruit Tree Sciences Institute of HorticulturE Volcani Centre.	+972 3 9683 407 +972 3 638 9555	+972 3 966 9583	*vhyuvale@volcani.agri.gov.il*
Mr. Barush Glasner Israel.	Hadiklaim, Tel Aviv.	+972 50 552 9744 (Mobile)	-	*buki@hadiklaim.co.il*
Dr. Mohan Jain IAEA/Austria.	Dept. Applied Biology, Horticulutre Section, PL-27 (Latokartanonkaari 5), 00014 Helsinki University, Finland.	+358 9 191 58428	+358 9 191 58582	*Mohan.jain@helsinki.fi*
Dr. Danielle Donnelly Canada.	Plant Scieces Dept. Mc Donald Campus of Mc Gill University 21, 111 Lakeshore Rd., H9X 3V9, Canada.	+514 398 7851 Ext. 7856	+514 398 7897	*Danielle.donnelly@mcgill.ca*
Dr. Ait Chitt Morocco.	Domaines Royaux, Meknes.	+212 378 31 624 +212 6817 9803 (Mob.)	-	*aitchitt@hotmail.com*
Mr. Anjarne Morocco.	Tissue Culture Laboratory INRA, Marrakech	+212 6827 2824	-	*inramrk@iam.net.ma*
Eng. Abdelouahhab Rajhi Rajhi Laboratories/KSA.	Director General, P.O.Box. 55155 Riyadh 11534.	+966 5 5464482 (Mobile)	+966 1 456 2600	*abdulwahab@shabakah.com*
Mr. P.F. de Wet Namibia.	Namibia Development Corporation, Windhoek.	+264 61 24 6464	+264 61 25 3814	*Pieter.DeWet@ndc.org.na* *Date.Support@ndc.org.na*

Country Full Name / or Institution	Mailing Address	Tel. No.	Fax. No.	E-mail address
Dr. Abdel Rahman Al Wasel KSA.	King Saud University. Al Qaseem, KSA.	+966 6 364 7375	+966 6 3800 310	*awasel@yahoo.com*
Dr. Abdullah Ali Al-Baiz KSA.	National Agriculture Center for Research P.O.Box. 17285, Riyadh, 11484, KSA.	+966 1 457 67 80, Ext. 2237 +966 50 425 57 505 (mobile)	+966 1 458 49 79	*aaabaiz@yahoo.com*
Mr. M. Ferry Spain	Estacion Phoenix, Apartado 996, 03201, Elche, Spain.	+34 965 421 551	+34 965 423 706	*m.ferry@wanadoo.es*
Dr. Sami S.M. Adawy Egypt.	AGERI, ARC, Egypt.	+202 573 44 24 / 572 9057	+202 568 9519	*sami_adawy@yahoo.com*
Dr. Mobasher Omar Iraq.	Head of Genetic Engineering & Biotechnology, Ministry of Sciences & Technology Baghdad, Iraq.	-	-	*mobasheromar@yahoo.com*
Mr. Javouhey Marc France.	Marrionet GFA, Soingsen Sologne 41, France.	+254 987 103		*marc.javouhey@marionnet.com*

PROGRAMME

SUNDAY, 22 MAY 2005

02:00 p.m. – 09:00 p.m. : **Registration for early arrivals.**

MONDAY, 23 MAY 2005

07:30 a.m. – 09:00 a.m. : **Registration for late arrivals.**

09:00 a.m. – 09:30 a.m. : **Welcoming Remarks by**

- *General Coordinator of Date Palm Global Network*
- *Hon. FAO Resident Representative &*
- *Hon. INRA General Secretary.*

09:30 a.m. – 10:00 a.m. : Coffee & Tea Break.

SESSION I : ABNORMALITIES IN T.C. PLANTS AROUND THE WORLD (10:00 A.M. – 01:00 P.M.)

Moderator : **Dr. E.J. Arias.**

10:00 a.m. – 10:30 a.m. : **Plant-off-types in tissue culture-derived date palm (Phoenix dactylifera L);**

By H.S Al Kaabi, A. Zaid & C. Ainsworth (Presented by Prof. A. Zaid / UNDP – UAE).

10:30 a.m. – 11:00 a.m. **Abnormalities of tissue culture derived date palm (Phoenix dactylifera L.) cultivated in Namibia and Niger;**
By Prof. A. Oihabi / FAO - KSA.

11:00 a.m. – 11:30 a.m. : **Radiation-induced mutations for developing bayoud disease resistant date palm in North Africa;**
By Dr. Mohan Jain / Austria.

11:30 a.m. – 12:00 p.m. : **"Barhee" fruit setting problems at Kingdom of Saudi Arabia: Research approaches to understand the physiological and physical events of the phenomenon;**
By Dr. Hassan A. Dinar / KSA.

12:00 p.m. – 12:30 p.m. : **Some documented abnormalities of tissue culture-derived date palms and their economical impact on the growers' revenue;**
By Dr. M. Aaouine / Morocco.

12:30 p.m. – 01:00 p.m. : **A survey study on: Somaclonal variations in In vitro-derived date palm trees;**
By Dr. Al Wasel / KSA.

01:00 p.m. – 03:00 p.m. : *Lunch Break.*

SESSION II: CASE PRESENTATION PER COUNTRY
(03:00 P.M. – 06:00 P.M.)

Moderator : **Prof. A. Oihabi**

03:00 p.m. – 03:30 p.m. : **Micropropagation and Genetic Risk: Securing Clonal Fidelity.**
By Dr. D. Donnelly / Canada.

03:30 p.m. – 04:00 p.m. : **True-to-Type date palms obtained through tissue culture using the axillary branching techniques.**
By Dr. M. Ait Chitt / Morocco.

04:00 p.m. – 04:30 p.m. : **A presentation about Date Palm Global Network;**
By Dr. A. Zaid / UAE.

04:30 p.m. – 05:00 p.m. : *Coffee & Tea Break.*

05:00 p.m. – 05:30 p.m. : **Phenotypic and molecular characterization of date palm off-types;**
By Dr. Y. Cohen / Israel.

05:30 p.m. – 06:00 p.m. : Mixed inflorescence vegetative axillary development: a trait of rejuvenation in the date palm from tissue culture;
By Mr. M. Ferry / Spain.

TUESDAY, 24 MAY 2005

SESSION III: AVAILABLE TECHNIQUES TO VERIFY THE TRUE-TO-TYPENESS (09:00 A.M. – 01:30 P.M.)

Moderator : **Dr. F. Taher**

09:00 a.m. – 09:30 a.m. : **Status of Tissue Culture Date Palms in Namibia;**
By Mr. P.F. de Wet / Namibia.

09:30 a.m. – 10:00 a.m. : **In Vitro propagation of date palm.**
By Dr. Mobasher S. Omar / Iraq.

10:00 a.m. – 10:20 a.m. : AFLP variation in tissue culture-derived date palm plants;
By H.S. Al Kaabi, A. Zaid & C. Ainsworth (Presented by Dr. C. Ainsworth / UK).

10:20 a.m. – 10:30 a.m. :	**A presentation** By Eng. Abdelwahhab Rajhi.
10:30 a.m. – 11:00 a.m. :	*Coffee & Tea Break.*
11:00 a.m. – 11:30 a.m. :	**AGERI experience with date palm molecular fingerprinting techniques.** By Dr. Sami Said M. Adawy / Egypt.
11:30 a.m. – 12:00 p.m. :	**Comparison of somatic embryos with offshoot origin in two cultivars of date palm (Phoenix dactylifera L.)**; By Dr. Abdullah A. Al Baiz / KSA.
12:30 p.m. – 01:00 p.m. :	**DNA Markers for the Detection of Genomic Integrity.** By Dr. C.A. Cullis / USA.
01:00 p.m. – 01:30 p.m. :	**A presentation** By Mr. Anjarne.
01:30 p.m. – 03:00 p.m. :	*Lunch Break.*

SESSION IV: BRAINSTORMING SESSION

(03:00 P.M. – 05:00 P.M.)

Moderator : **Prof. A. Zaid**

03:00 p.m. – 04:00 p.m. :	**Around Table Discussion.**
04:00 p.m. – 04:30 p.m. :	**Conclusions and Recommendations.**
04:30 p.m. – 05:00 p.m. :	**Closing Ceremony.**

WEDNESDAY 25 MAY, 2005

09:00 a.m. – 10:00 a.m. :	**Visit to the INRA Tissue Culture Laboratory.**
10:00 a.m. – 11:00 a.m. :	**Visit to the INRA Molecular Biology and Pathology Department.**
11:00 a.m. – 01:00 p.m. :	**Visit to the Molecular Biology Units of the Faculty of Sciences-Semlalia.**
01:00 p.m. – 03:00 p.m. :	*Lunch Break.*
03:00 p.m. – 05:00 p.m. :	**Meeting of the DPGN Coordination Board (General Coordinator, Technical Secretariat, the Regional Coordinators and the Working Group Coordinators).**
05:00 p.m. – 09:00 p.m. :	**Visit to the Old City of Marrakech (on a voluntary basis).**

PRESENTATIONS

PLANT-OFF-TYPES IN TISSUE CULTURE-DERIVED DATE PALM (*PHOENIX DACTYLIFERA L*).

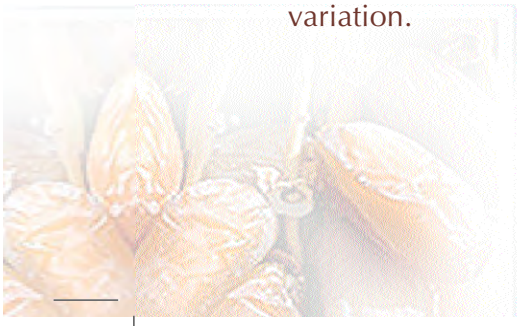
H.H. Al Kaabi [(1)], A. Zaid [(2)] and C. Ainsworth [(3)]

(1) National Project Director, Date Palm Research & Development Program, UAE University, P.O.Box. 81908, Al Ain, UAE. Tel: +971 3 7832334, Fax: +971 3 7832472. E-mail: helalk@uaeu.ac.ae
2) Chief Technical Advisor, Date Palm Research & Development Program, UAE University - United Nations Development Program, P.O.Box. 81908, Al Ain, UAE. Tel: +971 3 7832334, Fax: +971 3 7832472. E-mail: zaid@uaeu.ac.ae
(3) Dept. of Agricultural Sciences (MCR), Imperial College at Wye, Wye - Ashford, Kent, TN 25 5AH, UK. Tel: +44 7815 731 198, Fax: +44 2075 942 640. E-mail: c.ainsworth@imperial.ac.uk

ABSTRACT

True-to-typeness and appearance of abnormalities are the most serious problems associated with date palm tissue culture. The aim of the present investigation is to study the morphological abnormalities in tissue culture-derived date palms. A survey of embryogenesis-derived trees in the field identified various abnormalities including abnormal leaves and inflorescences, dwarfing, leaf bleaching, deformed offshoots, delayed flowering time, pollination failure and abnormal fruiting. Some varietal specificity was noted. The identification and early detection of these plant-off-types will contribute to avoiding such shortcoming in the future and avoid the occurrence and propagation of such abnormalities.

Key words: Date palm, phoenix dactylifera L., tissue culture, somaclonal variation.



1- INTRODUCTION

More than 500 articles have described the existence of genetic variability in plant tissue cultures (Orton, 1983). The terminology used for plant off-types is diverse. The name "phenovariant" was first coined by Sibi (1971). Sibi (1989) also proposed the terms "vitro variants" and "vitro variations" as new general and practical terms for this type of variation. The term "somaclonal variation", which is commonly used, was proposed by Larkin and Scowcroft (1981).

Somaclonal variation can be an epigenetic or genetic change, sometimes expressed as a new trait, resulting from in vitro culture of higher plants (Pierik, 1987; Zaid et al., 1999). An easy differentiation between the two types of variation can be carried out by the study of offspring. In comparison to epigenetic variation, genetic variation follows Mendelian segregation rules.

A field survey, covering most UAE date palm orchards recently planted with tissue culture-derived plants, was conducted during two growing seasons, 2001 / 02, 2002 / 03. These plants were obtained from several different commercial laboratories worldwide out of which used the embryogenesis technique for generating date palm plantlets. The survey targeted all abnormalities and off-types that appeared in these date palm plantations. These included plants with abnormal morphology and structure such as twisted inflorescences and broader leaves, excessive vegetative growth, leaf variegation, dwarfing, leaf bleaching, bastard offshoots, delayed flowering time, pollination failure and abnormal fruiting (Tables 1, 2).

2- DATE PALM MORPHOLOGY AND STRUCTURE

Morphological abnormalities were detected at the hardening phase in the nursery and included the following:

- absence of an onion like base;
- thin stem with leaves being juvenile and weak;
- abnormal phyllotaxy;
- very low growth of the root system with no more than three to four thin roots per plant (about 2 mm in diameter).

The occurrence of abnormalities clearly depends on plant producers and

their respective production / selection processes (Table 2). For example, abnormalities at a rate of up to 63 % in a date palm nursery containing 2,000 hardened in vitro Medjool plants was observed in UAE (Fig. 1a). In contrast, a recently established date palm orchard in Namibia contained only 5 % of Medjool plants with abnormal characteristics (Zaid and Arias, 1999). A case of abnormal phyllotaxy was observed with Barhee (Fig.1b).

3 EXCESSIVE VEGETATIVE GROWTH

An abnormality frequently found in the date orchards surveyed is an excessive degree of vegetative growth. These plants were found to have broader leaves, compact growth and a different spine structure (Figs. 1c, 1d). Out of 2,000 Barhee palms surveyed, which were derived from asexual somatic embryogenesis, only two plants (0.1 %) that showed this abnormal vegetative growth were found (Table 2). In contrast, McCubbin et al. (2000b) found a much higher ratio of 1.4 % in a survey carried out in South Africa on Medjool plants that had been produced by three separate tissue culture laboratories using somatic embryogenesis.

4 LEAF VARIEGATION

Some plants had leaves with a creamy-coloured stripe running parallel to the leaf margin (Fig. 1e, 1f). The abnormality occurred on all leaves but there was some variation in stripe width. Although the plants seemed healthy, they clearly suffered from reduced growth rate. Twelve out of 50,000 greenhouse plants (0.024 %) of several date palm varieties showed variegation (Table 2).

Variegation as an off-type is common among date palm tissue culture–derived plants and has also been reported with other plant species such as banana (Al Wasel, 2001). Variegation might depend on factors such as virus / microbial contamination, in vitro media nutrient deficiency or genetic variation (McCubbin et al., 2000a). Leaf variegation can occur because of mutations in the photosynthetic apparatus. Furthermore, since many genes are involved in chlorophyll synthesis, many independent mutations can cause the variegated phenotype.

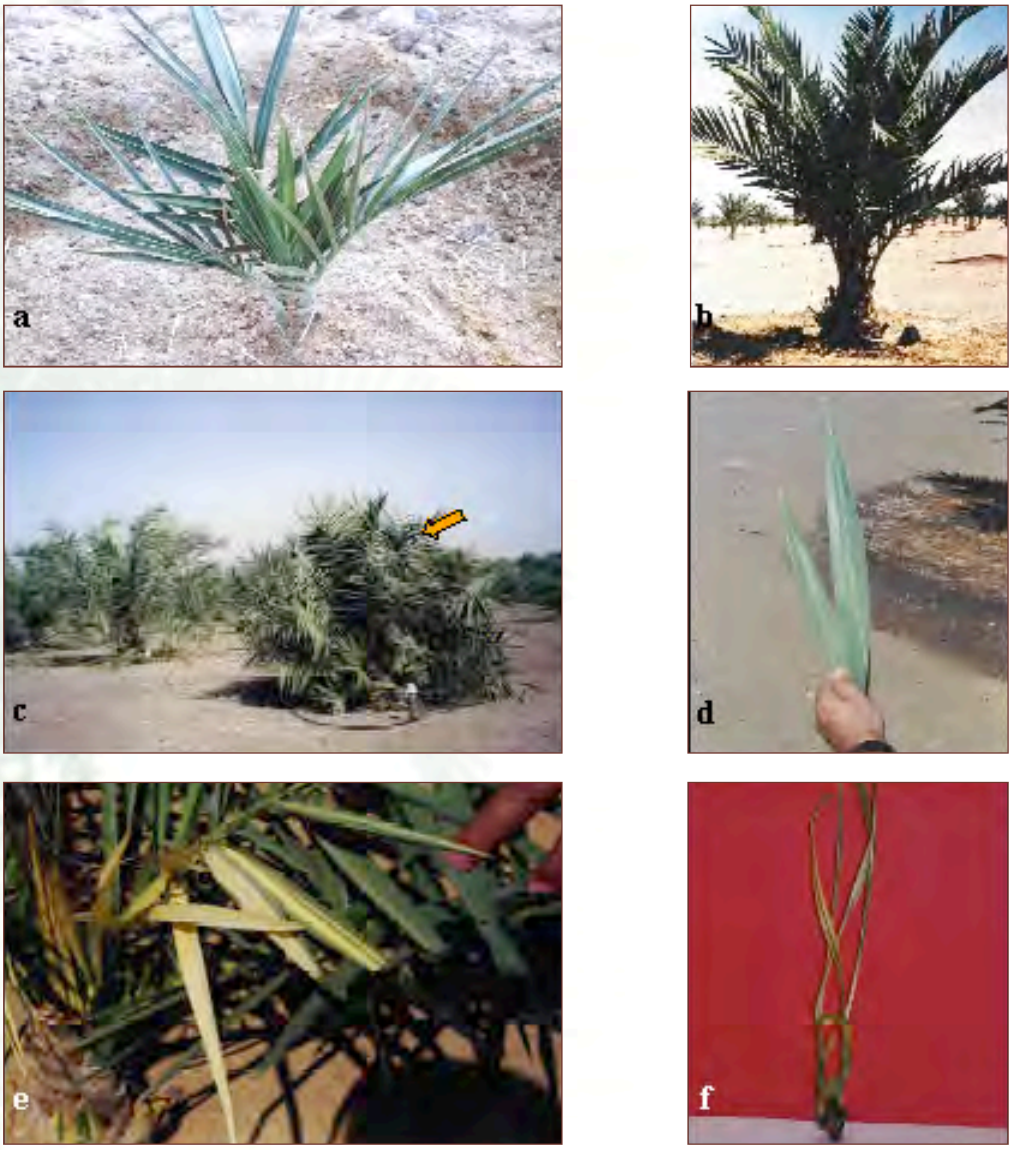


Figure 1. Abnormal date palm tree morphology:

a. Abnormal morphology and structure of a two year old date palm (Medjool) produced via somatic embryogenesis; b. Seven year old date palm (Barhee) derived from somatic embryogenesis showing abnormal leaf structure and size; c. A Barhee tree showing excessive vegetative growth (arrowed) with a normal tree in the background; d. Abnormally large leaf of a Barhee tree (right: abnormal Barhee; left: normal Barhee leaf); e. Variegation observed on one year old Sukkari at the Liwa area in UAE. f. Leaf variegation on a Barhee tissue culture-derived plantlet at the elongation stage.

5 DWARFISM

The dwarfing phenomenon in tissue culture–derived plants is manifested as a severe restriction in growth. Dwarf date palm plants are less than one metre in height after four to five years in the field, in comparison to a normal date plant of the same age with an average height of 3 m (Fig. 2a). However, most dwarf date palm trees are only identified during the first or second year after field planting, when stem elongation occurs (Fig. 2b). The old leaves are sometimes normal and dwarfism starts affecting the younger leaves (Fig. 2c). In some cases, dwarfism only affects the outer (older) leaves (Fig. 2d). Dwarfism reduces the leaf length (up to 2/3 shorter than a normal leaf) and also affects the leaflets, which are severely reduced in size (Fig. 2e). This reduction in leaf structure and canopy size affects leaf function resulting in low photosynthesis and consequently in greatly reduced growth. Dwarfism also severely weakens date palm plants and reduces offshoot production.

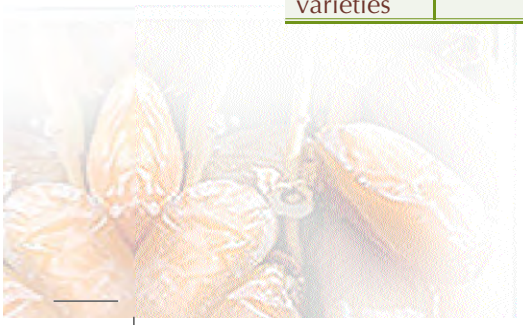
The survey was carried out in the western region of UAE, where date palm varieties, such as Sukkari, Barhee, Sultana, Khlass and Oum Dahn, were found showing dwarfism with Sukkari being the most affected. In one orchard, 20 dwarf trees were identified among 50 Sukkari plants (40 %); while at another orchard 15 Sukkari plants out of 42 (35 %) were affected. In contrast, Barhee and Sultana plants were less affected and only 17 out of 300 Barhee plants (5.6 %) and 2 out of 200 Sultana plants showed dwarfism (1 %), (Table 1). Khlass was even less affected with only 20 dwarf plants out of 5,000 plants (0.4 %) detected in the survey.

Dwarfism has also been described in the varieties Ajoua (Al Wasel, 2000 a, b), Sultana and Nabt Saif (Abo El Nil, personal communication) with the frequency of dwarfism varying from 0 to 30 %, depending on variety (Al Wasel, 2001). Dwarfism has also been commonly observed with embryogenesis-derived date palm plants (Cohen et al., 2003).

Table 1. Dwarfism in asexual embryogenesis-derived date palms.

Variety	Plants scored	Number of dwarf plants	% Plants affected	Author
Sukkari	50 42	20 15	40 35	Present study
Khlass	5,000	20	0.4	Present study
	218	53	24.3	Al-Wasel (2001)
Barhee	300	17	5.6	Present study
	234	42	17.7	Al-Wasel (2001)
Sultana	200	2	1	Present study
Various varieties	N.S	N.S	4	McCubbin et al. (2000b)

N.S: Not specified.



The causes of dwarfism in date palms are not known. A dwarf phenotype is also associated with black scorch disease (Fig. 2g). Black scorch, also called Medjnoon or Fool's disease is caused by the pathogen Ceratocystis paradoxa (Amira et al., 2000). Black scorch has been observed on a total of 19 date palm varieties, including Thoory, Hayani, Amhat, Saidy, Halawy, Medjool and Barhee (Djerbi, 1983; Zaid and Al Kaabi, 2001). While genetically dwarf plants will not recover after planting into the soil, and remain dwarf even after 3 consecutive years of chemical treatment, black scorch-affected date palm trees can recover from the disease after repeated chemical treatment. Tissue culture-derived date palm plants are apparently more susceptible to this disease than offshoots and immediately after the attack the meristem of tissue culture-derived plants shows restricted development. In this study, several genetic dwarf trees were examined and showed normal root systems (Fig. 2f).

6 LEAF BLEACHING

The bleaching of leaves of certain date palm varieties, such as Khlass, Sultana, Barhee and Nabt Saif was observed (Fig. 3a) and was due to partial or total loss of chlorophyll. Usually, 2 to 4 leaves were affected per tree. However, this abnormality was found to be rare in orchards (affecting 53 out of 864 plants surveyed) and is therefore of no great economic significance. Normally, the affected tree grows out of this phenotype and the photosynthesis process restarts in the affected leaves which slowly turn back to green (Fig. 3d, e, f). This phenomenon has not been previously described.

Figure 2. Dwarfism in date palm asexual embryogenesis-derived plants:

a. Dwarf Barhee date leaf (arrowed) in comparison to a normal grown leaf; b. Dwarf Oum Dahn date tree showing a variegation abnormality (arrowed); c. Dwarfism effect on a four year old Barhee plant derived from somatic embryogenesis with older leaves not showing any dwarfism; d. Dwarfism effect on a four year old Barhee plant derived from somatic embryogenesis. Outer, older leaves affected; e. Dwarfism effect on young fronds of a one year old somatic embryogenesis-derived Medjool plant; and f. Uprooted three year old dwarf Sukkari plant with a normal root system and g. Black scorch disease on a four year old Medjool plant.

7- DEFORMED OFFSHOOTS

It is well known that tissue culture–derived date palm plants have a better growth habit and produce uniform date palm orchards than offshoot-derived plants (Al Wasel, 2000b). They also produce more primary and secondary offshoots. However, this fast growing habit and the abundance of offshoot production is sometimes accompanied by the appearance of abnormal offshoots and twisted inflorescences. Deformed vegetative buds and their conversion to floral buds (Fig. 3b, c) are commonly observed. The frequency of these abnormal offshoots was approximately 1 in 20 trees but was only observed in trees in their first year of flowering. However, frequency estimates are difficult as abnormal offshoots are removed by the grower and in subsequent years are replaced by normal offshoots. This deformed condition can be caused by an infestation with the date palm bud mite (Makiella phoenicis K.) (Cohen et al., 2003), or may be due to reduction in growth caused by an inequilibrium of endogenous growth regulators accumulated during in vitro propagation (Cohen et al., 2003). Better knowledge is required of the cytokinin and auxin levels within the plant and the level of plant growth regulators that are needed to induce a response during in vitro propagation.

8- DELAYED FLOWERING TIME

Although tissue culture plants are known for their faster growth compared to offshoots (Al Wasel, 2000b), there is increasing concern about possible delay in their first fruit production. In one instance, in an orchard of 10 hectares of Barhee date palm trees derived from somatic embryogenesis (about 2,420 plants) it took more than 7 years for 50 % of the trees to reach the floral stage. Delayed flowering time may be caused by the prolific vegetative growth as a result of juvenile vigour in tissue culture-derived plants (Cohen et al., 2003).

9- POLLINATION FAILURE AND ABNORMAL FRUITING

An observation made during the course of this survey was of pollination failure and very low fruit set with asexual embryogenesis tissue culture-derived plants of Barhee and Medjool (Table 2). All pollinated bunches showed 80 to 100 % of parthenocarpic fruits during the first year of production (Figs. 4a, b, c) and sometimes the development of more than 3 carpels (Figs. 4d, e).

This phenomenon has also been found in somatic embryogenesis-derived Barhee date palm orchards around the world, including Namibia, South Africa, Kingdom of Saudi Arabia, and the United Arab Emirates (Djerbi, 2000; McCubbin et al., 2000b). Other varieties such as Khlass, Sukkari, Ajoua and Deglet Nour also have been reported to be affected, but to a lesser degree (Djerbi, 2000; Al Wasel, 2001).

Pollination failure of this type appears to be associated with plants produced via somatic emryogenesis (Djerbi, 2000; McCubbin et al., 2000b). The normal parthenocarpic fruits are not suitable for consumption, causing economic loss

(Al Wasel, 2000a; Cohen et al., 2003). This phenotype appears similar to the "Mantled" phenotype of tissue culture-derived oil palms that were associated with epigenetic variations (Corley et al., 1986; Matthes et al., 2001; Jaligot et al., 2002).

More research needs to be carried out to identify the causes of pollination failure in tissue culture-derived date palm plants. It is possible that tissue culture-derived date palms require heavier pollination than plants derived from offshoots. However, there is evidence that alleviation of abnormalities such as the pollination failure and the subsequent low level of fruit setting may occur after several years (Cohen et al., 2003).

Table 2. Morphological abnormalities identified in date palm.

Abnormality	Variety	Plants scored	Plants affected	%	Author
Morphology and structure	Medjool	2,000	1,260	63	Present study
		1000	50	5	Zaid and Arias, 1999.
Excessive vegetative growth	Barhee	2000	2	0.1	Present study
	N.S	N.S	N.S	1.4	McCubbin et al., 2000b
Leaf variegation	Several varieties together	50,000	12	0.024	Present study
	Khlass	218	2	0.92	Al Wasel, 2001.
Pollination failure	Barhee & Khlass	100,000	100,000	100	Djerbi, 2000.
	Barhee	N.S	N.S	Up to 100	McCubbin et al., 2000b
	Khlass & Sukkari	1000	786	78.6	Al Wasel, 2001.
	Ajoua	500	430	86	

N.S: Not specified.

Although genetic abnormalities such as changes in chromosome number have been found in somatic embryogenesis-derived date palm plants, this contrasts with oil palm where somatic embryogenesis-derived plants show very few abnormalities (D'Amato, 1978; Corley et al., 1979; Brackpool et al., 1986)

The key difference between date palm and oil palm somatic embryogenesis may be that meristematic cells from oil palm do not require any exogenous cytokinins application which is known to induce genetic variation (Corley et al., 1981).

The morphological differences which were observed included albinism and floral sex differences (Corley et al., 1986; McCubbin et al., 2000b; Sharma et al., 1980; 1984).

In addition to date palm and oil palm, variation phenomena have been described in about 150 different plant species (Pierik, 1987).

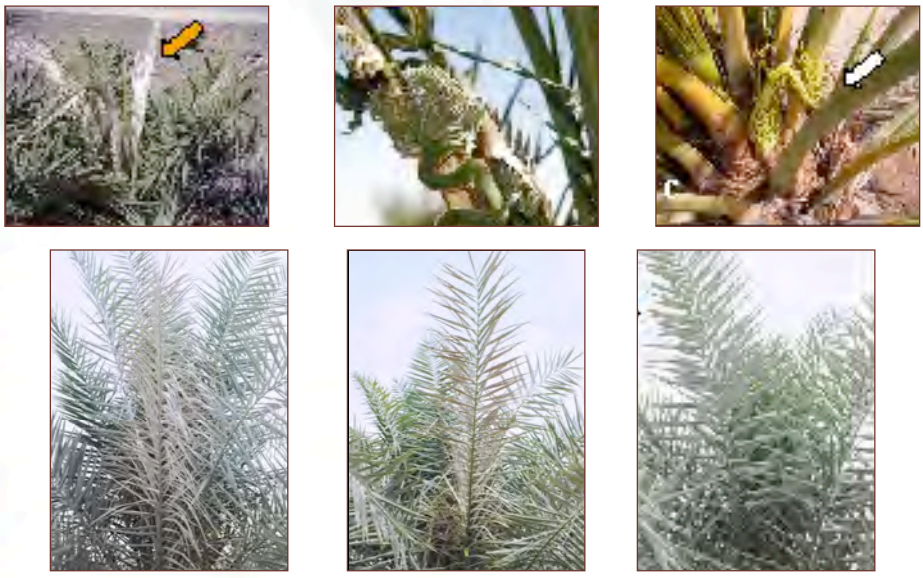


Figure 3. Date palm morphological abnormalities:

a. Abnormal white leaves on a three year old Barhee plant; b. Deformed offshoot on a somatic embryogenesis-derived Barhee plant (arrowed); c. Twisted inflorescence on Barhee four year old tissue culture plant (arrowed); d, e, f. Progressive stages of recovery of chlorophyll in the whitened leaves of a six year old Khlass tissue culture-derived tree. Note that the affected leaves turn back to the normal green colour (in the order d, e and f).

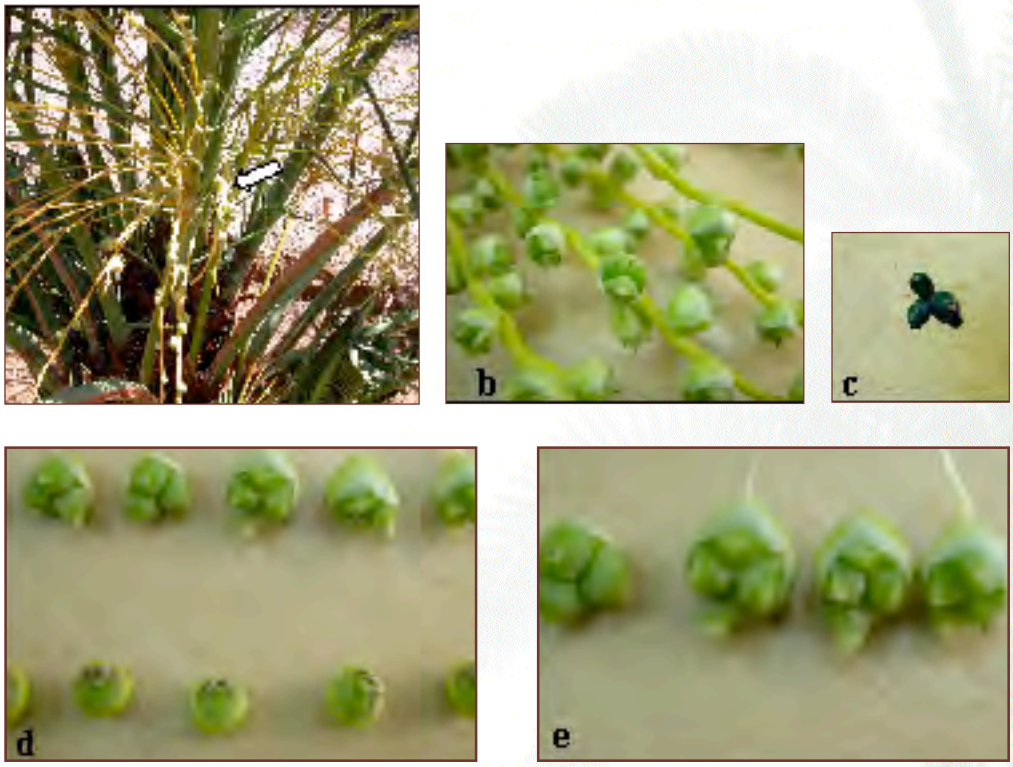


Figure 4. Fruiting abnormalities in date palm a sexual embryogenesis-derived plants:

a. Pollination failure on Khlass tissue culture-derived tree showing more than 90 % of loss in fruit set; b. A Khlass tissue culture-derived tree showing more than 80 % of parthenocarpic fruits; c. Development of three carpels instead of one; d. Parthenocarpic fruits of Khlass (top) compared to the normal simple carpel development (bottom of figure); and e. Parthenocarpic fruit showing the development of more than three carpels.

REFERENCES

Amira, M., A. Ilan and S. Freeman. (2000). Evaluation of symptoms caused by putative Thielaviopsis paradoxa infections and / or physiological disorders in date palm plants. Proc. Date Palm Inter. Symp. Windhoek – Namibia, 22–25 February 2000. 257–260.

Al-Wasel, A.S.A. (2000a). Tissue Culture Technique: Is it a safe method to micropropagate elite date palm (Phoenix dactylifera L.) cultivars? Arab J Biotech 3: 245–256.

Al-Wasel, A.S.A. (2000b). Vegetative and fruiting comparison of tissue culture-derived and conventionally propagated date palm (Phoenix dactylifera L.) cv. Barhee trees – College of Veterinary Medicine and Animal Resources. Twentieth annual meeting on Biological Sciences – King Faisal University Press, Saudi Arabia.

Al-Wasel, A.S.A. (2001). Phenotypic comparison of Tissue Culture-derived and Conventionally Propagated Date Palm (Phoenix dactylifera L.) cv. Barhee trees. I. Vegetative Characteristics. J. King Saud University, Riyadh. Vol. 13, Agric Sci 1: 65-73.

Brackpool, A., L. Branton and J. Blake. (1986). Regeneration in palms. In: Cell Culture and Somatic Cell Gen of Plants 3: 207–222.

Cohen, Y, R. Korchinsky, E. Tropler, G. Ziv, and R. Cohen. (2003). Fruit setting abnormalities in date palms of the cultivar Barhee originated from tissue cultu - re. Alon Hanotea (in Hebrew). 57:275-279.

Corley, R.H.V., K.C. Wooi, and C.Y. Wong. (1979). Progress with vegetative propagation of oil palm. The Planter 55: 377–380.

Corley, E.H.V., C.Y. Wong, and L.H. Jones. (1981). Early results from the first oil clone trials. Incorporated Society of Planters: 173–196.

Corley, R.H.V., C.H. Lee, I.H. Law, and C.Y. Wong. (1986). Abnormal flower development in oil palm clones. The Planter - Kuala Lumpur 62: 233–240.

D'Amato, F. (1978). Chromosome number variation in cultured cells and rege - nerated plants. In: Thorpe TA, ed. Frontiers of plant tissue culture. Inter Ass Plant Tiss Cult 197. Calgary, 287–295.

Djerbi, M. (1983). Diseases of the Date Palm. FAO Regional Project for Palm and Dates Research Centre in the Near East and North Africa.106.

Djerbi, M. (2000). Abnormal fruiting of the date palm derived from tissue cultu - re. Proceedings Of The Date Palm International Symposium, Windhoek, Namibia, 22-25 February 2000; 73.

Jaligot, E., T. Beule, and A. Rival. (2002). Methylation-sensetive RFLPs: charac - terisation of two oil palm markers showing somaclonal variation-associated polymorphism. Theor App1 Genet 104: 1263-1269.

Larkin, P.J. and W.R. Scowcroft. (1981). Somoclonal variation – a novel source of variability from cell culture for plant improvement. Theor Appl Genet 60: 197-214

Matthes, M., R. Singh, S.C. Cheah, and A. Karp. (2001). Variation in oil palm (Elaes guineensis Jacq.) tissue culture-derived regenerants revealed by AFLPs with methylation-sensitive enzymes. Theor App1 Genet 102: 971-979.

McCubbin, M.J., A. Zaid, and J. Van Staden. (2000a). The effects of sterilizing agents, plant growth regulators and explant type on somatic embryogenesis in date palm (Phoenix dactylifera L.) Proceedings of the Date Palm International Symposium, Namibia, 41-45.

McCubbin, M.J., J. Van Staden, and A. Zaid. (2000b). A Southern African sur - vey conducted for off-types on date palms produced using somatic embryoge - nesis. Date Palm International Symposium. Windhoek, Namibia. 22-25 February 2000.

Orton, T.J. (1983). Experimental approach to the study of somaclonal variation. Plant Mol Biol Rep 1: 67–76.

Pierik, R.l.M. (1987). In vitro culture of higher plants. Martinus Niijhoff Publishers. 343.

Sharma, D.R.; R. Kumari, and J.B. Chowdhry. (1980). In vitro culture of female date palm (P. dactylifera L.) tissues. Euphytica 29: 169-174.

Sharma, D.R., S. Dawra, and J.B. Chowdhury. (1984). Somatic embryogenesis and Plant regeneration in Date Palm (Phoenix dactylifera L.) cv. Khadravi through tissue culture. Indian J Exp Biol 22: 596 – 598.

Sibi, M. (1971). Création de variabilité par culture de tissus in vitro chez Lactuca sativa DEA. Amelior Pl Unir Paris, Sud, Orsay.

Sibi, M. (1989). Vitro variations ou variations somaclonales. Plantes viieres tro - picales. AUPELLF-URFF (ed.). John Libbey Eurotext. Paris. 27–49.

Zaid, A., and H.H. Al Kaabi. (2001). Second Technical Report – UAE Date Palm Research and Development Project. UAE / 2000 / 002; June 2001.

Zaid, A., and E.J. Arias. (1999). Date Palm Cultivation. FAO Plant Production and Protection Paper No. 156. ISBN: 92–5-104384–1. 287.

Zaid, A., H.G. Hughes, E. Porceddu, and F.W. Nicholas. (1999). Glossary of biotechnology and genetic engineering. FAO Research and Technology Paper No. 7 ISBN: 92–5–104369 – 8. 250.

DATE PALM ABNORMALITIES IN NAMIBIA AND NIGER

A. Oihabi [(1)], P.F. De Wet [(2)]

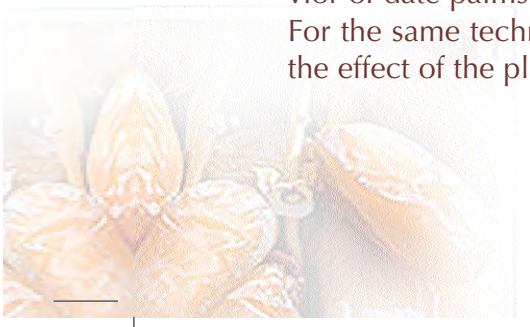
(1) FAO Programme Coordinator, Riyadh. abdallah.oihabi@ndc.org.na
2) National Project Director, Date Production Support Programme, Windhoek, Namibia. pieter.dewet@ndc.org.na

ABSTRACT

Date palm cultivated in the Namibia date plantations is mainly originated from tissue culture. Plant material was sourced from different date palm tissue culture laboratories representing the two main techniques currently used in the production of date palm, namely organogenesis and asexual somatic embryogenesis. Namibia is thus one of the best places in the world where a study on the abnormalities related to the origin of the plant material and the technique used can be done.

The aim of the present research is to summarize the main abnormalities observed on the field through several surveys and visits organized to different date plantations and nurseries in Namibia. Different cases of plant abnormalities are described and their origins are discussed in order to identify the cause of the described phenomenon.

It appears obviously that there is an important difference between the behavior of date palms produced by organogenesis and asexual embryogenesis. For the same technique, the effect of the laboratory is also clear as well as the effect of the plantation or nursery management.



Date palms planted in Namibia are originated from different commercial tissue culture laboratories using the two main propagation techniques (Asexual Embryogenesis and Organogenesis). (Annex1).

The Organogenesis is based on the use of meristimatic tissue and adillary buds as explants and doesn't present any callus phase. The Asexual embryogenesis is using various explants types and based on the production of somatic embryos from initiated callus (group of non organized and non specialized cells).

Beside the advantages of the mass-proparogation based on tissue culture techniques, the somaclonal variation causing the development of abnormal date palm is the main weakness of the micro propagation.

The somaclonal variations can be of two types:

- Epigenetic variation, which can be recovered and mainly owing to the environmental variations such as the use of inappropriate concentration of growth regulators. This variation is not hereditary (i.e. late flowering).
- Genetic variation, which is affecting the genom of the plant such as the change in the structure and the number of chromosomes this is a hereditary variation.

As mentioned above the plant material already planted in the commercial and private date plantations in Namibia is derived from both organogenesis and embryogenesis and purchased from different commercial laboratories around the world. Such diversity makes the Namibian date plantations the ideal field for a practical research on date palm abnormalities.

In accordance with the project document, the CTA of the DPSP undertook several field visits during April 2001 – October 2003 in order to assess the true-to-typeness of the tissue culture derived date palms planted in different areas of Namibia. The main cases of abnormalities observed are as follows:

1. DWARFISM

The dwarfism is characterized by an important growth restriction of date palms derived from tissue culture. The dwarfed are shorter than the normal ones.

The main dwarfism's symptoms are normal old leaves and dwarf young leaves. In few cases the opposite can be also observed; in this last case, date palm can sometimes recover from dwarfism and resume its normal growth.

Dwarfed date palms are often very weak plants and are frequently attacked by black scorch disease caused by Ceratocystis paradoxa (Holm) which is the perfect form of Thielaviopsis paradoxa.

In the special case of Medjool date palms produced by organogenesis technique coming from the same tissue culture laboratory; severe dwarfism associated to abnormal structure of the date palms is observed on about 100% of the trees. It was

observed that in this case the size of 4 year old plants do not exceed 1m. The same symptoms were observed on these plant material planted in Niger by the TCP/NER/0067.

The dwarfed date palms don't produce any flowers except some abnormal ones.

Dwarfism is the most common abnormality recorded at the Namibian date plantations. The most sensitive varieties to the dwarfism phenomenon are Medjool and Bonfeggons. At a private date plantation, Medjool date palms produced by somatic embryogenese and bought from at the same commercial tissue culture laboratory present a rate of 20% dwarfism phenomenon.

2. EXCESSIVE GROWTH

This abnormalities is present in few plantations and at a very low rate compared to the dwarfism. Leaves are presenting an atypical size doubling some times the length and wide of a normal leave. At the opposite of the dwarfed date palms, those presenting the excessive growth do produce and mature fruits.

3. ABNORMAL FLOWERING AND FRUITING

Very limited cases of abnormal fruiting were observed it includes abnormal inflorescence development, abnormal flowers, etc.

Variations of fruit shape and texture were observed mainly with the Medjool, Deglet Nour and Bou Feggous. It is worth mentioning that some of these abnormalities could be related to management problems (i.e. inadequate irrigation/fertilization programs) and/or environmental stresses.

4. OTHER CASES

Different other cases of minor importance were observed at a small scale such as production of abnormal offshoots, lopped leaflets, etc.

Conclusion and recommendations

- Despite the presence of these abnormalities, there occurrence is very limited and they come from the same sources.
- It is highly probable that these abnormalities resulted from improper handlings during the micro-propagation process.
- Some of the abnormalities could be due to environmental and/or management factors.
- With the worldwide increase of potential countries coming into commercial cultivation of date palm, TC is certainly the most appropriate tool to provide these countries with their needs of date palm plants. However it is highly recommended that all enterprises working with this commodity develop a safe tool that grantee a safe product to the end user.

Annex 1

Laboratory	Country	Technique	Main Varieties
Du Roi	RSA	Embryogenesis	Medjool
Date Palm Development	England	Embryogenesis	Barhee, Hilali, Ashrassi, etc.
Marionnet	France (UAE)	Embryogenesis	Medjool Bonfeggons
Palmdat	Namibia	Organogenesis	Medjool, Bonfeggons
Domaine Agricole	Marocco	Organogenesis	Bonfeggons
Keynoch Plant	RSA	Embryogenesis	Medjool

RADIATION-INDUCED MUTATIONS FOR DEVELOPING BAYOUD DISEASE RESISTANT DATE PALM IN NORTH AFRICA

S. Mohan Jain*,
FAO/IAEA Joint Division, Box 100, Wagramerstrasse 5, A-1400, Vienna, Austria.
*Current Address : University of Helsinki, Department of Applied Biology, Helsinki, Finland.
Email: Mohan.jain@helsinki.fi

ABSTRACT

FAO/IAEA inter-regional project RAF/5/035 was started in 1995 (completed in 2001) with a major objective to isolate Bayoud disease resistant date palm mutants by using in vitro propagation and selection, and gamma irradiation. This project continued as RAF/5/049 (active since 2001) with the main objective field evaluation of bayoud disease resistance date palm mutants. The major components of these projects are: in vitro culture of Deglet Nour, Tegaza, and Mejhool varieties, radiation induced mutations, screening of mutants and their field evaluation, and molecular marker analysis. Already, in vitro propagation methods, somatic embryogenesis and organogenesis, is developed and plants are ready for hardening under the controlled conditions before transferring to the field. Somatic embryogenesis technology seems to be promising for the isolation of mutants and their clonal propagation in large numbers by using bioreactor. Bayoud disease toxin can be isolated from the causal fungus FOA, and it can be used for in vitro selection against this disease. Our initial results have shown few putative date palm mutants showing tolerance to FOA toxin in all our participating countries Algeria, Morocco and Tunisia. These putative mutants will be micropropagated and tested with FOA before transfer to the field for final evaluation.

INTRODUCTION

The date palm (Phoenix dactylifera L.) belongs to monocot family Arecacea and classified as a dioecious tall evergreen. Date palm female trees bear fruits after 3-5 years and are fully matured at 12 years. It is called 'tree of life' in the bible and is very important fruit tree as a staple food and an export item in Saharan and Sub-Saharan regions of Africa and the Middle East. It also makes a significant contribution towards the creation of equable microclimates within oasis ecosystems thus enabling agricultural development to be sustained in many drought- and saline-affected areas. The rich fruit play an important role in the nutrition of human populations in these regions. In some areas, it provides food, shelter and fuel to the people. In North Africa, the total number of date palm trees is estimated to be 16 million, out of which 8 million are in Algeria, 4.5 million in Morocco and 4.25 million in Tunisia. The total date production is approximately 350,000 tones per year on an average yield of 20-30 kg per tree. Algeria and Tunisia export dates about 10,000 and 30,000 tones per year respectively. Major producers of date palm are Egypt, Iran, Saudi Arabia, Pakistan, Iraq, Algeria, United Arab Emirates Oman, Sudan, Libya, Tunisia, Morocco, Mauritiana and United States Al-Khayri 2005).

The propagation of date palm by offshoots has been done, a commercial method of vegetative propagation for multiplying the best varieties. These offshoots are produced from axillary buds situated on the base of the trunk during the juvenile life of the palm, and they develop slowly and their numbers are limited and are produced only within a certain period of time in mother palm's life. The off shoot number varies 10-30, depending on the genotype; and no field-based methods are as yet available for increasing the number of offshoots per plant.

Sexual propagation is the most convenient method for date palm propagation. However, this method can't be used commercially for propagating the cultivars of interest in a true-to-type manner for several reasons. The most obvious is the heterozygous characteristics of seedlings, which is related the dioecious nature of the date palm: half of the progeny are generally male, which produces no fruits, and large variations in phenotype can occur in progeny. Furthermore, no method is known at the present for sexing date palm at an early stage of tree development. It is therefore not possible to eliminate non-productive male trees in the nursery before plantation on the field scale. Another drawback of seed propagation is that the growth and maturation of seedlings is extremely slow. A date palm seedling may take 8-10 years or more before fruiting occurs.

Conventionally date palms are propagated from young off shoots, which appear as suckers at the base of the tree. Thus, it is possible to multiply popular good quality females and have the desired ratio of male to female trees in a plantation. However, bayoud disease of date palm is epidemic in Saharan region, which is caused by a soil-borne fungus Fusarium oxysporum sp. f. albedinis (FOA) (Fig.1).The conventional propagation methods have become unreliable and undesirable due to possible dissemination of Bayoud disease. By conventional method of seed propagation, it takes seven years to first flowering, which is too long, and the genotypes produced are completely differently from the parents. In vitro propagation of selected elite material is ideal for producing large number of high quality and disease free material in a short period of time.

Induced mutations have been applied for the past 70 years to produce mutants cultivars by changing the plant characteristic for a significant increase in plant production among both seeds and vegetatively propagated crops (see http://www-mvd.iaea.org/), and since then, over 2300 officially released mutant varieties in 59 countries have been listed (Jain, 2005). In date palm, there is hardly work done on induced mutations in the past due to poor in vitro culture system for plant regeneration. Now the recent developments of in vitro plantlet production via organogenesis and somatic embryogensis has made possible to induce mutations for the selection of beneficial mutants. Somatic embryogenic cell suspension cultures are being used for irradiation and induce mutant somatic embryos.

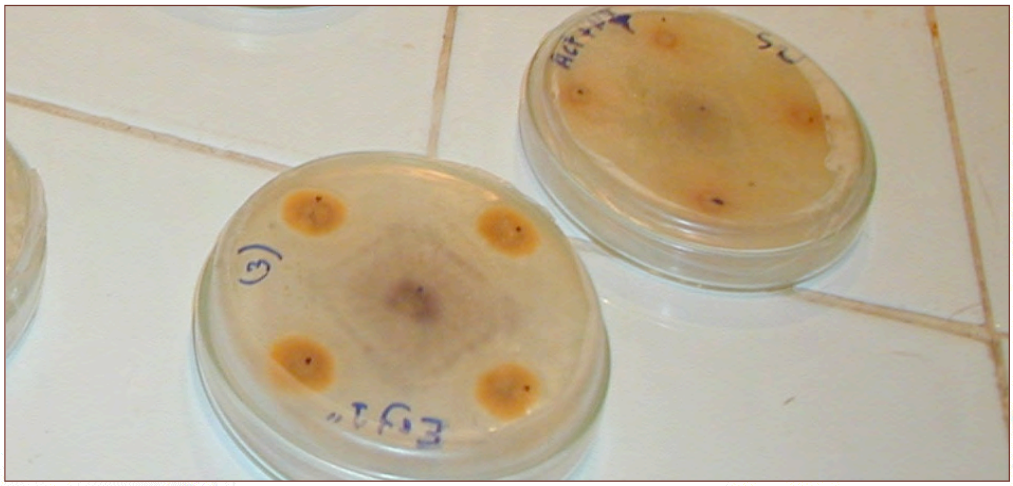
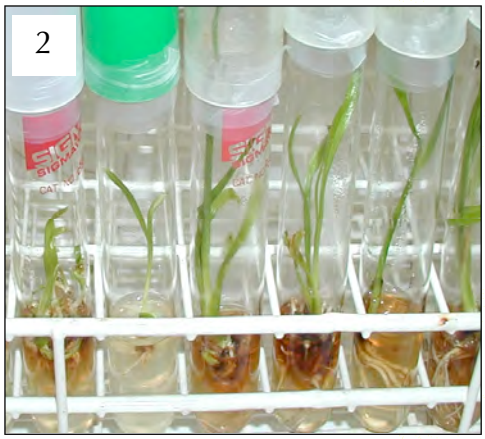


Fig.1. Cultures of Fusarium oxysporum sp. f. albedinis (FOA), which is the causal agent of Bayoud disease.

PLANT MULTIPLICATION WITH MICROPROPAGATION

Until now, micropropagation of date palm has had very limited success. The world market needs about 1-2 million date palms per year. Regardless of the significance of date palms in dry regions, demand may not increase due to water scarcity and urban migration from dry regions. Of the approximately 25 known groups active in date palm research worldwide, private companies are located in Israel, UAE, USA, UK, France and Morocco, and Saudi Arabia. Some of them multiply date palm plantlets through somatic embryogenesis. This technique involves generating embryos from cells not stemming from reproductive organs of the plant, and relatively easy to produce plantlets on large-scale. However, the required high level of plant growth regulators in the media make the plantlets vulnerable to somaclonal variation; these changes are detectable only after 6-8 years of planting in the field, and farmers pay the price for these losses. The Moroccan company, El Bassatine, has probably the most successful in date palm micropropagation by adopting organogenesis approach, i.e. plant regeneration from single cell via organ structures, often shoots. The Institute National de Recherche Agronomique (INRA) of Moroccan government, in close collaboration with EL Bassatine, has found protocols to scale up few varieties with organogenesis. INRA has also bred Bayoud resistant varieties that produce good quality fruit. However, for these varieties specific protocols have not yet been developed. Most of the over 3000 date palm varieties grown worldwide require specific protocols for large-scale micropropagation. For example, some varieties need more sugar in the medium, while others require more vitamins, nitrogen or calcium. Basic research to tackle these differences systematically is scarce. However, Smith and Aynsley (1995) reported results on field performance of tissue cultured date palm clonally produced by somatic embryogenesis, fruited with 4 years from field planting of small plants with leaf length 100 cm and 1.5 cm diameter at the base. Fruit from the tissue culture derived plants, cultivar Barhi, was indistinguishable from fruit of plants, which had originated from suckers (offshoots). These results justify commercial scale up of the micropropagation procedure using somatic embryogenesis to provide rapid cost-effective means of obtaining elite



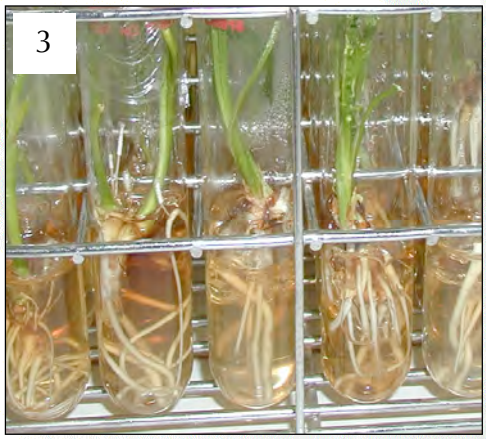


Fig. 2. Well developed shoots with roots. Fig. 3. Shoots with well developed roots.

date palm planting material. Recently, Date palm Research Centre (DPRC), Basrah University, Iraq, scientists have developed in vitro cloning system for Iraqi date palm varieties. This technology will make possible to produce up to 60,000 clones from one parent that can reach fruiting maturity in four years, compared to 8-10 years using traditional methods. Before the war, Iraq was home to 40 million trees of about 624 varieties. Now the number dwindled to 10 million, and new clonal propagation system will help them to replace destroyed date palm trees in around a decade.

There is no information available profit gain per plant by the companies, which is rather hard to obtain. Certainly, micropropagated date palm trees have not shown any decline in yield. Prof. Drira, Safax, Tunisia has 8-year-old Deglect Nour date palm variety, derived from tissue culture, in the field, showing better yield and 4-year early fruiting. Fki et al (2003) developed somatic embryogenesis protocol of date palm var Deglect Noor, and the regenerated plants did not show any changes in ploidy level, which opens the way for further scaling up this process for mass clonal propagation. They could produce 10,000 somatic embryos per litre per month, and obtained 85% somatic embryo germination rate by partial desiccation of embryos.

Influence of Culture Medium

The most common culture medium MS (Murashige and Skoog, 1962) is being used for micropropagation via somatic embryogenesis. Rarely B5

medium has been used. Several groups have modified the medium composition by adding, vitamins, adenine sulfate, thiamine, glycine, glutamine, myo-inositol, and activated charcoal (Fki et al 2003; Al-Khayri 2005). Among plant growth regulators, 2, 4-D (10-100 mg/l) is commonly added either alone or in combination with cytokinin (kinetin, 2-isopentyl phosphate) in the culture medium. In few cases, 2, 4-D is replaced by napthalene acetic acid (NAA). The role of vitamins in date palm somatic embryogenesis is not well defined except for thiamine and biotin positive influence on callus growth and somatic embryo production. Sucrose, 20-30g/l, is generally used as the major source of carbon and the energy in tissue culture media. Often in combination with sucrose, other sugars such as mannitol, maltose, and sorbitol have been added in the culture medium for enhancing somatic embryogenesis. In addition for being major carbon source, sorbitol and mannitol act as osmotic agents to alter osmotic potential of the medium. Sorbitol-induced osmotic stress hastened induction of somatic embryogenesis. The addition of polyethyl glycol (PEG 8000) in the culture medium enhanced somatic embryo maturation and germination. Addition of silver nitrate, ethylene inhibitor, has also a stimulatory influence on somatic embryogenesis, and its response is genotypic dependent.

Explant used

Various explants have been used to initiate date palm in vitro cultures, and their response to various plant growth regulators have been studies. The explants used were: mature and immature zygotic embryos, leaf segments excised from seedlings and young offshoots, leaf and meristmatic tissues excised from in vitro plants, and inflorescence tissue. The most frequently used explants are apical shoot tips and lateral buds since they are most responsive to in vitro culture.

Advantages of date palm micropropagation

a) High quality plants: Tissue culture plants are of a known, selected origin; they are uniform and of superior quality, and are available at any time without any special preparation in the plantation; b) Large quantity planned planting: Date palm tissue culture micropropagation enables the supply of large quantities of plants at a specific, planned date; c) A large and profitable plantation: The use of tissue culture plants permits to rapidly attain a large scale and economically viable plantation unit; d) Maximum receptivity: Field establishment is close to 100%, and transportation of plants from the nursery to the field is simple and easy; e) Healthy plants: Tissue culture

plants leave the laboratory and nursery completely clean of pests and diseases– a fact of special importance when shipping plants from one country to another; f) Early production: Tissue culture plants produce rapidly and give fruit as 3-4 years after planting; and g) Plant on demand: With tissue culture propagation it is possible to propagate and supply planting of rare or highly wanted plants

Major limitations of tissue culture in plant propagation

One of the major limitations of tissue culture in plant propagation is genetic variability or somaclonal variation (SCV). SCV has the characteristic of induced mutagenesis and that it may be strongly influenced by oxidative stress at excision of the tissues, genotype-dependent and influenced by both the explant source and tissue culture protocol, callus phase, age of the donor plant, type and concentration of plant growth regulators in the culture medium, culture conditions applied such as solid and liquid medium; number of in vitro subcultures used for propagation including age of cultures; genetic stability of mother plant material including chimeras (Jain, 2001). However, it is necessary to produce true-to-type micropropagated date palm plants for the survival of tissue culture commercial company and growers.

Fig. 4. Somatic embryogenic callus initiated from off shoot. (Left)
Fig. 5. Cell suspension developed from fraible callus. (Right)

The in vitro production of date palm via somatic embryogenesis requires the application of relatively high concentration of an auxin-type plant growth regulator such as 2,4-D or NAA, for process initiation. However, these

auxins are known to associate with genetic instability in plants, and could become cause of concern of genetic variability in date palm. Furthermore, variation in DNA methylation may be an important factor for initiating genetic variation, and also activation of retrotransposable elements, e.g. Tnt1A retrotransposon expression. A question is whether tissue culture processes activate retrotransposons, or is aberration connected to cell division program activation or to stress responses activation or both (Jain, 2001). Djerbi (2000) reported the abnormal fruiting of date palms cv Barhee derived from somatic embryogenesis, where more than 100,000 date palms planted in the beginning and middle of the 1990s in the Saudi Arabia showed 80-100% of parthenocarpic fruits sometimes with the development of more than 3 carpels. Saker et al (2000) detected somaclonal variation in tissue culture-derived date palm plants by using isozymes and RAPD fingerprinting. Smith and Aynsley (1995) had no obvious abnormalities in somatic embryogenesis derived Barhee plants. Similary, Al-Ghamdi (1996) also observed no significant difference in flowering and fruit setting when two cultivars Thoory and Zahdi were investigated. Therefore, it has become essential to develop diagnostic molecular markers to detect plant off-types at the early stage of plant development, before transfer date palm plants in the field (Azeqour et al, 2002; Saker et al 2000). So far, date palm somaclones showed changes in traits such as morphology and structure, excessive vegetative growth, leaf variegation, dwarfism, higher susceptibility to disease such as black scorch, delayed in flowering time, production of bastard offshoots, leaf whitening, pollination failure and abnormal fruiting, seedless fruits.

Somatic embryogenesis

Conventially date palm is propagated from off shoots, which are limited in quantity, e.g. 10-15 in the whole life of date palm cv Deglet Nour (Fki et al, 2003). The field performance of seed-derived palm plants varies because of highly genetic heterozygosity. For clonal propagation, tissue culture appraoch is most suited, especially production of somatic embryos. Somatic embryogenesis has been induced in a wide range of plants including angiosperms, gymnosperms (Jain and Gupta, 2005), and date palm (Fki et al 2003; Al-Khayri, 2005). In conifers, some commercial companies are using this approach for clonal production of elite genetic material. For example, Weyerhaeuser Inc, USA is producing somatic embryos of Douglas fir and field tests are being conducted for somatic embryo-derived plants (somatic seedlings). In date palm, somatic embryos have been produced from highly proliferating cell suspension (Fki et al 2003). Friable embryogenic calli were

initiated from both leaf and inflorescence explants. The overall production of somatic embryos reached 10,000 units per liter per month. The flow cytometry analysis of the somatic seedlings showed no variation in ploidy level.

Fig. 6. Initiation of somatic embryo development (Left).
Fig. 7. Well developed somatic embryos (Right)

Major advantages of somatic embryogenesis

a) Capable of producing unlimited number of plantlets under controlled conditions; b) Cost effective large-scale production of plantlets in liquid medium, e.g. bioreactor that can lead to automation for somatic embryo production; c) Cryopreservation for long-term storage; d) somatic embryos have both shoot and root meristem that develop in the same step of the process, and this enables direct plant regeneration like seed germination; d) Encapsulation for artificial seed production; e) Genetic transformation.

Major disadvantages of somatic embryogenesis

a) Low number of field-plantable clonal plantlets are produced per embryo culture; b) highly genotypic dependent for high number of plantlet production; c) plantlets may have a risk of inducing mutations which may not be detectable at the early stage of plant development and may appear at the later stage, that may cause severe economic loses to growers; and d) Gradual fluctuation and eventual decline in embryogenic culture productivity.

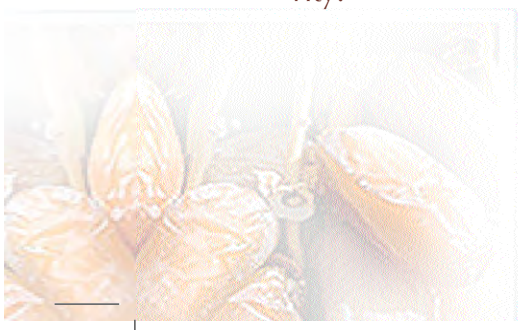


Fig. 8. Plantlets derived from germinated somatic embryos (Left)
Fig. 9. Hardened in vitro plants in the nursery (Right).

FAO/IAEA TECHNICAL COOPERATION PROJECTS

In Algeria, Morocco and Tunisia, two FAO/IAEA Regional Technical Cooperation projects are operational, namely: a) control of Bayoud disease in date palm RAF/5/035 started in 1995 and completed in 2001, and b) field evaluation of Bayoud disease resistance date palm mutants RAF/5/049, active since 2001 after the completion of earlier project.

The major components of these projects are: a) in vitro culture of Deglet Nour, Tegaza, and Mejhool varieties. Both organogenesis and somatic embryogenesis are being used for plant regeneration and multiplication; b) radiation-induced mutations by gamma irradiation of somatic embryogenic cell suspension; c) screening of mutants mainly showing tolerance to Bayoud disease by using toxin, isolated from FOA fungus that causes the Bayoud disease, both at the cellular and whole plantlet levels; and d) molecular techniques for the discrimination of susceptible and resistant types, and also to identify trait specific molecular markers for the developing diagnostic kit for marker-assisted selection and breeding.

The Moroccan group has been successful in isolation of FOA toxin in small quantity which is not sufficient enough for large-scale isolation of Bayoud disease tolerant mutants and efforts are being made to upscale FOA toxin production for the continuous supply and selection of mutants. Testing of different concentrations (0, 25 and 50 µg/ml) of FOA toxin sensitivity was done on regenerated plants from the irradiated material. The results showed that 50 µg/ml toxin concentration was highly effective for the selection of Bayoud disease toelrant plant by using detached leaf method. The selected

putative mutant date palm plants are being further screened with FOA spores for narrowing down the number selected putative mutants before transfer them to the field for the final evaluation. So far, all three countries have putative Bayoud tolerant date palm mutants, and are being evaluated in the field. The recent results have shown all putative mutants (about 1-year-old) have survived against Bayoud disease, and are being further monitored.

Fig. 10. Selection of Bayoud disease tolerant date palm by FOA toxin treatment, control- dead plant (left) and survived plant (right) after the toxin treatment. (Left).
Fig. 11. Putative bayoud tolerant date palm plant in the greenhouse before transfer to the field. (Right).

Major project achievements

1. Cultures from somatic embryogenesis and organogenesis have been established for plant regeneration. These technologies can routinely be used for plant regeneration and multiplication
2. The whole process of plant regeneration via somatic embryogeneiss is not fully controlled, e.g. acclimatization is still a problem, and still requires further assistance, e.g. training, knowhow, equipments
3. Isolation and production of Bayoud toxin in Morocco. Selection against bayoud toxin is available by using detached leaves, and the whole plantlet .
4. Molecular marker technology is fully adopted by laboratories in Morocco and Tunisia

5. Better tools are available for monitoring against bayoud diseases during selection pressure for selecting resistant lines
6. As a spin off project activities, gamma radiation treatment has improved duration, maintenance and capacity of somatic embryogenesis, and plant regeneration. This observation is novel in maintenance of somatic embryogenic cultures. Otherwise somatic embryogenic cultures have tendency to lose embryogenic property.
7. The outcome of the project that all three participating countries have well trained man power , tissue culture facilities, and molecular biology facilities.
8. Close collaboration among national institutions concerned was established

Opportunities

1. An opportunity to interact with Arab Center for Studies on Arid and Dry lands (ACSAD). The project is date palm Research and development network.
2. Look for date palm mutants with other desirable traits
3. Development of AFLP and microstallite techniques
4. Temporary Immersion System technology for large scale plant multiplication
5. Encapsulation of somatic embryos for synthetic or somatic seed production
6. Cryopreservation for long-term storage of elite germplasm including mutants

Regional priority needs

1. Acclimatization of in vitro grown date palm plantlets. To acquire the methodology of acclimatization and upgrade the laboratory facilities
2. In vitro selection of mutants against Bayoud toxin (produced by Morocco group).
3. In addition to Bayoud disease resistance, selection of mutants with other desirable traits, e.g. dwarf, early maturing type, low temperature and humidity types,

4. Use of molecular marker technology for screening mutants and genetic variability.
5. To streamline of legal and technical procedures for the transfer of biological substances, and in vitro genetic material.
6. Collaboration with extension services and all other national partners as well as regional and international organizations for the implementation of the project.
7. Performance assessment of selected mutants on the “hot spots” in the field on multi-location.

CONCLUSIONS

There is a great potential of induced mutations for the improvement of date palm in combination with conventional methodologies and biotechnology. This can be achieved with the proper use of tissue culture techniques, e.g. organogenesis and somatic embryogenesis, for clonal propagation with minimal somaclonal variation (acceptable at the commercial level) and prevent economic losses to the growers. It is highly desirable to modify micropropagation protcols for each elite genotype and determine the upper limit of subcultures before somaclonal variation sets in and initiate new fresh cultures.

In our FAO/IAEA TC projects, RAF/5/035 and RAF/5/049, we have made substantial progress by developing reliable date palm plant regeneration protocols via organogenesis and somatic embryogenesis (Fki et al, 2003) which could be further modified depending on the genotype. Radiosensitive tests have been made to determine Lethal dose 50 (LD 50) by irradiating with gamma rays. The optimal radiation dose 20Gy is used to irradiate somatic embryogenic callus of var. Deglet Nour. Plantlets coming from irradiated material are also being evaluated for morphological variation such as plant height, plant growth, leaf morphology in the acclimatization chamber and greenhouse.

GENERAL RECOMMENDATIONS

Robust date palm micropropagation - organogenesis and somatic embryogenesis- system and cost effective DNA-based detection technique are needed

for both cultivar identification and detection of somaclonal variants. Such techniques must be affordable and be easily applied by commercial growers. The most common approach has been somatic embryogenesis, which is very much dependent on genotype and culture medium for plant multiplication, even though there is a risk of genetic variability among regenerated plants. The following recommendations could be considered

- To identify genotypes respond to somatic embryogenesis and organogenesis for plant multiplication, and develop molecular marker system for each genotype and establish database of all available genotypes. In addition, "DNA fingerprints" as "signatures" could be used for legal purpose in order to protect patented varieties.
- To optimise tissue culture conditions for specific genotype (s): controlled growing condition of the mother plant, explant age, type and source, culture medium modifications, liquid vs. solid medium, type and concentration of plant growth regulators including ethylene inhibitors, determination of number of subcultures, light quality, measurement of osmotic potential of the medium. The germination rate of somatic embryos should be improved in the range of over 80-85% for commercial feasibility. The rooting could be done in vivo under high humidity, and save one tissue culture step. Attempts should be made to reduce concentration of plant growth regulator in the culture medium, especially of 2, 4-D.
- A number of biological functions have been associated with 5-methylcytosine (5mC) in DNA. Changes in plant development have resulted from inhibition of DNA methyl transferase, which suggest that the involvement of DNA methylation in cell programming and differentiation (Joyce et al 2003). The type and concentration of plant growth regulator influences 5mC, and it should be determined to determine the level of genetic variation.
- To establish cryopreservation facilities for storing embryogenic cell cultures and also to prevent somaclonal variation and contamination
- To develop reliable diagnostic molecular marker kit for early detection of genetic variability, e.g. TILLING, AFLP, SNPs. Also develop, molecular marker assisted selection and breeding of date palm.
- To develop temporary immersion system for shoot multiplication/rooting and somatic embryo production, e.g. RITA bioreactor

- To develop low cost tissue culture technology for reducing cost of production/plantlet without compromising the quality.
- To determine the quality of date palm fruit produced from micropropagated plants
- To identify male plants at early stage with specific molecular markers to discard at the early stage of development.
- It is highly recommended to establish a close collaboration among the North African countries for the development of date palm improvement programs to facilitate exchange of genetic material, transfer of technology, organising training courses, and regional symposia on date palm improvement.
- Farmer's participation should be encouraged in date palm improvement and also direct interaction with the agro-based food industries for providing the raw material.
- The off type date palm plants could be used for various purposes that may provide extra income to the small farmers, e.g. parthenocarpic fruits could be used as animal feed .

ACKNOWLEDGMENT

I am grateful to my former colleagues and counterparts of FAO/IAEA TC projects, RAF/5/035 and RAF/5/049, from Algeria, Morocco, and Tunisia, for their contribution in project implementation and the information provided in this article is from their results.

REFERENCES

Al-Ghamdi, AS. 1996. Field evaluation of date palm (Phoenix dactylifera L.) cultivars produced through tissue culture techniques. 4. Fruit chemical properties. Bulletin of Fac. Agric., Univ. Cairo 47: 167-177.

Al-Khayri, JM 2005. Date palm, Phoenix dactylifera L. In: Protocol for somatic embryogenesis in woody trees. SM Jain & PK Gupta (Eds.). Springer, Dordrecht, The Netherlands, pp 309-319.

Azeqour, M., K Majourhat & M Baaziz 2002. Morphological variations and isozyme polymorphism of date palm clones from in vitro culture acclimatised and established on soil in Southe Morocco. Euphytica 123: 57-66.

Djerbi, M. 2000. Abnormal fruiting of date palm derived from tissue culture. Proceedings of Date palm International Symposium, Windhoek, Namibia, 22-25 February 2000. p 73.

Fki, L. R Masmoudi, N Drira & A Rival 2003. An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, Phoenix dactylifera L., cv. Deglect Nour. Plant Cell Reports 21: 517-524

Jain SM. 2001. Tissue culture-derived variation in crop improvement. Euphytica 118: 153-166.

Jain SM. 2005. Major mutation-assisted plant breeding programs supported by FAO/IAEA. Plant Cell, Tissue & Organ Culture, 82: 113-123.

Jain SM & PK Gupta 2005. Protocol for somatic embryogenesis in woody plants. Springer, Dordrecht, The Netherlands.

Joyce, SM, AC Cassells & SM Jain. 2003. Stress and aberrant phenotypes in in vitro culture. Plant Cell Tiss. Org. Cult. 74: 103-121.

Saker, M., SA Bekheet, HS Taha, AS Fahmy & HA Moursy 2000. Detection of somaclonal variations in tissue culture-derived date palm plants using isozyme analysis and RAPD fingerprints. Biol Plant. 43:347-351

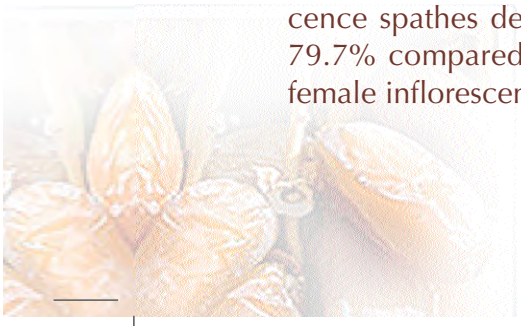
Smith RJ & JS Aynsley 1995. Field performance of tissue cultured date palm (Phoenix dactylifera) clonally produced by somatic embryogenesis. Principes 39 (1): 47-52.

'BARHEE' FRUIT SETTING PROBLEMS AT KINGDOM OF SAUDI ARABIA: RESEARCH APPROACHES TO UNDERSTAND THE PHYSIOLOGICAL AND PHYSICAL EVENTS OF THE PHENOMENON

Dr. Hassan M. Ali-Dinar
FAO Programme Coordination Office, Riyadh,
Kingdom of Saudi Arabia.

ABSTRACT

The failure of fruit set locally known as 'sheiss' in 'Barhee' date palm cultivar (Phoenix dactylifera L.) at Gassim area of Kingdom of Saudi Arabia was investigated with a series of experiments dealing with the hormonal and sexual processes and the receptivity of the stigma during two cropping seasons, namely: 1421 and 1422 H. The interrelated events of the sexual process and changes in endogenous levels of gibberellic acid (GA3), indoleacetic acid (IAA) and abscisic acid (ABA) for 'Barhee' trees from vegetative offshoots and tissue culture 'Barhee' trees with few and acute 'sheiss' problems from anthesis to early stages of seed development were investigated. The receptivity of the stigma and pollination approach studies were conducted at 2 sites in Gassim area, namely: Algayzailya (Site 1) and Alfeyha (Site 2) date palm plantations. Tissue culture trees in 'Algayzailya' date palm plantation are relatively older (9 years). At 'Alfeyha' date palm plantation, experiments were conducted with younger tissue culture trees (4-5 years) and older ones (13 years and older). Pollination of forced open female inflorescence spathes designated as (P0) in Site (1), increased normal fruiting to 79.7% compared to the control (farmer practice) (51.4%). Pollination of female inflorescence 3-5 days after natural opening of spathe, designated as



(P1), in the same Site gave 81.9% normal fruiting. Normal fruiting in Site (1) was relatively low in the first year and increased gradually during the following 2 cropping seasons. In Site 2, (P0) pollination of younger tissue culture trees reduced abnormal fruiting to 41.5% and 47.9% in the first and second seasons of study compared to the control (farmer practice) (89.8%, 76,6%). On the other hand, (P1) pollination of similar aged trees reduced abnormal fruiting to 38.3% and 42.7%. In the same Site (P0) and (P1) pollination of older trees gave 97.6% and 93.1% normal fruiting, respectively while the control (farmer practice) 90.8%. Photos of fruit setting of younger trees in Site 2 during 1423 H showed a substantial improvement in normal fruiting even with farmer pollination. This may probably indicate the relatively longer juvenility period of these trees induced by unstable interrelated factors and it seems that tree age plays a central vital role in these events.

SOME DOCUMENTED ABNORMALITIES OF TISSUE CULTURE-DERIVED DATE PALMS AND THEIR ECONOMICAL IMPACT ON THE GROWERS' REVENUE.

Dr. Aaouine Mohamed
IAV Hassan II-CHA, Agadir, Morocco.
E-mail: maaouine@menara.ma

ABSTRACT

The present needs for planting material in the Arab world, estimated at more than 45 million trees, can be satisfied only by the use of in vitro techniques using either somatic embryogenesis or organogenesis techniques. Callus regeneration followed by the induction of somatic embryogenesis, plant regeneration and acclimatization to ex vitro controlled environment was first achieved with the date palm. However, the use of callus in masspropagation often results in genetic variation in fruit quality, disease resistance and other morphological and horticultural characters with great economic impact on the growers. For this reason, an alternate system of regeneration has been developed via organogenesis. The author used such method at large scale to mass propagate date palm with hundreds of thousands of plants in the field and tens of thousands of them already bearing fruits with no genetic variation for all the genotypes produced. Thus, while no differences have been observed in the ease of field establishment of the plants obtained by the two techniques, great differences do exist in terms of multiplication rate, of the time required to produce salable plants, of true-to-typeness, and of production cost. Because of the high price of the date palm vitroplants, because it

takes many years for such plants to come into production, because of the high investment required to establish a date palm orchard, because of the low planting density used for the date palm, and because of the economical impact the off-types can have on the revenue of the growers, true-to-typeness is of prime importance.

In this presentation, the documented abnormalities of tissue culture-derived date palms that have been observed in many countries and the extremely severe economical impact they can have on the growers' revenue will be presented.

A SURVEY STUDY ON: SOMACLONAL VARIATIONS IN *VITRO-DERIVED* DATE PALM TREES

Abdelrahman S. A. AL-Wasel
Plant Production and Protection, AL-Qassim University,
P. O. Box 1482, AL-Qassim, Saudi Arabia.
E.mail: awasel@yahoo.com

ABSTRACT

In a survey study on some tissue culture-derived date palm cultivars (Medjool, Barhee, Sukkary, Toory, Deglat-Noor, Khalas, and Nabtat-Saif), somaclonal variations were observed. Dwarfism, slow growth, morphological abnormality, terminal bud bending, fruit set failure (shees), and supernumerary carpels were the most common phenomena. The type and percentage of variations differ among cultivars. The fruit set failure and dwarfism highly occurred and caused an economical loss. The Occurrence of fruit set failure varied between 20-100%, and number of carpels was between 4-9 carpels, except cvs. Medjool and Toory which were highly produced fuits. Whereas dwarfism was between 3-25% depending on cultivars. Morphological abnormality and terminal bud bending were the highest in Sukkary (10-50%). Albino and leaf variegation were very low (1-3%).

Many offshoots were detached from Tissue cultured-failed to set fruit trees of Barhee behaved like their mother and failed also to set fruit and had supernumerary carpels.

MICROPROPAGATION AND GENETIC RISK : SECURING CLONAL FIDELITY

D.J. Donnelly[1] and E. Ortiz-Medina[2]

ABSTRACT

Genetic risk associated with single-node culture and axillary micropropagation systems can usually be controlled in culture. Axillary shoot multiplication can occasionally be confounded by adventitious shoot proliferation. This is more prevalent for some cultivars in commercial situations. For many plant species, axillary shoot culture systems are not an option. The genetic risk associated with adventitious culture systems varies with the plants involved; relatively low (1 to 3 % per regeneration cycle) for adventitious shoots and much greater (up to 10 % per regeneration cycle) for adventitious somatic embryoids. Shoots or embryoids may show variation that reflects normal source-tissue variation. In chimeral species, somaclonal variation results from disassembly of the component genotypes and may approach 100 % of regenerants, completely undermining attempts of tissue culturists to achieve clonal fidelity. How can clonal fidelity be maintained when adventitious tissue culture systems are employed? This can only be done through rigorous choice of methodology, understanding of the type of variation inherent in the system, especially chimeral status of the explant, and careful screening of propagules. It will take a collaborative approach among plant anatomists, tissue culturists and molecular geneticists to solve clonal fidelity issues.

Additional Index Words : adventitious, axillary, chimera, genetic change, somaclonal variation

INTRODUCTION

Micropropagation technology is at work in laboratories all over the world due to the advantages over conventional methods of propagation. Micropropagation is used to increase a diverse range of vegetatively-propagated plants; many fruit species for temperate orchards and tropical plantations, ginger, potato, bulbous species and other rhizomes and geophytes, many types of vegetables and spices, trees for forestry, and a long list of ornamental species (reviewed by Rana and Raina, 2000). Clonal fidelity is the single most critical issue faced by propagators. It has profound biological and commercial implications. Understanding the forces that work against clonal fidelity challenges our knowledge of plant anatomy and genetics and has the potential to impact on many aspects of the commercial plant industry.

In vitro propagation is achieved through different methods, depending on the species and the commercial choices made. The full range of factors that affect clonal integrity in different types of culture systems is not completely understood. It is known that variation is inherent within the explant, and the frequency of variant propagules is affected by choice of pre-culture and culture techniques. Some of these choices, and their impact on clonal fidelity, are reviewed. Possible strategies to modulate variation are proposed.

Clonal Fidelity in Single Node Cuttings and Axillary Shoot Multiplication Systems

Propagation from single-node cuttings or axillary shoots has been used for a large number of plant species. These methods are believed to be least susceptible to mutations and phenotypic variation due to the presence of preexisting meristems within the explant, from which all in vitro growth derives (George, 1993; Pierik, 1997 and Kane, 1996; 2005). For example, commercial micropropagation of potato involves single-node cuttings, while for most temperate fruit species, including apple, blackberry, blueberry, cherry, grape, raspberry, strawberry, etc. axillary shoot multiplication is used. Named cultivars of potato or temperate fruit species are available from North American germplasm repositories. Requesting laboratories receive duplicate or triplicate test tubes of specific pathogen tested (SPT) plantlets. Often, the original explants were meristem tips, dissected following thermotherapy of virus-infected source (stock) plants. Therefore, the distributed plantlets are meristem tip-source clones. When SPT source plants are avai-

lable, the explants are apical or lateral shoot buds or single-node cuttings, and distributed plantlets are shoot tip-source clones.

Potato micropropagation facilities in North America use media devoid of growth regulators, relying on single-node cuttings rather than axillary shoot multiplication for increase in plantlet numbers. Despite this supremely cautious approach to micropropagation, routine practices among the germplasm repositories that supply the propagation facilities may result in the distribution of intraclonal variants. An explanation for this involves the method by which germplasm repositories regularly "audit" their cultivar collections (Figure 1). Every 1-several year(s), representative potato plantlets from a few test tubes are planted into the field for a grow-out test. Visual ratings of stem and tuber characteristics, and especially yield and maturity factors, are

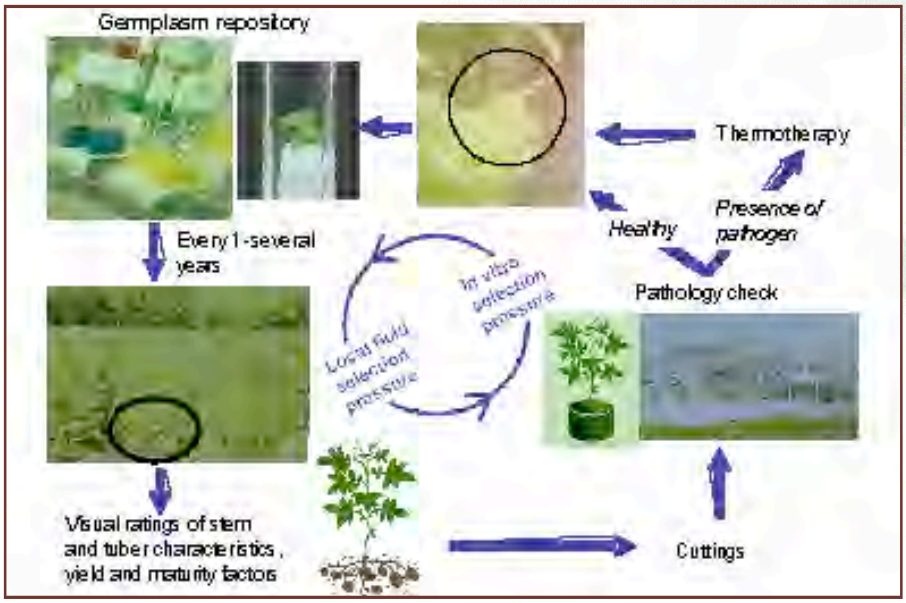


Figure 1. Cycle of activities involved in auditing cultivars held at a germplasm reposi - tory for trueness-to-cultivar. Some pre-micropropagaton activities, such as thermothe - rapy and meristem tip culture for virus elimination and in vitro germplasm storage, may serve to decrease the amount of genetic diversity present within a clonal cultivar. Local field selection pressure is followed by selection for growth in culture. The method of maintenance of clonal germplasm has changed a great deal over the years. The older the clonal cultivar the greater the range of genetic mutation that has accu - mulated within the clone. If a clonal cultivar is now represented by one meristem tip-source clone, inherent variation is reduced.

evaluated. If these plants are considered true-to-cultivar, cuttings are taken for transfer to the greenhouse, where plants undergo a pathology check, for presence of virus. If plants are healthy, shoot tip explants are used for tissue culture. If the clone is now virus-infected, plants receive thermotherapy before meristem tip explants are placed into culture. One or a limited number of shoot tip- or meristem tip-source clones are then used to represent the cultivar in the germplasm repository. The audit procedure relies on experienced nurserymens' and growers' subjective decisions on trueness-to-cultivar, for a limited number of plants, usually at one geographic location. This imposes local field selection pressure, based on performance in that geographic local. In vitro selection pressure follows, for acceptable performance in culture. The cycle repeats at site-specific intervals over decades. For old cultivars like Russet Burbank, held in several repositories in North America, this process repeated at several geographic locations over half a century has resulted in the emergence of suspected intraclonal differences.

So, how does the maintenance of germplasm affect plant genetics? If a cultivar is represented by fields of plants, accumulating genetic mutations with each field season, the older the cultivar, the greater the range of genetic variation that has accumulated within the clone. However, in the current reality, a cultivar may be represented by one or a few meristem tip- or shoot tip-source clones. The amount of inherent genetic variation that has accumulated in the clone is reduced during the pre-micropropagation process. For example, in the past, it was possible for potato breeders to identify superior plants from large field-grown populations of clonal members. These intraclonal variants, (strains or geographical clones) were renamed as new cultivars, for their superior performance in specific regions (Leever et al., 1994). Will breeders still be able to do this when the repository sends them a cultivar represented by germplasm that has received repeated cycles of geographic selection and meristem tip culture?

How different is the clonal germplasm maintained in different locations? Ten clones of potato cultivar Russet Burbank that had been geographically isolated for 25 years or more (some for >60 years), or subject to systematic selection (by breeders) were gathered for a yield comparison (Love et al., 1992). Yields in Idaho (mid-western U.S.), were not the same for all the clones, and the first alarm bells were heard over possible emerging intraclonal differences. Ten years later, a comparison in Eastern Canada showed that yield and maturity factors between 11 of these clones were not substantially different (Coleman et al., 2003). Nevertheless, geographical biases were evident in

chemical maturation rates and storage performance; and some phenotypic differences were apparent. Although Single Sequence Repeats (SSR) and Random Amplified Polymorphic DNA (RAPD) analysis could not detect DNA polymorphisms to distinguish these intraclonal strains, more sensitive techniques may resolve these differences in the future.

It is extremely rare to hear of "variants" or "off-type" plants, among temperate fruit species micropropagated through axillary shoot multiplication. However, this does occur. Occasionally, certain cultivars, for which the media employed are not ideal, may have a tendency to form callus at the base of axillary shoot cultures. Where this occurs, adventitious shoots may become mixed and difficult to distinguish from the axillary shoots. For example, strawberry cultures may contain a mixture of adventitious and axillary shoots, unless callus is stringently removed at each subculture. In a recent North American law suit, dozens of commercial strawberry growers were compensated when a provincial certification agency distributed a micropropagated strawberry cultivar that fruited abnormally. It is not a simple matter, even for certification authorities, to avoid these litigious situations. Commercial laboratories may not have the experience, or may not take the time, to optimize "generic" medium formulations for the needs of individual cultivars. Technicians working in laminar air flow units may not have the training to distinguish adventitious shoot clusters or may feel too pressured, to harvest as many shoots as possible per culture cycle, to rogue the adventitious shoots.

Economic pressures to maximize the productivity of axillary shoot multiplication systems sometimes leads to excessive use of growth regulators (especially certain auxins, such as 2,4-D and cytokinins), or the practice of "pulsing"; increasing the growth regulator level for one or more monthly culture cycles followed by decreasing the concentration. Prolonged use of elevated levels of growth regulators are suspected of causing mutations. However, there is not a clear relationship between growth regulator concentration and frequency of somaclonal variation (van Harten, 1998). Still, it is common to see recommendations to limit growth regulator exposure by reducing the total number of culture cycles following explantation. For example, in commercial strawberry production, 8-10 months of culture (8-10 subcultures) following explantation is the recommended limit. For many species, new isolations are recommended annually (Skirvin et al., 1994; Rana and Raina, 2000).

Clonal Fidelity in Adventitious Multiplication Systems

Somaclonal variation is a term introduced by Larkin and Scowcroft (1981) to describe genetically novel shoots or plantlets derived from tissue culture systems. It is not always known if these shoots arise from genetically variant cells that are present prior to culture or if variant cells are induced by the culture process due to environmental stress and/or chemical mutation from exposure to growth medium ingredients (Skirvin et al., 1994). In vitro stresses of environment or chemistry could cause mistakes during nuclear and cell division processes. It is usually unknown if individual changes are heritable or not – for clonally propagated species this is rarely of interest.

Pre-existing Chimeral Variation

Vegetatively propagated clones are known to accumulate mutations over time. This comes about through microenvironment effects on plant apical and lateral shoot meristems. When more than one genotype is present within a plant, the plant is known as a chimera. Probably all plants are chimeral to some extent, since during normal organ formation, mistakes in nuclear and cell division may lead to chromosomal changes, both small (point mutations) or large (aneuploidy, polyploidy). In some cases, variant cells within the shoot apical meristem may occur in discrete sectors (sectorial chimera), portions of the tunic (outer histogenic layer) (mericlinal chimera) or an entire tunic layer (periclinal chimera) (reviewed by Hartmann et al., 2002). While the sectorial and mericlinal chimeras are transient, the periclinal chimera is a stable arrangement, also known as a hand-in-glove chimera, involving a mutation in the outer histogenic layer(s) or tunic surrounding a wild-type (non-mutated) core or corpus. There are many well known examples of chimeras, of various complexity, such as 'Russet Burbank' potato (Davis, 1975; Tilney-Bassett, 1986), 'Bartlett' pear, 'Delicious' apple and thornless Rubus species (Loganberry or Thornless blackberry) (Skirvin, 1977).

If a chimeral cultivar, such as Loganberry or Thornless blackberry is propagated through callus and adventitious shoot or embryoid formation, then chimeral disassembly can occur. The individual cells or small groups of cells that contribute to shoot initiation may have only one genotype – in which case the shoot is no longer chimeral. The same is true when single cells develop into somatic embryoids. If an established chimeral cultivar is disassembled, then cultivar status is irrevocably altered in some adventitious propagules. Reversal to chimeral status can only occur if the original mutation is repeated – the likelihood of this is unknown. In the case of Thornless

blackberry, 100% of the regenerants were thornless; some were chimeral like the source tissue and some were genetically thornless derived entirely from the mutated L1 histogenic tissue layer (Skirvin et al., 1994; 2000; Figure 2). Following field-selection among a population of thornless plants, a commercially interesting genetically thornless (non-chimeral) plant was selected and named, cultivar Everthornless.

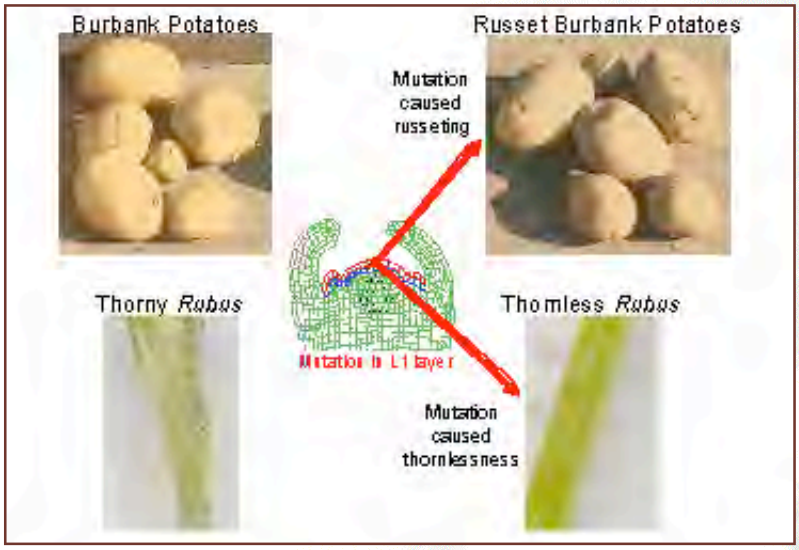


Figure 2. Two examples are illustrated where periclinal mutation of the L1 tunic layer lead to improved cultivars. The potato cultivar Russet Burbank is a sport of cultivar Burbank. Russet Burbank is a periclinal chimera in which the L1 tunic layer has a mutation that causes the russeted periderm phenotype. Thornless Rubus species are periclinal chimeras with a mutated gene for thorniness in the L1 tunic layer. Through tissue culture, a non-chimeral, genetically thornless Rubus cultivar was produced by Skirvin's group in the 1970s.

Reducing Genetic Risk in Micropropagation of Chimeral Species

If the chimeral status of a tissue cultured plant is unknown, a process of "uncovering" of the chimeral genotypes may occur (van Harten, 1998). When adventitious culture systems are used for putative chimeral species, there may be ways to minimize genetic variation through a better understanding of chimeral structure. For example, most Angiosperm dicotyledonous plants have shoot meristems composed of two tunic layers surrounding the corpus (Figure3). The outer (L1) and inner (L2) tunic layers generally develop into the

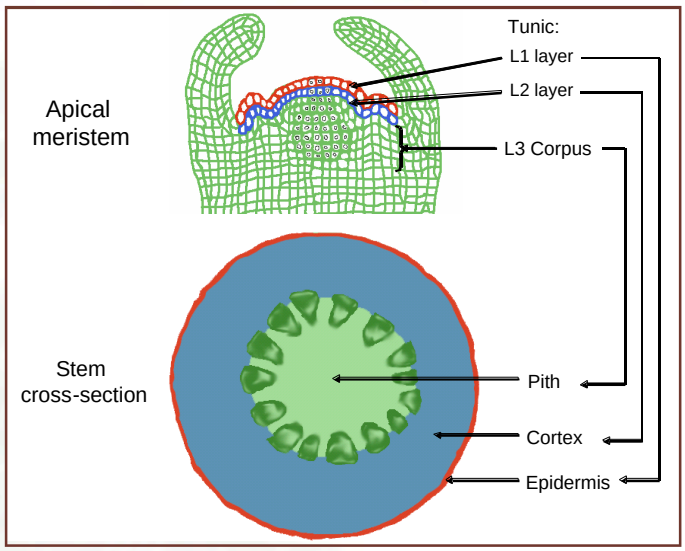


Figure 3. Shoot tip organization, in Angiosperm dicotyledons, involves two tunic layers, designated L1 (outer layer) and L2 (inner layer) and the corpus, designated L3. As stem development occurs, the L1 layer differentiates into the epidermis, the L2 layer grows into the cortex (outer cortex in some species) and the L3 layer becomes the pith (and inner cortex in some species). In the central corpus area is a group of cells that divide infrequently, while their derivatives divide many times. In this way, the genetic integrity of these central corpus cells (stem cells) is conserved.

stem epidermis and cortex (or outer cortex), respectively (Lineberger, 2005). The pith or medulla of the stem (sometimes also the inner cortex) develops from the corpus (L3) of the meristem. Mutations are more likely to accumulate towards the outer periphery of the meristems, within the tunic layers (Bäurle and Laux, 2003). This occurs due to the relatively small number of divisions that occur in the cells in the central zone of the corpus and the greater number of divisions within their derivative cells. For this reason, pith sections may hold fewer variant cells than epidermal or cortical tissues or whole stem sections. However, somaclones derived from the pith are by definition non-chimeral, so 100% somaclonal variation results from this chimeral disassembly.

Does it matter, if a chimeral cultivar is separated into its component genotypes, in terms of plant growth and productivity? For thornless Rubus, a mixed population of chimeral shoots and somaclonal variants from L1 tunic tissue were compared, and the best non-chimeral clone selected was just as good as the original chimeral cultivar for commercial fruiting attributes. So

the answer is that tissue selection is important, non-chimeral clones can be just as good as chimeral clones, but field evaluation is the only guaranteed way at the moment to test the commercial acceptability of these non-chimeral somaclones.

Culture-Induced Chimeral Variation

Somaclonal variation is associated with callus or wound-tissue proliferation and adventitious shoot regeneration systems. The process of accumulation of mutations in callus is said to result from asynchrony between nuclear and cell division that occurs in callus. Contributing to this could be mutation events that result from in vitro selection pressures. If meristems that are initiated in callus accumulate mutations in vitro in the same way as in the field, adventitious chimaeral shoot tips could arise. These could have transient sectorial or mericlinal chimeral arrangements or the stable periclinal arrangement. These shoots may appear identical to the source plant tissue, unless the genes involved affect some obvious phenotypic trait. The genetic risk associated with adventitious culture systems varies with the species involved. The risk is estimated to be relatively low (1-3 %) for adventitiously regenerated plants (Skirvin et al., 2000). However, off-types are usually visually assessed and real numbers of clonal variants may be far greater.

Many types of mutations are seen in clones derived adventitiously with or without callus. Are these variants from pre-existing variant cells or culture-induced variants? One way to distinguish the relative frequency of pre-existing and culture-induce variant cells would be through comparison of the incidence of somaclonal variants from indirect and direct shoot regeneration systems of the same explant. However, these studies are not easily controlled, as different regeneration systems require the use of different growth regulators and growing conditions. Some combinations may favour growth of variant cells or adventitious differentiation from them. Furthermore, genetic analysis may not readily distinguish between them.

Molecular techniques are not yet capable of fully characterizing adventitious shoots or embryoids to establish the degree of clonal fidelity. When somaclones present to growers as phenotypically identical or similar to the source plant and to each other, it is difficult to know what the actual genetic picture really is. Clearly, some plant species, pre-culture and culture protocols and some explants have the potential to yield much greater frequencies of somaclonal variants. For example, somaclonal variation reported in different bananas and plantains ranged from 0-69 %, with 6-38 % among Cavendish cultivars (Martinez et al., 1998 and Hwang and Tang,

2000 cited in Sahijram et al. 2003). Additional confusion may arise when chromosome or gene mutations occur, but are not stable (Karp, 1995). Plants may outgrow some types of mutations, for example sectorial and mericlinal arrangements where reversion to the stable periclinal or to the wild-type occurs (Hartmann et al., 2002). The incidence of this is unknown and may differ among species. To determine the incidence of reversion to wild-type, genetic analysis of adventitious propagules may have to be repeated at intervals.

Epigenetic Variation

Confounding pre-existing and culture-induced somatic variation, is a complex of epigenetic characteristics associated with the culture-induced phenotype. This is developmental variation that has been well characterized in temperate fruit species. It includes a suite of environmentally-dependent anatomical and physiological changes characteristic of in vitro-grown plants (Donnelly and Tisdall, 1993). These result from exposure to the culture environment, which imposes: saturated atmosphere, low medium water potential, low light level, low rate of gas exchange, high and constant temperature, presence of sugars and exogenous growth regulators in the medium. Some of the many features of the culture-induced phenotype include: miniaturization, mixotrophic nutrition, reduced epicuticular and cuticular wax deposition, reduced and altered trichome population and altered stomatal function. All of these features affect acclimatization of ex vitro transplants. However, the new tissues formed ex vitro exhibit the control phenotype in response to the climate outside of the culture containers. The culture-induced phenotype is quickly outgrown.

SUMMARY

In summary, single-node cuttings and axillary shoot proliferation techniques have been extensively used for micropropagation of potato and temperature fruit species, respectively. These are believed to be “safe” means of micropropagation, with little opportunity for introduction of genetic variation due to plant derivation from preexisting organized meristems. Nevertheless, at the germplasm repositories, field selection during cultivar audit followed by thermotherapy and in vitro selection of a representative meristem or shoot tip source clone may impose a series of selection pressures on cultivars, and have resulted in the emergence of suspected intraclonal strains or geogra-

phic clones. Axillary shoot multiplication can occasionally be confounded by adventitious shoot proliferation and this is more prevalent for specific cultivars of some fruit species and in some commercial situations. In addition, overuse of growth regulators may interfere with normal meristematic growth. Reducing the amount of growth regulators used, and the number of subculture cycles from the time of explantation, may reduce the risk of variation in these cultures.

In adventitious culture systems, the risk of somaclonal variation is much greater than in single-node or axillary shoot multiplication systems. It is not known how much preexisting variation occurs in plant tissues and how much is introduced by adventitious culture processes. Probably all plants are chimeral to some extent. Older cultivars may have accumulated significant numbers of mutations over the years. Some of these mutations may be distributed in stable periclinal arrangements. When adventitious culture systems are used for putative chimeral species, there may be ways to minimize genetic variation through a better understanding of chimeral structure. In the case of thornless Rubus species, an L1-derived genetically thornless somaclonal variant had satisfactory yield characteristics and was commercialized. However, for non-breeding purposes, selection of tissue derived from the corpus may be inherently less genetically variable than tissues derived from the tunic or especially the outer tunic layer (L1). The relative somatic variation derived from tissues of different histogenic layers should be evaluated, especially for plant species where somatic variation has been particularly troubling. At the present time, only field-evaluation can determine whether disassembled, non-chimeral clones can perform satisfactorily; a lengthy and costly activity for perennial species. Nevertheless, it is possible that new non-chimeral cultivars may propagate adventitiously with a reduced incidence of somaclonal variation. Molecular techniques cannot yet fully characterize adventitious shoots or embryoids to determine their clonal status but this era is approaching rapidly. It will take a collaborative approach among plant anatomists, tissue culturists and molecular geneticists to solve clonal fidelity issues.

ACKNOWLEDGEMENTS

The authors would like to thank Ms. Béatrice Riché for preparation of the figures, Dr. Ismahane Elouafi for proof reading the manuscript and the Natural Science and Engineering Research Council of Canada (NSERC) for research support to D.J. Donnelly for investigation of chimaeras in potato (Solanum tuberosum L.) cultivars and Rubus species.

LITERATURE CITED

Coleman, W.K., X.-Q. Li, E. Tremblay-Deveau and S. Coleman. 2003. Chemical maturation and storage performance of eleven Russet Burbank clones. Can. J. Plant Sci. 83:893-902.

Davis, J.W. 1975. Aristocrat in Burlap: A History of the Potato in Idaho. Idaho Potato Commission. Idaho Publications. 167 p.

Donnelly, D.J. and L. Tisdall. 1993. Acclimatization Strategies for Micropropagated Plants. In: Micropropagation of Woody Plants, Ahuja, M.R. (Ed.), Kluwer Academic Publishers. The Netherlands. pp. 153-166.

Bäurle, I. and T. Laux, 2003. Apical meristems: the plant's fountain of youth. BioEssays 25: 961-970.

George, E.F. 1993. Plant Propagation by Tissue Culture. Part 1. The Technology. Exegetics Ltd. England. pp. 37-66.

Hartmann, H.T., D.E. Kester, F.T. Davies, Jr. and R.L. Geneve. 2002. Principles and Practices of Clonal Selection. In: Plant Propagation Principles and Practices. Prentice Hall, 7th ed. New Jersey, U.S. pp. 592-635.

M.E. Kane. 1996. Propagation from Preexisting Meristems. In: Plant Tissue Culture Concepts and Laboratory Exercises. Trigiano, R.N. and D.J. Gray (Eds.) CRC Press. Boca Raton, Florida. pp. 61-72.

Kane, M.E. 2005. Shoot Cultures Procedures. pp.145-158. In: Plant Development and Biotechnology, Trigiano, R.N. and D.J. Gray (Eds.) CRC Press. Boca Raton, Florida.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement. Euphytica 85: 295- 302.

Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Applied Genet. 60:197-214.

Leever, G., W.A. Trank, G. Shaver, J.C. Miller, Jr. and A.D. Pavlista. 1994. Norgold Russet, Superior and Red La Soda strains selected for potato cultivar improvement in Nebraska. Am. Potato. J. 71:133-143.

Lineberger, R.D. 2005. Origin, development, and propagation of chimeras. Web ref:http://aggie-horticulture.tamu.edu/tisscult/chimeras/chimeralec /chime - ras.html

Love, S.L., A.L. Thompson, T.P. Baker and D.L. Corsini, 1992. Comparison of Russet Burbank clones from various geographical regions of the United States and Canada. Am. Pot. J. 69:299-307.

Pierik, R.L.M. 1997. In Vitro Culture of Higher Plants. Martinus Nijhoff Publishers. The Netherlands. 348 p.

Rani, V. and S.N. Raina. 2000. Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. In Vitro Cell. Dev. Biol. 36:319-330.

Sahijram, L., J.R. Soneji and K.T. Bollamma. 2003. Invited review: analyzing somaclonal variation in micropropagated bananas (Musa spp.). In Vitro Cell. Dev. Biol. 39:551-556.

Skirvin, R.M. 1977. Fruit improvement through single cell culture. Fruit Varieties Journal. 31:82-85.

Skirvin, R.M., K. D. McPheeters and M. Norton. 1994. Sources and frequency of somaclonal variation. HortScience 29:1232-1237.

Skirvin, R.M., M. Coyner, M.A. Norton, S. Motoike and D. Gorvin. 2000. Somaclonal variation: do we know what causes it? AgBiotechNet 2000 2:1-4.

Tilney-Bassett, R.A.E. 1986. Plant Chimeras. Edward Arnold. London. 193 p.

van Harten, A.M. 1998. Mutation Breeding Theory and Practical Applications. Cambridge University Press. Cambridge, U.K. 350 p.

TRUE-TO-TYPE DATE PALMS OBTAINED TROUGH TISSUE CULTURE USING THE AXILLARY BRANCHING TECHNIQUE

Dr. M. Aitchitt
Laboratoire de Biotechnologie appliquée
Domaine El Bassatine, BP: 299, Meknes 50 000, Morocco.
Tel: + 212 55 50 04 30, Fax: + 212 55 50 07 30
E-mail: aitchitt@datepalm.org

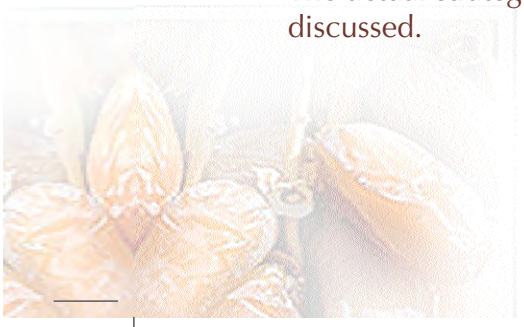
ABSTRACT

In Morocco, reviving the oases by extensive plantation of date palms with resistant and high quality producing plants is the only way to restore, to expand and to revitalise this ecosystem devastated by the Bayoud disease.

Tissue culture is an ideal alternative to the conventional propagation techniques which are limited. Date palms can be propagated following two main routes ie somatic embryogenesis and the axillary branching technique known as organogenesis.

The commercial laboratory El Bassatine (Domaines Agricoles) plays a key role within the National Date Palm Programme, by providing farmers with thousands of elite date palm plants obtained in vitro. This communication presents the role of Domaine El Bassatine Laboratory in the development of the oasis ecosystems and explains the reasons behind the technique used in order to have the highest chances of obtaining true to type plantlets.

The actual strategy and future prospects of date palm development are also discussed.



DATE PALM GLOBAL NETWORK

A. Zaid [1], E.J. Arias [2] and T. Fawzi [3]

(1) General Coordinator, Date Palm Global Network, United Arab Emirates University. P.O.Box: 81908, Al Ain, UAE, Email: zaid@uaeu.ac.ae

(2) FAO – AGPC, Room C – 785, Via delle, Termi di Caracalla 00100, Rome, Italy - Email: Enrique.Arias@fao.org

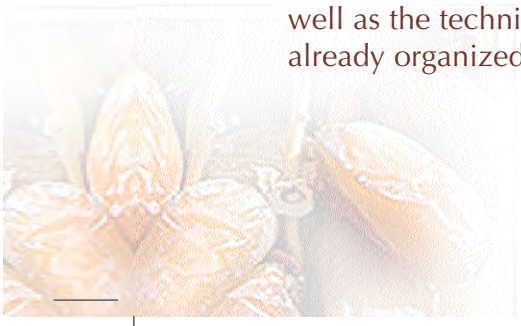
(3) T. Fawzi, FAO - RNE, Regional Office for the Near East, 11 El Eslah El Zerai Street, Dokki, Cairo, P.O.Box. 2223, Egypt. Email: Fawzi.Taher@fao.org

ABSTRACT

Several efforts are continuously made to group the date palm research and development activities either within a geographical region or between several regions. The past half century has seen the implementation of a few date palm projects such as the Regional Project for Palm & Date Research Centre in the Near East & North Africa, the Date Palm Research & Development Network, the Bayoud Disease Regional Project, the Date Palm Subregional TCP Project, the Bio-control project of Red Palm Weevil in the Arabian Gulf region, and the recently launched - Maghreb Date Palm Regional Project.

FAO, as well as other international institutions such as CARDA, IPGRI, IFAD, IDB, IAEA, and AFESD are always technically assisting the date growing countries in their development efforts. The recently established Date Palm Global Network (DPGN) is under the aegis of FAO with a head office in the United Arab Emirates.

Since its establishment, the DPGN did structure its Technical Secretariat as well as the technical coordinating board. Several regional workshops were already organized.



INTRODUCTION

The date palm culture in the world is continuously characterized by its social and economical roles. Due to the growing need of increasing communication and exchange of experiences among date producing countries, the creation of a functional mechanism for technical co-operation was always a matter of urgency. The creation of a date palm global network is justified by the following:

- There is an economic and social importance of date growing in many countries in North Africa, the Middle East and even Southern Africa, Australia and the USA;
- There is insufficient research and information exchange within and between the date producing countries in both the old and new date worlds; and
- There is a need of date exporting countries to follow a co-ordinated policy in relation to international date markets and to exchange statistics on the world date production and trade.

Several efforts are continuously made to group the Date Palm Research and Development activities, either within a geographical region or between several regions. The following are a few date palm projects / networks that were implemented during the last 20 years.

- The Regional Project for Palm and Dates Research Centre in the Near East and North Africa (NECP/REM/521/MUL);
- The Date Palm Research and Development Network (ACSAD);
- The Maghreb Date Palm Regional Project (GEF, UNDP, IPGRI);
- The North African Regional Date Palm Network (IAEA / FAO);
- The Bio-control project of Red Palm Weevil in the Arabian Gulf Region (AOAD);
- The Sahel Regional Project; and
- The Date Palm Subregional TCP project.

Technical cooperation networks have become an increasingly important means of action and are initiated and supported through the Headquarters and Regional Office Regular and Field Programs. These networks have become a generic model for the establishment of functional mechanisms for collaboration and enhancement of communication and exchange of expe-

riences among different countries in one region and/or different regions of the world.

Networks are found to reduce duplicative efforts among national institutions in several countries and may provide a cost-effective instrument for information exchange and institution building (including training). When the resources are limited, networks become a more effective means for the optimal utilization of indigenous expertise and available resources among the countries themselves.

Given the current status of the date agro-industry in the different date growing countries, and in the absence of a coordinating body for the promotion of cooperation among these countries for the optimal utilization of the limited resources available for the development of the date agro-industry, the establishment of a Technical Cooperation Network on Date Palm is a matter of urgency.

FAO and since the 60's is always technically assisting the date growing countries in their development efforts. The present Date Palm Global Network will be under the aegis of FAO in collaboration with the UAE University, the UNDP and UNOPS and the above mentioned organizations as well as the other national and international institutions.

1. THE REGIONAL PROJECT FOR PALM AND DATES RESEARCH CENTRE IN THE NEAR EAST AND NORTH AFRICA (NECP/REM/521/MUL) CALLED NENADATES.

The Regional Project for Palm & Dates Research Centre in the Near East & North Africa was a Trust Fund Project of the Food and Agriculture Organization of the United Nations composed of the following seventeen member countries: Algeria, Bahrain, Iraq, Kuwait, Mauritania, Morocco, Pakistan, Yemen (at the time People's Democratic Republic of Yemen and the Yemen Arab Republic), Qatar, Saudi Arabia,

Somalia, Sudan, Sultanate of Oman, Tunisia and United Arab Emirates. The Project was governed in technical matters by a Technical Coordinating Board composed of one representative of each member country.

The NENADATES Date Palm Network was successful in providing information and development initiatives that strengthened the date industry in these

countries.

The NENADATES regional project, lasted about 10 years (1978 – till December 1987), then it was followed by two Technical Cooperation Projects (FAO – TCPs); their respective references were TCP/RAB/84/018 implemented by FAO and TCP/RAB/88/024 implemented by FAO and UNDP.

2. DATE PALM RESEARCH & DEVELOPMENT NETWORK (ACSAD).

The establishment of this network was in 1990 after the technical consultancy supervised by IFAD and UNDP which assessed the date palm situation in various selected date countries. An agreement document was then approved and financed by three donors: IFAD, AFESD and IDB. The implementation of the Network activities was given to ACSAD.

There are 12 Arab member countries, with four of the hosting the regional network offices. The network did go so far through two phases of five years each and it seems that 2002 is its last year of the extended period.

3. PARTICIPATORY MANAGEMENT OF DATE PALM GENETIC RESOURCES IN OASIS OF THE MAGHREB.

The project RAB/98/G31 (GEF-UNDP-IPGRI) is designed to remove barriers to genetic erosion of date palm in the Maghreb region (Tunisia, Algeria and Morocco), namely:

- The replacement threat from national programmes varieties that are multiplying and distributing only a few varieties of trees,
- Market forces that are encouraging a farmer's preference to grow only a few high value varieties of date palm to the exclusion of a wide range of other varieties. Together with the number of baseline programmes described, the project will form an integrated ecosystem approach to the management of the oasis sites.
- The project will focuse on activities that will serve to broaden the number of date palm varieties that will be grown in-situ by comparison to

baseline projections, rather than promote higher yields or an expansion of market demand, which are not incremental activities.

4. NORTH AFRICA REGIONAL DATE PALM NETWORK.

Another regional network on Bayoud Disease of Date Palm was established by the Joint FAO/IAEA (International Atomic Energy Agency) in 1995 and was implemented by the Division of Nuclear Techniques in Food and Agriculture (Vienna). Three countries (Algeria, Morocco and Tunisia) were the network members.

5. BIO-CONTROL PROJECT OF RED PALM WEEVIL IN THE ARABIAN GULF REGION.

A specialized regional project on the control of the Date Palm Red Palm Weevil was launched in July 1997 between 6 Gulf countries: UAE, KSA, Kuwait, Kingdom of Bahrain, Qatar and Sultanate of Oman. The project's first phase will end in June 2002 but preparations for its second phase are underway.

The project's objectives were to strengthen national programmes through training, provision of laboratory equipments and developing applicable bio-control technology as important component of IPM for the management of Red Palm Weevil.

The project is having two office locations; one in UAE and the other in KSA; while its budget was provided by IDB (US$ 1.73 million) and the IFAD (US$ 1.00 million).

6. THE SAHEL DATE PALM REGIONAL PROJECT.

The Sahel Date Palm Regional Project is an initiative of FAO, International Programme for Arid Agriculture (IPALAC) and the Desert Margins Programme (DMP). The targeted countries are Burkina Faso, Cameron, Mali, Mauritania, Niger, Senegal and Chad. The project is still as a document but already different FAO Technical Cooperation Projects (TCPs) were already born from it (example: Burkina Faso and Niger TCPs).

7. DATE PALM SUBREGIONAL TCP PROJECT: DATE PRODUCTION SUPPORT PROGRAMME IN SOUTHERN AND EASTERN AFRICA.

The regional TCP was in favor of Botswana, Namibia, Republic of South Africa, Tanzania, Uganda and Zimbabwe.

During the regional workshop which was held in Keetmanshoop (05 – 08 October 1999), countries delegates from the Southern and Eastern Africa sub-region (Botswana, Namibia, RSA, Tanzania, Uganda and Zimbabwe) did insist on the well prioritisation of date palm research and development efforts and on the elaboration of a sub-regional project. Indeed, the advantages in favour of this sub-regional project were summarized as follows:

- The sub-regional project will benefit from reduced costs by sharing technical inputs (CTA, consultants, training, research, etc.) and facilities (tissue culture laboratory, regional offices, etc.) among countries.
- Sharing information and expertise will also be an outcome of this sub-regional project.
- Safe exchange of selected date palm plant material between member countries. The establishment of control measures at the sub-regional level will protect and strengthen the positive phytosanitary status.
- The sub-regional project will implement a participating and multi dimensional approach taking into account the socio-economic characteristics of each member country.
- The export marketing potential of the sub-region is very promising as it is situated in the Southern Hemisphere as opposed to that of all the other major date producing countries that are located in the Northern Hemisphere. The sub-region will thus will be able to produce and supply dates to all the major markets during the traditional off-season.

8. THE DATE PALM GLOBAL NETWORK (DPGN).

The idea of the establishment of the Date Palm Global Network was born within the framework of the Date Production Support Programme (UTF/NAM/004/NAM) with the Coordination and assistance from the FAO – AGPC / Division (Mr. E.J. Arias).

An experts consultation, to study the feasibility of establishing a DPGN for technical cooperation on date palm and to draft the objectives and guidelines of the network, was first held in Tehran, Iran, during the period 13 – 14 October 1999, with the participation of scientists from the following countries: Egypt, Iran, Libya, Morocco, Namibia, Tunisia, U.A.E., Sultanate of Oman, Saudi Arabia, Sudan and Pakistan. The consultation was hosted by the Iranian Government and supported technically and financially by FAO (AGP, RNE) and AARINENA.

In the meeting, common problems and interests were defined and a document was prepared summarizing the outcome of the consultation and was presented in the Date Palm International Symposium that was held in Namibia, February 22 – 25, 2000. the program included a special session to discuss the Network under the auspices of the FAO.

It was an important component of the Date Palm International Symposium, which was held in Namibia, 22 to 25 February 2000, and was organized by the Date Production Support Programme in Namibia with the technical and financial support of AGPC/FAO.

The meeting was attended by 130 participants from Algeria, Australia, Egypt, England, Eritrea, France, India, Iraq, Israel, Kenya, Kingdom of Saudi Arabia, Libya, Mali, Morocco, Namibia, Niger, Nigeria, Palestine, Peru, Republic of South Africa, Senegal, Swaziland, Tunisia, U.A.E., U.S.A. and Zambia, and representatives from the following international organizations: AOAD, DMP, FAO, ICRISAT and Proclima International.

The Network's establishment meeting, held during 07 – 09 April 2002 in Al Ain / UAE, did finalize the terms of reference of each of the Co-ordinating Board, the General Co-ordinator, the working groups and regional co-ordinators (Annex I). During the same meeting the structure of the Network was also adopted (Annex II).

The constitution document as well as the project document of the DPGN were discussed and adopted during the first technical meeting of the Coordinating Board, which was held in Cairo, Egypt during 10 – 11 June 2003.

9. OBJECTIVES OF THE DPGN

1. Collection and dissemination of information on production and planting, marketing, research, post-harvest and processing technologies of dates and date palm by-products and residues.

2. Collection, conservation, evaluation and utilisation of date palm germplasm.
3. Promotion of the ecological and social benefits of Date Palm.
4. Exchange of experiences, expertise, and information as well as organise training courses, workshops and meetings of experts.
5. Contribute to the formation of national networks in each country to increase collaboration among national institutions and particularly propitiating increased communication between scientific institutions and growers.
6. Promote the analysis of common problems, their study and search of solutions, particularly through the elaboration of joint research/development projects.

10. TECHNICAL WORKING GROUPS

Specific tasks were identified to be addressed by the DPGN, forming the basis for technical working groups. For each working group, specialized institutions in different countries were identified to participate. Participation in the working groups is open to any interested scientists, organisations and associations, as well as to researchers from the private sector.

The profile and arrangements for the 4 working groups were established as follows:

10.1. Germplasm/propagation

- Promotion of collection, characterisation, conservation (in situ and ex situ), exchange and utilisation of the genetical variability in different geographical regions, and preparing germplasm catalogues.
- Elaboration of a list of potential varieties as source of primary germplasm and conservation of varieties in the centres of origin and diversification, and identification of sources of safe material.
- Define sites and institutions for the establishment of regional and international germplasm collections.
- Study the possibility to establish a system for genetic material exchange among participating countries to the DPGN and report on the adaptability and results of germplasm exchange.

- Harmonisation procedures for certification for production of standard planting materials.
- To prepare a manual on propagation of date palm, which describe the classical propagation methods and modern techniques.
- Address legal aspects for the testing and propagation of materials.
- Biosystematic research on date palm for establishing a more coherent identification and classification of most varieties.

10.2. Postharvest physiology and processing

- Study postharvest quality of fruit in relation to varieties, stages of maturity at harvest and storage conditions
- Study postharvest variations due to different production conditions.
- Develop useful techniques to control postharvest decay on fruits.
- Study harvest and handling techniques that could reduce mechanical injuries; look for packaging options for national and export markets.
- Study the postharvest behaviour of lightly processed fruits (chopped and packaged).
- Periodical compilation and dissemination of information regarding progress and problems of postharvest handling of dates in various countries.
- Definition of research priorities, that will contribute to solve common problems on date palm processing especially to the production of juices as well as the development of appropriate rural technologies.
- Review cultural practices that are commonly used to increase productivity that may be related with the quality of the products and processing technologies.
- Promotion of research in technology oriented to fruit processing.
- Documentation and circulation of success stories on processing of dates.
- Promotion of production of date byproducts.

10.3. Productivity and Economics and Production and Commercialization

- Promotion of research concerning the promotion of flowering/fruit set and studies connected to fruit quality.

- Definition of water and nutrients requirements.
- To optimise planting systems and the technical itinerary.
- Definitions of breeding strategies for date palm using both classical methods and biotechnology tools, oriented to overcome limitations for adaptation (e.g. drought, salinity), productivity (e.g. insect pests and diseases) and fruit quality.
- The advanced genetic materials will be examined in different agroclimatic conditions.
- Development of appropriate cultural techniques that will result in both yield increase and environmental protection according to specific problems in a given area.
- To facilitate basic research into the responses of date palms to the environment as well as to provide physiological support for applied aspects of their uses.
- Future research emphasis will be on interactions of stresses, predicting the influences of thermoperiod and photoperiod on organ development, predicting environmental responses of fruiting, and other aspects that are physiologically important.
- The reinforcement of basic physiological studies in order to support the work of agronomists and horticulturists, in aspects related to orchard management, fruit quality and postharvest.
- To improve the collection and dissemination of economic information regarding future development, current plantings, expected supply, internal demand and world demand.
- Study marketing problems and explore potential for future demand expansion.
- International marketing studies may involve research on particular aspects related to quality requirements of food and non-food products and pesticides regulations, among others.

10.4. Pests and diseases

- Collection and exchange of information of current pests and diseases which limit yields and quality of fruit. The relevant aspects include biology, incidence, and severity of damage and current methods of control.

- Promotion of research on integrated control of pests, weeds and diseases
- Publication of a poster or manual for the practical identification in the field of the major pests and diseases.
- Formulate a project with an integrated pest management focus for the most important diseases of date palm.
- Participate in the newsletter of the network with short articles on phyto-sanitary problems of importance in different areas and control methods (e.g. Bayoud disease, red palm weevil, etc.).
- Extending information on phytosanitary standards applicable to the fruit for export markets.

11. NETWORK ACHIEVEMENTS

- Establishment Meeting (June 2003, Cairo);
- The Network Project Document;
- The Network Constitution Document;
- A web-site for the Network;
- Organization Chart of the Network;
- Nominations / elections of the General Coordinator, Technical Secretariat, Four Technical Group Coordinators, Network Regional Representatives and Focal persons per member countries.
- An office with a secretary and tel., fax, e-mail,…
- First Regional Workshop on Tissue Culture: (14 – 16 January, 2003).
- Regional Workshop on Red Palm Weevil: (28 – 30 March, 2004).
- Second Regional Workshop on Tissue Culture and Investment Opportunities: (01 – 03 May, 2005)
- First International Date Palm Exhibition: Al Ain, UAE; (14 – 16 December 2004).
- International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants: (23 – 25 May, 2005).

12. PARTICIPATION IN THE FOLLOWING EVENTS / MEETINGS

- 9th General Conference of AARINENA Muscat, Oman: 11 - 13 April 2004.
- Conference on Dates Processing and Marketing with beneficial by-products in Arab countries: Medina, KSA / 08 – 10 June 2004.
- AARINENA Global Planning Meeting: Global Post Harvest Initiative: Linking Farmers to Markets; Antalya / Turkey, 24 – 25 March 2005.

13. COUNTRIES THAT APPLIED FOR THE NETWORK MEMBERSHIP (TILL 01 MARCH 2005).

Country	Date of Letter	Remarks
Yemen	06/03/2004	-
UAE	23/03/2004	-
Palestine Authority	13/06/2004	Free membership
Lebanon	17/06/2004	Paid
Bahrain	15/06/2004	-
Somalia	07/07/2004	Free membership
KSA	24/08/2004	Paid
Syria	30/11/2004	-
Oman	-	Waiting for official letter
Qatar	19/09/2004	Apologized

I.B.D & UNEP: Expect request of assistance.

ANNEX I

TERMS OF REFERENCE

A. Co-ordinating Board

- Facilitate communication among the different working groups and regional Date Palm networks.
- Help the General Co-ordinator in promoting technical activities both at the regional and global level.
- Facilitate adaptation of the working arrangements of the Network to meet member requirements and to ensure efficiency of operations.
- Cooperate to identify and obtain funding assistance from donors and financing agencies for strengthening network activities.

B. General Co-ordinator

- Guarantee communication and feedback between the working groups.
- Guarantee divulgation of information among members.
- Elaboration of regional project proposal to address questions of global concern for submission through FAO to potential funding agencies or donors.
- Arrange meetings every 2 years of network focal points, to review progress and priorities.
- Arrange regular (2 yearly) meetings of working group co-ordinators to review activities, progress and priorities for ongoing and future work (combined with international workshops, congresses, etc., in order to minimise costs).
- Assist in planning and organising training activities in relation to needs of subgroups and working groups.
- Prepare publications every 2 years integrating the information coming from the subgroups.
- Guarantee regular publication of a newsletter and other instruments (e.g. website/web pages) for enhancing information dissemination and exchange.

- Promote co-ordinating board meetings, as well as specific meetings on technical subjects in collaboration with working group co-ordinators.
- Guarantee that the information system will be accessible for world benefit.
- Promote efforts to obtain funding assistance from donors and financing agencies for strengthening network activities.
- Stimulate collaboration among members, in close co-ordination with FAO, to elaborate strategies for Date Palm expansion, rehabilitation or reconversion, or to assist in defining diversification strategies.

C. Working Group Co-ordinators

- Develop programmes and guide activities for the working group in accordance with national strategies and priorities.
- Ensure communication between group members.
- Elaborate technical bulletins for dissemination of information.
- Assist in establishing agreements and procedures for exchanging information/material.
- Provide the General Co-ordinator with regular information on progress, results and needs of working groups.
- Prepare and disseminate annual progress reports on working group activities and promote appropriate scientific contributions to be included in the Network newsletter.
- Assist in preparation of regional or subregional technical programmes of assistance for submission to funding agencies/donors.
- Promote specific meetings on technical subjects within their groups' area of activity.
- Develop communications, through electronic and other media, to facilitate sharing of information and intra-group contacts.

D. Focal Points

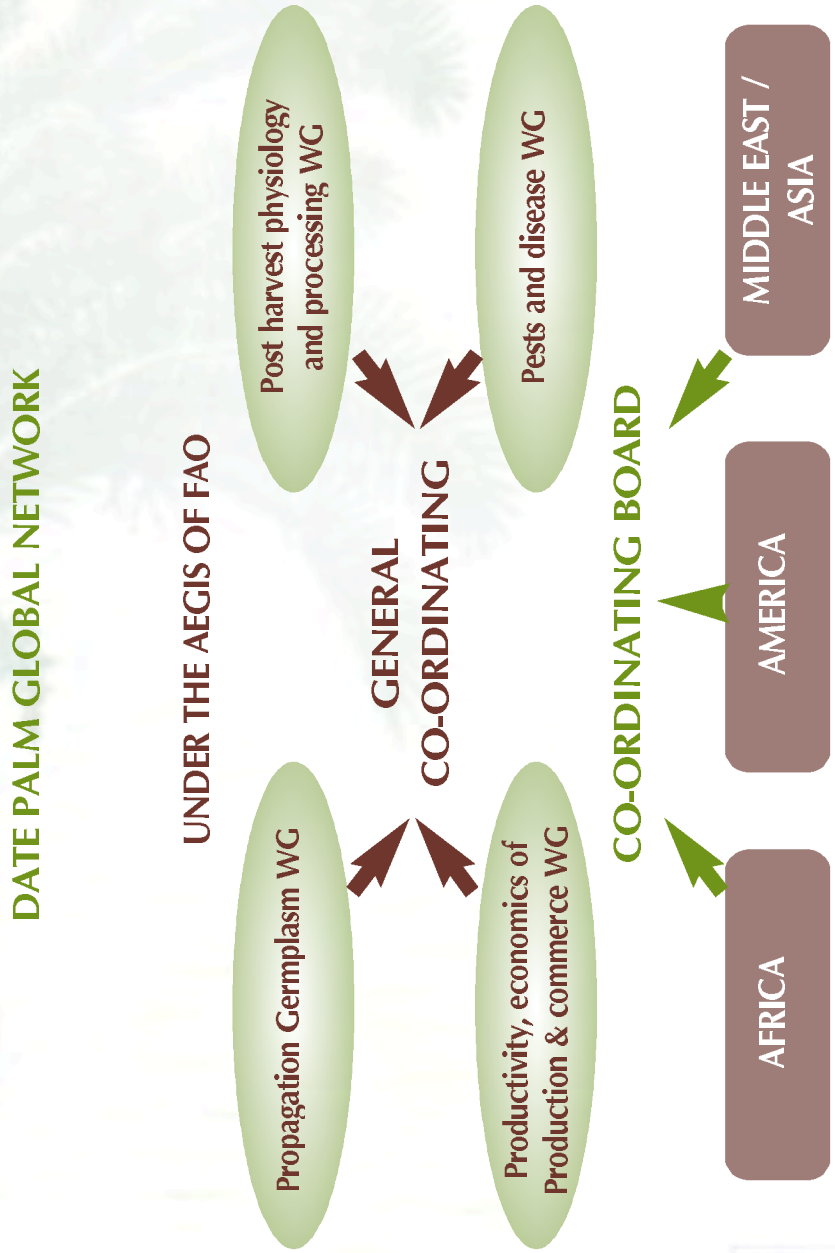
- To guarantee feedback to countries of information generated by the different components of the network.

- To ensure communications within each country on matters related to the GDPN.
- To guide overall co-ordination of network through regular meetings of focal points (Governing body).

E. Regional Co-ordinators

- Promote and encourage the establishment of joint projects on Date Palm research/development among countries that share common geographical and ecological situations in regard to arid lands.
- Ensure communication between group members, the focal points and the General Co-ordinator.
- Arrange regular meetings of their members to review activity progress, problems and formulate regional work plans.
- Assist the overall co-ordination in the elaboration of project proposals to be addressed to FAO for submission to donors.
- Assist the overall co-ordination in planning and organising regional training activities, workshops and meetings of experts.

ANNEX II



PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF DATE PALM OFF-TYPES

Cohen Y.[1], Gurevich V [1], Korchinsky R[1], Tripler E.[2], and Lavi U.[1]

1Deparetment of Fruit Tree Science, Agriculture Research Organization, Volcani Research Center, Bet Dagan, 50250, Israel. Tel: 972-3-9683407; FAX: 972-3-9669583, Email: vhyuvalc@volcani.agri.gov.il

2- Southern Arava Research Station, M. P. Hevel Eilot 88820, Israel.

ABSTRACT

Propagation of date palms using tissue culture techniques is very advantageous. However, among the normal trees, many abnormal, off-type trees are generated. The present study applies Amplified Fragment Length Polymorphism (AFLP) to characterize three common date palm off-type phenotypes widely spread among tissue culture originated trees in Israel, and in other countries. These phenotypes include variegation, "low level fruit setting" and dwarfism. While some off-types are easily detected in young plantlets, these off-types are commonly detected in the field years after planting. The last two, tend to occur in mass numbers mainly in plantlets of specific tissue culture laboratories. Therefore, both preventing the generation and developing methods for early detection of these off-types are necessary. Within the variegated trees multiple mutations seems to occur. In contrast, although relatively low number of mutations was detected in the fruit setting and the dwarf trees, no specific mutation was found to be associated with these phenotypes. However, differences in DNA-methylation patterns seem to characterize these off-types. Reduction in the overall DNA methylation level seems to be associated with both the "low fruit set" and the dwarf phenotypes.

Additional Index Words: AFLP, DNA-Methylation, dwarfism, flower-abnormalities, somaclonal-variation.

INTRODUCTION

Date palm (Phoenix dactylifera L.) is a major tree crop in arid regions of the Middle East and North Africa, having an important impact on the economy of many countries in these regions. Date palms are traditionally propagated through offshoots. The development of propagation methods through tissue culture resulted in massive expansion of date palm plantations. Several methods for in-vitro propagation through tissue culture were developed for date palm. Tissue culture generated trees provide many advantages over traditional trees propagated by offshoots. Tissue culture propagation has the potential to generate a large number of homogenous plants, has no seasonal effect on plant source, and enables easy and safe exchange of plant material. This method can provide tools to fight the expansion of date pests and diseases such as 'Bayoud' or 'Red Palm Weevil', by providing disease free material, propagation of resistant clones and in the future, by incorporation of resistant traits into elite date palm cultivars. Tissue culture propagation is therefore, crucial for the future development of date palm plantations.

In vitro propagation requires that plants will be true-to-type, i.e., will be identical to their progenitors and to those propagated by traditional methods. Currently, the process of tissue culture propagation results in the production of off-types, which differ from the original cultivar. The process of formation of these abnormalities is called somaclonal variation. It is caused by mutations and/or epigenetic changes occurring during the in vitro process (Kaeppler et al., 2000). Somaclonal variation can be induced by environmental conditions in the culture (such as light, temperature, etc.). Among tissue culture produced date palms, several typical off-type phenotypes are detected, including variegation in leaves, variation in leaf structure and in overall plant growth patterns, trees that do not form inflorescences and trees that produce seedless parthenocarpic fruits (Zaid and Al Kaabi, 2003). In Israel, about a third of planted tissue culture originated date palms were found to be abnormal(Cohen et al., 2004; Gurevich et al., 2005). When large quantities of off-types with similar symptoms are generated, it is assumed that processes occurring during the tissue culture propagation are the cause for this phenomenon.

Some off-type phenotypes can only be detected several years after their planting in the field. Since off-types are quite common among tissue culture originated date palms, efforts are made, to elucidate the mechanism of their generation and restrict their formation (Kunert et al., 2003; Zaid and Al Kaabi, 2003). The massive use of tissue culture plantlets can continue only

if efficient methods for prevention of off-type generation, as well as methods for detection and early removal of the unwanted off-types, are developed.

Molecular information on date palms is quite limited. Differences between varieties were detected by iso-enzyme analysis, RFLP, RAPD, AFLP (Cao and Chao, 2002; Devanand and Chao, 2003) and RDA (Vorster et al., 2002). While high levels of variation were detected between varieties, significant variation was also detected within trees from the same variety (Devanand and Chao, 2003; Gurevich et al., 2005). Some analyses of abnormal trees originated from tissue culture were also previously carried out. These included phenotypic and iso-enzyme characterizations (Azeqour et al., 2002) RAPD (Saker et al., 2000) and AFLP analyses (Gurevich et al., 2005). The data published so far, does not provide effective methods for early detection of these abnormalities.

The current methods do not detect epigenetic changes caused during the tissue culture stage. In several plant species, it was shown that changes in DNA methylation can occur at the tissue culture phases (Kakutani et al., 1996). In other plants, these changes were shown to cause reduced fertility and structural flower abnormalities. For example, Arabidopsis seedlings with reduced methylation are quite infertile and their flowers have additional carpels (Kakutani et al., 1996). Tissue culture originated oil palms generate a typical off-type phenotype called 'Mantled' sharing many similarities to the date palm off-type with having low levels of fruit setting and supernumerary carpels. In most of these trees, there is alleviation of the symptoms during the maturation of the trees and many of them revert to normal phenotypes after several years in the field. The 'Mantled' phenotype was found to be related to altered DNA methylation (Jaligot et al., 2000; Matthes et al., 2001). The present study combines phenotypic and genetic analyses at both the sequence level and the pattern of DNA methylation for the elucidation of the mechanisms associated with the generation of these off-types.

MATERIALS AND METHODS

Phenotypic analysis of date palms

A survey of date palm off-types in orchards in Israel was performed during 2000-2004. The age of the trees and the Tissue Culture (TC) producing laboratories were recorded. In certain orchards, of 'Barhee' tissue culture originated trees, detailed analysis of fruit setting over 2-4 years (2000-2004) was

preformed. The survey included the percentage of normal fruitlets, single carpel parthenocarpic fruitlets, parthenocarpic fruitlets with three carpels, or abnormal fruitlets with additional supernumerary carpels. The levels of flowers and young fruitlets dropout were also recorded. This survey resulted in detailed multi-seasonal maps of fruit setting levels of individual trees in several orchards.

Analysis of genetic variation

Leaf samples from twenty-seven 'Barhee' and twenty-seven 'Medjool' trees were collected from different orchards and nurseries in Israel. The various trees included offshoots and tissue culture propagated trees (from several tissue culture laboratories), having both normal and off-type phenotypes. Leaf samples of two variegated trees, from the normal (green) and variegated (yellow) sections as well as root samples, were also collected .Total DNA was isolated from leaves and roots using the Hexadecyl Trimethyl- Ammonium Bromide (CTAB) extraction method (Aitchitt et al., 1993). DNA was restricted with EcoRI and MseI restriction endonucleases, ligated to specific adaptors and PCR amplified using 5 different primer-sets (one primer in each set being radio-labeled). The resulting products were separated on acryl-amide gels, to generated AFLP band patterns (Gurevich et al., 2005).

Analysis of DNA-methylation patterns

DNA samples were isolated from leaves of 15 'Barhee' trees and 13 'Medjool' trees, originated either from offshoots or from tissue-culture. These included normal as well as trees of the common off-types ('Barhee' trees expressing low level of fruit set and abnormal fruitlets, and 'Medjool' trees with extremely restricted growth). 'The AFLP procedure was modified to enable detection of DNA methylation patterns. Two parallel restriction reactions were performed for each DNA sample, one with EcoRI/MspI and the other with EcoRI/HpaII. MspI and HpaII are two isoschizomers recognizing a similar restriction site but differing in their sensitivity to cytosine methylation in these restriction sites. AFLP band patterns from the two reactions were generated using six primer sets as described above and the patterns of the two reactions were than compared.

Genetic analysis

AFLP band patterns were converted into binary matrices - 1 for presence, 0 for absence of a band (only the clear and reliable bands were scored). Analysis of Molecular Variance (AMOVA) was performed using Arlequin

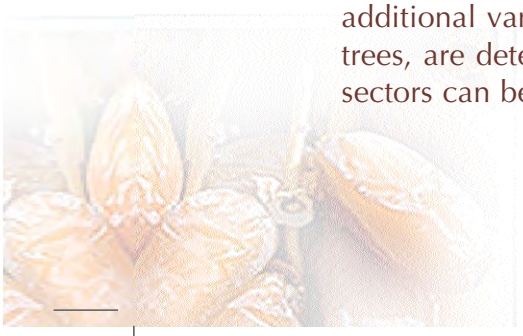
software (Schneider et al., 2000). Genetic variation within populations was estimated by the percent of polymorphic bands of all individuals within each population, and by the Average Gene Diversity (AGD) -, the average number of polymorphic bands in all pair-wise comparisons within populations.

RESULTS

Phenotypic characterization of off-types

We have preformed an extensive survey on date palm off-types in several date palm grooves in Israel, as well as in a nursery producing TC plants. Approximately 10% of the entire planted trees (35,000 out of 350,000 trees in Israel, and most of the young ones, were propagated in tissue culture. They originated from both local and foreign laboratories. Most of these trees are of the 'Medjool' cultivar. Several thousands 'Barhee' trees, as well as fewer trees from other cultivars and male clones were also planted. Several common abnormalities were detected: The two most abundant abnormal phenotypes detected in the field were: (a) 'Medjool' trees having very slow growth rate and (b) 'Barhee' and 'Hallas' trees characterized by low level of fruit setting. Other off-types include: (c) plants with variegated leaves, (d) distortion of leaf structure similar to the symptoms of black scorch (Thielaviopsis paradoxa), (e) plants with abnormal leaf structure, mainly with short rachis and very large leaflets, (f) plants that do not flower and (g) plants that form very high offshoots instead of inflorescences. These phenotypes are similar to previously reported off-types detected in other field surveys (McCubbin et al., 2000; Zaid and Al Kaabi, 2003). We focused on the characterization of the three most common off-type phenotypes in Israel: date palms with leaf variegation, trees with low levels of fruit settings, and trees characterized by dwarfism or restricted vegetative growth. The last two occur in large numbers, and exhibited common phenotypes sometimes detected in entire tree batches comprising many thousands of trees in several Mediterranean countries (Al-Wasel, 2000; Djerbi, 2000; Zaid and Al Kaabi, 2003; Cohen et al., 2004).

1. Leaf variegation: This phenotype is detected in many plantlets in the hardening stage. Although these are removed and not planted in the fields, additional variegated trees, which may reach up to 3% of the planted trees, are detected during the first years after planting. The variegated sectors can be relatively small, comprising several leaflets, or covering



large regions in several leaves. Although in several instances, normal looking fruits were produced by such variegated trees, farmers usually remove them upon the detection of the abnormality.

2. Low level of fruit setting: This phenotype is common in trees of the 'Barhee' and 'Hallas' cultivars. They are characterized by low level of fruit setting, and by supernumerary carpels which result in multi-carpel parthenocarpic fruits (Al-Wasel, 2000; Djerbi, 2000; Cohen et al., 2004). The severe cases occurred in about 2,000 (out of about 4,000) trees planted in Israel, originated in one Tissue Culture Laboratory. However; off-types with abnormal fruit setting were found in trees produced in five different commercial Tissue Culture Laboratories in Israel. Similar patterns were obtained in trees originated from at least two laboratories in Jordan. However, the severity of problem varied between the different tree batches and sources (Cohen et al., 2004). In some of them, the level of parthenocarpic fruitlets, and abnormal fruitlets was low, while others did not produce any commercial yield. In all studied cases, fruit setting improved during maturation of the trees. In severe cases, the tendency for lower level of fruit setting and formation of multi-carpel flowers and fruitlets was detected also in offshoots of the abnormal plants. The level of these abnormalities in the off-shoots was lower than in the "mother trees" when they reach the same size (Cohen et al., 2004). Additional flower distortions resulting in twisted stigma were recorded. These were found to be responsible for the inability of pollen tubes to grow in the stigma and carpel toward the ovule (Cohen et al., 2004).

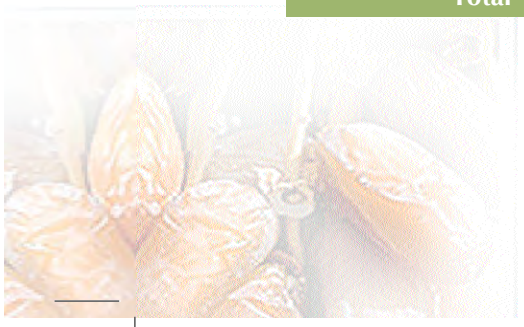
3. Dwarfism: This phenotype is detected in mass numbers in about 10,000 young 'Medjool' trees planted in 1999-2001. In several orchards comprising thousands of trees all of them exhibit the specific "dwarf" phenotype. These trees were produced by the organogenesis procedure by a single Tissue-Culture Laboratory. These trees developed poorly, showing an extremely recessed growth rate and developed much fewer leaves. After four years in the field their growth is delayed by 2-3 years relative to trees of the same age from other (tissue culture or offshoot) sources. Several of these dwarf trees (4-5 years old), produced first flower bunches. However, similarly to the phenotype of the “low level fruit setting”, the pollinated flowers did not develop to normal fruits, but develop parthenocarpic fruits, many times containing supernumerary carpel.

Genetic variation at the sequence level

We applied Amplified Fragment Length Polymorphism (AFLP) to characterize the genetic variation in the above mentioned three common date palm abnormal phenotypes (Gurevich et al., 2005). We compared the band patterns of 27 'Medjool' and 27 'Barhee' trees which including offshoots and tissue culture originated trees, normal and off-type trees phenotypes. For each cultivar, a low level of variation was detected between offshoots. The results are summarized in Table I. A comparable level was detected between various 'Barhee' trees from tissue culture, including the trees exhibiting the fruit setting abnormality. Genetic variation between 'Medjool' trees originated from tissue culture was significantly higher. However, among these trees some yield normal 'Medjool' fruits. Although among dwarf trees a considerable level of variation was detected, no specific bands associated with this phenotype were detected.

Table I: Characterization of the AFLP band pattern of 'Barhee' and 'Medjool' trees originated from tissue-culture (TC) or from offshoots (OS). Total of 317 clear bands were obtained by 5 different primer pairs.

Cultivar	Source of origin	phenotype description		Number of analyzed trees	Number of Polymorphic bands	% of polymorphic bands	AGD values
		Fruit setting	Multi-carpel fruitlets				
'Barhee'	OS	+++	-	8	2	0.63	0.78
'Barhee'	TC (1)	-	+++	7	2	0.63	0.57
'Barhee'	TC (2)	+	+	4	1	0.31	0.50
'Barhee'	TC (3)	+	+	3	0	0	0.00
'Barhee'	TC (4)	++	-	5	0	0	0.00
Total 'Barhee'				**27**	**4**	**1.26**	**0.79**
		Fruit setting	Normal Flowers and fruits				
'Medjool'	OS	++	+	10	3	0.94	0.75
'Medjool'	TC (5)	+++	+	4	15	4.73	7.83
'Medjool'	TC (2)	+++	+	8	27	8.52	9.32
'Medjool'	TC (6)	- -	-	3	11	3.47	7.33
'Medjool'	TC (7)	++	+	2	3	0.94	3.00
Total 'Medjool'				**27**	**43**	**13.56**	**5.97**



In contrast to the relatively low genetic variation detected in the two abnormal phenotypes, AFLP analysis of variegated trees detected differences in their AFLP band patterns (Gurevich et al., 2005). Moreover, differences in band patterns were detected between various tissues: Normal (Green) and Variegated (Yellow) regions of leaves, as well as root samples of the same variegated trees, and between different trees. This variation suggests that the trees are mosaic not only to a single mutation (easily detected by variegation), but to several other mutations as well. No specific bands associated with this phenotype were detected.

Epigenetic variation – patterns of DNA methylation

Using a modification of the AFLP procedure, we also analyzed differences in DNA methylation patterns in trees of different sources and phenotypes. The results of this analysis are summarized in Table II. Methylation patterns were found to vary between cultivars. Relatively low levels of polymorphism in methylation patterns were detected within 'Barhee' trees of the various groups and between the various sources and phenotypes. Much higher variation was detected between various sources of 'Medjool', mainly within the group of the growth retarded plants. Our results (Table III) also suggest that changes in the DNA methylation levels occur during TC. DNA methylation seems to be reduced by about 1%, in the “fruit setting” phenotype, compared to normal trees.

Table II: Methylation patterns in normal and off-type 'Barhee' and 'Medjool' trees originated from tissue-culture (TC) or from offshoots (OS). Total of 359 and 331 clear bands were obtained from the EcoRI/MspI and EcoRI/HpaII AFLP analyses respectively by 6 different primer pairs. 58 bands differing between the two analyses are consequences of differences in methylation of the analyzed MspI/HpaII sites. The number of polymorphic bands in these sites in each group, their percentage from the total differing bands and the calculated AGD values are presented.

Cultivar	Source of origin	phenotype description		Number of analyzed trees	Number of Polymorphic bands	% of poly-morphic bands	AGD values
		Fruit	Multi-carpel				
'Barhee'	OS	+++	-	6	1	1.72	0.33
'Barhee'	TC (2)	+	+	2	0	0	0
'Barhee'	TC (1)	-	+++	7	0	0	0
Total 'Barhee'				**15**	**3**	**5.17**	**1.4**
		Fruit setting	Normal Flowers and fruits				
'Barhee'	OS	+++	-	6	1	1.72	0.33
'Medjool'	OS	++	+	2	1	1.72	1
'Medjool'	TC (2)	+++	+	5	1	1.72	0.4
'Medjool'	TC (6)	- -	-	6	19	32.76	8.13
Total 'Medjool'				**13**	**35**	**60.34**	**10.1**

Table III: Percentage of methylated sites in normal and off-type 'Barhee' and 'Medjool' trees propagated from tissue culture (TC) or offshoots (OS). Only monomorphic sites (in which no polymorphism in the DNA sequence was detected between all in the samples of the same cultivar) were included in this analysis. Values are presented as averages ± standard errors.

Cultivar	Source	phenotype description	Number of analyzed trees	Average percentage of methylated sites	
'Barhee'	OS	Normal	7	12.58±0.06	12.58±0.05
	TC (1)	Normal	2	12.61±0.02	
	TC (2)	Low fruit setting	6	11.72±0.08	11.72±0.08
'Medjool'	OS	Normal	2	7.34±0.16	8.51±0.34
	TC (2)	Normal	5	8.98±0.21	
	TC (6)	Dwarf	5	8.42±0.18	8.42±0.18

DISCUSSION

The present study combines morphological and molecular analyses to elucidate the mechanism of formation of off-types in tissue-culture. We analyzed three common phenotypes and characterized two different forms of molecular variation. The first type, represented by the variegated trees, is characterized by genetic variation at the sequence level. Variegation is a phenotype clearly detected, which could be generated by many independent mutations in the photosynthetic apparatus or in chloroplast biogenesis. However, additional mutations are identified in these variegated trees. Therefore, batches in which variegated trees are common are probably relatively widespread with other undetected mutants. The occurrence of high level of genetic variation in certain batches of tissue culture (Gurevich et al., 2005), supports these results. Tissue culture conditions enhance the frequency of random mutations. Phenotypic screening, together with genetic variation analyses can probably provide tools to detect such "highly mutated" batches.

We could not detect any specific molecular variation, associated with the "fruit setting" and/or the "retarded growth" phenotypes. However, these phenotypes are characterized by changes in their methylation patterns. In the dwarf 'Medjool' trees, high variation in DNA methylation patterns was detected, and in the 'Barhee' fruit setting phenotype we detected a decrease in DNA-methylation. It was previously suggested that the hormonal balance, mainly the auxin - cytokinin ratio may be involved in the formation of similar off-types (Zaid and Al Kaabi, 2003). DNA methylation patterns are important for conservation of gene expression patterns and for development schemes. This may be the reason why many of these off-types are detected only late in plant development, years after the trees are planted in the fields, sometimes only following transformation from their juvenile to the reproductive stage. Early detection and prevention of these off-types, requires the development of new and more efficient diagnostic tools.

ACKNOWLEDGEMENTS

The research was funded by a research grant of the Chief Scientist of the Israeli Ministry of Agriculture and the Israeli Fruit Board.

LITERATURE CITED

Aitchitt M, Ainsworth CC, Thangavelu M (1993) A rapid and efficient method for the extraction of total DNA from mature leaves of the date palm (Phoenix dactylifera L.). Plant Molecular Biology Reporter 11: 317-319.

Al-Wasel AS (2000) Vegetative and fruiting comparison of tissue culture derived and conventionally propagated date palm (Phoenix dactylifera L.) cv. Barhi trees. In Vitro Biology Cellular and deveolopmental biology 36: P-1010.

Azeqour M, Majourhat K, Baaziz M (2002) Morphological variations and isoenzyme polymorphism of date palm clones from in vitro culture acclimatized and established on soil in South Morocco. Euphytica 123: 57-66.

Cao BR, Chao C-CT (2002) Identification of date cultivars in California using AFLP mar - kers. HortScience 37: 966-968.

Cohen Y, Korchinsky R, Tripler E (2004) Flower abnormalities cause abnormal fruit set - ting in tissue culture propagated date palm (Phoenix dactylifera L.). Journal of Horticultural Sciences & Biotechnology 79: 1007-1013.

Devanand PS, Chao CT (2003) Genetic Variation within 'Medjool' and 'Deglet Nur' date (Phoenix dactylifera L.) cultivars in California detected by fluorescent-AFLP mar - kers. Journal of Horticultural Sciences & Biotechnology 78: 405-409.

Djerbi M (2000) Abnormal fruiting of the date palm derived from tissue culture. In the date palm international symposium, Windhoek, Namibia, pp 73.

Gurevich V, Lavi U, Cohen Y (2005) Genetic variation in date palms propagated from offshoots and tissue culture. Journal of the American Society for Horticultural Science 130: 46-53.

Jaligot E, Rival A, Beule T, Dussert S, Verdeil JL (2000) Somaclonal variation in oil palm (Elaeis guineensis Jacq.): the DNA methylation hypothesis. Plant Cell Reports 19: 684-690.

Kaeppler SM, Kaeppler HF, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Molecular Biology 43: 179-188.

Kakutani T, Jeddeloh JA, Flowers SK, Munakata K, Richards EJ (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. Proc. Natl. Acad. Sci. USA 93: 12406-12411.

Kunert KJ, Baaziz M, Cullis CA (2003) Techniques for determination of true-to-type date palm (Phoenix dactylifera L.) plants: a literature review. Emirates Journal of Agricultural Sciences 15: 1-16.

Matthes M, Singh R, Cheah SC, Karp A (2001) Variation in oil palm (Elaeis guineensis Jacq.) tissue culture-derived regenerants revealed by AFLPs with methylation-sensitive enzymes. Theoretical and Applied Genetics 102: 971-979.

McCubbin MJ, Van Staden J, Zaid A (2000) A Southern African survey conducted for off-types on date palms produced using somatic embryogenesis. In Proceeding of the Date Palm International Symposium, Windhoek, Namibia, pp 68-72.

Saker MM, Bekheet SA, Taha HS, Fahmy AS, Moursy HA (2000) Detection of somaclo - nal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biologia Plantarum 43: 347-351.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, a software for populations gene - tic data analysis. In, Ed 2.0.

Vorster BJ, Kunert KJ, Cullis CA (2002) Use of representational difference analysis for characerization of sequence between date palm varieties. Plant Cell Reports 21: 271-275.

Zaid A, Al Kaabi H (2003) Plant-off types in tissue culture-derived date palm (Phoenix dactylifera L.). Emirates Journal of Agricultural Sciences 15: 17-35.

MIXED INFLORESCENCE VEGETATIVE AXILLARY DEVELOPMENT: A TRAIT OF REJUVENATION IN THE DATE PALM FROM TISSUE CULTURE

M. Ferry
Estación Phoenix, Spain, E-mail: m.ferry@wanadoo.es

ABSTRACT

During the first year and more infrequently the two years that precede the development of the first inflorescences, date palms from seeds usually produce an organ that is constituted of mixed vegetative and floral characters. An identical phenomenon occurs frequently with the date palms from tissue culture. It does not correspond to an abnormal morphogenesis consequence of the in vitro process but to a rejuvenation phenomenon similar to the phenomenon that characterizes the first leaves produced by the vitroplants.

Additional index words: Phoenix dactylifera, true to typeness, juvenile inflorescence, axillary bud, mature inflorescence, Medjool, Confitera

INTRODUCTION

The issue of abnormalities in tissue culture propagated date palms is crucial for the date palm sector. This issue has the same relevance for other palms like oil palms (Rival 1997) and more generally for all the plants for which propagation by tissue culture is realized at the commercial stage.

For date palms, the issue of abnormality has taken a great relevance when, contrary to the first analysis (Booij et al 1993, Al-Ghamdi 1996, Javouhey 2000,), it was found that, in some places, numerous date palms from tissue culture presented serious type of abnormalities (Djerbi 2000, McCubbin 2000, Al-Wasel 2001, Zaid 2003, Cohen 2004).

Various types of abnormalities have been listed and described: leaves dwarfism, plant dwarfism or with seriously abnormal low growth, pollination failure, abnormal fruiting, distorted leaves and inflorescences, leaf variegation, delayed first flowering time, bastard offshoots, apparition of black scorch or black scorch like symptoms.

The question is: should all these symptoms be called abnormalities?

The objective of this present study is to answer to this question for at least one of the so called abnormalities: the bastard offshoots.

MATERIAL AND METHODS

The material was constituted of two groups of date palms:

- Date palms from seeds.
- Date palms from tissue culture of two varieties Medjool and Confitera. Confitera is a new variety obtained from a original genotype selected in the palm grove of Elche for the quality of its fruit. Medjool has been propagated by organogenesis from an initial offshoot. For the Confitera, the tissue culture process is mixed with an initiation phase by somatic embryogenesis and a propagation phase through organogenesis (Ferry 2000).

Around 100 trees of each two groups have been studied.

The palms of the two groups were planted in 2002 in Elche (Spain) and observed three and four years after plantation.

RESULTS AND DISCUSSION

Three or four years after plantation, the palms of the two groups produce two types of inflorescences:

- "normal" or "mature" inflorescences with only one pedoncular bract (the spathe or prophyll).
- other type of inflorescence that I call juvenile. I will explain further the reason of this term. At the difference of the "normal" type, they present various bracts, either pedoncular or fertile. The number of these bracts and their shape vary from one inflorescence to another. The pedoncular bracts present transition from leaf like with leaflets to bracts reduced to an unique axis. The fertile bracts can envelop totally each rachilla (spikelet) like a prophyll. Very often, the leaf like, the bracts and the rachilla presents partially or totally a zigzag form. The rachillas bear some flowers that can evaluate to normal date if they are pollinated.

In the following table, I give the repartition of each type of inflorescences for the two groups.

		Juvenile inflorescences	Mature inflorescences
From seeds		65%	35%
From tissue culture	MJH	98%	2%
	Confitera	0%	100%

As it can been concluded from these results, the production of juvenile type inflorescences is very common in date palm from seeds. In the case of our study, it has concerned more than 50% of the shoots.

With the tissue culture palms, we have obtained a result that is very contrasted:

- No production of juvenile type inflorescences with the variety Confitera
- 98% with the variety Medjool: in fact, it is quite probable that for the 2% of shoots that has been noted with mature type inflorescence, the first inflorescences were of juvenile type but not detected the first flowering year.

From this result, it seems that the development of juvenile type inflorescences is a genotype dependant trait.

The production of juvenile type inflorescences usually occurs during the first year of flowering. During this first year, all the inflorescences, part of them or no one can be of that type. It has also to be noted that some offshoots can appeared in a upper position that juvenile or mature type inflorescences. Very often, they present also the zigzag symptoms either at the leaf level or at the shoot level.

For date palms from tissue culture, the juvenile type inflorescences use to be called bastard offshoots (Zaid 2003) and they are considered to represent a symptom of abnormality due to the tissue culture process. This development has been interpreted as hapaxanthic (Sudhersan 2001) but this interpretation does not seem right as no vegetative growth phase precedes the inflorescence development.

In fact, as demonstrated by the frequency of juvenile type inflorescences in date palms obtained from seeds, this development is quite normal. It corresponds to a transitory development phase between the juvenile one and the mature one. During the juvenile one, the axillary buds have a vegetative development. During the adult one, they have generally a floral development. During the transitory phase, their morphogenesis is often of this intermediate type that I call juvenile by analogy with the leaves development. It illustrates the classical transformation model that links vegetative organs morphogenesis to the floral organs one. In the case of the date palm, these two types of organs have the same origin, the undifferentiated bud (Ferry 2002).

It is well known that the first leaves of the date palms obtained from seeds are of a juvenile type: from entire leaves to pinnate leaves passing by partially pinnate leaves. With date palm from tissue culture, the same succession of leaves of different morphology occur (Ferry 1986) and it is genotype dependant (Ferry 1988). This juvenile morphology trait corresponds to the classical phenomena of rejuvenation created by the tissue culture process.

CONCLUSIONS

Very often date palms from tissue culture as well as from seeds produce during their first flowering year a type of inflorescence different from the ones that they will produce the following years. It corresponds to a transitory and juvenile morphogenetic model. For date palms from tissue culture, it should not be considered as an abnormality but to a classical rejuvenation phenomenon.

Juvenile inflorescences like juvenile leaves production correspond to traits of the rejuvenation phenomenon. But this phenomenon can affect also others aspects that are usually considered as abnormalities. Deformed leaves with particularly the zigzag shape should also be considered as normal as it is very common in date palms from seeds. Leaves dwarfism is also rather frequent. In fact, rejuvenation phenomenon could be the cause of other so-called abnormalities like the black scorch like symptom or delay in flowering.

In some cases, the consequences of the rejuvenation phenomenon can cause serious prejudices to the farmers. As this phenomenon is linked to the tissue culture process, the importance of its consequences will depend of each laboratory tissue culture protocol. As the origin of this phenomenon is epigenetic, the use of molecular techniques that detects polymorphism does not give any information and guarantee on the quality of the material produced regarding this issue. In fact, in the case of rejuvenation as, very often, in the case of real abnormalities, only the comprehensive and precise analysis of tissue culture protocol will allow to look for solutions.

LITERATURE CITED

- *Al-Ghamdi A. S. 1996. Field evaluation of date palm (Phoenix dactylifera L.) cultivars produced through tissue culture technique. 2. Flowering and fruit setting. Bulletin of Faculty of Agriculture, University of Cairo 47 (1): 141-151 1996*
- *Al-Wasel S. Abdelrahman S., 2001. Field Performance of Somaclonal Variants of Tissue Culture-derived Date Palm (Phoenix dactylifera L.). PTC, 11(2): 97-105.*
- *Booij I., Piombo G:, Risterucci J.M., Thomas D., Ferry M., 1993. Sugar and free amaino acid composition of five cultivars of date palm from offshoots or vitroplants in open field. J. Agric. Food Chem., 41.*
- *Cohen Y.; Korchinsky R.; Tripler E, 2004. Flower abnormalities cause abnormal fruit set - ting in tissue culture-propagated date palm (Phoenix dactylifera L.). Journal of Horticultural Science and Biotechnology 79 (6): 1007-1013 2004*
- *Djerbi M., 2000. Abnormal fruiting of the date palm derived from tissue culture. Proceedings of the date palm international symposium, Windhoek, Namibia, 22-25/02/2000. Date Production Support Programme.*
- *Ferry M., Louvet J.M., Desfonds V., 1986. Resurgence of juvenile foliage characters among in vitro date palm plants. Second symposium on date palm. King Faisal University. March 1986.*
- *Ferry M., Toutain G., Desfonds V., Louvet JM, 1988. Morphological differences in the development of date palm vitroplants of various varieties. Symposium Irako-Français sur la culture de tissus de palmier dattier, Baghdad, Iraq, 26-29/09/88.*
- *Ferry M., Navarro J., Ruiperez E., Jimenez E., 2000. An embryogenesis-organogenesis mixed system for in vitro date palm durable mass propagation. Date palm International Symposium, Windhoek, Namibia, 22-25/02/2000. Book of abstracts.*
- *Ferry M., Ruiperez E., Navarro J., 2002. The date palm axillary productions: morphoge - nesis and interest for in vitro propagation in the Date Palm. Abu Dhabi. ECSSR. pp.87-94*
- *Javouhey M.; Daguin F.; Letouze R., 2000. Somatic embryogenesis, an efficient tool for date palm (Phoenix dactylifera L.) industrial micropropagation. Characterization and genetic stability of original offshoots and regenerated plantlets by RAPD markers. International symposium on methods and markers for quality assurance in micropropa - gation, Cork, Irish Republic, 24-27 August, 1999. Acta Horticulturae (No. 530): 237-241*
- *McCubbin M.J., Van Staden J., Zaid A., 2000. A southern African survey conducted for off-types on date palms produced using somatic embryogenesis. Proceedings of the date palm international symposium, Windhoek, Namibia, 22-25/02/2000. Date Production Support Programme.*
- *Rival A., Aberlenc-Bertossi F., Morcillo F., Tregear J., Verdeil J-L., Duval Y., Durand-Gasselin T., Donan E.K., Kouame B., 1997. Micropropagation clonale par embryogenè - se somatique du palmier à huile. Programmes de recherche liés au transfert d'échelle. 6èmes J. Scient. Réseau Biotech. Vég. Orsay, juillet 1997. AUPELF-UREF.*
- *Sudhersan C., Abo El Nil M., Hussain J., 2001. Hapaxanthic axillary shorts in date palm plants grown in vivo and in vitro. Palms, 45(2), 84-89*
- *Zaid A.; Al-Kaabi H. 2003. Plant-off types in tissue culture-derived date palm (Phoenix dactylifera L.). Emirates Journal of Agricultural Sciences 15 (1): 17-35*

STATUS OF TISSUE CULTURE DATE PALMS IN NAMIBIA

P.F. de Wet (1), A. Ben Abdallah (2),
(1) National Project Director, Date Production Support Programme, Windhoek, Namibia. pieter.dewet@ndc.org.na
(2) Chief Technical Advisor, FAO/UN. Windhoek, Namibia. Ben.Abdallah@ndc.org.na

ABSTRACT

All the commercial cultivated date palms in Namibia derive from in vitro culture. Two main varieties (Barhee, Medjool) are present in the commercial plantations and it is envisaged that future expansion will be based on these two varieties. In general it can be stated that the palms produce satisfactorily and the quality of the dates are such that Namibia has been successful in entering the export market with the main varieties. The presence of a large number of date palm vitro plants that are in production, coming from various laboratories, give Namibia a unique opportunity to analyse any variability which could be attributed to the in vitro cultivation process. The analysis of the variability due to in vitro culture was started recently and concentrates on the two principal varieties. The first analysis concerning the Barhee variety derive from somatic embryogenesis produced at the laboratory of Date Palm Development in the United Kingdom (DPD) and consists of 8 blocks with a total of 2700 palms. The following was noted and are subject to further study:

- Variability noted on 4 aspects (Big size of leaves, Problem of fruit set, Restrictive growth, Strange shape of the palm)
- This variability is relatively small (3.2% only on the total of 2700 palms analyzed)

- The economic impact is relatively small.
- Variation to be subjected to further study to determine if it could be as a result of other factors (Physical factors such as soil, irrigation, hardening, management practices etc. as well as probable pest and disease effect on the palms)

Our analysis in progress on the Barhee and Medjool varieties will allow us, in the immediate future, to analyse and quantify the impact on production results. The origin of the vegetative material (laboratory, technique, genetic factor, physical factor or disease) influences the type of variability and initial results show the following:

- variability seems to be strongly related to the conditions of regeneration of the vitro plants (Mother material, technical protocol, personnel capacity, laboratory conditions, etc) and to a lesser degree to the technique (somatic embryogenesis or organogenesis)
- Possible origins of variability observed could also be related to the following factors:
  - Epigenetic variability (Phenotype abnormal of the plant but normal fruits) Physical factors (different type of stress, environment, climate, ground, water, fecundation, receptivity of the female flowers, and viability of pollen...)
- Diseases or pests.

INTRODUCTION

Publications dealing with the assessment of the relative variability experienced with date palm tissue culture plants are limited. It is generally accepted that variability with vitro plants could be generated during the;

i) callus formation process,

ii) generated by the use of relative high levels of growth hormones, and

iii) prolonged periods of material exposed to hormones.

It was however also found in a study, of numerous species multiplied through somatic embryogenesis, that no variability is noted (Cotton, Voo and al 1991; Soy, Komatsuda and coll. 1992).

With date palms certain authors noted that homogeneity was achieved with somatic embryogenesis vitroplants assessed through morphological, physiological and biochemical methods. (Alghamdi AlKhatani and, 1993).

Different publications are available relating to date palm somaclonal variation and especially the use of biochemical (Baaziz and Saaidi, 1988) and molecular biology techniques to detect the variation (RAPD, AFLP and Microsatellite: Ben Abdallah and Coll., 2000; Billotte and coll., 2004). It can however be noted that there is only limited information available where reference is made with regard to the behaviour of vitro date palm in the field.

It is deemed necessary to note that although it is theoretically regarded that organogenesis vitroplants should be less likely to have any variation it is also a reality that certain desired date varieties, such as Medjool, are not commercially available.

All the commercial cultivated date palms in Namibia derive from in vitro culture. Two main varieties (Barhee, Medjool) are present in the commercial plantations and it is envisaged that future expansion will be based on this two varieties. In general it can be stated that the palms produce satisfactory and the quality of the dates were such that Namibia was successful to enter the export market with the main varieties. The presence of a large number of date palm vitro plants that are in production, coming from various laboratories, give Namibia an unique opportunity to analysis any variability which could be attributed to the in vitro cultivation process.

The analysis of the variability due to in vitro culture was started recently and concentrates on the two principal varieties. These plants coming from different tissue culture laboratories were hardened and planted at three (3) pro-

ject sites. The date palms were acquired from 5 different laboratories, 3 laboratories using somatic embryogenesis and 2 laboratories using organogenesis as production technique. Since 1994 more than 17 000 vitro date palm were imported to Namibia. This situation presents us with an ideal opportunity to evaluate the performance of the palms in the field and study the effect of tissue culture techniques on the date palm variability.

Our aim is to identify and quantify this variability and to evaluate its impact on the production of the palms. It is also important that on field investigation take note of all factors that could influence the growth and production of the palms (Climatic, environmental, pest/diseases, etc).

The first analysis concerning the Barhee variety was done recently and the analysis concerning Medjool is currently in progress.

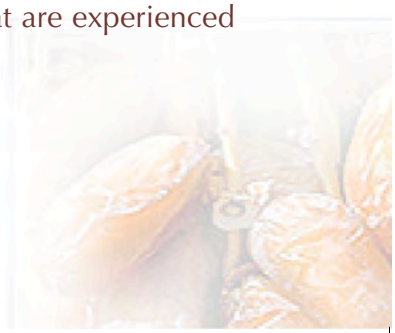
INVESTIGATION

The Naute project is situated in the Karas region ± 50 kilometres south-west of Keetmanshoop. The development started during 1990 and 9 840 tissue culture date palms have been planted to date at the plantation. The plantation consist of 2 700 Barhee date palms, 4 180 Medjool date palms while the remainder (2 960) consist of various other date varieties that were initially planted to evaluate their performance under local conditions.

The Eersbegin project is situated in the Kunene region ± 90 kilometres north-west of Khorixas. Development was initiated during 1987 with the planting of local date clones. It was later realised that the clones will not produce as expected and the plantation was gradually replaced with Medjool date palms. The plantation currently consists of 3 400 tissue culture date palms of mainly the Medjool and Barhee varieties.

The Aussenkehr project is situated in the Karas region ± 50 kilometers north-west of Noordoewer next to the Orange River. Development started during 1997 with the planting of date palm offshoots on 5 hectare and 2 200 Medjool tissue culture date palms was planted thereafter.

The first action is based on identifying all date palms that show variation in respect of growth and production. The follow up action will be to identify the possible reason/s that contributes to the variations that are experienced for each identified date palm.



The first detailed evaluation of the tissue culture date palms was done on the Barhee variety at the Naute project. The Barhee dates are planted in 2 plots (Plot 1 and Plot 5) and each plot consist of 4 blocks. To analyze the date palms, a morphological study was carried out on a total of 2 700 Barhee palms. A similar analysis is currently in progress for 4 180 Medjool palms at the Naute plantation (Plot 3, 4, 6 & 7) as well as for the Medjool palms at Eersbegin and Aussenkehr

RESULTS

The analysis of the variability due to in vitro culture was started recently and concentrates on the two principal varieties. The first analysis concerning the Barhee variety derives from somatic embryogenesis consist of 4 blocks with a total of 2 700 palms.

- The results of date palms on the first plot (P1) showed that only 65 palms are abnormal among a total of 1 200 palms and the abnormality rate is thus 5,4%.
- The results of date palms on plot five (P5) show that only 22 palms of the 1500 palms does show abnormalities and the abnormality rate is thus 1,4%.
- Average abnormality rate for the 2 700 palms is thus 3,2%

The variability was noted on 4 aspects regarding the date palms analyzed (Fig 4, 5, 6 & 7):

1. The first observation was on the very low level or no fruit set (Fig 4). A low fecundation was noted and virtually all of the fruits are parthenocarpic. This observation is also associated to variation with regards to the shape of the trunk of the palm that is much thicker than normal.
2. The second aspect of variability observed was on the abnormal size of leaves and a general reduction of fruit production we do however still have some bunches with normal fruit and others with problems of late maturation.
3. The third aspect of abnormality observed was the strange shape of the palm without real production.

The analysis on the Medjool dates indicated a fourth variation. (Variation not observed in the Barhee date palms)

4. The fourth aspect was the restrictive growth and all the vitro plants coming from the same laboratory showed many growth problems compared with the other plants coming from other laboratories. (This phenomena is a real problem and the financial consequences can be huge)

The results of the Barhee and Medjool analysis in progress showed that the behaviour of tissue culture plant material can be closely related to the production laboratory (L). We can easily distinguish between the laboratories and the initial observation can be summarized as follow:

- L1 (Somatic embryogenesis) Variability is very small – Less than 2%
- L2 (Somatic embryogenesis) Variability is relative small – Between 2% and 5%
- L3 (Somatic embryogenesis) Variability is relative high – Approximately 15%
- L4 (Organogenesis) Variability is very high (especially the restrictive growth)
- L5 (Organogenesis) Plants are still young but the variation observed is not high.

Variation was also detected between different batches of plants received from a specific laboratory.

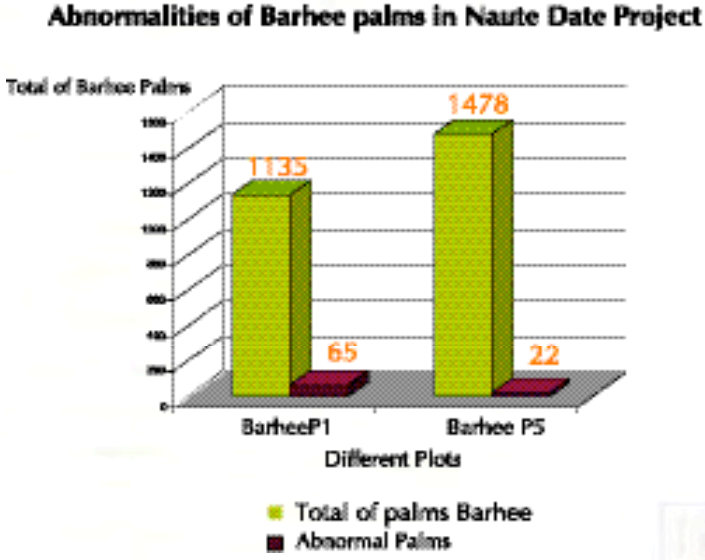


Fig 1: The number of Barhee abnormal palms in Naute Date Project (different plots)

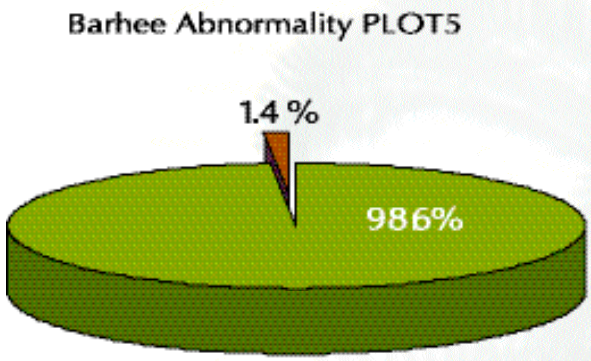


Fig 2: Percentage of Barhee abnormal palms in Naute Date Project (plot 5)

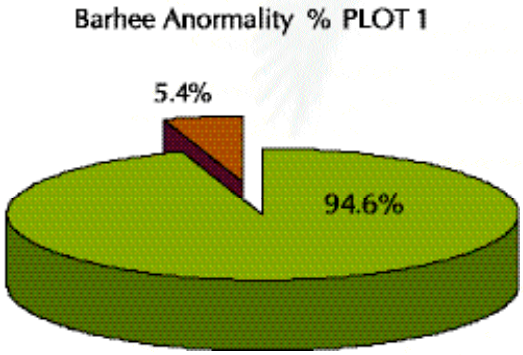


Fig 3: Percentage of Barhee abnormal palms in Naute Date Project (plot 1)

Fig 4: Problem experienced with fruit set (Low fecundation of Barhee at the Naute Date Project)

Fig 5: Abnormal size of leaves (Reduction of the production of Barhee at the Naute Date Project)

Fig 6: Strange Shape of the Palm

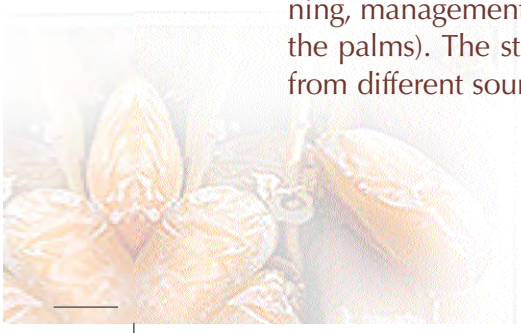
Fig 7: Restrictive growth

DISCUSSION

Our analysis on the Barhee and Medjool varieties will allow us in the immediate future to analyse and quantify the impact on production results. Only 87 palms of the 2700 Barhee palms showed variations and this comprises only 3.2% of the Barhee plantation. This variability is relative small and its economic impact is also small. The origin of the vegetative material (laboratory, technique, genetic factor, physical factor or disease) influences the variability and initial results show the following:

- Possible origins of variability observed could also be related to the following factors:
  - Epigenetic variability: - Only the phenotype is affected and the possibility exists that normal fruit production could be achieved if more care is taken during pollination and during the bunch management phase
  - Physical factors - Different type of stress, environment, climate, ground, water, fecundation, receptivity of the female flowers, and viability of pollen can affect the normal growth and production of the date palm.
    * It will be important to verify if the fecundation problems experienced are not due to mistakes made with the pollination of the flowers
    * It was also observed that two generations of flowers were produced by the palms with fertility problems. (Relatively big time gap between the two batches of flowers) The phenomena indicate a strong possibility that it has been as a result of physical factors.
    * Diseases or pests that could negatively influence the growth and / or production of the date palm.
- Variability seems to be strongly related to the conditions of regeneration of the vitro plants (Mother material, technical protocol, personnel capacity, laboratory conditions, etc) and to a lesser degree to the technique (somatic embryogenesis or organogenesis). Our initial conclusion indicates that the variability is related more to the specific production laboratory than to the techniques used.

Variations identified will be subjected to further study to determine if it could be as a result of other factors (Physical factors such as soil, irrigation, hardening, management practices etc. as well as probable pest and disease effect on the palms). The study will enable us to quantify the impact of plant material from different sources on the production results of a specific plantation.



BIBLIOGRAPHY

ALGHAMDI A.S., ALKAHTANI M.S., 1993. True to type date palm (Phoenix dactylifera L.) produce through tissue culture technique flowereing and fruit set. In: Al Hoseiny M.P., Ed. Abstract of the third symposium on date palm. Riadh: King Faisal University. Al-Ahsa Press: 58-59.

BAAZIZ M. & SAAIDI M., 1988. Preliminary identification of date palm culti - vars by esterase isoenzymes and peroxidases activities. Can J Bot. 66: 89-93.

BEN ABDALLAH A., STITI K., LEPOIVRE P. & DU JARDIN P., 2000. Identification de cultivars du palmier dattier (Phœnix dactylifera L.) par l'ampli - fication aléatoire d'ADN (RAPD). Cahiers Agricultures 9: 103-107.

Billotte N., and coll., 2004. Nuclear microsatellite markers for date palm (Phœnix dactylifera L.): Characterization and utility across the genus phoenix and in other genera. Molecular Ecology Notes 4: 256-258.

KOMATSUDA T., LEE W. & OKA S., 1992. Maturation and germination of somatic embryos as affected by sucrose and plant growth regulators in soy beans (Glycine gracilis skvortz and glucine max) (L.) Merr. Plant Cell, Tissue and Organ Culture 28: 103-113.

VOO K.S., RUGH C.L. & KAMALAY J.C., 1991. Indirected somatic embryoge - nesis and plant recovery from cotton (Gossypium hirsutun L.). In Vitro Cell. Dev. Biol. 27 : 117-124.

IN VITRO PROPAGATION OF DATE PALM

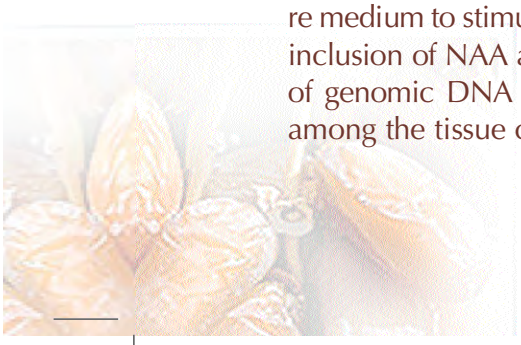
Mobasher S. Omar and Mohammad K. Hameed
Genetic Engineering and Biotechnology Center,
Ministry of Science and Technology, Baghdad, Iraq.

ABSTRACT

In order to improve the technique of the vegetative propagation of date palm through tissue culture, different explants were excised from 3-4 years old offshoots of six cultivars. Nutrient media for each stage of propagation was standardized. Different concentrations of various growth regulators were tested.

Three major methods for propagation of date palm were achieved. These methods included callus initiation from cultured shoot tips and lateral buds, followed by induction of somatic embryogenesis, and their subsequent development into complete plants. It was also possible to stimulate transformation of some cells at the cut base of the cultured shoot tips into meristemoids, which subsequently developed into a cluster of adventitious buds. Such buds were developed into shoots, which were further rooted to produce complete plants. The third method of propagation based on the stimulation of growth of pre-existing bud primordia in the leaf axils of cultured shoot tips. Such enhancement of axillary branching resulted in the formation of shoots, which were rooted individually.

The results also revealed that the various cultivars showed different responses to the propagation methods. Low concentration of GA3 was included in the culture medium to stimulate elongation of shoots, while best rooting was achieved by inclusion of NAA and relatively elevated sucrose concentration. Finger printing of genomic DNA conducted in this investigation confirmed the resemblance among the tissue culture-derived seedlings as well as with their parents.



INTRODUCTION

The date palm (Phoenix dactylifera) is considered as one of the most ancient plant that was cultivated in the Mesopotamia some 4000 years ago (Albakr, 1972). The high nutritional value of dates makes it one of the most important souse for human nutrition, and it comprises a constant source of the national income of Iraq. The date palm could be propagated either sexually by seeds or vegetatively by offshoots. The first method is used for production of large number of offshoots, regardless of their sex, cultivar or fruit quality. However seed propagation is not usually used since many disadvantages are usually associated with it. The number of male tree is often equivalent to the number of female trees among those derived from the seeds, which is unwanted practice in date palm orchards. Propagation by offshoots, on the other hand, is always preferred since the resultant palms are true to type. However still other problems are associated with propagation by offshoots, such as the low number of new offshoots

that are produced from each palm , especially in high quality cultivars, as well as the long period in which the offshoot must remain attached to its mother to develop a good root system before it could be detached, in addition to the high percentage of mortality

if detached with a poor root system (Shabana and Alshariri, 2000).Therefore, the most serious obstacle that hampers commercial expansion in date palm cultivation relies in the slow method of vegetative propagation. It is therefore necessary to develop a new method for clonal propagation of date palm that maintains the cultivar characteristics. Plant tissue culture technology may help in solving such a problem. Application of this technique to date palm propagation may have several advantages since it will reduce the dependence on the offshoots which are produced during a specific period of a palm life span. The resultant plants are usually disease and pathogen – free, so they could be used for local cultivation as well as exportation. The continuous decrease in date palms as a result of infectious diseases, reduced cultivated areas, difficult field management processes, in addition to the slow growth rate of date palms are factors that confirm the need for application of tissue culture technology for in vitro propagation of date palm.

MATERIALS AND METHODS

To execute a complete program for vegetative propagation of date palm by tissue culture technique, it was necessary to determine the optimal conditions throughout the program. Three- four years old offshoots of seven commercial cultivars were selected. The leaves were removed from the offshoots, and available buds were individually collected. The shoot tip was finally removed with part of the mantle meristem its future growth. Due to the presence of phenolic compounds usually involved with date palm tissue culture, tee shoot tips and lateral buds were placed in a cooled antioxidant solution to prevent browning. Surface sterilization was then carried out through immersion in a disinfestant, coupled with continuous agitation to assure the distribution and penetration of the disinfestant through the explants to control all bacteria, fungi and other microbes. The explants were then thoroughly washed with sterile deionized water to get rid of the harmful effect of the disinfestant.

All subsequent steps were carried out under sterile conditions of a laminar air flow hood. The explants were transferred to culture vessels containing nutrient medium of Murashige and Skoog (1962) inorganic salts, sucrose and other medium components. The effects of different growth regulators, vitamins and amino acids, as well as the physical conditions of culture medium on growth and development of the cultured explants were investigated. All practices of media preparation, sterilization, pH adjustment and dispensing were strictly followed throughout the investigation. Incubation conditions of cultured explants, including light and temperature regimes, varied according to the objective of each experiment.

The genetic stability of the tissue culture – derived plants was determined through the randomly amplified polymorphic DNA technique. This part was conducted in the Agricultural Genetic Engineering Research Institute (AGERI) in 2001, during a scientific visit of the senior author to this center.

RESULTS AND DISCUSSION

The results showed that the date palm could be propagated by three major methods. The first method included the formation of asexual embryos in cultures supplemented with high auxins. Callus initiation was induced on the cut basis of the cultured explants. Each callus cell had the potentiality to

develop into an embryo, and subsequently a complete plant if the required nutritional and incubation conditions are available. Callus

initiation was observed on the cut margins of the explants following 4-6 months, which included a single transfer to a fresh medium of the same composition. It was observed that the optimal nutrient medium suitable for callus initiation and growth contained a high auxin and low cytokinin concentrations, especially in the presence of activated charcoal. This usually adsorbs the toxic phenolic compounds excreted by cultured explants, but it also adsorbs the growth regulators alike (Omar, 1988a).

Callus induction ability of the explants varied among the varieties under investigation. The Maktoom cultivar for example, showed callus initiation within 4 months, while it took 6 months in Barhi. Callus color and texture also varied among cultivars. Some produced white nodular calluse, while others produced yellow callus. It was also observed that callus initiation was induced under dark as compared to the light condition. The callus was further propagated by dividing into almost 4 equal parts and subcultured onto fresh medium of the same composition and incubated under the same condition. When the desired amounts of calli were obtained, it was subdivided and cultured onto fresh medium tacking some of the original hormones and incubed under a 16 hrs of 1000 lux followed by 8 hr dark period. Asexual embryos formed after 3-4 weeks of incubation under the previous conditions. The present combination of nutrient medium and incubation conditions apparently stimulated the transformation of cultured cells from the differentiated into the meristimatics state, the redifferentiated into embryonic cell with genetic code identical with the " mother" plant from which it was originally isolated. The number of asexuals embryo which could be obtained from each tube depended on the amount of cultured callus.

The embryos commenced growth, where leaves developed following 4 weeks, and roots were also developed gradually from most of the cultured embryos. It was now possible to isolate individual shoots and re-culture them on a new medium to support further root growth to plants suitable for acclimization and transfer to greenhouse (Fig.1). It was also possible to excise the shoot tip from the newly formed plants and reculture onto fresh medium to induce vegetative multiplication for further plant production. These results confirm previous observations by several authors regarding initiation and development of asexual embryos in date palms (Omar, 1988b; Tisserat, 1982; Jassim, 1999).

Fig 1. Regeneration of date palm through asexual embryogenesis.

The second method of propagation based on the enhancement of axillary branching in the cultured shoot tips. Following 6-8 months of incubation of cultured shoot tips, the bud primordial which exists in the axils of leaf primoridia within the shoots tip, starts to grow and develops into new shoots (Fig 2). The process of stimulation of axillary branching depends on the combination of nutrient medium and the presence of specific concentration of certain growth regulators. The numbers of newly formed shoots from each shoot tip ranges from 4-8 shoot depending on the cultivars. Following

2-3 additional months, it was possible to isolated the shoot tips from the newly formed shoots, and reculture for a second cycle for further proliferation of shoots, and thus it was possible to produce another 4-8 new shoots. This process can be repeated as much as needed, i.e. the number of new shoots could be duplicated every 2-3 months (4, 16, 64, 256, 1024 etc.). When the desired of shoots is reached, transferring to culture medium supplemented with specific concentration of auxin to induce initiation and development of roots, and subsequently in production to complete plant with good root and systems suitable for acclimization. These results are in agreement with other investigators (Bakheet and Saker, 1998; Al-Maarri and Al-Ghamdi, 1995).

Fig 2. Enhanced axillary branching and rooting.

The third method of propagation based on the production of a base of adventitious buds on the cultured shoot tips. By this technique , it was possible to stimulate some superficial cells to re-differentiate into vegetative buds through inclusion of specific growth regulators, vitamins and amino acids in the culture medium necessary for the

Fig. 3. Initiation of buds and subsequent elongation.

process of redifferentiation (Fig. 3). These buds can be further propagated to produce a large base of buds. The number of newly initiated buds ranges between 4-8 buds, according to cultivar. The highest number reached 32 buds in Maktoom cultivar. In this technique, the number of new buds can be duplicated exponentially as described previously. When the desired number of buds is reached, another stage was required, namely elongation stage, to produce shoots, which were further rooted and acclimatized. The phenomenon of adventitious bud formation is common in date palms (Abjman, 1999; Albogrfawi and Anjran, 1999; Omar et al. 1992).

There are several advantages and disadvantages associated with each method of propagation, regarding the number of plants that could be produced, as well as the genetic stability of such plants. In general, several methods of propagation were combined to achieve the goal. Another step was added to the production program which included the DNA finger print of the resulted plants, which confirmed the genetic stability among the regenerated plants, as well as their parents (Fig. 4).

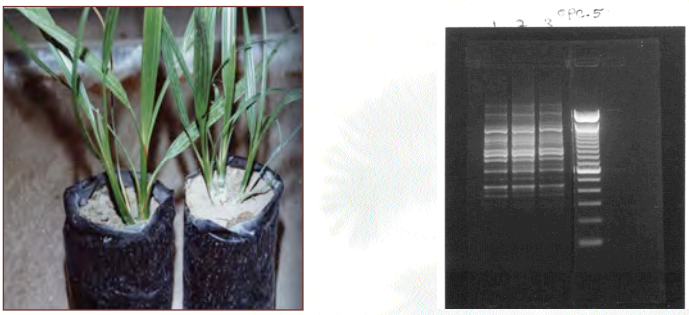


Fig. 4. Tissue culture derived plants from different methods and DNA finger prints.

Experiment conducted in this investigation revealed the response of different date palm cultivar for production of new plant by these methods. More research is directed towards the improvement of such techniques to increase its efficiency, as well as introduction of new cultivars.

The final step of the program included the transfer of the regenerated plants to the nursery. The tissue culture derived plants were acclimatized through a series of step for gradual adaptation to natural conditions and transfer from the heterotrophic to the autotrophic condition more than 10,000 plants were produced from different varieties by such techniques and successfully established in the nursery (Fig. 5), as well as the establishment of stock cultures sufficient for production of 1 million plant/ year in the near future.

Fig 6. Tissue culture derived date palms well established in the orchard.

COST-BENEFIT ANALYSIS:

Use of tissue culture technology in mass propagation of date palm could actively contribute to the national economy. Several companies around the world have started investments in such activities. According to cost analysis study conducted in our center during the year 2000, the cost of production of each plant of date palm equals to 25 cents. There was negotiation to sell date palm plants to Jordanian company at a rate of 10 USD/plant, however, the negotiations were stopped due to the war circumstances. It is worth to mention here that Jordan imported date palm plants from France at a rate of 20-40 USD/plant during the years 1996-2002 from France to sell them later on to the Emirates and the Gulf at 75 USD/plant, and this visualize the extent of marketing in Jordan and the Gulf area.

The production of date palm plants at an economical rate will start at the fourth or the fifth year, since it is very important to establish a good base of stock cultures during the first two years that could be used for the large scale production. In other words, its essential to establish 40-50 k stock cultures for the production of the first 3000 plants at the fourth year, followed by 10000 plants in the fifth year and so on. The work will proceed in two directions simultaneously, i.e. propagation of stock cultures and regeneration of plants to reach the final goal of annual production of 25000 plants.

In order to illustrate the economical feasibility of the project, one may consider the production of 10000 plants/year as an example, and selling price at 10 USD/plant. This means a total of 100000 USD gross from date palm. A total cost of production of 10000 plants at a rate of 25 cents/plant equals 2500 USD. This implies that a net profit of 97,500 USD will be gained. This profit will increase in the future with increased production.

The techniques described in this investigation will contribute for the production of a large number of elite palms in a relatively short period of time. It will help in creation of new job opportunities for younger generations and reduces the gap between developing and developed countries.

LITERATURE CITED

1. *Al-Maarri, K.W. and Al-Ghamdi, A.S. 1995. Effect of culturing date on in vitro micropropagation of date palm (Phoenix dactylifera L.) CV Hilaly J. Agric. Sci., Ain Shams Univ. 3:151-167.*
2. *Abjman, A. 1999. Rapid propagation of date palm through floral tissue. Proc. Int,l Conf. Date Palm. Nov. 1999. Asuit Univ. Egypt. Pp 385-388.*
3. *Al- Bakr, A. J. 1972. Date plam, Past, Present and New Trends in Its Industry and Commers. Al – Ani Press, Baghdad, Iraq.*
4. *Albougrfawi, M. and Anjran, M. 1999. Rapid propagation of date palm through vegetative budding in offshoot tissues. Ibid, Pp: 389-392.*
5. *Bakheet, A. S. and Saker, M.M. 1998. In vitro propagation of Egyptian date palm. II. Direct and indirect shoot proliferation from shoot tip explants of Phoenix dactylifera L. cv.Zaghlool. In: 1st Int.Conf.Date Palm, March 1998, Al-Ain, UAE, p 28.*
6. *Jasim, A.M. 1999. Response of different date palm cultivars (Phoenix dacty - lifera L.) to in vitro culture. Basrah J. Agric. Sci. 12: 9-17.*
7. *Murashige, T. and F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 437-497.*
8. *Omar, M.S. 1988a. Callus initiation, asexual embryogenesis and plant rege - neration in Phoenix dactylifera L. Date Palm J. 6:265-275.*
9. *Omar, M.S. 1988b. In vitro response of various date palm explants. Date Palm J. 6:371-389.*
10. *Omar, M.S., Hameed, M.K. and Al-Rawi, M.S. 1992. Micropropagation of date palm (Phoenix dactylifera L.). In: Y.P.S. Bajaj (Ed.): Biotechnology in Agriculture and Forestry, Vol. 18: High Tech and Micro propagation II. Springer-Verlag, Berlin, Heidelberg. Pp 471-492.*
11. *Shabana, H. A. and Al- Sharifi, R. M. 2000. Palms and Date Production in The UAE. Ministry Agric. & Fish. UAE.*
12. *Tisserat, B. 1982. Factors involved in the production of plantlets from date palm cultures. Euphytica 31:201-214.*

AFLP VARIATION IN TISSUE CULTURE-DERIVED DATE PALM PLANTS

H.H.S. Al Kaabi [1], A. Zaid [2] and C. Ainsworth [3]

(1) National Project Director, Date Palm Research & Development Program, UAE University, P.O.Box. 81908, Al Ain, UAE. Tel: +971 3 7832334, Fax: +971 3 7832472. E-mail: helalk@uaeu.ac.ae

(2) Chief Technical Advisor, Date Palm Research & Development Program, UAE University - United Nations Development Program, P.O.Box. 81908, Al Ain, UAE. Tel: +971 3 7832334, Fax: +971 3 7832472. E-mail: zaid@uaeu.ac.ae

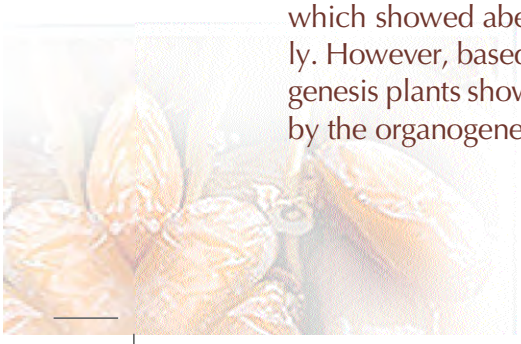
(3) Dept. of Agricultural Sciences (MCR), Imperial College at Wye, Wye - Ashford, Kent, TN 25 5AH, UK. Tel: +44 7815 731 198,Fax: +44 2075 942 64, E-mail: c.ainsworth@imperial.ac.uk

ABSTRACT

ITwo tissue culture methods, involving organogenesis or embryogenesis, were compared when applied to ten United Arab Emirates date palm varieties (Phoenix dactylifera L.). The frequency of somaclonal variation in the resultant plants was compared and related to the levels of variation at the DNA level, estimated by AFLP analysis (Amplified Fragment Length Polymorphism).

The incidence of somaclonal variation in plants regenerated through organogenesis tissue culture was low whilst a survey of embryogenesis-derived trees in the field identified a relatively high level of abnormalities.

The experimental conditions for the generation of reproducible AFLP markers were optimised using EcoRI and MseI primers. All ten date palm varieties were able to be distinguished by AFLP fingerprinting. Variability amongst 40 plants produced by organogenesis and embryogenesis, based on numbers of plants which showed aberrant patterns, was found to be 5 % and 12.8 %, respectively. However, based on the total numbers of variant DNA fragments, the embryogenesis plants showed a much higher level of variability (0.6 %) than that shown by the organogenesis plants (0.038 %).



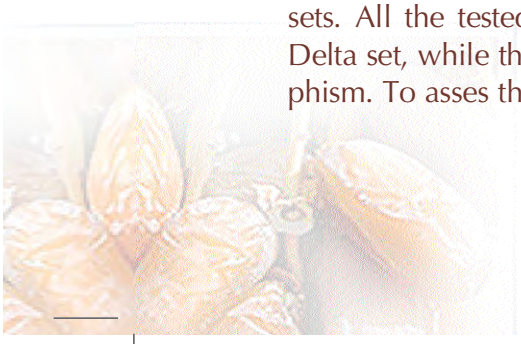
AGERI EXPERIENCE WITH DATE PALM MOLECULAR FINGERPRINTING TECHNIQUES.

Sami S. Adawy,[1] Ebtissam H.A. Hussein,[1'2] Mahmoud, M. Saker[3], Dina El-Khishin[1], Amina A.Mohamed[1] and Hanaiya, A. El-Itriby[1]

1. Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.
2. Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.
3. Plant Cell and Tissue Culture Dept., Genetic Engineering and Biotechnology Division, NRC, Cairo, Egypt.

ABSTRACT

Date palm (phoenix dactylifera L.) is one of the most important fruit crops in North Africa including Egypt and in Middle East. Egypt lies in the fruit largest producer among Arab countries. However, little is currently known about the molecular characterization of date palm cultivars. Determination of genetic variability and proper cultivar identification in date palm would be of major importance in improvement programs and in germplasm characterization and conservation to control genetic erosion. In an attempt to determine a molecular fingerprint characterizing each of the Egyptian date palm cultivars, three types of PCR based markers, i.e., RAPD, ISSR and AFLP's were applied on two sets of five cultivars cultivated in two different regions, Delta and Upper Egypt .Intravarietal variations were investigated using ten random decamer primers on seven to ten individual trees representing each of the five cultivars of the two sets. All the tested primers exhibited intravarietal polymorphism among the Delta set, while the Upper Egypt set revealed negligible intravarietal polymorphism. To asses the genetic polymorphism and to develop fingerprint for each



of these cultivars RAPD,ISSR and AFLP analysis were conducted on bulked DNA samples composed of the DNA of the different trees representing each cultivar. Fingerprinting of the Delta cultivars (Zaghloul, Samany, Hayani, Siwi and Amhat) was conducted using 8 ISSR and 6 AFLP primer /primers combinations. This revealed a total of 53 and 433 amplicons, respectively and a level of polymorphism of 64.1% and 53.81%, respectively. DNA profiling of the five Upper Egypt cultivars (Bertmoda, Gandila, Malikaby, Shameia and Sakkoty) was carried using 41 RAPD, 19 ISSR and 28 AFLP primer/primers combinations, Thus exhibiting 259, 159 and 1135 amplicons representing a level of polymorphism of 18.9%, 34.6% and 41.6%, respectively. The genetic similarity matrices were estimated for the two sets and used to develop dendrograms revealing the genetic relationships. Moreover, unique markers characterizing each cultivar were identified. Furthermore, the genetic stability of tissue culture derived plants from cultivars Zaghloul (Delta) Bertmoda, Gandila, and Sakkoty (Upper Egypt) were studied using RAPD and AFLP's. The DNA profiles exhibited non significant polymorphism indicating the true to type nature of these plants.

Additional Index Words: RAPD; AFLP; ISSR; Fingerprinting; Genetic relationships; Genetic stability.

INTRODUCTION

Date palm (Phoenix dactylifera L.) is one of the most important fruit crops in North Africa including Egypt and in Middle East. In Egypt, it is a source of income to Oases inhabitants. In addition to the dates high nutritive value, it provides protection to under –crops from the harshness of the climate and reduces the damage from sand storms and wind erosion. It is cultivated for food .fuel, shelter and fiber. It is a dioecious perennial monocotyledon diploid (2n=36), vegetatively propagated from offshoots. There are 3 main types of dates based on fruit moisture content i.e., Soft, semi-dry, and dry cultivars. Little is currently known about the molecular characterization of date palm cultivars. The improvement of date palm varieties has not been approached using molecular breeding tools, although in vitro propagation of selected varieties is beginning to play an important role in the breeding process. Most of this effort has been directed toward a program to identify resistance to Bayoud, a fungal disease caused by Fusarium oxysporum Schlechtend Fr. Sp. albedinis. The use of Polymerase Chain Reaction (PCR) procedures as a molecular screening tool has yielded a number of strategies for the identification of economically important traits in various crops. DNA marker analysis techniques such as RAPD, ISSR and AFLP have all been successfully employed for the selection of parents for conventional breeding and hybridization programs. However, the recovery of high-quality DNA represents one of the limiting steps in utilizing PCR-based molecular marker technology. Determination of genetic variability and proper cultivar identification in date palm would be of major importance in improvement programs and in germplasm characterization and conservation to control genetic erosion. The objectives of this study were to produce a catalogue revealing the DNA profiling of the Egyptian Date palm cultivars. To investigate the genetic variation at the intra and intervarietals levels in Egyptian date palm. To assess the genetic relationships among the Egyptian date palm cultivars. To identify unique markers characterizing each cultivar. To evaluate the genetic stability of tissue culture derived plantletes. This will have its impact in germplasm collection preservation date palm breeding and improvement programs.

MATERIALS AND METHODS

Plant material

Plant material was obtained from the Egyptian Ministry of Agriculture experiment station at Aswan governorate. The date palm cultivars studied were five,

i.e., Bertmoda, Gandila, Shameia, Malikaby and Sakkoty and other five Delta Egypt date palm i.e. Zaghloul, Samany, Hayany, Siwi and Amhat.

DNA extraction

Total DNA was extracted from young leaves of adult trees for RAPD ISSR and AFLP analyses following the method described by Porebski et al. (1997). DNA quantitation was performed by comparison of (Gibco BRL) used as standards 1 Kb and 1Kb plus DNA Ladder.

RAPD analysis

RAPD was performed using 41 and 15 ten-mer oligonucleotide primers as described by Williams et al., (1990) with few modifications in Upper and Delta Egypt date palm respectively.. PCR reactions were carried out in 25 ul volumes containing 25 ng of total genomic DNA, 20 pmoles of primer, 100 mM of each dNTP, 2 mM MgCl2, 1x PCR buffer and 0.4 ul Ampli Taq Polymerase (RTS Taq DNA polymerase). Amplification was performed in a Perkin Elmer Cetus Thermal cycler 9600 with the following

program: 94 C for 5 min, 94 C for 1 min, 36 C for 1 min, 72 C for 90 sec for 40 cycles. A final extension cycle was performed at 72 C for 7 min. The PCR products were electrophoresed on 1.4% ethidium bromide- stained agarose gels and visualized with a UV transilluminator.

ISSR analysis

Fingerprinting using ISSR was carried out as described by Adawy et al. (2002a) and Hussein et al. (2003). Nineteen and seven oligonucleotides composed of defined, short tandem repeat sequences with or without anchor and representing different microsatellites have been used as genetic primers in PCR amplifications in Upper and Delta Egypt date palm cultivars. PCR was performed in 25 ml reaction volume containing 1x PCR buffer, 2 mM MgCl2, 0.2 mM of each dNTP, 1 mM primer, 25ng genomic DNA and 1 unit hot start Taq DNA polymerase (Qiagen). Amplification reactions were subjected to a thermocycling profile composed of an initial hot start and denaturation step at 94C for 10 min, followed by 40 cycles of 1 min denaturation at 94 C, 1 min annealing at (60C to 46 C)), 2 min extension at 72 C and a final extension step of 10 min at 72C . The PCR products were separated on 2% agarose gels and /or 8% polyacrylamide gels, stained with ethidium bromide and photographed.

AFLP analysis

AFLP method was carried out as described by Vos et al., (1995) using the GIBCO BRL AFLP analysis system1, and the AFLP starter primer Kit (Cat No. 10544-013 and 10483-014, respectively). Primers with 3 selective nucleotides comprised 8 Mse 1 primers (CAA/CAC/ CAG/CAT/ CTA/ CTC/ CTG/CTT) and 7 EcoR1 primers (AAC/ACA/ACC/ACG/ACT/AGC/AGG) for a total of 28 primer combinations were employed in Upper Egypt date palm while 6 primer combinations were employed in delta region. To determine the size of the AFLP fragments (100-2000bp range),100bp DNA ladder Level of from GIBCO BRL was used.

Data analysis

Only distinct reproducible, well-resolved fragments were scored as present (1) or absent (0). The genetic similarity between different pairs of cultivars was estimated according to Dice coefficient (Sneath and Sokal, 1973). A dendrogram was constructed for each type of molecular markers using the unweighted pair group arithmetic average (UPGMA) method. Levels of diversity were estimated at the percentage of polymorphic bands out of the total scored.

RESULTS AND DISCUSSION

Due to the dioceous nature of date palm trees, intra-varietal variations are expected. Therefore, a preliminary study was carried out to investigate intra and inter-varietal variations among the ten date palm cultivars using the selected primers in RAPD reactions with sixteen samples from each cultivar. Polymorphism was detected among individuals within the same cultivar. From the sixteen DNA samples examined in each cultivar, ten samples were chosen which gave consistent and reproducible results with the fifteen primers.

Polymorphism among date palm genotypes as revealed by molecular markers

1 - Intravarietal polymorphism.

Intravarietal polymorphism has been investigated among 5 date palm cultivars from the Delta region and 5 other cultivars from Upper Egypt, Using RAPD markers.

To estimate the intravarietal polymorphism, DNA samples from seven individual trees representing each of the five cultivars (Malikaby, Sakkoty, Gandila, Shameia and Bertmoda) were subjected to RAPD analysis using 12 primers.

The amplification profiles of the seven samples of each cultivar were monomorphic and none of the studied primers revealed intravarietal polymorphism. These results are contradictory to those previously obtained by the same authors on date palm cultivars from the Delta region of Egypt (Adawy et al., 2002a).They reported that all the tested primers exhibited intravarietal polymorphism. This discrepancy suggests that the date palm cultivars from Upper Egypt are genetically more homogenous than those from Delta (Fig. 1 & 2) .

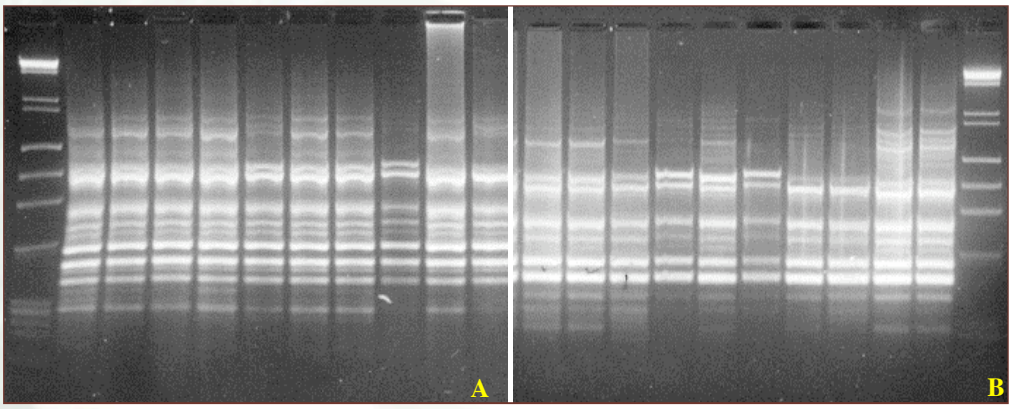


Fig. (1): RAPD profiles for date palm cultivars using different primers OP B20, (A) Hayany, (B), M Lambda Hind III digest, Phi x 174, Hae II digest

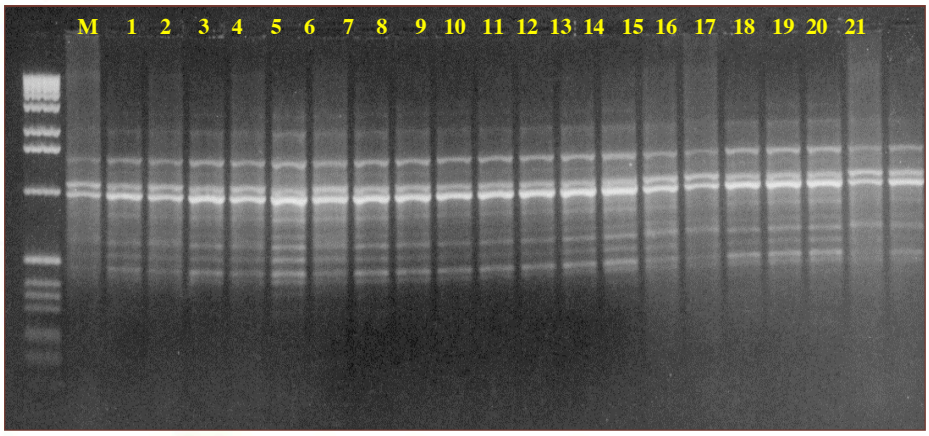


Fig. (2): RAPD profiles of the date palm cultivars 7 individual trees of each of the cultivars Malikaby (lanes 1-7), Sakkoty (lanes 8-14) and Shameia (lanes 15-21) as amplified by (OP) B11 (a). M. 1Kb plus DNA ladder.

2 - Intervarietal polymorphism among the five Upper Egypt date palm.

To investigate intervarietal polymorphism among the five Upper Egypt date palm cultivars, RAPD, ISSR and AFLP analysis have been conducted on bulked DNA samples composed of 7-10 trees representing each cultivar. The five bulked samples were assayed using 41 RAPD primers (Fig. 3). All the tested primers generated reproducible and easily scorable RAPD profiles. These primers produced multiple band profiles with a number of amplified DNA fragments ranging from 2 to 13. Fingerprinting revealed a total number of 259 unambiguous DNA fragments with an average of 6.30 fragment/primer. The number of polymorphic bands ranged from 0 to 5 per primer with an average of 1.2 polymorphic bands per primer. The total number of polymorphic amplicons produced by the 41 primers was 49, thus, representing a level of polymorphism of 18.92% Table (1).

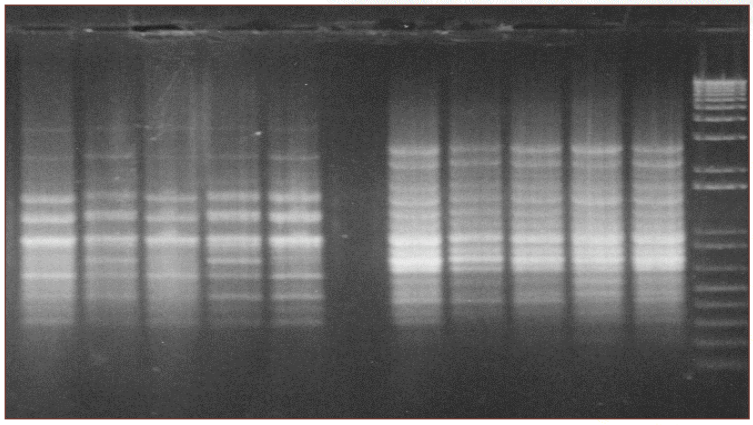


Fig. (3): Bulked samples representing the five date palm cultivars Malikaby (1), Gandila (2), Sakkoty (3), Shameia (4) and Bertmoda (5) as amplified by (OP) B3 and B4. M refers to DNA standards.

Table I: Level of polymorphism detected by 41 RAPD primer

primers	total no. primers	total no. of amplicons	polymorphic amplicons	polymorphism %
total	41	259	49	18..9
average	**6.3**		**1.2**	

The genetic similarity between cultivars was assessed on the basis of the Dice similarity coefficient and complemented with the UPGMA cluster analysis. Pairwise comparisons of RAPD profiles were resulted in a similarity matrix. The highest similarity value was between Gandila and Sakkoty (97.7). While the lowest value for this coefficient was found between Gandila and Bertmoda (93.1). The dendrogram clustered the five cultivars into two main clusters, where Gandila and Sakkoty constituted one cluster correlated with Malikaby while Shameia and Bertmoda formed the second cluster.

ISSR analysis

The ISSR analysis was performed on the bulked DNA samples representing the five cultivars using 19 ISSR primers composed of short tandem repeat sequences with or without anchor. Figure (4) illustrates the ISSR profiles generated by primers A9, A10, A mic 2 and A mic 4. Only one primer failed to produce scorable banding profiles. As shown in Table (2), a total of 159 amplicons were generated by the tested primers with an average number of 8.4 amplicons/primer. Primer A8 exhibited the highest number of fragments (16 amplicons), while primers Amic 8 revealed the least number (3 amplicons). The total number of polymorphic bands was 55 with an average of 2.9 polymorphic amplicons per primer. This represents a level of polymorphism of the results of the present study revealed that the polymorphism detected by the ISSR assay, although it was lower than that detected among other Egyptian date palm cultivars (Adawy et al., 2002a), however, it was higher than the polymorphism detected by the RAPD assay 34.6% .

Table 2: Level of polymorphism detected by 19 ISSR primer .

no. of primers	total no. of bands	polymorphic amplicons	% of polymorphism
19	159	55	34.59
average	8.4	2.9	

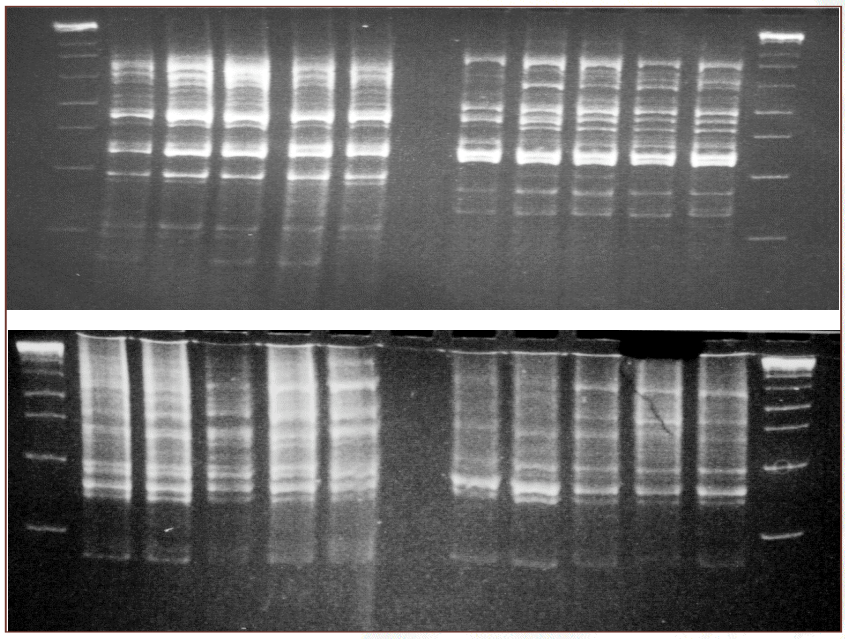


Fig. (4): ISSR profiles of the five date palm cultivars Malikaby (1), Sakkoty (2), Shameia (3), Gandila (4) and Bertmoda (5) as detected by different ISSR primers. A9 (a), A10 (b), Amic2 (c) and Amic4 (d). M. 1Kb plus DNA ladder.

The total number of unique ISSR markers was 31.The cultivar Malikaby was characterized by 3 positive and 2 negative markers. Gandila was distinguished by 2 positive and 3 negative markers. Sakkoty was characterized by 3 positive and 4 negative markers. Shameia revealed one positive and 7 unique negative markers. While Bertmoda exhibited 2 positive and 4 unique negative markers. In this context, Adawy et al., (2002a) were able to characterize four other Egyptian date palm cultivars by unique ISSR markers. The genetic distance estimates based on ISSRs ranged from 80.2% to 89.0%. The lowest similarity value was between Malikaby and Shameia. While, the highest value was between Shameia and Bertmoda. A dendrogram constructed by cluster analysis using ISSR based genetic distance. The overall tree topology suggested a rather weak grouping association except for the cultivars Shameia and Bertmoda which clustered together.

Combining RAPD and ISSR data

The two applied marker techniques (RAPD and ISSR) amplify different parts of the genome. This was partially reflected on the topology of the phyloge-

netic trees drawn from the data of the two assay. Therefore, to obtain more balanced values for genetic similarity among cultivars and an equilibrated dendrogram representation of the relationships among the five studied date palm cultivars, the data of RAPD and ISSR analysis were combined. As shown in Fig. (10), the relationships revealed by the combined data- based dendrogram were close to those revealed by the RAPD-based dendrogram. However, the three dendrograms confirmed the close relationship between Shameia and Bertmoda, which always clustered together. The three dendrograms also revealed the divergence of Malikaby which formed a separate group. Therefore, the observed different topology and reshuffling of the three cultivars Malikaby, Sakkoty and Gandila in the ISSR dendrogram could be attributed to the narrow genetic diversity among these cultivars. Nevertheless, the present study confirmed the usefulness of the two applied molecular marker types (RAPD and ISSR) in characterizing and fingerprinting date palm cultivars. This information will have great impact in date palm germplasm collection preservation and improvement programs.

AFLP Analysis

The technique of AFLP required initial optimization to identify primer combinations that yield reproducible and discernible patterns .Therefore, 40 primer combinations were initially tested with the five bulked DNA samples representing the five Upper Egypt date palm cultivars. Among the tested primer combinations, 28 EcoRI/MseI selective combinations yielded reproducible and discernible profiles. The rest of primer combinations did not yield any amplification. The 28 primer combinations produced 1135 bands, 41.59% being polymorphic across cultivars. The average number of polymorphic bands was 16.86 per AFLP primer combination (Table 3). The size of the AFLP amplified fragments ranged from 2600 bp to 50 bp (Fig. 5). The primer combination (5/5) showed the highest percentage of polymorphic bands (81.15%), while the lowest polymorphism (16.95%) was revealed by primer combination (8/1). The number of amplified bands per primer combination ranged between 17 and 69. The similarity matrices were used to generate a dendrogram using the UPGMA method, as shown in Fig. (10). The genetic similarity estimates ranged from 83.2% to 90.9%. The highest genetic similarity 90.9% was between Shameia and Sakkoty, this was followed by 90.2% between Sakkoty and Malikaby, while the lowest genetic similarity (83.2%) was detected between Gandila and Bertmoda. The dendrogram confirmed that the cultivar Bertmoda does not cluster with any other cultivar tested and is easily distinguishable, while the cultivars Gandila

and Malikaby were the most genetically similar among the studied cultivars, with Shameia and Sakkoty next.h an average of 40.5 bands.

Table 3: Level of polymorphism detected by 28 AFLP primer combinations.

no. of primers	total no. of bands	polymorphic amplicons	% of polymorphism
28	1135	472	41.59
average	40.5	16.9	

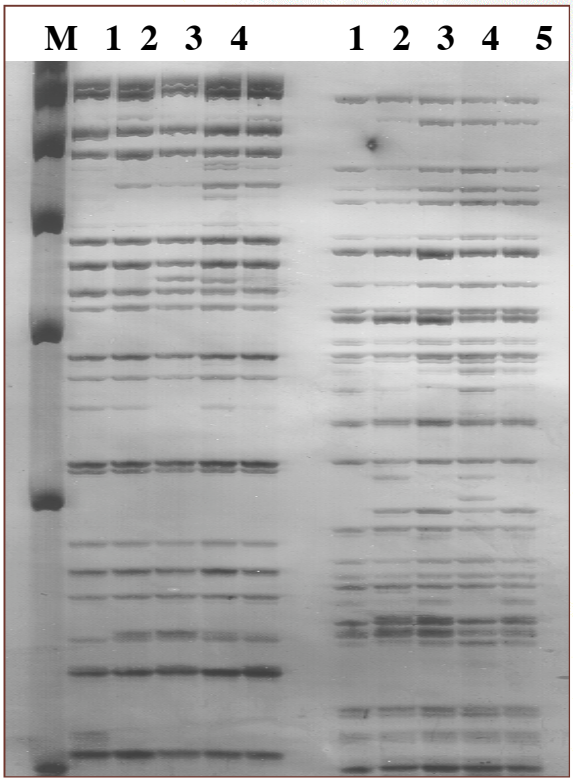


Fig. (5): AFLP profiles of the five date palm cultivars Malikaby, Gandila, Sakkoty, Shameia and Bertmoda as detected by primer combinations (A) 1/3, (B) 1/8, (C) 7/2, (D) 4/1 and (E) 3/7. M.DNA molecular weight standard (100 bp ladder).

Cultivar identification by unique markers

The markers are useful for cultivar identification and fingerprinting. As shown in (Table 4), the total number of unique bands across the five cultivars was 58 positive (UPM) and 133 negative markers (UNM) in the different cultivars .The number of UPM ranged from 6 to 22 and the number of UNM ranged from 15 to 57 in the different cultivars.

Table 4 : Unique positive and negative AFLP markers, markers size and total no. of markers characterized each of the five Upper Egypt date palm.

Primer combination	Malikaby		Gandila		Sakkoty		Shameia		Bertmoda	
	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp
Total UPM & UNM	11	15	22	24	6	16	11	21	8	57
Grand total	26		46		22		32		65	

Combined RAPD, ISSR and AFLP data

The authors used different molecular markers,i.e., RAPD and ISSR's on the same cultivars of Upper Egypt date palm A summary of the effectiveness of the different markers is given in (Table 5). AFLP were the most effective in that all primer pairs tested detected polymorphism. AFLP showed 16.9 as average polymorphism per primer combination compared with 2.9 and 1.2 in ISSR and RAPD, respectively. While the percentage of polymorphism detected was 41.6% in AFLP compared with 34.6% and 18.9% in ISSR and RAPD, respectively.

The dendrogram constructed on the basis of the combined data from RAPD, ISSR and AFLP analyses showed the same grouping pattern as generated by AFLP (Fig. 10) and Thus confirming that AFLP is the most effective.The use of RAPD and ISSR and AFLP techniques on the date palm genome enabled us to generate many polymorphic markers ensuring a good coverage of the genome.

Table 5 : Effectiveness of RAPD, ISSR and AFLP markers in detecting Polymorphism in data palm cultivars

	RAPD	ISSR	AFLP
total band detected	259	159	1135
polymorphism detected	49	55	472
% of total	18.9	34.6	41.6
no. of primers used	41	19	28
average polymorphisms per primer/primer pair	1.2	2.9	16.9

Intervarietal polymorphism among the five Delta Egypt date palm.

To investigate intervarietal polymorphism among the five Delta Egypt date palm cultivars, RAPD, ISSR and AFLP analysis have been conducted on bulked DNA samples representing each cultivars. The cultivars were assayed using 10 RAPD primers, 7 ISSR primers and 6 AFLP primers combinations.

Genetic polymorphism as detected by RAPD markers.

To investigate intervarietal polymorphism among the five Delta Egypt date palm cultivars out of the 15 tested primers, only ten revealed reproducible and discernible RAPD profiling. In general, the size of the amplified DNA fragments ranged from 2800 to 310 bp. The number of bands varied, from 5 to 13 bands. The primers revealed 12 polymorphic RAPD markers ranging from 2100 bp to 770 bp (Fig. 6).

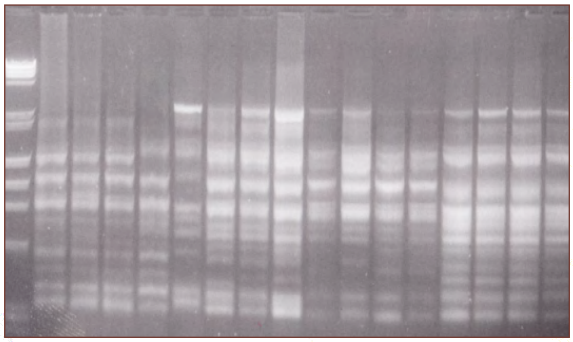


Fig. (6): RAPD profiles of the four date palm cultivars Hayany lanes 1-4, Zaghloul 5–8, Siwi 9–12 and Samany 13–16, M DNA molecular marker, Lambda Hind III digest, Phi x 174, Hae II digest, amplified by OP B1 primer.

Genetic polymorphism as detected by ISSR's

The bulked samples of the 5 date palm cultivars were analyzed using 7 Inter simple sequence repeat (ISSR) primers. These included five anchored primers at the 5` end with 3 base pairs and 2 primers composed of non- anchored repetitive sequences. The 7 ISSR studied primers produced good reproducible and scorable patterns and the amplification profiles were screened for the presence of polymorphisms among the 4 date palm cultivars (Fig. 7). a total of 53 fragments were generated by the 7 primers, with primer Amic 1 yielding the highest number of products (11 amplicons) and primer A mic7 the least (4 amplicons).The number of polymorphic markers also varied between primers, with primer Mic3 generating only polymorphic bands (100% polymorphism) and primer Amic1 revealing 10 polymorphic bands out of 11 and Amic7 yielding only monomorphic bands.

In the present investigation AFLP analysis has been adapted to assay the level of polymorphism and to produce a fingerprint for the five studied date palm cultivars. Eight primer combinations were initially tested with the five bulked DNA samples representing the five date palm cultivars. Among the eight tested primer combinations, six EcoR1/Mse1 selective primer combinations yielded discernible reproducible profiles (Fig. 7).

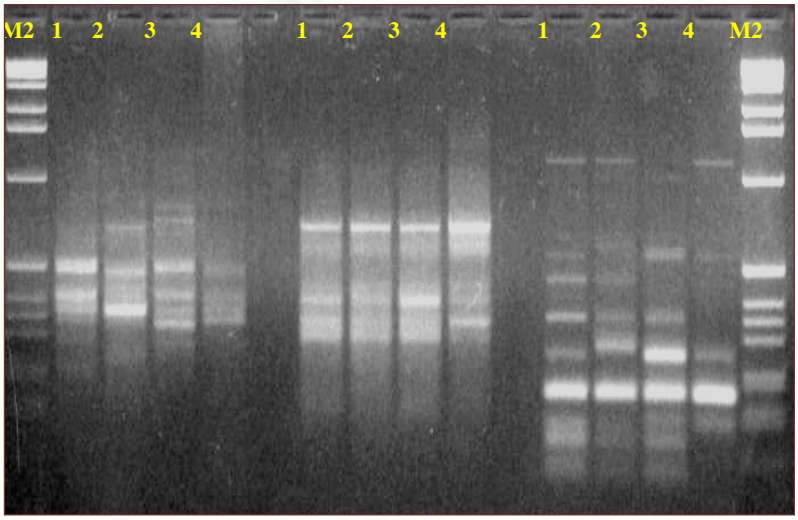


Fig. (7): ISSR profiles of the four date palm cultivars as detected by different ISSR primers (1) Samany, (2) Hayany, (3) Siwi and (4) Zaghloul as detected by (A) Mic3, (B) Mic9, (C) Amic1, M1 and M2, 1 kb plus ladder and 1 kb ladder, respectively.

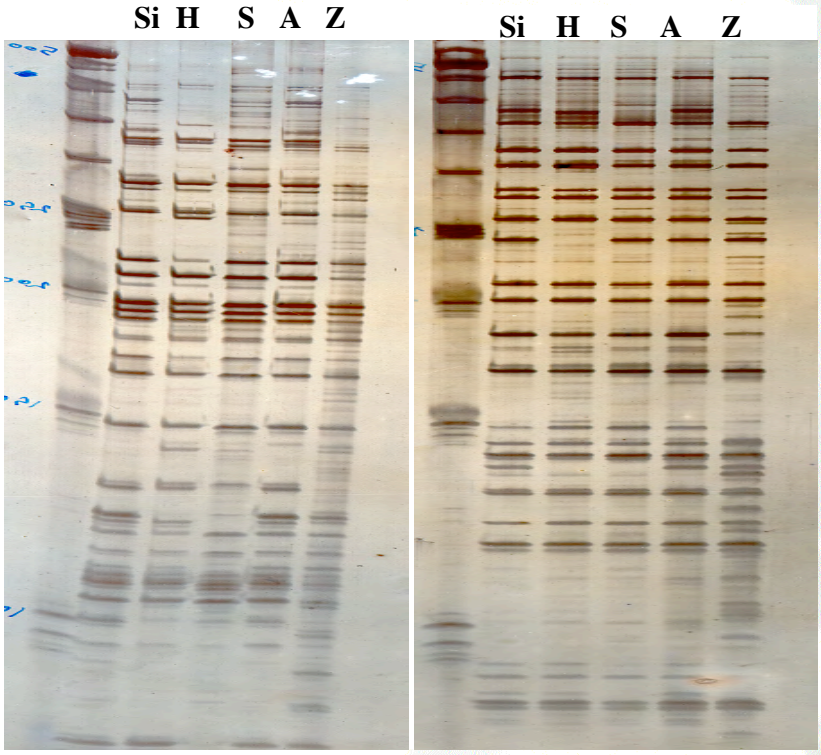


Fig. (8): AFLP profiles of the five date palm cultivars represent Upper Egypt Cultivars ; Malikaby, Gandila, Sakkoty, Shameia and Bertmoda as detected by primer combinations (A) 7/7, (B) 8/8. M.DNA molecular weight standard (100 bp ladder).

Figure (8) shows examples of two of the six combinations used in the present study. In this respect, similar findings were reported by Han et al., (2000) studying tea species, they found that the number of amplified AFLP bands per assay ranged from 32 to 150 with a mean of 84.7 and an average of 10.5 polymorphic bands per primer combination. Le Febvre et al. (2001) analyzed 47 pepper inbred lines with 10 AFLP primer combinations and revealed 863 selectively amplified fragments of which 378 were polymorphic (34.8%). Matthes et al. (2001) used ten AFLP primer combinations with oil palm and reported that the average number of bands per primer combination was 82 which is in agreement with our results.

The number of distinguishable bands detected after selective amplification varied among the different primer combinations. The highest number of amplicons (106) was exhibited by the primer combination (4.4), whereas the lowest number was 33 as revealed by primer combination (6.6). In this

concern, Goulao et al. (2001) reported a range of 34 to 66 fragments per primer pair in apple cultivars.

The level of polymorphism ranged from 42.42% to 59.02% in primer combinations 6.6 and 7.7, respectively. Similarly, Cervera et al., (1998) applied the AFLP technique to characterize 67 different grapevine accessions. They obtained an average of 100 amplified fragments per primer combination, of which 49% were polymorphic. Moreover, Hussein et al., (2002) reported that the level of polymorphism revealed by six AFLP primer combinations on cotton ranged from 38% to 65%.

Genetic relationships among the five date palm cultivars

To determine the genetic relationships among the five Delta Egypt date palm cultivars, the scoring data (1 for presence and 0 for absence) resulting from the six primer combinations were used to compute the similarity matrices according to Jaccard (Jaccard, 1908). These similarity matrices were used to generate a dendrogram using the UPGMA method. As shown in Table (6) the genetic similarity estimates ranged from 64.4% to 76.7%. This revealed moderate levels of genetic similarity among the studied cultivars. The highest genetic similarity (76.7%) was between Siwi and Hayani. This was followed by 75.2% between Samany and Amhat, while the lowest genetic similarity (64.4%) was detected between Zaghloul and Samany. These genetic relationships were reflected on the dendrogram which represents the graphical illustration of the genetic distances among the five date palm cultivars. The dendrogram confirmed that the cultivars Siwi and Hayani were the most genetically similar among the studied cultivars, with Amhat and Samany next, while Zaghloul was the most distinct cultivar (Fig. 9).

Table 6: Level of polymorphism detected by 6 AFLP primer combinations.

no. of primers	total no. of bands	polymorphic amplicons	% of polymorphism
6	433	233	53.81
average	54.1	29.1	

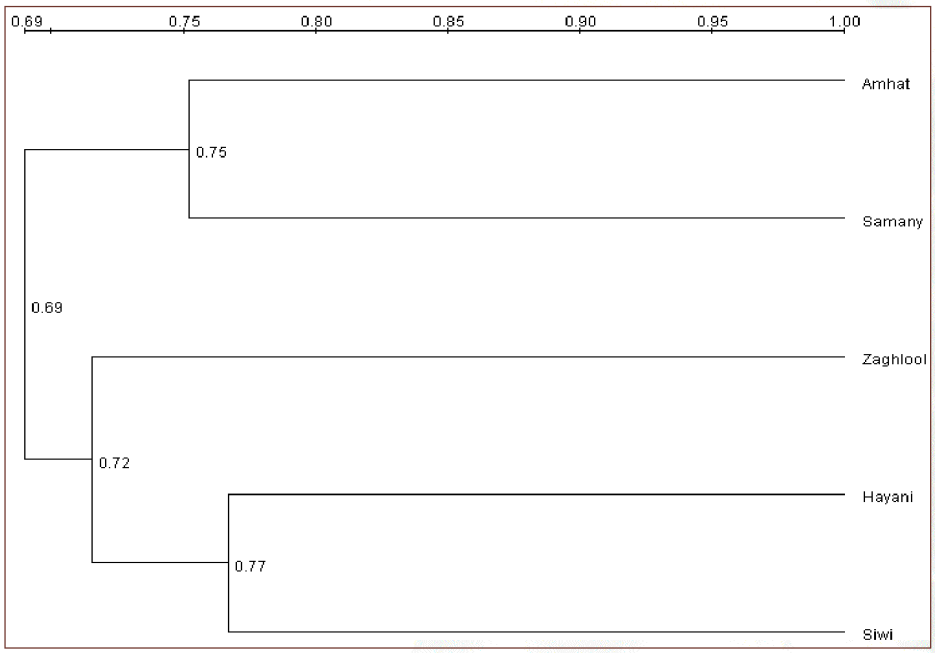


Fig. (9): Dendrogram of 5 date palm cultivars from Delta Egypt based on similarity index.

Cultivar identification by unique markers

As shown in Table (7), the AFLP analysis permitted the distinction among the five studied date palm cultivars and the characterization of each cultivar by specific unique markers. A total of 78 positive and 48 negative markers were identified by the six AFLP primer combinations. The total number of unique markers per genotype ranged from 13 to 51. The cultivar Zaghloul was characterized by the highest number of unique positive markers (48), in addition to 3 unique negative markers. Siwi was identified by 9 positive and 7 negative markers. Hayany exhibited a total of 23 unique markers among which 10 were positive. Samani also exhibited a total of 23 unique markers, seven of which were positive while the other 16 were negative. While, Amhat revealed the lowest number of unique markers (13) with 4 positive and 9 negative markers.

Table 7 : Unique positive and negative AFLP markers for the five date palm cultivars.

Primer combination	Siwi		Hayany		Amhat		Samany		Zaghloul	
	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp	UPM/bp	UNM/bp
Total UPM & UNM	9	7	10	13	4	9	7	16	48	3
Grand total	16		23		13		23		51	

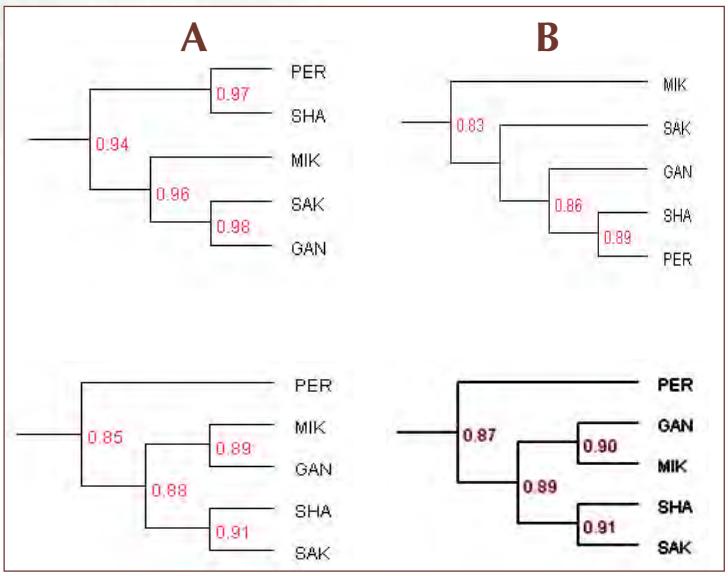


Fig. (10): Dendrogram of five date palm cultivars based on similarity index by AFLP (A) and combined data (B).

Genetic stability of somatic embryo- derived plantlets

The tissue culture overcomes the problem of date palm cultivation with traditional methods which prevent rapid crop improvement of the date palm trees. However, the tissue culture protocol should maintain the genotype of the original cultivar, i.e . we must prove that tissue culture – derived plants are true to type This problem could be avoided if an accurate identification method was utilized at an early stage of propagation. To investigate the genetic stability (true to type) of the clones derived from date palm tissue

culture, ten seedlings derived from in vitro culture of the cultivar Zaghloul were subjected to molecular analysis. The genetic stability of tissue culture derived plants was assayed using RAPD and AFLP. The DNA profiles of the tissue culture derived plants exhibited non significant polymorphism indicating the true to type nature of these plants. AFLP profiles of the date palm cultivars Sakkoty, Gandila and Bertmoda as amplified by primer combination.

Analysis of RAPD banding patterns generated by PCR amplification using 37 random primers gave no evidences for somaclonal variations and the percentage of polymorphic band in a total of 259 scored bands was zero. Meanwhile, analysis of AFLP banding patterns generated using 13 primers combinations pointed to minor genetic variations in the AFLP banding patterns. The percentage of genetic variations (polymorphism) in tissue culture-derived date palm off shoots belonging to cultivars Sakkoty, Gandila and Bertmoda was 2.6 , 0.79 and 1 % respectively, as revealed by AFLP analysis. The low percentage of genetic variations confirms the genetic stability of tissue culture derived dry date palm cultivars (Fig. 11 & 12 and Table 8 & 9).

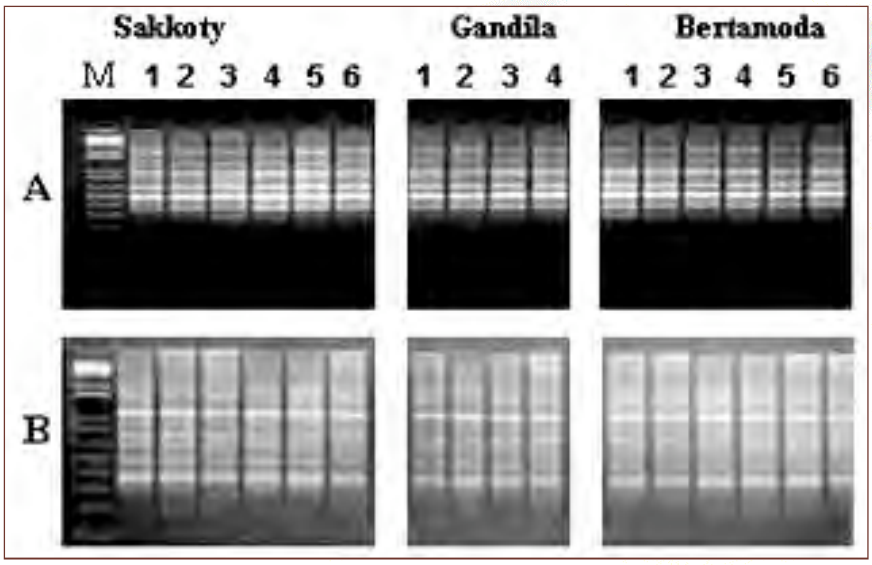


Fig. (11): RAPD banding patterns of donor mother trees (lane 1) And different tissue culture-derived date palm clones (lane 2-6), belonging to cultivars Sakkoty, Gandila and Bertmoda. The patterns generated by PCR amplification using the random primers OPD8 (A) and OPZ11 (B) and electrophoresed in TAE buffer using 2% agarose gel.

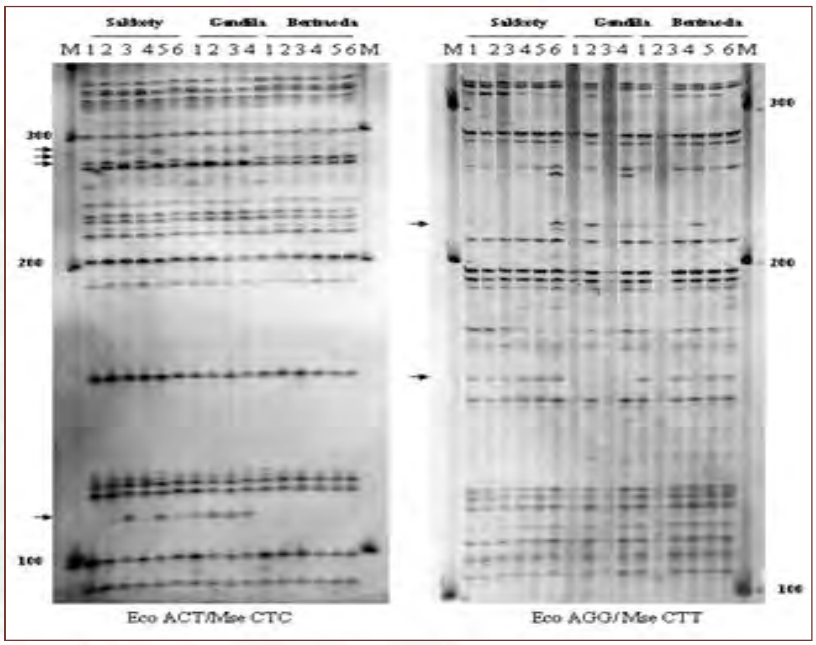


Fig. (12): AFLP banding patterns of donor mother trees (lane 1) And different tissue culture-derived date palm clones (lane 2-6), belonging to cultivars Sakkoty, Gandila and Bertmoda. The patterns generated by PCR amplification using primer combina - tions Eco ACT/Mse CTT.

Table 8 : RAPD and AFLP primers or primer combinations, total number of amplification products (amplicons), number of polymorphic amplicons and percentage of polymorphism among the three date palm cultivars and tissue culture-derived clones.

Type of analysis	RAPD			AFLP		
Parameter	Sakkoty	Gandila	Bertmoda	Sakkoty	Gandila	Bertmoda
Number of primers	37	37	37	13	13	13
Total no. of bands	259	259	259	380	380	380
No. of polymorphic bands	0.0	0.0	0.0	10	3	4
% of polymorphism	0.0	0.0	0.0	2.6	0.79	1.0

Table 9 : Distribution of informative AFLP polymorphic bands (markers) among certified donor mother trees of cultivars Sakkoty, Gandila and Bertmoda (Inter cultivars polymorphism) and among tissue culture-derived clones of the three cultivars (Intra cultivar polymorphism).

Markers	Cultivars															
	Sakkoty						Gandila				Bertmoda					
	C	Tissue culture clones					C	Tissue culture clones			C	Tissue culture clones				
	1	2	3	4	5	6	1	2	3	4	1	2	3	4	5	6
Eco ACA/Mse CTG-325	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco ACA/Mse CTG-195	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+
Eco AGC/Mse CTG-150	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+
Eco AAC/Mse CTT-300	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco AAC/Mse CTT-80	-	+	-	+	-	+	+	+	+	+	-	-	-	-	-	-
Eco ACT/Mse CTC-290	+	+	-	+	-	+	-	-	-	-	+	+	+	+	+	+
Eco ACT/Mse CTC-120	-	-	+	-	+	-	+	+	+	+	-	-	-	-	-	-
Eco AGG/Mse CTT-230	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-	-
Eco AGG/Mse CTT-170	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+

(C): control mother tree, certified donor mother tree (+): presence of marker (-): absence of marker

SELECTED REFERENCES

Adawy, S.S., Hussein, Ebtissam H.A., Saker, M.M. and El-Itriby, Hanaiya A. (2004a). Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (Phoenix dactylifera L.): I. As revealed by RAPD and ISSR markers. Proceed. Int. Conf. Genet. Eng. & Appl., Sharm El-Sheikh, South Sinai, Egypt (April, 8-11, 2004): 165-179.

Adawy, S.S., Saker, M.M., Mohamed, Amina A. and El-Itriby, Hanaiya A. (2004b). Genetic relationships of Upper Egypt date palm cultivars (Phoenix dac - tylifera L.): II. As revealed by AFLP markers. Proceed. Int. Conf. Genet. Eng. & Appl., Sharm El-Sheikh, South Sinai, Egypt. (April, 8-11, 2004): 181-195.

Adawy, S.S., Assem, Shireen K., Hussein, Ebtissam H.A. and El-Itriby, Hanaiya A. (2004c) Development of AFLP markers and genotyping of elite maize inbred lines. Arab J. Biotech., 7(1): 53-64.

Adawy, S.S., Hussein, Ebtissam H.A., El-Khishin, Dina, Moharam, H. and El-Itriby, Hanayia A. (2002). Genetic variability studies and molecular fingerprin - ting of some Egyptian date palm (Phoenix dactylifera L.) cultivars: II. RAPD and ISSR profiling. Arab J. Biotech., 5(2): 225-236.

Ben Saleh, M., and El-Helaly, R. (2003). Using ISSR to investigate the similari - ties and dissimilarities between some Tunisian date palm (Phoenix dactylifera L.) cultivars. (in Arabic and abstract in English). International Conference on Date Palm, Kingdom of Saudia Arabia, Ministry of Higher Education, King Saud University, Qaseem branch, College of Agriculture and Veterinary Medicine (16-19 September 2003).

Bornet, B., and Branchard, M. (2001). Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol. Biol. Rep., 19: 209-215.

Bellin, D., Velasco, R. and Grando, M.S. (2001). Intravarietal DNA polymor - phisms in grapevine (Vitis vinifera L.). Acta Hort. (ISHS), 546: 343-349.

Cao, B. R. and Chao, C. T. (2002). Identification of date palm cultivars in California using AFLP markers. HortScience, 37: 966-968.

Chao, C.T. and Devanand, P.S. (2003). Genetic variation within 'Medjool' and 'Deglet Noor' date (Phoenix dactylifera L.) cultivars in California detected by fluorescent-AFLP markers. J Hort Sci Biotech, 78(3): 405-409.

Devanand, P.S. and Chao, C.T. (2003). Identification of genetic strains of Medjool and Deglet Noor date palm cultivars in California using amplified frag - ment length polymorphism (AFLP) markers. Acta Hort. (ISHS), 623: 333-340.

Diaz, S., Pire, C., Ferrer, J. and Bonete, M.J. (2003). Identification of Phoenix dactylifera L. varieties based on amplified fragment length polymorphism (AFLP) markers. Cell. Mol. Biol. Lett., 8(4): 891-899.

El-Khisin, Dina A., Adawy S.S., Hussein, Ebtissam H.A. and El-Itriby, Hanaiya A. (2003). AFLP fingerprinting of some Egyptian date palm (Phoenix dactylifera L.) cultivars. Arab J. Biotech., 6(2): 223-234.

El-Seme'a, Ezz El-Din G.H.G. (2003). Propagation of dry varieties of date palm. Ph.D. Thesis, Department of Pomology, Faculty of Agriculture, Cairo University.

Henry, R.J. 1998. Practical applications of molecular markers to tropical and subtropical species. Acta Hort. (ISHS), 461: 107-112.

Hussein, Ebtissam H.A., Adawy, Sami S., El-Khishin, Dina, Saker, M.M. and El-Itriby, Hanayia A. (2002). Genetic variability studies and molecular fingerprin - ting of some Egyptian date palm (Phoenix dactylifera L.) cultivars: I. A prelimi - nary study using RAPD markers. Arab J. Biotech., 5(2): 217-224.

Hussein, Ebtissam H.A., Adawy, S.S., Ismail, S.E.M and El-Itriby, Hanaiya A (2005. Molecular characterization of some Egyption Date Palm germplasm using RAPD and ISSR markers. Arab J. Biotech., (under publication).

Motawei, M.I., Al-Moshileh, A., Al-Wasel, A., and Abdel-Ltif, T. (2003). Identification of some date palm (Phoenix dactylifera L.) cultivars in Saudi Arabia using RAPD fingerprints. International Conference on Date Palm, Kingdom of Saudia Arabia, Ministry of Higher Education, King Saud University, Qaseem branch, College of Agriculture and Veterinary Medicine (16-19 September 2003).

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J. , Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatel - lite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Porebski, S. Bailey, L.G. and Baum, R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Reporter, 15(1): 8-15.

Sakka, H., Trifi, M., Ould, M.S.A., Rhouma, A., and Marrakchi, M. (2000). Rapid construction of a random genomic library from date palm (Phoenix dac - tylifera L.). Plant Mol. Biol. Rep., 17: 1-7.

Sedra, M.H., Lashermes, P., Trouslot, P., Combes, M.C., Hamon, S. (1998). Identification and genetic diversity analysis of date palm (Phoenix dactylifera L.) varieties from Morocco using RAPD markers. Euphytica, 103: 75-82.

Soliman, S.S., Bahy Ahmed Ali, Mohamed Morsy Mohamed Ahmed. (2003). Genetic comparisons of Egyptian date palm cultivars (Phoenix dactylifera L.) by RAPD-PCR. African Journal of Biotechnology, 2(4): 86-87.

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy. Freeman, San Francisco, California.

Trifi, M., Zehdi, S., Sakka, H., Ould Med Salem, A., Rhouma, A. and Marrakchi, M. (2003). Molecular classification of date palm genetic stocks in the Tunisian oases using fingerprinting techniques. (in Arabic and abstract in English). International Conference on Date Palm, Kingdom of Saudia Arabia, Ministry of Higher Education, King Saud University, Qaseem branch, College of Agriculture and Veterinary Medicine (16-19 September 2003).

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau M. (1995). AFLP: a new tech - nique for DNA fingerprinting. Nucleic Acids Research, 23(21): 4407-4414.

Williams, J.G.K., Kublick, A.R., Livak, K.J. , Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as gene - tic markers. Nucleic Acids Research, 18: 6531-6535.

COMPARISON OF SOMATIC EMBRYOS WITH OFFSHOOT ORIGIN IN TWO CULTIVARS OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

Dr. Abdullah Ali Al-Baiz

Tissue culture laboratory / National Agriculture Center
for Research and Animal Resources
P.O.Box 17285 Riyadh 11484
Ministry of Agriculture – Saudi Arabia.

ABSTRACT

This study was carried out to compare the genetic stability of somatic embryos with that of offshoot origin of two highly economical cultivars namely Sukkary and Barhee of date palm (Phoenix dactylifera L .) in the Kingdom of Saudi Arabia.

The somaclones were micropropagated from shoot tips that were isolated from offshoots of palms and cultured under aseptic conditions on suitable nutrient media supplemented with various growth regulators , sucrose , amino acids and vitamins impregnated in phytagel as solid matrix . Somatic embryos were produced from embryogenic callus when cultured under suitable incubation conditions. Twelve subcultures (generations) had been performed for both cultivars . During the subcultures of somatic embryos , random samples had been collected from the somaclones in each generation for the analysis of fingerprinting of DNA by Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (ISSR) techniques for the comparison of the genotypic characteristics of the somatic embryos in each subculture with offshoot (maternal plant) origin to determine which generation of the somatic embryos in which they start to be different in genotypic characteristics when compared with offshoot origin in order to set a rule for true – to – type micropropagation in commercial mass production .

The results showed that the first four generations of somatic embryos of Sukkary were genetically identical with their mother offshoot , whereas the rest of the subsequent generations (5 – 12) had shown genetic variability .

In Barhee the analysis had shown that the first and second generations of somatic embryos were the only genetically identical generations with their offshoot mother plant .

The recently developed ISSR – PCR fingerprinting technique had been tested in this study to confirm the results of RAPD analysis using the single unanchored primer to detect polymorphism. Thus the ISSR-PCR technique confirmed the aforementioned analysis of DNA fingerprints by RAPD-PCR. The remaining generations of somaclones showed polymorphic DNA as compared to their mother plants in both cultivars .

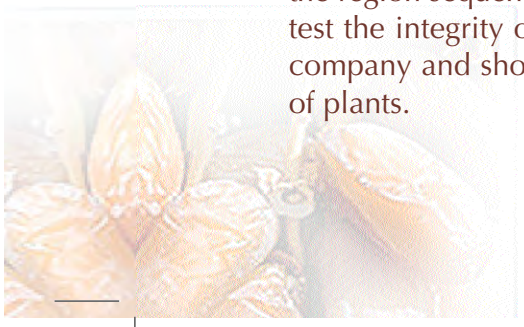
SOMACLONAL VARIATION: BANANA AS A MODEL FOR IDENTIFYING GENOMIC CHANGES.

Christopher A. Cullis[1,2], T homas J. Oh[1], Margaret A. Cullis[1], Karl Kunert[3], Iris Engelborghs[4], and R Swennen [4]

1NovoMark Technologies, LLP.; 2Department of Biology, Case Western Reserve University;
3Forestry and Agricultural Biotechnology Institute & Botany Department, University of Pretoria;
4 Kotholieke Universiteit Leuven

ABSTRACT

The molecular basis of somaclonal variation is not known. However, evidence points towards the existence of labile portions of the genome that can be modulated when the cells undergo the stress of tissue culture. Based on these observations a reasonable conclusion is that a set of variable sequences associated with the occurrence of somaclonal variation can be identified and used for the early detection of genome instability. This hypothesis, that there are identifiable, predictable markers diagnostic of somaclonal variation has been tested. Representational difference analysis (RDA) was used to isolate DNA differences between culture-induced off-types and normal banana plants. The results obtained are consistent with the hypothesis that there is a particularly labile portion of the genome that is especially susceptible to stress with higher rearrangement and mutation rates than other portions of the genome. One of the labile sites has shown a high degree of polymorphism where the variation can occur in up to 10% of the nucleotides in the region sequenced. The primers developed for this site have been used to test the integrity of 300 individuals supplied by a commercial propagation company and shown to predict the level of off-types observed in this batch of plants.



INTRODUCTION

Somaclonal variation is the genetic variation that is sometimes observed when plants are regenerated from cultured somatic cells, compared to the plant used as a source of the culture. During the micropropagation of valuable elite clones, this variation can result in problematic off-types that diminish the commercial value of resultant plants. Similarly, genetically enhanced (transgenic) plants need to be carefully screened to avoid unwanted and unintended somaclonal variation, so that the commercially released clone is identical to the starting material except for the effect of the added transgene. Various types of mutations have been described in somaclonal variants, including point mutations, gene duplication, chromosomal rearrangements, and chromosome number changes. Transposable element movement and changes in DNA methylation have also been implicated as possible mechanisms behind some somaclonal variation.

The appearance of somaclonal variants may not be a process unique to in vitro propagation, but may occur naturally in plant somatic and reproductive tissues (Cullis, 2005). The trigger for all of these types of changes can be described as genomic shock or plasticity, which occurs after the plant has exhausted its ordinary physiological responses to environmental stress (Cullis, 1999). This genomic shock response may be a radical, but limited genomic reorganization which is an adaptive mechanism that activates when ordinary physiological responses are insufficient. The occurrence of hot-spots of mutation and recurring menus of alternative alleles is consistent with this response being limited to a sub-fraction of the genome.

The sometimes massive but reproducible genetic changes observed with somaclonal variation offers the possibility for identifying specific DNA modifications that are associated with the release of this genetic variation and therefore that can used to monitor the genome through the process of in vitro propagation. Such DNA markers could also be used to screen plants to determine the possibility of their being true-to-type.

The micropropagation of horticultural species (and clonally propagated crops such as banana) is intended to produce chosen elite individuals in mass. Somaclonal variation is problematic under these circumstances, where even a low percentage of off-types is unacceptable for commercial use; a high percentage can be very costly, as has been proven in both the banana and oil palm industries (Larkin 2004). In a similar vein, somaclonal variation can be problematic in the genetic modification of crops where

hundreds of individual transgenics would need to be exhaustively tested so that only proven elite individuals are chosen to become commercial releases.

A number of different molecular techniques are currently available to detect sequence variation between closely related genomes including any between source plants and somaclones. These include random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) (Linacero et al. 2000; Labra et al. 2001). Both techniques are useful in comparing the DNA from any number of different samples for the differentiation of plants due to sequence variation by identifying random polymorphisms. These comparisons are usually made on the basis of the presence or absence of a DNA band rather than directly on any DNA sequence variation. Representational Difference Analysis (RDA) has been used to detect variation in a very limited number of plant species (Donnison et al., 1996; Cullis, and Kunert, 2000; Zoldos et al., 2001; Vorster et al., 2002; Oh and Cullis, 2003). RDA technology has been used to detect genomic losses, rearrangements, amplifications, point mutations and pathogenic organisms between two genomes (Lisitsyn et al., 1993; Ushijima et al., 1997; Michiels et al., 1998; Lisitsyn, 1995). The advantage of RDA is that a complexity of about 5x108 base pairs of DNA can be scanned in each subtraction, which is greater than can be achieved with other commonly used differentiation techniques, such as RFLPs, RAPDs, AFLPs or microsatellites. Further, RDA can also be performed using bulked amplicons derived from different individual plants of a specific group and can thus be used to identify polymorphisms that are restricted to a particular group of individuals.

DNA subtractions (RDA) have been performed using the tall (off-type) and dwarf forms of Curare Enano. A series of difference products have been isolated. Some of these difference products have been sequenced and primers designed that have been used in a PCR-based system to monitor changes in genomic integrity of in vitro produced banana plants.

MATERIALS AND METHODS

Representational difference analysis (RDA)

DNA from the two phenotypes was supplied by R. Swenen of the Katholic University, Leuven.

The general outline for the RDA procedure described by Lisitsyn et al. (1993) and Vorster et al. (2002) was followed. Amplicons (representations) were prepared by the digestion of 2 µg of DNA with 80 units of the restriction enzyme HpaII, ligating adaptor sequences to the digestion and amplifying the ligation products by the polymerase chain reaction (PCR) to generate the first round amplicons. The first hybridization reaction used 20 µg of driver DNA amplicons and 0.5 µg of tester DNA amplicons (40:1 driver/tester ratio). The mixed amplicons were dried and redissolved in 4 µl of hybridization buffer (30 mM EPPS [(2-hydroxyethyl piperizine)-N'-(3-propene sulfonic acid)], pH 8, and 3 mM EDTA) and overlayed with mineral oil. The DNA was denatured at 100oC for 10 minutes, 1 µl of sodium chloride (5 M) added to a final concentration of 1 M, and the reaction was incubated at 67oC for 16 hours. The hybridization mixture was then diluted to 300 µl and an aliquot amplified. Following all hybridizations, the first round of amplification was for 10 cycles followed by digestion of the products with mung bean nuclease to remove single-stranded DNA. The nuclease-treated product was then amplified for an additional 20 cycles. From the resulting amplicons (first subtraction product) the adaptors were removed by HpaII digestion and a second pair of adaptors were ligated onto the amplicons used as tester DNA for the second hybridization. The second hybridization used 20 µg of driver DNA amplicons and 0.01 µg of tester DNA amplicons (2000:1 driver/tester ratio) and following hybridization the products were amplified. Adaptors were removed from the resulting amplicons and the subtraction products were filled in, blunt-end ligated into the pCRScript cloning vector (Stratagene), transformed into E. coli competent cells. Plasmid-containing colonies carrying an insert were selected for plasmid isolation and determination of the insert size and sequence.

Flanking sequence isolation

A two-step PCR reaction technique was applied using 4 different flanking primers (FP) and the two primers designed from the difference product sequence according to the technique outlined by Sorensen et al. (1993). These primers were used to amplify from the banana genome the 3' and 5'-end DNA flanking sequences of one of the RDA subtraction products ToBac1. For amplification of the two flanking sequences, the 5'-end biotinylated primers complementary to sections of the RDA subtraction product were used in combination with a mixture of all 4 flanking primers to isolate a sequence complementary to the flanking regions of the RDA subtraction product. After PCR amplification, biotinylated DNA fragments were isolated

by mixing 40 ml of the PCR mixture with 40 ml of 200 mg pre-washed Dynabead M280-streptavidin as recommended by the supplier (Dynal Biotech, Norway) and removing all biotinylated fragments from the mixture using a Dynal magnetic particle concentrator. Binding and washing steps were done in the presence of a buffer consisting of 10 mM Tris-HCl (pH 7.5), 1 mM EDTA and 2 M NaCl. After incubation for 15 minutes to remove the biotinylated DNA fragments with the particle concentrator from the mixture and washing the beads in buffer, the bead-bound DNA fragments were treated in 8 ml of 100 mM NaOH for 10 minutes to remove the beads and the biotin label. The resulting supernatant containing non-biotinylated DNA strands was then neutralized with 4 ml of 200 mM HCl and 1 ml 1 M Tris-HCl, pH 8 diluted to 30 ml with distilled H2O.

A second PCR reaction with 2 ml of this produce was used to amplify the isolated flanking sequence with an internal primer. All PCR reactions were carried out using a standard PCR protocol with 42 cycles of amplification and primer annealing at 62C. Amplified and agarose gel-purified DNA fragments were cloned into the vector PCRScript and sequenced.

Primer design and testing

Pairs of primers were designed using the program, Primer3 (http://www.basic.nwu.edu/biotools/Primer3.html). The primer pairs were used in a PCR reaction using banana DNA as template at various annealing temperatures to optimize the PCR reaction. The PCR reactions were carried out in 25 µl volumes containing 25 ng of total genomic DNA, 15 ng of primer, 100 mM of each dNTP, 10 mM Tris-HCl, pH 8.3, 2 mM MgCl2 and 0.5 units of Taq polymerase (Takara, Japan). Amplification was performed using a Perkin Elmer GeneAmp PCR system 9600. The following standard amplification program was used: (i) 94C for 5 minutes x 1 cycle; (ii) 94C for 1 minute, 55C or 60C for 1 minute depending on the primer pair, 72C for 1 minute x 35 cycles; (iii) 72C for 5 minutes x 1 cycle, and extension at the last cycle was at 72C for 7 min followed with an optional soak period at 4C. The PCR products were separated on a 1.5 % agarose gel, stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

Twenty clones were identified from the subtraction between normal and dwarf of C. Enano. The average size of difference products was about 200 bp, of which the largest fragment was 297 bp. Six different sets of primers have been derived from the sequences of these clones. The result of PCR using these primers with C. enano normal and dwarf DNAs is given in Figure 1.

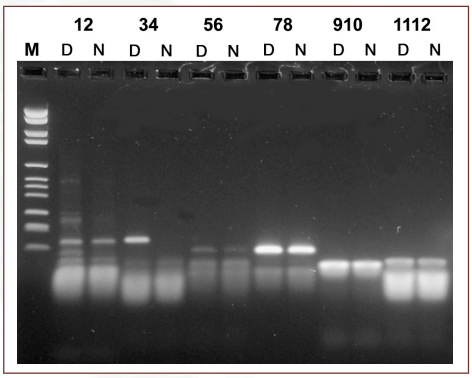


Figure 1. Gel electrophoresis of PCR products with various sets of primers designed from the difference products. Top number indicates a set of primers used in PCR, and N and D stand for normal and dwarf, respectively. Marker used (M) is Molecular Weight Marker VI (MWM VI, Roche).

The two primer sets, one consisting of primers 1 and 2, the other of primers 3 and 4 showed differences between the two starting DNAs. The primer set (3 and 4) revealed a polymorphism between normal and dwarf with a band being produced with one DNA but not with the other suggesting a possible structural change. The use of these primers (3 and 4) on other pairs of normal and dwarf banana pairs also distinguished these pairs (Figure 2). Therefore this particular subtraction product was subjected to additional characterization.

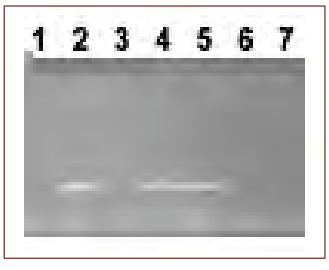


Figure 2. PCR products using primers 3 and 4 with target DNAs from: 1. Figue Rose naine (dwarf); 2. Figue Rose (normal); 3. Prata ana (dwarf); 4. Prata (normal); 5. Giant Cavendish; 6. Dwarf Cavendish; 7. Dwarf Parfitt (extra dwarf Cavendish).

The flanking regions of this difference product were isolated resulting in the region depicted in figure 3.

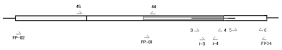


Figure 3. Isolation and organization of the exten - ded labile genomic region indicating the posi - tions of the various primers used for amplifica - tion. The region between primers 3 and 4 indi - cate the position of the original difference pro - duct.

Primers were designed from the flanking regions and again used to amplify from the banana dwarf and normal pairs. The PCR products are shown in figure 4.

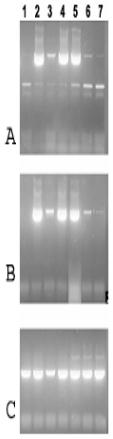


Figure 4. PCR products using primers 45 and i4 (A), 45 and 4 (B) 5 and 6 (C) (as indicated in Figure 3) with target DNAs from: 1. Figue Rose naine (dwarf); 2. Figue Rose (normal); 3. Prata ana (dwarf); 4. Prata (normal); 5. Giant Cavendish; 6. Dwarf Cavendish; 7. Dwarf Parfitt (extra dwarf Cavendish).

PCR reactions performed using additional primers within these flanking regions showed the extent of the variation within this region. These observations strongly suggest that this whole region of the genome is highly unstable.

Second generation diagnostic primers have also been derived from both the LHS and RHS sides of the difference clone. These were used to monitor their identification of differences within the starting material. The pattern observed (shown in Figure 5) ranges from differences similar to those shown by the primer pair 3 & 4 (e.g.13 & R2), others do not differentiate between these two DNA at all (e.g. 52 & 53), while others have different efficiencies of amplification leading to intensity polymorphisms (e.g.i3 & R4).

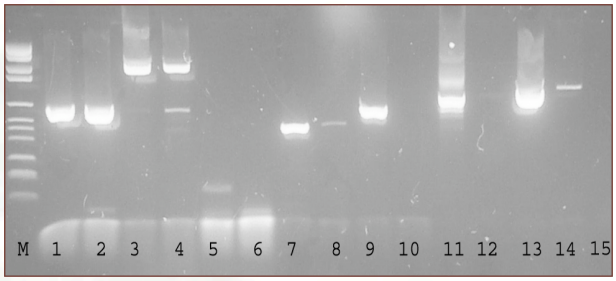


Figure 5. PCR products using primers 52 & 53 (lanes 1 & 2), 45 & i4 (lanes 3 & 4), 3 & 4 (lanes 5 & 6), i3 & R1 (lanes 7 & 8), i3 & R2 (lanes 9 & 10), i3 & R3 (lanes 11 & 12), i3 & R4 (lanes 13 & 14) (as indicated in Figure 3), M - Molecuolar weight Marker VI, Lane 15 – no DNA control

Primers 3 and 4 were used in PCR reactions with 300 individual plants from a commercial in vitro propagation facility. The result was that 14 of the 300 showed no amplification with the primer pair 3 and 4. However, all showed amplification with the primer pair 5 and 6. The frequency of observed non-amplification was approximately equal to that expected for the rate of dwarf types observed in this batch of plants.

CONCLUSION

The findings are consistent with the notion that there is a labile fraction of the genome that is modified during the generation of somaclonal variation. The difference product described here has all the characteristics of a representative of this labile region of the genome. The sequence of this region has been used to design sets of primers that differentiate between the two starting phenotypes as well as between other pairs of normal and dwarf plants arising from culture. The region contains an large number of mutations present which arise frequently during in vitro propagation and can be used as diagnostics for identifying off types in banana plants produced through in vitro propagation. This is the first reported diagnostic DNA marker for somaclonal variation. This type of marker, and as noted this is the first representative, will be useful to monitor the regenerated plants in a commercial setting to set limits to the proportion off-types present in any given batch. They will also be a tool to monitor the cultures to determine if genomic variation has occurred and to make decisions about continued use of such a culture for the regeneration of plants. Finally they will also be useful to monitor genomic change so that growth conditions can be optimized for rates of propagation and plant production while still minimizing off-type production.

REFERENCES

Cullis, CA (2005) Mechanisms and control of rapid genomic changes in flax. Annals of Botany 95: 201-206.

Cullis, CA (1999) Environmental Stress - a generator of adaptive variation? In: Lerner H.R. (ed) Plant Adaptations to Stress Environments. Marcel Dekker, New York, pp 149-160.

Cullis, CA, Kunert, KJ (2000) Isolation of tissue culture-induced polymorphisms in bananas by Representational Difference Analysis. Acta Hort 530:421-428.

Donnison, IS, Siroky, J, Vyskot, B, Saedler, H, Grant, SR (1996) Isolation of y chromosome-specific sequences from silene latifolia and mapping of male sex-determining genes using representational difference analysis. Genetics 144: 1893-901.

Labra, M, Savini, C, Bracale, M, Pelucchi, N, Colombo, L, Bardini, M, Sala, F (2001) Genomic changes in transgenic rice (Oryza sativa L.) plants produced by infecting calli with Agrobacterium tumefaciens. Plant Cell Rep 20: 325-330.

Larkin, P (2004) Somaclonal Variation: Origins and Causes Encyclopedia of Crop and Plant Sci. pp1158-1161. Marcel Dekker Inc. New York.

Linacero, R, Alves, EF, Vazquez, AM (2000) Hot spots of DNA instability revea - led through the study of somaclonal variation in rye. Theor Appl Genet 100: 506-511.

Lisitsyn, N (1995) Representational difference analysis: finding the differences bet - ween genomes. Trends Gen 11: 303-307.

Lisitsyn, N, Lisitsyn, N, Wigler M (1993) Cloning the differences between two complex genomes. Science 259: 946-951.

Michiels, L, Van Leuven, F, Van Den Oord, JJ, De Wolf-Peeters, C, Delabie, J (1998) Representational difference analysis using minute quantities of DNA. Nucl Acids Res 26: 3608-3610.

Oh, TJ Cullis CA (2003) Labile DNA Sequences in Flax Identified by Combined Sample Representational Difference Analysis (csRDA) Plant Mol Biol 52: 527-536.

Sorensen, AB, Duch, M, Jorgensen, P, Pedersen, FS (1993) Amplification and sequence analysis of DNA flanking integrated proviruses by a simple two-step polymerase chain reaction method. Am Soc Microbiol 67: 7118-7124.

Ushijima, T, Morimura, K, Hosoya ,Y, Okonogi, H, Tatematsu, M, Sugimura, T, Nagao, M (1997) Establishment of methylation-sensitive representational differen -

ce analysis and isolation of hypo- and hypermethylated genomic fragments in mouse liver tumors. Proc of the Nat Aca of Sci, USA 94: 2284-2289.

Vorster, BJ, Kunert, KJ, Cullis CA (2002) Use of representational difference analy - sis for the characterization of sequence differences between date palm varieties. Plant Cell Rep 21: 271-275.

Zoldos, V, Siljak-Yakovlev, S, Papes, D, Sarr, A, Panaud, O (2001) Representational difference analysis reveals genomic differences between Q. robur and Q. suber: implications for the study of genome evolution in the genus Quercus. Mol Genet Genomics 265: 234-41.

DATE PALM MICROPROPAGATION: SOME OBSERVATIONS ON TRUE-TO-TYPNESS OF TISSUE CULTURE-DERIVED PLANTS

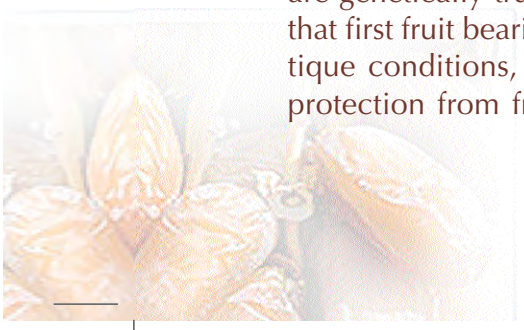
Anjarne Mohamed
Laboratoire de Recherche en Biotechnologie Vegetale,
Centre regionale de la recherche agronomique de Marrakech
B.P : 533, Marrakech 40 000, E-mail : m.anjarne@caramail.com

ABSTRACT

Development of oasien ecosystem is hampered by many constraints linked to Bayoud disease, desertification and drought. The use of micropropagation techniques is the best mean that can allow fast reconstitution of destroyed palm groves using suitable plant material. At international level, two techniques can be used for date palm micropropagation: organogenesis or somatic embrogenesis.

Organogenesis is based on the regeneration of vegetative buds from the bottom of the young leaves isolated from the offshoot. These buds can be multiplied to give rise to whole date palm plantlets that can be acclimatized and transferred to soil. Neo-formation of first buds occurs directly from meristematic zone around axillary buds. This technology was developed in the Plant Physiology Laboratory at Marrakech INRA center and already transferred to the private sector where it allowed the production of more than 350000 plants of more than 30 different varieties and selected clones.

Observations on plant development, fruit quality and many other morphological characters had shown that vitroplants produced via organogenesis are genetically true to type and show no abnormities. It is however, noted that first fruit bearing occure after 4 to 8 years depending on edapho-climatique conditions, water quality (salinity), availability of irrigation water, protection from frost and other cultural techniques. It is also shown that



vitroplants produce more offshoots than traditional plant material. These offshoots can be rooted and used for further propagation.

Somatic embryogenesis is based on callus initiation and regeneration from meristematic tissues. This callus can be multiplied and produce somatic embryos that germinate and transform into complete plantlets. The use of this technology is easier than organogenesis. However, for many plant species, it is noted that the use of callus is often linked with the apparition somaclonales variations. It is also important to note that protocols used for producing date palm via embryogenesis are different according to laboratories leading to different level of true- to- typness.

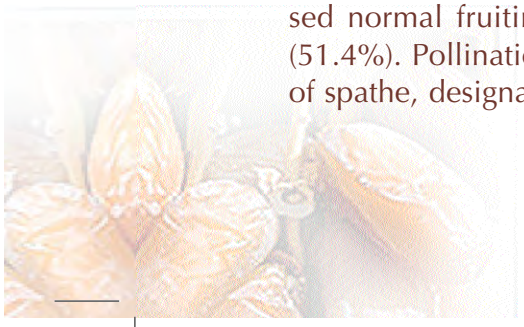
Key words: Date palm, Phoenix dactyliféra, in vitro culture, micropropagation. Somaclonale variations.

'BARHEE' FRUIT SETTING PROBLEMS AT KINGDOM OF SAUDI ARABIA : RESEARCH APPROACHES TO UNDERSTAND THE PHYSIOLOGICAL AND PHYSICAL EVENTS OF THE PHENOMENON

Hassan M. Ali-Dinar*, A.A.Alkhateeb**
* FAO, Riyadh, Kingdom of Saudi Arabia.
** Date Palm Research Center, King Faisal University, Kingdom of Saudi Arabia

ABSTRACT

The failure of fruit set locally known as 'sheiss' in 'Barhee' date palm cultivar (Phoenix dactylifera L.) at Gassim area of Kingdom of Saudi Arabia was investigated with a series of experiments dealing with the hormonal and sexual processes and the receptivity of the stigma. The interrelated events of the sexual process and changes in endogenous levels of gibberellic acid (GA3), indoleacetic acid (IAA) and abscisic acid (ABA) for 'Barhee' trees from vegetative offshoots and tissue culture 'Barhee' trees with few and acute 'sheiss' problems from anthesis to early stages of seed development were investigated. The receptivity of the stigma and pollination approach studies were conducted at 2 sites in Gassim area, namely: Algayzailya (Site 1) and Alfeyha (Site 2) date palm plantations. Vegetative offshoot trees in 'Algayzailya' date palm plantation are relatively older (9+ years). At 'Alfeyha' date palm plantation, experiments were conducted with younger tissue culture trees (4-5 years) and older ones (9+ years). Pollination of forced open female inflorescence spathes designated as (P0) in Site (1), increased normal fruiting to 79.7% compared to the control (farmer practice) (51.4%). Pollination of female inflorescence 3-5 days after natural opening of spathe, designated as (P1), in the same Site gave 76.6% normal fruiting.



In Site (2), (P0) pollination of younger tissue culture trees reduced abnormal fruiting to 41.5% in controlled pollination method and only 18.5% in farmers practice. On the other hand, (P1) pollination of similar aged trees gave relatively similar results. In the same Site (P0) of older tissue culture trees gave 95.3% and 90.2% normal fruiting under controlled pollination and farmer method, respectively. (P1) pollination of similar trees in same site gave 92.3% and 86.1% normal fruiting. The pollination technique results were much lower and inconsistent during the second season. The results cast some doubts on the use of the technique as a successful approach to solve the fruit set problem. However, photos of fruit setting of younger trees in Site 2 during the following cropping seasons showed a substantial improvement in normal fruiting even with farmer pollination. This may probably indicate the relatively longer juvenility period of these trees induced by unstable interrelated factors and it seems that tree age plays a central vital role in these events.

INTRODUCTION

A large number of tissue culture 'Barhee' trees at 'Al-Qassim' area of Kingdom of Saudi Arabia failed to produce normal fruits. The phenomenon is locally known as 'sheiss'. Several hypotheses have been postulated as causes of fruit failure. To define fruit set, many authors considered the collective changes that mark the transition of flower into a young fruit as fruit set (Crane, 1964; Leopold, 1964; Krezdorn, 1969; Albrigo and Sauco, 2004). These changes are illustrated by the burst of growth and development of the ovary. Pollination and fertilization of the ovary usually trigger the rapid growth of the ovary. The physiology and nature of fruit set have been studied over the years for many agricultural crops (Gustafson, 1936; Nitsch, 1950; Crane, 1964; Marcelle, 1984; Fankhauser and Schumacher, 1984; Martinez-Fuentes et al., 2004). Parthenocarpic and non-parthenocarpic fruits were previously exploited to study the hormones role in fruit set during the early events of reproduction (Gustafson, 1939; Hassaballa, 1964; Goldwin, 1984; Webster, 1984; Martinez-Fuentes et al., 2004). Suspecting that the growth stimulation following fruit-set could be related to auxin, Gustafson (1936) found that fruit set of many species in the absence of pollination and fertilization can actually be induced with auxin application. Gibberllins and cytokininis (Leopold, 1964; Marcelle, 1984) were also found to induce fruit set in different plant species. Other hormones such as ABA were negatively correlated to fruit set since they accelerate flower and fruit abscission (Leopold, 1964). Apparently, the nature of fruit set depends on an interrelated and complicated regulatory mechanism that involves hormones, carbohydrates, nutritional elements and environmental factors.

MATERIALS AND METHODS

A series of experiments were conducted to determine the possible causes of fruit set failure locally known as 'sheiss' in 'Barhee' date palm cultivar (Phoenix dactylifera L.) during two cropping seasons at the Kingdom of Saudi Arabia. Three types of 'Barhee' trees were selected, namely: trees from vegetative offshoots with normal fruits (9-years-old trees), tissue culture trees with low abnormal fruiting (9 years and older) and tissue culture trees with high abnormal fruiting (4-5 years old trees). The study was conducted at two sites at 'Gassim' area of Kingdom of Saudi Arabia (Algayzailya/ Site 1 and Alfeyha/ Site 2). The reproductive process from anthesis to early stages of seed development was microscopically studied in collected samples of pis-

tils, ovaries and young fruit at 2- day intervals during the first 3 weeks after anthesis and pollination and at weekly intervals thereafter. At the same intervals of sample collection for microscopic studies, enough samples were collected and immediately frozen in liquid nitrogen and stored at - 20° C until determination of IAA, GA3 and ABA (Wheaton and Bausher, 1977).

The pollination and the receptivity of the stigma studies were conducted using the following approaches: pollination of forced open female inflorescence spathe (P0) and pollination of female flowers 3-5 days after natural opening of spathe (P1). Vegetative and tissue culture trees at Site 1 and Site 2 were used under this study. Percent fruit set was determined by counting the normal fruits on certain bunch strands after the fruit setting period.

RESULTS AND DISCUSSION

Events of the reproductive process showed that pollens grew normally on the stigmatic surface and the pollen tubes were clearly progressing within the style 2- 4 days after pollination in tissue culture and vegetative offshoots 'Barhee' date palm trees (Table 1). Six to 10 days after pollination, pollen

Table 1 : Duration time for the occurrence of the different reproductive events at early stages of fruit growth.

Reproductive events	Days after pollination (No.)		
	Vegetative offshoot	Old tissue culture	Young tissue culture
Pollen growth on stigma	1	1	1
Pollen tubes in the style	2	2-3	3-10
Entrance of pollen tube into ovary	6-8	6-8	Still in style
Structure of integuments	Intact (After 10 days)	Intact (After 10 days)	Separation of inner and outer integu ments After 10 days)

tubes of vegetative offshoots and old tissue culture trees (with few incidences of abnormal fruiting) had already entered the ovary while those of young tissue culture trees (with high percent of abnormal fruiting) were slowly progressing within the style. Ovule fertilization as estimated by the initial endo-

sperm division was observed 2 weeks after pollination in vegetative offshoots and old tissue culture trees (Table 1). However, inner and outer integuments in ovary of young tissue culture trees became less intact and separated from each other reflecting a possible subsequent failure of normal fruit setting due to failure of the fertilization process. Fruit development was quite normal in offshoots and old tissue culture trees 6 weeks after pollination. Differences in pollen tube growth and the fertilization process between 'Barhee' date palm trees may reflect possible physical or hormonal related factors that prevent normal progress of the reproductive process in young tissue culture trees (Sastry and Muir, 1963; Krezdorn, 1969; Marcelle, 1984; Albrigo and Sauco, 2004).

IAA contents in ovaries and young fruits during early fruit growth stage of young and old tissue culture trees and vegetative offshoots trees are shown in Fig. (1). The IAA contents in all date palm tree types rose sharply after pollination, however, they declined sharply one week after pollination in fruit tissues of young tissue culture trees and continued to decline thereafter. Although the IAA contents started to decline 2 weeks after pollination in fruits of other 'Barhee' trees, they were relatively higher than those from young tissue culture trees throughout the 9 weeks sampling period. GA contents followed a relatively similar pattern, however, the increasing trend in old tissue culture and vegetative offshoots trees continued up to the 4 week and then declined thereafter (Fig. 2). The GA decline in young tissue culture trees started 2 weeks after pollination. ABA contents in the other hand, in young tissue culture trees continued to increase up to the 3rd week after pollination then declined (Fig. 3). The ABA contents, however, started to decline in old tissue culture and vegetative offshoot trees 2 weeks after pollination. IAA, GA and ABA contents were always low in abnormal fruits of young tissue culture trees. It was reasonable to assume that the slow progress of the pollen tubes and hence the subsequent failure of the fertilization process had led to the disintegration of the ovary tissues that might be responsible for production of enough hormonal quantities to sustain normal fruit growth. This assumption was supported by previous research work which indicated the importance of the presence of functional ovules to sustain normal fruit set (Sastry and Muir, 1963; Leopold, 1964; Marcelle, 1984; Albrigo and Sauco, 2004). It is noteworthy to mention that the relatively high contents of ABA compared to GA and IAA in ovary tissues of fruits from young tissue culture trees during early stage of fruit growth, might have slowed down the normal progress of pollen tubes within the style and subsequently led to fruit set failure.

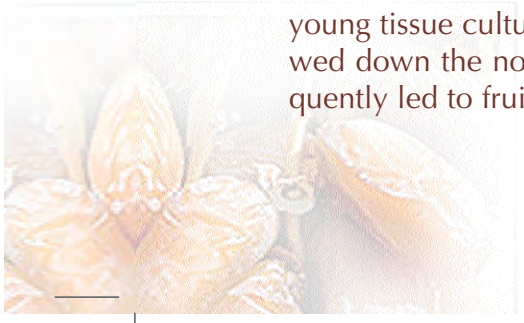


Fig. 1: IAA content in 'Barhee' during early fruit growth

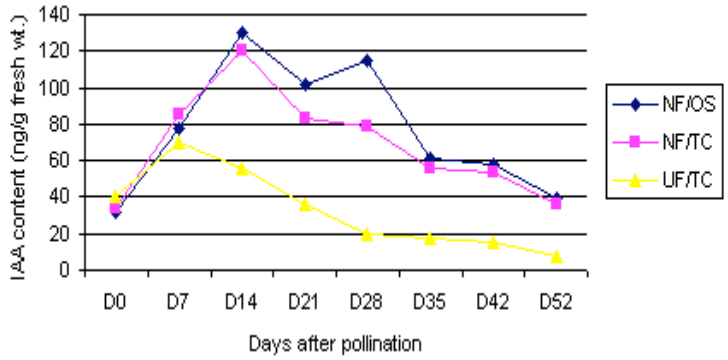


Fig. 2: GA content in 'Barhee' during early fruit growth

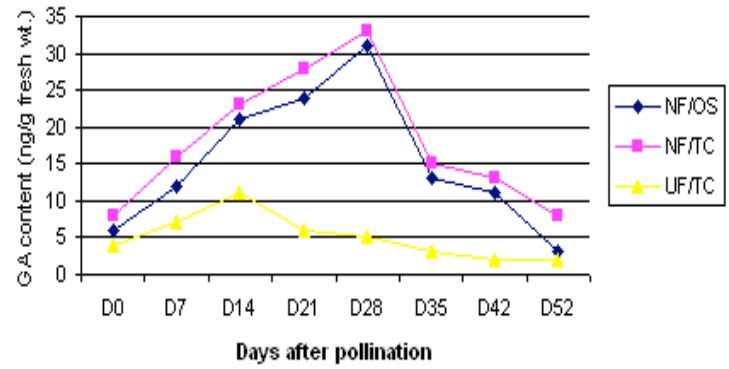
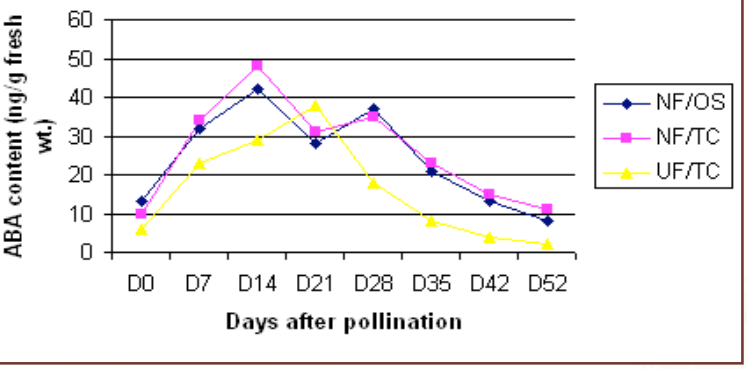
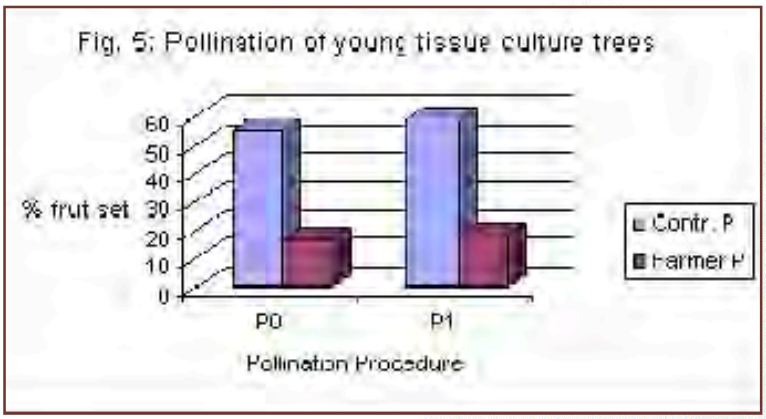
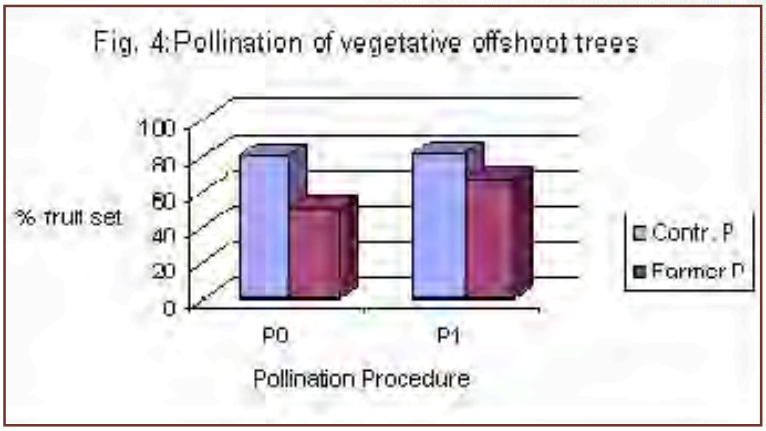
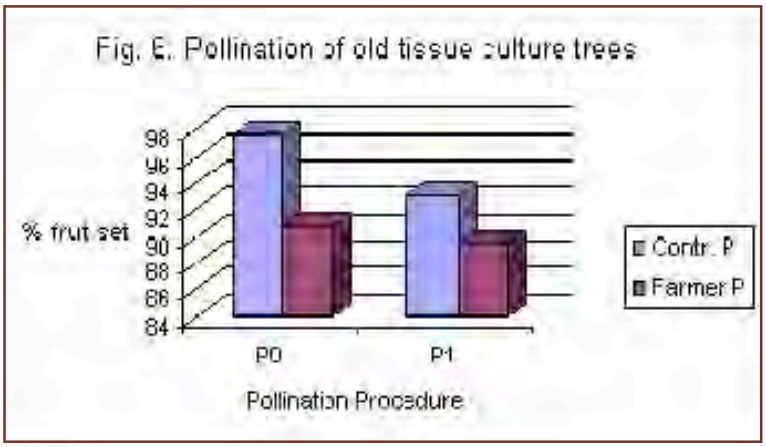


Fig. 3: ABA content in 'Barhee' during early fruit growth









Pollination of forced open female spathe (P0) in vegetative offshoot trees in site (1) increased normal fruiting to 79.1% compared to farmer practice (51.4%) (Fig. 4). Pollination 3-5 days in same site after natural opening of spathe (P1) provided 76.4% and 61.2% normal fruiting by controlled and farmer practices, respectively. Forced pollination (P0) in young tissue culture trees reduced abnormal fruiting by controlled and farmer practices 41.5% and 18.5%, respectively (Fig. 5). Relatively similar results were observed when pollination was conducted 3- 5 days after female spathe natural opening. Older tissue culture trees in site (2) under forced opening of spathe by controlled method and farmer practice resulted in 95.3% and 90.2% normal fruiting, respectively (Fig. 6). Pollination after 3- 5 days of naturally opened spathes (P1) of similar trees, provided 92.3% and 86.1% normal fruits with controlled method and farmer practice, respectively. These results were obtained during the first season of the study. During the second season, the results are much lower for all trees. Such inconsistency has cast some doubt on the reliability of such approach.

An overall relation between the hormonal contents and the different reproductive stages after pollination apparently exist. However, the failure of normal fruiting in young tissue culture trees was probably due to many interrelated events that lead to a slow growth of pollen tube at early stages of fruit growth and which may possibly be accentuated by the relatively high ABA contents during this period.

LITERATURE REVIEW

Albrigo, L. G. and V. G. Sauco, 2004. Flower bud induction, flowering and fruit set of some tropical and subtropical fruit tree crops with special referen - ce to citrus. Acta Horticulturae 632. XXVI International Horticultural Congress: Citrus and other subtropical and tropical fruit crops: Issues, advan - ces and opportunities.

Crane, J. C., 1964. Growth substances in fruit setting and development. Ann. Rev. Plant Physiol., 15: 303-326.

Frankhauser, F. and R. Schumacher, 1984. Influence of growth regulators on fruit development. Acta Horticulturae, 149: 121- 134.

Goldwin, G. K., 1984. Factors affecting hormone-assisted setting of Cox's apple. Acta Horticulturae, 149: 96-108.

Gustafson, F. G., 1936. Inducement of fruit development by growth promo - ting chemicals. Proc. Nat. Acad. Sci. 22: 628-636.

Gustafson, F. G., 1939. The cause of natural parthenocarpy. Amer. J. Bot. 24:102-107.

Hassaballa, I. A., 1964. Growth substances as related to fruit set and develop - ment of seedy and non-seedy cultivars of citrus. Ph.D. Dissertation, Univ. of Fla., Fla, 131pp.

Krezdorn, A. H., 1969. The use of growth regulators to improve fruit set in citrus. Proc. First Int. Citrus Symposium, 3:1113- 1119.

Leopold, A. C., 1964. Plant growth and development. McGraw-Hill Book Co., Inc., New York., pp. 271, 305, 349, 375 and 466.

Marcelle, R. D., 1984. Effects of GA3 and growth retardants on fruit set in pear cultivar 'Doyenne Du Comice'. Acta Horticulturae, 149: 151- 158.

Martinez-Fuentes, A., C. Mesejo, M. Juan, V. Almela and M. Agusti, 2004. Restrictions on the exogenous control of flowering in citrus. Acta Horticulturae 632. XXVI International Horticultural Congress: Citrus and other subtropical and tropical fruit crops: Issues, advances and opportunities.

Nitsch, J. O., 1950. Growth and morphogenesis of the strawberry as related to auxin. Amer. J. Bot., 37: 211-215.

Sastry, K. S. K. and R. M. Muir, 1963. Gibberellin: Effect on diffusible auxin in fruit development. Science, 140: 491-495.

Webster, A. D., 1984. The effect of plant growth regulator sprays and summer shoot tipping on the fruit set and yield of young plum trees. Acta Horticulturae, 149: 189-197.

Wheaton, T. A. and M. C. Bausher, 1977. S

2005

Proceedings of The International Workshop on True-To-Typeness of Date Palm Tissue Culture-Derived Plants