جامعة الدول العربية
المنظمة العربية للتنمية الزراعية
League of Arab States
Arab Organization For Agricultural Development

تقرير فني
عن
زراعة انسجة نخيل التمر
بدولة الكويت

الخرطوم — يونيو (حزيران) 1995

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المحتويات

الصفحة

1- تقديم

دعما لمسيرة التنمية الزراعية فى الوطن العربى وتلبية للطلب الذى تقدمت به الهيئة العامة لشئون الزراعة والثروة السمكية بدولة الكويت قامت المنظمة العربية للتنمية الزراعية بإيفاد الدكتور عبدالوهاب زايد استاذ بقسم البيولوجيا بكلية العلوم جامعة القاضى عياض بالمملكة المغربية وذلك لتقديم الإستشارة الفنية فى مجال زراعة الأنسجة النباتية فى دولة الكويت .

هذا وقد قام الخبير بزيارة دولة الكويت خلال الفترة من 8 ابريل وحتى 8 مايو 1994 حيث اجرى خلالها العديد من المقابلات مع المسئولين للتعرف على الوضعية الحالية فى مجال زراعة انسجة النخيل وكذلك المشاكل التى تواجه الإكثار السريع.

وفى ختام زيارته اعد تقريرا وافيا تضمن لمحة عن زراعة الأنسجة وأهمية زراعتها بالنسبة لنخيل التمر والمراحل الخاصة بزراعة انسجة النخيل ، كما قدم التوصيات الخاصة بالإكثار السريع بنخيل التمر والذى أكد فيه على اهمية الزيارات للمختبرات الدولية المختصة فى هذا المجال بالمملكة المغربية وفرنسا وإنجلترا وكذلك ارسال تقنيين للتدريب على اساليب العمل فى تلك المختبرات ونقل تلك التجارب بما يتناسب والواقع الزراعى فى دولة الكويت .

كما قام الخبير بزيارة اخرى لدولة الكويت خلال الفترة من 28 سبتمبر وحتى 14 اكتوبر 1994 للتعرف على الإمكانات المتاحة للأنشطة المختبرية فى تلك المجالات الحيوية وتحديد معوقات العمل وإعداد مجموعة من التوصيات للتغلب عليها .

وفى الختام اود ان اعرب عن شكرى وتقديرى لمعالى المهندس فهد عبدالله الحساوى رئيس مجلس الإدارة والمدير العام بالهيئة العامة لشئون الزراعة والثروة السمكية والمسئولين لما قدموه من معلومات وتسهيلات ساعدت على إنجاز مهمة الخبير .

والشكر للخبير الدكتور عبدالوهاب زايد على الجهد الذى بذله فى القيام بالمهمة.

المدير العام

الدكتور يحيى بكور

المقدمة

تلبية لطلب الهيئة العامة لشئون الزراعة والثروة السمكية – دولة الكويت ، قامت المنظمة العربية للتنمية الزراعية بإيفاد الدكتور عبدالوهاب زايد الخبير فى مجال زراعة الأنسجة النباتية ، للقيام بمهمة إستشارية فى مجالات الإكثار السريع لنخيل التمر عن طريق زراعة الأنسجة وقد تمثلت أهداف المهمة الإستشارية فيما يلى :

- الوقوف على الوضعية الحالية فى مجال زراعة انسجة النخيل .
- التعرف على المشاكل التقنينية المواجهة واقتراح الحلول الناجحة من اجل الإكثار السريع .

فبعد وصول الخبير الى مدينة الكويت استقبل مباشرة السيد نائب المدير العام لشئون التنمية الزراعية بالهيئة العامة لشئون الزراعة والثروة السمكية ، حيث تم إطلاعه على الأولويات المرجوة من هذه الزيارة وكذلك استمع الى المشاكل التى تواجه نخيل التمر .

حضر هذا اللقاء مدير ادارة البحوث النباتية ، و مدير ادارة العلاقات الخارجية ، مراقب النخيل والأشجار المثمره ، رئيس المختبر لزراعة الأنسجة ، والباحثات بنفس المختبر .

بدأ العمل بالمختبر يوم السبت 9 ابريل 1994 حيث تم اعطاء تدريب خاص يشمل كل مراحل الزرع الأولية من تكوين الوسط الزراعى المناسبة ، وعملية التعقيم والزرع داخل الأنابيب ... الخ) . وذلك بإستعمال فسائل ماخوذة من الأصناف الموجودة فى الكويت (خلاص – جبجاب ، برحى ، قنطار ، سعمران ، ساير ، خضراوى ، زاهدى ، شكر) .

ولقد تم إعداد المواصفات التقنية لكل مرحلة كما هو موضح فى الملحق باللغة الإنجليزية .

وفى نهاية المهمة الإستشارية قام الخبير بتقديم تقريره الفنى الذى تمت مناقشته والموافقة عليه من قبل المسئولين قبل رفعه بصفة نهائية الى الإدارة العامة بالمنظمة العربية للتنمية الزراعية .

2- لمحة عن زراعة الأنسجة النباتية :

زراعة الأنسجة أو الإكثار الخضرى السريع هى مصطلحات لعملية الإكثار السريعة بواسطة زراعة أعضاء أو أنسجة او خلايا معزولة على أوساط زرعية خارج الكائن الحى مختبريا وتحت ظروف محكمة ومعقمة .

تتطلب زراعة الأنسجة توفير عوامل محددة كطبيعة الأوساط الزرعية المستعملة ، كما تسمح بدراسة التغيرات التلقائية ، أو التى تحدث تحت تأثير بعض العوامل كالعناصر الغذائية ، أو محفزات النمو أو الضوء أو درجة الحرارة ... الخ .

ومزايا زراعة الأنسجة كثيرة يمكنها ان تحقق الأهداف التالية :

* تكثير الأصناف ذات الجودة العالية أو النباتات التى فى طريق الإنقراض .
* الحصول على نباتات سليمة من الأمراض .
* التهجين والحصول على أصناف جديدة لم تكن موجودة سابقاً .
* وضع مقاييس للتأكد من التطابق الوراثى للنبات الناتج عن زراعة الأنسجة.

لقد نال موضوع زراعة الأنسجة اهتماما كبيرا فى السنوات الأخيرة وللوصول الى الأهداف المرجوة سواء كان الهدف التكاثر او انتاج سلالات خالية من الأمراض فإن هناك ثلاثة مراحل للوصول الى زراعة ناجحة :

أ - الحصول على مزرعة معقمة وتعتبر أهم مراحل زراعة الأنسجة .

ب - زيادة عدد الخلايا داخل المزرعة ويتم ذلك عن طريق تكوين أعضاء عرضية أو تشجيع نمو الخلايا الى أجنة عرضية .

ج - الأعداد لزراعة النباتات فى التربة .

3- أهمية زراعة الأنسجة بالنسبة لنخيل التمر :

إن تعميم الأنواع الجيدة من نخيل التمر يبقى مرتبطاً بطريقة اكثارها ، فالفسائل المنتجة طبيعيا لا تفى بالحاجة الماسة والكبيرة التى تتطلب اعدادا هائلة لغرس المساحات الشاسعة وتستغرق وقتا طويلا على مدى اجيال . وهكذا اصبح من الأولويات ايجاد طريقة سريعة وفعالة لإكثار النخيل بواسطة زراعة الأنسجة التى تسمح وفى وقت قصير بإنتاج اعداد كثيرة من الفسائل ذات الجودة العالية والخالية من الأمراض دون أى خطر للعدوى التى يمكن ان تحصل عند اتباع الطرق التقليدية .

رغم أن مختبرات متعددة حاولت اكثار النخيل بإستعمال تقنيات زراعة الأنسجة ، فإن نجاحا محدودا قد حصل عليه حيث اصبح من الممكن اكثار النخيل عن طريق تكوين الجنين اللاجنسى (Asexual embryogenesis).أو بواسطة تكوين الأعضاء Organogenesis.

يجب الإشارة هنا أن طريقة الجنين اللاجنسى المستعملة من طرف بعض الشركات تمر بمرحلة الكالس المحصل عليه بإستعمال محفزات نمو مثل (2,4 - D) وذلك بكميات عالية غير انها معروفة بخصائلها كمبيدة للنبات (Herbicide) وذات قدرة عالية فى مجال التغيير الجنيني (Mutations) .. فالكالس هو مجموعة من الخلايا غير متميزة وغير منتظمة ويمكنها ان تعطى طفرات غير مطابقة للأصل .. وهذا طبعاً يشكل خطراً على عملية الإكثار السريع لنخيل التمر .

أما طريقة تكوين الأعضاء (Organogenesis) فهى لا تستعمل الكالس فى عمليتها وتتجنب كل محفزات النمو المعروفة بقدرتها المتغيرة ، فمن الناحية الفسيولوجيه فإننا نساعد فقط على نمو البراعم الموجودة فى اسفل الوريقيات المحيطة بالقمة النامية لنخيل التمر وهذا طبعا يعطينا تطابقا للأصل بدون أى احتمال للتغير كما قد يحدث فى طريقة الجنين اللاجنسى .

4- مراحل زراعة انسجة النخيل عن طريق تكوين الأعضاء (Organogenesis):

يجب الإشارة هنا ان هذه المراحل تم تفصيلها ودراستها بدقة فى التقرير الفنى (المرفق طيه) المعد باللغة الإنجليزية ، فهنا سنكتفى بسرد كافة المراحل وتعريفها بإيجاز

قد دلت النتائج الأولية ان الخلايا المرستيمية المعزولة من القمة النامية هى أكثر اجزاء النخلة صلاحية لإجراء عملية الإكثار السريع .

بدء الزراعة يحصل عليه عن طريق جزء مأخوذ من قلب الفسيلة ، وهذا الجزء البدائى يتمثل اما فى اسفل الأوراق البدائية او النسيج الرطب او البراعم العرضية او البرعم القممى .

زرع الجزء البدائى يتم على وسط زراعى معزز بعدة أوكسينات وسيتوكينات ، وعندما يبدأ هذا الجزء فى إعطاء براعم فإننا نقوم بمرحلة الإكثار حيث يقطع الى أجزاء صغيرة تعطى هى أيضاً براعم اخرى وهكذا ، تجزئة الأطراف الإبتدائية (الى اكثر من 4 أجزاء) تتم على فترات منتظمة (اربع اسابيع تقريبا) وكل جزء تعاد زراعته يمكن ان يعطى 4 براعم وهذه تعطى بدورها اربعة اخرى جديدة وهكذا .

بعد مرحلة الإكثار يتم عزل كل البراعم وزرعها فى وسط زرعى مخالف للأول وذلك من أجل تقويتها وإنتاج جذور كافية من أجل نقل النبات المحصل عليه الى التربة حيث يتم تأقلمه ، ويجب الإشارة هنا أن التكاثر عن طريق زراعة الأنسجة يعتبر ناجحا اذا انتهى بنجاح نقل نبات الأنبوبة المعقمة الى التربة .

5- الخلاصة والتوصيات :

1- يعتبر مشروع الإكثار السريع لنخيل التمر عن طريق زراعة الأنسجة مشروعا اساسيا وضروريا للنهوض بالزراعة والقفز بها الى الأمام فى دولة الكويت فالمجهودات المبذولة من طرف الهيئة العامة لشئون الزراعة والثروة السمكية فى هذا المجال تدعو الى الإطمئنان وتبشر بالخير حيث انه وقع اخيرا رصد ما يناهز مائة ألف دولار أمريكى لتوسيع المختبر زيادة عن المعدات ولوازم زراعة الأنسجة الموجودة حاليا او التى هى فى طريق التسليم ، يجب الإشارة هنا ان جهود المسئولين فى الهيئة ستسهل من مهام الباحثين الموجودين فى المختبر وذلك من أجل الوصول الى الأهداف المنشودة فى المستقبل القريب عبر انتاج وتلبية الطلب الوطنى من فسائل ذات جودة عالية وخالية من الأمراض.

2- كما سبق ذكره فإن اكثار نخيل التمر يمكن ان يتم بإستعمال طريقتين مختلفتين اولهما الجنين اللاجنسى (Asexual embryogenesis) وطريقة تكوين الأعضاء (Organogenesis)كما بجب التأكيد هنا أن الطريقة الأولى تمر بمرحلة الكالس (Callus) ويستعمل محفز النمو (2,4-D) المعروف بدوره فى إنشاء الطفرات (Mutations) فالكالس الذى هو مجموعة من الخلايا غير المتميزة وغير المتخصصة يمكن ان يعطينا نبات غير مطابق للأصل ، وهذا يشكل خطرا على عملية الإكثار السريع لنخيل التمر .

أما الطريقة الثانية (Organogenesis) المتبعة حاليا من طرف مختبر الهيئة العامة لشئون الزراعة والثروة السمكية والتى دأب الخبير على تلقينها للباحثين فى المختبر فهى لا تمر بالكالس وتتجنب استعمال محفزات نمو ذات قدرة على تغير المركبات الجينية ، فمن الناحية الفيسيولوجيه هذه الطريقة

تساعـــد البراعم الموجودة اصليا فى اسفل الوريقات المحيطة بالقمة النامية لنخيل التمر على النمو وهذا طبعا يعطينا تطابقا للأصل بدون أى احتمال للتغير كما قد يحدث عند استعمال طريقة الجنين اللاجنسى .

يجب الإشارة هنا أن اى استيراد لنبات نخيل التمر المحصل عليه عن طريقة الجنين اللاجنسى يشكل خطراً من حيث التطابق الوراثى الا اذا تم الأدلاء بشهادة علمية تؤكد مطابقته للنخلة الأم .

3- قد تم القضاء نهائيا على مشكلة التلوث الذى كانت تواجه المختبر ، فلمدة شهر كامل لم يحصل اى تلوث لكل الأنواع وهذا ناتج عن الإحتياطات الجديدة المقترحة من طرف الخبير (استعمال تقنية التعقيم تحت الضغط) . أنواع النخيل التى تم زرعها من " البرحى ، قنطار ، الخلاص ، سكر ، سعمران ، زهدى ، شبشاب ، وخضراوى .

4- برنامج العمل :

بعد الدراسة والمناقشة اتفق الخبير مع رئيسة المختبر والكوادر المساعدة لها على برنامج عمل يمكنهم من الوصول الى الأهداف التالية :

أ - بحث تقنيات زراعة الأنسجة وتطبيقها على الأصناف والأنواع المحلية المختارة

ب - وضع مقاييس للتأكد من التطابق الوراثى للنباتات المحصل عليها .

ج - تكثير الأصناف والانواع ذات الجودة العالية .

د - دراسة مرحلة التأقلم والأعداد لزراعة النباتات فى التربة .

5- التدريب والتعاون :

للوصول الى الأهداف السابقة الذكر وخاصة الإكثار السريع لنخيل التمر حتى يتم تلبية الطلب الهائل من الفسائل فى دولة الكويت ، يقترح الخبير الزيارات ومجالات التدريب التالية :

أ- قيام المسئول عن برنامج النخيل برفقة رئيسة المختبر بزيارة لبعض المختبرات الدولية المختصة فى إكثار النخيل بالمملكة المغربية ، فرنسا وإنجلترا وذلك من أجل :

* الإطلاع على نتائج التقنيات المستعملة وخاصة عمليات دراسة التطابق الوراثى والطرق العلمية المستعملة من أجل ذلك .
* ربط علاقات تعاون مع أحسن هذه المختبرات للإستفادة من تجربتها وذلك من أجل ارسال تقنيين اليها من اجل التدريب .

ب - توظيف تقنيين وارسالهما الى المختبر المنتقى من طرف البعثة الأولى حتى يتمكنا من التدريب فى هذا المجال .

ج - التعامل مع خبير فى زراعة انسجة النخيل وذلك لفترات قصيرة حيث تمكنه من إتباع كل المراحل الأساسية لطريقة تكوين الأعضاء (Oganogenesis) والإكثار السريع لأجود الأنواع الموجودة بدولة الكويت.

د - تشجيع المسوؤلين والباحثين فى مجال زراعة أنسجة النخيل للمشاركة فى الندوات الجهوية والدولية كما يجب تزويدهم بمكتبة ونشرات علمية متخصصة فى هذا المجال .

6- بعد عملية التوسيع التى يجب ان يراعى أثنائها المواصفات المقترحة ، وبعد أقتناء كل المعدات والمواد اللازمة ، وأيضا بعد تتبع كل التوصيات التقنية المشار اليها فى التقرير سيصبح المختبر من أحسن الوحدات الموجودة حاليا وبإمكانه انتاج ما بين 50,000 و 75,000 نخلة فى السنة .

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Report
Arab Organization for Agricultural Development Technical
On Date Palm
In Vitro Progpagation
In The State of Kuwait

Khartoum

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Report On Consultancy Mission On Date Palm In Vitro Propagation In The State of Kuwait

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A list of all persons met is to be found in Annex 1. They were helpful in taking time to give the consultant most relevant information.

Introduction:

In response to the request of the Government of Kuwait, Date Palm Tissue Culture Specialist, Dr. A. Zaid was recruited by the Arab Organization for Agricultural Development, to undertake a consultancy mission on the In Vitro Propagation of Date Palm in PAAF. More specifically his terms of reference were to :

- Assess the present situation of Date Palm in vitro propagation.

- Identify key technical constraints and make appropriate recommendations to overcome them.

- Toward the end of the mission, prepare a consultancy report, to be technicaly cleared with PAAF prior to consultant departure. The report to be forwarded to AOAD Headquarters for final technical clearance and acceptance prior of official submission to the Government of Kuwait.

The mission was scheduled for the period from April 8, 1994 till May 8, 1994. Shortly after arrival, the consultant, was briefed by DDG. PAAF. Mr. A. Al-Nakeeb and other officials.

The laboratory work started on April 9, 1994 at the Tissue Culture Laboratory located at PAAF in Al-Rabiya District. On May 2, 1994, a technical Seminar was held about the tissue culture techniques under the auspices of Eng. Fahad A. Al-Hasawi the Chairman & Director General for the Public Authority for Agriculture & Fish Resources and his deputies and PAAF Technical staff and from KISR as well as from private sector involved in agricultural activities in Kuwait.

The main findings, conclusions and recommendations were presented in a debriefing session, to the DDG. PAAF as well to other officials and seniors members of PAAF. Attached in Annex 1 listing of all people the consultant met.

Main Findings

1- Tissue Culture Laboratory

1.1 General:

The Tissue Culture Laboratory located in Al-Rabiya District is the only laboratory belonging to PAAF that is in charge of carrying out research on Date Palm Propagation using In Vitro techniques. However, there are also two units, one in Kuwait Institute for Scientific Research (KISR) and the other one in Kuwait University (K.U.) which are exclusively doing asexual embryogenesis. The research work started in the early 1987. Although the efforts are really impressive, they have not yet resulted in the production of Date Palm Plantlets derived from Tissue Culture. The following reasons are behind this situation:

- The Iraqi Invasion to Kuwait was deeply harmful to the programme .

- The use of inappropriate media for Date Palm and adequate techniques for in vitro propagation are lacking or not well practised.

- The lack of a solid background in plant tissue culture.

The consultant, accopmanied all the time by the Head of the Tissue Culture laboratory Miss. Salwa Sultan, and all the laboratory researchers, undertook a deep study of the laboratory facilities, scientific equipment and techniques used to in Vitro propagate Date Palm.

The study was carried out according to a well organised programme which allowed a precise understanding of major constraints and provided a good opportunity for fruitful and interesting discussions with the Head of Lab. and scientists regarding the improvement of techniques used to mass propagate Date Palm.

1.2 Tissue Culture Facility:

For a Tissue Culture mass production unit, the Al-Rabiya laboratory is too small even though it does have the basic installation and equipment needed. A project to build an extension work space is underway. After a review by the consultant, the design of the extension facility is presented in Annex 2, with the assistance of the Project Manager of Al-Wafra Centre, Eng. Aziz Abou-Amarah for the most adopted specifications and requirements.

The actual facility does not have a reception room, once the new aseptic block is functional, a restoration of the old building is highly recommended (see annex 2). The reception room as proposed is necessary and will allow to work in semi sterile conditions. Plant material (Date Palm offshoots) will be cut and dissected without any harm to the aseptic conditions required in the new laboratory.

The central room in the new facility, is one of the most important part of this unit as the basis for the whole tissue culture will be prepared in. It should be provided with ample sinks, hot and cold water, double distilled and deionized water, equipments needed to prepare and distribute the nutrient media and several cupboards to store sterilising agents, glassware and instruments. Vacuum pump and well designed work benches are essential.

The water system for the new facility should pass first through a sandy filter with softener and purifier, which is actually missing in the old building. Such recommendation will improve the water quality and consequently will increase the life span of filters used in various equipment's (double distilled water apparatus, autoclave, ... etc.) as the water in Kuwait is very active and contains ingredients which are not wanted for such research work.

The new transfer room should be kept clean and dust proof in order to prevent any contamination. The room should be entered only through an air lock. It should be fitted with a sliding door with no windows to ensure that no drafts air to circulate . The room should also be air conditioned and equipped with laminar flow hoods. It should be fitted with cupboards to store instruments for incubation or transfer. A special cupboard would be useful to store media to incubate. A work bench is essential.

The two new culture rooms should be completely isolated and maintain constant temperature. The whole laboratory should be positive air pressure in order to prevent any contamination. Dust was found abundant in the old laboratory, mainly in the existing culture room, air conditioning filters to be changed and cleaned frequently. The consultant had checked such situtation by placing open sterilised agar petri-dishes for various periods in the working space of the laminar hood and in the existing culture room. Fungi were easily detected within 72 hours. Filters of the existing central air condition unit need to be changed as soon as possible.

There is one horizontal autoclave in good working conditions. It was noticed by consultant that glassware and test tubes were cleaned in the actual central room. In order to provide aseptic conditions in the facility, it is recommended that all washing to be done in the future autoclaving room, which hence could be provided with two large sinks for cold and hot water. Clean and dry glassware and instruments could then be transferred to the central room.

Every room in the old and new facility will have its own function and unnecessary traffic between rooms should be avoided in order to reduce the risk of wide-spread contamination. A high standard of cleanliness requires not only a high standard of work by scientists, but adequate daily cleaning of the facility. Visits and access to facility should be restricted and smoking prohibited.

As mentioned earlier, the new laboratory should be equipped with a positive air pressure which will not allow the entrance of any particle from the main entrance.

1.3 Staff of the Tissue Culture laboratory:

The staffing sturcture is adequate for the actual required activities. However within one year from now, when the multiplication phase is researched for most Date Palm varieties, the hiring of two technicians is recommended.

Except the head of the unit and another scientist, the two remaining staff need first hand training on in Vitro Culture Techniques of Date Palm.

2- Techniques for Date Palm Micropropagation:

Though several attempts have been made by the Tissue Culture Laboratory to in vitro propagate date palm, results were not satisfactory, because of difficulties due to specific characteristics of date palm and high contamination rate obtained (mainly after invasion).

At the time of the consultant's visit, there was no culture at all of date palm in the laboratory. With the advise of the consultant, the laboratory staff was able to introduce various date palm varieties with no contamination problems (up to the end of the mission, 8/5/1994).

Date palm plantlets may be produced through either asexual embryogenesis, i.e. initiation and germination of somatic embryos from callus (or directly on the explant), or Organogenesis. i.e. the flush of buds from meristematic potential of the axil buttom of young leaves.

Maintaining genetic stability is crucial for date palm propagation; hence organogenesis technique is described and consequently proposed by the consultant . In fact, plantlets regeneration from somatic embryos may not be a consistent method for date palm mass production, because of the risk of somaclonal variation induced in vitro.

Organogenesis technique is made of four steps: Initiation of meristematic buds, multiplication, elongation and rooting. The success of such technique is tremendously dependent on the success of the first step (initiation). Furthermore, various problems met at other steps levels have their origin at the initiation phase.

Discussions and interactions with the head of the laboratory and her staff pertaining to their experience and technical problems encountered, has led the consultant to study and initiate the date palm micropropagation (nutrient media composition, varietal response,explant type, desinfection technique and incubation conditions). The following steps and recommendations are to be correctly applied.

2.1 Source of explant material:

Offshoots taken off adult palm trees of selected varieties are the chosen plant material. The offshoot's weight should be higher than (or at least equal to) five (5) kilograms. Using a knife of tapestry maker, or sometimes a hatched, bottom of palms (and offshoot's roots if existing) are gently cut and discarted. We should end up with an offshoot of 15 to 20 cm in diameter for its larger part (the base) and 40 to 50 cm in length.

The best period for starting in vitro culture with date palm offshoot is from the end date of fruits harvesting, to just before the beginning of the flowering (it vary from a variety to another; however, we can safely work between December till March).

When separating offshoots from their mother plant, be careful not to damage the offshoot's base and not to make it explode when pulling it. In such cases, bacteria and other microorganisms could penetrate to the " Heart " of the offshoot (the meristematic area) and all in vitro explants taken form that damaged offshoot will be contaminated regardless of any used desinfection technique.

When you reach the meristematic area (the soft part) be careful not to break it (mainly between the lighnified part of the base and the soft tissue). Hence, you must leave this small lignified base in order to avoid such " break". The final sizes are about 3 to 4 cm in width and 6 to 8 cm in length (see fig.2).

2.2 Desinfection tehniques:

The soft part with its lignified base, is then soaked in anti-oxidant solution of 150 mg/1 of citric acid and 100 mg/L of citric acid and 100 mg/L of ascorbic acid while waiting for the other steps (this is only one offshoot, you can skip it). This pre-soaking is mainly to avoid tissue browning.

The next step is desinfection of the offshoot's heart: 4g/1 of Mancozan (or any other fungal solution) during 20mn with few drops of teepol, then rinse it 3 times with autoclaved-distilled water, then dip it into 9% sodium hypochlorite solution under a weak vacuum during 5 mn but with some sudden breaks of vacuum, and the under normal pressure during 20 mn.

2.3 Explants culture:

Under an air laminar flow-hood, with triple rinsing, the offshoot's heart is dissected and put on the Initiation culture medium. The bottom of young leaves, mostly the axillary part between the leave and underlaying tissue, is put in culture. The top end of these leaves is cut and discarted.

At the bottom of young leaves some very little axillary buds are often visible. It happens also to find some axillary buds when the older leaves are cut out. All these buds are put in culture, and should be cut in two parts.

When the cluster of very little leaves of the apex is reached, it may be difficult, without use of binocular, to take off leaf by leaf. So, at this point of the process, the little leaves are taken off several together. At the end, the cluster of leaves gets so little, that is better to cut it at the right angle in two or four parts, and put in culture.

One offshoot, as described here, may give young leaves or axillary buds sufficiently for starting with fifteen (15) to twenty five (25) culture tubes.

2.4 Conditions of cultures incubation:

At the beginning, cultures are put in darkness, but after two to three months from introduction's date, a weak light should be used (16/8 hours photoperiod).

The cullture room temperature would be 27 c +1 c during 16 hours of light, and 20 to 22 c, during the 8 remaining hours. Thermoperiod is better than constant temperature. It seems expedient to transfer cultures on a fresh medium each month, mostly in the beginning, even if no growth is visible.

The culture process as mentioned earlier, includes the four following steps: Initiation, Multiplication, Elongation and rooting. Each step requires a special medium composition mainly growth regulators (nature and concentrations).

2.5 Initiation Medium:

This point is very important , and if we have knowledge of the main works on in vitro culture of date palm, we Know that a great number of different media have been tested. however, few have given some interesting results.

The initial medium used was Murashige and Skoog (1962) at full strength; the high level of ammonium salts in such medium has caused several problems with date palm (mainly the formation of vitrified buds). To avoid such problem, half MS strength was tried but with no improvement. The best results are actually obtained with a special formula (cf.Annex 3).

When your purpose is to obtain a rapid growth of undifferenciated tissues, the use of growth regulators is without problems. But, when you must obtain the proliferation of cuttings by in vitro culture, the use of such substances is not so easy. For the date palm initiation phase we must avoid to obtain cuttings showing no conformity to the mother plants. The aim is to simulate the flush of young buds from meristematic areas. All growth substances used should have their concentration level between 0.1 to 3 mg/1 (cf. Annex 2) . The PH adjusted to 5.7+0.1 (after adding the agar and just before autoclaving).

We have to mention that each variety of date palm requires little modification concerning the growth substances balance.

Normally after four to six months (rarely sooner and sometimes more, depending on the variety), the bottom of young leaves put in culture, gives some signs that budding is beginning (not yet buds) (cf. figure 3).

Hence, and immediately after the appearance of these budding signs, cultures are transferred to the multiplication medium and young plantlets are obtained (cf. Figure 4).

2.6 Problem of browning of tissues and media:

During the course of growth and development in vitro, mainly at the initiation phase, date palm explants not only deplete the nutrients that are furnished in the medium, but also release substances that accumulate in the cultures. These substances, such as phenols, have profound physiological effects on the cultured cultures. Browning of the tissues and the adjacent medium is assumed to be due to the oxidation of polyphenols and formation of guinones which are highly reactive and toxic to the tissues.

Taken together the results obtained by date palm tissue culturists, we recommend the following in order to overcome such problem:

- Pre- soaking of explants in antioxidant solution of 150 mg/1 citric acid and 100 mg/1 ascorbic acid.

- Employing small explants, and reculturing them to fresh medium after a short period of incubation.
- If necessary, use activated charcoal (2 to 3 g/1) for a short period. supply a high concentration of growth regulators since activated charcoal reduce their availability to the explants.

Conclusions and Recommendations

The following brief account summarizes the main observations made, conclusions drawn and recommendations framed.

1- Situation of date palm plantations:

Date palm plantations of Kuwait are estimated of about 300,000 trees and there is a huge space to increase the number of palms. an urgent need of approximately one million palms is well recognized by both Government Officials and Farmers.

According to data collected during the consultant's visit, all date palm plantations used to be free of important diseases and from serious pests. However and after the invasion, some problems such as the black scorch and Parlatoria blanchardii arised.Hence, special attention should be given in order to eliminate such hinderance and good sanitation is the first step in the control of black scorch disease. The affected fronds, leaf bases and inflorescences should be pruned, collected and immediately burned. Spraying with the following fungicides (Bordeaux mixture, lime- suplhur solution, dichlone, Thiram) is recommended. It is also highly recommended that no offshoot importation should take place from infected countries. Careful attetion should also be given to the origin of soil and sand imported from neighbouring countries.

2- Importance of in vitro mass propagation of date palm:

The rapid propagation of date palm, as well as propagation from a mature specimen, is impossible due to the limited number of offshoots and the fact that offshoot production is limited to a certain period in the palm's life span. Seed propagation is impractical for several reasons. Half of the progeny will be males and half will be females. Such seedling females usually produce late maturing fruits of variable and generally inferior quality to that of established clonal trees.

Micropropagation in vitro appears to be the sole solution that will have considerable potential for date palm propogation and production in the State of Kuwait. In fact, it will solve the problem of offshoots availability and will allow the rapid establishment of large scale commercial date production in Kuwait.

Importing date palm plantlets from foreign tissue culture laboratories proved to be very expenssive with a certain amount of RISK as to the quality and survival of plants. Hence the development and functioning of the Al-Rabiyah tissue culture laboratory constitute the only, but wise way to satisfy the large demand of date palm offshoots in Kuwait.

3-Tissure Culture Laboratory:

The tissue culture laboratory situated at Al-Rabiya, is the only facility of the Public Authority for Agriculture and Fish Resources that is in charge of carrying out research on date palm mass production using in vitro techniques. Although, the efforts are impressive, they have not yet resulted in the production of date palm plantlets. The laboratory still needs some scientific equipment and supplies. In order to have such facility working efficiently, the work on the new extension (Annex 2) is urgently recommended as well as the purchase of items described in Annex 4.

Based on the already existing facility, and when the new building is functional, the consultant estimates a yearly production of approximately 75,000 date palm plantlets ready for planting in the nursery (cf. figure 1).

A research programme focussing on the organogenesis technique was already initiated by the consultant. Various varities such as Barhee, Saamaran, Kentar, Khadraoui, Lakhless, sugar, chabchab and Zahdi were introduced into tissue clture conditions.

4- Tissue Culture Techniques:

A major technical problem which is hindering the well functioning of the facility and consequently the rapid propogation of date palm in Kuwait, is the lack of Know-how and practical in vitro techniques of date palm propogation. None of the people in the laboratory, except the head of the unit and one scientist, have been trained in date palm micropropagation.

Regarding this matter, and since adequate date palm in vitro techniques are lacking or not well practiced in the Al-Rabiya Laboratory, the consultant has presented in this report, full detailed techniques and recommendations to follow.

5- Training and cooperation:

Because of the above, and in order to successfully establish the date palm mass propagation in Kuwait, the following study tour and training are recommended:

- The supervisor of date palm and fruit trees programme accompanied with the head of tissue culture laboratory, should undertake a study tour in some of the foreign date palm laboratories (Morocco, France, England and Canada) in order:

  * To establish relations with researchers and institutions and discuss the possibility of collaboration and material exchange.

* To select most suitable foreign laboratory where Kuwaiti scientists and technical staff could be trained.

- Two technicians need to be hired and trained in a foreign tissue culture laboratory (where commercial production of date palm is established) for a period of at least one month.

- The services of a date palm tissue culture specialist for a period of two months, in order to give the staff first hand training on all steps, are necessary.

- Laboratory staff should be encouraged to attend regional and international symposia on date palm tissue culture. A library containing specialised journals and books should also be provided in the laboratory.

Finally, it is strongly urged that the date project in Kuwait be vigorously encouraged. The prospects for the mass propagation of the date palm are good. AOAD assistance should focus on providing both technical and scientific skills.

Annex 1 : List of the Government and AOAD Staff met:

The Government of Kuwait

- Eng. Fahad A. Al-Hasawi
  Chairman & Director General

The Public Authority for Agriculture Affairs and Fish Resources.

- Mr. Ahmad Al-Nakeeb

Deputy Director General for Agriculture Development
The Public Authority for Agriculture Affairs and fish Resources.

- **Mr. Amir Zalzala**

  Director of the Plant Research Department

- **Mr. Amir Marafi**

  Director of foreign Relations Department

- **Dr. Jasim Al-Midairas**

  Consultant to Director General

Scientists and Researchers:

- Mr. Abbas Hussein Abdul - Radha

Supervisor Date Palm and Fruit Trees

- Miss Salwa Sultan

Head of the Plant Tissue Culture Laboratory

- Mrs. Mona Karram
- Mrs. Mona Mohammed
- Mrs. Parveen Akhtar

Scientists at the tissue culuture laboratory

- Mr. Jawed Ikbal

Plant Pathologist

- Mr. Abdelkahar Mardoud

Agriculture Engineer

- Mr. Aziz A. Abou- Amarah

Senior Civil & Industrial Engineer
Project Manager for Al- Wafra & Al-Abdally projects

AOAD

Mr. Najem Ben Mohammed
AOAD Regional Representative
Rabat, Morocco

Mr. Aissa Alarbi
Assistant AOAD representative
Rabat, Morocco

Annex 3: composition of the Initiation Medium:

(Upon the request of the Head of Tissue culture Laboratory, the exact concentrations are not shown here).

Stock solutions (1 Liter, concentrated 100 times).

* Solution A	* Solution B	* Solution C
NH4No3	CaC12. 2H2O	MgSO4.7H20
KNO3	CoC12. 66H20	MnSO4.4H20
	KI	ZnSO4. 7H20
		CuSO4.5H20

* Solution D	* Solution E	* Solution Fe EDTA
H3BO3	Na H2PO4. 2H20	Na2 EDTA
Na2MoO4.2H20	NaH2PO4. OH20	FeSO4.7H20

* Skoog's Vitamins (100 ml as a stock solution, concentrated 100 times)

Thiamine HCL
Nicotinic Acid
Pyridoxine B6
Biotion

To Measure one Liter Take :

.10 ml of stock solution A
.10 ml of stock solution B
.10 ml of stock solution C
.10 ml of stock solution D
.10 ml of stock solution E
.10 ml of stock solution Fe EDTA
.10 ml of stock solution of skoog's vitamins
.40 mg Adenine
.2 g Polvinylpyrrolidone (pvp)
.200 mg of Glutamine
.100 mg of Inositol
.75 mg of Vitamin C
.30 g of sucrose
.8 g of Agar
. Growth regulators: NAA, NOA ,IAA and 2.IP

Annex 4: Equipment, Accessories, Chemical and Glassware Needed

1- Equipment:

- 1 Vacuum pump (Desinfection of plant material)
- 3 Air sterilizer, 200 V. electronic type.
- 1 microwave oven, rotating plate, minimum internal dimension 40 x 30 cm, 220 V. window type.
- 2 Burners with electronic ignition (Type Hoffman)
- 1 Microscope dissecting and lamp (olympus)
- 1 Microscope inverted (olympus)
- 1 Photo equipment adaptable to microscope.
- 1 Sand filtration system with Purifier and Softner.

2- Accessories and supplies:

- 02 Hygrothermographs with wound drive (Cole-Parmer)
- 05 Celsius chart - 10 to + 50 degrees C. seven day rotation (100/pk)
- 01 Replacement pens (6/pk)
- 50 Culture tubes racks, stainless steel, 40 space for test tubes, 25 mm diameter (Bellco)
- 50 Slant culture racks, polypropylene, to hold ten (25 mm X 150 mm) tubes.
- 04 Max and Min thermometer, scale ranging from -2 to + 50 degrees C.
- 04 scalpel Handle number 7, narrow blade.
- 10 Surgeons scalpel no. 10, 11 and 15 (100 blades/box each)

- 01 Label marker for printing self adhesive plastic tapes, 9 mm wide.
- 03 Plastic self adhesive red tape for label marker.
- 04 Trays, rigid PVC, with end handels, 57 x 41x 16 cm
- 04 Replacement filters for LAF cabient (gelaire, HF72)
-10 Replacement pre filters for LAF cabinent(Gelaire,HF72)
- 02 Graflter Knifes, stainless steel, Medium size
- 01 Laboratory Ladder, 3 steps with casters, metal 1,5 meter high
- 02 Ergomatic task chair, adjustable with foot ring.
- 02 (60 min). clock (to time desinfection).
- 100 Aseptic surgical cover hair
- 100 Non toxic dust mask (2packs of 50 each)
- 02 Parafilm boxes
- 04 Batteries for hygrothermograph (sato, 4 speed model R-704)

3- Glassware:

- 50 Petri dishes, 25 x 200 mm, pyrex.
- 10 Volumetric flasks, large mouth, 1000 ml capacity
- 10 Volumetric flasks, large mouth , 500 ml capacity
- 10 volumetric flasks, large mouth, 250 ml capacity
- 05 Erlenmeyers, Clear glass with graduations, 5 Liters capacity.

4- Chemicals and Reagents:

Manganese sulphate seven hydrate (MnSO4. 7H20)	2x100 g
Abscicic Acid (ABA)	1x100 g
L-Cystein Hydrochloric, anhydrous, crystaline	1 x10 g
Chloranphenicol	1 x 25 g
Gentamicine sulphate	1 x 25 g
Hydrochloric Acid (HCI)	1x1000 g
Electrolyate solution for PH Meter	1x1000 g
difco - Bacto - Agar	4x500 g
Polyethylene Glycol (PEG); M.W.= 10.000	4x 250 g
Dimethyl sulfoxide (DMSO)	1x100 g
Polyvinylpyrrolidone (pvp)	2x50 g
Ethanol (95%)	4x 2.5 L

5- Books And Journals:

- Recent books on plant tissue culture (a list will be defined later)
- Journal of " Plant cell, Tissue and Organ culture".
- Journal of " Plant cell Reports".

6- Addresses of suppliers:

Scientific Equipment

1- Dutch Agri-Products
P.O. Box. 1016
1620 KA Hoorn
Netherlands

2- Olympus corporation
4, Nevada drive
New Hyde Park
NY 11042, USA

3- Welch Scientific Corporation
1617 East Ball Road
Anahiem, CA 92803
Tlx.65-5425

- New Brunswik Scientific Co
44 Talmadge Road, P.O. Box 986
Edison, Nj 08817, USA
(201)-27 1200

Glassware and Lab Needs

1- Magenta corporation
Second & Mallinkrodt Div.
St. Louis Mo. 63160, USA
(314) - 231 8980

2- Ballco Glass Inc.
340 Endrudo Road
Vineland, NJ 08360, USA

3- Scientific Products
140 Waukegan Road
Mc Caw Park
Illinois 60085, USA

4- Fisher scientific
50 Faden Road
Springfield, NJ 07081, USA
(201) 379 1400

Reagents and Chemicals

1- Sigma Chemical Co.

P.O. Box 14508
St. Louis MO 63178, USA
(314) - 771 5765

2- Life Science Group

25201 Miles Road
Cleveland, OH 44128 USA
(216) 381 3000

3- Merk Co.

44, Allec Dela Robertsan
67000 Strasbourg

4- Difco Laboratories

Detroit, Michigan, 48232, USA

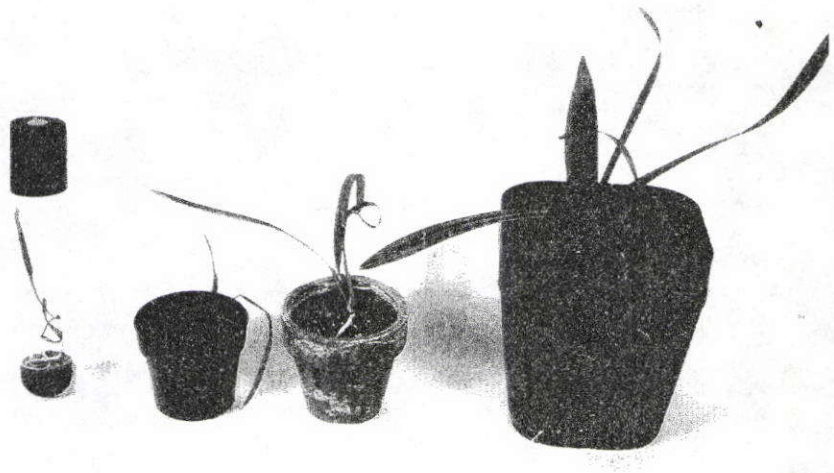

Figure 1 : A summary of itinerary of date palm in vitro propagation

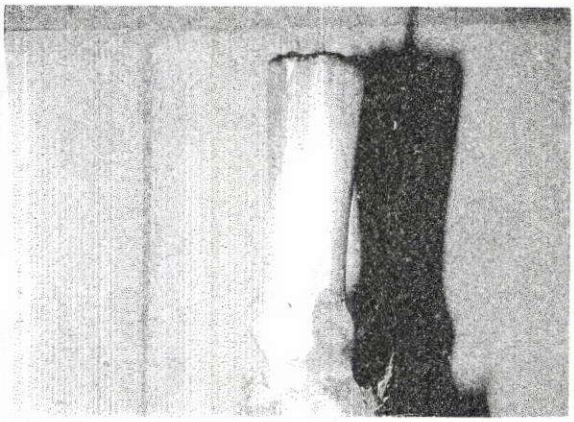

Figure 2 : Offshoot meristematic area to be used as a source of explants

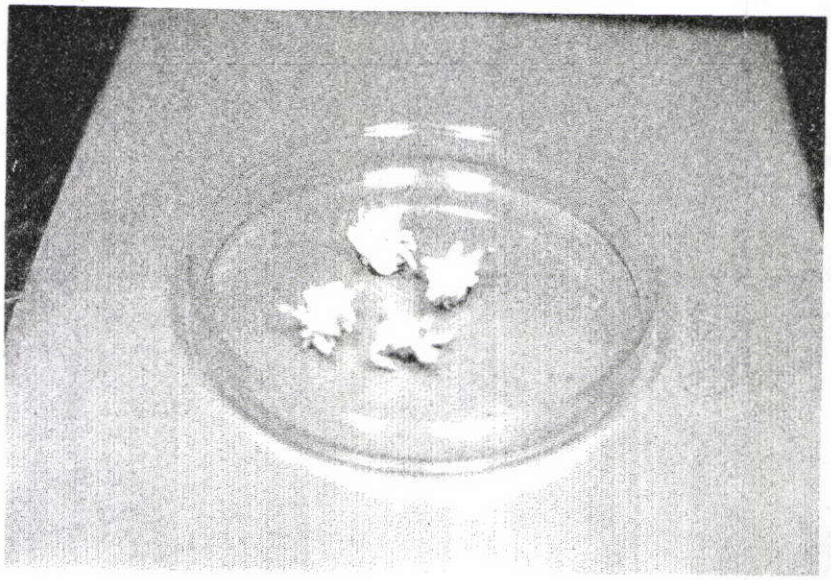

Figure 3: Type of multiple shoot formation obtained on the initiation medium.

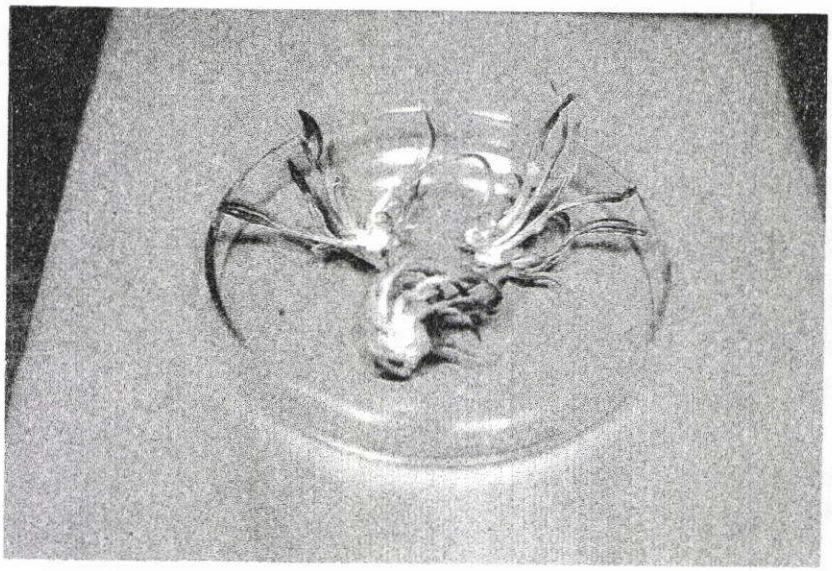

Figure 4: Young plantlets obtained from the multiplication process; Ready to be separated and cultured on elongation medium.

Report On Consultancy Mission On Date Palm In Vitro Propagation In The State of Kuwait

(Second Technical Report)

Table of Contents

Acknowledgments:

The writer of this report is indebted to Eng. Fahad A. Al-Hasawi Chairman & Director General for the Public Authority for Agriculture and Fish Resources, Mr. Ahmad Al-Nakeeb Deputy Director General for Agriculture Development, Mr. Jasim Mohammed Habib Al-Bader, Deputy Director General for the Plant Protection of the Public Authority for Agriculture and Fish Resources and Mr. Amir Zalzalah, Director of Plant Research Department, for their keen interest in the execution of the mission as well as the development of the Date Palm Tissue Culture in The State of Kuwait .

The consultant wishes to express his deep gratitude and appreciation to Mr. Abbas Hussaein, Supervisor Date Palm and Fruit Trees Propagation and Mr. Amir Marafee Director of foreign relations Department and Miss Salwa Sultan, Head of the Tissue Culture Laboratory, Mrs. Mona Karam, and Mrs. Perveen Akhtar researchers at the laboratory for their valuable assistance which enabled him to carry out his duty in the most efficient manner. Engineer Aziz A. Abou- Amarah, Project Manager of Al-Wafra center, is kindly acknowledge for his assistance.

For the organization of both missions the support and guidance of AOAD Headquarters in Khartoum (Sudan) and also of AOAD Representative in Rabat (Morocco) was appreciated in providing the basic information and establishing contacts and missions programmes. The consultant wishes to thank Dr. Yahia Bakour, AOAD General Director and Mr. Najem Ben Mohammed, Regional AOAD Representative in Rabat (Morocco).

The list of all persons met is similar to the one presented in the first technical report . They were helpful in taking time to give the consultant most relevant information .

Introduction :

In response to the request of the Government of Kuwait, a Date Palm Tissue Culture Specialist, Dr. A. Zaid was recruited by the Arab Organization for Agriculture Development, to undertake two consultancy missions on the In Vitro Propagation of Date Palm in PAAF., more specifically his terms of reference were to :

- Assess the laboratory activities and techniques proposed since the first visit.
- Identify key technical constraints and make appropriate recommendations to overcome them.
- Toward the end of the mission, prepare a consultancy report, to be technically cleared with PAAF prior to consultant departure.

The first mission was scheduled for the period from April 8, till May 8, 1994 . A first technical report was prepared by the consultant, forwarded to AOAD Headquarters and received technical clearance. However, Date Palm Micropropagation techniques is made of several phases and follow-up is a must. In response to a second request of the Government of Kuwait, the same consultant was recruited by AOAD to undertake a second counsultancy mission which was then scheduled from September 28, 1994 till October 14, 1994 .

Shortly afer his arrival, the consultant, was briefed by DDG.PAAF.Mr Jasim Mohammed Al-Bader and other Officials.

The laboratory work started on October 1 St., 1994 at the tissue culture laboratory located at PAAF in Al-Rabiyah District. On October 8,1994 a technical seminar was held about in vitro acclimatization of date palm plantlets, the head of the laboratory accompanied by her technical staff attended the Seminar .

The main findings, conclusions and recommendations were presented in a debriefing session, to officials and senior members of PAAF.

Main findings

1- Summary of the first technical report:

The following brief account summarizes the main observations made, conclusions drawn and recommendations framed during the first counsultancy mission . Date palm plantations of Kuwait are estimated of about 300,000 trees and their is a huge space to increase the number of palms. An urgent need of approximately one million palms is well recognized by both government Officials and Farmers.

According to data collected during the consultant's first visit, all date palm plantations used to be free of important diseases and from serious pests. However and after the invasion, some problems such as the black scorch and Parlatoria blanchardii arised. Hence, special attention should be given in order to eliminate such hinderance and good sanitation is the first step in the control of black secorch disease. It is highly recommended that no offshoots importation should take place from infected countries. Careful attention should also be given to the origin of soil and sand imported from neighboring countries.

Micropropagation in vitro appears to be the sole solution that will have considerable potential for date palm problem of offshoots availability and will allow the rapid establishment of large scale commercial date production in Kuwait.

Importing date palm plantlets from foreign tissue culture laboratories proved to be very expensive with a certain amount of RISK as to the quality and survival of plants. Hence the development and functioning of the Al-Rabiyah tissue culture laboratory constitute the only, but wise way to satisfy the large demand of date palm offshoots in Kuwait.

The tissue culture laboratory situated at Al-Rabiya, is the only facility of the Public Authority for Agriculture and Fish Resources that is in charge of carrying out research on date palm mass production using in vitro techniques. Although, the efforts are impressive, they have not yet resulted in the production of date palm plantlet. The laboratory still needs some scientific equipment and supplies. In order to have such facility working efficently, the work on the new extension is urgently recommended. Based on the already existing facility, and when the new building is functional, the consultant estimates a yearly production of approximately 75,000 date palm plantlets ready for planting in the nursery.

A research program focusing on the organogenesis technique was already initiated by the consultant. Various varieties such as Barhee, Sammaran, Kentar, Khadraoui, Lakhless, sugar, chabchab and Zahdi were introduced into tissue culture conditions.

A major technical problem which is hindering the well functioning of the facility and consequently the rapid propagation of date palm in Kuwait, is the lack of knwo-how and practical in vitro techniques of date palm propagation. None of the people in the laboratory, except the head of the unit and one scientist, have been trained in date palm micropropagation. Regarding this matter, the consultant has presented in his first technical report, full detailed techniques and recommendations to follow; Several study tours and training were also recommended by the consultant.

2- Evaluation of Date Palm Existing Cultures:

As previously mentioned in the summary of the first technical report, the consultant, with the help of the laboratory's head and researchers, has introduced into tissue culture conditions various varieties such as Barhee, Saamaran, Kentar, Khadraoui, Lakhlass, sugar, Chabchab and Zahdi. The date of explants introduction varied from 12 till 28 April, 1994 depending on the variety's offshoots availability.

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The composition of initiation media was previously detailed and discussed with the head of the laboratory during the consultant first visit. Since then, cultures were incubated in darkness at a temperature of 27c.+ 1 c. Cultures were then subculture on the same initiation medium at a monthly basis. At the consultant's second visit, cultures were at their sixth subculture, and some of them were ready to be transplanted to the multiplication medium.

The following account summarize the main observations made and conculsions drawn for each variety existing in the laboratory.

2.1 Saamaran cv:

Most explants were found to be positively responding to the initiation medium; Bottom of young leaves put in culture, did give signs that budding is beginning. for all 4 offshoots introduced, with a total of approximately 90 original explants, 13 explants mothers were selected to be subcultured on the multiplication medium.

However, it is worthwhile mentioning, that during the initiation phase (approximately six months), a slight browning occurred since the first introduction, but with no noticed effect on the initiation phase. Callus formation on some explants (approximately 10/90) did appear after two months from the initial introduction . Such callus was eliminated from the organogenesis process.

Even though, some aerial root development did appear on few cultures during the fifth subculture, no effect on the budding phase was noticed. Of course, and this is true for all varieties, a level of 4% contamination was never crossed and this is internationally accepted as a safety level.

During this study, offshoots weigh, which is related to its physiological age, had a great effect on the response to the initiation medium. Offshoots referenced (S3) with 7.3 kgs. did give arise to 09 positive explant mothers as compared to 01 positive explant given by S4 with 14 kgs. as original offshoot's weight. Such results were found with all varieties tested in PAAF laboratory.

2.2 Kentar cv:

As mentioned above, offshoots weight had a great effect on the response to the initiation medium. The four kentar's offshoots used in this study had their weight varying from 09 to 09.5 kgs. and did eventually gave the same results. They all reacted positively with 16 mother explants showing budding signs after 06 months of introduction's date.

Browning met with other varieties (mainly with Zahdi cv.) was almost absent for Kentar's cultures. However, some aerial roots did appear on some cultures (approximately 08%) after 04 months of the initiation phase. No callus signs were found in all cultures. It is worth to mention that Kentar was the best variety that responded to the organogenessis technique with a success rate of this initiation phase of approximately 20%.

2.3 Chebchab cv:

As Saamaran and Kentar, Chebchab was the third cultivar that responded positively to the initiation phase with two mother explants cultures showing some signs of budding. The main problem with Chebchab variety was the original weight of used offshoots which ranged between 10 and 20 kgs. While no contamination was observed, some cultures of Chebchab did show some browning that could also be a factor of low rate of multiple shoot formation . Little callus developed after one month of culture and some aerial roots were noticed during the second month.

2.4 Response of the remaining varieties:

It is well known that the response to the initiation phase, and mainly to the initiation medium's composition, depends on several factors including genotype, age of offshoot, time of introduction, media composition, browning phenomena,.. etc. It is now clear that for Zahdi, Khadraoui, Khlass, sugar and Barhee cvs. the change of the growth regulators is a must; recommendations were made by the consultant to work with an adjusted initiation medium containing half of the original concentration used for NAA and IAA. Concentrations of NOA and 2IP were left unchanged.

It is worthed to mention that for Barhee, sugar and Khadraoui cvs. there was a delay of more than two weeks in the subculturing process; such delay has caused a severe browning and consequently death of cultures (mainly Barhee cv.) . The advanced reason given by the head of the laboratory regarding this matter was an un-proper planning of staff vacations. Similar reason was presented regarding lack of data information for khlass and sugar cvs. Recommendations were made in order to avoid such problems in the future.

3. Multiplication phase:

As mentioned in the first technical report, organogenesis technique is made of four steps: Initiation of meristematic buds, multiplication, elongation-rooting, and acclimatization. The success of such technique is tremendously dependent on the success of the first step (initiation). Furthermore, various problems meet at other steps levels have their origin at the initiation phase.

Cultures that start showing budding signs were cut in 2 to 3 pieces and subcultured on the multiplication medium that is similar to the initiation one, except that IAA and 2IP are substituted by BAP and kinetin. The exact concentrations of all growth regulators were given, in a confidential way, to the head of the laboratory. Cultures of Sammaran, Kentar, and chebchab were Subcultured, by the consultant on the multiplication medium.

A weak light was recommended at this stage and the culture room temperature should be 27+1c, during 16 hours of diffuse light and 20 to 22 c during the 8 remaining hours. Thermoperiod is better than constant temperature.

Leave will start sprouting in this medium, but many of them won't give good shaped plantets, often obtained plantlets are similar to rosette plants. It is recommended that such leaves will be cut and multiple buds formation should be kept under control. Cutting gently between buds is recommended while dividing the explant (full of buds) into 2,3, or sometimes 4 pieces. Such pieces are put on a multiplication medium and take again the sequence of culture stages. Such multiplication process is done monthly and when a well developed plantets seem to take over, it is necessary to separate it from the remaining budding tissues without sprouting, because it seems that some correlative inhibitions exit between enlarged buds (plantlet) and others parts of budding tissues.

4. Rooting elongation and acclimatization of date palm in vitro plantlets:

When enough buds are obtained and most of them start resembling to a young plantlet, it is necessary to transfer those budding tissues on rooting-elongation medium with gibberellin. NAA, BAP, Kinetin and GA3 are used at the respective following concentrations: 1,5.0, 0.5 and 1-3 mg/1. It is highly recommended to keep young plantlets on this medium only for 10 to 15 days. If left longer, leaves will look like grass (vitrified). Then the budding tissues are again transferred on a " swelling" medium, which is the same as the multiplication medium, except that the sucrose level is 100 to 150 g per liter. The good shaped plantlets may be left on this swelling medium for several months without any transfer. They get vigorous and often rooted plantlets are obtained. No special rooting medium is used since date palm plantlets root easily and even, without roots, they may be transferred into conditioned greenhouse without problems, as unrooted cuttings dipped in rooting hormones solution.

The basic requirements involved in the acclimatization of date palm in vitro-derived plantlets were fully explained to the laboratory staff.

The regenerated date palm plantets with roots generally about 3 to 5 cm in length, should be carefully removed from the test tubes, washed thoroughly to remove nutrient medium and transplanted to polycarbonate culture vessels (25 X 65 X 110 mm: plantox) containing sterile vermiculite. After one week or two, old leaves are clipped and plants immersed in 0.2% Baristin (or any other fungicide) for 10 to 20 min, and then carefully transferred (avoid root damage) to a mixture of 2

peat: I sponge rock: I vermiculite in the greenhouse. Two to three times during the first week, irrigation should be realized using a half strength inorganic salts of MS(or Hoagland) solution, and subsequently with tap water. Covering plantlets with transparent plastic tunnels will increase the relative humidity and consequently avoid plant dessication. The equipment of the greenhouse with strip lights placed over the central bunches interspersed with incandescent bulbs, will enhance growth. Acclimatization should be reached less than 5 weeks . Five months later, when plantlets wither up, the form of these laters will be similar to that of onion bulbs.

Acclimatization of in vitro cultured plantlets of date palm should meet the most stringent phytosanitary measures to prevent bacteria and other microorganisms infestations.

The consultant is highly recommending the building and the equipment of at least 100m2 greenhouse (air conditioned , desert cooler, fog system,... etc.) and also to have humidity and solar insolation controlled. Such facility is a key step in the date palm micropropagation, once in vitro plantlets are produced in a large numbers as is obviously intended.

In vitro hardening, using osmotica such as polyethylene glycol, could also be envisaged prior to transfer to soil. The consultant did give a seminar on this subject in order to have the laboratory staff well prepared when this phase is reached.

Conclusions and Recommendations

The following account summarizes the main conclusions drawn and recommendations framed .

There is no question about the benefits the in vitro techniques will bring to the date palm program actually underway in the Public Authority for Agriculture and Fish Resources (PAAF). The building of an extension facility as well as the purchase and delivery of all equipments (Cf first technical report) is vigorously recommended in order to facilitate the rapid build -up of the tissue culture activities in thePAAF's unit.

Since the consultant's first visit, encouraging results were obtained and several date palm varieties, such as Saamaran, Kentar, and chebchab, are already entering the multiple shoot formation's phase. A contamination rate of less than 4% was obtained and is internationally considered as a safety level. Cultures of the above mentioned varieties were subcultured by the consultant, with the help of the unit's staff, into amultiplication medium.

In the present report, the consultant has prepared a full detailed evaluation of date palm existing cultures with a special interest on the multiplication phase (medium composition, incubation conditions,... etc.) and on the rooting-elongation, and acclimatization phase . Some abnormalities at the subculturing timing and medium composition were also found by the consultant who proposed , to the Head of the laboratory, adequate means to correct them.

According to data collected during the consultant's second visit, most varieties which were introduced in vitro during the first visit, were found to be positively responding to the initiation phase. The remaining varieties, such as Barhee. Sugar and Khlass, were subcultured on a different initiation medium, in order to improve their responses.

It is worthwhile mentioning that taken together the recommendations made by the consultant during his first visit , no severe browning problem had occurred during the six months of culture. However, the results also showed that the date of introduction of explants into tissue culture conditions (12 to 28 April 1994) was not appropriate for some cultivars (Barhee, Khadraoui, Zahdi), the consultant is highly recommending to initiate new cultures during the period between December 1994 and March 1995.

As mentioned in the first technical report a study tour in some of the foreign date palm laboratories is highly recommended for the supervisor of date palm and fruit trees program as well as for the head of the laboratory, in order to establish relations with researchers and institutions dealing with date palm mass propagation.

Building and equipment of a 100 m2 greenhouse, to be used as an acclimatization unit is urgently recommended. Such facility is a key step in the process of date palm in vitro propagation, once plantlets are produced in large numbers as is obviously intended. The technique of acclimatization for date palm tissue culture-derived plantlets is fully described by the consultant .

The information contained in both technical reports and rcommendations made by the consultant, if correctly applied, are ample to result in the successful establishment of rapid clonal propagation and acclimatization of date palm in the State of Kuwait.