Phenological Descriptors and Molecular Markers for the Determination of True-to-type of tissue culture-derived plants using organogenesis of some Moroccan Date Palm (*Phoenix dactilyfera L.*) Varieties

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Abstract

The date palm (Phoenix dactilyfera L.) is a dioecious perennial monocotyledonous fruit tree with long gener-ation time. It is conventionally regenerated by seeds and offshoots. Naturally, the reproduction of varietal authenticity is nearly assured to 100% by the offshoots. The genetic conformity of large numbers of date palm plants produced by several commercial laboratories using in vitro culture techniques can be affected in different degrees from nearly null to very important according to the pressure and intensity of in vitro parameters. In order to identify the true--typeness of in vitro plants, two approaches have been developed, one based on phenological descriptors and the second on molecular markers. Thus, 13 out of 120 or more phenological descriptors and 44 RAPD molecular markers were selected and tested on three Moroccan varieties (Mejhool, Sairlaylate and Najda (INRA-3014)) and 30 palm trees derived from in vitro organogenesis technique. The phenological descriptors which were already demonstrated as distinctive descriptors between date palm varieties, can be quantitative or qualitative, agro-morphological or organo-structural. Results didn't detect a polymorphism between the populations and their palm trees of origin. Although these results are encouraging, the research will continue to confirm them on a large number of varieties and to identify other molecular markers and other phenological descriptors. This will be useful to produce better in vitro plants true--typeness control at different development stages with high quality of the final products. The advantages and disadvantages of the use of these approaches as a quality assurance tool to be used by commercial plant micro propagation laboratories are discussed. Currently and due to the difficulty to assure the control of the total conformity, the question that arises for the date producers concerns the adoption of a genetic conformity or a phenotypical one.

Key words : Date palm, *Phoenix dactilyfera* L, Phenological descriptors, DNA molecular markers, Somatic embryogenesis, Organogenesis, *In vitro* propagation, True-typeness, Morocco.

Résumé

Le palmier dattier (Phoenix dactilyfera L.) est une espèce dioïque, monocotylédone et pérenne, qui se reproduit par graines et rejets. Naturellement, la reproduction de l'authenticité variétale est assurée presque à 100% par les rejets. La conformité génétique de grands nombres de plants du palmier produits dans plusieurs laboratoires commerciaux par les techniques de culture in vitro peuvent être affectées à des degrés différents de presque nul à très important en fonction de l'intensité de la pression des paramètres exercées in vitro. Pour identifier la conformité des plants, deux approches basées sur les descripteurs phénologiques et les marqueurs moléculaires ont été développées. Ainsi, 13 descripteurs phénologiques et 44 marqueurs moléculaires RAPD ont été testés sur trois variétés marocaines (Mejhoul, Sairlaylate et Najda (INRA-3014)) et 30 palmiers produits in vitro par la technique d'organogenèse. Les descripteurs phénologiques sont des caractères quantitatifs ou qualitatifs, agro-morphologiques intéressant les phoéniciculteurs et les producteurs de dattes ou organo-structural, lesquels ont déjà été démontrés comme descripteurs distinctifs entre les variétés du palmier. Les résultats comparés n'ont pas détecté de polymorphisme entre les populations et leurs palmiers d'origine. Bien que ces résultats obtenus soient encourageants, les recherches continueront à les confirmer sur un grand nombre de variétés et identifier plus de marqueurs moléculaires variés et complémentaires aux descripteurs phénologiques. Ce qui permettrait de mieux apprécier la conformité des plants produits in vitro à différents stades du développement et assurer une bonne qualité des produits. Les avantages et les inconvénients des approches adoptées comme outil permettant l'assurance de la qualité au profit de laboratoires commerciaux de micropropagation des plantes sont discutés. A l'état actuel et vu la difficulté pour assurer le contrôle de la conformité totale, la question pertinente qui survient pour des producteurs de dattes concerne l'adoption d'une conformité génétique ou phénotypique.

Mots clés : Palmier dattier, *Phoenix dactilyfera* L., Descripteurs Phénologiques, Marqueurs moléculaires d'ADN, Embryogenèse somatique, Organogenèse, Propagation *in vitro*, Conformité, Maroc.

ملـخص

تنتسب شجرة نخيل التمر (فنيكس داكتيليفيرا) إلى النباتات المستديمة و وحيدة المسكن وذات الفلقة الواحدة، التي يتم إكثارها بواسطة النواة و الفسائل. طبيعيا، تعطي هذه الأخيرة هوية صنف النخلة بنسبة حوالي 100 ٪. يتأثر التطابق الوراثي لأعداد الشتلات النسيجية المنتجة من طرف عدة مختبرات تجارية عن طريق زراعة الأنسجة بدرجات مختلفة تتراوح من حوالي صفر إلى جد مهمة حسب شدة أثر عوامل إنتاج الشتلات في المختبر. من أجل معرفة تطابق النبتات، تم تطوير تقانة تعتمد على الخصائص المورفلوجية التوصيفية و على البصمات الجزيئية. أجريت التجارب على 3 أصناف النخيل (المجهول، ساير لعيلات و النجدة) (INRA-3014) و على 30 نخلة منتجة عن طريق الزراعة النسيجية العضوية باستخدام 13 خاصة مورفلوجية توصيفية مهمة مختارة من بين 120 و 44 بصمة جزيئية .RAPD تشمل الخصائص المورفلوجية التوصيفية المستخدمة الصفات الكمية و الكيفية و الزراعية التي تهم المزارعين و المنتجين كما تتصف هذه الخصائص لكونها مميزة بين أصناف النخيل. أبانت مقارنة النتائج عن عدم وجود اختلاف جوهري بين الأشجار النسيجية و الأشجار الأصلية. على الرغم من أن هذه النتائج جد مشجعة، تتابع البحوث على عدد كبير من الأصناف و في مجال معرفة بصمات جزيئية أخرى مختلفة و مكملة للخصائص المورفلوجية التوصيفية. هذا يمكن من تقييم أفضل لتطابق الشتلات النسيجية في كل أطوار نموها ويعطي تأمين أفضل لجودة المنتج. و في هذه الورقة تمت مناقشة إيجابيات و سلبيات الطرق المتبعة كوسيلة تمكن من تأمين الجودة لفائدة المختبرات التجارية لإكثار النخيل عن طريق زراعة الأنسجة. و في الوضع الراهن ونظرا لإشكالية تأمين مراقبة التطابق الكلي للنبتات، يبقى السؤال من طرف منتجي التمور هو تبني فكرة التطابق الوراثي أو التطابق النباتي و الزراعي.

الكلمات المفتاحية : نخلة التمر، *Phoenix dactilyfera L*، الخصائص المورفلوجية التوصيفية، البصمات الجزيئية للحامض النووي، الزراعة النسيجية الجسدية، الزراعة النسيجية العضوية، الإكثار على الوسط الإصطناعي، التطابق، المغرب.

Introduction

The date palm (*Phoenix dactilyfera L.*) is a dioecious perennial monocotyledonous plant with a long gener-ation life time (a period of 4 to 5 years is necessary to reach the first flowering). In Morocco, the date palm is one of the most important traditional crops of the oases. In addition to its important ecological and social roles, this tree plays a significant role in human consumption and animal feeds, and is used to produce a wide range of end-products. In Morocco, around 4.7 million of date palm trees are cultivated over an area of approximately 48 000 ha. 223 known varieties are represented by two million trees and the remaining 2.7 million trees are originated from natural seed and are commonly known as "khalts" (Sedra *et al.*, 1996).

The major constraints of date palm production are drought, production techniques ; post harvest techniques, pests and diseases. The Bayoud is the most serious fungal disease of the date palm which occurs in the major date palm growing areas of Morocco, in large part of western and southern parts of Algeria and in some areas of Mauritania (Sedra, 2003). It is a serious disease in these North African countries and represents a serious threat to the countries which are still Bayoud free.

Since 1963, the Moroccan National Agricultural Research Institute (INRA) has carried out in collaboration with its national and international partners several scientific and applied investigations in order to serve date palm farmers and preserve the ecosystem of oases.

The genetic control of the Bayoud disease using resistant varieties is up to date the most privileged alternative. Several performing clones and resistant to the Bayoud were selected (Sedra, 1995, 2001, 2003, 2005), but they are represented by only one to a few trees. In order to produce enough nursery plants that are necessary for the reconstitution of date palm groves destroyed by the Bayoud, the mass micropropagation of the selected resistant date palms clones is essential.

Date palm can be propagated via seeds, vegetatively propagated via offshoots or by mass micro production using *in vitro* techniques. Palm trees derived from seeds can never be genetically similar to the mother plant. Vegetative propagation via offshoots derived from the axillary buds of the palm tree has been traditionally used as the main process for nursery plant production. Unfortunately, this traditional procedure is hampered by the limited number of offshoots produced from each selected tree. Offshoots quantity of highly requested cultivars can not satisfy the market demand. This affects severely the re-plantation programs, that are planned to compensate plant losses caused by Bayoud disease.

Throughout the world, *in vitro* production using either the process of somatic embryogenesis or organogenesis has been established in recent years. The techniques are used as a routine procedure in several commercial laboratories to produce large numbers of in vitro date palm plants at a competitive cost. Somatic embryogenesis is currently the most efficient technique used regarding its rates of multiplication and production. Most commercial companies carry out micropropagation production of date palm via somatic embryogenesis, also called asexual embryogenesis. In this process, embryo-like structures, called somatic embryos, are produced from explants, which can be somatic cells, tissues or organs derived from a single mother plant. In Morocco, the mass micropropagation is based on organogene-

sis process. This technique allows the production of seedlings using axillary buds (in the basis of the leaves) as explants. Therefore, being commercially attractive, the micropropagation has completely replaced the traditional vegetative propagation practices. Large-scale commercial plant production processes are subject to a number of risks related to the production of off-types, i.e. non true-to-type which are genetically not identical to the mother plant.

Few reports about tissue culture-derived plant off-types in date palm are controversial. In the Kingdom of Saudi Arabia, Djerbi (2000) reported the abnormal fruiting (specifically in the case of Barhee cultivar) of date palms derived from somatic embryogenesis. These fruit abnormalities reached 80 to 100% of parthenocarpic fruits. Other abnormalities such as the lack of pollination were observed in Southern Africa (McCubbin *et al.*, 2000), while Smith and Ansley (1995) found that somatic embryogenesis- derived Barhee plants had no obvious abnormalities and produced fruits of commercial quality indistinguishable from fruits of trees which were originated from offshoots. Al-Ghamdi (1996), using two varieties, Thoory and Zahdi, also observed no significant difference in flowering and fruit setting. Abnormalities such as leaves variegation, seedless fruit, broader leaves, different spine structure, bending of stem and compact growth appeared to be almost insignificant as types of variation (Mc Cubbin *et al.*, 2000). According to these findings, it is vital that commercial producers can guarantee to growers the identity of the plants that they are purchasing. This requires the application of appropriate quality assurance tests to ensure both the true-to-typeness of a variety with the detection of phenotypic uniformity and agronomical performance variation.

Although variety identification by morphological characters represents the easiest and least complex technique, it appears necessary to look for other alternatives that are less influenced by the environment and plant growth stage (Sedra, 2001). More than 120 phenological and agro-morphological characters were determined and can be quantitative or qualitative descriptors (Sedra, 2001) and either distinctive between varieties or common for date palm species. However, this approach doesn't allow the detection of plant off--types at the juvenile stage. In the contrary, molecular markers may identify the change in either the production of plant proteins, which are expressed from certain regions of the DNA, or the total composition of DNA.

Commercial producers cannot ignore the progress made in plant biotechnology including the application of DNA-based markers for quality assurance. DNA-based tests for date palm identification include techniques such as RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplification Fragment Length Polymorphism) and RDA (Representational Difference Analysis) (Powell *et al.*, 1996 ; Cullis *et al.*, 1999).

The objective of the present investigation is to develop the tools permitting to detect the true-typeness or the differences between the in vitro plant populations derived from organogenesis process and the date palms of the variety of origin. This investigation aims to study the efficiency of some phenological descriptors and some molecular markers and to detect phenotypic uniformity and agronomic performance variation.

Materials & Methods

Plant materials

Three date palm varieties were studied: 1) Najda (INRA-3014) which is the first highly performing and Bayoud resistant clone selected by the INRA (Sedra, 1993, 1995, 2003, 2005). Until now, more than 200 000 Najda in vitro plants derived from organogenesis process have already been distributed to the farmers in order to reconstitute the orchards destroyed by the Bayoud. 2)Mejhool is a famous variety of a high commercial value but extremely susceptible to the Bayoud disease. 3) Sairlyalate is a common variety of middle quality but resistant to the Bayoud disease. The diffusion of this variety has been relatively limited.

In vitro multiplication of these varieties using organogenesis techniques were initiated by INRA laboratory at Marrakech (Anjarne *et al.* 2005) and then taken over for mass production by a private laboratory at Meknès (Ait Chitt, 2005).

Methods

Set objective is to compare samples of the date palm trees produced by organogenesis technique with the mother palm trees of origin or with the palm trees which were grown from offshoots. This comparison is based on the appreciation of two diagnostic markers developed to detect off-type plants:

- Agro-morphological markers

Among many descriptive characters, 13 quantitative and qualitative descriptors of the date palm were used: date appearance, date colour at stage 'bleh' and stage 'tmar', date consistency, fruit shape, date weight, pulp percentage in the fruit, presence of small wings or bumps or distortions on the seed, seed shape, percentage of spiny part /palm leave, total number of spines, angle of the spine in the middle of rachis and leaflet consistency. Calculated mean of date weight represents the average of the weights of 100 dates chosen at random. These qualitative and quantitative descriptors are defined by Sedra (2001). For the palm trees of origin, a total of 12 samples, generally four trees per genotype and three samples per tree, were used with the exception of Najda variety which is only represented by two trees. For the date palm trees produced by tissue culture, at least ten trees by variety were sampled, with the exception of Mejhool variety which is represented by eight trees only.

The quantitative data were presented as averages. The qualitative data are compared on the basis of the average of the frequencies and the most frequent character was noted. The significance of the differences was determined using the statistical Duncan test at $p = 0.05$.

- Molecular markers

DNA extraction

Genomic DNA of each genotype was extracted from 0.2 g of lyophilised leaflets. The leaves were first ground into fine powder. DNA was extracted in 5 ml of ex-traction buffer (50 mM CTAB, 100 Mm Tris HCl, 20 mM EDTA, 1.4 Mm NaCl) to which we added 5 µl of ß-mercaptoethanol. The solution was mixed and incubated for 30-40 minutes at 65°C with occasional mixing. After cooling for about 2-4 minutes at room temperature, the extract was adjusted to 8.25 ml by adding chloroform/isoamyl alcohol (24/1 v/v). The mixture was homogenised by gentle inversion before being centrifuged at 5000 g for 20 minutes. The aqueous supernatant was recovered in clean tube in which we added 2 µl of 100 mg/ml RNase (Boehringer Mannheim) before incu-bating for 30-60 minutes at 37°C. The solution was distributed on clean tubes (600 µl/tube).The DNA was precipitated with an equal volume of isopropanol and 60 µl of sodium acetate (3M, pH 8.0) after well mixing. Optionally, each tube can be incu-bated at -20 °C for at least one hour. The solution was centrifuged at 10000 g for 20 minutes (preferably at 4°C). The precipitated DNA was recovered in 500 µl of 70% (v/v) ethanol (stored at – 20°C). The DNA pellet of each tube was grouped in a single tube, dried and dissolved in 200-300 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8) or sterile water. Depending on the leaf samples, yield in DNA varied from 100 to 200 pg using this protocol.

RAPD analysis

Oligonucleotide primers (10 mers) were pur-chased from Operon Technologies Inc. (Alameda, CA) and The University of British Columbia, (Vancouver, CA) (UBC). Ten primers were used: eight (OP) were already selected by Sedra *et al.* (1998) and two (UBC) were chosen among 80 interesting primers which were selected among more than 320 primers (results not yet published). PCR reactions were carried out in 25 µl volumes containing 25 ng of total genomic DNA, 15 ng of a single primer, 150 µM of each dNTP, 1X Taq buffer, 3 mM MgCl2 and 1unit AmpliTaq polymerase (Perkin-Elmer). Amplification was perform-ed in a Techne-Touchgene Thermal Cycler TTGO5TD with the following program: (i) 95°C for 5 minutes x 1 cycle ; (ii) 94°C for 1 minute, 34°C for 1 minute, 72°C for 2 minutes x 45 cycles ; (iii) 72°C for 8 minutes x 1 cycle, and an optional soak period at 4°C. The RAPD products were frac-tionated according to size on agarose gel (1.8% w/w) containing ethidium bromide and subjected to electrophoresis (80-100 V for 4.5 hours) in 1 X TBE buffer. Fragment length was estimated by comparison with standard size markers (a phage double digested with HindIII and EcoRI).

Results

Phenological identification

In the limit of the studied samples and the used 13 characters, it was shown that the palm trees derived from tissue culture technique present statistically the same phenological characters and quantitative and qualitative descriptors of the dates (Table 1) and of the trees (Table 2) as their date palm trees of origin. It seems that these characters are among those which present an interest for the farmers and producers.

Molecular identification

The number of amplified bands per primer varied between two and six, with a mean of four major bands per primer. The selection of the used bands was based on their intensity and size. Several preliminary tests on three DNA samples showed that these bands were reproducible upon repeated experimentation. Analysis of the bands obtained from ten such primers among the three original varieties generated 44 bands, 24 of which were polymorphic (table 3). The comparison of total bands generated on each variety and on the samples of palm trees derived from tissue culture process didn't show any difference when the important bands were considered. In fact, to ensure reproducibility and genetic pertinence of RAPD marker data, the weak or complex bands were discarded. The example of Mejhool variety and eight samples of plants derived from in vitro micro propagation is presented in Figure 1.

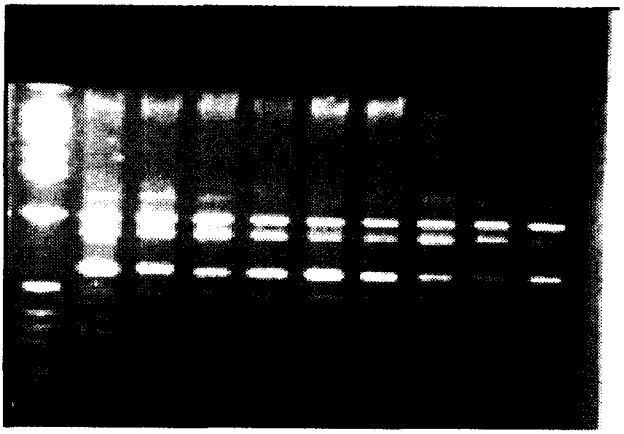

Figure 1. Examples of DNA monomorphism detected between Mejhool variety derived from offshoot and palm trees derived from *in vitro* multiplication. Ethidium bromide-stained agarose gel of amplification fragments produced with primer UBC-302. Lane 1 contains fragments of molecular weight markers, lane 2 : Mejhool variety, from lane 3 to lane 10 : palm trees derived from *in vitro* multiplication.

Table 1. Identification of the true-typeness of plants of date palm varieties derived from *in vitro* multiplication in comparison with palm trees of origin on the basis of some quantitative and qualitative descriptors of the dates.

Variety	Najda (INRA-3014)			Mejhool			Sairlayalate		
Character	Palm trees of origin	Palm trees issued from the vitroplants	Significant difference	Palm trees of origin derived from offshoots	Palm trees issued from the vitroplants	Significant difference	Palm trees of origin	Palm trees issued from the vitroplants	Significant difference
Date appearance	Good	Good	NS	Excellent	Excellent	NS	Medium	Medium	NS
Date colour - Stage 'bleh' - Stage 'tmar'	- Orange yellow - Clear brown	- Orange yellow - Clear brown	NS NS	- Yellow - Pale brown	- Yellow - Pale brown	- NS - NS	- Orange yellow - Clear brown	- Orange yellow - Clear brown	- NS - NS
Date consistency	Semi-soft	Semi-soft	NS	Semi-soft	Semi-soft	NS	Semi-dry	Semi-dry	NS
Fruit shape	Cylindrical	Cylindrical	NS	Ovoid stretched	Ovoid stretched	NS	Cylindrical	Cylindrical	NS
Date weight	17.7 a	17.9 a	NS	25.0 a	25.4 a	NS	10.9 a	11.1 b	NS
Pulp percentage in the fruit	92.1 b	92.2 b	NS	93.6 b	93.5 b	NS	90.0 b	90.5 b	NS
Presence of small wings on the seed	Quasi-frequent	Quasi-frequent	NS	Quasi-frequent	Quasi-frequent	NS	Absence	Absence	NS
Seed shape	Ovoid stretched	Ovoid stretched	NS	Elliptic	Elliptic	NS	drop	drop	NS

NS : Non significant

Table 2. Identification of the true-typeness of plants of the varieties derived from *in vitro* multiplication in comparison with palm trees of origin on the basis of phenological characters and quantitative and qualitative descriptors of the trees.

Variety	**Najda (INRA-3014)**			**Mejhool**			**Sairlayalate**		
Character	Palm trees of origin	Palm trees issued from the vitroplants	Significant difference	Palm trees of origin derived from offshoots	Palm trees issued from the vitroplants	Significant difference	Palm trees of origin	Palm trees issued from the vitroplants	Significant difference
Percentage of spiny part /palm leave	20.9 a	20.7 a	NS	20.8 a	20.7 a	NS	22.1 a	22.2 a	NS
Total number of spines	41.5 b	42.0 b	NS	34.0 b	34.6 b	NS	30.5 b	31.0 b	NS
Angle of the spine in the middle of rachis	20.0 c	20.5 c	NS	20.0 c	19.8 c	NS	15.0 c	16.0 c	NS
Leaflet consistency	Stiff	Stiff	NS	Very flexible	Very flexible	NS	Medium	Medium	NS

NS : Non significant

Table 3. RAPD markers used and their sequences of primer with the number of DNA bands amplified, number of polymorphic bands and sizes of some selected bands which allows differentiating the date palm varieties.

Primers	Sequences of nucleotids	Number of amplified DNA bands [1]	Number of polymorphic fragments between varieties [2]	One example for size of polymorphic band (Kb) [3]
OP-D3	5'-GTCGCCGTCA-3'	5	1	0.15
OP-D12	5'-CACCGTATCC-3'	5	3	1.58
OP-D16	5'-AGGGCGTAAG-3'	3	3	1.06
OP-J14	5'-CACCCGGATG-3'	5	2	1.47
OP-J19	5'-GGACACCACT-3'	4	3	0.93
OP-L6	5'-GAGGGAAGAG-3'	6	2	1.35
OP-M11	5'-GTCCACTGTG-3'	5	4	1.16
OP-X4	5'-CCGCTACCGA-3'	2	2	2.62
UBC-302	5'-CGGCCCACGT-3'	4	1	2.52
UBC-173	5'-CAGGCGGCGT-3'	5	3	3.53
Total		**44**	**24**	

1: Amplified DNA fragments used for checking and comparing the molecular markers profiles of the palm trees derived from in vitro multiplication and those of palm trees of origin

2: Amplified Polymorphic DNA fragments which allow distinguishing between several varieties including original studied ones.

3: Examples of polymorphic fragment size which have been used for distinguishing the original studied varieties and the palm trees derived from in vitro multiplication

Discussion & Conclusion

According to the limited parameters and plant material used in this study, we didn't detect any phenological nor molecular variability between in vitro palm trees and their palm trees of origin. However, for the descriptor "capacity of production of offshoots and of 'rkebs'", a difference was observed on the trees derived from in vitro technique that abnormally produce the offshoots especially during the first seven years of cultivation. Described phenological characters can identify the off-types varieties at fruit bearing stage but are helpless in the case of juvenile stages of the date palm. Molecular marker techniques are therefore required for cultivar identification and off-type plant detection at early juvenile growth stages. Corniquel and Mer-

cier (1994) found polymorphisms among the cultivars Barhee, Deglet Noor and Mejhool after the extraction of total DNA from offshoot leaves of different individuals and amplifcation of DNA segments using three commercially available random primers. They also indicated that RAPD allowed detecting difference, via changed banding patterns, between individuals of the same cultivar. In our opinion, the selection of molecular markers should be based on intensive and reproducible bands. The number of primers and bands must be high in order to select the best ones. Thus, all 43 analyzed different genotypes, including the present studied varieties, were distinguishable by their band patterns using 37 RAPD markers generated by 19 selected random primers among 123 used ones (Sedra et al., 1998). Thus, RAPD technology appears effective and may contribute to identifying date palm cultivars (Sedra et al., 1998, Sedra, 2000) and determining true-to-type trees. In this study, the molecular markers used can not detect a polymorphism between trees of origin derived from offshoots and the trees derived from in vitro multiplication. Therefore, it was not necessary to estimate the genetic distances between all genotypes and their derived vitroplants. In any case, the estimation of the genetic distances between 43 Moroccan accessions, including the three studied genotypes and other foreign accessions showed a relatively very weak level (Sedra et al., 1998). To detect somaclonal variation, Saker et al. (2000) analyzed tissue culture-derived date palm plants and showed that genetic variations occurred in approximately 4% of the 70 analyzed regenerated plants. Gurevich et al., (2005) fonded a very distinct AFLP band patterns from "Barhee" and "Mejhoul" cultivars. A significant level of genetic variation was detecected among "Mejhoul" plants generated from tissue culture. As also found for POD isozyme patterns, polymorphic bands in RAPD profiles were only detected in 6-12 months old plantations. This detected polymorphism may be due to the somatic embryogenesis in vitro technique used by the author. In our case, it seems that the organogenesis technique didn't generate a genetic variation detectable by the techniques we used.

The ALFP technique, was also used to confirm variety status of tissue culture-derived date palms (Lacaze and Brackpool, 2000). Representative DNA samples from Barhee and Khalas production lines were subjected to AFLP fingerprinting analysis and comparison with the variety standard DNA samples. This analysis indicated that all tissue culture lines were identical to the variety standards. However, the primer combinations only sample a very small portion of the genome.

Microsatellites are small arrays (typically <100bp) of simple di- and tri-nucleotide repeats (Scribner and Pearce, 2000). Microsatellites might have a great potential. In order to characterize several date palm varieties using this technique, 8 microsatellite primers only on 27 tested permitted an amplification of the fragments of DNA of the varieties. Six of these primers generated a polymorphism between the varieties at the level of some alleles like $((ATC)_5$, $(AG)_{12}$, $(CTCACA)_4$, (Sedra, not yet published).The development of a set of microsatellite markers would be useful not only for date palm variety identification but also as complementary tool to detect off-type plants. If the RDA (Representational Difference Analysis) was applied to identify differences between Barhee and Medjool (Kunert et al, 2000, Vorster et al., 2002), its use has not been yet applied for developing markers for culture-induced variation in date palm. The particular advantage of RDA in this context is the ability to screen a much greater fraction of the total genome in a single experiment (Cullis et al., 1999).

However, if molecular techniques can, until now, help to determine the true-to-type plants in monitoring somaclonal variation, they only sample a very small portion of the genome. Therefore, some genomic variation could easily be missed. The advancements made in plant biotechnology including the application of DNA-based markers will permit in vitro plants quality assurance. Given the difficulty to assure, in the present state, the control of the total conformity, the question that arises for a producer concerns the adoption of a genetic conformity or a phenotypical one.

Acknowledgements

Thanks are due to all the persons who helped me locating farmer's fields and orchards, to our laboratory technicians (Mrs Nour S. and Boussak Z.) and to INRA-Zagora experimental station staff and to my colleagurs of tissue culture laboratory at INRA Marrakech

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