

## REVIEW ARTICLE

# Recent achievements in date palm (*Phoenix dactylifera* L.) micropropagation from inflorescence tissues

L. Abahmane\*

Plant Biotechnology Lab, INRA, Regional Centre of Agricultural Research of Marrakech, P.O. Box 533, Marrakech, 40 000, Morocco

## Abstract

The Moroccan demand for date palm plants over the next decade is estimated to be 2.9 million. To fulfill this huge demand, the use of rapid micropropagation techniques is an objective of great interest. Generally, date palm micropropagation is performed from offshoot tissue. However, in the case of some rare genotypes, this technique is inefficient because only limited numbers of offshoots are available. To overcome this problem, a new technique based on the use of emerged inflorescence tissue was developed. The research activities resulted in a completely successful process starting from plant material excision to plant acclimatization and field planting. The technique was used for the micropropagation of 16 date palm genotypes with good fruit quality. To date, hundreds of well-acclimatized plants belonging to 9 genotypes have been produced. In addition, clusters of buds of 3 selected genotypes and Medjool cv. were produced and transferred to a private laboratory for mass propagation. In vitro plants needed to test for bayoud disease resistance of 6 selected genotypes were produced using this technique. Since 2001, well-acclimatized in vitro plants were gradually transferred to the field to study their behavior. Inflorescence-derived plants have shown normal growth and no abnormalities. Fruit set of planted palms started in 2005. In the present paper, micropropagation with inflorescence tissue as well as the related major achievements are presented.

**Key words:** floral explants, in vitro, organogenesis, selected genotypes, vitro plant

## Introduction

Date palm, *Phoenix dactylifera* L. is a perennial long-lived, dioecious, monocot which is highly heterozygous. It is considered the key agricultural species in the preservation of oasis ecosystems. Date palm cultivation is also one of the most economically-important activities in the arid zones of the Middle East and North Africa (El Hadrami and Al-Khayri, 2012). In this region, 62 million of the 105 million date palm trees worldwide grow on over 1.2 million hectares. World production of date fruits is approximately 7.5 million metric tons and generates important commercial activities (FAOSTAT, 2011).

In many countries, date palm cultivation is suffering from serious biotic and abiotic constraints (Sedra, 2011a). In fact, bayoud disease, a soil-borne fungus (*Fusarium oxysporum* f. sp. *albedinis*), is the most dangerous threat to date palm groves in North Africa (Fernandez et al., 1995; Sedra, 2011b). In this region, the disease has destroyed more than 13 million palms in Morocco and Algeria (Sedra, 2011a; Djerbi, 1988). A second important threat is the red palm weevil (*Rhynchophorus ferrugineus* Oliver) which has become the most serious insect pest of date palm in the world (Gómez and Ferry, 1999; Faleiro, 2006). The high rate of spread of this pest is human-caused, by transporting infested young or adult date palm trees and offshoots from contaminated to uninfested areas (Ferry and Gómez, 2002). Among the abiotic constraints, desertification is the most serious factor reducing palm grove production in most of the countries where date palm is cultivated. This phenomenon is aggravated by drought conditions which hamper successful production of the crop. Taking into account those constraints, the need to select palms with high commercial value to replace senescent and destroyed date palm groves has increased steadily in recent decades. Hence,

Received 17 March 2013; Revised 28 April 2013; Accepted 29 April, 2013; Published Online 24 July 2013

\*Corresponding Author

L. Abahmane  
Plant Biotechnology Lab, INRA, Regional Centre of  
Agricultural Research of Marrakech, P.O. Box 533, Marrakech,  
40 000, Morocco

Emails: abahmanel@yahoo.fr

the exploitation of all available means of date palm propagation is required to meet the huge demand for date palm *in vitro* plants.

Plant tissue culture techniques have been used to clone a wide range of date palm cultivars worldwide. Using these techniques, date palm can be micropropagated either by somatic embryogenesis in which embryos are produced from embryogenic callus and then germinated to regenerate complete plantlets (Letouze et al., 2000; McCubbin et al., 2000; Fki et al., 2011; Al-Khayri, 2013) or through organogenesis in which plantlets are produced from multiplied buds without passing through the callus stage (Al Khateeb, 2006; Abahmane 2011a). The organogenesis technique is based on exploitation of the meristematic potentialities of shoot tip explants to form new shoots. The growth regulators incorporated into the media are used at the lowest possible concentrations. Since vegetative buds come directly from mother plant tissue, the plantlets produced are identical to the mother tree. However the success of this technique is highly dependent on the success of the first multiplication step (initiation) which requires a well-trained staff. Furthermore, date palm shoot tip explants are sometimes highly contaminated with internal bacteria. These contaminants are introduced *in vitro* with cultured explants even if they have been well disinfected. Many studies have confirmed the existence of internal bacteria in apparently healthy offshoot tissue. The incidence of this contamination affects 20-50% of cultures (Abahmane, 2011a). Moreover, in the case of some rare or select genotypes, micropropagation is hampered by limited numbers of available offshoots. In fact, some selected genotypes with good fruit quality and presumed resistant to bayoud disease are usually represented by only one or two individual palms. Hence, little plant material (shoot tip explants) is available for development of their micropropagation protocols. In such situations, the use of inflorescence tissue remains the only way to launch *in vitro* multiplication of those genotypes. The use of this technique permits preserving the multiplied trees, especially when rare genotypes are involved. The plant material is abundant and produced every year.

In addition, floral plant material is free from internal bacterial contaminations usually associated with shoot tip derived explants. Recently, interesting research related to this technique has been published (Abahmane 1998, 2003, 2005a, 2005b, 2007, 2010, 2011b, 2011c; Abul-Soad and Mahdi, 2010; Abul-Soad, 2011, 2012; El-Korchi, 2007; Drira and Benbadis, 1985; Drira, 1985; Loutfi, 1989, 1999; Loutfi and Chlyah, 1998). However, more research must be undertaken before routine use of this technique at a commercial level is feasible for date palm mass propagation.

### **Micropropagation protocols via organogenesis from inflorescence explants**

#### **Plant material preparation**

Most research on floral explants has been done on plant material excised from immature inflorescences before emergence. However, tissue excised from newly-emerged inflorescences, before spathe open, can be a valuable source of plant material, particularly in the case of rare or selected genotypes of unique palm trees or those of limited populations. In fact, inflorescences are easy to collect and can be removed without serious damage to the mother tree. The inflorescences are harvested during the flowering period, in the Northern Hemisphere from February to April (Figure 1a). The spathe dimensions are genotype-dependent and consequently are variable, measuring 15-25 cm. The florets are 1.5-2 mm in diameter (Figure 1a, c).

Disinfection of plant material can be done in several steps; first, the spathe is immersed for 10 minutes in a fungicide solution containing 3 g/l of Moncozan (mancozeb). Then, it is opened under aseptic conditions and the spikelets carefully immersed for 15 minutes in a solution of sodium hypochlorite 50% (Figure 1b). Finally the spikelets are washed three times with sterile distilled water before transfer onto culture medium. An antioxidant solution (ascorbic acid: 100 mg/l and citric acid: 150 mg/l) can be used to protect the plant material until its inoculation on culture media. Spikelet fragments with at least 2 or 3 flowers were used as explants to start the micropropagation process from inflorescence tissue (Figure 1c).

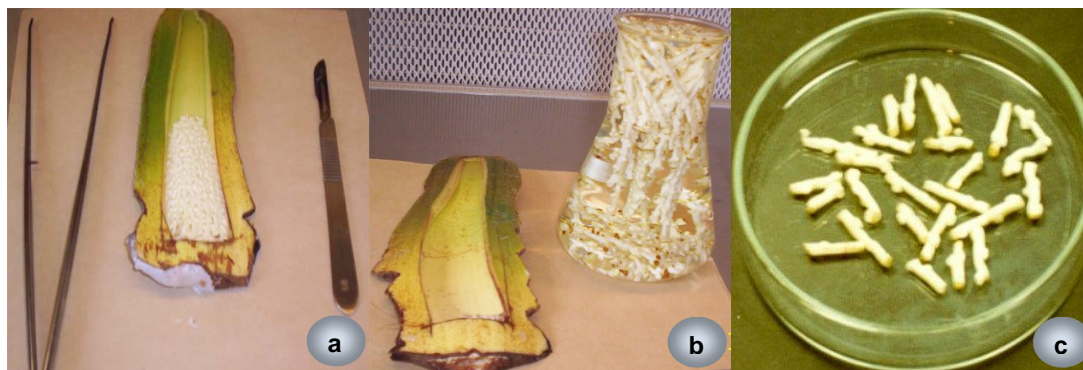


Figure 1. Plant material employed for date palm micropropagation protocols using inflorescence tissue: (a) Emerged inflorescence opened under aseptic conditions, (b) Plant material disinfection in sodium hypochlorite solution, (c) Inflorescence explants (1.5-2 cm) ready to be inoculated on culture media.

Table 1. Culture media used for date palm micropropagation from inflorescence explants.

Media components	Starting stage	Multiplication and elongation stages
Macro elements	Greshoff & Doy (1979)	Murashige & Skoog (1962) (1/2 strength)
Micro elements	Gamborg & Eveleigh (1968)	Murashige & Skoog (1962) (1/2 strength)
Iron source	Murashige & Skoog (1962)	Murashige & Skoog (1962)
Vitamins	Murashige & Skoog (1962)	Murashige & Skoog (1962)
Myo-inositol	100 mg.l <sup>-1</sup>	100 mg.l <sup>-1</sup>
Adenine	25 mg.l <sup>-1</sup>	25 mg.l <sup>-1</sup>
Sucrose	40 g.l <sup>-1</sup>	30 g.l <sup>-1</sup>
Agar	8 g.l <sup>-1</sup>	8 g.l <sup>-1</sup>
PVP-40	2 g.l <sup>-1</sup>	0

### Culture medium

After disinfection procedures, floral explants should be transferred immediately to a culture media. According to the multiplication stage, different mineral salts and other compounds are used in culture establishment, bud multiplication and shoot elongation (Table 1).

The culture media were dispensed in test tubes (150 x 25 mm) at 15-20 ml in the starting stage or in culture jars (170 ml baby food jars) at 50 ml in the multiplication stage. Media were autoclaved at 121°C and 1 bar pressure for 15 or 25 minutes according to the culture medium volume in the containers.

### Incubation conditions

During the first 6 months of incubation, cultures were incubated under complete darkness to avoid polyphenol oxidation which is catalyzed under light conditions. In the succeeding stages, explants were transferred gradually under illuminated conditions with a photoperiod of 16 hours a day. Light intensity varied from 3,000- 6, 000 lux illumination according to the stage of multiplication. The growth room temperature was

maintained at 27±1°C during the illumination period and 22±1°C during the dark period.

### Establishment of initial cultures

Research results on plant material from date palm inflorescences have shown that this kind of tissue is very sensitive to growth regulators, particularly auxins. In fact, tolerable concentration of the most used auxins (NAA, 2,4-D and IBA) is about 2 mg.l<sup>-1</sup>. In the case of NOA, a concentration of 1 mg.l<sup>-1</sup> causes 50% of culture necrosis during the first 3 months of culture. Furthermore, NAA has been shown to be the most effective auxin in bud formation. In the case of cytokinins, 2-iP was more effective than zeatin when combined with BA (Loutfi, 1999). Moreover, the combination of one auxin and two cytokinins was found to be more effective on tissue growth and bud formation (Abahmane, 2005a; Loutfi, 1999; Drira, 1985).

Concerning growth regulator balance, it was shown that high auxin/cytokinin ratios [NOA (0.5 mg.l<sup>-1</sup>), IBA (0.5 mg.l<sup>-1</sup>), BA (0.1 mg.l<sup>-1</sup>)] permitted a high percentage of tissue reactions, but root formation was the most frequent. In the opposite situation [NAA (0.5 mg.l<sup>-1</sup>), BA or 2-iP (2 mg.l<sup>-1</sup>)], budding was obtained either directly from cultured

explants or following floral piece multiplication. Similar results were reported in the literature on inflorescence explants of some Moroccan cultivars (Abahmane, 2005b; Loutfi, 1999).

Regarding sugar concentration, 30 g l<sup>-1</sup> has shown good growth and morphogenesis of the floral explants excised from emerged inflorescences. However, some authors reported that sucrose at 40 g.l<sup>-1</sup> was also optimal for growth and morphogenesis (Loutfi, 1989; Drira, 1985). Furthermore, the absence of sucrose in the culture medium leads to culture browning and necrosis. At concentrations of 10-90 g/l, sucrose permits tissue reversion in a proportion of 6-16% when immature inflorescences were used. Moreover, the percentage of cultures showing root formation increased when sucrose concentrations were augmented (Drira, 1985).

### Types of observed responses

Different tissue responses were observed on floral plant material cultured *in vitro*. The kind of tissue reactions were influenced by many factors but mostly by medium composition and the level of tissue differentiation and lignification. In fact, culture media containing low ratios of auxins/cytokinins: NAA (0.5 mg.l<sup>-1</sup>), BA or 2-iP (2 mg.l<sup>-1</sup>) enhance floral piece multiplication, mostly petals, usually followed by shoot formation (Figure 2a,b). Explants showing petal multiplication were about 5% of the total cultured tissue. In contrast, the use of culture media with high auxins/cytokinins ratios: NOA (0.5 mg.l<sup>-1</sup>), IBA (0.5 mg.l<sup>-1</sup>), BA (0.1 mg.l<sup>-1</sup>) leads to root formation (Figure 2c) and carpel development (Figure 2d). The percentage of explants showing carpel development was about 30%. This percentage decreased when cytokinins (BA or 2-iP) concentrations were augmented.



Figure 2. Floral explant responses on culture media used: (a) Shoot initiation, (b) Shoot multiplication, (c) Root formation and (d) Carpel development.

Similar results were reported in the literature. Drira (1981) reported that cultured floral explants, excised from emerged inflorescences taken from date palm trees 50 years of age, produced more roots, and a fast growth of carpels was observed on culture media containing NAA ( $2.5 \cdot 10^{-6} \text{M}$ ) and BA ( $5 \cdot 10^{-8} \text{M}$ ). Callus formation was also induced on floral explants excised from immature inflorescences when cultured on media containing 2,4-D ( $0.5 \text{ mg.l}^{-1}$ ), IBA ( $0.5 \text{ mg.l}^{-1}$ ) and BA ( $0.2 \text{ mg.l}^{-1}$ ) (Drira and Benbadis, 1985).

Reversion of date palm floral tissue to a vegetative state can take place under tissue culture conditions in one of the following pathways:

- Induction of inflorescence buds reversion: this method requires the use of floral tissue excised from immature inflorescences before spikelet formation. It has the disadvantage of the limited number of inflorescence buds available per tree (Drira, 1985).
- Induction of floral buds reversion: this method has the advantage of working with floral buds at different stages of inflorescence development (Drira, 1985).
- Induction of vegetative budding from floral parts: it presents the major advantage of working with plant material which is abundant and easy to collect from the mother tree (Abahmane, 2005a, 2007; Loutfi, 1989).

- Regeneration of vegetative buds (or somatic embryos) from callus induced from floral parts (Abahmane, 2010; Loutfi, 1999).

### Shoot multiplication

Different culture media have been tested to multiply vegetative buds obtained from inflorescence tissue. Working on inflorescence-derived shoots regenerated from some selected female genotypes, the use of a culture medium containing MS salts at half strength supplemented with NAA ( $0.2 \text{ mg.l}^{-1}$ ), 2-iP ( $0.5 \text{ mg.l}^{-1}$ ) and BA ( $0.5 \text{ mg.l}^{-1}$ ) has attained a satisfactory rate of multiplication varying between 1.5 and 2.2 depending on multiplied genotypes (Figure 3). This culture medium was developed in a previous study on shoot multiplication of some selected genotypes (Abahmane, 2005a). According to recorded data, a different behavior was noticed among genotypes, but in general, the rate of multiplication was beyond 2.2 after the fourth subculture in the case of selected clones INRA-3003 and INRA-B11.

Furthermore, using the developed culture medium, clusters of buds of 3 selected genotypes (INRA-1443, INRA-1445 and INRA-1782) and Medjool cv. were produced and transferred to a private laboratory for mass propagation. From 980 clusters of buds, more than 30,000 vitro plants were produced and acclimatized. This work substantiated the utility of the process developed for date palm mass propagation at a commercial scale.

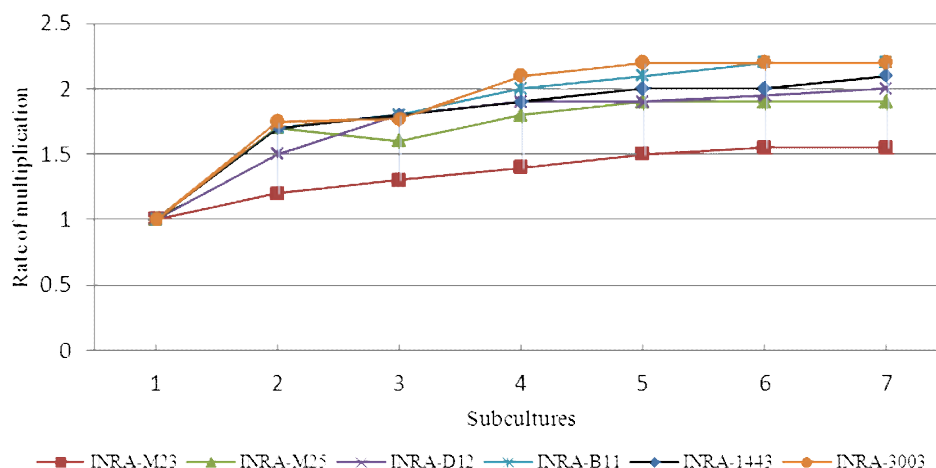


Figure 3. Evolution of multiplication rate of 6 selected date palm genotypes multiplied from inflorescence tissue.

Similar results were reported by Loutfi and Chlyah (1998) in date palm micropropagation from inflorescence tissue. In fact, working on several female cultivars and some palm males, they achieved a satisfactory rate of multiplication of 2 when culture medium containing MS salts supplemented with NAA ( $0.5 \text{ mg.l}^{-1}$ ), BA ( $2 \text{ mg.l}^{-1}$ ) and 2-iP ( $1 \text{ mg.l}^{-1}$ ) was used. However, a diminution in the rate of multiplication was observed after some subcultures. Reduction of BA concentration to  $1 \text{ mg.l}^{-1}$  permitted sustainable bud multiplication even after many subcultures. In addition, Drira (1985) used a culture medium containing NAA ( $0.2 - 0.5 \text{ mg.l}^{-1}$ ) and BA ( $2-3 \text{ mg.l}^{-1}$ ) to multiply inflorescence-derived vegetative buds of the Tunisian cv. Allig. In contrast, a different behavior was reported in the literature for offshoots derived from bud proliferation. Hence, shoot multiplication (Sukary cv.) was found to be better on culture media containing low hormone concentrations, as new bud formation was promoted. In addition, high concentrations resulted in abnormal growth without any observed signs of budding or shoot formation. According to this study, the best combination that gave a good multiplication rate was ( $\text{mg.l}^{-1}$ ): Kin ( $0.2$ ), 2-iP ( $0.1$ ), BA ( $0.1$ ), IAA ( $0.1$ ), NOA ( $0.1$ ) and NAA ( $0.1$ ) (Al Khateeb, 2006).

### Shoot elongation and rooting

At this stage, the use of modified MS salts at half strength supplemented with BA ( $0.05 \text{ mg.l}^{-1}$ ) and NAA ( $0.1 \text{ mg.l}^{-1}$ ) produced well-formed plantlets. Rooting of elongated shoots occurred in parallel with leaf elongation on the previous medium. In the case of immature inflorescences, Drira and Benbadis (1985) reported that well-formed plantlets can be obtained on MS medium supplemented with BA and NAA at  $1 \text{ mg.l}^{-1}$  each, but good rooting of elongated shoots was obtained on culture medium containing BA at  $1 \text{ mg.l}^{-1}$  and IBA at  $1 \text{ mg.l}^{-1}$ . Furthermore, Loutfi (1999) reported good shoot elongation on culture medium containing MS salts supplemented with  $2 \text{ mg.l}^{-1}$  NAA,  $0.5$  to  $1 \text{ mg.l}^{-1}$  2-iP. In addition, gibberellins (GA3) at  $0.01 \text{ mg.l}^{-1}$  were used to enhance elongation of the shoots obtained.

In the case of shoot-tip derived shoots, the most effective plant growth regulators at the elongation stage are NAA or IBA for auxins and Kin and 2-iPA or BA for cytokinins. A combination of NAA ( $1 \text{ mg.l}^{-1}$ ), BA ( $0.5 \text{ mg.l}^{-1}$ ) and Kin ( $0.5 \text{ mg.l}^{-1}$ ) enhances shoot growth and elongation. Gibberellins at  $2 \text{ mg.l}^{-1}$  can also be incorporated into the culture medium in this stage but for no more than 15 days (Beauchesne et al., 1986).

Among rooting auxins, NAA added at  $0.1 \text{ mg/l}$  gave the maximum percentage of root formation, numbers and length (Al Kaabi et al., 2001; Taha et al., 2001). They also reported that use of MS salts at  $\frac{3}{4}$  strength gave the best results on root formation as compared to  $\frac{1}{4}$ ,  $\frac{1}{2}$  and full strength. In the same study, light intensity of  $8,000 \text{ lux}$  and sucrose at  $40 \text{ g.l}^{-1}$  produced the best results in terms of root number and length.

### Plant acclimatization

The regenerated plantlets were transferred in plastic bags under greenhouse conditions. The substrate consisted of a mixture of peat moss and small gravel at equal volume (v/v). The greenhouse temperature was maintained at  $28 \pm 2^\circ\text{C}$  by using heat pumps. The relative humidity was maintained at 70% with a fog system. To increase relative humidity around the newly transferred plantlets, a micro-tunnel covered by transparent plastic was used. Every 2 to 3 days, plantlets were sprayed with a fungicide (Pelt 44) to prevent crown and leaf rot. Under such conditions, plantlets having 2 to 3 leaves, a well formed and closed crown and 3 to 4 roots showed a high survival rate of about 80-90%, depending on the multiplied genotype.

Using this protocol, hundreds of well-acclimatized vitro plants were produced in 9 genotypes: INRA-1007, INRA-1394, INRA-1782, INRA-1443, INRA-1445, INRA-B11 and INRA-3003, INRA-954 and Medjool cv. (Figure 4a,b).

During the acclimatization stage, water supply must be monitored very carefully during the first months. Too much water can lead to plantlet rot and too little moisture in the substrate can decrease the relative humidity around the plants and cause their rapid wilt. After 6-8 months, acclimatized vitro plants were transferred to a shade house for further hardening before transfer to open field.





Figure 4. Well-acclimatized inflorescence-derived vitro plants regenerated from genotypes (a) INRA-B11 and (b) INRA-1443.

More details on date palm plantlets acclimatization data were reported previously (Abahmane, 2011a). In fact, the research done on date palm acclimatization has shown that plantlets to be transferred ex vitro should have some important characteristics that enable them to succeed in the greenhouse. Multiplied date palm genotypes produced plantlets of different quality. In fact, some selected genotypes produced vigorous and well-formed plantlets of desired characteristics (INRA-3003, INRA-B11, INRA-1394, INRA-1007) while others produce plantlets of inferior quality (open crown, weak plantlets, etc). In the latter situation, plantlets should be kept in the lab until they acquire the desired characteristics. In fact, the artificial conditions during in vitro culture resulted in formation of plantlets with abnormal morphology, anatomy and physiology (El Bahr et al., 2003; Saker et al., 2000). After ex vitro transfer, they may be easily impaired by sudden changes in environmental conditions and so need a period of acclimatization to overcome these abnormalities (Pospisilova et al., 1999). Accordingly, the acclimatization phase is the most important stage in the protocol of date palm micropropagation, because if not controlled, the whole process can be inefficient. Hence, major differences exist between the environment of plants growing in tissue culture and those in the greenhouse; in particular, differences in light (both quantity and quality), relative humidity, nutrients and other growth promoters, the gaseous composition and the medium substrate (Seelye et al., 2003).

Multiple soil mixtures have been used to transfer plantlets ex vitro. The main mixture characteristic that influences plant growth is moisture, which should not be excessive to avoid

fungi attack (rot) and not too low to avoid plantlet desiccation. Tisserat (1981) reported that the best survival rate was recorded for 10-12 cm date palm plantlets transferred to a mixture composed of peat moss and vermiculite (v/v) and covered with transparent plastic.

Regenerated plants have shown a normal behavior similar to that of shoot-tip derived plants in terms of growth and development. Cultures are usually kept in the lab for 18-24 months and then plant material is renewed. Within this period, no abnormalities were observed. Currently, only field evaluation is used to study genetic fidelity of produced vitro plants. However, some parameters (ploidy level, DNA analysis) must be examined to confirm field observations and confirm true-to-typeness of regenerated plants.

#### Uses of inflorescence-derived vitro plants Bayoud resistance tests

One of the main objectives of the development of this multiplication process was the production of vitro plants needed to perform bayoud resistance tests on some selected genotypes. These tests are necessary to complete the studies on selected genotypes, mainly in terms of tolerance to bayoud disease, before their large scale propagation and distribution to farmers. For this reason, 40 well-acclimatized vitro plants, with 4 to 5 leaves and belonging to INRA-1007 and INRA-1394, were transferred to the plant pathology laboratory to complete those tests. This work was done under greenhouse-controlled conditions by artificial inoculation of vitro plants with *Fusarium oxysporum* f. sp. *albedinis* spores. Beyond the tested genotypes, vitro plants of Black Boushami cv. were used as the

bayoud resistant control and Boufeggous cv. as the sensitivity control.

Currently, vitro plants of four genotypes: INRA-954, INRA-1782, INRA-1443 and INRA-B11 are available and ready to be tested against bayoud disease in the coming months.

### Transfer of vitro plant to the field

Vitro plants, at a 1-3 pinnate leaf stage, belonging to INRA-954, INRA-1782 and INRA-1007 genotypes were transferred to the field of the experimental stations in Marrakech (March 2001), Zgora (March 2004) and Errachidia (April 2006). The main objectives of this work were:

- **Study of agronomic behavior:** Field evaluation of planted vitro plants has shown normal growth in the three localities for all planted genotypes. Growth rate is slightly different among vitro plants in soil according to their origin genotypes (Figure 5). No abnormalities in growth or development were observed after 10 years in the field (Figure 6a). Morphological traits between regenerated plants and their origin trees were similar.

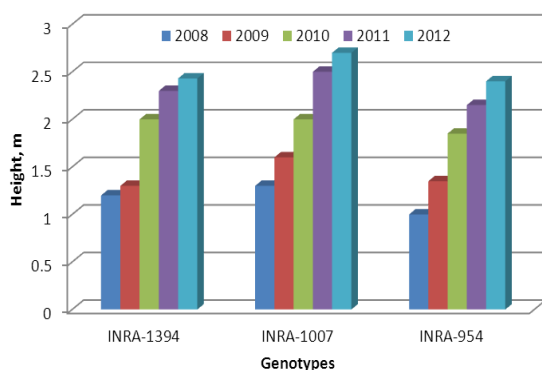


Figure 5. Growth over 5 years, of regenerated vitro plants in the field at Errachidia experimental station.

- **Evaluation of true-to-typeness:** The vitro plants of INRA-1007, transferred to the field at Marrakech station, fruited for the first time in 2005 (Figure 6b). The vitro plants of the second genotype, INRA-1394, produced fruit in 2007 (Figure 6c). Fruit set of vitro plants transferred to an open field at Errachidia and Zagora experimental stations was observed, respectively, in 2009 and 2011. Actually more than 45% of planted vitro plants have produced fruit. In order to evaluate true-to-typeness of inflorescence-derived plants, their fruits were

compared to those of the mother trees. No differences were found between vitro- plant derived fruits and fruit of the mother plants growing in Zagora and Errachidia experimental stations. All fruit traits (color, shape, weight, dimensions) were comparable. Results obtained provide strong indications that the inflorescence-derived plants are true-to-type. A previous study done on root tips of inflorescence-derived shoots, at the multiplication stage, showed that all samples studied were diploid ( $2n = 26$ ). The regenerated plantlets have the same number of chromosomes as the mother trees (Loutfi, 1999).

- **Study of planted vitro plants resistant to bayoud disease:** This work was undertaken at Zagora station which has soil which is highly contaminated with bayoud fungus. After 7 years in contact with fungus spores, no symptoms of bayoud disease were observed on planted vitro plants, suggesting bayoud tolerance of the genotypes studied. Bayoud tolerance confirmation must be done using artificial inoculation by fungal spores.

- **Enrichment of date palm gene banks:** Two field gene banks were established at the Zagora and Errachidia experimental stations. They contain all selected date palm genotypes for their fruit quality and tolerance to bayoud disease. However, some interesting genotypes were lost because of their high sensitivity to bayoud disease. So, two other gene bank collections were newly-created at Saada and Tassaut experimental stations in Marrakech. Those new collections are in areas free of bayoud disease and hence will be suitable sites to preserve bayoud-sensitive genotypes. They were gradually enriched by newly multiplied genotypes. Currently, vitro plants belonging to INRA-1394, INRA-1443, INRA-1007, INRA-954, INRA-1782 and INRA-B11 are available and ready to be transferred to those gene banks (Table 2).

It is worth mentioning that this was the first time in Morocco that hundreds of vitro plants have been produced from inflorescence tissue, and derived plants transferred to the field and fruited. The fruiting palms give good indications of true-to-typeness and consequently attest to the possibility of using this new process for mass propagation of date palm cultivars.





Figure 6. inflorescence-derived vitro plants at INRA experimental stations: (a) Vitro plants of good growth at Zagora station, (b) Fruit set of INRA-1007 vitro plants at Marrakech station and (c) Fruit set of INRA-1394 vitro plants at Errachidia station.

Table 2. Well acclimatized vitro plants regenerated from inflorescence tissue.

Genotypes	Produced numbers at:		Stage
	Research level	Commercial level	
INRA-1007	270	-	Hardening + field <sup>b</sup>
INRA-1394	300	-	Hardening + field <sup>b</sup>
INRA-0954	20	-	Hardening + field <sup>b</sup>
INRA-1782	100	3, 000 <sup>a</sup>	Hardening
INRA-1443	150	4, 000 <sup>a</sup>	Hardening
INRA-1445	10	3, 000 <sup>a</sup>	Hardening
INRA-3002	180	-	Acclimatization (Greenhouse)
INRA-3003	500	-	Acclimatization (Greenhouse)
Medjool variety	20	20, 000 <sup>a</sup>	Hardening
TOTAL	1, 550	30,000 <sup>a</sup>	

<sup>a</sup> Vitro plants produced in partnership with a private laboratory.

<sup>b</sup> Samples of regenerated vitro plants were transferred to the field.

## Conclusions and prospects

The use of emerged inflorescence tissue, as a novel source of plant material, is a powerful alternative method to micropropagate rare or selected date palm genotypes. In addition, the protocol used for inflorescence removal is very safe and does not affect adversely the mother tree. Results achieved make clear the possibility of shoot initiation from this kind of plant material. Shoots obtained can be mass propagated on suitable culture media in the same manner as offshoot-derived buds. Hundreds of complete plantlets were produced and successfully acclimatized under controlled conditions in the greenhouse. Well-acclimatized vitro plants were produced and transferred to the field in three experimental stations in Marrakech, Zagora and Errachidia, in order to study their agronomic behavior at different localities. After more than 10 years in the field, the inflorescence-derived plants have shown normal growth with no observed abnormalities. Moreover,

fruits produced were exactly comparable to those of the mother trees. A thousand clusters of buds were transferred to a private laboratory that managed to produce from them about 30,000 vitro plants. All those encouraging results attest to this technique as a promising useful tool for future mass propagation of date palm in Morocco. Additional research is planned, in particular to determine the ploidy of in vitro regenerated plants via the flow cytometry technique.

## References

- Abahmane, L. 1998. Micropropagation of date palm (*Phoenix dactylifera* L.) selected clones by using inflorescence tissues. In: Proceedings of the international conference on date palm. ACSAD, Syria. pp. 256-260.
- Abahmane, L. 2003. Date palm micropropagation by using inflorescence tissues. In: Proceedings of the international symposium on date palm. King Saud University, Kingdom of Saudi

- Arabia. pp. 911-924.
- Abahmane, L. 2005a. Les tissus inflorescentiels: Une nouvelle source de matériel végétal pour la micropropagation des clones sélectionnés du palmier dattier (*Phoenix dactylifera* L.). In: Proceedings of the international symposium on sustainable agricultural development of oasian systems. National Institute for Agricultural Research, Morocco. pp. 99-105.
- Abahmane, L. 2005b. Micropropagation par tissus inflorescentiels du palmier dattier (*Phoenix dactylifera* L.): Un outil efficace pour la sauvegarde des génotypes rares. Al-Awamia 113:49-60.
- Abahmane, L. 2007. Micropropagation of selected clones from inflorescence tissues and its role in date palm (*Phoenix dactylifera* L.) improvement program. In: Proceedings of the fourth international symposium on date palm. Al Hassa, King Faisal University, Saudi Arabia. p. 107. (Abstr.).
- Abahmane, L. 2010. Date palm (*Phoenix dactylifera* L.) micropropagation from inflorescence tissues by using somatic embryogenesis technique. Acta Hort. 882:827-832.
- Abahmane, L. 2011a. Date palm micropropagation via organogenesis. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 69-90. Date Palm Biotechnology, Springer, Dordrecht.
- Abahmane, L. 2011b. Multiplication à partir des inflorescences: une voie prometteuse pour la micropropagation des génotypes sélectionnés de palmier dattier (*Phoenix dactylifera* L.). In Proceedings of the first international date palm symposium, Algiers. p. 55. (Abstr.).
- Abahmane, L. 2011c. Importance of inflorescence tissues in micropropagation of selected date palm (*Phoenix dactylifera* L.) genotypes. In: Proceedings of the first scientific conference for the development of the palm and dates sector in Arab world, Saudi Arabia. p. 39. (Abstr.).
- Abul-Soad, A. A. 2011. Micropropagation of date palm using inflorescence explants. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 91-118. Date Palm Biotechnology. Springer, Dordrecht.
- Abul-Soad, A. A. 2012. Influence of inflorescence explant age and 2,4-D incubation period on somatic embryogenesis of date palm. Emir. J. Food Agri. 24(5):434-443.
- Abul-Soad, A. A. and M. S. Mahdi. 2010. Commercial production of tissue culture date palm (*Phoenix dactylifera* L.) by inflorescence technique. J. Gen. Eng. Biotech. 8(2):39-44.
- Al Kaabi, H. H., A. Rhiss and M. A. Hassan. 2001. Effect of auxins and cytokinins on the *in vitro* production of date palm bud generative tissues and on the number of differentiated buds. In: Proceedings of the second international conference on date palm. Al Ain University, UAE. p. 47-86.
- Al Khateeb, A. A. 2006. Role of cytokinin and auxin on the multiplication stage of date palm (*Phoenix dactylifera* L.) cv. Sukry. Biotechn. 5(3):349-352
- Al-Khayri, J. M. 2013. Factors affecting somatic embryogenesis in date palm (*Phoenix dactylifera* L.) In: J. Aslam, P. S. Srivastava and M. P. Sharma (Eds.), pp. 15-38. Somatic Embryogenesis and Genetic Transformation in Plants, Narosa Publishing, New Delhi.
- Beauchesne, G., A. Zaid and A. Rhiss. 1986. Meristematic potentialities of bottom of young leaves to rapidly propagate date palm. In: Proceedings of the second symposium on date palm. King Faisal University, Saudi Arabia. pp. 87-94.
- Djerbi, M. 1988. Les maladies du palmier dattier. Projet régional de lutte contre le Bayoud. FAO (RAB/84/0/8), Alger.
- Drira, N. 1981. Multiplication végétative et micropropagation du palmier dattier (*Phoenix dactylifera* L.) à partir d'organes prélevés sur la phase adulte cultivés *in vitro*. Specialty doctorate thesis. Fac. Sc. Tunis, Tunis.
- Drira, N. 1985. Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) par les néoformations induites en culture *in vitro* sur des organes végétatifs et floraux prélevés sur la phase adulte. Es-Sciences doctorate thesis. Fac. Sc. Tunis, Tunis.
- Drira, N. and A. Benbadis. 1985. Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) par réversion, en culture *in vitro*, d'ébauches florales de pieds femelles. J. Plant Phys. 119:223-235.
- El Bahr, M. K., Z. A. Ali and H. S. Taha. 2003. *In vitro* propagation of Egyptian date palm cv.

- Zaghlool: II. Comparative and anatomical studies between direct acclimatized and *in vitro* adapted pre-acclimatized plantlets. Arab Univ. J. Agr. Sci. 11(2):701-714.
- El Hadrami, A. and J. M. Al-Khayri. 2012. Socioeconomic and traditional importance of date palm. Emir. J. Food Agri. 24 (5):371-385.
- El-Korchi, B. 2007. Large scale *in vitro* propagation of a rare and unique male date palm (*Phoenix dactylifera* L.) using inflorescence technique. Acta Hort. 736:243-254.
- Faleiro, J. R. 2006. A review on the issues and management of red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. 26(3):135-154.
- FAOSTAT. 2011. <http://faostat.fao.org>
- Fernandez, D., M. Lourd, M. Ouinten, A. Tantaoui and J. P. Geige. 1995. Le Bayoud du palmier dattier: Une maladie qui menace la phoeniculture. Phytoma - La Défense des végétaux No 469:36-39.
- Ferry, M. and S. Gómez. 2002. The red palm weevil in the Mediterranean area. Palms 46(4):172-178.
- Fki, L., R. Masmoudi, W. Kriaa, A. Mahjoub, A. B. Sghaier, R. Mzid, A. Mliki, A. Rival and N. Drira. 2011. Date palm micropropagation via somatic embryogenesis. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 47-68. Date Palm Biotechnology, Springer, Dordrecht.
- Gamborg, O. L. and D. Eveleigh. 1968. Culture methods and detection of glucanases in suspension cultures of wheat and barley. Can. J. Biochem. 46:417-421.
- Gómez, S. and M. Ferry. 1999. Attempts at biological control of date palm pests recently found in Spain. In: Proceedings of the 1<sup>st</sup> Symposium for Applied Biological Control in Mediterranean Countries. Publisher Sacco, France. pp. 121-125.
- Gresshoff, P. M. and C. H. Doy. 1972. Development and differentiation of haploid *Lycopersicon esculentum*. Planta 107:161-170.
- Letouze, R., F. Daguin, L. Hamama, K. Paquier, F. Marionnet and J. Javouhey. 2000. Mass propagation of date palm through somatic embryogenesis. Histological study of embryo formation and cultivar identification by RAPD markers. In: Proceedings of date palm international symposium, Windhoek, Namibia. pp. 55-64.
- Loutfi, K. 1989. Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) à partir de la culture *in vitro* d'explants inflorescentiels. Doctorate thesis, Cadi Ayyad University, Morocco.
- Loutfi, K. 1999. Organogenèse et embryogenèse somatique à partir des tissus floraux du palmier dattier (*Phoenix dactylifera* L.) cultivés *in vitro*. Aspects histologiques et caryologie des vitro plants. Es-Sciences doctorate thesis, Université Cadi Ayyad, Morocco.
- Loutfi, K. and H. Chlyah. 1998. Multiplication végétative du palmier dattier à partir de segments d'inflorescences cultivés *in vitro*: effet de différentes combinaisons hormonales et capacités organogénétiques de divers cultivars. Agron. 18(8-9):573-580.
- McCubbin, M. J., J. Van Staden and A. Zaid. 2000. A southern African survey conducted for off-types on date palm production using somatic embryogenesis. In: Proceedings of date palm international symposium, Windhoek. p. 68-72.
- Murashige, T. and F. Skoog F. 1962. A revised medium for rapid growth and bioassays with tissues culture. Phys. Plant. 15:473-497.
- Pospisilova, J., I. Ticha, P. Kadlec, D. Haisel and S. Plzakova. 1999. Acclimatization of micropropagated plants to *ex vitro* conditions. Biotech. Plant. 42(4):481-497.
- Saker, M., M. Bekheet, H. S. Taha, A. S. Fahmy and H. A. Moursy. 2000. Detection of seasonal variations in tissue culture derived date palm plants using isozyme analysis and RAPD fingerprints. Biol. Plant. 43:347-351.
- Sedra, M. H. 2011a. Development of new Moroccan selected date palm varieties resistant to bayoud and of good fruit quality. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 513-532. Date Palm Biotechnology, Springer, Dordrecht.
- Sedra, M. H. 2011b. Molecular markers for genetic diversity and bayoud disease resistance in date palm. In: S. M. Jain, J. M. Al-Khayri and D. V. Johnson (Eds.), pp. 533-550. Date Palm

- Biotechnology, Springer, Dordrecht.
- Seelye, J. F., G. K. Burge and E. R. Morgan. 2003. Acclimatizing tissue culture plants: reducing the shock. Proc. Int. Plant Prop. Soc. 53:85-90.
- Taha H. S., S. A. Bekheet and M. M. Saker. 2001. Factors affecting *in vitro* multiplication of date palm. Biol. Plant. 44(3):431-433.
- Tisserat, B. 1981. Production of free-living date palms through tissue culture. Date Palm J. 1(1):43-54.