

Effect of Liquid Media and *In Vitro* Pre-acclimatization Stage on Shoot Elongation and Acclimatization of Date Palm (*Phoenix dactylifera* L.) cv. Najda

Mouaad Amine Mazri*

INRA, CRRA-Marrakesh, UR Agro-Biotechnologie, Avenue Mohammed VI, B.P. 533, Marrakesh, Morocco.

Received: 6 June 2012

Accepted: 15 October 2012

*Corresponding author's email: m.a.mazri@gmail.com

The effect of stationary liquid media to improve shoots elongation in date palm cv. Najda and an *in vitro* pre-acclimatization stage to increase the survival percentage of plantlets in the greenhouse was investigated. Two basal liquid media were tested: full strength and half strength Murashige and Skoog medium (MS). Media were devoid of plant growth regulators (PGRs), containing 0.5 mg/L 6-benzylaminopurine (BAP) or 0.5 mg/L indole-3-butyric acid (IBA) and 0.5 mg/L BAP. After three months of culture, all liquid media were efficient and allowed to overcome slow growth problem. The maximum shoot elongation was 11.3 cm, obtained in MS supplemented with 0.5 mg/L BAP and 0.5 mg/L IBA. The shoots were isolated and transferred either to the greenhouse or to one month *in vitro* pre-acclimatization stage in a PGRs-free solid medium (MS or MS/2) then to the greenhouse. A higher surviving rate (ranging from 70 to 86 %) was obtained after one month pre-acclimatization compared with the direct transfer to the greenhouse (12–28 %).

Abstract

Keywords: Acclimatization, BAP, Elongation, IBA, Liquid media, Pre-acclimatization, Rooting.

INTRODUCTION

Najda is a Moroccan date palm cultivar (*Phoenix dactylifera* L.) resulting from selection programs of the National Institute of Agronomical Research (INRA-Morocco), and characterized by *Fusarium oxysporum* f. sp. *Albedinis* (bayoud) resistance and high fruit quality (Ferry, 2011; Sedra, 2011). In order to reconstitute groves destroyed by bayoud disease, the use of a large-scale multiplication technique producing true-to-type plantlets of this cultivar was primordial. According to Kunert et al., (2003) and Ferry (2011), *in vitro* organogenesis guarantees the true-to-typeness since the callus phase is avoided. Thus, this technique was chosen for Moroccan date palm cultivars propagation (instead of somatic embryogenesis) and many culture media were subject to experiments.

The success of organogenesis is highly genotypic dependent (Jain, 2012), and investigations are needed to perform and adapt culture media considering the genotype used (Abohatem et al., 2011). For date palm organogenesis, the elongation phase is necessary in the conversion of bud shoot to plantlets (McCubbin and Zaid, 2007). Bud and shoot susceptibility to elongation depends on the cultivar used; and some assays to improve this phase, based mainly on PGRs concentrations, were performed (Khierallah and Bader, 2007; Mazri and Meziani, 2012).

Regarding the acclimatization phase, some authors have suggested the use of a liquid medium before the transfer of plantlets to the greenhouse (Fki *et al.*, 2011) while others suggested the use of a solid medium (Preil, 2005). Hence, the effect of the physical status of the medium on plantlets acclimatization depends also on the genotype in study.

Considering cv. Najda and according to our experiments (Mazri and Meziani, 2012), after each transfer to an elongation medium, 15 to 20 % of organogenic cultures showed recalcitrance to elongation (Fig. 1a), instead, they continued multiplication or showed a very slow growth. In order to resolve this problem, the effect of liquid media to improve shoots elongation was investigated. Furthermore, in order to optimize acclimatization, the effect of one month pre-acclimatization stage in a solid medium to strengthen plantlets was also evaluated.

MATERIALS AND METHODS

Plant material and culture conditions

Two liquid media were prepared, full strength MS as it was described by Murashige and Skoog (1962), and half strength MS (MS/2). Media were supplemented with 30 g/L sucrose and 1 g/L Polyvinyl-pyrrolidone (PVP) then with different concentrations of IBA and BAP:

1. M1: MS/2
2. M2: MS
3. M3: MS/2 + 0.5 mg/L BAP
4. M4: MS + 0.5 mg/L BAP
5. M5: MS/2 + 0.5 mg/L IBA + 0.5 mg/L BAP
6. M6: MS + 0.5 mg/L IBA + 0.5 mg/L BAP

Eight milliliters of each medium was dispensed into jars (300 ml volume, 7 cm diameter) then autoclaved at 121°C for 25 minutes. Under a laminar flow hood, the organogenic cultures that showed recalcitrance to elongation (Fig. 1a) were collected and transferred into the jars. The jars were then transferred to a growth chamber with a photoperiod of 16 h light and 8 h dark at 25 ± 1°C. Media were refreshed every month.

After three months of culture, 50% of shoots of or exceeding 10 cm in length were transferred directly to the greenhouse for acclimatization, and 50% were cultured for one month into solid MS or MS/2 media (solidified with 6 g/L plant agar) before acclimatization.

Prior to acclimatization, the root system of isolated shoots was washed gently under running tap water then treated with fungicide (1 g/L pelt 44 PM) for 15 minutes. The plantlets were then transferred to plastic pots containing peat and gravel (1:1, v/v) for acclimatization. Pots were placed in a polyethylene tunnel within the greenhouse. After two weeks, we started a gradual opening of

the tunnel and the polyethylene was totally removed after four weeks.

Inside the tunnel, the relative humidity was 98% while in the greenhouse it was 70%. In both cases, the temperature was 27°C + 1.

Observed data and statistical analysis

Each jar was containing three explants, which was considered as one repetition, for each medium we used forty repetitions.

At the end of each month of culture, the following data were recorded: shoot length, shoot greening, root number and length. Three months after acclimatization, percentage of survival plantlets was calculated.

All data were subjected to an analysis of variance (ANOVA); the adopted model was CRD (Completely Randomized Design) at 5% significance level. Prior to analysis, percentage data were arcsin transformed. Means were separated according to the Student-Newman-Keuls test (SNK) at a 0.05 level of probability (SPSS 16.0).

RESULTS AND DISCUSSION

Table 1 shows data of all observed parameters during the elongation phase, after one, two and three months of culture. The first thing to notice is that liquid media were very efficient to overcome the recalcitrance of shoots to elongation, since 100% shoots showed an immediate and fast elongation, which was consistent with Fki *et al.*, (2011), who reported the beneficial effect of liquid media on shoot elongation of date palm, cv. barhee. According to statistical analysis, there was no significant difference between shoot length in hormone-free media (M1 and M2); however, they were significantly different ($p \leq 0.05$) to those on media supplemented with PGRs. Shoots on media M3, M4, M5 and M6 didn't show a significant difference in shoot length among them. Those results lead to the conclusion that media strength (full strength and half-strength) didn't have an effect on shoots elongation; however, PGRs did. The effect of PGR to increase shoot length in date palm was also reported by Khierallah and Bader (2007) and Mazri and Meziani (2012). The effect of media strength was observed only on leaf greening, which was more important in MS full strength media (Table 1).

When IBA was supplemented to the culture medium (M5 and M6), we didn't observe an efficient effect on plant length, since there was no significant difference among media with and without this hormone (M3, M4, M5 and M6). Shoots in elongation onto media supplemented with IBA showed the formation and proliferation of new bud shoots in their basis (Fig. 1b), which is undesirable during the elongation stage. Thus, only the physical status of the medium (liquid) and BAP (cytokinin) were responsible on accelerating shoot elongation. The beneficial effect of BAP on shoot elongation was also reported in other species such as *Musa acuminata* cv. Berangan (Jafari *et al.*, 2011) and *Aquilaria hirta* (Hassan *et al.*, 2011). According to Khierallah and Bader (2007), solid and agitating liquid media had increased significantly shoot proliferation, which is undesirable during the elongation stage, while a stationary liquid medium didn't promote shoot proliferation. This result explained why shoot length increased when shoots are cultured in a stationary liquid medium, since the formation of new bud shoots was rare and all absorbed elements were used to increase shoot length and rooting. As reported afore, the only case in which new shoot formation and proliferation occurred was in media supplemented with IBA. Furthermore, IBA didn't accelerate elongation; therefore, IBA could be omitted since the only benefit it showed was increasing the number of roots per shoot. Nevertheless, this effect didn't increase the survival percentage of plantlets during the acclimatization (Table 2).

Root formation during the elongation phase was depending on PGRs in the culture medium. Media supplemented with BAP and IBA showed the highest number of roots per explants (4.8), followed by media supplemented by the sole BAP then hormone-free media. This showed the

effect of both BAP and IBA on root formation, and confirmed the findings of Othmani *et al.* (2009), who mentioned that a pronounced decrease in root formation was observed on media that lacked growth regulators in date palm cv. Deglet Nour. Concerning roots length, there was no significant difference between the studied media.

The positive effect of IBA on rooting was previously mentioned by Aslam and Khan (2009) and Hegazy and Aboshama (2010) on date palm cv. Khalas and cv. Medjool, respectively. Fki *et al.*, (2011) reported the presence of a significant positive correlation between IBA concentrations and rooting frequencies in *Phoenix dactylifera* L. Barhee cv. Concerning BAP, despite that it is categorized as cytokinin, it has succeeded to improve root formation, which is generally stimulate by auxins as it was reported by many authors (Khierallah and Bader, 2007; Hegazy and Aboshama, 2010; Fki *et al.*, 2011). Nevertheless, culturing plantlets in media supplemented with BAP during elongation did not increase the survival percentage in acclimatization (Table 2).

The pre-acclimatization stage was very efficient to improve shoot quality (Table 2, Fig. 1c). Shoots that have been isolated and cultured in a solid medium showed an increase in their length (reached 14.4 cm for those transferred from M6 to MS) and a development in their root system (Table 2). They showed also an enlargement and a thickness increase in their leaves and the increase of their greening intensity (Fig. 1c). Moreover, these shoots showed a high survival percentage during acclimatization, exceeding 70 % (Fig. 1d), while it was less than 30 % within those transferred directly from the liquid media to the greenhouse (Table 2). Our findings confirms the statement of Preil (2005) who suggested to avoid the use of liquid media just before acclimatization; but disagrees with Othmani *et al.*, (2009) who suggested the use of liquid media before the acclimatization of date palm Deglet Nour plantlets. These different findings showed that, going through a solid or a liquid medium just before acclimatization depends on the genotype. In our case, going through a solid medium as a final stage just before acclimatization allowed plantlets to turn vigorous with good root system and foliage, ready for acclimatization. Other authors have also used a pre-acclimatization stage to ensure high survival percentage in acclimatization of date palm (cv. Bartomuda) plantlets (Darwesh and Mohamed, 2009; Darwesh *et al.*, 2011). Elbahr (2011) reported that 30 days of pre-acclimatization was the best period to obtain the highest survival percentage for cultivars Bartamoda and Sakkoty.

In conclusion, liquid media were very efficient to overcome shoot recalcitrance to elongation. However, shoots elongated in liquid media showed some difficulties during the acclimatization; and a pre-acclimatization stage in a solid medium seemed primordial to increase their vigor then their survival percentage in the greenhouse. Adding BAP or IBA to media was not necessary. Yet these two hormones have accelerated elongation and improved rooting, respectively; however, they didn't improve the survival percentage of plantlets during acclimatization.

Literature Cited

- Abohatem, M., Zouine, J. and El-Hadrami, I. 2011. Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. *Sci. Horticult.* 130: 344–348.
- Aslam, J., Khan, S.A. 2009. *In vitro* micropropagation of khalas date palm (*Phoenix dactylifera* L.), an important fruit plant. *J. Fruit Orn. Plant Res.* 17:15–27.
- Darwesh, R.S.S. and Mohamed, H.F. 2009. The adverse effect of GA3 (gibberellic acid) on salinity of *Phoenix dactylifera* L. plantlets *in vitro* rooting stage. *Proc. 4th Conference on Recent Technologies in Agriculture*; Cairo, Egypt 3-5 November. p 663-669.
- Darwesh, R.S.S., Zaid, Z.E. and Sidky, R.A. 2011. Effect of ammonium nitrate and GA3 on growth and development of date palm plantlets *in vitro* and acclimatization stage. *Res. J. Agric. Biol. Sci.* 7(1): 17-22.

- El-Bahr, M.K. 2011. Application of *in vitro* culture technique for propagation of two cultivars of Egyptian date palm. <http://arabpalm.org/2011/eng/day2.asp>.
- Ferry, M. 2011. Potential of date palm micropropagation for improving small farming systems. p. 15-28. In: S.M. Jain, J.M. Al-Khayri and D.V. Johnson (eds.), *Date Palm Biotechnology*. Springer Science+Business Media B.V.
- Fki, L., Bouaziz, N., Kriaa, W., Benjemaa-Masmoudi, R., Gargouri-Bouazid, R., Rival, A. and Drira, N. 2011. Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. *J. Plant Physiol.* 168:1694–1700.
- Hassan, N.H., Ali, N.A.M., Zainudin, F. and Ismail, H. 2011. Effect of 6-benzylaminopurine (BAP) in different basal media on shoot multiplication of *Aquilaria hirta* and detection of essential oils in the *in vitro* shoots. *Afr. J. Biotechnol.* 10(51): 10500-10503.
- Hegazy, A.E. and Aboshama H.M. 2010. An efficient novel pathway discovered in date palm micropropagation. *Acta Hort.* 882:167-176.
- Jafari, N., Othman, R.Y. and Khalid, N. 2011. Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. *Afr. J. Biotechnol.* 10(13): 2446-2450.
- Jain, S.M. 2012. Date palm biotechnology: Current status and prospective-an overview. *Emir. J. Food Agric.* 24 (5): 386-399.
- Khierallah, H.S.M. and Bader, S.M. 2007. Micropropagation of date palm (*Phoenix dactylifera* L.) var. Maktoom through direct organogenesis. *Acta Hort.* 736:213–224.
- Kunert, K.J., Baaziz, M. and Cullis, C.A. 2003. Techniques for determination of true-to-type date palm (*Phoenix dactylifera* L.) plants: a literature review. *Emirates J. Agric. Sci.* 15:1–16.
- Mazri, M.A. and Meziani, R. 2012. An improved method for micropropagation and regeneration of date palm (*Phoenix dactylifera* L.). *J. Plant Biochem. Biotechnol.* doi: 10.1007/s13562-012-0147-9.
- McCubbain, M.J. and Zaid, A. 2007. Would a combination of organogenesis and embryogenesis techniques in date palm micropropagation be the Answer? *Acta Hort.* 736: 255-259.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15:473–497.
- Othmani, A., Bayoudh, C., Drira, N., Marrakchi, M. and Trifi, M. 2009. Regeneration and molecular analysis of date palm (*Phoenix dactylifera* L.) plantlets using RAPD markers. *Afr. J. Biotechnol.* 8(5): 813–820.
- Preil, W. 2005. General introduction: a personal reflection on the use of liquid media for *in vitro* culture. p 1-18. In: A.K. Hvoslef-Eide and W. Preil (eds), *Liquid Culture Systems for in vitro Plant Propagation*. Springer, Dordrecht, The Netherlands.
- Sedra, M.H. 2011. Development of new Moroccan selected date palm varieties resistant to Bayoud and of good fruit quality. p 513-531. In: S.M. Jain, J.M. Al-Khayri and D.V. Johnson (eds.), *Date Palm Biotechnology*. Springer Science+Business Media B.V.

Tables

Table 1. Effect of different media on shoot length and development during the elongation phase, data are reported after one, two and three months of culture.

		Shoot length (cm)						
		M1	M2	M3	M4	M5	M6	
Elongation stage	Medium							
	Month 1	3.5 + 0.3	3.4 + 0.2	4.2 + 0.4	4.5 + 0.2	4.6 + 0.3	4.8 + 0.3	
	Month 2	5.2 + 0.5	4.9 + 1.0	8.3 + 0.6	7.7 + 0.7	8.6 + 0.7	8.5 + 0.3	
	Month 3	8.3 + 0.8 a	8.2 + 0.9 a	11.2 + 1.9 b	10.9 + 2.0 b	11.1 + 1.1 b	11.3 + 1.5 b	
			Shoot greening					
	Medium	M1	M2	M3	M4	M5	M6	
	Month 1	+	+	+	+	+	+	
	Month 2	++	++	++	++	++	++	
	Month 3	++	+++	++	+++	++	+++	
			Root number					
	Medium	M1	M2	M3	M4	M5	M6	
	Month 1	1.4 + 0.5	1.1 + 0.3	2.3 + 0.6	2.4 + 0.5	2.7 + 0.4	2.6 + 0.8	
Month 2	2.3 + 0.7	2.6 + 0.7	3.6 + 0.7	3.7 + 0.8	4.1 + 0.8	4.3 + 0.7		
Month 3	2.5 + 0.5 a	2.9 + 0.2 b	3.9 + 0.2 c	4.1 + 0.3 c	4.8 + 0.4 d	4.8 + 0.2 d		
		Root length (cm)						
Medium	M1	M2	M3	M4	M5	M6		
Month 1	1.3 + 0.4	1.3 + 0.5	1.4 + 0.8	1.6 + 0.7	1.3 + 0.6	1.4 + 1.0		
Month 2	2.4 + 0.6	2.5 + 0.7	2.7 + 0.7	2.6 + 0.6	2.3 + 1.0	2.8 + 1.0		
Month 3	3.0 + 0.3 a	3.1 + 0.5 a	3.5 + 0.8 a	3.6 + 0.8 a	3.5 + 1.5 a	3.8 + 1.3 a		

Means within columns having different letters are significantly different at 0.05 level of probability. Intensity of shoots greening: +++++ very high, +++ high, ++ moderate, + low

Table 2. Effect of one month pre-acclimatization stage on shoot development and plantlet acclimatization

		Elongation medium	M1	M2	M3	M4	M5	M6					
Pre-acclimatization stage	Pre-acclimatization medium	MS/2	- MS	- MS/2	- MS	- MS/2	- MS	-					
	Shoots length (cm)	11.5	11.7	14.3	14.1	14.0	14.4	-					
	Shoots greening	+ 0.8 a	+ 2.3 a	+ 0.7 b	+ 1.1 b	+ 0.9 b	+ 1.1 b	-					
	Roots number	2.8	3.1	4.2	4.3	5.0	5.3	-					
	Roots length (cm)	+ 0.5 a	+ 0.6 a	+ 0.4 b	+ 0.4 b	+ 0.5 c	+ 0.4 c	-					
		3.6	3.5	4.5	4.4	4.3	4.4	-					
Acclimatization stage	Percentage of plant survival (%)	86.0 + 8.4a	25.0 + 17.1b	83.0 + 11.5a	28.0 + 7.8b	76.0 + 17.7a	18.0 + 16.8b	73.0 + 11.5a	16.0 + 20.1b	70.0 + 11.5a	15.0 + 12.6b	72.0 + 7.8a	12.0 + 9.1b

Means within columns having different letters are significantly different at 0.05 level of probability. Intensity of shoots greening: +++++ very high, +++ high, ++ moderate, + low

Figures



Fig. 1. From undeveloped shoots to acclimatization: a: shoots after 45 days onto elongation solid medium, arrow indicates recalcitrant shoots used in this study. b: Shoots after three months in liquid media (On the left MS/2 + BAP, on the right MS/2 + IBA + BAP). c: Plantlets after three months in MS/2 liquid medium and one month in MS/2 solid medium. d: Plantlets survival after 90 days in the greenhouse. Bars correspond to 3 cm.