Impact of culture medium formulation and texture, and plant growth regulators on in vitro shoot bud multiplication and plantlet regeneration in date palm (*Phoenix dactylifera* L.) cv. Bouskri

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Abstract

Bouskri cv. is one of the most important date palm (*Phoenix dactylifera* L.) cultivars in Morocco. Unfortunately, cv. Bouskri is highly sensitive to the bayoud disease. In order to preserve this cultivar, developing a rapid and large-scale propagation system is highly desirable. Herein, the effects of different factors on cv. Bouskri organogenesis were evaluated. Organogenic cultures were cultured on Murashige and Skoog (MS) medium, Woody Plant Medium (WPM) or Nitsch and Nitsch Medium (NNM) for 3 months. Afterwards, the impact of different plant growth regulator (PGR) combinations and medium texture (solid, stationary liquid or agitated liquid) was examined. It was found that the use of MS medium resulted in a significantly higher number of shoot buds per explant (13.1) than WPM and NNM. Plant growth regulators also significantly affected the multiplication rate of shoot buds. The highest multiplication rate (17.4) was observed on the medium containing 0.9 µM 2-naphthoxyacetic acid (NOA), 1.1 µM indole-3-acetic acid (IAA), 1.9 µM 6-(dimethylallylamino) purine (2iP) and 1.8 µM kinetin, while the other PGR combinations exhibited multiplication rates ranging from 13.1 to 16.2 shoot buds per explant. The use of liquid media did not improve shoot bud multiplication, but instead resulted in very high hyperhydricity (75-90%). Shoot elongation and rooting were performed on PGR-free MS medium and plantlet acclimatization was successfully archived with a survival rate of 90% after 3 months in the glasshouse. The protocol established will contribute to the propagation and preservation of this date palm cultivar, highly appreciated by the Moroccan consumer.

Keywords: Phoenix dactylifera L., cv. Bouskri, medium formulation, medium texture, organogenesis, plant growth regulators.

Effet du milieu de culture, de sa texture et des régulateurs de croissance sur la multiplication in vitro et la régénération des plantules du palmier dattier (*Phoenix dactylifera* L.) cv. Bouskri

Résumé

Bouskri cv. est l'une des variétés du palmier dattier (*Phoenix dactylifera* L.) les plus appréciées par le consommateur marocain. Cependant, cette variété est très sensible à la maladie du bayoud. Afin de la préserver, le développement d'un schéma de propagation rapide est primordial. La présente étude permet d'évaluer l'effet de plusieurs facteurs sur l'organogenèse du cv. Bouskri. Des souches bourgeonnantes ont été cultivées sur le milieu de Murashige et Skoog (MS), le milieu Woody Plant Medium (WPM) et le milieu de Nitsch et Nitsch (NNM) pendant 3 mois. Par la suite, l'effet de différentes combinaisons hormonales ainsi que celui de l'état physique du milieu ont été évalués. Les résultats ont montré que l'utilisation du milieu de base MS a produit le nombre le plus élevé de bourgeons adventifs par souche (13.1). Les régulateurs de croissance ont également affecté de manière significative la multiplication des bourgeons. En effet, le taux de multiplication le plus élevé (17.4) a été observé sur le milieu additionné de 0.9 µM de l'acide 2-naphthoxyacetique (NOA), 1.1 µM de l'acide indole-3-acetique (IAA), 1.9 µM du 6-(dimethylallylamino) purine (2iP) et 1.8 µM de kinétine, tandis que les autres combinaisons hormonales ont donné des taux de multiplication allant de 13.1 à 16.2 bourgeons par souche. L'utilisation des milieux liquides n'a pas amélioré la multiplication des bourgeons, mais elle a entraîné une vitrification accentuée (75-90%). L'élongation et l'enracinement des bourgeons ont été effectués sur le milieu MS dépourvu d'hormones et, après le transfert des plantules sous serre, le taux de survie observé était de 90% après 3 mois. Le protocole développé dans la présente étude sera utilisé pour la multiplication rapide et la conservation de la variété Bouskri.

Mots-clés : Phoenix dactylifera L., cv. Bouskri, milieu de base, état physique du milieu, organogenèse, régulateurs de croissance.

تأثير طبيعة وبنية وسط النمو وهرمونات النمو على التكاثر الدقيق للبراعم داخل الأنابيب وتكون نباتات نخيل التمر (*Phoenix dactylifera* L.) صنف بوسكري

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ملخص

يعتبر بوسكري أحد أهم أصناف نخيل التمر (*Phoenix dactylifera* L.) والمفضلة لدى المستهلك المغربي. لسوء الحظ، فإن صنف بوسكري هو أيضا أحد أكثر الأصناف عرضة للإصابة بمرض البيوض. من أجل حماية هذا الصنف من مرض البيوض والمحافظة عليه، أصبح من الواجب والضروري تطوير تقنية تكاثر سريعة وفعالة. في هذا الإطار، قمنا في هذا البحث بدراسة تأثير عدة عوامل على تكوين وتكاثر براعم صنف بوسكري داخل الأنابيب. قمنا إذا بزراعة البراعم في ثلاثة أوساط نمو مختلفة وهيMS، WPM وNNM لمدة 3 أشهر. بعد ذلك، قمنا بدراسة تأثير هرمونات النمو وبنية وسط النمو (صلب أو سائل) على تكاثر البراعم. أظهرت نتائج هذا البحث أن استخدام وسط النموMS أنتج أكبر عدد من البراعم (13.1). إضافة إلى ذلك، أظهرت نتائجنا أن لهرمونات النمو تأثير جد فعال على تكاثر البراعم، حيث لوحظ أعلى معدل تكاثر للبراعم (17.4) عند استعمال الوسط المحتوي على kinetin 1.8 Mµ، 2iP Mµ 1.9 ، IAA Mµ 1.1، NOA Mµ 0.9 ، في حين أظهرت التركيبات الهرمونية الأخرى معدلات تكاثر تراوحت بين 13.1 و 16.2 برعم. من جهة أخرى، لم تقم الوسائط السائلة بتحسين تكاثر البراعم بل على العكس أدى استعمالها إلى ارتفاع كبير لمعدل التزجيج (90-75%). تمت استطالة وتجدير البراعم على وسط MS خالٍ من هرمونات النمو، وبعد نقل الشتلات إلى البيت الزجاجي، أظهرت 90% منها نموا طبيعيا بعد 3 أشهر. ستمكن نتائج هذه الدراسة من إنتاج عدد كبير من شتلات نخيل التمر صنف بوسكري وكذا الحفاظ عليه.

الكلمات المفتاحية: نخيل التمر، وسط النمو، تكاثر البراعم، هرمونات النمو، بنية وسط النمو.

Introduction

Date palm (*Phoenix dactylifera* L.) is a fruit species that plays important socioeconomic, agronomic and ecological roles (Al-Khayri et al., 2018). In fact, in arid and semi-arid areas, date palm significantly contributes to population incomes, creates suitable microclimates for agriculture, preserves biodiversity and helps in controlling desertification (Sedra, 2015). Besides, date palm produces a highly delicious and nutritious fruit known as date, which is consumed either fresh or after processing into different products (Al-Khayri et al., 2018).

Date palm cultivation is hampered by the very dangerous disease called bayoud, which is caused by the fungus *Fusarium oxysporum* f. sp. *Albedinis.* Bayoud has killed millions of date palm plants of the best cultivars and, up to date, is still the main biotic factor that threatens this plant species (Jaiti et al., 2007). Furthermore, the best date palm cultivars such as Mejhoul, Boufeggous, Bouskri and Jihel are highly susceptible to bayoud (Sedra, 2011). In order to preserve these cultivars and to satisfy the high demand of consumers in terms of fruits, a new strategy was launched by the Moroccan government in 2010, aiming at large-scale propagating and planting these cultivars in extension zones, which are bayoud-free areas (Meziani et al., 2015; Mazri et al., 2016).

Large-scale propagation of date palm could be achieved through in vitro culture, namely by somatic embryogenesis or organogenesis (Mazri and Meziani, 2015). In Morocco, the technique used is organogenesis as it preserves the genetic conformity of regenerants (Sedra, 2005; Mazri et al., 2019). Organogenesis refers to the technique in which adventitious buds are induced and developed in vitro to form complete plants that are thereafter acclimatized to ex vitro conditions (Mazri and Meziani, 2013). In date palm, successful regeneration through organogenesis is highly genotype-dependent (Jain, 2012). Accordingly, it is necessary to optimize the culture medium components depending on each genotype. Along this line, many studies were carried out to evaluate the effects of plant growth regulators during date palm organogenesis (e.g. Mazri and Meziani, 2013; Mazri, 2014; Mazri, 2015; Meziani et al., 2015). In fact, exogeneous PGRs play a major role during plant micropropagation as they interact with the endogenous plant hormones. This affects cell division, differentiation and growth (Gaspar et al., 1996; Feher et al., 2003; Gaj, 2004). Auxins and cytokinins are the main groups of PGR used during plant micropropagation, and they are generally used in combination. Auxins are involved in maintaining the plant polarity and in the plant signaling systems, which allow plant cells to interact among them in response to different factors and culture conditions (Libbenga and Mennes, 1995; Friml, 2003). Regarding cytokinins, they promote protein synthesis and chloroplast maturation, delay the senescence of isolated leaves and contribute to the control of the cell cycle (van Staden et al., 2008).

Developing an efficient regeneration system through organogenesis for date palm may be hampered by several factors. In fact, tissue browning, hyperhydricity and precocious rooting are physiological disorders that may cause important losses during the large-scale propagation of date palm through organogenesis (Mazri and Meziani, 2015). Tissue browning is caused by the high content of caffeoylshikimic acids in date palm explants (Loutfi and El Hadrami, 2005). Hyperhydricity is the physiological state

in which explants become water-soaked and glassy (Debergh et al., 1992), whereas precocious rooting is the production of adventitious roots during shoot bud multiplication, which is undesirable since it significantly reduces the multiplication potential of explants. The occurrence and intensity of these phenomena depend strongly on culture medium components, culture conditions and the genotype.

Bouskri cv. is one of the most important date palm cultivars and is highly appreciated by the Moroccan consumer. The fruits of this cultivar are dry and characterized by a dark brown color (Sedra, 2011). Unfortunately, cv. Bouskri is very sensitive to the bayoud disease (Sedra, 2011). To the best of our knowledge, there is no report on cv. Bouskri micropropagation through organogenesis. Accordingly, developing an efficient and reproducible regeneration system for this cultivar through organogenesis is of high importance.

The purpose of the present investigation was to evaluate the effects of different basal formulations of culture medium (MS, WPM and NNM), PGR combinations and medium texture (solid, stationary liquid and agitated liquid) on adventitious shoot bud multiplication, hyperhydricity, tissue browning and precocious rooting of date palm cv. Bouskri. Besides, the growth and development of adventitious shoots and plantlet acclimatization were monitored.

Materials and Methods

Plant material and culture conditions

Organogenic cultures (i.e. explants with 3-4 adventitious buds) of date palm (*Phoenix dactylifera* L.) cv. Bouskri were obtained in vitro according to the protocol of Beauchesne et al. (1986). Briefly, offshoots of cv. Bouskri were detached from mother plants, and the shoot tips were extracted and disinfected by immersion in 0.03 % potassium permanganate in commercial liquid bleach for 20 min, followed by three washes in sterile distilled water. Shoot tip explants were cultured for 9 months on half-strength Murashige and Skoog (1962) medium (MS/2) supplemented with 3 mg/L, 2-naphthoxyacetic acid (NOA), 1 mg/L 1-naphthaleneacetic acid (NAA), 1 mg/L indole-3-acetic acid (IAA), 0.1 mg/L 6-(dimethylallylamino) purine (2iP), 0.2 g/L L-glutamine, 0.1 g/L myo-inositol, 2 g/L polyvinylpyrrolidone (PVP), 30 g/L sucrose and 8 g/L agar. The organogenic cultures were then cultured on PGR-free MS medium solidified with 8 g/L agar for 3 months in order to avoid the effect of previous culture media on our experiments.

In all experiments, the media were dispensed into jars before autoclaving at 121 °C for 25 min. All cultures were maintained under a 16/8h photoperiod and 25 $\pm$ 1 °C, and were subcultured onto a fresh medium at one-month intervals.

The experiments were carried out in 2018 at the Regional Center of Agronomic Research of Marrakech (CRRA-Marrakech).

Experiment 1: effect of the basal formulation of culture medium on shoot bud multiplication

In this experiment, the effect of three different basal formulations on shoot bud multiplication of date palm cv. Bouskri was evaluated. The organogenic cultures were cultured on either MS medium, Woody Plant Medium (WPM; Lloyd and McCown, 1980) or Nitsch and Nitsch Medium (NNM; Nitsch and Nitsch, 1969) for three months. All media were supplemented with 1.2 µM NOA, 1.2 µM kinetin, 1 g/L PVP, 30 g/L commercial granulated sugar and were solidified with 8 g/L agar.

Experiment 2: effect of plant growth regulators on shoot bud multiplication

Organogenic cultures of date palm cv. Bouskri were cultured on MS medium supplemented with different PGR combinations: (i) 2.5 µM indole-3-butyric acid (IBA) and 2.5 µM 6-benzylaminopurine (BAP); (ii) 3 µM IBA and 3 µM BAP; (iii) 2.4 µM NOA and 2.3 µM kinetin, and (iv) 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin. These PGR combinations were previously suggested for other date palm cultivars (Mazri, 2014; 2015; Mazri and Meziani, 2013; Meziani et al., 2015). Besides, the combination of 1.2 µM NOA and 1.2 µM kinetin was used as control. All culture media were supplemented with 1 g/L PVP, 30 g/L commercial granulated sugar and solidified with 8 g/L agar.

Experiment 3: effect of medium texture on shoot bud multiplication

Based on the results of the second experiment, organogenic cultures of date palm cv. Bouskri were cultured for 3 months on MS medium supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP, 1.8 µM kinetin, 1 g/L PVP and 30 g/L commercial granulated sugar. Furthermore, three different culture conditions were evaluated: MS supplemented with 8 g/L agar (i.e. solid MS medium), stationary liquid MS medium and agitated MS liquid medium (60 rpm).

Shoot elongation, rooting and plantlet acclimatization

At the end of multiplication experiments, shoots with a single root were isolated and transferred to solid PGR-free MS medium for elongation and rooting. After 3 months of culture on the elongation-rooting medium, shoots with three leaves and a good root system were transferred to the glasshouse according to the protocol described by Mazri et al. (2016). After 3 months in the glasshouse, the plantlet survival percentage was calculated.

Data collection and statistical analysis

In all experiments, two organogenic cultures were used per jar, which was considered as one replicate, and in each treatment, 10 replicates were made.

After 3 months of culture on multiplication media, the following data were recorded: the average number of shoot buds per organogenic culture, the percentage of tissue browning, the intensity of tissue browning, the percentage of hyperhydricity, the

intensity of hyperhydricity, the percentage of precocious rooting and the average number of roots per organogenic culture. The intensity of tissue browning and hyperhydricity was visually estimated and noted as low (+), moderate (++) or high (+++).

Data were subjected to a completely randomized design and analyzed with ANOVA. Mean data were separated by using the Student-Newman-Keuls test. Prior to analysis, all percentage data were arcsine transformed. All analyses were made by SPSS version 26 for windows.

Results and Discussion

Effect of the basal formulation of culture medium on shoot bud multiplication

The results of this experiment revealed that MS basal medium is the most appropriate for shoot bud proliferation of date palm cv. Bouskri. In fact, the use of this basal formulation resulted in significantly higher average number of shoot buds per explant (13.1). Culturing organogenic cultures on WPM resulted in an average number of 11.0 shoot buds per explant while NNM gave an average of 11.6 shoot buds per explant (Table 1). The effects of basal culture medium on shoot bud multiplication of date palm were reported in other genotypes. For example, in date palm genotype 16-bis, the use of MS basal formulation resulted in significantly higher shoot bud multiplication than WPM and NNM (Mazri, 2013). In cv. Al-Fayda, the strength of MS medium (full strength, half-strength and one-third-strength) significantly influenced the multiplication rate of shoot buds (Mazri et al., 2019). In cv. Najda, the use of MS basal formulation at full strength or half-strength showed significantly higher shoot bud multiplication rates than this same basal formulation at one-third-strength, or Beauchesne medium (Beauchesne et al., 1986) at full strength, half-strength and one-third-strength. The impact of basal medium on in vitro propagation of plants was reported by other researchers (Ramsay et al., 2003; Wang et al., 2008; Hassan et al., 2011). This could be explained by the specific mineral requirements for efficient shoot bud multiplication. In date palm cv. Bouskri, it was found that MS medium is the most appropriate for shoot bud multiplication. This basal formulation has been widely used in date palm (Taha et al., 2001; Khierallah and Bader, 2007; Khan and Bi Bi, 2012; Al-Mayahi, 2014).

Regarding hyperhydricity and tissue browning, they ranged from 40 to 60 % and from 40 to 50 %, respectively. The precocious rooting percentage ranged from 25 to 30% while the average number of roots per organogenic culture ranged from 0.9 to 1.2 (Table 1). Hyperhydricity, tissue browning and precocious rooting are the main physiological disorders that hamper date palm propagation through organogenesis. Hyperhydricity is reflected in water-soaked and glassy tissues and is caused by many factors, including the high level of mineral salts of culture medium (Debergh et al., 1992; Al-Khateeb, 2008). Tissue browning is caused by the high concentration of caffeoylshikimic acids in date palm tissues (Loutfi and El Hadrami, 2005) while precocious rooting is mainly caused by high auxin concentrations in the multiplication

medium (Al-Khateeb, 2008). All these phenomena cause important losses for commercial laboratories during the organogenesis process of date palm and thus is highly recommended to well optimize the components of the culture medium to reduce their incidences. According to table 1, there was no significant difference in terms of hyperhydricity, tissue browning and precocious rooting among the three basal formulations. However, high hyperhydricity (40-60%) and tissue browning (40-50%) percentages were observed and thus more experiments should be performed to reduce their incidence. Based on these results, MS basal formulation is recommended for shoot bud multiplication of date palm cv. Bouskri.

Table 1: Effect of basal media on shoot bud multiplication, tissue browning, hyperhydricity and precocious rooting in date palm cv. Bouskri

Culture medium	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Intensity of tissue browning	Precocious rooting (%)	Average number of roots per explant
MS	13.1 ± 0.6 a	40 ± 12.4 a	+	45 ± 8.9 a	+	25 ± 8.3 a	1.1 ± 0.3 a
WPM	11.0 ± 0.6 b	40 ± 14.5 a	+	50 ± 12.9 a	+	30 ± 13.3 a	1.2 ± 0.4 a
NNM	11.6 ± 0.5 b	60 ± 12.4 a	++	40 ± 10.0 a	+	30 ± 11.0 a	0.9 ± 0.3 a

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% level of the Student-Newman-Keuls test. MS, Murashige and Skoog; NNM, Nitsch and Nitsch Medium; WPM, Woody Plant Medium. Intensity of tissue browning and hyperhydricity: (+) low, (++) moderate.

Effect of plant growth regulators on shoot bud multiplication

In the second experiment, the effects of five PGR combinations on shoot bud multiplication were evaluated. It was found that the use of the combination of 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin resulted in significantly higher average number (17.4) of shoot buds per explants (Table 2; Fig. 1A) than the other combinations. In fact, the other PGR treatments resulted in an average number of shoot buds per explant ranging from 13.1 to 16.2 (Table 2). Regarding hyperhydricity, it ranged from 40% in the medium containing 1.2 µM NOA and 1.2 µM kinetin to 60% in that supplemented with 2.4 µM NOA and 2.3 µM kinetin, with no significant difference among the five treatments. Tissue browning ranged from 45 to 60%, with low intensity in all culture media. The lowest percentage of tissue browning was observed in the medium containing the combination of 1.2 µM NOA and 1.2 µM kinetin while the highest percentage was observed in the medium containing 3 µM IBA and 3 µM BAP. Regarding precocious rooting, it ranged from 20 to 40%, with no significant difference among the 5 treatments. On the other hand, the average number of roots per explant varied from 0.5 to 1.8 (Table 2).

The use of PGRs is primordial for in vitro plant cell and tissue culture. In fact, PGRs interact with the endogenous hormones and modify their levels, which results in cell division and morphogenesis (Gaspar et al., 1996; Feher et al., 2003; Gaj, 2004).

Similarly to mineral salts, the requirements for PGRs for efficient shoot bud multiplication vary among date palm genotypes. Thus, Mazri and Meziani (2013) suggested the combination of 2.4 µM NOA and 2.3 µM kinetin for cv. Najda, Mazri (2015) recommended the combination of 3 µM IBA and 3 µM BAP for cv. Boufeggous. For cvs. Mejhoul and Al-Fayda, the combination of 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin, and that of 2.4 µM NOA and 2.3 µM kinetin were suggested, respectively (Meziani et al., 2015; Mazri et al., 2019). In the present investigation, the combination of 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin gave the highest average number of shoot buds per explants (17.4) and thus is recommended for date palm cv. Bouskri. This same PGR combination resulted in the production of up to 18.7 shoot buds per explant in cv. Mejhoul (Meziani et al., 2015; Mazri et al., 2016). In cv. Al-Fayda, the combination of 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin gave 25.2 shoot buds per explant (Mazri et al., 2019). However, better results (28.4 shoot buds per explant) were observed when the culture medium was supplemented with 2.4 µM NOA and 2.3 µM kinetin. All these findings highlighted the different requirements and responses of date palm cultivars to exogeneous PGRs. On the other hand, there was no significant difference among the different PGR combinations in terms of hyperhydricity, tissue browning and precocious rooting, even though some previous studies on date palm revealed that PGRs may affect these phenomena. For example, Mazri (2015) reported that thidiazuron was a causal factor of tissue browning in date palm cv. Boufeggous. Based on the results of this experiment, the combination of 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin is recommended for shoot bud multiplication of date palm cv. Bouskri.

Table 2: Effect of PGRs on shoot bud multiplication, tissue browning, hyperhydricity and precocious rooting in date palm cv. Bouskri

Plant growth regulator combination	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Intensity of tissue browning	Precocious rooting (%)	Average number of roots per explant
• 2.5 µM IBA + 2.5 µM BAP	14.8 ± 1.1 ab	45 ± 11.6 a	+	50 ± 14.9 a	+	20 ± 11.0 a	0.5 ± 0.2 a
• 3 µM IBA + 3 µM BAP	16.2 ± 0.9 ab	50 ± 12.9 a	+	60 ± 14.5 a	+	20 ± 13.3 a	0.7 ± 0.5 a
• 2.4 µM NOA + 2.3 µM kinetin	16.0 ± 1.0 ab	60 ± 14.5 a	++	55 ± 15.7 a	+	40 ± 14.5 a	1.8 ± 0.6 a
• 0.9 µM NOA + 1.1 µM IAA + 1.9 µM 2iP + 1.8 µM kinetin	17.4 ± 0.6 b	45 ± 11.6 a	+	55 ± 15.7 a	+	30 ± 13.3 a	1.0 ± 0.4 a
• 1.2 µM NOA + 1.2 µM kinetin	13.1 ± 0.6 a	40 ± 12.4 a	+	45 ± 8.9 a	+	25 ± 8.3 a	1.1 ± 0.3 a

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% level of the Student-Newman-Keuls test. 2iP, 6-(dimethylallylamino) purine; BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NOA, 2-naphthoxyacetic acid. Intensity of tissue browning and hyperhydricity: (+) low, (++) moderate.

Figure 1: Shoot bud multiplication and plantlet regeneration in date palm cv. Bouskri. A. Shoot bud multiplication on solid MS medium supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM. B. Plantlets ready for acclimatization after 3 months on PGR-free MS medium

Effect of medium texture on shoot bud multiplication

In the last experiment, the effect of medium texture on shoot bud multiplication was evaluated. Three different media were used, solid MS medium (containing 8 g/L agar), stationary liquid MS medium and agitated liquid (60 rpm) MS medium. It was found that the use of solid MS medium resulted in significantly higher number of shoot buds per explants (17.4) than liquid media. The use of stationary liquid MS medium showed an average number of shoot buds per explant of 11.3 while the agitated one gave 13.1 shoot buds per explant (Table 3). Besides, the use of the liquid state drastically increased hyperhydricity to 75 and 90 % in agitated and stationary liquid media, respectively, with moderate to high intensity. This high level of hyperhydricity could be the main reason behind the decreased proliferation of shoots buds. These results are in good agreement with those of Mazri et al. (2019), who reported that the use of liquid media increased hyperhydricity in date palm cv. Al-Fayda, to reach 100%. Al-Khateeb (2008) also indicated that the liquid state of culture medium is a causal factor of hyperhydricity in date palm, as it may promote water accumulation within the cultured tissues. On the other hand, liquid media slightly reduced tissue browning percentages to 35-40%, while it was 55% in solid MS medium (Table 3). The use of liquid media also decreased the percentage of precocious rooting (10%) and the average number of roots per explant (0.3-0.5).

Table 3: Effect of medium texture on shoot bud multiplication, tissue browning, hyperhydricity and precocious rooting in date palm cv. Bouskri

Medium texture	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Intensity of tissue browning	Precocious rooting (%)	Average number of roots per explant
Solid	17.4 ± 0.6 a	45 ± 11.6 a	+	55 ± 15.7 a	+	30 ± 13.3 a	1.0 ± 0.4 a
Stationary Liquid	11.3 ± 0.4 c	90 ± 6.6 b	+++	35 ± 10.6 a	+	10 ± 6.6 a	0.3 ± 0.2 a
Agitated liquid	13.1 ± 0.4 b	75 ± 8.3 b	++	40 ± 14.5 a	+	10 ± 6.6 a	0.5 ± 0.3 a

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% level of the Student-Newman-Keuls test. Intensity of tissue browning and hyperhydricity: (+) low, (++) moderate, (+++) high.

Shoot elongation, rooting and plantlet acclimatization

After the multiplication experiments, shoots were singled out and transferred to solid and PGR-free MS medium for elongation and rooting. After 3 months of culture, all the shoots exhibited normal growth and development, with an average shoot length of 13.5 cm, an average number of 3.6 roots per shoot and an average root length of 3.1 cm (Fig. 1B). The use of PGR-free medium for shoot elongation and rooting was suggested for many date palm cultivars such as cvs. Najda, Boufeggous and Mejhoul (Mazri and Meziani, 2013; Mazri, 2015; Meziani et al., 2019). However, in some other cultivars, for example, cv. Al-Fayda, the use of PGRs was necessary since this cultivar is characterized by recalcitrance to elongation and rooting (Mazri et al., 2019). All these results highlight the fact that all the phases of date palm organogenesis are genotype-dependent. After transferring the plantlets to the glasshouse, the survival rate observed was 90% after 3 months.

Conclusions

The effects of different factors on shoot bud multiplication of date palm cv. Bouskri were studied in order to select the optimal ones for efficient in vitro propagation. The use of solid MS medium supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin resulted in the highest number of shoot buds per explant, surpassing the other basal formulations, PGR combinations and liquid media. Unfortunately, high hyperhydricity and tissue browning were observed. Accordingly, further study should be carried out in order to reduce the incidence of these undesirable phenomena that significantly affect the organogenesis process of date palm.

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