Effects of medium strength, carbon source and antioxidants on adventitious bud proliferation and plantlet development of date palm cv. Bouskri

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Abstract

The Bayoud disease, caused by the fungus *Fusarium oxysporum* f. sp. *albedinis*, is a very dangerous date palm disease that killed millions of plants during the last century and threatens the best Moroccan cultivars, including cv. Bouskri. In order to fight Bayoud and to preserve the best date palm cultivars, rapid and large-scale propagation through organogenesis followed by planting regenerants in Bayoud-free areas is being in use in Morocco. In the present work, the effects of medium strength, carbon source and antioxidants were investigated in order to develop an efficient regeneration system through organogenesis for cv. Bouskri. Four different strengths of Murashige and Skoog medium were evaluated: full-strength (MS), half-strength (MS/2), one-third strength (MS/3) and one-quarter strength (MS/4). In addition, different concentrations of sucrose, commercial granulated sugar (10, 30 or 50 g/l), polyvinylpyrrolidone (PVP) and activated charcoal (1, 2 and 3 g/l) were tested. It was found that the optimal medium strength for the multiplication of adventitious shoot buds of cv. Bouskri is MS/2, while the use of 30 g/l sucrose and 2 g/l activated charcoal improved the proliferation rate. In fact, under these culture conditions, the average number of shoot buds per explant was 23.3, and the percentages of tissue browning, hyperhydricity and precocious rooting were 25.0, 30.0 and 25.0 %, respectively. On the other hand, the average number of precociously formed roots per organogenic culture was 0.9. Shoot growth and development were carried out on plant growth regulator (PGR)-free MS/2 medium and resulted in an average shoot length of 13.9 cm, an average root number of 4.1 and an average root length of 3.2 cm. After transferring the plants to the glasshouse, the survival rate observed was 95%.

Keywords: Phoenix dactylifera L., medium components, multiplication, organogenesis, plant regeneration.

Effet du milieu de culture, de la source carbonée et des antioxydants sur la prolifération des bourgeons adventifs et le développement des plantules chez le palmier dattier cv. Bouskri

Résumé

Le Bayoud, maladie causée par le champignon *Fusarium oxysporum* f. sp. *albedinis*, est la menace la plus sérieuse qui pèse sur le secteur phoenicicole marocain. En effet, le Bayoud a tué des millions de plantes du palmier dattier au cours du dernier siècle, et menace actuellement les meilleures variétés marocaines, dont Bouskri. Afin de préserver ces variétés, la multiplication massive par organogenèse et la plantation dans les zones indemnes du Bayoud est la méthode utilisée au Maroc. Dans le présent travail, l'effet de la concentration du milieu de culture, de la source carbonée et des antioxydants a été évalué afin de développer un système de régénération efficace par organogenèse pour la variété Bouskri. Ainsi, quatre différentes concentrations du milieu de culture de Murashige et Skoog ont été évaluées : milieu concentré (MS), milieu dilué de moitié, (MS/2), milieu dilué au tiers (MS/3) et milieu dilué au quart (MS/4). De plus, différentes concentrations de saccharose, de sucre granulé commercial (10, 30 ou 50 g/l), de polyvinylpyrrolidone (PVP) et de charbon actif (1, 2 et 3 g/l) ont été testées. Les résultats ont montré que le milieu optimal pour la multiplication des bourgeons adventifs de la variété Bouskri est MS/2, tandis que l'utilisation de 30 g/l de saccharose et de 2 g/l de charbon actif a amélioré significativement le taux de prolifération. En fait, dans ces conditions de culture, le nombre moyen de bourgeons par explant était de 23,3, les taux de brunissement, de vitrification et d'enracinement précoce étaient respectivement de 25,0 ; 30,0 et 25,0%; tandis que le nombre moyen de racines par souche était de 0,9. La croissance et le développement des pousses ont été effectués sur le milieu MS/2 dépourvu de régulateurs de croissance, ce qui a donné une longueur moyenne des pousses de 13,9 cm, un nombre moyen de racines par pousse de 4,1 et une longueur moyenne des racines de 3,2 cm. Au cours de l'acclimatation, le taux de survie des plantules était de 95%.

Mots-clés *:* Phoenix dactylifera L., composition du milieu de culture, multiplication, organogenèse, régénération.

دراسة تأثير بنية وسط النمو، مصدر الكربون ومضادات الأكسدة على تكاثر البراعم ونمو نباتات نخيل التمر صنف بوسكري

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ملخص

يعتبر مرض البيوض، الناتج عن فطر *Fusarium oxysporum* f.sp. *albedinis* ، أحد أخطر أمراض نخيل التمر، إذ أنه تسبب في مقتل ملايين الأشجار خلال القرن الماضي ، كما أنه يهدد أفضل الأصناف المتواجدة بالمملكة المغربية ، بما في ذلك صنف بوسكري. من أجل محاربة مرض البيوض والحفاظ على أفضل أصناف نخيل التمر، يتم في المغرب استعمال تقنية التكاثر السريع من خلال تكوين الأعضاء في المختبر، متبوعًا بزراعة النباتات المنتجة في المناطق الخالية من البيوض. في هذا البحث، قمنا بدراسة تأثير بنية وسط النمو، مصدر الكربون ومضادات الأكسدة من أجل تطوير بروتوكول تكاثر فعال عن طريق تقنية التكوين العضوي لصنف بوسكري. في هذا الإطار، تم تقييم أربعة مستويات مختلفة لوسط النمو Skoog وMurashige : بنية كاملة القوة (MS)، بنية مخففة إلى النصف (MS/2)، بنية مخففة إلى الثلث (MS/3) و بنية مخففة إلى الربع (MS/4). بالإضافة إلى ذلك، تم اختبار تركيزات مختلفة من السكروز، السكر التجاري (10 ،30 أو 50 جم/لتر) ، البوليفينيل بيروليدون (PVP) والفحم المنشط (1 ،2 أو 3 جم/لتر). أظهرت النتائج التي توصلنا إليها أن وسط النمو المثالي لتكاثر براعم صنف بوسكري هوMS/2 ، في حين أن استخدام 30 جم/لتر من السكروز و2 جم/لتر من الفحم المنشط يحسن بشكل ملحوظ من معدل التكاثر. أدى استخدام هذا الوسط إلى تكوين أعلى معدل تكاثر للبراعم 23.3، فيما كانت النسب المئوية لاسمرار الأنسجة، التزجيج والتجدير المبكر هي 25.0 و30.0 و25.0٪ على التوالي؛ بينما كان متوسط عدد الجذور هو0.9. تمت بعد ذلك استطالة وتجدير البراعم على وسط MS/2خالٍ من هرمونات النمو، والذي أدى إلى متوسط طول النبتة بلغ 13.9 سم، متوسط عدد جذور بلغ 4.1 ومتوسط طول جذور بلغ 3.2 سم. بعد نقل النباتات إلى البيت الزجاجي، أظهرت 95% منها نموا طبيعيا.

الكلمات المفتاحية: *نخيل التمر، مكونات وسط النمو، تكاثر، التكوين العضوي.*

Introduction

Date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae and is one of the oldest cultivated fruit species in the arid and semi-arid regions of the world (Al-Khayri et al., 2018). This species is native to Iraq, but today is widely distributed throughout the Middle east and North African region (Johnson et al., 2013). Date palm is also found in other countries such as USA, Peru, Mexico and Spain (Al-Khayri et al., 2018). It is mainly cultivated for its fruit which is rich in nutritional compounds such as carbohydrates, dietary fibers, minerals and vitamins and have health-promoting properties (Bouhlali et al., 2016, 2017, 2018). Date palm also serves to create favorable microclimate for vegetable growth in the arid and semi-arid regions, to control desertification and to preserve biodiversity (Sedra, 2015; Mazri et al., 2019). Date palm seeds, leaves and trunks are also used for other purposes. For example, to feed animals, as additives for food, to make handicrafts, paper and fibers and as a source of fuel (Al-Khayri et al., 2018).

In Morocco, date palm is threatened by the wilt disease known as Bayoud and that is caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Jaiti et al., 2007; Sedra, 2011). Unfortunately, the best date palm cultivars that are highly demanded by the consumer, including cv. Bouskri, are very sensitive to the Bayoud disease (Sedra, 2011). In order to protect these cultivars against the Bayoud disease, tissue culture technologies, particularly organogenesis, have been used, and the plantlets produced through these technologies are planted in Bayoud-free areas (Mazri, 2015; Meziani et al. 2015, Mazri et al., 2016; Meziani et al., 2016).

The organogenesis technique allows to significantly accelerate the process of multiplication, and offers the possibility for large-scale production of true-to-type plants (Sedra, 2005; Mazri et al., 2019). In fact, micropropagation through organogenesis can be used to produce millions of disease-free and genetically uniform plants annually without any seasonal variation. However, developing a reproducible regeneration system through organogenesis and overcoming the different physiological problems that hamper the efficiency of this technique are primordial to reach such purpose (Mazri, 2015). On the other hand, it is well known that the efficiency of an *in vitro* regeneration system depends strongly on the genotype and explant type. Thus, not only is it important to induce adventitious buds, but also to promote their proliferation and development into complete plantlets. To do so, the *in vitro* culture conditions must be well optimized.

During the *in vitro* organogenesis process, some physiological disorders affect the cultures and result in important losses of plant material. These physiological disorders are hyperhydricity, tissue browning and precocious rooting. Hyperhydricity is caused by high concentrations of ammonium nitrate and plant growth regulators (PGRs) in culture medium, as well as the use of liquid systems (Al-Khateeb, 2008a). Tissue browning is caused by a high level of caffeoylshikimic acid in date palm tissues (Loutfi and El Hadrami, 2005), while precocious rooting is the result of high concentrations of auxins in culture medium (Al-Khateeb, 2008a). The occurrence of these three phenomena affects the proliferation potential of adventitious buds and depends strongly on the date palm genotype. Reducing the occurrence of these undesirable disorders represents a useful tool to significantly increase the production of adventitious shoot buds and thus date palm plants through organogenesis.

The aim of the present study was to assess the influence of medium strength, carbon source type and concentration and antioxidants in order to improve the proliferation of adventitious shoot buds of date palm cv. Bouskri, and to reduce the incidence of undesirable phenomena, namely hyperhydricity, tissue browning and precocious rooting. As a result, an efficient and reproducible regeneration system through organogenesis could be developed.

Materials and Methods

Origin of explants

Shoot tips (7-8 cm long) of date palm (*Phoenix dactylifera* L.) cv. Bouskri were excised from 3-year-old offshoots. The shoot tips were treated with a solution 0.03 % potassium permanganate in commercial liquid bleach for 20 min, then rinsed three times with sterile distilled water. Afterwards, the shoot tip explants were cultured on half-strength Murashige and Skoog (1962) medium (MS/2) containing 30 g/l sucrose, 8 g/L agar, 2 g/l polyvinylpyrrolidone (PVP), 0.2 g/l L-glutamine, 0.1 g/l myo-inositol, 3 mg/l 2-naphthoxyacetic acid (NOA), 1 mg/l 1-naphthaleneacetic acid (NAA), 1 mg/l indole-3-acetic acid (IAA) and 0.1 mg/l 6-(dimethylallylamino) purine (2iP) for 9 months (Beauchesne et al. 1986). The organogenic cultures were then used in proliferation experiments.

Effects of medium strength on adventitious shoot bud proliferation

The organogenic cultures (each containing 3-4 adventitious buds) of date palm cv. Bouskri were cultured for 3 months on four different basal formulations all derived from the culture medium of Murashige and Skoog: full-strength (MS), half-strength (MS/2), one-third strength (MS/3) and one-quarter strength (MS/4). All culture media were supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP, 1.8 µM kinetin, 30 g/l commercial granulated sugar, 1 g/l PVP and gelled with 8 g/l agar. The PGR combination used in the present investigation was based on the results of a previous study (data not shown).

Effects of carbon source on adventitious shoot bud proliferation

In the second experiment, organogenic cultures of date palm cv. Bouskri were cultured for 3 months on MS/2 medium containing 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP, 1.8 µM kinetin and 1 g/l PVP. To evaluate the effects of carbon sources on adventitious shoot bud proliferation, the culture medium was supplemented with either 10, 30 or 50 g/l commercial granulated sugar, or with 10, 30 or 50 g/l sucrose. All culture media were gelled with 8 g/l agar.

Effects of PVP and activated charcoal on adventitious shoot bud proliferation

Organogenic cultures of date palm cv. Bouskri were cultured for 3 months on MS/2 medium supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.9 µM 2iP and 1.8 µM kinetin, 30 g/l sucrose, 8 g/l agar and different concentrations of PVP or activated charcoal: 1, 2 or 3 g/l, to evaluate their effects on adventitious shoot bud proliferation and tissue browning.

Shoot elongation, rooting and plantlet acclimatization

Shoots larger than 4 cm length were singled out and transferred to PGR-free MS/2 medium supplemented with 30 g/l sucrose and solidified with 8 g/l agar for elongation and rooting. After 3 months of culture, the developed plantlets were transferred to the glasshouse for acclimatization according to the protocol of Mazri et al. (2016). Briefly, the shoots were removed from the jars and their root system was genteelly rinsed with tap water to remove remaining agar. The shoots were placed in plastic pots containing a mixture of peat and gravel (1:1, w:w) then transferred to the glasshouse, inside a tunnel covered with a polyethylene bag to keep high relative humidity. After 15 days, the polyethylene bag was opened gradually to ensure adaptation to the glasshouse conditions (27°C, 70% relative humidity).

Culture conditions

The pH of all culture media was adjusted to 5.7 before autoclaving at 121°C for 25 min. The cultures were maintained under a 16/8h photoperiod at 25 $\pm$ 1 °C, and transferred to a fresh medium at one-month intervals.

Data collection and statistical analysis

In all experiments, four organogenic cultures were placed per jar, which was considered as one replicate, and 10 replicates were performed. At the end of each experiment (i.e. after 3 months of culture), data were collected for the average number of shoot buds per explant, percentage and intensity of hyperhydricity, percentage and intensity of tissue browning, percentage of precocious rooting and the average number of roots per explant. The intensity of hyperhydricity and tissue browning was visually estimated as low (+), moderate (++) and high (+++).
All experiments were carried out in a completely randomized design at the 5 % significance level. Data were analyzed by ANOVA followed by the Student-Newman-Keuls post hoc test at $p<0.05$. All percentage data were arcsine transformed before analysis. The SPSS software (v.26, IBM-SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results and discussion

Effects of medium strength on adventitious shoot bud proliferation

In the first assay, the effect of four different strengths of MS medium on shoot bud proliferation was evaluated: MS, MS/2, MS/3 and MS/4. It was found that medium strength significantly affects the multiplication of shoot buds of date palm cv. Bouskri. The highest multiplication rate (16.9 shoot buds per explant) was observed when organogenic cultures were placed on full strength MS medium. However, statistical analysis showed that there was no significant difference with MS/2 medium, which showed an average of 16.3 shoot buds per explant (Table 1). The use of MS/3 and MS/4 media resulted in the production of 13.6 and 11.9 shoot buds per explant, respectively, which highlights the significant impact of medium strength on the proliferation of cv. Bouskri adventitious buds. The effects of medium strength on shoot bud proliferation were observed in other date palm cultivars. For example, in cv. Mejhoul, it was found that the concentrations of ammonium nitrate, potassium nitrate, calcium chloride dehydrate, magnesium sulfate heptahydrate and potassium dihydrogen phosphate all affect the proliferation of adventitious shoot buds and should be well optimized for efficient multiplication (Mazri et al., 2016). In cv. Al-Fayda, the effects of MS, MS/2 and MS/3 were evaluated and the findings showed that the use of MS/2 significantly increase the average number of shoot buds per explant (Mazri et al., 2019). In cv. Najda, MS, MS/2 and MS/3 were compared and the results suggested to use MS/2 medium (Mazri and Meziani, 2013). This is in good agreement with the present study. On the other hand, the findings of the present investigation revealed that the use of full-strength MS basal formulation resulted in the highest hyperhydricity percentage, with 55.0% hyperhydric cultures. This was followed by MS/2 (37.5%), MS/3 (27.5%) and MS/4 (25.0%). This highlights the impact of medium strength on hyperhydricity, and is in good agreement with the findings of Mazri et al. (2019), according to which increasing medium strength increased the frequency of hyperhydricity. According to Al-Khateeb (2008a) and Mazri (2015), this phenomenon may be caused by many factors such as a high ammonium concentration in the culture medium, PGRs and liquid systems. Therefore, it is highly important to choose a medium able to improve the proliferation potential of shoot buds without increasing hyperhydricity. On the other hand, the occurrence of tissue browning ranged from 40 to 55%, with no significant difference among the four culture media. However, these percentages are high and should be attenuated. Tissue browning is caused by a high level of caffeoylshikimic acid in date palm tissues (Loutfi and El Hadrami, 2005). It has been widely reported in date palm explants, and compounds such as PVP and activated charcoal were used to control it (Meziani et al., 2016). Al-Khayri (2005) suggested to add citric and ascorbic acids during surface sterilization while Abohatem et al. (2011) recommended to shorten the period between subcultures to reduce it. According to Mazri (2015), some PGRs such as thidiazuron (TDZ) may significantly increase tissue browning in date palm explants. In this experiment, all culture media were supplemented with 1 g/l PVP. However, it appears that this concentration is not sufficient to avoid or at least reduce the occurrence of this phenomenon to an acceptable rate. Thus, it is important to evaluate higher PVP concentrations as well as activated charcoal, which has been suggested to control tissue browning in many plant species.

During shoot bud proliferation, adventitious rooting may be observed. Adventitious rooting is undesirable during the multiplication phase since the formed roots absorb the nutrients of culture medium, which negatively affect the multiplication of adventitious shoot buds (Al-Khateeb, 2008a). This phenomenon is mainly caused by a high auxin concentration in the culture medium. During the first experiment of the present study, adventitious rooting percentage ranged from 40.0 to 62.5%. Statistical analysis showed that there was no significant impact of medium strength on precocious rooting. However, it was noticed that decreasing medium strength increased precocious rooting percentage. Besides, the average number of roots per organogenic culture ranged from 0.6 when MS medium was used to 3.7 in MS/3 medium. The findings of this experiment suggest the use of MS/2 medium for shoot bud multiplication of date palm cv. Bouskri.

Table 1: Effect of medium strength on adventitious shoot bud multiplication of date palm (*Phoenix dactylifera* L.) cv. Bouskri

Culture medium strength	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Browning intensity	Precocious rooting (%)	Average number of roots per explant
MS	16.9 ± 1.0 a	55.0 ± 8.9 a	++	50.0 ± 13.4 a	+	40.0 ± 16.3 a	0.6 ± 0.2 a
MS/2	16.3 ± 1.3 a	37.5 ± 6.7 ab	+	45.0 ± 13.3 a	+	40.0 ± 12.4 a	1.8 ± 0.5 ab
MS/3	13.6 ± 1.1 ab	27.5 ± 10.1 b	+	40.0 ± 11.3 a	+	52.5 ± 14.6 a	3.7 ± 0.9 b
MS/4	11.9 ± 1.1 b	25.0 ± 5.2 b	+	55.0 ± 15.7 a	+	62.5 ± 15.4 a	3.1 ± 0.8 b

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% significance level. Intensity of hyperhydricity and tissue browning were visually estimated as: + low or ++ moderate.

Effects of carbon source on adventitious shoot bud proliferation

The findings of the second experiment revealed that carbon source type and concentration significantly affect shoot bud proliferation and hyperhydricity. Indeed, the highest average number of shoot buds per explant (19.7) was observed on MS/2 medium supplemented with 30 g/l sucrose. This was followed by the medium containing 50 g/l sucrose (18.8 shoot buds per explant). The use of commercial granulated sugar at concentrations ranging from 10 to 50 g/l resulted in an average number of shoot buds per explant ranging from 11.3 to 16.7 (Table 2). The beneficial effect of sucrose at 30 g/l on shoot bud proliferation in date palm was observed in other cultivars such as Mejhoul and 16-bis (Mazri, 2014; Mazri et al., 2016). According to Al-Khateeb (2008b), glucose, fructose and maltose at 30 to 60 g/l have almost the same effect as sucrose on shoot bud proliferation of date palm cv. Khanezi. On the other hand, the findings of this study showed that the use of sucrose resulted in lower hyperhydricity percentages when compared to commercial granulated sugar. In fact, when the culture medium was supplemented with sucrose, the hyperhydricity percentage was 17.5, 20.0 and 25.0% at 10, 30 and 50 g/l, respectively. Besides, the use of commercial granulated sugar resulted in hyperhydricity percentages ranging from 27.5 to 42.5%. In date palm cvs. Mejhoul, 16-bis and Al-Fayda, carbon source type and concentration did not significantly affect the occurrence of hyperhydricity (Mazri, 2014; Mazri et al., 2016, 2019). These conflicting results show that the

response of date palm explants to medium components, including the carbon source, is genotype dependent. Regarding tissue browning and precocious rooting, the findings of the present study showed that carbon source type and concentration do not significantly affect their occurrences. Indeed, the percentage of tissue browning ranged from 40% in the medium containing 10 g/l commercial granulated sugar to 55% in that containing 50 g/l commercial granulated sugar. This is in good agreement with the results reported in date palm cvs. Mejhoul and Al-Fayda (Mazri et al., 2016, 2019). However, in cv. 16-bis, the carbon source type and concentration significantly influenced the occurrence of tissue browning, with sucrose showing lower percentages than sorbitol and mannitol (Mazri, 2014). On the other hand, the percentage of precocious rooting ranged from 30% in the medium containing 50 g/l commercial granulated sugar to 45% in that supplemented with 50 g/l sucrose, while the average number of roots per organogenic culture varied from 1.3 when 50 g/l commercial granulated sugar was used to 2.0 when 10 g/l sucrose was used. This confirms previous results on cvs. Mejhoul and Al-Fayda (Mazri et al., 2016, 2019). However, different results were observed in other date palm cultivars. For example, in cvs. 16-bis and Khanezi, increasing carbon source concentration increased the rooting of organogenic cultures (Al-Khateeb, 2008b; Mazri, 2014). Based on the findings of this experiment, the use of sucrose at 30 g/l is recommend.

Table 2: Effect of carbon source type and concentration on adventitious shoot bud multiplication of date palm (*Phoenix dactylifera* L.) cv. Bouskri

Culture medium	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Browning intensity	Precocious rooting (%)	Average number of roots per explant
MS/2 + 10 g/l commercial granulated sugar	11.3 ± 0.7 a	27.5 ± 10.1 ab	+	40.0 ± 4.0 a	+	40.0 ± 13.0 a	1.4 ± 0.4 a
MS/2 + 30 g/l commercial granulated sugar	16.3 ± 1.3 bc	37.5 ± 6.7 a	+	45.0 ± 13.3 a	+	40.0 ± 12.4 a	1.8 ± 0.5 a
MS/2 + 50 g/l commercial granulated sugar	16.7 ± 0.9 bc	42.5 ± 11.2 a	++	55.0 ± 7.2 a	+	30.0 ± 8.9 a	1.3 ± 0.3 a
MS/2 + 10 g/l sucrose	14.4 ± 1.0 ab	17.5 ± 5.3 b	+	50.0 ± 12.3 a	+	32.5 ± 8.3 a	2.0 ± 0.5 a
MS/2 + 30 g/l sucrose	19.7 ± 1.0 c	20.0 ± 6.2 b	+	50.0 ± 13.4 a	+	35.0 ± 6.6 a	1.6 ± 0.3 a
MS/2 + 50 g/l sucrose	18.8 ± 1.3 c	25.0 ± 9.1 ab	+	47.5 ± 5.8 a	+	45.0 ± 8.1 a	1.9 ± 0.4 a

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% significance level. Intensity of hyperhydricity and tissue browning were visually estimated as: + low or ++ moderate.

Effects of PVP and activated charcoal on adventitious shoot bud proliferation

In the third experiment, different concentrations of PVP and activated charcoal were evaluated in order to reduce the occurrence of tissue browning. The findings showed that the use of activated charcoal at a concentration ranging from 2 to 3 g/l resulted in the lowest tissue browning rates (20-25%). The use of activated charcoal at 1 g/l showed a tissue browning percentage of 40% while adding PVP to culture medium showed tissue browning percentages of 50.0, 37.5 and 30.0% at 1, 2 and 3 g/l, respectively (Table 3). In all cases, the intensity of tissue browning was low. Regarding the other parameters evaluated, it was found that the different concentrations of PVP and activated charcoal used do not significantly affect them. Indeed, the use of activated charcoal at 2 g/l resulted in the highest average number of shoot buds per explant (23.3), with no significant difference with the other culture media (19.7-21.7 shoot buds per explant). The percentages of hyperhydricity and precocious rooting ranged from 20 to 30%, and from 22.5 to 35%, respectively; while the average number of roots per organogenic culture ranged from 0.9 to 1.6. All these results suggest the use of activated charcoal at 2 g/l, which significantly reduce tissue browning of explants. Very few studies were carried out to reduce the incidence of browning in date palm organogenic cultures. According to Meziani et al. (2016), in cv. Mejhoul, the use of date stone-based activated carbon at 1.5 g/l resulted in the lowest tissue browning percentage (7.5%) while activated charcoal was not efficient for this cultivar (62.5-65.0% tissue browning). Besides, the use of PVP showed a tissue browning range of 17.5-20.0%. In addition, a positive correlation was observed between tissue browning and the peroxidase activity of explants (Meziani et al., 2016). The results of the present study suggest the use 2 g/l activated charcoal for cv. Bouskri to reduce tissue browning, which highlights again the different responses of date palm genotypes to medium components.

Table 3: Effect of PVP and activated charcoal on adventitious shoot bud multiplication of date palm (*Phoenix dactylifera* L.) cv. Bouskri

Culture medium	Average number of shoot buds per explant	Hyperhydricity (%)	Intensity of hyperhydricity	Tissue browning (%)	Browning intensity	Precocious rooting (%)	Average number of roots per explant
MS/2 + 1 g/l PVP	19.7 ± 1.0 a	20.0 ± 6.2 a	+	50.0 ± 13.4 b	+	35.0 ± 6.6 a	1.6 ± 0.3 a
MS/2 + 2 g/l PVP	20.5 ± 0.9 a	30.0 ± 8.1 a	+	37.5 ± 9.3 ab	+	27.5 ± 2.5 a	1.2 ± 0.1 a
MS/2 + 3 g/l PVP	20.7 ± 1.4 a	30.0 ± 5.0 a	+	30.0 ± 5.0 ab	+	30.0 ± 6.2 a	1.0 ± 0.2 a
MS/2 + 1 g/l activated charcoal	20.0 ± 1.2 a	30.0 ± 6.2 a	+	40.0 ± 4.0 ab	+	35.0 ± 5.5 a	1.1 ± 0.1 a
MS/2 + 2 g/l activated charcoal	23.3 ± 0.6 a	30.0 ± 13.3 a	+	25.0 ± 6.4 a	+	25.0 ± 3.7 a	0.9 ± 0.1 a
MS/2 + 3 g/l activated charcoal	21.7 ± 0.8 a	30.0 ± 8.1 a	++	20.0 ± 5.0 a	+	22.5 ± 4.4 a	0.9 ± 0.1 a

Data are means ± standard error. Data in the same column followed by the same letter are not significantly different at the 5% significance level. Intensity of hyperhydricity and tissue browning were visually estimated as: + low or ++ moderate.

Shoot elongation, rooting and plantlet acclimatization

Shoot elongation and rooting were performed on PGR-free MS/2 medium as suggested for many date palm cultivars. In fact, in cvs. Najda, Boufeggous and 16-bis, the use of PGR-free media before acclimatization resulted in high survival rates after transferring the plantlets to the glasshouse, reaching 100% (Mazri and Meziani, 2013; Mazri, 2014, 2015). In cv. Najda, the effects of media with and without PGRs on shoot elongation and rooting were evaluated, and the findings showed that PGRs resulted in longer plantlets with more roots. However, the plantlets were fragile and their survival rate during acclimatization was low (Mazri and Meziani, 2013). Meziani et al. (2019) evaluated the effects of different medium additives, including PGRs, during shoot elongation and rooting of cv. Mejhoul on subsequent acclimatization, and suggested against the use of PGRs since they did not improve the survival rate during acclimatization, while they increase the production cost of the plants. In the present study, the use of PGR-free MS/2 medium during shoot elongation and rooting showed an average length of shoots of 13.9 cm, an average root number of 4.1 and an average root length of 3.2 cm. Besides, the survival rate of plantlets after 3 months in the glasshouse was 95%.

Conclusions

Developing an efficient regeneration system through organogenesis is highly important to preserve cv. Bouskri, which is one of the most preferred date palm cultivars by the Moroccan consumer. The findings of this study suggested to use MS/2 medium containing 30 g/l sucrose and 2 g/l activated charcoal for efficient proliferation of adventitious shoot buds of this date palm cultivar. These results will be of great benefit for rapid and large-scale propagation of cv. Bouskri, and will significantly contribute to the preservation of this endangered date palm cultivar.

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