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Comparison of vegetative buds formation in two date palm cultivars, Medjool and Mazafati through direct organogenesis

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ABSTRACT: Micro-propagation through direct organogenesis is widely used for rapid propagation of superior cultivars of date palm.In the present study, the shoot tip explants were cultured on Murashige and Skoog (1962) (MS) mediasupplemented with different plant growth regulator (PGR) treatmentsfor inducing buds regeneration. The experiment was set up in a factorial based withtwo factors.Factor A was PGR treatments in four levels (1- 2iP: 1mg/L, BAP: 1mg/L, 2- 2iP: 0.5mg/L, BAP: 0.5mg/L, 3- 2iP: 0.2mg/L, BAP: 0.2mg/L, 4- 2iP:0.1mg/L, BAP:0.1mg/L) and factor B was two date palm cultivars. The results showed that the PGR treatments had significant effects on vegetative buds formation and the best treatment was 0.5mg/L1-naphthylacetic acid, 0.5mg/L2-naphthyloxyacetic acid,1mg/L N6-(2-isopentyl)adenine, 1mg/L6-benzylaminopurine. Also, significant difference observed between cultivars and Medjool produced more vegetative buds (2.62) thanMazafati cultivar.

***Keywords*:** *Phoenix dactylifera* L., Regeneration, Initiation step, Cytokinins

INTRODUCTION

World dates production is approximately 7.51 milliontons whichcreatedlarge commercial activities (FAO, 2012). Date palm culture is one of the important and economic activities which is used not only for fruits but also as fuel, fiber and shade for ground crops (Duke James, 1983).

Phoenix dactilifera L. from *Palmaceae* family is a dioecious, perennial, monocotyledonous plant that has an important role in the economics of Middle East and North Africa areas (Gurevich, 2005). Traditional method of date palm propagation is through offshoots that usually grows under ground or near the surface beside the trunk of tree (Al Khateeb and Ali-Dinar,2002). Some cultivars donot produce offshootat all or have very few cultivable offshoots (10 to 30) (Heselmans, 1997) and less than 60% of them survive(Saaidi, 1979; Al Khateeb, 2006). Other disadvantages of this method are spreading of fungi diseases and red palm weevil pestwhich canbe transferred by infected offshoots. Also, rooting of these offshoots is very difficult (Asemota, 2007; Eke , 2005).

Tissue culture multiplication technique can be a very efficient tool forthe development of the date palm culture.The benefits of using this technique are production of many plantlets from one date palm mother tree, pathogen-free, true to type, fruit bearing after 4 years, easy to transport and growth and viability up to 100% compared to plants produced through traditional offshoot culture.Micro-propagation of date palm is done through twodifferent methods,Somatic embryogenesis anddirect organogenesis,each has advantages and disadvantages (Alkhateeb and Ali- Dinar, 2002).In embryogenesis method, vegetative embryos are produced from embryogenic callus. In this technique, date palm meristem is cultured as the explants on the medium containing high concentrations of auxin and consequentlycallusis formed (Alkhateeb, 2006).

Exponential increase in callus production is achieved by steady subculturinginthe fresh culture medium (Alkhateeb, 2006).

In direct organogenesis, plantletsare produceddirectly from mother plant tissue without a callus phaseintervening and theyare completely similar to mother plant (Aaouine, 2000; Alkhateeb and Ali-Dinar, 2002; Al Khateeb, 2008). The advantages of this method are shortening culture stages (no callus stage), nosomaclonal variations(Khierallah and Bader, 2007) inplantlets produced which is very important, incubation and propagation of the explants only under light conditions, propagation for research and commercial production (Alkhateeb and Ali-Dinar, 2002; Al Khateeb 2008).

Direct organogenesis has four stages including: induction of vegetative buds, propagation and proliferation of buds, lengthening of shoots and rooting. Success in this method depended mainly on success in the first stage (Abahmane, 2011).

Recently, protocols were published for mass propagation of different cultivars of date palm by organogenesis (Hegazy and Aboshama, 2010). Al Khateeb (2006) reported that low concentrations ofauxins and cytokininsplant growth regulators (PGRs) causednew buds formation.

Micro-propagation of date palm through direct organogenesis has been done using different explants such as shoot tips or lateral buds in several cultivars (Zaid and Tisserat, 1983; Tisserat, 1984; Al Khateeb, 2006). At the present time, mass propagation of date palm through direct organogenesis has been performed for 7 cultivars including Zaghlool, Samany, Hayany, Amhat, Siwy, Selmy and Malakaby (Hegazy, 2008; Hegazy and Aboshama, 2010). Bekheet and Saker (1998) investigated the effects of PGRs on production of direct or indirect budsfrom shoot tips of Zaghloulcultivar. Taha(2001)obtained vegetative buds by culturing shoot tips in the medium containing high concentrations of 2iP. Khierellah and Bader (2007) also produced vegetative buds through direct organogenesis.Therefore the aim of this study was to determine the best combination of PGRs and commercial cultivars (Medjool and Mazafati) to stimulate the initiation of vegetative buds directly from shoot tips.

MATERIALS AND METHODS

Offshoots of Medjool and Mazafaticultivars (3-5 kg weight and 3-4 years old) were separated from selected healthy mother plants and weretransferred to Agricultural Biotechnology ResearchInstitute of Iran (ABRII). Medjool date palm isnot native to Iran and originated from North Africa. The preference of this cultivar to Iranian date palm cultivars is its considerable large fruits. Mazafati date palm is the third economically important dates in Iran after Staemaran and Shahani, cultured in different areas of this country but it originated from Bam area in Kerman province. This cultivar is counted as soft (or moist) and semi-dry dates group and is black in colour (Hashempoor, 2001).

Large leaves and fibrousparts were cut away (Fig 1. A.) and finally upper part of the stem accompanied withprimary leaves was taken to laboratory forsurface sterilization and culture (Fig 1. B). Samples were washed usingsterile distilled water first and transferredto antioxidant solution consisting of 100 mg/Lcitric acidand 100 mg/L ascorbic acid and after 15 minutes,they were immersed inBenomyl as fungicide (5g/L)for 15 minutes. Then, they were sterilized using 70% ethanol solution for 60 seconds and finally were immersed in5% sodium hypochlorite for 20 min followed by three times rinsing with sterile distilled water. Explants of apical meristems with theirsurrounding tissues (Shoot tips) were cut into four segments under laminar air flow (Fig 1. C and D), andwere separately cultured on medium.The culture media consisted of MS salts, supplemented withMyo-Inositol (100 mg/L), Glutamine (200 mg/L), Thiamine-hydrochloride (1 mg/L), nicotinic acid (1 mg/L), Pyridoxine-hydrochloride (1 mg/L), sucrose (30 g/L),activated charcoal (1.5 g/L) and agar (7 g/L)and different PGRsto induce vegetative buds. The pH was adjusted to 5.7-5.8 and then the media were autoclaved at 121°C for 20min.Cultures were incubated in the dark condition at 22±1ºCfor the first month and were thentransferred to 16/8 light/dark at 27±1 ºCfor the rest of the incubation period. At this stage, a factorial experiment was conducted based on completely randomized designinfour replications. Treatments were four media (combination of four PGRs) (A) as follows,(all treatments have 0.5 mg/L NAA and 0.5 mg/L NOA):

1- 2iP: 1mg/L, BAP: 1mg/L
2- 2iP: 0.5mg/L, BAP: 0.5mg/L
3- 2iP: 0.2mg/L, BAP: 0.2mg/L
4- 2iP:0.1mg/L, BAP:0.1mg/L

Andtwo cultivars Medjool and Mazafati(B).

In this experiment the numbers of regenerated buds were counted after 8months (The explants were sub-cultured each month). The data were statistically analyzed using SAS Version 9.1and mean separation was done usingDuncan's multiple range test at 5%.

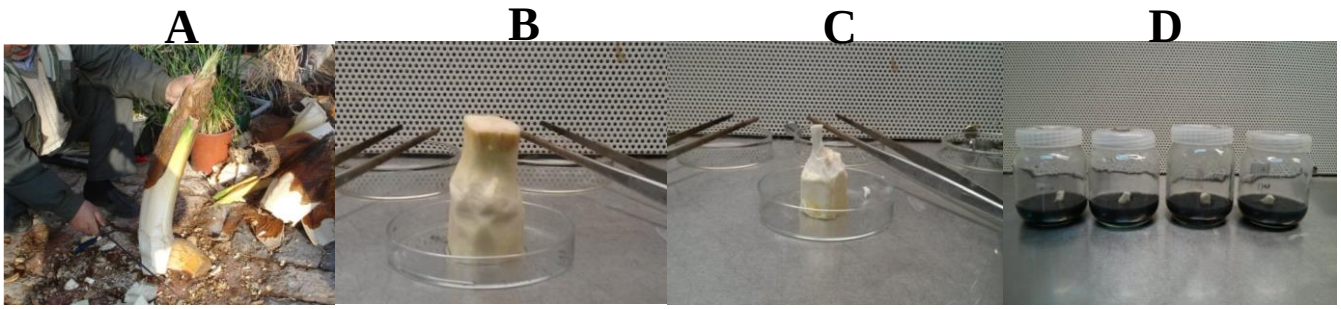

Figure 1. Date palm explant preparation. A) Removing large leaves and fibrous parts, B) Upper part of the stem, C) Surrounding shoot tip with primary leaves, D) Four segments of shoot tip cultured on MS media

RESULTS AND DISCUSSION

According to the results presented invariance of analysis (Table 1),the effectsofPGRs (A) on number of regenerated buds is significant(1%). Also, cultivars of Mazafati and Medjool had significant effects(1%) on number of regenerated buds. There is also a significant (5%) interaction effect between two tested factors. It means that explants from the two cultivars, Medjool and Mazafati, responded differently to PGRs treatments consideringregeneration of buds.

Mean comparisonof plant media (PGRs treatments) based on Duncan's multiple range test (Fig. 2) showed that the highest number of regenerated buds (4.51 buds) was achieved in medium 1.Bekheet and Saker (1998) reported that cytokinins like 2iP and BAP are essential components in culture medium used for production of vegetative buds,causing cell division and inhibition of apical dominance.Khierallah and Bader (2007) found that no vegetative bud regenerated in the medium without cytokinins and they revealed that medium supplemented with 2.0 mg/L 2ip, 1.0 mg/L BA, 1.0 mg/L NAA and 1.0 mg/L NOA was the best for bud formation fromshoot tip. Khan and Bibi (2012) reported that addition of 0.5 mg/L BAP to the culture medium increasedbud regeneration. In the present study, the best treatment was the medium containing 1mg/L 2iP, 1mg/L BAP, 0.5 mg/L NAA and 0.5 mg/L NOA.

Also, results in Fig. 3 show that Medjool cultivar produced more vegetative buds (2.62) compared to Mazafati (1.85). Based on several reports (e.g. Beauchesne, 1983; Al Maari and Al Ghamdi, 1998; Hussain, 2001; Jain, 2007), the number of regenerated vegetative buds, is depended on the cultivar. Belal and El Deeb (1997) conducted some researches on Egyptian cultivars (Zaghloul and Samani) and cultured these cultivars on MS medium containing auxins and cytokininsfor direct organogenesis and their results showed that different PGRs treatments have different effects on Egyptian date palm cultivars.Researches done by Al-Khayri and Al-Bahrany (2004) also showed significant effects of cultivars on number of vegetative buds regenerated which is in agreement with the results of the current study.

According to the means presented (Fig.4) on the interaction effects of cultivars and PGRs treatments, eightdifferent treatments could be compared.It shows that the treatment number 1 and Medjool cultivarhad the highest number of average bud formation (5.05). In this study Mazafati cultivar with treatment number 4 had the lowest number of average bud formation (0.12).

Table 1. Analysis of variance of the effects of different treatments on average bud formation in Medjool and Mazafati cultivars of date palm

	Mean Square	
Source of variation	df	Average bud formation
Growth regulator treatment (A)	3	27.7187 **
Cultivars (B)	1	4.7278 **
A*B	3	0.1087 *
Error	24	0.0393
Total	31	
		CV% 8.86

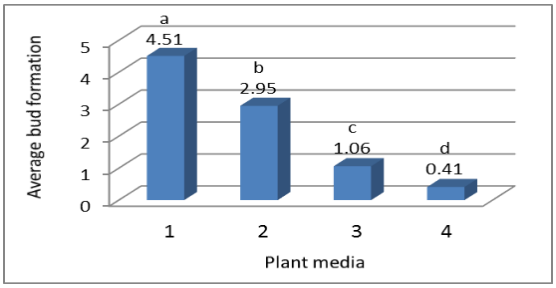

Figure 2. Comparison of different treatments on regenerated buds

Medium 1: 2iP: 1mg/L, BAP: 1mg/L; Medium 2: 2iP: 0.5mg/L, BAP: 0.5mg/L; Medium 3: 2iP: 0.2mg/L, BAP: 0.2mg/L; Medium 4: 2iP: 0.1mg/L, BAP: 0.1mg/L (all media have 0.5 mg/L NAA and 0.5 mg/L NOA).

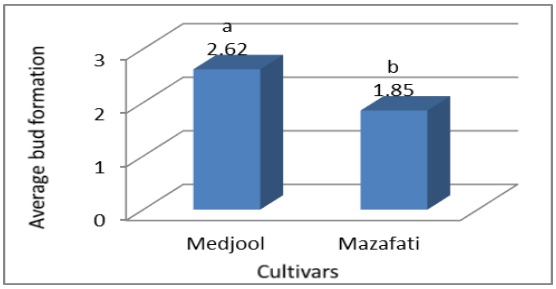

Figure 3. Comparison of regenerated buds from explants of Medjool and Mazafati cultivars.

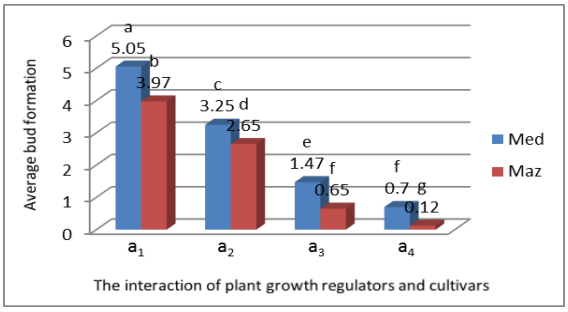

Figure 4. The interaction effects of different plant growth regulators (a) and cultivars of date palm (b) (Medjool and Mazafati) on number of regenerated buds

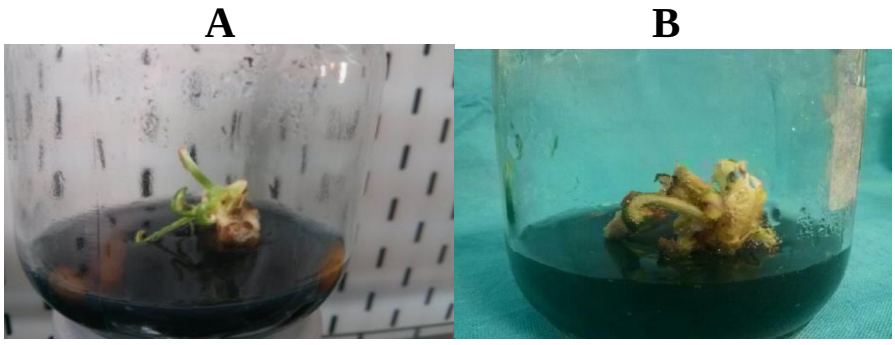

Figure 5. Direct vegetative buds formation from quarter of shoot tip. Vegetative buds of A) Medjool and B) Mazafati on MS medium containing (mg/L) 2ip 1, BAP 1, NAA 0.5 and NOA 0.5 after 8 months

Conclusion

Results of the present study showed that number of regeneratedbuds is depend on the cultivars and Medjool cultivar has better result than Mazafati (Fig. 5). The presence of cytokininssuch as BAP and 2ip in plant media,causing budsregeneration andby reducing them, initiationof vegetative buds was reduced.

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