Pak. J. Bot., 52(6): 2021-2030, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-6(13)

AN EFFICIENT MICROPROPAGATION PROTOCOL VIA INDIRECT ORGANOGENESIS FROM CALLUS OF ECONOMICALLY VALUABLE CROP DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS "SAGAI AND KHALAS"

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Abstract

Regeneration of date palm cultivars (Khalas and Sagai) was achieved from callus by indirect organogenesis using different plant growth regulators (PGRs) on the modified MS culture medium. The best PGRs combination for the indirect induction of adventitious bud formation was observed at 4 mg BA/L + 0.5 mg/L of NAA in Khalas (4.50 buds) and 2 mg BA/L + 1 mg/L NAA in Sagai (8.75 buds). The multiplication of adventitious bud was found to be greater at a combination of PGRs (1 mg/L of 2iP + 0.5 mg/L of NAA) in Khalas (8 buds); and 4 mg 2iP/L + 0.5 mg NAA/L in Sagai (12.75 buds). The shootlet length (6.16 cm) was achieved at 5 mg/L GA_3 as compared to that at other GA_3 concentrations used in both cultivars. The highest number of roots (4.14 roots) from shootlets was achieved at 0.5 mg/L of NAA in cv. Sagai, whereas, in Khalas, the highest number of roots (3.28 roots) was formed at 1.5 mg/L of IBA. The highest root length (4.10 and 3.57 cm) was formed at 1.5 mg/L of IAA in both cultivars, respectively. Thus, the protocol developed through this investigation could be used at a wide scale for the production of high-quality plantlets to meet the demand of these date palm cultivars.

Key words: Regeneration, Date palm, Indirect organogenesis, Plant growth regulators (PGRs), Khalas, Sagai.

Introduction

Date palm (*Phoenix dactylifera* L.) is considered as an important fruit tree worldwide. It is diploid (n = 18; 2n = 36) tree of family Arecaceae. It contains around 200 genera and more than 2,500 species (Jain *et al*., 2011). It is monocotyledon, perennial and acclimated to the harsh environmental conditions. Different cultivars of the palm are found in various countries (Chao & Krueger, 2007).

Phoenix dactylifera (*P. dactylifera*) is regarded as the main source of income and food for the local population in North Africa and the Middle East. The different parts of the date palm are used for the treatment of various diseases (Chao & Krueger, 2007). Date fruits are used to cure many infectious diseases and cancer (El-Far *et al*., 2016). Fruits of date palm have important nutrients and vitamins (Mrabet *et al*., 2008). Syrup prepared from dates has traditionally been used to treat various diseases including angiogenesis and inflammations (Taleb *et al*., 2016). The extracts of dates showed to have potent antioxidant and antimutagenic activity (Saafi *et al*., 2008).

Different biotic and abiotic stresses affect the yield and quality of date palm fruits. Biotic factors such as red palm weevil (*Rhynchophorus ferrugineus*) and fungus, *Fusarium xysporum* f. sp. Albedinis (Jain, 2012) that affect date palm (Ferry & Gomez, 2002). Consequently, there is need of biotechnological approaches for their propagation, breeding, and conservation of the germplasm of date palm (Fki *et al*., 2011a).

The propagation by seeds gives half of the progeny female, and the other half will be male (Al-Khateeb, 2006b; Eke *et al*., 2005). However, the seeds of palm are heterozygous; therefore, the progeny will not be true-to-type (Naik & Al-Khayri, 2016). Moreover, the palm produced by seeds required 4-7 years to produce dates (Zaid, 2002). Using offshoots for the propagation of date palm produce plants which are identical to their mothers (true-to-type) (Abahmane, 2011). However, palms produce a limited number of offshoots (20 to 25 at most) in whole life and it depends on the cultivar, and some of them do not produce offshoots (Naik & Al-Khayri, 2016). Moreover, the rooting of date palm offshoot is difficult (Asemota *et al*., 2007). In fact, the successful growth of offshoots is up-to 60% (Saaidi *et al*., 1979). Furthermore, this may result in transfer of pathogens such as red palm weevil or Bayoud (Abahmane, 2011).

Therefore, there is a need of biotechnology approaches for the multiplication of date palm. Mazri & Meziani, (2015) stated that there are two methods for the micropropagation of the date palm by organogenesis or somatic embryogenesis to speed up and large-scale production. However, the tissue culture technique is an easy way to obtain an adequate number of vigorous and healthy (disease-free) plants to full fill the requirement of the local and international demand (Khokhar & Teixeira, 2017).

Plants can be regenerated by indirect organogenesis from the callus. This technique involves five stages viz., induction of callus, induction of vegetative buds, multiplication of buds, elongation of shoot and rooting stage (Abahmane, 2011). However, date palm propagation by tissue culture is still challenging for recalcitrant genotypes. Jain, (2007) reported limited success in propagation of the palm. Therefore, we aimed to develop an effective protocol for micropropagation of cultivars Sagai and Khalas of date palm through indirect organogenesis.

Materials and Methods

Plant material: The offshoots were obtained from Agricultural Research station of Dirab (Riyadh) and farm of Al-Rajhi (Al-Qassim), Kingdom of Saudi

Arabia. Young offshoots of cultivars Khalas and Sagai (2-3 years old) were separated from the mother tree. The fibrous tissues with outer leaves surrounding the base of offshoots were removed until to get shoot tips. The separated shoot tips were chopped into small pieces (10 cm) having 3 cm diameter in a circular shape. The shoot tips were cleaned with tap water for 10 min. Thereafter, they were transferred into a chilled antioxidant solution contain ascorbic acid 150 mg/L and citric acid 100 mg/L; then kept at 4°C in a refrigerator for 24 h to reduce browning (Fig. 1A). Further, shoot tips were cleaned with distilled water and kept in ethanol (70%) for 1 min. The explant surface sterilization was performed by 30% v/v Clorox, followed by commercial bleach (sodium hypochlorite 1.6%) for 20 minutes, supplied with Tween 20, two drops per 100 ml. Further, the explants were washed three times with sterile distilled water for 15 minutes to eliminate traces of sterilization solution. The leaf primordial was divided into couple of pieces and the apical meristem into 5-7 pieces, and used as explants (Fig. 1B).

The MS media (Murashige & Skoog, 1962) supplemented with 120 mg/L of myo-inositol, 2 mg/L of glycine, 0.5 mg/L of pyridoxine, 0.5 mg/L of nicotinic acid, 0.1 mg/L of thiamine, 170 mg/L of sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$). 40 mg/L of adenine sulfate, 200 mg/L of glutamine and 30 g/L of sucrose was used for explant culture. Agar at a concentration (6-7 g/L) was added to MS medium after making the pH (5.8) of the media and then autoclaved for half an hour at 121°C and 1x105 Pa (1.1 kg cm-2).

Callus induction from leaf primordia and shoot tip: The modified MS media was supplemented with several combinations of PGRs as following: (30 mg/L NAA and 3 mg/L 2iP, 10 mg/L 2,4-D and 3 mg/L 2iP, 30 mg/L NAA and 3mg/L BA, 80 mg/L 2,4-D and 5 mg/L BA, and MS free of PGRs. The shoot tips and leaf primordial of Khalas and Sagai cvs. were utilized for callus induction. The explants were put on MS media then incubated in the darkness at 27±2°C for ten weeks and sub-cultured every after four weeks on same media.

Effect of different combinations of phytohormones on the formation of adventitious buds from callus and its multiplication: The modified MS media supplemented with NAA at concentrations (0.0, 0.5, 1 and 1.5 mg/L) and BA (0.0, 1, 2 and 4 mg/L) was used for the formation of the adventitious buds. The initial callus (each 50 mg) from cultivars Sagai and Khalas was cultivated on MS medium for 10 weeks with sub-culturing every after four weeks on the same media composition. The number of produced adventitious buds was recorded.

Further, the modified MS media supplemented with NAA at concentrations (0.0, 0.1, 0.5 and 1mg/L) and 2iP (0.0, 1, 2 and 4 mg/L) was utilized for multiplication of adventitious buds. The adventitious buds that formed in the previous experiment from cultivars Sagai and Khalas were divided into small lumps, each one containing at least two buds. The sub-culturing was continued every after four weeks on MS medium containing the same components. After 12 weeks of the culture, the number of multiplexed buds were recorded.

Effect of phytohormone on root and shoot length: In this study, we used the MS media that contained different concentrations of GA_3 (0.0, 0.1, 1, 2, 3 and 5 mg/L). The shoots of cultivars Sagai and Khalas derived from adventitious buds were selected, and they were equal in size (2 cm), and age. Twelve plantlets for each treatment were planted on the medium. The shoot lengths were recorded after 20 days.

The modified media contained different concentrations of NAA (0.5, 1 and 1.5 mg/L) or IBA (0.5, 1 and 1.5 mg/L) or IAA (0.5, 1 and 1.5 mg/L) along with MS medium free of PGRs. Some of the shoots of cultivars Sagai and Khalas derived from adventitious buds were selected as they were equal in size (3 cm), and age. Four shoots were put on the MS media (8 replicates per treatment) at the first culture. The second culture was put after 3 weeks on the previous media, where each shoot was individually cultured in culture tubes. The number of roots and root length, were recorded after one and a half months culturing.

Statistical analysis

All experiments were conducted as factorial experiments according to a completely randomized factorial design. The data were analyzed by using the statistical analysis of variance (ANOVA) Averages of the main effects, and their interactions were compared by using Duncans's test at $p<0.05$ level to compare means (values were expressed as means ± SE) (Crawley, 2005). All experiments were performed in triplicate. The analysis of data was achieved using SPSS (version 11, SPSS Inc. Chicago, USA) software package.

Results and Discussion

The combination of phytohormones (3 mg 2iP/L + 30 mg NAA/L) gave a higher rate of induction of callus in Khalas as 47% in leaf primordia and 95% in shoot tip. Whereas, in cultivar Sagai, the higher rate of induction of callus was 41% in leaf primordia and 92% in shoot tip on the same medium that contained (3 mg 2iP/L + 10 mg 2,4-D/L). Further, the calli were cultivated on calli proliferation medium contained 0.5 mg 2,4-D/L for two months, until enough amount of calli were formed.

Formation of adventitious buds from callus with different phytohormone combinations: The calli were transferred to the MS media containing PGRs for adventitious bud formation. Different phytohormone combinations produced the adventitious buds on MS media as shown in Table (1). The significant effect was observed among the concentrations of BA irrespective of the effect of NAA**.** The BA (4 mg/L) gave the highest mean value (3.62 buds per culture) in Khalas cultivar, whereas, the highest mean value (6.87 buds per culture)

was found in Sagai at 2 mg /L, respectively. However, the lowest number of adventitious buds were observed on the medium without BA in both cultivars. Our results are in line according to Al-Taha *et al*., (2011) who reported that callus without PGR did not produce any buds. However, addition of cytokinins to the media play an important role as it stimulates the formation of adventitious buds through an increase in DNA replication, and finally promotes cell division (Auge, 1984). The results of our study are dissimilar with Saheem *et al*., (2008) who reported that the best treatment of cytokinin 2iP (5 mg/L) stimulated buds formation (9.15 buds) of callus, which was significantly different from the other concentrations. Furthermore, they reported the best concentration of cytokinin (BA) at 5 mg/L induced buds formation (7.3 buds) from callus, which was significantly different than other concentrations.

The significant differences were also found at different concentrations of NAA (Table 1) for the formation of buds (regardless of the effect of BA). The highest mean of adventitious bud formation was observed at 1 mg NAA/L (3.50 buds in case cv. Khalas, and 6.81 buds in cv. Sagai). Whereas, the lowest mean of adventitious bud formation (1.93 buds in Khalas and 2.75 buds in Sagai) was recorded on medium without application of NAA. It is worth mentioning that the highest and the lowest mean values in terms of adventitious bud formation were at the same concentrations of NAA in both cultivars, but there was a difference observed in the number of buds produced in both cultivars. Bader *et al*., (2007) found the difference between two cultivars Barhee and Maktoum in the number of adventitious buds formed which were 11.2 and 24.8 buds, respectively with liquid agitated medium supplemented with 10.0 µM 2iP and 5.0 µM NAA.

Table 1. Effect of different combinations of the PGRs on the neoformation of buds from calli of date palm.

Number of adventitious buds mean ± SE						
Con. NAA (A)	**Cultivar**	**Con. BA mg/L (B)**				
		0.0 mg	**1 mg**	**2 mg**	**4 mg**	**Average A**
0.0 mg	Sagai	0.00 a*	2.00 ± .408 b	5.00 ±.408 def	4.00 ± .408 cd	2.75 a
	Khalas	0.00 ± .000 a	1.75 ± .000 b	3.00 ±. 408 cde	3.00 ± .250 cde	1.93 a
0.5 mg	Sagai	3.00 ± .408 bc	8.50 ± .866 i	7.75 ±.478 hi	5.75 ± .853 efg	6.25 bc
	Khalas	2.25 ± .478 bc	2.75 ± .288 bcd	3.25 ± .288 cde	4.50 ± .629 f	3.18 bc
1 mg	Sagai	5.75 ± .472efg	6.75 ±.471gh	8.75 ±.478 i	6.00 ± .408 fg	6.81 c
	Khalas	2.25 ± .250 bc	4.00 ± .500 ef	3.75 ± .250 def	4.00 ± .250 ef	3.50 c
1.5 mg	Sagai	4.25 ± .47 cde	5.50 ±.288 defg	6.00 ±.408 fg	7.00 ± .408 gh	5.68 b
	Khalas	1.75 ± .478 b	3.00 ± .288 cde	3.50 ± .408 def	3.00 ± .478 cde	2.81 b
Average B	Sagai	3.25 a	5.68 b	6.87 c	5.68 b	
	Khalas	1.56 a	2.87 b	3.37 bc	3.62 c	

**Data that have the same alphabets are not significantly different, according to the test of Duncan at level (p<0.05)

The findings of our research are in agreement with Al-Taha *et al*., (2011) as genesis of adventitious buds in date palm was achieved from primary calli cultivated on MS media supplied with single or in combination of low auxins level (IAA and NAA). Moreover, Saheem *et al*., (2008) indicated that the best treatment of auxin NAA was 1 mg/L to stimulate bud formation from callus, which was significantly different than 2 and 3 mg/L of NAA in Barhee of date palm cultivar. However, Fki *et al*., (2011a) stated that the buds of date palm 'Barhee' were successfully formed from young leaves (1cm) using 0.2 mg/L of 2,4-D.

Various NAA concentrations (0.0, 0.5, 1 and 1.5 mg/L) and BA (0.0, 1, 2 and 4 mg/L) produced adventitious buds from callus and best result was found at combination (4 mg BA/L + 0.5 mg/L of NAA) in Khalas. While, in Sagai cultivar, the combination of BA and NAA at 2 mg /L and 1mg/L, respectively, produced the highest number of buds significantly compared with the other combinations of different concentrations of NAA and BA cvs. Khalas and Sagai (Table 1) Figs. (1 & 2). These findings of our study are in agreement with Al-Taha *et al*., (2011), who stated that the MS medium including the mixture of 1.5 mg NAA and 3 mg 2iP was superior for the formation of buds in some rare palm. As well as, no bud was produced on MS medium without PGRs. However, Al-Khateeb, (2006a) reported that in the propagation of date palm cv. Sukry, low PGRs concentrations promoted the neoformation of buds while higher concentrations lead to abnormal growth.

As the data exhibited in Table (1) show the best results for neoformation of buds obtained with combination of BA and NAA as compared with NAA or BA which were used separately Figs. (1 & 2). Our result is consistent with Jasim, (2002) and Saheem *et al*., (2008) who showed that using a low concentration of the auxin and a relatively higher concentration of the cytokinin produced the neoformation of adventitious buds in date palm.

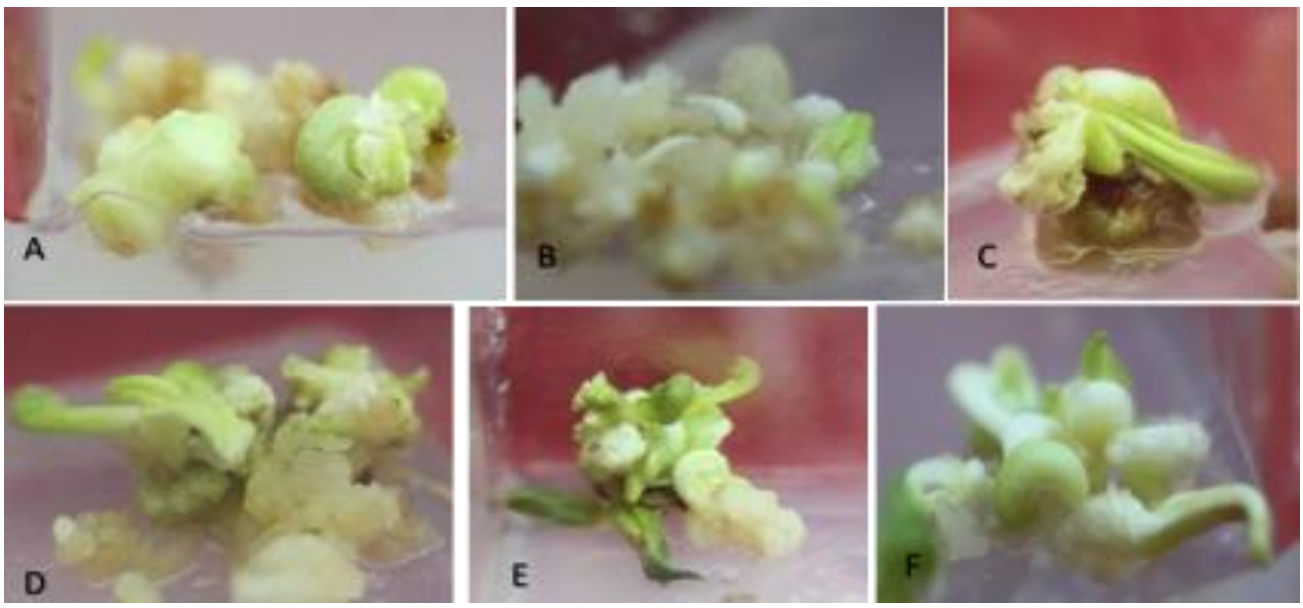

Fig. 1. Formation of adventitious buds from callus (cv. Khalas); (a) On modified MS medium supplemented with 1 mg BA/L + 0.5 mg NAA/L. (b) On modified MS medium supplemented with 2 mg BA/L + 0.5 mg NAA/L. (c) On modified MS medium supplemented with 1 mg BA/L + 0.0 mg NAA/L. (d) On modified MS medium supplemented with 1 mg BA/L + 1.5 mg NAA/L. (e) On modified MS medium supplemented with 4 mg BA/L + 1 mg NAA/L. (f) On modified MS medium supplemented with 4 mg BA/L + 0.5 mg NAA/L.

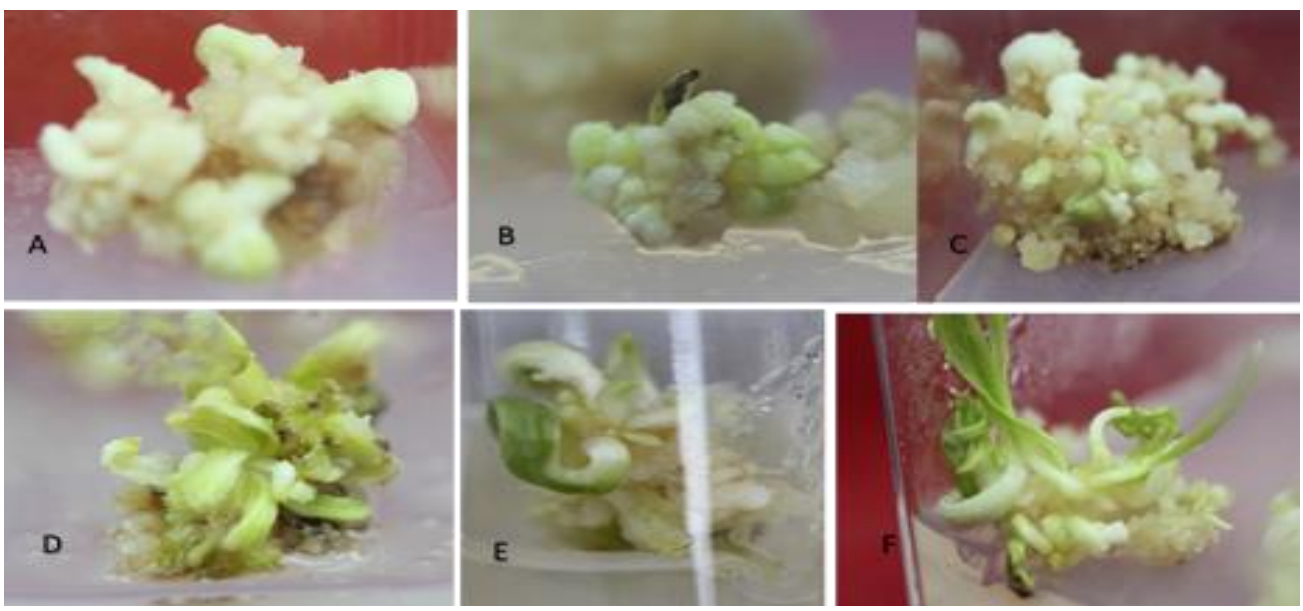

Fig. 2. Adventitious buds formation of from callus (cv. Sagai); (a)Modified MS medium supplemented with 4 mg BA/L + 0.0 mg NAA/L. (b) Modified MS medium supplemented with 1 mg BA/L + 1 mg NAA/L. (c) Modified MS medium supplemented with 4 mg BA/L + 0.5 mg NAA/L (d) Modified MS medium supplemented with 2 mg BA/L + 1.5 mg NAA/L. (e) Modified MS medium supplemented with 2 mg BA/L + 0.5 mg NAA/L. (f) Modified MS medium supplemented with 2 mg BA/L + 1 mg NAA/L.

Effect of different concentrations of PGRs on multiplication of indirect adventitious buds: Significant differences were found on adventitious bud multiplication under different combinations of concentrations NAA and 2iP (Table 2). The highest mean value of adventitious buds was obtained at 0.5 mg/L of NAA (6.37 buds in Khalas and 9.87 buds in Sagai) or 2 mg/L of 2iP (5.43 buds) in Khalas, and 4 mg 2iP/L (9.50 buds) in Sagai. The obtained results were close with Al-Taha *et al*., (2011), who observed that the 1 mg/L of NAA and 4 mg/L of 2iP gave more numbers of buds at the multiplication stage when used separately. However, in terms of multiplication of buds, Saheem *et al*., (2008) found that the concentration of NAA at 2 mg/L produced non-significant results as compared to other treatments of NAA, while 5 mg/L of 2iP produced best results significantly compared to other treatments of 2iP.

The combined effect of various concentrations of NAA (0.0, 0.1, 0.5 and 1 mg/L) and 2iP (0.0, 1, 2 and 4 mg/L) on multiplication of buds Table (2) Figs. (3 & 4) showed that significant differences were found in Sagai whereas non-significant difference was found in Khalas. However, the combination of PGRs (1 mg/L 2iP + 0.5 mg/L NAA) gave the higher number of buds (8 buds) in Khalas. Whereas in

Sagai, the best combination of PGRs (4 mg 2 iP/L + 0.5 mg NAA/L) gave the highest number of buds (12.75 buds) compared with other treatments. These results demonstrate the superiority of Sagai on Khalas cultivar to produce multiplexed buds in all treatments and it depends on PGRs and genotypes (Mazri, 2012). The results found in this investigation agree with studies done by Saker *et al*., (1998) and Jasim, (2002) as NAA at low concentrations relatively with 2iP had stimulated the adventitious bud proliferation. Al-Khateeb & Alturki, (2014) stated that the largest production of buds was in Reziz and Sukary cultivars, whereas the lowest was observed in Medjool. However, combined effect of NAA and 2iP is close with Al-Taha *et al*., (2011), who stated that the MS medium supplemented with 1 mg/L NA and 4 mg/L 2iP gave the highest amount of bud multiplications (11.4 buds). Moreover, Saheem *et al*., (2008) indicated that the mixture of the cytokinin (2iP and BA at 5 mg/L) and 1 mg/L NAA induced the formation of buds, and higher number of buds (15.3 buds) with same combination. Contrary to our findings, Bekheet, (2013) stated that the medium supplied with a combination of 5 mg/L 2iP and 2 mg/L Kin produced the maximum buds proliferation.

Effect of various concentrations of GA_3 on shootlets length of Sagai and Khalas cultivars: GA_3 plays a potential role in elongation of buds. However, the data present in Table (3) show significant differences on length of shootlets under various GA_3 concentrations (0.0, 0.1, 1, 2, 3 and 5 mg/L) as increasing concentrations of GA_3 resulted in a steady increase in shootlets length. Furthermore, highest length of shootlets (6.16 cm) was obtained with GA_3 at 5 mg/L whereas, the lowest shootlets length (3.53 cm) was observed on MS medium free from GA_3 (Figs. 5 & 6). Thus, it was obvious from the result that GA_3 increased the shootlets length as compared to the control. The cause for the elongation of shootlets possibly due to the impact of GA_3 on the flexibility of the cell wall which promotes cell elongation (Saheem *et al*., 2008). Moreover, GA_3 converts various carbohydrates into simple forms (Al-Taha *et al*., 2011). The outcomes of the current study are compatible with Khierallah & Bader, (2006) and Saheem *et al*., (2008), where, elongation of shootlets derived from adventitious buds of the date palm, required the addition of GA_3 to the culture media. Contrary to the findings of our study, Al-Taha *et al*., (2011) stated that the medium supplied with GA_3 at 0.5 mg/L and NAA at 0.1 mg/L for 6 weeks stimulated the elongation of plantlets with broad leaves. However, Mazri, (2012) and Fki *et al*., (2011b) pointed out that using a liquid medium (MS) free from PGRs overcome the slow growth in date palm.

Table 2. Effect of different concentrations of PGRs on multiplication of indirect adventitious buds of date palm cvs. Sagai and Khalas.

Multiplication buds mean ± SE						
Con. NAA (A)	**Cultivar**	**Con. 2iP (B)**				
		Average A	**4 mg**	**2 mg**	**1 mg**	**0.0 mg**
0.0 mg	Sagai	4.00 a	5.75 ±.478 cd	6.00 ± .707 cde	3.25 ± .478 b	1.00 ± .408 a*
	Khalas	1.93 ± .67 a	1.75 ± .742	3.75 ± .467	2.25 ± .478	0.00 ± .00
0.1 mg	Sagai	9.31 b	11.50 ±.645 j	8.75 ± .629 fgh	11.50 ± .288 ij	5.50 ± .645 cd
	Khalas	4.43 ± .37 b	3.25 ± .471	6.00 ± .652	6.25 ± .853	2.25 ± .624
0.5 mg	Sagai	9.87 b	12.75 ±.478 j	10.50 ±.645 hi	9.75 ± .478 ghi	6.50 ± .2886 de
	Khalas	6.37 ± .07c	5.75 ± .623	6.75 ± .47	8.00 ± .816	5.00 ± .566
1 mg	Sagai	7.18 c	8.00 ±.531 efg	9.25 ± .853 fgh	7.50 ± .645 ef	4.00 ± .408 bc
	Khalas	3.87 ± .48 b	4.50 ± .468	5.25 ± .470	3.75 ± .750	2.00 ± .408
Average B	Sagai		9.50 c	8.62 bc	8.00 b	4.25 a
	Khalas		3.81 ± .44 b	5.43 ± .34 c	5.06 ± .52 c	2.31 ± .47 a*

**Data that have the same alphabets are not significantly different, according to the test of Duncan at level (p<0.05)

Table 3. Effect of different concentrations of GA_3 on shootlets length of date palm cvs. Khalas and Sagai.

Con. GA_3 mg/L (B)	Means ± SE. (cv. Khalas) (A)	Means ± SE. (cv. Sagai) (A)	Average (B)
0.1 mg	3.64 ± .404	4.07 ± .446	3.85 ab
1 mg	4.42 ± .260	4.36 ± .260	4.39 b
2 mg	5.09 ± .169	5.00 ± .242	5.04 c
3 mg	5.43 ± .318	5.07 ± .212	5.25 c
5 mg	6.35 ± .294	5.97 ± .366	6.16 d
MS	3.70 ± .182	3.37 ± .234	3.53 a
Average (A)	4.77 a*	4.64 a	

*Data have the same alphabets are not significantly different, according to the test of Duncan at level (p< 0.05)

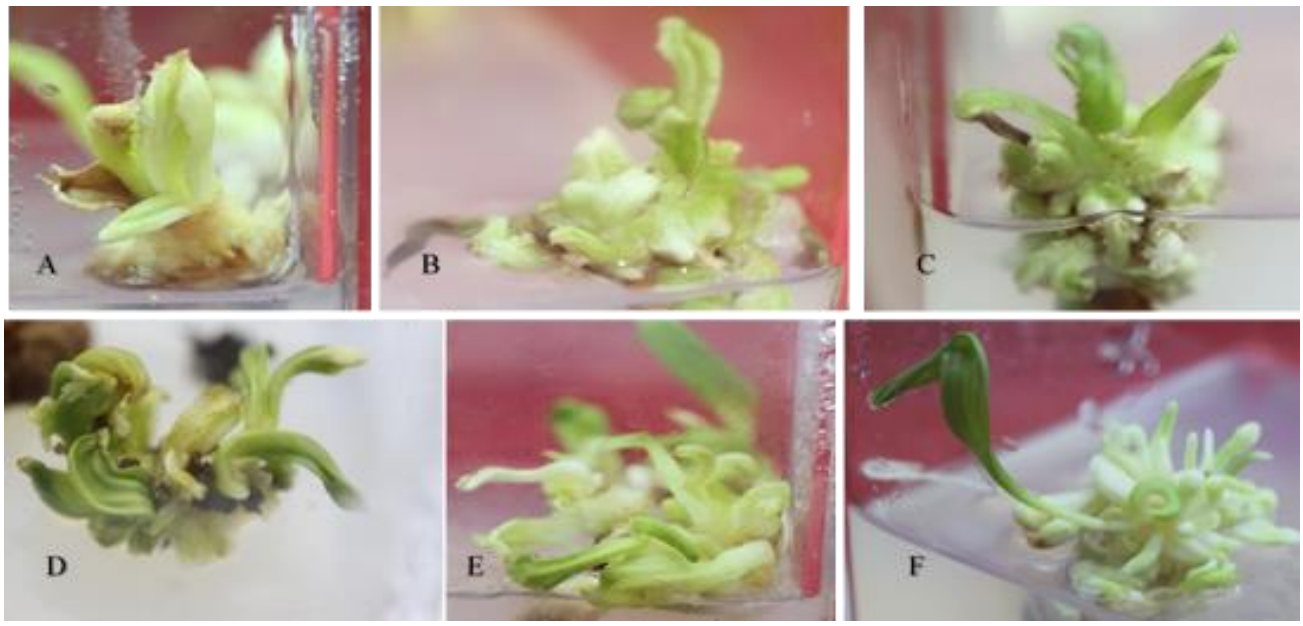

Fig. 3. Multiplication of adventitious buds (cv. Khalas); (a) On modified MS medium supplemented with 2 mg 2iP/L + 0.0 mg NAA/L. (b) On modified MS medium supplemented with 1 mg 2iP/L + 1 mg NAA/L. (c) On modified MS medium supplemented with 4 mg 2iP/L + 1 mg NAA/L. (d) On modified MS medium supplemented with 1 mg 2iP/L + 1mg NAA/L. (e) On modified MS medium supplemented with 2 mg 2iP/L + 0.5 mg NAA/L. (f) On modified MS medium supplemented with 1 mg 2iP/L+ 0.5 mg NAA/L.

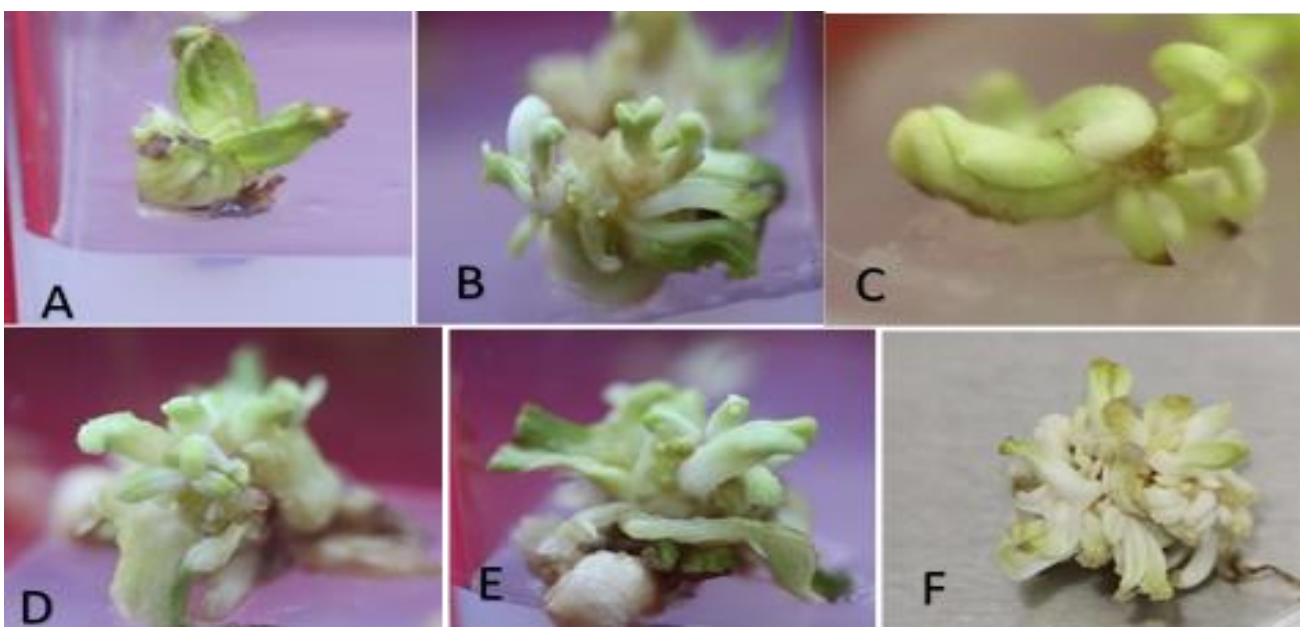

Fig. 4. Multiplication of adventitious buds (cv. Sagai); (a) On modified MS medium supplemented with 1 mg 2iP/L + 0.0 mg NAA/L. (b) On modified MS medium supplemented with 1 mg 2iP/L + 1 mg NAA/L. (c) On modified MS medium supplemented with 0.0 mg 2iP/L + 1 mg NAA/L. (d) On modified MS medium supplemented with 1 mg 2iP/L + 0.5 mg NAA/L. (e) On modified MS medium supplemented with 2 mg 2iP/L + 0.1 mg NAA/L. (f) On modified MS medium supplemented with 4 mg 2iP/L + 0.5 mg NAA/L.

Effect of various auxin concentrations on the root number and length of plantlets derived from adventitious buds of date palm: The protocol for any plant micropropagation needs successful and effective rooting (AL-Qurainy *et al*., 2015). Therefore, individual shootlet was subjected to different auxins concentration for the enhancement of rooting. The variations were observed non-significantly in root number or root length between Khalas and Sagai (Tables 4 & 5). However, Sagai gave a higher root number (2.24) as compared to Khalas (2.11), whereas Khalas gave higher root length (2.78) as compared to Sagai (2.43). These differences in root number and root length between cultivars might be due to the differences in genotype. Al-Khateeb *et al*., (2006) pointed out that rooting varies widely and depending on genotypes and culture protocols. Our result is consistent with Al- Taha *et al*., (2011), who reported that the cultivar Sharifi was superior over others including Khsab, Um Al-Dihin and Auwaidy in the average length and number of formed roots. Furthermore, Al Khateeb and Alturki, (2014) stated that a variation was found among cultivars in terms of number of roots as well as in root length *In vitro* in date palm cvs. Sukry and Reziz.

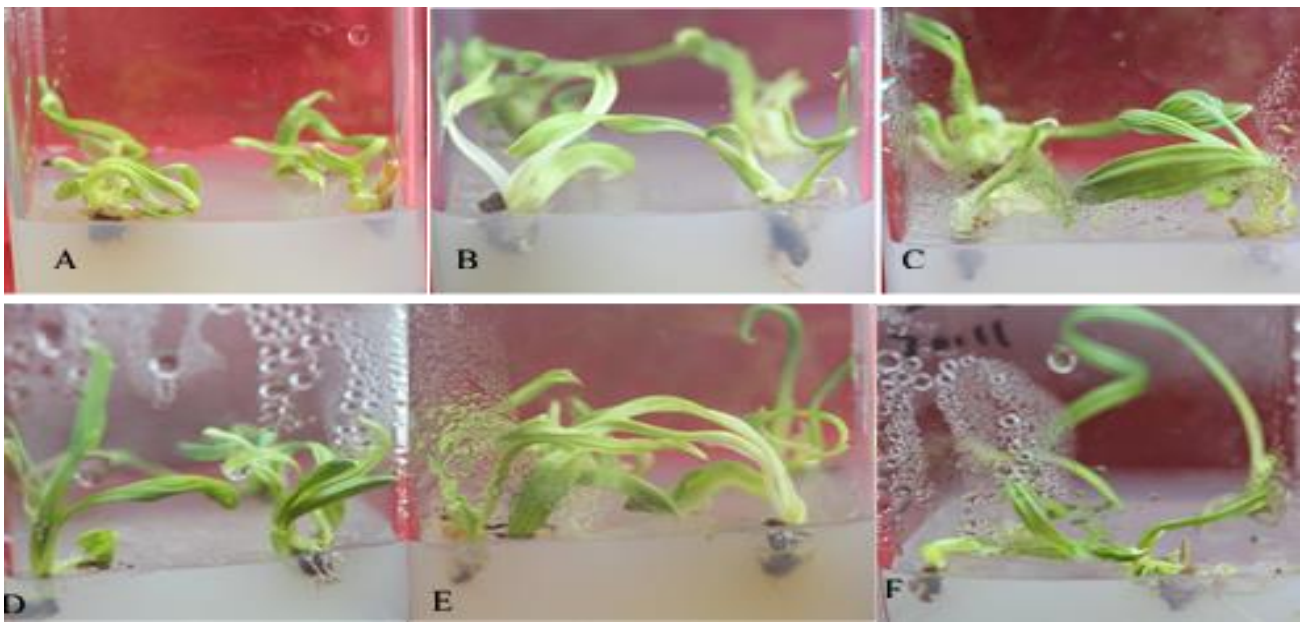

Fig. 5. Shootlets elongation (cv. Khalas); (a) on modified MS medium free of PGRs (control). (b) On modified MS medium supplemented with 0.1 mg GA_3/L. (c) On modified MS medium supplemented with 1 mg GA_3/L. (d) On modified MS medium supplemented with 2 mg GA_3/L. (e) On modified MS medium supplemented with 3 mg GA_3/L. (f) On modified MS medium supplemented with 5 mg GA_3/L.

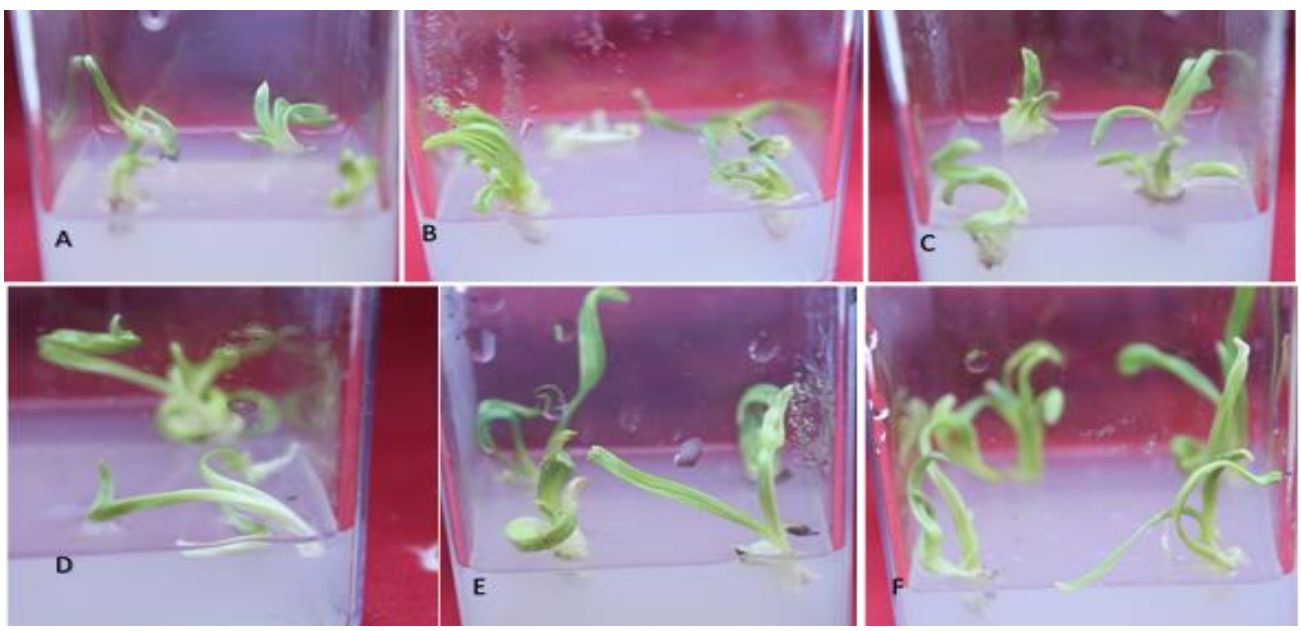

Fig. 6. Shootlets elongation (cv. Sagai); (a) On modified MS medium free of PGRs (control). (b) On modified MS medium supplemented with 0.1 mg GA3/L. (c) On modified MS medium supplemented with 1 mg GA3/L. (d) On modified MS medium supplemented with 2 mg GA3/L. (e) On modified MS medium supplemented with 3 mg GA3/L. (f) On modified MS medium supplemented with 5 mg GA3/L.

In addition, the non-significant effect had been seen between the effect of different PGRs including, IBA, IAA and NAA (regardless of the effect of different concentration) on root number formed from *In vitro* cultured shootlets. However, different concentrations of PGRs (IBA, IAA, and NAA) showed a significant impact on root length in these cultivars Tables (4 & 5) and Figs. (7 & 8). These results are in disagreement with Bekheet, (2013) who mentioned that the NAA was more effective than IAA or IBA at the equal concentration in root number formation *In vitro* of cv. Zaghlool. Furthermore, Saheem, (2008) reported that the auxin NAA was important for rooting induction in shootlets that produced from adventitious buds and 0.5mg/L of NAA gave the higher number of roots and length compared with the equal concentration of IBA. Different responses have been observed in rooting of date palm and it was depending on concentration and type of auxins (El-Sharabasy *et al*., 2001).

Table 4. Effect of various concentrations (0.5, 1 and 1.5 mg/L) of IBA, IAA, and NAA on formed roots number (mean ± SE) of cultivars Khalas and Sagai.

Type and Con. mg/L (PGRs)	Roots/plantlet cv. Sagai	Roots/ plantlet cv. Khalas	Average of roots/ plantlet (A)	Average of roots/ plantlet (A)
IB 0.5	1.28 ± .18	1.57 ± .20	1.42 ab	
IB 1	1.57 ± .48	1.85 ± .26	1.71 ab	2.23 b
IB 1.5	3.85 ± .26	3.28 ± .28	3.57 c	
IAA 0.5	2.71 ±.71	2.14 ± .40	2.42 b	
IAA 1	1.42 ± .42	2.71 ± .52	2.07 b	2.07 b
IAA 1.5	1.85 ± .63	1.57 ± .36	1.71 ab	
NAA 0.5	4.14 ±.40	3.14 ± .26	3.64 c	
NAA 1	2.71 ±.77	2.14 ± .45	2.42 b	2.66 b
NAA 1.5	2.00 ± .75	1.85 ± .50	1.92 b	
MS	0.82 ±.26	0.88 ± 26	.85 a	.85 a
Average (B)	2.24 a*	2.11 a		

*Data that have the same alphabets are not significantly different, according to the test of Duncan at level (p 0.05)

Table 5. Effect of different concentrations (0.5, 1 and 1.5 mg/L) of PGRs (IBA, IAA, and NAA) on the length of roots formed (mean ± SE) of date palm (cvs. Khalas and Sagai).

Type and Con. mg/L (PGRs) (A)	Root length (cm) cv. Sagai (B)	Root length (cm) cv. Khalas (B)	Average of root length (A)	Average of root length (A)
IB 0.5	2.64 ± .678	2.91 ± .67	2.77 cde	
IB 1	2.91 ± .085	3.44 ± .16	3.17 de	3.12 c
IB 1.5	3.25 ± .392	3.58 ± .34	3.42 de	
IAA 0.5	1.21 ± .315	1.41 ± .38	1.31 a	
IAA 1	2.28 ± .420	2.67 ± .50	2.47 abcd	2.54 ab
IAA 1.5	3.57 ± .349	4.10 ± .60	3.83 e	
NAA 0.5	3.15 ± .349	3.22 ± .26	3.19 de	
NAA 1	2.20 ± .400	2.84 ± .50	2.52 bcd	2.39 ab
NAA 1.5	1.14 ± .196	1.78 ± .33	1.46 ab	
MS	1.92 ± .579	1.90 ± .57	1.91 abc	1.91 a
Average (B)	2.43 a	2.78a		

*Data that have the same alphabets are not significantly different, according to the test of Duncan at level (p0.05)

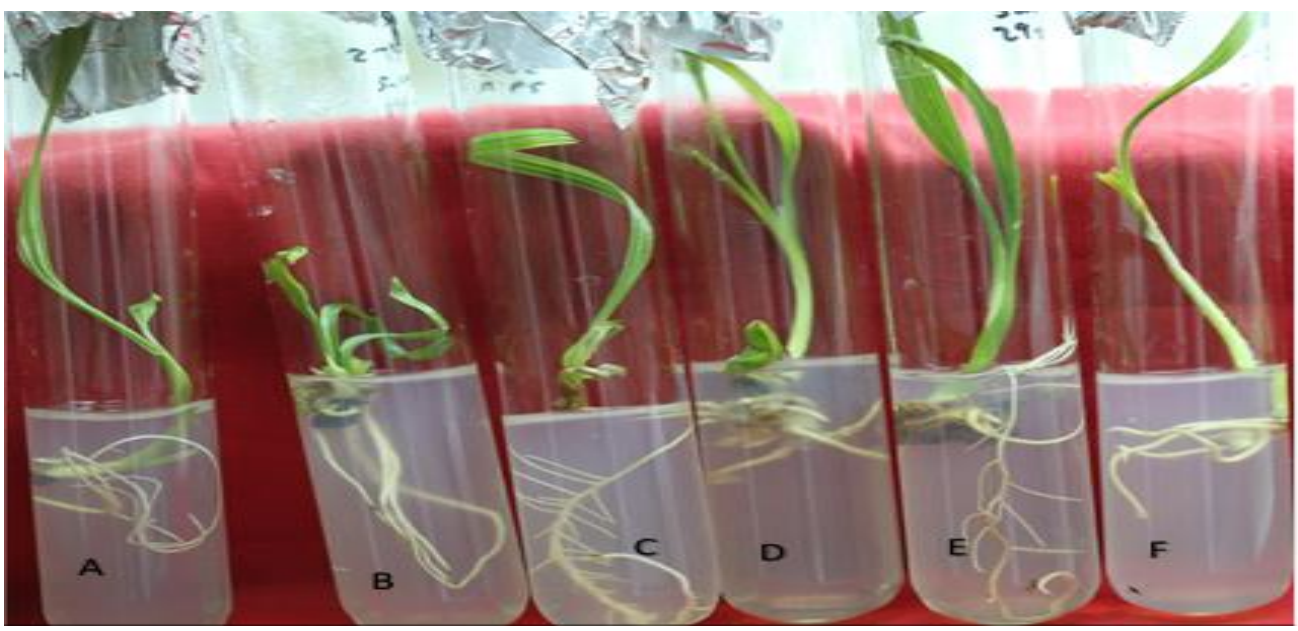

Fig. 7. Rooted plantlets obtained from adventitious buds (cv. Sagai); (a) On modified MS medium supplemented with 0.5 mg IBA/l. (b) On modified MS medium supplemented with 0.5 mg NAA/l. (c) On modified MS medium free of PGRs. (d) On modified MS medium supplemented with 1.5 mg IAA/l. (e) On modified MS medium supplemented with 1.5 mg IBA/l. (f) On modified MS medium supplemented with 1 mg NAA/l.

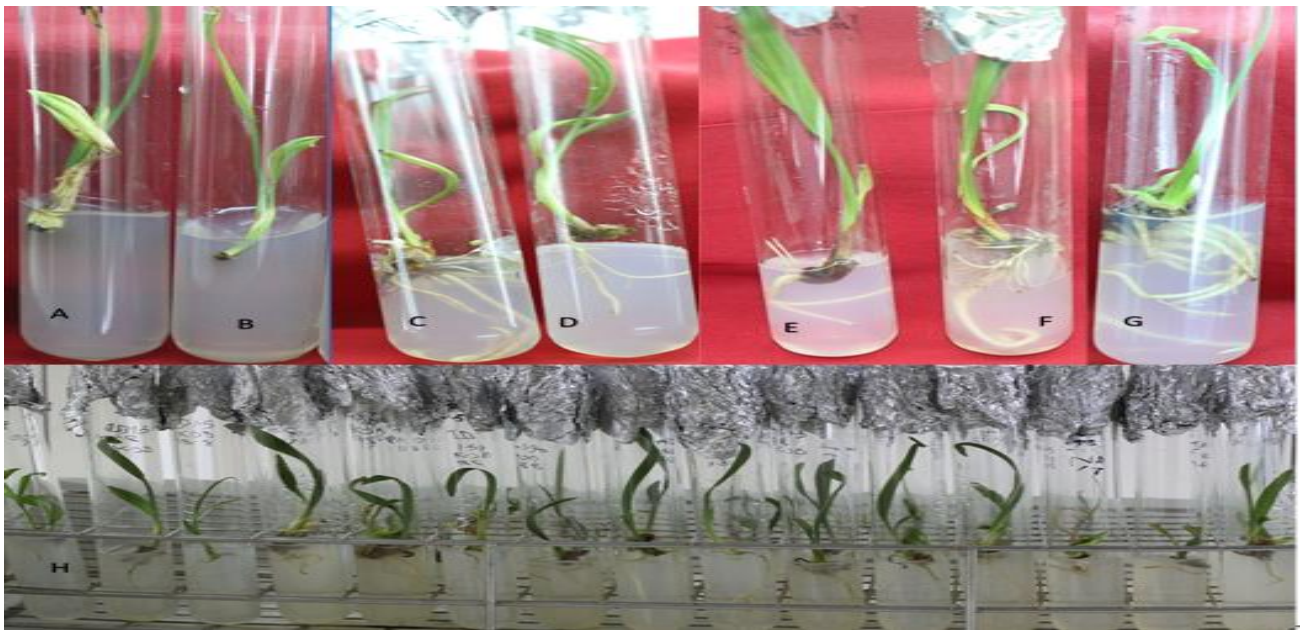

Fig. 8. Rooted plantlets obtained from adventitious buds (cv. Khalas); (a), (b) On modified MS medium free of PGRs. (c) On modified MS medium supplemented with 1.5 mg IBA/L. (d) On modified MS medium supplemented with 0.5 mg NAA/L. (e) On modified MS medium supplemented with 1 mg NAA/L. (f) On modified MS medium supplemented with 1 mg IBA/L. (g) On modified MS medium supplemented with 1.5 mg IAA/L. (h) plantlets in rooting stage in room culture.

However, the largest root number (2.66) was obtained with NAA, at 0.5 mg/L (3.64 roots), whereas, the highest length of root (3.12 cm) was obtained with IBA at (1.5 mg/L) which gave the largest root length (3.42 cm) Tables (4 & 5). This result illustrated the role of NAA in plant rooting in tissue culture, which gave the highest rate of root formation per plantlet as compared to the same concentrations of IBA and IAA was used. Saheem *et al*. NAA and 2iP (Table 2). Whereas, 0.1 mg/L NAA gave the longest roots. Bekheet, (2013) reported that 1 mg/L NAA gave the best root formation *In vitro* as compared to IBA and IAA at same concentration, in cv. Zaghlool. Al-Taha *et al*., (2011) stated that MS medium supplimented with 0.5 mg/L NAA with 1 g/L activated charcoal gave the highest rate of root numbers and lengths in cv. Sheraify. Furthermore, Sidky *et al*., (2007) stated that plantlets cultivated on half strength of medium with 0.1 mg/L of NAA, 4 mg/L of paclobutrazol 40 or 50 g/L of sucrose and 1 g/L of activated charcoal, induced the formation of roots and also accelerated the formation of secondary roots. Mazri, (2012) pointed out the positive effect of IBA on rooting of date palm cv. Najda. Fki *et al*., (2011a) stated the presence of a significant positive correlation between rooting frequencies and IBA concentrations in *Phoenix dactylifera* L. cv. Barhee. However, Al-Qurainy *et al*., (2015) reported that rooting induction was achieved and improved when shoots of *Tamarix nilotica* were cultivated on medium supplied with 100 µM IBA for a brief time 5, 10 & 15 days. Furthermore, when shoots were transferred to a medium free of PGRs for one month duration, the percentages of rooting were found to be 50-86 %.

Conclusion

The micropropagation protocols were developed for two potential date palm cultivars viz., Sagai and Khalas through indirect organogenesis. The response between both cultivars was found to be different at various stages of micropropagation. However, in most cases, the cultivar Sagai was found to be superior over Khalas during micropropagation.

Acknowledgement

Researchers supporting project number (RSP2019/73), King Saud University Riyadh, Saudi Arabia.

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(Received for publication 18 March 2019)