

Optimizing Micropropagation Protocol for *Rosa hybrida* cv. Eiffel Tower with Improved *in vitro* Rooting Ability

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IN VITRO propagation techniques were studied to establish a protocol for rapid and mass production of *Rosa* spp. cv. Eiffel Tower, an important and endangered cultivar. Several experiments were conducted through a two-stage procedure: multiplication stage and rooting of microshoots stage. For multiplication, the best results were obtained when nodal segments were cultured on MS medium supplemented with BAP at 1mg/l and/or Kinetin at 0.5 mg/l. The highest shoots number (3.17 shoots/explant) was obtained when 0.5 mg/l BAP was added to the medium. Kinetin at 0.5 mg/l produced the highest shoot number/explant (3.67 shoots), and shoot length (3.67 cm). The interaction between BAP at 1 mg/l plus kinetin at 0.5 mg/l gave the highest shoot number/explant (4 shoots). For rooting, the best interaction effect on rooting percentage (61.00%), highest number of roots/shoot (5.12) and root length (3.33 cm) were observed when the shoots were cultured on hormone-free MS medium and pH level was adjusted to 5.5. Moreover, adjusting the medium pH to 5.5 formed roots faster in 17.67 days followed by medium with pH level 4.5 (18 days). The medium with pH at 5.5 supplemented with 50 g/l sucrose produced the highest significant value of rooting (85%) and enhanced root formation in 7.5 days. The rooted plantlets were transplanted into the potting substrate and successfully acclimatized in the laboratory then transferred to the field with a survival rate of 55%. After 6 month, the plantlets reached ~ 22 cm in length and 5.50 leaves/plant

Keywords: Rose, MS medium pH, Nodal segments, Sucrose, Kinetin, BAP

Introduction

The genus *Rosa* belongs to family Rosaceae and includes more than 100 species and thousands of cultivars of economical and commercial perennial shrubs and climbers. Of the several major classes belonging to garden roses, hybrid tea is the best-known and most-popular class. It is the source of the majority of rose cultivars grown in greenhouses, gardens and sold in the florist market, which are known for their beauty and mostly fragrant flowers. One of the most popular and preferable cultivars of hybrid tea roses in the Egyptian market is 'Eiffel Tower'. This cultivar was obtained via hybridization by David Armstrong and Herbert Swim. It was registered and introduced in 1963 (Rose Society, 2012). It is characterized by its tolerance to hot and dry Mediterranean climate in addition to its fragrant pink flowers which appear throughout the entire growing season.

Like many other rose cultivars, 'Eiffel Tower' is propagated by vegetative means (grafting and stem cuttings) even though it does not ensure mass production of healthy and disease-free plants (Kroin, 2016 and Manners, 2014). Different species are commercially propagated using tissue culture techniques. Micropropagation is a fast propagation method and offers a potential to deliver enormous quantities of true-to type and disease-free plants within a short span of time (Brunda et al., 2016). So, tissue culture can be used as substitute to traditional production methods.

Since the first report of Hasegawa (1979) on micropropagation of *Rosa hybrida* L. by proliferation of axillary buds, different methodologies have been applied for *in vitro* propagation of many species and cultivars of roses (Ibrahim & Debergh, 2001, Schum et al., 2001, Pati et al., 2006, Kumud et al., 2015, Uzunova,

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2015, Ambros *et al.*, 2016 and Kumari *et al.*, 2017) and many commercial laboratories have been established all over the world for mass production of such important ornamental cut flowers to meet the market demand. The success of micropropagation involves several factors viz., the composition and type of the culture medium (Tawfik *et al.*, 1992 and Bagheri *et al.*, 2015), culture environment (Schum *et al.*, 2001 and Tawfik and Mohamed, 2005), genotype (Tawfik & Mohamed, 2006 and Hamama *et al.*, 2015), explant type (Tawfik & Noga, 2001 and Maurya *et al.*, 2013).

In plant micropropagation, type and concentration of plant growth regulators play a major role in cell division, differentiation and morphogenesis (Tawfik, 1995 and 1997). In addition, the successful regeneration of shoots and their subsequent rooting is essential for the commercial exploitation of micropropagation technology (McGaw & Burch, 1995 and Mahmood & Hauser, 2015).

Nevertheless, there is scarce available information about the use of micropropagation technique for *Rosa hybrida* cv. Eiffel Tower. Thus the aim of this study is to encourage the expansion of cultivation of such cultivar and the production of high quantities of genetically homogenous plants by tissue culture techniques through the establishment of an efficient protocol for *in vitro* propagation of such important and cultivar with special emphasis on enhancing *in vitro* rooting ability.

Materials and Methods

Plant material

Young and healthy shoots of *Rosa hybrida* cv. Eiffel Tower were collected from mature shrubs growing in the farm of Floriculture and Medicinal plants, Faculty of Agriculture, Assiut University during 2017 and 2018 seasons. The subterminal part of the shoots (10-15 cm in length) were defoliated and used to prepare the explants as single nodes (1.5-2 cm).

Culture establishment

The excised explants were thoroughly washed with running tap water, then an experiment was carried out to investigate the effect of disinfection with various concentrations and duration of commercial bleach ~ 5% sodium hypochlorite (data are not shown). As a result of a preliminary trial 100% of the commercial bleach for 10 minutes

was applied. The containers which contained the explants and the bleach were thoroughly shaken. Then the explants were rinsed 4 times with sterile distilled water. After the surface disinfection, the explants were cultured into 350 ml sterilized glass jars, containing 50 ml of assigned medium MS nutrient medium (Murashig and Skoog, 1962) was used in all experiments conducted in the course of the current study. The media were solidified with 8 g agar/l, sucrose was added at the rate of 30 g/l and the pH was adjusted to 5.8 ± 0.1 in all experiments except where different levels of pH and sucrose were investigated. All the tested media were autoclaved at 121°C and 1.2 kg/cm² for 20 min. All the cultured jars were incubated in a plant growth room at 25 ± 2 °C under constant fluorescent light of 1500 Lux for 16/8 h (light/dark) photoperiod.

Multiplication stage experiments

An experiment was conducted to study the effect of different plant growth regulators on proliferation of the *Rosa hybrida* cv. Eiffel Tower explants transferred from 4-week old culture on the same medium from the previous stage. The experiment was conducted to test the effect of 6-Benzylaminopurine (BAP) at different concentrations (0.0, 0.5, 1.0 and 2 mg/l) and Kinetin at 0.0 and 0.5 mg/l on proliferation ability. The experiment was designed as a factorial experiment in a randomized complete block design (RCBD) with 3 replicates and 15 jars for each treatment each jar containing 2 explants. Data were measured after 4 weeks on shoot proliferation, shoot number per explant, shoot length (cm), shoot fresh weight (g) and shoot dry weight (g).

Rooting stage

The main goal at this stage was to enhance the micro-shoots (from the multiplication stage cultured on the same medium) to induce roots and obtain complete plantlets to be hardened and transferred to the field with a minimum loss. So, shoots (2-3 cm in length) were transferred to basal MS medium for root induction. Three experiments were carried out. In the first experiment, MS medium was used with 5 levels of pH (4.0, 4.5, 5.0, 5.5 and 5.8) supplemented with NAA at different concentrations (0.0, 0.1 and 0.2 mg/l). It was designed as a factorial (5x3) in a completely randomized design (CRD) with 3 replicates and 15 jars for each treatment. The second experiment was designed to test the effect of different concentrations of sucrose (0.0, 30, 40

and 50 g/l) on rooting of *Rosa hybrida* cv. Eiffel Tower. Half-strength MS medium was solidified with 8 g/l agar (w/v) and the pH of the medium was adjusted to 5 ± 0.01 . A completely randomized design (CRD) was used with 3 replicates and 15 jars for each treatment. In the third experiment, the effect of MS medium pH (5 and 5.5) and sucrose concentrations (30, 40 and 50 g/l) on rooting was studied. This experiment was designed as a factorial experiment in randomized complete block design with 3 replicates and 15 jars for each treatment. Data were recorded starting from the second week after culturing and followed up until the fourth week on rooting%, roots number per shoot, root length (cm) and number of days to form roots.

Acclimatization stage

Healthy and well rooted plantlets of *Rosa* 'Eiffel Tower' obtained from the previous stage were selected and taken away from jars, then their roots were thoroughly washed with tap water to eliminate the agar. These plantlets were transferred to plastic pots filled with peat moss and perlite 1:1 (v/v). The pots were covered with clear polyethylene bags to maintain high relative humidity and then kept in the growth room for ~ 3 weeks. The plants were watered with tap water each 2 days. Then the polyethylene bags were removed and the plants were transferred to the green house. Data were recorded on survival percentage, shoot length and leaves number per shoot.

Statistical analysis

Data of all experiments were subjected to analysis of variance (ANOVA) and mean comparison were performed using the least significant difference (LSD) methods with a significant level of 5% according to (Gomez and Gomez, 1984)

Results and Discussion

Multiplication stage

Preliminary trials were conducted to determine the most proper concentration of both BAP and Kin for shoot multiplication (data not shown). It was found that BAP at 0.5 and 1 mg/l and Kin at 0.5 mg/l showed the best proliferation rate of *in vitro* grown explants of the cultivar 'Eiffel Tower'. Both cytokinin types were involved together in order to study their solitary and combined effect.

As shown in Tables 1 and 2 and Fig.1, the interaction effect of kinetin and BAP on all the growth measurements instigated significant

differences. Shoot multiplication and their fresh and dry weights of shoots were significantly enhanced when nodal segments were cultured on MS medium supplemented with BAP at 1 mg/l plus Kin at 0.5 mg/l. The same combined treatment gave the highest shoot number per explant (4 shoots) followed by the medium supplemented with Kin and BAP both at 0.5 mg/l which produced 3.67 shoots per explant. These results are consistent with the previous observations reported by Kanchanapoom et al. (2009), Hegde et al. (2011) and Kharde & Kshirsagar (2014). MS medium supplemented with Kin at 0.5 mg/l and BAP at 1 mg/l gave the highest fresh and dry weights of shoots (0.35 g and 0.08 g respectively). Tawfik (2002), has shown an increased efficacy of medium supplemented with both BAP and TDZ (thidiazuron, a cytokinin-like substance) in regeneration of *Salvia officinalis*. The stimulation effect has been attributed to possible complementary action. However, both BAP and Kin decreased the shoot length (Tables 1 and 2). These results could be interpreted according to the general fact that cytokinins stimulate shoot proliferation and inhibit their elongation. Moreover, strong cytokinins such as BAP depress shoot length by an increase in number of axillary buds (Hameed et al., 2006) where the formation of lateral shoots utilize all the nutrients (Yakimova et al., 2000). Other investigators such as Ahmad et al. (2003) concluded that the high cytokinin concentration causes an increase in the ethylene level in plants which block the basipetal transport of endogenous auxin in the shoots resulting in the minimum shoot length.

Rooting stage

Shoots obtained from the multiplication stage were transferred to MS basal medium for rooting induction.

Effect of medium pH, NAA concentrations and their interaction

It could be inferred from the data presented in Table 3 that the influence of NAA was affected by the medium pH, where no response was observed on a medium with pH 4. While adding NAA at different concentrations to a medium with pH level of 5.8 (the standard level recommended by Murashig and Skoog, 1962) led to the formation of callus. Data presented in Tables 3 and 4 indicate that adding NAA to the rooting medium decreased all the measured growth parameters. This is maybe attributed to the change of the medium pH to 4.5, 5 and 5.5.

Concerning the effect of medium pH on the
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rooting measurements, it is clear that at medium pH 4 no response was observed while at the standard pH level (5.8) recommended for MS medium, callus was formed on the shoot base (Table 3 and 4). Adjusting the medium pH to levels 5 and 5.5 produced the highest root percentage (55.22 and 53.44%), root number (3.20 and 3.48 roots per shoot) and root length (2.08 and 2.40 cm) respectively with no significant differences between these two levels of pH. However, medium with pH 5.5 significantly enhanced forming roots in 21.89 days compared to medium pH at 5 where roots were formed in 25.11 days. Similar results were reported by Martins *et al.* (2011) on *Plantago lmogravensis*, Kovacevic *et al.* (2013) on poplar. Also, Ahmed *et al.* (2014) reported that *Musa* spp. produced the longest roots in the shortest time when the medium pH was adjusted to 5.5. The reasons behind the stimulating effect of the pH levels lower than 5.8 may be attributed to the increased initial uptake of micronutrients at low pH as suggested by Van Winkle and Pullman

(2003) and the increased initial uptake of auxins at low pH according to the results demonstrated by De Klerk *et al.* (2008). Other investigators, such as Shang *et al.* (1991), stated that the effect of the initial pH on the developmental fate of the cell culture indicates that complicated action-reaction processes between the cells and the extracellular pH must occur.

As shown in Tables 3 and 4, the highest rooting percentages (62.67% and 61.00%) were recorded when the shoots were cultured on free-hormone-MS medium and pH level was adjusted to 5 and 5.5, respectively. Moreover, adjusting the medium pH to 5.5 produced the highest number of roots (5.12 roots per shoot) and root length (3.33 cm). Shoots cultured on hormone-free medium with pH adjusted to 5.5 formed roots faster in 17.67 days followed by medium with pH level 4.5 (18 days).

TABLE 1. Effect of Kin and BAP on shoot number/explant and average shoot length during *in vitro* multiplication stage of *Rosa hybrida* cv. Eiffel Tower nodal segments

BAP mg/l	Kin mg/l	Shoot number/explant			Shoot length (cm)		
		0.0	0.5	Mean	0.0	0.5	Mean
0.0		0.97	1.67	1.32	5.40	3.60	4.50
0.5		2.00	3.67	2.84	3.00	3.00	3.00
1.0		2.00	4.00	3.00	2.00	1.67	1.84
Mean		1.66	3.11		3.57	2.76	
LSD 5%							
			0.57			0.17	
			0.69			0.21	
			0.98			0.30	

TABLE 2. Effect of Kin and BAP on shoot fresh and dry weights (g) during *in vitro* multiplication stage of *Rosa hybrida* cv. Eiffel Tower nodal segments

BAP mg/l	Kin mg/l	Fresh weight (g)			Dry weight (g)		
		0.0	0.5	Mean	0.0	0.5	Mean
0.0		0.15	0.12	0.14	0.02	0.02	0.02
0.5		0.24	0.27	0.26	0.05	0.03	0.04
1.0		0.18	0.35	0.27	0.04	0.08	0.06
Mean		0.19	0.25		0.04	0.04	
LSD 0.05							
			0.02			0.01	
			0.02			0.02	
			0.03			0.02	

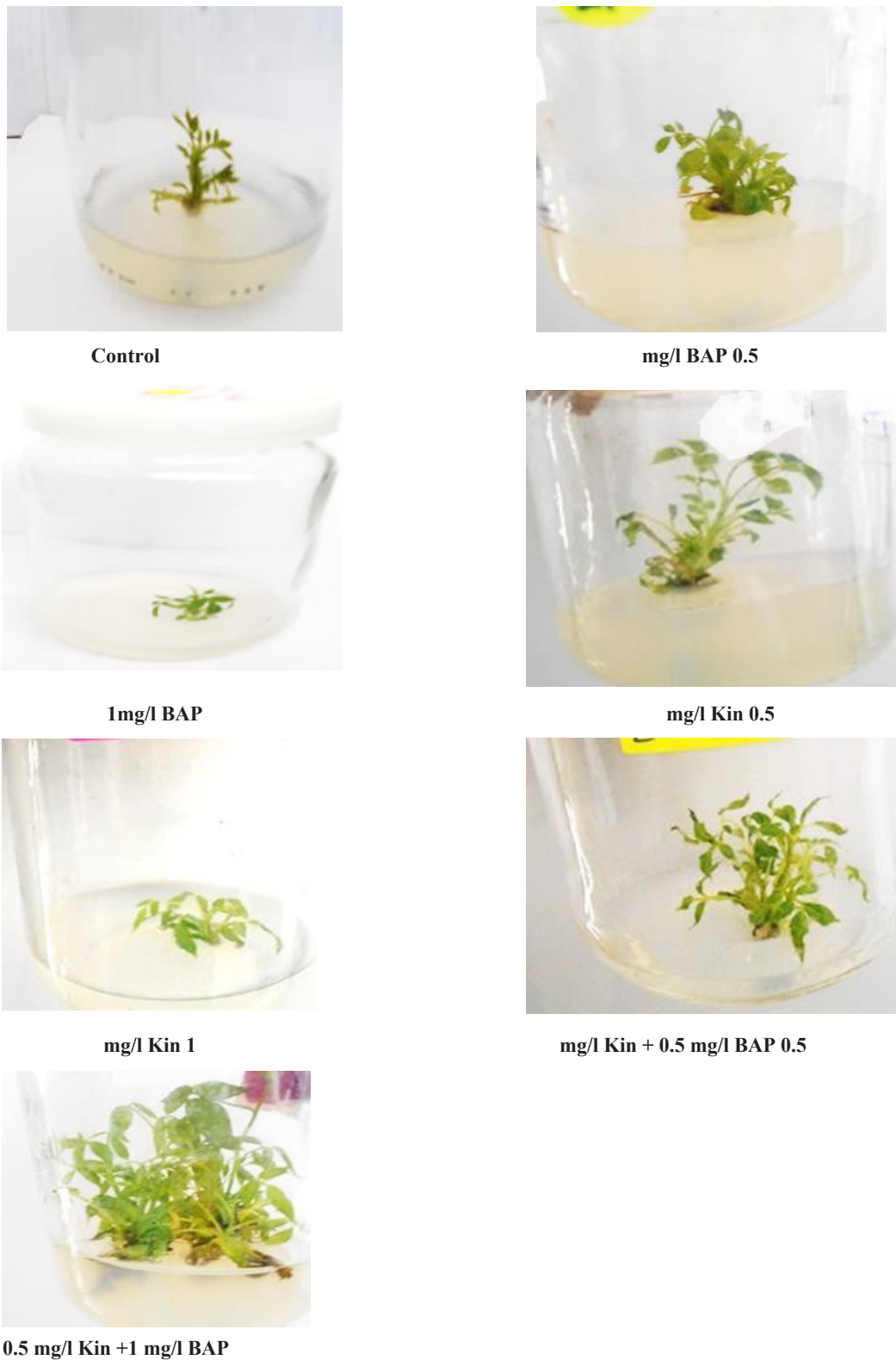


Fig. 1. Effect of interaction between BAP and kinetin at different concentrations on proliferation of *Rosa hybrida* cv. Eiffel Tower nodal segments

TABLE 3. Effect of medium pH and NAA on rooting% and number of roots during *in vitro* rooting stage of *Rosa hybrida*. cv. Eiffel Tower

pH \ NAAmg/l	Rooting%				Number of roots			
	0.0	0.1	0.2	Mean	0.0	0.1	0.2	Mean
4.0	-	-	-	-	-	-	-	-
4.5	42.33	47.33	52.67	47.44	5.33	4.17	2.00	3.83
5.0	62.67	49.00	54.00	55.22	4.33	3.67	1.60	3.20
5.5	61.00	47.33	52.00	53.44	5.12	3.33	2.00	3.48
5.8	Callus	Callus	callus		callus	callus	callus	
Mean	55.33	47.89	52.89		4.93	3.72	1.87	
LSD 0.05								
pH		2.05				0.42		
NAA		2.05				0.47		
pH x NAA		3.55				0.72		

TABLE 4. Effect of medium pH and NAA on root length and number of days to form roots during *in vitro* rooting stage of *Rosa hybrida*. cv. Eiffel Tower

pH \ NAAmg/l	Root length (cm)				Number of days to form roots			
	0.0	0.1	0.2	Mean	0.0	0.1	0.2	Mean
4.0	-	-	-	-	-	-	-	-
4.5	1.33	0.83	0.83	0.99	18.00	22.33	27.67	22.67
5.0	3.33	2.07	0.83	2.08	27.00	27.00	21.33	25.11
5.5	3.33	2.00	1.87	2.40	17.67	20.67	27.33	21.89
5.8	callus	callus	callus		callus	callus	callus	
Mean	2.66	1.63	1.18		20.89	23.33	25.44	
LSD 0.05								
pH		0.35					2.51	
NAA		0.14					2.51	
pH x NAA		0.61					4.35	

Effect of different concentrations of sucrose on rooting behavior

The response of the microshoots of *Rosa hybrida* cv. Eiffel Tower cultured on half-strength MS medium with pH 5 at different concentrations of sucrose is illustrated in Fig. 2. Elimination of sucrose completely from the rooting medium cause a significant reduction in rooting percentage, number of roots and root length, while number of days to form roots significantly increased. Similar results were reported by Tawfik (1995), who stated that thin and weak roots were formed after 35 days on the basal end of *Melaleuca armillaris* explant cultured on sucrose-free MS

medium. Raising the sucrose concentration up to 50 mg/l significantly increased rooting %, root number, root length and, moreover, reduced the time to form roots. It is obvious that 82.33% of cultures formed roots in 7.33 days when 50 g sucrose/l was added to the medium comparing with the medium containing 30 g sucrose/l which had rooting percentage (62.33 %) formed within 14.67 days. Similar results were found by Hasan *et al.* (2015) and Gabryszewska (2015) who reported that using 50 g/l sucrose strongly simulated the number of roots per shoot (5.8-6.0 roots) in *Helleborus niger*.

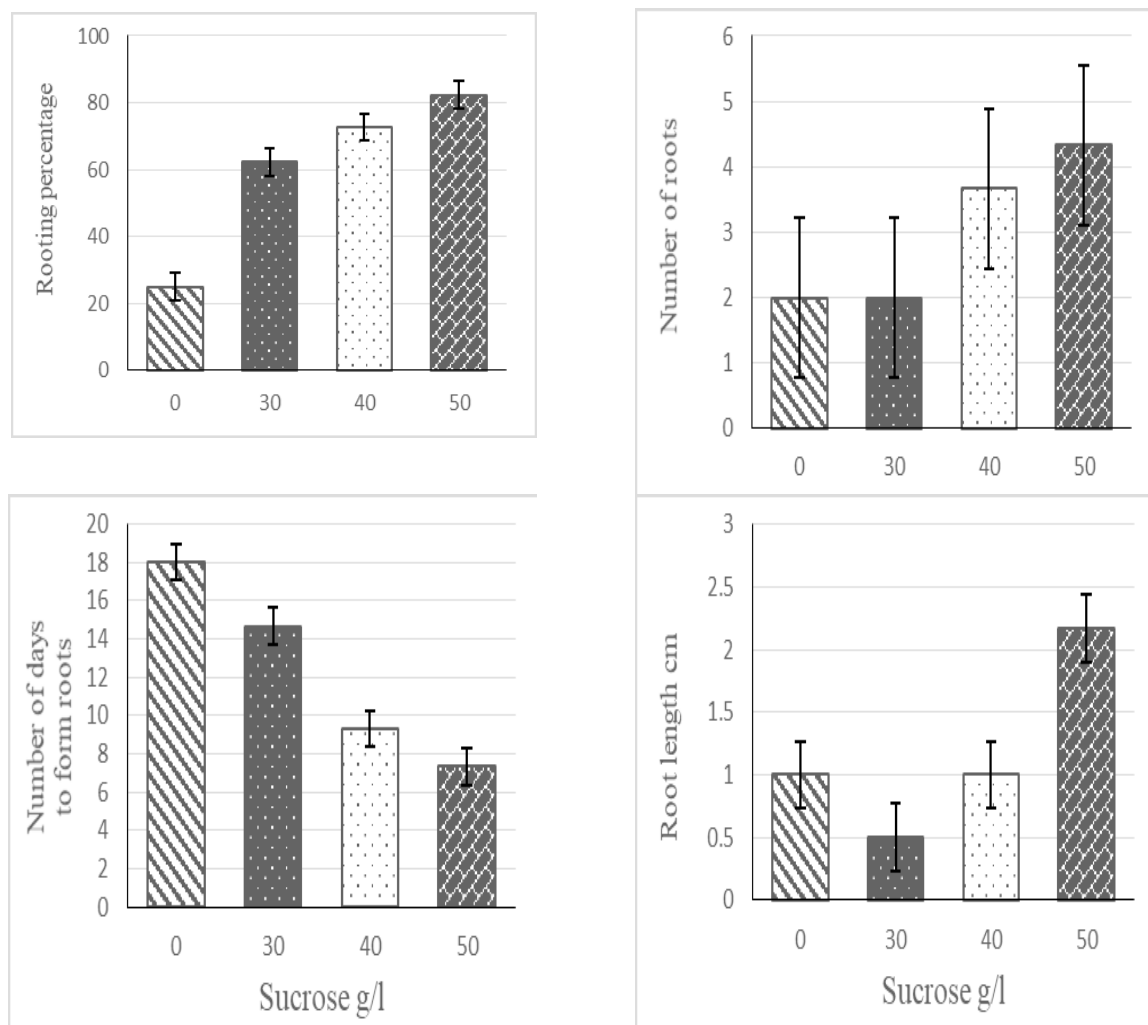


Fig. 2. Effect of sucrose concentrations on rooting behavior of *Rosa hybrida* cv. Eiffel Tower shoots. Vertical bars indicate LSD at $P = 0.05$

Effect of medium pH and sucrose concentrations on rooting behavior

This experiment was conducted to test the effect of pH medium at 5 and 5.5 and different concentrations of sucrose (30, 40 and 50 mg/l) as well as their interactions on rooting percentage, root number, root length and number of days to form roots of *Rosa* cv. Eiffel Tower shoots. The results are shown in Tables 5 and 6.

Data in Tables 5 and 6 indicate that culturing the shoots on a medium at a pH level of 5.5 significantly increased the rooting percentage up to 72 % and accelerated root formation (11.50 days). No significant effect of pH medium was observed on root length, nevertheless, medium pH at 5.5 reduced the root number to 4.61 roots per shoot in comparison to shoots cultured on pH medium of 5 which produced 6 roots per shoots.

The obtained data in Tables 5 and 6 indicate that increasing sucrose concentrations up to 50 g/l significantly increased rooting percentage (80%), root length (1.92 cm) and enhanced the root formation in 7 days compared to medium supplemented with 30 g/l (16.17 days). No significant effect of sucrose was observed on number of roots.

It appears from the data presented in Tables 5 and 6 and Fig. 3 that the medium with pH 5.5 supplemented with 50 g/l sucrose produced the highest significant value of root percentage (85%) and enhanced root formation in 7.5 days. No significant interaction effects were observed on root number and root length.

Acclimatization and field establishment

Well-developed *in vitro*-rooted healthy plantlets with 1-2 leaves were successfully acclimatized according to the protocol followed under the conditions of the current experiment. After the plantlets were transferred to plastic pots containing mixture of peat: perlite (1:1 v/v), they were kept in a growth chamber under 16/8 h. photoperiod, at a temperature of

20/25°C (night/day) and 80% humidity. After one month, the survival rate of 55% was recorded. Survived plantlets were placed under plastic greenhouse conditions (25 °C and 80 % RH) for acclimatization. After 6 month, the plantlets with shoot length ~ 22 cm and 5.50 leaves/shoot, on average, were transferred to big plastic pots containing clay and kept outside the green house (Fig. 4).

TABLE 5. Effect of medium pH and sucrose on rooting% and number of roots during *in vitro* rooting stage of *Rosa hybrida*. cv. Eiffel Tower

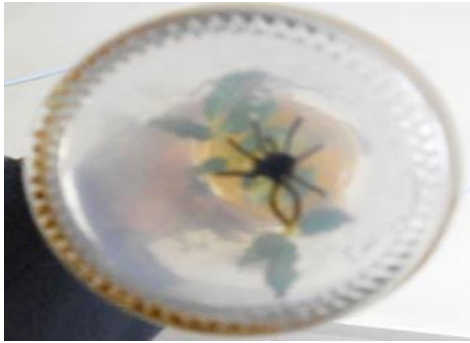
sucrose g/l	pH	Rooting %			Number of roots		
		5	5.5	Mean	5	5.5	Mean
30		62.00	61.00	61.50	7.33	5.00	6.17
40		65.00	70.00	67.50	5.67	3.67	4.67
50		75.00	85.00	80.00	5.00	5.17	5.09
Mean		67.33	72.00		6.00	4.61	
LSD 0.05							
	pH		1.18			1.07	
	Sucrose		1.44			N.S*	
	pH x sucrose		2.04			N.S	

* NS= not significant at $p < 0.05$ by LSD

TABLE 6. Effect of medium pH and sucrose on root length and number of days to form roots during *in vitro* rooting stage of *Rosa hybrida*. cv. Eiffel Tower

sucrose g/l	pH	Root length (cm)			Number of days to form roots		
		5	5.5	Mean	5	5.5	Mean
30		1.00	1.00	1.00	17.33	15.00	16.17
40		1.50	1.33	1.42	12.33	12.00	12.17
50		2.00	1.83	1.92	6.50	7.50	7.00
Mean		1.50	1.39		12.05	11.50	
LSD 0.05							
	pH		NS*			0.68	
	Sucrose		0.19			0.48	
	pH x sucrose		NS			1.18	

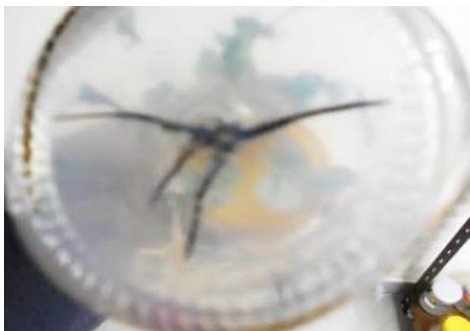
* NS= not significant at $p < 0.05$ by LSD



30 g/l sucrose and medium pH 5.5



40 g/l sucrose and medium pH 5



50 g/l sucrose and medium pH 5



50 g/l sucrose and medium pH 5.5



Fig. 3. Effect of interaction between pH and sucrose at different concentrations on rooting behavior of *Rosa hybrida* cv. Eiffel Tower shoots



Fig. 4. Acclimatization of in vitro-derived plantlets of *Rosa hybrida* cv. Eiffel Tower

Conclusion

Nodal segments of *Rosa* spp. cv. Eiffel Tower were successfully established and multiplied on MS medium supplemented with BAP at 1 mg/l plus kinetin at 0.5 mg/l. High rooting percentage and fast rooting were obtained through adjusting auxin concentration, sucrose and medium pH value. Rooted shoots were successfully acclimatized.

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Conflicts of interest: the authors declare that there are no conflicts of interest related to the publication of this study

References

- Ahmad, T., Hafeez-ur-Rahman, Ahmed, C.M.S. and Laghari, M.H. (2003) Effect of culture media and growth regulators on micro-propagation of peach rootstock GF 677. *Pak. J. Bot.*, **35**, 331-338.

- Ahmed, S., Sharma, A., Bhushan, B., Singh, A.K. and Wali, V.K. (2014) Effect of carbohydrate source, pH and supporting media on *in vitro* rooting of banana (*Musa* spp.) cv. Grand Nain plantlets. *Afr. J. Agric. Res.*, **9**, 1135-1140.
- Ambros, V.E., Vasilyeva, Y.O. and Novikova, T.I.V. (2016) Effects of *in vitro* propagation on ontogeny of *Rosa canina* L. micropropagated plants as a promising rootstock for ornamental roses. *Plant Cell Biotechnol. Mol. Biol.*, **17**, 72-78.
- Bagheri, M.S., Saidi, A., Jari, S.K., and Goodarzi, G. (2015) Effects of different hormonal concentrations on damask rose (*Rosa damascena* Mill.) micro-propagation in liquid tissue culture medium. *Int. J. Biosci.*, **6**, 10-16.
- Brunda, S. M., Rani, C. L., Rajendran, P., Smitha, R., and Priya, L. (2016) *In vitro* propagation of *Rosa hybrida* 'Golden Fairy' through nodal explants. In International Symposium on Succulents and Other Ornamentals, Ambalavayal, Wayanad, Kerala, India, 2016, Rajendran, P., Revi, S., Vishnu Savanth, V. (Eds.), *ISHS Acta Hortic.*, **1165**, 87-90.
- De Klerk, G., Hanecakova, J. and Jasik, J. (2008) Effect of medium-pH and MES on adventitious root formation from stem disks of apple. *Plant Cell Tissue Organ Cult.*, **95**, 285-292.
- Gabryszewska, E.A. (2015) Effect of different sucrose and nitrogen salt levels in the medium and temperature on *in vitro* propagation of *Helleborus niger* L. *Acta Agrobot.*, **68**, 161-171.
- Gomez, K.A. and Gomez, A.A. (1984) *Statistical Procedures for Agricultural Research*, 2nd ed. John Wiley, NY., 680 p.
- Hamama, L., Cesbron, D., Voisine, L., Lecerf, M., Jareno, C., Dorion, N., and Oyant, H.S. (2015) Effects of carbohydrate sources and BAP concentrations on *in vitro* morphogenesis of four rose genotypes. *Acta Hortic.*, **1083**, 75-83
- Hameed, N., Shabbir, A., Ali, A. and Bajwa, R. (2006) *In vitro* micropropagation of disease free rose (*Rosa indica* L.). *Mycopath.*, **4**, 35-38.
- Hasan, N.A., Hussein, S.B. and Ibrahim, R. (2015) Effect of medium pH and sucrose concentrations on adventitious roots induction of *Labisia pumila*. *Proceedings of the IRES 14th International Conference, Paris, France, 7th November, 2015*, 44-48.
- Hasegawa, P.M. (1979) *In vitro* propagation of rose. *Hortic. Sci.*, **14**, 610-612.
- Hegde, V., Kag, B., Sathyanarayana, B.N. and Sharath, R. (2011) Rapid *in vitro* multiplication and shoots regeneration from nodal explants of rose. *Journal of Cell and Tissue Research*, **11**, 2961-2965.
- Ibrahim, R. and Debergh, P.C. (2001) Factors controlling high efficiency adventitious bud formation and plant regeneration from *in vitro* leaf explants of roses (*Rosa hybrida* L.). *Sci. Hortic.*, **88**, 41-57.
- Kanchanapoom, K., Posayapisit, N. and Kanchanapoom, K. (2009) *In vitro* flowering from cultured nodal explants of rose (*Rosa hybrida*). *Not. Bot. Hort. Agrobot. Clu. J.*, **37**, 261-263.
- Kharde, A.V. and Kshirsagar, A.B. (2014) Effect BAP and Kinetin on nodal culture of *Rosa hybrida* L. *Bionano Frontier*, **7**, 254-257.
- Kovacevic, B., Miladinovic, D., Katanic, M., Tomovic, Z. and Pekee, S. (2013) The effect of low initial medium pH on *in vitro* with poplar growth. *Biblid*, **108**, 67-80.
- Kroin, J. (2016) Propagation of Roses: Stenting - Simultaneously Cutting and Grafting. *Hortus USA Article*. Available online: http://www.rooting-hormones.com/IBAarticles/Propagation_of_roses_by_Stenting.pdf (accessed in May 2018).
- Kumari, S., Singh, K. P., Singh, S. K., Kumar, S. and Sarkhel, S. (2017) Establishment of *in vitro* propagation protocol for Hybrid Tea rose cv. Raktagandha. *The Horticultural Society of India (Regd.)*, **74**, 245-250.
- Kumud, S., Hem, P., and Vijay, R. (2015) Micropropagation of rose cultivars: Biotechnological application to floriculture. *Journal of Environmental Research and Development*, **10**, 40.
- Mahmood, S. and Hauser, B. (2015) Influence of cytokinins on the shoot proliferation and subsequent rooting in rose. *Asian Journal of Agricultural Research*, **9**, 259-267.
- Manners, M. M. (2014): Propagation roses from cuttings, Florida Southern College. Available online: <http://www.rkdn.org/roses/propagate.asp> (accessed in May 2018).
- Martins, N., Goncalves, S., Palma, T. and Romano, A. (2011) The influence of low pH on the *in vitro* growth and biochemical parameters of *Plantago almogravensis* and *P. algarbiensis*. *Plant Cell Tissue Organ Cult.*, **107**, 113-121.

- Maurya, R.P., Yadav, R.C., Godara, N.R. and Beniwal, V.S. (2013) *In vitro* plant regeneration of rose (*Rosa hybrida*) cv. Benjamin Paul through various explants. *Journal of Experimental Biology and Agricultural Sciences*, **1**, 111-119.
- McGaw, B.A. and Burch, L.R. (1995) Cytokinin Biosynthesis and Metabolism. In: Davies, P.J. (Ed.) *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, Kluwer Academic Publishers, Dordrecht, 98-117.
- Murashig, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.*, **15**, 473-497.
- Pati, P.K., Rath, S.P., Sharma, M., Sood, A. and Ahuja, P.S. (2006) *In vitro* propagation of rose -a review. *Biotechnol. Adv.*, **24**, 94-114.
- Rose Society (2012): Eiffel Tower. Available online: <http://sarose.org.au/rose-month/eiffel-tower/> (Accessed in May 2016).
- Schum, A., Hofmann, K., Ghaalib, N., and Tawfik, A.A. (2001) Factors affecting protoplast isolation and plant regeneration in *Rosa* spp. *Gartenbauwissenschaft*, **66**, 115-122.
- Shang, X.M., Huang, J.Y., Haigler, C.H. and Trolinder, N.L. (1991) Buffer capacity of cotton cells and effects of extracellular pH on growth and somatic embryogenesis in cotton cell suspensions. *In Vitro Cell. Dev. Plant*, **27**, 147-152.
- Tawfik, A.A., Read, P.E. and Cuppett, S.L. (1992) Effect of some nutritional factors on monoterpene synthesis in *Rosmarinus officinalis* cultured *in vitro*. *Acta Hort.*, **319**, 189-191.
- Tawfik, A.A. and Noga, G. (2001) Adventitious shoot proliferation from hypocotyl and internodal stem explants of cumin. *Plant cell Tissue organ cult.*, **66**, 141-147.
- Tawfik, A.A. (2002) Adventitious meristem organogenesis and shoot proliferation of *Salvia officinalis* L. in split-nodal culture. *Assiut J. Agric. Sci.*, **33**, 99-114.
- Tawfik, A.A. and Mohamed, M. F. (2005) Organogenic response of *Salvia officinalis* L. to dark preconditioning, thidiazuron and benzyladenine. *Plant Growth Regul. Soc. Amer. Quarterly*, **33**, 125-133.
- Tawfik, A.A. and Mohamed, M. F. (2006) *In vitro* cloning of two cumin landrace lines via shoot-tip culture. *J. Hort. Sci. & Biotech.*, **81**, 246-268.
- Tawfik, A.A. (1995) *In vitro* propagation of *Melaleuca armillaris*. *Assiut J. Agric. Sci.*, **26**, 137-149.
- Tawfik, A.A. (1997) Micropropagation and plant regeneration of neem tree (*Azadirachta indica* Juss). *Assiut J. of Agric. Sci.*, **28**, 3-14.
- Uzunova, K. (2015) Comparative analysis of different rose cultivars (*Rosa hybrida* L.) rooting using conventional and biotechnology approaches. *Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie*, **100**, 191-199.
- Van Winkle, S. and Pullman, G.S. (2003) The combined impact of pH and activated carbon on the elemental composition of plant tissue culture media. *Plant Cell Rep.*, **22**, 303-311.
- Yakimova, E., Toteva, V.K., Groshkoff, I. and Ivanova, G. (2000) Effect of BA and CPPU on protease and α amylase activity of *in vitro* cultured explants of *Rosa hybrida* L. *Bulg. J. Plant Physiol.*, **26**, 39-47.

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عمل بروتوكول إكثار دقيق لنبات الورد صنف برج إيفل يتميز بقدرة تجذيرية عالية داخل الأنابيب

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تمت دراسة بعض تقنيات الإكثار الدقيق بهدف تعظيم بروتوكول للإكثار على نطاق واسع وسريع لنبات الورد صنف برج إيفل وهو أحد الأصناف الهامة. أجريت العديد من التجارب على مرحلتين وهما مرحلة التضاعف ومرحلة التجذير. تم الحصول على أفضل النتائج في مرحلة التضاعف عند زراعة العقل الساقية على بيئة موراشيچ وسكوج مضافاً إليها إياً من ١ ملجم/لتر بنزيل أمينوبيورين أو ٠,٥ ملجم/لتر كينتين منفردين أو مجتمعين معاً. فمن بين التركيزات المختلفة المستخدمة من البنزيل أمينوبيورين، كان التركيز ٠,٥ ملجم/لتر هو الأفضل حيث أعطى أكبر عدد للفروع/قطعة ساقية (٣,١٧). في حين أن إضافة الكينتين للبيئة بمعدل ٠,٥ ملجم/لتر تفوق على باقي التركيزات من حيث عدد الفروع/عقلة ساقية (٣,٦٧) وكذلك طول الساق (٣,٦٧ سم). وأدت التداخل بين البنزيل أمينوبيورين بمعدل ١ ملجم/لتر والكينتين بمعدل ٠,٥ ملجم/لتر إلى الحصول على أكبر عدد للفروع (٤ فروع/عقلة ساقية). وبالنسبة لمرحلة التجذير، فقد لوحظت أفضل النتائج فيما يتعلق بنسبة التجذير (٦١,٠٠٪)، عدد للجذور (٥,١٢ جذر/ساق) وطول الجذر (٣,٣٣ سم) وذلك عند زراعة الأفرع على بيئة موراشيچ وسكوج خالية من الهرمونات مع ضبط الرقم الهيدروجيني للبيئة على ٥,٥. كما أن ضبط الرقم الهيدروجيني على ٥,٥ أدى إلى الإسراع في تكوين الجذور في ١٧,٦٧ يوماً يليه البيئة ذات الرقم الهيدروجيني ٤,٥ (١٨ يوماً). في حين أن البيئة ذات الرقم الهيدروجيني ٥,٥ والمضاف إليها السكرز بمعدل ٥٠ ملجم/لتر أعطت أعلى قيم معنوية لنسبة التجذير (٨٥٪) كما أدت إلى تحسين سرعة إنتاج الجذور في ٧,٥ يوماً. كما لم تلاحظ أية تأثيرات معنوية لمعاملات التداخل على كل من عدد الجذور وطول الجذر. وتم أقلمة النباتات المكونة للجذور بنجاح بعد نقلها إلى أصص مملوءة ببيئة زراعة وذلك داخل المعمل ثم نقلت إلى الحقل بمعدل بقاء وصل إلى ٥٥٪. وبعد مرور ٦ أشهر فإن متوسط طول النبات قد وصل إلى ٢٢ سم، وعدد الأوراق إلى ٥,٥ ورقة/نبات.