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Clonal Propagation of Rangoon Creeper (*Quisqualis indica* Linn.) Via Nodal Explant Culture

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A PROTOCOL was developed for micropropagation of *Quisqualis indica* (Combretaceae). Media for culture establishment, multiplication, rooting and hardening were studied. For culture establishment and multiplication, MS medium supplemented with BAP 1.5 mg/l plus GA_3 1 mg/l produced the highest number of shoots/explant (3.43) among the various tested concentrations of BAP and GA_3. For rooting, ½ MS medium without any growth regulators was effective and formed more number (6.00) and longer roots (13.00 cm). Plantlets were successfully acclimatized in a mixture of perlite, peat-moss and vermiculite 1:1:1 v/v/v under ex-vitro conditions with a survival rate of 73.3 %.

Keywords: Micropropagation, Medicinal creeper, Ornamental plant, Salt strength MS medium.

Introduction

Quisqualis indica linn is an ornamental plant related to the family (Combretaceae) and commonly known as rangoon creeper or red jasmine due to its bright flowers with pleasant fragrance (Fig. 1). It's grown as a woody ornamental climber for outdoor gardens. It has horizontally orientation to pendulous white, pink and red flowers that give out distinct perfume (Eisikowitch and Rotem, 1987). *Quisqualis* is a small genus of woody climbers comprising more or less than 17 species, distributed in Africa, India, Asia (China) and Malesia. The plant grows best in tropical areas (De Padua et al., 1999).

Rangoon creeper is generally used as an ornamental plant but due to presence of phytoconstituent, it was usually used for medicinal purposes. Generally, the parts which are traditionally used of this plant are leaves, flower, fruits, seeds, and roots. These parts of *Quisqualis* have some active constituents (tannins, flavonoids, steroids, carbohydrates, protein, amino acids, saponins and phenolic compounds) which are responsible for giving its particular pharmacological activities such as anti-bacterial, anti-oxidant, antifungal, anti-helmintic, anti-inflammatory, anti-diabetic, acetyl-cholinesterase inhibition and immunomodulatory (Sahu et al., 2012 and Aher & Mahajan, 2017).

Quisqualis indica is conventionally propagated through seeds, sukers and cuttings. Propagation through seeds lead to render undesirable variation. Moreover, the plant does not produce seeds in our climate conditions. The plant produces few numbers of suckers. So using suckers produce small number of plants and need skilled workers. Cuttings are difficult to develop roots and may take more than 36 days to develop adventitious roots. The percent of success is very low because using cuttings are affected by many factors such as type of cutting and time of planting (Elgimabi, 2009 and Ahmed et al., 2021). These difficulties may be overcome using tissue culture techniques. Nowadays, and since Murashig and Skoog have been released their famous medium (MS medium) in 1962, plant tissue culture techniques has been used for commercial production of many ornamental and medicinal plants as well as for academic research.

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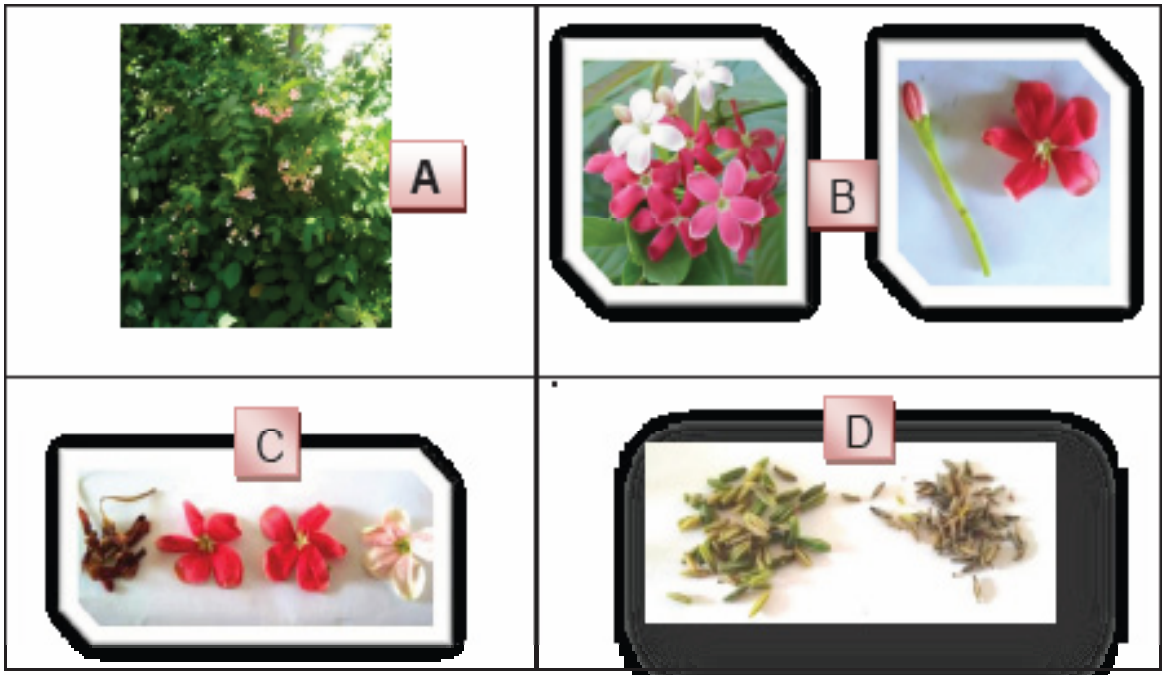

Fig.1. Parts of *Quisqualis indica;* (A) *Q.indica* grown in Faculty of Agriculture Campus of Assuit University; (B) and (C) flowers and (D) seeds.

Thus, the aim of this study was to develop an efficient protocol to micropropagate *Q. indica* and produce high number of genetically homogenous plants.

Materials and Methods

This work was carried out at plant tissue culture laboratory, Department of Ornamental Plants and Landscape Gardening, Faculty of Agriculture, Assiut University. The stages of plant micropropagation technique were conducted as follow:

Culture Establishment Stage

Plant materials and explant preparation

During the preliminary trials to propagate *Q. indica in vitro*, different type of explants (shoot tips, petal of the flowers, leaves, seeds and nodal segments) were tested and it was found that the nodal segments had a better response (data not shown) and therefore, they were used as an explant for further experiments. Young healthy shoots of *Q. indica* were obtained in spring from 5 - year- old shrubby plants grown in the campus of Assuit University. Nodal segments (1-1.5 cm containing 2 nodes) cut after 5 nodes on branch tip were used. Trimmed explants were washed in tap water with few drops of tween 20 for 30 min and disinfected by submerging them in 70% (v/v) ethanol for 1min. Then in the laminar air flow-hood, the ends of the nodal segments were cut and all the explants were rinsed three times with sterile distilled water before culturing into baby food jars 250 ml containing 30 ml of plant growth regulators (PGRs) -free basal salt MS medium. After 4-5 days, clean explants were cultured on MS medium supplemented with different concentrations of 6-Benzlaminopurine (BAP) 0 , 0.5 , 1 , 1.5 and 2 mg/l with or without 1 mg/l gibberellic acid (GA_3) with 3 replicates. After 4 weeks, the regenerated axillary shoots were excised and used for the next stage.

Media and culture conditions

Murashig and Skoog (MS) basal salt medium (Murashig and Skoog, 1962) was used in all the experiments. Unless otherwise stated, the medium was supplemented with 30 g/l sucrose, solidified with 7.5 g agar /l (w/v) and the pH of the medium was adjusted to 5.8 ± 1 using NaOH or HCL 1N prior to autoclaving. All the jars (250 ml) containing 30 ml of medium were autoclaved at 121º C and 1.5 kg/cm^2 for 25 min. The cultures of all experiments were incubated at 25 ±2°C under constant cool white fluorescent light for 16 hours.

Multiplication stage

Effect of 6-Benzlaminopurine (BAP) and gibberellic acid (GA_3) on shoot multiplication of Q. indica.

Microshoots (1 - 1.5 cm) were excised from the previous stage after 4 weeks and cultured on MS medium supplemented with different concentrations of BAP (0, 0.5, 1, 1.5 and 2 mg/l) with or without 1 mg/l GA_3. A factorial experiment (2X5) in a completely randomized design (CRD) was used with 3 replicates for each treatment and 4 jars per each replicate (total 12 jars for each treatment). Means separation was done according to the L.S.D. at 5% level of probability. Data were recorded after 4 weeks for: Responded explants % = (Number of grown explants/number of the cultured explants x 100. Number of shoots per explant. Shoot length (cm) and leaves number per explant.

Effect of basal medium salt strength and sucrose concentrations on shoot multiplication of Q. indica.

Shoots (~ 1.5 cm) were cultured on three different strengths of MS basal salt medium (full MS, ½ MS and ¼ MS strength) in combination with three concentrations of sucrose (20, 30 and 40 g/l). All media were supplemented with BAP at 1.5 mg/l with 1 mg/l GA_3 (the best concentration from multiplication stage). A factorial experiment (3x3) in a CRD was used with 3 replicates for each treatment and 5 jars per each replicate (total of 15 jars for each treatment). Data were recorded after 4 weeks for: Shoot numbers per explant; Shoot length (cm); and leaves number per explant.

Rooting stage

Effect of basal salt strength and sucrose concentration on root formation of Q. indica

To test the effect of basal salt strength and sucrose concentration on rooting of shoots of *Q. indica*, uniform individual shoots (~3-4 cm long each) derived from the multiplication stage were transferred to different rooting media comprising three different strengths of MS basal salts (full, ½ and ¼ MS) in combination with four concentrations of sucrose (15, 20, 25 and 30 g/l). A factorial experiment (3x4) in a CRD was used with 3 replicates for each treatment and 3 of 250 ml baby-food jars per each treatment (total of 9 jars for each treatment). Means separation was done according to the L.S.D. at 5% level of probability. Data were recorded after three weeks on rooting percentage; roots number per shoot; and roots length (cm).

Acclimatization stage

An experiment was conducted to study the effect of transplanting media on the growth and survival percentage of the well rooted plantlets. These plantlets were carefully removed from the culture medium and the roots were gently washed with sterile distilled water to remove the agar. The plantlets where then transferred into plastic pots (15-cm in diameter) containing one of the following autoclaved media: clay or peatmoss + perlite (1:1 v:v) or peat moss + vermiculite + perlite (1:1:1 by v/v/v) Three replicates were employed and each treatment consisted of 3 pots and each pot contained one plantlet covered with transparent polyethylene plastic sheet to maintain high relative humidity around plantlets. The plastic cover was gradually removed after one week of transplanting in order to get rid of excess humidity as well as expose the plantlets to *ex vitro* conditions. The plantlets were irrigated with tap water whenever needed. Data were recorded after 4 weeks for survival percentage and average increase in shoots length (cm).

Statistical analysis

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

Results and Discussion

Establishment of in vitro culture (Initiation stage)

A clean culture with the maximum number of shoots of *Q. indica* was obtained using the nodal segment as an explant cultured on MS medium supplemented with BAP and GA_3. Several studies reported that nodal segments used as explants were the best to induce proliferation from axillary buds in different plant species such as *Quisqualis indica* (Mandal, 2013), *Terminalia bellirica* Roxb (Ramesh et al., 2005), *Terminalia arjuna* Roxb. (Pandey et al., 2006), *Terminalia bellirica* (Phulwaria et al., 2012) and *Rosa* spp (Tawfik et al., 2018). Concerning the interaction effect of BAP and GA_3 concentrations, it was cleared that culturing the nodal segments into MS medium supplemented with BAP at 1.5 mg/l plus GA_3 at 1.0 mg/l significantly affected the

proliferation of the explant (Table 1, Fig.2-B). It produced the highest percentage (90%) of responded explants, shoots number (2.67 shoots/ explant), shoot length (3.62 cm). These results are supported by Mandal (2013), he reported that the best multiplication response was obtained when nodal explants of *Q. indica* were cultured on MS medium supplemented with 1 mg/l BAP plus 0.5 mg/l GA_3.

Axillary shoot multiplication

The regenerated adventitious shoots from the previous step were multiplied on MS medium supplemented with BAP and GA_3 concentrations shown in Table 2 and fig. 2 B and C. A significant interaction effect between GA_3 and BAP concentrations was noticed. It is cleared that culturing the micro-shoots on MS medium supplemented with BAP at 1.5 mg/l plus 0.5 mg/l

TABLE 1. **Effect of different concentrations of BAP and GA_3 on nodal explants proliferation of *Quisqualis indica* after 4 weeks in culture.**

Treatment (mg/l)			**BAP**			
GA_3	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**Mean**
			Responded explants %			
0.0	33.00	50.00	65.00	66.70	58.30	**54.60**
1.0	30.00	65.00	80.30	90.00	65.00	**66.10**
Mean	**31.50**	**57.50**	**72.70**	**78.4**	**61.70**	
			Shoot number/explant			
0.0	1.33	1.47	1.75	1.83	1.22	**1.52**
1.0	1.67	2.00	2.11	2.67	1.23	**1.94**
Mean	**1.50**	**1.74**	**1.93**	**2.25**	**1.23**	
			Shoot length(cm)			
0.0	3.00	1.50	2.00	2.17	2.01	**2.14**
1.0	2.50	2.50	2.81	3.62	2.50	**2.79**
Mean	**2.75**	**2.00**	**2.41**	**2.9**	**2.26**	
			Leaves number /shoot			
0.0	5.00	3.10	3.25	3.80	2.67	**3.56**
1.0	3.25	4.00	5.17	5.33	3.50	**4.25**
Mean	**4.13**	**3.55**	**4.21**	**4.57**	**3.09**	

L.S.D 5 %				
	Responded explants %	**Shoot number / explants**	**Shoot length (cm)**	**Leaf number /shoot**
BAP	5.80	0.37	0.45	0.61
GA_3	3.70	0.23	0.29	0.39
BAP*GA_3	8.20	0.52	0.64	0.87

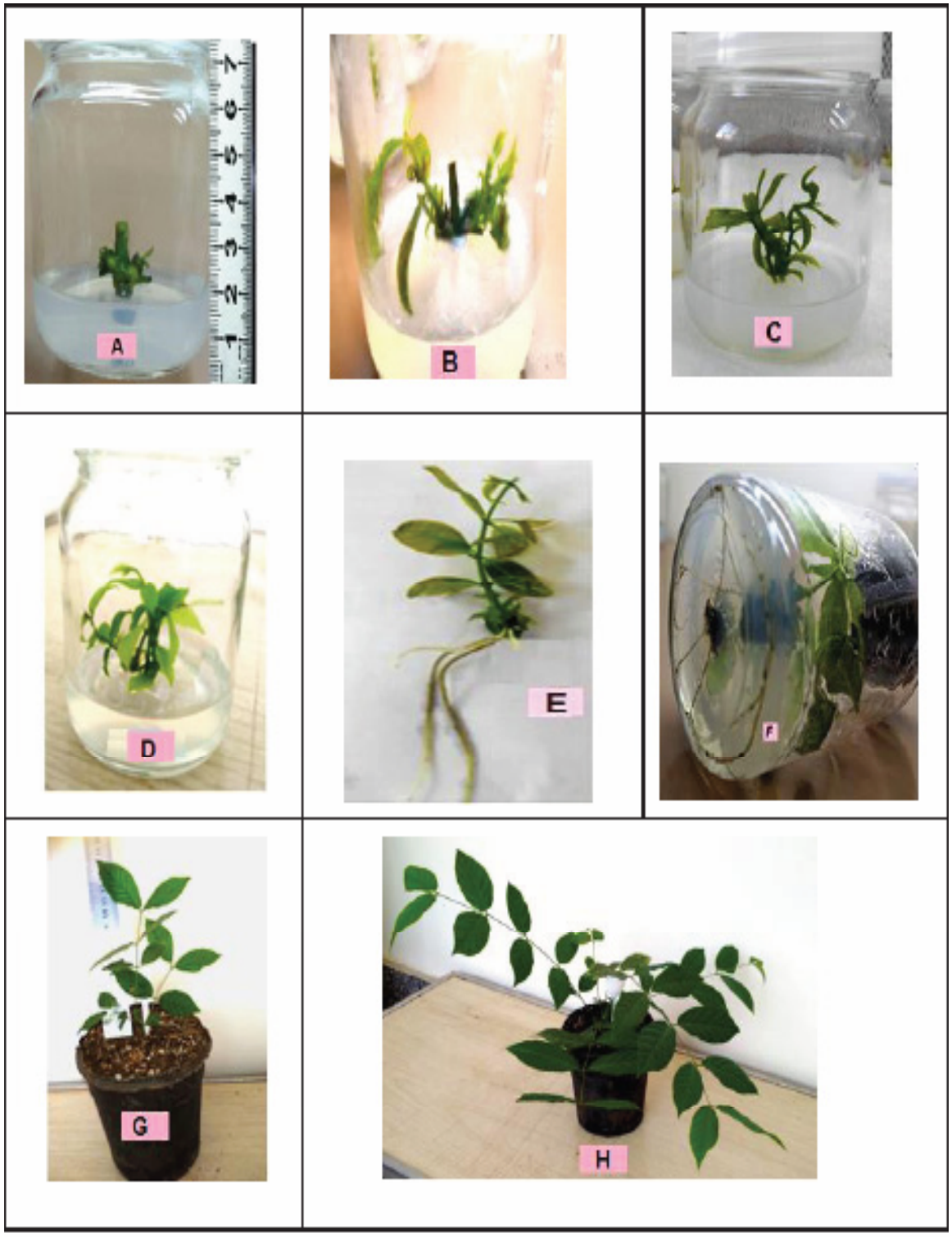

Fig. 2. Stages of micropropagation of *Quisqualis indica*; (A) aseptically cultured a nodal explant on PGRs-free MS medium (B) induction of axillary shoots on medium containing 1.5 mg/l BAP and 1 mg/l GA_3 after 4 weeks; (C) shoot multiplication on medium containing 2 mg/l BAP and 1 mg/l GA_3 after 4 weeks ; (D) Shoot multiplication on medium containing 1.5 mg/l BAP and 1 mg/l GA_3 with 30 g sucrose/l after 4 weeks; (E and F) Rooting of isolated single shoot on 1/2 MS medium with 15 g sucrose/l after 3 weeks (G) Acclimatized plantlets transferred to plastic pots filled with Peat moss + vermiculite + perlite (1:1:1) after one month; (H) plants after 3 months.

TABLE 2. Effect of different concentrations of BAP and GA_3 on axillary shoots multiplication of Quisqualis indica after 4 weeks in culture.

Treatment (mg/l)			BAP			
GA_3	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**Mean**
			Shoot number/explants			
0.0	1.00	1.60	1.90	2.00	1.25	**1.55**
1.0	1.63	2.00	2.50	3.43	2.00	**2.31**
Mean	**1.32**	**1.80**	**2.20**	**2.72**	**1.63**	
			Shoot length(cm)			
0.0	3.20	1.75	2.20	2.67	1.50	**2.26**
1.0	3.60	2.70	3.40	6.00	2.50	**3.64**
Mean	**3.40**	**2.23**	**2.80**	**4.34**	**2.00**	
			Leaves number /shoot			
0.0	2.90	3.35	3.70	3.75	2.90	**3.32**
1.0	4.00	4.67	6.00	9.10	4.50	**5.65**
Mean	**3.45**	**4.01**	**4.85**	**6.43**	**3.7**	**4.49**

L.S.D 5 %

Meas. / Treat.	Shoot numbers / explants	Shoot length (cm)	Leaf numbers /shoot
BAP	0.37	0.28	0.43
GA_3	0.23	0.18	0.27
BAP*GA_3	0.52	0.39	0.61

GA_3 significantly increased the shoot number (3.43 shoots/explant), shoot length (6 cm) and leaves number (9.10 leaves/shoot) compared to the other combination treatments. Many investigators reported that GA_3 at low concentrations ranging from 0.5 to 1.5 mg/l in combination with BAP improved the multiplication rate (Gonbad et al., 2014; Mandal and Laxminarayana, 2012; Saini et al., 2010; and Mulwa and Bhalla, 2000).

Effect of basal salt strength of MS medium and sucrose concentration on multiplication of regenerated shoots of Q. indica after 4 weeks cultured in vitro.

As shown in Table 3 and Fig. 2 D, reducing the media strength to quarter strength (¼ MS medium) decreased all the measured parameters. Similar results were reported by Frett (1987), Hidayah et al. (2012) and Rezali et al. (2017). It is also clear, that the sucrose concentration was effective up to 30 g/l for all the measured parameters (Table 3). Gurel and Gulsen (1998) stated that the highest rate of shoot tip multiplication of *Amygdalus communis* was obtained when cultured on a medium supplemented with 3 or 4% sucrose. However, the interaction effect of salt strength of MS medium and sucrose concentrations on axillary shoots proliferation of *Q. indica* indicated that full MS medium supplemented with 30 g/l sucrose gave the highest value of shoot numbers (3.0 shoots/explant), shoot

TABLE3. Effect of basal salt strength and sucrose concentration on multiplication of regenerated shoots of *Quisqualis indica,* after 4 weeks in culture.

Treatment	Sucrose g/L			
Media Strength	**20**	**30**	**40**	**Mean**
		Shoot number/explants		
Full MS	1.50	3.00	2.50	**2.33**
½ MS	1.00	1.25	1.00	**1.08**
¼ MS	1.00	1.00	1.00	**1.00**
mean	**1.17**	**1.75**	**1.5**	**1.47**
		Shoot length(cm)		
Full MS	2.80	4.80	3.80	3.80
½ MS	1.90	2.40	2.20	2.17
¼ MS	1.60	1.80	1.50	1.63
mean	**2.10**	**3.00**	**2.50**	**2.53**
		Leaves number /shoot		
Full MS	3.43	8.67	5.33	**5.81**
½ MS	3.00	3.77	3.00	**3.26**
¼ MS	2.17	2.50	2.43	**2.37**
mean	**2.87**	**4.98**	**3.59**	3.81

L.S.D 5 %

Meas. / Treat.	Shoot numbers /explants	Shoot length (cm)	Leaf numbers /shoot
MS strength	**0.20**	**0.29**	**0.4**
Sucrose	**0.20**	**0.29**	**0.4**
MS*sucrose	**0.35**	**0.50**	**0.7**

length (4.8 cm) and leaf numbers (8.67 leaves/ shoot). It could be concluded that full MS medium supplemented with 30 g/l sucrose was the best for shoot proliferation of *Q. indica*. These results were similar to that reported by Abo-Dahab et al*.,* (2005), who found that using MS medium at full strength contained 30 or 40 g/l sucrose produced the highest number of shoots from buds of *Ruscus hypoglossum* rhizomes.

In vitro rooting of microshoots

As shown in Table 4 and Fig.3, half-strength MS medium supplemented with 15 g/l sucrose significantly affected root formation on the microshoots of *Q. indica.* Such auxin-free medium produced the highest rooting percentage (100%), the highest number of roots (6 roots/shoot) and the longest roots (13 cm). Similar results were reported by Tawfik, (1995) on *Meulaleuca* where the explants were formed roots in auxin-free medium, and Tawfik et al., (2018) who reported that the best effect on rooting percentage (61.00%), highest number of roots/shoot (5.12) and root length (3.33 cm) were observed when the shoots of *Rosa* spp cv. Eiffel Tower were cultured on hormone-free MS medium. These values were significantly reduced when ¼ MS strength was used. Using ¼ MS with 25 and 30 g/l sucrose gave the lowest values of root numbers (1.00 root/ shoot) and length of roots was 1.5 and 1.4 cm respectively**.** The interaction between the basal salt strength MS medium and sucrose concentrations indicated no significant differences among

full MS and half-strength MS media with any of sucrose concentrations regarding rooting percentage. It could be concluded that the lowest concentrations of sucrose (15 g/l) and half MS was the best for root formation. These results confirm previous findings reported by Meziani, et al. (2019) on date palm and Tawfik et al. (2019) on petunia. This could be interpreted by the fact that media with small osmotic potential are favorite for root induction and growth under in vitro condition (Goerg et al. 2008)

Acclimatization stage

Acclimatization of plantlets is a crucial step in the micropropagation of any plant species. Selecting the proper growing medium helps achieving good results. In the present study the effect of transplanting media on growth of the plantlets of *Q. indica* is shown in Table 5 and

TABLE 4. Effect of basal salt strength MS medium and sucrose concentration on root characteristics of *Quisqualis indica* 3 weeks after culture *in vitro*.

Treatment	Sucrose g/l				
Media Strength	**15**	**20**	**25**	**30**	**Mean**
			Rooting %		
Full MS	100.00	100.00	93.33	86.67	**95.00**
½ MS	100.00	100.00	100.00	93.33	**98.33**
¼ MS	53.33	40.00	40.00	40.00	**43.33**
Mean	**84.44**	**80.00**	**77.78**	**73.33**	
			Roots number/shoot		
Full MS	4.00	3.50	2.00	1.80	**2.83**
½ MS	6.00	5.20	3.00	3.20	**4.35**
¼ MS	1.56	1.33	1.00	1.00	**1.22**
Mean	**3.83**	**3.37**	**2.00**	**2.00**	
			Root length(cm)		
Full MS	2.80	3.20	3.40	3.00	**3.10**
½ MS	13.00	12.40	10.80	10.30	**11.63**
¼ MS	3.70	3.00	1.50	1.40	**2.40**
Mean	**6.50**	**6.20**	**5.23**	**4.90**	

L.S.D 5 %

Meas. / Treat.	Rooting %	Root numbers/shoot	Root length (cm)
MS	5.62	0.17	0.29
Sucrose	6.49	0.20	0.33
MS*sucrose	11.23	0.34	0.58

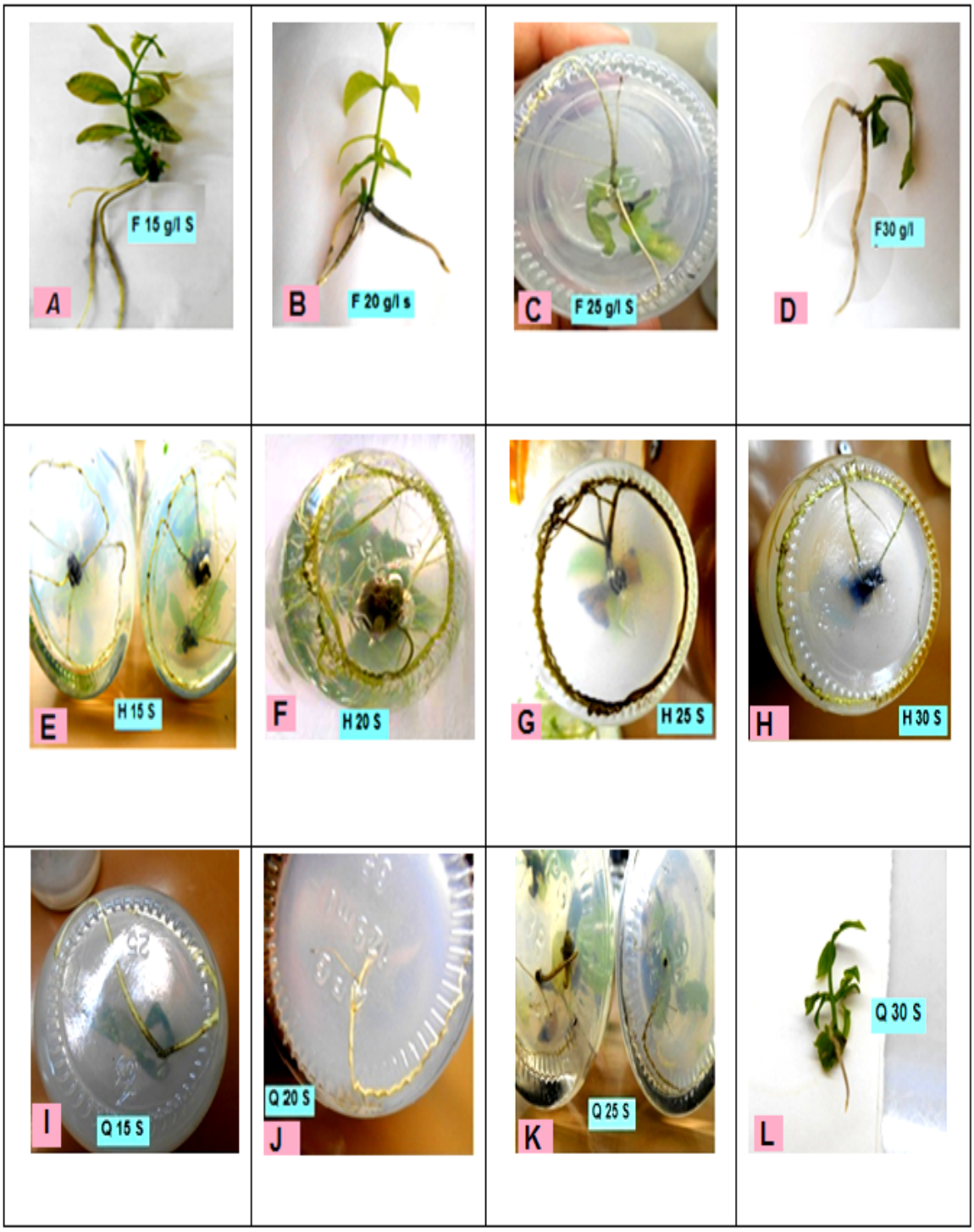

Fig. 3. Effect of basal salt strength MS medium and sucrose concentration on root characteristics of *Quisqualis indica* after 3 weeks of culture in vitro. F: full MS (A,B,C and D) – H: half-strength MS (E,F,G and H) – Q: quarter-strangth MS (I,J,K and L) – S: sucrose (15,20.25 and 30) (g/l).

TABLE 5. Effect of growing medium on regenerated plantlets of Quisqualis indica plantlets after 4 weeks of transplanting.

Transplanting media (v)	Survival %	Shoots length (cm)
- Clay	20.00	3.80
- Peat moss + perlite (1:1)	40.00	4.90
- Peat moss + vermiculite + perlite (1:1:1)	73.30	9.70

Fig. 4. Effect of transplanting medium on *ex vitro* growth of *Quisqualis indica* plantlets after 4 weeks. (1) Clay, (2) Peat moss + perlite (1:1; v/v), (3) Peat moss + vermiculite + perlite (1:1:1; v/v/v).

Fig.4. Transferring the plantlets into a soil mixture of peat moss + vermiculite + perlite (1:1:1) by volume, significantly increased the survival percentage (73.3 %) and the shoot length (9.7 cm) in comparison with the plantlets transferred to clay (the soil of the nursery). The clay soil gave a survival rate of 20% and a shoot length of 3.8 cm**.** On contrary to this result, Tawfik et al., (2019) reported that clay soil recorded the highest survival rate (89 %) and the longest shoots (8.80 cm) among the all three media tested for petunia plantlets.

Conclusions

Nodal segments of *Quisqualis indica* were successfully established and multiplied on MS medium supplemented with BAP at 1.5 mg/l plus GA_3 at 1 mg/l. Full strength MS medium supplemented with sucrose 30 g/l gave the best number of shoots (3.0 shoots/explant). High rooting percentage (100%) was accomplished on PGRs-free half strength MS medium supplemented with 15 g/l sucrose. Plantlets were successfully acclimatized on a mixture of peat moss + vermiculite + perlite (1:1:1; v/v/v) with a survival rate of 73.3%.

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the publication of this study

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الإكثار الدقيق لنبات الكويسكوالس كنبات زينة متسلق من خلال القطع العقدية الساقية

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لتطوير برتكول اكثار دقيق لنبات الكويسكوالس تم دراسة بيئة التأسيس والتضاعف والتجذير والاقلمة .حيث اشارت النتائج إن افضل بيئة لمرحلة التأسيس و التضاعف كانت المحتوية على ١,٥ملليجرام/ لتر بنزيل امينوبيورين مع ١ ملليجرام/لتر حمض الجبريليين حيث أعطت أفضل عدد للأفرع (٣,٤٣/) من بين التركيزات التى تم إختبارها. وللتجذير كانت البيئة المحتوية على نصف تركيز الاملاح بدون اى منظمات نمو كانت مناسبة لأعطاء اكبر عدد من الجذور (٦,٠٠) وكذلك طول الجذر ١٣,٠٠ سم . وتم اقلمة النباتات الناتجة فى خليط من البيرليت والبيت موس والفيرمكيوليت بنسبة ١:١:١ (بالحجم) تحت الظروف الخارجية مع نسبة بقاء ٧٣ ٪ .