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Propagation Protocol of *Gardenia Jasminoids* 'Varigata' Ellis through Plant Tissue Culture

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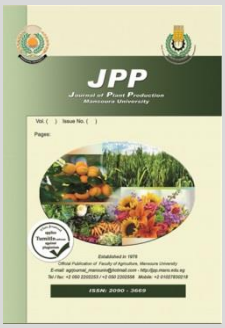

ABSTRACT

Study of *Gardenia jasminoids* 'Varigata' took place over the course of two years (2021–2023) in the Tissue Culture Laboratory -Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. This study sought the micropropagation protocol for this promising cultivar of Gardenia used as an indoor plant and recently imported. The aim of this study is to achieve the best result for the various stages of propagation (establishment, multiplication under the influence of cytokinins and fluorescent lights, rooting and acclimatization stages). Since it is imported cultivar. Shoot tip explants sterilized with Mercury chloride (MC) at 0.1% for 10 min produced the best free-contaminated and survival %. Establishing explants on full Murashige and Skoog (MS) basal medium supplemented with 2.0 mg/l BAP produced the largest shootlet number/explant, while ½ MS salt strength free hormone (control) recorded the longest shootlet (cm). For florescent lights, a white florescent lamp recorded the highest shootlet number/explant and leaf number/shootlet, while the shootlet length increased under a yellow florescent lamp. For rooting, culture shootlet on ½ MS salt strength recorded the longest plantlet, roots (cm), and largest plantlet, leaf, and root number/shootlet. Acclimatization on peat moss recorded very good survival and growth.

Keywords: Micropropagation, Cytokinins, MS salt strength, Fluorescent lights

INTRODUCTION

The Rubiaceae family includes *G. jasminoides* cv. 'Varigata'. The common gardenia, also known as the cape jasmine, and the East Asian gardenia. There are more than 200 species of gardenia genus, with *G. jasminoides* being the most significant and common throughout the tropics and subtropics (Rezam *et al.*, 2014). The leaves and fruit of *G. jasminoides* are often used to treat inflammation, demulcent, and diuretic. It was used in medicating jaundice, fever, swelling, headache, high blood pressure, and problems with the liver Hayashi *et al*., 1992; Koo *et al*., 2006).

Ornamental plants are now being propagated utilizing tissue culture technology, leaving behind many well-known traditional methods (Aysar *et al.*, 2020). For many plant species, it has been possible to use *in vitro* methods to grow new plants, keep them alive, and change how they grow. Micropropagation of *G. jasminoides* using *in vitro* organogenesis by modified MS medium (Murashige and Skoog (1962)) records a higher rate of growth from the starting plant (Suprasanna and Bapat, 2005; Wu *et al*., 2012). For obtained aseptic *in vitro* growth, the choice of time and chemical agents depend on the sensitivity of the explant to be sterilized (Örge *et al*., 2018). There is much common sterility for the surface disinfection of plant material. Popular disinfectants are sodium hypochlorite, mercuric chloride, calcium hypochlorite, silver nitrate, hydrogen peroxide, and bromine water (Ishfag, 2016). Cytokinins are types of PGRs generally known to activate the formation of buds and differentiation of shoots in micropropagation. Cell division intercede factors such as development shoot. (Youssef *et al*., 2021; Sayed *et al*., 2023). Light plays important role in a plant's life, not only for photosynthetic power production but also for its regulatory role of biochemical, molecular, and morphological processes that over plant growth and development (Morini and Muleo, 2012 and Kozai, 2016). Controlling light quality, photoperiod, and irradiances is unavoidable in the production of plants with needed characteristics (Valeria *et al*., 2022). Lamps fluorescent have been the important popular in rooms of *in vitro* culture and consume approximately 65% of total electricity in labs of tissue culture (Yeh and Chung, 2009). The fluorescent lamps have large amounts of photons in the infrared and red ranges, gradually falling toward blue (Dutta Gupta and Jatothu, 2013). The concentration of macro-and microelements of culture medium (strengths of medium) without or with activated charcoal (AC) remarkably affected rooting behavior as showed (Sayed *et al.*, 2010 and Sayed *et al*., 2023).

Therefore, the objective of the present study was to establish a protocol for the propagation of *G. jasminoids* 'Varigata' plant through tissue culture by examination of plant growth regulators (PGR), florescent light, and different MS salt strength medium on different stages of micropropagation.

MATERIALS AND METHODS

This study was carried out at the Tissue Culture Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt during 2021 to 2023 years on *G. jasminoides* 'Variegata'.

Sterilization of explants

G. jasminoides 'Varigata' shoot tips and lateral buds were obtained from "My Garden" company and excised to use on examination. These explants were soaked in water

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with 2:3 drops of liquid soap and stirred for 20 min, before being put under running tap water for 1 hour. Then, under aseptic conditions, explants were transferred into the laminar air flue and were given one of the following treatments:

- Commercial Clorox solution at 5% (sodium hypochlorite "NaOCl" 5%) for 5, 10, or 15 min.
- Mercuric chloride (MC) solution ($Hgcl_2$) at 0.1% at 5, 10 or15 min.

Explants were properly washed three times with distilled water after treatments to remove any excess sterility. After that, three explants with fife replicates were cultured on MS medium-free hormones in all treatments for 3 weeks. At the end of the period, the survival and free contamination % were calculated

Culture medium and condition

The culture medium was supplemented with 30 g/l sucrose and 7 g/l agar. The pH medium was adjusted at 5.7±0.1 and autoclaved at 121°C and 1.2 kg/cm2 for 20 min. The explants were placed vertically in 200 ml capacity glass containers containing 25 ml medium. The cultures were incubated at 24±1° C under fluorescent illumination of 2000-2500 lux at 16/8 (daylight/dark) of white light during all stages. For the effect of light, a Philips fluorescent lamp, white (1360 lux), yellow (1270 lux), blue (1310 lux), green (1300 lux), or red (1320 lux) were used to study the second experiment in the multiplication stage.

Multiplication stage:

1. Effect of MS strength medium and concentration of BAP on shooting growth.

In this experiment the explants survival and free contamination culture on different salt strengths of MS medium Murashige and Skoog (1962) at (full, ¾ or ½ salt strength) with different concentrations of BAP (6-benzylaminopurine) at (1.0, 1.5 or 2.0 mg/l) for one month in three subculture. In all treatments, five replicates were used. At the end of the third subculture, shootlet number/explant, shootlet length (cm), and leaf number/shootlet were studied.

2. Effect of florescent lights on shooting growth

The best medium in the previous experiment (full MS +2.0 mg/l BAP) were used to culture explant to investigate different light effects (white, yellow, blue, green, and red) for shooting growth got one month in three subculture. In all treatments five replicates were culture. Shootlet number/explant, shootlet length (cm), and leaf number/shootlet were recorded after the third subculture.

Rooting stage:

In the study of rooting behavior, shootlet length (2-2.5 cm) was cultured on different salt strengths of MS medium at (full, ¾, or ½). In this step, after one month look at length of the plantlet (cm), number of plantlet, number of leaves, number of roots, and length of roots (cm).

Acclimatization stage

The produced rooted shootlets obtained from rooting treatments were transferred to plastic pots containing peat moss irrigated with a solution of fungicide topsin-M-70 (0.2 g/l) and covered by transparent polyethylene bags. Then, plantlets were kept in an acclimatized glass house for four weeks before transplanting-of-door, after that survival capacity was recorded.

Experimental design and data analysis

The layout of the experiment was designed in a Completely Randomized Design (CRD) and comparing between means was achieved by the Least Significant Difference (L.S.D.) method at $p \leq 0.05$ (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Establishment stage: Effect of disinfection kind and explant type on survival and free contaminated percentage of *G. jasminoides* 'Variegata'

Data in Table 1. demonstrated a significant effect on survival and free contamination percentage when using different kinds and different types of explants. For survival%, no significant differences were found between shoot tip or nods on the survival percentage. Applying 0.1% MC ($HgCl_2$) produces the highest survival percentage (94.45%). As for the interaction between explant types and disinfections kind, nods sterilized with 0.1% MC for 5 min recorded the highest survival percentage (100%), while shoot tip disinfected by Clorox at 5% for 15 min produced the lowest survival percentage (22.22%).

Table 1. Effect of disinfection and explants type on survival and free contaminated percentages of *G. jasminoides* 'Variegata'

	Survival%			Free contamination%		
	Shoot tip	Nods	Mean B	Shoot tip	Nods	Mean B
Clorox 5% 5min	11.80	77.80	77.80	33.33	22.22	27.78
Clorox 5% 10min	44.45	55.58	50.10	55.58	33.33	44.45
Clorox 5%15min	22.22	33.33	27.78	77.80	55.58	66.69
MC 0.1% 5min	88.90	100.0	94.45	44.45	33.33	38.89
MC 0.1% 10min	77.80	66.70	72.25	77.80	40.79	59.29
MC 0.1% 15min	44.45	33.33	38.89	88.80	88.90	88.90
Mean A	59.27	61.12		62.98	45.69	
$LSD_{0.05}$		A = NS B = 17.94 A×B = 25.37			A = 12.28 B = 21.27 A×B = 30.08	

LSD 0.05; Least Significant Different at 0.05 level of probability; MC: Mercuric Chloride; NS: Non-Significant.

Using the shoot tip explant produces a good percentage of free contamination compared to the nods explant. For the disinfection, using 0.1% MC for 15 min recorded the highest free contamination percentage. In interaction, sterilized shoot tip or nods explants with 0.1% MC for 15 min recorded the best result for free contamination percentage (88.80 or 88.90%, respectively) in comparison with sterilized nods explant with 5% Clorox for 5 min which decreased the free contamination percentage to the lowest value (22.22%). The suitable type of explant and kind of disinfection was the shoot tip which was immersed in 0.1% MC for 10 min, since recorded 77.80% survival and free contamination percentages.

The outcomes of this study are consistent with those of Khatun *et al*. (2016) on *Zingiber officinale* who examined the effects of four different sterilizing agents on the rhizome buds,

and revealed that 0.1% $HgCl_2$ for 15 minutes was the best procedure for obtaining the highest percentage of contamination-free cultures. According to Mahmoud and Al-Ani (2016), sterilizing nodal segments of *Cestrum nocturnum* for 7 minutes while continuously rinsing with $HgCl_2$ at 0.05–0.20% resulted in good free contamination (90–100%), survival percentages, and shoot development percentages (50–60%). NaOCl at 50% for 5 minutes of non-stop rinse produced acceptable viability and shoot development results (at 50%).

Multiplication stage:

1.Effect of MS salt strength media and concentrations of BAP on shooting characteristics of *G. jasminoids* 'Varigata'.

The illustrated data in Table 2. and Fig. 1. indicated that shootlet multiplication of *G. jasminoids* 'Varigata' was influenced by the salt strength of MS medium, BAP concentrations, and interaction recorded significant effects.

Table 2. Effect of salt strength MS media and concentrations of BAP on shooting growth of *G. jasminoids* 'Varigata'.

	Shootlet number/explant				Shootlet length (cm)				Leaf number/shoot			
Mg/l BAP	Full MS	¾ MS	½ MS	Mean (B)	Full MS	¾ MS	½ MS	Mean (B)	Full MS	¾ MS	½ MS	Mean (B)
0.0	1.45	1.90	1.11	1.49	1.06	1.59	2.71	1.79	6.45	7.24	5.61	6.43
1.0	4.44	4.00	3.34	3.93	1.48	1.84	1.67	1.66	5.15	5.37	4.18	4.90
1.5	3.22	3.80	3.11	3.38	1.00	1.74	2.04	1.59	6.44	5.76	4.58	5.59
2.0	4.50	3.44	3.80	3.91	1.43	1.67	1.44	1.52	6.00	6.31	4.28	5.53
Mean (A)	3.403	3.285	2.84		1.242	1.712	1.965		6.009	6.172	4.66	
LSD0.05	A= NS B =0.688 A×B =1.191				A=0.358 B = NS A×B =0.716				A=1.095 B =1.364 A×B =2.190			

LSD 0.05 = Least Significant Difference at 0.05 level of probability; BAP: 6-enzylaminopurine; MS: Murashige and Skoog (1962) basal medium; NS: Non-Significant.

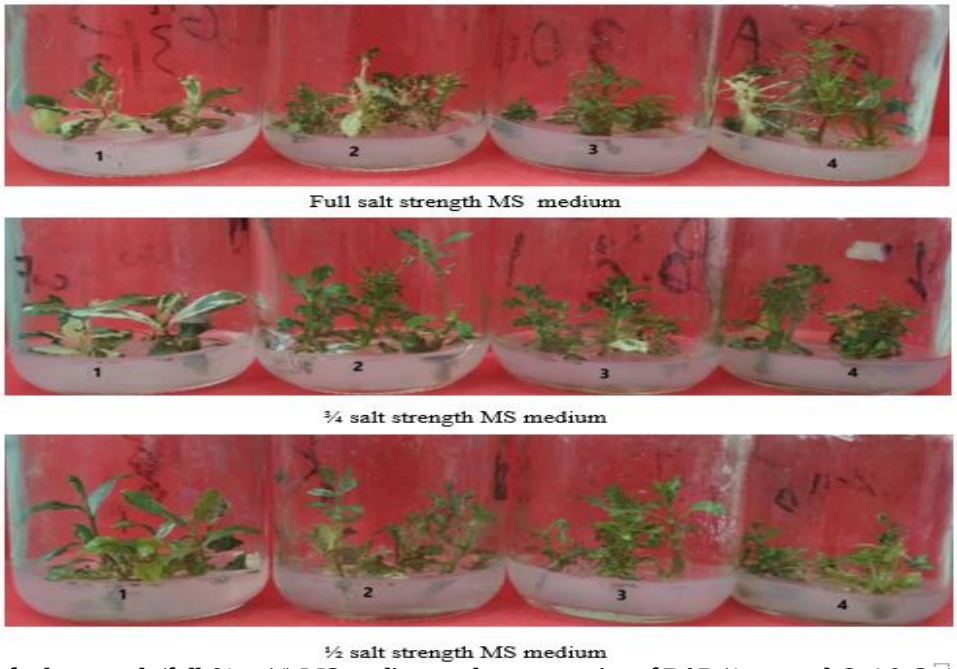

Fig. 1. Effect of salt strength (full, ¾ or ½) MS medium and concentration of BAP (1- control; 2- 1.0; 3- 1.5; 4- 2.0 mg/l) on shooting growth of *G. jasminoids* 'Varigata'.

For shootlet number/explant, using different salt strength MS medium recorded a non-significant effect. In different concentrations of BAP, all concentrations (1.0, 1.5, or 2.0 mg/l) produced many shootlets (3.93, 3.38, or 3.91 shootlet/explant, respectively) compared to the control (MS free hormone) which gave (1.49 shootlet/explant. In the interaction between the strength of MS medium and concentrations of BAP, full salt strength of MS medium concluding 2.0 mg/l BAP recorded the highest shootlet number (4.50 shootlet/explant) in comparison with different salt strengths of MS medium without hormone (control) which gave the lowest number shootlet number (1.45, 1.90 or 1.49 shootlet/explant, respectively).

For shootlet length, medium ¾ or ½ salt strength recorded longer shootlet (1.712 or 1.965 cm, respectively) compared to full salt strength (1.242 cm). In interaction, ½ salt strength medium free hormone (control) recorded the longest shootlet length (2.71 cm) while using full strength MS supplemented 1.5 mg/l BAP observed the shortest shootlet (1.0 cm). For leaves number, using full or ¾ MS salt strength medium gave a large number of leaves (6.009 or 6.172 leaf/shootlet, respectively). In concentration of BAP, MS free BAP recorded the largest leaf number (6.43 leaf/shootlet) compared to 1.0 mg/l BAP which decreased leaf number to (4.90 leaf/shootlet). In combination, ¾ MS free BAP recorded the largest leaf number (7.24 leaf/shootlet) while ½ MS strength supplemented with 1.0 mg/l BAP decreased leaf number to (4.18 leaf/shootlet).

In this respect, Sayed *et al.* (2010) on *Gardenia jasminoids* observed that medium supplemented with 2 or 3 mg/l BAP in the shooting stage recorded the highest shootlet number/explant. Also, El-Afry *et al.* (2017) reported that the longest shoots of *Phytolacca dioica* plant were observed when adding BAP at 3 ppm compared to the control.

2.Effect of fluorescent lights on shooting growth of *G. jasminoids* 'Varigata'.

Data in Table 3. and Fig. 2. showed that shootlets growing under white light recorded the largest number of

leaves (2.733 leaves/shootlet), while red or green decreased the leaf number (1.50 or 1.43 leaves/shootlet). Shootlet length increased when growth under yellow light (2.67 cm) compared to shootlet growth under green light which was recorded (1.30 cm). For leaf number, explants grow under all light colors gave no significant effect.

In this respect, the specific roles observed by green light in plant physiology, such as in chloroplast orientation in Mougeotia Lechowski and Bialczyk (1987) and stomatal aperture Frechilla *et al*., (2000). In addition, green light increased shootlet number, fresh and dry weight. However, exposure to yellow light increases shootlet length and leaf number on *Ceratonia siliqua* (Sayed *et al*., 2020). On the other hand, Cavallaro *et al*. (2022) pointed out that using red light significantly affected shooting behavior, especially on plantlet quality, i.e., shoot length as well as fresh and dry weigh

Table 3. Effect of fluorescent lights on shooting growth of *G. jasminoids* 'Varigata'

	Shootlet number/explant	Shootlet length (cm)	Leaf number/shootlet
White	2.73	2.50	5.60
Red	1.50	1.70	5.60
Green	1.43	1.30	6.27
Blue	2.33	1.17	5.87
Yellow	2.13	2.67	5.33
LSD 0.05	1.052	0.9601	NS

LSD 0.05 = Least Significant Difference at 0.05 level of probability; NS: Non-Significant.

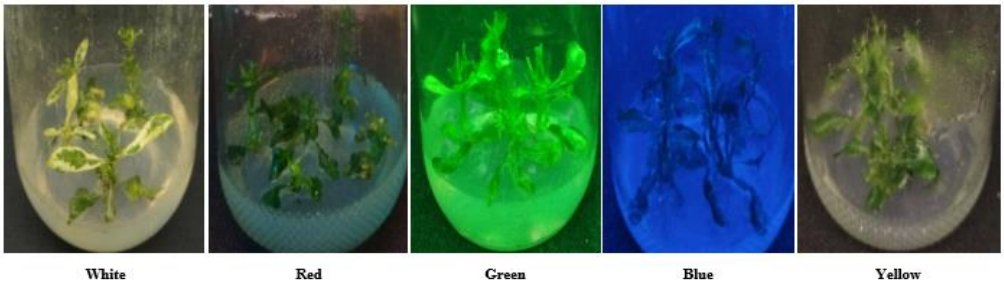

Fig. 2. Effect of florescent lights on shooting growth of *G. jasminoids* 'Varigata'.

Rooting stage: Effect of MS salt strength medium on rooting behavior of *G. jasminoids* 'Varigata'.

The data in Table 4. and Fig. 3. show that the longest plantlet and largest leaves were produced by culture shootlet in ½ salt strength MS medium which recorded (3.89 cm and 11.56 leaf/shootlet) while decrease on full or ¾ salt strength MS medium (2.61, 2.56 cm and 8.89, 9.56 leaf/shootlet, respectively). For plantlet length and root number, no significant differences were recorded. In root length culture shootlet in ¾ salt strength of MS medium observed long root (3.56 cm) compared to full or ½ MS salt strength which was recorded (2.11 or 2.33 cm, respectively).

Table 4. Effect of salt strength MS medium on rooting behavior of *G. jasminoids* 'Varigata'.

	Plantlet length (cm)	plantlet number	Leaves number	Roots number	Root length (cm)
Full MS	2.61	1.67	8.89	1.22	2.11
¾ MS	2.56	1.78	9.56	2.00	3.56
½MS	3.89	1.00	11.56	4.11	2.33
$LSD_{0.05}$	1.031	NS	1.743	NS	1.113

LSD 0.05 = Least Significant Difference at 0.05 level of probability; MS: Murashige and Skoog basal medium; NS: Non-Significant.

Fig. 3. Effect of salt strength MS medium on rooting characteristics after the acclimatization process of *G. jasminoids* 'Varigata'.

In this respect, Woldeyes *et al*. (2021) indicated that the maximum root number and root length of *Abelmoschus esculentus* were observed at ½ MS strength. Also, Dönmez *et al*. (2022) revealed that all *Spathiphyllum* plants rooted when grown in full, half, and quarter strength MS medium containing 1 mg/l IBA. However, there are notable variations in the number of roots and root length across different strength media.

Acclimatization

All the rooted shootlets were cultured on peat moss. The survival of plantlet after one month were calculated and gained 98% percentage with excellent growth behavior.

CONCLUSION

The present work describes the micropropagation protocol of *G. jasminoids* 'Varigata'. For this protocol, shoot tip explants sterilized with 0.1% MC ($HgCl_2$) for 10 min appears to be a good percentage of survival and free contamination. Moreover, culture explant on full MS medium supplemented 2.0 mg/l BAP under white light recorded good shooting behavior. After that, the culture shootlet on 1/2 MS strength medium recorded good rooting behavior. Finally, acclimatization on peat moss observed excellent growth behavior.

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برتوكول إكثار الجاردينيا جاسمينويدس المبرقشة عن طريق زراعة الأنسجة

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قسم بحوث نباتات الزينة، معهد بحوث البساتين، مركز البحوث الزراعية، الدقي، الجيزة، مصر

الملخص

أجريت هذه الدراسة على مدار عامين (2021/ 2023)علي نبات الجاردينيا جاسمينويدس المبرقشة بمعمل زراعة الأنسجة - معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر. هدفت هذه الدراسة إلى تحديد بروتوكول للإكثار الدقيق لهذا الصنف الواعد من نبات الجاردينيا المستخدم في التنسيق الداخلي و المستورد حديثا. تهدف هذه الدراسة إلى تحقيق أفضل نتيجة لمراحل التكاثر المختلفة (التأسيس، الاكثار تحت تأثير السيتوكينينات وأضواء الفلورسنت، مرحلة التجذير والأقلمة). تعقيم المنفصلات النباتية المأخوذة من البراعم الطرفية بكلوريد زئبقيك 0.1% أعطى أفضل نسبة بقاء وخلو من التلوث. أدى زراعة الأجزاء النباتية على بيئة كاملة القوى من أملاح موراشيج وسكوج مزوده 2.0 مجم/لتر بنزيل امينو بيورين الى تحقيق أعلى عدد من الأفرخ بينما نصف تركيز الأملاح الخاليه من الهرمون (الكنترول) سجل أطول الأفرخ. على العكس من ذلك ثلاثة أرباع تركيز الأملاح الخاليه من الهرمون (الكنترول) زاد من عدد الأوراق . أدى التعرض للضوء الأبيض الفلورسنت الي تسجيل أعلى عدد من الأفرخ والأوراق بينما طول الأفرخ زاد عند الزراعه تحت الفلورسنت الأصفر. في مرحلة التجذير، زراعة الأفرخ على بيئه نصف تركيز من أملاح موراشيج وسكوج سجل أطول النبيتات والجذور وأكبر عدد من النيتات والأوراق والجذور. الاقلمه فى بيئة بيت موس سجلت نسبه عالية من البقاء ونمو ممتاز.