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In Vitro Propagation of *Jasminum Sambac* L.

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Annotation: To obtain highly locally desired Iraqi jasmine (Jasminum sambac L.) seedlings in a large scale and free of pathogens to meet the local demand for this plant, this work was done in the Laboratory for Plant Cell and Tissue Culture/Department of Plant production techniques/Agricultural Technical College/Northern Technical University. Included In vitro planting of jasmine single nodes during the multiplication stage on MS (Murashige and Skoog) basal medium enriched by (0, 0.5, 1, 1.5, and 2.0 mg L-1) of BA and Kin. At the rooting stage, the plantlets that obtained were planted on full and half of MS salt's strength medium enriched with IBA at a concentration of (0, 0.5, 1, 1.5, and 2 mg L-1). The results showed that the shoot numbers were not affected significantly by CK type. In contrast, the number of leaves was significantly affected, as nodes were planted in media containing Kin recording the largest leaves number (6.24 leaf/shoot) compared to those planted in media containing BA. While, the nodes planted on MS nutrient medium equipped with (1 mg L-1) BA gave the largest new shoots formed number and the greatest number of leaves (2.6 and 7.40 respectively) with significant superiority over all other treatments. As for the rooting stage a highest rooting percentage (80%) and the largest roots number (4.8) was recorded in MS with half salt's strength medium enriched by 0.5 mg L-1 IBA, the plantlets were successfully transferred to the greenhouse with a survival percentage of (85%). The results of the study can be adopted as a successful

protocol for the propagation of the high aesthetic value Razaki plant used as an ornamental plant.

Keywords: Jasminum sambac L., Micropropagation, regeneration, plant growth regulators.

Introduction

Razaki or Jasmine (*Jasminum sambac* L.) the popular ornamental Shrub plant belongs to Oleaceae family, it distributed in Asia, Africa and Europe (Ramdas *et al*., 1993). Jasmine is distinguished by its white blossoms and fragrant aroma many uses of jasmine make it one of the most important ornamental plants, as it is used as pots or cut flowers and outdoor ornamental plant, room decoration, in addition to its use in in beauty products, conventional therapy, a source of raw materials for the fragrance industry (Suryowinoto, 1997), its extracts contain many medically active substances. thus, it is used in the pharmaceutical industry (Kumar *et al*., 2007).

Jasmine species are propagated via both asexual and sexual techniques (Shahmoradi and Naderi, 2018). Due to the high rate of seedling death and low seed germination in natural settings, sexual propagation is uncertain (Reddy and Gupta, 2013). layering and/or cutting propagation methods restrict the plant quantity produced, are cumbersome, time-consuming and have a low multiplication rate. Plant propagated by cutting or layering continuously was found to result in reduced flowering, cultivar degeneration, and resistance lacks (Cai et al., 2007). Also, plants propagated by the seeds may not show the clonal reliability because of genetic variability (El-Sadat and Hewidy, 2020).

Biotechnology including tissue culture technology is one of the most important means that has helped improve crops, food, and medicine production. Practical biotechnology applications rely on simple methods and do not require complex and expensive equipment, so it taken agricultural sciences outside their traditional field (Al-Hadidi, 2002).

In vitro multiplication is considered a rapid and true-to-type method to produce a large scale of uniform plantlets in a short time (Khan et al., 2020). In addition, it is also a successful way producing low-cost plant material (Chaitanya et al., 2018). Numerous earlier research has used tissue culture techniques to propagate various jasmine species by direct or indirect shoot proliferation utilizing various plant parts, shoot tips **(**Ai **et al., (2024),** leaves (Sapra and Pandya, 2017), nods and internodal segments (Salim, 2016), and apical/ axillary shoots (Biswal et al., 2016). However, no studies were found on the micropropagation of jasmine, known locally as Razqi, Hence, the present study was conducted.

Material and Methods:

this work was done in Laboratory for Plant Cell and Tissue Culture /Dep. of Plant production techniques/Agriculture Technical College/Northern Technical University, Iraq. Newly grown nodal segment of 3-5 cm length, containing at least two nodes, were taken from one-year-old mother plants of jasmine from local nurseries in Nineveh Governorate.

The nodal segments were transferred to the laboratory where they defoliated, and placed for 15 min under running water, and under air flow cabinet chamber the surface sterilization was done by immersing in 10% (V/V) Chlorox solution containing 5% sodium hypochlorite $NaOCl_3$ for 20 min. Then washed with distilled and sterile water four times in succession with shaking for 10 min each time and then in sterile Petri dishes the nodules in length of approximately 1 cm were prepared. Single nodules were planted in MS medium (4.25 g/L salts, 30 g/L Sucrose, and 6 g/L agar) free of growth regulators for two weeks (Saleh and Abbood 2018; Abbood and Ahmed 2019).

First: Multiplication stage: The healthy explants were transferred to be planted on MS medium

enriched with different concentrations (0.0, 0.5, 1, 1.5, and 2 mg L^{-1}) BA or Kin, each separately. After four weeks, the explants were replanted on MS medium containing the same growth regulators at the previous concentrations for another four weeks, then the following data were taken: shoot number, number of leaves, and length of the longest shoot.

Second: Rooting stage: The shootlets resulting from the multiplication stage were replanted in MS medium content (0, 0.5, 1, 1.5, and 2.0 mg L^{-1}) IBA. After four weeks, the following data were taken: rooting response percentage, roots number, and length of the longest root.

During every investigation phase, the cultures were kept in a growth chamber at the light period of sixteen hours of light/day a light intensity of 2000 lux, and a temper of 25 ± 1 ºC,

The studies were conducted as factorial studies utilizing CRD at ten replicates. Duncan's multiple range test was utilized to compare the means at 5% probability level for all the studied treatments (Al-Rawi and Khalaf, 1980; Alrawi and Ahmed 2024) and the statistical analysis was carried out using a computer by analyzing them with the SAS program (V9.2) (Anonymous, 2002; Saleh 2020).

Results:

Multiplication stage:

1- BA and Kin Effect in number of the new formed shoots:

Data represented in Table 1 indicated that cytokinin types had no appreciable impact on the number of newly formed shoots after 8 weeks of nodes cultivating, while its concentrations made a major impact on this characteristic since treatments enriched by 1 mg L^{1} CKs got the highest shoots number (1.80 shoot /node) with a considerable superiority over all other concentrations used except the treatment of 1.5 mg L. However, BA interaction treatment with the concentration of 1 mg L^{-1} recorded the highest number of new shoots formed on the nodes (2.60 shoots/node) and noteworthy superiority over other interaction therapies except for the interaction of BA with the concentration of 1.5 mg L-1. In contrast, the lowest number of new shoots formed on the nodes (1.4 shoots/node) was recorded in medium free of growth regulator (control).

with a notable advantage over other forms of interaction therapy.

Table 1: effect of cytokinin type, concentrations and its interaction on the shoots number formed on single nodes of jasmine plant

CON. / CK. type	0.0 mg L^{-1}	0.5 mg L^{-1}	1.0 mg L^{-1}	1.5 mg L^{-1}	2.0 mg L^{-1}	CK. Type mean
BA	1.4 bc	1.80 bc	2.6 a	2.0 ab	1.2 c	1.80A
Kin	1.4 bc	1.6 bc	1.80 bc	1.6 bc	1.80 bc	1.64A
Con. mean	1.40 B	1.70 B	2.20 A	1.80 AB	1.50 B	

* Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

2- BA and Kin effect in the leaves number:

The results of Table 2 shows BA & Kin effect on leaves number emerged on the nodes of the jasmine plant after 8 weeks of cultivation. It is noted from the table that Kin significantly outperformed BA in the leaves number, as the media enriched with Kin recorded a number of 6.24 leaves/node. The concentrations of BA and Kin also significantly effect on this trait, as the mediums that enriched with these two growths regulators significantly outperformed the control treatment. As for the largest leaves number (7.00 node leaves), was recorded at the concentration of 1 mg/L, significantly outperforming all the studied concentrations. As for the effect of the interaction treatments of the type of cytokinin and its concentrations, the largest number of new leaves formed was recorded in the nodes planted on the medium enriched with 1 mg L-1BA, which

recorded a value of 7.40 node leaves, which was equal to the value that recorded in the interaction treatment of Kin at 1.5 mg/L concentration.

Table 2: effect of cytokinin type, concentrations and its interaction on the leaves number formed on single nodes of jasmine plant

CON. / CK. type	0.0 mg L^{-1}	0.5 mg L^{-1}	1.0 mg L^{-1}	1.5 mg L^{-1}	2.0 mg L^{-1}	CK. Type mean
BA	4.40 d	5.0 cd	7.40 a	4.8 d	4.40 d	5.20 B
Kin	4.80 d	6.0 bc	6.60 ab	7.40 a	6.40 ab	6.24 A
Con. mean	4.60 C	5.50 B	7.00 A	6.10 B	5.4 B	

* Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

3 - Effect of BA and Kin in the shoots lengths (cm):

The results of Table 3 show that the lengths of new shoots formed on single nodes of jasmine plant that were planted on MS medium were significantly affected by the type of cytokinin prepared with it, as the medium containing Kin significantly outperformed those enriched with BA, while the concentrations of growth regulators used did not affect this trait, as the control treatment recorded the highest shoot length (4.50 cm), significantly outperforming all the used concentrations. The interaction treatments also had no significant effect on the shoot's length, as none of the interactions achieved a value higher than the control treatment (4.50 cm), in contrast, a gradual decrease in shoot lengths was observed with increasing growth regulators concentrations, and the lowest shoot's length (2.70 and 3.30 cm) were recorded in the media that contained high concentrations (2.0 mg/L) of both BA and Kin, respectively.

Table 3: effect of cytokinin type, concentrations and its interaction on the shoots length (cm) formed on single nodes of jasmine plant

CON. / CK. type	0.0 mg L^{-1}	0.5 mg L^{-1}	1.0 mg L^{-1}	1.5 mg L^{-1}	2.0 mg L^{-1}	CK. Type mean
BA	4.50 a	3.56 cd	3.32 d	3.26 d	2.70 f	3.46 B
Kin	4.50 a	3.92 b	3.88 bc	3.5 d	3.30 d	3.81 A
Con. mean	4.50 A	3.74 B	3.60 BC	4.38 C	2.98 D	

* Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

Rooting stage

1- MS salt strength and IBA concentration effect in rooting percentage:

The results of the rooting stage showed that MS medium salt strength had a significant effect on the percentage of response of the shoots resulting from the multiplication stage to rooting after four weeks of planting, as the MS media with half salts strength response to rooting at a percentage of 48% compared to the rate of 20% that recorded in MS media with full salt strength (Table 4), also IBA concentrations significantly affected the percentage of rooting response. As for the highest rooting percentage (80%), it was recorded in the shoots planted on the MS medium with half salt's strength enriched with 0.5 mg L-1IBA, which significantly outperformed the rest of the studied interactions.

Table 4: effect of IBA concentration, MS salt strength and their interaction on the percentage of jasmine shoots' response to rooting.

IBA Con. / MS salts strong	0.0 mg L^{-1}	0.5 mg L^{-1}	1.0 mg L^{-1}	1.5 mg L^{-1}	2.0 mg L^{-1}	MS salts strong mean
full MS	00 e	20 ce	40 bd	30 be	10 de	20 B

1/2MS	20 ce	80 a	60 ab	50 ac	30 be	48 A
Con. mean	10 C	50 A	50 A	40 AB	20 BC	

Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

2 - MS salt strength and IBA concentration effect in number of formed roots.

Table 5 shows the effect of MS medium salt's strength and IBA concentrations and their interaction in the roots formed number on the shoots induced from the nodes of the jasmine plant. The data in the table show that the MS medium with half strength of its salts stimulated the formation of roots with high efficiency compared to the MS medium with full salts strength. Also, the shoots planted in media enriched with 0.5 mg L-1IBA gave a high number of roots (3 roots/shoot) compared to growth regulator-free medium. The largest roots number (4.80 roots/shoot) was recorded in the medium in which the shoots were planted on the MS medium with half salt's strength interacted with 0.5 mg L-1IBA, with a significant superiority over all other interaction treatments.

Table 5: effect of IBA concentration, MS salt strength and their interaction on roots number of jasmine shootlets.

IBA Con. / MS salts strong	**0.0 mg L^{-1}**	**0.5 mg L^{-1}**	**1.0 mg L^{-1}**	**1.5 mg L^{-1}**	**2.0 mg L^{-1}**	**MS salts strong mean**
full MS	00 g	1.20 ef	1.80 de	1.70 de	1.00 f	1.14 B
1/2MS	2.0cd	4.80 a	2.80 b	2.50 bc	1.60 df	2.74 A
Con. mean	1.0 C	3.0 A	2.30 B	2.10 B	1.30 C	

* Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

3- MS salt strength and IBA concentration effect on roots length.

In general, MS medium with half salt strength recorded high root lengths (2.78 cm) with a clear significant superiority compared to full salt strength MS medium (0.98 cm) (Table 6). Media enriched with 0.5 mg L-1IBA also significantly outperformed all other concentrations in this trait by recording root lengths of 2.55 cm.

As for the effect of interaction coefficients between MS salt strength and IBA concentration on root lengths, the highest root length (4.56 cm) was recorded in shoots planted on MS medium with half salt strength and free of IBA (control) with a significant superiority over the rest of the interaction coefficients.

Table 6: effect of IBA concentration, MS salt strength and their interaction on root lengths (cm) of jasmine shootlets.

IBA Con. / MS salts strong	**0.0 mg L^{-1}**	**0.5 mg L^{-1}**	**1.0 mg L^{-1}**	**1.5 mg L^{-1}**	**2.0 mg L^{-1}**	**MS salts strong mean**
full MS	00 h	1.74 e	1.38 f	0.89 g	0.73 g	0.94 B
1/2MS	4.56 a	3.36 b	2.57 c	2.10 d	1.40 f	2.798 A
Con. mean	2.28 B	2.55 A	1.975 C	1.495 D	1.065 E	

* Dunkin multi-range test indicates that, at the 5% likelihood range, numbers with identical letters in a single column are not statistically different.

Rooted plantlets were removed from vessels after four weeks and washed with sterile water, then transferred into pots containing rooting media (perlite and pet mouse in a ratio of 1:1) for acclimatization. with maximum plant survival of 85% observed in plants.

Discussion:

growth parameters (shoots and leaves number) (Tables 1 , 2) were statistically superior in most of cytokinin treatments compared to control treatment, which may attributed to the cytokinin role in

regulating meristems activity, morphological creation, development of chloroplast, and leaf expansion (Fahmy, 2003), Since cytokinin eliminate apical dominance and promote cell division and expansion, they favorably influence the development of lateral buds during vegetative propagation processes (Van Staden et al., 2007). Additionally, it performs a part in drawing metabolites to lateral buds, aggregating them, and promoting the transfer of nutrients and other growth materials to initiate bud development. (Devlin and Witham, 1983).

The statistical superiority of BA over Kin in the effectiveness for multiplication inducing maybe because of the BA molecule‘s internal structure and the quantity of double bonds its side chain (Mohammed, 1985). It has a side chain with three double bonds compared to two double bonds in Kinin, as many double bonds exist on the side chain, the more potent cytokinin becomes (Krishnamoorthy, 1981). In addition, the benzene ring existing in the BA structure raises the activity and makes it one of the most effective cytokinin's (Wasfi, 1995).

Adding IBA to the rooting medium caused an increase in the rooting percentage (Table 4) and roots number that formed on detached shoots (Table 5) may attributed to the physiological action of the auxins on adventitious root formation (Robinson, 1983), in addition to its functions in accelerating root production, decreasing the duration of time needed for rooting, and improving the rate of rooting (Hartmann et al., 2002). Tawagine (1987) and Wasfi (1995) stated that IBA's function in encouraging adventitious root production by enhancing cambium cell proliferation and root elongation causes the increase in rooting rate.

Cultivation of shoot tips on MS medium with half the salt concentration was better than cultivation of branch tips on MS medium at full salt concentration, as it offers a higher rooting percentage than MS medium with full salt concentration. This is due to the increase in N/C ratio, an increase in the energy source (carbohydrates) that is necessary for rooting (Hartmann et al., 2002).

Conclusion:

The type and concentrations of growth regulators significantly affect the shoots and leaves number, shoot length, percentage of rooting, roots number, and length of longest root. BA was the best growth regulator for jasmine node multiplication, and for the rooting stage, MS with half salt's strength enriched with 0.5 mg L^{-1} IBA was preferred.

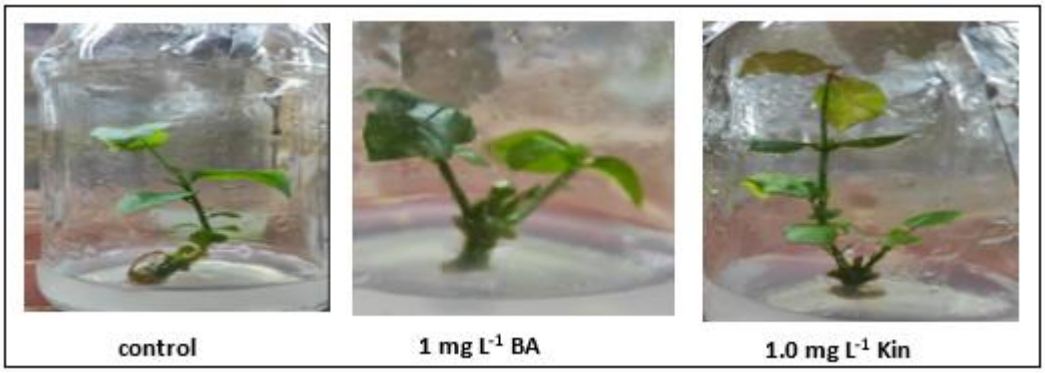

Figure (1): **Multiplication stage.**

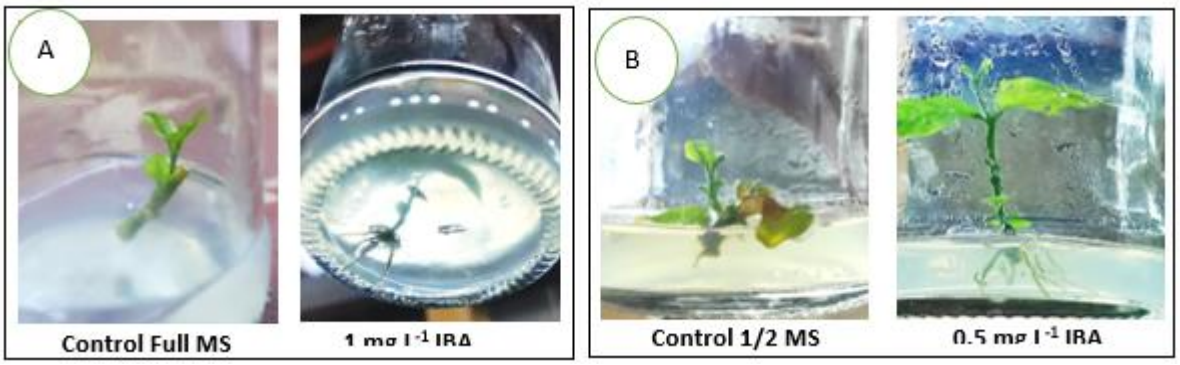

Figure (2): Rooting stages, A: full MS, B: 1/2MS.

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