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Direct Plant Regeneration from Nodal Explants of Grapefruit (*Citrus paradisi* Macfad.)

Cheranda Muddappa Bhavishya [a], Puttanaik Venkatesha Murthy [a*] and Munirangaiah Shanthala [a]

[a] Department of Horticulture, University of Agricultural Sciences, Bangalore, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was undertaken to develop a protocol for in vitro regeneration and multiplication of disease-free quality planting material of Grapefruit (Citrus paradisi Macfad.).
Study Design: Completely Randomized Design (CRD)
Place and Duration of Study: The study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru, from 2020 to 21.
Methodology: Nodal segments from the young shoots of field growing plants were used as explants to conduct the experiment. The explants were sterilized and placed on MS medium supplemented with different concentrations and combinations of growth regulators, namely BAP, Kinetin and Gibberellic acid.

**Corresponding author: E-mail: pvenkateshamurthy@gmail.com;*

Results: Among the different combinations of growth regulators, the combination of BAP 1.0 mgL-1 and GA3 3.0 mgL-1 produced maximum number of shoots (7) from nodal segments
Conclusion: The use of growth regulators such as BAP and GA3 is reliable for shoot regeneration even when the field explants are used.

Keywords: In-vitro; BAP; kinetin; gibberellic acid; IBA.

1. INTRODUCTION

A member of the genus citrus, Grapefruit (*Citrus paradisi* Macfad), has an interesting history, its one-of-a-kind origin is shrouded in mystery. Hughes in 1750 [1] described a tree growing as the "forbidden fruit" in Barbados, although Browne (1756) [2] reported the same and associated it to pummelo (*Citrus maxima*), which was called "smaller shaddock". Later, Macfadyen (1830) [3] classified the “forbidden fruit” reported by both Browne and Hughes as *Citrus paradisi*, also known as “Barbados grapefruit”. Chemotaxonomy [4] and molecular data [5] suggest that accidental hybridization produced an interspecific hybrid of pummelo (*Citrus grandis*) and sweet orange (*Citrus sinensis*), which is now classified as grapefruit.

The *in vitro* regeneration of grapefruit plants offers the benefit of producing disease-free planting material and a source for transformation experiments. The traditional method of propagation is either through budding or grafting which have posed the problem of graft transmissible diseases [6]. However, crop improvement is hindered by the biological characteristics of woody plants, such as nucellar polyembryony, high heterozygosity, long juvenile period, and auto incompatibility [7].

In vitro regeneration is one of the most important steps in plant transformation experiments, and it enables crop improvement. Several studies have been conducted to establish a protocol for the *in vitro* regeneration of grapefruit using explants such as seeds [8] and leaves [9], nodal segments [10], internodal segments [11], epicotyl [12] and roots.

Plant growth regulators play a pivotal role in plant tissue culture which influences the growth and development of plants in vitro. BAP is one of the cytokines that can encourage the division of meristemic cells so that the formation of shoots can take place [13]. Kinetin also promotes proliferation of shoots and helps in promoting plant height [14]. Gibberellic acid (GA3) has long been known to regulate shoot and root growth. Many reports suggests that GA3 and cytokinins act antagonistically. The lower dose of of GA3 ameliorate the activity of many cytokinins including during in vitro developmental processes like shoot and root elongation, multiple shoot induction and cell differentiation [15].

Tissue culture studies have been conducted in various citrus species such as in Kinnow mandarin, where, BAP at 1mg/L have shown a promising result for early shoot induction in a time period of 4 weeks, whereas Kinetin at 1.5mg/L showed highest number of shoots (7), but it took a longer duration for the results [16]. An experimental report on shoot organogenesis in *Citrus jambhiri* Lush, mentions about the high multiple shoot proliferation with an average of 34.3 shoots per root explant when inoculated on the MSN (MS medium with Nitsch vitamins) medium supplemented with BAP (1.0 mgL^{-1}) and GA_3 (1.0 mgL^{-1}) [17]. IAA has influenced root formation in citrus plants with maximum rooting when MS medium was supplemented with 2.5 mgL^{-1} IAA. The root system were well formed and successfully hardened and acclimatized [18]. IBA at 10 μM concentration was said to influence rooting in Regenerated shoots of *Citrus chrysocarpa* [19].

This study aimed to develop a protocol for *in vitro* regeneration of grapefruit (*Citrus paradisi* Macfad) using different plant growth regulators such as BAP, Kinetin and GA3 for promoting shoot agrowth and IBA, IAA for root gowth.

2. MATERIALS AND METHODS

The study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru, from 2020 to 21. The media used in the in vitro study were full-strength solid MS (Murashige and Skoog, 1962) [20] media for both shoot and root initiation studies.

Young nodal segments from field-grown trees were used as explants in the experiments. The field explants (nodal segments) were subjected

to running tap water for 20 minutes and further sterilization was carried out under aseptic conditions. The explants were treated with 0.5 per cent bavistin followed by three sterile water washes (5 minutes each). The explants were treated with 0.3 per of mercuric chloride ($HgCl_2$) for 4 minutes, washed with sterile distilled water three times (5 minutes each), and transferred to 70 per cent alcohol for 1 minute. The explants were then cut to a length of 1 cm, comprising one node each, and transferred to a solution containing 0.1 per cent streptomycin for half an hour. After treating the explants with streptomycin, the cut explants were placed on the media after the moisture on the explant surface dried. Each treatment consisted of ten explants.

The explants were placed on medium consisting of MS salts supplemented with 3 per cent (w/v) sucrose, and the media were solidified by 0.6 per cent agar with different concentrations of growth regulators, viz., cytokinin with concentrations of 0.5, 1.0, 1.5, and 2.0 mg L-1 BAP (6 Benzyl amino purine), KIN (Kinetin) with concentrations of 0.5, 1.0, 2.0, and 3.0 mg L-1. In another experiment BAP was combined with Kinetin, also BAP and GA_3 (0.5, 1.0, 2.0, and 3.0 mg L-1) combinations were tried to analyse the combination effects. IBA (Indole-butyric acid) and IAA (indole-acetic acid) with concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mgL^{-1} for root initiation.

Therefore, the experiments conducted in the study are as follows:

1. Influence of BAP and Kinetin on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant
2. Influence of BAP and Kinetin combinations on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant
3. Influence of BAP and GA_3 combination on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant
4. Influence of IBA and IAA alone on days for root initiation

The culture bottles were maintained in a growth room at a temperature of 24 ± 2^0C. A light intensity of 2000 lx was provided using white fluorescence tubes for eight hours of dark and 16 h of light, which was the general set-up of the laboratory. The chamber was fumigated with potassium dichromate and formaldehyde weekly.

The observations such as number of days taken for shoot initiation, number of shoots and shoot length were recorded. The days taken for shoot initiation were observed at weekly intervals to record the exact time taken. Later the total number of shoots and shoot length were recorded at 90 days after shoot initiation.

The experiments were laid out in completely randomized design (CRD) to test the significance of variation in experimental results obtained from the various treatments. In all the experiments five replications were taken to record the data. The data was recorded to find out the effect of PGRs on different parameters like No of days taken for initiation, no of shoots, shoot length, no of roots and root length from nodal explants of Citrus paradisi Macfad. The complete data was analyzed using CRD. The critical difference of the experimental data was tested by using F test at 1 per cent level of confidence. The analysis was done using OPSTAT online analysis tool (http://14.139.232.166/opstat/).

3. RESULTS AND DISCUSSION

3.1 Effect of BAP and Kinetin on Different Regeneration Parameters

In the first experiment, we observed the effects of BAP and Kinetin on shoot regeneration. The nodes were cultured separately on MS medium supplemented with BAP and Kinetin**.**

When nodes were cultured on the media, BAP 1 mgL^{-1} showed early shoot initiation (39.4 days), and the number of shoots was higher in BAP 0.5 mgL^{-1} (2.6) and BAP 1 mgL^{-1} (2.4). Shoot length (1.08 cm) was also the highest at BAP 1 mgL^{-1} (Table 1 and Fig. 1). The variation in the days taken for shoot initiation at higher concentrations of BAP might be due to the increased level of BAP, which shows a significant effect on shoot initiation, and the low concentration may be insufficient to promote or accelerate shoot growth.

After the initial development of buds, the shoots did not regenerate for 30 to 60 days, but bud proliferation continued. None of the buds regenerated into shoots; some buds were arrested at the bud proliferation phase, along

with callus formation, and did not form shoots. These observations were also reported previously in sweet orange (*Citrus sinensis*) [21,22]. They suggested that the small size of regenerated buds may be the reason for this problem. Similar observations were recorded in nodal explants of *Citrus indica* [23].

It was noticed that, among all the treatments the concentration of BAP at 1 mgL^{-1} showed increased shoot length. There was no production of shoots at 30 and 60 days, but at 90 days, the maximum shoot length noticed was 1.08 cm after shoot initiation, and the lowest was recorded when BAP was used at a higher concentration of 2 mgL^{-1}. Reports state that, when kinetin was used alone, it exhibited the lowest percentage of shoot induction and number of multiple shoots in Meyer lemons (*Citrus meyeri*), which supports the results of the present study. The synergistic and inhibitory interactions of exogenous and endogenous plant growth regulators are specific, as different species, genotypes, and explant sources largely influence the responses of plant cells and tissues [24]. Many other research findings in rough lemon and cleopatra [25], Pumello (*Citrus grandis* L.) [26] and *Citrus macroptera* [27] also suggest that 1 mgL^{-1} is superior for shoot regeneration.

Table 1. Influence of BAP and Kinetin on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant

Treatments	Days taken for shoot initiation	Number of Shoots (at 90 days)	Shoot Length (at 90 days)
T_1 - Basal medium (Control)	50.0	0.0	0.00
T_2 -BAP 0.5	42.2	2.6	0.34
T_3 -BAP 1.0	39.4	2.4	1.08
T_4 -BAP 1.5	41.2	1.4	0.98
T_5 -BAP 2.0	49.6	1.0	0.24
T_6 -KINETIN 0.5	46.8	1.8	0.54
T_7 -KINETIN 1.0	45.8	2.2	1.00
T_8 -KINETIN 2.0	46.2	1.2	0.70
T_9 -KINETIN 3.0	46.4	1.0	0.50
F-test	*	*	*
S.Em±	**0.722**	**0.277**	**0.277**
CD (1%)	**2.055**	**0.789**	**0.789**

Fig. 1. Shoot formation in BAP 1.0 mgL^{-1}

3.2 Effect of BAP and Kinetin Combination on Different Regeneration Parameters When Nodal Segments were Used as Explants

The effects of BAP and Kinetin levels on the number of days required for shoot initiation are presented in Table 2. The number of days taken for shoot initiation was significantly different between treatments. The earliest shoot initiation was observed at 35.20 days in the media containing BAP 1.5 mgL^{-1} and Kinetin 1 mgL^{-1}. The maximum duration (79.80 days) for shoot initiation was seen in the media with BAP 2.0 mgL^{-1} and Kinetin 3.0 mgL^{-1} (Fig 2 A). Media with BAP 2.0 mgL^{-1} and different combinations of kinetin did not show any response. The explants in such media remained green, but failed to regenerate. This may be due to the supraoptimal concentration of BAP, which was detrimental for shoot proliferation, and also due to the endogenic levels of cytokinin in different species. *In vitro* propagation of *Citrus reticulata* Blanco and *Citrus limon* Burm showed that, the mean minimum number of days required for regeneration was directly dependent on the species and medium combination in which the explants were placed [28].

Although there was proliferation of buds on the explants, none of the buds formed regenerated into shoots. This may be due to the recalcitrant nature of the explants and the small size of the buds. The maximum number of shoots (5.0) at 90 days after shoot initiation was obtained in BAP 1.0 mgL^{-1} with Kinetin 1.0 mgL^{-1} (Fig. 2 B). In Nagpur mandarin when explants were inoculated with MS medium supplemented with BAP (8.88 µl) and (2.32 µl) kinetin maximum shoots (9.11) had regenerated [29]. Similarly, highest number of shoots per explant was found in 1.5 mgL^{-1} Kinetin with 0.5 mgL^{-1} BAP in *Citrus reticulata* [30]. Highest number of multiple shoot formation per explant was observed (4.4 ± 0.4 shoots) in media containing 1.0 mgL^{-1} BAP and 0.50 mgL^{-1} Kinetin from shoots tip explants of *C.megaloxycarpa* [31].

The combination of BAP 2.0 mgL^{-1} with Kinetin 0.5 mgL^{-1} showed highest shoot length of 1.54 cm (Fig. 2 C). The number of shoots produced per explant was inversely proportional to shoot length. The combination of BAP 0.5 mgL^{-1}+ Kinetin 2.0 mgL^{-1}showed the highest shoot length when nodal segments were used as explants for Cleopatra mandarin [32].

Table 2. Influence of BAP and Kinetin combinations on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant

Treatments	Days taken for shoot initiation	Number of Shoots (at 90 days)	Shoot Length (at 90 days)
T1 - Basal medium (Control)	40	0.0	0.00
T2 -BAP 0.5 + Kinetin 0.5	42.6	2.0	1.14
T3 -BAP 0.5 + Kinetin 1.0	35.8	4.2	0.00
T4 -BAP 0.5 + Kinetin 2.0	43.6	2.6	1.12
T5 -BAP 0.5 + Kinetin 3.0	00.0	0.0	0.46
T6 -BAP 1.0 + Kinetin 0.5	36.2	2.2	0.94
T7 -BAP 1.0 + Kinetin 1.0	37.6	5.0	0.44
T8 -BAP 1.0 + Kinetin 2.0	43.0	4.0	0.76
T9 -BAP 1.0 + Kinetin 3.0	00.0	0.0	0.00
T10 -BAP 1.5 + Kinetin 0.5	00.0	0.0	0.00
T11 -BAP 1.5 + Kinetin 1.0	35.2	4.0	1.18
T12 -BAP 1.5 + Kinetin 2.0	41.0	2.2	1.80
T13 -BAP 1.5 + Kinetin 3.0	37.2	3.2	1.08
T14 -BAP 2.0 + Kinetin 0.5	45.8	4.2	1.54
T15 - BAP 2.0 + Kinetin 1.0	00.0	0.0	0.00
T16 - BAP 2.0 + Kinetin 2.0	00.0	0.0	0.00
T17 - BAP 2.0 + Kinetin 3.0	46.4	3.0	1.10
F-test	*	*	*
S.Em±	**1.19**	**0.417**	**0.134**
CD (1%)	**3.36**	**1.18**	**0.378**

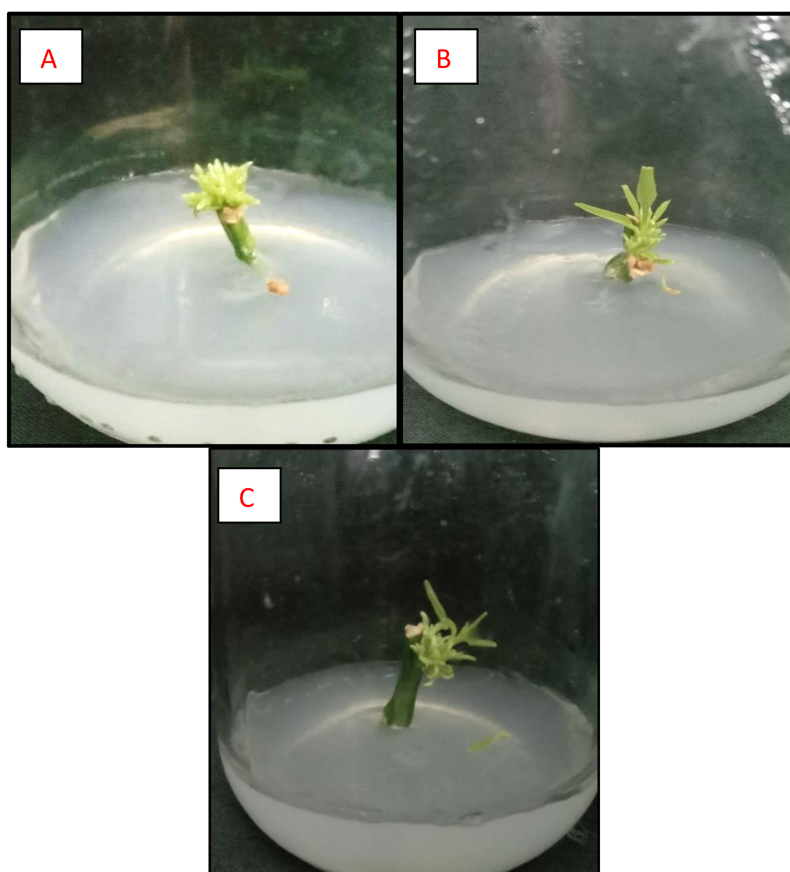

Fig. 2. Influence of BAP and Kinetin on shoot induction from nodal segments. A. Bud proliferation in BAP 0.5 mgL^{-1} + Kinetin 2.0 mgL^{-1} B. Multiple shoot formation at BAP 1.0 mgL^{-1} with Kinetin 1.0 mgL^{-1} C. Shoots formed in BAP 1.5 mgL^{-1} with Kinetin 2 mgL^{-1}

3.3 Effect of BAP and GA_3 on Different Regeneration Parameters When Nodal Segments are Used as Explants

The time taken for shoot initiation from nodal segments when BAP and GA_3 were used is shown in Table 3. Significant differences were observed among the treatment combinations. Among the different combinations, the culture media which had BAP 2.0 mgL^{-1} and GA_3 3.0 mgL^{-1} showed early shoot initiation (31.8 days) compared to all the other combinations and control (Fig. 3A). Lower concentrations required a longer period for shoot initiation. Bud proliferation occurred before complete shoot formation. All the buds that proliferated did not regenerate into shoots. The highest number of shoots (7) was recorded for BAP 1.0 mgL^{-1} and GA_3 3.0 mgL^{-1} (Fig. 3B). The results showed that lower concentrations of BAP and higher concentrations of GA_3 were suitable for producing a greater number of shoots. The number of shoots formed was dependent on the BAP and GA_3 concentrations and the best results with 2 mgL^{-1} BAP and 1 or 2 mgL^{-1} GA_3 were reported in lemon (*Citrus limon*) [33]. Exogenous addition of 4.44 μM BAP in combination with 1.54 μM GA_3 enhanced shoot multiplication rate significantly (17.73±1.69 shoots/explant) in comparison to control (0.00±0.00 shoots/explant) in *Citrus sinensis* (L.) Osbeck [34]. Also, the number of shoots was dependent on the BAP and GA3 concentrations, and the best result was 1 mgL^{-1} BAP + 1.0 mgL^{-1} GA3, and the shoot length was greater with increasing concentrations of GA3 in carrizo (*Citrus carrizo*) [35].

The influence of BAP with GA3 combinations differed significantly among the different concentrations used on the shoot length at 90 days after shoot initiation (Table 3). The maximum length of shoots (1.7 cm) was observed in a medium containing BAP 1.0 mg/L and GA3 2.0 mg/L (Fig. 3C). The treatment with GA3 had the effect of first promoting the multiplication of adventitious shoots and then stimulating their elongation.

Table 3. Influence of BAP and GA_3 combination on number of days taken for shoot initiation, number of shoots and shoot length using nodal segment as explant

Treatments	Days taken for shoot initiation	Number of Shoots (at 90 days)	Shoot Length (at 90 days)
T_1 - Basal medium (Control)	40.0	0.0	0.00
T_2 -BAP 0.5 +$GA_3$0.5	42.8	3.6	1.08
T_3 -BAP 0.5 + GA_3 1.0	37.4	3.0	0.30
T_4 -BAP 0.5 + GA_3 2.0	38.8	3.4	1.16
T_5 -BAP 0.5 + GA_3 3.0	40.0	5.4	1.00
T_6 -BAP 1.0 + GA_3 0.5	34.4	5.0	1.60
T_7 -BAP 1.0 + GA_3 1.0	44.2	3.8	1.00
T_8 -BAP 1.0 + GA_3 2.0	37.4	4.8	1.70
T_9 -BAP 1.0 + GA_3 3.0	61.0	7.0	0.64
T_{10} -BAP 1.5 + GA_3 0.5	38.2	1.4	0.60
T_{11} -BAP 1.5 + GA_3 1.0	52.8	3.0	0.22
T_{12} -BAP 1.5 + GA_3 2.0	60.4	2.4	0.80
T_{13} -BAP 1.5 + GA_3 3.0	59.0	3.2	0.80
T_{14} -BAP 2.0 + GA_3 0.5	60.4	3.4	0.64
T_{15} - BAP 2.0 + GA_3 1.0	61.4	3.0	0.68
T_{16} - BAP 2.0 + GA_3 2.0	39.4	3.4	0.42
T_{17} - BAP 2.0 + GA_3 3.0	31.8	2.8	0.70
F-test	*	*	*
S.Em±	**1.36**	**0.459**	**0.125**
CD (1%)	**3.86**	**1.298**	**0.355**

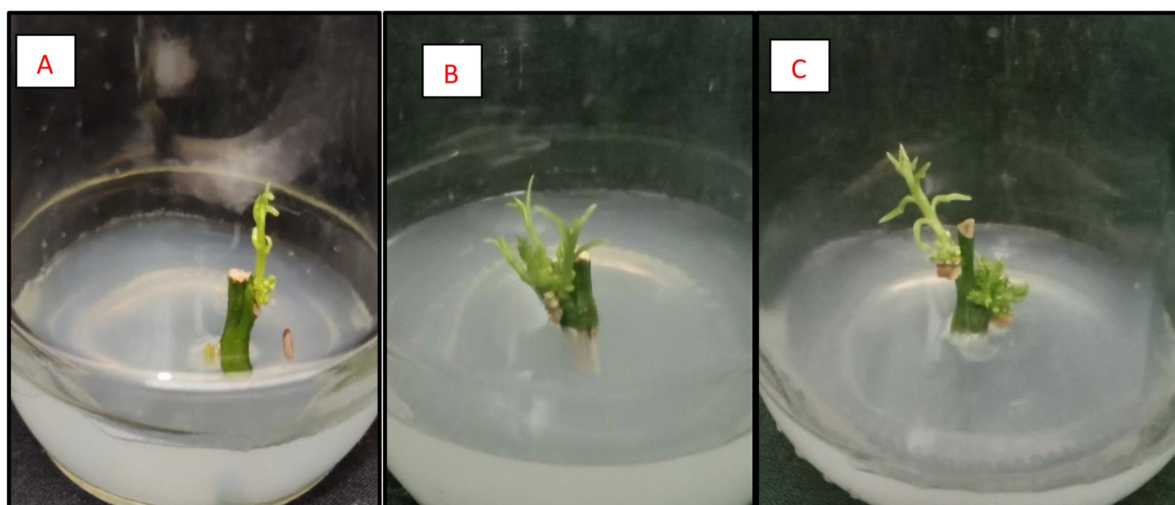

Fig. 3. Influence of BAP and GA_3 on shoot induction from nodal segments. A. Shoot formation in BAP 1.0 mgL^{-1} + GA_3 2.0 mgL^{-1} ,B. Multiple shoot formation at BAP 1.0 mgL^{-1} + GA_3 3.0 mgL^{-1}, C. Shoots formed in BAP 1.0 mgL^{-1} and GA_3 0.5 mgL^{-1}

3.4 Effect of IBA and IAA Alone on Root Regeneration Parameters

For root regeneration, the shoots that were regenerated in the trials were placed on medium enriched with IBA and IAA at varying concentrations from 0.5 to 2.5 mg/L. The effect of different concentrations of IAA and IBA on the number of days taken for root initiation did not show any significant difference among treatments (Table 4). The media supplemented with IAA 0.5 mgL^{-1} showed a better response for the production of roots (1.6). Significant differences were observed in root length after 45 days, and IBA 1 mgL^{-1} showed its supremacy over all the other auxins used in the experiment. It was found that, IBA 1 mgL^{-1} showed a maximum root length of 8.6 cm at 45 days. IBA

Table 4. Influence of IBA and IAA alone on days for root initiation

Treatment	Days for root initiation	Number of roots at 45 Days	Root length (cm) at 45 Days
T_1 - Basal medium (Control)	42.4	0.2	1.0
T_2 – IBA 0.5	12.4	1.0	5.70
T_3 – IBA 1.0	22.4	1.2	8.60
T_4 – IBA 1.5	31.4	1.4	5.96
T_5 - IBA 2.0	12.8	1.0	6.10
T_6 – IBA 2.5	27.0	1.2	6.84
T_7 – IAA 0.5	11.2	1.6	4.32
T_8 – IAA 1.0	19.2	1.2	4.22
T_9 – IAA 1.5	20.0	1.4	6.06
T_{10} – IAA 2.0	23.0	1.0	5.02
T_{11} – IAA 2.5	36.6	1.4	5.10
F-test	**NS**	*	*
S.Em±	**NS**	**0.191**	**2.29**
CD (1%)	**NS**	**0.545**	**0.80**

was better for rooting, as this might be due to the fact that IAA is photo-oxidised rapidly than IBA in tissue culture media. So, IAA degrades soon after initial root induction in the rooting medium. IBA, even at a lower concentration, remained active in the medium for a longer period of time, which positively affected the root length. Some of the reports with similar findings where 1 mgL^{-1} of IBA was used and reported a maximum number of roots, 3.19 per shoot on MS + 1 mgL^{-1}l NAA + 1 mgL^{-1} IBA and a minimum of 0.75 on MS + 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} IBA [36]. Also, higher shoot length at 3 mgL^{-1} IBA were also reported [37].

4. CONCLUSION

The present study reveals that, the combination of BAP 1.0 mgl^{-1} and GA_3 3.0 mgl^{-1} produced maximum number of shoots (7) in nodal segments, which could be used for producing a greater number of planting material. IBA 1 mgl^{-1} produced the longest roots of 8.6 cm at 45 days after root initiation and could be recommended for in vitro rooting of grapefruit plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hughes G. The natural history of Barbados. Book V. Printed for the author, London, UK. Reprinted in 1972 by Arno Press, New York, NY; 1750. Available:https://doi.org/10.5962/bhl.title.47069
2. Browne P. The civil and natural history of Jamaica: In three parts. P. Browne, London, UK; 1756.
3. Macfadyen J. Some remarks on the species of the genus *Citrus* which are cultivated in Jamaica. Bot. Misc. The flora of Jamaica; a description of the plants of that island. 1837;1:295-304.
4. Scora RW, Kumamoto J, Soost RK, Nauer EM. Contribution to the origin of the grapefruit *Citrus paradisi* (Rutaceae). Systematic Botany. 1982;7:170-177. Available:https://doi.org/10.2307/2418325
5. Wu GA, Terol J, Ibanez V, Lopez Garcia A, Perez Roman E, Borreda C, Domingo C, Tadeo FR, Carbonell-Caballero J, Alonso R, Curk F. Genomics of the origin and evolution of *Citrus*. Nature. 2018; 554(7692):311-316. Available:https://doi.org/10.1038/nature25447
6. Saponari M, Zicca S, Loconsole G, Navarro B, Di Serio F. Detection of *Citrus tristeza* virus and coinfecting viroids. *Citrus Tristeza* Virus: Methods and Protocols. 2019;67-78.
7. Salonia F, Ciacciulli A, Poles L, Pappalardo HD, La Malfa S, Licciardello C. New plant breeding techniques in citrus for the improvement of important agronomic

traits. A Review. Frontiers in Plant Science. 2020;11:1234.

8. Niedz RP, Evens TJ. Mixture screening and mixture-amount designs to determine plant growth regulator effects on shoot regeneration from grapefruit (*Citrus paradisi* macf.) epicotyls. *In Vitro* Cellular and Developmental Biology-Plant. 2011; 47(6):682-694.
   Available:https://doi.org/10.1007/s11627-011-9381-4
9. Ye W, Guo X, Liu H, Wang L, Liu P, Wu H. Establishment of rapid propagation for sterile seedlings of grapefruit by tissue culture. Guangxi Zhiwu/Guihaia. 2015; 35(6):891-898.
10. Ghorbel R, Navarro L, Durán-Vila N. Morphogenesis and regeneration of whole plants of grapefruit (*Citrus paradisi*), sour orange (*C aurantium*) and alemow (*C. macrophylla*). The Journal of Horticultural Science and Biotechnology. 1998;73(3): 323-327.
    Available:https://doi.org/10.1080/14620316.1998.11510981
11. Marutani-Hert M, Bowman KD, Mccollum GT, Mirkov TE, Evens TJ, Niedz RP. A dark incubation period is important for Agrobacterium-mediated transformation of mature internode explants of sweet orange, grapefruit, citron, and a citrange rootstock. Plos One. 2012;7(10): e47426.
    Available:https://doi.org/10.1371/journal.pone.0047426
12. Lu R, Han M, Huo X, Yang Y, 2013. Study on regeneration system of adventitious bud from grapefruit. Acta Agriculturae Jiangxi. 25(6):35-38.
13. Handayani RS, Yunus I, Tillah N, Handayani I. Effect of cytokines on the in vitro of sweet kaffir lime (*Citrus hystrix* Dc). Journal of Tropical Horticulture. 2020;3(2): 60-64.
14. El-Gedawey HI, Abido AI, Gaber MK. Impact of Kinetin and Benzyladenine on growth performance of croton *In vitro*. Alexandria Science Exchange Journal. 2020;41(July-September):381-391.
15. Ahmad A, Ahmad N, Anis M, Alatar AA, Abdel-Salam EM, Qahtan AA, Faisal M. Gibberellic acid and thidiazuron promote micropropagation of an endangered woody tree (*Pterocarpus marsupium* Roxb.) using in vitro seedlings. Plant Cell, Tissue and Organ Culture (PCTOC). 2021;144: 449-462.
16. Hussain M, Raja NI, Rashid H, Mashwani Z, Mehmood A, Iqbal M. Establishment of an efficient protocol for plantlets regeneration via direct and indirect organogenesis in Citrus reticulate blanco (kinnow mandarin). Pakistan Journal of Botany. 2018;50(3):1203-1210.
17. Devi TR, Dasgupta M, Sahoo MR, Kole PC, Prakash N. High efficient de novo root-to-shoot organogenesis in *Citrus jambhiri* Lush. Gene expression, genetic stability and virus indexing. Plos One. 2021;16(2): e0246971.
18. Bhalero SR, Kumre KR. Multiple shoot formation and plant regeneration of a commercially useful tropical plant Citrus aurantifolia (Swingle). Research Journal of Biotechnology. 2019 Nov:14:11.
19. Prodhan SH, Hasan N, Hoque H, Alam SS, Hasan MR, Gupta A, Khatun MUS, Parvin A, Joy ZF. Development of an efficient in vitro regeneration system for endangered wild orange *Citrus chrysocarpa* L. International Journal of Sciences: Basic and Applied Research. 2016;4531:187-96.
20. Murashige I, Skoog F. A revised medium for rapid growth and bioassays with tobacco culture. Physiologia Plantarum. 1962;15:473-9.
    Available:https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
21. Almeida WAB, Mourao Filho FAA, Pino LE, Boscariol RL, Rodriguez APM, Mendes BMJ. Genetic transformation and plant recovery from mature tissues of Citrus sinensis L. Osbeck. Plant Science. 2003; 164(2):203-211.
    Available:https://doi.org/10.1016/S0168-9452(02)00401-6
22. Kobayashi AK, Bespalhok JC, Pereira LFP, Vieira LGE. Plant regeneration of sweet orange (*Citrus sinensis*) from thin sections of mature stem segments. Plant Cell Tissue and Organ Culture. 2003;74: 99-102.
23. Sangma SYA, Pereira LS, Dang JC Mathew B. Evaluation of explants for *In vitro* propagation of Citrus indica Tanaka-An Endangered Species. Plant Tissue Culture and Biotechnology. 2020;30(1): 87-96.
    Available:https://doi.org/10.3329/ptcb.v30i1.47794

24. Haradzi NA, Khor SP, Subramaniam S, Chew BL. Regeneration and micropropagation of Meyer lemon (*Citrus x meyeri*) supported by polymorphism analysis via molecular markers. Scientia Horticulturae. 2021;286:110225. Available:https://doi.org/10.1016/j.scienta.2021.110225
25. Mwaniki WI, Lubabali AH, Asava KK, Agwanda CO, Anami SE. Effects of genotype and plant growth regulators on callus induction in leaf cultures of Coffea arabica L. F1 hybrid. African Journal of Biotechnology. 2019;18(31):1004-1015. Available:https://doi.org/10.5897/AJB2019.16913
26. Sharma S, Prakash A, Ajinath TELE. In vitro propagation of Citrus rootstocks. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2009;37(1):84-88.
27. Begum F, Amin MN, Islam S, Azad MAK. A comparative study of axillary shoot proliferation from the nodal explants of three varieties of Pumello (*Citrus grandis* L.), Biotechnology (Pakistan), 2004;3(1): 56-62. Available:https://doi.org/10.3923/biotech.2004.56.62
28. Miah MN, Islam S, Hadiuzzaman S. An improved protocol for multiple shoot regeneration from seedlings and mature explants of *Citrus macroptera* Mont. Plant Tissue Culture and Biotechnology. 2008; 18(1):17-24. Available:https://doi.org/10.3329/ptcb.v18i1.3246
29. Singh S, Roy BK, Bhattacharya S, Deka PC. *In vitro* propagation of *Citrus reticulata* Blanco and *Citrus limon* Burm. Hort Science. 1994;29(3):214-216. Available:https://doi.org/10.21273/HORTSCI.29.3.214
30. Mukhtar R, Khan MM, Fatima B, Abbas M, Shahid A. *In vitro* regeneration and multiple shoots induction in *Citrus reticulata* (Blanco). International Journal of Agriculture and Biology. 2005;7(3):414-416.
31. Haripyaree A, Guneshwor K, Sunitibala H, Damayanti M. *In vitro* propagation of *Citrus megaloxycarpa*. Environmental and Experimental Botany. 2011;9:129-132.
32. Kumar R, Kaul MK, Saxena SN, Singh AK, Singh J, Lohora SK. In vitro propagation studies of virus tolerant citrus rootstock Cleopatra mandarin (*Citrus reshnii Tanaka*). Progressive Horticulture. 2014; 46(2):202-208.
33. Perez-Tornero O, Tallon OC, Porras I. An efficient protocol for micropropagation of lemon (*Citrus limon*) from mature nodal segments. Plant Cell Tissue and Organ Culture. 2010;100(3):263-271. Available:https://doi.org/10.1007/s11240-009-9643-6
34. Pandey A, Tamta S. Efficient micropropagation of *Citrus sinensis* (L.) Osbeck from cotyledonary explants suitable for the development of commercial variety. African Journal of Biotechnology. 2016;15(34):1806-1812. Available:https://doi.org/10.5897/AJB2015.14986
35. Kanwar J, Godara S, Kaul MK, Srivastava AK. Micro propagation of carrizo (*Citrus carrizo*) through mature bud culture. Agricultural Science Digest- A Research Journal. 2013;33(2):109-113.
36. Saini HK, Gill MS, Gill MIS. Direct shoot organogenesis and plant regeneration in rough lemon (*Citrus jambhiri* Lush.). Indian Journal of Biotechnology. 2010; 9(4): 419-423.
37. Gurnani C, Choure K, Mukhija S, Kumar V. *In vitro* conservation of Citrus paradisi Macfd, redblush through clonal propagation. World Journal of Pharmaceutical Research. 2015; 5(01): 1082-1091.

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