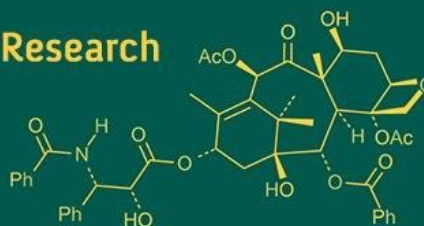


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Effect of growth regulators on multiple shoot induction from *in vitro* single node of Nagpur mandarin (*Citrus reticulata* Blanco)

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Abstract

Nagpur mandarin is the most important commercially grown citrus cultivar in the central part of India. It holds a dominant position due to its suitability for consumption as fresh fruit, dessert fruit, and for its excellent juice with a pleasant flavor (Karwa, A., 2003). However, conventional propagation of Nagpur mandarin is limited due to its susceptibility to various diseases, genetic variability, labor-intensive processes, and risk of poor-quality fruit production. Therefore, the present study aimed to develop an *in vitro* regeneration protocol for the rapid multiplication of mandarin (*C. reticulata*). Single nodes from *in vitro* plantlets derived from seeds were used as explants. These explants were cultured on full-strength MS medium supplemented with various concentrations and combinations of plant growth regulators, including BAP, Kinetin, and GA₃ for shoot regeneration and half MS medium supplemented with various concentrations of IBA and NAA for root regeneration. The combination of BAP (0.5 mg/L) + GA₃ (1.0 mg/L) produced the highest number of shoots (4.8) at 90 days after shoot initiation. The greatest shoot length was recorded with GA₃ (1.5 mg/L), while the highest number of roots (3.20) was observed with NAA (2.5 mg/L), and the longest root (10.52 cm) was recorded with IBA (2.0 mg/L).

Keywords: Nagpur mandarin, growth regulator, *in vitro* regeneration, single nodes

Introduction

Among the fruit crops grown in India, mandarin (*Citrus reticulata* Blanco) ranks third in terms of area and production, following mango and banana. The “Nagpur Mandarin” is considered one of the most important and finest mandarin varieties, particularly suited for cultivation in subtropical climates. In India, it is widely grown in the states of: Maharashtra (Nagpur, Akola, Amravati, Wardha), Rajasthan (Jhalawar, Kota), Karnataka (Chikmagalur, Kodagu, Hassan), Madhya Pradesh (Chhindwara, Mandsaur, Betul, Ujjain, Shajapur), Nagaland (Wokha, Tuensang), Mizoram (Aizawl), Assam (Tinsukia, North Cachar Hills, Karbi Anglong), Meghalaya (East & West Khasi Hills, Ri-Bhoi, Garo Hills, Jaintia Hills), Tamil Nadu (Dindigul, Salem, Nilgiris), West Bengal (Darjeeling). Nagpur Mandarin is a well-recognized citrus variety, primarily cultivated in Maharashtra's Nagpur region. The fruits are small to medium in size, slightly flattened, with a thin, loose peel that separates easily from the segments. The flowers are small to medium-sized, and the fruits are typically depressed-globose or sub-globose in shape. Seeds are small, pointed, and possess green cotyledons (Singh *et al.*, 2021)^[30].

Nagpur mandarin is prized for its distinctive taste, juiciness, and vibrant color, making it one of the most popular and highly sought-after citrus varieties in India with GI tag. It is a commercially significant cultivar, contributing approximately 41% of the country's total citrus production, with an average yield of 6.0 tons per hectare. The Vidarbha region of Maharashtra, along with adjoining areas of Madhya Pradesh and Rajasthan particularly the Jhalawar district shares similar agro-climatic conditions, supporting the successful and expanding cultivation of this variety (Karwa, 2003)^[14].

In vitro regeneration is one of the most important steps in plant transformation experiments, as it enables crop improvement. Several studies have been conducted to establish protocols for the *in vitro* regeneration of mandarin using explants such as seeds, leaves, nodal segments, internodal segments, and roots (Singh *et al.*, 2018; Hasan *et al.*, 2016; Kawtar

2019; Pérez and Ochoa, 1997)^[30, 10, 15, 21].

Plant growth regulators play a pivotal role in plant tissue culture, influencing the growth and development of plants *in vitro*. BAP is one of the cytokinins that encourages the division of meristematic cells, thereby facilitating shoot formation (Handayani *et al.*, 2020)^[9]. Kinetin also promotes the proliferation of shoots and contributes to increased plant height. Gibberellic acid (GA₃) has long been recognized for its role in regulating shoot and root growth. Many reports suggest that GA₃ and cytokinins act antagonistically. A lower dose of GA₃ can enhance the activity of various cytokinins during *in vitro* developmental processes such as shoot and root elongation, multiple shoot induction, and cell differentiation (El-Gedawey *et al.*, 2020)^[6]. Tissue culture studies have been conducted on various citrus species, including Kinnow mandarin, where BAP at a concentration of 1 mg/L has shown promising results for early shoot induction within a period of four weeks. In contrast, kinetin at 1.5 mg/L produced the highest number of shoots (7), although it required a longer duration to achieve results (Hussain *et al.*, 2018)^[12]. Additionally, IBA at a concentration of 10 µM was reported to promote rooting in regenerated shoots of *Citrus chrysocarpa* (Prodhan *et al.*, 2016)^[10].

This research paper aims to establish a standardized micropropagation protocol for Nagpur mandarin, with a specific focus on using single-node segments as explants. It also explores the potential of individual and combined plant growth regulators to enhance and potentially revolutionize the cultivation of this economically important citrus variety.

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, during the years 2022 to 2024. Single node collected from seedling raised through seeds were used as explant at this experiment, the seeds were collected from healthy and ripe fresh fruits, by cutting the fruit horizontally without causing any damage to the seeds. The seeds were then removed from the fruits and soaked in water for 2 to 3 hours in tap water. The extracted seeds were washed thoroughly to remove the sticky pulp from the surface to enable easy handling without any slip. Surface sterilization was carried under the aseptic condition just before inoculation. The chemicals concentration and the duration of their use is presented in Table 1.

Table 1: Chemicals used in the sterilization process of seeds

Chemicals	Concentratio (%)	Duration (minutes)
Bavistin	0.2	15
Sodium hypochlorite + Tween 20	5	15
Mercuric chloride (HgCl ₂)	0.1	3
Alcohol	70	1

The seeds were transferred to bottle containing 0.2 percent of Bavistin (Carbendazim 50% WP) and continuously shaken for 15 minutes and rinsed with sterile water for 3 times (5 minutes each). The seeds were transferred to bottle containing 5 percent sodium hypochlorite along with Tween 20, a wetting agent and were continuously shaken for 15 minutes followed by 3 sterile water wash (5 minutes each). After this the seed coat was removed using forceps and scalpel followed by treatment with 0.1 percent Mercuric chloride (HgCl₂) for 3 minutes. The seeds were then again washed with sterile water for 3 times (5 minutes each). Then the seeds were transferred to 70 percent alcohol for a period of 1 minute after which they were placed on the basal medium. Removal of seed coat is necessary to facilitates easy germination when compared to intact seed coat because seeds cultured with the seed coat may take longer to germinate or may not germinate at all.

The *in vitro* single nodes explants were placed on medium consisting of MS salts supplemented with 3 percent (w/v) sucrose and the media were solidified by 0.6 percent agar having different concentrations of growth regulators *viz.*, cytokinin with concentration of 0.5, 1.0, 1.5, and 2.0 mg/L⁻¹ BAP, Kinetin with concentration of 0.5, 1.0, 1.5 and 2.0 mg/L⁻¹ and Gibberellin such as GA₃ concentration of 0.5, 1.0, 1.5, and 2.0 mg/L⁻¹ were used for shoot proliferation.

Results and Discussion

Effect of BAP, Kinetin and GA₃ on different regeneration parameters when single node segment is used as explant

In vitro single-node segments were cultured separately on MS medium supplemented with BAP, Kinetin and GA₃ in

different concentrations. Among the treatments, BAP at 1 mg/L produced the highest number of shoots after 90 days (Table 2). Better shoot initiation observed at low concentrations of BAP may be attributed to the delicate hormonal balance required for optimal organogenesis in citrus species, which are known to be sensitive to elevated cytokinin levels. Paudyal and Haq (2000)^[20] highlighted the importance of BAP as a key plant growth regulator for promoting shoot induction. In alignment with their findings, the current study found that lower concentrations of BAP led to the highest shoot development, indicating that excessive BAP concentrations may hinder shoot growth. These observations align with the work of Costa *et al.*, (2004)^[5], who reported variation in shoot production at a 1 mg/L BAP concentration in Foster Grapefruit, and Haripyaaree *et al.*, (2011)^[8], who found the greatest shoot proliferation on MS medium with 0.25 mg/L BAP in *Citrus megaloxycarpa* Lush. Similar experiments have also been conducted on seedless lemon (*Citrus limon* L. cv. Kaghzi Kalan), and grapefruit. All GA₃ treatments showed positive effects on shoot elongation., GA₃ at 1.5 mg/L resulted in the greatest shoot length, reaching 2.52 cm Table 3. This observation aligns with findings by (Perez-Tornero *et al.*, 2008)^[22] in lemon, where GA₃ was found to significantly enhance shoot elongation. Similarly, (Cohen *et al.*, 1987)^[4] reported the role of GA₃ in promoting shoot growth in citrus, emphasizing its contribution to shoot elongation and improved canopy structure. GA₃ acts specifically on internodes the segments of the stem between nodes stimulating their elongation, which leads to an overall increase in shoot length Hedden and Phillips (2000)^[11].

Table 2: Influence of Cytokinin (BAP, Kinetin and GA₃) on number of shoots at 30, 60 and 90 days from nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Number of shoots produced from Nodal segment		
	30 (day)	60 (day)	90 (day)
T ₁ Basal medium (Control)	1.80	2.20	2.20
T ₂ BAP 0.5	1.60	1.80	3.20
T ₃ BAP 1.0	1.60	2.60	2.40
T ₄ BAP 1.5	1.80	2.40	2.00
T ₅ BAP 2.0	1.00	1.40	1.40
T ₆ Kinetin 0.5	1.00	1.40	1.40
T ₇ Kinetin 1.0	1.00	1.20	1.60
T ₈ Kinetin 1.5	1.40	1.80	1.80
T ₉ Kinetin 2.0	1.00	1.20	1.40
T ₁₀ GA ₃ 0.5	1.00	1.00	1.40
T ₁₁ GA ₃ 1.0	1.20	1.20	1.40
T ₁₂ GA ₃ 1.5	1.00	1.20	1.20
T ₁₃ GA ₃ 2.0	1.00	1.00	1.60
F-test	*	*	1.11
S.Em±	0.21	0.38	0.52
CD (1%)	0.81	1.45	NS



(A)



(B)



(C)

Fig 1: Influence of BAP, KIN and GA₃ on shoot induction from single node. A. Shoot formation in BAP 0.5 mg/L⁻¹ at 30 days B. Shoot formation in BAP 0.5 mg/L⁻¹ at 60 days C. Shoot formation in BAP 0.5 mg/L⁻¹ at 90 days**Table 3:** Influence of cytokinin (BAP, Kinetin) and GA₃ on shoot length at 30, 60 and 90 days from nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Length of shoots in (cm)		
	30 (day)	60 (day)	90 (day)
T ₁ Basal medium (Control)	1.20	1.54	1.74
T ₂ BAP 0.5	1.16	1.32	1.42
T ₃ BAP 1.0	0.86	1.28	1.30
T ₄ BAP 1.5	1.12	1.16	1.22
T ₅ BAP 2.0	0.54	0.58	0.58
T ₆ Kinetin 0.5	1.48	1.66	1.64
T ₇ Kinetin 1.0	1.10	1.46	1.62
T ₈ Kinetin 1.5	1.40	1.18	1.22
T ₉ Kinetin 2.0	1.08	1.22	1.48
T ₁₀ GA ₃ 0.5	0.60	0.78	1.26
T ₁₁ GA ₃ 1.0	1.30	1.70	2.26
T ₁₂ GA ₃ 1.5	1.48	1.70	2.52
T ₁₃ GA ₃ 2.0	0.48	0.94	2.02
F-test	*	1.78	*
S.Em±	0.22	0.26	0.24
CD (1%)	0.86	NS	0.93

Effect of BAP and GA₃ on different regeneration parameters when single nodes are used as explants

Among the various combinations of BAP and GA₃ tested, the highest number of shoots was recorded with 0.5 mg/L BAP and 1.0 mg/L GA₃ (Table 4). These results suggest that lower concentrations of BAP combined with higher concentrations of GA₃ were optimal for promoting shoot proliferation. Shoot formation was significantly influenced by the concentrations of both BAP and GA₃. (Sarker *et al.*, 2015) [26] reported optimal shoot proliferation and elongation in lime when explants were cultured on MS medium supplemented with BAP and GA₃. Similarly, (Syamal *et al.*, 2007) [31] and (Pérez-Tornero *et al.*, 2009) observed the highest number of shoots from nodal segments of *Kagzi lime* and *Citrus limon*, respectively, using various combinations of BAP and GA₃. (Navarro-García *et al.*,

2016) [19] demonstrated that in *Citrus limon*, the highest development of adventitious buds occurred when nodal segments were treated with MS medium containing 1-3 mg/L BAP and 1 mg/L GA₃.

The effect of different combinations of BAP and GA₃ on shoot length varied significantly across the concentrations tested. The maximum shoot length (2.28 cm) was observed in a medium containing 1.0 mg/L BAP and 1.5 mg/L GA₃ (Table 5). GA₃ promoted the multiplication of adventitious shoots and stimulated their elongation. When combined with BAP, GA₃ enhanced the shoot multiplication rate, and their synergistic effect resulted in more vigorous and elongated shoots. However, as the number of shoots increased, the shoot length tended to decrease, as observed by (Upadhyay *et al.*, 2010) [32].

Table 4: Influence of BAP and GA₃ on production of multiple shoots at 30 60 and 90 days from Nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Number of shoots produced from Nodal segment		
	30 (day)	60 (day)	90 (day)
T ₁ Basal medium (Control)	1.80	2.20	2.20
T ₂ BAP 0.5 + GA ₃ 0.5	2.00	2.80	4.20
T ₃ BAP 0.5 + GA ₃ 1.0	2.00	4.00	4.80
T ₄ BAP 0.5 + GA ₃ 1.5	2.40	3.80	4.00
T ₅ BAP 0.5 + GA ₃ 2.0	2.00	2.80	3.40
T ₆ BAP 1.0 + GA ₃ 0.5	1.40	2.00	2.00
T ₇ BAP 1.0 + GA ₃ 1.0	1.60	3.80	4.00
T ₈ BAP 1.0 + GA ₃ 1.5	2.40	3.40	3.80
T ₉ BAP 1.0 + GA ₃ 2.0	1.00	1.20	2.60
T ₁₀ BAP 1.5 + GA ₃ 0.5	1.40	1.40	1.80
T ₁₁ BAP 1.5 + GA ₃ 1.0	1.00	1.00	1.00
T ₁₂ BAP 1.5 + GA ₃ 1.5	1.00	1.00	1.00
T ₁₃ BAP 1.5 + GA ₃ 2.0	2.40	2.40	2.40
F-test	1.60	3.81	4.06
S.Em±	0.42	0.93	1.06
CD (1%)	NS	NS	NS

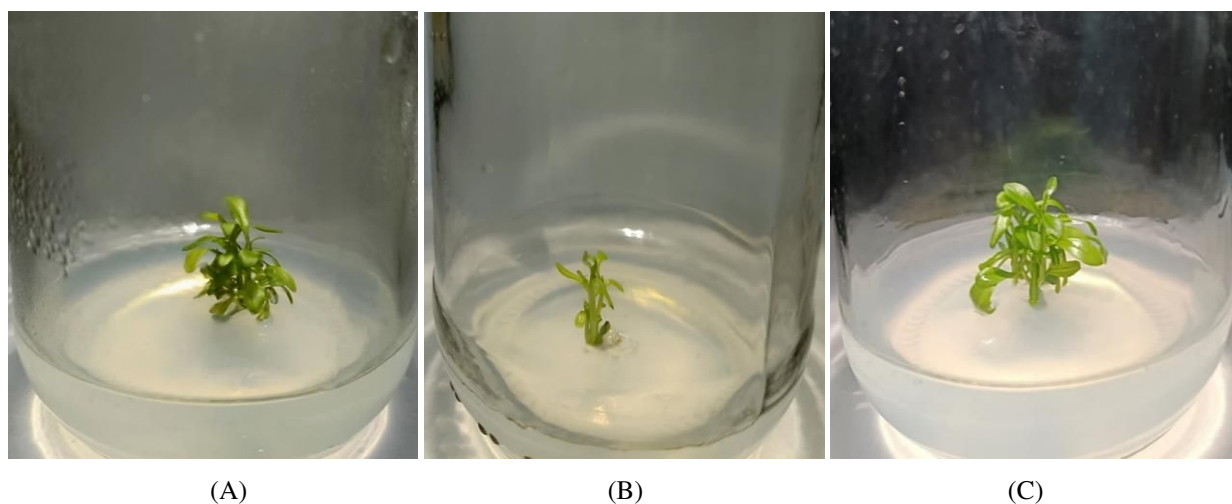


Fig 2: Influence of BAP and GA₃ on shoot induction from single node. A. Shoot formation in BAP 0.5 + GA₃ 1.0 mg/L⁻¹ at 30 days B. Shoot formation in BAP 0.5 + GA₃ 1.0 mg/L⁻¹ at 60 days C. Shoot formation in BAP 0.5 + GA₃ 1.0 mg/L⁻¹ at 90 days.

Table 05: Influence of BAP and GA₃ on shoot length at 30, 60 and 90 days from nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Length of shoots in (cm)		
	30 (day)	60 (day)	90 (day)
T ₁ Basal medium (Control)	1.20	1.54	1.74
T ₂ BAP 0.5 + GA ₃ 0.5	0.72	1.46	1.96
T ₃ BAP 0.5 + GA ₃ 1.0	1.42	1.70	1.82
T ₄ BAP 0.5 + GA ₃ 1.5	1.56	2.02	2.10
T ₅ BAP 0.5 + GA ₃ 2.0	1.40	1.64	1.70
T ₆ BAP 1.0 + GA ₃ 0.5	0.92	1.06	1.10
T ₇ BAP 1.0 + GA ₃ 1.0	0.70	1.02	1.14
T ₈ BAP 1.0 + GA ₃ 1.5	1.54	2.12	2.28
T ₉ BAP 1.0 + GA ₃ 2.0	0.58	0.88	1.40
T ₁₀ BAP 1.5 + GA ₃ 0.5	1.48	1.58	1.66
T ₁₁ BAP 1.5 + GA ₃ 1.0	0.52	0.54	0.68
T ₁₂ BAP 1.5 + GA ₃ 1.5	0.42	0.42	0.48
T ₁₃ BAP 1.5 + GA ₃ 2.0	1.28	1.42	1.52
F-test	1.85	*	*
S.Em±	0.31	0.37	0.37
CD (1%)	NS	1.41	1.41

Effect of BAP and Kinetin combination on different regeneration parameters when single node is used as explant

Maximum number of shoots at 90 days was recorded with a combination of 1.0 mg/L BAP and 1.0 mg/L Kinetin. In a related study, explants cultured on MS medium supplemented with 8.88 mg/L BAP and 2.32 mg/L Kinetin produced the highest number of shoots in Nagpur mandarin. Similarly, (Mukhtar *et al.*, 2005) [18] reported that a combination of 1.5 mg/L Kinetin and 0.5 mg/L BAP resulted in the greatest number of shoots per explant in *Citrus reticulata*. Hariyaree *et al.*, (2011) [8] found that the combination of 0.25 mg/L BAP and 0.50 mg/L NAA led to the highest formation of multiple shoots, followed by a combination of 1.0 mg/L BAP and 0.50 mg/L Kinetin, resulting in more shoots per explant from citrus shoot tips. Additionally, Kanwar *et al.*, (2016) [13] reported successful micropropagation of Sour Orange (*Citrus aurantium* L.) using nodal explants from mature trees, with the highest

shoot formation 7.4 shoots per node achieved on MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L Kinetin. Shoot length varied across different combinations of BAP and Kinetin treatments, with the maximum shoot length being observed in the combination of 1.0 mg/L BAP and 1.0 mg/L Kinetin, as shown in Table 7. This effect can be attributed to the synergistic action of both growth regulators in promoting cell division and elongation. Interestingly, an inverse correlation was observed between shoot length and shoot number, where an increase in the number of shoots corresponded with a reduction in shoot length. A similar trend was reported by Upadhyay *et al.*, (2010) [32] in the micropropagation of sweet orange, where the longest shoots were obtained using a combination of 2.0 mg/L BAP, 1.0 mg/L Kinetin, and 0.1 mg/L NAA. Likewise, (Kumar *et al.*, 2014) [16] found that 0.5 mg/L BAP combined with 2.0 mg/L Kinetin resulted in the longest shoots when nodal segment explants were used in *Cleopatra mandarin*.

Table 6: Influence of BAP and Kinetin on production of multiple shoots at 30 60 and 90 days from Nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Number of shoots produced from Nodal segment		
	30 (day)	60 (day)	90 (day)
T ₁ Basal medium (Control)	1.80	2.20	2.20
T ₂ BAP 0.5 + Kinetin 0.5	1.40	1.40	2.00
T ₃ BAP 0.5 + Kinetin 1.0	1.20	1.40	1.60
T ₄ BAP 0.5 + Kinetin 1.5	1.60	2.20	2.60
T ₅ BAP 0.5 + Kinetin 2.0	1.80	2.40	2.80
T ₆ BAP 1.0 + Kinetin 0.5	1.80	2.20	2.60
T ₇ BAP 1.0 + Kinetin 1.0	2.60	3.00	3.00
T ₈ BAP 1.0 + Kinetin 1.5	2.00	2.00	2.00
T ₉ BAP 1.0 + Kinetin 2.0	2.40	2.60	2.60
T ₁₀ BAP 1.5 + Kinetin 0.5	2.00	2.00	2.00
T ₁₁ BAP 1.5 + Kinetin 1.0	1.40	2.20	2.40
T ₁₂ BAP 1.5 + Kinetin 1.5	2.20	2.40	2.40
T ₁₃ BAP 1.5 + Kinetin 2.0	1.80	2.20	2.40
F-test	1.56	1.00	0.58
S.Em±	0.32	0.42	0.50
CD (1%)	NS	NS	NS

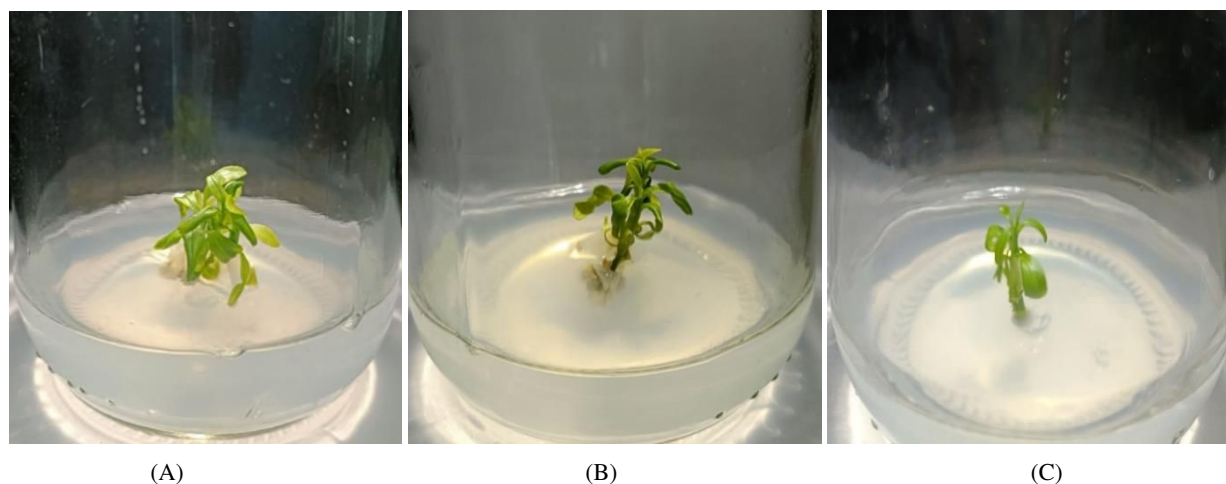


Fig 3: Influence of BAP and Kinetin on shoot induction from single node. A. Shoot formation in BAP 1.0 + Kinetin 1.5 mgL⁻¹ 30 Days B. Shoot formation in BAP 1.0 + Kinetin 1.5 mgL⁻¹ 60 Days C. Shoot formation in BAP 1.0 + Kinetin 1.5 mgL⁻¹ 90 Days

Table 7: Influence of BAP and Kinetin on shoot length at 30, 60 and 90 days from nodal segment of Nagpur mandarin (*C. reticulata* Blanco)

Treatments (mg/L)	Length of shoots in (cm)		
	30 (day)	60 (day)	90 (day)
T1 Basal medium (Control)	1.2	1.54	1.74
T2 BAP 0.5 + Kinetin 0.5	0.7	0.92	1.02
T3 BAP 0.5 + Kinetin 1.0	0.76	1.16	1.24
T4 BAP 0.5 + Kinetin 1.5	0.82	1.24	1.38
T5 BAP 0.5 + Kinetin 2.0	1.2	1.54	1.64
T6 BAP 1.0 + Kinetin 0.5	1.36	1.32	1.42
T7 BAP 1.0 + Kinetin 1.0	1.52	1.9	1.96
T8 BAP 1.0 + Kinetin 1.5	1.16	1.02	1.08
T9 BAP 1.0 + Kinetin 2.0	1.16	1.42	1.44
T10 BAP 1.5 + Kinetin 0.5	1.32	1.42	1.44
T11 BAP 1.5 + Kinetin 1.0	1.12	1.52	1.58
T12 BAP 1.5 + Kinetin 1.5	1.22	1.52	1.56
T13 BAP 1.5 + Kinetin 2.0	1.14	1.32	1.46
F-test	1.14	1.30	1.35
S.Em±	0.22	0.22	0.21
CD (1%)	NS	NS	NS

Effect of IBA and NAA from *in vitro* shoot on root regeneration

For root regeneration, NAA and IBA were used in this experiment. Both IBA and NAA were included at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L⁻¹ in half-strength MS media. the regenerated shoots derived from shoot tip during the shoot multiplication trials were placed on media enriched with the aforementioned levels of NAA and IBA. Roots development at 15, 30, and 45 days after root induction in the media, are described below.

Number of roots

The influence of different concentrations of IBA and NAA on the number of roots produced per shoot at intervals of 15, 30, and 45 days is presented in Table 8. The medium supplemented with 2.5 mg/L NAA exhibited the highest rooting response, producing an average of 3.2 roots per shoot. Root formation remained relatively consistent across

all treatments throughout the 15-to 45-day observation period. The lowest number of roots (1.0) was observed in the untreated control medium. Among the IBA treatments, the best response was achieved with a half-strength MS medium containing 2.0 mg/L IBA, which produced an average of 1.8 roots per shoot. Supporting these results, Al-Khayri and Al-Bahrany (2001) [2] reported the highest rooting response in a medium supplemented with either 1.5 mg/L NAA alone or a combination of 0.5 mg/L NAA and 2.0 mg/L IBA. Similarly, (Savita *et al.*, 2010) [28] observed the best rooting response (71%) with 0.5 mg/L NAA; however, they noted that although callus formation occurred, no root regeneration was observed in *Citrus jambhiri*. Further studies by (Savita *et al.*, 2011) [27] demonstrated a maximum rooting response of 91.67% on half-strength MS medium enriched with 0.5 mg/L NAA, while developing an optimized plant regeneration protocol from callus cultures of *Citrus jambhiri* Lush.

Table 8: Influence of IBA and NAA on number of roots formed at 15, 30 and 45 days

Treatments (mg/L)	Number of Roots		
	15 (day)	30 (day)	45 (day)
T1 Basal medium (Control))	0.40	0.80	1.00
T2 IBA 0.5	0.20	1.00	1.20
T3 IBA 1.0	0.40	1.00	1.20
T4 IBA 1.5	0.40	0.60	1.80
T5 IBA 2.0	1.00	1.00	1.80
T6 IBA 2.5	1.40	1.40	1.20
T7 NAA 0.5	0.80	1.80	2.80
T8 NAA 1.0	1.40	2.80	3.00
T9 NAA 1.5	1.00	2.20	2.60
T10 NAA 2.0	0.80	2.20	2.80
T11 NAA 2.5	0.40	2.60	3.20
F-test	1.32	*	*
S.Em±	0.35	0.45	0.53
CD (1%)	NS	1.71	2.02

**Fig 4:** Influence of treatment NAA 2.5 mg/L⁻¹ on root development after 45 days

Root length

The influence of various concentrations of IBA and NAA on root length at 15, 30, and 45 days showed significant differences among treatments (Table 9). The maximum root length (10.52 cm) at 45 days was observed with 2.0 mg/L IBA, while the shortest root length (3.8 cm) was recorded in the half-strength medium without any growth regulators. Among the NAA treatments, 0.5 mg/L NAA produced the longest roots (6.38 cm) at 45 days, indicating that lower concentrations of NAA were more effective in promoting root elongation.

Similar findings have been reported in previous studies. Saini *et al.* (2010) [25] observed the maximum number of roots (3.19 per shoot) with MS medium supplemented with 1 mg/L NAA and 1 mg/L IBA, whereas the minimum number of roots (0.75) was recorded with 0.5 mg/L of both NAA and IBA. (Gurnani *et al.*, 2015) [7] also reported increased shoot length with 3.0 mg/L IBA. Additionally, Pérez-Tornero and Porras (2008) [22] found that root elongation was more pronounced with 3.0 mg/L IBA, and explants showed improved overall appearance, including greener and larger leaves

Table 09: Influence of IBA and NAA on root length at 15, 30 and 45 days after root initiation

Treatments (mg/L)	Length of Roots		
	15 (day)	30 (day)	45 (day)
T1 Basal medium (Control))	0.10	1.08	3.80
T2 IBA 0.5	0.30	1.02	5.02
T3 IBA 1.0	0.14	1.74	9.56
T4 IBA 1.5	0.10	1.50	4.50
T5 IBA 2.0	0.34	1.44	10.52
T6 IBA 2.5	0.32	3.28	7.78
T7 NAA 0.5	0.10	0.84	6.38
T8 NAA 1.0	0.08	0.86	6.00
T9 NAA 1.5	0.08	0.80	5.90
T10 NAA 2.0	0.06	0.56	4.82
T11 NAA 2.5	0.08	0.90	5.60
F-test	0.62	1.33	*
S.Em±	0.13	0.65	1.53
CD (1%)	NS	NS	5.83



Fig. 5. Influence of treatment IBA 2.0 mg/L⁻¹ at root elongation after 45 days

Conclusion

The present study demonstrates that the combination of 0.5 mg/L BAP and 1.0 mg/L GA₃ resulted in the highest number of shoots (4.80), making it the most effective treatment for producing a greater quantity of planting material. Additionally, the maximum number of roots (3.20) was observed with 2.5 mg/L NAA, while the longest root (10.52 cm) was recorded with 2.0 mg/L IBA. Notably, treatments with IBA also exhibited a higher survival percentage compared to those with NAA.

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