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Comparative Effects of IAA with BAP and Kinetin on *In Vitro* Callus Induction and Shoot Regeneration in Tomato (*Solanum lycopersicum* L.)

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Abstract

Tomato (*Solanum lycopersicum* L.) is a nutritionally and economically important crop, widely cultivated and recognized as a model plant in genetic and physiological studies. Despite its significance, tomato productivity is limited by biotic and abiotic stresses, underscoring the need for efficient in vitro regeneration systems to facilitate crop improvement. In this study, tomato variety 'Arka Sourabh' was used to evaluate the effect of different hormone combinations on callus induction and shoot organogenesis. Murashige and Skoog (MS) medium was supplemented with indole-3-acetic acid (IAA) in combination with two different cytokinins, 6-benzylaminopurine (BAP) and kinetin (Kn), to evaluate their comparative effects on callus induction and shoot regeneration. The combination of IAA (0.4 mg/L) and kinetin (0.2 mg/L) induced profuse callus formation without subsequent organogenesis, while IAA (2.5 mg/L) with BAP (2.5 mg/L) promoted direct shoot regeneration after 10-15 days of culture initiation. Among the tested treatments, the IAA and BAP combination was found to be most effective for shoot induction from stem nodal cuttings. These results demonstrate that the cytokinin type plays a critical role in determining morphogenic responses in tomato, with BAP favoring shoot differentiation and kinetin enhancing callus proliferation. The optimized protocol can be applied in future studies involving genetic transformation and large-scale clonal propagation of tomato.

Keywords: 6-benzylaminopurine (BAP), callus induction, Indole-3-acetic acid (IAA), In vitro regeneration, Kinetin (Kn), shoot organogenesis, stem nodal cuttings, *Solanum lycopersicum* L.

1. Introduction

Tissue culture is a fundamental technique in plant biotechnology that enables the regeneration of whole plants from small explants under controlled *in vitro* conditions. It is widely used for large-scale clonal propagation, production of disease-free plants, germplasm conservation, and genetic improvement. Among its diverse applications, callus induction and plant regeneration are critical steps, as they create a platform for cellular differentiation, mutagenesis, and gene transfer experiments. The success of tissue culture largely depends on the choice of explant and the balance of plant growth regulators[1], which govern callus formation and subsequent shoot or root development. This technique is applied to a wide range of plant species, including cereals, legumes, vegetables, fruits, and ornamentals. Within this context, tomato (*Solanum lycopersicum* L.) represents one of the most widely studied and commercially significant vegetable crops. It is not only valued for its nutritional composition, being a rich source of vitamins, minerals, antioxidants, and lycopene, but also serves as a model species for genetic and physiological research due to its relatively small genome and well-characterized genetics.

In India and many other countries, tomato occupies a central position in vegetable cultivation, contributing substantially to both the fresh market and processing industries. However, its productivity is frequently challenged by biotic and abiotic stresses, which necessitate the development of efficient regeneration and genetic improvement protocols[2].

Tissue culture-based regeneration systems in tomato are indispensable for the success of transformation, genome editing, and functional studies. The explants, such as cotyledons, hypocotyls, and leaves can be used with varying plant growth hormone combinations to induce callus and promote shoot organogenesis. Therefore, optimizing the hormonal composition of culture media remains a key challenge in tomato tissue culture. Nevertheless, the regeneration response remains highly genotype-dependent and often requires optimization.

In the present study, we aimed to establish a simplified and reproducible regeneration protocol for tomato using a single explant source. Tomato variety 'Arka Sourabh', a pure line variety developed through pure line selection and known for its high yield and disease resistance, was used as the experimental material for tissue culture studies. This streamlined protocol reduces complexity in media preparation and offers a reliable platform for downstream applications such as transgenics and genome editing in tomato improvement programs.

2. Materials and Methods

2.1 Plant Material and Sterilization

Tomato (*Solanum lycopersicum* L.) variety 'Arka Sourabh' was used as the experimental material for tissue culture studies. The seeds were procured from Krishi vigyana Kendra, Taliparamba, Kannur. The seeds were cleansed twice with running water, followed by disinfection using a 0.1% Mercuric chloride solution for 5 minutes. Two drops of Tween 20 were added to the mixture, which was then rinsed thrice with sterilized distilled water. The sanitized seeds were then placed in sterilized petri dishes. The seeds were then inoculated in test tubes containing ½ Murashige and Skoog[3] medium enriched with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The medium's pH was adjusted to 5.8 using either 1 N NaOH or 1 N HCl before autoclaving at 121 °C for 15 min. These cultures were kept in dark conditions for three days. Subsequently, the seedlings were exposed to a 16-hour photoperiod. The germinated seedlings served as explants for subsequent tissue culture experiments. Under aseptic conditions, stem nodal cuttings (1-2 cm) were excised from 10-12 days old plants. These explants were then cultured in the media. All cultures were transferred to a growth room for 4-6 weeks.

2.2 Culture Media and Growth Regulators

For callus induction, MS basal medium was supplemented with 0.2 to 1.0 mg/L indole-3-acetic acid (IAA) and 0.2 to 1.0 mg/l kinetin. Shoot induction was performed on MS medium containing 0.5 to 2.5 mg/l IAA and 0.5 to 3.0 mg/L 6-benzylaminopurine (BAP). For root induction, elongated regenerated shoots were excised and transferred to MS medium supplemented with 1.0 mg/L indole-3-butyric acid (IBA), which was found optimal for promoting healthy and profuse root development.

2.3 Statistical Analysis

All experiments were performed in a completely randomized design with three replicates per treatment. The data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of plant growth regulator concentrations and their interactions. Observations were recorded for each treatment at regular intervals, including explant response, callus formation, shoot regeneration, and root development.

3. Results

3.1 *Callus Induction*

The effect of indole-3-acetic acid (IAA) and kinetin (Kn) concentrations on callus induction

from stem nodal cuttings of tomato is presented in Table 1. Two-way analysis of the treatments revealed significant effects of the hormone combinations on callus formation. The interaction between IAA and kinetin concentrations influenced both the extent and quality of callus production. Lower concentrations favored friable, actively growing callus, whereas higher concentrations resulted in compact, less proliferative callus.

Among the tested combinations, MS medium containing 0.4 mg/L IAA + 0.2 mg/L kinetin yielded the best response, showing the highest mean values for callus induction (Figure 1). Increased levels of both hormones generally reduced callus formation efficiency, producing compact and less viable tissue, indicating the importance of maintaining an optimal hormonal balance. No shoot initiation was observed in these media, suggesting that the tested combinations were more conducive to callogenesis rather than organogenesis. The calli obtained were pale green and friable, though some cultures exhibited browning and necrosis due to excessive phenolic accumulation, which is known to hinder callus growth and viability. Root formation was occasionally observed in some calli (Figure 1c).

Table1.ShowingtheeffectsofIAAandkinetinconcentrationsoncallusinductionfromtomato Stem nodal cuttings

IAA/Kinetin (mg/L)	**IAA (0.2)**	**IAA (0.4)**	**IAA (0.6)**	**IAA (0.8)**	**IAA (1.0)**
Kn**(**0.2**)**	7.40(±2.92)	7.81(±2.97)	7.38(±2.89)	7.34(±2.89)	6.90(±2.81)
Kn**(**0.4**)**	7.21(±2.87)	7.19(±2.86)	7.15(±2.86)	7.15(±2.86)	6.96(±2.81)
Kn**(**0.6**)**	6.81(±2.80)	7.05(±2.84)	7.05(±2.84)	7.04(±2.84)	6.90(±2.81)
Kn**(**0.8**)**	6.78(±2.76)	7.09(±2.84)	6.53(±2.74)	6.54(±2.75)	6.34(±2.71)
Kn**(**1.0**)**	6.20(±2.68)	6.55(±2.75)	6.66(±2.77)	6.15(±2.67)	6.09(±2.66)

Data represent mean ± standard error (SE) of three replicates. Analysis of variance (ANOVA) revealed significant effects of IAA ($F = 248.62$, $p < 0.001$), Kinetin ($F = 11.92$, $p < 0.001$), and their interaction ($F = 41.14$, $p < 0.001$). All hormone concentrations are expressed in mg/L.

Figure1: a) Callogenesis from stem nodal cuttings, **b)** Two-week-old callus, **c)** Root initiation from callus

3.2 *Shoot organogenesis*

The response of stem nodal cuttings on regeneration medium supplemented with varying concentrations of IAA and BAP revealed significant differences in shoot organogenesis. The two-way analysis of the data is presented in Table 2. Visible shoot primordia appeared between 10-15 days after culture initiation (Figure 2a), depending on hormonal composition.

The medium containing 2.5 mg/L IAA + 2.5 mg/L BAP produced the optimum response, where 90% (18 out of 20) of the explants regenerated shoots. Lower BAP levels resulted in fewer shoots, indicating the key role of BAP in promoting shoot morphogenesis in tomato. The

regenerated shoots were healthy, elongated, and suitable for further growth and rooting.

Table 2: Effect of IAA and BAP concentrations on shoot regeneration from stem nodal cuttings of tomato

BAP/IAA (mg/L)	IAA 0.5	IAA 1.0	IAA 1.5	IAA 2.0	IAA 2.5
BAP 0.5	1.50 (±1.22)	2.25 (±1.50)	2.40 (±1.55)	2.80 (±1.67)	3.00 (±1.73)
BAP 1.0	4.20 (±2.05)	4.42 (±.10)	4.78 (±2.19)	5.03 (±2.24)	5.00 (±2.24)
BAP 1.5	5.12 (±2.26)	5.50 (±2.35)	5.40 (±2.32)	5.57 (±2.34)	5.58 (±2.36)
BAP 2.0	5.87 (± 2.42)	5.82 (±2.41)	5.98 (±2.45)	6.90 (±2.63)	6.92 (±2.63)
BAP 2.5	7.10 (±2.66)	7.10 (±2.66)	7.17 (±2.67)	7.20 (±2.68)	7.25 (±2.69)
BAP 3.0	7.00 (±2.65)	6.85 (±2.62)	6.93 (±2.63)	6.02 (±2.45)	5.70 (±2.39)

Data represent mean ± standard error (SE) of three replicates; figures in parentheses indicate square root–transformed means. Analysis of variance (ANOVA) revealed significant effects of BAP ($F = 5562.65$, $p < 0.001$), IAA ($F = 12.35$, $p < 0.001$), and their interaction ($F = 139.51$, $p < 0.001$). All hormone concentrations are expressed in mg/L

The data support the classical concept that a balanced auxin–cytokinin ratio promotes organogenesis, while deviations toward either hormone alone reduce morphogenic potential. The success of 2.5 mg/L IAA + 2.5 mg/L BAP further validates the suitability of stem nodal cuttings as a standard explant source for tomato tissue culture.

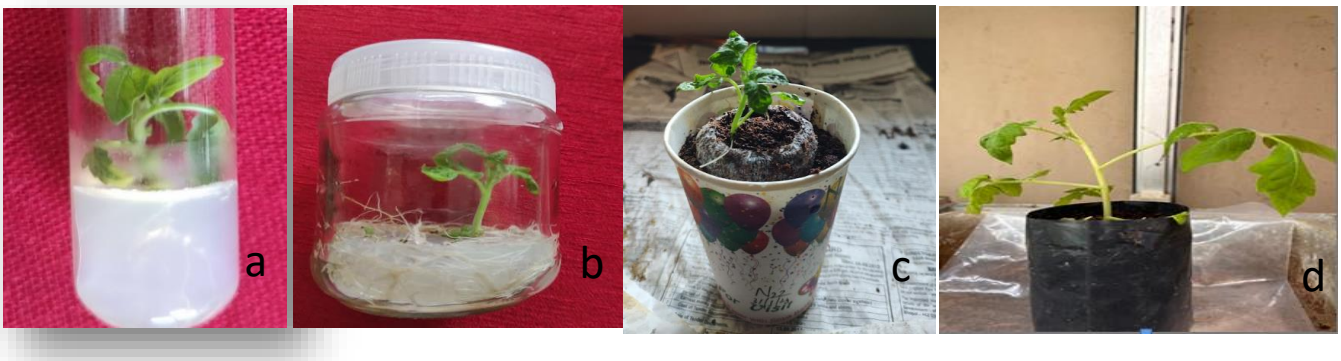

Figure 2: a) Shoot regeneration on medium, **b)** Root formation in regenerated tomato plant, **c)** Acclimatization, and **d)** Hardening

3.3 *Rooting*

Regenerated shoots were successfully rooted when transferred to MS medium supplemented with indole-3-butyric acid (IBA). Maximum rooting (87%) with the highest root number and root length was observed at 1 mg/L IBA (Table .3). Root initiation occurred within 5-7 days and full elongation was visible after one week (Figure 2b). Both lower and higher concentrations resulted in reduced rooting efficiency, indicating that IBA at 1.0 mg/L was optimum for root induction of tomato shoots, ensuring vigorous root development and subsequent acclimatization success.

Table 3: Effect of different IBA concentrations on rooting of tomato shoots.

IBA (mg/L)	% Rooting (Mean ±SD)	Root Number (Mean ± SD)	Root Length (cm, Mean ± SD)
0.5	47 ± 2.5	4.5 ± 0.5	2.0 ± 0.5
0.75	65 ± 3.0	6.0 ± 1.0	2.75 ± 0.25
1.0	87 ± 2.1	9.0 ± 1.0	3.6 ± 0.6
1.25	67 ± 3.5	6.5 ± 0.5	3.25 ± 0.25

Data represent the mean of three replicates with five explants per treatment. SD = standard deviation.

3.4 *Acclimatization and Hardening*

The regenerated tomato plants with well-developed roots were acclimatized in the laboratory using a 1:1:1 mixture of sand, cow dung, and soil. This acclimatization process allowed the plants to gradually adjust from *in vitro* conditions to *ex vitro* environments. Following acclimatization, the plants underwent a hardening phase under controlled environmental conditions, which helped them develop tolerance to external stresses such as variations in temperature, humidity, and light intensity. This phase was crucial for improving their survival rate and ensuring successful establishment under field conditions (Figure 2c-d). Once transferred to the field, the hardened plants exhibited robust growth, with healthy vegetative development.

4. Discussion

The present study demonstrated the differential effects of IAA in combination with kinetin and BAP on the morphogenic responses of tomato stem nodal cuttings. The combination of IAA and kinetin induced profuse callus formation but failed to promote organogenesis, while IAA with BAP effectively triggered direct shoot regeneration.

These findings emphasize that even subtle changes in cytokinin type can redirect the developmental pathway of cultured tissues. Auxin (IAA) primarily stimulates cell division and dedifferentiation, leading to callus formation, whereas BAP, a more active cytokinin than kinetin, promotes cell differentiation and shoot induction. This observation aligns with earlier studies highlighting that BAP plays a crucial role in shoot morphogenesis in tomato[4, 5]. Previous work has also demonstrated that auxin-mediated dedifferentiation is crucial for callogenesis, while an optimum cytokinin concentration promotes tissue proliferation.

The superior response of nodal cuttings corroborates previous reports indicating their high regenerative potential due to the presence of pre-formed meristems[6] Phenolic accumulation and tissue browning observed in some cultures may have limited callus proliferation. The phenolic-induced necrosis is a major barrier to sustained callus growth in tomato tissue cultures. Furthermore, the friable nature of callus obtained at optimal IAA and kinetin concentrations is advantageous for Agrobacterium-mediated transformation, protoplast isolation, and mutagenesis studies.[7]

Overall, the results support the classical concept that the auxin-to-cytokinin balance determines morphogenic outcomes. Kinetin favored callus proliferation, while BAP enhanced shoot induction, validating the decisive role of cytokinin type in morphogenesis. These outcomes agree with the conclusions of Long et. al.[8], Pruski et al.[9], and Raza et al. [10], who also reported that the ratio, concentration, and nature of plant growth regulators critically influence the direction of morphogenic responses.

In addition, recent studies further strengthen these observations. Cytokinin-specific signaling has been shown to reprogram cell fate and accelerate meristem establishment in tomato explants[11], while auxin–cytokinin crosstalk has been identified as a key regulator of shoot induction efficiency in Solanaceae cultures[12]. Moreover, comparative morphogenic analyses consistently demonstrate that BAP outperforms kinetin in promoting shoot organogenesis across diverse tomato genotypes, supporting the superior response observed in the present study. Recent advancements in tomato regeneration research further reinforce these findings: efficient shoot and root formation from cotyledon and protoplast-derived tissues has been achieved through optimized culture systems, confirming that the cytokinin–auxin balance is a central to directing morphogenic transitions[13]. Together, these evidences validate the present results and emphasize the decisive role of cytokinin type and auxin–cytokinin interactions in determining morphogenic outcomes in tomato tissue culture.

5. Conclusion

This study established a simple and reproducible regeneration protocol for tomato using stem nodal cuttings. Optimal callus induction was achieved using IAA with kinetin, whereas IAA combined with BAP promoted efficient shoot organogenesis. IBA (1.0 mg/L) was most

effective for rooting regenerated shoots. These results confirm that variations in the type and combination of growth regulators profoundly affect morphogenic responses in tomato. Overall, the protocol developed here minimizes medium complexity, enhances reproducibility, and provides a reliable platform for downstream applications such as genetic transformation, mutation breeding, and genome editing. By offering high regeneration efficiency with a single explant type, the method can contribute significantly to future tomato crop improvement and biotechnology research.

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Author contributions

All the authors contributed equally to the work and have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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Nothing to declare.

Data Availability

Data supporting this study is available from the corresponding author upon reasonable request.

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