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In vitro clonal propagation of papaya (*Carica papaya* L.) cv. 'Taiwan-786' through lateral bud culture

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Abstract

An experiment titled "*In vitro* Clonal Propagation of Papaya (*Carica papaya* L.) cv. 'Taiwan-786' through Lateral Bud Culture" was conducted at the Laboratory and Experimental Station, Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh, during the year 2022. The objective of this study was to investigate the morphogenetic responses and root formation phenomena of papaya plants propagated under Murashige and Skoog (MS) medium supplemented with five different concentrations (0.0, 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L) of 6-benzylaminopurine (BAP) and 0.5 mg/L α -naphthalene acetic acid (NAA) with each level of BAP. The shortest duration for shoot emergence was observed with 0.5 mg/L BAP and 0.5 mg/L NAA, while the highest number of leaves and shoots were recorded with a combined medium solution of 1.0 mg/L BAP and 0.5 mg/L NAA. A higher concentration of BAP (1.5 mg/L) resulted in a rapid shoot induction. Among the seven levels (0.0, 0.65, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) of indole-3-butyric acid (IBA), the maximum number of roots was achieved with 1.5 mg/L, and the greatest root length per shoot was observed with a 2.0 mg/L concentration. Successfully rooted plantlets were hardened and acclimatized to greenhouse conditions. The developed protocol is efficient, genotype-specific, and suitable for the large-scale production of disease-free, true-to-type papaya planting materials.

Keywords: Papaya, *in vitro* culture, BAP, IBA, Lateral buds, Micropropagation

Introduction

Papaya (*Carica papaya* L.) is the most economically important fruit crop grown in tropical and subtropical regions of the world and belongs to the small family *Caricaceae* (Jaime *et al.*, 2007) [6]. Papaya is cultivated in more than seventy-one countries over an area of 4.40 million hectares, producing 13.02 million tons of papaya (FAOSTAT, 2017). Being a major papaya producer, India grows papaya over an area of 1.34 million hectares and produces a yield of about 5.94 million tonnes, followed by Brazil, Mexico, Indonesia, the Dominican Republic, and Nigeria (FAOSTAT, 2017). In Uttar Pradesh, papaya is cultivated over an area of 2.689 ha, with a production of 12,052.19 (000 MT) (APEDA, 2023-24). Papaya is mainly grown and consumed in developing countries and is becoming popular internationally as a fresh fruit and as a processed product (Jaime *et al.*, 2007) [6]. Owing to its high yield, nutritional value, and year-round fruit production, the importance of this crop worldwide is undeniable. Papaya contains carbohydrates and other secondary metabolites, such as alkaloids, phytosterols, tannins, phenols, and flavonoids, which have high remedial potential. In addition to seeds, papaya is also propagated asexually by cutting and grafting; however, these methods are tedious and impractical for commercial use, and there is a possibility of disease transmission, which could be a source of direct yield losses (Nguyen *et al.*, 2018) [8]. Therefore, the requirement for quality planting material is a priority. Over the last few decades, plant tissue culture techniques have shown immense potential in producing true-to-type plantlets and obtaining quality planting materials to fulfil farmers' demands. To date, several *in vitro* clonal propagation protocols have been reported by Ahmed *et al.* (2025) [14], Dewanto *et al.* (2024) [15], and Kumar *et al.* (2024) [16]; however, genotype dependency in plant tissue culture is a major problem. Therefore, the present study was undertaken to evaluate the potential of a high-yielding papaya variety under *in vitro* conditions and to develop an efficient protocol for papaya clonal propagation.

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Materials and Methods

Plant Materials and Tissue Culture

For *in vitro* aseptic establishment of shoot meristems, all the lateral buds were carefully excised from papaya twigs with the help of a sharp knife and washed thoroughly under running tap water for 30 minutes containing 2-3 drops of the liquid detergent Tween-20. Thereafter, the explants were surface sterilized with 0.1% (w/v) aqueous solution of mercuric chloride (HgCl₂) for 5 min, followed by treatment for one minute with 0.1 % (w/v) fungicide Bavistin 50 DF, 0.1 % (w/v) antibiotic Cefotaxime, and 70% ethanol for 45 s. Finally, the explants were rinsed 4-6 times with pre-sterilized double-distilled water under aseptic conditions to remove traces of the surface sterilization agents. The lateral buds containing apical dome along with 1-2 leaf primordia were carefully excised from the sterilized segments and immediately inoculated onto the full-strength MS medium (Murashige and Skoog) fortified with 100 mg/L Myo-inositol, 1.0 mg/L BAP + 0.2 mg/L NAA, 50 mg/L ascorbic acid, 30 g/L sucrose, and solidified with 8.0 g/L agar. For shoot multiplication, *in vitro* regenerated shoots were subcultured onto the same culture medium combination. The culture media pH was adjusted to 5.8 before autoclaving at 121 °C and 1.06 kg cm⁻² pressure for 20 min. All cultures were incubated at culture room temperature 25 ± 2 °C under 40 µmol m⁻²s⁻¹ light intensity for 16h/8h light/dark photoperiod, provided by cool white fluorescent tubes (Figure 1).

The present study evaluated the efficacy of various surface sterilization agents, including mercuric chloride (HgCl₂), ethanol, and sodium hypochlorite (NaOCl), either individually or in combination, at varying concentrations and treatment durations. The objective was to determine the optimal concentrations of these agents for the successful aseptic *in vitro* establishment of plant materials, while minimizing toxicity and mortality rates in the established cultures.

Acclimatization and Hardening of *In vitro* Raised Plantlets

In vitro raised plantlets with well-developed shoot and root systems were carefully removed from the test tubes and gently washed under running tap water to remove traces of the culture medium. The plantlets were dipped in a 1% aqueous solution of Bavistin, a systemic fungicide (BASF, India), for 10-15 min, followed by rinsing with running tap water. Thereafter, the fungicide-treated plantlets were transferred to the pots containing a mixture of soil: sand: compost (1:1:1). The pots were covered with polypropylene bags and kept in a greenhouse. The plants were watered weekly. The top corners of the polypropylene bags were cut after two weeks to gradually expose the plants to the outside environment. After 3-4 weeks, the polypropylene bags were completely removed (Figure 1).

Experimental Design and Statistical Analysis

All experiments were conducted using a completely randomized design (CRD), with a minimum of six replicates (*n* = 6) per treatment, and each experiment was repeated three times. A single replicate consisted of one culture vessel. Observations were recorded for all cultures based on

several parameters, including the number of explants exhibiting shoot induction, the number of shoots per explant, shoot length (cm), number of leaves, number of roots, and root length per shoot (cm). For the statistical analysis of data generated from tissue culture experiments, the effects of different treatments on various parameters were quantified. The significance of differences among means was determined using analysis of variance (ANOVA) with SPSS Statistics version 19.02 software [SPSS, Chicago, USA].

Results and Discussion

Shoot Initiation and Proliferation

The combined application of cytokinin (BAP) and auxin (NAA) significantly influenced shoot induction and subsequent growth parameters in papaya cv. 'Taiwan-786' (Table 1). All evaluated morphogenetic traits, namely days to shoot emergence, number of leaves, number of shoots, and shoot length, showed significant variation among the treatments (*p* < 0.01). The earliest shoot emergence was recorded on MS medium supplemented with 0.5 mg/L BAP + 0.5 mg/L NAA, where shoots emerged within 9.4 days, followed by 1.0 mg/L BAP + 0.5 mg/L NAA (13.2 days). In contrast, media containing higher concentrations of BAP in combination with 0.5 mg/L NAA required the longest duration for shoot emergence, with a maximum of 16.3 days. The number of leaves was also significantly influenced by the combined application of BAP and NAA. The maximum number of leaves (21.8) was observed on MS medium supplemented with 1.0 mg/L BAP + 0.5 mg/L NAA, whereas the minimum (16.4) was recorded under 0.5 mg/L BAP + 0.5 mg/L NAA. The treatment comprising 1.5 mg/L BAP + 0.5 mg/L NAA produced a comparable response, yielding 19.6 leaves per explant. All media combinations significantly affected shoot multiplication in papayas. The highest number of shoots (17.6) was obtained on MS medium fortified with 1.0 mg/L BAP + 0.5 mg/L NAA. Higher concentrations of BAP in combination with NAA resulted in a comparatively poor response and produced the minimum number of shoots (9.6), indicating that excessive BAP inhibited shoot proliferation. Shoot length varied significantly among the treatments. The maximum shoot length (2.94 cm) was recorded on MS medium supplemented with 1.5 mg/L BAP + 0.5 mg/L NAA, followed by 1.0 mg/L BAP + 0.5 mg/L NAA. Although all treatments enhanced shoot elongation to varying degrees, the shortest shoots were observed under the MS medium containing 2.0 mg/L BAP + 0.5 mg/L NAA.

These results suggest that lower to intermediate concentrations of BAP in combination with NAA are more effective for shoot induction in papaya than higher concentrations. Higher cytokinin levels may cause hormonal imbalance, leading to disrupted auxin transport and signalling, which can reduce shoot differentiation and proliferation. Similar findings have been reported in papaya and other tropical fruit crops, where moderate cytokinin levels in combination with auxin promoted optimal shoot multiplication and morphogenesis, while supra-optimal cytokinin concentrations hindered regenerative responses (Zhao *et al.*, 2021; López-Encina *et al.*, 2023; Batool *et al.*, 2024; Patil *et al.*, 2007; Efendi & Putra, 2025) [13, 7, 2, 10, 3].

Optimization studies on other fruit and horticultural crops have also emphasized that precise auxin-cytokinin ratios are

critical for maximizing shoot proliferation and elongation *in vitro* (Nguyen, 2022; Getnet *et al.*, 2017)^[9, 5].

Table-1: Effects of different concentrations of BAP on the different morphogenetic responses of papaya variety “Taiwan-786”

BAP (mg/L)	NAA (mg/L)	Duration for shoot emergence	Number of leaves (n)	Number of shoots(n)	Shoot length (cm)
0.0	0.0	0.00	0.00	0.00	0.00
0.5	0.5	9.40	16.40	14.80	1.84
1.0	0.5	13.20	21.80	17.60	2.62
1.5	0.5	14.60	19.60	12.40	2.94
2.0	0.5	16.30	16.70	9.60	1.72
LSD	---	2.57	3.24	1.92	0.32
CV	---	11.14	7.96	9.84	9.43
Mean	---	13.38	18.62	13.6	2.28

Root Induction

The rooting response was significantly influenced by the IBA concentration (Table 2). No root induction was observed on MS medium devoid of auxin (0.0 mg/L IBA), confirming the essential role of IBA in adventitious root formation. The maximum number of roots per shoot (16.43) was recorded on MS medium supplemented with 1.5 mg/L IBA, followed by 2.0 mg/L IBA (11.70). In contrast, the minimum number of roots was observed at the highest concentration tested (3.0 mg/L IBA). Roots are an important part of the plant that provides food material to the leaves, where food is produced for the proper growth and development of the whole plant. The data presented in Table 2 for root length showed significant variation among all treatments. The longest (4.65 cm) root was obtained with

the use of 2.0 mg/L IBA, followed by 1.0 mg/L IBA (3.15 cm). The shortest root (2.80 cm) was recorded on MS medium supplemented with 0.65 mg/L IBA. A further increase in IBA concentration reduced both root number and length. This reduction may be due to the inhibitory effect of excess auxin, which can disturb the internal hormonal balance of plantlets and negatively affect root initiation and elongation. Similar results have been reported in recent studies on papaya and other fruit crops, where moderate concentrations of IBA were found to be most effective for inducing healthy and profuse roots, while higher concentrations either failed to improve rooting or caused a decline in root quality (Zhao *et al.*, 2021; López-Encina *et al.*, 2023; Samanmalie *et al.*, 2023; Al-Drissi *et al.*, 2023; Batool *et al.*, 2024; Waidyaratne *et al.*, 2025)^[13, 7, 11, 1, 2, 12].

Table 2: Effects of various concentrations of IBA for the formation of roots on the *in vitro* grown micro-shoots of papaya variety “Taiwan-786”

IBA (mg/L)	Number of roots (n)	Root length per Shoot (cm)
0.00	0.00	0.00
0.65	9.60	2.80
1.00	10.80	3.15
1.50	16.43	3.90
2.00	11.70	4.65
2.50	9.20	3.04
3.00	6.54	2.46
LSD	3.68	0.84
CV	19.36	16.34
Mean	10.71	3.34

Treatment means within a column followed by the same letter are not significantly different at the 1% level. (Where, n= number, CV= Coefficient of Variation (%), expl. = explants, cm= centimetre, LSD =Least Significant difference)

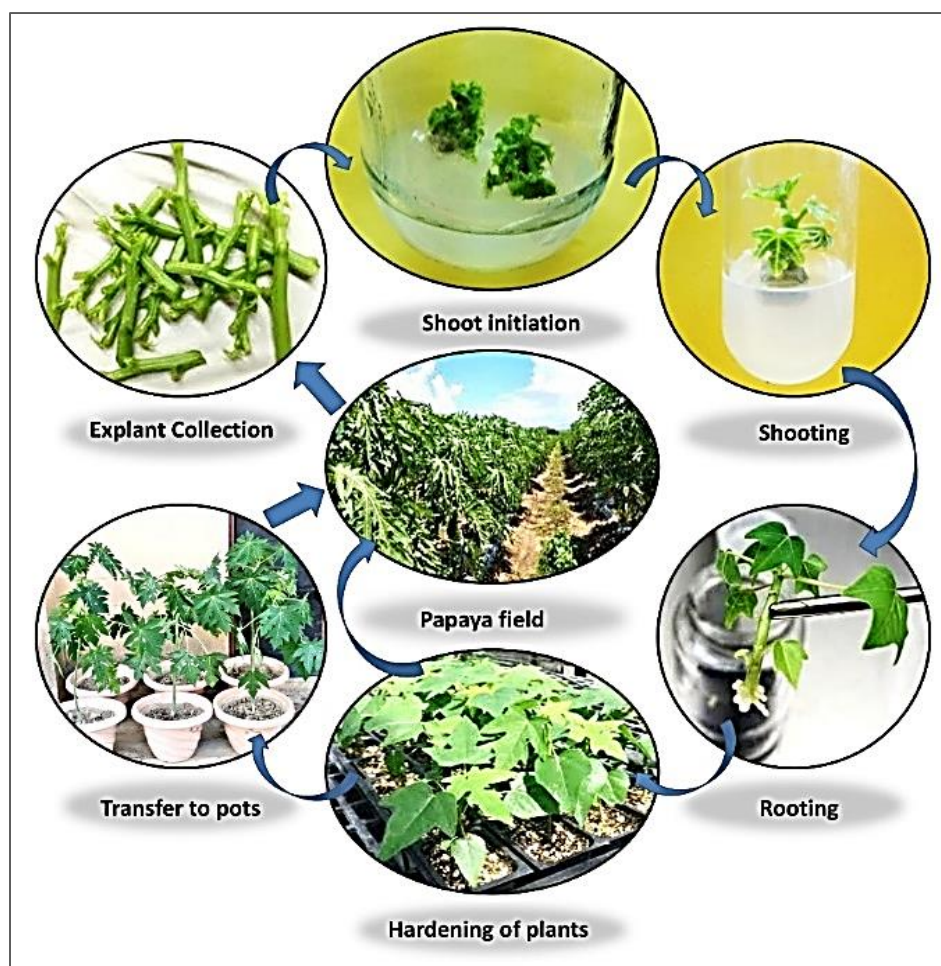


Fig 1: Micropropagation protocol using lateral bud culture for mass multiplication of true-to-type papaya plants

Conclusion

The present study successfully standardized an efficient *in vitro* clonal propagation protocol for papaya (*Carica papaya* L.) cv. 'Taiwan-786' using lateral bud explants. MS medium supplemented with 0.5 mg/L BAP + 0.5 mg/L NAA was found to be optimal for rapid shoot induction during the duration of shoot emergence and multiplication, whereas 1.0 mg/L BAP + 0.5 mg/L NAA resulted in a higher number of shoots per explant. The number of leaves and adequate shoot elongation were observed with 1.5 mg/L BAP + 0.5 mg/L NAA. MS medium containing 1.5 mg/L IBA was proven to be the most effective for rooting, that is, the number of roots, and 2.0 mg/L IBA was proven to be the most effective for root length per shoot. The absence of callus formation and successful acclimatization highlight the reliability of this protocol for producing true-to-type, disease-free planting material. This protocol can be effectively utilized for large-scale commercial propagation and germplasm conservation of elite cultivars.

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