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In vitro regeneration of avocado (*Persea americana* Mill.) through growth regulator-induced shoot induction

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Abstract

Avocado (*Persea americana* Mill.), the only major fruit of the Lauraceae family, is valued for its nutrition. Traditional methods like seed propagation and grafting face issues of variability and low success. *In vitro* regeneration offers a better alternative for clonal uniformity and mass propagation. This study examined sterilizing techniques to reduce contamination and optimize shoot induction from nodal segments and apical meristems using different doses of plant growth hormones. Surface sterilization with 2.0% sodium hypochlorite (NaOCl) for 5 minutes significantly reduced microbial contamination (12% in nodal segments and 23% in apical meristems) while maintaining excellent survival rates (82% and 69%, respectively). Explants were cultivated on full-strength Murashige and Skoog (MS) medium that was enhanced with various BAP, kinetin, and TDZ combinations. A study of explants from the nodal segments and apical meristem showed that the nodal segments reacted better to BAP 1.5 mg/L treatment. Compared to apical meristem explants, nodal segment explants induced early shoots more quickly (34.43 and 36.67 days). At 40, 60, and 90 days following initiation, nodal segment explants had more shoots per explant (1.67, 2.00, and 2.33, respectively) than apical meristem explants (1.33, 1.77, and 2.00, respectively). Similarly, the shoot length of nodal segment explants was longer than that of apical meristem explants, measuring 0.63 cm, 1.20 cm, and 1.57 cm at 40, 60, and 90 days after initiation, respectively, as opposed to 0.53 cm, 1.00 cm, and 1.13 cm. These findings demonstrate the exceptional capacity for regeneration of nodal segment explants and offer important information for refining avocado micropropagation methods for large-scale plant production.

Key words: Avocado, sodium hypochlorite, BAP, nodal segment, apical meristem, shoot induction

Introduction

The avocado (*Persea americana* Mill.), the sole fruit in the Lauraceae family with economic significance, is prized for its high nutritional value. Originally from tropical America, it is currently grown extensively in tropical and subtropical climates (Chen *et al.,* 2009; Geethu, and Gomez, 2022). Mexican (*P. americana var. drymifolia*), Guatemalan (*P. americana var. guatemalensis*), and West Indian (*P. americana var. americana*) are the three horticultural races that make up this species (Galindo-Tovar *et al.,* 2008). An American missionary brought avocado to India in the 19th century, and it is now produced intermittently in areas such as the Nilgiris, Kodaikanal, Coorg, and portions of Tamil Nadu and Karnataka (Singh *et al.,* 2022). 8.05 million metric tons of avocados are produced worldwide, with Mexico producing the most at 2.3 million tonnes, followed by Colombia, the Dominican Republic, and Peru (FAO, 2023). The crop is still only grown on a modest scale, and no commercial orchards have been formed, despite India having appropriate agroclimatic zones (Kumar *et al.,* 2020).

The avocado is a subtropical fruit crop that grows up to 20 meters tall and is humid and evergreen. The exquisite blooms are carried on terminal panicles, and the leaves are elliptical and alternate. Due to its peculiar reproductive biology, protogynous diurnally synchronous dichogamy, which encourages cross-pollination, mostly by honeybees, fruit set is incredibly low (~0.1%) despite abundant flowering (Salazar-García *et al.,* 2021). The fruit ripens into a buttery pulp and is a huge berry with a single seed (Abraham *et al.,* 2018). A climacteric fruit, avocados are picked when they reach physiological maturity and ripen after harvest. With a calorific value of 245 kcal/100 g, it is regarded as one of the most nutrient-dense fruits in the world. It is high in fat (30 %), protein (4 %), fiber (10 g/100 g), and carbs (13 %). Additionally, it has vital minerals (K, Na, P, Mg, Mn, Fe, Cu, Ca, S) and vitamins (A, B-complex, C, E, and K) (Singh *et al.,* 2022).

The major method of propagating avocados is by seeds, which are difficult to grow and barely remain viable for two to three weeks. Because they are cultivated from non-standardized local seed sources, seedlings usually take 5-6 years to bear fruit and exhibit substantial genetic variability (Gazit and Lavi, 2020). Low success rates, high prices, and a shortage of good-quality grafted plants are the main obstacles to vegetative propagation techniques such as veneer and cleft grafting (Kumar *et al.,* 2020). Current propagation techniques are unable to meet the increasing need for consistent, superior planting material, which is being driven by the fruit's remarkable nutritional and therapeutic potential.

Effective *In vitro* regeneration technologies remain scarce, despite growing interest in avocado farming. Because tissue culture can quickly proliferate elite genotypes in a controlled, disease-free environment, it presents a promising alternative for the large-scale production of true-to-type plants (Barceló

& Pliego-Alfaro, 2012; Rout *et al.,* 2023). To overcome these bottlenecks, *In vitro* micropropagation has emerged as a critical tool for ensuring clonal uniformity and rapid mass multiplication of elite germplasm. Recent studies in horticultural biotechnology have underscored the versatility of tissue culture, ranging from the conservation of endangered orchids like *Paphiopedilum dianthum* and *Dendrobium moschatum* (Pham *et al.*, 2025; Sarkar *et al.*, 2024) to the large-scale production of fruit crops such as *Ficus carica* (Baby *et al.*, 2024; Labidi *et al.*, 2023). However, achieving successful regeneration requires the optimization of surface sterilization protocols and the precise calibration of plant growth regulators (PGRs), such as BAP, Kinetin, and TDZ, which are pivotal in overcoming recalcitrance and inducing shoot organogenesis (Rehman *et al.*, 2025).

Building upon these advancements, the present study aims to standardize an efficient *In vitro* regeneration protocol for avocado by evaluating the synergistic effects of various PGRs and explant types on shoot induction and survival.

Materials and methods

Experimental site: The present investigations on *In vitro* regeneration of avocado (*Persea americana* Mill.) were carried out at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Bangalore, GKVK (Gandhi Krishi Vignana Kendra) Campus, Bengaluru, during the year 2023-24.

Plant material and explant source: The explants were collected from the scion bank of the fruit nursery, Department of Horticulture, UAS, GKVK, Bengaluru. It was selected as a mother tree for explant material. The explants, nodal segment, and apical meristem were collected from the West Indian race avocado tree cultivar Hass (Fig. 1). The young and healthy shoots were selected as explants, and shoots (10-15 cm) were freshly harvested. The explants were carried to the Plant Tissue Culture Laboratory.

Fig. 1. Explant types. a) Nodal segment., b) Apical meristem.

Surface sterilization: To remove surface impurities, the collected explants were first washed for 30 minutes under running tap water. They received three washes with sterile distilled water after being treated with 0.5% Bavistin for 30 minutes under aseptic circumstances. After that, the surface was sterilized for five minutes using different concentrations of mercuric chloride (0.1-0.3%) and sodium hypochlorite (1-3%). Following three further rinses with sterile water, the explants were trimmed to size in sterile bottles, treated with 70% isopropyl alcohol for one minute, and then immersed in 0.1% streptomycin for twenty minutes. Lastly, the culture media were inoculated with the five explants for each treatment.

Media preparation and culture conditions: MS (Murashige and Skoog, 1962) solid medium containing 6 g/L agar and 30 g/L sucrose was used for raising the explants. The medium's pH was kept between 5.70 and 5.78 before the agar was added. Plant growth regulators BAP (6-benzylaminopurine) 1, 1.5, and 2 mg/L, kinetin 1, 1.5, and 2 mg/L, and TDZ (thidiazuron) 1, 1.5, and 2 mg/L were added to the medium in different doses, along with a control (MS only) for shoot initiation. The culture bottles were maintained in the growth room with a 16:8 hour light:dark cycle and at a temperature of 24 ± 2°C for a period of 90 days.

Experimental design and statistical analysis: A completely randomized design (CRD) with five replicates was used to evaluate all of the data. The significance of treatment effects was assessed using the F-test. To assess the dependability of each individual therapy, standard errors of variance and crucial differences were computed for each treatment at the 1 % significance level. The online analysis program OPSTAT was used to do the analysis**.**

Results and discussion

Effect of sterilization: The effectiveness of sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) as sterilizing agents was assessed based on their impact on contamination rates and survival rates of nodal segments and apical meristems (Table 1). The results indicate that while both disinfectants reduce contamination, their effectiveness varies with concentration. Sodium hypochlorite was tested at different concentrations (1 %, 2 % and 3 %). The 1 % NaOCl treatment resulted in high contamination rates (32 % for nodal segments and 23% for apical meristems) with low survival rates (22 % and 25 %, respectively), suggesting insufficient disinfection. Increasing the concentration to 2% significantly reduced contamination (5% in nodal segments and 10 % in apical meristems), leading to the highest survival rates (86 % and 79 %) among all treatments. However, further increasing NaOCl to 3 % led to an increase in contamination (10 % for nodal segments, 16 % for apical meristems) and a sharp decline in survival rates (20 % and 15 %). The decrease in survival at higher concentrations suggests that prolonged exposure to NaOCl may cause tissue damage. Mercuric chloride was tested at different concentrations (0.1 %, 0.2 %, and 0.3 %). The lowest concentration (0.1 % $HgCl_2$) had the highest contamination rates (44 % in nodal segments, 48 % in apical meristems), with poor survival (12 % and 17 %). Increasing the concentration to 0.2 %

Table 1. Effect of sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) on contamination and survival rates of nodal segments and apical meristems for shoot induction

Treatment	Concentration (%)	Nodal segments		Apical meristem	
		Contamination (%)	Survival rate (%)	Contamination (%)	Survival rate (%)
Control	0	100	0	100	0
NaOCl	1	32	22	23	25
NaOCl	2	5	86	10	79
NaOCl	3	10	20	16	15
$HgCl_2$	0.1	44	12	48	17
$HgCl_2$	0.2	6	26	11	24
$HgCl_2$	0.3	8	13	12	10

$HgCl_2$ drastically reduced contamination (6 % in nodal segments, 11 % in apical meristems), leading to a moderate survival rate (26 % and 24 %). However, further increasing the concentration to 0.3 % $HgCl_2$ did not improve contamination control significantly (8 % for nodal segments, 12 % for apical meristems) and reduced survival rates (13 % and 10 %), indicating cytotoxic effects at higher concentrations. The highest contamination (100 %) was observed in both nodal segments and apical meristem of untreated explants (control). This revealed that nodal segments performed better than the apical meristem as a source of explants for *In vitro* regeneration.

Among all treatments, 2 % NaOCl was the most effective, achieving the lowest contamination rates (5-10 %) while maintaining the highest survival rates (79-86 %). Although 0.2 % $HgCl_2$ was effective in reducing contamination, its survival rates were lower compared to NaOCl, suggesting that it is more toxic to plant tissues. Tissue damage was observed at higher concentrations of both sterilants (3 % NaOCl and 0.3 % $HgCl_2$), suggesting that disinfectant strength optimization is crucial to strike a balance between tissue viability and microbiological control**.** Since sodium hypochlorite is a potent oxidizing agent that can denature microbial proteins by interacting with amino acids and nucleic acids, it is frequently employed for surface sterilization. Rabuma *et al.* (2020) reported that the axillary bud explants treated with 1 % NaOCl for 5 minutes recorded the minimum percentage of contamination (10 %) in avocado. The higher the concentration, the lower the survival percentage was because of the burning and death of the explants. EL-Dengawy *et al.* (2017) also recorded the highest survival rate (66.7 %) in the nodal segment of guava treated with NaOCl. According to Alizadeh *et al.* (2022), *Melia azedarach* L. leaf explants' disinfection and *In vitro* response were markedly improved by a two-step sterilization procedure that involved 0.5% NaOCl with 3 mg/L benomyl and 2% NaOCl for 12 minutes.

Effect of cytokinin (BAP, kinetin, and TDZ) on shoot induction

Number of days taken for shoot initiation for nodal segment explants: The data recorded on the number of days taken for shoot initiation showed significant differences among the different concentrations of cytokinins (BAP, kinetin, and TDZ), as shown in Table 2**.** The nodal segment as an explant in the experiment recorded the least number of days (34.33) for shoot initiation when treated with the cytokinin at 2.0 mg/L BAP when compared to other treatments. The basal medium took the maximum number of days (39.00) when compared with other treatments supplied with cytokinins. This reflects that the growth regulators are accelerating the explant for early shoot initiation. The lower concentration of BAP, kinetin, and TDZ treatments took the maximum number of days for shoot initiation. The variation in days taken for shoot initiation at lower concentrations of cytokinins may be due to insufficient cytokinins to promote shoot initiation. According to Kumar and Meena (2023), the maximum shoot regeneration frequency of 53.33 % was obtained in grapes (*Vitis vinifera*) when 1.5 mg/L BAP and 0.5 mg/L NAA were used together. This suggests that the concentration is helpful in encouraging shoot start. The response for Kinetin and TDZ was lower when compared to BAP, resulting in a similar response being reported in guava by Sharma *et al.* (2018). The pale yellow to light green shoot initiation was observed on the day of shoot initiation. The regenerated shoot in TDZ was different when compared to other treatments. The shoots in TDZ were wrinkled and twisted. Both TDZ and Kinetin, on the other hand, evoked comparatively poor responses when compared to BAP. Similar results were observed by Qasrawi (2019).

Table 2. Effect of different combinations of BAP, KIN and TDZ on shoot initiation, number of shoots per explant, and shoot length when using a nodal segment as an explant

Treatments (mg/L)	Days taken for shoot initiation	Number of shoots per explant			Shoot length (cm)		
		40 days	60 days	90 days	40 days	60 days	90 days
T_1 - Control	39.00	0.67	1.00	1.00	0.37	0.63	0.67
T_2 - BAP 1.0	35.67	1.67	2.00	2.00	0.57	1.13	1.30
T_3 - BAP 1.5	34.33	1.67	2.00	2.33	0.63	1.20	1.57
T_4 - BAP 2.0	35.33	1.00	1.00	1.00	0.50	1.00	1.50
T_5 – KIN 1.0	37.00	1.67	1.67	1.67	0.53	0.97	1.47
T_6 - KIN 1.5	36.67	1.00	1.00	1.33	0.60	1.17	1.53
T_7 - KIN 2.0	36.33	1.00	1.00	1.00	0.43	1.03	1.10
T_8 - TDZ 1.0	38.33	1.33	1.33	1.67	0.40	0.67	0.77
T_9 - TDZ 1.5	37.67	1.00	1.00	1.00	0.37	0.67	0.87
T_{10}- TDZ 2.0	37.33	1.00	1.00	1.00	0.37	0.77	0.90
SEm±	0.60	0.24	0.15	0.21	0.06	0.07	0.06
CD (P=0.01)	2.40	0.95	0.60	0.85	0.24	0.30	0.25

Significant at $P< 0.01$

Number of shoots per explant from nodal segment explants: The observed results on the number of shoots per explant from each nodal segment are presented in Table 2. The significant difference was observed in the number of shoots per explant at different intervals of 40, 60, and 90 days after culture initiation. In the present study, explants cultured on MS medium fortified with 1.5 mg/L of BAP resulted in a maximum number of shoots per explant (1.67, 2.00, and 2.33) at 40, 60, and 90 days after culture (Fig. 2). Followed by explants cultured on MS medium containing 1 mg/L of BAP, which showed 1.67, 2.00, and 2.00 shoots per explant at 40, 60, and 90 days after culture, respectively. The lowest numbers of shoots per explant (0.67, 1.0, and 1.0) at 40, 60, and 90 days after culture, respectively, were formed from the explants inoculated on the media without any growth regulators (control). Shoot proliferation was highest in explants grown on MS medium supplemented with 1.5 mg/L BAP. This implies that the ideal BAP concentration for encouraging axillary shoot proliferation in the examined plant species is 1.5 mg/L. Since BAP is a strong cytokinin that encourages cell division and the development of shoot meristems, its intermediate concentration probably created a hormonally balanced environment that supported long-term shoot proliferation. Following closely behind were explants treated with 1.0 mg/L BAP. This concentration considerably outperformed the control and other cytokinin treatments, despite being marginally less effective than the 1.5 mg/L treatment. This suggests that even lower concentrations of BAP can have a positive impact on multiple shoot induction, albeit not as much as the ideal concentration. On the other hand, the control treatment (T_1) produced the fewest shoots per explant because it did not contain any cytokinin supplementation. Because the explants' endogenous hormone levels were insufficient to initiate or sustain strong shoot initiation and multiplication, this emphasizes the need for exogenous cytokinin administration for effective shoot proliferation. Interestingly, there was no increase in the number of shoots in the media supplemented with higher doses of cytokinins (2.0 mg/L BAP, Kinetin, and TDZ). These increased concentrations seemed to inhibit rather than promote shoot proliferation in certain instances. Excess cytokinins may have cytotoxic or inhibitory effects, upsetting the hormonal balance required for well-organized shoot development. Reduced or deformed shoots can be the result of physiological stress, aberrant tissue differentiation, or suppression of apical dominance caused by high cytokinin levels. Similar findings were also reported by Sholi and Qasrawi (2022), who observed the maximum number of shoots per explant (3.2) in medium supplemented with BAP (1.5 mg/L) from axillary buds of avocado. Safeer *et al.* (2020) in Litchi observed the large number of shoots produced in the medium supplemented with BAP (1 mg/L) and kinetin (1 mg/L). According to Alam *et al.* (2015), the best response was obtained in cucumber (*Cucumis sativus*) when MS was supplemented with 1.5 mg/L BAP. This resulted in an 87% shoot production rate from nodal explants.

Mean shoot length from nodal segment explants: Table 2 shows that the significance of exogenous cytokinins in regulating vegetative growth *In vitro* was highlighted by the statistically significant changes in mean shoot length seen at different intervals (40, 60, and 90 days after culture). At 40, 60, and 90 days following culture, the explants grown on MS media supplemented with 1.5 mg/L BAP showed the longest shoots, measuring 0.63 cm, 1.20 cm, and 1.57 cm, respectively (Fig. 2). These findings show that BAP is the most efficient cytokinin among those examined and that its concentration is ideal for elongating regenerated shoots. The capacity of BAP to promote cell division and elongation in meristematic tissues, which directly contributes to shoot length, may be the reason for its excellent performance in elongating shoots. The lowest shoot lengths (0.37 cm, 0.63 cm, and 0.67 cm) were consistently observed in the basal media (control) without any additional growth regulators. This suggests that the explants' endogenous hormone levels were insufficient for substantial vegetative development. This demonstrates how crucial exogenously administered cytokinins are for encouraging shoot growth in *In vitro* settings. Conversely, the results for shoot duration were less encouraging for kinetin and TDZ treatments. Even though kinetin is a naturally occurring cytokinin, it frequently shows less effectiveness in causing elongation. This could be because it has a reduced binding affinity to cytokinin receptors or transfers more slowly within tissues. Because of its high cytokinin-like activity and sustained action, which may impede proper elongation, TDZ is frequently linked to compact and stunted shoot forms despite its robust morphogenetic activity. Pradhan *et al.* (2015) provided support for these findings by showing that BAP was superior to kinetin in encouraging shoot elongation in *Stevia rebaudiana*. Furthermore, BAP produced better shoot elongation in *Psidium guajava* than kinetin and TDZ, according to Jain *et al.* (2014). Yadav *et al.* (2010) found that BAP at 1.5 mg/L caused the maximum shoot elongation in *Withania somnifera*. Ashrafuzzaman *et al.* (2012), who found that jackfruit with nodal segments grown on MS medium supplemented with 2.0 mg/L BAP had the longest shoots (3.84 cm), while those with lesser concentrations had shorter shoots, further corroborate the efficiency of BAP as observed. These results support the findings of the current study and highlight how crucial it is to optimize BAP concentrations for the desired shoot elongation.

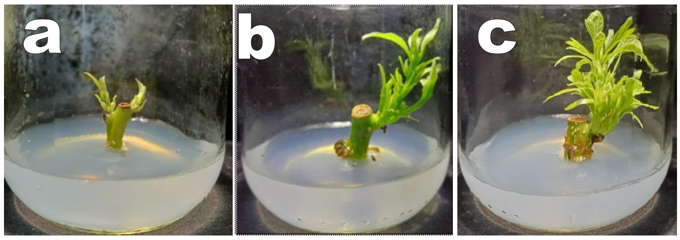

Fig. 2. Effect of BAP 1.5 mg/L on shoot induction response on the explant nodal segment at a) 40 days., b) 60 days., c) 90 days.

Number of days taken for shoot initiation for apical meristem explants: Table 3 data on the number of days needed for shoot initiation from apical meristems showed statistically significant differences between the treatments. Explants grown on MS medium supplemented with 1.5 mg/L BAP had the shortest initiation time (36.67 days), followed closely by 2.0 mg/L BAP (37.33 days). The control group, which was basal medium without growth regulators, had the longest duration for shoot initiation, at 43.00 days. The kind and concentration of exogenously applied cytokinins have a considerable impact on shoot initiation from apical meristems, according to the study's findings. Explants grown on MS media supplemented with 1.5 mg/L BAP showed the quickest time to shoot initiation, with 2.0 mg/L BAP coming in second. In contrast, explants on the basal medium without growth regulators (control) needed the most days, highlighting how important cytokinins are for speeding up the formation of shoots in apical meristems. This finding indicates that the use of growth regulators promotes early shoot induction by increasing meristematic cell division and metabolic activity. Additionally, it was observed that the number of days needed for shoot initiation decreased as cytokinin concentration increased up to 2.0 mg/L. implies that in apical meristem explants, increased cytokinin concentrations are advantageous for promoting early morphogenic responses. Despite having actively proliferating cells, apical meristems are frequently less sensitive than nodal segments because of apical dominance and increased endogenous auxin levels, which, if not properly regulated, might prevent cytokinin-mediated shoot induction. Higher quantities of BAP and kinetin may work in concert to overcome this apical dominance, enabling efficient cytokinin activity and the consequent development of shoots (Pathak & Dhawan, 2021). According to Safeer *et al.*

Table 3. Effect of different combinations of BAP, KIN and TDZ on shoot initiation, number of shoots per explant, and shoot length using an apical meristem as an explant

Treatments (mg/L)	Days taken for shoot initiation	Number of shoots per explant			Shoot length (cm)		
		40 days	60 days	90 days	40 days	60 days	90 days
T_1 - Control	43.00	0.33	1.00	1.00	0.03	0.47	0.57
T_2 - BAP 1.0	37.67	1.33	1.67	1.67	0.47	0.97	1.07
T_3 - BAP 1.5	36.67	1.33	1.67	2.00	0.53	1.00	1.13
T_4 - BAP 2.0	37.33	0.67	1.00	1.00	0.43	0.87	1.03
T_5 – KIN 1.0	38.67	1.00	1.33	1.33	0.40	0.70	0.93
T_6 - KIN 1.5	38.33	0.67	1.00	1.00	0.47	0.83	0.97
T_7 - KIN 2.0	38.00	0.67	1.00	1.00	0.37	0.77	0.93
T_8 - TDZ 1.0	41.33	0.33	1.00	1.00	0.30	0.60	0.83
T_9 - TDZ 1.5	41.00	0.33	1.00	1.00	0.10	0.47	0.77
T_{10}- TDZ 2.0	40.33	0.33	1.00	1.00	0.07	0.37	0.73
SEm±	0.84	0.37	0.18	0.15	0.06	0.08	0.08
CD (P=0.01)	3.39	1.47	0.73	0.60	0.22	0.31	0.34

(2020), MS media supplemented with 2 mg/L BAP and 2 mg/L kinetin considerably increased shoot induction and regeneration frequency (51.10 %) from apical meristems. These results are consistent with those of that study. Similar patterns were noted by Ansari *et al.* (2021) in *Rosa indica*, where shoot induction from apical buds was enhanced by greater BAP concentrations (up to 2.0 mg/L). Further supporting the beneficial impact of increased cytokinin concentrations on shoot organogenesis, Kumar *et al*. (2022) also showed that shoot start in *Capsicum annuum* was noticeably earlier and more prolific in medium supplemented with higher BAP and kinetin levels.

Number of shoots per explant from apical meristem explants: The data recorded on the number of shoots per explant from apical meristem with different concentrations of cytokinins are furnished in Table 3. The maximum number of shoots per explant (1.33, 1.67, and 2.00) at 40, 60, and 90 days after culture, respectively, was in the MS medium fortified with BAP 1.5 mg/L (Fig. 3) and followed by explants cultured on MS medium containing 1 mg/L of BAP, which showed 1.33, 1.67, and 1.67 shoots per explant at 40, 60, and 90 days after culture, respectively. The lowest numbers of shoots per explant (0.33, 1.0, and 1.0) at 40, 60, and 90 days after culture, respectively, were formed from the explants inoculated on the media without any growth regulators (control), TDZ 1.00, 1.5, and 2.00 mg/L. When cultivated on MS media supplemented with BAP, particularly at 1.5 mg/L, apical meristems showed the maximum shoot multiplication, demonstrating the efficacy of BAP in boosting axillary bud proliferation and shoot induction. BAP consistently produced better results than TDZ and kinetin; however, TDZ treatments were less successful, perhaps as a result of their propensity to cause compact growth and upset hormonal balance. Similar results in avocado were reported by Sholi and Qasrawi (2022), who found that BAP considerably increased the number of shoots from both apical and nodal explants. Because increased endogenous auxin levels can prevent cytokinin-induced shoot development, apical meristems often respond less than nodal segments. Because of their latent axillary buds and advantageous cytokinin-to-auxin ratio, nodal segments usually react faster. A balanced hormonal environment is necessary for optimal shoot proliferation; hence, the relationship between exogenously applied cytokinins and internal hormone levels is critical (Schaller *et al.,* 2015; Zhao *et al.,* 2021). For shoot multiplication, BAP is still a dependable cytokinin, particularly in explants with greater apical dominance.

Mean shoot length from apical meristem explants: The influence of different cytokinins was statistically significant, and the observations recorded on shoot length from the apical meristem at 40, 60, and 90 days after culture are presented in Table 3. MS medium fortified with BAP (1.5 mg/L) showed the highest shoot length (0.53, 1.00, and 1.13) at 40, 60, and 90 days after culture, respectively (Fig. 3). The basal medium without growth regulators (control) showed the lowest shoot length (0.03, 0.47, and 0.57) at 40, 60, and 90 days after culture, respectively. Significant differences were observed in the shoot length data obtained from apical meristems following various cytokinin treatments. Out of all of them, BAP at 1.5 mg/L generated the longest shoots at every observation interval, proving to be more effective at encouraging elongation than kinetin and TDZ. This is explained by the strong cytokinin activity of BAP,

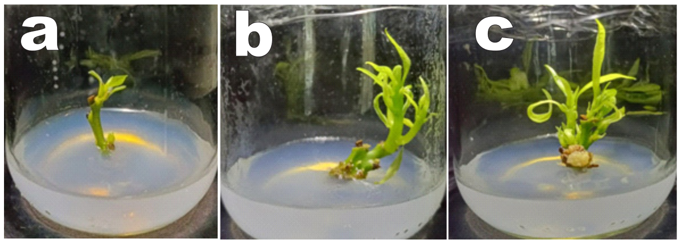

Fig. 3. Effect of BAP 1.5 mg/L on shoot induction response on the explant apical meristem at a) 40 days, b) 60 days, c) 90 days.

which promotes meristematic tissue cell division and elongation activities. Conversely, the shortest shoots were seen in the basal media (control), indicating that exogenous cytokinin addition is required to support vegetative growth *In vitro*. Patel *et al.* (2020), for example, showed that *Psidium guajava* nodal and shoot tip explants grown on MS media with 1.5 mg/L BAP produced the longest shoots (5.4 cm). In contrast, kinetin and TDZ treatments produced noticeably shorter lengths of 3.6 cm and 3.2 cm, respectively. Similarly, when *Rheum emodi* were cultivated on media containing 1.5 mg/L BAP, the highest shoot elongation was found to be 6.1 cm by Bhatt and Dhar (2021), as opposed to 4.0 cm and 3.7 cm in media fortified with kinetin and TDZ, respectively. In *Ficus religiosa*, the combination of 1.5 mg/L BAP and 0.15 mg/L IBA in the culture medium produced the highest shoot regeneration frequency (96.66 %) and maximum shoot length (1.83 cm), according to Sharma *et al.* (2022). This suggests that the treatment is more effective than kinetin and TDZ at promoting shoot development.

In conclusion, this work shows how *In vitro* regeneration might improve avocado micropropagation by overcoming the drawbacks of conventional propagation techniques, such as poor grafting success rates and genetic variability. Apical meristems and nodal segments were used as explants to optimize shoot induction; nodal segments performed better in terms of both shoot length and induction. According to the findings, surface sterilizing with 2.0% sodium hypochlorite for five minutes successfully reduces microbial contamination while preserving explant survival rates. Nodal segments produced an average of 2.33 shoots per explant and reached a maximum shoot length of 1.57 cm at 90 days post-culture, making MS medium supplemented with 1.5 mg/L BAP the most effective growth regulator combination tested for shoot induction and elongation. These results offer a trustworthy method for avocado micropropagation, which can greatly aid in the mass production of high-quality, genetically homogeneous seedlings. The procedure will be improved for commercial use and conservation initiatives with further rooting and acclimation studies.

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