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EFFECTS OF CYTOKININS ON AXILLARY SHOOT TIP CULTURE OF *AGLAONEMA* 'HUNGARY PINK' DURING MICROPROPAGATION

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ABSTRACT

The present study investigated the influence of various cytokinins on axillary shoot tip culture of *Aglaonema* 'Hungary Pink' under *in vitro* conditions. The shoot tips obtained from axillary shoots of *Aglaonema* 'Hungary Pink' were cultured on MS medium supplemented with different concentrations of BA (0.0, 2.0, 4.00 and 6.00 mg L^{-1}), TDZ (0.0, 0.5, 1.0 and 2.0 mg L^{-1}) and kinetin (0.0, 2.0, 4.0 and 6.0 mg L^{-1}). The MS medium devoid of growth regulator served as control. Significant differences were observed in response percentage, days to respond, days to shoot initiation, shoot number, shoot length and fresh weight. The highest response was obtained with TDZ 2.0 mg L^{-1} (80.63%) and BA 4.0 mg L^{-1} (80.42%), both statistically on par and superior to all other treatments. TDZ 0.5–1.0 mg L^{-1} also promoted higher response, while kinetin at 6.0 mg L^{-1} recorded the lowest value (48.58%) for response per cent. The earliest response (5.50 days) and rapid bud initiation (7.27 days) were observed in TDZ 0.5 mg L^{-1} and BA 4.0 mg L^{-1}, respectively. Maximum shoot proliferation (4.46 shoots/explant) and fresh biomass (4.98 g) were recorded with TDZ 2.0 mg L^{-1}, whereas kinetin at 2.0 mg L^{-1} enhanced shoot elongation (2.67 cm) which was on par BA 4.0 mg L^{-1}. Though, shoot tips cultured on TDZ 2.0 mg L^{-1} recorded highest number of shoots per explants and fresh biomass per explants but shoots produced on medium supplemented with TDZ were small and vitrified. Overall, BA at 4.0 mg L^{-1} proved most effective plant growth hormone for shoot induction. While kinetin was more suitable for improving shoot elongation. These findings highlight the differential roles of cytokinins in optimizing micropropagation protocols for *Aglaonema* 'Hungary Pink'.

***Key words*: *Aglaonema*, Cytokinins, Micropropagation, Shoot proliferation**

Introduction

Aglaonema (Chinese evergreen) is a genus of ornamental plants in the family Araceae, valued for its attractive, variegated foliage and adaptability to low light and humidity (Henny, 2000; Chen *et al.*, 2003). Native to Southeast Asia, it comprises about 26 species (Kew Garden, 2025) and has been cultivated in Asia for centuries as a lucky plant before its introduction to the West in 1885 (Brown, 1885). Apart from its aesthetic appeal, it is also recognized as an effective indoor air purifier (Wolverton *et al.*, 1989).

The genus has a base chromosome number of x = 6, though polyploidy is common (Nicolson, 1969). Propagation is mainly vegetative through cuttings or division, as sexual reproduction is hindered by non-simultaneous flowering and short pollen viability (Chen *et al.*, 2002; Henny *et al.*, 2003). However, traditional methods are slow and prone to transmitting pathogens from stock plants (Chase, 1997).

Tissue culture (micropropagation) provides an efficient alternative for producing large numbers of uniform, disease-free plants. It offers rapid multiplication, reduces greenhouse space need for stocking mother plants for propagation and speeds up the release of new cultivars (Razdan, 2003; George *et al.*, 2008). Yet, progress in *Aglaonema* micropropagation remains limited due to difficulties in culture establishment, low multiplication rates and the lack of standardized protocols (Chen and Yeh, 2007; Zhang *et al*., 2004). Plant growth regulators, especially cytokinins, are key to improving shoot multiplication efficiency in this genus (Hussein, 2004). Therefore, the present study was conducted to optimise the type and concentration of cytokinin required for initiation shoots from the shoot tips of axillary shoots.

Materials and Methods

The present study was conducted at ICAR-Directorate of Floricultural Research, (ICAR-DFR) Regional Station, Vemagiri, Andhra Pradesh and ICAR-National Institute for Research on Commercial Agriculture (ICAR-NRCA), Rajahmundry, Andhra Pradesh during academic year 2024-2025.

Plant material

One-year old plants were procured from local nursery at Kadiyam. The plants were grown in shade net house (50% shade) with standard cultivation practices. For inducing the axillary shoots, the plants were sprayed with 200 ppm Benzyl aminopurine (6-BAP) at weekly interval. After two months of spray, the shoots measuring 3-4 cm were removed using clean scalpel blade during morning hours and packed in sterile container. The shoots were then transported to tissue culture laboratory at ICAR-NIRCA, Rajahmundry from the ICAR-DFR, RS Vemagiri, Rajahmundry immediately for further processing and tissue culture.

Chemicals

The present study employed a range of chemicals for culture media preparation. The basal medium was Murashige and Skoog (MS) medium supplemented with $CaCl_2$ and vitamins. Plant growth regulators used included N^6-Benzyladenine, Thidiazuron and Kinetin. Sucrose served as a carbon source and Agar agar Type I was used for medium solidification. To prevent microbial contamination, antibiotics such as streptomycin sulphate and tetracycline hydrochloride were applied, along with the fungicide carbendazim. Tween-20 was incorporated as a surfactant. For surface sterilization of explants, mercuric chloride and sodium hypochlorite were used. Sodium hydroxide and hydrochloric acid were also used for adjusting the pH of culture medium.

Culture vessels and equipment

The study utilized essential equipment such as an analytical balance, magnetic stirrer, pH meter, glass bead sterilizer, heating vessels, microwave oven, autoclave and laminar air flow cabinet. Tools like a Bunsen burner, ethanol dip, ethanol spray, scalpels and forceps were also used. Glassware included borosilicate bottles, test tubes with caps, beakers, conical flasks, cylinders, pipettes and glass rods. The autoclave was used to sterilize culture media, instruments and glassware.

Washing and sterilization of experimental material

Glassware vessels were cleaned with germitol, rinsed with distilled water and dried in an oven at 140°C for 2 hours. For sterilization, glassware and instruments such as forceps and scalpels were autoclaved at 121°C for 15 minutes under 15 psi. The laminar airflow cabinet was disinfected with 70% ethanol and UV light and its blower and heater were kept on during culturing. During transfer of explants the dissecting instruments were further sterilized in a glass bead sterilizer and frequently flamed after dipping in 90 percent ethanol to maintain aseptic conditions.

Basic culture medium

Murashige and Skoog (MS) medium (1962) was used as the basal medium. For preparation, 4.4 g of MS powder (Himedia, PT021) was dissolved in double-distilled, deionized water along with 30 g L^{-1} sucrose as a carbon source. The culture media was supplemented with different concentrations of BA (0.0, 2.0, 4.0 and 6.0 mg L^{-1}), TDZ (0.0, 0.5, 1.0 and 2.0 mg L^{-1}) and Kinetin (0.0, 2.0, 4.0 and 6.0 mg L^{-1}). The MS medium devoid of growth regulator served as control. The pH was adjusted to 5.6 using 1N NaOH or HCl. Solidifying agent, Agar was added at 8 g L^{-1}, and the medium was heated until properly dissolved. The final volume was adjusted to a desired level. The culture medium then sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. The sterilized medium was dispensed into sterilized glass bottles (50 ml) or test tubes (20 ml), sealed with autoclavable polypropylene caps and wrapped with shrink film. The test tubes or bottles containing culture medium were cooled to room temperature and kept under observation for three days to confirm sterility before culturing the explants.

Culture conditions

Aseptic conditions were maintained using a laminar airflow chamber equipped with UV light, HEPA filters and fluorescent lighting. The chamber was disinfected with 70% ethanol and UV light was run for 20–30 minutes before culturing. Hands were sterilized with 70% ethanol and instruments were dipped in 95% ethanol, flamed and kept in a glass bead sterilizer when not in use.

Culture techniques

Selection of explant and sterilization

Axillary shoots were collected early in the morning hours and prepared with removing the leaf sheaths and washing in the running water for 30 minutes to remove dust and other surface contaminants and these shoots were placed in solutions of carbendazim @ 3 g L^{-1} for 20 minutes, streptomycin sulphate @ 0.5 g L^{-1} for 10 minutes, tetracycline hydrochloride @ 0.5 g L^{-1} for 10 minutes. After that they are rinsed three times with autoclaved distilled water in laminar air flow chamber. Then they were dipped in 70% ethanol for 30 seconds then dipped in 0.1% mercury chloride for 5 minutes. After each step of sterilization, the explants were rinsed three times with sterile distilled water to reduce the toxicity of the chemicals.

Explant preparation

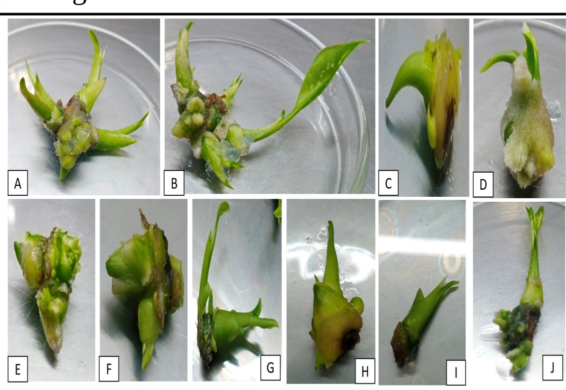
After sterilization, explants were placed on sterilized tissue paper to dry and the shoot tips measuring 3-4 mm in length were isolated. The shoot tips were then transferred aseptically into test tubes containing culture medium and gently pressed into the medium using sterilized forceps. The tubes were sealed with polypropylene caps and wrapped with parafilm to maintain sterility.

Transfer of explants

After an initial 30-day culture period, healthy, contamination-free explants were aseptically shifted to fresh culture bottles with the same medium, where they were maintained for another 30 days to support further growth.

Incubation of cultures

Cultures were maintained under controlled conditions at a temperature of 27 ± 2°C and relative humidity between 70% and 80%. A photoperiod of 16 hours light and 8 hours dark was provided, with illumination supplied by cool white LED lamps. The light intensity was maintained in the range of 2500–3000 lux to support optimal growth.



(A) elongated shoots, MS medium + BA 2 mg L^{-1} **(B)** elongated shoots, MS medium + BA 4 mg L^{-1} **(C)** shoots, MS medium + BA 6 mg L^{-1} **(D)** vitrified rosette shoots, MS medium + TDZ 0.5 mg L^{-1} **(E)** vitrified rosette shoots, MS medium + TDZ 1.0 mg L^{-1} **(F)** vitrified rosette shoots, MS medium + TDZ 2.0 mg L^{-1} **(G)** elongated shoots, MS medium + Kinetin 2.0 mg L^{-1} **(H)** elongated shoots, MS+ Kinetin 4.0 mg L^{-1} **(I)** elongated shoots, MS Medium + Kinetin 6.0 mg L^{-1} **(J)** MS medium devoid of growth regulator.

Effects of Cytokinins on culture initiation from axillary shoot tip of Aglaonema 'Hungary Pink' after 2 months of *in vitro* culture

Observations recorded

The response percentage was calculated as the proportion of explants showing callus induction or shoot primordia to the total cultured explants, recorded at the end of the culture period. The days taken to respond was noted as the number of days from inoculation to the first visible response, while days to shoot initiation was recorded as the time from inoculation to the appearance of the first visible shoot primordium ($\geq$0.5 mm). The number of shoots per explant was counted visually and shoot length was measured from the base to the tip of the longest shoot at the end of the culture period, with mean shoot length calculated for explants producing multiple shoots. At the end of the culture period, plantlet clumps were carefully removed from the culture vessels. The adhering medium was gently washed off with distilled water and excess surface moisture was removed using sterile blotting paper. The fresh weight of each plantlet clump was immediately measured using a digital electronic balance with milligram sensitivity.

Results and Discussions

Response percentage

Cytokinin type and concentration significantly affected shoot tip response in *Aglaonema* 'Hungary Pink'. The highest response percentage were recorded with TDZ 2.0 mg L^{-1} (T_7, 80.63%) and BA 4.0 mg L^{-1} (T_3, 80.42%), statistically superior to other treatments (CD at 5% = 2.34). TDZ 1.0 mg L^{-1} (T_6, 78.42%) and 0.5 mg L^{-1} (T_5, 76.97%) also showed high responses, while kinetin 6.0 mg L^{-1} (T_{10}, 48.58%) was the lowest. The mean response was 67.07%.

The superior response recorded with BA and TDZ can be attributed to their strong cytokinin activity in breaking apical dominance and stimulating axillary meristems. Cytokinins regulate cell division and bud outgrowth by activating cytokinin-responsive genes, enhancing cell cycle activity, and mobilizing assimilates to dormant buds (Muller and Leyser, 2011). BA is one of the most commonly used cytokinins in ornamental tissue culture and has consistently been reported to induce high shoot multiplication in *Aglaonema* (Henny, 1985; Chen *et al.*, 2003), *Dieffenbachia* (Rahman *et al.*, 2006), and *Anthurium* (Rout and Das, 1997). As kinetin was less effective it might be due to producing the lowest response percentage. Its weaker biological activity in promoting cell division and shoot differentiation has also been documented in other ornamentals such as *Chrysanthemum* (Sahoo and Chand, 1998) and *Anthurium* (Rout *et al.*, 2001). BA's performance aligns with earlier studies in *Aglaonema* (Henny, 1985; Chen

Table 1: Effect of media fortification with different cytokinins on response percentage, days taken to respond, days to shoot initiation, number of explants per plant, shoot length (cm) and fresh weight (g).

Treatments	Response percentage	Days taken to respond (d)	Days to shoot initiation (d)	Number of shoots per explant	Shoot Length (cm)	Fresh weight (g)
T_1: MS Devoid of growth regulators (control)	56.15 (48.53)	9.52	13.63	1.27	2.48	1.34
T_2: MS+ BA 2.0 mg L^{-1}	72.42 (58.33)	6.70	8.50	3.11	2.17	1.85
T_3: MS+ BA 4.0 mg L^{-1}	80.42 (63.76)	5.83	7.27	3.93	2.52	3.15
T_4: MS+ BA 6.0 mg L^{-1}	66.33 (54.54)	8.23	11.77	2.31	2.42	2.43
T_5: MS+ TDZ 0.5 mg L^{-1}	76.97 (61.33)	5.50	7.37	3.54	0.76	2.37
T_6: MS+ TDZ 1.0 mg L^{-1}	78.42 (62.34)	6.47	8.43	4.23	0.76	3.33
T_7: MS+ TDZ 2.0 mg L^{-1}	80.63 (63.91)	6.50	8.53	4.46	0.73	4.98
T_8: MS+ Kin 2.0 mg L^{-1}	58.33 (49.80)	10.90	14.93	1.60	2.67	1.98
T_9: MS+ Kin 4.0 mg L^{-1}	52.54 (46.46)	10.52	15.03	1.27	1.88	1.79
T_{10}: MS+ Kin 6.0 mg L^{-1}	48.58 (44.19)	12.93	17.63	1.09	1.43	1.45
Mean	**67.07 (55.31)**	**8.31**	**11.30**	**2.68**	**1.78**	**2.46**
SEm±	0.79	0.43	0.45	0.08	0.06	0.18
CD @ 5%	2.34	1.28	1.33	0.24	0.19	0.52

et al., 2003), *Dieffenbachia* (Rahman *et al*., 2006), and *Anthurium* (Rout and Das, 1997). TDZ, a phenylurea derivative, also enhanced shoot induction in *Syngonium* (Gantait *et al*., 2011) and Aglaonema hybrids (Henny and Chen, 2003). These results confirm BA and TDZ as effective cytokinins for axillary shoot induction in Aglaonema 'Hungary Pink'.

Days taken to respond

A significant variation was observed in the number of days required for explants to respond among different cytokinin treatments (CD at 5% = 1.10) . The earliest response was recorded in TDZ 0.5 mg L^{-1} (T_5) (5.50 days), which was statistically comparable to BA 4.0 mg L^{-1} (T_3) (5.83 days) and TDZ 2.0 mg L^{-1} (T_7) (6.50 days). Explants under these treatments responded considerably faster than the control (9.52 days) and all kinetin treatments, which required 10.52-12.93 days. The maximum number of days to response was observed in kinetin 6.0 mg L^{-1} (T_{10}) (12.93 days), significantly higher than all other treatments. The overall mean across treatments was 8.31 days, indicating a clear influence of cytokinin type and concentration on the rate of explant response.

The faster response seen with BA and TDZ is likely due to their strong cytokinin activity, which promotes early cell division and reactivates dormant meristematic tissues. Cytokinins enhance gene expression related to cell cycle regulation and bud initiation, accelerating processes like callus induction and shoot primordia formation (Huetteman and Preece, 1993). TDZ, a potent phenylurea derivative, mimics cytokinins and boosts their accumulation in explant tissues, leading to quicker bud break and shoot emergence. BA also stimulates cytokinin-responsive pathways, improving nutrient mobilization at the bud formation site.

Conversely, kinetin shows a delayed response, indicating a weaker effect on early morphogenic events. Its lower biological activity results in slower initiation of shoot primordia, as seen in previous studies (Sahoo and Chand, 1998). Research on *Anthurium* (Rout and Das, 1997) and *Syngonium* (Gantait *et al*., 2011) confirms that TDZ and BA significantly reduce response times, while kinetin's effects are often delayed.

Days to shoot initiation

BA and TDZ significantly reduced the number of days required for shoot bud initiation (CD at 5% = 1.33). The earliest initiation was observed in BA 4.0 mg L^{-1} (T_3, 7.27 days), which was statistically on par with TDZ 0.5 mg L^{-1} (T_5, 7.37 days), both being significantly faster than the control (13.63 days) and all kinetin treatments. The longest durations were recorded with kinetin 6.0 mg L^{-1} (T_{10}, 17.63 days) and kinetin 4.0 mg L^{-1} (T_9, 15.03 days), with the mean duration across treatments being 11.30 days.

Cytokinin type had a pronounced effect on shoot bud initiation in *Aglaonema*. BA and TDZ promoted earlier shoot initiation, likely due to their strong influence on meristematic activity and organogenic differentiation, which accelerates cell division and bud formation. These findings are consistent with earlier reports in *Aglaonema commutatum* (Henny, 1985), *Aglaonema* hybrids (Chen *et al*., 2003), and *Philodendron* (Chen and Henny, 2006). In contrast, kinetin required longer durations (14.93-17.63 days), reflecting its relatively lower efficiency in stimulating shoot initiation, as also reported in other ornamentals (Sahoo and Chand, 1998). The results further confirm that cytokinins, particularly BA and TDZ, positively influence shoot proliferation, shoot length and fresh weight in *Aglaonema*, underscoring their utility in optimizing micropropagation protocols.

Number of shoots per explant

A significant variation (CD at 0.5 = 0.22) was observed among the treatments for the number of shoots per explant. The highest shoot proliferation (4.46 shoots/explant) was recorded in MS medium supplemented with TDZ at 2.0 mg L^{-1} (T_7), which was significantly superior to all other treatments, followed by TDZ 1.0 mg L^{-1} (4.23 shoots/explant). Among BA treatments, BA 4.0 mg L^{-1} (T_3) induced a higher number of shoots (3.93 shoots/explant), whereas a higher concentration (6.0 mg L^{-1}) caused a significant decline (2.31 shoots/explant). In contrast, kinetin treatments were less effective, with the lowest shoot induction observed at 6.0 mg L^{-1} (T_{10}, 1.09 shoots/explant), which was statistically comparable to the control (1.27 shoots/explant).

The results indicate that BA at moderate concentrations effectively enhanced shoot proliferation per explant, while excessive concentrations led to reduced multiplication. These findings are in agreement with previous studies on *Aglaonema* and related aroids. For instance, Abass *et al.*, (2016) reported that BA at 8 mg L^{-1} produced the highest number of shoots (4.08/explant), which further increased to 6.70 shoots/explant when combined with 0.5 mg L^{-1} NAA. Similarly, El-Mahrouk *et al.*, (2016) achieved 2.5 shoots per explant on 3.0 mg L^{-1} BA, and Barakat and Gaber (2018) emphasized the essential role of BA in shoot multiplication in *A. commutatum*, particularly when supplemented with NAA. Fang *et al.*, (2013) also demonstrated that in vitro-derived nodal sections cultured on MS medium containing 0.5 mg L^{-1} NAA and 2 mg L^{-1} TDZ produced an average of 10.9 adventitious shoots per stem segment.

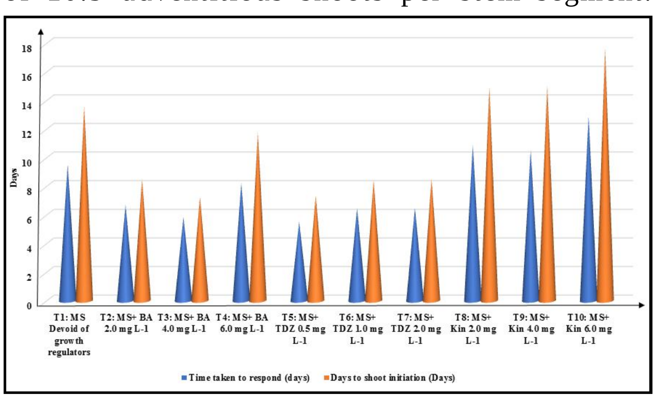


Fig. 1: Effect of different cytokinins on days taken to shoot initiation and time taken to respond.

Collectively, these results confirm the pivotal role of BA and TDZ in stimulating axillary bud break and shoot proliferation in *Aglaonema*.

Shoot length (cm)

Shoot elongation was significantly influenced by cytokinin type and concentration (CD at 0.05 = 0.19). The longest shoots were observed with kinetin at 2.0 mg L^{-1} (T_8, 2.67 cm), followed by BA 4.0 mg L^{-1} (T_3, 2.52 cm) and the control (2.48 cm). In contrast, TDZ treatments markedly restricted shoot elongation, with lengths remaining below 1.0 cm (0.73-0.76 cm). Higher concentrations of kinetin (6.0 mg L^{-1}, T_{10}) also led to a significant reduction in shoot length (1.43 cm), indicating that excessive cytokinin levels can negatively affect elongation.

Shoot elongation was significantly affected by cytokinin type and concentration (CD at 0.05 = 0.19). The longest shoots were observed with kinetin at 2.0 mg L^{-1} (T_8, 2.67 cm), followed closely by BA at 4.0 mg L^{-1} (T_3, 2.52 cm) and the control (2.48 cm). In contrast, TDZ treatments limited elongation to below 1.0 cm. Higher Kinetin concentrations (6.0 mg L^{-1}, T10) also reduced shoot length to 1.43 cm.

These results highlight the critical role of cytokinin type and concentration in shoot induction and architecture. BA at moderate levels promoted reasonable elongation and axillary shoot proliferation, whereas higher concentrations inhibited elongation due to excessive meristematic activity. TDZ, while effective for high-frequency shoot induction, produced compact shoots with minimal elongation (Chen and Yeh, 2007).

Although Kinetin is generally less effective for multiple shoots (El-Mahrouk *et al.*, 2016; Labasano, 2018), it enhanced shoot length at moderate levels, surpassing both BA and TDZ. Similar findings in Aglaonema 'Red Valentine' indicated kinetin's role in improving shoot quality. Thus, optimizing cytokinin type and concentration is essential for balancing shoot multiplication with desired

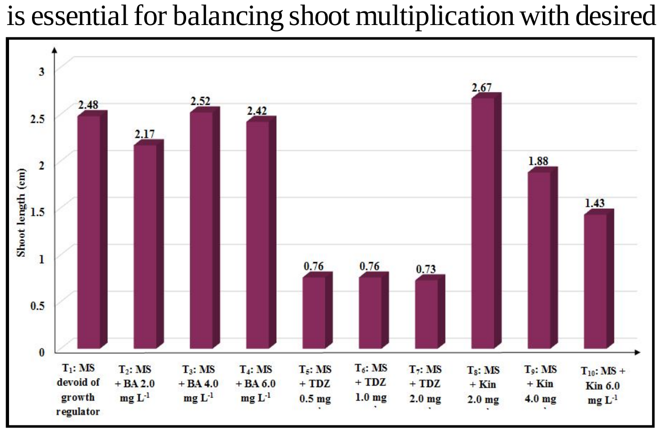


Fig. 2: Effect of different cytokinins on shoot length (cm).

architecture and quality, crucial for acclimatization and large-scale propagation.

Fresh weight (g)

Various cytokinins and their concentrations significantly influenced fresh biomass accumulation (CD at 0.05 = 0.52). The highest fresh weight (4.98 g) was recorded in MS medium supplemented with TDZ at 2.0 mg L^{-1} (T_7), which was significantly superior to all other treatments, followed by TDZ 1.0 mg L^{-1} (3.33 g) and BA 4.0 mg L^{-1} (3.15 g). In contrast, the lowest fresh weights were observed in the control (1.45 g) and kinetin 6.0 mg L^{-1} (1.34 g), both statistically comparable.

Fresh weight, representing overall biomass accumulation, was closely associated with treatments that promoted higher shoot proliferation and elongation. TDZ treatments, particularly at 2.0 mg L^{-1}, favored rapid multiplication of multiple shoots, thereby contributing to increased biomass. However, BA treatments, while slightly less effective in producing shoot numbers and vigor, which also contributed substantially to overall fresh weight. These observations align with earlier studies in *Aglaonema* and related ornamentals. For instance, El-Mahrouk *et al*., (2016) reported that TDZ (1.5 mg L^{-1}) combined with NAA (1.0 mg L^{-1}) produced the highest proliferation rate (5.0 shoots/explant) in *Aglaonema* 'Valentine', whereas BA treatments were more effective in improving shoot elongation and quality. Thus, the choice of cytokinin not only affects shoot multiplication but also influences biomass accumulation, highlighting the importance of balancing proliferation with shoot vigor for successful micropropagation.

Conclusion

The present study demonstrates that cytokinin type and concentration significantly influence axillary shoot induction, shoot bud initiation and shoot elongation in Aglaonema 'Hungary Pink'. TDZ (2.0 mg L^{-1}) and BA (4.0 mg L^{-1}) were most effective in enhancing response percentage, reducing the days to shoot initiation and fresh weight confirming their strong role in overcoming apical dominance and promoting shoot proliferation. In contrast, kinetin showed lower efficiency in shoot multiplication but was effective in improving shoot elongation, particularly at 2.0 mg L^{-1}. Overall, BA and TDZ are recommended for maximizing shoot proliferation, while kinetin can be used to improve shoot quality and elongation, providing a balanced approach for in vitro propagation of Aglaonema 'Hungary Pink'.

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