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# DEVELOPMENT OF IN-VITRO PROPAGATION PROTOCOL FOR THE MASS PROPAGATION OF TURMERIC (*Curcuma longa* L.) USING TISSUE CULTURE TECHNIQUES

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**ABSTRACT.** The growing demand for high-quality, disease-free turmeric (Curcuma longa Linn.) planting materials in the Philippines is limited by traditional propagation methods and the high cost of micropropagation. This study aimed to develop an efficient, cost-effective in vitro propagation protocol by optimizing sterilization, hormonal treatments, culture systems, and acclimatization methods. Explants from disease-free rhizomes were sterilized using varying sodium hypochlorite (NaClO) concentrations (40–60%) and soaking times (10–20 min). Results showed that 60% NaClO for 10–20 minutes provided optimal sterilization and survival. Murashige and Skoog (MS) medium supplemented with Thidiazuron (TDZ) at 2.0–2.5 mg/L promoted bud emergence, while 0.01 mg/L enhanced root development. The double-phase culture system (DPS) produced longer, heavier roots than the conventional system (CMS), though with higher contamination (35%) and lower survival (65% vs. 85%). Hydroponic acclimatization outperformed soil, yielding superior shoot and root growth with 100% survival. Additionally, hydroponic supplementation with 1.00 mL seaweed extract produced the most vigorous growth, while both low (0.50 mL) and excessive (1.50 mL) levels were less effective. Overall, the optimized sterilization, TDZ application, and hydroponic acclimatization significantly improved turmeric micropropagation efficiency, offering a practical approach for large-scale, high-quality plantlet production.

**Keywords:** *Turmeric, micropropagation, thidiazuron (TDZ), double-phase system, hydroponics, acclimatization, seaweed extract, sterilization*

## INTRODUCTION

Turmeric (*Curcuma longa Linn.)* is a rhizomatous perennial plant native to tropical South Asia and a member of the Zingiberaceae family. It is widely cultivated in tropical climates ranging from sea level up to 1500 meters in elevation, thriving in temperatures between 20–30 °C and with annual rainfall of 1500 mm or more [18]. This commercial crop is primarily grown for its tuberous rhizomes, which contribute to the flavor and color of foods and are extensively used as a spice, natural colorant, and ingredient in cosmetics, textiles, and pharmaceuticals. Turmeric also holds significant cultural and therapeutic value, largely due to its active compound curcumin, which exhibits antioxidant, antibacterial, antifungal, anti-inflammatory, anti-mutagenic, and anticancer properties [5, 20, 23, 37].

India dominates global turmeric cultivation, accounting for approximately 82% of the total area, followed by China, Myanmar, Nigeria, and Bangladesh [3, 48]. The Philippines has begun small-scale commercial cultivation mainly to supply the herbal and nutraceutical industries [11]. Traditional propagation through rhizomes is hindered by

slow multiplication, high costs, and susceptibility to diseases such as soft rot and bacterial wilt [4, 35].

In vitro micropropagation presents a promising alternative for rapid production of disease-free, high-quality planting materials [6, 40]. This technique commonly uses Murashige & Skoog (MS) medium supplemented with essential nutrients and plant growth regulators like auxins and cytokinins, which regulate root and shoot development [27, 33, 49]. Thidiazuron (TDZ), a cytokinin-like compound, has been increasingly used in turmeric micropropagation due to its high efficacy in shoot induction, somatic embryogenesis, and callus formation, though its optimal concentration varies depending on plant species and explant type [8, 13, 25, 48].

While conventional micropropagation relies on semi-solid agar media, the dual-phase culture system (DPS), which combines solid and liquid media, reduces contamination risks and production costs by minimizing subculturing [36, 44, 46]. DPS shows potential to enhance propagation efficiency and lower the time required for mass production of planting materials [24].

This study aims to develop an optimized and cost-effective in vitro protocol for turmeric propagation, including establishing aseptic culture, TDZ supplementation in MS medium, applying the DPS during root proliferation to reduce contamination, and evaluating hydroponic acclimatization to produce healthy, robust plantlets suitable for field planting [29, 41].

## MATERIALS AND METHODS

The study was conducted at the Tissue Culture Laboratory, Isabela State University, Echague, Isabela, to develop an optimized in vitro propagation and acclimatization protocol for Curcuma longa. Five interrelated studies were carried out, each addressing a key stage of the propagation process.

### *Surface Sterilization and Establishment of Aseptic Culture*

Rhizomes of Curcuma longa were collected from the College of Agriculture, Isabela State University. Disease-free explants (25–30 cm) were cleaned by removing roots and leaves, washed with soapy water, peeled, and sterilized using 20% hypochlorite, followed by varying sodium hypochlorite (NaClO) concentrations (40%, 50%, 60%) for 10, 15, and 20 minutes. Explants were then exposed to UV light for 20 minutes. All procedures were conducted under aseptic conditions, with disinfection of the laboratory, inoculation chamber, and sterilization of glassware by pressure cooking. Explants were inoculated onto Murashige and Skoog (MS) basal medium and cultured vertically under 25°C with fluorescent light. The experiment followed a Completely Randomized Design (CRD) with factors of NaClO concentration and soaking time, replicated three times. Data on contamination and survival rates were collected after 30 days.

### *Effect of Thidiazuron (TDZ) Concentrations on In Vitro Propagation*

An experiment was conducted to determine the effect of varying TDZ concentrations on bud induction, shoot proliferation, and rooting. MS medium enriched with macronutrients, micronutrients, vitamins, 6-Benzylaminopurine (6-BAP), Indole-3-acetic acid (IAA), Naphthaleneacetic acid (NAA), and different TDZ levels was prepared, adjusted to pH 5.8, solidified with agar, and sterilized. Surface-sterilized explants were inoculated aseptically and maintained at 23 ± 2°C with a 16-hour photoperiod. Bud

multiplication was achieved through three subcultures (S1–S3) at 30-day intervals. After S3, shoots were transferred to rooting media containing NAA and varying TDZ concentrations. The experiment followed a CRD with nine treatments (0–3.0 mg/L TDZ) and three replications. Growth parameters were evaluated after 30 days.

### *Comparison of Conventional and Double-Phase Culture Systems*

This experiment compared the Conventional Micropropagation System (CMS) and Double-Phase Culture System (DPS) for root induction and elongation. Forty uniform in vitro plantlets (9 cm) were divided equally: Group A (CMS) used solidified MS rooting media with vitamins, organics, sucrose, coconut water, and NAA; Group B (DPS) used non-agarized liquid MS media for root proliferation. Both groups were incubated at 23°C under a 16-hour photoperiod for 30 days. Root length, weight, and survival rates were measured. Differences between treatments were analyzed using an unpaired Student's t-test.

### *Acclimatization in Soil and Hydroponic Systems*

Acclimatization performance of in vitro plantlets was evaluated in two systems: (A) soil media composed of sun-sterilized loam soil and vermicompost, and (B) the Simple Nutrient Addition Program (SNAP) hydroponics system, a passive non-circulating setup. Plantlets were rinsed, treated with fungicide, and planted in either medium using Styrofoam cups filled with coconut coir dust (hydroponics) or a soil mixture. Polyethylene covers maintained humidity, which was gradually reduced. Data on growth and survival were collected at 30 and 60 days after planting. An unpaired Student's t-test was used to determine significant differences between treatments.

### *Hydroponic Acclimatization Using Seaweed Extract-Enriched Nutrient Solutions*

To optimize hydroponic acclimatization, uniform plantlets (4–5 cm height, 3–4 leaves) were acclimatized using a passive Kratky hydroponic system under four seaweed extract concentrations. Plantlets were placed in net pots with sterile coconut coir and immersed in nutrient solutions (pH 5.5–6.5; EC 1.8–2.5 mS/cm). The setup was maintained at 25–30°C and 70–80% humidity, with weekly nutrient replenishment and daily monitoring. Growth metrics, including plant height, stem diameter, leaf count and area, root development, and survival rate, were recorded. The experiment followed a CRD with four treatments and three replications. Data were analyzed using ANOVA and Tukey's HSD test.

## RESULTS AND DISCUSSION

### *Effect of Sodium Hypochlorite (NaClO) Solutions and Soaking Duration on Explant Contamination and Survival*

Table 1 shows the effect of concentration of sodium hypochlorite (NaClO) solutions and soaking durations applied singly and in combination on the contamination and survival rate of the explants. The data reveal the significant effect of NaClO concentration and soaking duration on contamination and survival rates of explants. As the concentration of NaClO increases from 40% to 60%, contamination decreases markedly from 55% to just 7%, while survival rates improve from 44% to 92%. This trend highlights the effectiveness of higher NaClO concentrations in reducing contamination and promoting explant viability. Similarly, soaking duration influences these outcomes,

with a 20-minute soak resulting in the lowest contamination (13%) and the highest survival rate (91%), compared to shorter soaking times.

When considering the interaction between concentration and duration, treatments with 60% NaClO combined with either 10 or 20 minutes of soaking yield the best results, completely eliminating contamination (0%) and achieving 100% survival. Conversely, the 15-minute soak with 60% NaClO shows a slight decrease in survival (78%), suggesting potential stress from prolonged exposure. Lower concentrations of 40% NaClO, regardless of soaking time, consistently exhibit higher contamination and lower survival rates, indicating less effective sterilization. At 50% NaClO, soaking for 15 to 20 minutes provides a good balance of contamination control and explant survival rates. Overall, the optimal treatment appears to be soaking explants in 60% NaClO for either 10 or 20 minutes to maximize survival and minimize contamination effectively.

Increasing NaClO (sodium hypochlorite) concentration generally leads to a marked reduction in contamination rates and an improvement in explant survival, but only up to a certain threshold. For example, soaking peach rootstock explants in 20% NaClO for 15 minutes resulted in minimal contamination (0.24%) and high survival (96.6%) [17]. In strawberry, 1% NaClO for 5 minutes yielded the lowest contamination (10%) and highest viability (60%) [34]. However, excessively high concentrations can damage tissues and reduce survival, as seen in some studies where higher NaClO levels increased explant mortality [17].

Longer soaking times with NaClO typically further reduce contamination, but overexposure can stress or injure explants, lowering survival rates. For instance, a 15-minute soak at optimal NaClO concentrations was effective for both peach and strawberry explants [17, 34]. In other species, extending NaClO treatment up to 50 minutes further reduced specific fungal contamination, but did not affect germination rates [31]. The optimal duration varies by species and tissue type, with 10–20 minutes often balancing contamination control and survival [17].

Optimal NaClO concentration and soaking duration are critical for minimizing contamination and maximizing explant survival. While higher concentrations and longer soaks are generally more effective, excessive exposure can harm plant tissues. The best results are typically achieved with moderate-to-high NaClO concentrations (1–20%) and soaking times of 10–20 minutes.

***Table 1.*** *Contamination and survival rate of the explants as affected singly and in combination with the concentration of sodium hypochlorite (NaClO) solutions and soaking durations*

| Treatments | Contamination (%) | Survival Rate (%) |
|---|---|---|
| **Factor A - NaClO Concentration** | | |
| C1 - 40% | 55$^{a}$ | 44$^{b}$ |
| C2 - 50% | 18$^{b}$ | 85$^{a}$ |
| C3 - 60% | 7$^{b}$ | 92$^{a}$ |
| **Factor B - Soaking Duration** | | |
| T1 - 10 minutes | 30$^{ab}$ | 70$^{b}$ |
| T2 - 15 minutes | 39$^{a}$ | 61$^{b}$ |
| T3 - 20 minutes | 13$^{b}$ | 91$^{a}$ |
| **Factor A x Factor B** | | |
| $C_1T_1$ (40 percent x 10 minutes) | 67$^{a}$ | 33$^{c}$ |
| $C_1T_2$ (40 percent x 15 minutes) | 78$^{a}$ | 22$^{c}$ |
| $C_1T_3$ (40 percent x 20 minutes) | 67$^{a}$ | 78$^{b}$ |
| $C_2T_1$ (50 percent x 10 minutes) | 22$^{b}$ | 78$^{b}$ |
| $C_2T_2$ (50 percent x 15 minutes) | 17$^{b}$ | 83$^{a}$ |

| | | |
|---|---|---|
| $C_2T_3$ (50 percent x 20 minutes) | $17^b$ | $94^a$ |
| $C_3T_1$ (60 percent x 10 minutes) | $0^c$ | $100^a$ |
| $C_3T_2$ (60 percent x 15 minutes) | $22^b$ | $78^b$ |
| $C_3T_3$ (60 percent x 20 minutes) | $0^c$ | $100^c$ |

Means followed by common letters are not significantly different

***Effect of Thidiazuron (TDZ) Concentration on In vitro Propagation***

This study investigates the effect of varying levels of TDZ on the *in vitro* propagation of turmeric. During bud proliferation, higher numbers of buds are obtained in S1 and S3 when the culture media is supplemented with TDZ in the range of 1.0 to 2.5 mg/L of water. In S2, a TDZ concentration of 2.5 mg/L induces higher bud proliferation. These findings suggest optimal bud proliferation can be achieved by supplementing the MS medium with 1.0 to 2.5 mg/L of TDZ.

Regarding shoot growth parameters, the study demonstrates that the optimal shoot fresh weight is observed when the MS medium is supplemented with 1.0-2.5 mg/L of TDZ. Additionally, higher stem girth is obtained when 1.5-2.5 mg/L of TDZ is added to the medium. Longer shoot length and a higher number of leaves are observed when TDZ is added in media ranging from 0.01-2.5 mg/L. These results indicate that varying amounts of TDZ enhance the growth performance of turmeric during in vitro culture. Furthermore, *in vitro*-grown turmeric exhibits a greater leaf area when the TDZ concentration is between 2.0 and 2.5 mg/L, suggesting that higher TDZ concentrations promote leaf growth during micropropagation.

However, in terms of root growth, an inverse relationship is observed between the amount of TDZ and the number and fresh weight of roots produced. Lower TDZ concentrations (0.01 mg/L) in the MS medium lead to more roots and heavier fresh roots. Conversely, higher TDZ concentrations promote longer root growth. These findings indicate that lower TDZ concentrations (0.01 mg/L) facilitate root regeneration, resulting in a higher number and weight of roots, while higher TDZ concentrations encourage longer root growth. The study demonstrated the effect of TDZ on the in vitro bud proliferation of turmeric. The result of the study shows that higher concentrations of TDZ, specifically 2.5 mg/L (T7) and 2.0 mg/L (T6), consistently led to the highest bud emergence across all subculture stages. This suggests that a concentration of 2.5 mg/L of TDZ is particularly effective in promoting bud initiation and development.

The data demonstrates a dose-response relationship between TDZ concentration and bud emergence. As the TDZ concentration increased, there was a corresponding increase in the number of buds. The concentration of TDZ appears to significantly impact the proliferation of buds, indicating the importance of carefully optimizing the concentration in tissue culture protocols. Findings show that T7 (2.5 mg/L) consistently outperformed other treatments, and T6 (2.0 mg/L) ranked second across all subculture stages. This consistency shows that these particular TDZ doses effectively stimulate and induce bud development. The control group (T0) consistently exhibited the lowest bud emergence, emphasizing the necessity of TDZ supplementation for optimal results. This reinforces the notion that TDZ plays a crucial role in stimulating bud formation, and the absence of TDZ (or lower concentrations) results in a significantly lower number of buds.

The increasing trend in bud emergence from S1 to S3 indicates a cumulative effect of TDZ on the proliferation of buds with successive subcultures. This temporal pattern suggests that the effects of TDZ on bud emergence may become more pronounced over time. The study also investigated the effects of varying TDZ concentrations on shoot regeneration and elongation in turmeric meriplants. The stem length exhibited a clear

dose-dependent relationship with TDZ concentration. The treatment with 0.5 mg TDZ/L (T3) produced the longest stems, emphasizing the stimulatory effect of TDZ on stem elongation. Interestingly, the treatments with higher TDZ concentrations (T6 and T7) closely followed, indicating that while an optimal concentration promotes elongation, excessively high concentrations may not further enhance this trait.

Similarly, stem girth was significantly influenced by TDZ, with T7 (2.5 mg TDZ/L) displaying the highest girth. This suggests that TDZ influences stem length and contributes to the development of thicker stems. In terms of the number of leaves, the study revealed that T7 had the highest mean number of leaves, indicating a positive correlation between TDZ concentration and leaf production. This result has implications for plants, which are crucial factors in turmeric crops' overall health and productivity.

With regards to leaf area, T7 displayed the highest mean leaf area, affirming the role of TDZ in promoting more considerable leaf development. In terms of shoot fresh weight, T7 again emerged as the treatment with the highest mean weight. This result implies that TDZ is pivotal in enhancing overall biomass production in turmeric meriplants.

The results indicate that the optimal shoot fresh weight is achieved when the MS medium is supplemented with 1.0-2.5 mg/L of TDZ, while a higher stem girth is observed when 1.5-2.5 mg/L of TDZ is added to the medium. Longer shoot length and a higher number of leaves are observed when TDZ is added in concentrations ranging from 0.01-2.5 mg/L. Additionally, a larger leaf area is observed in in vitro-grown turmeric when the TDZ concentration is between 2.0-2.5 mg/L. These findings suggest that using varying amounts of TDZ enhances the growth performance of turmeric during *in vitro* propagation, compared to the absence of TDZ. Thus, TDZ promotes shoot regeneration and elongation in the micropropagation process of turmeric.

However, in terms of the number and fresh weight of the roots, an inverse relationship was revealed. Specifically, lower TDZ concentrations (MS Medium supplemented with 0.01 mg/L of TDZ) result in more roots and heavier fresh roots. Conversely, longer root length is observed with higher TDZ concentrations in the MS medium. This observation could be attributed to the fact that plantlets with fewer total roots exhibit longer individual roots. Overall, the findings suggest that shoot and root regeneration are facilitated by lower TDZ concentrations, specifically at 0.01 mg/L in the MS medium. The study shows that different levels of TDZ significantly affect the growth of turmeric plants in controlled environments. Higher TDZ concentrations, especially at 2.5 mg/L and 2.0 mg/L, consistently result in more buds, indicating their effectiveness in promoting bud development. The study highlights a clear connection between TDZ concentration and the number of buds, emphasizing the need for precise control in tissue culture practices. Treatment with 2.5 mg/L TDZ consistently performs best, emphasizing its efficacy in stimulating both bud and shoot growth. Overall, the findings suggest specific TDZ concentrations optimize various growth aspects in turmeric, showcasing its role in improving overall plant development during in vitro propagation. Additionally, the study demonstrated a relationship between TDZ levels and root characteristics, indicating the influence of TDZ on both shoot and root regeneration.

The findings of the study align with previous research conducted on the effect of TDZ on the in vitro propagation of turmeric and other plant species. TDZ has been extensively studied for its efficacy in promoting shoot and root regeneration and bud formation in various plant species. It is a widely recognized plant growth regulator commonly utilized in tissue culture techniques to enhance axillary bud proliferation and facilitate shoot

organogenesis and morphogenesis. However, the optimal concentrations of TDZ can vary depending on the specific plant species.

For instance, research focused on turmeric micropropagation revealed that using 1.0 mg/L TDZ resulted in an average of 3 shoots per explant [48]. In the case of *Curcuma caesia* Roxb., the highest mean number of shoots/buds was achieved with a TDZ concentration of 0.1 mg/L. Similarly, in *Curcuma zedoaria* Roscoe, the most effective shoot multiplication occurred in an MS medium supplemented with 1.5 mg/L TDZ, leading to an average of 5.3 ± 0.24 shoots per explant during the regeneration of in vitro plantlets from explants.

Similar favorable effects of TDZ have been observed in other plant species, such as *Zingiber officinale* Rosc. Var. Rubrum, *Capsicum annuum* L, *Artemisia annua* L., *Casuarina cunninghamiana* Miq., and *Jatropha curcas*. Furthermore, TDZ has shown efficacy in inducing morphogenesis in plant *in vitro* propagation, as observed in *Echinacea purpurea* L. [25], African violet, *Vitex trifolia* L, *Curcuma caesia* Roxb., *Musa* sp., almond "Beldi" ecotypes, *Alstroemeria aurantiaca* cv. 'Rosita' [21].

In addition, the effectiveness of TDZ in inducing shoot multiplication and bud formation has been observed in various plant species, such as *Aloe vera* (L.) Burm. f., tea plant, *Tulipa edulis*, *Myrtus communis* L, *Lilium monodelphum* M. Bieb, var. Armenum, *R. decorum*, *Uraria picta*, *Astragalus cariensis* Boiss, *Etlingera coccinea*, *Bauhinia tomentosa*, *Hydrangea quercifolia* Bartr, *Ocimum basilicum*, and others.

Studies have also shown that pre-treatment with TDZ can enhance multiple shoot formation in certain plant species before in vitro propagation. Researchers such as Prathanturarug et al. [38] and Gou et al. [19] have observed that TDZ pre-treatment increases the chances of tissues responding to other inductive stimuli and improves the efficiency of in vitro propagation.

TDZ, a synthetic cytokinin, is widely used in plant tissue culture to promote shoot regeneration and in vitro multiplication. It mimics the natural plant hormone cytokinin, which regulates plant cell division and differentiation. TDZ interacts with cytokinin receptors in plant cells, triggering biochemical reactions that activate cell division genes and promote the formation of new plant tissue [25, 55]. Moreover, TDZ increases endogenous cytokinin levels and inhibits cytokinin-degrading enzymes, enhancing cell division and proliferation. Its mode of action also involves inhibiting adenylate isopentenyltransferase (IPT), the enzyme responsible for endogenous cytokinin biosynthesis, leading to the accumulation of cytokinin precursors. These precursors can be converted into active cytokinins, triggering adventitious bud formation and shoot regeneration.

Furthermore, TDZ affects shoot elongation by regulating gene expression in cell expansion and differentiation. It upregulates genes associated with cell wall component synthesis and auxin/gibberellin signaling pathways, resulting in longer and more robust shoots [55]. However, TDZ can inhibit root proliferation and elongation in some plant species [45]. High concentrations of TDZ in the culture medium can impede root development, while low concentrations may initially enhance root growth but inhibit it at higher concentrations. The rooting process with TDZ is complex, and its mechanism involves the modulation of auxin-cytokinin balance and the production of endogenous auxins. TDZ is often used with other rooting hormones, such as IBA or NAA, to improve rooting. However, the optimal concentration and duration of TDZ treatment depend on the plant species and tissue culture protocol. Excessive TDZ treatment can hinder rooting or result in shoot proliferation instead. Hence, TDZ effectively promotes shoot

regeneration, bud formation, and in vitro multiplication by mimicking cytokinins and stimulating cell division. It also influences shoot elongation through gene regulation. However, its impact on root proliferation varies with concentration and species, and careful optimization of TDZ treatment is necessary to achieve successful in vitro rooting in plant micropropagation.

***Table 2.*** *In Vitro growth performance of turmeric (Curcuma longa Linn.) as affected by varying concentrations of TDZ.*

| Treatments | Number of Buds Emerged 30 Days after Bud Induction and 30 days thereafter | | | Shoot Proliferation taken 30 Days after Shoot Induction | | | | |
|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | Stem Length (mm) | Stem Girth (mm) | Number of Leaves | Leaf Area ($mm^2$) | Shoot Fresh Weight (g) |
| $T_0$ – MS Medium with no TDZ (Control) | 3.25$^{c}$ | 4.25$^{e}$ | 4.23$^{d}$ | 70.55$^{c}$ | 0.38$^{c}$ | 3.10$^{b}$ | 320.63$^{d}$ | 0.75$^{d}$ |
| $T_1$ – MS Medium Supplemented with 0.01 mg of TDZ/L of water | 3.21$^{c}$ | 4.30$^{de}$ | 4.27$^{d}$ | 87.23$^{b}$ | 0.40$^{c}$ | 3.70$^{ab}$ | 445.37$^{cd}$ | 0.95$^{c}$ |
| $T_2$ – MS Medium Supplemented with 0.1 mg of TDZ/L of water | 3.60$^{c}$ | 4.32$^{de}$ | 4.73$^{c}$ | 87.46$^{a}$ | 0.42$^{c}$ | 3.70$^{ab}$ | 449.73$^{cd}$ | 0.96$^{c}$ |
| $T_3$ – MS Medium Supplemented with 0.5 mg of TDZ/L of water | 4.05$^{b}$ | 4.40$^{d}$ | 5.53$^{b}$ | 88.90$^{a}$ | 0.47$^{bc}$ | 3.83$^{a}$ | 449.37$^{cd}$ | 1.01$^{bc}$ |
| $T_4$ – MS Medium Supplemented with 1.0 mg of TDZ/L of water | 5.03$^{a}$ | 5.06$^{c}$ | 6.73$^{a}$ | 88.47$^{a}$ | 0.47$^{bc}$ | 3.83$^{a}$ | 538.57$^{bc}$ | 1.06$^{ab}$ |
| $T_5$ – MS Medium Supplemented with 1.5 mg of TDZ/L of water | 5.13$^{a}$ | 5.05$^{c}$ | 6.73$^{a}$ | 88.47$^{a}$ | 0.57$^{ab}$ | 3.85$^{a}$ | 583.07$^{bc}$ | 1.07$^{ab}$ |
| $T_6$ – MS Medium Supplemented with 2.0 mg of TDZ/L of water | 5.18$^{a}$ | 5.76$^{b}$ | 6.83$^{a}$ | 88.83$^{a}$ | 0.57$^{ab}$ | 3.90$^{a}$ | 677.03$^{ab}$ | 1.09$^{a}$ |
| $T_7$ – MS Medium Supplemented with2.5 mg of TDZ/L of water | 5.26$^{a}$ | 6.32$^{a}$ | 6.83$^{a}$ | 88.63$^{a}$ | 0.58$^{a}$ | 4.05$^{a}$ | 761.27$^{a}$ | 1.11$^{a}$ |
| **Grand Mean** | **4.34** | **4.93** | **5.74** | **86.07** | **0.38** | **3.75** | **528.13** | **1.00** |
| **ANOVA** | ** | ** | ** | * | ** | ** | ** | ** |

Means followed by common letters are not significantly different

### *Comparison of Conventional Micropropagation System (CMS) and Double-Phase System (DPS)*

*Root Growth Performance*

The root growth performance of turmeric roots (*Curcuma longa* Linn.) was measured in terms of the number of roots developed, root length (mm), and root fresh weight (g).

***Table 3.*** *Root growth performance of turmeric (Curcuma longa Linn.) 30 days after in vitro root initiation.*

| Treatments | Number of Roots | Root Length (mm) | Root Fresh Weight (g) |
|---|---|---|---|
| Conventional Micropropagation System (CMS) | 6.32[b] | 50.73[b] | 0.36[b] |
| Double Phase Culture System (DPS) | 6.97[a] | 61.75[a] | 0.68[a] |

Means followed by common letters are not significantly different

Turmeric shoots subjected to DPS developed more roots than those inoculated in CMS, with a mean of 6.97 roots per meriplant and 6.32 roots per meriplant, respectively. The result reveals significant differences between the treatments at a 5% significance level. Meriplants inoculated in DPS produced longer roots with a mean of 61.75 mm, which is longer compared to meriplants inoculated in CMS with a mean of 50.73 mm (Table 3). DPS significantly varies with CMS regarding turmeric root lengths grown *in vitro*. On the other hand, the fresh weight of *in vitro*-grown turmeric is heavier following the DPS, with a mean of 0.54g. The root fresh weight of 0.38g was obtained in cultures grown using the CMS. The result of the study shows that the DPS induces the development of more roots than CMS in *in vitro* root induction in turmeric. This indicates that the utilization of DPS in the induction of roots in turmeric is advantageous over the CMS. The findings in the study corroborate the findings of some researchers, where DPS was more advantageous than CMS. The use of DPS has provided equal or greater micropropagation efficiency for many plant species, such as pineapple, banana, potato, and sugarcane [43].

In the study of Senapati [44] on *in vitro* propagation of *Rauwolfia serpentine* L. Bent., the number of roots was higher in DPS than in the CMS. Meanwhile, Mehrotra *et al*. [30] demonstrated the efficiency of DPS on plant micropropagation and that better root growth was observed in liquid media than in semi-solid media. Scherwinski-Pereira *et al*. [42] also claimed that better root induction and elongation in *in vitro* culture of pineapple were obtained in DPS. The number of roots was also higher in DPS than in CMS in the *in vitro* root induction of *Malus sylve*stris (L.) Mill., [46].

The increased number of roots in the micropropagation of turmeric may be due to the increased contact surface area of plantlets, which ultimately increases the rate of diffusion/absorption. According to Scherwinski-Pereira & Fortes [42] and Be & Debergh [7], the *in vitro* proliferation rate of plant cultures in a liquid medium tends to increase compared to systems using semi-solid media due to the greater ease with which the plants absorb nutrients and regulators. The liquid medium allows close contact with the tissue, which stimulates and facilitates the uptake of nutrients and phytohormones, leading to better shoot and root growth [55].

Moreover, De La Viña *et al*. [14] assert that the media in DPS provide a higher quantity of nutrients readily usable by the explants being grown because there is no physical barrier present in the medium in these systems, which facilitates increased availability of the nutrients to the explants. In addition, the media allows rapid replenishment of nutrients and adjustment or change of pH, hormones, or other parameters without disturbing the tissue [39].

In terms of root length, the DPS yields longer roots during the root proliferation and elongation stage. This may mean that DPS induces longer roots during root development in turmeric grown *in vitro*. Some studies on *in vitro* root proliferation and elongation in various plant species yielded similar results. In the study of Senapati et al. [44] on the in vitro culture of R. serpentine, the root length and root thickness were higher in DPS than in CMS. They claimed that in DPS, well-developed roots were observed after eight weeks of initial culture, whereas in CMS, it takes approximately 12 weeks after initial culture for root development. Senapati *et al*. [44] reported similar findings. De Klerk and Brugge [13] also claimed that the root growth of dahlia in DPS was much more than the root growth in solid medium. Vyas *et al*. [51] also reported that the *Chlorophytum borivilianum* plant in liquid media showed a higher rate of multiplication and root growth than in solid media. Kuria *et al*. [27] also reported a lower rate of root development of plantlets in solid media than in liquid media. Sota *et al*. [46] observed the highest value of root length in DPS in *in vitro* propagation of *Malus sylvestris* (L.) Mill.

The dual phasic culture system has shown an excellent rooting response in the same culture vessel. The increased root development in DPS may be due to the increased contact surface area of explants with the media, which leads to increased diffusion, absorption, and replacement of growth medium components. It may also be due to better translocation of nutrients to the regions of utilization in the liquid medium than in the solid medium, which ultimately improved the growth responses in DPS. Similarly, the direct contact of plantlets with the liquid phase of the medium contents causes ease in the availability of nutrients, resulting in better root plantlet growth compared to solid media, where nutrient uptake is comparatively difficult due to the solidification of the medium. Based on the result of the present study, solid-liquid media (DPS) were superior to solid-solid media (CMS) in the root development of turmeric. The better aeration of cultures in the liquid medium benefited root formation. In addition, DPS was cheaper than the CMS; hence, in micropropagation, DPS cultures simplify plant material transfer, sterilizing and filling vessels, and allow for more rapid growth**.**

The DPS influences relatively better root growth in turmeric than CMS. The heavier root fresh weight of turmeric *in vitro* culture using the DPS can be attributed to the increased length and number of roots observed in the study. Similarly, Scherwinski-Pereira *et al*. [43] found that pineapple plantlets grown *in vitro* using DPS had rooting values close to 100% higher than those grown in an agar-solidified medium. Sota *et al*. [46] tested the DPS propagation system compared to CMS on *in vitro* propagation of *Malus sylvestris* (L.) Mill. They found that DPS was highly influential during *in vitro* shoot regeneration and rooting.

It can be argued that in a liquid medium, the proliferation rate tends to increase in comparison to systems using semi-solid media, following the CMS, due to the greater ease with which the nutrients and regulators are absorbed by the plants [42]. According to De La Via *et al*. [14], the DPS provides a higher amount of nutrients that are easily utilizable by the explants being grown because there is no physical barrier present in the medium in these systems, which facilitates increased availability of the nutrients to the explants.

Another essential purpose for using a DPS is to reduce inoculation number manipulation during the *in vitro* multiplication stages, since there is no need to carry out constant subcultures, as the plantlets can be maintained for several months under these conditions. Therefore, using DPS potentially results in decreased expenditures on gelling agents for the growth medium and a reduced need for manpower during transplanting.

The DPS was effective for the growth of plantlets and, at the same time, for their rooting. During the rooting stage, the DPS allowed the simultaneous formation of roots and new shoots at a higher rate, thus reducing the micropropagation cost by combining the last stage of subculturing and the rooting phase [46].

*Contamination Rate and Plantlet Survival*

After 30 days of incubation, the number of contaminated cultures was counted and recorded, and the percentage was computed. A higher contamination rate was obtained in DPS with 35.00%. Only a 15.00% contamination rate was noted in CMS (Table 4).

***Table 4.*** *In vitro survival rate of plantlets and observed contamination rate of cultures.*

| Treatments | Contamination Rate | Survival Rate |
|---|---|---|
| Conventional Micropropagation System (CMS) | 15.00 | 85.00 |
| Double Phase Culture System (DPS) | 35.00 | 65.00 |

A higher survival rate of plantlets after 30 days of root induction is obtained in CMS with 85.00%, while 65.00% was observed in DPS. The survival rate of plantlets was due to contamination observed in the cultures. Contaminated cultures were discarded and were not considered in counting the survival rate of plantlets.

Based on visual observation, the contaminants were of fungal origin due to their greyish-to-blackish color. The oozing milky-like appearance in the cultures also characterized bacterial contamination. It was also noted that contamination started from the point farther from the base of the explants. This may suggest that contamination may be introduced during tissue culture manipulation or handling of cultures. Although the cause of the contaminants in the cultures under investigation was not determined, it is safe to assume that aseptic practices were not followed correctly. When liquid rooting media was poured into the culture vessel for DPS or when cultures were transferred into fresh media for CMS, there is a possibility that aseptic conditions were not observed adequately during the handling of the cultures.

The main difficulty with cell and tissue cultures is their propensity to contamination, whether by microbes or stray cells from other cultures in the laboratory. Strosse *et al*. [47] stated that contamination in tissue cultures might be caused by an endogenous microorganism that escapes initial disinfection or by microorganisms introduced during tissue-culture manipulations. Both contaminants may persist for several subculture cycles and extended periods in the plant material without showing any symptoms in the tissue or visible signs in the medium.

In addition, Cassells [9] stated that plant-associated, environmental, and human-associated bacteria, yeasts, and micro-arthropods cause laboratory contamination. Most microbial contaminants are expressed (i.e., grow) on plant tissue culture media, but some may be latent (i.e., not expressed) or subliminal. Biological contamination in plant cells and tissue cultures originates from the tissue used to initiate the culture and the laboratory environment. Contaminants transferred in or on the plant material include plant pathogens and environmental microorganisms. Aseptic cultures may become contaminated in the laboratory due to failure of sterile technique or equipment malfunction.

Detection and at least partial identification are prerequisites for controlling and eliminating laboratory contamination. Some microorganisms are biomarkers for system failures. It may not be possible to control fast-growing microorganisms before they overrun the culture, but slow-growing, or latent microorganisms, can be treated with

antibiotics [9]. Preventing or avoiding microbial contamination of plant tissue cultures is critical to successful micropropagation. Every plant tissue culture procedure stage should be considered to prevent contamination. These procedures cover handling stock plants and different types of explants, preparing media, sub-culturing, incubating, and storing sterile culture vessels, media, and plant cultures. Cultures may be recontaminated and are believed to be the primary cause of losses during commercial micropropagation.

A higher survival rate of plantlets after 30 days of root induction is obtained in CMS than in DPS. The survival rate of plantlets was due to contamination observed in the cultures. Contaminated cultures were discarded and were not considered in counting the survival rate of plantlets. The result of the study is quite contrary to the results of some micropropagation studies using DPS. For example, Oliveira *et al*. [36] claimed that shoots of vanilla (*Vanilla planifolia*) rooted *in vitro* in DPS, even without growth regulators, showed 100% survival. Scherwinski-Pereira *et al*. [42] studied the shoot proliferation of the pineapple (*Ananas comosus* L.) and showed that shoots taken from DPS were rooted within four weeks and survived with 100% success. It was not stated in the studies previously cited whether the contamination was considered when calculating the survival rate. As previously mentioned, the lower survival rate of the turmeric plantlets grown in DPS was due to the cultures' higher contamination rates. Contaminated vessels were discarded and were not factored into the calculation of the survival rate.

### *Acclimatization of In Vitro Plantlets in Soil and Hydroponic Systems*

The growth performance of the plantlets was taken 30 days after planting (30 DAP) and 60 days after planting (60 DAP), as shown in Table 5.

***Table 5.*** *Growth performance and survival rate of the plantlets at 30 DAP and 60 DAP.*

| Treatments | Stem Length (mm) | | Stem Girth (mm) | | Number of Leaves | | Leaf Area ($mm^2$) | | Shoot Fresh Weight (g) | | Root Length (mm) | | Root Fresh Weight (g) | | Survival Rate (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP | 30 DAP | 60 DAP |
| Soil Media | 53.78 | 90.62 | 3.8 | 5.38 | 4.90 | 5.15 | 508.2 | 897.06 | 1.1 | 1.55 | 50.7 | 81.53 | 0.46 | 2.47 | 100 | 100 |
| Hydroponic | 63.25 | 124.58 | 4.82 | 7.22 | 5.85 | 5.95 | 591.27 | 3331.41 | 1.62 | 5.3 | 82.32 | 163.07 | 1.47 | 6.23 | 100 | 100 |
| **t-test** | ** | ** | ** | ** | ** | ** | ns | * | ** | ** | ** | ** | ** | ** | ns | ns |

* - significant at a 5% level of significance; ** - significant at 1% level of significance; ns – non-significant

Table 5 presents the growth performance and survival rates of acclimatized turmeric plantlets in hydroponic and soil media 30 and 60 days after planting (DAP). The turmeric plantlets showed improved growth performance in both media from 30 DAP to 60 DAP. Furthermore, a 100% survival rate was observed in plantlets cultivated in the hydroponic system and soil media. Comparing the two media, it is evident that the plantlets grown in the hydroponic system displayed better growth performance than those in the soil media. Significant differences were noted between the two media in various growth parameters, except for leaf area at 60 DAP and survival rates at 30 DAP and 60 DAP, where no significant differences were observed. At both 30 days after planting (DAP) and 60 DAP, the turmeric plantlets in the hydroponics system displayed higher growth parameters, including stem length, stem girth, number of leaves, leaf area, shoot fresh weight, root length, and root fresh weight. Significant differences between the hydroponics and soil media were evident in all measured parameters, except for leaf area at 30 DAP and survival rates at 30 DAP and 60 DAP.

The analysis of growth performance and survival rates of acclimatized turmeric plantlets 30 and 60 days after planting (DAP) in hydroponic and soil media revealed noteworthy trends and implications. Over the acclimatization period, there was a consistent increase in growth performance for turmeric plantlets in both media, suggesting the positive impact of the acclimatization process. Notably, the hydroponic system demonstrated a distinct advantage, consistently outperforming soil media in various growth parameters, including stem length, stem girth, number of leaves, leaf area, shoot fresh weight, root length, and root fresh weight at 30 DAP and 60 DAP. The findings suggest that the hydroponics system positively influences the growth performance of turmeric plantlets, leading to the development of better above-ground (stem, leaves, shoot) and below-ground (roots) components.

Plantlets grown through tissue culture are typically fragile due to their cultivation under low-intensity artificial light and high humidity conditions. In addition, the roots of the *in vitro* propagated plants are fragile and susceptible to mechanical damage. For this reason, the plants can die a few days after they are transplanted into the pots. Consequently, they lack sufficient "hardening off" and may easily experience water loss upon exposure to the surrounding environment. When tissue culture plants are transferred from the laboratory to the soil, they are exposed to abiotic stresses, like altered temperature, light intensity, and humidity conditions, hence the need to acclimatize the plants under nursery conditions before field planting.

The methodology employed in this study involves a gradual reduction in relative humidity through periodic punching of the covering, which may likely trigger changes in leaf structure and function in response to altered environmental conditions. Furthermore, the hydroponic system supplies essential nutrients for plant growth [35]. These conditions support the revitalization of *in vitro* roots and the absorption of minerals for the autotrophic growth of turmeric plants.

The proliferation of the above-ground (stem, leaves, shoot) and below-ground (roots) components of the turmeric may be attributed to the gradual changes in relative humidity. According to Zapata et al. [53], changes in relative humidity may significantly affect some of the events that occur during the acclimatization of plants in the greenhouse, such as the regulation of the stomatal opening and closing, the biosynthesis of the epicuticular wax and the development of the internal structure of the leaf. In addition, the favorable response of the plants to the hydroponic system may also be attributed to the availability of necessary nutrients for plant growth. Plant growth highly depends on the availability of all required nutrients, ideally in well-balanced ratios. These conditions favor the invigoration of the *in vitro* roots and the absorption of minerals for the growth of *C. longa* plants.

The result agrees with Trejo-Téllez and Gómez-Merino [50], who discovered that the nutrient solution formula highly influences crop growth, yield, and quality. According to them, hydroponic nutrient solutions require well-balanced quantities of critical macro- and microelements. In the Duan et al. [15] study, a far greater survival rate (100%) was reached when the *in vitro*-grown plants were supplied with nutritional solutions. These plants outperformed the others, showing remarkable growth and significantly higher biomass. Because a nutrient solution of the hydroponic system can supply enough nutrients and compensate for water loss for plant growth compared to soil conditions, it can harden micro-propagated plants effectively and conveniently. The SNAP Hydroponic solution used in this study contained essential elements such as nitrogen (6.10% wt./vol), calcium (4.245% wt/vol), potassium (3.09% wt/vol), phosphorus (0.376% wt/vol), magnesium

(0.494% wt/vol), and iron (0.151% wt/vol). It also contains trace amounts of boron, manganese, and molybdenum.

The turmeric plantlets responded well to the SNAP solution, as shown by the increased growth of the above-ground and below-ground parts. This underscores the importance of various nutrients, such as potassium (K), nitrogen (N), phosphorus (P), magnesium (Mg), iron, boron (B), and manganese (Mn), for the growth and development of *in vitro*-grown turmeric plants. Potassium (K) significantly enhances plant height, tiller number, and rhizome production. Phosphorus (P) is a fundamental building block for biomolecules, crucial in energy production. Nitrogen (N) affects photosynthesis. Nitrate transporters, such as NRT3.1 and NRT2.5, are crucial for increased $NO_3^-$ and $NH_4^+$ uptake, promoting seedling growth. Moreover, Potassium (K+) is an enzyme activator, regulating water utilization and participating in ATP and protein synthesis. Magnesium (Mg) is essential for photosynthetic $CO_2$ assimilation, playing a key role in chlorophyll pigment. Iron is vital for metabolic activities, maintaining chloroplast structure, and participating in chlorophyll synthesis. Boron (B) contributes to cell wall formation, plasma membrane integrity, and various physiological processes. Manganese (Mn) is a cofactor for the photosynthetic machinery.

These findings underscore the importance of nutrients in plant growth, such as potassium (K), phosphorus (P), nitrogen (N), magnesium (Mg), iron (Fe), boron (B), and manganese (Mn) for their roles in various physiological processes, such as photosynthesis, enzyme activation, metabolism, chlorophyll synthesis, and overall plant development. The study emphasizes the significance of nutrient balance in hydroponics, providing precise control over nutrient levels for optimal plant growth. The results demonstrate the potential of hydroponics as a cultivation method for optimizing the growth of turmeric plantlets, offering advantages over traditional soil-based cultivation. The findings of the study are aligned with previous research results. These researches encompass a variety of plant species, including *Solanum tuberosum* L., *Stemona curtisii* Hook., Grammatophyllum *speciosum* Blume, *Colocasia esculenta* spp.

More recently, researchers explored the feasibility of acclimatizing micro-propagated plants through hydroponic systems in crops such as cassava, wasabi and caladium. The findings from these studies collectively demonstrate that hydroponics is a viable alternative for acclimatizing *in vitro* plantlets cleanly and saving water. The studies on hydroponic systems across various plant species reveal notable physiological responses, emphasizing the impact of nutrient availability, cultivation techniques, and environmental conditions on plant development. These studies focused on the physiological responses of different crops, such as tomatoes, lettuce, cucumbers, and *Stevia rebaudiana*, to various aspects of hydroponic systems. Nguyen et al. [35] discussed how hydroponics allows precise control over nutrient levels in tomatoes, influencing processes like photosynthesis and enzyme activity.

Ahmed et al. [1] emphasized the importance of optimal nutrient balance in lettuce hydroponics, affecting essential element uptake and determining growth. Giban and Salas [18] explore how different hydroponic systems impact cucumber physiology, considering factors like root oxygenation and nutrient availability. Kumar et al. [28] focused on a novel nutrient film technique (NFT) system for lettuce and spinach, highlighting improved nutrient uptake and water-use efficiency. Janakiramudu et al. [22] investigated the hydroponic cultivation of *Stevia rebaudiana*, examining physiological responses tied to nutrient concentrations and harvest frequencies, including changes in leaf morphology, photosynthetic rates, and metabolite production. The research then highlights the potential

of hydroponics for plant acclimatization since results demonstrated that there is better growth and development of turmeric plantlets in a hydroponic system compared to soil due to the nutrient-rich hydroponic solution that plays a crucial role in promoting robust growth and achieving a 100% survival rate. The balance of nutrition and availability in the system is also essential in plant physiology and the advantages of hydroponics in providing a controlled and nutrient-rich environment for plant cultivation.

***Hydroponic Acclimatization with Seaweed Extract-Enriched Nutrient Solutions***

The growth performance of acclimatized turmeric plantlets varied significantly across treatments, reflecting the influence of nutrient solution concentration on plant development (Table 6). Plantlets exposed to the high concentration treatment exhibited the best growth performance, with the tallest average height (138.40 mm), largest stem diameter (5.40 mm), highest leaf number (6.30), and largest leaf area (917.80 $cm^2$), indicating optimal nutrient availability for vigorous growth. Medium concentration treatments also promoted good growth, with plantlet height (115.33 mm), stem diameter (5.07 mm), and leaf area (842.90 $cm^2$) performing better than the low concentration treatment but lower than the high concentration. Excessive concentration resulted in suboptimal growth compared to the high treatment, with a moderate plant height (123.50 mm), slightly reduced leaf number (6.07), and leaf area (885.60 $mm^2$), suggesting that excessive nutrients may induce mild toxicity, impairing optimal growth. The low concentration treatment consistently showed the least growth, with the smallest plant height (107.93 mm), stem diameter (4.90 mm), leaf number (5.50), and leaf area (799.87 $cm^2$), likely due to insufficient nutrient availability. Overall, the results highlight that while moderate to high concentrations of seaweed extract enhance growth performance, excessive levels may slightly inhibit growth, emphasizing the importance of balanced nutrient application for successful acclimatization.

The root development of acclimatized turmeric plants showed significant variation across the different treatments, reflecting the influence of nutrient concentration on root growth dynamics (Table 1). Plants treated with a high concentration of seaweed extract exhibited the most robust root development, producing the greatest number of roots (20.0) with the longest average root length (95.73 mm). This indicates that the high concentration provided an optimal balance of nutrients and bioactive compounds that stimulated root proliferation and elongation. Medium concentration also supported substantial root development, with 16.0 roots and an average root length of 76.53 mm, suggesting a slightly less pronounced, but still effective, nutrient availability for root growth. In contrast, low concentration resulted in the least root development, with only 13.0 roots and a shorter average root length of 68.40 mm, likely due to insufficient nutrient supply limiting growth. Interestingly, excessive concentration produced a high number of roots (19.0) but with reduced length (84.90 mm) compared to the high concentration treatment. This suggests that excessive nutrients may promote root proliferation but inhibit elongation, potentially due to nutrient toxicity or osmotic stress. Overall, the results underscore the importance of optimizing nutrient concentrations to balance root proliferation and elongation for successful acclimatization in hydroponic systems.

The uniform 100% survival rate observed across all treatments indicates that *in vitro*-cultured turmeric plantlets acclimatized effectively in the hydroponic system regardless of the nutrient solution concentration. This finding suggests that hydroponics provides an inherently supportive environment for plantlet establishment, characterized by optimal aeration, consistent moisture availability, and controlled nutrient delivery. The seaweed

extract concentrations, while influential on growth parameters like shoot height, root length, and physiological attributes, did not appear to significantly impact the plantlets' ability to survive. This uniformity might be attributed to the bioactive compounds in the seaweed extract, which could have enhanced stress tolerance across all concentrations. The hydroponic system's ability to minimize common stressors such as water deficit or nutrient imbalances likely played a critical role in achieving universal survival. However, while survival rates were unaffected, the growth and vigor of the plantlets varied with the nutrient concentration, underscoring the importance of optimizing treatment levels for enhanced productivity. This highlights hydroponics' potential as a robust acclimatization method, even under varied nutrient conditions.

***Table 6.*** *Growth Performance and Survival Rate of Acclimatized Turmeric*

| Treatments | Plantlet Height, mm | Stem Diameter, mm | Leaf Number | Leaf Area, $cm^2$ | Number of Roots | Root Length, mm | Survival Rate, % |
|---|---|---|---|---|---|---|---|
| 0.50 mL | 107.93d | 4.90c | 5.50b | 799.87d | 13.0c | 68.40d | 100% |
| 0.75 mL | 115.33c | 5.07bc | 5.77b | 842.90c | 16.0b | 76.53c | 100% |
| 1.00 mL | 138.40a | 5.40a | 6.30a | 917.80a | 20.0a | 95.73a | 100% |
| 1.50 mL | 123.50b | 5.20ab | 6.07a | 885.60b | 19.0a | 84.90c | 100% |
| **Mean** | **121.29** | **5.14** | **5.91** | **861.54** | **17.0** | **81.39** | |
| **CV (%)** | **2.23** | **2.25** | **2.63** | **1.87** | **5.88** | **3.85** | |

Means with the same letter(s) are not significantly different.

## CONCLUSION

The study successfully developed an optimized, cost-effective in vitro propagation protocol for turmeric (Curcuma longa Linn.) to produce high-quality, disease-free planting materials. Soaking explants in 60% sodium hypochlorite for 10–20 minutes effectively minimized contamination and maximized survival. Supplementing Murashige and Skoog (MS) medium with Thidiazuron (TDZ) at 2.0–2.5 mg/L promoted optimal bud and shoot proliferation, while lower concentrations (0.01 mg/L) enhanced root formation. The Double-Phase System (DPS) outperformed the Conventional Micropropagation System (CMS) in root induction and elongation but exhibited higher contamination rates, highlighting the need for stricter aseptic control. During acclimatization, hydroponic systems provided superior growth and a 100% survival rate compared to soil media. Incorporating 1.00 mL/L seaweed extract into hydroponic nutrient solutions further enhanced shoot and root development. Overall, integrating optimized sterilization, TDZ regulation, DPS culture, and hydroponic acclimatization offers an efficient, scalable, and sustainable method for turmeric micropropagation. This protocol supports large-scale production of quality planting materials, benefiting farmers and strengthening the country’s growing turmeric industry.

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