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In vitro propagation of turmeric (Curcuma longa Linn.) using Murashige and Skoog (MS) media supplemented with varying concentrations of thidiazuron (TDZ)

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ABSTRACT

This study investigates the use of Thidiazuron (TDZ) as a supplement to Murashige and Skoog (MS) media for the *in vitro* propagation of turmeric (*Curcuma longa* Linn.). The study aimed to determine the best concentration of TDZ to facilitate efficient micropropagation of turmeric. Results showed that supplementing the MS medium with TDZ at levels ranging from 1.0 to 2.5 mg/L of water can result in higher bud proliferation, optimal shoot fresh weight, and higher stem girth in *in vitro*-grown turmeric. Moreover, adding TDZ in media ranging from 0.01 to 2.5 mg/L of water promotes longer shoot length, a higher number of leaves, and a greater leaf area in *in vitro*-grown turmeric. However, higher TDZ concentrations led to longer roots but fewer total roots. The study concludes that TDZ can facilitate shoot and root regeneration in turmeric during micropropagation, providing several advantages for commercial production.

KEYWORDS: root regeneration, plant growth regulators, explant inoculation, bud initiation, *in vitro* shoot regeneration

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1. INTRODUCTION

Turmeric (*Curcuma longa* Linn.) is a highly valued plant known for its culinary uses and potential applications in the medicinal and cosmetic industries. Its rhizome, containing volatile oil and curcumin, has gained attention for its anti-inflammatory, antioxidant, and anticancer properties[1,2]. However, the limited flowering and sterile triploid nature (2n = 3x = 63) of turmeric make its propagation challenging[3]. Traditional methods, such as rhizome division, are time-consuming, inefficient, and susceptible to rhizome rot diseases[4,5].

To overcome these limitations and ensure a consistent supply of disease-free planting material, *in vitro* propagation techniques have been explored. Tissue culture methods offer a means to rapidly replicate turmeric plants, increasing multiplication rates and reducing disease risks[6]. This approach provides a steady supply of high-quality, true-to-type plantlets, ultimately enhancing crop yield potential.

Extensive research has been conducted on the *in vitro* proliferation of turmeric and its related species. Researchers have investigated various tissue or organ explants and different combinations and concentrations of plant growth regulators (PGRs) in the culture media from 1978[7] to recent studies in 2020[8,9]. PGRs and vitamin supplements play a crucial role in plant regeneration and multiplication rates. Commonly used PGRs for micropropagation include 6-benzyl amino purine (BAP), 1-naphthalene acetic acid (NAA), and 6-furfurylaminopurine [kinetin (KT)][10]. BAP is favored for shoot elongation and multiplication, while NAA stimulates rooting. Different combinations of PGRs, such as BAP and NAA or KT and BAP, have been explored [11, 12].

In recent decades, Thidiazuron (TDZ) has gained significant interest for its applications in plant cell and tissue culture. TDZ exhibits auxin and cytokinin-like actions despite its chemical dissimilarity to regular auxins and cytokinins. It has been found to induce or enhance various cell biological activities[13]. In the micropropagation of turmeric (*Curcuma longa* L.), TDZ has been used in several studies. For instance, one study demonstrated that TDZ effectively promoted shoot proliferation in turmeric cultures, achieving a maximum of 20 shoots per explant at a concentration of 1.0 mg/L TDZ[14].

Another study compared TDZ with other cytokinins like kinetin and BA. TDZ outperformed them in shoot induction, with a maximum of 22.9 shoots per explant observed at a concentration of 2.0 mg/L

TDZ[15]. Additionally, TDZ has been shown to enhance rooting in turmeric shoots when combined with IAA (indole-3-acetic acid)[16].

Despite the promising results of TDZ in promoting shoot proliferation and enhancing rooting, further research is necessary to optimize its concentration and determine the most effective dosage in the MS medium for efficient micropropagation of turmeric. This study aims to contribute to the existing knowledge in this area and provide insights into the commercial production of disease-free turmeric planting material.

2. METHODOLOGY

The Murashige and Skoog (MS) medium served as the base medium, adding vitamins, organic compounds, 20g/L sucrose, and 100 ml coconut water. This medium contains micronutrients, vitamins, and plant growth regulators to support cell division, growth, and differentiation. Specific plant growth regulators like 6-BAP, IAA, and varying levels of TDZ are added to the proliferation medium to initiate culture, promote multiplication, and facilitate shoot development. NAA and different concentrations of TDZ are included in the rooting media to encourage root growth. The pH of the medium is adjusted to 5.8±0.1, and agar is added as a solidifying agent. The prepared media are then autoclaved at 121°C for 20 minutes to ensure sterility.

Collected disease-free turmeric rhizomes were washed, and the apical meristems were carefully excised. Surface sterilization was performed using a hypochlorite solution, followed by rinsing with sterile water to remove any remaining disinfectant. The sterilized shoot tips are inoculated into separate bud proliferation media and maintained under controlled conditions, including temperature and lighting, for 30 days. Sub-culturing is performed to multiply buds. Buds are excised from the initial culture and transferred to freshly prepared proliferation media. This process is repeated up to three times with a 30-day interval.

After the third subculture, the regenerated shoots are excised and transferred to agarized proliferation media to develop for 30 days under controlled conditions. Successfully regenerated shoots are transferred to a rooting medium solidified with agar, containing NAA and TDZ at varying concentrations, and maintained for four weeks under controlled conditions to establish roots. After each transfer, spent media and used plant specimens are autoclaved at 121°C for 60 minutes to ensure decontamination. They are then disposed of using standard waste disposal methods.

The study follows a Completely Randomized Design (CRD) with three replications, each comprising 16 culture bottles. Various treatments with different concentrations of TDZ are employed. Data are analyzed using one-way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test for variations among treatments, using the Statistical Tool for Agricultural Research (STAR 2.0.1).

3. RESULTS

Bud proliferation of turmeric (Curcuma longa Linn.) grown in vitro as affected by varying concentrations of TDZ.

After two weeks of induction, bud emergence was observed, and sub-culturing was performed 30 days after bud induction. In the first subculture (S1), the number of buds varied among different treatments(Table 1). The highest mean number of buds (5.26) was observed in T_7 (MS Medium with 2.5 mg of TDZ/L), followed closely by T_6 (2.0 mg of TDZ/L), T_5 (1.5 mg of TDZ/L), T_4 (1.0 mg of TDZ/L), T_3 (0.5 mg of TDZ/L), T_2 (0.1 mg of TDZ/L), and T_0 (Control with no TDZ) with means ranging from 3.25 to 5.18. The lowest number of buds (3.21) was found in T_1 (0.01 mg of TDZ/L).Analysis of variance (ANOVA) showed a highly significant effect of TDZ concentration on bud emergence at S1. According to Tukey's HSD test, T7 and T6 significantly differed from all other treatments, while T_5 and T_4 did not significantly differ from each other but differed from T_3, T_2, T_1, and T_0. T_2, T_1, and T_0 did not significantly differ from each other.

At S2, a similar trend was observed, with T_7 producing the highest number of buds (6.32), followed by T_6 (5.76), T_4 (5.06), T_5 (5.05), T_3 (4.40), T_2 (4.32), T_1 (4.30), and T_0 (4.25). Once again, T_7 and T_6 significantly differed from all other treatments, while T_4 and T_5 did not significantly differ from each other but differed from the rest. T_2, T_1, and T_0 did not significantly differ from each other.

At S3, the average number of buds was 5.74, with the highest mean in T_6 and T_7 (6.83 each), followed by T_4 and T_5 (6.73 each), T_3 (5.53), T_2 (4.73), T_1 (4.27), and T_0 (4.23).ANOVA indicated a significant effect of TDZ concentration on bud proliferation at S3. According to the HSD test, no significant differences existed among the means of T_6, T_7, T_4, and T_5. However, these treatments significantly differed from T_3, T_2, T_1, and T_0. Additionally, T_3 significantly differed from T_2, T_1, and T_0, while T_2 did not significantly differ from T_1 and T_0.

Table 1. Bud proliferation performance of in vitro - grown as turmeric affected by different concentrations of TDZ.

Treatments	Number of Buds Emerged 30 Days after Bud Induction and 30 days thereafter		
	S1	S2	S3
T_0 – MS Medium with no TDZ (Control)	3.25^{c}	4.25^{e}	4.23^{d}
T_1 – MS Medium Supplemented with 0.01 mg of TDZ/L of water	3.21^{c}	4.30^{de}	4.27^{d}
T_2 – MS Medium Supplemented with 0.1 mg of TDZ/L of water	3.60^{c}	4.32^{de}	4.73^{c}
T_3 – MS Medium Supplemented with 0.5 mg of TDZ/L of water	4.05^{b}	4.40^{d}	5.53^{b}
T_4 – MS Medium Supplemented with 1.0 mg of TDZ/L of water	5.03^{a}	5.06^{c}	6.73^{a}
T_5 – MS Medium Supplemented with 1.5 mg of TDZ/L of water	5.13^{a}	5.05^{c}	6.73^{a}
T_6 – MS Medium Supplemented with 2.0 mg of TDZ/L of water	5.18^{a}	5.76^{b}	6.83^{a}
T_7 – MS Medium Supplemented with2.5 mg of TDZ/L of water	5.26^{a}	6.32^{a}	6.83^{a}
Grand Mean	**4.34**	**4.93**	**5.74**
ANOVA	**	**	**
C.V.	3.55	1.01	1.47

Means with the same letters are not significantly different from each other
*** significant at 1% level of significance*
** significant at 5 % level of significance*

Shoot Regeneration and Elongation

In terms of stem length, T_3 (MS Medium with 0.5 mg of TDZ/L of water) exhibited the longest average stem length at 88.90 mm, while T_0 (MS Medium with no TDZ - Control) had the shortest stem length at 70.55 mm. The T_6 (MS Medium with 2.0 mg of TDZ/L of water) had the second-longest stems, closely followed by T_7 (MS Medium with 2.5 mg of TDZ/L of water), T_5 (MS Medium with 1.5 mg of TDZ/L of water), T_4 (MS Medium with 1.0 mg of TDZ/L of water), T_2 (MS Medium with 0.1 mg of TDZ/L of water), and T_1 (MS Medium with 0.01 mg of TDZ/L of water). The ANOVA showed a significant effect of TDZ concentration on stem length. T_3 significantly differed from the Control (T_0) but did not significantly differ from other treatments. There were no significant differences among T_6, T_7, T_5, T_4, T_2, and T_1.

Regarding stem girth (mm), T_7 (MS Medium with 2.5 mg of TDZ/L of water) had the highest mean stem girth at 0.58 mm, followed by T_6, T_5, T_3, T_4, T_2, and T_1 in descending order. The lowest stem girth was observed in T_0 (MS Medium with no TDZ - Control) at 0.38 mm. The ANOVA indicated a highly significant effect of varying TDZ levels on stem girth, with T_7 significantly differing from T_3, T_4, T_2, T_1, and T_0 but not from T_6 and T_5. There were no significant differences among T_6, T_5, T_4, T_3, T_2, T1, and T_0.

The T_7 (MS medium with 2.5 mg of TDZ/L of water) produced the highest mean number of leaves (4.05), followed by T_6, T_5, T_4, T_3, T_2, and T_1 in descending order. The lowest number of leaves was observed in T_0 (MS medium with no TDZ - Control). The ANOVA showed a highly significant effect

of TDZ on the number of leaves. T_7 significantly differed from the T_0 (Control), but no significant differences were observed among the other treatments.

Table 2 also shows that T_7 (MS medium with 2.5 mg of TDZ/L of water) exhibited the highest mean leaf area at 761.27 mm², while the lowest mean leaf area was observed in T_0 (MS medium with no TDZ - Control) at 320.63 mm². T_6 had the second-highest leaf area, followed by T_5, T_4, T_3, T_2, and T_1 in descending order. The ANOVA indicated a highly significant effect of TDZ on leaf area, with T_7 significantly differing from T^5, T_4, T_2, T_3, T_1, and T_0 but not from T_6. No significant differences were observed among T_6, T_5, T_4, T_3, T_2, T_1, and T_0.

In terms of shoot fresh weight, T_7 (MS Medium with 2.5 mg of TDZ/L of water) had the highest mean shoot fresh weight (1.11g), followed by T_6, T_5, T_4, T_3, T_2, and T_1 in descending order. The lowest shoot fresh weight was observed in T_0 (MS Medium with no TDZ - Control). A highly significant effect of TDZ on shoot fresh weight was revealed by ANOVA, with T_7 significantly differing from T_3, T_4, T_2, T_1, and T_0 but not from T_6. No significant differences were observed among T_6, T_5, T_4, T_3, T_2, T_1, and T_0.

Table 2.Shoot regeneration and elongation of in vitro-grown turmeric in response to varying concentrations of TDZ

Treatments	**Shoot Proliferation taken 30 Days after Shoot Induction.**				
	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)	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	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	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	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	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	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	88.83^{a}	0.57^{ab}	3.90^{a}	677.03^{ab}	1.09^{a}
T_7 – 2.5 mg of TDZ/L of water	88.63^{a}	0.58^{a}	4.05^{a}	761.27^{a}	1.11^{a}
Grand Mean	**86.07**	**0.38**	**3.75**	**528.13**	**1.00**
ANOVA	*	**	**	**	**
C.V.	2.11	7.43	6.78	11.60	2.92

Means with the same letters are not significantly different from each other
*** p < 0.01*
** p < 0.05*

Root Proliferation and Elongation

Table 3 presents the root proliferation and elongation response of turmeric as affected by the different amounts of TDZ that supplemented the MS medium for *in vitro* culture of turmeric. This is measured in terms of the number of roots, root freshweight (g), and root length (mm).

In terms of the number of roots, T_1 (MS Medium with 0.01 mg of TDZ/L of water) had the highest average number of roots (6.39), closely followed by T_0 (MS Medium with no TDZ - Control) with 6.36 roots. The treatments with fewer roots, ranked in descending order, are T_2, T_3, T_4, T_5, T_6, and T_7. The lowest number of roots was observed in T_7. The ANOVA demonstrated a highly significant effect of varying TDZ levels on the number of roots. T_0 significantly differed from most treatments, indicating a significant difference in root numbers. However, there was no significant difference between T_0 and T_1. Significant differences were also found among some other treatments.

Regarding root fresh weight, T_0 (MS Medium with no TDZ - Control) had the highest mean root fresh weight at 1.07 g, followed by T_1, T_2, and T_3 in descending order. Slightly lower mean values were obtained for T_4, T_5, T_6, and T_7. The lowest root fresh weight was observed in T_7. The ANOVA results indicated no significant variation among the means of T_0, T_1, T_2, and T_3. However, these treatments significantly differed from T_4, T_5, T_6, and T_7. Additionally, T_4 and T_5 showed significant variation compared to T_6 and T_7.

Regarding root length, T_7 (MS Medium with 2.5 mg of TDZ/L of water) had the highest mean root length (6.36 mm), followed by T_6, T_5, T_4, T_3, T_2, and T_1 in descending order. The lowest mean root length was observed in T^0 (MS Medium with no TDZ - Control). The ANOVA results indicated a highly significant effect of TDZ on root length. T_7 significantly differed from most other treatments, except T_6. Furthermore, T_6 varied significantly from several other treatments, while T_2 and T_1 did not differ significantly from each other.

Table 3. Root regeneration and elongation of in vitro-grown turmeric in response to varying concentrations of TDZ

Treatments	Root Proliferation 30 Days after Root Induction		
	Number of Roots	Root Fresh weight (g)	Root Length (mm)
T_0 – MS Medium with no TDZ (Negative Control)	6.36[a]	1.07[a]	3.67[f]
T_1 – MS Medium Supplemented with 0.01 mg of TDZ/L of water	6.39[a]	1.05[a]	3.77[ef]
T_2 – MS Medium Supplemented with 0.1 mg of TDZ/L of water	6.09[b]	1.04[a]	4.00[e]
T_3 – MS Medium Supplemented with 0.5 mg of TDZ/L of water	5.58[c]	1.02[a]	4.42[d]
T_4 – MS Medium Supplemented with 1.0 mg of TDZ/L of water	4.42[d]	0.92[b]	5.58[c]
T_5 – MS Medium Supplemented with 1.5 mg of TDZ/L of water	4.00[e]	0.91[b]	6.09[b]
T_6 – MS Medium Supplemented with 2.0 mg of TDZ/L of water	3.77[ef]	0.72[c]	6.39[a]
T_7 – 2.5 mg of TDZ/L of water	3.67[f]	0.71[c]	6.36[a]
Grand Mean	**5.04**	**0.93**	**5.04**
ANOVA	**	**	**
C.V.	1.79	1.78	1.79

Means with the same letters are not significantly different from each other
*** p < 0.01*
** p < 0.05*

4. DISCUSSION

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 2.0-2.5 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.

This study provides evidence of the positive impact of TDZ (thidiazuron) on the *in vitro* growth performance of turmeric. The results indicate that more buds can be achieved in S1 and S3 when the culture media is supplemented with TDZ at concentrations ranging from 1.0 mg/L to 2.5 mg/L. In S2, 2.5 mg/L of TDZ induces more significant bud proliferation. TDZ at concentrations of 1.0-2.5 mg/L promotes increased bud proliferation. Therefore, to achieve optimal bud proliferation, it is recommended to supplement the MS medium with 1.0-2.5 mg/L of TDZ.

The study also examines the impact of different levels of TDZ on various growth parameters of *in vitro*-grown turmeric. 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 micropropagation, compared to the absence of TDZ. Thus, TDZ promotes shoot regeneration and elongation in the micropropagation process of turmeric.

The study also reveals an inverse relationship between the concentration of TDZ and the number and weight of roots produced. 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 meriplants with fewer total roots exhibiting 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 findings of the study align with previous research conducted on the impact of TDZ on 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[17, 13]. However, the optimal concentrations of TDZ can vary depending on the specific plant species.

For instance, research on turmeric micropropagation revealed that using 1.0 mg/L TDZ resulted in an average of 3 shoots per explant[18]. 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[19]. 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[20].

Similar favorable effects of TDZ have been observed in other plant species, such as *Zingiber officinale* Rosc. Var. Rubrum[21, 22, 23, 24], *Capsicum annuum* L[25], *Artemisia annua* L.[26], *Casuarina cunninghamiana* Miq.[27], and J*atropha curcas*[28]. Furthermore, TDZ has shown efficacy in inducing morphogenesis in plant micropropagation, as observed in *Echinacea purpurea* L.[29, 13],African violet[30], *Vitex trifolia* L[30], *Curcuma caesia* Roxb.[19],*Musa* sp.[31], almond "Beldi" ecotypes[32], *Alstroemeria aurantiaca* cv. 'Rosita'[33].

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.[34], tea plant[35],*Tulipa edulis*[36], (*Myrtus communis* L[37],*Lilium monodelphum* M. Bieb, var. *Armenum*[38], *R.decorum*[39], *Uraria picta*[40], *Astragalus cariensi*s Boiss[41], *Etlingera coccinea*[42], *Bauhinia tomentosa*[43], *Hydrangea quercifoli*a Bartr[44],and *Ocimum basilicum*[25].

Studies have also shown that pre-treatment with TDZ can enhance multiple shoot formation in certain plant species before *in vitro* propagation. Researchers have observed that TDZ pre-treatment increases the chances of tissues responding to other inductive stimuli and improves the efficiency of micropropagation[45, 46, 47, 48, 49, 50].

TDZ, a synthetic cytokinin, is widely used in plant tissue culture to promote shoot regeneration and *in vitro* multiplication[51]. It mimics the natural plant hormone cytokinin, which regulates cell division and

differentiation in plants[52]. TDZ interacts with cytokinin receptors on plant cells, triggering biochemical reactions that activate cell division genes and promote the formation of new plant tissue[53, 54, 55].

Moreover, TDZ increases endogenous cytokinin levels and inhibits cytokinin-degrading enzymes, enhancing cell division and proliferation[29, 54, 56]. 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, more robust shoots[57, 58, 59].

However, TDZ can inhibit root proliferation and elongation in some plant species[60, 61, 62]. 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[63, 19]. 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 shoot proliferation instead[46, 64].

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 depending on the concentration and species, and careful optimization of TDZ treatment is necessary to achieve successful in vitro rooting in plant micropropagation.

5. CONCLUSION

It can be concluded that supplementing the MS medium with TDZ at levels ranging from 1.0 to 2.5 mg/L of water can result in higher bud proliferation, optimal shoot fresh weight, and higher stem girth in *in vitro*-grown turmeric. Moreover, the addition of TDZ in media ranging from 0.01 to 2.5 mg/L of water promotes longer shoot length, a higher number of leaves, and a greater leaf area in *in vitro*-

grown turmeric. However, there is an inverse relationship between the amount of TDZ present and the number and weight of roots produced. Specifically, lower TDZ concentrations (0.01 mg/L) led to a more significant number of roots and heavier fresh roots, while higher TDZ concentrations (2.5 mg/L) led to longer roots but fewer total roots. Overall, the study concludes that TDZ can facilitate shoot and root regeneration in turmeric during micropropagation.

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