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RESEARCH ARTICLE – PLANT PROPAGATION

Synergistic effect of TDZ and IBA on rooting efficacy of *Butea monosperma* L.

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Abstract

Butea monosperma L. commonly called “Flame-of-the-forest” belongs to family Fabaceae. *Butea* holds a significant position in the pharmaceutical domain due to its extensive medicinal properties, which result from the presence of several classes of secondary metabolites. Considering its importance as a medicinal plant, the present investigation was conducted to propagate this precious plant on a large scale using various growth regulators under field conditions. The present regeneration protocol provides an effective system for utilizing Thidiazuron (TDZ) and Indole-3-butyric acid to enhance the rooting efficacy of *Butea monosperma* L. A pot experiment was planned using stem cuttings with two nodes, which were planted in plastic pots filled with well-drained soil. Before planting, the cuttings were dipped in solutions of various growth regulators (TDZ and IBA). At the conclusion of the experiment, various growth parameters were recorded to evaluate the growth of the cutting. The best rooting responses were obtained when the cuttings were applied with 10 ppm TDZ and 2000 ppm IBA. They showed the highest shoot and root length with broader leaves as compared to other treatments. Considering its medicinal importance, it was concluded that vegetative propagation of *Butea monosperma* using growth regulators is a significant approach.

Keywords: *Butea monosperma*, indole-3 butyric acid, rooting efficacy, thidiazuron

Key message: TDZ 10 ppm and 2000 ppm IBA improved rooting frequency in *Butea monosperma* shoot cuttings.

Abbreviations: TDZ Thidiazuron, **IBA** Indole-3-butyric acid, **PPM** Parts per million

1. Introduction

Butea monosperma L. is a deciduous tree that belongs to the family Fabaceae. This is a medium-sized tree, also known as Flame of the Forest, and is referred to by several other names in local languages, including Palas, Palash, Chichra, Dhak, Bijasneha, Bengal kino, Khakara, and Mutthuga. It is a naturally occurring ethno-medicinal tree in tropical and subtropical climates (Kaur *et al*., 2018). It is often a non-timber forest species of the plains, creating dominant patches in open spaces and grazing pastures, and it avoids extinction because of its resistance (Rai *et al*., 2019). *B. monosperma* plays a crucial role in the environment because it has extensive root systems that help in preventing soil erosion and making it valuable for conservation efforts in regions prone to landslides. Therefore, it can thrive in swampy soils, saline soils, alkaline conditions, black cotton soils, poorly drained soils, barren lands, and waterlogged conditions. In terms of soil erosion management, land rehabilitation, water conservation, and soil carbon sequestration, wood of the genus *Butea* provides ecological and environmental benefits.

Due to its extensive therapeutic capabilities resulting from the presence of various secondary metabolites, *Butea* holds a significant position in the pharmaceutical industry (Yara *et al*., 2018). It has been practiced in Ayurveda and Unani for a very long time (Chauhan and Mahish, 2020). Bark, leaves, flowers, seeds, and roots of *B. monosperma* are significant in terms of medicine (Dhakad *et al*., 2023). Alkaloids are found in *Butea* leaves, which possess aphrodisiac, tonic, diuretic, and astringent qualities, and are valued for their medicinal properties (Patil *et al*., 2018). Flavonoids and glucosides have been found in the flowers of *B. monosperma,* while the primary phytochemicals found in flowers such as

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monospermoside, aurones, isobutyine, palasitrin, coreopsin, isocoreopsin (butin 7-glucoside), and triterpene steroids (Sutayria and Saraf, 2015). *B. monosperma* was said to have antibacterial, antifungal, hypoglycemic, and anti-inflammatory properties in both crude extracts and pure isolates of its various components (Dhakad *et al*., 2023). There is a long history of using various techniques of vegetative propagation to create woody dicots with one or more desired genetic features. By using rooted cuttings, layering, budding, or grafting in accordance with the species' response, utility, and requirements, numerous species of ornamental, fruit, or nut trees from various genera have been successfully cloned. Plants can grow continuously throughout their lifetimes because their roots and branches contain relatively undifferentiated cells with unconstrained developmental potential (Leakey, 1985). Vegetative propagation refers to the reproduction of plants through asexual means, such as using plant parts like stems, roots, or leaves in *B. monosperma* (Kumar, 1989). Long generation durations, erratic fruiting/flowering, and outbreeding are obstacles to domestication that can be easily addressed through vegetative propagation. A variety of tropical tree species from both moist and dry regions are increasingly being propagated vegetatively. Even in species where these characteristics are not currently restricting adventitious root formation, identifying the relevant components is essential for sustained and economical propagation (Leakey *et al*., 1994). Plant growth regulators (PGRs) in plant tissue culture play a vital role in plant growth and development. Two important PGRs commonly used in plant propagation and growth are Indole-3-butyric acid (IBA) and Thidiazuron (TDZ). While IBA is widely used to promote root development, TDZ is known for its cytokinin-like activity, which stimulates shoot growth and branching (Mozafari *et al*., 2018). IBA application significantly enhances the rooting success of *Butea* cuttings. It stimulates the formation of adventitious roots, enabling successful propagation of the plant (Sharma *et al*., 2015). IBA treatment has been found to improve the stress tolerance of plants of genus *Butea*. It helps in better nutrient uptake and enhances the plant's ability to withstand adverse environmental conditions. TDZ application can result in compact and bushy growth of genus *Butea*. *B. monosperma* is desirable in certain ornamental or landscaping applications where a more compact and well-branched form is preferred (Chiruvella *et al*., 2011). High rate of axillary shoot proliferation is known to be stimulated and enhanced by TDZ, a substituted phenylurea derivative with a non-purine structure and vigorous cytokinin-like activity in many plant species. It causes a wide range of morphogenic and cultural reactions. Compared to other regularly used amino purine cytokinins, TDZ induces stronger axillary shoot bud growth at concentrations lower than 1M (Mok *et al*., 1982). TDZ induces the breaking of lateral bud dormancy and has the potential to increase or decrease endogenous cytokinin production (Kumari *et al*., 2018). Up to 4 weeks of incubation, TDZ had a positive impact on shoot induction, multiplication, and proliferation (Hussain *et al*., 2018). TDZ was found to be more effective at regenerating new shoots. The results of the current study demonstrated that shoots from leaf explants were significantly improved when TDZ and IBA were combined (Thiruvengadam *and* Chung, 2015). TDZ in general at higher concentrations has adverse effects on shoot length which increases with increased TDZ concentration. IBA resulted in 100% root induction (Aasim *et al*., 2018). IBA was the most effective in terms of percentage response for rooting of regenerated shoots, at 84.5 ± 0.98%, in the micropropagation of *B. monosperma* (Rathnaprabah *et al*., 2017).

Although studies regarding the use of TDZ and IBA to enhance the asexual propagation of plants have been carried out by several researchers and reported in previous literature. However, their use in the propagation of the highly valuable plant *Butea* is currently restricted and warrants further investigation. The present investigation is stepping towards the use of these growth hormones. Considering the aforementioned facts regarding its importance, *B. monosperma* aims to conserve the genetic resources of this endangered species via propagation. The research primarily focuses on selecting and propagating *Butea* plants with desirable growth characteristics, including faster growth rates, increased resistance to pests and diseases, or enhanced flower production.

2. Materials and Methods

2.1 Procurement of Plant Material

The experiment was conducted in the Botanical Garden, Institute of Botany, University of the Punjab, Lahore. The growth conditions of the garden during the experimental period (March to May) were mainly humid, with an average temperature of 24.8 °C and an average humidity of 76%. The healthy cuttings (ca.9 cm) of *B. monosperma* L. were obtained from healthy (30 years old) tree grown at the Botanical Garden. Cuttings were selected of uniform in size and having two nodes on it for further plantation.

2.2 Preparation of Rooting Medium

The rooting medium was prepared by mixing 40% compost and 60% garden soil. The garden soil and compost were taken from the Botanical Garden. After removing straws and stones, the mixture was passed through a sieve (RETSCH Stainless-Steel, 5 mm pore size) to maintain homogeneity. After thoroughly mixing all the constituents of the medium, pots measuring 10 inches were filled with the medium.

2.3 Preparation of Stock Solutions for Plant Growth Regulators

Stock solutions of 3000 ppm of Indole-3 butyric acid (IBA, Merck) and 3000 ppm of Thidiazuron (TDZ, Sigma) were prepared. Stock solutions were stored in amber colored bottles at 4 °C. From the stock solutions, working solutions of 1000, 2000, and 3000 ppm were prepared for each treatment. The prepared solutions were kept in small Erlenmeyer flasks (Pyrex) covered with aluminum foil and stored in a refrigerator (PEL).

2.4 Transplantation and Watering of Cuttings

The cuttings were dipped in the respective solutions of IBA and TDZ separately before transplantation for a few hours. The cuttings were transplanted into dry soil in earthen pots (5 × 12 inches) and watered with fully saturated soil. Watering was done according to the plant's growth requirements. Various concentrations of IBA and TDZ alone or in combinations were used.

2.5 Data Collection

The effects of TDZ and IBA were observed on the root and shoot parameters of the plant for each treatment, including germination percentage, days to bud initiation, number of buds, number of shoots, number of leaves, shoot length, and root length, which were also recorded at the conclusion of the experiment (after 90 days).

2.5.1 Germination percentage:

The germination percentage was evaluated by counting the number of successfully rooted and established cuttings or vegetative propagules. The final evaluation was done at the time of harvesting. Germination percentage calculated by using the formula:

Germination Percentage (%) = (Germinated cuttings) / (total number of cuttings) × 100

After planting these cuttings into the pot, the data were recorded for up to one month for the timing of bud initiation. The number of buds produced in each replicate of each treatment was counted at the time of plant harvesting. The number of shoots counted in each replicate. The first shoot emerges after 45 days. The final counting was done at the time of harvesting. The numbers of leaves were also counted for each replicate. The final counting was done at the time of harvesting when side branches emerged. The final measurement of shoot length was taken at the time of maturation using a measuring scale for each replication of the treatment. The root length of propagated cuttings was analyzed to evaluate the extent of root development. The root length was also measured after three months, using a measuring scale for each replicate.

2.6 Statistical Analysis

Completely randomized design was used. Three replicates were taken for each treatment of TDZ & IBA. All the calculated data were analyzed by SPSS version 26.0 to find out mean values and standard error by applying one-way ANOVA and using Duncan's multiple range tests to find the significant level among the means within the column at P ≤0.05.

3. Results

The current research work investigated the effect of TDZ and IBA on the rooting efficacy of *B. monosperma*. Assessments were made for morphological parameters grown in the field. Various concentrations of TDZ and IBA were used for rooting and shoot initiation. The data was recorded after 90 days of plantation.

3.1 Germination percentage of cuttings under the influence of TDZ and IBA

The results indicated varying germination percentages across different treatments in plant species. Maximum germination percentage recorded in all treatments (100%), indicating the successful rooting and subsequent growth of the vegetative propagules. While in the case of control plants, a 66% germination percentage was recorded (data not given).

3.2 Effect of TDZ and IBA on days to bud initiation

Rapid bud initiation began in treatments that combined IBA and TDZ. In treatment T11, bud initiation took a minimum of 18 days, whereas in T12, it took 21 days. In the case of treatment, the T8 results showed that bud initiation occurred in 22 days, whereas in T8 and T4, it took 23 and 25 days, respectively. In treatments T2 and T3, 24 and 27 buds were recorded, respectively. Conversely, in the case of T7 (35) and T5, bud initiation occurred after 36 days, whereas in T6, it occurred after 38 days. The results showed that in the control, the maximum number of days (45) was recorded for bud initiation (Figure 1).

All Figures and tables should be cited in the main text as Figure 1, Table 1, etc.

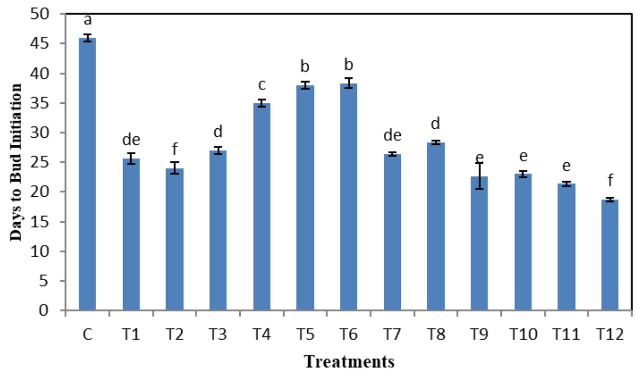

Figure 1. Effect of IBA and TDZ on days to bud initiation in the cutting of *B. monosperma* L. (C=Control, T1-T3 (1000-3000 ppm IBA), T4-T6 (1000-3000 ppm TDZ), T7-T9 (10 ppm IBA + 1000-3000 ppm TDZ), T10-T12 (10 ppm TDZ + 1000-3000 ppm IBA). Data is the mean of three replicates of each treatment. Small alphabets represent significant differences (p ≤ 0.05) among treatments according to Duncan's multiple range test (DMRT).

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3.3 Effect of IBA and TDZ on number of Buds

The viability and productivity of cuttings were checked by counting the number of buds in each concentration. The maximum number of buds was recorded in treatments used in combinations of IBA and TDZ. In treatment T11, the number of buds was 11, while in T12, there were nine buds. The number of buds was also high in T2 (8) and T3 (9). While 6 buds were recorded in T8, and 7 buds were recorded in T4 treatment. Conversely, 5 buds were recorded in T7 and 4 in treatment T6. The results showed that 2 buds were recorded in control plants (Figure 2).

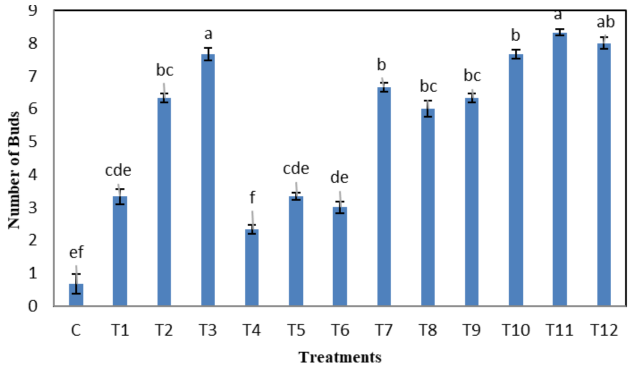

Figure 2. Effect of IBA and TDZ on number of buds in the cutting of *B. monosperma* L. (C=Control, T1-T3 (1000-3000 ppm IBA), T4-T6 (1000-3000 ppm TDZ), T7-T9 (10 ppm IBA + 1000-3000 ppm TDZ), T10-T12 (10 ppm TDZ + 1000-3000 ppm IBA). Data is the mean of three replicates of each treatment. Small alphabets represent significant differences ($p \leq 0.05$) among treatments according to Duncan's multiple range test (DMRT).

3.4 Effect of IBA and TDZ on the number shoots and leaves

The maximum number of shoots (12) and leaves (38) were recorded in treatment T11. In comparison, in the case of T10, the number of shoots (9) and leaves (25) was also relatively high in combination of IBA and TDZ (Figure 3). In treatment T8 number of leaves were 33 and overall, 6 shoots were observed. In case of T7 the number of shoots was 9 and 36 leaves were observed. Rapid production of shoots (12) and leaves (37) were recorded in treatment T8- conversely, the number of shoots (8) and leaves (30) in treatment T6. The minimum number of shoots (3) and leaves (11) were recorded in treatment T4 (Figure 5 A-K). In the case of control plants, only one shoot was observed (Figure 3) (Figure 5A).

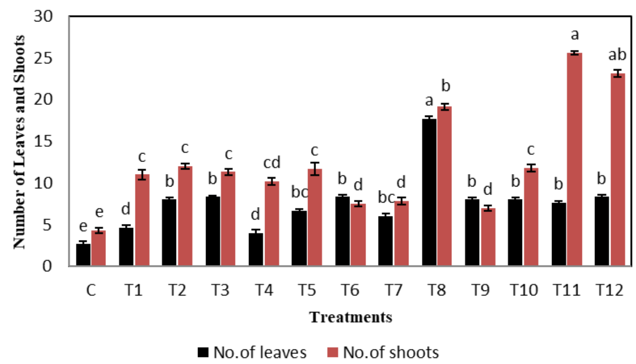

Figure 3. Effect of IBA and TDZ on number of leaves and shoots in the cutting of *B. monosperma* L. (C=Control, T1-T3 (1000-3000 ppm IBA), T4-T6 (1000-3000 ppm TDZ), T7-T9 (10 ppm IBA + 1000-3000 ppm TDZ), T10-T12 (10 ppm TDZ + 1000-3000 ppm IBA). Data is the mean of three replicates of each treatment. Small alphabets represent significant differences ($p \leq 0.05$) among treatments according to Duncan's multiple range test (DMRT).

3.5 Effect of IBA and TDZ on the length of the shoot and root

After statistical analysis, data on comparisons of the mean number of shoots and roots are presented (Figure 6 A-D). Maximum shoot (25.6 cm) and root length (8.6 cm) were recorded in treatment T11 after 90 days (Figure 5 K, Figure 6 D). For treatment T10, the shoot length was 12.5 cm, and the root length was 7.1 cm. In case of treatment T8, the shoot length was 12.5cm and root length was 6.2 cm. Conversely, in treatment T2 the shoot (18 cm) and root length (7.1 cm) were also high and in treatment T3 shoot length (13 cm) and root length (6.2 cm). The minimum number of shoots (4.2 cm) and roots (3.2 cm) was observed in T4 (Figure 5E). In control plants, shoot length (4.3 cm) and root length (2.5 cm) also increase gradually (Figure 4).

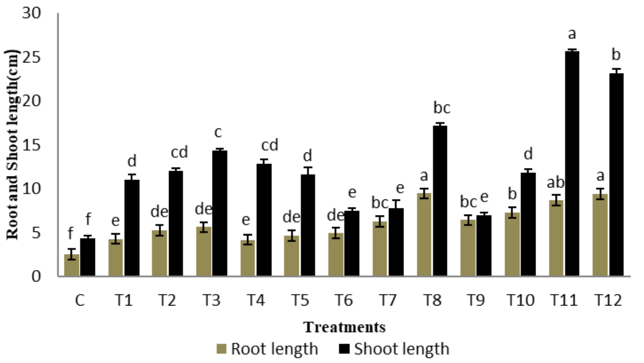

Figure 4. Effect of IBA and TDZ on shoot length and root length in the cutting of *B. monosperma* L. (C=Control, T1-T3 (1000-3000 ppm IBA), T4-T6 (1000-3000 ppm TDZ), T7-T9 (10 ppm IBA + 1000-3000 ppm TDZ), T10-T12 (10 ppm TDZ + 1000-3000 ppm IBA). Data is the mean of three replicates of each treatment. Small alphabets represent significant differences ($p \leq 0.05$) among treatments according to Duncan's multiple range test (DMRT).

4. Discussion

The present study demonstrated that TDZ, in combination with auxin, was quite effective in improving rooting and shoot development. The effect of TDZ varies across different plant species, explants, and exposure durations (Deepa *et al*., 2018). The morphological parameters, including germination percentage, number of leaves, number of shoots, shoot length, and root length, all showed an increase in growth under the treatment of IBA and TDZ. However, IBA is the most effective PGR for rooting stem cuttings. Rooting hormone types significantly influence the production of rooting and vegetative growth of cuttings. Moreover, the rooting potential depends upon the plant type, juvenility of the cuttings, growing conditions, and seasons (Kaushik and Shukla, 2020).

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Figure 5. Shoot growth of *B. monosperma* after treatment with different PGRs. **A)** Control, plants not treated with TDZ and IBA. **B)** Plant grown in 60 % garden soil and 40% compost and treated with T1 (IBA 1000 ppm). **C)** Plant grown in 60 % garden soil and 40 % and treated with T2 (IBA 2000 ppm). **D)** Plant grown in 60 % garden soil and 40% and treated with T3 (IBA 3000 ppm). **E)** Plant grown in 60 % garden soil and 40 %and treated with T4 (TDZ 1000 ppm). **F)** Plant grown in 60 % garden soil and 40 % compost and treated with T5 (TDZ 1000 ppm). **G)** Plant grown in 60 % garden soil and 40% compost and treated with T6 (TDZ 3000 ppm). **H)** Plant grown in 60 % garden soil and 40% compost and treated with T7 (IBA 10ppm + TDZ 1000ppm). **I)** Plant grown in 60 % garden soil and 40% compost and treated with T8 (IBA 10 ppm + TDZ 2000ppm). **J)** Plant grown 60 % garden soil and 40% compost and treated with T9 (TDZ 10 ppm + IBA 1000 ppm). **K)** Plant grown in 60 % garden soil and 40% compost and treated with T11 (TDZ 10 ppm + IBA 2000 ppm).

The results of the present research demonstrated that the highest rooting rate of 98.5% with 100% survival was obtained in cuttings treated with 3000 IBA ppm on sand. In contrast, the lowest rooting and survival were obtained in untreated cuttings in the same plant species (Singh, 2012). In the present study, the highest root growth was observed at 3000 ppm IBA. Root initiation was observed after 30 days in May, July, and August treatments only. However, the best rooting was observed in May, which coincided with the peak propagation season. The best hormone treatment was IBA (1000 ppm), which resulted in 20-60% rooting, depending on the twig diameter class and month (Kumar, 1989). In the present study, root initiation starts at the end of April, and best rooting occurs in May. The treatments supplemented with IBA have the best rooting.

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Figure 6. Rooting of cuttings of *B. monosperma*. **A)** Plants grown in T8 (10 ppm IBA + 2000 ppm TDZ) showing the best rooting growth. **B)** Plants grown in T9 (IBA 10 ppm + TDZ 3000 ppm) show the best rooting growth. **C)** Plant grown in T11 (10 ppm TDZ + 2000 ppm IBA showing best rooting growth. **D)** Plants grown in T12 (10 ppm TDZ + 3000 ppm IBA) showing best root growth.

The minimum number of shoots was observed in TDZ; however, when used in combination, more rooting and growth occurred in the present study. Multiple shoots were produced on all the concentrations of TDZ (Parveen and Shahzad, 2010). In the present study, the maximum number of shoots was observed in T11 (10 ppm TDZ + 2000 ppm IBA). The treatment is present in combination T8 (10 ppm IBA + 3000 ppm TDZ). But a relatively low number of shoots were observed in TDZ (2000 ppm).

The Maximum number of buds was produced in treatments T10 (10 ppm TDZ + 1000 ppm IBA) and T11 (10 ppm TDZ + 2000 ppm). Capelleti *et al*. (2016) demonstrated the best regeneration efficiency for the leaves of the strawberry cultivar obtained by culturing in a medium supplemented with thidiazuron (TDZ). However, the present study investigated that the maximum number of leaves was present in treatments T11 and T12. Aasim *et al*. (2018) suggested that a combination of TDZ and IBA (0.40+0.10 mg L^{-1}) induced maximum shoot regeneration frequency (61.11%) and maximum number of leaves. Present studies have also investigated how the interaction of IBA and TDZ enhances leaf production.

When PGRs IBA and TDZ were used in plant tissue culture studies, 80% rooting was achieved on MS medium supplemented with 0.3 µM IBA (Hussain *et al*., 2018). This did not apply to the present study because best rooting also occurred when IBA and TDZ were combined, as in T11 (10 ppm TDZ + 2000 ppm IBA) and T12 (10 ppm TDZ + 3000 ppm). The maximum root length was recorded in this treatment as compared to other treatments. Murti *et al*. (2012) suggested that the effects of TDZ and IBA concentrations, as well as their interactions, were significant for plant regeneration rates in *Fragaria ananassa*. The best rooting present in *Fragaria* occurs when TDZ and IBA were combined. These results are similar to those of the present study. It is well known that TDZ is a potent cytokinin (Kumari *et al*., 2018), its prolonged exposure decreases the quality of shoots, as observed in the present investigation.

Conclusion

The current study effectively evaluated the role of TDZ and IBA in positively influencing the rooting efficacy of *B. monosperma.* TDZ promotes cell division and differentiation, leading to the formation of adventitious roots, while IBA stimulates root initiation and development. Developing a vegetative propagation system is crucial for increasing the production and productivity of plants, as well as for conserving rare and threatened medicinal plants like *Butea*. By using these hormones appropriately, it is possible to enhance the rooting success and overall growth of *B. monosperma* cuttings. Plants grown in medium supplemented with TDZ and IBA showed the best results for bud initiation, number of buds, number of leaves, shoot length, and root length. The results clearly indicated that morphological parameters were significantly affected by the treatment with TDZ and IBA. However, it is essential to note that the concentration and application method of these hormones should be optimized based on specific experimental conditions and plant responses.

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Author(s) contribution Conceptualization, **Z.A.S** methodology, software, validation, formal analysis and investigation, data curation, **F.K** writing—original draft preparation, **F.K** writing—review and editing, supervision **Z.A.S**, All authors have reviewed and approved the manuscript.

Conflict of interest The authors declare no conflict of interest.

Data availability All data supporting the findings of this study are available within the paper. We do not have any research data outside the submitted manuscript file.

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Declarations

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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