

Optimization of Cytokinin–Auxin (BAP–IBA) Regimes for *In Vitro* Shoot and Root Regeneration in Tomato (*Solanum lycopersicum* L.) hybrids

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ABSTRACT

Tomato (*Solanum Lycopersicum* L.) is one of the valuable and nutritious crop. It has antioxidants such as vitamin C, vitamin A, flavonoids, fibers, lutein, β -carotenes and lycopene. Plant tissue culture is a technique utilized to obtain suitable cultivars in a shorter time, produce disease-free plants, introduce desirable traits into selected plants and multiply elite selections. This research experiment was conducted during 2023-24 in PTCC Lab at the IHS, University of Agriculture, Faisalabad to evaluate the effect of different concentrations of BAP and IBA on shoot multiplication and rooting. Experiment was laid out in Completely Randomized Design (factorial) with three replications. Data was evaluated for different parameters such as number of days taken to root and shoot induction, root and shoot induction percentage, shoot length, root length, plant height, number of roots and shoots per explant. To-1057 hybrid showed maximum days to induce shoot in control and T5 in Sandal hybrid indicated the minimum days to induce shoots. The maximum no. of shoots was observed in the sandal hybrid. Maximum shoot length (2.8 cm), shoot induction percentage (73%), roots per explant (3.8), plant height (2.75 cm) and survival percentage (73%) was observed in T5 in Sandal hybrid. While root length (0.8 cm) showed maximum values in To-1057 at T5. It was observed that days to root induction is maximum at control in both genotypes and minimum at 3 BAP + 2.5 IBA mg/L concentrations. Our findings suggest that Sandal hybrid performed better than T0-1057 in T5 (3 mg/L BAP +2.5 mg/L IBA). This study will be helpful in transformation technique of tomato crop for development of varieties resistant to biotic and abiotic stresses.

Keywords: Plant tissue culture; hybrids; *in-vitro* propagation; tomato; auxin; cytokinin; Plant growth regulators (PGRS)

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the

valuable and nutritious crops which is grown in almost all over the world (Mallick, 2021).

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It has antioxidants such as vitamin C, vitamin A, flavonoids, fibers, lutein, β -carotenes and lycopene (Collins et al., 2022). In the top 10 tomato producing countries in the list China, India and Turkey are three top leading countries in the world. China being top ranked in tomato production of about 61.7 M tons occupying around 1.1 M hectares of land. Pakistan is 30th in list of world tomato grower by producing 0.8 M tons occupying 0.068 M hectares (FAOSTAT, 2023). Tomato is perennial herbaceous plant but typically grown as an annuals, although its biennial and perennial forms are also available. In both temperate and tropical regions, tomatoes are grown in

open fields as well as in greenhouses (Ro *et al.*, 2021). Tomato cultivation is more challenging as change in environment and abiotic factors like heat, salinity and drought which has a significant impact on crop productivity. The most common diseases are tomato leaf curl virus, early and late blight, fusarium wilt, tomato mosaic virus, fruit rot which are caused by fungal or viral attack. Insects can also cause yield loss. Production of tomato can be increased by managing these diseases (Esquivel-Cervantes *et al.*, 2022).

Tissue culture, also referred to as *in vitro* cultivation, involves growing plant cells, tissues or organs in a controlled laboratory environment (Long *et al.*, 2022). It is an important biotechnology tool that can be utilized to increase productivity in agriculture by quickly providing superior planting material (Malabadi *et al.*, 2025). This technique involves the term totipotency (ability of each cell to develop into living organism). In tomato tissue culture, a single plant is multiplied and tissues which are known as explants are used to develop new plants while maintaining the genetic purity (Bhardwaj *et al.*, 2025). Plant hormones are chemicals found naturally within plant tissues that regulate growth and development (Bajguz and Piotrowska, 2023). PGRs are synthetic compounds that have physiological functions similar to those of these molecules. PGRs are applied from outside the tissues, indicating they have been applied exogenously. There are many classes of PGRs including abscisic acid, auxins, ethylene, cytokinins and gibberellins. Auxins and cytokinins play crucial roles in controlling growth and development in plant tissue and organ cultures. Mostly in combination with cytokinins, auxins control the direction of morphogenesis and promote the development of calli, cell suspensions, and organs (Zhumanova *et al.*, 2024). Different hormonal combinations are used for plant regeneration and callus induction like BAP, IAA, IBA, NAA *etc* (Saleem *et al.*, 2022).

The technique of plant tissue culture is used in plant to obtain suitable cultivars in a limited time, to produce disease-free plants, to introduce desirable traits in selected plants and to multiply elite selections. Moreover, process is independent of season and can be performed throughout the year. If this technique is used in conjunction with conventional breeding, an efficient system can be developed to accelerate the program of cultivar development (Khalil *et al.*, 2023). Effect of plant growth regulators to different sources of explants varies depending on their use in combination or separate (Martini *et al.*, 2022). The regeneration response varies depending on the cultivars and PGRs used in culture medium (Akter *et al.*, 2025). Many cultivars have established an efficient tissue culture system using proffer explants and PGRs,

enhancing callus induction and regeneration with optimal levels of cytokinin and auxin in MS media (Abd El-Hameid, 2021; Yaroshko *et al.*, 2023; Akter *et al.*, 2025; Sidik *et al.*, 2024).

Disease control is critical during tomato cultivation. Few pathogens can destroy the entire crop. Tissue culture provide a potential solution by enabling the production of disease free planting material (Panno *et al.*, 2021). Tissue culture also provide a way for genetic manipulation, paving the way for development of tomatoes with novel traits. Agrobacterium mediated transformation and gene gun can be used to introduce foreign gene in tomato tissue for resistance of biotic and abiotic stresses (Vu *et al.*, 2020).

The current study was conducted to establish a protocol for regeneration and to evaluate the influence of various concentrations of plant growth regulators on the growth and development of tomatoes.

MATERIALS AND METHODS

The study was carried out in the PTCC Lab at the IHS, University of Agriculture, Faisalabad, focusing on the *in vitro* regeneration of tomato plants. Experiment was laid out in Completely Randomized Design (factorial) with having three replications.

Explant Source

Seeds from two different hybrids, To-1057 and Sandal, were obtained from the Ayub Agriculture Research Institute in Faisalabad.

Media Preparation: The basal medium used for shoot proliferation, culture and subsequent regeneration of tomato (*Solanum lycopersicum* L.) consisted of MS salt mixture supplemented with varying concentrations of BAP and IBA (Murashige and Skoog, 1962). Solutions of growth regulators and essential salts were prepared using distilled water, then adjusted to achieve the necessary concentrations for preparing the medium. A carbon source, consisting of 30 g/L of sugar, was added, and the medium's pH was adjusted to 5.6. Agar, at a concentration of 8 grams per liter, served as a solidifying agent. Each test tube was filled with 10 ml of the medium, sealed with plastic sheets and placed in an autoclave for sterilization at 121°C for 20 minutes under a pressure of 15 psi.

Explant Sterilization

To ensure surface disinfection, the seeds were immersed in a 70% ethanol solution (v/v) with 1-2 drops of Tween-20 for 2-3 minutes. They were then rinsed three times with sterilized double-distilled water. Following this, the seeds were treated with a 5% sodium hypochlorite solution (v/v) for another 2-3 minutes and subsequently rinsed three times with sterilized double-distilled water. The explants were then cultured on a modified Murashige and Skoog medium.

Seed Culture

MS basal media was used for the culturing of seeds. The seeds were cultured on MS medium for germination.

Micropropagation of in vitro grown seedlings

In vitro developed seedlings were cultured on an MS basal medium enriched with different concentrations of BAP and IBA i.e. T0 (Control), T1 (1 mgL⁻¹ BAP + 0.5 mgL⁻¹ IBA), T2 (1.5 mgL⁻¹ BAP + 1 mgL⁻¹ IBA), T3 (2 mgL⁻¹ BAP + 1.5 mgL⁻¹ IBA), T4 (2.5 mgL⁻¹ BAP + 2 mgL⁻¹ IBA), T5 (3 mgL⁻¹ BAP + 2.5 mgL⁻¹ IBA) were used to culture the explant and boost shoot initiation.

Sterilization of Media

The media sterilized in an autoclave set for 20 minutes at 121°C and 15 psi. Following sterilization, the media were transferred to growth room facilities where light intensity was set at 2500 lux and the temperature was kept at 25°C ± 2.

Root and Shoot Regeneration

MS media was used to culture nodal explant for shoot regeneration and then transferred to root regeneration medium.

Data was recorded for following parameters

Number of days taken to induce shoots, Shoot Length (cm), Number of shoots per explant, Shoot induction (%): The number of days taken to induce shoots from explants was recorded after culturing them on a shoot induction medium. The total shoot length was measured using a measuring scale. The number of shoots induced per explant was recorded after culturing on the shoot induction media.

The shoot induction percentage was evaluated by using formula

$$\text{Shoot induction \%} = \frac{\text{Total no. of shoots per explant}}{\text{total no. of cultures induced shoots}} \times 100$$

Number of roots per explant, Root Length (cm), Root induction (%): The number of induced roots per explant was noted after cultivation on media designed for root induction.

Measuring scale was used to measure total root length. The root induction percentage were calculated by using following formula.

$$\text{Root induction \%} = \frac{\text{Total no. of Roots per explant}}{\text{total no. of cultures induced shoots}} \times 100$$

Height of Plant (cm): The height of plant was measured with a measuring scale from base of plant to the tip of the highest leaf in centimeters (cm).

Plant survival percentage (%): The plant survival percentage were calculated by using following formula.

Plant survival%

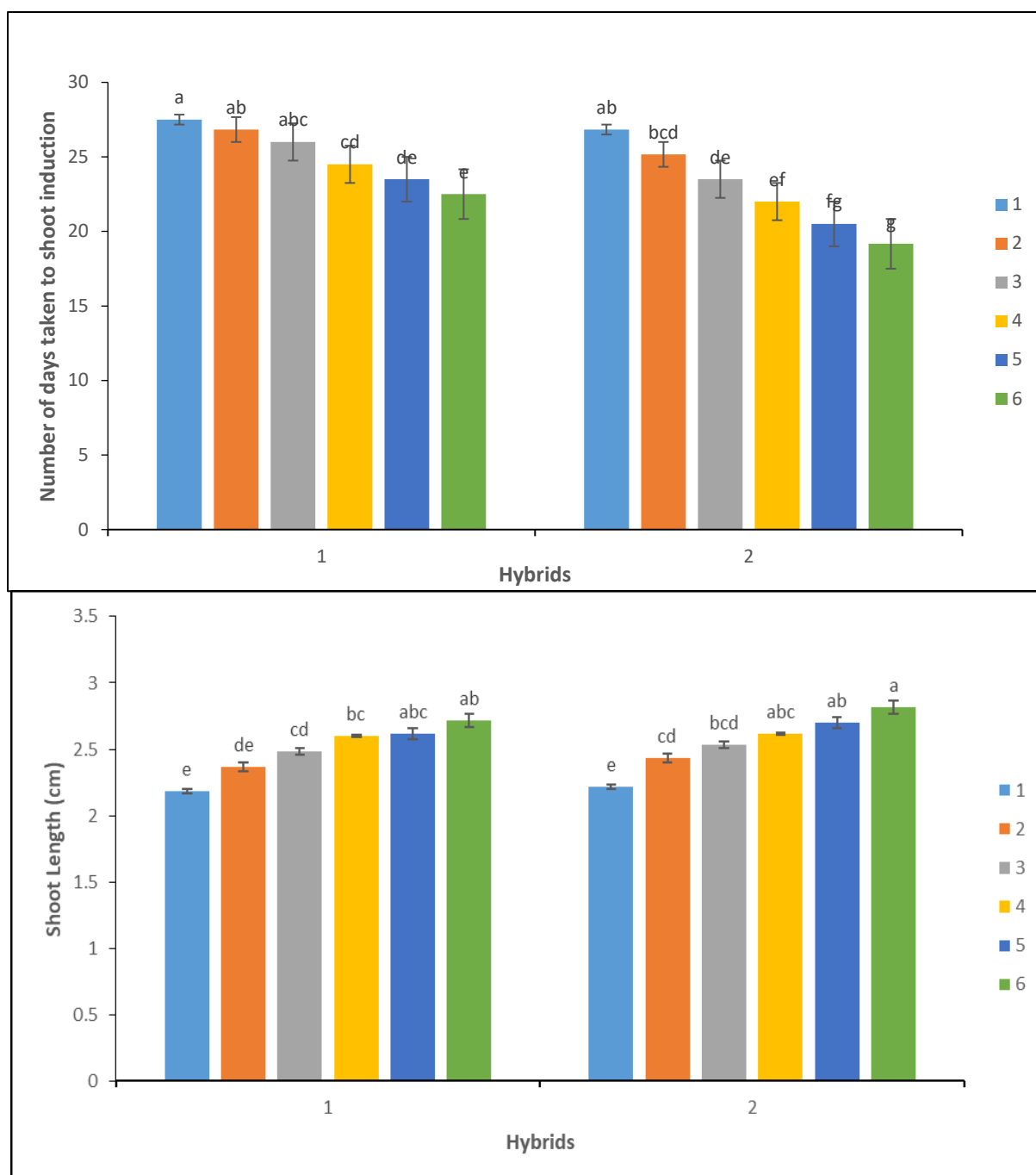
$$= \frac{\text{Total no. of plant survived}}{\text{total no. of plant cultured}} \times 100$$

Statistical analysis: Data were analyzed using a two-way ANOVA followed by Tukey's test ($p \leq 0.05$) with Statistics software version 8.1 (Steel et al., 1997). Additionally, a correlation heat map analysis (using the Pearson correlation coefficient) and Principal Component analysis (PCA), utilizing Origin Pro 2024 software from Origin Lab Corporation in Northampton, MA, USA.

RESULTS

Days taken for Shoot Induction, Shoot length, number of shoots per explant and Shoot induction percentage: Micro propagation in tomato hybrids 'To-1057' and 'Sandal' was successfully established and analysis of variance showed highly significant impact of PGRs, hybrids and their interaction on number of days taken to induce shoots. The result depicted substantial decrease in number of days taken to induce shoots with increase in plant growth regulators concentration. Minimum No. of days taken to induce shoots in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 22 days and 19 days respectively. Meanwhile to induce shoots the maximum days taken by To-1057 hybrid and Sandal hybrid were observed in control i.e. 27 and 26 days respectively.

Shoot length, number of shoots per explant and shoot induction percentage increased meaningfully in higher concentrations of BAP and IBA. ANOVA showed highly significant impact of PGRs and hybrids on shoot length, number of shoots per explant and shoot induction percentage. However, when examining their combined effect, there was no statistically significant interaction observed. Maximum shoot length in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 2.71 cm and 2.81 cm respectively. Whereas minimum No. of days taken to induce shoots were observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 2.18 and 2.21 cm. Maximum number of shoots in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 2.6 and 3.6 respectively. Whereas minimum No. of shoots were observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 1.667. Maximum shoot induction percentage in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 53.33 % and 73.33 % respectively. Whereas minimum No. of days taken to induce shoots were observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 23.33 %.



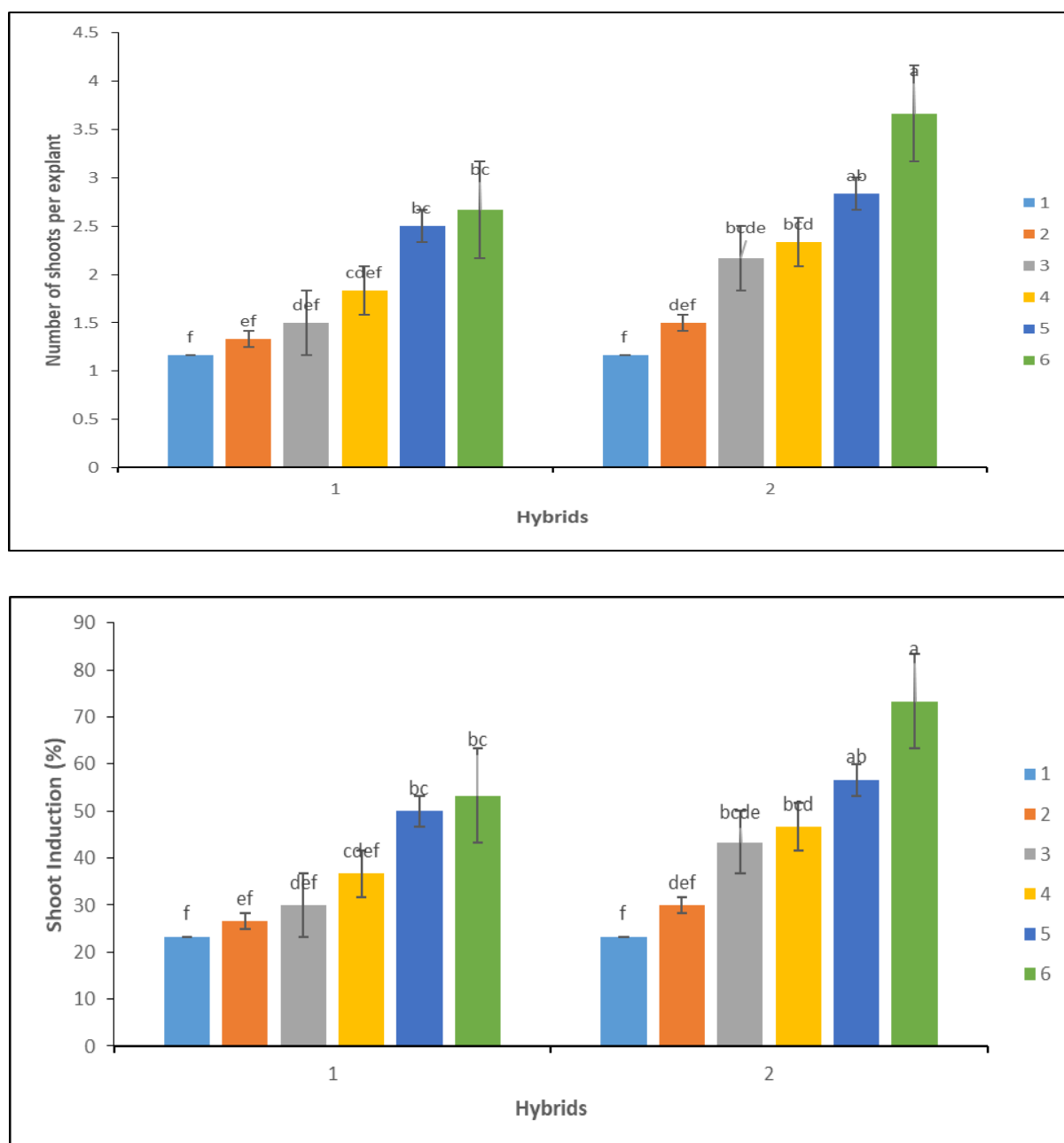
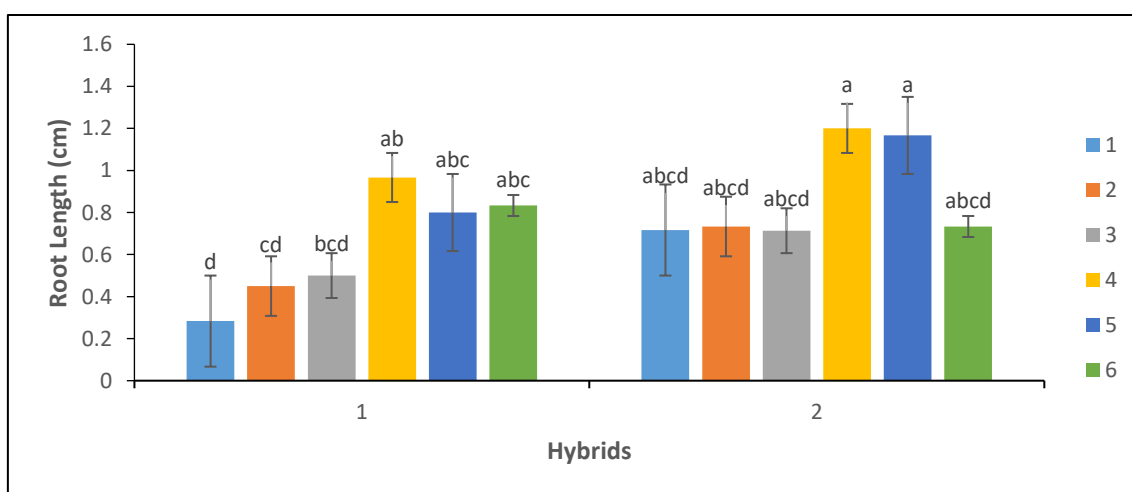
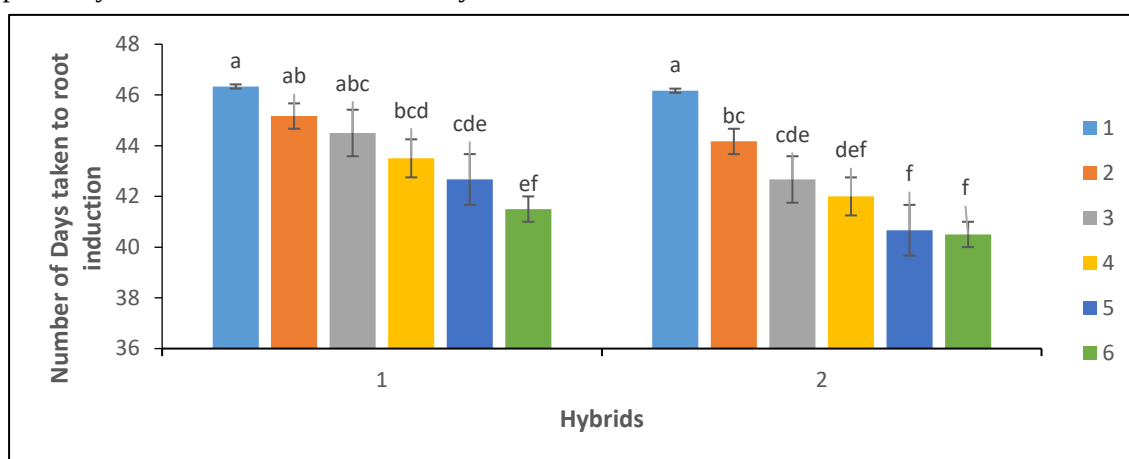


Figure (1a). Days taken for Shoot Induction, **(1b)** Shoot Length, **(1c)** Number of Shoots per explant, **(1d)** Shoot Induction Percentage in two hybrids i.e. (To-1057) and (Sandal) hybrids. T0= control, T1= 1 BAP + 0.5 IBA, T2= 1.5 BAP + 1 IBA, T3= 2 BAP+ 1.5 IBA, T4= 2.5 BAP+ 2 IBA, T5= 3 BAP+ 2.5 IBA. Mean followed by different superscript letters differs significantly ($P \leq 0.05$).

Days taken to root induction, Root Length, Number of roots per explant and Root induction percentage: Micro propagation in tomato hybrids ‘To-1057’ and ‘Sandal’ exhibited that days taken to root induction gradually decreased in higher concentrations of BAP and IBA. While root length, number of roots per explant and root induction percentage gradually increased in higher concentrations of BAP and IBA. Analysis of variance showed highly significant impact of PGRs and hybrids on root induction percentage, root length, number of roots per explant and root induction percentage. However, when examining their combined effect, there was no statistically significant interaction observed. Maximum number of days in root induction in To-1057 hybrid and Sandal hybrid were observed at control i.e. 46.33 and 46.167 respectively. Whereas minimum No. of days taken

to induce root were observed in To-1057 hybrid and Sandal hybrid were observed in T5 i.e. 41.50 and 40.50 respectively. Maximum root length in To-1057 hybrid and Sandal hybrid were observed at T3 i.e. 0.96 cm and 1.20 cm respectively. Whereas minimum root length was observed in To-1057 hybrid and Sandal hybrid in control i.e. 0.28 and 0.71 cm. Maximum No. of roots in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 2.83 and 3.83 respectively. Whereas minimum No. of roots were observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 1.667. Maximum root induction percentage in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 56.66 % and 76.66 % respectively. Whereas minimum root induction percentage was observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 23.33 %.



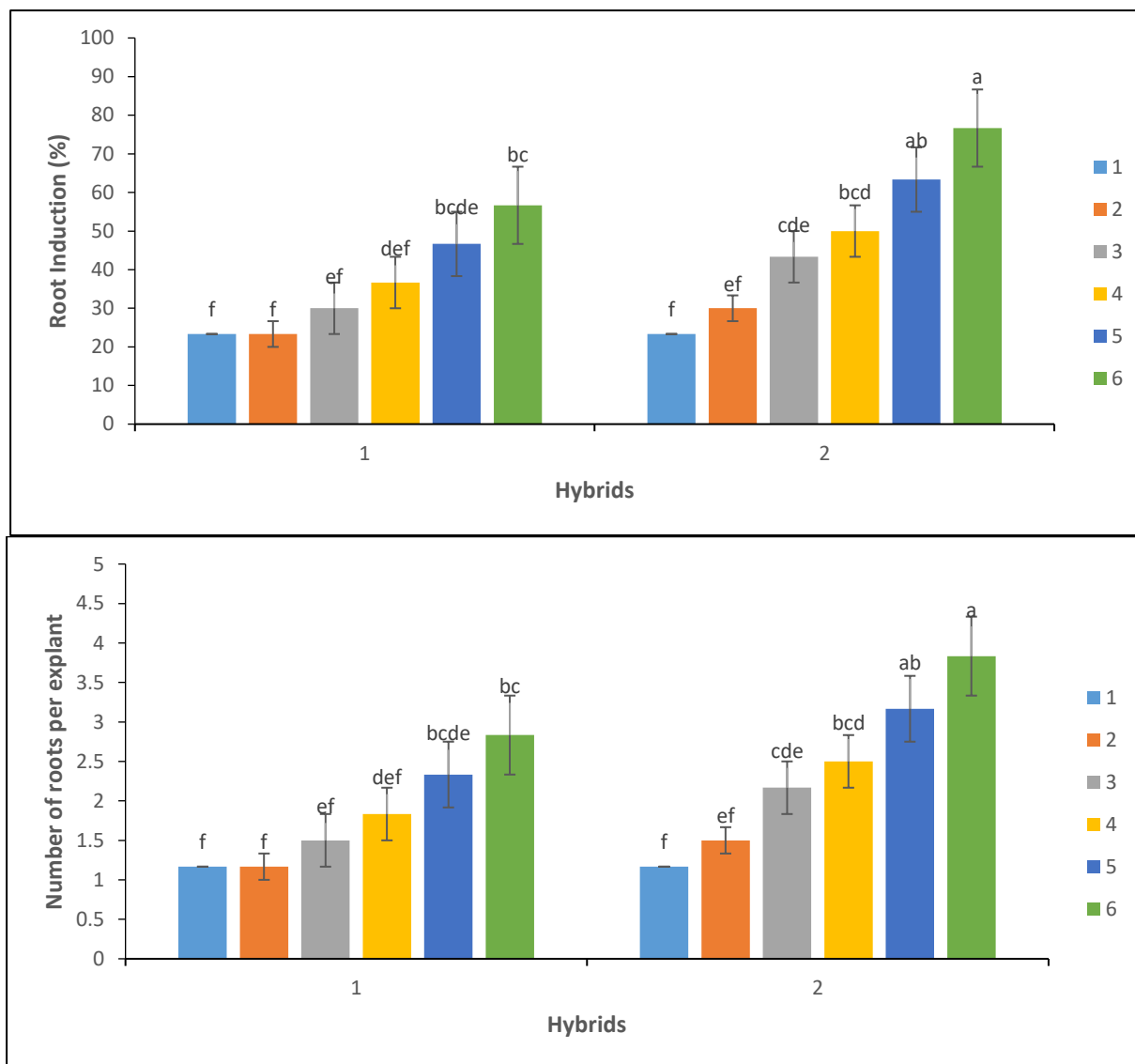


Figure. 2a (Days taken for Root Induction), **2b** (Root Length), **2c** (Number of Roots per explant), **2d** (Root Induction Percentage) in two hybrids i.e. (To-1057) and (Sandal) hybrids. T0= control, T1= 1 BAP + 0.5 IBA, T2= 1.5 BAP + 1 IBA, T3= 2 BAP+ 1.5 IBA, T4= 2.5 BAP+ 2 IBA, T5= 3 BAP+ 2.5 IBA. Mean followed by different superscript letters differs significantly ($P \leq 0.05$).

Survival Percentage and Plant Height: Micro propagation in tomato hybrids ‘To-1057’ and ‘Sandal’ showed that survival percentage and plant height potentially increased in higher concentrations of BAP and IBA. Analysis of variance showed highly significant impact of PGRs on survival percentage. However, when examining hybrids and combined effect of PGRs and hybrids, there was no statistically significant interaction observed. Maximum survival percentage in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 66.67 % and 73.33 %

respectively. Whereas minimum survival percentage in To-1057 hybrid and Sandal hybrid were observed in control i.e. 23.33 and 20 %. In case of plant height analysis of variance showed highly significant impact of PGRs, hybrids and their interaction. Maximum plant height in To-1057 hybrid and Sandal hybrid were observed at T5 i.e. 2.71 cm and 2.75 cm respectively. Whereas minimum plant height was observed in To-1057 hybrid and Sandal hybrid were observed in control i.e. 1.56 cm and 1.55 cm.

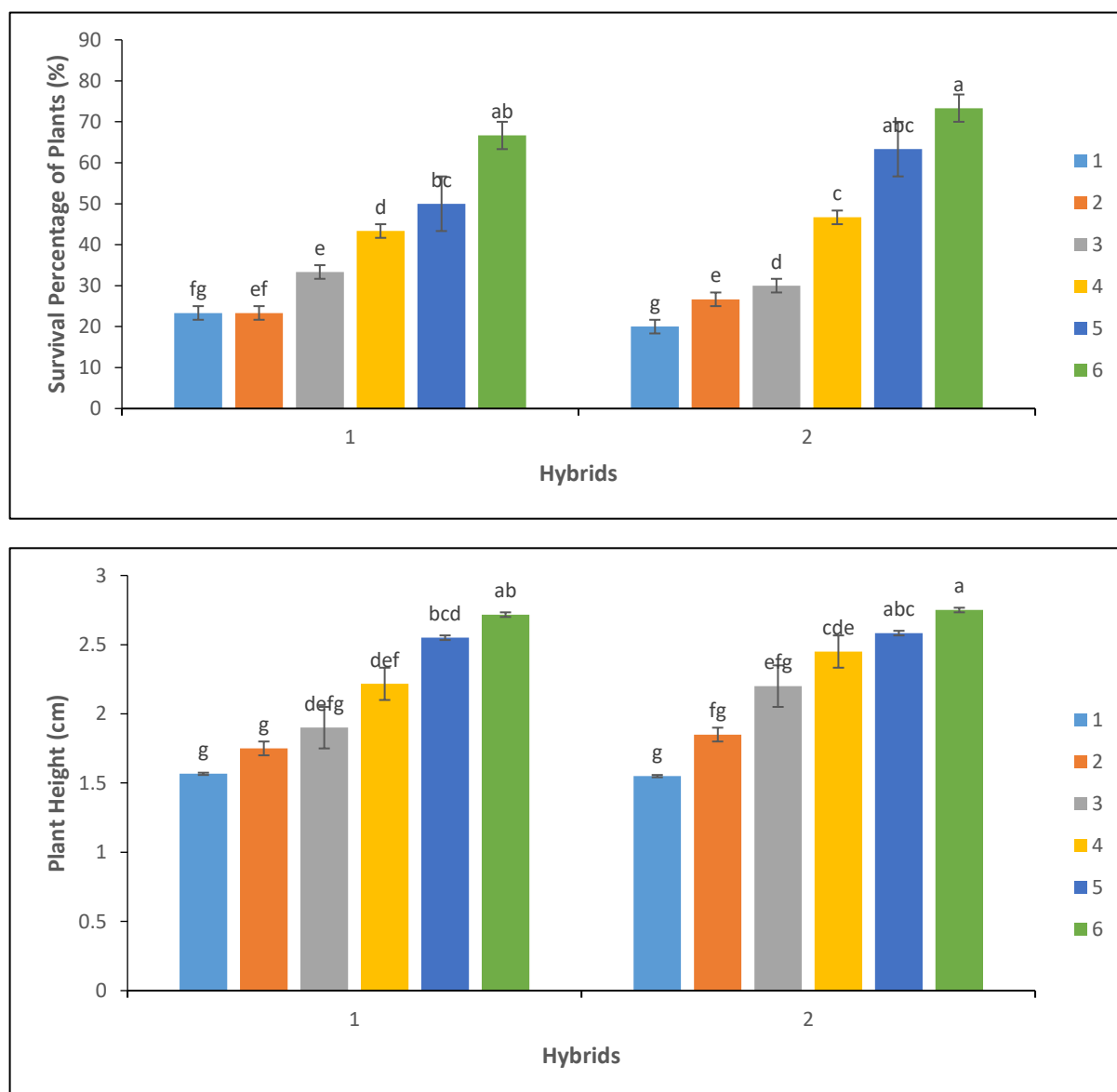


Figure. 3a (Survival Percentage), **3b** (Plant Height) in two hybrids i.e. (To-1057) and (Sandal) hybrids. T0= control, T1= 1 BAP + 0.5 IBA, T2= 1.5 BAP + 1 IBA, T3= 2 BAP+ 1.5 IBA, T4= 2.5 BAP+ 2 IBA, T5= 3 BAP+ 2.5 IBA. Mean followed by different superscript letters differs significantly ($P \leq 0.05$).

Pearson Correlation of Tomato Hybrids

The correlation analysis of To-1057 hybrid showed that days taken for shoot induction (DSI) exhibit strong negative correlations with all other parameters (shoot length (SL), number of shoots per explant (NSE), shoot induction percentage (SI), root length (RL), number of roots per explant (NRE), root induction percentage (RI), survival percentage (SP) and plant height (PH)) except DRI. Days taken for shoot induction (DSI) had strong positive correlation with days taken for root

induction DRI. This indicates that genotypes requiring more time for shoot initiation generally showed poorer performance in both shoot proliferation and subsequent growth stages. In contrast, shoot-related traits including SL, NSE and SI were positively correlated with each other as shown in figure 4. The correlation analysis for the second tomato hybrid “Sandal” revealed strong correlation among shoot induction, rooting and acclimatization traits. Days taken for shoot induction (DSI) showed a strong negative

correlation with shoot length (SL), number of shoots per explant (NSE), shoot induction percentage (SI), number of roots per explant (NRE), root induction percentage (RI), survival percentage (SP) and plant height (PH) as shown in figure 5. This indicates that genotypes taking more time for shoot initiation exhibited reduced efficiency in shoot multiplication, rooting, survival and overall plant growth. Moreover, DSI showed a strong positive correlation with days taken to root induction (DRI) suggesting that

delayed shoot initiation is associated with delayed rooting as well. Rooting traits also demonstrated strong association with shoot characteristics. Root length (RL) showed moderate positive correlations with SL, NSE, SI and SP while number of roots per explant (NRE) and root induction percentage (RI) exhibited very strong positive correlations with NSE and SI indicating that better shoot formation supports improved rooting efficiency.

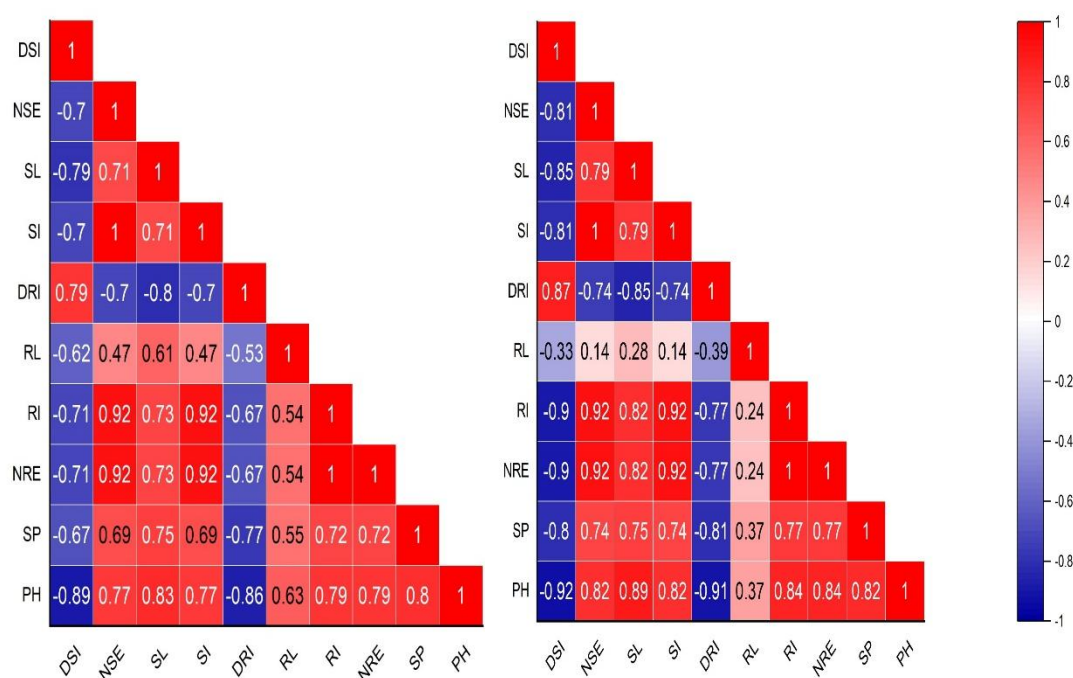


Figure 4. Pearson Correlation of Tomato Hybrid 'To-1057' (Red color shows strong positive correlation and Blue color shows strong negative correlation Figure. 5 Pearson Correlation of Tomato Hybrid 'Sandal' (Red color shows strong positive correlation and Blue color shows strong negative correlation.

Moreover, RI and NRE were perfectly correlated, reflecting their close functional relationship in root development. Survival percentage (SP) was positively correlated with both shoot and root traits, particularly SL, RI, NRE, and PH, suggesting that well-developed shoots and roots contribute significantly to successful acclimatization. Plant height (PH) also showed strong positive correlations with most growth traits including SL, RI, NRE and SP.

Shoot-related traits including shoot length (SL), number of shoots per explant (NSE) and shoot induction percentage (SI) were strongly and positively correlated with each other. Similarly, these shoot traits also showed strong positive correlations with rooting traits, particularly number of roots per explant (NRE) and root induction percentage (RI) indicating that better shoot development supports effective root

formation. The correlation pattern observed in the both tomato hybrids was largely similar indicating a consistent trend.

Principal Component Analysis (PCA)

The PCA biplot shows how different traits contribute to variation among the samples in a simple visual way. The first principal component (PC1) explains most of the variation while the second component (PC2) explains a smaller portion for both hybrids indicating that most differences among samples are captured along PC1 as shown in figure 6 and 7 respectively.

Traits such as shoot length (SL), shoot induction percentage (SI), plant height (PH), survival percentage (SP), root length (RL), number of shoots per explant (NSE) and number of roots per explant (NRE) are grouped on the positive side of PC1, showing that these traits are positively associated with each other and contribute to better growth and performance.

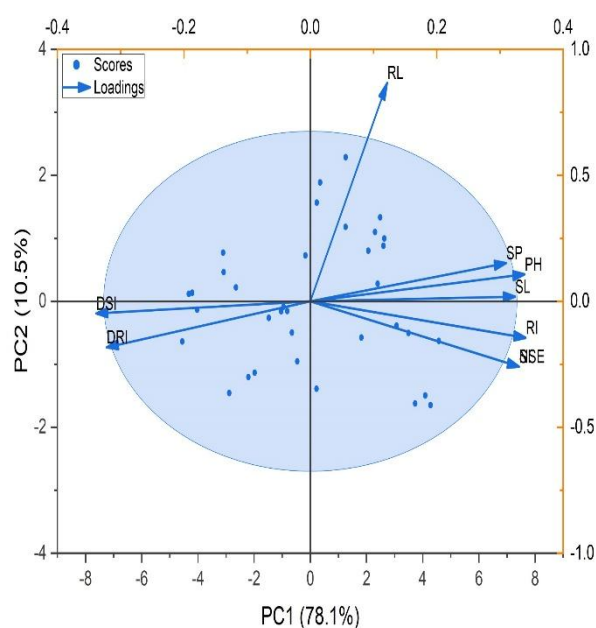
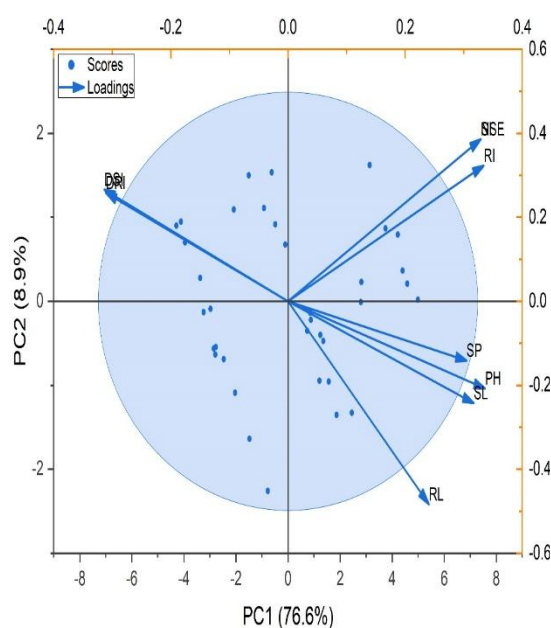


Figure 6. PCA Biplot Illustrating the Relationship Among Different Traits in Tomato Hybrid 'To-1057'. **Figure 7.** PCA Biplot Illustrating the Relationship Among Different Traits in Tomato Hybrid 'Sandal'. Label each figure in the figures.

DISCUSSION

The present study was conducted to evaluate the influence of various concentrations of plant growth regulators (PGRs) and to establish a protocol for regeneration on the growth and development of tomatoes. Multiple morphological characteristics were evaluated, such as the length of shoots, the number of shoots per explant, days to shoot induction, root length, the number of roots per explant, the percentage of shoot induction, the survival rate, height of the plants, days to root induction and the percentage of root induction. The hormone combinations had a significant effect on shoot induction, root development and overall plantlet survival. The development of the shoot apex depends on auxin-cytokinin interactions (Tran et al., 2021).

Minimum days for shoot induction in To-1057 and Saandal hybrids were noted with the treatment of 3 mg/L BAP combined with 2.5 mg/L IBA, resulting in 22 and 19 days, respectively. While it is possible to achieve shoot regeneration using only BAP, the combination of 3 mg/L BAP with 2.5 mg/L IBA decreased the time required for shoot induction. In this study, the percentage of shoot initiation decreased as BAP concentration increased. This may be due to higher callus production at elevated BAP levels. Specifically, at these higher concentrations, explants produced excessive callus, which hindered the efficiency of shoot multiplication (Abebe et al., 2024). This is in line with previous studies that higher cytokinin concentrations promote cell division and meristematic activity (Ak et al., 2021).

Shoot length and number of shoots per explant were also increased at this concentration, confirming the synergistic effect of BAP and IBA in multiple shoot formation as reported by Frary and Earle (1996). Our findings are consistent with the results of Rashid and Bal (2010), where maximum shoot length was recorded at 3 mg/L BAP + 2.5 mg/L IBA in both tomato hybrids. The increase in shoot length and number may be attributed to the stimulatory effects of auxins on shoot proliferation. These results are also supported by Elfil et al. (2023) and Ishag et al. (2009), who reported that BA levels positively influenced the number and length of tomato shoots under tissue culture conditions. Furthermore, higher concentrations of BAP and IBA led to the maximum shoot induction percentage, which aligns with the findings of Jehan and Hassanein (2013). Auxin and cytokinin have been shown to function synergistically in a number of solanaceous crops, such as *Capsicum annum*, *Solanum melongena* and *Solanum tuberosum* (Akhtar et al., 2025).

Similarly, the shortest time to root induction, longest root length, maximum root induction percentage and

greater roots per explant and were observed at 3 mg/L BAP + 2.5 mg/L IBA. This is in agreement with Jehan and Hassanein (2013) who demonstrated the efficacy of this hormonal combination in enhancing root formation in tomato cultures. IBA works well as an auxin to encourage root elongation and induction. It can be used with other growth regulators to increase the effectiveness of rooting (Frick and Strader, 2025). The current study's findings show that high doses of IBA are highly effective at promoting rooting and root development in shoots, even when paired with BAP. The higher plant height and survival percentage in T5 could be due to establishment of strong shoot-root system which contributes to plantlet vigor and acclimatization potential. Highest plant height in both To-1057 and Saandal was recorded at 3 mg/L BAP + 2.5 mg/L IBA, BAP and IBA at higher concentration promotes plant height. Raza et al. (2020) also reported higher plant heights under similar conditions; however, their results showed greater values than those in our study, possibly because they recorded data at the 6-leaf stage, whereas our measurements were taken at the 2–3 leaf stage. Maximum survival percentage was also observed at 3 mg/L BAP + 2.5 mg/L IBA, and our results are partially consistent with the findings of Ajenifujah-Solebo et al. (2012). Interestingly Sandal performed better in vitro than To-1057 in most parameters. This is genotype dependent response to micropropagation protocols as earlier reported by Compton and Veilleux (1991), who found varying regeneration capacity among tomato genotypes.

CONCLUSIONS

Overall MS medium with 3 mg L⁻¹ BAP and 2.5 mg L⁻¹ IBA was the best combination for in vitro propagation with high shoot proliferation, root development and survival in both tomato hybrids. This protocol can be used as a basis for large scale propagation and genetic enhancement programs of *Solanum lycopersicum*.

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Not Applicable.

Authors' Contributions

Maham Sajid conducted the study, collected data and contributed in developing initial draft of the paper. Muhammad Ammar Amjad, Javaria Ashraf and Hammad Sajid analyzed the data; Rashida Bibi and Reena Batool prepared the final manuscript. Azeem Iqbal and Bilquees Fatima guided for experimenting and also reviewed the paper. Muhammad Ammar Amjad, Tayyaba Sajid and Muhammad Qadeer critically reviewed the paper. All authors approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not Applicable

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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