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Enhancing in vitro seed germination and seedling vigour in lisianthus (Eustoma grandiflorum shinn) through optimised plant growth regulator combinations

S Y Geethanjali
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
S. Y. Champa
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
C. K. Pramila
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
M R Namratha
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
Hemla Naik
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
B. Brijesh
Keladi Shivappa Nayaka University of Agriculture and Horticultural Sciences
A. S. Vignesh
University of Agricultural Sciences, Bangalore
P. V. Puneeth
puneethicar25@gmail.com

ICAR - Central Island Agricultural Research Institute

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Abstract

Efficient commercial production of lisianthus (*Eustoma grandiflorum* Shinn), an economically important ornamental crop, requires uniform seed germination and successful seedling establishment. However, the irregular and delayed seed germination associated with dormancy-related constraints remains a major limitation in obtaining uniform and vigorous planting material. The combination of plant growth regulators (PGRs) with suitable nutrient media has considerable potential to improve the germination performance and early seedling growth in vitro. The present study was designed to standardise an efficient in vitro seed germination protocol by testing different combinations of auxin, cytokinin and gibberellin in full- and half-strength Murashige and Skoog (MS) media in relation to the germination kinetics, seedling vigour, survival and early development traits in lisianthus. The working hypothesis was that the optimum MS medium strength, along with the concurrent application of auxin, cytokinin and gibberellin, would improve germination efficiency and promote balanced seedling growth as compared to the basal medium and other PGR combinations. The most efficient treatment among the tested treatments was the treatment with full-strength MS medium supplemented with indole-3-butyric acid (IBA, 0.5 mg L^{-1}), 6-benzylaminopurine (BAP, 3.0 mg L^{-1}) and gibberellic acid (GA_3, 0.5 mg L^{-1}). This combination showed the highest germination percentage (95.37%), germination energy (82.02%) and germination value (6.58), earliest initiation (6.08 days) and completion (16.08 days) of germination and lowest mean germination time (6.80 days). Besides, it resulted in an increased seedling survival rate (67.27%) and vigour index (1213), and maximum lengths of plumule and radicle (6.10 and 6.62 mm, respectively). Early developmental responses were also improved, with first, second and third pairs of true leaves appearing at 6.27, 20.30 and 34.10 days, respectively. This treatment also resulted in the highest seedling fresh weight (1.24 g) and dry weight (0.19 g). The better response could be associated with complementary physiological functions of IBA, BAP and GA_3 in root and shoot development, cell division and metabolism related to germination. The optimised treatment provides a promising basis for standardised in vitro germination and early seedling production of Lisianthus.

1. INTRODUCTION

Lisianthus (*Eustoma grandiflorum* Shinn.) belonging to the family Gentianaceae is an economically important ornamental crop which has gained considerable prominence in the world floriculture industry. Its attractive rose-like flowers, diversity of vibrant and pastel colours and excellent postharvest vase life have contributed to its recognition as one of the leading cut flowers traded internationally (Harbaugh, 2007; Hutchinson et al., 2011). Besides its importance as a cut flower, dwarf and compact cultivars are becoming increasingly important as potted plants, thereby increasing its commercial prospects. Lisianthus is gaining importance, but poor, delayed and non-uniform seed germination often hinders commercial growing of Lisianthus, negatively affecting seedling establishment and the availability of uniform, high-quality planting material (Ecker et al., 2006).

In Lisianthus, seed dormancy is linked to a range of factors such as immature embryos, physiological barriers and a particular temperature range for germination. They are very small seeds, producing about

19,000–20,000 seeds per gram, which makes them difficult to handle, sow and establish (Harbaugh, 2007). This, combined with irregular germination, leads to asynchronous emergence of seedlings, variable seedling vigour and uneven establishment of the crop. Traditional approaches such as stratification and seed priming have been used to enhance germination and to overcome dormancy-related constraints (Ecker et al. 1994). However, their efficiency is often genotype-, environment- and seed quality-dependent, highlighting a need for more consistent and reproducible approaches to seed germination. Ultimately, this variability in seedling emergence can lead to poor crop uniformity, affect production scheduling and subsequent flowering, thus reducing the efficiency of large-scale commercial Lisianthus production.

In vitro culture techniques are a promising approach to improve seed germination and early seedling growth under controlled conditions, especially when used in combination with appropriate plant growth regulator (PGR) combinations (Kumar, N., & Reddy, 2011). Plant growth regulators, such as gibberellic acid (GA_3), cytokinins like 6-benzylaminopurine (BAP) and auxins like naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), play significant roles in the regulation of germination-related physiological processes, embryo growth, cell division and further seedling development (Mousavi et al., 2012; Uddin et al., 2017). Gibberellins are mainly associated with activation of germination-related metabolic processes. Auxins and cytokinins are involved in coordinated root and shoot development. Hence, the best combination of these growth regulators with the optimum nutrient environment may enhance the germination efficiency and promote uniform seedling establishment. However, individual and combined applications of PGRs may have different responses depending on their concentration, interaction and nutritional composition of the culture medium (Gaspar et al. 1996). Thus, the choice of appropriate combinations of PGRs and basal medium strength is crucial to the development of an efficient and reproducible in vitro germination protocol.

Previous studies have established the role of plant growth regulators in Lisianthus tissue culture and regeneration (Rezaee et al., 2012); however, further optimisation of PGR combinations for improving seed germination kinetics and subsequent seedling establishment is still relevant (Paek et al., 2000: Griesbach et al., 1988). In particular, the combined evaluation of auxin, cytokinin and gibberellin on different strengths of Murashige and Skoog (MS) medium provides the opportunity to identify treatment combinations that simultaneously enhance the percentage of germination, speed of germination, survival of the seedlings and early vegetative growth. The present work is novel in the comparative evaluation of these hormonal combinations in full- and half strength MS media for their effect on germination performance and subsequent seedling development. It was hypothesised that the combined application of auxin, cytokinin and gibberellin at appropriate concentrations, with a suitable MS medium strength, would enhance germination efficiency, reduce time required for germination and promote superior seedling vigour and establishment compared with basal MS media and other PGR combinations.

The present study was conducted to evaluate the effect of different PGR combinations and MS medium strengths on in vitro seed germination, germination kinetics, seedling survival and early growth

characteristics of lisianthus (*Eustoma grandiflorum* Shinn.) for standardisation of an efficient protocol for improved seed germination and uniform seedling production.

2. MATERIAL AND METHODS

2.1. Experimental setup

The experiment was conducted in the tissue culture laboratory of Department of Biotechnology, College of Agriculture, Navile, Shivamogga during 2024–2026 under controlled environmental conditions. The experimental material consisted of seeds of lisianthus (*Eustoma grandiflorum* Shinn.) var. 'Nozomi White' obtained from Sakata Seed, Bangalore. The experiment was conducted to evaluate the effect of different strengths of MS medium and PGRs combinations on in vitro seed germination and subsequent seedling development in Lisianthus. Seeds were used as explants and inoculated under aseptic conditions on respective treatment media. The inoculated cultures were maintained under above controlled environmental conditions for seed germination and growth of seedlings. Entire experimental workflow is depicted in Fig. 1. Ten treatments of three replication containing 50 seeds per replication totalling to 150 seeds per treatment are used in full and half-strength Murashige and Skoog (MS) media supplemented with different combinations of plant growth regulators (PGRs) (Table 1).

Table 1
Composition of Murashige and Skoog (MS) media and plant growth regulator combinations used for in vitro seed germination and seedling development of lisianthus (*Eustoma grandiflorum* Shinn.).

Treatment	MS medium strength	NAA (mg L^{-1})	IBA (mg L^{-1})	BAP (mg L^{-1})	GA_3 (mg L^{-1})
T1	Full strength	–	–	–	–
T2	Half strength	–	–	–	–
T3	Full strength	0.5	–	3.0	–
T4	Half strength	0.5	–	3.0	–
T5	Full strength	–	0.5	3.0	–
T6	Half strength	–	0.5	3.0	–
T7	Full strength	0.5	–	3.0	0.5
T8	Half strength	0.5	–	3.0	0.5
T9	Full strength	–	0.5	3.0	0.5
T10	Half strength	–	0.5	3.0	0.5

2.2. Preparation of media

MS medium was prepared according to the formulation of Murashige and Skoog, 1962. The required amounts of MS stock solutions (MS-A, MS-B, MS-C and MS-D) were dissolved in a small volume of

double-distilled water (DDW) on a magnetic stirrer. Depending on the respective treatment, the required concentrations of plant growth regulators and antioxidants were added in the medium. Medium was supplemented with 30 g L^{-1} sucrose and 7–8 g L^{-1} agar and was adjusted to DDW. Growth regulators were precisely measured and dissolved before addition to the respective media. The pH of medium was adjusted to 5.7–5.8 by 1 N HCl or NaOH. Prepared medium was heated on hot plate and required quantity of agar was added while heating. The culture medium was supplemented with antioxidants at recommended concentrations to decrease phenolic oxidation and browning of explants during in vitro culture. The medium was dispensed in culture bottles and covered with polypropylene cap after complete dissolving of agar.

2.3. Sterilisation of culture media

The prepared culture media were autoclaved at 121^0C and 15 psi pressure for 20 min. The media were placed in culture bottles in perforated baskets. The caps of the culture bottles were marked with the respective medium numbers and the date of preparation. The bottles were removed from the autoclave after sterilisation and were allowed to cool at room temperature. The media were allowed to set for 24 h and then stored for 3 to 4 days to ensure no microbial contamination before inoculation. Sterilised culture media were stored at 25°C until use.

2.4. Aseptic Inoculation and Seed Surface Sterilisation

The lisianthus seeds were packaged in small packets constructed of filter paper due to the very small size of the seeds and the possibility of loss during handling with conventional wire strainers. The surface sterilisation was carried out by dipping the packets in 20% commercial sodium hypochlorite solution for 15 min. Three drops of Tween-20 (0.2%) were added to the disinfectant solution to ensure uniform contact with the surface of the seed.

Seed packets were surface sterilised and rinsed 3 times for 5 min with sterile distilled water. After the last rinse, excess water was removed by filtration. The filter paper packets containing seeds were placed on sterile blotter paper to remove excess moisture. Then paper clips were opened gently, and seeds adhered to the filter paper were placed on the surface of the nutrient medium following the procedure described by Aktas et al. (2023). All seed sterilisation and inoculation steps were carried out under aseptic conditions in a laminar airflow cabinet.

2.5. Culture conditions and observations

The cultures were incubated at 25°C with a 16 h photoperiod, using cool white fluorescent lamps providing a light intensity of 40–50 µmol m^{-2} s^{-1} and 60–70% relative humidity. The following observations were recorded to determine the germination performance, seedling survival and early growth characteristics.

2.6. Experimental observations

2.6.1. Germination percentage (%)

The germination percentage was calculated by counting the number of seeds showing visible emergence of the plumule with cotyledon development in each replication. The percentage of germinated seeds in relation to the total number of seeds inoculated in the respective treatment was calculated (Germination (%) = (Number of germinated seeds/Total number of seeds inoculated) *100.

2.6.2. Germination energy (%)

Germination energy is calculated based on the percentage of seeds germinated within a fixed early period. It is the ratio of the percentage of seeds germinated at the first count to the total number of seeds germinated at the end of the test. This was used to evaluate the early germination performance of the seeds.

2.6.3 Germination value (GV)

was calculated according to Djavanshir and Pourbeik (1976) based on daily germination speed (DGS) and maximum value. The germination rate per day was calculated by dividing the cumulative percentage of germination by the number of days after the beginning of germination. The germination value was calculated using the cumulative daily germination speed and the peak value. The peak value was defined as GP × 10, where GP is the final germination percentage and 10 is the constant used in the calculation.

2.6.4. Time required for the start and completion of germination

The number of days to initiation (DTIG) and completion (DTCG) of germination was recorded for each treatment. A seed was considered germinated only when the sprouted plumule was visible along with cotyledons.

2.6.5 Mean Germination Time (days):

The mean germination time (MGT) was calculated to estimate the average time needed for seed germination (Bonner, 1983). It was calculated by multiplying the number of seeds germinated each day by the number of days from the beginning of the test to the time each seed was counted as germinated, summing these products and dividing the sum by the total number of seeds germinated at the end of the test. Seeds that did not germinate at the end of the test were assigned the value n + 1, where n is the total number of days of the germination test, and these values were used in the calculation of the mean.

2.6.6. Seedling survival (%)

The survival rate of the seedlings was recorded as the percentage of the germinated seedlings that were alive, healthy and actively growing under the experimental conditions after the given period of observation. It was calculated as the percentage of the number of surviving seedlings to the total number of germinated seeds multiplied by 100.

2.6.7. Vigour index:

The total length of seedling (length of plumule + length of radical) were multiplied by total germination per cent (Abdul-Baki and Anderson, 1973) (Vigor index (VI) = Total seedling length x germination per cent)

2.6.8. Length of Plumule and Radicle (mm)

Seedling growth was assessed by measuring and recording plumule and radicle lengths separately in millimetres (mm) at the end of the germination test, to the nearest two decimal places.

2.6.9. Days to the appearance of pairs of true leaves

The number of days to emergence of the first, second and third pairs of true leaves were recorded for each treatment. The appearance of true leaves was separated from the formation of cotyledons and the third distinct pair of true leaves on the main stem was considered for noting the respective observation.

2.6.10. Fresh and dry weight of seedlings (g)

Seedlings from the germination test were harvested and their fresh weights were recorded. The seedlings were dried in a hot-air oven at 80°C for 48 h and dry weight was recorded and expressed in grams.

2.7 Statistical analysis

The experiment was arranged in a Completely Randomised Design (CRD) with ten treatments, three replications per treatment and 50 seeds per replication. Experimental data were subjected to analysis of variance (ANOVA) appropriate for a Completely Randomised Design (CRD). Treatment means were compared using the Critical Difference (CD) test at the 1% level of significance ($P \leq 0.01$). Pearson's correlation coefficient was used to analyse the correlation of germination, seedling growth and developmental traits across the treatments. The correlation matrix was visualised as a heatmap with hierarchical clustering to group traits by their correlation patterns. The analysis and visualisation were done in Metaboanalyst *v 6.0*.

3. Results and Discussion

3.1 Effect of plant growth regulators on in vitro seed germination kinetics of lisianthus

In vitro seed germination and subsequent seedling development of lisianthus was significantly impacted by different combinations of plant growth regulators incorporated in Murashige and Skoog (MS) medium. Results indicated that the type and combination of growth regulators and the strength of the basal medium were important factors in determining germination performance and early seedling growth. The increased response in PGR supplemented media suggests the significance of preserving an appropriate hormonal balance to overcome dormancy related constraints, in promoting uniform

germination and in facilitating vigorous seedling establishment. Adhikari and Pant (2019) reported similar improvements in in vitro germination and seedling development by the application of suitable growth regulators in Dendrobium orchid.

Gibberellic acid (GA3) is an important hormone in seed germination and activates hydrolytic enzymes such as α-amylase which breakdown stored starch into soluble sugars required for embryo growth and development. Increased availability of metabolisable carbohydrates may facilitate embryo expansion, germination speed and subsequent seedling growth. Other hormones such as indole-3-butyric acid (IBA), a type of auxin, are also involved in root initiation and development, while cytokinins such as 6-benzylaminopurine (BAP) stimulate cell division and shoot development. Therefore, combined application of these growth regulators may create more favourable hormonal environment for the coordinated development of root and shoot, resulting in better germination and establishment of the seedlings. These results are in agreement with the findings of Mahajan et al. (2012) and Shintiavira et al. (2015) who reported improved germination and uniform growth of seedlings in MS media with suitable concentrations of PGR. Similar responses have been reported by Pradhan et al. (2013) in Cymbidium aloifolium and Akhtar et al. (2016) in Rosa centifolia, signifying the importance of suitable combinations of growth regulators in promoting in vitro growth and development of ornamental plants.

T9 consisting of full strength MS medium supplemented with IBA (0.5 mg L^{-1}), BAP (3.0 mg L^{-1}) and GA 3 (0.5 mg L^{-1}) recorded the highest germination percentage of 95.37% among the treatments evaluated in the present investigation (Table. 2). T9 exhibited better performance indicating that the combined application of auxin, cytokinin and gibberellin at the tested concentrations created a favourable hormonal environment for seed germination. The relatively better response under T9 than its corresponding half-strength MS treatment (T10) also suggests the potential importance of adequate basal nutrient availability in supporting germination and early seedling development. The better response might be attributed to the presence of essential mineral nutrients in full strength MS medium and complementary physiological functions of the three growth regulators. Thus, successful in vitro germination depends not only on individual effects of PGRs but also on its combined interaction with the nutritional environment of the culture medium.

The differences observed between treatments with NAA and IBA further indicate that the choice of auxin could influence the germination and subsequent developmental responses of Lisianthus seedlings (Uddin et al. 2017). The relatively better response of IBA containing combination in T9 indicates its potential in combination with BAP and GA_3 for induction of germination and early seedling growth under the conditions tested. Overall, the results indicate that optimisation of PGR combinations and basal medium strength is an important aspect for improving the efficiency and uniformity of in vitro seed germination in Lisianthus.

The various plant growth regulator (PGR) treatments also significantly impacted germination energy and germination value (Table. 2). These parameters are indicators of the speed, uniformity and overall performance of seed germination. Highest germination energy (82.02%) and germination value (6.58)

were recorded in T9 followed by T10 (80.31% and 5.67 respectively). The best performance of these treatments suggests that the combination of IBA, BAP and GA_3 provided a favourable environment for fast and relatively synchronised seed germination. Germination energy refers to the percentage of seeds that germinate at the first counting. Germination value is a combination of the rate of germination and the percentage of germination for an overall assessment of the germination performance. Higher values were recorded under T9 and T10 which indicates that the combined hormonal treatments not only improved the extent of germination but also enhanced faster and uniform germination. This response may be due to GA3-induced activation of hydrolytic enzymes, especially α-amylase, which helps in hydrolysis of stored reserves to soluble sugars that are needed for embryo growth and radicle emergence (Yang et al., 2026). The increased available energy may explain the faster germination and more synchronous seedling emergence. Similar observations on the beneficial effect of growth regulators on in vitro germination and subsequent development of seedling were also reported by Dawande and Gurav (2015). Moreover, combined use of IBA and BAP might have resulted to coordinated cell division, differentiation and development of early roots and shoots leading to better seedling establishment. Similar responses were observed by Bhowmik and Rahman (2017) who demonstrated the importance of appropriate basal media and PGR combinations for better in vitro seed germination and seedling development. Similarly, Kang et al. (2020) reported that gibberellin and cytokinin combination application enhanced in vitro propagation responses in Orchidaceae.

The time of start and finish of germination also showed the effect of PGR combinations on germination kinetics (Table 2). The minimum days for initiation of germination was recorded in T9 (6.08 days) followed by T10 (6.11 days) but control recorded maximum duration of 12.84 days. GA_3-treated plants initiated germination relatively earlier because of stimulation of germination-related metabolic activities, better mobilisation of stored food reserves and enhanced embryo growth potential. Gibberellins are known to encourage the synthesis and activity of hydrolytic enzymes and therefore aid the mobilisation of stored carbohydrates needed for embryo development and germination. These physiological responses can lead to a reduced germination time and a greater seedling emergence. Roni et al. (2017) reported similar findings on the effect of GA_3 on germination behaviour and dormancy-related responses. The same trend was observed for completion of germination which was fastest in T9 (16.08 days) followed by T10 (16.11 days). The relatively shorter period of germination in these treatments indicates that the combined application of IBA, BAP and GA_3 could have facilitated more synchronised germination and early seedling development. The coordinated progression of germination and seedling growth may have been facilitated by gibberellin-mediated mobilisation of stored reserves, the involvement of cytokinin in cell division, and the involvement of auxin in early root development. In particular, BAP may have promoted active cell proliferation and then shoot formation, while IBA may have been involved in the initiation and establishment of roots. The complementary physiological functions of these two treatments may be the reason for the better performance of T9 as compared to other treatments. Bhatia and Sindhu (2019) also reported similar findings in their studies on Lisianthus, emphasising the significance of cytokinin induced responses in promoting vegetative growth. In general, higher germination energy and germination value and shorter time for germination initiation and

completion indicate the potential of the combined treatment of IBA, BAP and GA3 for increasing the germination efficiency and synchrony in Lisianthus.

Mean germination time (MGT) is an important measure of germination speed and synchronisation, with lower values of MGT usually indicating faster germination. In the present investigation, the minimum MGT was recorded in T9 (6.80 days) followed by T10 (6.96) indicating comparatively rapid and uniform germination under this treatment. The reduction in the time of germination might be due to the stimulating effect of gibberellic acid (GA_3) on germination related metabolic processes, especially the activation of hydrolytic enzymes involved in the mobilisation of stored food reserves. The conversion of stored starch to soluble sugars supplies readily available metabolic energy for the growth of the embryo and the emergence of the radicle, which in turn results in faster germination. In addition, the combined application of IBA and BAP may have resulted in a synchronous early development of roots and shoots, which favoured later establishment of the seedlings. These results are in agreement with Mateen (2019) in Gladiolus and Kaviani et al. (2014) in Lisianthus who reported reduced mean germination time with GA_3 application.

Table 2
Effect of Plant growth regulators on in vitro seed germination and its attributing characters (DTIG- Days taken for initiation of germination, DTCG- Days taken for completion of germination, MGT- Mean germination time).

Treatment	Germination percentage (%)	Germination energy (%)	Germination value	DTIG (Days)	DTCG (Days)	MGT (days)
T_1	87.17	65.38	3.42	6.97	17.96	8.08
T_2	85.40	62.34	2.99	6.93	17.93	8.07
T_3	91.75	71.56	4.48	6.87	16.86	7.13
T_4	90.42	68.72	4.01	6.83	16.83	7.10
T_5	93.93	75.14	5.00	6.52	16.49	7.13
T_6	90.63	69.79	4.01	6.22	16.44	7.12
T_7	94.33	79.18	5.64	6.18	16.18	7.15
T_8	93.82	78.81	4.72	6.15	16.15	7.14
T_9	95.37	82.02	6.58	6.08	16.08	6.80
T_{10}	94.48	80.31	5.67	6.11	16.11	6.96
S. Em ±	**1.16**	**1.06**	**0.07**	**0.10**	**0.23**	**0.13**
C.D. @ 1%	**4.67**	**4.25**	**0.27**	**0.40**	**0.92**	**0.54**

3.2 Effect of plant growth regulators on seedling vigour, survival and growth characteristics

The survival and vigour of seedlings are important indicators of the performance of culture media and growth regulator combinations to support sustained seedling development. The highest seedling survival rate (67.27%) and vigour index (1213) were recorded in T9 followed by 66% and 1126 respectively in T10, demonstrating the superior performance of this treatment compared with the other combinations (Fig. 3 and Table 3). The enhanced survival under combined application of GA 3, BAP and IBA might be ascribed to improved coordination of root and shoot development, efficient nutrient acquisition and sustained seedling growth. A well-developed root system and a balanced development of the shoot could contribute to a better utilisation of the nutrients available in the culture medium and to the establishment of healthy plantlets. Similar increase in seedling survival following the application of plant growth regulators has been reported by Baskaran and Misra (2007) in Gladiolus and Sekhukhune and Maila (2025) in Actinidia chinensis. The higher vigour index in T9 also reflects the combined effect of improved germination percentage and increased seedling growth. This response may be due to GA3-mediated cell elongation, auxin-related root development, and cytokinin-mediated cell division and shoot proliferation. Similar improvements in seedling vigour under appropriate growth regulator treatments have been reported by Bhandari et al. (2022) in China aster and Zahedi et al. (2012) in *Dianthus barbatus*.

True leaves are another sign that the seedlings have successfully established themselves and are moving out of the germination phase and into active vegetative growth. The present investigation revealed that the application of suitable PGR combinations influenced the duration of successive true leaf emergence. Superior treatment recorded earliest emergence of first, second and third pairs of true leaves at 6.27, 20.30 and 34.10 days respectively (Table 3). The early development of leaf pairs in the succession leads to a better progression of vegetative growth after germination. This response may be attributed to the synergistic effect of GA3 and BAP on cell elongation, cell division and shoot growth which helps in induction and expansion of new leaves. Thus, better leaf development would help set the photosynthetic capacity and ongoing growth of the seedling. Similar observations have been reported by Kaya et al. (2006) in sunflower and Zare et al. (2011) in asafoetida, where early leaf initiation was recorded due to GA_3 mediated seed priming. Similar trends of better early vegetative development were also reported by Pangtu et al. (2018) in China aster.

The combined PGR treatment also positively influenced seedling morphological characteristics, with significant changes in shoot and root development. The maximum length of plumule (6.10 mm) and radicle (6.62 mm) was recorded in T9 followed by T10 (5.35mm and 6.55mm) (Table 3). The enhanced shoot elongation may be attributed to the function of GA3 in stimulating cell elongation and cell wall loosening, and the presence of BAP may have induced cell division and shoot formation. Simultaneously, IBA could have contributed to root initiation and elongation leading to better root development and possibly higher capacity for nutrient acquisition. Thus, the complementary effect of these growth regulators may have resulted in balanced growth of root and shoot in the developed plantlets. Farooq et

al. (2021) and Hesami and Daneshvar (2018) reported similar improvements in shoot and root development under the optimised PGR combinations in Petunia hybrida and Ficus religiosa respectively.

Biomass accumulation is an integrated measure of seedling growth and the efficiency of resource use throughout development. The highest fresh weight (1.24 g) and dry weight (0.19 g) of in vitro plantlets were recorded in T9, followed by 1.16 and 0.17 respectively in T10 (Table 3). The higher biomass accumulation in these treatments indicates that the combined application of IBA, BAP and GA_3 supported improved seedling growth under both full- and half-strength MS media. The increase in fresh weight may be indicative of enhanced tissue development and biomass accumulation, whereas higher dry weight is indicative of greater accumulation of structural and stored dry matter. These responses might be due to the increased mobilisation of carbohydrates, better nutrient use and continuous cell growth due to the effects of GA3 and BAP, and the development of roots caused by IBA. The possible role of sufficient nutrient supply in favour of seedling development in combination with appropriate PGR concentrations is also indicated by the relatively higher biomass in T9 than T10. Khan et al. (2017) and Pyati (2019) reported similar improvements in seedling biomass and growth characteristics in Gerbera and orchid, respectively.

The findings collectively show that the combined application of IBA (0.5 mg L-1), BAP (3.0 mg L-1) and GA3 (0.5 mg L-1) in full strength MS medium (T9) resulted in the most favourable response for several germination and seedling growth parameters. The enhanced germination performance, survival, vigour, leaf development, root and shoot growth and biomass accumulation highlighted the importance of optimising the combinations of hormones along with basal nutrient strength for successful in vitro establishment of Lisianthus seedlings.

Table 3
Effect of Plant growth regulators on *in vitro* seedling vigour and seedling morphology

Treatment	Vigour index (length)	First true leaf pair (days)	Second true leaf pair (days)	Third true leaf pair (days)	Plumule length (mm)	Radicle length (mm)	Seedling fresh weight (g)	Seedling dry weight (g)
T_1	599	7.00	21.00	35.92	2.97	3.90	0.59	0.06
T_2	541	7.03	20.03	36.05	2.80	3.54	0.55	0.07
T_3	874	6.83	21.20	35.71	4.37	5.16	1.12	0.11
T_4	800	7.14	20.92	36.03	4.38	4.47	1.09	0.12
T_5	949	6.79	21.02	35.43	4.45	5.65	1.13	0.13
T_6	883	7.17	21.17	35.10	4.57	5.17	1.07	0.12
T_7	1104	6.78	20.93	34.93	5.33	6.37	1.15	0.16
T_8	1004	6.93	21.10	35.30	5.07	5.63	1.13	0.14
T_9	1213	6.27	20.30	34.10	6.10	6.62	1.24	0.19
T_{10}	1126	6.55	20.90	34.95	5.37	6.55	1.16	0.17
S. Em ±	**10**	**0.11**	**0.26**	**0.20**	**0.05**	**0.07**	**0.02**	**0.01**
C.D. @ 1%	**44**	**0.46**	**0.54**	**0.81**	**0.22**	**0.29**	**0.12**	**0.06**

Correlation analysis revealed significant positive association among germination percentage, germination energy, germination value, plumule length, radicle length and seedling vigour index. Also, several germination and growth parameters were positively correlated with both fresh and dry weights of the seedlings (Fig. 4). Conversely, germination timing traits, such as days to initiation of germination (DTIG), mean germination time (MGT) and days to completion of germination (DTCG), were mostly negatively correlated with germination and seedling growth traits. Generally, the clustering pattern separated germination and growth attributes from time-related developmental traits, suggesting a link between faster germination and better seedling performance (Hossain et al., 2010).

The identified treatment provides a promising base for the development of a reproducible and efficient propagation protocol for commercial production of Lisianthus. Future studies should be performed to validate this protocol on a variety of genotypes and seed lots.

Conclusion

The present investigation demonstrated the effectiveness of plant growth regulator combinations for enhancing in vitro seed germination and early seedling growth of Lisianthus (*Eustoma grandiflorum* Shinn.). Full-strength Murashige and Skoog (MS) medium supplemented with IBA (0.5 mg L^{-1}), BAP (3.0 mg L^{-1}) and GA_3 (0.5 mg L^{-1}) (T9) was the best among the treatments evaluated, showing the highest germination percentage (95.37%) and vigour index (1213) with improved germination performance and seedling growth. The superior response emphasises the importance of an adequate auxin–cytokinin–gibberellin combination in inducing germination and synchronised seedling development in vitro. The optimised treatment offers a potential basis for developing reproducible seed germination and seedling production protocols with implications for the improvement of the availability of uniform and vigorous planting material for commercial Lisianthus cultivation. Future investigations should validate the improved process in multiple Lisianthus genotypes and seed lots, reproduce it under varied growth conditions, and acclimatise and establish it ex-vitro. Integrating physiological, biochemical, and molecular techniques could potentially develop more efficient, scalable, and economically viable Lisianthus propagation systems.

Declarations

Declaration of Competing Interest

The authors declare no competing interests.

Ethical Approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Declaration on Plant Material

All plant materials used in this study were obtained, maintained, and utilized in accordance with relevant institutional, national, and international guidelines and legislation.

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Author Contribution

G.S.Y. contributed to conceptualization, methodology, data collection and original manuscript preparation. B.V.C. provided supervision. M.R.N. performed data analysis. C.K.P. contributed to manuscript review and editing. B.N.H. carried out statistical analysis. A.S.B. contributed to culture validation. N.V.V. was responsible for media preparation and culture maintenance. P.V.P. contributed to manuscript review and editing.

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Data Availability

The datasets used and/or analysed during the current investigation are included in this article. Additional data supporting the findings of this study are accessible from the corresponding author upon reasonable request.

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Figures

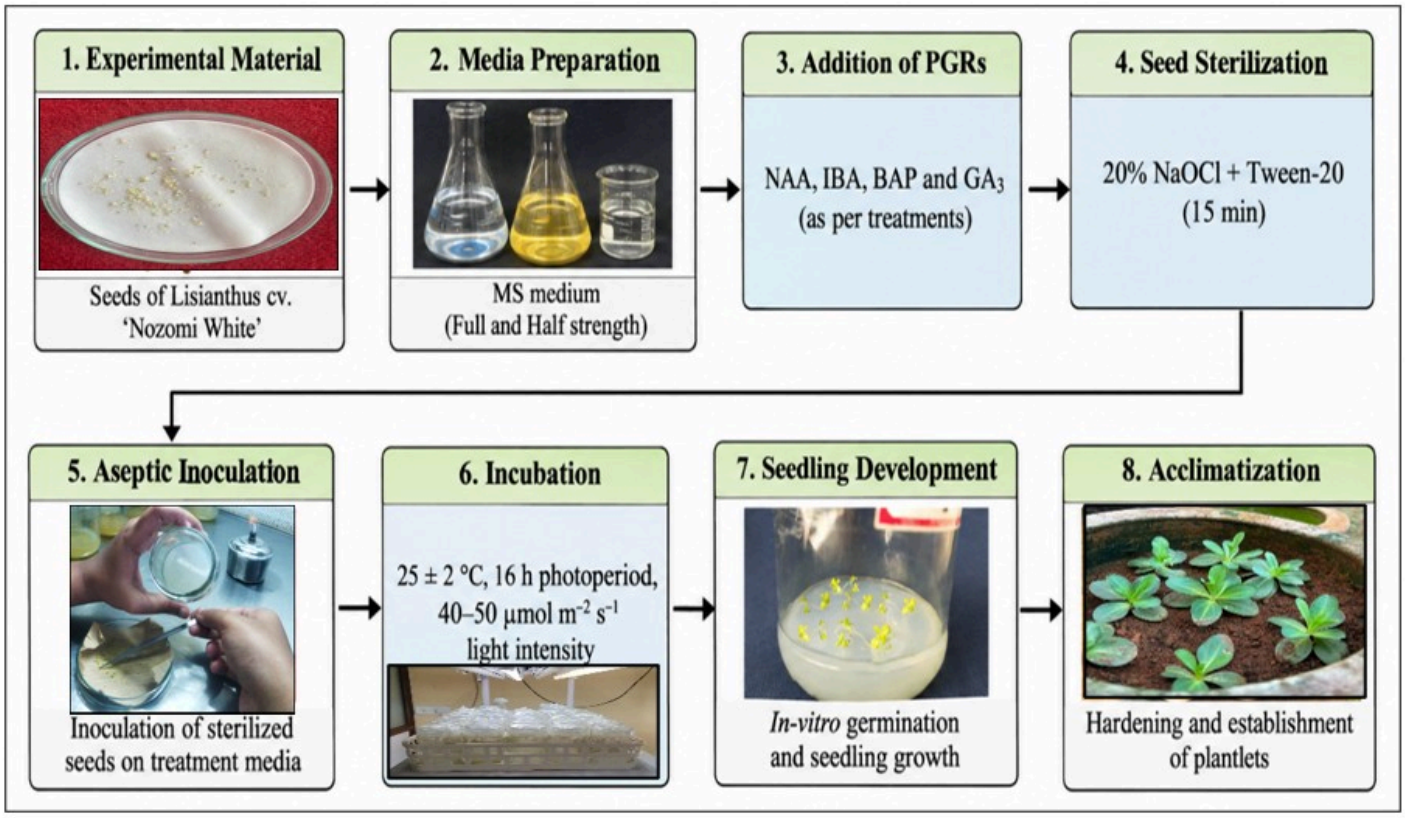

Figure 1

Flow chart showing the methodology adopted for in vitro seed germination and seedling development of lisianthus.

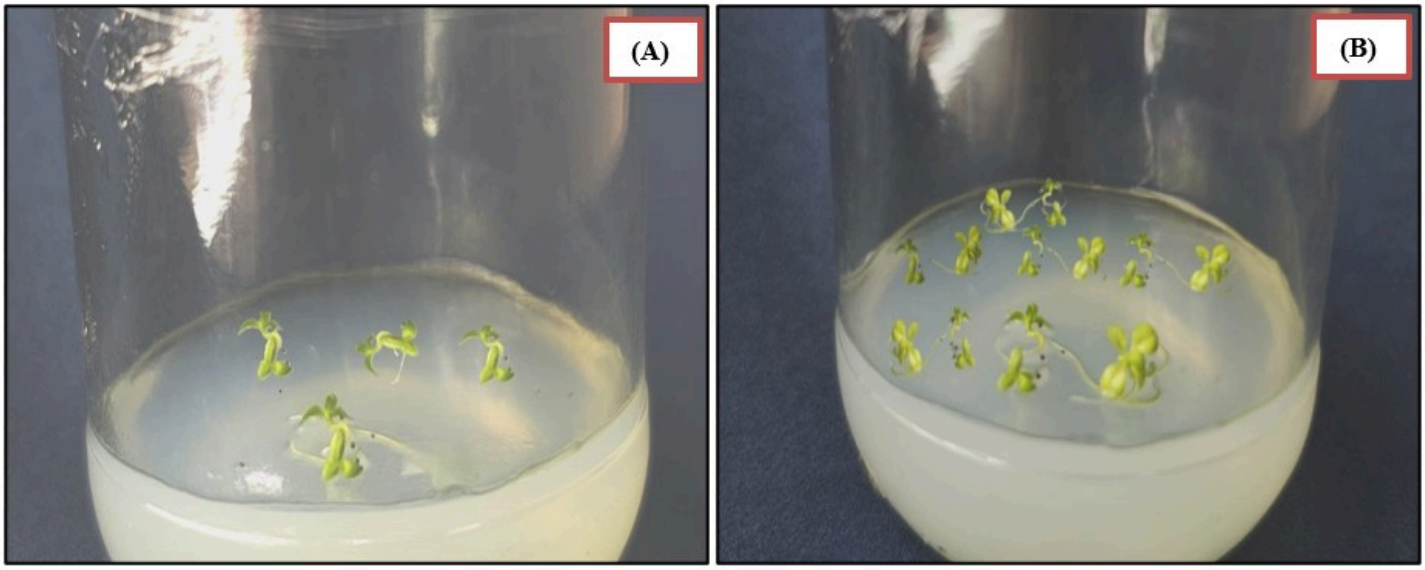

Figure 2

In vitro seedling establishment of Lisianthus (*Eustoma grandiflorum* Shinn.) under different culture media: (A) Full-strength MS medium (T1) and (B) Full-strength MS medium supplemented with IBA (0.5 mg L^{-1}) + BAP (3.0 mg L^{-1}) + GA_3 (0.5 mg L^{-1}) (T9).

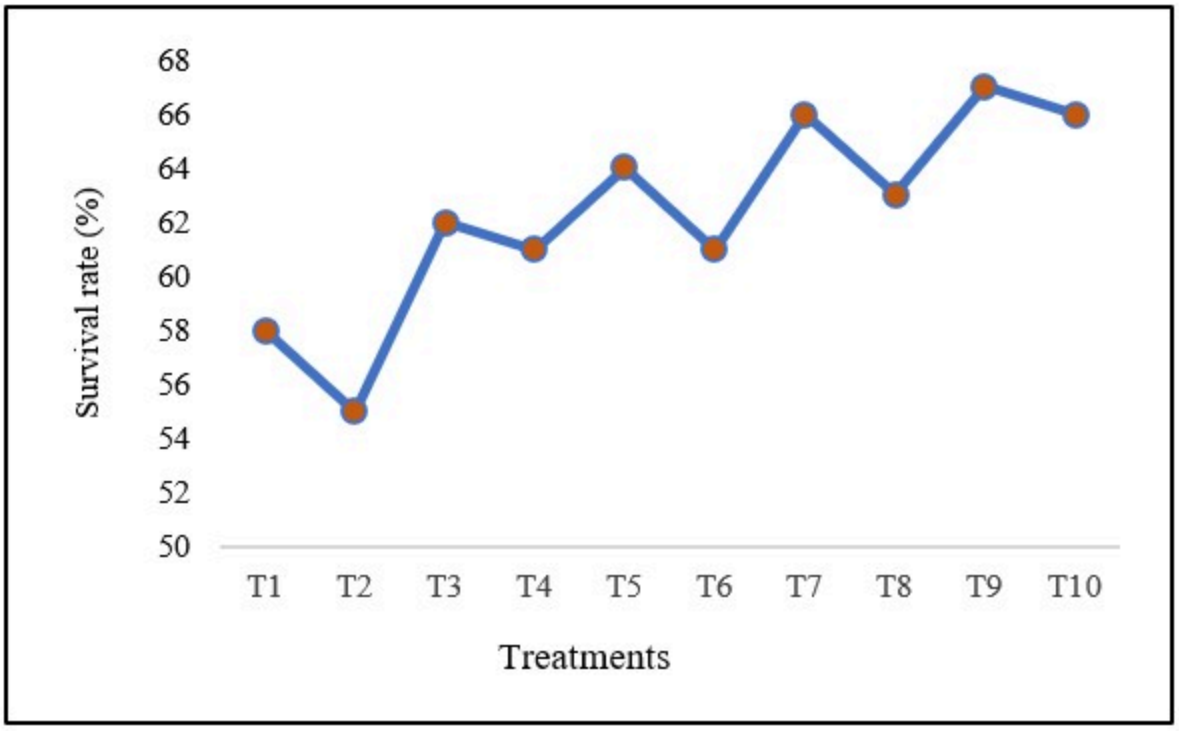

Figure 3

Effect of different plant growth regulator combinations on the survival rate (%) of in vitro-grown lisianthus (*Eustoma grandiflorum* Shinn.) seedlings under different culture treatments (T1–T10).

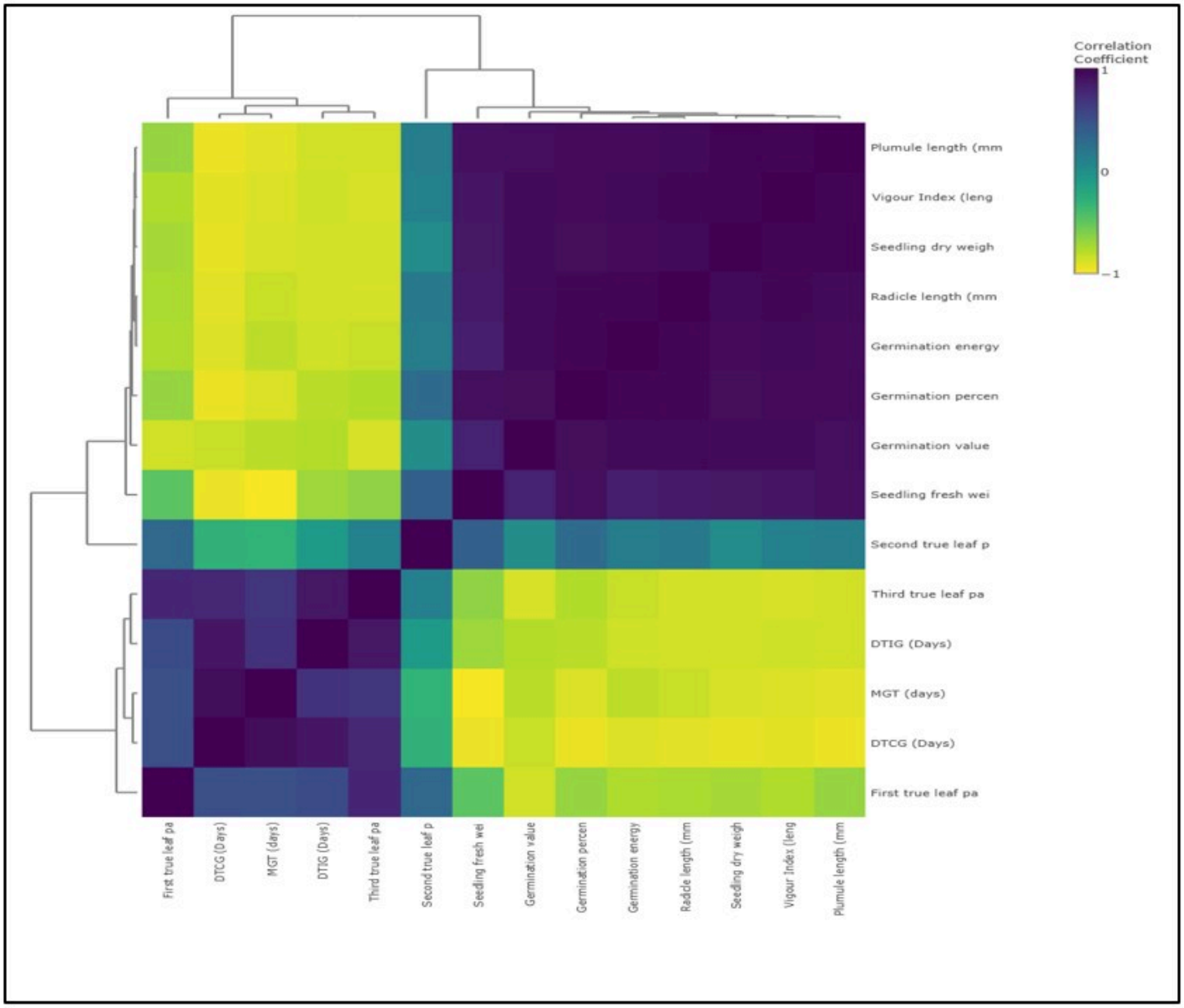

Figure 4

Hierarchical clustering heatmap showing the correlation matrix among germination, seedling growth and developmental parameters of lisianthus (*Eustoma grandiflorum* Shinn.) under different plant growth regulator treatments.