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Effect of seed priming on seed germination in Lisianthus (*Eustoma grandiflorum* Shinn.)

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Abstract
Seed priming with chemicals and plant growth hormones enhances the germination efficiency of Lisianthus. The present study evaluated the influence of different priming agents *viz*., thiourea (0.2 ppm, 0.4 ppm and 0.6 ppm), potassium nitrate (0.1%, 0.2% and 0.3%), and plant growth hormones such as gibberellic acid (GA_3 at 500 ppm, 600 ppm and 700 ppm) and Ethrel (15 ppm, 25 ppm and 35 ppm) on seed germination and associated traits in Lisianthus. Among the treatments, priming with GA_3 at 700 ppm showed superior performance across most germination and seedling parameters, including highest germination percentage (97%), germination energy (92.15%), and germination value (6.46). It also resulted in the shortest time for initiation and completion of germination (6.0 and 15.3 days, respectively), reduced germination time (6.55 days), highest survival rate (80.70%), and maximum vigour index (1399). Seedlings from this treatment exhibited the longest plumule (6.20 mm) and radicle length (6.80 mm)**,** required the least time for emergence of first (6.19 days), second (19.83 days) and third (34.13 days) true leaf pairs, and recorded the highest fresh (2.22 mg) and dry weight (0.21 mg). From this study, it can be concluded that GA_3 at 700 ppm is an effective priming agent for improving germination and seedling growth of Lisianthus**.**

Keywords: Lisianthus, Seed priming, Dormancy, Germination, Thiourea, Gibberellic acid, Potassium nitrate, Ethrel

Introduction
Lisianthus (*Eustoma grandiflorum*), a member of the family Gentianaceae (Reid, 2009) [18], is native to North America and is predominantly found in the southern United States, particularly in moist meadows spanning Nebraska, Colorado and Texas (Ohkawa *et al*., 1991) [16]. Over the years, Lisianthus has gained global prominence as an ornamental plant and a highly valued cut flower. The plant typically grows to a height of 50-70 cm and produces 20-40 flowers, blooming mainly during the summer season (Cantor *et al*., 2013) [6]. It is an herbaceous species with bluish-green foliage and may bear either a single upright stem or branched stems. The flowers, which can reach up to two inches in diameter, are available in a wide range of colours. Beyond its ornamental appeal, Lisianthus has notable medicinal properties, with its flowers and extracts traditionally used for digestive ailments, anti-inflammatory and antifungal purposes, and for reducing fever and treating malaria.
Lisianthus is also employed in cosmetics, fragrances, weight-management products, air-purifying formulations and various homeopathic preparations (Sreelatha *et al*., 2006) [23]. The seeds exhibit cold-induced dormancy (Ecker *et al*., 1994) [9] and possess tolerance to soil acidity, pathogen infection and high-temperature stress. However, seed size is extremely small approximately 19,000 seeds per gram or 545,000 seeds per ounce making sowing and handling particularly difficult (Rezaee *et al*., 2012) [19]. Additionally, the species is characterized by slow and often poor germination, with some batches showing little to no germination even under otherwise favourable conditions (Ecker *et al*., 1994) [9].
Seed germination in Lisianthus is strongly influenced by environmental factors such as temperature, light and moisture. The seeds are photoblastic, requiring light for successful germination, and both suboptimal and supra-optimal temperatures can suppress the process. The physiological dormancy displayed by Lisianthus seeds is mainly associated with incomplete embryo development and the presence of inhibitory substances that restrict

metabolic activation during the initial imbibition phase. Consequently, achieving uniform and rapid germination poses a major challenge for commercial growers, particularly under greenhouse and field conditions.

The species' extended germination period and uneven seedling emergence further complicate nursery management and transplanting schedules. To address these limitations, several pre-sowing treatments including hormone application, temperature conditioning and chemical priming have been investigated. These treatments aim to break dormancy, enhance enzymatic activity and improve the physiological readiness of seeds for rapid and uniform germination. Among them, seed priming is recognized as a reliable, cost-effective technique that promotes controlled hydration and activates metabolic processes prior to radicle emergence.

Seed priming has proven effective in improving germination traits such as seed vigour, uniform seedling emergence and early seedling growth across various species, particularly under stress conditions (Bajehbaj, 2010) [2]. Primed seeds generally demonstrate higher germination percentages, improved reserve mobilization and reduced time to sprouting compared to non-primed seeds (Tabatabaei, 2014) [24]. Considering the germination challenges associated with Lisianthus, the present study was undertaken to evaluate the effect of growth hormones and chemical treatments at different concentrations on seed germination and its associated attributes.

Material and Methods

The present study was conducted during 2024-25 in the Department of Floriculture and Landscaping, College of Horticulture, Mudigere, under Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga. Seeds of Lisianthus var. Nozomi White were generously provided by Sakata Seeds, Japan. Then seeds were soaked in aqueous solutions of various priming treatments, including chemicals *viz*., thiourea (0.2 ppm, 0.4 ppm and 0.6 ppm), potassium nitrate (0.1%, 0.2% and 0.3%) and plant growth regulators *viz*., gibberellic acid (GA_3 at 500 ppm, 600 ppm and 700 ppm) and Ethrel (15 ppm, 25 ppm and 35 ppm). Seeds and solution were maintained in a 1:2 (w/v) ratio at a constant temperature of 25 ± 1°C for 24 hours.

Germination Assay: After priming, seeds were removed from the solutions, surface-dried and transferred to 9 cm sterile Petri dishes lined with Whatman No. 1 filter paper moistened with 5 ml of distilled water. Incubation was conducted in a germinator for 30 days under a 12-hour photoperiod provided by fluorescent light (40 µmol m^{-2} s^{-1}) at 25 ± 1°C. Germinated seeds were counted every 48 hours for 20 days (Millaku *et al*., 2012) [14]. Seeds were considered germinated upon appearance of a radicle > 2 mm (Sharma and Sharma, 2010) [22]. Primed seeds were later sown in trays filled with a sterile cocopeat: vermicompost (3:1) medium and maintained under controlled conditions to ensure uniform seedling growth for further evaluation at true leaf emergence stages.

Germination percentage (%): Germination test was conducted in glass petri plates on top of a germination paper. Total number of seeds germinated were counted and expressed into percentage.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Germination energy (%): The percentage of seeds germinated within a fixed early period was recorded.

$$\text{Germination energy} = \frac{\text{Number of seeds germinated at first count}}{\text{Total number of seed germinated by test end}} \times 100$$

Germination value: Germination value was **c**alculated as per Djanvanshir and Pourbeik (1976) [8].

$$GV = \frac{\sum DGS}{N \times (GP \times 10)}$$

Where,

$$\text{DGS (Daily germination speed)} = \frac{\text{Cumulative germination per cent}}{\text{Number of days since sowing}}$$

GP = Germination per cent at the end of test and 10 as a constant.

N = total number of daily counts, starting from the date of first germination.

Days taken for initiation (DTIG) and completion of germination (DTCG): Number of days taken for initiation and completion of germination was recorded for each treatment. The seeds were considered as germinated only when the sprouted plumule was found along with the cotyledons.

Mean Germination Time (days): It is an expression of total germination at the end of test period with time taken to complete germination (Bonner, 1983).

$$MGT = \frac{\sum (\text{daily germinated on each day} \times \text{number of days from start of the test})}{\text{Total number of seeds that germinated at the end}}$$

Un-germinated seeds at the end of the test were given value of n+1

Where n = Number of days in the test and these values will be included in the calculation of means.

Survival rate (%): The percentage of germinated seedlings that remain alive, healthy, and actively growing after a specific period of observation under experimental conditions. It is an important indicator of seedling vigour and the effectiveness of the growth medium or treatment.

$$\text{Survival rate} = \frac{\text{Number of surviving seeds}}{\text{Number of Germinated seeds}} \times 100$$

Vigour index I: For this total length of seedlings were multiplied by total germination per cent (Abdul-Baki and Anderson, 1973) [1].

Vigor index I = Total seedling length x germination per cent

Plumule and radicle length (mm): Length of the plumule and radicle was measured up to two decimal places and presented in mm at the end of the germination test.

First, second and third true leaf pair (days): The emergence of the first, second and third pair of true leaves (not cotyledons) from the seedling were recorded.

Seedling fresh and dry weight (mg): The fresh weight and dry weight of the seedlings from the germination test were weighed using electronic balance and expressed in milligram.

Statistical Analysis

Data obtained from the experiment were analyzed using ANOVA, and treatment differences were tested for significance at the 1% level using critical difference (CD) values.

Results and Discussion

Seed companies must maintain high seed quality and longevity to ensure superior product performance. Rapid and uniform field emergence is key prerequisites for maximizing crop yield, quality and profitability. Slow or uneven seedling emergence often results in smaller plants, reduced field stand and increased susceptibility to soil-borne diseases. To mitigate these challenges, seed priming using chemicals and growth hormones has become a widely adopted practice to enhance seed performance. Over the past two decades, seed enhancement through priming has significantly improved growers' ability to achieve reliable germination in both field and greenhouse environments, and many ornamental species have been successfully primed.

Erken and Kaleci (2010) [10] as well as Millaku *et al*. (2012) [14] reported improved germination in yellow gentian seeds primed with GA_3 at 600 ppm and 1000 ppm, respectively. In the present study, similar results were observed in Lisianthus, where seeds primed with gibberellic acid recorded significantly higher germination (Fig 1) and its attributing characters in Lisianthus (Table 1) compared to other treatments and the control (Plate 1 and Plate 2). The highest germination percentage (97%) occurred with GA_3 at 700 ppm. This improvement may be attributed to enhanced hydrolytic activity and amylase induction, which improve carbohydrate mobilization and energy availability for embryo growth. These findings are consistent with earlier reports by Rouhi *et al*. (2010) [20] and Zare *et al*. (2011) [28]. In contrast, Ethrel treatments resulted in lower germination, possibly due to toxic effects at the concentrations used.

Seed priming with GA_3 at 700 ppm also resulted in the highest germination energy (92.15%) and germination value (6.46), followed by GA_3 at 600 ppm (87.26% and 5.56, respectively). Seeds treated with GA_3 germinate more rapidly and uniformly, as higher concentrations promote enhanced radicle protrusion and early seedling vigour. These results agree with Mohammadi *et al*. (2020) [15], who reported that GA_3 accelerates germination processes, and with Shah *et al*. (2018) [21], who noted that improved speed and final germination percentages contribute to higher germination values.

Seeds primed with GA_3 at 700 ppm and 600 ppm took the shortest time for initiation (6.00 and 6.33 days, respectively) and completion of germination (15.33 and 18.02 days, respectively). Faster initiation may be due to seed coat softening induced by GA_3, reducing mechanical resistance to radicle emergence. Thiourea also enhanced membrane permeability and enzyme activation, supporting faster germination. These findings align with Rouhi *et al*. (2010) [20], who reported improved germination in *Tulipa kaufmanniana* following dormancy-breaking treatments. Further, GA_3 stimulates enzymatic activities such as α-amylase, enhancing starch breakdown, while thiourea facilitates reserve mobilization (Sharma and Sharma, 2010) [22].

The lowest mean germination time (6.55 days) was recorded in seeds primed with GA_3 at 700 ppm. Enhanced metabolism, increased protein synthesis (globulins and cruciferin) and improved membrane integrity due to GA_3 priming likely contributed to this improvement (Varier *et al*., 2010) [26]. Similar reductions in germination time were reported by Erken and Kaleci (2010) [10] in yellow gentian and by Millaku *et al*. (2012) [14], who found enhanced MGT in *Gentiana lutea* with GA_3 and potassium nitrate priming. Ghaleh-Shahi *et al*. (2017) [11] also reported reduced germination time in cock's comb (*Celosia cristata*) with potassium nitrate priming.

Seedling survival was significantly affected by priming treatments. The highest survival rate (80.70%) occurred with GA_3 at 700 ppm, followed by GA_3 at 600 ppm (72.75%). GA_3 likely enhanced nutrient mobilization and reserve use, resulting in vigorous, stress-tolerant seedlings. These findings correspond with previous work by Varier *et al*. (2010) [26] and Millaku *et al*. (2012) [14] in yellow gentian.

Gibberellic acid also proved highly effective in enhancing seedling vigour. The highest vigour index (1399) was recorded with GA_3 at 700 ppm, due to stimulated cell division, elongation and increased radicle and plumule growth (Fig. 2). Similar results were reported by Bhandari *et al*. (2022) in China aster, Rouhi *et al*. (2010) [20], Baskaran and Misra (2007) [3] in gladiolus and Zahedi *et al*. (2012) [27] in sweet William.

The data on the effect of priming treatments on seedling characters is presented in Table 2. GA_3 at 700 ppm resulted in the earliest emergence of the first true leaf pair (6.19 days), followed closely by GA_3 at 600 ppm (6.72 days), whereas Ethrel at 25 ppm resulted in substantial delay (25.17 days). These findings agree with Kaya *et al*. (2006) and Zare *et al*. (2011) [28] in *Ferula asafoetida*, who reported faster vegetative initiation due to enhanced metabolic activity. GA_3 also promoted faster emergence of the second true leaf pair, with the earliest appearance at 19.83 days (700 ppm) and 21.40 days (600 ppm). Delayed emergence in Ethrel at 25 ppm (38.98 days) corroborates findings by Pangtu *et al*. (2018) [17].

Priming also significantly affected development of the third true leaf pair. Seeds primed with GA_3 at 700 ppm showed the earliest emergence (34.13 days), followed closely by 600 ppm (35.47 days), indicating strong vegetative vigour (Fig 2). Delayed development in Ethrel treatments (50.42 days) suggests inhibitory effects at higher concentrations, consistent with Chouhan (2018) [7].

Seedling fresh and dry weight increased significantly with GA_3 priming. Enhanced water uptake, cell elongation and improved mobilization of stored reserves likely contributed to higher biomass. Similar observations were reported by Thakur and Dhatt (2024) [25] in sunflower, Khan *et al*. (2017) [13] in gerbera and zinnia, reinforcing the role of GA_3 and hormonal regulation in promoting seedling growth and biomass accumulation.

Table 1: Effect of priming treatments on germination and its attributing characters

Treatment	Germination Energy (%)	Germination value	DTIG (Days)	DTCG (Days)	MGT (days)	Survival rate (%)
Control	36.40	1.13	7.00	19.67	10.60	35.87
Thiourea @ 0.2 ppm	44.59	1.31	6.37	19.00	11.14	38.72
Thiourea @ 0.4 ppm	46.53	1.56	6.39	19.00	8.64	39.89
Thiourea @ 0.6 ppm	21.34	0.46	6.34	18.33	9.22	21.97
GA_3 @ 500 ppm	85.03	5.28	6.67	18.67	9.76	64.42
GA_3 @ 600 ppm	87.26	5.56	6.33	18.02	8.55	72.75
GA_3 @ 700ppm	92.15	6.46	6.00	15.33	6.55	80.70
KNO_3 @ 0.1%	38.24	2.06	7.33	17.00	9.26	43.27
KNO_3 @ 0.2%	44.42	2.32	7.67	18.33	8.71	39.85
KNO_3 @ 0.3%	55.37	2.27	7.33	19.67	8.74	46.69
Ethrel @ 15 ppm	7.80	0.04	19.33	22.00	20.14	6.60
Ethrel @ 25 ppm	11.90	0.08	20.33	20.33	20.53	9.11
Ethrel @ 35 ppm	11.22	0.03	20.67	21.67	19.63	17.21
S. Em ±	0.66	0.05	0.18	0.27	0.19	0.65
CD @ 1%	2.58	0.18	0.69	1.06	0.73	2.67

Note: DTIG-Days taken for initiation of germination., DTCG- Days taken for completion of germination., MGT-Mean germination time

Table 2: Effect of priming treatments on seedling characters

Treatments	Length of (mm)		Days taken for emergence of			Seedling weight (mg)	
	Plumule	Radical	First true leaf pair	Second true leaf pair	Third true leaf pair	Fresh	Dry
Control	2.50	3.29	7.78	21.46	35.71	1.20	0.12
Thiourea @ 0.2 ppm	3.20	4.12	6.78	21.51	36.05	1.66	0.15
Thiourea @ 0.4 ppm	3.97	5.20	6.74	21.43	35.70	1.86	0.15
Thiourea @ 0.6 ppm	4.20	5.20	6.95	21.41	36.03	1.76	0.16
GA_3 @ 500 ppm	4.50	5.60	6.99	21.47	35.48	1.99	0.18
GA_3 @ 600 ppm	5.10	6.10	6.72	21.40	35.47	2.03	0.20
GA_3 @ 700ppm	6.20	6.80	6.19	19.83	34.13	2.22	0.21
KNO_3 @ 0.1%	3.80	4.80	6.81	21.30	35.30	1.48	0.13
KNO_3 @ 0.2%	4.40	5.40	6.78	21.50	35.57	1.78	0.14
KNO_3 @ 0.3%	4.90	5.82	6.80	21.57	35.49	1.80	0.15
Ethrel @ 15 ppm	3.40	4.41	19.97	33.66	46.42	1.62	0.13
Ethrel @ 25 ppm	3.90	4.90	25.17	38.98	50.42	1.61	0.15
Ethrel @ 35 ppm	3.10	4.00	20.90	33.33	45.42	1.60	0.14
S. Em ±	0.04	0.08	0.19	0.40	0.59	0.02	0.01
CD @ 1%	0.18	0.33	0.75	1.57	2.31	0.09	0.03

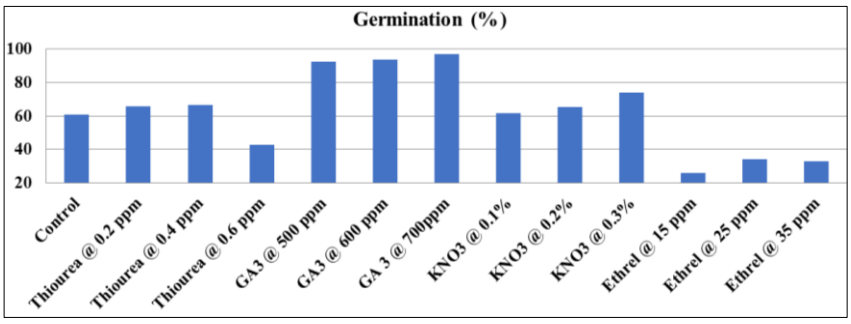

Fig 1: Effect of seed priming on seed germination in Lisianthus

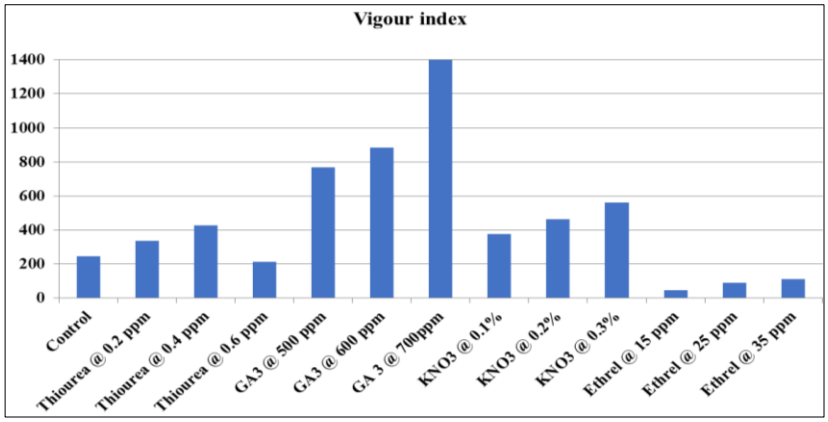

Fig 2: Effect of seed priming on seed vigour index in Lisianthus

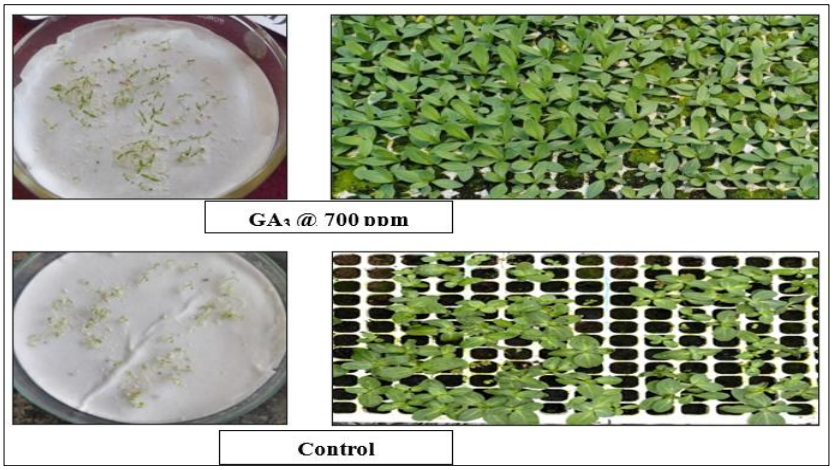

Plate 1: Emergence of seedlings in GA_3 treatment and control

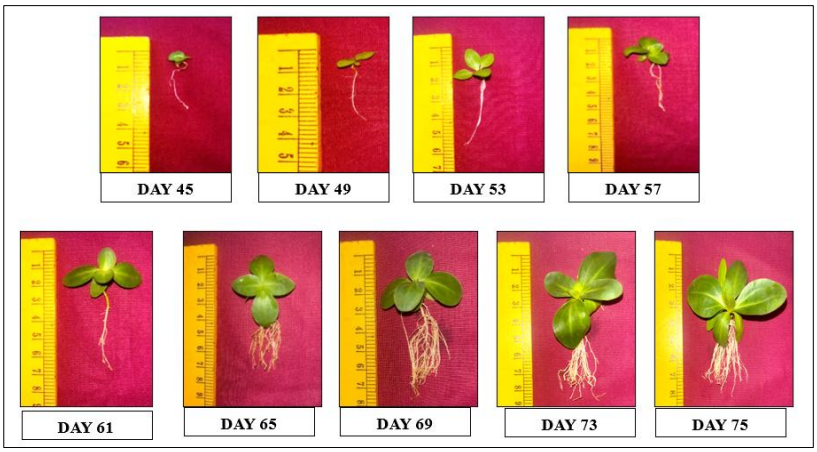

Plate 2: Comparison of seedling establishment in control & GA_3 treatment

Conclusion

The study demonstrated that seed germination and early seedling growth in Lisianthus were markedly enhanced by gibberellic acid (GA_3) priming. Among all treatments, seeds primed with GA_3 particularly at higher concentrations—consistently exhibited superior performance, including the highest germination percentage, vigour index and mean germination rate, as well as greater plumule and radicle elongation. These results underscore the pivotal role of GA_3 in promoting rapid, uniform and vigorous seedling establishment. Overall, GA_3 priming proves to be an effective, economical and practical pre-sowing treatment for improving Lisianthus seed performance, offering significant benefits for both nursery management and commercial cultivation.

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