

Short communication

Effect of *in vivo* and *in vitro* seed germination and performance of *Lisianthus* seedlings

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ABSTRACT

An experiment was conducted under controlled conditions to standardize the temperature, substrates and seed quality on germination in *Lisianthus* cv. Blue Magic using both *in vivo* and *in vitro* propagation. *In vivo* seed germination was conducted at three temperature levels ($20 \pm 1^\circ\text{C}$; $25 \pm 1^\circ\text{C}$ and ambient room temp.) and eight substrate media {cocopeat, sand, perlite, blotting sheet, germination paper, cocopeat + perlite (1:1); (2:1) and (1:2)}. For *in vitro* germination, seeds were inoculated on the MS medium supplemented with 0.5 mg/l IBA and BAP and cultured at controlled conditions ($25 \pm 1^\circ\text{C}$ with 16/8 h light/dark photo period). In one-year-old seeds, maximum seed germination (38.66%) were observed in cocopeat: perlite (1:2) followed by cocopeat: perlite (1:1) at room temperature (34.0%). Under *in vitro* conditions, maximum seed germination (63.89%) was observed on MS medium supplemented with 0.5 mg/l IBA for fresh seeds

Key words: *Lisianthus*, seed germination.

Lisianthus (*Eustoma grandiflorum* Raf.) is an exotic and promising ornamental plant. Its belongs to the family Gentianaceae (Halevy *et al.*, 4). It is a wild flower of North America, native to Central and Southern US and mainly inhabits the moist prairies from Nebraska to Colorado and Texas (Wood *et al.*, 6 and Halevy *et al.*, 4). The common name comes from the Greek words lysis, meaning "dissolution" and anthos meaning "flower". *Lisianthus* is grown as a cut flower, pot and border garden plant. Due to its large flowers, long stems, attractive colour of flower and good vase-life, interest is increasing as commercial cut flower. *Lisianthus* is propagated by seeds. It was introduced in the country as a new flower crop. However, there is not scientific and technical information on *Lisianthus* crop, particularly on its seed germination, growth and flower production. Therefore, the present investigation was carried out to standardize the temperature, substrate and quality of seeds for seed germination by *in vivo* and *in vitro* propagation and performance evaluation of seedlings under polyhouse conditions.

An experiment was conducted in randomized complete block design with three replications at Institute of Himalayan Bioresource Technology (CSIR), Palampur (Himachal Pradesh) during 2008-2010 under controlled conditions to standardize the temperature, substrates and quality of seeds on seed germination of *Lisianthus* cv. Blue Magic by *in vivo* and *in vitro* propagation. The experimental site

was located at an altitude of 1290 m msl and $32^\circ 6' \text{N}$ latitude & $75^\circ 5' \text{E}$ longitude. In studies of *in vivo* seed germination, three levels of temperature ($20 \pm 1^\circ\text{C}$; $25 \pm 1^\circ\text{C}$ and ambient room temperature) and eight substrates media {cocopeat, sand, perlite, blotting sheet, germination paper, cocopeat + perlite (1:1); (2:1) and (1:2)} were used. For *in vitro* seed germination, seeds were inoculated on the MS media supplemented with 0.5 mg/l IBA and BAP, respectively and zero basal media and petri-plates were kept in culture room under control conditions at $25 \pm 1^\circ\text{C}$ with 16/8 h photoperiod (light/dark). In both experiment one year old and fresh seeds were used. Seeds were sterilized with 0.1% HgCl_2 for 1 min. and rinsed with distilled water 3-4 times & soaked in distilled water for 24 h before inoculation on various media. Hundred seeds were inoculated in each petriplate in different media of *in vivo* and *in vitro* propagation. Petriplates were observed regularly and date of root and shoot initiation were recorded. Number of germinated seeds was counted after 2 weeks. In the month of March, seedlings were transplanted under polyhouse at spacing of 15 cm \times 15 cm between rows and plants, and maintained uniform cultural practices. Before planting, mixture of fine sand @ 1 cft/m² and decomposed dry FYM @ 5 kg/m² as well as NPK @ 15:10:15 g/m² were applied as basal dose. The growing medium was fertilized manually with a nutrient solution containing 200 (mg/l/week/plant) through calcium ammonium nitrate (CAN) and potassium nitrate. In the laboratory, soil from the bulk sample from the plot of *Lisianthus*

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Table 1. Studies on *in vivo* seed germination of Lisianthus.

Media	Days to root initiation						Days to shoot initiation						Germination (%)											
	Old seed			Fresh seed			Old seed			Fresh seed			Old seed			Fresh seed								
	20 ± 1°C	25 ± 1°C	RT	20 ± 1°C	25 ± 1°C	RT	20 ± 1°C	25 ± 1°C	RT	20 ± 1°C	25 ± 1°C	RT	20 ± 1°C	25 ± 1°C	RT	20 ± 1°C	25 ± 1°C	RT						
Cocopeat	15.00	9.66	11.00	12.66	6.33	10.00	16.00	10.00	12.00	13.66	7.33	11.00	9.66	18	25.00	39.30	54.30	23.66	(17.86)*	(25.08)*	(29.94)*	(38.49)*	(47.47)*	(29.07)*
Sand	14.66	8.66	12.00	11.33	4.66	10.00	15.66	9.66	13.00	12.33	5.66	11.00	11.33	23.60	22.33	19.00	58.00	25.00	(18.92)	(29.54)	(28.14)	(24.72)	(49.60)	(31.27)
Perlite	13.66	9.00	13.00	12.00	5.33	13.00	14.66	10.00	14.00	13.00	6.33	14.00	25.00	22.00	12.33	48.00	63.00	25.66	(29.87)	(27.94)	(20.50)	(43.78)	(52.55)	(30.39)
Cocopeat : Perlite (1:1)	15.66	9.33	12.00	14.33	7.66	10.00	16.66	10.33	13.00	15.33	8.66	11.00	19.66	28.60	34.00	17.00	45.00	18.33	(25.98)	(32.35)	(35.64)	(24.20)	(42.49)	(25.30)
Cocopeat : Perlite (2:1)	15.33	9.00	-	14.66	7.66	9.00	16.33	10.00	-	15.66	8.66	10.00	15.33	21.30	-	11.60	59.66	19.33	(23.01)	(27.48)	-	(19.94)	(50.57)	(25.97)
Cocopeat : Perlite (1:2)	14.66	8.00	11.00	14.00	7.33	10.00	15.66	9.00	12.00	15.00	8.33	11.00	21.66	22.30	38.66	20.00	71.30	24.00	(27.56)	(27.72)	(38.40)	(26.30)	(57.57)	(29.27)
Blotting Sheet	12.00	6.00	10.00	9.00	6.66	9.00	13.00	7.00	11.00	10.00	7.66	10.00	22.33	26.30	27.00	41.00	79.00	25.00	(28.18)	(30.86)	(31.21)	(39.78)	(61.78)	(29.95)
Germination paper	13.33	8.66	11.00	10.00	6.33	10.00	14.33	9.66	12.00	11.00	7.33	11.00	23.33	24.30	30.00	55.60	69.00	27.00	(28.78)	(29.54)	(33.18)	(48.25)	(56.58)	(31.27)
CD at 5%	1.867	0.714	1.302	1.357	1.28	1.384	1.867	0.714	1.302	1.357	1.288	1.384	9.742	NS	6.978	18.327	7.541	NS						

The value in brackets done after Arc sine transformation

Table 2. Studies on *in vitro* seed germination of lisianthus.

Media	Days to root initiation		Days to shoot initiation		Germination (%)	
	Old seed	Fresh seed	Old seed	Fresh seed	Old seed	Fresh seed
Basal	10.98	8.50	11.80	9.50	11.63 (19.81)	43.34 (40.92)
MS + 0.5 mg/l IBA	9.60	7.40	10.60	8.40	17.95 (24.99)	63.89 (53.26)
MS + 0.5 mg/l BAP	8.75	7.20	9.75	8.20	12.91 (20.78)	49.67 (44.78)
CD at 5%	NS	NS	NS	NS	NS	7.868

The value in brackets done after Arc sine transformation

during planting was used for analysis. Soil pH 6.12, electrical conductivity 0.80 mmhos/cm, available N (252.45 kg/ha) and available P 44.27 kg/ha were measured by standard method (Gupta *et al.*, 3). Available K 224.84 kg/ha was determined by digital flame photometer (Kanudsen *et al.*, 5), while the method described by (Chopra *et al.*, 1) was used to determine the percentage of organic matter (1.27%) in the soil sample. The study of performance evaluation of seedlings was carried out under ambient conditions and flowers data were recorded. The data were analysed using the analysis of variance (ANOVA) technique outline by (Gomez *et al.*, 2).

Mean pooled data of *in vivo* and *in vitro* in respect percentage of seed germination and days required for root and shoot initiation studied in different temperature, substrates and quality of seeds are presented in Tables 1 & 2, respectively. Seed germination percentage was influenced by temperature during growing period, substrate and quality of seeds. Minimum days for root and shoot initiation were 4.66 and 5.66 days, respectively recorded in sand at 25 ± 1°C in fresh seeds. In one-year-old seeds, minimum 6 and 7 days were recorded for root and shoot initiation on blotting sheet at 25 ± 1°C. Maximum seed germination percentage (79%) was observed on blotting sheet at 25 ± 1°C followed by cocopeat: perlite (1:2) at same temperature in fresh seeds (71.30%). In one year old seeds, maximum seed germination percentage (38.66%) was observed in cocopeat: perlite (1:2) followed by cocopeat: perlite (1:1) at room temperature (34.0%). *In vitro*

seed germination was influenced by plant growth regulator (Table 2). Minimum days (7.20 and 8.20) and (8.75 and 9.75) were recorded for root and shoot initiation on media supplemented with 0.5 mg/l IBA in fresh seeds and one year old seeds, respectively. However, days to root and shoot initiation in old and fresh seeds under *in vitro* condition were non-significant. Maximum seed germination percentage (63.89%) was observed on media supplemented with IBA 0.5 mg/l in fresh seeds and minimum germination (11.63%) was recorded in basal medium in one year old seeds.

Date in Table 3 indicates the various vegetative and reproductive growth parameters of *in vivo* and *in vitro* seedlings under polyhouse conditions. Maximum length of flowering shoot (60.90 cm), numbers of leaves per shoot (33.66), width of leaves (3.53 cm), diameter of flowering shoot (0.31 cm), total number of flowers (5.86) and number of petals per bud (9.86) were recorded in tissue culture raised seedlings. *In vitro* seedlings performed better as compared to *in vivo* seedlings.

REFERENCES

1. Chopra, S.L. and Kanwar, J.S. 1991. *Analytical Agricultural Chemistry*, New Delhi, pp. 178-81.
2. Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedure for Agricultural Research* (2nd Edn.) John Willey and Sons, Singapore.
3. Gupta, V.P., Thakur, R.C., Raj, D., Sharma, P.K., Sharma, O.P. (Eds.) 1997. *Soil Science. Practical Mannual Series*, College of Agriculture.

Table 3. Performance evaluation of *in vitro* germinated seedings of lisianthus under polyhouse conditions.

Treatment	Length of flowering shoot (cm)	No. of leaves\ shoot	Leaf length (cm)	Leaf width (cm)	Size of flower (cm)	Dia. of flowering shoot (cm)	Total No. of flowers	Flower Size (cm)	No. of petals\ bud
<i>In vivo</i> seedlings	54.27	28.73	5.71	3.14	7.15	0.299	4.48	7.15	9.13
<i>In vitro</i> seedlings	60.90	33.66	5.78	3.53	7.43	0.315	5.86	7.43	9.86
CD at 5%	6.596	0.939	NS	0.194	NS	NS	0.174	NS	0.599

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Page, A.L. *et al.* (ed.), Madison, USA, Part 2, pp.
225-46.

4. Halevy, A.H. and Kofranek, A.M. 1984. Evaluation of *Lisianthus* as a new flower crop. *HortSci.* **19**: 845-47.
5. Knudsen, D., Mehlich, A. and Sommers, L.E. 1982. Lithium, sodium and potassium. **In**: *Method of Soil Analysis*, American Society of Agronomy,
6. Wood, C.E., and Weaver, R.E. 1982. The genera of Gentianaceae in the Southern United States. *J. Arnold Arbor.* **63**: 441-48.

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