D-5347
[1-5]

RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Effect of Different Media on *in vitro* Shoot Regeneration from Various Explants of *Lilium longiflorum* Cv. Elite, Brunello, Cordelia

Manoj Kundu, Suresh Kumar, Rajesh Lathar, Sakshi

10.18805/ag.D-5347

ABSTRACT

Background: Lilium (*Lilium longiflorum Thunb*.) belongs to the family Liliaceae and is a native of Northern Hemisphere (up to South Canada and Siberia). Conventionally, lilium can easily be propagated by sexual and asexual methods of propagation but these prevalent methods are not capable of meeting the increasing demand in domestic and global market. Generally, lilium is propagated through bulbs which has bottlenecks like high cost of planting material, infected from seed borne and other diseases like mosaic, grey mould, *etc*. So, *in vitro* multiplication of lilium is utmost important. Lily can be easily regenerated from explants The objective of present study was to identify the best media and the explant among scales and nodal segments belonging to the three different cultivars *viz*., Elite, Brunello, Cordelia where maximum number of shoots could be regenerated.

Methods: The present investigation was carried out in the tissue Culture Laboratory of the Centre for Research and Application in Plant Tissue Culture. Twenty-five media combinations were used to study their effect on in vitro shoot regeneration from various explants of *Lilium longiflorum* cultivars. Two type of explants *viz*. scales and nodal segments were aseptically excised from Elite, Brunello, Cordelia cultivars of Lilum and cultured on Murashige and Skoog (MS) medium containing different concentrations and combinations of 6-benzyl amino purine (BAP), kinetin and Naphthalene Acetic Acid (NAA).

Result: In cultivars Elite and Brunello, the maximum number of shoots were observed when nodal segments were used on media combination *i.e*., LM-8 (MS+BAP 3.0 mg/l+NAA0.1mg/l). In cultivar Cordelia, scales showed the maximum response on media LM-24 (MS+Kinetin 2.0 mg/l+NAA 0.1 mg/l).

Key words: 6-benzyl amino purine (BAP), Kinetin, Murashige and Skoog (MS) medium, Naphthalene acetic acid (NAA), Nodal segments, Scales.

INTRODUCTION

Lilium (*Lilium longiflorum Thunb*.) belongs to the family Liliaceae and is a native of Northern Hemisphere (up to South Canada and Siberia). It also have the southern limits in Florida and the Nilgiri mountains of India. The diploid chromosome number of *L. longiflorum* is 24. Lilium is one of the top ten ornamental bulbous crops in the world (Anonymous, 1996). Due to large flowers, diverse array of colours, wide range of variability, long vase life and capacity to rehydrate after long transportation it has become an economically important floricultural crop. Hybrid lilies are exceptionally useful for use as cut-flowers and pot-plants.

Conventionally, lilium can easily be propagated by sexual and asexual methods of propagation but these prevalent methods are not capable of meeting the increasing demand in domestic and global market. Generally, lilium is propagated through bulbs which has bottlenecks like high cost of planting material, infected from seed borne and other diseases like mosaic, grey mould, *etc*. Limited number of bulbs per plant, long dormancy period of bulbs which again results into non-availability of planting material throughout the year. So, *in-vitro* multiplication of lilium is utmost important. Plant can be easily regenerated from explants such as flower organs and scales *via* organogenesis (Gupta *et al*., 1978; Stimart and Asher, 1981; Niimi 1984; Nhut, 1998 and Nhut *et al*., 2001). Since, not a single variation among regenerated plants has been reported so, lily is also known to be genetically stable (Qu *et al.,* 1988 and Bacchetta *et al.,* 1998). The objective of present study was to identify the best media and the explant among scales and nodal segments belonging to the three cultivars *viz*., Elite, Brunello, Cordelia where maximum number of shoots could be regenerated.

Department of Horticulture, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India.

Corresponding Author: Sakshi, Department of Horticulture, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India. Email: sakshigrewal6@gmail.com

How to cite this article: Kundu, M., Kumar, S., Lathar, R. and Sakshi (2022). Effect of Different Media on *In-vitro* Shoot Regeneration from Various Explants of *Lilium longiflorum* Cv. Elite, Brunello, Cordelia. Agricultural Science Digest. DOI: 10.18805/ag.D-5347.

Submitted: 15-04-2021 **Accepted:** 28-01-2022 **Online:** 02-05-2022

MATERIALS AND METHODS

The present investigation was carried out in the Tissue Culture Laboratory of the Centre for Research and

Application in Plant Tissue Culture, Department of Science and Technology, Government of Haryana, located at CCS Haryana Agricultural University, New Campus, Hisar. The experiment was laid out in a C.R.D. (Factorial) with three replications.

Culture medium

MS media was prepared by mixing the stock solutions as given in Table 1. Distilled water was used in preparation of stocks as well as other solutions. The stock solutions No. 1-5 of macro-elements, micro-elements, vitamins and Fe-EDTA were prepared by dissolving the required amount of salts in measured quantity of distilled water. The basal MS (1962) medium was prepared by mixing the required quantity of each of the five stock solutions in 500 ml of distilled water by continuous stirring with magnetic stirrer. Myo-inositol (100 mg/1) and sucrose (3%), unless mentioned otherwise, were added to this solution. The basal MS medium was modified by adding measured quantity of growth regulators, wherever required. The stock solutions of these were prepared separately. The final volume was made to one litre by adding distilled water. The pH of the medium was adjusted to 5.8 using 1N HC1 or 1N KOH/NaOH. Agar (BDH) @ 8 g/1 was melted by placing the medium on hot plate. The medium was stirred regularly to avoid formation of agar clumps till boiled thoroughly. It was allowed to cool for few minutes at room temperature. Twenty millilitres of medium was poured into each of the culture vessel and plugged with non-absorbent cotton wrapped in muslin cloth. The medium was autoclaved at 15 1b per square inch pressure for 20 minutes and solidification was done at room temperature.

Preparation of stock solution of growth regulators and antibiotics

The stock solutions of auxin was prepared by dissolving 100 mg of auxin in few drops of absolute alcohol. The final volume was made to 100 ml by adding distilled water. Likewise, the stock solution of cytokinin (BAP) was prepared by dissolving 100 mg of BAP in few drops of 1N HCI and finally the volume was made to 100 ml by adding distilled water. The stock thus prepared was stored at 4-5°C in refrigerator in glass bottles and used whenever needed.

Twenty-five different media used in the present investigation were prepared using the MS basal medium. The chemical compositions of different media used for plantlet regeneration are described in Table 2. Media were sterilized by autoclaving and were poured in culture and vessels.

Glassware in general were sterilized by dry heat in an oven at 18°C ± 2°C for 2-3 hours. Scalpels, forceps, *etc.*, were sterilized in oven and flame sterilized prior to use. Glassware like Pasteur pipettes were sterilized by autoclaving at 15 lb for 15 minutes.

Storage of stock solutions and culture media

Stock of macro and micro nutrients and growth regulators were prepared and stored in a refrigerator at 4°C. Amino acids and other chemicals like glycine, MS major, MS minor, *etc.*, were stored at 4°C. Repeated freezing and thawing was avoided. Vessels containing autoclaved media were kept at low temperature (9°C) in an incubator and used within a week.

Table 1: Composition of stock solutions of Murashige and Skoog (1962) medium.

Strength	Constituent salts	Quantity (per 2 litre)	Used in the culture medium (mg/1)	Actual amount (mg/1)
X20	Ammonium nitrate (NH_4NO_3)	66.0 g	50	1650
	Potassium nitrate (KNO_3)	76.0 g		1900
	Potassium dihydrogen phosphate (KH_2PO_4)	6.800 g		170
	Manganese sulphate ($MnSO_4.7H_2O$)	0.248 g		6.2
	Zinc suplphate ($ZnSO_4.7H_2O$)	0.344 g		8.6
	Potassium iodide (K1)	0.033 g		0.825
(a)*	Copper sulphate ($CuSO_4.5H_2O$)	1.0 ml		0.025
(b)*	Sodium molybdate ($Na_2MoO_42H_2O$)	1.0 ml		0.250
(c)*	Cobalt chloride ($CoCl_26H_2O$)	1.0 ml		0.025
X50	Calcium chloride ($CaCl_2.2H_2O$)	11.0 g	20	440
X50	Magnesium sulphate ($MgSO_4.7H_2O$)	9.250 g	20	370
X100	Ferrous sulphate ($FeSO_4.7H_2O$)	2.780 g	10	27.8
	Ethylene diamine tetra acetic acid and disodium salt (Na_2EDTA)	3.728 g		37.28
X100	Thiamine HCI	0.010 g	10	0.1
	Nicotinic acid	0.050 g		0.5
	Pyridoxine HCI	0.050 g		0.5
	Glycine	0.200 g		2.0
	Myo-inositol			100.0
	Sucrose			30,000.0

Plant material

Scales-The scales were excised from the healthy and disease free bulbs. Leaving the outermost layer of bulbs, the basal portion of subsequent scales were dissected into 4-6 mm size to be used as explants. Nodal segment - Healthy stalks were cut 15 cm above the soil, leaves removed and stalk sectioned into 8-10 mm size bearing at least two buds to be used as explants.

Explants *i.e.*, scales and nodal segments were thoroughly washed in running tap water for 30 minutes and then dipped in Teepol (1%) for 10 minutes. The explants were then rinsed in distilled water. The explants were treated with 0.1% mercuric chloride ($HgCl_2$) for different time intervals (1-10 minutes) and rinsed 4-5 times with sterilized distilled water. The explants were then inoculated on to the culture media containing different concentrations of growth regulators under sterilized condition and incubated in the culture room.

Culture conditions and establishment of cultures

Different auxins and cytokinins were initially tried for culture establishment. NAA and BAP were found better hence used for further regeneration. Cultures were maintained at 25± 2°C under dark and light photoperiod (8/16 h) light intensity 50 u^2 Ems^{-1} as required. Maesato *et al.* (1994) reported that the optimum temperature for micropropagation of lilium from bulb scales lies between 20°C to 25°C.

Table 2: List of different media used.

Media code	Growth regulators (mg/1)			Media strength
	BAP	Kinetin	NAA	
LM-1	1.0	-	-	Full
LM-2	2.0	-	-	Full
LM-3	3.0	-	-	Full
LM-4	4.0	-	-	Full
LM-5	5.0	-	-	Full
LM-6	1.0	-	0.1	Full
LM-7	2.0	-	0.1	Full
LM-8	3.0	-	0.1	Full
LM-9	4.0	-	0.1	Full
LM-10	5.0	-	0.1	Full
LM-11	1.0	-	0.5	Full
LM-12	2.0	-	0.5	Full
LM-13	3.0	-	0.5	Full
LM-14	4.0	-	0.5	Full
LM-15	5.0	-	0.5	Full
LM-16	-	0.5	-	Full
LM-17	-	1.0	-	Full
LM-18	-	1.5	-	Full
LM-19	-	2.0	-	Full
LM-20	-	2.5	-	Full
LM-21	-	0.5	0.1	Full
LM-22	-	1.0	0.1	Full
LM-23	-	1.5	0.1	Full
LM-24	-	2.0	0.1	Full
LM-25	-	2.5	0.1	Full

RESULTS AND DISCUSSION

Two explants *viz.*, scale and nodal segments were aseptically excised from Elite, Brunello and Cordelia cultivars of lilium and cultured over different media (Table 3). Maximum number of shoots were obtained using nodal segment as explant source in cultivars Elite and Brunello. Addition of NAA 0.5 mg/l in both the media in combination with BAP or kinetin increased the number of shoots per explant in both the explants used and in all three cultivars studied. This may be attributed to the fact that auxin and its distribution within the tissue are important factors in the process of bud regeneration. These results were in confirmation with that of (Aartrijik and Barnhoorn, 1981). A differential effect of growth regulators, under a similar culture environment was also observed by Maesato *et al.* (1994).

In-vitro shoot regeneration from various explants of *Lilium longiflorum* cv. Elite.

Table 3 exhibited the effect of different media on *in-vitro* shoot regeneration from various explants of *Lilium*

Table 3: Effect of different media on *in vitro* shoot regeneration from various explants of *Lilium longiflorum* cv. Elite.

Media	Average number of shoots formed		Mean
	Scale	Nodal segment	
LM-1	5.2±0.5	5.7±0.8	5.4
LM-2	5.7±0.4	7.5±0.6	6.6
LM-3	5.8±0.1	10.2±0.5	8.0
LM-4	5.8±0.8	8.6±0.4	7.2
LM-5	5.6±0.6	5.7±0.4	5.6
LM-6	5.5±0.5	5.9±0.6	5.7
LM-7	6.7±0.4	7.8±0.2	7.2
LM-8	9.3±0.4	11.4±0.4	11.4
LM-9	7.2±0.3	9.2±0.8	8.2
LM-10	5.4±0.5	5.5±0.4	5.4
LM-11	4.9±0.6	4.5±0.4	4.7
LM-12	5.0±0.4	7.7±0.8	6.4
LM-13	7.0±0.4	8.2±0.5	7.6
LM-14	5.7±0.3	7.5±0.6	6.6
LM-15	6.3±0.5	8.4±0.4	7.4
LM-16	6.7±0.3	8.0±0.2	7.4
LM-17	6.1±0.6	8.3±0.4	7.2
LM-18	6.0±0.1	7.8±0.5	6.9
LM-19	5.8±0.1	10.2±0.5	8.0
LM-20	4.6±0.4	5.0±0.4	4.8
LM-21	5.3±0.2	6.3±1.0	5.8
LM-22	6.2±0.3	8.2±0.8	7.2
LM-23	7.8±0.2	8.9±0.4	8.4
LM-24	9.0±0.4	10.8±0.3	9.9
LM-25	52.0±2.7	4.9±0.6	4.7
Mean	6.1	7.7	

C.D. at 5% level

Media = 0.78

Cultivar = 0.27

Media × cultivar = 1.34

Table 4: Effect of different media on *in-vitro* shoot regeneration from various explants of *Lillium longiflorum* cv. Brunello.

Media	Average number of shoots formed		Mean
	Scale	Nodal segment	
LM-1	5.1±0.6	5.4±0.9	5.2
LM-2	5.4±0.5	7.2±0.4	6.3
LM-3	8.5±0.5	11.0±0.8	9.8
LM-4	5.9±0.6	6.9±0.6	6.4
LM-5	5.8±0.6	4.2±0.5	5.0
LM-6	5.4±0.5	5.4±0.2	5.4
LM-7	5.1±0.6	8.5±0.8	6.6
LM-8	13.9±1.5	14.6±1.7	14.2
LM-9	7.8±0.5	7.3±0.6	7.6
LM-10	7.3±0.6	5.3±0.4	6.3
LM-11	4.5±0.4	2.9±0.7	3.7
LM-12	4.4±0.1	7.4±0.3	5.9
LM-13	7.6±0.7	8.3±0.7	8.0
LM-14	4.4±0.7	3.7±0.7	4.0
LM-15	3.4±0.4	2.8±0.5	3.1
LM-16	4.5±0.3	2.9±0.7	3.7
LM-17	7.5±0.4	5.4±0.4	6.4
LM-18	8.8±0.2	10.9±0.7	9.8
LM-19	5.2±0.6	8.5±0.8	6.8
LM-20	7.2±0.6	7.5±0.4	**7.4**
LM-21	5.8±0.7	6.9±0.5	6.4
LM-22	5.6±0.3	7.5±0.4	6.6
LM-23	7.2±0.5	12.1±1.5	9.6
LM-24	13.6±1.2	14.1±1.7	13.8
LM-25	3.8±0.1	2.8±0.4	3.3
Mean	6.5	7.4	

C.D. at 5% level
Media = 0.78
Cultivar = 0.99
Media × cultivar = N.S.

longiflorum cv. Elite. Two types of explants *i.e.*, scales and nodal segments and twenty-five types of media were used. The maximum (11.4%) number of shoots were observed when nodal segments were used as explants. When scales were used as explant, the highest (9.3) number was less than that of the nodal segments on same media combination *i.e.*, LM-8 (MS+BAP 3.0 mg/1+NAA 0.1 mg/1). The minimum (4.5) number of shoots were observed on media LM-11 (MS +BAP 1.0 mg/1+NAA 0.5 mg/1) when nodal segment was used as explant and number of shoots were minimum (4.6) on media LM-20 (MS+Kinetin 2.5 mg/l) by using scales as an explants. The nodal segments also responded well on media LM-3 (MS+BAP 3.0 mg), LM-19 (MS+Kinetin 2.0 mg/1) and LM-24 (MS+Kinetin 2.0 mg/1+NAA 0.1 mg/1). Scales also responded well on media LM-23 (MS+ Kinetin 1.5 mg/1+NAA 0.1 mg/1), LM-24 (MS+Kinetin 2.5 mg/1+NAA 0.1 mg/1) and LM-9 (MS+BAP 4.0 mg/1+NAA 0.1 mg/1).

In-vitro shoot regeneration from various explants of *Lilium longiflorum* cv. Brunello

Table 4 revealed the effect of different media combinations on *in-vitro* shoot regeneration from various explants. Scales and nodal segments were used as explants and twenty-five media combinations (LM-1 to LM-25) were used. In nodal segments the maximum (14.6) number of shoots were formed on media LM-8 (MS+BAP 3.0 mg/1+NAA 0.1 mg/1) and minimum (2.8) on LM-15 (MS+BAP 5.0 mg/1+NAA 0.5 mg/1).The maximum (13.9) number of shoots were observed on media LM-8 (MS+BAP 3.0 mg/1+NAA 0.1 mg/1) when scales were used as explants and media LM-1 (MS+BAP 5.0 mg/1+NAA 0.5 mg/1) showed the minimum (3.4) number of shoots. Medium LM-24 (MS + Kinetin 2.0 mg/1+NAA 0.1 mg/1), LM-18 (MS+ Kinetin 1.5 mg/1) and LM-3 (MS+BAP 3.0 mg/1) also responded well in both the explants used. Hence, it is concluded from the table that media LM-8 (MS+BAP 3.0 mg/1+NAA 0.1 mg/1) is most efficient and media LM-15 (MS+BAP 5.0 mg/1+NAA 0.5 mg/1) is the least

Table 5: Effect of media on *in-vitro* shoot regeneration from various explants of Lillium longiflorum cv. Cordelia.

Media	Average number of shoots formed		Mean
	Scale	Nodal segment	
LM-1	5.7±0.3	6.3±0.4	6.0
LM-2	8.4±0.6	5.7±0.5	7.0
LM-3	8.3±0.7	8.8±0.5	8.6
LM-4	8.3±0.8	6.8±0.2	7.6
LM-5	6.6±0.9	6.7±0.4	6.6
LM-6	6.5±0.9	5.9±1.1	6.2
LM-7	8.1±0.7	6.6±0.8	7.4
LM-8	11.8±0.7	10.2±0.6	11.0
LM-9	7.0±0.6	8.3±0.6	7.6
LM-10	6.5±0.3	6.6±0.4	6.5
LM-11	4.9±0.5	5.7±0.5	5.3
LM-12	6.6±0.4	6.2±0.4	6.4
LM-13	8.2±0.6	7.4±0.5	7.8
LM-14	7.9±0.6	7.9±0.6	7.9
LM-15	5.7±0.2	6.0±0.3	5.8
LM-16	5.7±0.3	6.2±0.5	5.9
LM-17	6.6±0.3	6.6±0.4	6.6
LM-18	8.2±0.5	7.5±0.5	7.8
LM-19	9.10.9	8.6±0.4	8.8
LM-20	6.9±1.0	6.0±0.5	6.4
LM-21	6.6±0.4	6.2±0.4	6.4
LM-22	8.2±0.7	6.7±0.8	7.4
LM-23	8.4±0.8	6.8±0.2	7.6
LM-24	12.4±0.8	10.3±0.6	11.4
LM-25	5.6±0.2	6.1±0.6	5.8
Mean	7.5	7.0	

C.D. at 5% level
Media = 1.00
Cultivar = 0.35
Media × cultivar = 1.74

effective for *in-vitro* shoot regeneration from various explants.

In-vitro shoot regeneration from various explants of *Lilium longiflorum* cv. Cordelia

Table 5 presented the data pertaining to the effect of different media combinations on *in-vitro* regeneration from various explants of lilium. Two type of explants *viz*., scales and nodal segments were used and twenty-five types of media were taken. The scales showed the maximum (12.4) response on media LM-24 (MS + Kinetin 2.0 mg/1)+NAA 0.1 mg/1) and minimum (4.9) on LM-11 (MS + BAP 1.0 mg/1+NAA 0.5 mg/1). The trend of media combination was same when nodal segments were used as explants but the number of shoots formed were different. The nodal segment showed the maximum (10.3) and minimum (5.7) number of shoots. So, it is evident from the table that the media LM-24 (MS+ Kinetin 2.0 mg/1 + NAA 0.1 mg/1) is most efficient and media LM-10 (MS+BAP 5.0 mg/1+NAA 0.1 mg/1) is least effective in cultivar Cordelia.

CONCLUSION

Among the three cultivars used, two cultivars namely Brunello and Elite responded best on medium LM-8 (MS+ BAP 3.0 mg/1+NAA 0.1 mg/1). But cultivar Cordelia responded best on medium LM-24 (MS+ Kinetin 2.0 mg/1 +NAA 0.1 mg/1).

Conflict of interest: None.

REFERENCES

Aartrijk, J. Van and Blom Barnhoon, G.J. (1981). Growth regulator requirementsfor adventitious regeneration from Lilium bulb scale tissue in vitro in relation to duration of bulb storage and cultivar. Sci Hort. 14: 261-68.

Anonymous. (1996). International Flower Trade Show. Flower Auction Marker, Aalsmeer, Holland 8-12 Nov.

Bacchetta, L., Di., Lollo, M., Bernardini, C., Tucci, M. and Remotti, P.C. (1998). Increasing of genetic variability in Lilium spp. By mutagenesis and in vitro techniques. XV Eucarpia, 1998. Genetic and breeding for crop quality and resistance. 20-25 September, Viterbo, Italy.

Gupta, P., Sharma, A.K. and Charturvedi, H.C. (1978). Multiplication of *Lilium longiflorum thumb*. by aseptic culture of bulb-scales and their segments. Indian J. Exp. Biol. 16: 940-942.

Maesato, K., Sharda, K., Fukui, H., Hara, T. and Sarma, K.S. (1994). *In vitro* bulblet regernation from bulb scale explants of *Lilium japonicum Thunb*. Effect of plant growth regulators and culture environment. J. Hort. Sci. 69(2): 289-297.

Nhut, D.T. (1998). Micropropagation of lily (*Lilium longiflorum*) *via in vitro* stem node and pseudo-bulblet culture. Plant Cell Rep. 17: 913-916.

Nhut, D.T., Le, B.V., Fukai, S. Tanaka, M. and Van, T.T. (2001). Effects of activated charcoal, explant size, explant position and sucrose concentration on plant and shoot regeneration of *L. longiflorum via* young stem. Plant Growth Reg. 33: 59-65.

Niimi, Y. (1984). Bulblet productivity of explants from scales, leaves, stems and tepals of *Lilium rubellum* Baker. Sci Hort. 22: 391-394.

Qu, Y., Mok, M.C., Mok, D.W.S. and Stang, J.R. (1988). Phenotypic and cytological variation among plants derived from anther cultures of *Lilium longiflorum*. *In Vitro* Cell Dev. Biol. 24: 471-476.

Stemberg, N.G., Chen, C.H. and Ross, J.G. (1977). Regeneration of plantlets from leaf cultures of *Lilium longiflorum Thumb*. Proc SD Acad. Sci. 56: 152-158.

Stimart, D.P. and Asher, P.D. (1981). Foliar emergence from bulblets of *Lilium longiflorum* as related to *in vitro* generation temperatures. J. Am. Soc. Hortic. Sci. 106: 446-450.