



A novel regeneration protocol for LA *Lilium*

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Received: 27 August 2019; Accepted: 24 September 2019

Key words: 6-BAP, 2,4-D, *Lilium*, NAA, PLB's, t TCL

Lilium is one of the economically important flowers grown as cut flower, pot plant and bedding plant. The demand of *Lilium* particularly LA hybrid is growing day by day due to its attractive colour and excellent vase life. Multiplication rate is very poor by conventional method. So, to meet the market demand micropropagation using thin cell layer system is an effective approach to obtain a higher frequency of propagules in a short time.

tTCL makes a large number of cells responsive for regeneration as explant are composed of few layer of cells. So, the interaction of tissue or organ is avoided. The actual regeneration capacity of t TCL explants is often much higher than thicker conventional explants partly due to having a higher ratio of morphogenic cells and better transport between the medium and these cells. Thin sections made transversely from either pseudo-bulblets, young stems, stem segments or receptacles have also been used for manipulation of the morphogenetic pathways and bud regeneration in *L. longiflorum* (Van Le *et al.* 1999 and Nhut *et al.* 2001 a,b). tTCL opens up the possibility of producing superior plants of high quality and also have the ability to manipulate and control organogenesis with efficient transformation. Therefore, we considered use of the tTCL method as it is also known to be an excellent source of material for genetic transformation (Nhut *et al.* 2002).

Bulbs of LA *Lilium* hybrid cv. Brindisi was obtained from the Division of Floriculture and Landscaping, IARI, New Delhi and experiments were performed in the Central Tissue Culture Laboratory, IARI, New Delhi, India during 2015-2017. Bulb scales were carefully detached, cleaned for 1 h to remove soil particles. Thereafter, pre-treated with Carbendazim (0.2%) + Mancozeb (0.2%) + 8-HQC (200 ppm) for 3.0 h and then surface sterilized with 70% ethanol for 30 second followed by 0.1% HgCl₂ for 7 min to minimize microbial contamination. Scales were inoculated in MS medium supplied with 30 g/l sucrose and kept under light to regenerate bulblets from base of the scale. The regenerated *in vitro* bulblets after four sub-culturing was used as explant source for conducting TCL experiment.

Scales were excised to obtain thickness ranging 0.5-2.0 mm and cultured on MS medium (Murashige and Skoog 1962) containing 30 g/l sucrose, 0.8% agar and supplemented with different concentration of 6-BAP, NAA and 2, 4-D to see its response on plant regeneration. The inoculated t TCL were first kept in dark for 2 weeks then transferred to light. The t TCL in the petriplates was cultured for 40 days in the same media with subculturing at two weeks interval. Regenerated plants were proliferated in the proliferation media containing higher concentration of 6-BAP and low concentration of NAA. The data were analyzed for significance by analysis of variance with mean separation by DMRT.

Table 1 illustrated the effect of 2, 4-D and 6-BAP on plant regeneration from tTCL when supplied in MS medium. Half strength of MS medium could not show any response and died within 20 days. 2, 4-D was supplemented with 6-BAP in MS medium in order to see its response on callus formation from tTCL of *in vitro* bulb scale. But, surprisingly there was no callus or very little callus formation. PLB's was directly induced from t TCL sections which gradually developed into shoot. After four weeks of tTCL culture, the survival percentage was calculated. Maximum regeneration percentage (70.80%) was recorded when a combination of 6-BAP (0.50 mg/l) + 2, 4-D (2 mg/l) was fortified in MS medium. Callus formation was observed (Fig 1) only when 2, 4-D concentration was increased to 5 mg/l or above, otherwise, protocorm like bodies (Fig 1) were directly being induced. Early PLB's formation (18.36 days) was recorded with the treatment combination MS + 6-BAP (0.50 mg/l) + 2, 4-D (1 mg/l). The number of adventitious shoot per t TCL increased with increasing levels of 6-BAP. A statistically significant response was observed when different combination of 6-BAP and NAA was tried (Table 2). Maximum regeneration (74.24%) was observed with the treatment combination MS + 6-BAP (2 mg/l) + NAA (0.50 mg/l). The days to adventitious shoot bud initiation was recorded earlier (17.08 days) with the treatment combination MS + 6-BAP (5 mg/l) + NAA (0.25 mg/l). In the present study, a protocol was developed for controlling the type of morphogenesis which occurs in Ttcl explant. These growth hormones were shown to stimulate the direct formation of tissues or organs such as PLBs depending on the medium.

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Table 1 Effect of 6-BAP and 2, 4-D on PLB's induction and direct regeneration from t TCL explant in *Lilium* cv. Brindisi.

Treatment	Regeneration (%)	Days to PLB's induction	Number of shoot per explant	Shoot length (cm)
MS medium	10.88±0.72C	24.20±1.22A	1.16±0.08E	1.12±0.10E
MS + 6-BAP (0.25 mg/l) + 2, 4-D (1 mg/l)	53.72±3.84B	20.20±0.52AB	3.08±0.10D	2.72±0.10D
MS + 6-BAP (0.25 mg/l) + 2, 4-D (2 mg/l)	59.92±6.33AB	19.40±0.48C	3.24±0.08D	2.96±0.23CD
MS + 6-BAP (0.25 mg/l) + 2, 4-D (3 mg/l)	62.36± 5.82AB	19.32±0.39C	3.72±0.14C	3.24±0.13AB
MS + 6-BAP (0.25 mg/l) + 2, 4-D (4 mg/l)	70.64±4.89A	19.16±0.28C	3.80±0.11BC	3.20±0.09BCD
MS + 6-BAP (0.25 mg/l) + 2, 4-D (5 mg/l)	65.16±5.10AB	19.36±0.20C	3.12±0.08D	3.12±0.08BCD
MS + 6-BAP (0.50 mg/l) + 2, 4-D (1 mg/l)	65.96±1.49AB	18.36±0.56C	4.68±0.23A	3.84±0.24A
MS + 6-BAP (0.50 mg/l) + 2, 4-D (2 mg/l)	70.80±0.83A	19.00±0.34C	4.72±0.30A	3.76±0.22A
MS + 6-BAP (0.50 mg/l) + 2, 4-D (3 mg/l)	65.88±4.93AB	18.80±0.49C	4.52±0.26A	3.48±0.16AB
MS + 6-BAP (0.50 mg/l) + 2, 4-D (4 mg/l)	65.32±4.93AB	18.76±0.51C	4.32±0.13A	3.20±0.09BCD
MS + 6-BAP (0.50 mg/l) + 2, 4-D (5 mg/l)	61.12±6.47AB	18.88±0.49C	4.24±0.15AB	3.16±0.08BCD

Values represent the means ± S.E. Different letters within a column indicate significant differences at $P < 0.05$ by Duncan's multiple range test.

Using 0.2-0.4 mm thick pseudo-bulblets *in vitro* explants as tTCLs, successful regeneration in *L. longiflorum* was reported by Van Le *et al.* (1999). Nhut *et al.* (2001a) have developed an efficient *in vitro* regeneration in *L. longiflorum* using explant as young stems tTCLs and reported that 1 mm thickness of the explants from the apex layers of the shoot were best for directly inducing the highest number of shoots per explant. Whereas, Bakhshaie *et al.* (2010) reported formation of somatic embryo from bulblet microscale tTCLs. In the present study, no somatic embryos were developed instead; direct adventitious shoot bud formation

was recorded. Similar results have been reported by Nhut *et al.* (2001) in *Lilium longiflorum* and observed bulblet formation in presence of NAA media supplemented with 6-BAP. While 2,4-D at 2.2 μ M resulted in root formation was reported by Nhut *et al.* (2001).

Although 2, 4-D, NAA and 6-BAP has been effectively used to induce shoot regeneration through different explants of lily species (Bacchetta *et al.* 2003, Han *et al.* 2005 and Ling Fei *et al.* 2009), its response has not been reported in LA *Lilium* hybrids. While, Nhut *et al.* (2006) reported SE (somatic embryo) from tTCL in *Lilium longiflorum*; in contrast, our results showed that embryogenic callus could not be induced from tTCLs of bulblet microscales.. Direct adventitious shoot bud regeneration using tTCL technology have also been reported by Park (2002) in *Doritaenopsis* hybrid, Singh (2012) in *Eclipta* and Pereira Gomes (2015) in *Brasilidium* orchid. A survey of methods for the regeneration from tissue explants of *Lilium* showed that tTCL explants are highly re-generable and well suited for the large scale multiplication within short time and this technology can also be well utilized for transformation experiment.

SUMMARY

Lilium is a high value ornamental crop and its demand particularly LA hybrids is growing day by day. To utilize the high regeneration potential in *Lilium*, transverse thin cell layer sections were excised from *in vitro* bulb scales of LA *Lilium* hybrid cv. Brindisi and inoculated on MS medium with 3% sucrose, 0.8% agar and varied concentration of 6-BAP,

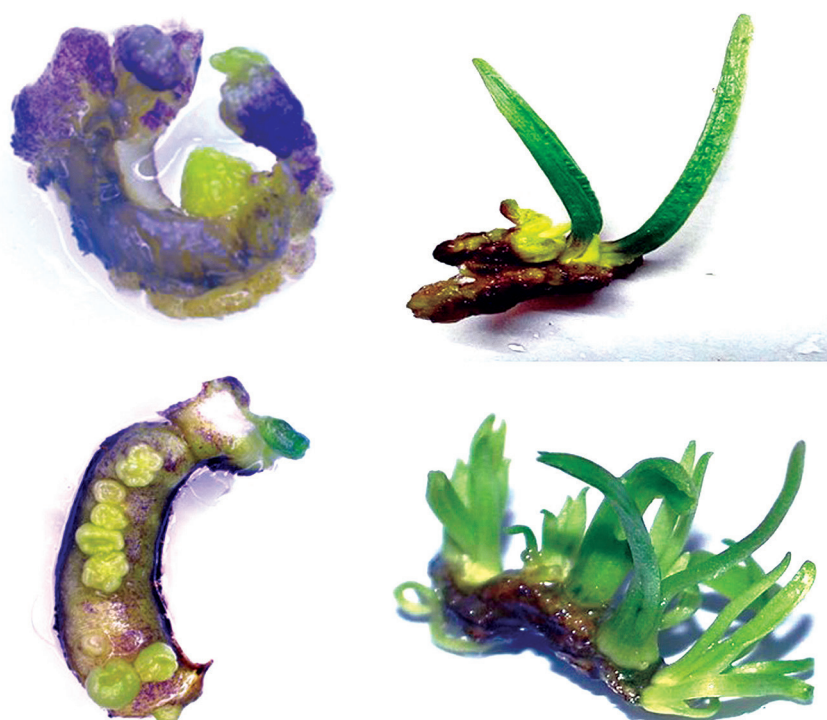


Fig 1 PLB's induction at 6-BAP (0.50 mg/l) + 2, 4-D (2 mg/l) and plant regeneration in *Lilium* LA hybrid cv. Brindisi.

Table 2 Effect of 6-BAP and NAA on direct regeneration from tTCL explant in *Lilium* cv. Brindisi.

Treatment	Regeneration (%)	Days to shoot initiation	Number of shoot per explant	Shoot length (cm)
MS medium	10.88±0.72D	24.20±1.22A	1.16±0.08D	1.12±0.10D
MS + 6-BAP (1 mg/l) + NAA (0.25 mg/l)	62.24±1.62BC	19.04±0.50B	3.12±0.13C	2.76±0.1C
MS + 6-BAP (1 mg/l) + NAA (0.50 mg/l)	60.84±4.35C	18.96±0.31B	3.28±0.15BC	3.08±0.15BC
MS + 6-BAP (2 mg/l) + NAA (0.25 mg/l)	71.08±3.44ABC	18.96±0.57B	3.48±0.23BC	3.28±0.10ABC
MS + 6-BAP (2 mg/l) + NAA (0.50 mg/l)	74.24±2.76A	18.72±0.21B	3.84±0.23B	3.32±0.16ABC
MS + 6-BAP (3 mg/l) + NAA (0.25 mg/l)	71.68±2.76AB	18.36±0.60B	3.44±0.35BC	3.24±0.15ABC
MS + 6-BAP (3 mg/l) + NAA (0.50 mg/l)	69.76±2.38ABC	18.00±0.74B	4.76±0.22A	3.68±0.27A
MS + 6-BAP (4 mg/l) + NAA (0.25 mg/l)	69.32±1.43ABC	18.12±0.49B	4.60±0.24A	3.56±0.25AB
MS + 6-BAP (4 mg/l) + NAA (0.50 mg/l)	66.40±4.43ABC	17.12±0.51B	4.72±0.22A	3.52±0.28AB
MS + 6-BAP (5 mg/l) + NAA (0.25 mg/l)	64.28±3.23ABC	17.08±0.34B	4.72±0.21A	3.24±0.17ABC
MS + 6-BAP (5 mg/l) + NAA (0.50 mg/l)	63.08±5.24BC	17.40±0.31B	4.52±0.29A	3.28±0.05ABC

Values represent the means ± S.E. Different letters within a column indicate significant differences at P<0.05 by Duncan's multiple range test.

NAA and 2,4-D. Results showed that Protocorm like Bodies (PLB's) were directly being induced from the surface of t TCL explant. These protocorm like bodies are unipolar structure forming shoot and further developed root when transferred to rooting medium. When NAA was fortified with 6-BAP in MS medium, its regeneration percentage was recorded maximum in the combination 6-BAP (2 mg/l) and NAA (0.50) mg/l), whereas, maximum number of adventitious shoots per explant were recorded in 6-BAP (3 mg/l) and NAA (0.50) mg/l). Although, adventitious shoot buds were also produced on hormones free medium but at a very slow rate. The regeneration percentage was enhanced in the presence of NAA as compared to 2, 4-D when supplemented with 6-BAP. The regenerated bulblets were best rooted in IBA supplemented with ½ MS medium and hardened in the glass jar.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR-IARI, New Delhi and UGC, Ministry of Human Resource Development; GOI for financial assistance.

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