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Callus mediated *in-vitro* plant regeneration and clonal fidelity assessment of *Lilium longiflorum* cv. "Pavia" by ISSR markers

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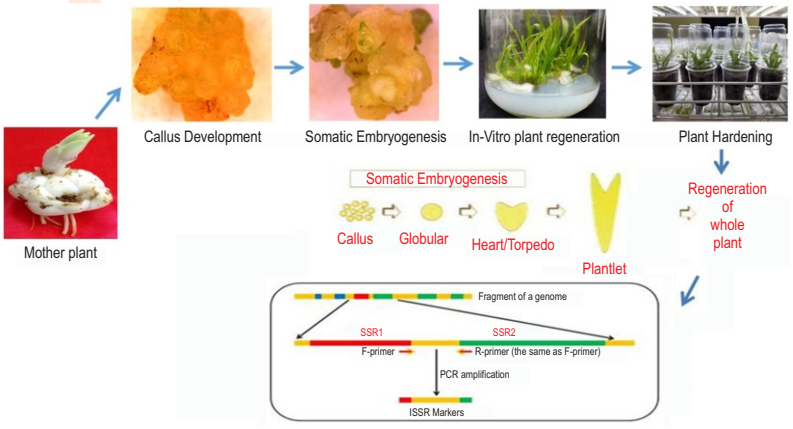
Abstract

Aim: Developing a protocol for mass multiplication of Lilium (*Lilium longiflorum*) bulbs via indirect organogenesis and somatic embryogenesis for *in-vitro* plant regeneration and ISSR marker-based mapping to assess the fitness of true to type.

Methodology: To induce organogenic callus, six combinations of 2,4-D and BAP were tested; while nine combinations of picloram and NAA were used for somatic embryogenesis. NAA and IBA were tested for root induction. The clonal fidelity of the *in-vitro* regenerated plantlets from both calluses were tested using polymerase chain reaction (PCR)-based ISSR markers analysis. All experiments were arranged in a complete randomized block design and replicated three times.



Results: Two methods (direct and indirect organogenesis) were investigated to assess the best callus mediated plant regeneration for Lilium bulb multiplication. The maximum organogenic callus induction and the highest regeneration percent was recorded with BAP (0.5 mg l^{-1}) + 2,4-D (3.0 mg l^{-1}). However, MS medium containing 0.50 mg l^{-1} picloram and 0.20 mg l^{-1} NAA was found best for initiation of embryogenic callus and proliferation and was found superior over direct organogenesis. Clonal fidelity was assessed through ISSR markers comparing the mother plant and regenerated plantlets.

Interpretation: Both organogenic callus andembryogenic callus are capable of developing true to type *in-vitro* plants and can be explored for mass multiplication of Lilium bulbs. Embryogenic callus can further be utilized in liquid suspension based bioreactor system. The present protocol has potential applications in micropropagation and genetic transformation studies in other *Lilium* spp.

Key words: Bulb scale, ISSR marker, *Lilium*, Organogenic callus, Somatic embryogenesis

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Introduction

Lilies are held high esteem by floriculturists because of their outstanding fragrance and large flowers (Bhandari *et al.,* 2016). The genus Lilium includes more than 110 species distributed throughout the cold and temperate regions of the Northern Hemisphere, particularly East Asia and North America. Many ornamental cultivars and hybrids (such as Oriental, LA, OT, Asiatic, LO, and Aurelian & Trumpet hybrids and *L. longiflorum*) are cultivated for their high ornamental value (Du *et al.,* 2017). A recent substantial increase in the demand for cut flowers and pot plants is urging for the development of novel lily cultivars. Most commercial cultivars are propagated through vegetative methods, such as use of stem bulblets, bulbils and adventitious bulblets formed on bulb scales (Dwipayani Lestari *et al.,* 2020). However, the conventional breeding cycle of lilies is long, the bulb production systems are underdeveloped, the multiplication efficacy via bulbs is low, plantlets are relatively susceptible to disease, and the overall production cost remains high. Moreover, the preservation of many wild lily resources is difficult, and the excellent germplasm resources available are underutilized (Wu *et al.,* 2019). Conventionally, lilies are propagated by vegetative means (Van Tuyl *et al.,* 1991), which is laborious and cannot satisfy the present market demands.

Regeneration of plants from individual cells is an important step in the application of biotechnology (Martinez *et al.,* 2019). However, *in-vitro* micropropagation provides an alternative for the rapid propagation of *Lilium*. Jin *et al.* (2014) pioneered regeneration via organogenesis in *L. pumilum*. An effective micropropagation protocol for mass production, multiplication and conservation of *Lilium candidum* was developed by Tokgoz and Altan (2020). The direct or indirect shoot organogenesis and somatic embryogenesis are the most frequently exploited techniques for mass multiplication (Bakhshaie *et al.,* 2010). Somatic embryogenesis is an *in-vitro* regeneration method, where somatic cells acquire the embryogenic capability to form a complete plant (Zimmerman, 1993). Somatic embryogenesis usually involves two stages: somatic cells first undergo dedifferentiation and redifferentiation to acquire the capability for embryogenesis, and embryogenic cells then form somatic embryos, and subsequently develop into complete plants (Yang and Zhang, 2010).

Plant regeneration through somatic embryogenesis has many advantages, such as the potential of developing an entire plant from a single cell and the possibility of achieving automatic large-scale production of embryos in a bioreactor and synthetic seeds planted in the field (Anjaneyulu *et al.,* 2004). Haensch (1996) reported somatic embryogenesis in Lilium hybrids, as one of the effective approaches for whole plant regeneration. somatic embryogenesis has several advantages, including reduced material requirements, high genetic stability, low mutation rates and high regeneration rates, and is often used for mass propagation *in vitro*. However, two major concerns include limited information on the genetic stability of regenerates (*in-vitro*) from somatic embryogenesis in *Lilium* spp. and genetic integrity of *in-vitro* raised plants concerning to the mother plant (Kędra and Bach, 2005; Prado *et al.,* 2010) owing to the occurrence of genetic variations during subsequent sub-cultures and regeneration process (Varshney *et al.,* 2001). Thus, it becomes crucial to assess the genetic integrity of all *in-vitro* raised progenies. Therefore, the present study aimed to devise an efficient protocol for the mass multiplication of *Lilium* cv. Pavia and further assess their genetic stability using ISSR markers.

Materials and Methods

Plant material and explant preparation: Bulbs of *L. longiflorum* cv. "Pavia" were collected from Division of Floriculture and Medicinal Crops, ICAR- Indian Institute of Horticultural Research, Hessaraghatta, Banglore, India. The inner bulb scales detached from mother bulb were pre-treated with Carbendazim (0.02%) + Mancozeb (0.1%) + Streptocyclin 300 ppm for 4 hrs and further exposed to 70% Ethanol for 120 sec to reduce microbial load (Fig.1.A). Subsequently, the pretreated inner bulb scales were sterilized with (3%) NaOCl for 5 min. Finally, the inner bulb scales were cut into transverse segments approximately of 1.0 cm^2 size and cultured in the media.

Culture medium and conditions: Murashige and Skoog medium (17) with 3% sucrose and 0.8% Agarose as a gelling agent with media pH adjusted to 5.8 was used throughout the study. The medium was sterilized in an autoclave for 20 min at 121°C under 100- kPa pressure. All cultures were incubated at 25 ± 1°C, with a photoperiod of 16 hrs.

Organogenic callus induction and shoot differentiation (indirect organogenesis): To induce organogenic callus, inner bulb scales segments (1.0 cm^2) were placed on MS basal medium supplemented with 2,4-D and BAP (Table 1, Fig.1.B). To regenerate adventitious shoots, 0.5 g green 4 weeks old were transferred to MS medium with BAP and NAA (Table 2, Fig. 1.C).

Embryogenic callus induction and regeneration of somatic embryogenesis: Inner bulb scales were placed on MS medium supplemented with picloram and NAA for callus (EC) induction (Table 4 and Fig. 1.E). After 8 weeks, PGR-free MS medium utilizes for EC culture.

Root induction in regenerated plantlets derived from direct and indirect organogenesis: The newly regenerated shoots (4-5 cm in height) derived from direct and indirect organogenesis were placed on MS medium containing different concentrations of NAA and IBA for rooting (Table 3). The frequency of rooted shoots, the number of roots per shoot and main root length were measured.

Acclimatization and hardening: *In-vitro* raised bulblets were thoroughly washed with sterile distilled water to remove the agarose gel. Each bulblet with well-formed roots was grown in plastic cups containing a sterile cocopeat and sand (1:1), further acclimatized at 25 ± 1°C and 90% relative humidity.

Testing clonal fidelity of regenerants: Clonal fidelity of *in-vitro* regenerated plantlets from callus was tested using PCR-based ISSR markers analysis. DNA from leaf tissue was extracted by Cetyl trimethylammonium bromide method and the quality of total DNA samples were asessed by UV spectrophotometer. Each sample was stored at -20ºC until further use.

Polymerase chain reaction: PCR reactions were conducted at 25 µl final volume, consisting of 2.5 µl of 10X Taq buffer, 2.2 µl $MgCl_2$, 2.5 µl of 1 mM dNTP mix, 0.3 µl of Taq polymerase, 1.5 µl of 10 µM primer, 3.0 µl of plant DNA (25 ng μl^{-1}), and sterile deionized water added to 13.0 µl final volume. The PCR cycling program as follows: initial DNA denaturation at 94°C for 4 min followed by 35 cycles of 60 second denaturation at 94°C, 45 sec annealing at 54°C and 2 min extension at 72°C with a final extension at 72°C for 8 min using an applied Biometra™ Thermal Cycler (PCR machine). Subsequently, the PCR amplicons were analyzed via gel electrophoresis.

ISSR analysis: Out of 50 primers with 100bp DNA ladder used to screen and select suitable primers for ISSR markers, only five primers (Table 6) indicating distinguishable multiple PCR bands were used for further analyses. The experiment was repeated twice to confirm reproducibility, and only reliable data was used for analysis.

Statistical analyses: All experiments were conducted in a complete randomized block design. For each treatment, at least five explants including mother plants were used and replicated thrice. The given data in percentages were subjected to arcsine transformation prior to statistical analyses, and Duncan's Multiple Range Test (DMRT, 1955) at 1% level of significance were used for ANOVA and statistically significant difference between means.

Results and Discussion

In this study, the organogenic callus (Green and compact) was induced from the inner bulb scales in varied treatments (T_1-T_6) of BAP and 2,4-D (Table 1). Significant differences in the percent callus induction were observed among all treatments ($p \leq 0.01$). The medium supplemented with T_3 treatment was found best for organogenic callus induction with compact and green color callus (Fig. 1 B) and the maximum percentage (84.89%) was recorded with T_3 treatment (Table 1; Fig. 1B). Additionally, the maximum fresh weight of callus (0.81 g) was significantly higher than other treatments (Fig. 1B). Besides, the average time taken for callus initiation varied from 26.46 to 31.76 days across all treatments.

The minimum number of days (26.46) to induce maximum callus (84.89%) was observed with T_3 treatment. The perusal of data showed a significant interaction between auxin and cytokinin that favors callus induction under *in-vitro* conditions. In previous studies, (Kanchanapoom *et al.,* 2011; Liu *et al.,* 2011; Tribulato, 1997) 2,4-D has been found as a good inducer of callus from the bulb-scale explants and cytokinins play a key role in cell division and differentiation of shoots from callus by favoring direct organogenesis in *Lilium* spp. Signaling of auxin-induced callus formation is transduced via auxin response factor, transcription factors and a family of transcription factors that plays a central role in cell cycle reentry. Tang *et al.*, (2014) reported highest percentage (98.3%) of callus induction from scales when MS medium supplemented with 0.5 mg l^{-1} BA and 3.0 mg l^{-1} 2,4-D. The following results are consistent with the studies conducted by Nhut *et al.* (2001) and Ault and Siqueira (2008).

Addition of auxin and cytokinin on culture medium plays an important role in triggering cell division and elongation that favors organogenic callus induction (Lestari *et al.,* 2019). Bhagat *et al.* (2020) also suggested that variation in nutritive factor resulted dynamic changes in response to plant growth. Thus, the present study establishes BAP (0.5 mg l^{-1}) + 2,4-D (3.0 mg l^{-1}) promotes highest callogenesis in *Lilium* cv Pavia. After organogenic callus induction, the calluses were sub-cultured to shoot regeneration medium containing different concentration of BAP and NAA (Table 2). The maximum percentage of regenerated organogenic callus into shoots (78.43%) was recorded in medium (T_7) containing (1.0 mg l^{-1} BAP + 0.20 mg l^{-1} NAA). Additionally, multiple shoots (7.50) per 0.5 g of callus mass was also recorded with T_7 medium. Relatively in a short span (19.83 days), the hormone combination in T_7 medium was found to be most responsive compared to other combinations tested.

Table 1: Effect of BAP and 2,4-D on organogenic callus induction from inner bulb scales of *Lilium longiflorum* cv. "Pavia"

Treatments	BAP	2,4-D	Frequency of inner bulb scales that induced OC (%)	Mean number of days to induce callus	Nature of callus
T_0	0.0	0.0	-	-	-
T_1	0.5	1.0	77.590 (61.757)^c	29.430 (32.854)^b	Pale yellowish
T_2	0.5	2.0	72.480 (58.362)^d	26.460 (30.954)^d	Pale yellowish
T_3	0.5	3.0	84.890 (67.126)^a	26.460 (30.954)^d	Compact green
T_4	1.0	1.0	79.850 (63.333)^b	30.230 (33.354)^b	Pale yellowish
T_5	1.0	2.0	67.760 (55.406)^e	27.460 (31.600)^c	Compact green
T_6	1.0	3.0	46.540 (43.073)^f	31.760 (34.364)^a	Compact green

Values with lower letters within the column indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.01$

Table 2: Effect of BAP and NAA on *in-vitro* adventitious shoot induction from organogenic callus of *Lilium longiflorum* cv. "Pavia"

Treatments	BAP	NAA	OC differentiation frequency into shoots (%)	Number of shoots per callus	Mean number of days to induce shoots
T_0	0.00	0.00	9.620 (18.067)^{h}	2.200 (8.529)^{i}	26.820 (31.190)^{a}
T_1	0.50	0.00	34.350 (35.880)^{g}	2.600 (9.279)^{h}	24.910 (29.940)^{b}
T_2	0.50	0.10	42.460 (40.663)^{f}	4.700 (12.519)^{f}	24.460 (29.642)^{bc}
T_3	0.50	0.20	57.620 (49.386)^{d}	4.900 (12.788)^{e}	22.760 (28.491)^{de}
T_4	0.50	0.30	49.920 (44.954)^{e}	5.200 (13.181)^{d}	23.490 (28.990)^{cd}
T_5	1.00	0.00	56.760 (48.885)^{d}	4.400 (12.108)^{g}	21.860 (27.874)^{ef}
T_6	1.00	0.10	63.920 (53.083)^{c}	6.500 (14.771)^{c}	21.920 (27.914)^{ef}
T_7	1.00	0.20	78.430 (62.350)^{a}	7.500 (15.893)^{a}	19.830 (26.442)^{g}
T_8	1.00	0.30	73.430 (58.973)^{b}	6.800 (15.116)^{b}	21.430 (27.567)^{f}

Values with lower letters within the column indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.01$

Table 3: Effect of NAA and IBA on rooting from 12-week-old shoots of *Lilium longiflorum* cv."Pavia".

Treatments	Treatment details	Frequency of rooted shoots (%)	Number of days taken for root initiation	Mean number of roots	Main root length (mm)
T_0	½ MS devoid of hormones	68.00 (55.552)^{f}	19.00 (25.841)^{b}	3.70 (11.090)^{h}	20.50 (26.920)^{h}
T_1	½ MS + NAA (0.5 mg l^{-1})	97.00 (80.220)^{a}	16.00 (23.575)^{d}	6.40 (14.654)^{e}	26.40 (30.915)^{e}
T_2	½ MS + NAA (1.0 mg l^{-1})	98.00 (81.892)^{a}	16.00 (23.575)^{d}	7.30 (15.673)^{d}	28.20 (32.076)^{d}
T_3	½ MS + IBA (0.5 mg l^{-1})	93.00 (74.810)^{b}	13.00 (21.134)^{e}	9.90 (18.339)^{b}	24.70 (29.800)^{f}
T_4	½ MS + IBA (1.0 mg l^{-1})	99.33 (81.249)^{a}	13.00 (21.134)^{e}	11.40 (19.732)^{a}	31.30 (34.019)^{b}
T_5	Full MS devoid of hormones	56.00 (48.446)^{g}	21.00 (27.273)^{a}	3.50 (10.782)^{h}	22.90 (28.589)^{g}
T_6	MS + NAA (0.5 mg l^{-1})	82.00 (64.938)^{de}	19.00 (25.842)^{b}	7.80 (16.217)^{c}	33.70 (35.486)^{a}
T_7	MS + NAA (1.0 mg l^{-1})	89.00 (70.664)^{c}	19.00 (25.840)^{b}	9.70 (18.145)^{b}	29.50 (32.896)^{c}
T_8	MS + IBA (0.5 mg l^{-1})	78.00 (62.042)^{e}	18.00 (25.104)^{c}	5.80 (13.934)^{f}	25.60 (30.392)^{ef}
T_9	MS + IBA (1.0 mg l^{-1})	84.00 (66.448)^{d}	18.00 (25.103)^{c}	4.30 (11.967)^{g}	22.20 (28.110)^{g}

Values with lower letters within the column indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.01$

The growth and developmental processes of *in-vitro* plants are influenced by the exogenous application of hormones, such as cytokinins and auxins (Jamwal *et al.,* 2016). Cytokinins, in general, are required for shoot induction and proliferation (Park *et al.,* 2008). These finding are also in accordance with Jayanthi *et al.* (2021) who reported that MS augmented with 4.5 µM 2,4-dichlorophenoxyacetic acid (2, 4-D), 5.4 µM α-naphthaleneacetic acid (NAA), and 2.2 µM 6-benzylaminopurine (BAP) favors direct organogenesis in *Lilium*. Also, these results corroborate with the findings of Kedra and Bach (2005) where they observed that the use of low concentrations of BAP promoted shoot proliferation in *Lilium* spp. Similarly, the findings of Tang *et al.* (2014) reported the highest shoot regeneration and bulblet formation in *Lilium leucanthum* (Baker) from *in- vitro* cultured scales.

Efficient root growth and development is a pre-requisite for successful *in-vitro* plant regeneration. In the present study, rooting was affected by various degrees of IBA and NAA in full and ½ strength of MS medium (Table 3). Among all treatments, T_4 treatment containing ½ MS + IBA (1.0 mg l^{-1}) was found best over other treatments for maximum rooting (99.33%). Root emergence was observed after 13.00 days of culture from *in-vitro* raised

Table 4: Effect of Picloram and NAA on embryogenic callus induction from inner bulb scales of *Lilium longiflorum* cv. "Pavia".

Treatments	Picloram	NAA	Picloram/ NAA	OC induction (%)	Mean number of days to induce callus	Status of embryogenic callus
T_0	0.0	0.0	-	-	-	-
T_1	0.50	0.10	5	25.610 (30.401)^f	31.460 (34.117)^b	Light yellowish, granular, friable
T_2	0.50	0.20	2.5	90.840 (72.616)^a	24.890 (29.926)^d	Light yellowish, granular, friable
T_3	0.50	0.30	1.6	65.530 (54.058)^c	27.540 (31.652)^c	Light yellowish, granular, friable
T_4	1.00	0.10	10	31.480 (34.129)^e	33.530 (35.384)^a	Pale yellowish-white, granular,
T_5	1.00	0.20	5	72.640 (58.462)^b	32.760 (34.915)^a	Light yellowish, granular, friable
T_6	1.00	0.30	3.3	64.330 (54.058)^d	27.140 (31.652)^c	Light yellowish, granular, friable

Values with lower letters within the column indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.01$

Table 5: Standardization of hardening techniques to develop stage I (1-4 g) bulblets of *Lilium* cv. 'Pavia'

Treatments	Treatments details	Per cent survival
T_0	Control; direct transplantation in plastic pots containing garden soil	47.36 (45.341)^d
T_1	Tissue culture bottles with Agarose based media	96.683 (80.786)^a
T_2	Plastic cups having cocopeat (100 %)	92.460 (74.184)^b
T_3	Pro-trays containing cocopeat + sand (75:25)	86.260 (68.263)^{bc}
T_4	Pro-trays containing cocopeat + sand (50:50)	81.629 (64.645)^c
T_5	Pro-trays containing cocopeat + sand (25:75)	79.760 (63.296)^c

Values with lower letters within the column indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.01$

shoots in ½ MS containing IBA (1.0 mg l^{-1}), which was significantly high than other concentrations of NAA and IBA tested (Table 3; Fig. 1 G). Root proliferation in terms of mean number of roots per explant (11.40) and mean length of roots (31.30 mm) was also observed to be higher in the medium supplemented with ½ MS containing 1.0 mg l^{-1} IBA. Bacchetta *et al.*, (2003) reported that lily plantlets (*Lilium* spp.) cultured on MS medium + 0.5 mg NAA performed better rooting then rest of the treatment. Similar findings were also reported by Taha *et al.*, (2018) in *L. longiflorum Thunb*. that low concentration of NAA (0.5 mg l^{-1}) had promotion effect on both number of roots/shootlet and the diameter of formedbulblets. To further explore the potential of somatic embryogenesis, the effects of different concentrations of picloram and NAA were examined on somatic embryo induction, somatic embryogenesis development, germination and seedling formation.

The reasonable application of PGRs is one of the determinants of successful induction and is also a major factor that can lead to high frequency of somatic embryos. Picloram is highly effective at promoting the induction of lily somatic embryos. Kamo *et al.* (2012) also reported that MS with 0.5 mg l^{-1} picloram was the best embryogenic callus induction medium for *L. longiflorum* "Nellie White", however, the frequency of embryogenic callus to shoots was not continuous. With aim to boost induction frequency and obtain a best regeneration pathway, the effect of picloram in combination with NAA was investigated. embryogenic callus and somatic embryogenesis was observed in inoculated scales just after three weeks of culture. The highest percentage of embryogenic callus induction (90.84%) with maximum fresh weight (0.24 g) and minimum days to induce embryogenic callus (24.89 days) were obtained with T_2 treatment 0.50 mg l^{-1} picloram and 0.20 mg l^{-1} NAA (Table 4). Also, light yellowish, granular and friable somatic embryogenesis were observed in T_2 treatment (Table 4; Fig. 1.D). somatic embryogenesis successfully converted into cotyledon embryos when sub-cultured in PGR-free medium, (Fig. 1.E) that further developed into plants after eight weeks of culture (Fig. 1.F). Qi *et al.* (2014) showed that the optimal medium for callus induction from bulb scales and filament tissue in *L. tenuifolium* (Oriental × trumpet) variety 'Robina' was MS medium supplemented with 1.0 mg l^{-1} PIC. Zhang *et al.* (2016) used a combination of PIC and NAA to induce somatic embryos in *Lilium pumilum*, and the induction rate reached 100%. These results corroborate with the studies of Nakano *et al.* (2000) and Ault and Siqueira (2008) where they obtained embryogenic calli from bulblet scales of *L. formosanum* on medium containing picloram. Furthermore, Mori *et al.* (2005) reported although calli were induced by picloram from bulblet scales of several *Lilium* species and hybrids, only the callus induced in *L. formosanum* was embryogenic. These results further confirm that PIC plays a key role in inducing somatic embryogenesis in lilier.

Acclimatization and hardening is pivotal step during *in-vitro* plant regeneration and *ex-vitro* re-establishment of plants. In

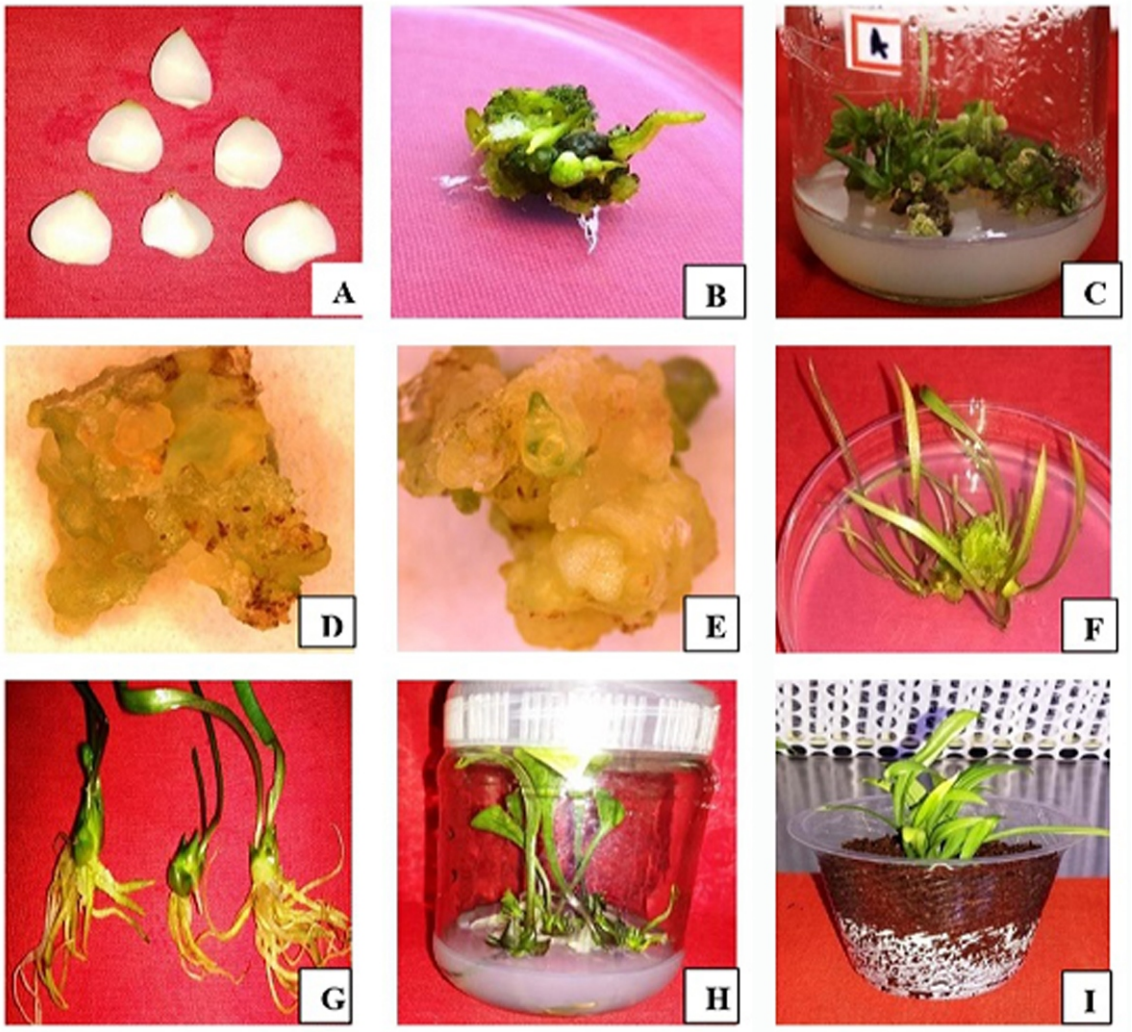

Fig.1.A: Bulb scale (inner) explants; B: Organogenic callus induced from inner bulb scales after 4 week of culture on medium containing BAP (0.5 mg l^{-1}) + 2,4-D (3.0 mg l^{-1}); C: Organogenic callus differentiated into shoots after 8 week of culture on medium containing BAP (1.0 mg l^{-1}) and NAA (0.20 mg l^{-1}); D: Embryogenic callus (EC) induction in picloram (0.50 mg l^{-1}) and NAA (0.20 mg l^{-1}) and somatic embryos development after three week of culture; E: Cotyledon embryos on the 4th week after culture on PGR-free medium; F: Regenerated plantlet with leaves on the 8th week after culture on PGR-free medium; G: Rooting on *in-vitro* raised shoots in half MS containing IBA (1.0 mg l^{-1}); H: Acclimatization in agarose gel under tissue culture bottle; I: Acclimatization in plastic cups having cocopeat: sand (1:1, v/v).

this study, *in-vitro* raised plantlets were acclimatized in tissue culture glass bottle containing hormone free agarose medium, followed by plastic cups containing sterile cocopeat and sand in hardening chamber at 25 ± 1°C with 90% relative humidity. The following acclimatization treatment (T_1 and T_2) resulted in higher survivability of 96.68% and 92.46% respectively (Table 5) compared to direct transplantation in plastic pots having garden soil (47.36%). Deb and Imchen (2010) reported 85-85% survivability of orchid seedlings when acclimatized and hardened on MS basal medium as an alternate substratum over the conventional hardening techniques for *invitro* raised plants. Further, acclimatized and transplanted plantlets grew normally under naturally ventilated polyhouse conditions. In this study, three *in-vitro* raised plantlets DNA (from both OC and EC derived) including two mother plant DNA was screened to test their genetic stability using ISSR marker. Out of 50 ISSR primers, initially screened, five produced strong, clear, multiple bands (Table 6), ranging from 200-900 bp in size (Fig. 2). The number of bands varied from four to eight across all primer tested. No deviation in banding pattern was found within five primers tested for progenies and mother plants genomic DNA of Lilium cv. "Pavia". (Fig. 2), this confirmed the genetic stability of *in-vitro* raised plantlets of Lilium cv. "Pavia". Studies on genetic stability in somatic embryo-derived plants in Lilium are limited. These results are similar to the

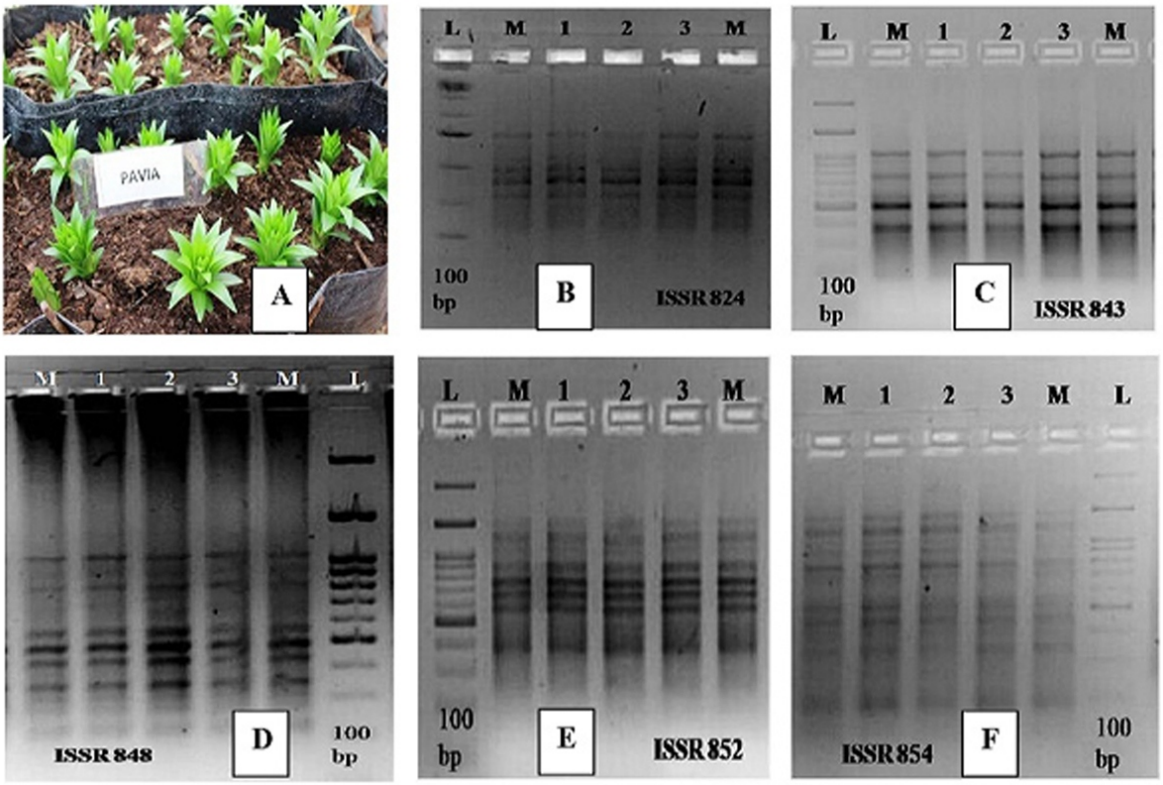

Fig. 2. A: Plantlets derived from organogenic callus and embryogenic callus; B-F:Clonal fidelity test using Inter-simple sequence repeat (ISSR) marker: ISSR 824, ISSR843, ISSR 848, ISSR 852 and ISSR 854 between the mother plant and plantlets derived from organogenic callus (OC) and embryogenic callus (EC). Lanes M represent mother plant, Lanes 1-3 represents plantlets -derived from OC and EC, Lane L represent 100 bp molecular (DNA) ladder).

findings obtained by Kędra and Bach (2005) who reported that Lilium plantlets regenerated via somatic embryogenesis can be considered genetically stable.

The present research establishes a simple, efficient and widely applicable protocol to regulate organogenesis and somatic embryogenesis in *Lilium* cv. "Pavia". The results indicated that PGRs combination is an essential factor in callogenesis and organogenesis of Lilium. Maximum callogenesis 90.84% (EC) was achieved with picloram (0.5 mg l^{-1}) and NAA (0.20 mg l^{-1}) that was superior over treatment containing 0.5 mg l^{-1} BAP and 3.0 mg l^{-1} 2,4-D that resulted 84.89% organogenic callus (OC) induction. This picloram and NAA combination found best to proliferate the embryogenic callus up to 90.84%. Somatic embryos induced from scale explants can be formed indirectly through embryogenic callus, pass through globular, heart-shaped, torpedo, and cotyledon embryo stages, and ultimately become seedlings. Tissue culture bottles with Agarose based media found best for hardening of rooted plants with 96.68 % survival ability. Clonal fidelity was assessed through ISSR markers comparing mother plant and in vitro raised regenerated plantlets. No deviation in banding pattern were detected among the daughter and mother plants. Further, callus induction with high regeneration ability can be used in genetic transformation studies targeting important traits. Besides, a successful *in-vitro* propagation system is indispensable for commercialization. To our knowledge, the present study would be the first report of either direct or indirect regeneration from bulb scale explants in Lilium cv. "Pavia, further, established protocol has the potential to be used for rapid mass propagation to meet out the current demand of commercial Lilium cultivars.

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Add-on Information

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