



Original article

Standardization of *in vitro* micropropagation procedure of Oriental *Lilium* Hybrid Cv. 'Ravenna'



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ABSTRACT

Micropropagation protocol of Oriental Hybrid *Lilium* cv. Ravenna was developed using bulb scale segments (Basal and Tip) as explants. Surface sterilization of healthy bulb scales with carbendazim 200 ppm for 30 min, then 0.1 percent mercuric chloride for 10 min, then 70% ethyl alcohol for 30 s was superior to all other treatments in recording highest culture asepsis (77.08%) and higher explant survival (86.12%). Explant survival was higher in basal segments (88.54%) compared to tip segments (85.52%). Highest culture establishment was recorded in basal scale segments (68.26%) followed by tip scale segments (55.21%). MS medium augmented with 0.50 mg l⁻¹ Naphthalene acetic acid and 2.0 mg l⁻¹ 6-Benzylamino Purine recorded maximum culture establishment (76.17%), highest bulblet number/explant (5.52) with maximum length of shoots (2.20 cm) and number of leaves (3.39). This treatment combination of growth regulators resulted in highest shoot proliferation (83.33%) along with maximum shoot number (2.41 explant⁻¹), shoot length (2.35 cm) and leaf number (5.44) of micro shoots during proliferation stage. Rooting of explants was superior with Indole-3-butyric acid compared to Naphthalene acetic acid. Highest rooting of 92.71% along with maximum number of primary roots shoot⁻¹ (12.06), maximum primary root length (3.17 cm) was documented in Murashige and Skoog medium added with Indole-3-butyric acid 1.50 mg l⁻¹ with best *ex vitro* survival rate (98.96%) of rooted plantlets during primary hardening in perlite + vermiculite (1:1) mixture.

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1. Introduction

Lilium is a genus of perennial herbaceous floral plants that grow from bulbs and are members of the Liliaceae family, grouped in 7 taxonomic sections (van Tuyl et al., 2018) comprising of 110 accepted species (GRIN, 2016). Lilies are the most important, beautiful, and economically viable blossoming plants found globally, as well as one among the three most significant commercial bulb crop, grown as pot or cut flowers, owing to their huge, fragrant, fas-

cinating, flowers with a wide range of colours and a long shelf life (Robinson and Firoozabady, 1993; Pobudkiewics and Treder, 2006; Van Tuyl et al., 2011; Younis et al., 2014; Bakhshaie et al., 2016). *Lilium* species and hybrids are also grown in gardens (Van Tuyl et al., 2011). *Lilium* is cultivated extensively in a wide range of climates. In the world floriculture trade, it is ranked fourth among the top ten cut flowers (Bhandari et al., 2018). *Lilium* is regarded as a popular floral trade cut flower, and several firms of breeding are working to develop new varieties of species from various divisions of the genus. Oriental *Lilium* of the Archelirion section is the most costly of the various lily types since their bulbs are extremely valued also their cultivation necessitates a unique procedure. They have one of the most economically valuable types of hybrid flowers that vary in height, form, colour, and other morphological characteristics (Roh, 2011; Younis et al., 2014; Lucidos et al., 2017; Youssef et al., 2019). The *in-vitro* protocol resulted in rapid large-scale production and rejuvenation of standardised disease-free planting material, recognised both as need, especially in future

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breeding and profit-oriented use of lily species (Pelkonen, 2005; Muhammad et al., 2013). Tissue culture methods aimed at rapid production have been found to be effective in different species and cultivars of *Lilium* viz., *L. longiflorum* (Bacchetta et al., 2003), Oriental Hybrid Lilies (Lian et al., 2002), and *Lilium* Asiatic Hybrid (Lian et al., 2003; Taha et al., 2018). However, in order to make *in vitro* culture a commercially viable production method, preparation procedures for each crop and cultivar must be developed separately.

To retain genetic purity, *Lilium* cultivars are propagated vegetatively (like shoot and root bulb development, bulb development on bulb scales, tissue culture and aerial axillary stalk bulbs) while new cultivars are produced sexually (seed production). The time it takes for crops to attain an appropriate bulb size to develop floral stalk, as well as undesirable variation and seasonal availability are the key drawbacks of using seed; however, it can be a valuable tool for maintaining diversity of genes and procurement of stock that is devoid of viruses. The majority of varieties as well as species that are grown using seed start blooming after three years, however, flowers may be produced as early as in 2nd (e.g., in *L. longiflorum*), 3rd (e.g., in Asiatic), or 4th year (e.g., in Orientals). Aimed at such purposes, it is common to raise new plant material in commercial production fields from the bulblets that emerge naturally just at the base of a fully developed plant's stem in the growth medium, besides specialist plant breeders and nurseries often use *in vitro* methods for plant and tissue growth to quickly generate a supply of new clones with suitable characteristics, and producing such cultivars that are uninfected and healthy. One of the most successful and productive asexual propagation techniques of *Lilium* is indeed *in situ* scale culture (Bahr & Compton, 2004). Micropropagation can produce a large number of good quality plants in a short time (George et al., 2008). Many tissues may be used in *Lilium* micropropagation, however bulb-scales being the most famous explants (Van Aartrijk and Van der Linde, 1986, Bahr and Compton, 2004, Han et al., 2005). In two years, tissue culture can produce one million small bulblets from a single large bulb (Langens-Gerrits, 2003). Depending on the size and variety, lily propagation normally yields 3–4 bulbs per scale. The bulb's multiplication efficiency is poor, and plantlets are much more disease-prone. As a result, procedure intended for its widespread dissemination is needed (Patil et al., 2021). *In vitro* culturing has a significant impact on current *Lilium* cultivars breeding and genetic enhancement programs; hence, the development of effective method for *in vitro* tissue & plant cell culturing that allow the lily breeders, bulb growers, and biotechnologists in producing viable, true to type plants quickly. *Lilium* is also given special consideration as a perfect model plant for the study of methods of *in situ* pollination and rescue of embryos in an *in situ* culture system (Van Tuyl et al., 1988, 1991; Bakhshaie et al., 2016).

Thus the current research was undertaken in order to standardise the explant sterilisation and micropropagation protocol for plantlet production from oriental lily bulb-scale explants. The aim of this research was to develop an effective *in-vitro* culture protocol for rapid propagation of oriental *Lilium* hybrid Cv. 'Ravenna' at the Floriculture and Landscape Architecture Division's Plant Tissue Culture laboratory, SKUAST-K, Shalimar, India.

2. Materials and methods

2.1. Collection of explant and surface sterilization

Mature, flowering sized bulbs Of Oriental *Lilium* Hybrid cv. 'Ravenna' was used as explant source in the current study. Scales of bulbs stored in coco-peat under normal storage conditions were collected from healthy bulbs. Initially explants were cleansed thor-

oughly with tap water to clear any clinging particles of dirt and debris. The outside diseased and damaged scales were discarded. Healthy outer scales were selected, removed and provided 30 min vigorous shakes in Tween-20 (a few droplets) augmented with the required fungicide dosage- Carbendazim. Under flowing tap water, the surfactant and fungicide were washed away, after that; a last rinse with distilled water was given. Two types of explants (basal and tip scale segments) and three sterilizing agents [mercuric chloride (0.1%), sodium hypochloride (1.0%) and ethyl alcohol (70%)] were used. The explants were cleaned before being put under a laminar to be treated with surface sterilants. The bulb scales after treatment with sterilants were given three final sterile water rinses. Percent asepsis and survival of explants was noted after 4th & 6th week's culture period, respectively.

2.2. Preparation of culture media and incubation conditions

During the research, Murashige and Skoog (1962) media comprising macro-elements, micro elements, and also vitamins were used. According to the treatment requirements, the necessary amount of sucrose (usually 30 g l⁻¹), myo inositol, and growth regulators were added. Then the medium's pH level was set to 5.7. In a microwave oven, the medium was slightly warmed, then the requisite amount of agar was added, stirred, and heated to boiling to fully dissolve the agar. After cooling for a few minutes, the media was distributed into culture vessels and non-absorbent cotton plugs were used to seal them. Media in test tubes or flasks was sterilised in an autoclave at 121 °C & 15 psi temperature and pressure, respectively. The cultures were kept at 24 °C in air conditioned laboratory condition having a light/dark cycle of 16/8 h plus 3500 lx intensity of light. For optimum culture establishment, proliferation, and rooting, different concentrations and formulations of PGR's were attempted. Basal and tip segments after sterilization were isolated from scales and placed on the semi-solid MS medium comprising different growth regulator combinations of NAA (Naphthalene acetic acid) and BAP (6-Benzylamino Purine) for establishment conforming to their original polarity. Data on per cent establishment, number of bulblets/explant, length (cm) and number of leaves on established shoots was documented after 4 weeks of culture. From establishment cultures, microshoots of the same size were isolated under aseptic conditions, from the clumps, and placed in proliferation media same as in establishment excluding two concentrations of NAA as there occurred callusing and direct rooting of bulb scales. Percent proliferation, shoot number/explant, length (cm) & number of leaves of proliferated cultures was recorded after 6 weeks of proliferation cycle. Microshoot rooting was standardised using rooting media containing various auxin concentrations (NAA and IBA). Under a laminar hood, uniform shoots were isolated from uniformly proliferated clumps and transferred to test tubes containing root growth media. After 4 weeks of culture, the percent rooting, number, and length (cm) of primary roots/shoot were measured.

2.3. Hardening media, sterilization and acclimatization

Primary hardening of rooted plantlets was done under incubation room conditions in Polystyrene glasses filled with media consisting of perlite + vermiculite (1:1). Equal volumes of perlite and vermiculite were mixed thoroughly and moistened with distilled water. It was then packed in white cloth bags and autoclaved at 121°C and 15psi for one hour. Media was then cooled before being processed till it was required. The media was then dispensed in propylene glasses under laminar cabinet. Rooted *Lilium* plantlets were transferred in the sterile conditions in the laminar air flow chamber, from *in vitro* rooting media in hardening containers. Each container was covered with another propylene glass i.e., inverted

on top of the first, and the rims were sealed with a parafilm strip to maintain proper humidity around the transferred *in vitro* developed plant. Hardening propylene containers containing rooted plantlets for hardening were put in the growth chamber. Once plants showed establishment signs (growth of new leaves), after 10 days of transmission, holes were made to the bottom of upper inverted glass then their subsequent removal after signs of establishment. During the transition process observations were made on an *ex-vitro* survival percent of plantlets after 4th week.

2.4. Statistical analysis

Data for various parameters collected during this study were subjected to the factorial CRD (Completely randomized Design) with 4 replicates per treatment.

3. Results

3.1. Aseptic culture and survival of bulb scale segments

The data presented in Table 1 shows that sterilants used individually or in combination had a significant influence on culture asepsis and survival.

On bulb scale, S₂ (0.1 percent mercuric chloride for 20 min) performed better than the other single sterilant treatments, yielding 54.17% aseptic cultures, while the performance of S₃ (1.0% sodium hypochlorite for 10 min) was poor yielding minimum aseptic cultures. Increase in sterilant treatment duration from 10 to 20 min resulted in increased culture asepsis. Combined treatment of underground Liliium bulb scales with different sterilants significantly increased the culture asepsis, recording maximum aseptic cultures (77.08%) with S₉ (Carbendazim 200 ppm for 30 min, then 0.1 percent mercuric chloride for 10 min, then 70% ethyl alcohol for 30 s). Maximum culture asepsis of 63.54% was recorded with tip scale segments and minimum of 56.46% with basal scale segments.

Table 1
Influence of various sterilant treatments and explant type on culture asepsis (%) and explant survival (%) in Oriental Liliium hybrid cv. 'Ravenna'.

Sterilant treatments	ASEPSIS			SURVIVAL		
	EXPLANT TYPE			EXPLANT TYPE		
	Scale Base* (E ₁)	Scale Tip* (E ₂)	Mean	Scale Base** (E ₁)	Scale Tip** (E ₂)	Mean
S ₁ Mercuric chloride 0.1% for 10 min	41.66 (6.53)	50.09 (7.14)	45.83 (6.84)	97.22 (9.91)	93.75 (9.73)	95.48 (9.82)
S ₂ Mercuric chloride 0.1% for 20 min	52.08 (7.28)	56.25 (7.57)	54.17 (7.42)	93.65 (9.73)	90.08 (9.54)	91.86 (9.64)
S ₃ Sodium hypochlorite 1.0% for 10 min	39.58 (6.36)	47.92 (6.99)	43.75 (6.68)	90.63 (9.57)	87.08 (9.38)	88.85 (9.48)
S ₄ Sodium hypochlorite 1.0% for 20 min	43.75 (6.68)	54.17 (7.43)	48.96 (7.06)	86.46 (9.35)	83.62 (9.20)	85.04 (9.27)
S ₅ Mercuric chloride 0.1% for 10 min followed by ethyl alcohol 70% for 30 s	58.33 (7.70)	66.67 (8.23)	62.50 (7.96)	90.97 (9.59)	87.74 (9.42)	89.36 (9.51)
S ₆ Mercuric chloride 0.1% for 20 min followed by ethyl alcohol 70% for 30 s	66.67 (8.22)	72.92 (8.60)	69.79 (8.41)	85.42 (9.30)	84.97 (9.27)	85.19 (9.28)
S ₇ Sodium hypochlorite 1.0% for 10 min followed by ethyl alcohol 70% for 30 s	56.25 (7.56)	64.59 (8.10)	60.42 (7.83)	85.56 (9.30)	82.89 (9.16)	84.22 (9.23)
S ₈ Sodium hypochlorite 1.0% for 20 min followed by ethyl alcohol 70% for 30 s	64.57 (8.09)	66.67 (8.23)	65.62 (8.16)	82.89 (9.16)	79.62 (8.98)	81.25 (9.07)
S ₉ Carbendazim (200 ppm) for 30 min followed by mercuric chloride 0.1% for 10 min followed by ethyl alcohol 70% for 30 s	72.92 (8.60)	81.25 (9.07)	77.08 (8.83)	88.42 (9.46)	83.82 (9.21)	86.12 (9.33)
S ₁₀ Carbendazim (200 ppm) for 30 min followed by sodium hypochlorite 1.0% for 10 min followed by ethyl alcohol 70% for 30 s	68.75 (8.35)	75.00 (8.72)	71.88 (8.53)	84.17 (9.23)	81.67 (9.09)	82.92 (9.16)
Mean	56.46 (7.54)	63.54 (8.01)		88.54 (9.46)	85.52 (9.30)	
C.D (P<0.05)	0.10		0.23	0.06		0.14

Letters in the parenthesis are square root transformed values of percentage data. *Data recorded after 4 weeks of culture**Data recorded after 6 weeks of culture.

Among the combined treatments, S₅ (0.1 percent mercuric chloride for 10 min, then 70% ethyl alcohol for 30 s) and S₉ (Carbendazim 200 ppm for 30 min, then 0.1 percent mercuric chloride for 10 min, then 70% ethyl alcohol for 30 s) recorded better explant survival rate of 89.36 and 86.12%, respectively. Both these treatments also recorded higher culture asepsis as well. Basal scale segments recorded maximum explant survival (88.54%) and minimum of 85.52% was observed with scale tip segments.

3.2. Culture establishment

All the eight plant growth regulator combinations tested during the investigation resulted in successful culture establishment (Table 2). MS media fortified with (0.50 + 2.0 mg l⁻¹ NAA + BAP) showed best culture establishment (Fig. 1a, b) in terms of better establishment percentage (76.17%), bulblet number per explant (5.52), established length of shoots (2.20 cm) and established shoots leaf number (3.39). Maximum and minimum explant establishment (68.26% and 55.21%), bulblets/explant (4.91 and 3.51), length of established shoots (2.05 cm and 1.81 cm), leaf number of established shoots (3.15 and 2.24) was observed with basal and tip scale segments, respectively.

3.3. Culture proliferation

Established cultures were divided and put into MS media augmented with various concentration of NAA + BAP for culture proliferation. Same growth regulator combinations involving NAA and BAP, which were used in culture establishment, were tried for further culture proliferation except two treatments viz., T₇ (0.50 mg l⁻¹ NAA) and T₈ (1.0 mg l⁻¹ NAA) were discarded because callusing and rooting was detected during establishment phase in these treatments (Fig. 1 c, d). MS- solid media fortified with (0.50 + 2.0 mg l⁻¹ NAA + BAP) showed best culture proliferation (Fig. 1 e, f) resulting in maximum proliferating cultures (83.33%), highest number of shoots/explant (2.41), maximum length (2.35 cm) and

Table 2
Influence of growth regulator combinations and explant type on culture establishment in Oriental Liliun hybrid cv. 'Ravenna'.

Treatment	Growth regulators	Concentration (mg/l)	Establishment (%)			Number of bulblets per explant			Length of established shoots (cm)			Leaf number of established shoots		
			EXPLANT TYPE			EXPLANT TYPE			EXPLANT TYPE			EXPLANT TYPE		
			Scale Base* (E1)	Scale Tip* (E2)	Mean	Scale Base* (E1)	Scale Tip* (E2)	Mean	Scale Base* (E1)	Scale Tip* (E2)	Mean	Scale Base* (E1)	Scale Tip* (E2)	Mean
T ₁	NAA + BAP	1.5 + 1.5	68.75 (8.35)	66.67 (8.2)	67.71 (8.29)	4.38	3.58	3.98	2.07	1.82	1.94	2.92	2.24	2.58
T ₂	NAA + BAP	2.0 + 1.5	54.17 (7.43)	62.50 (7.9)	58.34 (7.70)	3.36	3.44	3.40	2.22	1.95	2.08	3.31	2.66	2.99
T ₃	NAA + BAP	0.5 + 2.0	83.58 (9.20)	68.75 (8.3)	76.17 (8.77)	6.97	4.08	5.52	2.34	2.06	2.20	3.94	2.84	3.39
T ₄	NAA + BAP	1.0 + 2.0	72.92 (8.60)	50.00 (7.1)	61.46 (7.87)	4.92	3.79	4.35	2.32	1.89	2.11	3.60	2.33	2.96
T ₅	NAA + BAP	1.5 + 2.0	64.59 (8.10)	45.84 (6.8)	55.21 (7.47)	5.87	3.75	4.81	2.14	1.77	1.95	3.54	2.17	2.86
T ₆	NAA + BAP	2.0 + 2.0	56.25 (7.57)	43.75 (6.6)	50.00 (7.13)	5.33	3.41	4.37	1.64	1.71	1.68	3.39	2.05	2.72
T ₇	NAA	0.5	75.00 (8.72)	54.17 (7.4)	64.58 (8.07)	4.75	3.29	4.01	1.69	1.61	1.65	2.03	1.77	1.90
T ₈	NAA	1.0	70.84 (8.48)	50.00 (7.1)	60.42 (7.81)	3.75	2.79	3.27	1.95	1.71	1.83	2.45	1.84	2.14
Mean			68.26 (8.30)	55.21 (7.4)		4.91	3.51		2.05	1.81		3.15	2.24	
C.D (P<0.05) Interaction			0.06		0.12	0.31		0.63	0.03		0.06	0.18		0.37
			0.16			0.89			0.08			0.49		

Letters in the parenthesis are square root transformed values of percentage data. *Data recorded after 4 weeks of culture.

leaf number (5.44) of proliferated shoots. Increase in the concentration of auxin from 0.50 – 2.0 mg/l in media fortified with 2.0 mg/l BAP, resulted in the reduction of values of various proliferating characteristics (Table 3). Highest and lowest culture proliferation (75.35% and 68.40%), number of shoots/explant (2.37 and 1.74), length of proliferated shoots (2.21 cm and 1.87 cm), leaf number/proliferated shoot (5.07 and 4.32) was recorded with basal and tip scale segments, respectively.

3.4. Rooting and hardening

The rooting behaviour of microshoots of Oriental *Lilium* hybrid cv. Ravenna was significantly affected by varying combinations of IBA and NAA (Table 4). IBA was found superior than NAA (Fig. 1 g, h). The highest rooting cultures (92.71%), primary roots/shoot number (12.06) and length of primary roots (3.17 cm) were reported in medium augmented by 1.50 mg l⁻¹ IBA. In terms of different explants, highest and the lowest mean rooting (84.12 and 77.55) percentage, root number/shoot (10.02 and 9.08), length of primary roots (2.17 cm and 1.65 cm), was recorded in basal and tip scale segments, respectively.

Significant influence was noticed on the plantlet survival during primary hardening. Rooted plantlets were hardened in media containing perlite and vermiculite (1:1). Highest *ex vitro* survival of 98.96% was observed in plantlets which came from media fortified with IBA (1.5 mg/l). Also highest plantlet survival (93.49%) was recorded in basal scale segments and lowest (84.63%) in tip scale segments (Table 4). Plantlets which came from IBA treated cultures had higher survival rate as compared to NAA treated cultures (Fig. 2 a, b, c). Higher *ex vitro* survival of IBA treated cultures may be attributed to their better rooting characteristics like root number and root length.

4. Discussion

During the current investigation, various sterilants were used to enhance the culture asepsis in *Lilium* like mercuric chloride, sodium hypochlorite and ethyl alcohol individually or in

combination. In contrast to single-chemical sterilization, combined treatments of explants with two or even more sterilants have been found to be very useful for sterilising underground buds/explants (Rather, 2010). The findings show that fungicidal treatment followed by mercuric chloride and ethyl alcohol is necessary for obtaining higher culture asepsis in *Lilium* bulb scale explants. Mercuric chloride is highly antimicrobial with action against both fungi and bacteria. Concentrated ethanol/ethyl alcohol is a potent disinfectant that instantly dehydrates the majority of bacterial and fungal spores. The findings of this study are consistent with those of Taha et al. (2018), Sindhu et al. (2016), Mir et al. (2012), and Pandey et al. (2009) who reported better culture asepsis in *Lilium* with combined sterilization treatments. Also highest culture asepsis was observed in tip scale segments compared to basal scale segments, which recorded minimum. As basal segment of scale is closer to bulb roots and basal plate, while tip segment of bulb scale is away from basal plate and roots. Thus, close proximity to basal plate and roots may be the cause of lower culture asepsis of basal segment explants.

Explant survival data is utilised to get a sense of the negative impact of sterilants on explant establishment and growth (Rather, 2010). Although explant survival was highest with single sterilant treatments compared to combined sterilant treatments, but yielded lower aseptic cultures. These findings are in conformity with those of Rather et al. (2014) who also recorded reduced explant survival under combined treatments in comparison to single sterilant treatments with underground buds of herbaceous peony. Aslam et al. (2013) used a combined sterilization treatment regime including 96% ethanol, 70% commercial bleach and 0.1% HgCl₂ for developing micropropagation protocol of *Lilium orientalis* in addition to *Lilium longiflorum* cv. White Fox.

Overall results indicated that basal scale segments survived better than tip scale segments which may be due to difference in their maturity. Basal segment of the scale is more mature and thicker than tip segment which is thin and tender. Tender explants show more phytotoxic effects of sterilants compared to mature ones and hence less survival. Bhandari and Aswath (2018) used three different forms of explants to create an efficient *in-vitro* culture

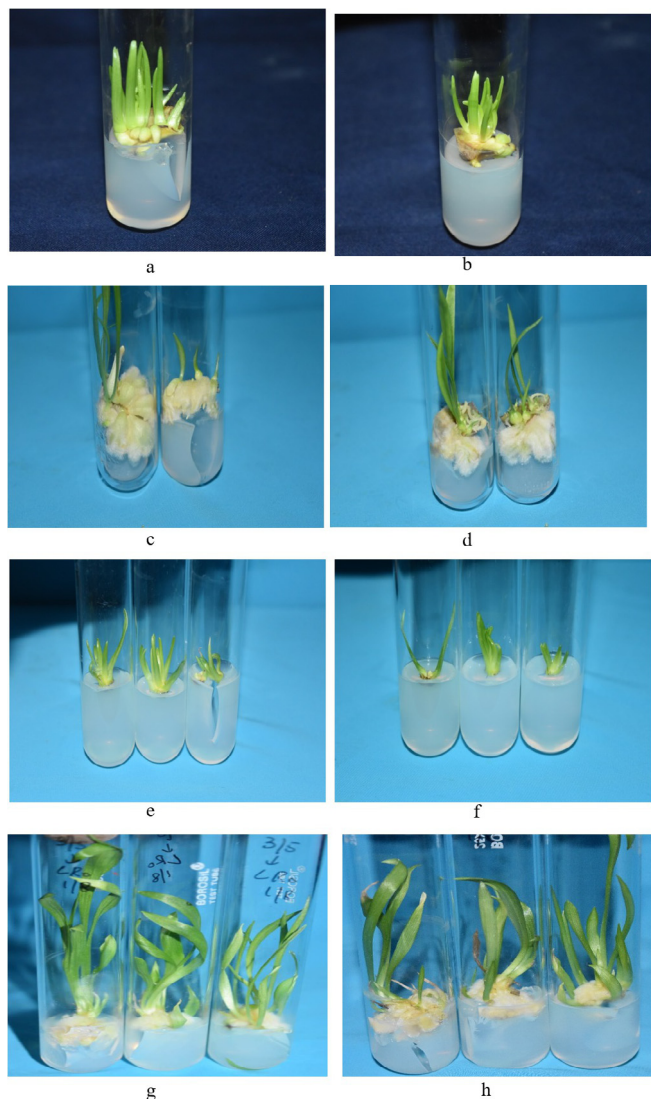


Fig. 1. a, b Establishment of basal and tip scale on MS media augmented with NAA + BAP ($0.50 + 2.0 \text{ mg l}^{-1}$) (c, d) Direct rooting and callusing in basal scale and tip scale segments (e, f) Proliferation of basal and tip scale on MS media augmented with NAA + BAP ($0.50 + 2.0 \text{ mg l}^{-1}$) (g, h) Rooting of Basal and Tip scale segments on MS media augmented with NAA (1.50 mg l^{-1}).

protocol for *Lilium longiflorum* Cv. 'Pavia', including leaves, outer and inner bulb scales. Across all growth parameters, he found that inner scales outperformed the other explants.

All the eight plant growth regulator combinations tested during the investigation resulted in successful culture establishment. MS media augmented with 0.50 mg l^{-1} NAA + 2.0 mg l^{-1} BAP proved best for culture establishment. Similar results were reported by Mir et al. (2012) while working with *Lilium longiflorum*. *In-vitro* effects of NAA & BA on cultural establishment & bulblet development in two lily hybrids, Pollyanna and Stargazer, were documented by Dilta et al. (2000). According to Jamwal et al. (2016), MS basal media augmented with 1.0 mg l^{-1} NAA resulted in the best culture establishment and bulblet multiplication of three

Asiatic *Lilium* hybrids (Prato, Brunello and Dreamland). Wang et al. (2017) recorded that MS + 1 mg l^{-1} (6-BA) + 1 mg l^{-1} (NAA) was appropriate medium for inducing shoots in the *Lilium Martagon* var. pilosiusculum.

Basal scale segments proved better explants recording higher values for establishment parameters as compared to tip scale segment explants. It may be attributed to optimum maturity and healthy status of basal scale segments. Tip scale segments are usually thin and hence weaker than basal scale segments. Superior performance of basal scale segments may also be attributed to the fact that they are close to basal plate of the bulb and contain meristematic cell groups, which develop into adventitious buds.

In the present study culture proliferation in *Lilium*, results indicated that low auxin to cytokinin ratio favored proliferation of *Lilium* cultures. Mir et al. (2012) found similar results in *Lilium longiflorum*. Micropropagation of *Lilium* using bulb scales as explants cultured on MS medium augmented with 0.50 mg l^{-1} NAA + 2.0 mg l^{-1} BAP resulted in maximum proliferation, according to Pandey et al. (2009). Sindhu et al. (2016) discovered that MS media augmented with 0.2 mg l^{-1} NAA and 1.0 mg l^{-1} BAP was most successful for shoot initiation and proliferation in Asiatic *Lilium* cv. Pollyanna. On MS medium augmented with 1.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA, the maximum number of shoots, leaves, and bulb-scales were found (Youssef et al., 2019). Cytokinin's are well-known to inhibit elongation of terminal buds & encourage proliferation of axillary shoots when present in sufficient amounts. As a result, determining the optimal cytokinin level for optimal shoot development is a prerequisite for rapid multiplication of any specie.

Effect of different dosages of IBA & NAA on root activity of Oriental *Lilium* hybrid cv. Ravenna was studied in the current investigation. IBA was found superior than NAA. Sindhu et al. (2016) also reported better performance of IBA than NAA in rooting of micro-shoots of Asiatic *Lilium* cv. Pollyanna with 1.0 mg l^{-1} IBA mixed in half-strength MS medium. The use of auxins, especially IBA, has been shown to improve rooting percentage and quality. Arbaoui et al. (2017) used IBA (0, 0.50, 1.0, 1.50 and 2.0 mg l^{-1}) to root *Lilium longiflorum* *in vitro* shoots and discovered that 1.50 mg l^{-1} IBA was the best concentration for different rooting characteristics.

The hardening system's principle goal is to preserve a low-evaporative-demand environment around a tissue cultured plant that has just been emancipated from a suffocating system. Before the hardening module begins, the plant can avoid large water deficits from tissues. Hardening medium is a crucial element in the *ex-vitro* establishment of micro propagated plants. Rooting media for hardening of tissue cultured plantlets had already been standardized in the Division's Plant Tissue Culture laboratory, which consists of perlite + vermiculite in the ratio of 1:1 as reported by Farooq et al. (2021). The standardized hardening media resulted in more than 80% *ex vitro* survival of rooted plantlets. Hardening *in-vitro* rooted bulblets of *Lilium* Cv. 'Toscana' was reported to be comparable with coco-peat, peat-moss, and coco-peat in conjunction with peat-moss offering 100 percent survival (Kaur et al., 2006). Kaviani and Javaheri (2021) observed that the plantlets which were shifted to a growing medium comprising cocopeat, peat moss and perlite in identical proportion for acclimatization following proliferation. Approximately, 90% of regenerated plantlets survived and were morphologically similar to the mother stocks.

Table 3
Influence of growth regulator combinations and explant type on culture proliferation in Oriental Liliium hybrid cv. 'Ravenna'.

Treatment	Growth regulators	Concentration (mg/l)	Proliferation (%)			Number of shoots/explant			Length of proliferated shoots (cm)			Leaf number of proliferated shoots		
			EXPLANT TYPE		Mean	EXPLANT TYPE		Mean	EXPLANT TYPE		Mean	EXPLANT TYPE		Mean
			Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base* (E ₁)	Scale Tip* (E ₂)	
T ₁	NAA + BAP	1.5 + 1.5	70.83 (57.35)	66.67 (54.74)	68.75 (56.04)	2.65	1.72	2.18	2.35	1.91	2.13	4.88	4.40	4.64
T ₂	NAA + BAP	2.0 + 1.5	81.25 (64.37)	72.92 (58.62)	77.08 (61.50)	2.70	1.86	2.28	2.43	1.94	2.19	4.93	4.94	4.93
T ₃	NAA + BAP	0.5 + 2.0	87.50 (69.54)	79.17 (62.83)	83.33 (66.18)	2.90	1.92	2.41	2.59	2.12	2.35	5.77	5.12	5.44
T ₄	NAA + BAP	1.0 + 2.0	77.08 (61.40)	70.84 (57.35)	73.96 (59.37)	2.34	1.72	2.03	2.23	1.85	2.04	5.13	4.38	4.76
T ₅	NAA + BAP	1.5 + 2.0	70.84 (57.35)	62.50 (52.23)	66.67 (54.79)	2.00	1.62	1.81	1.94	1.79	1.87	5.12	3.74	4.43
T ₆	NAA + BAP	2.0 + 2.0	64.59 (53.47)	58.34 (51.24)	61.46 (52.36)	1.67	1.58	1.62	1.74	1.63	1.68	4.57	3.37	3.97
Mean			75.35 (60.58)	68.40 (56.17)		2.37	1.73		2.21	1.87		5.07	4.32	
C.D (P<0.05)			1.40		2.42	0.07		0.12	0.08		0.13	0.28		0.48
Interaction			NS			0.17			0.19			NS		

Letters in the parenthesis are arcsine transformed values of percentage data. *Data recorded after 4 weeks of culture.

Table 4
Influence of various growth regulator combinations and explant type on rooting in Oriental Liliium hybrid cv. 'Ravenna'.

Treatment	Growth regulators	Concentration (mg/l)	Rooting (%)			Number of primary roots/shoot			Length of primary roots (cm)			Hardening%		
			EXPLANT TYPE		Mean	EXPLANT TYPE		Mean	EXPLANT TYPE		Mean	EXPLANT TYPE		Mean
			Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base* (E ₁)	Scale Tip* (E ₂)		Scale Base** (E ₁)	Scale Tip** (E ₂)	
T ₁	NAA	0.5	75.00 (8.72)	72.92 (8.60)	73.96 (8.66)	7.55	7.03	7.29	0.77	0.59	0.68	87.50 (9.41)	72.92 (8.59)	80.21 (9.00)
T ₂	NAA	1.0	77.08 (8.84)	75.00 (8.72)	76.04 (8.78)	8.30	7.65	7.98	0.84	0.66	0.75	89.58 (9.52)	77.08 (8.83)	83.33 (9.17)
T ₃	NAA	1.5	83.33 (9.18)	79.17 (8.95)	81.25 (9.07)	11.15	9.68	10.41	1.33	1.02	1.17	93.75 (9.73)	83.33 (9.17)	88.54 (9.45)
T ₄	NAA	2.0	66.67 (8.23)	64.59 (8.10)	65.63 (8.16)	9.78	8.80	9.29	1.86	1.49	1.67	89.58 (9.52)	75.00 (8.71)	82.29 (9.11)
T ₅	IBA	0.5	87.50 (9.41)	77.08 (8.84)	82.29 (9.12)	8.85	8.53	8.69	2.51	2.10	2.30	93.75 (9.73)	83.33 (9.18)	88.54 (9.45)
T ₆	IBA	1.0	93.75 (9.73)	82.92 (9.16)	88.33 (9.45)	10.05	9.55	9.80	3.22	2.27	2.74	97.92 (9.94)	91.66 (9.62)	94.79 (9.78)
T ₇	IBA	1.5	97.92 (9.94)	87.50 (9.41)	92.71 (9.68)	13.40	10.73	12.06	3.57	2.77	3.17	100.00 (10.05)	97.92 (9.94)	98.96 (10.00)
T ₈	IBA	2.0	91.67 (9.63)	81.25 (9.07)	86.46 (9.35)	11.08	10.65	10.86	3.28	2.35	2.81	95.83 (9.83)	95.83 (9.83)	95.83 (9.83)
Mean			84.12 (9.21)	77.55 (8.85)		10.02	9.08		2.17	1.65		93.49 (9.72)	84.63 (9.23)	
C.D (P<0.05)			0.08		0.16	0.45		0.91	0.08		0.15	0.18		0.37
Interaction			0.23			1.28			0.21			NS		

Letters in the parenthesis are square root transformed values of percentage data.*Data recorded after 4 weeks of culture. ** Data recorded after 8 weeks of culture.

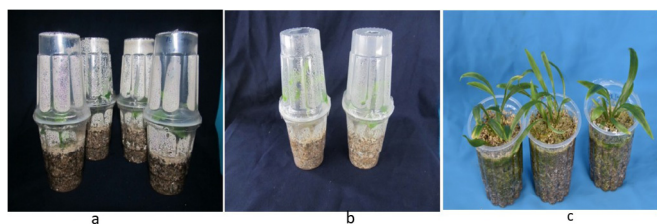


Fig. 2. a,b,c Hardening of rooted plantlets.

5. Conclusions

Combined sterilization of explants with Carbendazim 200 ppm for 30 min, then 0.1 percent mercuric chloride for 10 min, then 70% ethyl alcohol for 30 s lead to maximum culture asepsis and optimum explant survival. Best growth regulator combination for culture establishment was found to be 0.50 mg l⁻¹ NAA + 2.0 mg l⁻¹ BAP. Proliferation of developed shoots was also maximum in media augmented with 0.50 mg l⁻¹ NAA + 2.0 mg l⁻¹ BAP. MS media augmented with IBA 1.50 mg l⁻¹ showed the best rooting results. Basal

scale segment explants proved better than tip scale segments, showing superior performance in all establishment, proliferation and rooting parameters. Standardized rooting media (perlite and vermiculite in the ratio of 1:1) resulted in more than 80% rooted plantlets' *ex vitro* survival. Plantlets grown in media augmented with IBA (1.50 mg l^{-1}) had the best *ex vitro* survival.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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