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In Vitro Micropropagation of *Lilium candidum* Bulb by Application of Multiple Hormone Concentrations Using Plant Tissue Culture Technique

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ABSTRACT

The multiplication efficacy by bulb is low and the plantlets are more susceptible to disease, therefore, there is a need to develop a protocol for its propagation. *Lilium candidum* is listed in the saitma prefecture Red Data Book as a critically endangered plant and rescuing information regarding its micro-propagation is rather limited. On this regard, the application of in vitro micropropagtion procedure might help to obtain large numbers of uniform plants of endangered species of Lilium. Dried lilies are a rich source of fiber and also rich in sodium and carbs. Lily bulbs have proteins and starch and also small quantities of iron, calcium, phosphorous, and vitamin B1, B2, C. The health benefits of the lily for the heart are well known on account of the active cardiac glycosides as well as the flavonoids which tend to stimulate the arteries and can cause them to dilute. Another one of the therapeutic uses of the lily flower is in the case of treating burns and preventing the formation of scar tissue. One of the main health benefits of the lily flower is that it helps regulating the heart rate there by allowing the heart to function more efficiently and regular. Having multiple medicinal properties we decided to cultivate *Lilium candidum* using plant tissue culture so farming can be increased using this cost efficient techniques. In this research, we have studied various Effect of different concentration of BAP and NAA on the initiation of *Lilium candidum* from bulb and IBA, IAA and NAA on the rooting of shoots of *Lilium Candidum*.

Keywords- Micropropagation, Lilium bulb, *Lilium candidum*, medicinal plant, therapeutic, standardization of growth regulators.

I. INTRODUCTION

Lilium is a genus of herbaceous flowering plant growing from bulbs, all with large prominent flowers. Lilies are a group of flowering plant which are important in culture and culture and literature in much of the world .Most species are northern to temperate hemisphere , though their range extends into the northern hemisphere ,though their range extends into the northern subtropics. Many other plants have, 'Lily' in their common name but are not related to true lilies.

Turkey is very rich in bulbous, rhizome and tuberous plants (geophytes) in line with itsoverall crop wealth. Six different species of the genus *Lilium* grow in Turkey. *Lilium candidum*. One of these species is a rare plant and grows in southwest Anatolia, Turkey (Davis 1984). Genus *Lilium* is commonly used as cut flower and *L. candidum* has also been used as with aromatic andornamental plants and exported for years, but it is now an endangered plant (in the category of vulnerable plants-VU). Generally, in micropropagation of flower bulbs, it is noted that a low concentration of auxinpromotes the formation of plantlets and cytokinins stimulate the number of plantlets. Besides, highconcentrations of MS salts, vitamins and additional sucrose have positive effects and compared to the concentration used for other plants.

Lilium, an important genetic resource as an ornamental plant belongs to family liliaceae. It is one of the leading cut flower crops in world because of beautiful and fascinating form of flowers, long vase life and capacity to rehydrate after long transportation. Lilium propagation is usually produces 3-4 bulbs per scale depending on size and variety. The multiplication efficacy by bulb is low and the plantlets are more susceptible to diseases. Therefore there is need to develop a protocol for its mass propagation. With the advent of tissue culture technique, a new era has dawned the research and development of floriculture. Through tissue culture, there is not only a continuous supply of bulbets but true-to-type and disease free plants can be obtained. The genus of lilium approximately 220 species belongs to large family of the liliaceae.

At present 80 species of Lilium are found in the temperate and subtropical zones of the northern hemisphere (woodcock and stearn 1950; Feldmaier and McRae, 1982) and 47 species 18 varieties of Lilium are mainly for the edible oils and medicinal use. Its heterozygous state and self hamper convential breeding in the lily in compatibility among the species of the different Lilium groups (Van Tuy l et. al., 1990). The development of efficient systems for transformation could be accelerating the breeding process, but these techniques require an efficient and reproducible protocol for the induction of adventitious shoots. In lily, plant

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regeneration has been achieved from a vast array of explants ranging from flower organs to bulb scales (Han et al., 2002; Kumar et al., 2005).

Lilium species is one of the most important flower species bulb corps in commercial market. About 8000 species cultivars have been registered. They are classified into three groups, *Longiflorum*, Asiatic and Oriental hybrids. The lily bulb is mainly for vegetative propagation, breeding bulb quantity easily degenerated and their breeding cycle is longer. Tissue culture can not only solve the degradation of the lilies but also can solve the problem of detoxification and propagation. The tissue culture techniques have been successful for rapid propagation of some members of the genus *Lilium Candidum, L. Longiflorum (Nhut.1997), L. rubellum (Nillmii et al., 1997), L. lancifollium (Mariangeli and Curvetto, 1997), L. auratum (Takayama and Misawa, 1979, 1980, 1983), and L. testaceum (woziniewski et al., 1991).* The objective of present study was to establish cultural conditions where shoot organogenesis from bulb scales could be improved. Furthermore, the results of micropropagation that used bulb scales segments are Natural habitats of lilies are mostly on high altitudes, reaching up to over 2000 meters from the sea level. Their adaptation to extreme annual changes in temperature and humidity makes them suitable garden plants in a large climatic area from temperate to subarctic latitudes.

The botanical name Lilium is the Latin form and is a Linnaean name. The Latin name is derived from the Greek, Leirion, generally assumed to refer to true, white lilies as exemplified by the Madonna lily. The word was borrowed from Coptic hreri. From standard hreri, from Demotic hrery, Rom Egyptian *hr. rt* 'flower'. Millet maintains that both the Egyptian and the Greek word are possible loans from an extinct, substratum language of the Eastern Mediterranean. The Greeks also used the word albeit for non-white lilies. Many species are widely grown in the garden in temperature and sub-tropical regions. They may also be grown as potted plants. Numerous ornamental hybrids have been developed. They can be used in herbaceous borders, woodland and shrub plantings, and as patio plants. Some lilies, especially *Lilium longiforum*, form important cut flower crops. These may be forced for particular markets; for instance *Lilium longiforum* for the Eastertrade, when it may be called the Easter lily. Lilies are usually planted as bulbs in the dormant season. They are best planted in a south-facing (northern hemisphere), slightly sloping aspect, in sun or part shade, at a depth 2 times the height of the bulb (except *Lilium candidum*) which should be planted at the surface. Most prefer a porous, loamy soil, and good drainage is essentials. Most species bloom in July or August. The flowering periods of certain lily species begin in late spring, while others bloom in late summer or early actumn. They have contractile roots, which pull the plant down to correct depth; therefore, it is better to plant them too shallowly than too deep. Day lilies (Hemero callis, not true lilies) are excluded from this category. Plants can suffer from damage caused by mice, deer and squirrels. Slugs, snails and millipedes attack seedlings, leaves and flowers. Brown spots on damp leaves may signal botrytis. Various fungal and viral diseases can cause mottling of leaves and stunting off.

The dried bulbs are commonly used in the south to flavor soup. Lily flowers are also said to be efficacious in pulmonary affections, and to have tonic properties. Lily flowers and bulbs are eaten especially in the summer, for respective perceived ability to reduce internal heat. They may be reconstituted and stir- fried, grated and used to thicken soup, or processed to extract starch. Their texture and taste draw comparisons with the potato, although the individual bulb scales are much smaller. There are also species, which are meant to suitable culinary and herb uses. There are five traditional lily species whose bulbs are certified and classified as 'Vegetable and non- Staple foodstuffs' on the National geographical indications product of china. Dried lilies are a rich source of fiber and also rich in sodium and carbs. Lily bulbs have proteins and starch and also small quantities of iron, calcium, phosphorous, and vitamin B1, B2, C. One of the main health benefits of the lily flower is that it helps regulating the heart rate there by allowing the heart to function more efficiently and regularly. The lily flower has mainly therapeutic uses as it minimizes the irritability of the myocardium without actually resulting in an increase in the oxygen that is required to function by the heart muscle. The health benefits of the lily for the heart are well known on account of the active cardiac glycosides as well as the flavonoids which tend to stimulate the arteries and can cause them to dilate. Another one of the therapeutic uses of the lily flower is in the case of treating burns and preventing the formation of scar tissue. In this herbal remedy, the roots of the lily flower are used to prepare on ointment, which is then applied on burnt area. The therapeutic uses of the lily flower also include it being used as in case of weak contractions at the time of childbirth. The lily flower is also used as for herbal remedies for medicinal conditions such as leprosy, conjunctivitis, strokes, angina and so on. Another medicinal uses of the lily flower is that the dried flowers are used as a very effective laxative or diuretic. Additionally, the roots and the flowers of the lilies are also used to treat spider bites. In some parts of the world, roots of the wood lily variety of the flower are consumed as medicinal teas to treat coughs fevers, stomach disorders as well as for open sores, wounds and to wash bruises may have swollen. Another one of the health benefits and therapeutic uses of the lily flower include the juice of the fresh bulbs of the lily flower being used to cure dropsy naturally. The extract of the lily flower is also used in various cosmetics as cure for cuperosis.

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II. REVIEW AND LITERATURE

Successful in vitro culture depends of several factors such as; culture media, light conditions, concentration, combination of plant growth regulators, and other media constituents and explants type. Several Lilium species have been successfully propagated *In vitro* through direct regeneration or by intermediate callus formation Arzate- Fernandez (2002), Klerk (2012) Lilium (Lilium Spp.) were usually propagated in two ways. Sexually by seeds and by scaling. Propagation by seeds is not a practical method because of the extra ordinarily slow growth of bulbs until flowering and at large scale vegetative propagation; it is difficult to obtain a large number of bulbs in a short time. He used different hormones are based cytokinin, which is frequently used in micropropagation. Amaury-M. Arzate-Fernández *et al.*, (2007) reported that *in vitro* propagation of miyamasukashi-yuri (*Lilium maculatum* thunb.var. *bukosanense*), an endangered plant species *Lilium maculatum* var. *bukosanense* (Honda) Hara, called Miyamasukashi-yuri in Japanese, is a wild lily, considered as critically endangered in Japan. Before *L. maculatum* var. *bukosanense* is lost, attempts for *in vitro* propagation can be performed to provide information for its rescue, efficient *ex situ* conservation as well as for possibleuse in breeding programs to be used as ornamental plant. For bulblet induction, five scales from aseptic bulbs were used for each treatment and placed in the MS (half-strength) medium. Arzate *et al.*, (2003) reported, observe and analyzed the somaclonal variatioin micro propagated lilium and they also reviewed of the factors influencing the genetic micro propagated lilium. On the other hand, lily breeders are interested in producing a variety of flowers colours and shapes of Asiatic and Oriental lily types. Davis *et al*., (1984) reported the micropropagation of *Lilium candidum* l. : a rare and Native bulbous flower of turkey *Lilium candidum* bulb scales were cultured on MS containing different doses and combinations of NAA, BA, Kn and 2iP. Maximum bulblet formation was 88.2% in MS supplemented with 0.1 mg/l NAA + 0.01 mg/l BA

Jeong *et al.,*(2007) reported that conducted experiment on the bulb scale experiment, Lilium bulb culture for the propagation conservation of lilium and distribution of its growth stages. He concluded that bulb culture is a simple techniques. Kapoor *et al.*, (2007) to regenerate bulbets from ex vitro node explants of hybrid lilies. Node sections (3-4 mm) isolated from the middle part of the stem in hybrid lilies were cultured on Murashige and Skoog (MS) medium supplemented with several combinations of NAA and BAP. A significant increase in the percent of explant producing bulbet and number of bulbet per explants was observed when 0.2mg/l NAA was used in combination with 1.0 mg/l BAP. The heaviest bulbets were obtained with 0.2 mg/l NAA with 1.0mg/l BAP after 5-7 week of culture, 2.0 mg/l BAP and 0.5 mg/lNAA was most effective in producing root. The rooted bulbets were hardened with 66% Survival success after 30 days of transfer in the pots. Kantamaht Kanchanapoorm *et al.*, (2012) reported the effect of chitosan on regeneration of lily (*lilium longiflorum* thunb. 'Ester lily') from bulb scale explants cultured *in vitro.* Marco Polo *et al*., (1984) reported the it obtained from the Department of Floriculture and Landscaping, University of Horticulture & Forestry, Solan (H.P.) India. The bulbs were grown in earthenware pots (25 cm diameter) containing sand, soil and farmyard manure (FYM) mixed in the ratio m 1:1:1 under the bulblets were treated with 0.2% bavistin (Carbendazim, a fungicide) and stored in coco peat at2°C. The survival percentage of the bulblets was recorded after 30 days of storage. All data were analyzed using completely randomized design (Gomez and Gomez, 1984). Marijana skoric *et al.*, (2011) reported the rapid, one-step method for direct leafy and rooted bulblets regeneration of *Lilium martagon* var. *cattaniae* Vis. was established using seeds as the starting explants for *in vitro* culture initiation. Adventitious bulblets were regenerated from one scale explants on MS (Murashige and Skoog, 1962). Pejman Azadi and Morteza Khosh-Khui (2007) reported that and Standardized the protocol for the micropropagation in different harvesting time of *Liliumledebourii* (Baker) Boiss, Pandey *et al*., (2009) reported that micropropagtion of *Lilium, with* explants (bulb scales) Were surface sterilized with 0.1% $HgCl_2$ (4-5 min) and 1% Bavistin (10 min) and inoculate on MS medium supplemented with 0.5mg/l (NAA)+2.0mg/l (BAP) which resulted in maximum proliferation.

Ranjan Kapoor *et al*., (2008) reported the *in vitro* bulblet productivity in different explants of hybrid lilies *Lilium* is one of the most important floriculture crops and is in great demand as cut flower and potted plant. Van Aartrijk *et al*., (1990) reported the main constraints in conventional propagation of lilies include the inadequate availability of healthy, disease-free planting material, and slow multiplication rates. Thus scaling a bulb yields somewhere between 50 and 100 bulblets, far too few to meet the present demand for planting material (Varshney, 2000). (Jeong, 1996; Varshney *et al*., 2001; Bahr & Compton, 2004)One of the best and most prolific vegetative propagation methods for lilies is *in-vitro* scale culture. *In vitro* adventitious bud regeneration from scales of *Lilium* depends on factors such as concentrations of plant growth regulators employed, (Jeong, 1996; Varshney *et al*., 2000), concentrations of sucrose (Jeong, 1996; Varshney *et al*., 2000; Kumar *et al*., 1104 K.saifullah *et al*., 2005), light treatment scale position or kind of explant (Jeong, 1996; Varshney *et al*., 2000). The current study reports developing a micropropagation of regime for *L. regale* in which regeneration, followed by production of adventitious shoots on a large scale was optimized, and it is envisaged to yield showy, attractive flowers. Therefore micropropagation by means of *in-vitro o*f the axillary buds used asexplants, aiming at the

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maximization of the multiplication rate in order to make this a Blom- Barnhoorn, (G.J and J.V An Aartijk. 1985). The regeneration of plants free of LSV and TBV from infected *Lilium* bulb-scale explants in the presence of virazole

III. MATERIAL AND METHODS

Collection of Plant Material:

Healthy lilium plants were collected from Sanjay Nursery Solapur Road, Hadpsar, Dist- Pune 410 506.

The fresh middle scales of fresh bulbs and free from disease were separated from mother plant and washed with water thoroughly for sterilization and initiation.

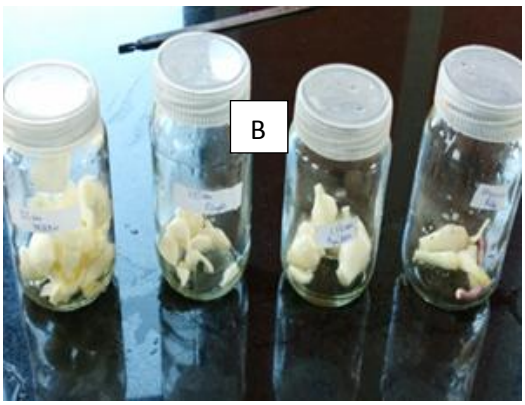

Plate 1: Lilium bulbs separated from mother plant for inoculation

Sterillization Glasswares

The glasswares such as culture bottles, measuring cylinders and the other equipments like forceps, cutting paper and blade holder were washed in running tap water using detergent followed by rinsing with double distilled water and making up the required volume with double distilled water.

Preparation of stock solution

Separate stock solution of macronutrients, micronutrients, potassium iodide, iron, Inositol glycine and various vitamins were prepared by dissolving each chemical separately in small quantity of double distilled water. The stock solutions of growth regulators were prepared by dissolving them in small quantity of appropriate solvents, heating gently and then making up the volume with double distilled water.

Media preparation

According to the available literature on *in vitro* propagation of lilium, Murashige and Skoog's (1962) medium is the most commonly used growing medium for lilium. The procedure for composition of 1 litre, MS medium was used.

Precautions in the preparation of media

1. All the stock solution was stored in glass bottles under refrigeration.
2. Iron stock was stored only in an amber coloured bottle.
3. The bottle must thoroughly washed by double distilled water.
4. Details about the date and concentration of all stock solution were labeled with glass marking pen.

Surface sterilization

Explant were selected (Bulbs) & were cut in appropriate size (2 to 3cm). Explant Bulbs washed under running tap water For 30 min. Bulbs treated with Bavistin 0.1% For 10 min. Explant washed with distilled water for 5-6 times. Explants bulbs treated with Tween-80 (2-3drops), After that Sodium hypochloride for 30 min. Explants bulbs washed with distilled water 5-6 times (30min) Again explant treated with Bavistin for 30min, In LAF. Bulbs treated with 70% IPA for 30 sec, In LAF. Explants washed with sterile distilled water 4-5 time. Explant bulbs treated with 0.1% $HgCl_2$ for 4 min. Explant bulbs were thoroughly washed with sterile distilled water for 4-5 times.

Surface Sterillized Lilium Bulbs

Inoculation of explants

All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. Before use, the working surface of the laminar air flow cabinet was cleaned by swabbing with 70% ethyl alcohol and switch on the U.V light for 20 min. to reduce the chances of contamination. After that, the UV light became switched "off" and switch "on" the ordinary light.

The instruments like scalpels, forceps were sterilized by an alcoholic dip followed by flaming inside

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the laminar airflow cabinet. Other requirements like bottles, conical flasks, cotton, distilled water etc. were sterilized by steam sterilization method. Before the onset of inoculation, hands were washed thoroughly by soap and then swabbing with 70% ethyl alcohol. Initiation media were divided in to 5 parts given below. Each part contains 1 litre MS medium. First part was free from growth regulators and remaining four parts contains different concentrations of growth regulators.

Table 1: Media Combinations for Initiation

Sr. No.	Medium+ Hormones	Code
1	MS Medium	L1
2	MS+1.0mg/l BAP+0.2mg/lNAA	L2
3	MS+2.0mg/l BAP+0.2mg/l NAA	L3
4	MS+3.0mg/l BAP+0.2mg/l NAA	L4
5	MS+5.0mg/l BAP+0.2mg/l NAA	L5

After surface sterilization, explants were transferred to large sterile glass plate having sterile cardboard paper on it, with the help of sterile forceps under strict aseptic conditions in laminar airflow cabinet. Then explants were cut into very small pieces (about 2.5cm) with sterile scalpel. The bottles containing initiation medium prepared as given in (Table 3.10), were unplugged by holding them over spirit lamp and inoculations were performed by placing explants on the surface of the medium with the help of flame sterilized long forceps and replacing the cap of the bottle. During inoculation, the explants were properly positioned on the media and were gently pressed with forceps to secure their firm contact with the media.

After vertically inoculating explants in to culture bottles the mouth of bottles were quickly flamed and bottles were tightly capped and properly sealed with kiln film to avoid entry of external air. After that properly labeled media, number of bottles and date of inoculation etc. The bottles were transferred to growth room. Data were recorded after five weeks of initiation in terms of average shoot length (cm), average number of shoots per explant and survival percentage.

Incubation of culture (6 week): The inoculated culture bottles were incubated at 25±2˚C temperature,24 hour dark period cool temperature in culture room, under the relative humidity maintain of about 70%,.

Shoot Multiplication (6week): Shoot multiplication was carried out using the 2-3 cm long and most vigorous bulb scale from the initiation stage. The bulb which had grown up to suitable sizes were excised and transferred to MS media supplemented with NAA and BAP and sucrose concentration 3%. Proliferated bulblets bud were cut and cultured on shoot multiplication media and incubated the culture room. Shoot multiplication media (5lit. as given in table 3.11) were divided into 5 parts as given below. Each contains 1 litre MS medium. First part was free from growth regulators and remaining four parts contains different concentrations of growth regulators (BAP and NAA).

Table 2: Media combinations for shoot multiplications

Sr. No.	Media Combination for Shooting	Codes
1	MS medium	LR1
2	MSmedium+0.5mg/l BAP +0.1mg/l NAA	LR2
3	MS Medium+1.0 mg/l BAP+0.2 mg/lNAA	LR3
4	MS medium+1.50mg/l BAP+0.3mg/lNAA	LR4
5	MS medium+2.0mg/l BAP +0.5 mg/l NAA	LR5

Within 40 days each pseudo bulb developed into a rosette of shoots. The number of shoots on each rosette varied within the amount of 6-BAP and NAA. Shoot proliferation was determined after 6 weeks of culture. Data were recorded in terms of average number of shoots per explants.

Rooting 6 (Weeks): For rooting shoots (length about 3cm) were excised, and placed vertically into strength MS medium containing NAA concentration 0.1mg/l with sucrose 6%without using, Activated charcoal, At a P^H5.7.The cultures were incubated for two months at 25±2˚C under 24 hours dark period. The root induction was observed within 6 week.

Table 3: Media combination for rooting

Sr. No.	Media + Hormone concentration	Code
1	MS+0.5mg/l IAA+0.5mg/l IBA+0.1mg/l NAA	LR1
2	MS+1.0mg/l IAA+0.5mg/l IBA+0.2mg/l NAA	LR2
3	MS+1.5mg/l IAA+0.5mg/l IBA+0.3mg/l NAA	LR3
4	MS+2.0mg/l IAA+0.5mg/l IBA+0.4mg/l NAA	LR4

After inoculation of shoots were transferred root induction media containing NAA, IBA, IAA. The culture bottles of rooting were transferred to growth room under controlled condition.

IV. RESULT AND DISCUSSION

1) Establishment of Lilium plant

Bulb shoot initiation and establishment from bulbs explants are cultured on MS basal and MS

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supplemented with different concentration of growth hormones. BAP 1.0 ,2.0,3.0,5.0 mg/l was successfully used. In present work same media concentrations has been used.

Data were obtained after 5 weeks of initiation of culture showed that sprouted buds of *LiliumCandidum*, could be established at all tested media including the control medium (free from growth regulators). The best result was obtained on MS medium supplemented with, 1.0 mg/l BAP + 0.2mg/l NAA (Table 4.1). The maximum sprouting of bulb scale number 4 or shoot length (average shoot length 2.85±0.59 was observed on medium supplemented with 1.0mg/l BAP +0.2mg/l NAA showed in (Table 4.1)

Table 4.1: Observation of various combination of hormone BAP and NAA on sprouting of bud by using bulbs scale as explants of *Lilium candidum* after 6 week

SR. NO	Media code	MS +Growth Harmones mg/l	No. of bulbet sprouting	Average shoot Length Mean+S.E(cm)	% Survival
1	LM1	MS Medium	1	2.75±0.575	40
2	**LM2**	**MS+1.0mg/lBAP+0.2mg/ l NAA**	**4**	**2.85±0.59**	**80**
3	LM3	MS+2.0mg/lBAP+0.2mg/l NAA	3	2.1±0.52	60
4	LM4	MS+3.0mg/l BAP+0.2mg/l NAA	2	1±0.42	30
5	LM5	MS+5.0mg/l BAP+0.2mg/l NAA	2	1.35±0.51	50

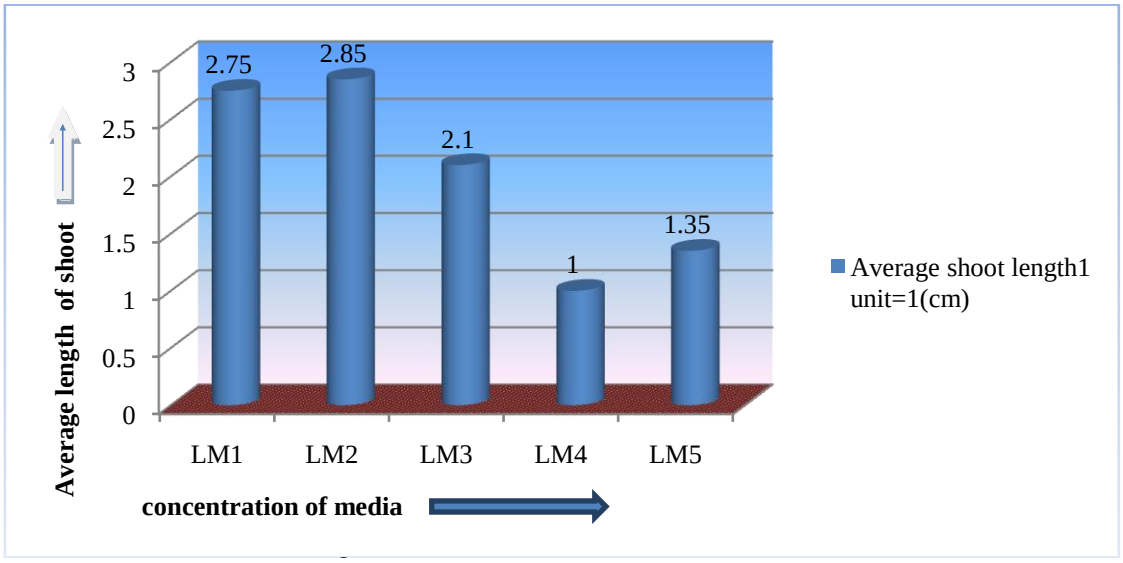

Figure 1: Effects of Various combinations of BAP and NAA on bulb

Plate 4.1: Initiation results

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2) Shoot multiplication (6 weeks)

The buds sprouting in the initiation were subjected to the multiplication on the different media and the growth was observed after 6 weeks of sub culturing. The media with all concentration of BAP and NAA showed multiple shoot generation and proliferation. The average number of shoots formed on all concentration of BAP and NAA. Average shoot length is 1.87±0.52 cm per pseudo bulbet of explant. Best growth observed on 2.0mg/l BAP+ 0.5 mg/l NAA. Where the, Mean number of shoots per pseudo bulbet 4.1±0.1.The result showed that no. of shoots formed per explants was increased with increase in concentration of the BAP and NAA. The BAP promotes cell division and shoot formation. Exceptionally media with 0.1mg/l NAA and 0.3 mg/l NAA were observed low growth.

Table 4.2: Observation of various combination of hormone BAP and NAA on sprouting of bud by using bulbs scale as explants of *Lilium candidum* after 6 week.

SR. No	Media code	Media concentration	Average no. of shoots per pseudo bulbets Mean± S.E	Average no. of shoot length Mean ± S.E	% survival
1	LM1	MS Medium	2.0±0.1	1.02±0.40	65
2	LM2	MS+0.5mg/l BAP+0.1mg/l NAA	2.1±0.1	1.08±0.52	75
3	LM3	1.0mg/l BAP+0.2mg/lNAA	3.1±0.1	0.4±0.89	70
4	LM4	1.5mg/l BAP+0.3mg/lNAA	3.7±0.1	0.28±0.28	65
5	**LM5**	**2.0mg/l BAP+0.4mg/l NAA**	**4.1±0.1**	**1.87±0.52**	**85**

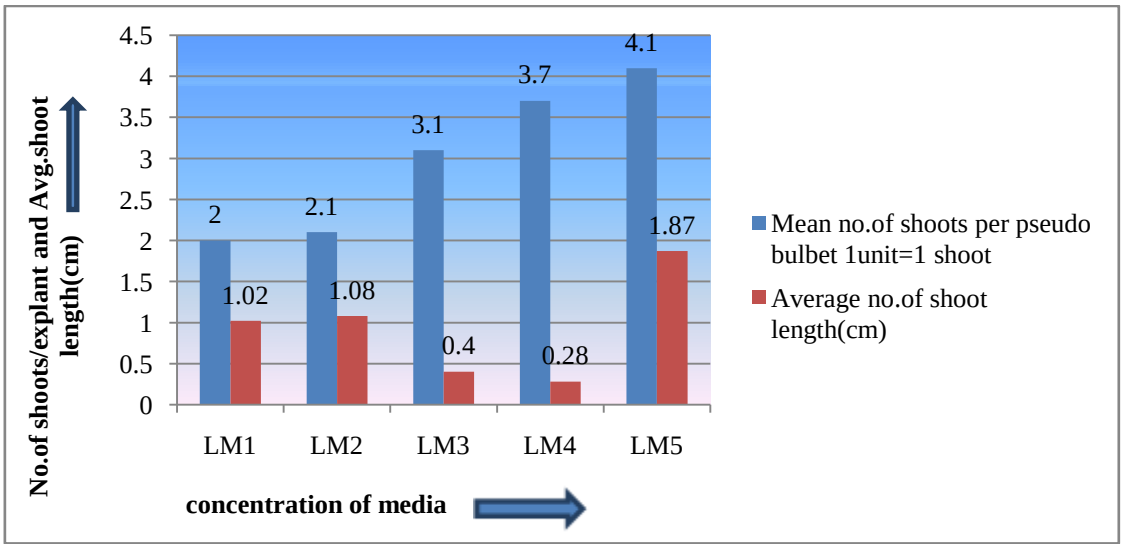
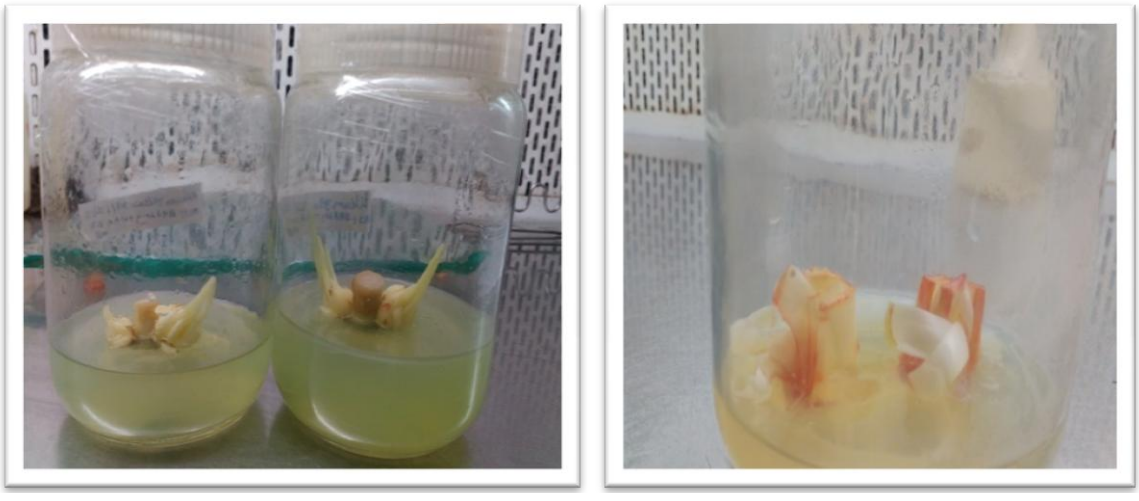


Figure 4.2: Effect of BAP and NAA on shoot multiplication



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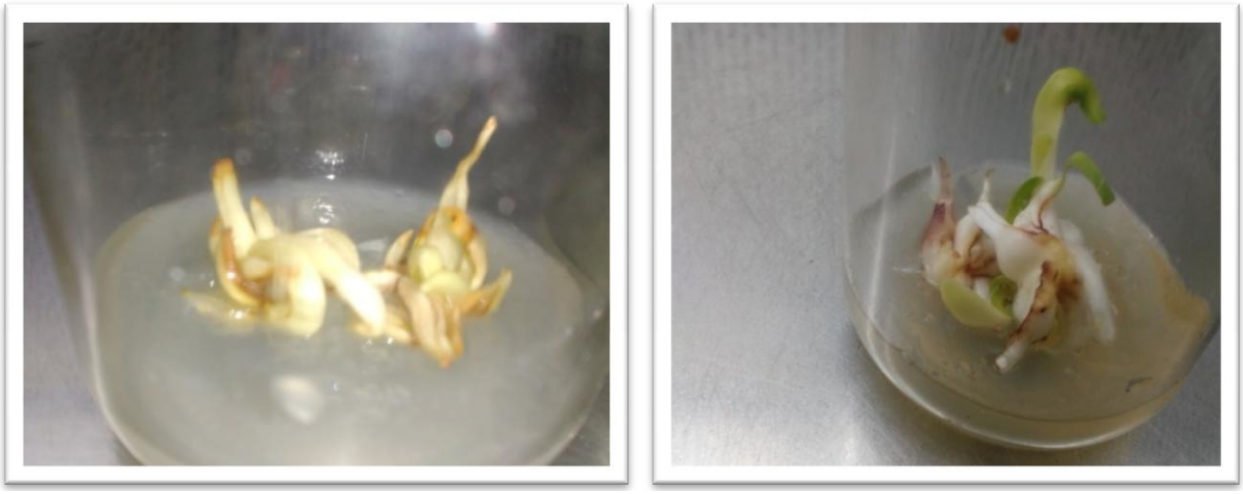

Plate 4.2: Result of multiplication

3) Rooting

The regenerated multiple shoots or buds achieve from bulbs were excised and individually transferred to the MS medium supplemented with different NAA, IAA, and IBA concentration. The shoots were successfully rooted after 6 weeks. The induced roots were hairy and short. The best result of rooting were observed in MS media containing 0.5mg/l IBA +0.5 mg/l IAA+0.1mg/lNAA.

Table 4.3: Observation of various combination of IBA, IAA and NAA on root induction from isolated multiple shoots of *Lilium candidum*

Sr.No	Media code	MS + Growth harmones	Avg.Number of roots per shoot Mean+S.E	Average root length Mean+S.E	%Survival
1	**LR1**	**MS+0.5mg/l IBA+0.5mg/l IAA+0.1 mg/l NAA**	**1.3±0.26**	**2.52**±0.35	**66**
2	LR2	MS+0.5mg/l IBA+ 1.0mg/l IAA+0.2mg/l NAA	0.9±0.17	1.46±0.31	50
3	LR3	MS+0.5mg/l IBA+1.5mg/l IAA+0.3mg/l NAA	0.83± 0.21	0.85±0.24	40
4	LR4	MS+0.5mg/lIBA+0.4mg/l IAA+	0.86 ±0.13	0.66±0.19	55

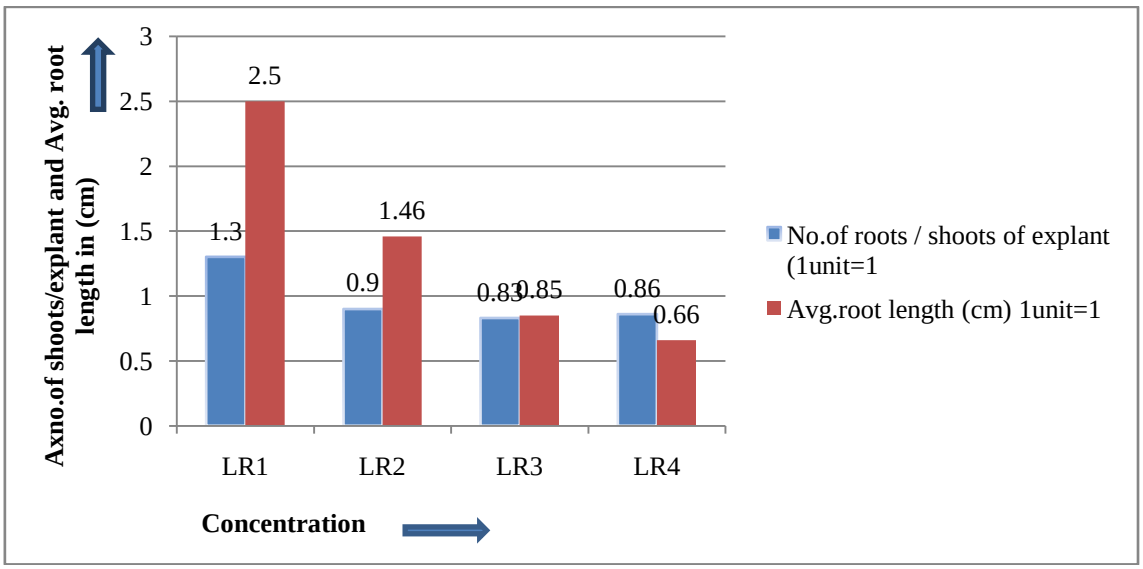

Figure 4.3: Effect of NAA, IBA, IAA on root induction

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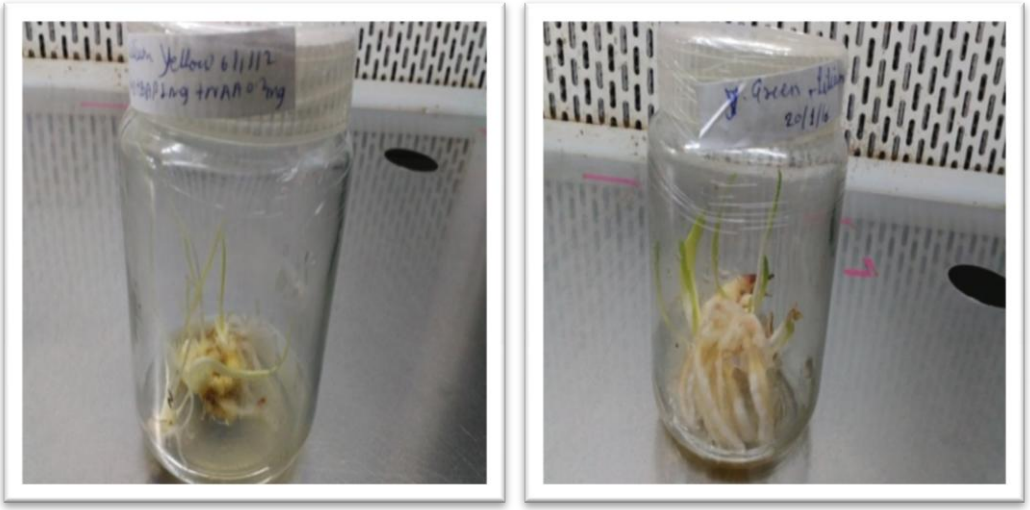

Plate 3 Result of Rooting

V. DISCUSSION

Effect of different concentration of BAP and NAA on the initiation of Lilium candidum from bulb:

The result revealed that 1.0mg/l BAP+0.2mg/l NAA showed its superiority among all the other treatments. No. of bulbet sprouting 4 percent of bud initiation, Average shoot length is 2.85±0.59cm.

Effect of different concentration of BAP and NAA on the multiplication of Lilium candidum from pseudo bulbet shoot:

The result revealed that 2.0mg/l BAP and 0.5mg/l NAA showed its superiority among all the other treatments. Average length of shoots length is 1.87±0.52 and cm and mean number of shoots per pseudo bulbet 4.1 ±0.1 and survival rate was 85% were recorded in 2.0mg/l BAP +0.5 mg/l NAA+3% Sucrose.

Effect of different concentration IBA, IAA and NAA on the rooting of shoots of Lilium Candidum and green var. from the shoot:

The result show superiority of MS+0.5mg/l IBA+0.5mg/lIAA+0.1mg/l NAA+6% Sucrose as compared to all the other treatment as it produce significantly maximum number of roots per shoot 1.3±0.26 and Average root length2.52±0.35cm is survival rate was 66% . Regenerated shoots have been rooted and these are the best rooting medium.

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