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MICRO PROPAGATION OF *LILIUM CANDIDUM* UNDER 2,4-DICHLOROPHENOXYACETIC ACID AND 6-BENZYLAMINOPURINE GROWTH REGULATORS

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ABSTRACT

Plant tissue culture is a technique, used to grow plant cell, tissue and organ under controlled and aseptic conditions, where the concentration of hormones is well defined. It is an important tool in both applied and commercial applications. *Lilium candidum* is an important ornamental plant also used for pharmaceutical purposes. In our present study lilies species *candidum* was used to grow under controlled conditions by using its leaf, shoot and root as explant to regenerate callus. The effects of change in the concentrations of growth hormones was accessed for callus regeneration that how the concentration of hormone caused a change in the growth of shoot and root from undifferentiated mass of cells or callus. It was concluded that direct organogensis occuried when we treated explant with 2-4-D (2,4-Dichlorophenoxyacetic acid) and BAP (6-Benzylaminopurine) growth regulators or hormones.

Keywords: Plant tissue culture, *Lilium candidum*, regeneration, explants, callus, growth hormones

INTRODUCTION

Tissue culture technique have key role in the improvement of crop variety or crop yield by producing some type of variation like soma clonal variation [1, 2] and to produced disease free plants. Through this technique we save plant germplasm free from all types of disease. Modern methods of tissue culture are grafting, cutting, air layering, and seed of plant use as a first source of plant production and micro propagation which plays an important role in the production of virus free plant and it is rapid method and less time consuming [3, 4]. *Coryodalis yanhusuo* is a plant having some medicinal use to produced disease free tuber from tuber callus [5, 6], was derived through somatic embryogenesis one of the plant tissue culture technique is somatic embryogenesis and by using meristem of banana produce BBTV and BMT free plant [7, 8]. The bigger benefit of plant tissue culture is to produce virus free plant along with 150% increased yield of crop plants [9]. The very first medium which used for plant growth was based upon the overall morphology of plant nutrients which is required for the growth of whole plant, but Knop's *Uspenski* and *Uspenskia* solutions used 200 times less of the total salt [10], based upon the study of carrot tissues with two time increased concentration of nutrients increase two times [11, 12]. During another study more concentration was increased but the result [13, 14] of these changes were not satisfactory and maximum growth of plant was not achieved [15]. After this Murashige and Skoog [16, 17] studied the ash of tobacco tissues relation with growth of plant tissues which was found as a new medium known as MS medium [18]. In MS medium the concentration of some salts increased 25 times as compared to knop's medium the concentration of macro nutrients nitrates and ammonium are increased (Harding K et al. 2005). The composition of MS medium increased the culturing rate of plant but some plants required only macro and micro nutrients for their production.

Organogenesis is about direct organ production of plant through root shoot and leaves or any reproductive part of a plant use as explant [19]. After the callus production direct induction or formation of shoot and leaves by changing the concentration of hormones in MS media. Callus production is an important step in plant tissue culture. In vitrocally produced a mass of undifferentiated cells under controlled condition from root shoot and leaf or meristem of plant under controlled condition [20, 21]. After callus production whole plant

can be regenerated by changing the concentration of hormone also regeneration of plants allows the isolation of soma-clonal varieties which has been seen in case of mutation. After callus regeneration increases the concentration of cytokinin, required for shoot regeneration or elongation and auxin required for root production. After roots and shoots regeneration the in vitrocally grown plant shifted into pot in lab conditions after this transferring the plants into green house and then goes to field trials with exposure of natural environment [22, 23]. Lilies are the ornamental plants links to the genus of *lilium* consist of 155 *lilium* plant species in whole world. Most available source of *lilium* plant present in china and cultivated commercially. Ornamental plant lily belongs to the genus *lilium* as most important plant grown in garden as cultured flower. There are 99 above species of *lilium* present all over the world and classified into different categories based on their culture period, color of flower and also depends upon the distribution of land bodies. Lilies have different morphology and physical characteristics but have same chromosomal or genetic makeup throughout the genus. The first lily plant use as a decorative plant is candidum. Lilies are also distributed on the bases of size of plant, and color of flower [19] leave size, leave position, linkage between the species of genus presences of bulb (start the germination process) [24, 25].

MATERIAL AND METHOD

Research work for callus regeneration and organogenesis was done in tissue culture lab of CRIMM (Center of Research in Molecular Medicine) in University of Lahore.

Preparation of MS media:

Murashige and *Skoog* medium was prepared by using MS powder 4.9g/l and sucrose 40g/l phytagel 4g/l for solidifying and pH of media was maintained 5.7 to 5.8 by using 1N KOH and 1N acetic acid and autoclaved media at 121^0C for 20 minutes at 15KPa for sterilization purpose [16, 17].

Components of MS media

Macronutrients, micronutrients, vitamins and sugar are present in MS media.

Different concentration of hormone

MS media supplemented with different hormone BAP, NAA. Kinetin and 2-4-D (2,4-Dichlorophenoxyacetic acid), made three different concentration of BAP (6-Benzylaminopurine) stock was (100mg/l), hormone concentrations were (1µM, 5µM, 10µM) and concentration of 2-4-D stock was (100mg/l), working concentration was (10µM) and also concentration of NAA stock was (0.5mg/l) and Kinetin for callus regeneration and organogenesis.

Sterilization through laminar flow cabinet

Sterilized the surface where the next process was done. Pipette, pipette tips, forceps, tongs, petri plates, test tube, test tube stand, paraffin, cotton, scissor, put in laminar flow cabinet and turn on UV light for 20 minute, turn off UV light, spray ethanol for disinfection purpose. After autoclaving of media, cool down media and add hormones in media with different concentrations and pour it in test tubes and petri plates, when media was solidify then store it at 4^{o}C to avoid media contamination.

Selection and Inoculation of explants

The size of root, shoot and leaves was 1-4mm selected from in vitro grown *Lilium* species *candidum* plant use as explant to regenerate callus. After media preparation and autoclaving of media and sterilization of hood the next step was inoculation of explant into freshly prepared media. All the process was done in laminar flow cabinet to avoid any type of contamination. After surface sterilization inoculate the explant into glass test tubes which contained 5 to 6 ml of MS media and round shape petri plate contained 30 to 35 ml of MS media cover with paraffin to avoid contamination. Inoculated culture was placed in growth chamber of tissue culture lab under optimized conditions where temperature was 25^{0}C and light scale was also optimized, provided with 16 hour's photoperiod and 8 hours dark periods.

RESULTS

Stage 1:

The stage 1 was inoculation of explant into test tubes and plates contained MS media with concentration of different hormone which was necessary for their growth as shown in fig 3.1 the concentration of hormone 2-4-D, NAA.

Stage 2

Callus induction

After two weeks callus induction started in test tubes from shoots of *Lilium* plant. And no results was observed in petri plates. Callus regeneration started in 5µM of BAP and 10µM of NAA hormonal concentration.

Stage 3

Organogenesis

Organogenesis is a process by which production of plant organ from callus and direct from explant. The big achievement of tissue culture process was to produced plant organ direct from explant by using different hormone to produced root and shoot. The hormone concentrations was BAP (0.5mg/ml) and 2-4-D (100mg/l).

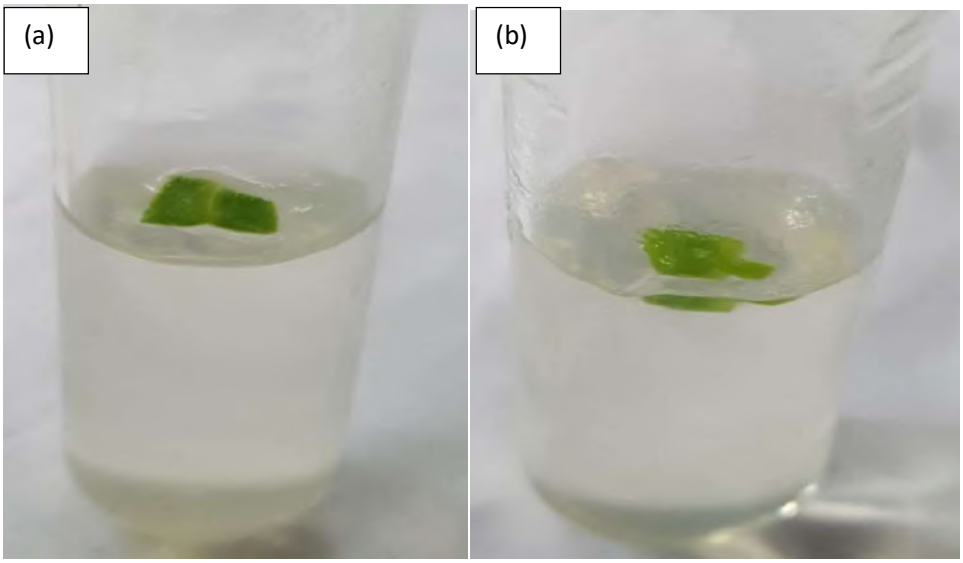


Fig 3.1: Root and shoot of invitro grown *lilium* plant are used as explant in test tubes

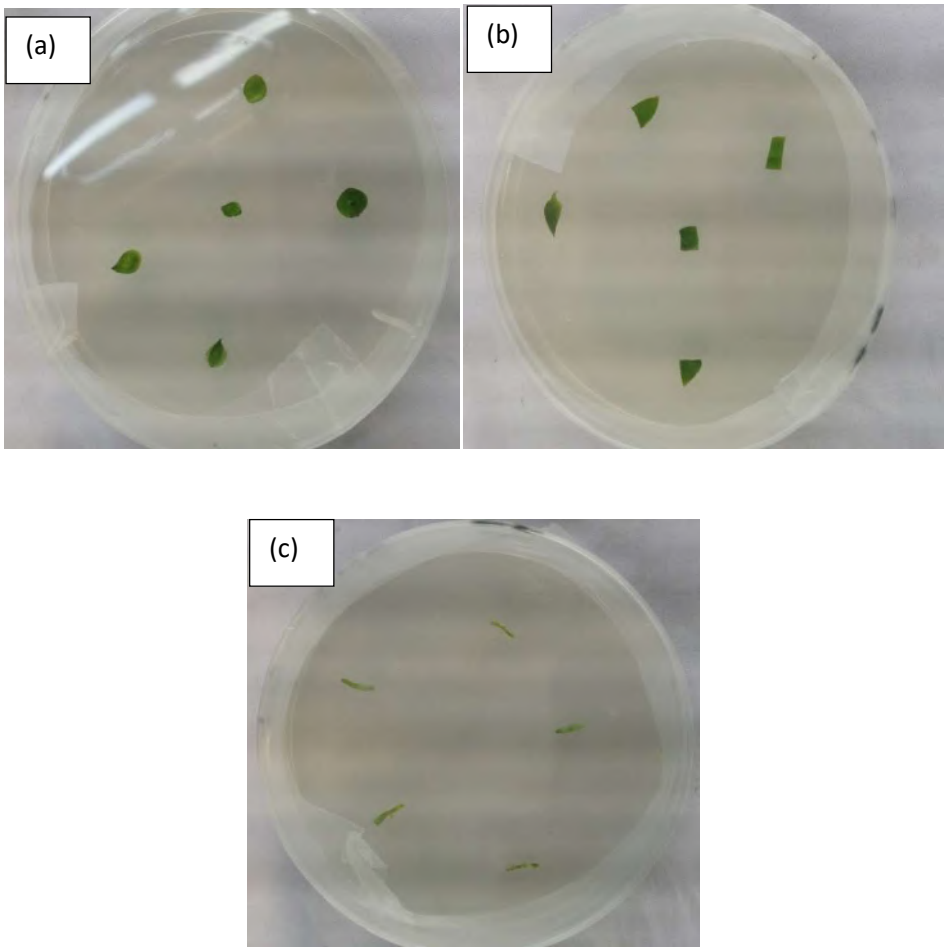


Fig 3.2: a shows shoot b shows leaves and c shows roots of a invitrocally grown lillum plant are used as a explant in petri plates for callus induction. Hormone was 2-4-D, BAP, NAA.

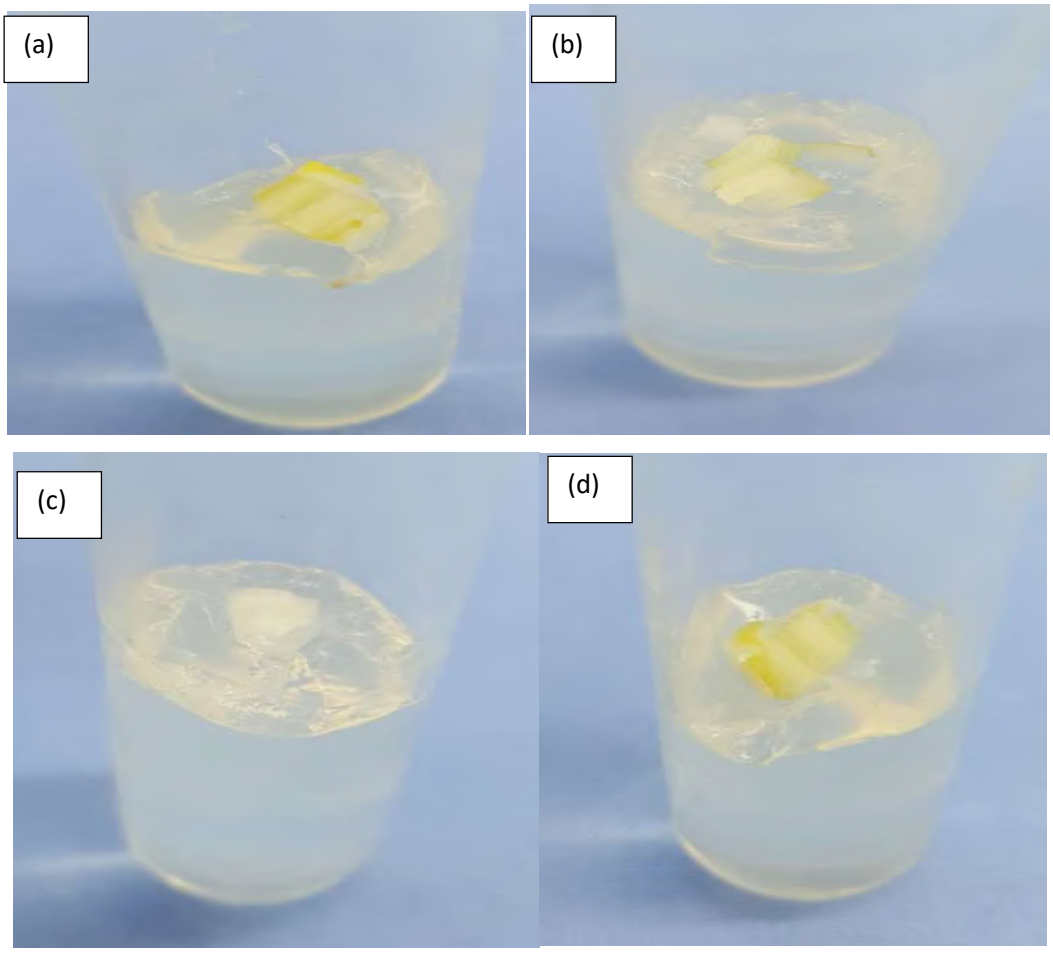
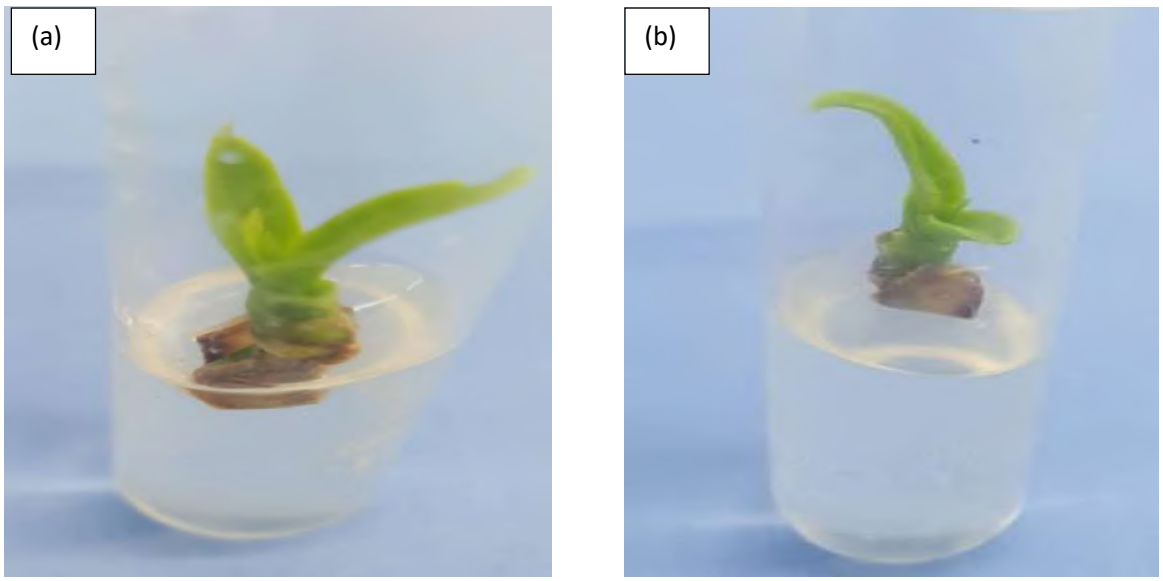


Fig 3.3: callus induction from shoots of *lilium* plant after 3 weeks at 22 temperature and 8 hour dark and 16 hour light period



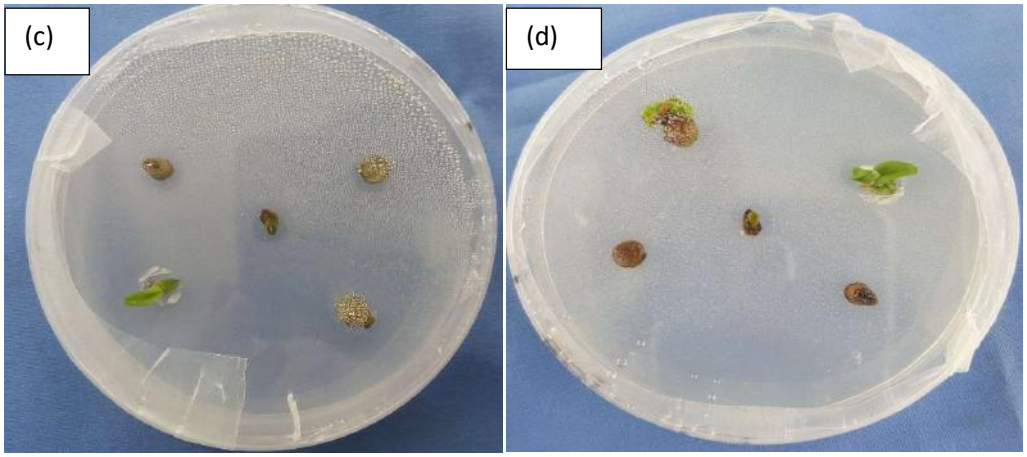


Fig 3.4: Direct organogenesis started from shoot of callus of in-vitro grown *Lilium* plant, hormone concentration was BAP (0.5mg/ml) and 2-4-D (100mg/l)

DISCUSSION

Tissue culture is a technique which is carried out through the culture of cells, organ, tissue and whole plant to grow in vitro under controlled environmental and nutritional requirement or to produce the plant copy from explant which has same genotype in vitro controlled condition have to provide with pH of medium, proper temperature nutrients and light supply. It was found from an experiment that the concentration of NAA gives an effieient result to grow callus under optimized and controlled lab conditions. But the concentration of two hormone BAP, NAA gives more effective result as compaired to NAA concentration [26, 17]. In this research BAP with different concentration (5µM,1µM) callus induction start no result of callus induction are observed in NAA containing media. It has been studied that high concentration of cytokinin were required their efficient results. The effects of sucrose as an important sugur for plant indicaed taht the by increasing the concentration of sucrose from 6-9% caused decrease in the growth of the plant [28, 29]. Whoever in another study no effects of sugar were studied or observed [30].

The size of the explant is also important for callus regenration time period [31], resulted that 5mm long explant take more time to callus regenration as compare to 3-4mm long explant. Same result has been observed our report that 3mm long leaf explant regenrated undifferentiated mass of cell after 1 month. It has been observed no callus regenration and organogensis after two month on MS containing media with (0.5mg/l) BAP hormone but some gives very poor response after one month. Same results were observed but the concentation of BAP hormone were

changed, the optimized concentration of BAP is 100mg/l [30]. In our study the callus regenration was observed under optimized conditions with two hormone concentration, BAP concentration was (100mg/l) and NAA concentration was (0.5mg/l). The colour of calli was yellow. And no result was observed in control which was contaning 2-4-D hormone concentration of (10µM) with stock concentration is (100mg/l)in figure (3.3).Verron and his coworker resulted that direct organogensis occur without callus regenration stage by using hormone NAA with IBA (0.5mg/l) concentration shoot regenration was observed from leaf explant after two weeks [25, 16]. Same results of organogensis were also observed but the concentration of hormone and combination of hormone was changed with resulted concentration and combination of hormone BAP stock concentration (0.5mg/l) and required concentration (5µM), 2-4-D stock concentration was(100mg/l) and required concentration (10µM) and explant is shoot under 16 hours photo and 8 hours dark period under temprature 25^{o}C was independent from NAA concentration as shown in figure (3.5). Now a days technique of the transformation is crossing its boundry towards the monocot plants, but most research work has been conducted on the plant belonged to the family of grass, due to that reason the plants of this family like maize, rice and wheat which use as are mostly food crops [32, 33].

CONCLUSION

The plant tissue culture is a technique which is used to grow plant invitrocally under optimized lab conditions and also culture condition. Large scale production of plant through micropropagation in which small part of plant root, shoot, leave use as a explant and treated with different hormonal concentration. In this report concluded that direct organogensis is occur when we treated explant with 2-4-D and BAP hormone.

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