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Plant Regeneration from Protoplasts of *Lilium ledebourii* (Baker) Boiss

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ABSTRACT

For half a century, the limitations of obtaining cross-combinations in lilies because of the incompatibility and incongruity between different varieties have been known. Somatic hybridization is one of the most powerful tools for achieving distant interspecific hybrids. For this purpose, protoplast preparation is a first and important step in efficient system for the regeneration of plants from protoplasts. Protoplast isolation method was previously developed in *Lilium ledebourii* (Baker) Boiss. In this study, several valuable experiments were done based on completely randomized design with 3 replications and also each experiment was repeated twice. The results revealed that cell wall and colony formation were better in a liquid medium than those on a semi-solid medium. The highest plating efficiency (1.34×10^6 per gr FW) and callus formation was obtained by using a medium containing 1 mg L^{-1} 2,4-D, 0.2 mg L^{-1} Kin and 2 g L^{-1} Yeast extract. Micro calli were formed after one month of culture. Many plantlets were formed on the calli after transfer of the proliferated calli to regeneration medium. The highest plantlet regeneration (91.66%) was obtained by using a medium containing 0.5 mg L^{-1} NAA, 1.5 mg L^{-1} BA. Means comparison revealed that the semi- solid MS medium containing 0.5 mg L^{-1} NAA and 1.5 mg L^{-1} BA had the highest percentage of regeneration (91.66%), bulb number (8.83), and length (0.7366 cm), root length (0.421cm) and leaf number (13.66) and length (0.5052cm).

Keywords: Callus formation, *In-vitro* culture, Medium, Proliferated calli.

INTRODUCTION

Among various types of lilies, asiatic, oriental and *L. longiflorum* hybrids have premium potentialities in the florist trade (Kumar *et al*., 2006). *Lilium ledebourii* Boiss is one of the important ornamental and odorant plants. This plant has high adaptability to Iran environmental condition. It has a wide applicability in floral industry as cut flower and potted plant. *Lilium ledebourii* is grown in the eastern slopes of Alburz Mountains, where it is now protected on a small area of degraded beech forest around Damash village, Khangah of Ardabil province and Kalardasht (Rechinger, 1989). Wild populations are currently at risk of rapid eradication because of irregular grazing and poaching (Azadi and Khosh-Khui, 2007).

Somatic hybridization is one of the most powerful tools for achieving distant interspecific hybrids (reviewed in Evans, 1983). In lilies, efficient methods for isolating large quantities of protoplasts from calli initiated from bulb scale tissues (Simmonds *et al*., 1979), pollen (Tanaka *et al*., 1987), and generative cells (Tanaka, 1988) have been reported. Plant regeneration from protoplasts has been achieved (Mii *et al*., 1994), and a modified protocol for the regeneration of plants subsequently established (Godo *et al*., 1996, 1998). Mii *et al*. (1994) first succeeded in regenerating fertile plants derived from the protoplasts of *Lilium formolongi* through

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successful cell division in a solidified medium containing picloram. However, we were not able to obtain divided cells from our preliminary culturing of protoplasts isolated from various kinds of oriental hybrid cultivars according to their protocol. Protoplasts can be isolated from plant tissues or cultured cells. The success of protoplasts isolation depends especially on the condition of the tissue and the combination of enzymes being used. The individual cell or tissue source may require special conditions for successful isolation or for culturing. Protoplasts isolated from cell suspension cultures, generally, are readily obtained and usually regenerate into dividing cells at a reasonable frequency. Moreover, if the plant regeneration is possible from the cells of the suspension culture, the same is often true for the cells regenerated from the protoplast derived from the culture (Tan *et al*., 1987). Leaf mesophyl cells of a wide range of plants also have been used as a protoplasts source with success by Keller *et al*. (1982) and Gleddie and Keller (1989).

Protoplasts have served as recipient hosts for DNA transformation and are required in somatic hybridization by protoplasts fusion (Stephen, l995). Protoplasts have been isolated from various genotypes of *petunia hybrida* (Izhar and Power, 1977), as well as from *P. inflata, P.violocea* and *P. axillaris* (Dulieu *et al*., 1983). Also, there are reports on isolation and culture of protoplasts from leaves and suspension in specious such as *Allium cepa* (Karim and Adachi, 1997), *Spathiphyllum* and *Anthurium* (Duquenne *et al*., 2007), *Lotus corniculatus* (Raikar *et al*., 2008).

To our knowledge, no report exists on protoplast technology in *Lilium ledbourii* (Baker) Boiss. In the present study, we report the induction of callus formation from protoplasts in *Lilium ledebourii* for the first time. To optimize the preparation of protoplasts and cell division frequency, factors such as donor material, composition of enzyme solutions, duration of incubation period, and culture system and the flowering of the regenerates through subsequent protoplast culturing were studied.

MATERIALS AND METHODS

Protoplast from young leaves of *L. ledebourii* were isolated from leaf explants, digested with different enzyme solutions and also purified as described previously (Chamani *et al*., 2012).

Protoplast Culture

Protoplasts were cultured at a density of 1×10^6 protoplasts ml^{-1}. Two culture systems were tested: liquid culture and semi-solidified culture. For the semi-solidified culture, protoplasts were plated as a thin layer on a MS medium containing 9% mannitol and solidified with 8 g L^{-1} agar in small petri dishes. For liquid culture (MS without agar, with 9% mannitol), protoplasts were suspended in 4 ml of media in small Petri dishes (5.5 cm diameter).

Five days after protoplast culture, the cells were transferred to Erlenmeyer flasks containing MS liquid medium and incubated at 120 rpm on a rotary shaker in the dark at 25 ± 2°C. After 10 days, every time, 5 ml of fresh medium was added to the culture medium. Star shaped microcalli developed within 15 days of culture. After the development of microcalli visible by naked eye, the cultures were transferred to the light. The plating efficiency was defined and measured as the ratio of cell number undergoing division to the total cultured protoplast number. After one month when the calli attained sizes of 0.5–1.0 mm in diameter, they were transferred to the semi-solidified MS medium at 23°C under fluorescent light (40 µmol per m^2 s^{-1}) in a 16:8 hour of day/night regime in the culture cabinets.

Experimental Designs, Data Collection, and Analysis

In this study, three separate experiments were done and each experiment was repeated twice. In the first experiment, in order to optimize the medium for protoplast

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growth and cell proliferation, the effect of various plant growth regulator combinations in MS medium (2 gr yeast extract (YE), 1 and 2 mg L^{-1} 2,4-D, 0.2 and 1 mg L^{-1} Kin) were tested as a suspension culture based on completely randomized design with factorial arrangement and three replications.

In the second experiment, to determine the growth possibility of protoplast-derived cells on the semi-solid medium, all of cells proliferated in suspension culture were sub-cultured on semi-solidified MS medium supplemented with various combinations of 2,4-D and kinetin (Kin) and yeast extract (1 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin; 1 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin; 1 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin and 2 gr Yeast extract; 1 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract, 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract, 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract, 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin). After callus formation, callus mass were counted.

In the third experiment, after 26 days of callus proliferation, the developed calli in suspension culture (1 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin; 1 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin; 1 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract; 1 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract) were transferred to regeneration medium consisting of semi-solidified MS medium supplemented with NAA (0 and 0.5 mg L^{-1}), BA (0, 0.5 and 1.5 mg L^{-1}) based on completely randomized design with factorial arrangement with three replications. The cultures were kept in light conditions of l6 hours/day at 25°C.

Cell density was estimated with a Nageotte hemocytometer. Results were expressed as yield per gr FW for leaves or calli. Length of bulbs, roots, and leaves were measured by ruler. Callus mass was evaluated by naked eye. Data analyses were performed using SPSS (SPSS Inc. Version 19.0) software. Mean comparisons were done using Duncan's multiple range test (DMRT) at a probability level of 0.05.

RESULTS

First Experiment: Effect of Different Hormones on Cell Growth and Deviation

The results of ANOVA showed that different concentrations of 2,4-D and Kinetin significantly ($P< 0.01$) affected proliferation of protoplast derived cells. Significant ($P\leq 0.01$) interaction effects of 2,4-D×Kin, Yeast extract×Kin, Yeast extract×2,4-D and Yeast extract ×2,4-D×Kin were found on cell proliferation (Table 1, Figure 1).

Means comparison by DMRT showed that the highest and lowest cell proliferations were produced in MS suspension medium containing 1 mg L^{-1} 2,4-D, 0.2 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract (1.34×10^{6} cells per

Table1. Analysis of variance of the effects of yeast extract and different plant growth regulators on the proliferation and growth of *Lilium ledebourii* protoplasts.

Source of Variation	df [a]	MS [b]
Kinetin	1	2.659×10^{10} **
2,4-D	1	9.191×10^{11} **
Yeast extract	1	8.911×10^{8} ns
2,4-D×Kinetin	1	2.025×10^{9} **
Yeast extract × Kinetin	1	9.460×10^{9} **
Yeast extract ×2,4-D	1	1.381×10^{10} **
Yeast extract ×2,4-D × Kinetin	1	1.152×10^{10}**
Error	16	3.552×10^{8}
CV%		1.74

ns, *,**: Non significant and significant at probability level of 5% and 1%, respectively. [a] Degree of Freedom, [b] Mean of Squares.

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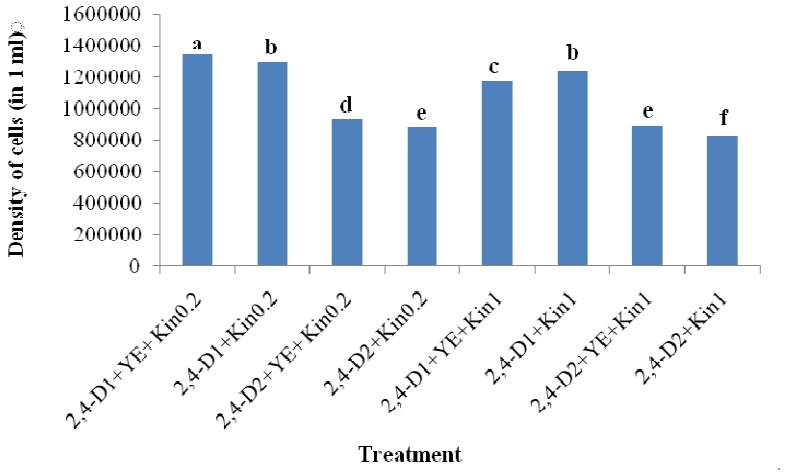

Figure 1. The mean effect of different combinations of hormones on density of cells in *Lilium ledebourii.* Columns with different letters are significantly different at $P \leq 0.05$.

ml), and 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin (8.28×10^5 cells per ml), respectively (Figure 1). However, other MS suspension media containing 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract, 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin, 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract and as well as 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kin produced significantly lower density of cells. Hence, the latest mentioned media was not used in the next experiments (Figure1).

Thus, the best treatment for proliferation and growth of *Lilium ledebourii* (Baker) Boiss cells was MS medium supplemented with 1 mg L^{-1} 2,4-D, 0.2 mg L^{-1} Kin and 2 gr L^{-1} Yeast extract. The first cell divisions were observed 48 hours after protoplast culture. Cell density was measured every five days and the first density measurement was done 15 days after protoplast culture (Figures 2 and 3).

Second Experiment: Callus Mass Formation from Plating of Cell Suspension on Solid MS Medium

The results of ANOVA showed that growth of plated cells and formation of calli (detectable by naked eye) on semi-solidified medium were significantly ($P\leq0.01$) influenced by different combinations of plant hormones and yeast extract (Table 2). Means comparison revealed that the highest and lowest callus induction from plated cell on semi-solidified MS medium were produced on media containing 1 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin with 2 gr L^{-1} Yeast extract (29.66) and 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} Kin (0), respectively (Table 3, Figure 4).

Table 3. The effects of different treatments on callus formation from plated cells of *Lilium ledebourii.*

2,4-D (mg L^{-1})	Yeast extract (gr L^{-1})	Kin (mgL^{-1})	Number of callus mass formed in each petridish[a]
1	-	0.2	11^{c}
1	-	1	7^{d}
1	2	0.2	29.66^{a}
1	2	1	22.66^{b}
2	2	0.2	4^{e}
2	2	1	1.66^{ef}
2	-	0.2	0^{f}

[a] Means followed by different letters in each column are significantly different at $P\leq 0.05$.

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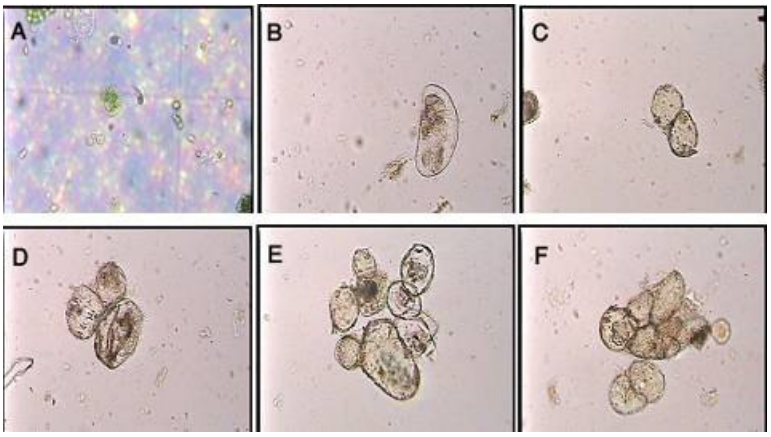

Figure 2. Growth and development of *Lilium ledebourri* protoplast in suspension culture: (a) The protoplast release; (b) The cell wall formation of protoplasts; (c) Initiation of cell division after 48 hours; (d) and (e) Subsequent cell divisions, and (f) Formation of cell aggregates.

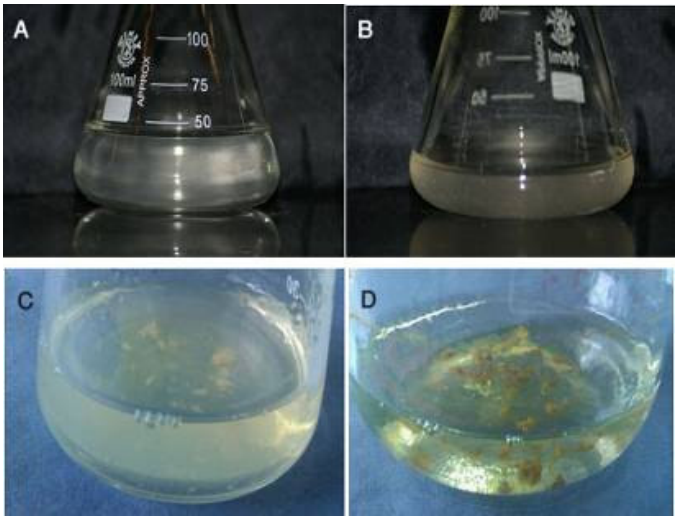

Figure 3. Developmental stage of protoplast in culture suspension: (A) Culture suspension contain release protoplast; (B) Cell proliferation and growth after two days and turbid suspension medium; (C) Formation of cell masses After 14 days, and (D) Cell mass enlargement and callus formation 20 days after culture.

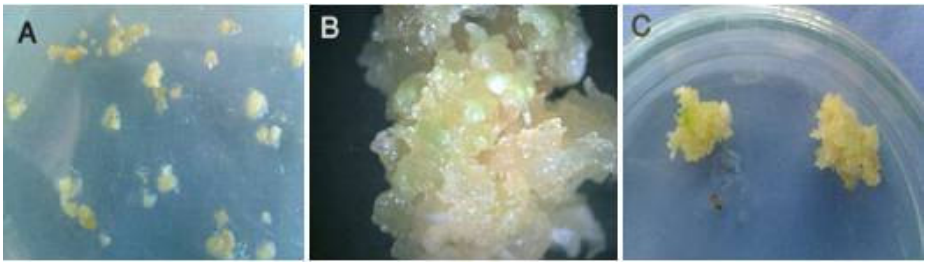

Figure 4. Callus induction of *Lilium ledebourii* plated cell suspension on solid MS medium: (A) Plate of cell suspension and callus formation can be detected with the naked eye after 10 days; (B) Formed callus differentiated into plantlets after 29 days, and (C) Regeneration of plantlet (arrow) from callus.

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Table 2. Analysis of variance of the effects of different treatments on growth efficiency of plated cells of *Lilium ledebourii* on solidified MS medium and formation of callus.

Source of variation	df [a]	MS [b]
Treatment	6	378/762**
Error	14	2.571
CV%		14.77

** Significant at probability level of 1%.
[a] Degree of Freedom, [b] Mean of Squares.

Third Experiment: Plant Regeneration

The results showed that there was no significant difference (P≤ 0.01) between initial treatments of cells proliferation suspension culture in all of the measured traits, except bulb length and number of leaves (Table 4). However, regeneration medium significantly (P≤ 0.01) affected the percentage of regeneration, bulb number and length, leaf number and length, and root number and length (Table 4). Means comparison (Table 5) revealed that the semi-solidified MS medium containing 0.5 mg L^{-1} NAA and 1.5 mg L^{-1} BA produced the highest percentage of regeneration (91.66%), bulb number (8.83) and length (0.7366 cm), root length (0.421cm) and leaf number (13.66) and length (0.5052cm). The results also showed that the highest root number was produced from MS medium containing 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BA (4.5). Moreover, hormone free MS medium had the lowest effects in all measured traits (Table 5). No significant differences were found between hormones free MS medium and suspension medium in case of bulb number and length, and root and leaf number. However, these two media significantly differed from each other in terms of root and leaf length and regeneration percent. MS medium supplemented with 0.5mgL^{-1} NAA+1.5 mg L^{-1} BA was the best medium for plant regeneration from L. *ledebourii* (Baker) Boiss protoplast-derived calli (Table 5).

Table 4. Analysis of variance of different treatments impacts on plant regeneration and measured traits from cultured protoplasts of *Lilium ledebourii*.

		MS						
Source of variation	df	Regeneration %	Bulb number	Bulb length(cm)	Root number	Root length(cm)	Leaf number	Leaf length(cm)
Cell proliferation medium	3	355.159 ns	0.096 ns	0.055 *	0.115 ns	0.009 ns	0.301 *	0.076 ns
Regeneration medium	3	18934.555 **	2.906 **	0.196 **	1.429 **	0.239 **	3.556 **	0.292 **
Cell proliferation mediumׯ Regeneration medium	9	147.206×10^8 ns	0.022 ns	0.007 ns	0.021 ns	0.012 ns	0.029 ns	0.011 ns
Error	32	274.888	0.051	0.015	0.052	0.026	0.080	0.043
CV%		25.04	8.27	30.69	11.93	25.70	6.59	31.89

ns, *, **: Non significant and significant at probability level of 5% and 1%, respectively.

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Table 5. The effect of different treatments on plant regeneration and measured traits from cultured protoplasts in *Lilium ledebourii.*[a]

Treatment	Regeneration %	bulb number	bulb length(cm)	root number	root length(cm)	leaf number	leaf length(cm)
MS media	0 [d]	0[c]	0[c]	0[c]	0[c]	0[c]	0[c]
The suspension medium	13.88[c]	0.25[c]	0.123[c]	0.333[c]	0.115[b]	0.34[c]	0.125[b]
0.5mg/l NAA+0.5mg/l BA	41.66[b]	1[b]	0.363[b]	4.5[a]	0.421[a]	1.75[b]	0.355[a]
0.5mg/l NAA+1.5mg/l BA	91.66[a]	8.83[a]	0.736[a]	1.583[b]	0.405[a]	13.66[a]	0.502[a]

[a] Means followed by different letters in each column are significantly different at $P \leq 0.05$.

DISCUSSION

No reports have been published on protoplast culture and regeneration in *Lilium ledebourii* (Baker) Boiss. In this study, plants were regenerated from *Lilium ledebourii* (Baker) Boiss protoplasts. The efficiency of protoplast isolation and growth depends on many factors, such as the enzyme mixture, the presence and type of growth regulator, and *in vitro* culture (Assani *et al*., 2001). A liquid medium was better than an agarose-solidified PCA medium for further growth of isolated protoplasts. Although in many crops agarose-solidified media were used. They showed that more frequent browning occurred in an agarose-solidified medium than in a liquid medium. This browning is probably caused by the oxidation of phenolic compounds, which are released from plant cells cultured into the medium (Saxena and Gill, 1986). Oxidation causes severe damage to plant cells or tissue and, consequently, stops their growth. In a liquid medium, this toxic compound might be diluted, thus showing less browning than an agarose-solidified medium does. Ochatt and Power, (1988a) reported that casein hydrolysate is needed for sustaining protoplast division of Williams pear as a source of amino acids. In these experiments, we used yeast extract instead of casein hydrolysate. These results are supported with the findings of Ochatt and Power (1988b) who used protoplasts of several woody fruit crops. Therefore, the positive effect of casein hydrolysate or yeast extract and amino acids on cell division proved its successful effect in protoplast cultures.

This result indicated that combination of Kin and 2,4-D in high concentration inhibited protoplast division, consistent with earlier findings that the combined optimal auxin and cytokinin were relatively effective for cell division in petal protoplast of Petunia hybrid (Oh and Kim, 1994), and in cell suspension protoplast of *Allium cepa* (Karim and Adachi, 1997). Another important factor for protoplast culture is the culture system. In these experiments, protoplasts were cultured either in liquid or solid MS medium comprising 1×10^5 and 1×10^5 protoplasts ml^{-1}. Division of protoplasts obtained in liquid MS medium at optimal density was 1.34×10^6 protoplasts ml^{-1}. The density of protoplasts influenced the initiation of cell divisions, as has been reported in oat by Hahne *et al*. (1990). The suspension-derived protoplasts of vetiver did not divide on gelrite-solidified medium. In contrast to published data (Kisaka *et al*., 1998) the same gelrite was successfully used for protoplast culture. There are some reports that agarose and phytagel have been used to improve protoplast culture in Medicago sp. and *Garcinia atroviridis* Griff., respectively (Te-chato, 1997).

During the present study, cell-wall regeneration, cell division, and callus formation were obtained (Figure5). Among the plant growth regulators we tested, only the combination of 2,4-D and Kin induced cell division. In earlier studies on rose mesophyll protoplasts, NAA and BA were the most efficient growth regulators for the regeneration of microcalli (Marchant *et al*., 1997). In lily protoplasts, the addition of

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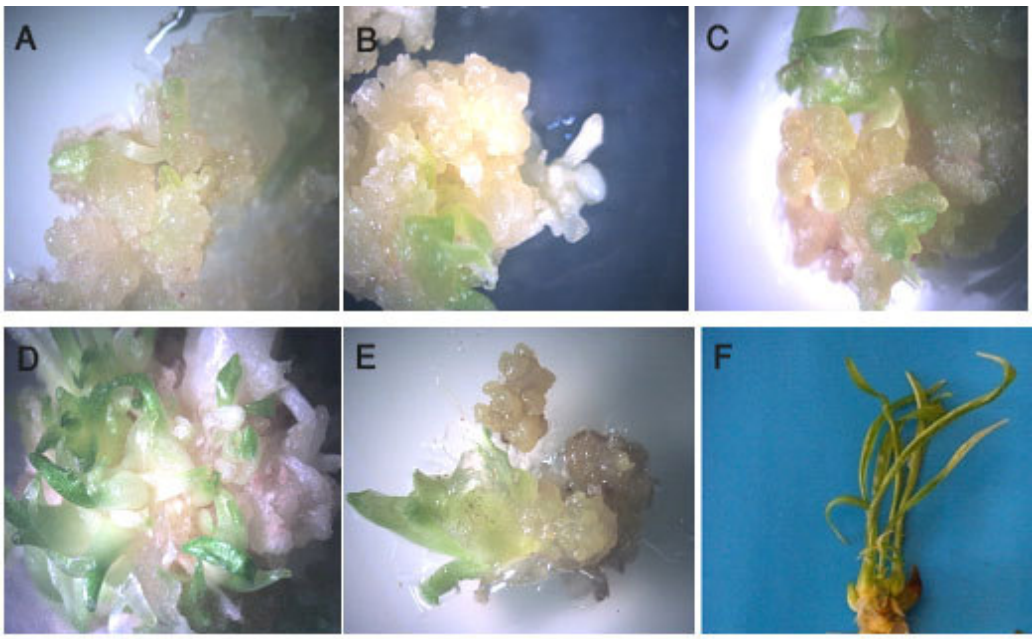

Figure 5. Callus growth stages and plantlet regeneration from protoplast culture in *lilium ledebourii.* (A, B, C) Growth and differentiation of callus and formation of small bulblet; (D and E) Growth and development of bulblet, and (F) Regenerated plantlets.

picloram to the culture medium was critical of development of microcalli (Horita *et al*., 2002).

The number of microcalli we obtained was close to those obtained in earlier studies in banana (Assani *et al*., 2001). Auxin is involved in cell division and callus formation. The high concentration of auxin, does not induce root formation but callus formation (Pierik, 1998).

Shoot organogenesis depends on many parameters, including the genotype, protoplast-derived material, plant growth regulators, culture system, and exposition time of protoplasts on nurse cells (Chabane *et al*., 2007). Previous investigations showed the impact of genotype on plant regeneration from protoplasts in apple and banana (Assani *et al.*, 2002).

Chang (1999) reported that optimum callus formation from inflorescence explants of *lilium* was obtained in medium containing 3 mg L^{-1} 2,4-D and 0.25 mg L^{-1} BA. In another experiment, Naik and Nayak (2005) reported that callus induction in scale explants of *ornithogalum virens* was obtained in medium containing 1-4 mg L^{-1} 2,4-D and 2 mg L^{-1} BA. Chen (2005) also stated that, the highest percentage of callus induction from another culture of Narcissus, was obtained in medium containing 1 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BA.

The main plant growth regulators such as auxin and cytokinin, alone or in combination, are generally essential for efficient protoplast division in plant systems (Davey *et al.*, 2005). Plant growth regulator concentrations and combinations need to be optimized for each protoplast development step. The following plant growth regulators were tested in our preliminary experiments: 2,4-D, Kin, BA, NAA, and Yeast extract. Only the combination of 2,4-D and Kin induced sustained cell divisions and callus formation. None of the plant growth regulators induced plant regeneration, which may be related to the negative interaction between those plant growth regulators and some metabolites produced by callus tissues. Nagata and Takede (1984) succeeded in

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isolating of protoplasts from *Nicotiana tabacun* L. leaves using enzyme solution. They isolated 10^7 protoplasts from 1 gr fresh weight tobacco leaves. After 3 weeks, shoots were induced in the colonies by transferring them into differentiation medium containing NAA and BA at 4 mg L^{-1} and Kin at 2.56 mg L^{-1}. Shoots were transferred to hormone free MS-medium to induce root formation.

Concentration of 0.2 mg L^{-1} 2,4-D, 1 mg L^{-1} NAA and 0.5 mg L^{-1} Zeatin, produced the highest protoplast regeneration and cell division (Pongchawee *et al*., 2006). According to Tamura *et al*. (1992) report, high concentration of glucose (0.5M) requested the best outcome for protoplast culture.

They also proved that addition of Zeatin (1 mM) and NAA (10 mM) gives the normal size of the colonies formed. Changing the protoplast culture medium to 5.4 mM NAA and 2.3 mM Zeatin was suitable for protoplast regeneration, therefore, that was the appropriate density of cells in the medium (Tian *et al*., 1999). In case of bulb number, Takayama *et al.* (1991) also achieved similar results. They reported that high ratio of cytokinin in culture medium produced high bulb number from lilium scale explants. Nhut (1998) reported that there was a direct relationship between the concentration of cytokinin and bulb formation. However, the use of cytokinin alone was more effective in bulb formation than the combination of cytokinin with auxin.

It is reported that solid MS medium containing 2 mg L^{-1} BA produced the optimal bulb number from scale of *L. pyrenacium* and *L. longiflorum* (Hassey, 1976). Azadi and khosh-khui (2007) revealed that the highest bulb number of *lilium ledebourii* was produced from solid MS medium containing 0.1 mg L^{-1} NAA and 1 mg L^{-1} BA. Hence, the mentioned results confirm our experimental findings, too. However, the ratio of Auxin to Cytokinin was related to root and shoot regeneration. In case of root number, it seems that decreasing BA concentration in the medium, increased the root number of explants. This result is consistent with the results of Izadi *et al*. (2011). Nhut (1998) also reported application of NAA significantly increased the root number in *L. longiflorum* compared to control. Pierik (1998) reported that cytokinin in low concentrations stimulates cell division, but usually the higher concentrations prevent the formation of root. The result of our experiment revealed that auxin by influencing cell elongation was able to produce more elongated shoots and also leaf length. Pierik *et al.* (1975) reported that auxins increased shoots length in hyacinth flower. Asoa (1992) showed that leaves formation occurred under *in vitro* culture of *Lilium japonicum* when the bulb was cultivated in a medium containing 2 mg L^{-1} BA or 9 mg L^{-1} GA3, but was stopped when transferred to a medium containing a low concentration of NAA (0.01 mg L^{-1}) and BA (0.001 mg L^{-1}), indicating that leaves emergence was directly affected.

CONCLUSIONS

In conclusion, we described here, for a first time, an efficient protocol for plant regeneration from protoplasts of *L. ledebourii* (Baker) Boiss. In this protocol, the efficiency of viable protoplast recovery, cell proliferation and callus formation, and finally plant regeneration were relatively high and reproducible. Thus, this protocol can be utilized in protoplast fusion projects, and also genetic manipulation of *Lilium* species.

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باززایی گیاه سوسن چلچراغ از طریق کشت پروتوپلاست

س. ک. تهامی، ا. چمنی، و ن. زارع

چکیده

نیم قرن است که محدودیت‌های موجود در تلاقی بین سوسن‌ها (دگرگرده‌افشانی) بدلیل وجود ناسازگاری و عدم تقارن ژنتیکی بین واریته‌های مختلف شناخته شده است. هیبرید سوماتیکی یا امتزاج پروتوپلاست‌ها یکی از ابزارهای اصلی در دورگ‌گیری بین‌گونه‌ای است، برای این منظور، اولین قدم وجود یک سیستم بهینه برای آماده‌سازی و باززایی گیاه از پروتوپلاست می‌باشد. روش جداسازی پروتوپلاست در سوسن چلچراغ قبلا بهینه شده است. این آزمایش بر اساس طرح کاملا تصادفی با ۳ تکرار انجام گرفت و هر آزمایش دو بار تکرار شد. نتایج حاصل از آزمایش نشان داد که تشکیل دیواره سلولی و کلونی در محیط مایع نسبت به محیط نیمه جامد آگارز بهتر بود. بیشترین تراکم کشت و تشکیل کالوس در محیط کشت حاوی یک میلی‌گرم در لیتر 2,4-D ، ۰/۲ میلی‌گرم در لیتر کینتین به همراه دو گرم در لیتر عصاره مخمر بدست آمد ($۱/۳۴\times۱۰^{۶}$). کالوس‌های کوچک بعد از یک ماه کشت تشکیل شدند. گیاهچه‌های زیادی پس از انتقال کالوس‌های رشد یافته به محیط کشت حاوی تنظیم کننده‌های رشد گیاهی تشکیل شدند. محیط حاوی ۰/۵ میلی‌گرم در لیتر NAA به همراه با ۱/۵ میلی‌گرم در لیتر BA با میانگین ۹۱/۶۶ درصد بیشترین باززایی گیاهچه را داشت. مقایسه میانگین بین داده‌ها نشان داد که در محیط کشت MS حاوی ۰/۵ میلی‌گرم در لیتر NAA به همراه با ۱/۵ میلی‌گرم در لیتر BA بیشترین باززایی با میانگین ۹۱/۶۶ درصد، تعداد پیازچه با میانگین ۸/۸۳، طول پیازچه با میانگین ۰/۷۳۶۶، طول ریشه با میانگین ۰/۴۲۱ ، تعداد برگ با میانگین ۱۳/۶۶، طول برگ با میانگین ۰/۵۰۵۲ تولید شد.

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