



## MORPHOGENESIS OF *LILIUM MARTAGON* L. EXPLANTS IN CALLUS CULTURE

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The effect of two plant growth regulators on the initiation of callus and regeneration of *L. martagon* was studied on modified MS medium. The cultures were initiated using seeds. The explants were isolated from different parts of seedlings (hypocotyls, seedling bulb, root) and adventitious bulblets. The growth regulators stimulated various types of callus. 4-amino-3,5,6-trichloropyridine-2-carboxylic acid (Picloram) induced a yellow, friable, granular callus, whereas benzyladenine (BA) alone induced a cream-white, compact callus. MS medium containing Picloram plus BA stimulated a yellow, compact, granular callus. The most useful explants for callus initiation were seedling bulbs and adventitious bulblet scales. The most efficient embryogenic callus was obtained on MS medium containing 5 µM Picloram and 5 µM BA. Histological studies showed that the embryogenic callus was formed at the epidermal cells and near vascular bundles of explants. Somatic embryos were solitary, whereas adventitious bulblets were closely connected with vascular tissue. No differences in the amount of DNA between scale tissue and callus were observed. *Lilium martagon* L. is an endangered species in Poland. In vitro culture techniques can play an important role in the future protection of this rare plant species.

**Key words:** *Lilium martagon* L., plant growth regulators, callus, regeneration, somatic embryogenesis.

### INTRODUCTION

The Turk's cap lily (*Lilium martagon* L.), which belongs to the Martagon section of the large Liliaceae family (McRae, 1998), is known as an endangered and protected species growing wild in Eurasia (Madalski, 1975; Meusel et al., 1965; Kolon et al., 1994).

Organogenesis and somatic embryogenesis have been studied in culture of *L. auratum*, *L. bulbiferum*, *L. japonicum*, *L. longiflorum*, *L. regale*, *L. rubellum*, *L. speciosum*, *Lilium* × *formolongi*, and Asiatic and Oriental hybrids (Simmonds and Cumming, 1976; Takayama and Misawa, 1982/83; Nimii, 1984; Priyadarshi and Sen, 1992; Maesato et al., 1994; Mii et al., 1994; Wickremesinhe et al., 1994; Famelaer et al., 1997; Tribulato et al., 1997a; Watad et al., 1998; Pelkonen and Kauppi, 1999). However, there are only a few reports on the role of cytokinins in early stages of bulblet formation and on induction of callus under the influence of NAA and BA in bulb scales and leaf ex-

plants of *L. martagon*. Pelkonen (1997) achieved callus induction of *L. regale* and *L. martagon* on media containing NAA plus BA. Rybczyński and Gomolińska (1989) described the influence of BA at low concentration on bulblet formation in *L. martagon*. In this study we investigated the optimum in vitro conditions for seed germination as initial pathogen-free explants. We examined the induction of embryogenic callus and organ regeneration in *L. martagon*. The goal of our work was to study its morphogenesis under the influence of plant growth regulators in callus cultures derived from either seedlings or adventitious bulblet scales.

### MATERIALS AND METHODS

#### PLANT MATERIAL

*Lilium martagon* seeds were collected from 12 plants (15 seed capsules, ~100 seeds each) in Biskupice (Pogórze Wielickie, southern Poland, 260–290 m a.s.l.).

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TABLE 1. Effect of different media on in vitro seed germination of *L. martagon*; seeds stored at +20°C; n=50

Medium	Germination efficiency [%]
MS, 30 g/l sucrose	44 a
1/2 MS, 15 g/l sucrose	72 b
MS+0.27 µM NAA, 30g/l sucrose	72 b
1/2 MS+0.27 µM NAA, 15 g/l sucrose	72 b

a, b... – values followed by the same letters do not significantly differ

Seeds before cold treatment (3 months at 20°C) and after cold treatment (at least 3 months at 4°C) were used. The seeds were surface-disinfected with 70% ethanol for 1 min followed by 7% Chloramine B solution plus a few drops of Tween 20 for 20 min. Then they were washed three times with sterile distilled water and soaked for a few sec in 0.7% Chloramine B solution. Sterilized seeds were aseptically plated on MS medium (Murashige and Skoog, 1962) with 30 g/l sucrose or on 1/2 MS with 15 g/l sucrose, with or without 0.27 µM  $\alpha$ -naphthalene acetic acid (NAA), solidified with 0.7% Difco Bacto-Agar. The pH was adjusted to 5.8 before autoclaving (121°C for 20 min). Each seed was placed individually in a tube containing 10 ml medium. The response was observed during 12 weeks. The cultures were kept at 20°C or 25°C under illumination with daylight fluorescent lamps (30 mol m<sup>-2</sup>s<sup>-1</sup>) under a 16 h photoperiod or in darkness.

#### CALLUS INDUCTION

Callus was induced on explants isolated from different seedling parts or from adventitious bulblet scales. In the initial experiment, 4–5-week-old seedlings were separated into explants (hypocotyl, 2 lengthwise bulb cuttings and root) which were placed in Petri dishes. The MS medium contained different growth regulators: 0.5–25 µM 4-amino-3,5,6-trichloropyridine-2-carboxylic acid (Picloram) and/or 0.5–25 µM benzyladenine (BA). The Petri dishes were kept at 20°C in darkness. The morphogenic response was observed after ~8 weeks. The percentage of explants forming callus and the number of bulbs and roots per explant were counted. The characteristics of the callus and the number of somatic embryos (at least ~200 µm long) were determined with a light microscope (Leica, Germany).

In the next experiment, scales from regenerated adventitious bulblets were used for callus induction under the same conditions as described above for seedling culture. Scale segments (apical and basal) were placed abaxial side down on the medium. After 8 weeks, embryogenic callus formation and the frequency of somatic embryos, bulblets and roots were assessed.

TABLE 2. Effect of media and culture conditions on seed germination of *L. martagon*; seeds stored at +4°C; n=50

Medium	Culture conditions	Disinfection efficiency [%]	Germination efficiency [%]
MS, 30 g/l sucrose	25°C/L*	90	10 a
	25°C/D	100	10 a
1/2 MS, 15 g/l sucrose	25°C/L	95	15 a
	25°C/D	95	45 b
MS, 30 g/l sucrose	20°C/L	100	70 bcd
	20°C/D	90	50 bc
1/2 MS, 15 g/l sucrose	20°C/L	85	85 d
	20°C/D	85	75 cd

a, b... – values followed by the same letters do not significantly differ

\*L – 16 h photoperiod; D – darkness

#### HISTOLOGICAL AND CYTOLOGICAL OBSERVATIONS

For histological study, tissue samples were fixed in formalin-acetic acid-ethanol (FAA) for 72 h. The tissue was dehydrated in an ethanol series (70%, 90%, 100%) for 10 min at each concentration and embedded in paraffin (56°C). Serial sections 8–10 µm thick were cut with a rotary microtome (RM 2145, Leica, Germany), stained with 1% safranin and 1% fast green (Jensen, 1962), and photographed under a light microscope.

#### FLOW CYTOMETRY

Cytometric analysis was performed with a PARTEC CA II flow cytometer (Münster, Germany) to determine the relative DNA content in scale and callus tissues. Tissue samples were prepared by a two-step procedure, using Partec solution for isolation of cell nuclei and DAPI (fluorochrome 4'6-diamidino-2-phenylindole) for staining. DNA content was estimated from a minimum 5000 cells per specimen. Ten plant tissue samples were tested.

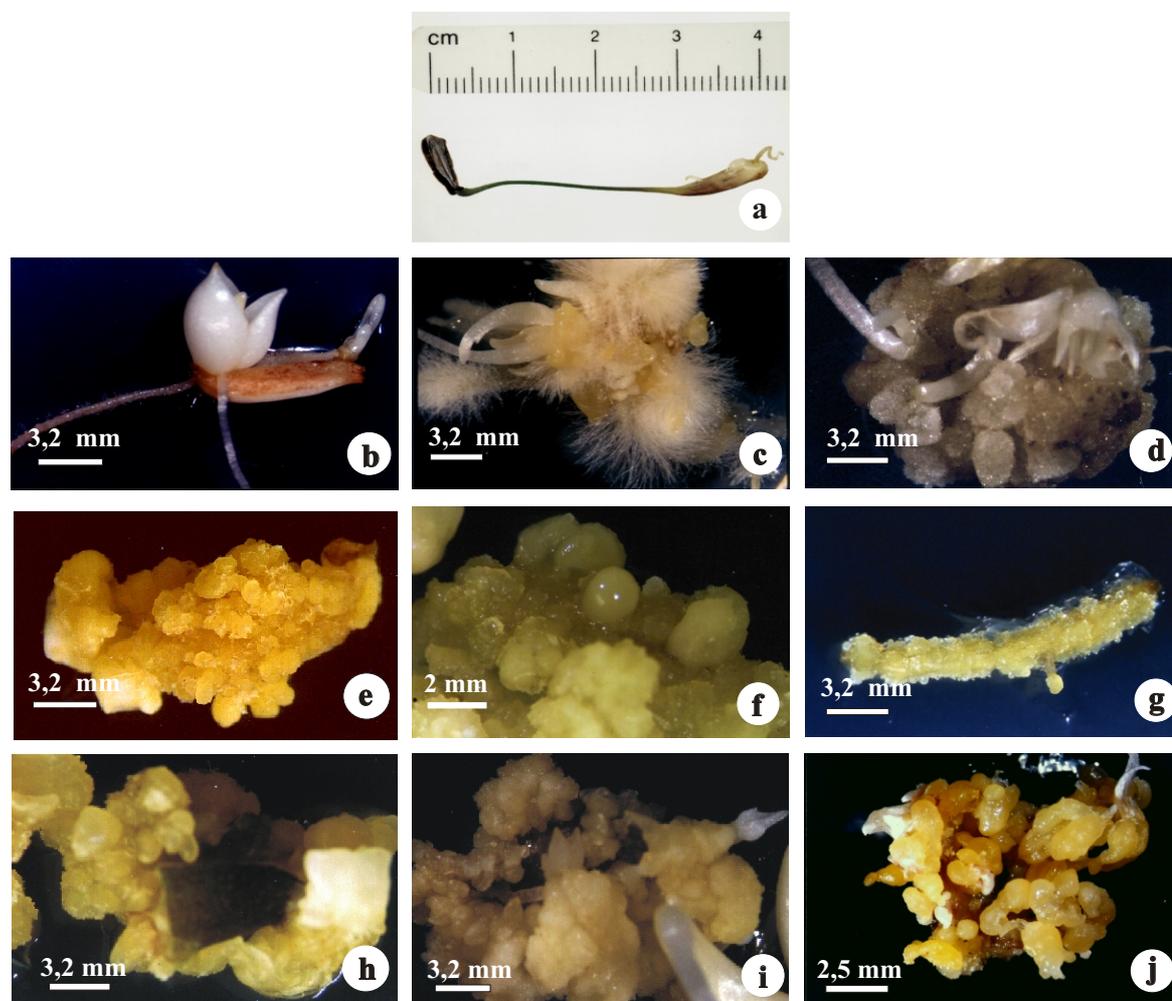
#### STATISTICS

The results were subjected to ANOVA, and the significance of differences between means was tested by Duncan's multiple range test ( $\alpha = 0.05$ ) using Stat (Skierniewice). There were five replicates of every combination of medium, each with 10 seeds or seedlings or scale explants, and each experiment was done twice.

## RESULTS

#### SEED GERMINATION AND SEEDLING DEVELOPMENT

Seed germination in vitro depended on the concentration of MS medium and the presence of auxin. Seeds



**Fig. 1.** Callus induction and plant regeneration in seedling culture (a–g) and bulb scale culture (h–j) of *L. martagon*: (a) Seedling obtained in vitro from a seed (half-strength MS medium, 20°C, 16 h photoperiod), (b) Bulblet initiation from seedling bulb (MS hormone-free medium), (c) Yellow, granular, friable callus, bulblets and hairy roots on bulb of seedling (MS medium + 0.5 µM Picloram), (d) Cream-white compact callus and bulblet on seedling bulb (MS medium + 5 µM BA), (e) Yellow, granular, friable callus on bulb of seedling (MS medium + 5 µM Picloram), (f) Yellow, granular, compact callus on bulb of seedling (MS medium + 5 µM Picloram + 5 µM BA), (g) Yellow compact callus on root of seedling (MS medium + 5 µM Picloram + 5 µM BA), (h) Yellow, granular, compact callus on bulblet explants (MS medium + 5 µM Picloram + 5 µM BA), (i) Cream-white compact callus and bulblets on bulblet explants (MS medium + 5 µM BA), (j) Yellow, granular, friable callus and somatic embryos on bulblet explants (MS medium + 5 µM Picloram).

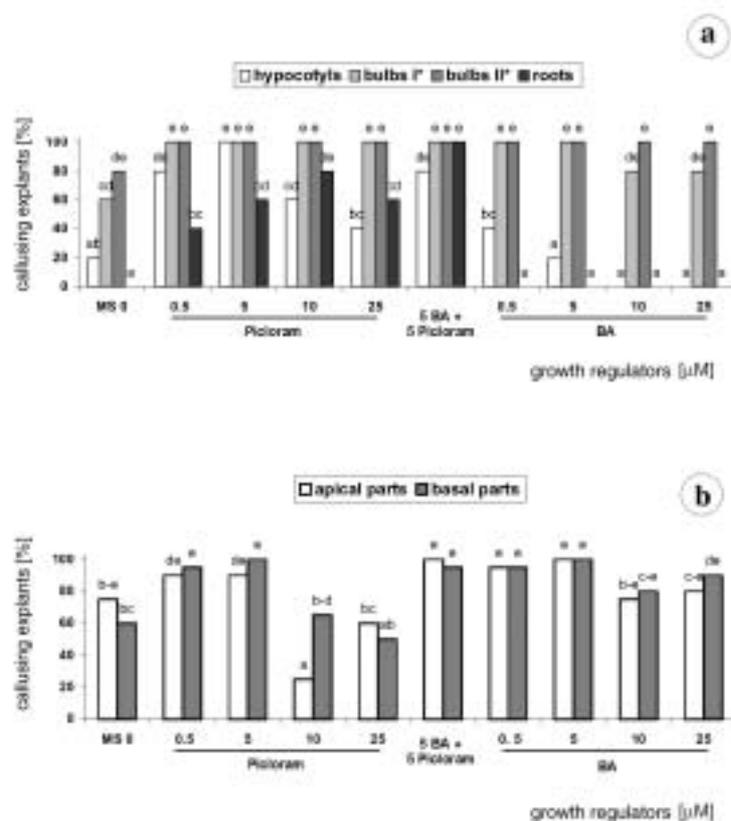
that had been stored at 20°C germinated within 4–12 weeks and formed seedlings with a single bulb, root and hypocotyl (Fig. 1a). The highest rate of germination (72%) was on MS or 1/2 MS in the presence of NAA or in 1/2 MS alone. MS medium without auxin yielded only 44% germination (Tab. 1).

In another series of experiments, seeds that had been cold-treated at 4°C for 3 months germinated at a higher rate at 20°C (50–85%) than at 25°C (10–45%) on both MS and 1/2 MS (Tab. 2).

The percentage of disinfected seeds was significantly high in all cases (85–100%) (Tab. 2).

#### CALLUS INDUCTION IN SEEDLING CULTURE

The callus formation response varied considerably between explant types. Explants started swelling after a week of culture. After 3–4 weeks, callus induction was observed on hypocotyls, bulbs and roots. The highest rate of callus development was on seedling bulb explants (80–100%) (Fig. 2a). Callus induction was achieved in 80–100% of the hypocotyl explants cultured on 0.5–5 µM Picloram and 5 µM Picloram plus 5 µM BA. Callusing occurred on seedling root explants in media containing Picloram (0.5–25 µM) alone or in combination with 5 µM Picloram and 5 µM BA. It did



**Fig. 2.** Effects of various concentrations of Picloram and BA on percentage of callusing explants in seedling culture (a) and bulblet scale culture (b) of *L. martagon* (\*bulbs I; bulbs II – 2 lengthwise bulb cuttings).

not occur in the absence of growth regulators or when only cytokinin was present (Fig. 2a).

Picloram induced yellow, friable, granular callus, whereas media containing BA resulted in the development of cream-white and compact callus. Callus tissue obtained in the presence of both Picloram and BA was yellow, compact and granular.

A considerable number of globular somatic embryos were observed on callus grown on medium with the addition of Picloram or Picloram with BA. Embryos grown on callus obtained in the presence of Picloram were white, whereas those obtained on media containing BA were transparent. A large number of elongated somatic embryos were observed on seedling bulb explants, but no embryos were found on root explants (Figs. 1b–g, 3a,b).

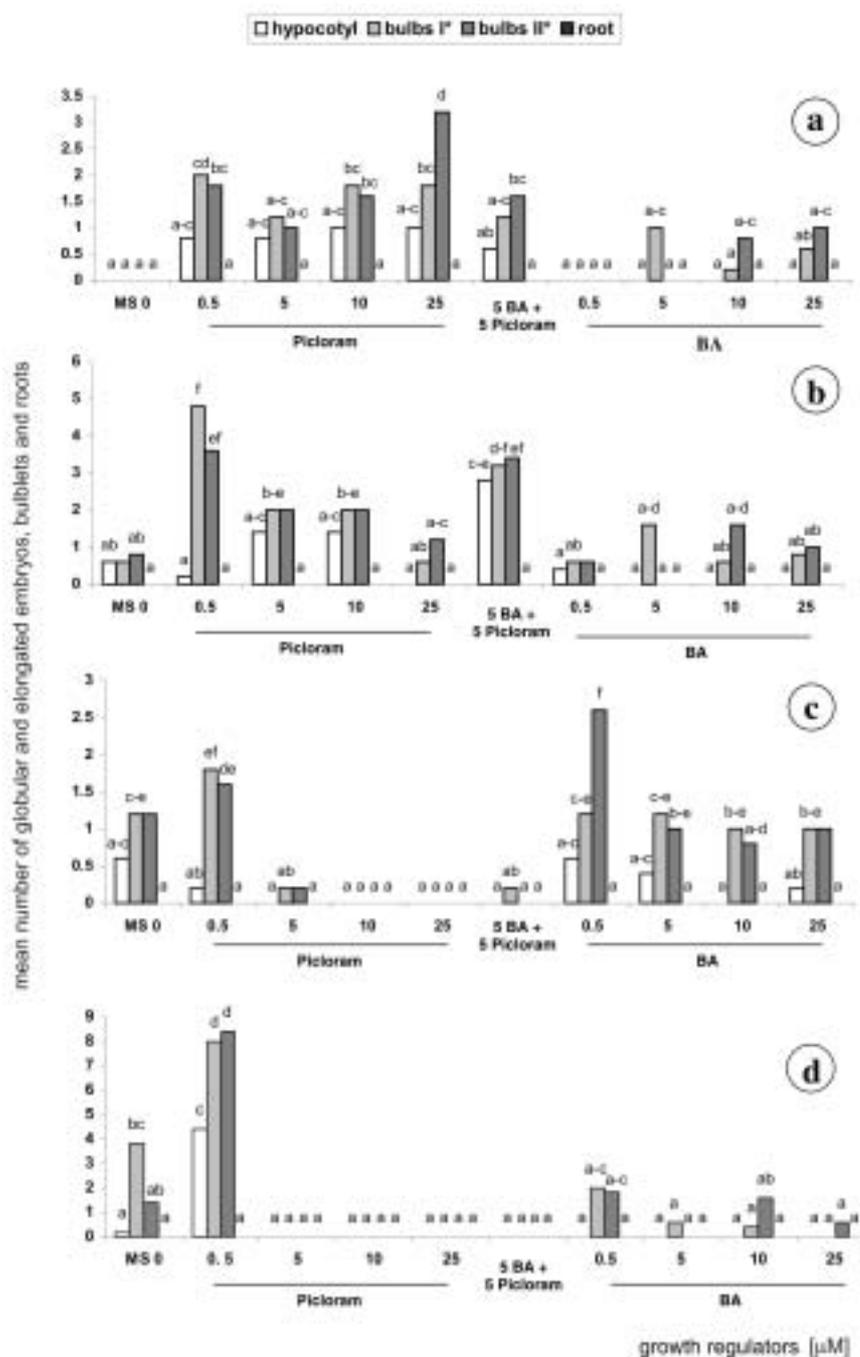
Besides somatic embryos, a great number of adventitious bulblets were observed on seedling bulb explants, especially on hormone-free MS medium or in the presence of 0.5 μM BA (Fig. 3c). Bulblets on media with BA were grown actively, whereas those on media containing Picloram or Picloram with BA were mostly dormant. No adventitious bulblets were observed on media containing high concentrations of Picloram (5–25 μM). The best rhizogenesis was observed on stand-

ard MS medium or MS with BA. Media with a low concentration of Picloram (0.5 μM) also favored the formation of thick and hairy roots from callus tissue (Fig. 3d).

#### CALLUS INDUCTION IN ADVENTITIOUS BULBLET SCALE CULTURE

Initiation of callus in adventitious bulblet scale culture was equally frequent on apical (79%) and basal parts (85%) of scales. Callus developed from the bulblet scale surface after ~2 months of culture in darkness (Fig. 1h–j). Higher concentrations of Picloram (10–25 μM) reduced the number of callusing explants from 85% to 25–65%. The highest amount of callus was obtained with 5 μM Picloram (95–100%; data not shown). BA stimulated the induction of callus through the entire concentration range. Hormone-free MS medium also induced callusing explants, at a lower frequency (Fig. 2b).

Different types of morphogenetic response were observed: globular and elongated embryos, adventitious bulblets and roots, depending on the concentration of growth regulators. A considerable number of globular embryos were obtained from the apical part of the

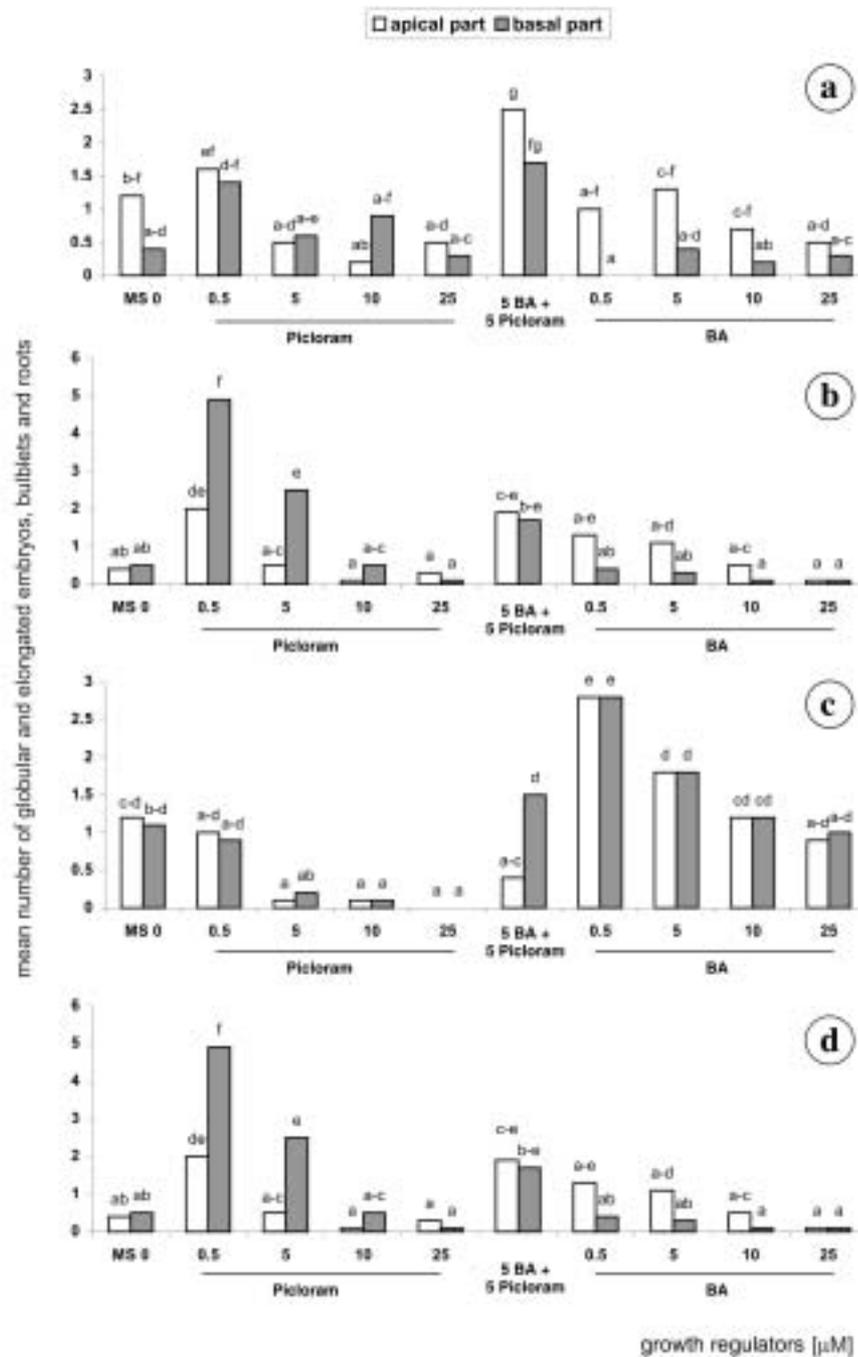


**Fig. 3.** Number of regenerated (a) globular and (b) elongated embryos, (c) bulblets and (d) roots of *L. martagon* in seedling culture at different concentrations of Picloram and/or BA (\*bulbs I; bulbs II – 2 lengthwise bulb cuttings).

scale bulbs on medium containing Picloram combined with BA. However, high concentrations of Picloram and BA added separately reduced the number of globular and elongated embryos (Fig. 4a,b).

The best media for adventitious bulblet formation were those including BA, especially at 0.5 μM concentration. Media with Picloram (5–25 μM) did not induce

the formation of bulblets. Basal segments of bulbs produced more roots than apical segments did. Short roots covered with dense hairy roots formed on medium with 0.5 μM Picloram. Roots that developed at the base of the bulb were longer than those formed directly from callus (Fig. 4c,d).

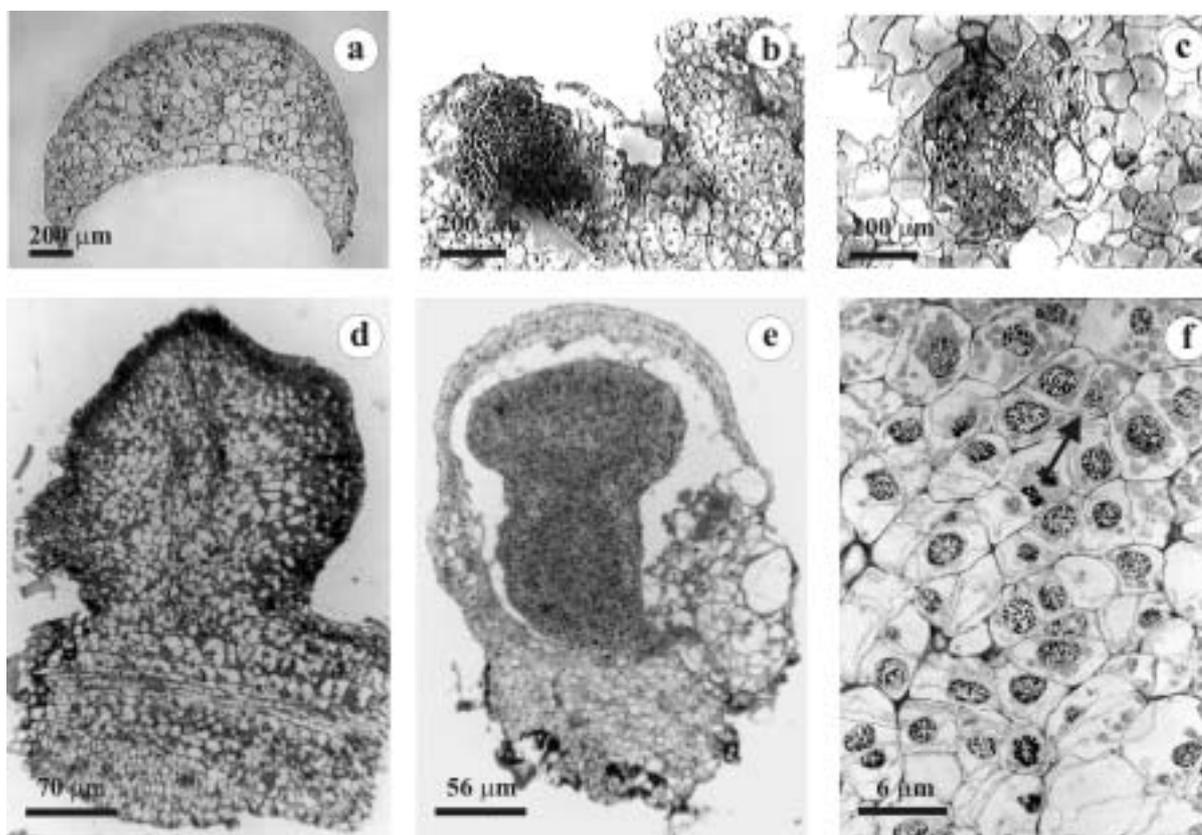


**Fig. 4.** Number of regenerated (a) globular and (b) elongated embryos, (c) bulblets and (d) roots of *L. martagon* in bulblet scale culture at different concentrations of Picloram and/or BA.

#### HISTOLOGICAL AND CYTOLOGICAL ANALYSES

Cytological analyses of single scales (control material) revealed the occurrence of epidermis and mesophyll with vascular bundles in the middle (Fig. 5a). The cells of the epidermal layer were smaller and more compact

than parenchyma cells. The dividing cells were located at the epidermal layer and around the vascular bundles. They were smaller than mesophyll cells, contained starch granules, and formed meristematic structures (Fig. 5b,c). Their morphology showed differences between somatic embryos and bulblets. Somatic embryos were solitary and loosely attached to the callus.



**Fig. 5.** Histological analysis of callus induction in *L. martagon*: (a) Cross-section of bulblet scale grown in vitro on MS medium (control material), (b) Cell proliferation at epidermal cells (MS medium + 5 µM Picloram), (c) Cell proliferation near vascular bundles (MS medium + 5 µM Picloram), (d) Longitudinal section of bulblet connected with tissue scale (MS hormone-free medium), (e) Longitudinal section of somatic embryo (MS medium + 5 µM Picloram), (f) Cell proliferation in callus tissue and starch accumulation in meristematic center.

Bulblets formed at the base of the explants and were closely connected by tissue (Fig. 5d,e). Cells with large nuclei, frequently in mitotic stage, and dense cytoplasm with great amounts of starch were observed on the surface of the callus. The inner cells were larger and loosely connected (Fig. 5f).

#### FLOW CYTOMETRY

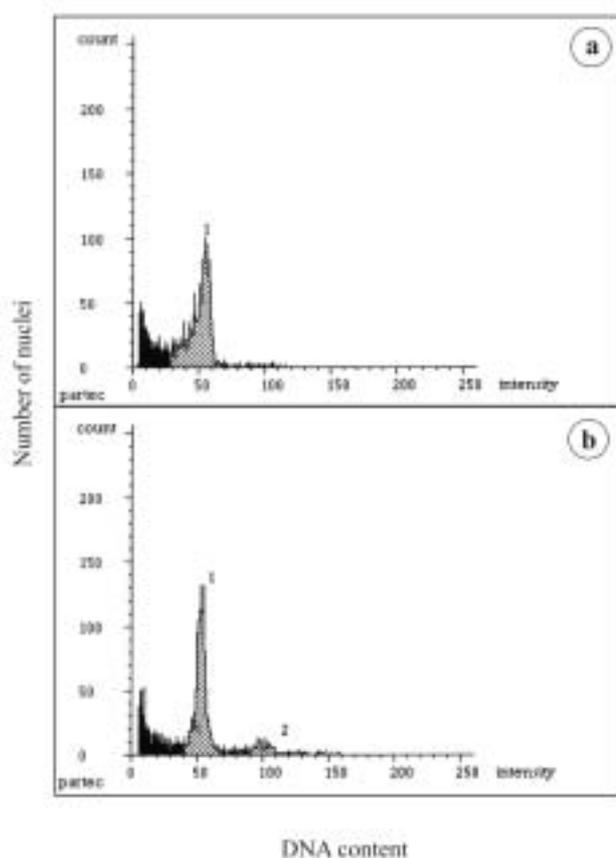
Flow cytometry was used to assess ploidy stability during micropropagation. The measurements showed no differences in the amount of DNA between the tissues of plants regenerated from callus and scale (Fig. 6). No haploids were found in the investigated samples.

#### DISCUSSION

The study presented here was focused on the initial stages of in vitro propagation of *Lilium martagon*, an endangered species legally protected in Poland. Despite strict protection, the population of *L. martagon* has

decreased over recent decades under the impact of human activity (Kolon et al., 1994). The use of tissue culture techniques can help in its restoration.

In vitro cultures of *Lilium* sp. are most frequently started from bulb scales. However, seeds are usually recommended for the production of pathogen-free plants (Pelkonen, 1997; McRae, 1998). We attempted to optimize the conditions for the germination of *L. martagon* seeds. In our experiments, germination was most efficient at lower temperature on 1/2 MS medium containing 15 g/l sucrose. The preference for lower temperature is characteristic of the germination process (Beattie and White, 1993). In earlier studies, seeds were used as explants by Mii et al. (1994) for *L. × formolongi* and by Pelkonen (1997) for *L. regale*, *L. martagon* and *L. bulbiferum* with germination rates similar to ours. Lower concentrations of macro- and micro-nutrients and sucrose stimulated seed germination in Pelkonen's (1997) work. Low concentrations of sucrose in medium have been suggested to stimulate the uptake of nutrients and decrease the level of abscisic acid required for the induction of dormancy in



**Fig. 6.** Flow cytometric analysis: (a) Bulblet scales (control material), (b) Callus tissue.

seeds (Stavarek and Rains, 1984; Vreugdenhil and Helder, 1992). Our work confirmed this suggestion.

In an attempt to increase the efficiency of micro-propagation through somatic embryogenesis, we investigated various factors influencing callus formation. In our experiments, callus induction was carried out on seedlings and adventitious bulblet scales using Picloram and/or BA at different concentrations. Callus induction was observed on each medium, but the best results were achieved on media with lower concentrations of Picloram and/or BA (0.5–5  $\mu\text{M}$ ). Hormone-free medium also stimulated the formation of callus on the explants, albeit less frequently. The highest percentage of callusing explants was obtained on bulb fragments from seedling culture and adventitious bulb scales.

We observed the formation of three types of callus tissue. The type of callus formed depended on the kind and concentration of growth regulators. Hormone-free media or MS medium containing BA induced cream-white, compact callus. Yellow, granular and friable callus appeared on media with Picloram, whereas the presence of both Picloram and BA resulted in callus

that was yellow, granular and compact. Yellow granular callus consists of cell aggregates with dense, easily dividing cytoplasm, most useful for cell culture in suspension. The embryogenic callus contains a large amount of starch, which is an important factor in embryogenic tissue. Starch grains appear when the cells become embryogenic, and also in meristematic cells of embryos (Thomas, 1972; Schwendiman et al., 1988).

The positive effect of Picloram on callus induction has been reported before for monocotyledons (Beyl and Sharma, 1983; Sellés et al., 1999). A low concentration of Picloram stimulated cream-white granular callus in seedling culture of *L.  $\times$  formolongi* (Mii et al., 1994) and *L. longiflorum* 'Snow Queen' and the Oriental hybrid 'Star Gazer' (Tribulato et al., 1997a,b). Tribulato et al. (1997a) reported that they obtained friable callus only on media with a low concentration of Picloram (2  $\mu\text{M}$ ) on styles, pistils and flower pedicels of *L. longiflorum* 'Snow Queen' and the Oriental hybrid 'Star Gazer.' Our results demonstrate that callusing can also be induced in seedlings and adventitious bulblet scales of *Lilium martagon* when Picloram is used at concentrations in the range of 0.5–25  $\mu\text{M}$ . The addition of BA to the medium with Picloram stimulates the formation of a more compact callus.

Treatment with growth regulators during callus induction can result in genetic modification of the plant material, most often chromosome number aberrations (Pierik, 1987). Although such variation can be used as a tool when creating new ornamental varieties, it is important to ensure that no changes are introduced when, as is the case here, species conservation is the ultimate goal. To test for chromosome stability, flow cytometry was used as a rapid method of determining DNA content in the individual cells in culture. In our experiments, the ploidy of the callus and regenerated plants was stable. Somaclonal variation is very rare in the genus *Lilium*. Cytological methods and observations by Sheridan (1974), Simmonds and Cumming (1976), Mii et al. (1994) and Wickremesinhe et al. (1994) confirmed chromosome stability at the diploid level in lilies. Van Harmelen et al. (1997) also found only slight variation in regenerants obtained from 3-year-old callus of *L. longiflorum* 'Gelria.'

Seeds of *L. martagon* are good material for obtaining virus-free plants. Different growth regulators stimulate different callus types. The results of this study suggest that Picloram or Picloram plus BA are favorable for embryogenic callus induction. The efficiency of morphogenesis (percentage of callusing explants, number of adventitious bulblets per explant) of *L. martagon* depends on the kind of explant, and is highest in bulb seedlings and adventitious bulblet scales. Initiation of callus in lily culture without the loss of morphogenic potency is important to achieve in transformation techniques. The presented procedure

could be applied to multiplication of this endangered species or to its genetic manipulation.

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#### REFERENCES

- BEATTIE DJ, and WHITE JW. 1993. *Lilium* – hybrids and species. In: De Hertogh AA, and Le Nard M [eds.], *The physiology of flower bulbs*, 423–454. Elsevier Science Publisher, B. V., Netherlands.
- BEYL CA, and SHARMA GC. 1983. Picloram induced somatic embryogenesis in *Gasteria* and *Haworthia*. *Plant Cell, Tissue and Organ Culture* 2: 123–132.
- FAMELAER I, BORDAS M, BALIU E, ENNIK E, and MEIJER H. 1997. The use of morphogenic suspension cultures for the development of a protoplast regeneration system in lily. *Acta Horticulturae* 430: 339–345.
- JENSEN WA. 1962. *Botanical histochemistry*. Freeman, San Francisco.
- KOLON K, KRAWCZYK A, and SZCZYREK B. 1994. Dynamika populacji lili złotogłów (*Lilium martagon* L.) w Karkonoszach. *Geoekologiczne Problemy Karkonoszy* 237–240.
- MAŁAŁSKI J. 1975. *Atlas Flory Polskiej i Ziemi Ościennych. (Florae Polonicae Terrarumque Adiacentium Iconographica). Liliaceae* (P. 2). Academia Scientiarum Polona, Institutum Botanicum, vol. II. (4), 26–27, 189. PWN, Warszawa-Wrocław-Krakow.
- MAESATO K, SHARADA K, FUKUI H, HARA T, and SARMA S. 1994. *In vitro* bulblet regeneration from bulb scale explants of *Lilium japonicum* Thunb. Effect of plant growth regulators and culture environment. *Journal of Horticultural Science* 69: 289–297.
- MCRAE EA. 1998. *Lilies*, 1–162, Timber Press, Portland, Oregon (USA).
- MEUSEL H, JAEGER E, and WEINERT E. 1965. *Vergleichende Chorologie der Zentraleuropäischen* 96, 119–120, 444. Flora, Karten, Jena.
- MII M, YUZAWA Y, SUETOMI H, and MOTEKI T, and GODO T. 1994. Fertile plant regeneration from protoplasts of a seed-propagated cultivar of *Lilium* × *formolongi* by utilizing meristematic nodular cell clumps. *Plant Science* 100: 221–226.
- MURASHIGE T, and SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15: 473–497.
- NIMII Y. 1984. Bulblet-productivity of explants from scales, leaves, stems and tepals of *Lilium rubellum* Baker. *Scientia Horticulturae* 22: 391–394.
- PELKONEN VP, and KAUPPI A. 1999. The effect of light and auxins on the regeneration of lily (*Lilium regale* Wil.) cells by somatic embryogenesis and organogenesis. *International Journal of Plant Sciences* 160: 483–490.
- PELKONEN VP. 1997. Tissue culture as means in the production of hardy lilies. *Acta Horticulturae* 447: 665–667.
- PIERIK RLM. 1987. *In vitro culture of higher plants*. Martinus Nijhoff Publishers, Dordrecht, Netherlands.
- PRİYADARSHI S, and SEN S. 1992. A revised scheme for mass propagation of Easter Lily. *Plant Cell, Tissue and Organ Culture* 30: 193–197.
- RYBCZYŃSKI JJ, and GOMOLIŃSKA H. 1989. 6-benzyladenine control of the initial bulblets formation of wild *Lilium martagon* L. *Acta Horticulturae* 251: 183–189.
- SCHWENDIMAN J, PANNETIER C, and MICHAUX-FERRIERE N. 1988. Histology of embryogenic formation of oil palm *Elaeis guineensis*. *Annals of Botany* 62: 43–52.
- SELLÉS M, VILADOMAT J, BASTIDA J, and CODINA C. 1999. Callus induction, somatic embryogenesis and organogenesis in *Narcissus confusus*: correlation between the state of differentiation and the content of galanthamine and related alkaloids. *Plant Cell Reports* 18: 646–651.
- SHERIDAN WF. 1974. Plant regeneration and chromosome stability in tissue cultures. In: Ledoux L [ed.], *Genetic manipulation with plant material*, 263–267. Plenum Publishing, New York.
- SIMMONDS JA, and CUMMING BG. 1976. Propagation of *Lilium* hybrids. I. Dependence of bulblet production on time of scale removal and growth substances. *Scientia Horticulturae* 5: 77–83.
- STAVAREK SJ, and RAINS DW. 1984. The development of mineral stress. *HortScience* 3: 377–381.
- TAKAYAMA S, and MISAWA M. 1982/83. A scheme for mass propagation of *Lilium in vitro*. *Scientia Horticulture* 18: 353–362.
- THOMAS T. 1972. The fine structure of the embryogenic callus of *Ranunculus*. *Journal of Cell Science* 11: 95–109.
- TRIBULATO A, REMOTTI PC, LOEFFLER HJM, and VAN TUYL JM. 1997a. Somatic embryogenesis and plant regeneration in *Lilium longiflorum* Thunb. *Plant Cell Reports* 17: 113–118.
- TRIBULATO A, REMOTTI PC, and LOEFFLER HJM. 1997b. Lily regenerative callus and cell cultures for transformation. *Acta Horticulturae* 430: 299–306.
- VAN HARMELLEN MJ, LOEFFLER HJM, and VAN TUYL JM. 1997. Somaclonal variation in lily after *in vitro* cultivation. *Acta Horticulturae* 430: 347–350.
- VREUGDENHIL D, and HELDER H. 1992. Hormonal and metabolic control of tuber formation. *Progress in Plant Growth Regulation*, 399–400. Kluwer Academic Publishers, Netherlands.
- WATAD AA, YUN DJ, MATSUMOTO T, NIU X, WU Y, KONONOWICZ AK, BRESSAN RA, and HASEGAWA PM. 1998. Microprojectile bombardment – mediated transformation of *Lilium longiflorum*. *Plant Cell Reports* 17: 262–267.
- WICKREMESINHE ERM, HOLCOMB EJ, and ARTECA RN. 1994. A practical method for production of flowering Easter lilies from callus cultures. *Scientia Horticulturae* 60: 143–152.