Acta Universitatis Latviensis, Biology, 2004, Vol. 676, pp. 163–166

Tissue culture for elimination of lily viruses depending on explant type

Stase Dapkūniene[1]*, Gitana Indrišiūnaite[2], Regina Juodkaite[2], Meletele Navalinskiene[3], Marija Samuitiene[3]

[1]Department of Plant Physiology and Microbiology, Vilnius University, Ciurlionio 21/27, Vilnius-9 LT-10301, Lithuania
[2]Botanical Gardens of Vilnius University, Kairenų 43, Vilnius-40 LT-10239, Lithuania
[3]Institute of Botany, Žaliųjų Ežerų 49, Vilnius-21 LT-08406, Lithuania
*Corresponding author, E-mail: stase.dapkuniene@gf.vu.lt

Abstract

The aim of the present work was to assess the potential of lily virus elimination by tissue culture *in vitro*. The medium used for explants was based on Murashige and Skoog medium. Plantlets regenerated from explants were examined by electron microscopy. Plantlets from basal parts of the petals and of basal pieces of leaves used as explants resulted in the best virus elimination. In conclusion, tissue culture is applicable for virus elimination in lily cultivars and hybrids but the success depends on the type of explants.

Key words: explant, lily *in vitro*, regeneration, virus elimination.

Introduction

Lithuanian breeders have created a great number of cultivars and hybrids of lilies, which constitute a part of the national treasure and ethnic culture (Dainauskaite, Indrišiūnaite 1997). Lilies introduced or created by local breeders are grown in special collection nurseries at the Department of Floriculture of Vilnius University Botanical Gardens (130 cultivars), Department of Plant Systematic and Geography (700 cultivars) and *in vitro* in the Laboratory of Plant Physiology and Biochemistry (48 cultivars). Seven viruses affecting lily have been isolated and identified in Lithuania: *Lily mottle potyvirus* (LMV), *Lily symptomless carlavirus* (LSV), *Tomato ringspot nepovirus* (ToRSV), *Cucumber mosaic cucumovirus* (CMV), *Tobacco rattle tobravirus* (TRV), *Tomato spotted wilt tospovirus* (TSWV) and *Lily X potexvirus* (LXV) (Dapkūnienė et al. 2000). According to some scientists, tissue isolation and cultivation leads to partial release of tissues from viruses (Allen 1974). It has been hypothesized that these cells may have a special system for eliminating viruses (Kubitz 1979). Different explants have been widely used for initial cultivation (Montezuma-de Carvalho, Quimares 1974; Niimi, Onozawa 1979; Globa-Mikhjlenko et al. 1986). The aim of the present work was to investigate the possibility of lily virus elimination by tissue culture *in vitro*, using micro bulbs from scale, bulbils from stem, and segments of the basal part of the petals and leaves as explants.

Materials and methods

The cultivars of the Asiatic hybrid lilies 'Aelita', 'Dzintars', 'Red Beauty' and 'Volchova' were used for cultivation in tissue culture. The bulbils from stem, bulblets from scale, basal pieces of leaves and basal pieces of the petals were used as the explants. The number of explants per variant was 20. Bulblets on the scales were obtained, separated, sterilized and were used as explants (Dapkūnienė et al. 2000). Other explants were sterilized with 0.1 % sublimate ($HgCl_2$) for 5 min and then three times rinsed in sterile distilled water for 15 min. The medium used for explants was based on Murashige and Skoog (1962; MS) medium: twice MS with addition of 1 mg l^{-1} 6-benzylaminopurine, 1 mg l^{-1} naphthaleneacetic acid for basal pieces of the petals by Takayama and Misawa (1982); for bulbils from stem – with addition of 5 mg l^{-1} naphthaleneacetic acid, 0.5 mg l^{-1} kinetin, 30 g l^{-1} sucrose and 10 g l^{-1} agar; MS with addition of 5 mg l^{-1} 6-benzylaminopurine, 0.1 mg l^{-1} naphthaleneacetic acid for bulblets from scale by Jakobsone and Andersone (1997) and MS with addition of 0.1 mg l^{-1} 6-benzylaminopurine, 1 mg l^{-1} naphthaleneacetic acid for the basal part of leaves as explants by Niimi and Onozava (1979). All mother material and plantlets were examined by electron microscopy (Robinson et al. 1987) and DAS-ELISA (Hagita 1989).

Results

The regeneration of explants differed depending on the type of explant and properties of the lily cultivar. Not all explants (bulblets from scale, bulbils from stem, basal pieces of the petals) regenerated at a full capacity (Fig. 1). The basal part of leaves and petals formed embryogenic calluses, from which regenerants were obtained.

The biggest number of regenerants were obtained from embryogenic calluses of basal pieces of leaves (from 166.7 to 309.1 %).

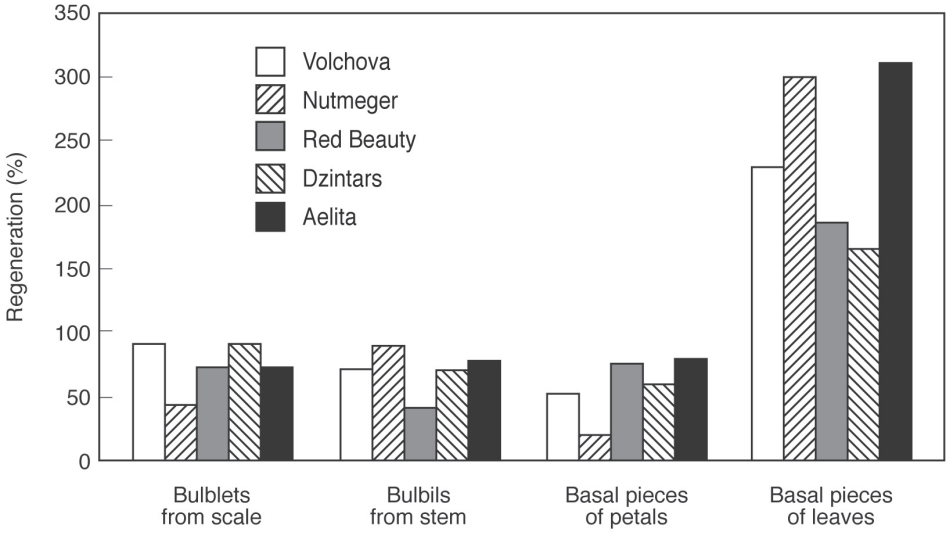

Fig. 1. Regeneration capacity of lily explants (%) of different cultivars.

Table 1. Electron microscopy observation of lily virus infection

Plant material	Morphology of virus particles in lily cultivars			
	'Aelita'	'Dzintars'	'Red Beauty'	'Volchova'
Scale of a mother plant	Isometric (30 and 80-120 nm in diameter), filamentous (lengths of 550, 640-650 and 750-770 nm)	Isometric (30 and 80-120 nm in diameter), filamentous (lengths of 550, 640-650 and 750-770 nm)	Isometric (30 and 80-120 nm in diameter), filamentous (lengths of 550, 640-650 and 750-770 nm)	Isometric (30 and 80-120 nm in diameter), filamentous (lengths of 550, 640-650 and 750-770 nm)
Plantlet of bulblets from scale	No particles	Isometric (30 nm in diameter), filamentous (lengths of 640-650 nm)	No particles	Isometric (30 nm in diameter), filamentous (lengths of 640-650 nm)
Plantlet of bulbils from stem	Isometric (30 and 80-120 nm in in diameter) filamentous (lengths of 750-770 nm)	Isometric (30 nm in diameter)	No particles	Isometric (30 and 80-120 nm in in diameter)
Plantlet of basal parts of the petals	No particles	Isometric (30 nm in diameter)	No particles	No particles
Plantlet of basal pieces of leaves	Isometric (30 and 80-120 nm in in diameter) filamentous (lengths of 750-770 nm)	No particles	No particles	No particles

All mother material and plantlets regenerated from different explants of the lily cvs. 'Aelita', 'Dzintars', 'Red Beauty' and 'Volchova' were examined by electron microscopy (Table 1). Some of the tested lily plantlets regenerated from basal pieces of leaves of cvs. 'Dzintars', 'Red Beauty' and 'Volchova', from the basal part of the petals of 'Aelita', 'Red Beauty' and 'Volchova', from bulb-scale explants of 'Aelita', 'Red Beauty' and from bulbils of 'Red Beauty', were found to be released from virus infection.

Discussion

In vitro propagation technology adopted for the floral industry was aimed initially to control virus and viroid disease problems in several important crops (Daub et al. 1997). For Asiatic hybrids, methods using adventitiously formed meristems on bulb-scale

explants resulted in 50 - 70 % LSV-free plants. No virus-free plants of *Lilium longiflorum* cultivars were obtained using this type of meristem. The culture of isolated vegetative stem apices of the bulbs can be used to release these lilies from LSV. Detection of LSV with ELISA during culture in vitro has a high reliability in contrast to the detection of LMV (Bloom-Batnhoorn, Van Aatrijk 1995). In our case, the bulblets from scale and bulbils from stem explants regenerated directly. The regeneration from the basal part of the petal and basal pieces of leaves proceeded through callus, but more plantlets from one explant were obtained from basal pieces of leaves (Fig. 1). According to virus particle morphology and symptom expression on test plants it was established that mother bulb scale material was infected by LSV, CMV, LMV, TSWV and LXV (Dapkūnienė et al. 2000). Using the tissue culture in vitro technique we obtained release from virus infection in some cultivars. Plantlets from basal parts of the petals and of basal pieces of leaves used as explants resulted in the best virus elimination. Tissue culture is suitable for virus elimination, but success depends on type of explants.

References

Allen T.C. 1974. Production of virus-free lily. *Acta Hortic*. 36: 235–240.

Dainauskaitė D.J., Indrišiūnaitė G. 1997. Genetic resources of ornamental field herb plants and their preservation problems in Lithuania. *Baltic Botanical Gardens in 1996 Estonia, Latvia, Lithuania*. Riga. pp. 54–57.

Dapkūnienė S., Šimonienė L., Navalinskienė M., Samuitienė M., Jakobsone G. 2000. Micropropagation of lily (*Lilium* L.) and virus elimination. *Horticulture and Vegetable Growing* 19: 499–506.

Daub M.E., Jones R.K., Mayer J.W. 1997. Biotechnology approaches for virus resistance in floral crops. In: Geneve R.L., Preece J.E., Merkle S.A. (eds). *Biotechnology of Ornamental Plants*. University Press. Cambrige. pp. 335-354.

Globa-Mikhjlenko I.D., Dmer I.F., Smirnova N.S. 1986. Propagation of lilies in tissue culture. *Commercial Floriculture in Southern Regions of USSR* 33: 115–123.

Hagita T. 1989. Detection of cucumber mosaic virus and lily symptomless virus from bulb scales of Maximowicz's lily by enzyme-linked immunosorbent assay (ELISA). *Ann. Phytopathol. Soc. Jap*. 55: 344–348.

Jakobsone G., Andersone U. 1997. Propagation of Latvian-selected lily *in vitro* in the National Botanic Gardens. *Baltic Botanical Gardens in 1996 Estonia, Latvia, Lithuania*. Riga. pp.76–83.

Kubitz K., Börner R., Richter P. 1979. Versuche zur Vermechrung von Lilien über Kalluskultur. *Arch. Gartenbau*. 27: 379–389.

Montezuma-de Carvalho J., Quimares M.M. 1974. Production of buds of plantlets from stamen's filament of *Lilium regale* cultivated *in vitro*. *Biol. Plant*. 16: 472–473.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant*. 15: 473–479.

Niimi V., Onozava J. 1979. *In vitro* bulblet formation from leaf segments of lily, especially *Lilium ruvellum* Baker. *HortScience* 11: 379–389.

Robinson D.G., Echlers U., Herhen R. 1987. *Methods for Electron Microscopy.* Springer-Verlag. Berlin. 190 p.

Takayama S., Misawa M. 1982. Regulation of organ formation by cytokinin and auxin in *Lilium* bulbscales grown *in vitro*. *Plant Cell Physiol*. 23: 67–74.