Micropropagation of Decorative Plants in Bulgaria

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INTRODUCTION

The use of micropropagation for clonal multiplication of ornamental species in Bulgaria began about 25 years ago. The earliest research explored the seasonal regenerative ability of isolated meristem tissues from *Dianthus* (Izvorska and Kacharmazov, 1977) and the obtaining of haploid plants from *Anemone hepatica* (Georgiev and Chavdarov, 1974). The in vitro propagation of ornamental tree species started later, with the first successful cloning of *Betula pendula* through callus cultures, introduced from apical and leaf segments (Iliev and Chavdarov, 1988).

By the 1990s laboratories were being built and equipped for in vitro propagation of ornamental, forest, and agricultural species and by the middle of the decade there were six large-scale laboratories in different research institutes and two on farms. Each of them has a considerable industrial capacity; they were designed to satisfy not only Bulgarian needs, but those of countries of the former Soviet Union and other eastern European countries. With its total of 19 laboratories, Bulgaria has the 12th largest micropropagation capacity in Europe (Riordain, 1997). The main purpose of the work in these laboratories is the investigation of propagation technologies of economically valuable plants.

In particular, methods have been developed and applied to cloning and multiplication of *Dianthus* (Yantcheva et al., 1997a), *Dahlia* (Nencheva and Protich, 1996), *Rosa* (Kornova and Angeliev, 1996; Uzunova, 1996), *Lilium* (Chavdarov and Denkova, 1994), *Hippeastrum* (Denkova et al., 1995), *Rhododendron* (Haralampieva and Gyuleva, 1996), *Betula* (Iliev and Chavdarov, 1988a), *Quercus* (Tsvetkov and Atanassov, 1992), *Populus* (Gyuleva and Atanassov, 1992), *Sequoiadendron* (Iliev and Iliev, 1996), *Sequoia* (Iliev and Trifonov, 1996b), *Metasequoia* (Iliev and Tsvetkov, 1995), *Albizia* (Iliev et al., 1993), *Robinia* (Iliev and Ganchev, 1991), *Paulownia* (Gyuleva and Garelkova, 1993), *Platanus* (Gyuleva and Atanassov, 1994), and others.

Bulgarian researchers, like those elsewhere, have two goals for their work on micropropagation techniques: multiplication of existing taxa and creation of genetic variability.

MULTIPLICATION OF EXISTING TAXA

Micropropagation to increase existing taxa is carried out by: (1) direct organogenesis; (2) adventitious bud-formation; and (3) somatic embryogenesis.

The most often used explants for inducing direct organogenesis are segments with axillary and/or apical buds. When obtained from tree and shrubs species they are taken from dormant specimens but juvenile (Iliev and Ganchev, 1991; Tsvetkov and Atanassov, 1992; Iliev et al., 1993; Iliev and Tsvetkov, 1995) or mature (Gyuleva and Atanassov, 1994) donors can be used. Micropropagation has also been used to effectively rejuvenate propagation material to improve speed and quality of production, for example *B. pendula*, *Sequoia sempervirens*, *Sequoiadendron giganteum* (Iliev, 1996; Iliev and Trifonov, 1996; Iliev and Iliev, 1996). The technique shows promise for the production of stockplants that will result in high yields of difficult-to-propagate plants produced by conventional vegetative propagation techniques.

Apical, nodal (Iliev, 1991, 1996a; Haralampieva and Gyuleva, 1994; Chavdarov and Denkova, 1994; Uzunova, 1996) or leaf segments (*B. pendula* cultivars Tristis, Youngii, Laciniata (syn. 'Dalecarlica); *P. tremuloides*) (Iliev, 1988, 1996, Iliev et.al., 1998; Gyuleva and Atanassov, 1992) have all been used successfully as initial explants for inducing adventitious bud formation. It was established that younger leaves have higher morphogenetic potential, which also depends on their position on the shoot. Induction of adventitious bud formation and the rate and extent of multiplication are determined to a great extent by the genotype, which imposes the use of different nutrient media and phytohormones.

Somatic embryogenesis has by far the highest multiplication coefficient of any of the micropropagation methods used in Bulgaria. Experiments are under way to study the effect of combinations of various hormones on induction of the process in immature embryos of common oak (*Quercus robur* L.) (Tsvetkov, 1998).

An essential stage in the production cycle of the in-vitro-cloned plants is their acclimatization to greenhouse and field conditions. Trials here have shown that basic factors that aid successful acclimatization are the preliminary washing of the plantlets' roots; high soil moisture content and atmospheric humidity, and the temperature of the greenhouses. Based on these results, techniques for industrial acclimatization have been elaborated for a number of species of *Gerbera, Dendranthema*(syn.*Chrysanthemum), Phillodendron, Rosa, Gypsophila, Cordyline*, and others. Trial plantations have been established from in-vitro-cloned plants from *B. pendula* at an altitude of 900 m above the sea level.

Micropropagation is currently being exploited in Bulgaria for the large-scale production of flowers. The need to obtain large quantities of virus-free planting material has seen the routine use of meristem culture coupled with contemporary methods for virus indexing, such as ELISA (Jankulova et al., 1983; Eskenazy, et al., 1983; Denkova et al., 1993; Denkova and Chavdarov, 1994) and ISEM (Kajtazova, 1983).

CREATION OF GENETIC VARIABILITY

Genetic engineering is one of the possible methods for obtaining new forms of plants with desirable ornamental features. At the present stage this technique is generally only being developed for agricultural species, for example by the Institute of Genetic Engineering, Kostinbrod.

Transgenic techniques, using transformation with *Agrobacterium tumefaciens*, have been used to produce new ornamental cultivars (Lena, Scania, Yanita, Regina, Nasslada, and Line 84) from *Dianthus caryophyllus* (Yantcheva et al., 1997b).

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