Multiplication and growth acceleration of *Lilium rhodopaeum* Delip. using *in vitro* and hydroponic technologies

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Introduction: *Lilium rhodopaeum* Delip. (Liliaceae) is a Balkan endemic, with limited distribution in Bulgaria and Greece, in The Rhodopes. The species is included in the Red Data Book of Bulgaria as critically endangered, and protected by the Biological Diversity Act. Its populations are fragmented, with low density of the individuals.

Propagation and planting of bulbs in the populations would be a good measure for their strengthening. According to our previous studies, the growth of the *in vitro* obtained bulblets and the *ex vitro* adaptation to soil were very slow. On the other hand, soilless cultivation was reported as a successful alternative for large-scale production of *Lilium* hybrids. The main advantages of hydroponics are rapid plant growth and development, and independence from seasons and soil type.

Aim: The aim of the present study was to accelerate the growth of *in vitro* multiplied *Lilium rhodopaeum* bulblets using 2 different hydroponic systems and to compare their effectiveness.

Fig. 1. *Lilium rhodopaeum* in the natural population near Sivino village.

Material and methods: Plant bulbs were taken from the largest population of the species in Bulgaria, in the protected area "Livadite" near Sivino village (Fig. 1). Bulb half-scales, used as initial material for *in vitro* clonal propagation, were disinfected by soaking in 70% ethanol for 1 min. and 50% commercial bleach for 60 min., then rinsed with sterile distilled water trice for 10 min. Explants were put on MS based media supplemented with different plant growth regulators, to form bulblets. Growth of 75 uniform *in vitro* bulblets evenly distributed in 3 variants (Fig. 2) has been tested: on Flood & Drain hydroponic system (with perlite as substrate), Cutting Board hydroponic system (deep water culture system, peat cubes as substrate), and Control (soil substrate, wick system).

Fig. 2. In vitro bulblets of *L. rhodopaeum* used as starting material for hydroponic experiment.

Fig. 3. *In vitro* clonal propagation of *L. rhodopaeum*. A) Initial native bulb; B) Formation of *in vitro* bulbets on a half-scale; C) Over 20 bulblets on a single explant; D) Growth of *in vitro* bulblets separated from the explant.

Fig. 4. Hydroponic experiment: A) Flood & Drain hydroponic system; B) Cutting Board hydroponic system; C) Control (soil substrate, wick system).

Results: *In vitro* micropropagation was successfully applied, resulting in an average of 4.7±1.8 bulblets per explant for 4 months. Some explants formed over 20 bulblets on the best nutrient medium (MS, supplemented with 0.1 mg/l kinetin and 0.5 mg/l NAA) (Fig. 3). Six months after the start of the experiment significant differences were noticed between variants concerning bulblets' survival and growth, and root development. Bulbs' growth was considerably accelerated on Flood & Drain hydroponic system owing to the favorable conditions for root system development (Fig. 4-A). In this variant all bulblets survived and are ready to be potted in soil mixture, and then gradually adapted to natural conditions. Roots of bulblets on Cutting Board hydroponic system were weak and bulblets hardly grew although almost all of them survived (Fig. 4-B). Worst results were observed in the control variant where 45.8% of the bulblets died (Fig. 4-C).

Conclusions: Flood & Drain hydroponic system was chosen as the suitable one for growth acceleration of the *in vitro* obtained *L. rhodopaeum* bulblets.

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