Application of cell and tissue culture for small-flowered clematises (genus Clematis L.) propagation using *in vitro* conditions

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Small-flowered clematis saplings are obtained by both vegetative and generative reproduction. Species of the group can be reproduced by seeds. The main method for mass vegetative propagation of forms and varieties is green cuttings, for small volume of production - bush division and layering. Selectionists usually use grafting for breeding new hybrids and rare varieties. In vitro culture methods are increasingly used for producing saplings in ornamental nursery.

Despite the wide range of research conducted by scientists from around the world, the question of small-flowered clematises clonal micropropagation is not fully disclosed and requires further research.

The aim of research was the obtaining of small-flowered clematises aseptic culture, through the selection of primary explants and establishing conditions for their introduction to the culture in vitro.

Seed, vegetative buds and leaves of small-flowered clematises species and varieties (Clematis viticella L., C. tibetana Kuntze., C. ispahanica Boiss. 'Zvezdograd', C. fargesii Franch. 'Paul Farges' and C. taxensis Buckl. 'Princess Diana') were used as the basic material.

In the experiment the following sterilizing substances had been used: sodium hypochlorite NaClO in concentrations of 3.3 and 5 %, hydrogen peroxide H_2O_2 – 17.5 %, solution of potassium permanganate $KMnO_4$ – 1% sulfuric acid H_2SO_4 – 0.1 %.

Modified nutrient medium Murashige and Skoog (MS) enriched with growth regulators (zeatin (Zea), 6-benzylaminopurine (BAP), hiberellin (GA) and naphthalene acetic acid (NAA)) had been exploited.

Seeds before germination and leaf explants prior to initial callus formation had been kept in the dark at 25… ± 2 ° C. After this cultivation was been continued at 2 kLk lighting and 16-hour photoperiod with temperature and humidity value 24…± 2 ° C and 70 % severally. Vegetative buds from the beginning had been being cultivated in the light.

Explants preparation and introduction to the culture in vitro was conducted according to accepted guidelines, providing compliance with asepsis principles.

Soaking during 20 minutes in hydrogen peroxide solution (17.5%) is useful for C. tibetana seeds disinfection. Five-minute soak in 0.1% sulfuric acid is effective for bacterial and fungal infection elimination on C. viticella seeds and accelerating their germination. The best mode for C. 'Paul Farges' and C. 'Princess Diana' leaf explants and C. 'Zvezdograd' vegetative buds sterilizing is soaking them for 5 minutes in a sodium hypochlorite solution (3.3% - for leaf segments, 5 - for vegetative buds).

C. tibetana and C. viticella sprouts are advisable to be carried to the nutrient medium enriched with zeatin (2 mg × L-1) to increase the intensity of their growth.

C. 'Zvezdograd' vegetative buds cultivation on MS medium with the addition zeatin (2 mg × L-1) leads to callus formation. It’s expedient to use hiberellin acid (0.5 mg × L-1)for this variety breeding by axillary meristems activation method.

Leaf explants cultivation of on MS medium supplemented with BAP (1 mg × L-1) and NOK (0.2 mg × L-1) in the dark for 17-20 days followed by transfer to light is recommended for C. 'Paul Farges' and C. 'Princess Diana' reproduction by method of indirect morphogenesis.