

# Scientific basis for *ex situ* conservation of rare species of genus *Centaurea* s.l. in Bulgaria

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PhD thesis abstract

*Centaurea* s. l. is the genus richest in endemic taxa in the Bulgarian flora. The objects of this PhD thesis are 9 rare and endemic *Centaurea* species native to Bulgaria - *C. caliacrae* Prodán, *C. davidovii* Urum., *C. diospolitana* (Bancheva& Stoyanov) Bancheva, *C. finazzeri* Adamović, *C. immanuelis-loewii* Degen, *C. mannagettae* Podp., *C. pseudaxillaris* Stef. & T. Georgiev, *C. trinervia* Willd. and *C. wagenitziana* Bancheva & Kit Tan. They have a few populations with generally small number of individuals, and are dependent upon their specific habitats. Habitat destruction and low reproductive capacity of these species, mainly due to weak seed germination and damage of seeds by insects, necessitate their conservation both *in situ* and *ex situ*. The objective of the present PhD thesis was to accumulate knowledge for a scientific base for the *ex situ* conservation of the mentioned species and to develop protocols for their *in vitro* cultivation as a means of conservation.

Within the present work, altogether 22 populations of these 9 species were visited and mapped and the data for them in terms of precise location, population size, conservation status, threats and interactions with co-habiting species was updated. The genetic diversity of 13 populations of 7 species was studied by isozyme analysis with 4 enzyme systems. This analysis demonstrated a high intrapopulation variability in most populations which makes all of them a suitable source of material for *ex situ* conservation in order to preserve their genetic diversity. The highest variability was found for phosphoglucosomerase in the two studied populations of *Centaurea mannagettae* – two loci with three alleles each.

Seeds were found to be the best starting material for *in vitro* cultivation with these species, despite the very low germination rate in most of them, with the remarkable exception of *C. caliacrae* (up to 94% germinating seeds). Twenty-five MS-based nutrient media compositions were tested, with or without added auxins and cytokinins in different concentrations.

*Centaurea caliacrae* and *C. davidovii* germinated, grew and formed roots on the phytohormone-free MS medium. New plants were regenerated also through callus from leaves (*C. caliacrae*, *C. davidovii*, *C. finazzeri*, *C. pseudaxillaris*) on a medium containing 1 mg/l benzylaminopurine (BAP) or N<sup>6</sup> – (Δ<sup>2</sup>-isopentenyl)- adenine (2iP) or 1.4 mg/l BAP and 3.5 mg/l thidiazuron (TDZ), and from roots (*C. pseudaxillaris*) on a medium containing 0.5 mg/l BAP. Once regenerated, the plantlets grew normally and rooted on the basal medium. The rooting was problematic only with *C. finazzeri*. A considerably higher propagation coefficient was achieved with adding 0.5 mg/l BAP to the propagation medium; however, this reduced rooting.

Micropropagated plants of *C. caliacrae*, *C. davidovii*, *C. finazzeri* and *C. pseudaxillaris* were successfully adapted *ex vitro* and acclimated to ambient conditions. The quality of the roots and the gradual decrease of humidity were the crucial factors for the adaptation process. With the use of a climate chamber with programmable temperature, light and humidity, a survival

rate of at least 93% was observed. An *ex situ* collection was established with the acclimated plants. They flowered and set seeds after their first winter. Altogether 75 *ex vitro* adapted plants were obtained from a single seed of *C. davidovii*; these plants produced fertile seeds.