IN VITRO FLOWERING OF DARK-GROWN CENTAURIUM PULCHELLUM. 1 2 Tijana Cvetić, 3 Snežana Budimir and 2 D. Grubišić. 1 Institute of Biology and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000, Belgrade, Serbia and Montenegro; 2 “Siniša Stanković” Institute for Biological Research, University of Belgrade, 29. novembra 142, 11060 Belgrade, Serbia and Montenegro; 3 Center for Multidisciplinary Studies, University of Belgrade, 29. novembra 142, 11060, Belgrade, Serbia and Montenegro

Flowering in vitro has been reported in intact plants obtained from seeds (Mitrović et al., 2000) or in explant culture (Culafić and Nešković, 1980). According to Scorza (1982), in vitro culture of intact plants might have an advantage over greenhouse-grown plants by virtue of precise control of environmental factors and composition of the culture medium. Krsć (1998) reported that C. pulchellum cultured in vitro on a medium lacking growth regulators flowered and produced viable seeds. The aim of the present study was to investigate the possibility of flower morphogenesis and seed production in C. pulchellum cultured in vitro in darkness.

Seeds of Centaurium pulchellum (Sw.) Druce were surface sterilized in 2% NaOCl for 3 min, rinsed three times in sterile deionized water, and transferred to MS (Murashige and Skoog, 1962) medium solidified with 0.7% agar and supplemented with sucrose in concentrations ranging from 0.01 to 0.3 M. The pH of the media was adjusted to 5.7 prior to autoclaving for 25 min at 115°C. Seeds were induced to germinate in 100-cm² flasks with 25 cm² of medium in a growth chamber (16-h photoperiod, light intensity of 5.0–7.2 W/m², temperature of 25±2°C) for five days. The control group was continuously cultured under these conditions, while the experimental group was transferred to darkness. The control group was continuously cultured under these conditions, while the experimental group was transferred to darkness. For histological analysis, material was fixed in formalin:acetic acid:ethanol (10:5:85), dehydrated in a graded ethanol series, and embedded in paraffin wax at 57°C. Sections 5–10 μm thick were stained with Alcian blue and Nuclear Fast Red.

Plants of C. pulchellum grown in the dark on MS medium supplied with 0.01 M, 0.03 M, 0.1 M, and 0.3 M sucrose were etiolated and produced only vegetative organs (Fig. 1a). The greatest shoot growth (Fig. 1b) and flower morphogenesis (Fig. 1c) was obtained on medium containing 0.2 M sucrose. During the first 6 weeks on medium of this composition, shoot elongation of dark-grown plants was enhanced compared to light-grown plants (Fig. 1d). After 8 weeks, node formation began increasing more in light-grown plants (Fig. 1e). Light-grown plants flowered in the 14th week, while flowering was delayed in dark-grown plants, where the first flowers were observed after 24 weeks.

Only plants that developed at least seven nodes produced flowers, regardless of the light regime. It was reported earlier that in the long-day plant Chenopodium murale, responsiveness to photoperiod signals in floral induction was dependent on age of the plant (Mitrović et al., 2000). However, flowering response to inductive treatment of the short-day plant C. rubrum was not correlated with plant age, but rather with the type of explant (Blazková et al., 2000).

Flowers of C. pulchellum grown in darkness showed no morphological malformations and contained ovules with female gametophytes (Fig. 1f). Development of male gametophytes was also observed, as well as bursting of antherae. Roldán et al., (1999) reported that development and flowering of Arabidopsis thaliana grown in darkness depended on sugar availability in the aerial part of the plant. Centaurium pulchellum grown in vitro produced seeds, but the seeds from dark-grown plants were not viable. We therefore suggest that either fertilization or apomixis has occurred, but certain nutritional or biochemical factors probably prevented seed development and maturation. Experiments to clarify the role of these factors in seed development are already in progress.

Acknowledgements: This work was supported by the Ministry of Science, Technology, and Development of Serbia, Grant No. 1716.


UDC 582.92331 : 581.145.1
Table 1. a — Morphology of six-week-old plants grown in the dark; b — Shoot length in plants grown in the dark for 17 weeks; c — Flowering plants grown in darkness; d — Shoot length in dark- and light-grown plants; e — Number of nodes in dark- and light-grown plants; f — Ovule with female gametophyte from dark-grown plants (o — ovule; ♀ — female gametophyte).