

Biotechnological approach for multiplication of *Glaucium flavum* Crantz. (Papaveraceae)

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Summary

Glaucium flavum Crantz. (yellow horned poppy) is a medicinal plant species used in the pharmaceutical industry as a source of the alkaloid glaucine, valuable for its antitussive and bronchodilator activity. In Bulgaria the yellow horned poppy is mainly distributed along the Black Sea coast.

The objective of the present PhD thesis was to study the particular requirements of *G. flavum* under the conditions of *in vitro* cultivation and *ex vitro* adaptation, and to compare the localities of the species according to their glaucine content, in order to choose the most appropriate ones as source for further *in vitro* multiplication.

Seeds and aboveground part of flowering plants, were collected from ten Bulgarian localities of the species. Seed germination was found to depend on the abiotic factors temperature and light regime, and the conditions of 15°C in the dark were proved to be optimal. Germination rate varied between localities as well.

G. flavum was recalcitrant for *in vitro* cultivation because of exudates leaching in the medium which resulted in soft and brown shoots. The addition of activated charcoal led to reduction of polyphenol leaching. Medium B5 with doubled macrosalts, 20 g/L of sugar and 0,5 mg/L of activated charcoal was the most suitable for *in vitro* plants growth.

Callus induction was scarce and slow growing. It was found to depend on the type of the explant and the medium composition. Seedling roots were the most appropriate explants, regarding callus induction and growth rate in *G. flavum*.

Indirect somatic embryogenesis was achieved for the first time in genus *Glaucium*, starting from the roots of *in vitro* obtained seedlings. All

stages of somatic embryogenesis were observed in MS medium supplemented with 1 mg/L 2,4-D, 0,5 mg/L TDZ and 0,2 mg/L BAP. The percentage of embryogenic calli varied between the localities (43,3±0,1% for Shkorpilovtsi and 11,6±0,1% for Varvara). A limited number of plants regenerated by somatic embryogenesis were *ex vitro* adapted due to the accrete roots of some plants. *In vitro* germinated plants survived two years after their acclimatization outdoor, flowered, and formed fruits.

The content of the alkaloid glaucine was evaluated in *G. flavum* plants from nine localities along the Bulgarian Black sea coast during two or three consecutive years, in order to select those with highest glaucine content. Some fluctuations of glaucine content were observed during the years. Pomorie and Ahtopol maintained high percentages of glaucine in the dry plant material, being 2,3%±0,1 for Pomorie in 2014 and for Ahtopol in 2015. The lowest percentages of glaucine were recorded in the plant material from Shkorpilovtsi.

Glaucine percentages in *ex vitro* plants were lower than those occurring in natural conditions due to the generally reduced amount of the biosynthetic alkaloids in the plant material.

Three alkaloid chemotypes of *G. flavum* in Bulgaria were suggested. The first one contains aporphines and protopines with a main alkaloid glaucine (Varvara and Sofia localities). The second chemotype contains also aporphine and protopine alkaloids, but the main alkaloid is isocorydine (Shkorpilovtsi), and the third chemotype contains, besides these two types of alkaloids and the morphinane alkaloid salutaridine (Durankulak).

The locality of Pomorie stood out because of both high content of glaucine and high seed germination rate, therefore it was chosen as the most appropriate source of plant material for further *in vitro* multiplication of the species.