

IN VITRO GERMINATION OF GENTIANA LUTEA L. VALUABLE GENOTYPES

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Abstract: *Gentiana lutea* L., is a protected medicinal plant species which in Romania is a listed among the threatened species due to human impact and also to irrational collecting from the wild. For medicinal use the rhizome is containing the well-known substances for their effects such as: tonic, digestive, stomachic, anti-parasitic, colagogue, emmenagogue, antipyretic and bitter. It is well know its effect in stimulating the development of leucocytes with anti-malaria effect. Genetic studies for genotyping the most valuable population in our country has been realized as well as for studying their biological cycle. For the purpose of this study the seeds from genotypes G2 and G4 have been collected for in vitro germination and cultivation. For G4 genotype for the same cultivation conditions it was registered the largest stem diameter (7,9 cm) and the longest root system was registered for G2 (98 cm). The root ramification system ranged between very tight limits and the largest was registered also for the G2 (1410 g). Regarding the stem characterization fro G3 the high was about 112 cm and for G1 it was registered the highest fruits yielded (117).

Key-Words: - *Gentiana lutea*, in vitro, germination, cultivation, culture media, MS

1 Introduction

The gentian roots known among pharmacists as šradix gentianaeö encompasses tonic properties such as bitter used in anorexia, dyspepsia, hepatic disorders, febrile disorders and paediatric debility stages [1].

Globally it is well known and used as an adjuvant in different treatments [2] being also recommended for asthenia, convalescence recovery, for combating guts parasites and also for supporting fermentation processes [3].

This study is referring to *Gentiana lutea* L. as it is a traditional medicinal plant in our country or for the fermentation stimulation in which the raw material is of this species origin [4].

Another reason is due to the extinction process of the species in order to find an alternative way for the sustainable us of the species and protecting it from being collected from the wild where it is considered as öendangered speciesö [5].

The scope of this study is on one hand to describe through morphometry the donor plants, the five genotypes and to set the major technical indicators for in vitro germination and cultivation of the five *Gentiana lutea* L. genotypes identified in our country and also.

2 Material and Methods

Biology of the species *Gentiana lutea* L. or yellow ghin ura galben , as the common name in Romanian language, is the most vigorous plant species belonging to the genus *Gentiana* and it live due to a mycorrhiza which is establishing in roots a symbiotic relationship [6].

It is a perennial species with a very slow development and which can vegetate for the rosette stage even for 12 years.

Occurrence In the massive of Post varului Mountains as part of the Bucegi Mountains in the altitude of 700 m it was collected the five genotypes from the areas belonging to Bra ov county. The plants have been collected at the maturity stage (Fig. 1).

Plant preparation The plants were measured for the aerial parts as well as for the rhizomes diameter and length which were removed and very well cleaned under a running tap water. After sliding the samples were dried to constant weight and prepared for analysis (Fig. 2).

Seeds preparation The collected seeds were washed in laboratory in tap water for 30 min. The well washed seeds were sterilized in sterile conditions through washing in HgCl solution 2% for 1 min and washed three times in distilled sterile water for removing the HgCl residues.

Seed inoculation for germination The seeds were inoculated into jars of 20 ml with 5 ml culture of modified Murashige-Skoog (MS) [7] for germination in sterile conditions. The original culture media was supplemented for variant 1 (V1) with acid naphthylacetic (ANA) 1mg/l and variant 2 (V2) with acid benzylaminopurine (BAP) 1mg/l.

3 Results and discussions

The collected plants of *Gentiana lutea* L. have been identified on the South Easter part of the Post varu Massive at 700 m altitude [8]. The results regarding the morphometrical studies of the plants are presented in table no. 1.



Fig.1 *Gentiana lutea* in Post varu Massiv

Mapping and monitoring the *Gentiana lutea* in the Post varul Massiv were very important in our country and revealed new features for the species [9]. Thus, executing some specific handcrafts on the plants such as cutting, collecting the flower stem,

transplanting the entire plant it was observed the stimulation of dormant buds at the limit of stem with root ensuring in this way the survey of the species. Probably this is an adaptation of the species to the cutting off process due to the strong winds from the mountains or for the frequent avalanche during early spring time.



Fig. 2 *Gentiana lutea* measurements

Regarding the maturation process it was observed that at least 20% of the individuals of the

population were able to produce fruits and seeds thus ensuring a good reproducibility of the species. In this way it was possible to easily collect only seeds and to make some primary analysis for the plant biology.

Table 1. Morphometry results in *Gentiana lutea* L.

Genotype	Roots				Stem			
	Ø (cm)	Length (cm)	Secondary root no	Wight (g)	heigh (cm)	Nodes no	Inflorescence	Fruits/plant
G1	6,2	91	4	1210	106	8	5	117
G2	7,0	98	3	1410	100	8	4	91
G3	7,1	93	4	1320	112	11	5	112
G4	7,9	72	4	680	102	8	4	54
G5	2,8	51	2	260	103	9	5	70

Studying the data presented in table no. 1 it results that the genotype G4 has the biggest diameter to the stem-root junction (7,9 cm), the longest root was recorded for G2 genotype (98 cm) (Fig. 3), the secondary ramification numbers were registered for G2 genotype and for the rest the variations were not significant (1410 g).



Fig. 3 Roots of *Gentiana lutea*

Regarding the stem characterization, the highest stem was registered for the G3 genotype with 112 cm, and for the G1 genotype it was registered the highest fruit yield respectively 117 fruits (Fig. 3).

For each genotype 25 seeds have been collected and inoculated on the MS modified culture media for germination (Fig. 4).

After 1 month of cultivation the best rate of response was registered for G1 genotype with 20 plantlets on the culture media supplemented with cu ANA 6 1mg/l and 17 plantlets on the culture media supplemented with BAP 6 1mg/l (Table no. 2).

Table 2. *Gentiana lutea* plantlets regenerated during the in vitro seed germination

Genotype	Hormones	
	V ₁ 1mg/l ANA	V ₂ 1mg/l BAP
G 1	20	17
G 2	18	14
G 3	16	12
G 4	16	11
G 5	15	11

After the in vitro cultivation, the obtained plants have been acclimatized in the greenhouse and tested for the active principles content. The entire process took 4 month.

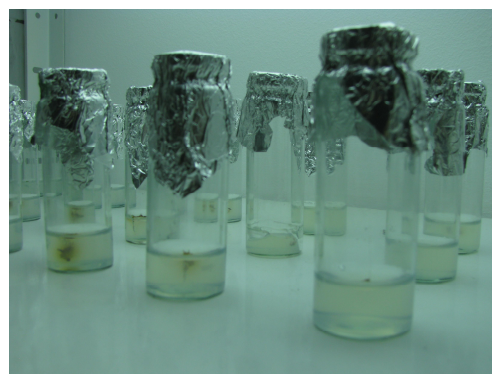


Fig. 4 A general view of the experiment for *in vitro* seed germination

The technical parameters considered as optimum for in vitro culture are as following: temperature 14°C (night) and 18°C (day) with a photoperiod of 14 hours of day and 10 hours of night and a humidity in the air of the culture room of 40%.

4 Conclusions

The study results revealed that for *Post varu* Massive exists a differentiation of individuals into the population which it is expressed also during the in vitro cultivation as a response to the stress conditions. This variation in response to the stress conditions in the *Gentiana lutea* may be supportive for the survival of the species. The in vitro cultivation of the species revealed that classical biotechnology can be used as a tool for further developing new mechanisms for the sustainable use and conservation of the species

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