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Ex Situ and In Vitro Conservation of Glicyrrhiza glabra L. – Crop Wild Relative from Fabaceae

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Abstract. *Glycyrrhiza glabra* L. (Licorice), belongs to the Fabaceae family. The species is included in the Crop Wild relatives list for Bulgaria and is close to beans and peas. Its origin is Southeast Europe, the Mediterranean and Asia, where Bulgaria falls. The roots of the plant contain glycyrrhizin, 30 to 50 times sweetener than the sucrose. As a CWR, as well as due to the high level of glycyrrhizin in the roots, the conservation and maintenance of the species is of interest, although it is not included in the list of threatened plant species yet. In the Institute of Plant Genetic Resources - Sadovo *Glycyrrhiza glabra* is maintained in situ in the Botanical garden. Besides the in situ conservation of the species, *in vitro* techniques are a reliable means of reproduction and long-term storage. After introduction of the raw cuttings from plant species in culture *in vitro*, the process of micropropagation is accomplished by single bud microcuttings in nutrient medium fitted with growth regulators, enabling the development of single-rooted stems with options of repeatedly subcultivating. Along with that the possibility for long term *in vitro* propagation by reduction of the composition of the nutrient medium was tested, where the period for conservation of the cultivated explants reaches 8 months.

Key words: *Glycyrrhiza glabra, ex situ* conservation, *in vitro* conservation, Fabaceae

Introduction

Glycyrrhiza glabra L. belongs to *Fabaceae.* The species is included in the Red Book of protected plants according to the Convention for biodiversity (Secretariat of the Convention on Biological Diversity, 2005.). Part of the population is in the protected area Dolni Vit. Othert part is in protected areas from NATURA 2000 (PEEV, 2011). The genera is CWR of beans and peas. For further protection of *G. glabra* are necessary additional investigations to show it`s potential to grow in different soil and climatic conditions, particularly in terms of Sadovo, which are quite different from the conditions in Danube plain.

The area of origin of *G. glabra* is the Mediterranean, where Bulgaria is situated (ZEVEN *et al*., 1982)

Other option for preservation of *G. glabra* is the *in vitro* techniques for long-term storage with opportunities for precultivation. Besides low temperatures, growth retardants, osmotic stress reduction of the growth potential *in vitro* can be easier obtained by reduction of the chemical compound of the nutrient substance (REED, 1999; PAULA, 2000; LAMBARDI *et al.*, 2006; PREVIATI *et al*., 2008; OZUDOGRU *et al*., 2010). Similar studies have been made for many plant species – potatoes, roses, humulus, ornamental plants etc. The reduction of

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mucro- and micro salts in the nutrient substance (MURASHIGE & SKOOG, 1962) up to 25, 50 and 75% the *in vitro* growth of tomatoes and carnations is suppressed. For *in vitro* preservation of vitis SHIBLI *et al.* (2006) makes investigation by reduction only of the amonium nitrate up to 6 and 25% of it's total quantity in the nutrient substance. The cultivated nodal segments (0.5mm) are kept 262-290 days with preserved vitality up to 70-80%. There are no investigations in that area for *G. glabra* up to now.

The purpose of the current study is to establish the possibilities for *ex situ* and *in vitro* preservation and propagation of *G. glabra* with the aim it`s preservation and practical use.

Material and Methods

G. glabra is kept *ex situ* in the Botanical garden of the IPGR – Sadovo. The species is collected during expeditions in Danube plain. In order to establish if the plant can succesfuly develop in the condition of Sadovo, different from the natuaral habitat, 3-years biomentrical investigations have been done as follows: lengh of the stem (cm), lengh and width of leaf (cm), dimensions of the blossom parts (cm) – corrola, flag and wings, lengh and width of the pod (cm) and diameter of seed (mm).

For the *in vitro* experimental work 10 mm microcuttings with one stem nod are used as starting material. The nutrient medium with mineral solution after Knop, modified after Mur, microelements after Berthelot and vitamins after Morel (GRENAN, 1979) is a relative good composition for root development and single leading shoot for a period of 25-35 days. To reduce the growth force of cultured explants by reducing the composition of the nutrient medium, the experimental work was displayed in the following variants:

1. Reduction of the Nitrogen compounds up to ¼ of their total quantity (KNO3 - 237 mg, NH4NO3 -90mg) (¼ PAC).

2. Reduction of macro- and micro elements up to ¼ of their composition (¼ PMM).

3. Reduction of the overall chemical composition of the medium to ¼ of the total intensity (¼ POXC).

Plants were grown under lights 1000 lx, photoperiod 16/8 hours day/night and temperature 24°C.

For each option were included 20 explants with 4-4-fold repetition of the experiments.

The survival rate (%) and the duration of maintaining in *in vitro* conditions (days) of the explants was recorded. After the storage period of the regeneration capacity of the stored material was determined by re-culturing in a standard culture medium, expressed in time (days) required for the formation of the leading shoot stem with 5 knots. For control were used unstored microcutings from *G. glabra.*

Results and Discussion

According to JORDANOV (1976) *G. glabra* in it1s natural habitats is perrenial shrub with thickq woody roodstock with shoots. Stems are erectedm 50-100 cm heigh, ribbed, reddish, highly branched, fibrous, rarely acute rough. Leaves longer than internodes, petals elliptical, curved on the top, (Fig. 1).

Fig. 1. *Glycyrrhiza glabra* L.

The inflorescence spherical to cylindrical, loose. The flowers sessile. Calyx shorter than the corolla. The corolla 8-10 mm, lilac. The flag eleptical, curved on the top, in the bottom concave in triangular claw. Wings equal to the flag or little shorter. Keel equal to the flag and wings, acute on the top, petiole twice longetr than the claw. Pods are

1-3 cm long and 4-5 mm wide, smooth. Seeds greenish, spherical. Blossoms from July to August. Grows in dry, grassy areas.

To study the growth behavior of *G. glabra* in nonspecific conditions for the plant species, namely, *ex situ* maintenance in the Botanical garden IPGR - Sadovo three-year biometric analyzes were carried out, summary results of which are shown in Table. 1.

Table 1. Summary results from three-year biometric analyzes of *G glabra* L.

Index \ Value		x±Sx	min	max	CV%
Lengh of the stem (cm)		65.06±2.99	53.0	85.0	89.19
Leaf	length (cm)	10.9±0.57	9.0	14.0	3.21
	width (cm)	3.71±0.26	2.5	4.6	0.7
Blossom	corrola length (cm)	9.24±0.37	7.5	11.0	1.33
	flag length (cm)	10.88±0.17	10.0	11.9	0.29
	wings length (cm)	9.43±0.14	8.9	10.1	0.2
Pod	length (cm)	2.7±0.15	1.5	2.9	0.22
	width (cm)	3.43±0.18	2.8	4.5	0.33
Seed diameter, mm.		3.06±0.06	2.9	3.5	0.033

The lengh of the stem is 65.06 cm ±2,99. The minimum is 53 cm and the maximum is 85 cm. The degree of variation of this indicator is very high (89.19%). Leaf size vary from 9-15 cm in length (10.9±0.57) and 2.5 – 4.6 cm width (3.71±0.26). The variation of that indexes is insignificant. The variation of the size of the flower is also very low. The corolla length is 7.5 – 11 cm (9.24±0.37), th flag length is 10.0-11.9 cm (10.88±0.17) and length of the wings is 8.9 – 10.1 cm (9.43±0.14). The pod is comparatively short, 1.5 – 2.9 cm (2.7±0.15) long and 2.8 – 4.5 cm (3.43±0.18) wide. The seed is small, spherical in shape with diameter 2.9 – 3.5 mm (3.06±0.06). These results confirm the data of other authors (IVANOV *et al.,* 1977) and shows that *G. glabra* can successfully grow in different climatic conditions.

The level of survival of the *in vitro* explants as well as the duration of their conservation are the twoo basic indexes, having equal importance for *in vitro* preservation. The results fro that study in Table 2 show some regularities and dependencies between both indexes under the influence of investigated factors. The main tendency, that comes out from the data from the three repetitions is elongation of the storage period under lower survival and the explants as well as the opposite, the more explants survived, the period for storage id shorter. That tendency is a result from the interaction between the compounds of the nutrient media, which is cleared by detailed analysis of the results for the different variants. In the variant of reduction of the Nitrogen compounds up to 1/4 was recorded comparatively high percent of vitality of the explants (76.5%), but the duration of their preservation in *in vitro* conditions was very short – 102.2 days.

In reduction of the macro- and micro elements up to 1/4 from the compound od the nutrient media the level of survival of the explants is reduced (61.6%) and the recorded period of storage is 137.7 days.

In the case of total reduction of the chemical compounds in the nutrient media up to 1/4, 59.9% from the cultivated microcuttings preserve their vitality, but the period of storage is elongated up to 239.7 days. That is due to the lower concentration of growth regulators and thus the growth process is suppressed and the time of preservation is elongated, (Fig. 2&3).

After the storage period, in recultivation of the explants to standard nutrient media (with full compound) was recorded that there is no difference in time, nececary

Table 2. Development of microcuttings from *G. glabra* in in vitro storage and after that. Legend: RNC - Reduction of Nitrogen Compounds, RMM - Reduction of Macro- and Micro elements, RNC - Reduction of the Nutrient Compound.

	Number of microcuttings	**Vitality of the explants**	**Duration of the storage**	**Time for regeneration**	**Control**
Variant 1 1/2 RNC	number	/%/	days	days	days
	80	76.5	102.2	33.4	32.7
Variant 2 1/2 RMM	number	/%/	days	days	days
	80	61.6	137.7	30.5	35.2
Variant 3 1/2 RNC	number	%	days	days	days
	80	59.9	239.7	34.6	31.9

Fig. 2. In vitro storage of *G. glabra*

Fig. 3. Control

for formation of the shoots up to 5- stem nod between the stored in ¼ PAC, ¼ RMM, ¼ RNC (30.5 – 34.6 days), as well as between them and unstored explants (control – 31.9 – 35.2 days). So in *in vitro* storage of microcuttings of *G. glabra* by reducing of the chemical compound af the nutrient media the explants do not change their regeneration and growing potential, which is realised in equal level with th eunstored explants.

Conclusions

1. *Glycyrrhiza glabra* can be succesfully grown ex sity in the botanical garden of the IPGR – Sadovo, which ensures the presrvation of the species as valuable plant genetic resources in different conditions.

2. The optimalway for *in vitro* preservation of explants from *Glycyrrhiza glabra* is the variant with 1/4 reduction of the whole chemical compound of the

nutrient media (RNC), where 8 month period for conservation in 60% preserved vitality is ensured, without any changes in their regeneration potential.

References

GRENAN S. 1979. Rhizogenese du bourgeons apicaux de boutures de vigne cultivees *in vitro*. - *Vigne Vin,* 13: 125-130

IVANOV I., I. LANDJEV, G. NESHEV. 1977. *[Herbs in Bulgaria and their use.]* Sofia. (in Bulgarian)

JORDANOV D. 1976. *Flora Republicae Populares Bulgarica [Flora of People's Repyblic of Bulgaria],* Vol. VI, Bulgaria, Aedibus Academiae Scientiarum Bulgaricae. (in Bulgarian)

LAMBARDI M., R. RONCASAGLIA, A. PREVIATI, A. DE CARLO, G. DRADI, F. DA RE, L. CALAMAI. 2006. *In vitro* slow growth storage of fruit rootstocks inside gas-tight or gas-permeable containers. - *Acta Horticulturae*,725: 483-488.

MURASHIGE T., F. SKOOG. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. - *Physiol. Plant,* 15: 473-497.

OZUDOGRU E.A., A. PREVIATI, M. LAMBARDI. 2010. *In vitro* conservation and cryopreservation of ornamental plants. - In: Jain S.M., S.J. Ochatt (eds), Protocols for *In vitro* Propagation of Ornamental Plants. *Methods in Molecular Biology,* vol. 589. Humana Press-Springer, New York, pp. 303-324.

PAULA W. 2000. *In vitro* storage of *Eucalyptus grandis* germplasm under minimal growth conditions. - *Plant cell organ and tissue cultures*, 61: 161–164.

PEEV D. (Ed.). 2011. *Red Data Book, Volume 1 – Plants & Fungi,* Institute of Biodiversity and Ecosystem Research – BAS, Sofia, Bulgaria, Available at: [http://e-ecodb.bas.bg/rdb/en/vol1/]

PREVIATI A., C. BENELLI, F. DA RE, E.A. OZUDOGRU, M. LAMBARDI. 2008. Micropropagation and *in vitro* conservation of virus-free rose germplasm. - *Prop. Ornam. Plants,* 8(2): 93-98.

REED B. 1999. *In vitro* storage conditions for mint germplasm. - *HortScience: a ication of the American Society for Horticultural Science,* 34(2): 350-352.

Secretariat of the Convention on Biological Diversity. 2005. Handbook of the Convention on Biological Diversity Including its Cartagena Protocol on Biosafety, 3rd edition, (Montreal, Canada).

SHIBLI R., M. SHAT NOWI, W. SUBAIH, M. AJLOUNI. 2006. *In vitro* conservation and cryopreservation of plant genetic resources: Review. - *World Journal Agricultural Science,* 2(4): 372-382.

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