Ex Situ and In Vitro Conservation of Glycyrrhiza glabra L. - Crop Wild Relative from Fabaceae

Katya Uzundzhaliieva*, Ruska Ruseva, Svetla Kachakova

Department of Plant Genetic Resources - Medical and Aromatic Plants, Institute of Plant Genetic Resources, 4122 Sadovo BULGARIA
* Corresponding author: k_spassova@abv.bg

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 sweeter 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. Other 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 its 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; Ozudoğru et al., 2010). Similar studies have been made for many plant species - potatoes, roses, humulus, ornamental plants etc. The reduction of
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 ammonium 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 successfully develop in the condition of Sadovo, different from the natural habitat, 3-years biometrical investigations have been done as follows: length of the stem (cm), length and width of leaf (cm), dimensions of the blossom parts (cm) – corolla, flag and wings, length 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 reculturing 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 microcuttings from G. glabra.

Results and Discussion

According to JORDANOV (1976) G. glabra in its natural habitats is perennial shrub with thick woody roodstock with shoots. Stems are erected dm 50-100 cm height, 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 eleyptical, 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 longer 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.**

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

The length 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) 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 two basic indexes, having equal importance for *in vitro* preservation. The results from 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 is 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 of 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, necceary
Table 2. Development of microcuttings from *G. glabra* in in vitro storage and after that.


<table>
<thead>
<tr>
<th>Variant</th>
<th>Number of microcuttings</th>
<th>Vitality of the explants</th>
<th>Duration of the storage</th>
<th>Time for regeneration</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant 1 1/2 RNC</td>
<td>number</td>
<td>76.5</td>
<td>days</td>
<td>days</td>
<td>days</td>
</tr>
<tr>
<td>Variant 1 1/2 RMM</td>
<td>number</td>
<td>61.6</td>
<td>days</td>
<td>days</td>
<td>days</td>
</tr>
<tr>
<td>Variant 1 1/2 RNC</td>
<td>number</td>
<td>%</td>
<td>days</td>
<td>days</td>
<td>days</td>
</tr>
</tbody>
</table>

![Fig. 2. In vitro storage of *G. glabra*](image1)

![Fig. 3. Control](image2)

Conclusions

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

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

for formation of the shoots up to 5- stem nod between the stored in 1/4 PAC, 1/4 RMM, 1/4 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 at the nutrient media the explants do not change their regeneration and growing potential, which is realised in equal level with the unstored explants.
nutrient media (RNC), where 8 month period for conservation in 60% preserved vitality is ensured, without any changes in their regeneration potential.

References

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


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