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The influence of phytohormones on indirect regeneration of goji (*Lycium barbarum* L.)

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Abstract Goji is a species with food, medicinal and ornamental importance, due to its many properties. In this study, some tissue culture activities were performed to induce callus from different explants of Goji, as well as in vitro regeneration from this tissue, indirectly. The leaves and stems were found to have given good responses on the Murashige Skoog (MS) medium supplemented with cytochinine BAP in the amount of 0.5 mg/l and giberelin GA3 in the amount of 0.5 mg/l determine slowest regenerative percentage of callus in the caulinary cells of 90%, and the remaining 10% gave regenerative response of the rooted shoots from the calus. Callus generated from meristem and foliar tissue was also subcultivated on the medium by 0.5 mg/l ANA + 0.5 mg/l GA3 and 0.5 mg/l ANA + 0.5 mg/l IBA, with a regression of callous cells and their death
The influence of various hormone concentrations on in vitro proliferation and multiplication at *Lycium barbarum* in experimental subcultures leads to the conclusion that good callus regeneration occurs only in the presence of BAP cintochinin in the amount of 0,5 mg/l and GA3 giberelin in the amount of 0.5 mg/l.

Key words

Lycium barbarum, callus, indirect regeneration, phytohormons

Medicinal plants are currently a very important remedy for the treatment of diseases, together with synthesis drugs [1]. Often, the use of the latter have led to numerous side effects. One of the causes that leads to people getting sick is precisely the excessive consumption of chemicals, which is another reason for our attention to turn more and more towards the use of the drugs that nature gives us [4, 16].
The species *Lycium barbarum* is part of the Solonaceae family and is a perennial shrub, which is found in temperate and subtropical regions of Tibet, China, Mongolia, Himalayas. Today plants are also spread in Europe, in countries such as Spain, Ireland, Kosovo, Romania, but also in America. The species *Lycium barbarum* is native to Tibet and China, and the use of miraculous fruits has a history of over 2500 years [12]. Goji is considered a food-medicine is recommended as an adjunct in treatments against: diabetes (hypoglycemic), heart disease (cholesterol regulation), tuberculosis, childhood pneumonia, anemia, general debility or vision disorders caused by malnutrition [6, 8, 9, 10, 13].
Contains significant amounts of vitamin B and C as well as polysaccharides. These fruits have about 10% protein content and provide 18 different amino acids of which 8 are essential [9, 12]. It is rich in glutathione which is among the most effective antioxidants with a role in the optimal functioning of human cells. The content of phenols and vitamin C determined in dehydrated goji fruits was higher than in fresh fruit [17].
In vitro micropropagation is a set of methods of plant propagation by the use of *in vitro* cultures of cells, tissues and plant organs [2, 3]. Using it, this technique allows a considerable increase in the propagation yield of the different species. In vitro micropropagation has great potential for biodiversity conservation, the artificial cultivation method can multiply rare, protected species, such as endemic species [5, 7, 11].
Callus are tissue formations consisting of an uneven mass of cells. A certain type of callus is also produced naturally in vivo, at the level of areas traumatized by cutting, having a role in wound healing, or is formed at the base of planted cuttings for rooting. Callus can also be used as a research tool in various experiments, in the study of morphogenesis, ultrastructural changes, biochemical analyses, in the dependence of the inoculus from which caulinary cells originate [13, 14, 15].
In this paper we tested the micropropagation capacity of *Lycium barbarum* (*Solanaceae*), an important medicinal species, spread in nature in wild form, but also cultivated for fruits rich in vitamins and other active principles. Using the Murashige-Skoog culture medium supplemented with different hormonal balances, we tested the regenerative capacity of several goji explants and their ability to produce callus. We have followed indirect regeneration, the formation of

shoots and roots, knowing that callus tissue can cause the appearance of somatic variability.

Material and Method

We took the biological material from the personal garden, consisting of young shoots of one year, from which buds were harvested, stem fragments and leaves from the species *Lycium barbarum*. The experiments were carried out within the in vitro Culture Laboratory of the Faculty of Horticulture and Forestry of USAMVB Timisoara.

The Murashige-Skoog culture medium was used, whose mineral and organic composition is balanced, ensuring good development of explants. Its composition is shown in the table.

Table 1.

Experimental variants used in *in vitro* culture of goji

Experimental variant	Phytohormons mg/l			
	GA3	BAP	ANA	IBA
V_1	0,5	0,5		
V2	0,5			0,5
V3	0,5		0,5	

Prepare the MS culture medium, add 30 g sucrose and sterile distilled water up to 1000 ml to obtain 1 litre of MS medium, correct pH to 5,8 and add agar 7 g/l, sterilise in autoclave at 120°C for 25 minutes. Add growth phytohormones: in three concentration variants 0.5 mg/l each hormonal variant, BAP, GA3, ANA, IBA.

After taking the biological material from the shoots harvested from the parent plant, it is sterilised with $HgCl_2$ solution (mercurial chloride). Explants at the internodes, meristems and leaves were introduced into the sterilizing solution and left to sterilize for 10 minutes, after which 4 baths of distilled water were prepared. The sterilized plant material was inserted into each distilled water bath for 5 minutes. At the flame of the spirtier, sterilize the brush together with the scalpel, then cool in advance, use to extract the explant from the last bath of distilled water and to indoculate the biological material in artificial environment, and with the help of the scalpel, which has previously been sterilized, both ends of sterile explants will be made and will be innoculated in the artificial medium in sterile containers.

After taking the biological material from the level of caulinary cells (callus) from the resulting callus was experimented the subcultivation of the callus resulting from the meristems of the species *Lycium barbarum*. This was used the MS culture medium supplemented with the hormonal balance (BAP, GA3, IBA) which supported the induction of callus.

At the flame of the spirtier, sterilise the brush together with the scalpel, after the instruments have cooled and are ready for use, with the help of the tweezer and sterile scalpel, make the callus remove the necrotic part, inoculating on the surface of the solidified medium, cover the Petri box with the lid and wrap the surrounding edge. The callus expeplants of *Licium barbarum* innoculated on artificial medium are exposed in the sterile growing room at 22°C, with 8 h of darkness and 16 h light at 4000 lux.

Results and Discussions

Our research has been carried out to establish the influence of phytohormones on indirect regeneration in vitro at *Lycium barbarum* in order to obtain planting material, which can be planted. Due to phytohormones that were suppressed in an artificial environment, the tissue fragments, on which the research was carried out, had different response. The results obtained are in the table below**.**

Table 2.

The translation of the resulting phytohormones and their response

Hormonal variantes	Number of inoculum	Response of explants *in vitro* (%)					
		Leaf explant	(%)	Meristem	(%)	Stem explant	(%)
V_1 0,5 mg/l BAP + 0,5 mg/l GA3	30	Callus	25	Shoots	25	Callus	100
V_2 0,5 mg/l GA3 + 0,5 mg/l IBA	30	Callus	0	Shoots	0	Callus	90
V_3 0,5 mg/l ANA + 0,5 mg/l GA3	20	Callus	10	Callus	5	Callus	50

The formation of non-differentiated cells at the level of goji explants takes place in all artificial cultures made, in which the three hormonal versions are added. (Figure 1.).

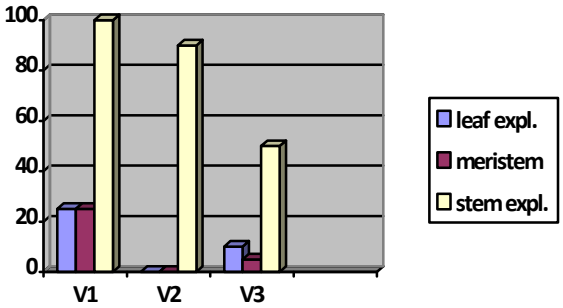

Figure 1. Increase of callus from *Licium barbarum* tissue (% from explant inoculum)

Figure 2. Culture of non-differentiated cells (callus) (Original photo)

By mixing auxine and cytochinin the balance is different in the proces of the formation of non-differentiated cells. In the fragments of foliar tissue resulted values of 30%-40% in the production of callus. In the culture of caulinary tissue at *Licium barbarum* cytochinine and gibereline led to the formation of callus in the proportion of 85%-100% of the inoculi. Meristematic calus resulted only from the use of the combination of auxine with giberelic acid. At the plant taken in research the result obtained shows us that the type of explant and hormonal balance is marked in the production of callus. The fragments of meristematic tissue studied are the only ones to which the phenomenon of direct organogenesis with the formation of shoots is highlighted.

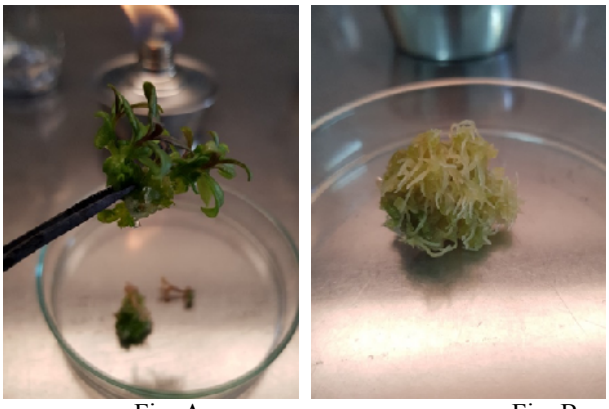

Fig. A Fig. B

Figure 3. In figure A, regenerated goji shoots from callus meristematic sallus are observed; in figure B callus with root formations is observed (Original photo)

The association of BAP cytochinins with GA3 gibereline in different amounts of 0.5 mg/l determines a high percentage of regeneration in caulinary cells, with values of 100%. The combination of cytochinin BAP and ANA auxines and IBA causes a low regenerative percentage in the foliar and caulinary callus.

In vitro, the non-different cells were harvested under aseptic conditions from the explants that gave birth to them and were innoculated on a fresh medium to which

were added growth phytohormones that conditioned the creation of this tissue. Explants with a size of 3-16 mm were grown for 90 days and their development in callus subculture was observed. Consequently, the callus obtained, following the increase of three repetitions, is shown in Table 3.

Table 3.

Development of non-differentiated cells in subculture on different hormonal balances

Experimental variant	Origin callus	Callus growth in subculture (mm)									
		week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10
V_1 (0,5 mg/l BAP + 0,5 mg/l GA3)	Leaf	0,4	0,5	0,7	1	1,2	1,5	1,1	0,7	0	0
	Stem	0,4	0.5	0,8	1,2	1,5	1,6	1,3	1	0,6	0,5
	Meristem	0,4	0,5	0,8	1	1,3	1,3	0,9	0,5	0,4	0
V_2 (0,5 mg/l ANA + 0,5 mg/l GA3)	Leaf	0,3	0,4	0,8	0,8	1	0,7	0,6	0,5	0	0
	Stem	0,3	0,7	1,2	1,1	1,4	1	0,7	0,5	0,4	0,3
	Meristem	0,4	0,5	0,7	0,9	1,2	0,8	0,4	0,4	0,2	0
V_3 (0,5 mg/l IBA + 0,5 mg/l GA3)	Leaf	0,4	0,4	0,7	1	0,8	0,6	0,5	0,3	0	0
	Stem	0,4	0,5	0,9	1	1,1	0,8	0,7	0,4	0,2	0
	Meristem	0,3	0,3	0,6	0,8	1	0,7	0,4	0	0	0

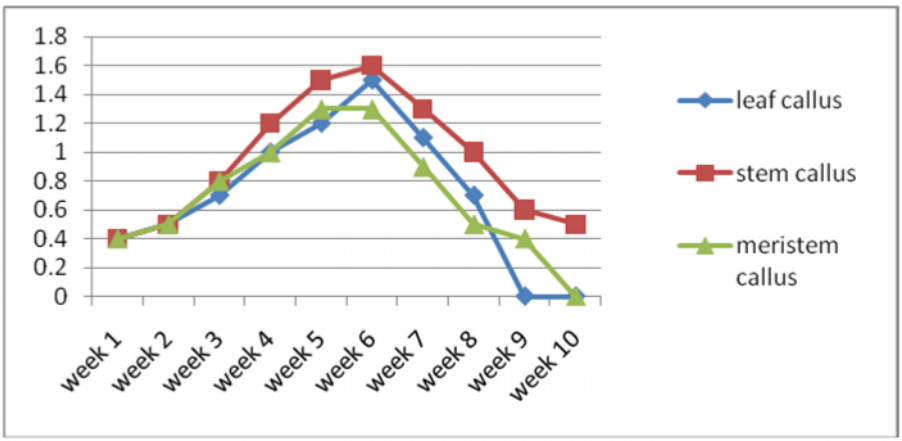

Figure 5. Growth of non-differentiated cells at *Licium barbarum* in subculture on Variant V_1 (0.5 mg/l BAP + 0.5 mg/l GA3).

From week 7 a slight regression is observed until week 9, in the development of the callus, but from week 10 stagnation is observed and even death occurs in the growth and development of undifferentiated cells.

In Figure 5 the resulting callus develops on the medium in the additional subculture with cytochinin BAP and GA3 gibereline different from the tissue origin. The best results were recorded in cells not differentiated with caulinary provenance. A sudden regression is observed in the other types of callus in weeks 7-8. In meristematic callus, stagnation is observed at week 4.

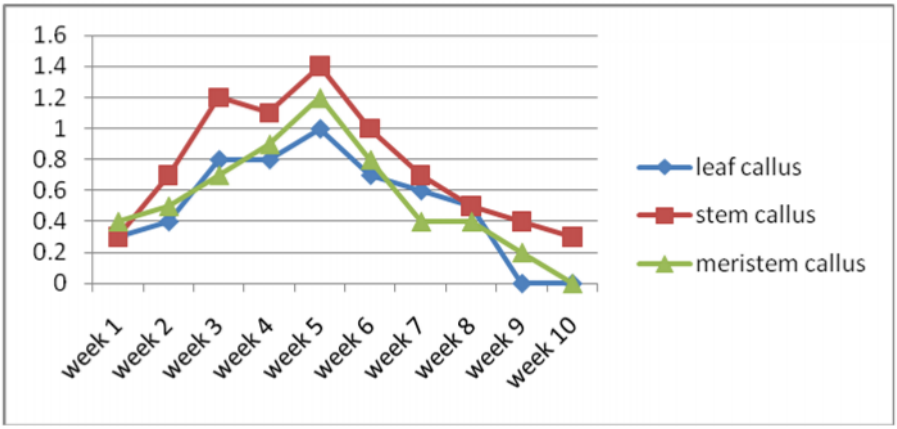

Figure 6. Development of non-differentiated cells at *Licium barbarum* on medium in Variant V_2 (0.5 mg/l ANA + 0.5 mg/l GA3)

Callus shows on medium added with the hormone balance V_2 a lower increase of gibereline GA3 (0.5 mg/l) and auxine ANA (0.5 mg/l) for each. Undifferentiated cells develop differently from tissue origin. In the development of the callus of 16mm caulinary origin, the best responses were recorded. In meristematic cells, stagnation from week 4 to week 6 and a slight regression at week 7 are observed.
As a result of the results obtained, it is noted that lycium barbarum callus develops in medium subculture added to V_1 in the content of which citochinine BAP is in good condition in proportion to (0.5 mg/l). After 6 weeks of cultivation the caulinary callus shows a significant increase of 2 mm. Values close to those of variant V_3 are maintained in the development of non-differentiated leaf and meristematic tissue.
In conclusion, it can be said that throughout the 10 weeks of subcultivation, the cytochinin BAP used, ensures a significant increase in the callus of *Lycium barbarum*. The proliferation of non-differentiated tissue in subculture is favourable in the presence of cytochinin, being supported by the association of giberelines and cytochinins.
The evolution of *Lycium barbarum* tissue is continuously introduced in the second subculture cycle, which is transferred to the medium MS freshly supplemented by 0.5mg/l BAP, the hormonal balance that best supports the development of callus. The phenomenon of organogenesis, i.e. the appearance and development of shoots in undifferentiated tissue, is intended.
The shoots were regenerated in the medium subculture by 0,5 mg/l BAP + 0,5 mg/l GA3, observing the formation of roots and callus (Figure 7).

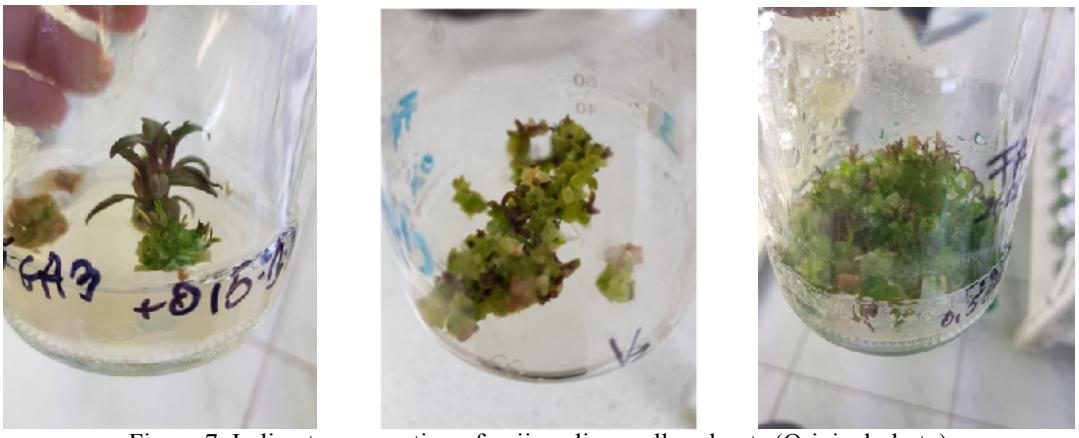

Figure 7. Indirect regeneration of goji caulinar callus shoots (Original photo)

To conduct an in vitro experiment with the different hormonal balances and types of explants at *Lycium barbarum*, it is important to test the in vitro response in order to establish a technique of differentiation and cell proliferation, regeneration and multiplication.

Conclusions

From our experiments under aseptic conditions we highlight the importance of hormonal balances and *Lycium barbarum*.

- The in vitro response to *Lycium barbarum* is influenced by the type of explant: caulinary and foliar explants are a callus producer, and meristematic explant is not a callus producer, but still regenerates shoots. - In small amounts cytochinine favors the growth of callus to *Lycium barbarum*, but this increase can also be achieved in the absence of this hormone.
- Following the results achieved, a slight increase in the inoculated callus on the medium added with hormonal balances is observed: IBA indolilbutiric acid (0.5mg/l) and Giberelin GA3 (0.5mg/l).
- The combination of GA3 0,5mg/l and BAP favours the induction of root organs in non-differentiated cells (meristematic callus).

To conduct an in vitro experiment using different hormonal balances and types of explants at *Lycium barbarum*, it is important to test the response of the selected plant material in order to establish a specific and correct working technique.

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