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In vitro regeneration at *Licium barbatum* L.

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Abstract *Lycium barbarum* L. (Goji) the important food and medicine plant contains numerous antioxidant and antidiabetic principles. In the present study, some tissue culture activities were performed to induce calus, *in vitro* regeneration from various Goji explants.
The optimal hormonal regimen and the most appropriate and responsive explants were identified. Leaves and strains were found to give good responses to Murashige and Skoog (MS) supplemented with cytokinin BAP in the amount of 0.5 mg / l causing the highest percent regenerative of meristems, 100%. Also, the regenerated shoots from meristems were subcultivated on the medium with 0.5 mg / l BAP, observing the formation of roots and calus. It is noted that undifferentiated tissue increases in the subculture on the medium supplemented with ANA auxin and BAP cytokinin differently, depending on the tissue origin. The best results are recorded in callus with meristematic origin. The influence of various hormone concentrations on the proliferation and *in vitro* multiplication of *Lycium barbarum* in the experimental subcultures leads to the conclusion that a good regeneration of the shoots results only in the presence of 0.5 mg / l BAP cintoquine. In these conditions the phenomenon of rhizogenes is induced at the level of the subcultivated shoots. The induction of callus in goji is favored by the presence of small amounts of auxine, but it is also achieved in its apse. BAP cytokine favors active proliferation of callus.

Key words

Lycium barbarum, regeneration, phitohormons, callus

Lycium barbarum l. (Goji) belongs to the Solanaceae family and is native to China. Rich in vitamins, mineral salts and other active principles, goji has been commonly used as food and medicine, but due to its beneficial effects on the cardiovascular system by reducing cholesterol and the antioxidant properties and antidiabetic (Feng et al., 2001; Jing and Yin, 2010; Luo et al., 2004). Some authors tested the *in vitro* response of different genotypes of goji, using various hormonal combinations (Fira et al., 2016; Hu et al., 2008; Nurul et al., 2013; Rosna et al., 2015). In the present work we tested the regenerative ability of *Lycium barbarum* explants. Using culture medium Murashige-Skoog supplemented with various hormonal balances, we tested the ability of some goji explants to produce callus with proliferative aptitude and differentiated tissue.

Material and Method

The culture medium, Murashinge-Skoog was used, whose mineral and organic composition is balanced ensuring a good development of explants. The growth have been added into the medium after his sterilization in 4 types of concentration (table1), using BAP cytokinins (benzylaminopurine) and Kin (kinetin) associated with ANA (maphthalene acetic acid) and 2,4-D (2,4 dichloraphenoxyacetic acid) auxins.

After taking the biological material from the harvested shoots from the mother plant, this is sterilized with $HgCl_2$ 0,1% solution. Explants from the internodes, meristems and leaves have been added in the sterilizing solution for 10 minutes and after that, they been washed with sterile sistilled water. The plant material have been wrought and inoculated on the solid medium in sterile recipients, in aseptic condition.

After the inoculation of the explants, these have been transfered in the growth room, in which the temperature is 25℃ and the light has an intensity of 4000 lux.

The fitoperiod of cultivationis was of 16 hours light and 8 hours dark.

Table 1

Experimental variants used in in vitro culture in Licium barbarum

Varianta experimentala	Fitohormoni mg/l		
	ANA	BAP	KIN
V_1	0,1	1	
V2	0,5	0,5	
V3		0,5	1
V_4		0,5	

The undifferention tissue, callus , has been activated an the culture medium, MS, supplemented with hormonal balance who sustained his induction. After the 7 days period from the inoculation in subculture, the stage of development of callus cells, recording the tisue dimmension in *mm*, weeklyin a 3 months interval.

Results and Discussions

1. The reaction of explants on MS mediumwith different hormonal balance

According to the hormonal additional balance in the culture medium, the explants taken in this study different reaction, which ca be found in 2 table.

Table 2

Hormonal variants and results

Variante hormonale	Nr. inoculi	The answer to the in vitro explants (%)					
		Leaves expl.	%	Meristem	%	Stem expl.	%
V_1(0,1 mg/L ANA+1,0 mg/L BAP)	20	Calus	75	Lăstari	10	Calus	25
V_2(0,5mg/L ANA+ 0,5mg/L BAP)	20	Calus	60	Lastari	5	Calus	20
V_3(0,5mg/l BAP+ 1mg/l Kin)	20	Calus	80	Calus	90	Calus	70
V_4 (0,5mg/l BAP)	20	Calus	90	Lastari	100	Calus	75

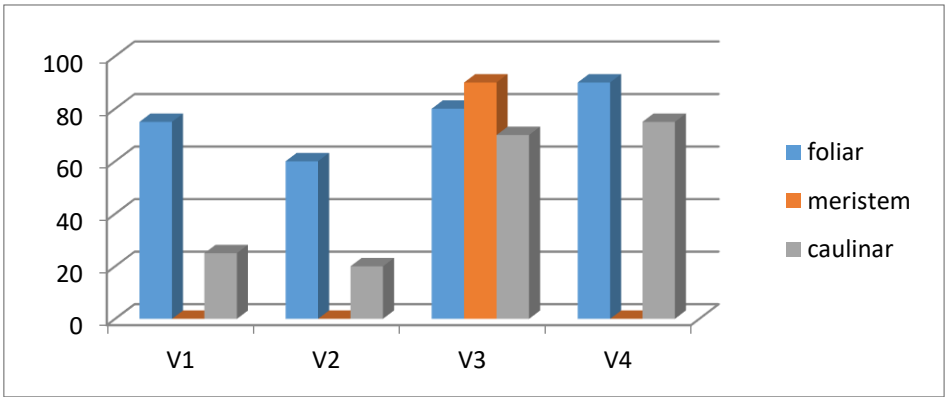

Fig. 1. Induction of callus from various explants of goji under the influence of hormonal balance

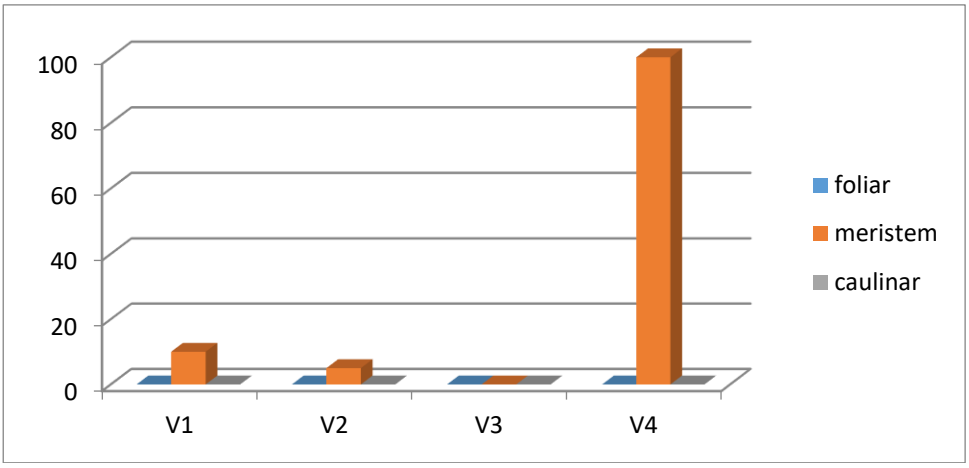

Fig.. 2. Regeneration of shoots from explants of goji under the influence of hormonal balance

The formation of the callus has been present at all explants, cultivated on MS, suplemented with those 4 hormonal types (2 table).

From the obtaimed results it is obvious the association of an auxin with determine processesof differention cytokinins and formations of the callus. Regarding the foliar explants, we obtained values of the of the reactions between 60-70% when it comes to the generation of the callus. Explants cultivated of *Licium barbarum* have produced callus in the presence cytokinins with value between 70-75% from inoculated meristem has produced callus only in the presence of the combination of cytokinin (V_3). These result indicate that the type of explant and the hormonal balance influences the production of the un differentiated tisue of the plant we study.


Fig. 3. Induction of callus from meristematic explants to goji (original photo)

The organogenesis have been observed to the explants of *Licium barbarum* in the condition we studied, only to the level of meristem. The asociation of the ANA auxine with BAP cytokinin, in different quantities determines a low proncentage of regenerate only of meristem, with value between 10% an V_1 and 5% V_2 . The combination between BAP cytokinins and Kin doesnt determine regenerative processes to the level of explants. Only the utilization of BAP in quantity of 0,5 mg/l determinesthe bigest regenative procentage from meristem 100%.

2. Raising calus in subculture on solid medium

After 6 weeks in culture, callus originating in different explants was harvested and transferred under aseptic conditions to the freshly added medium with hormonal balances having induced induction of this tissue. Calugal explants of the size of 0.4-0.6 cm were inoculated and their growth was monitored within 8 weeks (2 months) under the calus culture. The results obtained with respect to the growth of undifferentiated tissue, representing the average of three repetitions, are presented in Table 3.

Table 3

Calus growth in the subculture

Varianta experimentala	Originea calusului	Calus growth in the subculture (mm)							
		Sapt.1	Sapt.2	Sapt.3	Sapt.4	Sapt.5	Sapt.6	Sapt.7	Sapt.8
V1 (1 mg/l ANA+1,0 mg/l BAP)	Foliar	3	5	8	10	12	14	15	15
	Caulinar	2	4	6	7	8	9	9	9
	Merisrem	3	6	8	10	12	14	16	17
V2 (0,5mg/L ANA+ 0,5mg/L BAP)	Foliar	2	3	5	7	9	11	12	13
	Caulinar	1	3	4	6	7	9	10	10
	Merisrem	2	5	7	10	12	14	16	17
V3 (0,5mg/l BAP+ 1mg/l Kin)	Foliar	3	6	8	10	13	15	17	19
	Caulinar	2	5	7	10	12	14	16	18
	Merisrem	3	6	9	12	14	16	18	20
V4 (0,5mg/l BAP)	Foliar	2	5	8	10	11	13	14	15
	Caulinar	3	5	7	10	12	14	15	15
	Merisrem	3	6	8	11	15	17	19	21

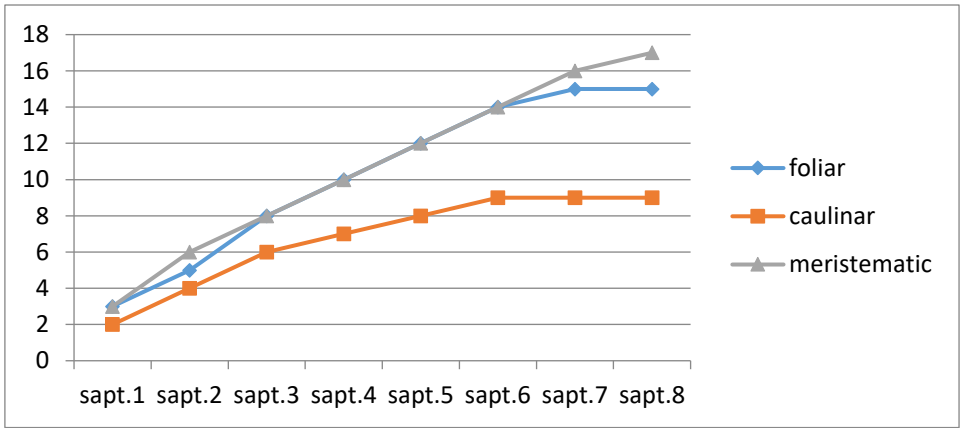

Fig.. 4. Growth of the Goji callus in subculture on variant V1 (0.1 mg / L ANA + 1.0 mg / L BAP)

From the results presented in Figure 4. it is noted that undifferentiated tissue increases in the subculture on the medium supplemented with the ANA auxin and the BAP cytokinin differently, depending on the tissue origin. The best results are recorded in merustematic calus (17mm). The other types of calus growth stagnate from week 6-7.

Medium supplemented with hormonal balance V2, differentiated tissue presents a better growth under the influence of smaller amounts of auxina Anne and citochinina BAP (0.5 mg/l) (Figure 5). Callus growth is different depending on the origin of tissue. The best results are recorded with the meristematic origin (17 mm). The callus is observed stagnation of caulinar from week 5.

From the results presented in Figure 6. it is observed that the undifferentiated tissue grows in the subculture on medium supplemented with two types of cytokines: BAP (0.5 mg / l) and Kin (1 mg / ml), showing values higher than in previous cases (V1 and V2), such as: 20 mm in meristematic origin, 19 mm in leaf origin; and 18 mm in the caulinar callus. The growth of leaf callus is somewhat slower, and caulinar callus stagnates from week 4 with a slight increase from week 5.

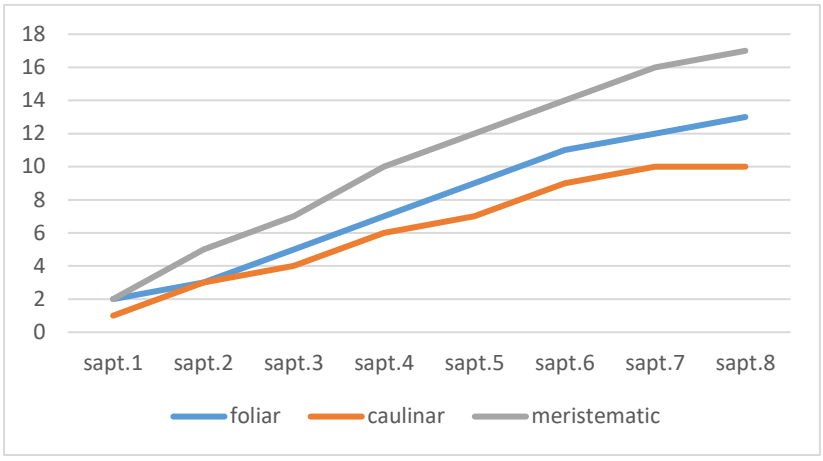

Fig..5. Growth of Goji callus in subculture on variant V2 (0.5mg / L ANA + 0.5mg / L BAP)

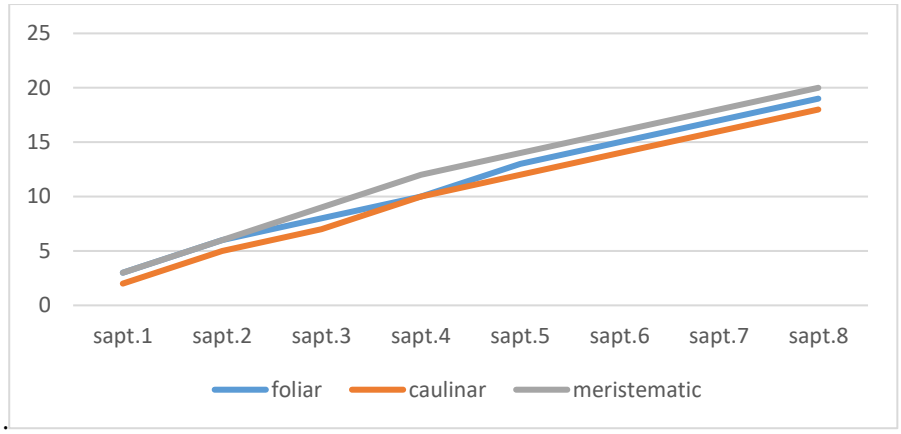

Fig. 6. Growth of the Goji callus in the V3 variant (0.5mg / l BAP + 1mg / l Kin)

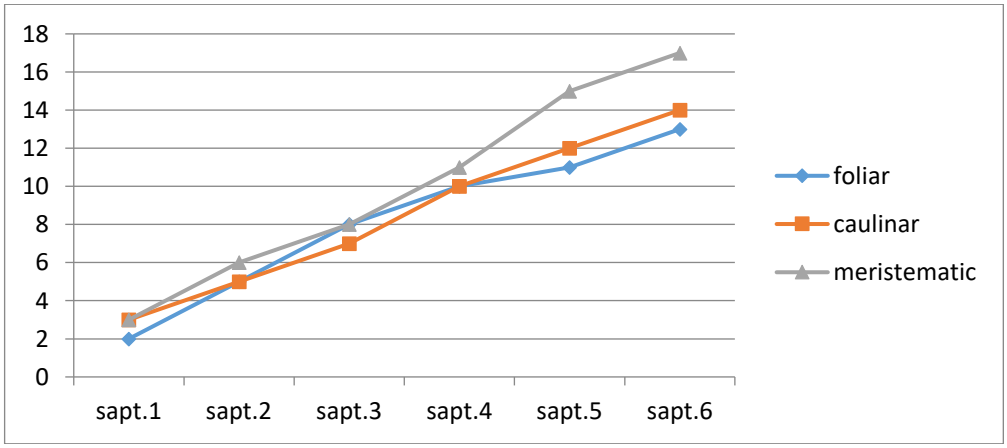

Fig. 7. Growth of Goji callus in subculture on variant V4 (0.5mg / l BAP)

From the results presented in Figure 7. it appears that the undifferentiated goji tissue grows in the subculture on V4-supplemented medium containing only BAP cytokinin (0.5mg / l) in good condition. The callus of meristematic origin shows a significant continuous increase, registering the value of 21 mm after 8 weeks of cultivation. The increase in foliar and caulinular callus is maintained at values close to those of variant V3.

We can conclude that the use of BAP cytokinin provides the best callus growth throughout the eight-week cycle of cultivation. Although the association of

auxin with cytokinin is favorable for the induction of undifferentiated tissue, its proliferation in the subculture is mainly supported by the presence of cytokinin.

Our experiments continued with the second round of Goji callus subculture cycle, which was transferred to fresh MS medium supplemented with 0.5mg / l BAP - the phytohormone that best supported the growth of this tissue. Both the increase in callus and the occurrence of organogenic phenomena were observed.

Also, the regenerated shoots from meristems were subcultivated on the medium with 0.5 mg / l BAP, observing the formation of roots and callus (Figure 8).

Fig. 8. Root formation at the level of subcultivated shells (original photo)

The influence of different concentrations of hormones on the proliferation and *in vitro* proliferation of *Lycium barbarum* in experimental subcultures, it is apparent that a good sprout regeneration occurs only in the presence of cintochininei BAP in amount of 0, 5 mg/l. under these conditions are induced and rizogenei phenomena at the level of the shoots subcultivați.

The callus induction to goji is favoured by the presence of small quantities of auxine, but it is performed and in her apsence. BAP citochinine favours active proliferation of callus. In the second cycle of the subculture was observed organogene phenomena at the level of this tissue (Figure 9).

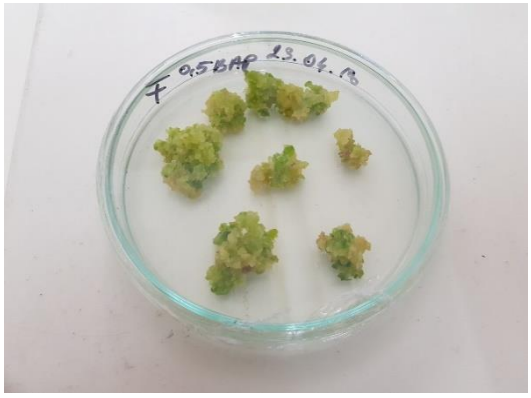

Fig. 9. Meristematic calus with organogenic tendency in the second subculture cycle (V4) (original photo)

The origin of callus influences its evolution in subculture. The undifferentiated stem tissue does not have regerative processes.

The use of the BMP 0.5mg / l combination with Kin 1mg / l determines the formation of roots in the cauline callus in the second subculture cycle. So the origin of callus and hormonal balance are important factors in determining organogenic processes in the goji undifferentiated tissue.

In an *in vitro* experiment, it is necessary to test the response of different types of explants and hormonal balances to goji genotypes in the aim to establish a tissue multiplication or proliferation protocol.

Conclusions

Based on the results obtained in our experimental conditions, we draw the following conclusions:

- The type of explant influences the in vitro response to goji: the leaf and caulinar explants produce calus, and the meristem produces callus and regenerates shoots.
- The induction of callus in goji is favored by the presence of small amounts of auxin, but it is also achieved in its apsence.
- From the callus resulting from dedifferentiation, tissue lines with proliferative aptitude can be selected by subcultivating the tissue on MS medium supplemented with BAP cytokinin.
- The use of the BMP 0.5 mg / l combination with Kin 1 mg / l determines the formation of roots in the cauline callus in the second subculture cycle.
- In an in vitro experiment, it is necessary to test the *in vitro* response of different types of explants and hormonal balances to the goji genotypes studied, to establish a tissue proliferation or proliferation protocol.

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