IN VITRO MODELING OF MORFOGENESYS PROCESSES IN SEQUOIA SEMPERVIRENS (D.DON) ENDL, VIA THE USE OF PHYTOHORMONES

Laslo Vasile, Agud Eliza* Maria, Vicaş Simona

* University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru, St., 410048 Oradea; Romania: vasilelaslo@yahoo.com; eliza_agud@yahoo.com

Abstract

The main objective of the study has been the realization of a screening for the role multiple types of phytohormones in different concentrations within the medium play in the initiation and unfolding of regeneration and organ-genesis processes for Sequoia sempernirens (D. Don) Endl. Our results show that explants constituting of apical and stem fragments react very well to the MS1/2 medium, with low content of macro and micro-element salts. The addition of auxins in the medium does not stimulate the root-genesis process; only the callus-genesis process is stimulated. A good multiplication percentage is obtained on mediums which have one auxin and BA -0.5mg/l in their composition.

Key words: Sequoia sempervirens, in vitro culturing, explants, phytohormones

INTRODUCTION

Sequoia sempervirens (D.Don). Endl is a tree which is part of the Sequoia genus. Its landscaping and the economic value of its wood makes this species a well-researched one, both *in vitro* and *ex vitro*. The *in vitro* culturing techniques of extracted plant tissue offer the possibility of controlling the morphogenesis processes under strict conditions, which may complement conventional methods (Cristea et. al. 2008; Agud, 2014). An important condition for the vegetative multiplication of plants is the strict control of regeneration and organogenesis processes. Morphogenesis and organogenesis processes are influenced both by endogenous and exogenous factors. Out of the endogenous factors, an extremely important role is played by the genetic background of the explant, acting as the substrate for regenerative potential. (Butiuc et al., 1996). Alongside this, an significant role in the regenerative processes is played by the endogenous content of growth regulators, their nature, and the enzymatic equipment involved. Some of the exogenous factors include the characteristics of the culturing medium, the added phytohormones and the culture conditions. Increasing the efficiency of the conditions for the unfolding of the somatic embryogenesis and organogenesis in Sequoia by using different combinations of phytohormones has been studied by Liu C, et al 2006. A

study referring to the possibilities of *in vitro* medium and long term conservation of softwood germplasm by using bud explants was initiated by Ozudogru, E.A et al. in 2012, and the efficiency of Sequoia sempervirens (D. Don) apical bud conservation techniques has been researched in depth by Ozudogru, E.A et al 2011. The optimization of *in vitro* culturing conditions of the species by using the addition of carbon microstructure materials as a substitute for active coal has been tested as part of a study organized in 2009 by R. Stoiculescu et al. The regenerative competency of multiple explants according to different hormonal balances were tested by Ill-Whan Sul, Schuyler S. Korban,2005, Timofte Adrian Ioan, Laslo V. et al. 2010. The process of *in vitro* rooting of cuttings, associated with the culturing medium and with the activity of certain enzymes has been studied by A.G. Fett-Neto et al. (1992). Different methods of juvenilization and a complex biochemical study on the process in this species have been studied by Li-Chun Huang 2002 and Ing-Feng Chang et al.2010.

The main objective of the study has been the realization of a screening for the role multiple types of phytohormones in different concentrations within the medium play in the initiation and unfolding of regeneration and organ-genesis processes for Sequoia sempernirens (D. Don)Endl.

MATERIAL AND METHOD

Explants with a length of 2o mm were extracted from the superior, apical part of plants grown *in vitro*. The base medium used was Murashige-Skoog, with sucrose 30 mg/L, agar 6,2mg/L , Ph 5,7 and phytohormone balances presented in Tab 1. After sterilizing the medium at 121^0 C for 15 minutes, phytohormones and vitamins were added through sterile Millipore filters of 0.025 μm (Merck Millipore), after which the medium was portioned in 25 ml transparent glass containers, 6 ml/container.

Table 1

Experimental variants

Variant	Phytohormones
Control	MS
1	MS +ANA 0.5 mg/L +BA 0.2mg/L
2	MS +AIB 0.5 mg/L+BA 0.5 mg/L
3	MS+ANA 1.0mg/L+BA -0.2mg/L
4	MS+AIB-1.0 mg/L+BA-0.5mg/L
5	MS 1/2+ANA-1.0mg/L+BA -0.5mg/L
6	MS 1/2+AIB 1.0 mg/L+BA -0.5mg/L
7	MS 1/2+2.4 D- 6 mg/L+BA -0.5mg/L
8	MS ½ + AIB 0.1 mg/L

MS –Murasige-Skoog ,BA-benzyladenine ,ANA –α-naftil acetic acid,AIA- indolil butyric acid,2,4D –2,4 dyclorfenoxiacetic acid

During inoculation, the explant polarity was respected. Each experimental variant had 3 repetitions, each repetition consisting of 6 inoculated tubes. In the Sanyo growth chamber, a photoperiod of 16 hours of light/8 hours of darkness, a temperature of 25-27C and a light intensity of 2600 lx achieved with fluorescent tubes were ensured. The evaluation of the explant regeneration capacity was performed 6 weeks after inoculation.

RESULTS AND DISSCUSIONS

The obtained results regarding the regeneration of Sequoia sempervirens (D.Don)Endl explants on the Murasige & Skoog culturing medium, with different phytohormone balances are presented in Figure 1.

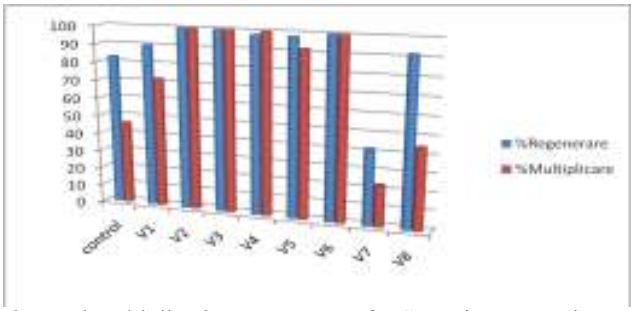

Fig.1 Regeneration and multiplication percentages for Sequoia sempervirens (D.Don) Endl on MS, with different phytohormone balances

We have obtained 100% or very close regeneration and multiplication percentages on the V_2,V_3,V_4 and V_6 variants. The lower multiplication percentages obtained on the control variant are explained by the lack of phytohormones in this culture medium. The V_7 variant, with 2.4 D, only generates callus.

Excepting the Mt control variant (MS medium with no added phytohormones), in all the other experimental variants the explants generated callus initially, out of which stems were formed. On the control variant, stem formation was approximately 2-3, with an average length of 10.5 cm. V_1 formed 3-5 stems/explant, with lengths between 3 and 12 cm. The evaluation we are presenting was performed 125 days after inoculation. V_3 (ANA 1.0mg/L+BA -0.2 mg/L) and V_8 MS1/2 (no phytohormones) exhibit an average formation of 4,4 roots, with an average length of 1.4 cm in V_3 and an average formation of 2,3 roots with an average length of 11.2 cm in V_8.

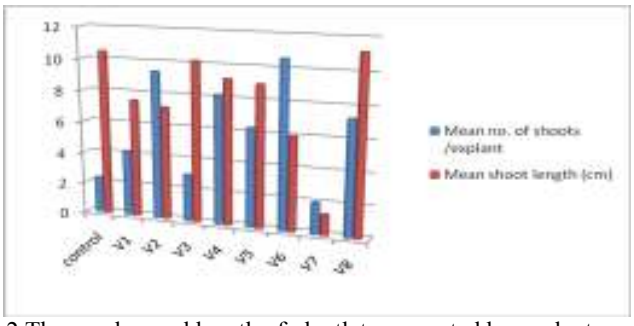

Fig.2 The number and length of plantlets generated by explant, in relation to the culture medium

The number of stems forming in V_3 is however low (3), with long lengths – between 6 and 15 cm – and very strong ramification in their superior half. The combination AIB-1 mg/L+BA-0,5mg/L ensures the best multiplication, with 8-10 stems/explant. Callus formation, excepting control variants, takes place in all medium variants which contain auxins, regardless of concentration. The V_8 variant offers a rich root system and a good multiplication percentage.

CONCLUSIONS

1. Explants consisting of apical stem fragments generate callus very easily in auxin enriched mediums.
2. The generated callus does not have root-generating potential.
3. The MS1/2 medium with a low content of macro and microelements ensures the formation of a very well-developed root system.
4. The optimal multiplication percentage is obtained on mediums which have an auxin and BA -0,5mg/L in their composition.
5. Regeneration percentages obtained are over 80%.

REFERENCES

1. Agud Eliza Maria, 2014, Campanula rotundifolia L. Species Threatened with Extinction, Preserved by in Vitro Techniques, ANALE 2014.B
2. Anca Livia Butiuc,Maria Zăpârţan,C. Deliu,1996, Influenţa unor citochinine în procesele de regenerare,morfogeneză şi organogeneză in vitro la Sequoia sempervirens(D. Don.) şi Chrysanthemum Morifolium Ramat,Institutul de Cercetări Biologice,Contribuţii Botanice 1995-1996,pg.161-164
3. Cristea Tina Oana, Flticeanu Marcela, Prisecaru Maria,2008,Considerations regarding the effects of growthregulators over the „in vitro" morphogeneticreaction at origanum vulgare L.,J. Plant Develop.15 (2008): 133–138
4. Fett-Neto A.G., S.L. Teixeira, , E.A.M. Da Silva, R. Sant' Anna,1992, Biochemical and Morphological Changes during in vitro Rhizogenesis in Cuttings of Sequoia sempervirens (D. Don) Endl ,Journal of Plant Physiology,Volume 140, Issue 6, November 1992, Pages 720–728
5. Ill-Whan Sul, Schuyler S. Korban,2005, Direct shoot organogenesis from needles of three genotypes of Sequoia sempervirens, Plant Cell, Tissue and Organ Culture,March 2005, Volume 80, Issue 3, pp 353-358
6. Ing-Feng Chang, Peng-Jen Chen, Chin-Hui Shen, Tsung-Ju Hsieh, Ya-Wen Hsu, Bau-Lian Huang, Ching-I Kuo, Yu-Ting Chen, Hsiu-An Chu, Kai-Wun Yeh and Li-Chun Huang ,2010,Proteomic profiling of proteins associated with the rejuvenation of Sequoia sempervirens (D. Don) Endl ,2010, , Proteome Science 2010, 8:64 doi:10.1186/1477-5956-8-64
7. Li-Chun Huang, Jui-Hsi Weng, Chiu-Hui Wang, Ching-I Kuo, and Yuh-Jang Shieh,2002,Photosynthetic potentials of in vitro-grown juvenile, adult, and rejuvenated Sequoia sempervirens (D. Don) Endl. Shoots, Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 11529,(Received June 7, 2002; Accepted November 7, 2002)
8. Liu C, Xia X, Yin W, Huang L, Zhou J.,2006, Shoot regeneration and somatic embryogenesis from needles of redwood (Sequoia sempervirens (D.Don.) Endl.), Plant Cell Rep. 2006 Jul;25(7):621-8. Epub 2006 Feb 22.

9. Ozudogru E .A., Kirdok, E., Kaya, E., Capuana, M. and Benelli, C. (2012). In vitro conservation of redwood (Sequoia Sempervirens) by slow growth storage and cryopreservation. Acta Hortic. 961, 291-296
10. Ozudogru E. A.; Kirdok, E.; Kaya, E.; Capuana, M.; Benelli, C.; Engelmann, F. - 2011-Cryopreservation of redwood (Sequoia sempervirens (D. Don.) Endl.) in vitro buds using vitrification-based techniques. -CryoLetters 32-p. 99-110
11. Stoiculescu R., Cogalniceanu G., Brezeanu A., Hristea G.,2009,Influence of microstructured carbon materials on Sequoia sempervirens (D. Don.) Endl. in vitro culture, Ann. For. Res. 52: 137-142
12. Timofte A., Laslo V., Zapartan M., Vicas S., Agud E., Bals C., 2010, In vitro morphogenesis and organogenesis of Sequoia sempervirens (D. Don) Endl specie, Analele Universitatii din Oradea, Fascicula Protectia Mediului, ISSN 1224-6255(Ed. română) ISSN 2065-3476, (Ed. engleză) ISSN 2065-3484, categorie B+.