IN VITRO MORPHOGENESIS AND ORGANOGENESIS OF *SEQUOIA SEMPERVIRENS* (D.DON)ENDL SPECIE

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Abstract

The morphogenesis and the organogenesis are processes which accomplish under the influence of endogenous and exogenous factors, among those the genetic dowry of the donor cells of explants represents the under layer of the regenerating potential, which makes so that the cellar totypotentiality be different from one specie to another. The paper proposes a study of in vitro regenerating capacity at Sequoia sempervirens (D.Don)Endl, starting with the explants taken from free adult material, the apex and the nodal detached from young sprouts of that year. The explants were grown on MS medium, in the following variants: S_o = MS1/2; S_1 = MS+ 0,5mg/l BA+ 0,1mg/l AIA; S_2= MS + 0,5mg/l BA; S_3 = MS + 2,0mg/l BA + 0,1 mg/l AIA; S_4 = MS + 2,0mg/l BA. The results obtained after 14 weeks have brought us to the following conclusions: both tissues (nodal and apex) of Sequoia sempervirens (D.Don)Endl, have a good reaction; the maximum concentration of BA (2,0 mg/l) regenerates in maximum percentage of 100%, determining the formation of the greatest number of plants; the small dose of BA - 0.5 mg/l (S_2 and S_1) favours a harmonious development of the neoplantlets, and the presence of naftil acetic acid (0,5 mg/l), stimulates the rooting of Sequoia sempervirens(D.Don)Endl plantlets generated in vitro. The acclimatization has realised in a great percentage on a layer of peat with temporary protection under a bell of glass.

Key words: *in vitro*, apex, *Sequoia sempervirens* (D.Don)Endl, phitohormons, cytokinine, auxine, regeneration, organogenesis, multiplication, acclimatization.

INTRODUCTION

The percentage of *in vitro* micropropagation of *Sequoia sempervirens* variety is related to the year ’90, when remarkable results were obtained concerning *in vitro* propagation of the variety (*Kyte*, 1990), but much more later, in 2007, a protocol for *in vitro* micropropagation was established for the woody varieties in general and for the Sequoia variety (*Korbal and Sul*., 2007). The same community of researchers, before establishing the protocol of proliferation of the specie, has tested different types of cytokinine, for the proliferation and elongation of the neoexplant detached from different Sequoia genotypes (*Sul and Korbal*., 1994), also following the organogenetic evolution of the stem explants (*Sul and Korbal*., 2005). The regeneration, the morphogenesis and the organogenesis are processes which take place under the influence of endogenous and exogenous stimulus. The genetic value of a specie, of the celles which compile the donor tissue of the explant are the most important endogenous factor which ensures the under layer of the plant’s regenerating capacity and of its *in vitro* reaction. The manifestation of the cellular totypotence differs from one specie to another and more than that, from one botanical family to another, the totypotentiality being different along the phylogenesis of vegetal species (*Liu et al*. 2001).

The present paper represents a study concerning the stimulation of the regeneration, the organogenesis and the *in vitro* multiplication of the tissues detached from the *Sequoia sempervirens* (S.Don)Endl plants. Specie originary from the American

continent (from the North California to the South of Oregon), it is one of the greatest confiers which tolerate a large variety of climates (*Encyclopédie de botanique et d'horticulture"* 1999). On Romanian territory, the specie can be seen in old parks and in botanical gardens created at the beginning of the last century. Even if it is not originary from our Romanian space, the specie is of interest for the architectural Romanian space, this is why we desire its multiplication on unconventional ways. The importance of vegetal biotechnologies and the role of micropropagation at some species, of interest for the economical sectors is presented and deeply analysed by *Cachiță and Ardelean,* 2009, in their work „Importanța biotehnologiilor vegetale şi aplicațiile lor" (The importance of vegetal biotechnologies and their application).

MATERIALS AND METHODS

The experiment was initiated at the beginning of May, following *in vitro* evolution of adult explants (Fig. 1), detached from *Sequia sempervirens* (D.Don)Endl samples, from The Botanical Garden (Cluj-Napoca). It is known that the juvenile tissues ensure the regeneration and the multiplication of the specie in a larger way (*Arnaud* et al., 1993), this is why we detached explants from young sprouts grown in the spring of the same year. Generally, the studies analysed have shown that the vegetal material obtained from these sprouts have manifested the highest capacity of regeneration (*Kane*., 2005), and the biomass arised from the material obtained *in vitro* at *Sequoia sempervirens* (D.Don)Endl, are superior qualities (*Busing and Fujimori*, 2005). The paroxidasyc activity of the biomass obtained from *in vitro* generated plants according to *Keul-Butic* et al., 1997 is superior to the biomass detached from free space. We have reached to the time when modern biotechnologies ensure, at this moment, the micropropagation of vegetal species, using automatic gadgets (*Cachiță*, 2007).

After sterilizing the young sprouts detached from the samples from the garden we passed to the selection of the top explants and nodal of about 0,5-1,0cm and to their pester on *Murashige – Skoog* 1972 medium, with the following variants: S_o = MS1/2; S_1 = MS+ 0,5mg/lBA + 0,1mg/l AIA; S_2= MS + 0,5mg/l BA ; S_3 = MS + 2,0mg/l BA + 0,1 mg/l AIA; S_4 = MS + 2,0mg/l BA.

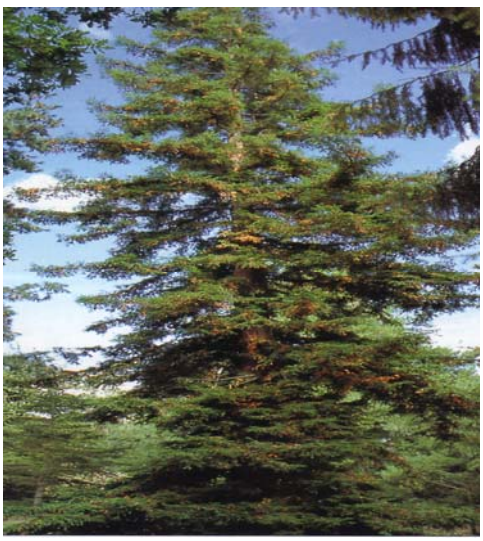

Fig. 1 *Sequoia Sempervirens* (D.Don)Endl
(the material from which we detached the explants)

RESULTS AND DISCUSSION

The presence of the cytokinines in the Murashige – Skoog basal medium ensures the best regeneration percentage, a complete *in vitro* morphogenesis and organogenesis to the *Sequoaia sempervirens* (D. Don)Endl specie (*Keul-Butiuc* et al., 1995 – 1996). From this point started the idea of testing the medium formulas presented above: S_o, S_1, S_2, S_3 and S_4. Among the analysed parameters, we recall: the percentage of regeneration, the number of plants according to their height and the percentage of roots' formation based on their length, all parameters compared to the medium variants. In table 1 we present the percentage of regeneration of the explants and the average of the number of plants and roots.

Table 1

The regenerating capacity and the evolution of Sequoia semperviren (D.Don)Endl explants

The variant	% of regeneration	No. plant/height (cm)	No. roots/length (cm)	Note
S_o	60	2,0/2,8	3,0/1,5	xxx
S_1	80	3,0/1,5	**18,0/1,8**	**xxxxx**
S_2	85	3,5/1,5	4,0/1,0	xxxx
S_3	100	**7,0/1,0**	**14,0/2,0**	**xxxxx**
S_4	100	6,0/1,2	5,0/1,0	xxxx

It is important to keep in mind that between the two types of explants (top and nodal) we did not see any significant differences, their evolution concerning the followed parameters being almost identical. The percentage of regeneration after about 14 weeks is shown in the 2nd fig., from which, we can see the percentage of 100% on S_3(MS + 2,0mg/l BA + 0,1 mg/l AIA) and on S_4(MS + 2,0mg/l BA), so on variants with high concentration of cytokinine. On S_1 and S_2 the percentage is also good, but it is smaller of about 80- 85%, and on witness, S_o of only 60%.

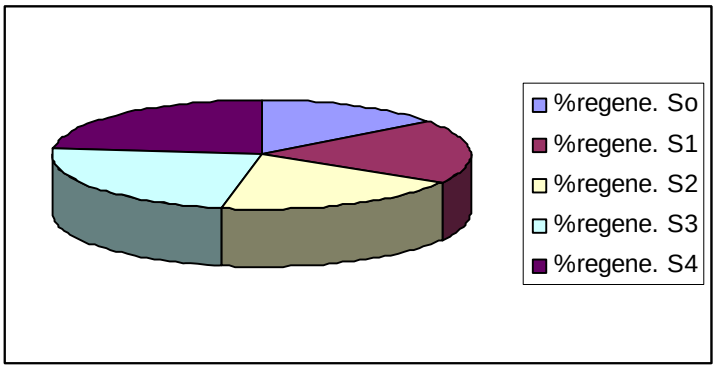

Fig. 2 The percentage of regeneration of *Sequoia sempervirens* explants, grown *in vitro* (after 14 weeks)

The number of detached neoplantlets and their height is shown in the 3rd fig., which includes the average of the number of sequoia neoplantlets and the average of their height. In this case of parameter too, the variants with big concentration of benzyladenine have proved the best evolution. If the average number of plantlets on those mediums reaches about 7-8 neoplantule of about 1 cm, on witness there can be obtained only 2 neoplantlets, and on the ones with low concentration of BA about 3 neoplantlets of 1,5cm.

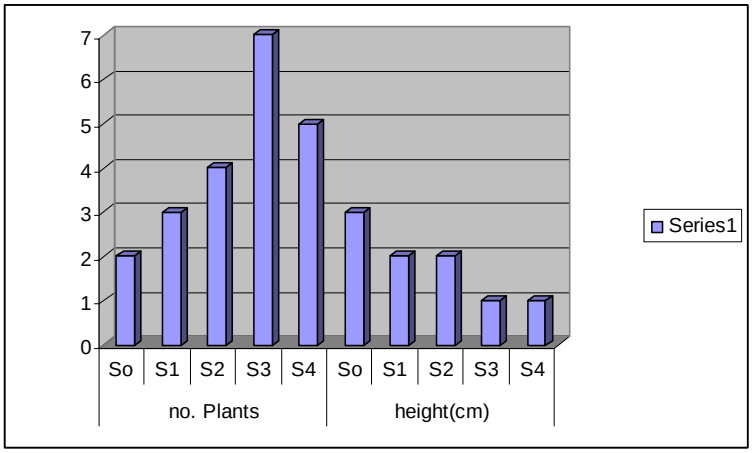

Fig. 3 *In vitro* evolution of *Sequoia sempervirens* explants (after 14 weeks)

The root system reaches the highest values as number and length on mediums with auxine (S_1 and S_3), about 14 – 18 roots with 1,8 – 2,0 cm length. Generally, there are no differences in what concerns the length of the roots, which at all variants situates between 1,0 – 2,0 cm.

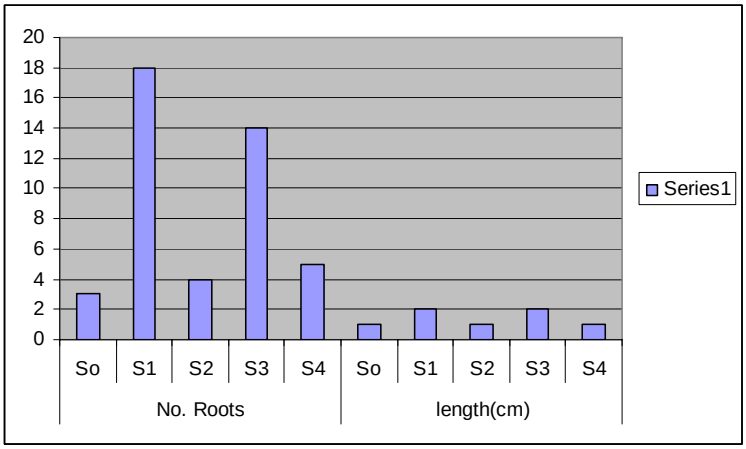

Fig. 4 The evolution of the root system at Sequoia
(after 14 weeks)

Fig. 5 The organization of *Sequoia sempervirens* (D.Don)Endl neoplantlet on medium with vegetal charcoal (after about 5 months)

Fig. 6 and 7 Aspects of acclimatization of *Sequoia sempervirens* (D.Don)Endl neoplantlets

CONLUSIONS

1. There are no differences concerning the evolution of the two tissues, nodal and apex, detached from young sprouts of *Sequoia sempervirens* (D.Don)Endl,.
2. The high dose of BA (2,0 mg/l) has generated the maximum percentage of regeneration, of 100% and the bigest number of plants.
3. The small concentration of cytokinine (0.5 mg/l BA) has lead to a harmonious development of Sequoia neoplantlets, both in (S_2) medium and in combination with an auxine (S_1).
4. The presence of auxine (acetic naftil acid), in concentration of 0,5 mg/l, was favorable for rooting stimulation.
5. The acclimatization of *Sequoia* neoplantlets can be accomplished in high percentage on a layer of peat and temporary protection under a bell of glass.

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