THE INFLUENCE OF DEUTERIUM LOW CONTENT WATER ON *SEQUOIA SEMPERVIRENS* L. HEAT RESISTANCE

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ABSTRACT. In this research we have examined the influence of deuterium low content water on *Sequoia* vitroplantlets, resulted from „in vitro" regeneration of this plant. The biological material was exposed to a 36-38°C (97-100°F) high environmental temperature, for 40 days. During this time we have made observations concerning plantlets growth and also their survival percentage. This experiment has shown that 50% to 75% proportion of deuterium low content water (DLCW), added in culture media, has a good stimulator effect to *Sequoia* sp. L vitroplantlets growth, and help vitroplantlets to pass thru a relative long warm period, protecting them from chlorosis induced by hyperthermia.

Keywords: *Sequoia*, deuterium, water, culture media, heat resistance

Abbreviations: DLCW, deuterium low content water; BM, basic medium; MS, Murashige and Skoog (1962)

INTRODUCTION

The water is one of the fundamental elements of biosphere, an essential condition of life on planet Earth, being the most important environment where all biochemical reactions and metabolic transformations happen. We can really see that, looking at the high water percent in all living beings, vegetal organisms and animal as well.

As we already know, water contains not only Hydrogen atoms (H), but also Deuterium (D), and their concentration ratio are determinating for the physiological processes, at cellular level. The Deuterium is needed for cell divisions, because it seems that a temporary higher D/H ratio is the trigger which starts mitosis process (Somlyai, 2001).

Water with Deuterium low content is a secondary industrial product, as a result of *heavy water* (D_2O) extraction. Deuterium low content water (DLCW) has the property to reduce the tumor size in animal organisms and, concerning the plants, the effect of DLCW is an inhibitory one, when refers to germination and growth (Somlyai, 2001; Blidar et all, 2006), but not when is about *Sequoia* sp. L. (Pop and Cachiţă, 2007).

Some effects of DLCW in vitroplantlets acclimatization process (Petruş et all, 2003), and in hyper-hydric control (Radoveţ et all, 2004), are also known.

Because of these interesting properties, in this experiment we have tested the influence of DLCW presence when *Sequoia sempervirens* L vitroplantlets were exposed to a higher than usual temperature, respectively 36-38°C, which represents double than optimal for this plant growth (18-19°C).

Sequoia sempervirens L. (Figure 1) is a special kind of tree, native on Californian coast, but also cultured in entire world as ornamental plant in many parks and gardens. Many researchers were preoccupied with the study of *Sequoia sempervirens* L. and *S. giganteum* L. (Bowlay, 1998), because of those species economical significance, their special aesthetical look, and because *Sequoia*'s survival depends of the human actions, as being considered a vulnerable genus (Farjon et all, 2006).

Redwoods are the highest trees in the world. *Sequoia sempervirens* L. of 60 m in height are common, and trees of 3.6 to 4.8 m diameter are very tall (tallest recorded in 1956: 110.61 m). It is a long-lasting evergreen tree and is mature when 400 to 500 years old (oldest so far found is 2200 years, determined by growth ring count) (Fowells 1965). The most *Sequoia* forests in the world are represented by young plantations, initiated from sprouts (Lindquist, 1974). Preservation of this specie needs human intervention, and the biotechnology is an optimal solution for this purpose, because its procedures need a very few biological material, can produce a high number of new plants, is environment friendly, and has a very low production cost. Using biotechnology we can select a genetic pure and strong line of clones, which can be the start point for new plantations, which later will become new *Sequoia* forests.

This beautiful tree is still missing in Romanian vegetation landscape, fact that motivates and encourages researches concerning its rejuvenation and micropropagation.

Fig.1 *Sequoia sempervirens* (Coast redwood): A-old tree, B-young plantlet

MATERIALS AND METHODS:

For this experiment we used *Sequoia sempervirens* L. vitroplantlets as biological material. We have cultured them on basic Murashige and Skoog (1962) media, but where the distilled water was replaced with DLCW (25 ppm), in different percents. The vitroplantlets were resulted from a previous experiment (Pop and Cachiţă, 2007) that lasted for 90 days.

The experimental variants were the following:

- V_0 – control variant – *Sequoia sempervirens* cultured on basic MS medium, where DLCW was missing. (150 ppm D)
- V_1 – *Sequoia sempervirens* vitroplantlets cultured on 25% DLCW MS medium (128.75 ppm D)
- V_2 – *Sequoia sempervirens* vitroplantlets cultured on 50% DLCW MS medium (87.5 ppm D)
- V_3 – *Sequoia sempervirens* vitroplantlets cultured on 75% DLCW MS medium (56.25 ppm D)
- V_4 – *Sequoia sempervirens* vitroplantlets cultured on 100% DLCW MS medium (25 ppm D)

The vitrocultures were exposed to white fluorescent light, at 1700 lux, and about 36-38°C (97-100°F), for 40 days along. The photoperiod was 16/24h light.

RESULTS AND DISCUSSIONS:

At the beginning of this experiment, the vitroplantlets were already grown on those media; healthy, dark green and strong (Figure 2).

Fig. 2 *Sequoia sempervirens* vitroplantlets before hyperthermic treatment (V_0 – control variant – *Sequoia sempervirens* cultured on basic MS medium, where DLCW was missing. (150 ppm D), V_1 – *Sequoia sempervirens* vitroplantlets cultured on 25% DLCW MS medium (128.75 ppm

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D), V_2 – *Sequoia sempervirens* vitroplantlets cultured on 50% DLCW MS medium (87.5 ppm D), V_3 – *Sequoia sempervirens* vitroplantlets cultured on 75% DLCW MS medium (56.25 ppm D), V_4 – *Sequoia sempervirens* vitroplantlets cultured on 100% DLCW MS medium (25 ppm D))

After 40 days of thermic stress, chlorosis spots occurred on most vitroplantlets (Figure 3), in the beginning at apexes, and then continuing at some inoculs to entire stalk. Many plantlets died (Fig.4) because of thermic stress (the optimal temperature for *Sequoia sempervirens* vitroplantlets growth is around 18-19°C, which means 64-66°F).

Fig. 3 *Sequoia sempervirens* vitro-plantlets after hyperthermic treatment (V_0 – control variant – *Sequoia sempervirens* cultured on basic MS medium, where DLCW was missing. (150 ppm D), V_1 – *Sequoia sempervirens* vitroplantlets cultured on 25% DLCW MS medium (128.75 ppm D), V_2 – *Sequoia sempervirens* vitroplantlets cultured on 50% DLCW MS medium (87.5 ppm D), V_3 – *Sequoia sempervirens* vitroplantlets cultured on 75% DLCW MS medium (56.25 ppm D), V_4 – *Sequoia sempervirens* vitroplantlets cultured on 100% DLCW MS medium (25 ppm D))

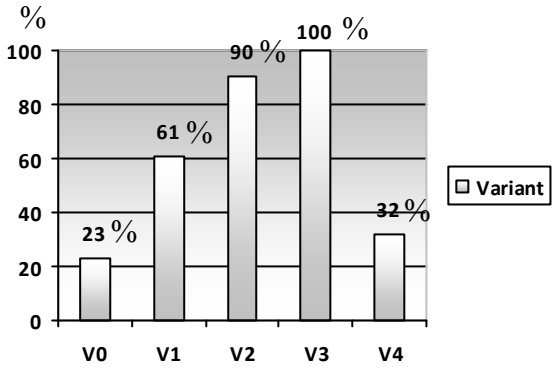

Fig.4 *Sequoia sempervirens* vitroplantlets survival percent (V_0 – control variant – *Sequoia sempervirens* cultured on basic MS medium, where DLCW was missing. (150 ppm D), V_1 – *Sequoia sempervirens* vitroplantlets cultured on 25% DLCW MS medium (128.75 ppm D), V_2 – *Sequoia sempervirens* vitroplantlets cultured on 50% DLCW MS medium (87.5 ppm D), V_3 – *Sequoia sempervirens* vitroplantlets cultured on 75% DLCW MS medium (56.25 ppm D), V_4 – *Sequoia sempervirens* vitroplantlets cultured on 100% DLCW MS medium (25 ppm D))

On control variant, where DLCW was missing (150 ppm D), the vitroplantlets got chlorosis on almost entire plant, being the most affected experimental variant, and having the smallest survival percent.

In the opposite, on V_3 (56,25 ppm D), where DLCW has substituted water in a 75% percent, all inoculs had a good development, looking healthy, with no chlorosis, and having a very impressive elongation, in comparison to any other experimental variant (Figure 5). The control variant didn't have any elongation, being the weakest one.

The rest of variants manifested chlorosis mostly at apexes, but all of them having a better survival percent than the control variant.

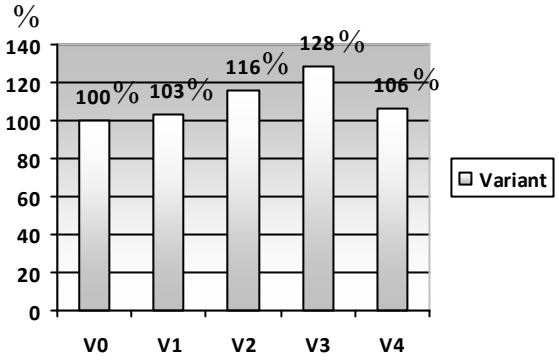

Fig.5 *Sequoia sempervirens* vitroplantlets elongation (percent) (V_0 – control variant – *Sequoia sempervirens* cultured on basic MS medium, where DLCW was missing. (150 ppm D), V_1 – *Sequoia sempervirens* vitroplantlets cultured on 25% DLCW MS medium (128.75 ppm D), V_2 – *Sequoia sempervirens* vitroplantlets cultured on 50% DLCW MS medium (87.5 ppm D), V_3 – *Sequoia sempervirens* vitroplantlets cultured on 75% DLCW MS medium (56.25 ppm D), V_4 – *Sequoia sempervirens* vitroplantlets cultured on 100% DLCW MS medium (25 ppm D))

CONCLUSIONS:

Some vitro-plantlets can pass easier thru a warmer period, if DLCW is added in their culture media.

Deuterium low content water (DLCW 25 ppm) can be used instead usual water, in a certain percent (75% in *Sequoia sempervirens* case, respectively 56.25 ppm), to increase plantlets heat resistance

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