

## SUGAR BEET (*BETA VULGARIS* L. VAR. *SACCHARIFERA*) VITROCULTURE INITIATION FROM ENCAPSULATED SEEDS

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**Abstract.** This study was conducted to identify the optimal method of sugar beet (*Beta vulgaris* var. *saccharifera*) seed sterilization to "in vitro" cultures initiation, and to found a cultivar that is suitable to growth in specific conditions of vitroculture. The study was necessary because the literature does not refer to the method of initiating vitrocultures from encapsulated beet seeds and to avoid any losses that may occur in a massive micropropagation. The most optimal method for beet encapsulated seeds asepsization, prior inoculation on the vitroculture medium consists in the their dipping in sodium hypochlorite solution for 15 minutes, and the best cultivar which was suited to micropropagation was *Evelina*, but *Diamant* too, with 90 - 95% germination rate and a very good ulterior growth.

**Keywords:** asepsization, *Beta vulgaris*, micropropagation, seeds

### INTRODUCTION

Beet (*Beta vulgaris* var. *saccharifera*) is one of the interest economic plants for sugar production, necessary in the food industry, in animal breeding, and to produce ethanol, and to improve his features is cloned through the micropropagation. To increase the productivity is used seed encapsulation, which means to cover the seeds with a pellicle, which contains a binder, the insecto-fungicides substances, herbicides, biostimulators, sometimes nutrients and micro-elements. Following this procedure, the seeds receive a regular, spherical form.

Micropropagation (plant vitrocultivation or "in vitro" plant culture) is a rapid plant multiplication, used for decades on industrial scale. Many laboratories are producing plants through micropropagation, by means of protocols based on past discoveries, but they hold the latest techniques, with important economic impact, for multiplication of both species grassy and woody plants, for the purpose of research or economic interest [7].

Vitroculture types related cormophytotechnology is referring to *explants* cultures which are varied nature (when it comes to primary culture) or inoculs culture - minicuttings, propaguls, cells or protoplasts (in the case of subcultures). To successfully initiate a *primary* vitroculture, a particularly important role is the plant material asepsization, prior to inoculation on the sterile growing medium.

The sterilization method of the future inocul, used to initiate a "in vitro" culture, is very important and sometimes difficult to establish, because a strong asepsization with too high concentration of disinfection agents or an excessive application of it on plant material, lead to embryo destruction and the inability of seeds to germinate. On the other hand, low concentrations or a short time in applying the disinfection fail to destroy all germs and causes infections, and after the placement of the culture their began to proliferate, destroying the cultures [6].

Because, there is not mentioned a procedures for asepsization the sugar beet encapsulated seed, to inoculate on the aseptically medium for the "in vitro"

cultures initiation, to carry out further research on these, we proposed to determine the optimal sterilization need them, depending on the cultivar, with sodium hypochlorite solution, the cheapest and the reach of anyone explants asepsization methods. These exploratory experiments is made with fewer seeds and are binding prior to initiation of "in vitro" cultures with a large number of cultures containers, as micropropagation prevent losses arising from an incorrect method of plant material sterilization, as is encapsulated seeds, which are costly.

### MATERIALS AND METHODS

Plant material used in "in vitro" germination experiments was either unencapsulated, normal sugar beet (*Beta vulgaris* var. *saccharifera*) seed or encapsulated *Beta vulgaris* var. *saccharifera* cultivar *Evelina* seed produced by the KWS SAAT AG from Germany [3], *Diamant* cultivar, produced by the Danisco Company [2] from Denmark and cultivation *Cronos*, made by Swiss firm Syngenta [4], which were submersion for 5, 10, 15 or 20 minutes in sodium hypochlorite with an active chlorine content of 42 G/DM3 and hydroxide free from 5-8 G/DM3, procured from trade, to which were added a few drops of Tween 20, for modifying surface tension at the surface of semincery material (breaking of air bubbles) and favoring sterilization.

After the hypochlorite submersion, seeds were passed through 5 sterile water baths, which were maintained 5 minutes in each bath, as the removal of chlorine is required, otherwise, it acts on the embryo, causing death of it. All these operations to sterilize plant material were made in sterile conditions in hood, under laminar flow of sterile air.

Sterile water was obtained by autoclaving to 121 °C for 25 minutes in large capacity vessels (800 ml) filled with ¾ water [8]. Dissolution of aluminum foil, after water sterilization was only a need to light.

During seed maintenance in hypochlorite for a period of more than 15 minutes, they have undergone a process of discoloration and disintegration of the

protective pellicle, particularly at *Evelina* and *Diamant* cultivars.

Growing medium used in the experiments was basal medium (BM) Murashige - Skoog (MS) ½ [5], which have brought some changes, namely: vitamins thiamine HCl, piridoxină HCl and nicotinic acid, instead 0.1 mg/l or 0.5 mg/l, as was provided in the original recipe, was added to each 1 mg/l and were removed amino acids, was introduced 20 g/l sucrose, instead 30 g/l and 7 g/l agar-agar, instead 10 g/l; culture medium pH was always adjusted to the 5.7 value, prior autoclaving. We proceeded to reduce to one half the nutrient concentration of the culture medium as was described on MS recipe (1962), because the seeds endosperm contain all the substances required to growth plantlets from germination to emergence first two leaflet that in natural life conditions do photosynthesis. In vitroculture conditions, there is a photoautotrophic nutrition, just one myxotrophic or heterotrophic (carbon source being taken from culture medium by vitroplantlets), and in our experiments, the aim being to find a optimal methods of plant material asepsization, the vitrocultures duration required to achieve the objectives was only 21 days.

Vitroculture containers consisted in colorless glass bottles with a 7 cm height and 2 cm diameter, containing 5 ml medium (Fig. 1). To prevent occurrence of rebel infections, before the culture media introduction, glassware was washed with water and detergent, and then fritter it was well under water jet, and finally was washed with distilled water. Since a number of organisms have resistant spores to the disinfectants, or to a short autoclaving, culture vessels were presterilized to dry (dry heat), in drying stove at 160 °C temperatures for 4 hours. The instruments used in inoculation process were sterilized by dry heat, every drying stove for two hours at 170 °C temperature. After the culture media preparation and their distribution in glass containers, and after their closing (each with a cork), they were sterilized by autoclaving to 121 °C, for 20 minutes times.



Figure 1. Germinated seed on aseptic medium.

To avoid infections, the condition itself understood for explants cultivation on aseptic medium, had ensured a perfect sterilization of all surfaces (tables,

instruments, containers) and, of course, the plant material for tissue sampling.

In inoculation were strictly adhered to the basic principles of the process [1], the operations are held in an asepsized room, in the perimeter of laminar hood, with horizontal sterile air, according to the aseptic regime.

After inoculation, the culture vessel were transferred on the growth room, being illuminated with white fluorescent tubes, with an 1700 lux intensity, a 16 h light/24 h photoperiod and to varied temperature between 20 – 23 °C.

At 21 days after inoculation, at those four different variety of sugar beet (*Beta vulgaris* var. *saccharifera*) vitroplantlet levels, which was regenerated "in vitro" following seeds germination on aseptic conditions were made following measurements: the percentage of seeds germination, roots number average/inocul, roots length average, total leaflets number average/inocul, green leaflets number average/inocul, stalks length average/inocul, stems length average, vitroplantlets size average.

## RESULTS

As can be seen in the graph in Figure 2, maintaining the sugar beet seed in sodium hypochlorite, for 5 minutes was not sufficient to achieve the asepsization, their germination percentage and plants formation being small, with values between 25% and 32.5%, from too the four varieties of sugar beet, the remaining seeds at least maintained in disinfectant agent, have developed infections (Fig. 2). Protective seed pellicle in this case had no effect on defense against specific infections of vitroculture conditions (in special, the culture medium rich in sucrose).

The asepsization time of 10 minutes led to higher percentages of germination and survival "in vitro", at 21 days after vitroculture initiation, percent was up from 50% to unencapsulated seeds, but with considerable percentages, 42% - 45%, in the case of *Diamant* and *Evelina* cultivars (Fig. 2).

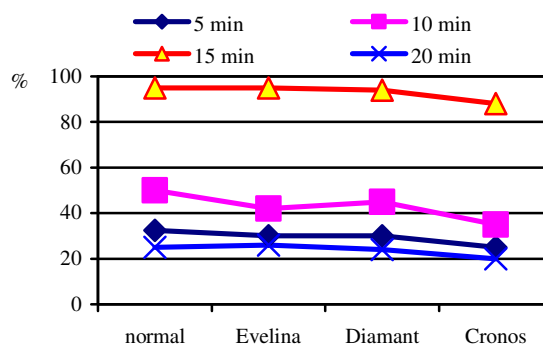


Figure 2. Sugar beet (*Beta vulgaris* var. *saccharifera*) vitroplantlets regeneration percents for unencapsulated (normal) seeds, *Evelina* cv., *Diamant* cv. or *Cronos* cv., at 21 days from their putting to germinated on aseptic BM – MS (1962) ½ solidified medium, depending on the time to maintain their in disinfectant agent.

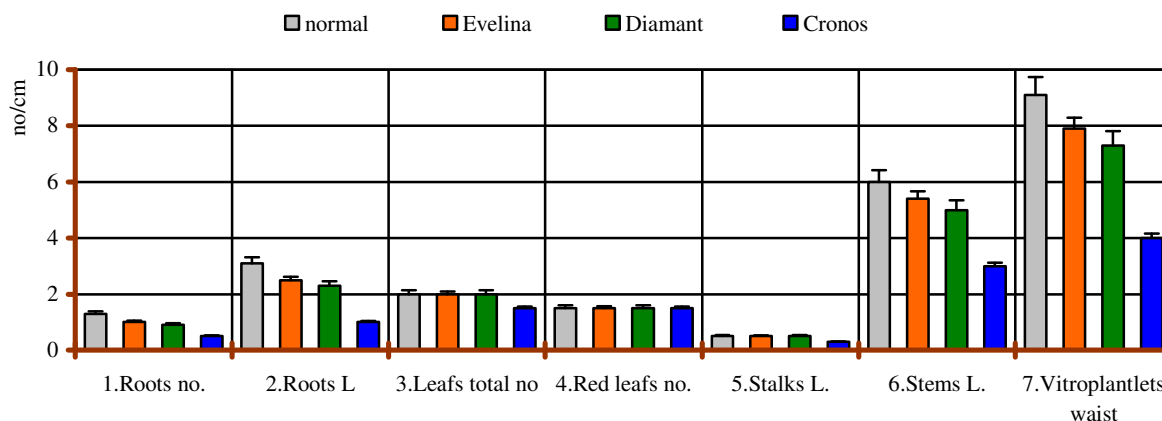
The highest percentage of plantlets generation after germination in the aseptic environment, 95%, was recorded to unencapsulated seeds, maintained in

disinfectant agent for 15 minutes, but at this time of sterilization, in the four cultivation have been obtained survival percent and regeneration of over 80%, including the *Cronos* variety, which proved to be more recalcitrant to the vitroculture initiation from seeds.

Seed maintain for 20 minutes in sodium hypochlorite advance in their inoculation on aseptic medium led to the destruction of embryos because, although the medium were not infected, only 20 - 26% of seeds have germinated. Since the optimum sterilization time was 15 minutes and that this variant

at all cultivars survived enough vitroplantlets, they were measured and statistical processed to justify the efficacy of treatment.

Regarding indices of growth (Fig. 3), from monogerm sugar beet protected seeds, after germination occurred a root which, at 21 days of "in vitro" seed at bay, their average height was approximately 2.5 cm to plantlets provided from unencapsulated (normal) seeds and *Evelina* cv. and only an average of 1 cm to *Cronos* cv. (Fig. 3), standard deviation marking normal limits.



**Figure 3.** Sugar beet (*Beta vulgaris* var. *saccharifera*) vitroplantlets growth provided from unencapsulated (normal) seeds, or *Evelina* cv., *Diamant* cv. or *Cronos* cv., at 21 days from their putting to germinated on aseptic BM – MS (1962) ½ solidified medium, with 15 minutes sterilization time in sodium hypochlorite (no – number, cm – centimeter, L – length; bars means standard deviation).

Above the two cotyledons, at 21 days of making the sugar seeds in aseptic conditions, appeared first leaflets, so that all vitroplantlets provided from unencapsulated seeds and *Evelina* cv., or *Diamant* cv. has two leaflets (Fig. 3), the standard deviation of this caulogenesis parameter was zero, and to *Cronos* cv., this situation was slightly different. Of the total number leaflet, ¾ of them show a green foliar limb, and the rest were unpigmented foliar limb, suffering from an early hyperhydricity, a tumoral phenomenon fervently appeared in the sugar beet vitrocultures.

The value of stalks average length of vitroleaflets ranged between 0.4 - 0.6 cm, which are relatively uniform in size (Fig. 3).

Vitroplantlet stems had 6.1 cm average length, where, the origin of their, was from unencapsulated seeds and only 2.5 cm, to the *Cronos* cv. (Fig. 3).

By adding the two average lengths, therefore embryo roots with that of stems size was calculated the waist of vitroplantlets, whole stressing - that once in addition - differences in growth between the four beet cultivation.

## DISCUSSIONS

According to investigations so encapsulated seed producers and the beneficiaries (Diamant Sugar Factory), on cultivation in natural conditions of life were not reported problems of germination at any of the 3 variety, at least in the number of plants remaining harvest (% density). Roots production/ha to *Cronos* was slightly below the level and variety *Evelina*, *Diamant*, but he compensated with the sugar content

(digestion). The germination in aseptic conditions is different given the natural conditions.

The optimal method for sugar beet (*Beta vulgaris* var. *saccharifera*) seeds asepsization, prior inoculation on aseptic medium, to launch the vitroculture, is to maintain them for 15 minutes in sodium hypochlorite, followed by rinsing the material sterilized by five baths of sterile water for 25 minutes.

Of sugar beet variety tested by us to be vitroculture initiated, most suitable proved to be *Evelina* cultivar and *Diamant* cultivar, both of terms of germination percentage, and in terms of vitroplantlets growth post - germination.

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