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NEW METHODS TO IMPROVE THE BIOECONOMIC AND ECOECONOMIC IMPACT BY PLANT BIOTECHNOLOGY

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ABSTRACT.
Sugar beet (*Saccharomices cerevisiae* var. Saccharifera) is a species of economic and food interest, which, in terms of clonally multiplication, is liable to hyperhydricity. In these studies was identified a method that prevents the hyperhydricity process installation, and at the same time, it increased *in vitro* plant biomass, using honey, as a replacement for sucrose, and deuterium depleted water (DDW), as a replacement for distilled water (DW), either culture in single-sided, or in the double layer system. Murashige-Skoog mineral culture media, solid, free of growth regulators, prepared with DW or DDW, with the supernatant consist in diluted honey either DW or in DDW were most beneficial in stimulating both the rhysogenesis and the caulogenesis process. The increase of plant biomass in the vitroculture and eliminating waste generated by hyperhydricity has a positive economic impact for producers in this branch.
Keywords: deuterium, honey, double system layer

INTRODUCTION

Plant biotechnology is a domain with a great practical value, it represent the solutions to solve the major social problems of humankind such as providing food and energy, health and environmental protection, in the current context of the bioeconomy, ecoeconomy and ecosanogenesis. Modern and traditional cultures is essential for further supporting food security and new policies (Bogdan et al., 2010; Ipate et al., 2010) in relation with biodiversity conservation and climate change responses such as adaptation and mitigation (Antofie et al., 2010 b).

Identification of new methods in plant biotechnology with applications in food and not only has an important step, predicts the ecoeconomic and bioeconomic impact of food safety and security in perspective the increased consumption of food and feed during 2030-2100. The dynamic of food's world population projection to 2050 year and consumption dynamic of vegetables in terms of respect international standards of food safety and security (Bogdan et al., 2010). In this framework, the food safety and security must be correlated with the respect of known principles of Hazard Analysis and Critical Control Points, based on actual international standards from ISO 9001-9002 series (Quality Management System), ISO 14001: 2004 (Environment Management), ISO 22000 (Food Safety).

Sugar beet (*Beta vulgaris* var. Saccharifera) has economic interest (Atanasov, 1986) for sugar production, required for both food and ethanol obtain, and which, for conservation [among other techniques developed *in situ* (Antofie, 2010; Antofie et al., 2010 a; Purcărea and Borbëly, 2011)] and for amelioration, is cloned by micropropagation procedures. Beet multiplications was achieved through various types of inoculs, namely the apex in the early seed-lobe, the buds from colet or from shoots (Sand and Cachiță, 1999; Sand and Cachiță, 2000), phytoinocula placed on different types of culture media with or without added growth regulators or in which sucrose was partially replaced, successful, with starch (Constantinovici, 1997; Constantinovici and Cachiță, 1999). In such practices, the hyperhydricity appearance on vitroculture is quite common for this species, which lowers production yield for the field of plant breeding, plant which are indispensable in rhysomania resistant genotypes selecting and their cloning. At phytoinocula, hyperhydricity is considered a complex neoplasic disease process, reflected not only by an abnormal accumulation of water in tissue, but also by the anatomical changes appearance and deep morpho-physiological organs affected by this syndrome, which is translated and a tissue hypertrophy and cells hyperplasia (Cachiță et al., 2008 a, 2009).

In some experiment, Cachiță and collaborators (2008 a, 2009) has highlighted the profound transformation suffered by sugar beet hyperhydrice vitroplantlets, both in altering terms of the chloroplasts structure and in a fast decrease of the assimilating pigments contents, especially in chlorophyll a. In hyperhydrice vitroplantlets leaflets foliar mesophyll cells this authors identified either chloroplasts flatten or volume increased, but with a content being disorganization; they were scattered in vacuolar juice mixed with cytoplasm (myxoplasm), a mixture resulting because the tonoplast disintegration. The hyperhydrice leaflets presented a low content of assimilating pigments, much lower than that determined in sugar beet greenhouse plantlet leaflets, and lower than that recorded in leaflets of „in vitro" nonhyperhydrice plantlets, which was treated with DDW. DDW can prevent and annihilate hyperhydricity (Cachiță et al., 2008 b and Cachiță et al., 2010).

In this research we intend to stimulate growth of sugar beet plant biomass, without hyperhydricity by replacing sucrose as carbon source, with acacia honey,

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either single-sided, or in the double layer system. Present research is an answer at the question that arises more and more, in present: whether and under what conditions the estimated future food demand can be met and how food security can be achieved.

MATERIAL AND METHODS

The phytoinocules consisted in sugar beet (*Beta vulgaris* var. Saccharifera) uninodal, apical minicuttings, which provided from aseptically germinated seeds, grown on basic medium (BM) Murashige-Skoog (MS) (1962) ½, solid, without growth regulators.

The culture medium used in these experiments was MS (1962), modified by us; the solidification was achieved by 7 g/l agar-agar; culture medium pH was adjusted to a value of 5.7 before its autoclaving.

The culture medium preparation was performed according to the following test:

Single-layer system

V_0 - BM-MS solid prepared with DW and sucrose (control);
V_1 - BM -MS prepared with DDW and sucrose;
V_2 - BM -MS prepared with DW and honey;
V_3 - BM -MS prepared with DDW and honey.

Double-layer system

V_4 - BM –MS prepared with DW and sucrose + supernatant DDW;
V_5 - BM –MS prepared with DW and sucrose + supernatant honey, mixed with DW;
V_6 - BM –MS prepared with DW and sucrose + supernatant honey, mixed with DDW.

We were taken into account that to each experimental variant, the carbohydrate content (regardless of its nature) to a total of 30 g/l, both for single layer and double layer cultures (Cachiță, 1987).

Vitrocultures vessels consisted of glass jars, 7 cm height and 4 cm diameter, each one with 20 ml of solid medium (first layer). The supernatant was 5 ml.

Incubation and growth was realised in growth chamber at 22 ° C ± 2 ° C, illuminated with white fluorescent tubes, 16h day length photoperiod of 24 h day light and 1700 lx intensity.

The biometrisation of plantlets growth indices were realised at 90 days after inoculation and the dry weight were weighed after maintenance in aluminium foil, in oven at 115 °C, for 3 days.

The dates obtained from measurements were interpreted statistically by analysis of variance, also the statistical significance was determined using *one sample t test* of statistical SPSS for Windows.

RESULTS AND DISCUSSIONS

At *90 days after inoculation*, the sugar beet vitroplantlets rhysogenesis was decreased to control lot (V_1) and those in single-layer, in which the DDW was replaced with DW (Table 1). Moreover, the phytoinocula submerged in the supernatant consisting of DDW (V_5) showed *in vitro* no regeneration processes.

The same rhysogenesis inhibition effect exerted by DDW was reported by Petruş-Vancea and Cachiță (2008 and 2009) in the case of chrysanthemum or African violets plantlets base watering with this type of water, throughout the period of acclimatization to the septic medium. Also, rhysogenesis to the *Tradescantia* cuttings, rooted in natural living conditions in perlite, was inhibited by watering them with DDW, compared with watering with DW (Petruş and Cachiță, 2008). At the *Cymbidium hybridum* and *Petunia* cultures, placed on MS medium (1962), in single-layer system, solid, without growth regulators, DDW was a timing effect of growth, with an important role in *in vitro* preserving cultures (Petruş et al., 2004). Also mixture of honey and DDW - but as supernatant - was beneficial in the caulogenesis stimulation, the longest stemlets, an average of 2.11 cm, and a large number of leaflets, few of them being necrotic were registered at vitroplantlets cultivated on experimental variant denoted V_6. Mediums containing honey and DDW generated vitroplantlets with fresh weight, respectively dry weight increased, on medium in which the mixture was consist in solid layer, on the base system (V_3), the rhysogenesis was stimulated, and at the regenerated vitroplantlets in the supernatant consisting of those two component (V_6), the caulogenesis being intensified (Table 1), while separate use of two natural compounds generated even total inhibition of *in vitro* regeneration, as in DDW case which was used individually as supernatant (V_4).

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Table 1.

Comparative analysis of average values of sugar beet (*Beta vulgaris* var. Saccharifera) vitroplantlets growth indices, at *90 days after inoculation*, as follows: V_0 - BM-MS solid prepared with DW and sucrose (control); V_1 - BM-MS prepared with DDW and sucrose; V_2 - BM-MS prepared with DW and honey; V_3 - BM-MS prepared with DDW and honey; V_4 - BM-MS, prepared with DW and sucrose + supernatant DDW; V_5 - BM-MS, prepared with DW and sucrose + supernatant honey, mixed with DW; V_6 - BM-MS, prepared with DW and sucrose + supernatant honeyl, mixed with DDW.

Biometri-sation	V_0		V_1		V_2		V_3		V_4		V_5		V_6	
	Mean± st.dev	Sig	Mean± st.dev	Sig	Mean± st.dev	Sig	Mean± st.dev	Sig	Mean± st.dev	Sig	Mean± st.dev	Sig	Mean± st.dev	Sig
Rootlet no.	2.85± 2.27	***	2.56± 1.82	***	5.38± 1.12	***	9.00± 1.61	***	-	-	8.50± 1.58	***	6.44± 3.24	***
Rootlet L. (cm)	1.96± 1.05	***	1.19± 1.29	**	0.73± 0.44	***	1.55± 0.52	***	-	-	1.75± 0.86	***	1.67± 1.00	***
Stem L. (cm)	1.46± 0.14	***	1.19± 0.23	***	1.23± 0.26	***	1.95± 0.15	***	-	-	1.95± 0.28	***	2.11± 0.49	***
Leaflets total no.	9.62± 1.80	***	7.50± 1.79	***	8.08± 1.26	***	9.27± 1.01	***	-	-	9.20± 1.03	***	9.33± 1.00	***
Necrosed leaflets no.	3.54± 2.22	***	2.50± 1.79	***	1.31± 0.48	***	1.64± 0.67	***	-	-	1.80± 0.92	***	1.56± 1,01	**
Petiole L.	3.19± 0.75	***	3.84± 0.93	***	4.15± 0.90	***	6.45± 0.52	***	-	-	5.50± 1.58	***	5.89± 1.05	***
Ramifica-tion no.	0.23± 0.44	*	0.06± 0.25	ns	0.31± 0.48	*	0.00± 0.00	***	-	-	0.10± 0.32	ns	0.00± 0.00	***
Fresh w.(g)	0.13± 0.01	***	0.24 ± 0.01	***	0.35 ± 0.01	***	0.96 ± 0.01	***	-	-	0.61± 0.20	***	0.93± 0.03	***
Dry w. (g)	0.02± 0.01	***	0.04 ± 0.01	***	0.04± 0.03	***	0.09 ± 0.01	***	-	-	0.06± 0.04	***	0.098± 0.01	***

Note: BM-MS – basal medium Murashige-Skoog (1962); DW – distilled water; DDW-deuterium depleted water; sig. – significance [ns – non significant ($p > 0.1$), ** significant ($p < 0.05$), *** very significant ($p < 0.01$)]; st.dev. – standard deviation; no – number; L – length; w – weight.

At *Cymbidium* protocorms (Vancea and Pătru, 2002) or at chrysanthemums and African violets plantlets (Petruş-Vancea et al., 2008) have been reported light inhibitions of *in vitro* organogenesis, being cultured on medium in which sucrose was replaced with acacia honey 20g/l, but when transferring the plantlets, regardless of species, living in septic environment, the survival of lot provided from honey was superior to that whose carbon source was sucrose.

In this research, the optimal culture medium for beet proved to be that which was prepared with honey, in which DW was replaced with DDW (V_3). Although the double layer media have generated intense growth of sugar beet vitroplantlets, as sparrow grass (personal dates), compared with this specie (which grows as a shrub), double layer system applied to beet has shown some mechanical drawbacks. Thus, the supernatant exerted a beneficial physiological effect on vitroplantlets growth, but, because the sugar beet leaflets, having long stalks, the plant arrived in the supernatant and it was overturned. Certainly, finding a support for plant (like luffa lignoskeleton described by Petruş - Vancea et al. 2004) as a solution to ensure the aerial part of vitroplantlets and thus the optimization of micropropagation on double layer system from physically point of view.

CONCLUSIONS

Economic implications of the processes identified in this research are important, because we managed to remove two of the most delicate issues of sugar beet *in vitro* culture, namely hyperhydricity and quantity of plant biomass, due to successful combination of DDW and honey. Combining honey with DDW we managed to achieve two major objectives in the micropropagation of sugar beet, namely increasing biomass on vitrocultures and hyperhydricity prevention, thus eliminating loss of production and reducing the costs of obtaining healthy plant material, the bioeconomical and ecoeconomical impact being certainly.

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