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NEW TECHNIQUES IN *ASPARAGUS OFFICINALIS* L. MICROPROPAGATION

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Abstract

In this study we aimed to identify new techniques for sparrow grass (Asparagus officinalis L.) micropropagation and to increase plant biomass, quantitatively and qualitatively. The objective was achieved by using deuterium depleted water (DDW), containing only 25 ppm deuterium, and honey, in double-layer system practice vitro-cultures. Thus, 90 days after the sparrow grass propaguls were cultivated on Murashige-Skoog medium (1962), solid, with no growth regulators, over which was applied a liquid layer consist in DDW (V_4), rhysogenesis and caulogenesis processes were significantly stimulated, as well as applying a mixture of honey 15 g/l and distilled water (V_5) over the solid layer. Both to the single and the double layer system, the honey and DDW combination (V_3, respectively V_6) caused rhysogenesis process inhibition to 100%, but in case of double layer system the caulogenesis was stimulated. Variants of culture medium in single-layer – in which distilled water was replaced with DDW (V_1) or in which sucrose was replaced with honey (30 g/l) (V_2) - are recommended to delay the growth of sparrow grass vitro-plantlets (vitro-conservation).

INTRODUCTION

Deuterium depleted water (DDW) - produced by National Research and Development Institute for Cryogenic Technologies from Râmnicu Vâlcea, Romania had many uses in cancer therapy [22]. In time, research was performed regarding their effect upon vegetal organisms, including the effect on phyto-inoculum [3, 13, 17, 19, 21]. Somlyai and his colleagues [23] found that a decreasing of the deuterium, from the content of the water used by animals, influenced the cell metabolism till to the halt of the development and even disappearance of the tumour cells. In this author's opinion, the cells are able to regulate deuterium (D)/hydrogen (H) ration and those changes in this rapport can trigger certain molecular mechanism having a key role in cell circle regulation. It is suppose that the concomitant increase of D/H ratio is the real trigger for the cells to enter in the S phase of division [24]. The decreases of D level can interfere in the signal transduction pathways thus leading to tumour regression. In the case of transplantable tumours, low-deuterium water treatment lead to a significant

inhibitory effect on the volume of all tumour patterns concerned: it delayed nodule formation at transplantation site.

On the other hand, on plants, DDW was used as tracers to characterize whole-tree water transport and storage properties in individual trees belonging to the coniferous species and on five tropical tree species and a bamboo species [8]. DDW tracing method appears suitable for answering some questions, such as relative differences in water use among trees, water redistribution among neighbours and internal water transport and storage processes in plants [25]. Also, the effects of combined soil physical stresses of compaction and drought on the production of fully hydrated mucilage (mucilage) and root border cells (RBCs) in maize was studied using deuterated water method [26].

DDW and double system layer for *in vitro* cultures are the solution that Petruş-Vancea and collaborators [16] found it to *Coleus* and *Petunia* vitroplantlets, suffering by hyperhydricity. After 30 days of vitrocultures in double layer the mentioned authors observed the following results: at the *Coleus* vitroplantlets a new formation of healthy apexes, which were subcultivated on fresh medium and finally the new regenerated vitroplantlets were ready for acclimatization, especially to those lots which were treated with a 1.5% glucose solution, prepared with DDW and to the *Petunia* vitroplantlets, the acclimatization surviving rate was 90% - 95% to the lots treated with DDW and was zero to the vitroplantlets submersed in distilled water (DW - control lot).

By replacing bidistilled water in the Murashige - Skoog (MS) [9] culture media, with DDW (with 25 ppm D), even in the presence of 6-Benzylaminopurine (BA) in 2.5 mg/l 6 concentrations was achieved callusogenesis, an undesirable phenomenon in some vitrocultures [15]. The presence of the DDW in this culture medium has exercised a 100% inhibitory effect of the callusogenesis, determining regeneration of vigorous and healthy plantlets.

As belonging, the genus *Asparagus* (*Monocotyledonatae, Liliaceae* family) plantlets are susceptible *in vitro* to hyperhydricity, a phenomenon that significantly decreases the quantity and quality of final plant mass obtained by micropropagation, in the present experiments, we aimed to study a new procedure for optimization this, by the double-layer culture system. Also, distillate water was tested the replacement from culture medium composition of DW with DDW and/or replacement of sucrose with honey (acacia honey). Procedures for replacing DW with DDW in *in vitro* culture have already been patented [5], and research regarding carbon source replacement from culture medium are numerous, but the use of honey is less studied [10, 17]. The optimisation of *Asparagus officinalis* L. micropropagation is a important objective of the research in this field, because, in bioindustry, there is an high economic and commercial interest, especially for megastores [6], and in the future, in bio-economic and eco-economic context, the human need for food will be considerable higher [4, 7]. The i*n vitro* conservation is

an important method of germplasm conservation, as traditional conservation of crop both plants of agricultural interest [1, 2, 20], as well as the medicinal [19].

MATERIAL AND METHODS

The plant material consisted of uniform asparagus propaguls (*Asparagus officinalis* L.), which are in laboratory phyto-vitrobase, grown on basic medium (BM) MS, solid, without growth regulators.

The culture medium used in these experiments was MS, modified by us, namely: thiamine vitamins HCl, pyridoxine HCl and nicotinic acid, instead of 0.1 mg/l or 0.5 mg/l, as provided in the original recipe, were added to each 1 mg/l and amino-acids were removed; 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 30 g/l (control);

V_1 - BM-MS prepared with DDW and sucrose 30 g/l;

V_2 - BM-MS prepared with DW and honey 30 g/l;

V_3 - BM-MS prepared with DDW and honey 30 g/l.

Double-layer system:

V_4 - BM-MS prepared with DW and 30 g/l sucrose + supernatant DDW;

V_5 - BM-MS prepared with DW and sucrose 15 g/l + supernatant honey 15 g/l, mixed with DW;

V_6 - BM-MS prepared with DW and sucrose 15 g/l + supernatant honey 15 g/l, 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.

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, 16 h day length photoperiod of 24 h day light and 1700 l x 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 vs.16.0. Software.

RESULTS AND DISCUSSION

90 days after inoculation, the rhysogenesis of sparrow grass vitroplantlets was absent to those vitroplantlets cultured on medium with honey and DDW mixture, either to simple layer (V_3) or at the supernatant (V_6) (table 1). The same inhibitory effect, exercised by DDW, was reported [13, 14] in case of watering, with this type of water, at the base of chrysanthemum or African violets plantlets, throughout the all period of acclimatization to the septic medium. Also the rhysogenesis, at the *Tradescantia* minicuttings level, rooted in perlite as natural living conditions, was inhibited by watering them with DDW, compared with DW [11].

In *Cymbidium hybridum* and *Petunia* vitroplantlets, grown on MS medium, in single layer system, solid, with no growth regulators, the DDW took effect on timing growth of phyto-inoculum, with an important role in preserving *in vitro* cultures [12].

Growth index values, registered on type V_1, were noted with statistically insignificant, because this lot has huge losses by the lack of regeneration process at the phyto-inoculum level, the deuterium depleted water, as an replacement of DW, on solid layer, exerted a powerful inhibition effect of organogenesis. To previous experiments were noticed light inhibition of *in vitro Cymbidium* organogenesis [27], or to the chrysanthemums and African violets plantlets [17], grown on culture media in which the sucrose was replaced with acacia honey, 20 g/l, but, when the plantlets (species regardless) were transferred to septic medium, the survival of the lot grown *in vitro* on medium with honey, was superior to that from the medium whose carbon source was sucrose.

Instead, caulogenesis, expressed by the total number of propaguls, was very high on double layer cultures medium (V_4 - V_6), especially to the vitroplantlets grown on culture medium prepared with DW and 30g/l sucrose, as the first layer, over which the DDW supernatant was applied (V_4) (Table 1). Additionally, the lots cultured in the double layer system (V_4-V_6) were also reported propaguls with larger sizes, up 3.4 cm.

Directly proportional with the organogenesis, highlighted to the phyto-inoculum's level, were the fresh weights vitroplantlets values, at the experimental tested variants. Instead, the dry weight of the vitroplantlets cultured on medium prepared with DW and sucrose 15 g/l with honey supernatant 15 g/l, mixed with DW (V_5) (whose fresh weight was the highest and statistically significant) marked values similar to those registered on the other two lots cultivated on medium with supernatant (V_4 and V_6) (Table 1).

Table 1

Comparative analysis of average values of sparrow grass (*Asparagus officinalis* L.) vitroplantlets growth indices, at 90 days after inoculation, as follows: V_0 - BM-MS solid prepared with DW and sucrose 30 g/l (control); V_1 - BM-MS prepared with DDW and sucrose 30 g/l; V_2 - BM-MS prepared with DW and honey 30 g/l; V_3 - BM-MS prepared with DDW and honey 30 g/l; V_4 - BM-MS, prepared with DW and 30 g/l + supernatant DDW; V_5 - BM-MS, prepared with DW and sucrose 15 g/l + supernatant honey 15 g/l, mixed with DW; V_6 - BM-MS, prepared with DW and sucrose 15 g/l + supernatant honey 15 g/l, mixed with DDW

Biometrisation	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
No. rootlets	1.86± 0.38	***	1.50± 0.71	ns	1.90± 0.31	***	0.00± 0.00	-	2.00± 0.63	***	2.00± 0.50	***	0.00± 0.00	-
Length rootlets (cm)	3.86± 0.69	***	1.50± 0.71	ns	1.60± 0.52	***	0.00± 0.00	-	4.00± 0.63	***	1.30± 0.48	***	0.00± 0.00	-
No. propaguls	11,3± 0.95	***	5.00± 0,7	ns	3.30± 0.82	***	3.90± 0.99	***	15.3± 1.27	***	10.0± 1.70	***	10.0± 0.94	***
No. propaguls with 0.0-0.9 cm length	1.67± 0.58	***	4.00± 0.01	ns	1.17± 0.41	***	1.00± 0.01	***-	1.00± 0.01	***	3.00± 0.94	***	1.11± 0.33	***
No. propaguls with 1.0-1.9 cm length	3.86± 0.38	***	1.00± 0.03	ns	1.00± 0.01	***	1.11± 0.33	***	2.09± 0.70	***	2.30± 0.48	***	1.80± 0.42	***
No. propaguls with 2.0-2.9 cm length	2.57± 0.98	***	0.00± 0.00	ns	1.00± 0.01	***	1.21± 0.43	***	6.18± 0.60	***	1.80± 0.92	***	4.20± 0.42	***
No. propaguls with 3.0-3.4 cm	3.00± 0.58	***	0.00± 0.00	ns	1.13± 0.35	***	1.56± 0.73	***	6.00± 0.63	***	2,90± 0.74	***	3.00± 0.47	***
Weight fresh (g)	1.94± 0.06	***	1.25± 0.01	ns	0.52± 0.01	***	1.63± 0.06	***	2,95± 0.01	***	5.27± 0.01	***	3.19± 0.01	***
Weight dry (g)	0.17± 0.01	***	0.22± 0.02	ns	0.04± 0.01	**	0.28± 0.01	***	0.35± 0.03	***	0.33± 0.01	***	0.36± 001	***

Note: BM-MS – basal medium Murashige-Skoog (1962); DW – distilled water; DDW-deuterium depleted water; sig. – significance [ns – no significant; ** *significant,* ***very significant*]; st.dev. – standard deviation; no – number; L – length; W – weight.

CONCLUSIONS

1. Double layer system improves quality and quantity of the aerial part of *Asparagus officinalis* L. vitroplantlets.
2. The replacement of distillate water from the sparrow grass culture medium with deuterium depleted water (with 25 ppm D) led to lower rhysogenesis, but with honey mixture this system improved organogenesis.
3. Replacing sucrose with honey caused an inhibition both to the rhysogenesis and to the caulogenesis, in case of single layer system, but in double layer system, this compound of culture medium improve quantity of biomass.

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REFERENCES

1. Antofie M., Mihaela Dana Constantinovici, M.R. Pop, Pompilica Iagrăru, Camelia Sand, G. Cirotea, 2010. *Theoretical methodology for assesing the status of conservation of crop landraces in Romania.* Analele Universității din Oradea - Fascicula Biologie, Tom. XVII (2) (pp. 313-317).
2. Antofie M. Mihaela, M.R. Pop, Camelia Sand, G. Ciotea, Pompilica Iagrăru, 2010. *Data sheet model for developing a red list regarding crop landraces in Romania.* Annals. Food Science and Technology, Vol. 11(1) (pp. 45-49).
3. Beleş Dana, Dorina Cachiță, 2007. *Pistia stratiotes L. exvitoplantlets acclimatization on medium prepared with deuterium depleted water.* The XVth Plant Cell and Tissue Culture National Symposium, Dorina C. Cachiță, (ed.). Cluj-Napoca, Risoprint Publish House (pp. 173-181).
4. Bogdan A.T., Iudith Ipate, V. Bara, D. Diaconescu, Cornelia Purcarea, Amalia Strateanu, 2010. *Ecoeconomic and bioeconomic impact of food safety and security in perspective increased consumption of food and feed during 2030-2100.* International Symposium "Risk Factors for Environment and Food Safety", Faculty of Environmental Protection, November 5-6, Oradea 2010 (pp. 1044-1056).
5. Cachita Dorina, Adriana Petrus-Vancea, Dorina Radovet, 2010. *Procedures for preventing or controlling hyperhidration in phyto-inoculi by using deuterium depleted water.* Patent RO 125649-A2, Published in 30.08.2010, International Patent Classification A01N-02500; C01B005/00.

6. Hurgoiu Floare, Dorina Cachiță, 2006. *Inițierea de vitroculturi la Asparagus, Plant vitroculture conservation.* The XIVth Plant Cell and Tissue Culture National Symposium, Cachiță, C.D. and Sand, C. (eds.), Alma Mater Publish House, Sibiu (pp. 217-222).
7. Ipate Iudith, A.T. Bogdan, M. Paraschivescu, M. Sandu, Simona Ivana, N. Ipate, Amalia Strateanu, G. Toba, M. Enache, 2010. *Use rare breed for genuine foods in Romanian rural tourism and possibility of traceability the traditional products.* Bulletin UASVM Animal Science and Biotechnologies, 67(1-2) (pp. 225-230).
8. Meinzer F.C., J.R. Brooks, J.C. Domec, B.L. Gartner, J.M. Warren, D.R. Woodruff, K. Bible, D.C. Shaw, 2005. *Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques.* Plant, Cell & Environment, Vol. 29 (1) (pp. 101-114).
9. Murashige T., F. Skoog, 1962. *A revised medium for rapid growth bioassays with tobacco tissue cultures.* Physiologia Plantarum, No. 15 (pp. 473-497).
10. Pătru Diana, Dorina Cachiță, 2001. *Research regarding Dianthus caryophyllus var. Tundra minicuttings in vitro grown on culture medium in which the sucrose was replaced total or partial with honey.* Analele Universității din Oradea - Fascicula Biologie, Tom. VIII (pp. 453-451).
11. Petruş C.M., Dorina Cachiță, 2008. *Foliar and radicular sprinkling of Tradescantia cuttings, with different types of water, and their effect to organogenesis and the epidermal formations of foliar limbs*. Analele Universității din Oradea - Fascicula Biologie, Tom. XV (pp. 73-78).
12. Petruş C.M., Adriana Petruş-Vancea, Dorina Cachiță, 2004. *Cymbidium and Petunia micropropagation on culture medium prepared with deuterium depleted water*. Plant cell physiopathology in vitroculture system - The XIIIth Plant Cell and Tissue Culture National Symposium, Cachiță, C.D. and Ardelean, A. (eds.), Daya Publish House, Satu - Mare (pp. 185-192).
13. Petruş-Vancea Adriana, Dorina Cachiță, 2008. *Regards on Chrysanthemum (Chrysanthemum morifolium Ramat var. Lamet), leafs epidermis which were sprinkle, during "ex vitro" acclimatization period, with Pi water and deuterium depleted water.* Studia Univ. Vasile Goldiş, Seria Șt. Vieții, Vol. 18 (pp. 81-86).
14. Petruş-Vancea, Adriana, Dorina Cachiță, 2009. *The effect of Pi or deuterium depleted water on foliar stomata of Saintpaulia ionantha L. exvitroplantlets sprinkled foliar or radicular.* Studia Univ. Vasile Goldiş, Seria Șt. Vieții, Vol. 19(2) (pp. 309-312).
15. Petruş-Vancea Adriana, Dorina Cachiță, 2011. *New methods for preventing the unwanted callusogenesis at sugar beet (Beta vulgaris var. Saccharifera).* Romanian Biotechnological Letter (accepted, in press).
16. Petruş-Vancea Adriana, Dorina Radoveț, Dorina Cachiță, 2008. *Hiperhydricity annihilation out of vitrocultures with deuterium depleted water and Pi water, using a double layer system.* Lucrări Ştiintifice. Serie F, Special Volume, Biotechnology (pp. 20-30).

17. Petruş-Vancea Adriana, G.E. Bandici, Dorina Radovet, C.F. Blidar, 2008. *The acclimatization capacity of the Saintpaulia and of the Chrysanthemum exvitroplantlets, derived from vitroculture medium in which the saccharose was replaced with honey.* Analele Universităţii din Craiova, Vol. XII (XLVIII), Secţiunea Biologie (pp. 209-214).
18. Petruş-Vancea Adriana, Dorina C. Cachiţă, C.F. Blidar, I. Ştefănescu, 2003. *The effect of dedeuterised water in acclimatization of Chrysanthemum vitroplantlets to septic medium.* Papers of the 5th International Symposium Young People and Multidisciplinary Research, Sudura Press, Timişoara (pp. 335-340).
19. Pop M.R., 2010. *Characters with multiple usages – phenotypic variability analysis at Echinacea purpurea (L.) Moench species.* Analele Universităţii din Oradea - Fascicula Biologie, Tom. XVII (2) (pp. 329-331).
20. Pop M.R., Camelia Sand, Dana Bobiş, Mihaela M. Antofie, H. Barbu, Petronela B. Pavel, L. Muntean, M. Savatti, 2010. *Studies concerning the production of volatile oil, rhizomes and roots, to different genotypes of Valeriana officinalis L.* Analele Universităţii din Oradea - Fascicula Biologie, Tom. XVII (2) (pp. 332-335).
21. Radoveţ Dorina, 2004. *Study of acclimatization capacity of Coleus blumei Benth. Vitroplantlets, which was obtined from vitroculture from hyperhydrice plantlets treated with deuterium depleted water.* Papers of the 5th International Symposium Young People and Multidisciplinary Research, Timişoara, Sudura Publish House (pp. 507-515).
22. Somlyai G., 2001. *Să învingem cancerul! Efectele biologice ale reducerii de deuterium.* Conphys Publish House, Râmnicu Vâlcea.
23. Somlyai G., G. Laskay, T. Berkenyi, Z. Galbacs, G. Galbacs, A.S. Kiss, G. Jakli, G. Jancso, 1998. *The biological effect of deuterium depleted water, a possible new tool in cancer therapy.* Z. Onkol./Z of Oncol., Vol. 304) (pp. 91-94).
24. Turusov V.S., I.E. Siniak, A. Grigorev, D.G. Zaridze, E.E. Antoshina, L.S., Trukhanova, T.G. Gorkova, 2005. *Low-deuterium water effect on transplantable tumors*. Vopr Onkol., Vol. 51(1) (pp. 99-102).
25. Schwendenmann L., D. Dierick, M. Köhler, D. Hölscher, 2010. *Can deuterium tracing be used for reliably estimating water use of tropical trees and bamboo?* Tree Physiology, Vol. 30(7) (pp. 886-900).
26. Somasundaram S., T.P. Rao, J. Tatsumi, M. Iijima, 2009. *Rhizodeposition of mucilage, root border cells, carbon and water under combined soil physical stresses in Zea mays L.* Plant Production Science, Vol. 12 (pp. 4443-4448).
27. Vancea Adriana, Dorina Cachiţă, Diana Pătru, 2002. *Adapted capacity of Cymbidium hybridum plantlets at ex vitro conditions, which was provided from culture medium with honey as sucrose replaced.* Analele Universităţii din Oradea - Fascicula Biologie, Tom. IX (pp. 213-218).