EX VITRO ACCLIMATIZATION AND CONSERVATION OF THE PLANT MATERIAL OBTAINED IN VITRO AT SOME FOREIGN AND AUTOCHTHONOUS VARIETIES OF SOLANUM TUBERSOSUM L

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Abstract

In vitro acclimatization and conservation of the plant material obtained in vitro is an important stage for the units of micro-propagation and production of the potato tubers this way. The capacity of acclimatization of the foreign variety of Desirée potato and of the autochthonous variety Super potato was followed, after a period of in vitro culture of about two months on mediums with or without phytohormones, stimulating or not the differentiation of mini-tubers. Acclimatization refered, on one hand, to the mini-tubers obtained in vitro, and on the other to the neoplantlets which did not differentiate tubers. The material obtained in vitro acclimatized ex vitro after about 3 months, the capacity of acclimatization depending on the completion of the stages of acclimatization, on the variety or on the potato population, on the preliminary preparation stage still in the in vitro conditions, through an additional light treatment, on the culture substratum and on the conditions of its sterilization against pathogens agents with antifungal treatment, also applied for ensuring a good nutrition, the size of the tubers (the diameter of the tuber) etc. Desirée variety acclimatized in a percentage of 80%, and the autochthonous one, Super, in a percentage of 95%, depending on the type of acclimatized material (tubers or rooted neoplantlets). After completing these stages the obtained material (tubers) corresponds to merchandising and to the internal and external exchange, being accompanied by a phytosanitary certification issued by the law of the country which produced the plant material.

Keywords: ex vitro, acclimatization, culture substratum, light, humidity and temperature regime, soil disinfection, antifungal treatment, phytosanitary certificate

INTRODUCTION

A large number of researches focused on stimulating the *in vitro* formation of tubers, a practical aspect particularly important for obtaining the plant material (tubers) in an infinite greater quantity and with a superior biological value (Zăpârţan, 1992). Some researches followed the stages of acclimatization of the material obtained in vitro to different species of plants from the spontaneous flora (Zăpârţan, 2000), and sometimes the induction of the *in vitro* formation of bulbils had as an experimental model some bulbous species to which the *in vitro* formation of the bulbils was stimulated (Zăpârţan et. al., 2006). It was highlighted that in vitro tuberization (both of potato and also to other bulbous species) took place on a hormonal balance well balanced in auxins, higher in cytokinins and at a higher dose of sucrose within the medium of culture (Butiuc-Keul et. al. 1997-1998).

To potato, the phenomenon of tuberization was induced depending on the time of the year when the culture was initiated (Agud et. al. 2013), the most favourable period proving to be March – April, and also on the nature of the explant, the meristem having the best reaction to

the *in vitro* culture (Agud et. al., 2013), but also depending on the variety, population and their origin (Agud et. al., 2013a). Due to the economic value of the *Solanum tuberosum* L specie and to some varieties or populations, our research were often directed towards the establishment of some technologies of advantageous, economical *in vitro* amelioration and tuberization (Agud, 2011), using small doses of phytohormones, substitutes, or culture mediums without hormones, in some situations resorting to hormone supplementation from the mediums with natural extracts (Agud, 2011a).

In the acclimatization research referring to the rose plants grown *in vitro*, it was observed that if the luminous intensity increases in the last period of *in vitro* life, the degree of hydration in the atmosphere of the vessel reduces and the stomata begin to function (Capellades, 1990), this is why the leaves at their passage *ex vitro* blur with different systems for protection against the direct action of the solar rays, a method currently applied by the majority of researchers who wish to obtain a high percentage of acclimatization (Laslo, 2011). Some researchers obtain an acclimatization of plants after about 6 weeks from the passage at large, but the duration can reach up to 4-5 months, depending on a lot of factors: the specie, the vigour of the neo plant, appropriate culture substratum, etc. (Ibañez and col., 2005, quoted by Laslo, 2013).

In vitro differentiated vegetal material suffers certain morphological and physiological changes in these controlled living conditions, changes which create a certain state of shock at their passage *ex vitro*, a state with repercussions on acclimatization (Laslo, 2013). The concerns regarding these issues of acclimatization were remarkable and very different; hence, in Table 1 we remind some of these morphological and physiological changes, the causes of their occurrence and the states of shock that can occur (Cachiță, 1987).

Some researchers sustain that when transferring plants at large; the morphological changes took place at the level of the pholiar system, for example at the tobacco leaves after a period of *ex vitro* acclimatization, stomata doubled its number due to the growth of the pholiar surface (Pospišilová and col., 1999).

Table 1

Some morphological and physiological changes of the neoplantlets obtained *in vitro* which can provoke a state of shock when transferring at large (Cachiță, 1987)

No. crt.	**Possible morphological and physiological changes at the *in vitro* plants**	**The causes of the occurrence of changes**
1.	The poor development of the cuticle of the cells	Due to the excessive perspiration of the cells
2.	Changes in the process of photosynthesis by reducing its intensity	*In vitro* plants have less chlorophyll, a smaller number of chloroplasts and a single cell layer
3	The foliar (biological) mass of the plant has the tendency of growth	The imbalance between the kind of ions and of their concentration
4	The Radicular System and the size of the *in vitro* tubers are sometimes less developed	In which case the passage of the neoplantlets *ex vitro* can be compromised
5	The occurrence of an infection *in vitro*	*In vitro* infections can compromise culture and lead to the death of the formed neoplantlets
6	The occurrence of vitrification (hyperhidricity of the plants and of the tissues from vitro)	Due to the accumulation of ethylene into the phial, and the forcefulness of the neoplantlets declines

MATERIAL AND METHOD

In this study there was followed the acclimatization percentage at the *ex vitro* transfer of the differentiated tubers and of the *in vitro* rooted plantlets from *Desireé* foreign potato variety and from the autochthonous one, *Super*. The vegetal material was obtained from the potato meristem cultivated *in vitro* on the Murashige – Skoog, 1972 medium, with the following variants of medium considered significant by us in terms of the followed parameters: $\mathbf{C_o}$= MS, basal medium; $\mathbf{C_1}$ = MS + 0,5mg/l AIB+ 1mg/l Z; $\mathbf{C_2}$ = MS + 0,5mg/l AIB+ 2mg/l Z (Table 2).

The material obtained *in vitro* can find itself in two situations: the first in which the rooted potato neoplantlets did not differentiate mini-tubers, in which case at the transfer stimulating substances must be applied and the second case in which minuscule tubers of about 1-2mmø or somewhat higher up to 5mmø, differentiated, which when transferred at large must be carefully watched and appropriate medium conditions must be found (humidity, soil substratum, luminous intensity). At the rooted neoplantlets, but to which mini-tubers did not differentiate, the state of shock which can appear during the first 5-7 days from the transfer at large must be avoided in order for the differentiation of mini-tubers *ex vitro* to take place. Acclimatization of the plantlets obtained *in vitro* to the utmost extent depends on the quality of the neoplantlets and to the quantity and number of differentiated roots.

RESULTS AND DISCUTIONS

It was followed the acclimatization of the vegetal material obtained on the variants of medium specified in Table 2, chosen after a number of experimental series and years. There were selected the variants which gave de best results at obtaining *in vitro* tubers over the time, in order to establish a working protocol, to which to have in mind the stages to be accurately followed concerning the *in vitro* culture of the potato varieties and the acclimatization of the material obtained *ex vitro*.

In the case of the potato we affirm that the percentage of acclimatization depends on the size of the mini-tubers, on the forcefulness of the Radicular System, on the potato variety or population and on the way in which the acclimatization stages are respected from the moment the plant material gets in touch with the in large conditions (Agud, et. al., 2010).

Table 2

Value of the *in vitro* differentiated plant material at *Desireé* and *Super* varieties cultivated on the variants (MS = Murashige-Skoog; AIB = indolil β acetic acid; Z = zeatin)

Variety	Var.	Composition of the *in vitro* culture medium	Average of the no. of roots/meristem	% of tubers / neoplantlets
Desirée	C_o	MS – control	3	0
	C_1	MS+0,5mg/lAIB+0,5mg/l Z	5	3
	C_2	MS+0,5mg/lAIB+2mg/l Z	**12**	**45**
Super	C_o	MS – control	3	0
	C_1	MS+0,5mg/lAIB+0,5mg/l Z	5	1
	C_2	MS+0,5mg/lAIB+2mg/l Z	**10**	**35**

Following Table 2 we see that *Desirée* variety reaches the best values under the aspect of the number of roots/explant and of the *in vitro* tuberization percentage. On the variant with

2mg/l Zeatin (C_2) the percentage of tubers/meristem reaches to 45%, while on the control sample (C_o) tuberization is missing and on a low dose of zeatin (C_1) it is barely 3%. To this variety the average of the number of roots is also the best on the medium with a high dose of cytokinin (C_2). At the *Super* autochthonous variety the followed values are similar to the ones of other foreign varieties, but slightly inferior, with a tuberization percentage of 35% and with an average of 10roots/explant. The evolution presented above is reproduced in Figure 1 in which we notice the superiority of the followed parameters on Murashige-Skoog (MS) medium, variant C_2, with a high dose of zeatin (Z) (MS+0,5mg/lAIB+2mg/l zeatin).

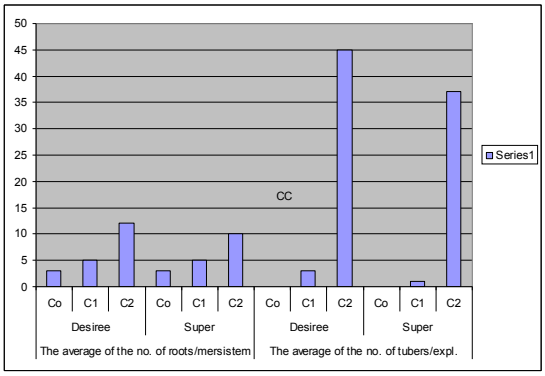

Fig. 1. Average of the number of roots and mini-tubers/meristem differentiated *in vitro*

Some autochthonous populations have a percentage of acclimatization double to the foreign ones (Agud et. al., 2013), we believe that due to the high adaptation capacity to the conditions of provenience of the autochthonous soil. The planting substratum must be first treated against pathogenic agents from the soil and it can be formed of peat, sand, Perlite, etc. and fertilized after the need of the specie (fertilization in straw). *Ex vitro* tuberization will take place in about a month if the plant is stimulated through using powders or auxinic solutions (in a concentration depending on the specie, on the plantlets' physiological status, etc.). The material obtained *in vitro* ensures an internal or external exchange of plants, governed by the law of the country, which imposes certain phytosanitary conditions (Fay, 1994). In this exchange of plant material the possibility of introducing pathogenic agents must be removed, hence according to the safety conditions of health of the plant material (Martin and Postman, 1999). It was followed the capacity to adapt and the losses at acclimatization expressed in percentages, of the mini-tubers and of the *in vitro* rooted neoplantlets at the two potato varieties cultivated in vitro after two months (photo 1, above).

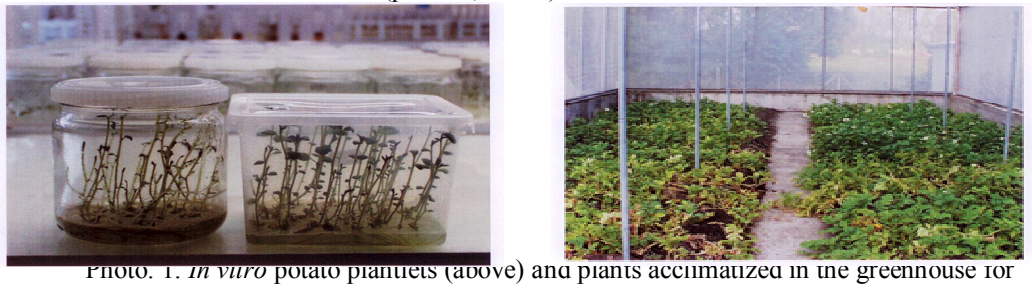

Photo. 1. *In vitro* potato plantlets (above) and plants acclimatized in the greenhouse for the differentiation of mini-tubers (below)

The mini-tubers differentiated in vitro at *Desirée* variety adapted in a percentage of 80%, the losses being of 20%; and at the rooted neoplantlets adaptability is of 43% with losses of over 55%. In the case of these latter plants, the tubers form *in vitro*, after about 3-4 months, depending of the forcefulness of the Radicular System (Photo 2).

Photo 2. Aspects of the plants with potato tubers

At *Super* autochthonous variety, mini-tubers differentiated *in vitro* adapt in a higher percentage than *Desirée* variety (95% adaptation and 3% losses), this better adaptation is, we believe, due to the greater capacity to adapt of the autochthonous varieties, towards the foreign ones. Rooted neoplantlets from the *Super* variety adapt *ex vitro* in a percentage of 42% with about 55% losses and form tubers at large in a shorter time than *Desirée*, after about three months from their planting (Photo 1, below). The percentage of adaptability and of losses at *Super* variety compared to *Desirée* variety is reproduced in figure 2, from which we also deduce the superior capacity to adapt of the mini-tubers differentiated *in vitro* towards the one of the rooted neoplantlets. To reduce further losses we must correlate certain conditions of temperature, light and soil – atmosphere humidity to the optimal values required by the specie.

We consider that it is necessary to remind some conditions that have to be completed for a successful acclimatization of the potato varieties. At the time of removal of the neoplantlets from the aseptic conditions they must be protected against dehydration by applying a transparent hand-glass over the plantlets (about 7-8 days), or of another system which also protects against air currents, temperature and light. In order to prevent infection (either from the soil or from the atmosphere) it was intervened through initial disinfection of the culture substratum of the plantlets. Soil can be disinfected with weak phytotoxic substances and thiram in a law dose of 5-10mg/l, TMTD (according to Boxus and col., 1995 – quoted by Laslo, 2013), in order not to damage the plants came from controlled medium conditions, which have certain degrees of sensitivity.

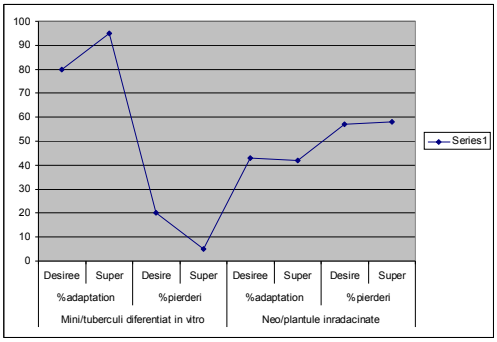

Fig. 2. Percentage of adaptability and of losses at the *ex vitro* transfer of the mini-tubers and of the rooted neoplantlets

The adaptation of the plant material differentiated *in vitro* is influenced by humidity which must be high in the first days from the transfer at large, by light whose intensity must be lower and by the condition of sterility which is imperative both at the level of the soil and of the plant. The tubers obtained *in vitro* can be directly transferred into the greenhouse or at large (Photo 3), with the mention of seeing what is the climate of the region in which the potato culture lends itself, with the maintenance of the environmental factors (temperature, light, humidity) at the optimum parameters and with the mandatory application of the successive irrigation (Photo 4).

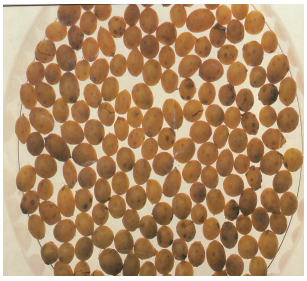

Photo 3. Mini-tubers obtained *in vitro* from potato meristem on Murashige – Skoog medium with zeatin

Photo 4. Plants formed in the greenhouse from tubers obtained *in vitro*

Therefore neoplantlets, at the transfer at large or in greenhouse must be first prepared, by administering a higher luminous intensity (of 3-10 times higher), even during *in vitro* life (Murashige, 1974), a very important treatment especially for the foliar system, because the leaves are the ones which suffer the first shock during the transfer and at the contact with the free air. During acclimatization it is recommended the application of antifungal treatments, treatments which, at the same time, ensure a good nutrition and reduce the stress conditions which are sometimes inevitable and even negative (Gianinzzi and col., 1989; Rapparini, and col., 1994).

CONCLUSIONS

1. Tubers obtained *in vitro* can acclimatize by direct transfer at large, solariums or greenhouses, taking into account the climate conditions necessary to the specie and the periodic administration of irrigation;

2. Acclimatization percentage depends on the successive following of the acclimatization stages and on the size of the tubers, but it also depends on the potato variety or population, some autochthonous populations acclimatize in a better percentage than the foreign ones;

3. New obtained plantlets must be first prepared, by administering a higher luminous intensity (of 3-10 times higher), even during *in vitro* life, treatment which ensures the reducement of the shock at contact with the conditions at large;

4. The essential condition in acclimatization is the maintenance of a clean culture substratum (soil) by the sterilization against pathogenic agents and the administration of the auxinic treatment for the stimulation of tuber differentiation in greenhouse, solarium or at large;

5. During acclimatization there can be administered antifungal treatments for a better nutrition and for reducing stress during *in vitro* transfer;

6. At the mini-tubers of *Desirée* variety the percentage of adaptability is of 80%, and the losses of 20%: at the plants to which there were not obtained *in vitro* tubers, the percentage of adaptability and differentiation of tubers in greenhouse or at large is of 43%, and the losses are of more than half;

7. *Super autochthonous* variety reaches a percentage of adaptability of the tubers of 95%, with only 5% losses: at neoplantlets is of 42%, with losses of more than half (58%), proving a superior percentage during the transfer at large;

8. The material obtained *in vitro* ensures an external and an internal exchange of plant material (tubers) certified by law from a sanitary point of view. Within the exchange of biological materiel between the countries, by this method there are ensured the health conditions of the material and any possibilities to introduce and transmit some pathogenic agents are eliminated.

9. The success of the *in vitro* transfer is conditioned by the value of the Radicular System and by the size (ø) of the tubers obtained *in vitro*, and the presence of zeatin in the culture medium of the explants (about 2mg/l) stimulates the formation of a larger number of *in vitro* tubers, with a diameter of over 5mm.

REFERENCES

1. Agud. E. M., Zăpârțan, M. Cap, Z., 2010, The in vitro tuberisation at the potato Desiree variety in mediums with phloroglucinol, Research Journal of Agricultural Science, Vol. 42(2)1-341, Agroprint Editorial, Timişoara, 191-196.
2. Agud, E., 2011, Economical methods of in vitro tuberization at Solanum Tuberosum L Variety, Analele Universității din Oradea, Fascicula: Protecția Mediului,vol.XVI B, Ed. Universității din Oradea, 1-6.
3. Agud, E., 2011a, The role of natural extracts in the in vitro culture of Solanum tuberosum L. Variety, Analele Univ. din Oradea, Fascicula: Protecția Mediului, vol. XVI A, Ed. Univ. din Oradea, 1-8.
4. Agud. E., Laslo V., Zăpârțan M., 2013, Factors with differentiated implication in the in vitro minituberization at some potato varieties (Solanum tuberosum L.), Book of Abst. UAB-B.E.N.A. Intern. Conf. Environmental Engineering and Sustainable Development, Alba-Iulia, pp. 169-170

5. Agud E., Zăpârțan, M., Savatti, M., and V. Laslo, V., 2013a, The influence of the moment of sampling of the potato meristem over the in vitro regeneration and diferentiation capacity, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, Vol. 70, Nr. 1, 1843-5262; Electronic, pp. 104-109.
6. Baciu, A., 2007, Studiul privind comportamentul in vitro a unor genotipuri de Solanum tuberosum L., sub influența nanocompozitelor magnetofluididice bioactive, Biotehnologii vegetale pentru secolul XXI, Cel de al XVI – lea Simp. Nati. de Culturi de Țesut. şi Cel. Veget., Buc., Ed. Risoprint, 2008
7. Boxus, P., Jemmali, A., Piéron, S., 1995, Micromultiplication végétative in: Biotehnology végétales (Ed. Demarly; Y., Picard, E., Boxus, P., CNED; Inst., de Rennes, France; 5-116
8. Cachiță-Cosma, D., 1987, Metode *in vitro* la plantele de cultură, Ed. CERES, Bucureşti
9. Cachiță-Cosma, D., Ardeleanu, A., Crăciun, C., 1998, Actualitate şi perspective în biotehnologiile vegetale, Ed. Vasile Goldiş, Arad
10. Cachiță-Cosma, D., Deliu, C., Rakosy-Tican, L., Ardeleanu, A., 2004, Tratat de biotehnologii vegetale, Vol. I., Ed. Dacia, Cluj – Napoca,
11. Cappelades, M., Fontarnau, R., Carulla, C., Debergh, P., 1990, Environment influences anatomy of stomata and epidermal cells in tissue-cultured Rosa multifl, J. Amer, Soc. Hort., Sci., 115, 141-145
12. Fay, M.E., 1994, In what situations is in vitro culture appropriate to plant conservation? Biodiversity and Conservations, 3, 176-183
13. Gianinazzi, S., Gianianzzi-Pearson, V., Trouvelot, A., 1989, Potentialities and procedures for the use of endomycorrhizas with special emphasis and high values crops. In: Biotehnologii of Fungi for Improving Plant Growth (ed. Whipps and Lumdesn), Cambridge Univ. Press. Cambridge, (England), 41-45
14. Ibañez, A., Valero, M., Morte, A., 2005, Establishment and in vitro clonal propagation of the Spanish autochthonous tabel grapevine cultivar Napoleao: an improved syste, where proliferating cultures alternate with rooting ones. Anales de Biologia, 27, 211-220
15. Laslo, V., Zăpârțan, M. Agud. E., "In vitro conservation of certain endangered and rare species of Romanian spontaneons flora", în: Analele Univ. din Oradea, Fascicula: Protecția Mediului,vol. XVI A, Ed. Univ. din Oradea, 2011, 247-252.
16. Laslo, V., 2013, BIOTEHNOLOGII VEGETALE şi aplicațiile lor, Ed. Univ. din Oradea
17. Martin, R. R., Postman, J. D., 1999, Phytosanitary aspects of plant germoplasm conservation. In: Benson E.E., (ed.) Plant Conservation Biotehnology, Zaylor and Francis Lrd. London, 63-82
18. Murashige T., Skoog, A**.,** 1972, Revised medium for rapid growth and bioassays with tabbacco tissue cultures, Physiol. Plant, 15, pp. 85-90
19. Pospišilová J., Tichá, I., Kadleček, P., Haisel, D., Plzáková, S., 1999, Acclimatization of micropropagated plants to ex vitro conditions, Biologia Plantarum, 42, 481-497
20. Rapparini, F., Baraldi, R., Bertazza, G., Brazanti, B., Predieri, S., 1994, Vesicular arbuscular mycorrhizal inoculation of micropropagation fruit trees, J. Hort. Sci. 69, p. 1101-1109
21. Zăpârțan, M 1992, „In vitro tuberization some potato cultivars" in: Studia Univ. Babeş – Bolyai, Biologia, XXXVII, (2), 85-90
22. Zăpârțan, M, 2000, Conservarea florei spontane prin înmulțire in vitro, Ed. ALC MEDIA GROUP, Cluj – Napoca, 135-141.