STUDY ABOUT THE ULTRASOUND EFFECTS ON POTATO PLANTLETS (*SOLANUM TUBEROSUM* L.), CONSERVED *IN VITRO* THROUGH *SLOW GROWTH* METHOD

Manuela IBANESCU[1], Dana CONSTANTINOVICI[1]*, Silvia STRAJERU[1]
[1]Banca de Resurse Genetice Vegetale Suceava

*** Correspondence:** Dana CONSTANTINOVICI, Banca de Resurse Genetice Vegetale Suceava, B-dul 1 Mai, nr 17, 720224, Suceava, Romania, tel +0230 52 41 89; e-mail: dana_constantinovici@yahoo.com
Received: march 2008; Published: may 2008

ABSTRACT. In this paper an attempt to investigate the effects of ultrasounds on potato conserved by *in vitro* technique is presented. All experiments were carried out at 35 KHz frequency of ultrasounds for 14 minutes exposure time and two different *slow growth* media. A combination of these parameters with a lower temperature in the conservation room allowed an extension of period between two subcultures from 18 to 40 months, on the same medium, without loosing the viability and vitality of the plantlets.

Keywords: anatomy, stem, leaf, woody climber, *Lonicera pallida*

INTRODUCTION

Potato (*Solanum tuberosum* L) is the fourth most important crop in the world, and the need for preservation of genetic resources belonging to these genotypes is in continuous increase. Traditionally, potato varieties have been and are still maintained in the field genebank's. The major disadvantage of a field collection is the risk to lose part of the collection through diseases, pests, weather damage or other accidents. Tissue culture allows the rapid clonal propagation of large numbers of plantlets in a short period and the conservation of potato germplasm under controlled conditions requiring reduced space and labor (Espinoza N et al., 1992).

Usually, the entire collection has to be subcultured on a fresh micropropagation medium in 2 – 2.5 months. The aim, for *in vitro* conservation, is to reduce growth, thus increasing intervals between two subcultures. In most cases, environmental conditions and/or culture medium have to be modified to induce a *slow growth*, and different methods were used to increase the vitality for a longer period (Cachita CD, 1987). The viability and vitality of shoot's apex and the capacity to produce new plantlets in subculture are the main criterions for the evaluation, after 16 – 18 months of conservation.

Lately, the influence of physical factors as microwave and laser radiation, magnetic field and ultrasounds treatment on plant development was reported. The goal of this experiment is to find out if the use of ultrasounds could enhance the period between two subcultures for potatoes conservation. It isn't exactly clear *how* ultrasounds influence plants growth. Seeds are sometimes treated with ultrasound to help start the germination process (Shors et al, 1999; Weinberger et al, 1981). It was established that the treatment with ultrasound irradiation could change the state of the substances and even accelerate the reactions. This fact motivated its application for stimulating the growth of different cultures (Aladjadjiyan A, 2007).

MATERIALS AND METHODS

Material

Shoot cultures of eight old local potato genotypes were established on a Murashige-Skoog (MS-1962) medium (Murashige et al., 1962). After 6-8 weeks, 40 nodal segments, for each variant, were excised aseptically from these cultures and subcultured on two different conservation media (Table 1).

Table 1

Composition of *in vitro* conservation culture media for potato (*Solanum tuberosum* L.)

Compounds/ 1 l medium	Amount (mg)	Compounds/ 1 l medium	Amount (mg)	
Medium	**A & B**	**Medium**	**A**	**B**
NH_4NO_3	825	Thyamine HCl	0.2	
$K NO_3$	950	Pyridoxine HCl	0.2	
$CaCl_2$ $6H_2O$	330	Nicotinic acid	0.2	
$MgSo_4$ $7 H_2O$	185	M-Inositol	100	
$K H_2PO_4$	85	Glycine	0.2	
KI	0.42	NAA	0,01	
H_3BO_3	3.1	Kinetin	0,01	
$MnSo_4$ H_2O	11.1	BA	0,01	
$ZnSo_4$ $7 H_2O$	4.3	Daminozide (B 9)	-	30
Na_2MoO_4 $2H_2O$	0.13			
$CuSo_4$ $5 H_2O$	0013	Sucrose **(g)**	30	
$CoCl_2$ $6H_2O$	0013	Mannitol **(g)**	30	-
$FeSo_4$ $7 H_2O$	27.80			
Na_2EDTA $2H_2O$	37.30	Agar-Agar **(g)**	7	

The basal conservation medium was, also, that of Murashige-Skoog (MS-1962) with different combinations of organic compounds. Media **A** & **B** were similar except the type of retardant agent. In the medium **A** was incorporated 30-g/l mannitol, in order to reduce the water availability to the culture (Westcott, 1981; Cachita CD, 1987). In the variant **B** was included daminozide (B_9, Alar) (Sigma Catalog: S2022), 30-mg/l. It is usually used as a foliar spray on ornamental plants, to produce more compact plants, increasing tuberization of potato, too (Westcott, 1981; Cachita CD, 1987). A higher concentration of daminozide could lead to *slow growth* of potatoes.

The media were adjusted to pH 5.6 – 5.8, before the addition of Agar-Agar, dispensed in culture vessels (glass jars) and sterilized by autoclaving at 121^0C, for 20 minutes (Toledo J, 1998).

For each medium and potato variant, 40 explants were distributed in 8 culture vessels, covered with polyethylene folia, fixed by rubber rings and transferred to the growing room. For three to four weeks all the culture vessels were maintained at 19-20ºC, with a photoperiod of 16 hours, in white fluorescent light, at 2000 lux. After this period, the jars were transferred to *in vitro* conservation room, at 8-10°C, (8ºC, during the dark period and 10ºC, during the photoperiod of 12 hours), in white fluorescent light, at 1000 lux.

The plantlets evolved in these conditions, on the two media, for a period of about 18 months.

Equipment

Ultrasonic (**US**) treatment was given by means of an ultrasonic generator, Bandelin Sonorex RK 31 type (35 kHz frequency, 120W maximum nominal power output and 0.2A intensity), designed by Bandelin Electronic (Berlin, Germany).

Experiments Design

In this study were selected 5 important effective parameters namely: the culture media, the conservation temperatures, the ultrasound intensity, the time of ultrasonic exposure and the frequency. The first factor was variable and the last four were fixed. After 18 months spent in conservation room at 8-10ºC half of glass jars with potato plantlets was exposed to ultrasounds. The other half of biological material was considered as control sample. All the samples were transferred in the cold room for another 22 months of *slow growth*.

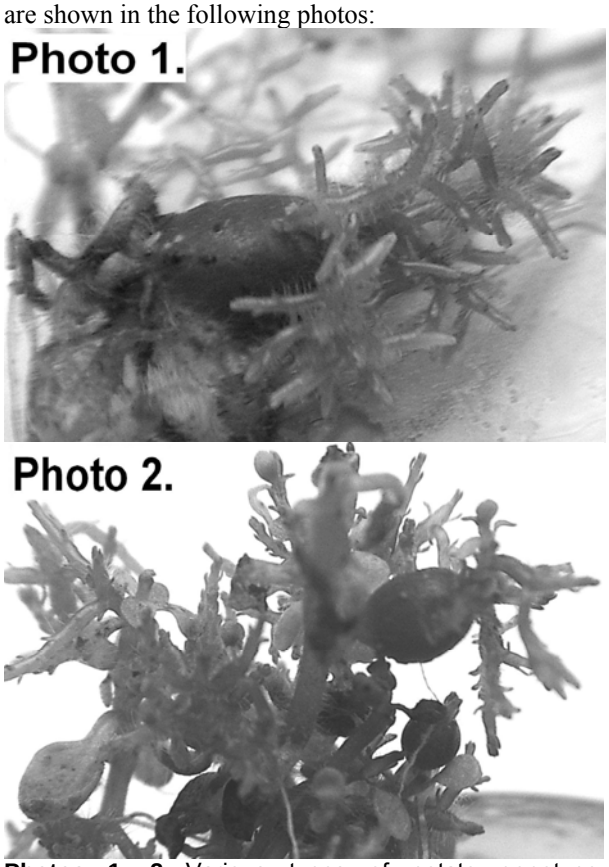
At the end of the experimental period of time the green shoots and the microtubers of ultrasonicated samples and of control samples, also, were counted.

Experimental work

The ultrasonication experiment was carried out at 35 kHz using an ultrasonic generator. The glass jars were introduced in the wire basket and immersed in the water from the ultrasonic bath. Based on the results of previous tests regarding the ultrasounds influence on the germination capacity in maize and wheat seeds as well as in the development of potato plantlets maintained under different periods of treatment it was found that 14 minutes of exposure is relevant time to reach the aim of this study.

RESULTS AND DISCUSSION

Observations of different characteristics (shoot length and vigor, leaves color and size, the degree of rooting and percentage of survived explants) showed a large variation between the populations in the collection, after 18 months of *slow growth*. Certain evolutions of the cultures developed on each medium, are shown in the following photos:



Photos 1, 2 Various types of potato genotype responses after 18 months of conservation on medium A and B, respectively

The **medium A**, containing mannitol, combined with temperature reduction, had effective *slow growth* action, with the most reduced shoot length, very short internodes, poor leaf and root development (Photo 1). The presence of B_9, in the **medium B,** seems to stimulate leaf growth in the first four to six months of the conservation period and the microtuber regeneration in the second phase (Photo 2) (Constantinovici D, 2005).

Eight varieties where the signs of senescence were observed after the *slow growth* were chosen for the 14 minutes US treatment, instead of their transfer on a fresh medium. Both, control samples (without US exposure) and the treated ones were preserved in the conservation room. The results of biometric analyzes, after a 40 months of *slow growth,* are presented in the figures 1 and 2.

As a result of the interaction between the factors involved (genotypes, composition of medium and exposure to ultrasounds), the intraspecfic variability was preserved and the values of the characteristics

under study (number of green shoots and microtubers) were improved.

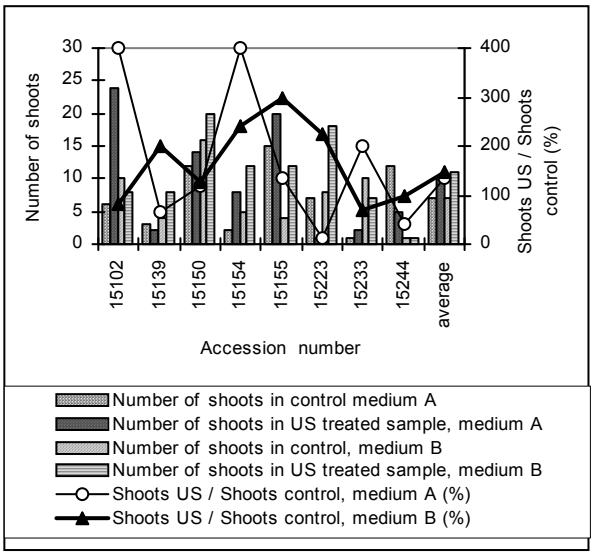


Fig. 1 Number of green shoots and average percentage in US treated samples vs. control after 40 months of conservation on medium A & B

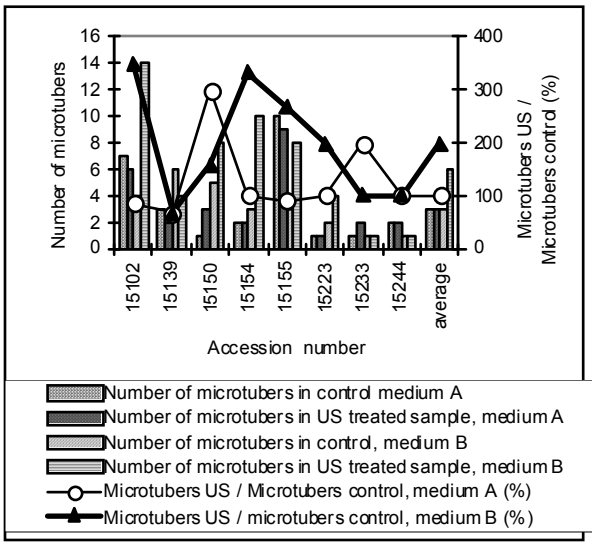


Fig. 2 Number of microtubers and average percentage in US treated samples vs. control after 40 months of conservation on medium A & B

The figure 1 shows that the positive effects of the treatment were expressed in increasing of the number of green shoots on both media, in comparison with the control. The percentage of this characteristic, related to the control, varied from 14% (acc. no 15223) to 400% (acc. no 15102 & 15154) on the medium A (with mannitol), and from 70% (acc. no 15233) to 300% (acc. no 15155) on the medium B (with B_9). Five genotypes from the medium A and six from the medium B revealed a higher survival rate of green shoots.

The average percentage of the viable shoots ranged from 133%, for the medium A, to 148%, for the medium B.

The figure 2 illustrates the influence of the treatment on the number of microtubers regenerated during the 40 months of the experiment, compared with the untreated biological material. There was recorded a large variation between genotypes which was reflected by the percentages resulted from the biometric data. Thus, this parameter ranged between 67% (acc. no 15139) to 300% (acc. no 15150) on the medium A (with mannitol), and from 67% (acc. no 15139) to 350% (acc. no 15102) on the medium B (with B_9). The most effective microtuber stimulation has resulted from the combination of 14 minutes ultrasound treatment, and the medium B, as shown by the number of genotypes with higher percentage of microtubers regenerated. The effects of these two factors can be seen in the photo number 3, compared with the control (Photo 4).

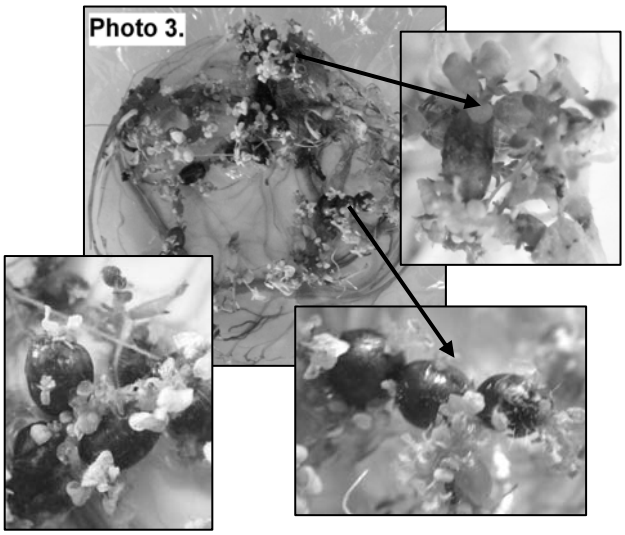


Photo 3. Sonicated potato genotype conserved for 40 months by *slow growth* and detail aspects

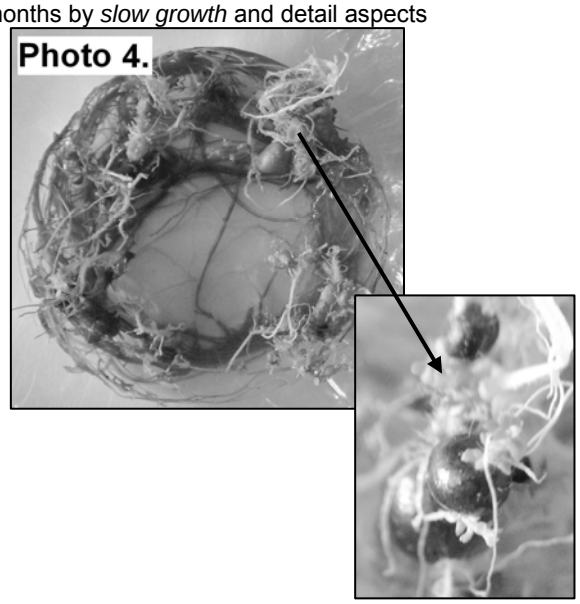


Photo 4. Control potato genotype conserved for 40 months by *slow growth* and detail aspect

CONCLUSIONS

The combinated action of ultrasounds (35 KHz, 14 minutes exposure time) and low temperature (8 – 10^0C), led to different effects on the potato conserved by *in vitro* technique, depending on genotypes and conservation media.

Compared to control samples the highest average values of the characteristics under study (number of green shoots, number of microtubers) was registered for B medium (with daminozide).

It seems possible to increase the period between two subcultures for the potato conserved on the medium with daminozide, by using ultrasounds exposure and low temperature.

REFERENCES

Aladjadjiyan A, The use of physical methods for plant growing stimulation in Bulgaria. Journal of Central European Agriculture Vol 8 (2007) No 3, pp. 369-380, 2007

Cachiţă CD, Metode ”in vitro” la plantele de cultură, Ed. Ceres, Bucureşti, 1987

Constantinovici D, *In vitro* growth responses and plant storage in old local potato populations, in „15 ani de învăţământ horticol în Banat”, Editura Agroprint Timişoara, pp. 53-58, 2005

Espinoza N, Lizarraga R, Siguenas C, Buitron F, Bryan J, Dodds HJ, Tissue culture: Micropropagation, conservation, and export of potato germplasm. International Potato Center (CIP) Research Guide 1, Lima, Peru, 19, 1992

Murashige T., Skoog F., A revised medium for rapid growth and bioassay with tobacco tissue cultures, *Physiologia Plantarum* 15, pp. 473-497, 1962

Shors JD, Soll DR, Daniels KK, Gibson DP, Method for enhancing germination. University of Iowa Research Foundation, Assignee, US patent 5,950,362. September 14, 1999

Toledo J, Espinoza N, şi Golmirzae, Tissue Culture Management of *in Vitro* Plantlets in Potato Seed Production, Trening Manual, Lima, Peru, 1998

Weinberger P, Burton C, The effect of sonication on the growth of some tree seeds. Can J Forestry Research, 11, pp. 840-844, 1981

Westcott R.J., Tissue culture storage of potato germplasm. 1. Minimal growth storage. *Potato Research*. 24, pp. 331-342, 1981

Westcott R.J., Tissue culture storage of potato germplasm. 2. Use of growth retardants. *Potato Research*. 24, pp. 343-352, 1981