Gayana Bot. 66(2): 127-133, 2009 ISSN 0016-5301

INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGIES AND A NATIVE DIAZOTROPHIC BACTERIA IN SURVIVAL AND TUBERIZATION OF *EX VITRO* POTATO PLANTS

INFLUENCIA DE HONGOS MICORRÍZICOS ARBUSCULARES Y UNA BACTERIA DIAZOTRÓFICA NATIVA EN LA SOBREVIVENCIA Y TUBERIZACIÓN DE PLANTAS DE PAPA EX VITRO

Gabriela Palacios[1], Miguel Abud[1], Miguel Salvador[2], Lourdes Adriano[2], Luc Dendooven[3] & Federico Antonio Gutiérrez[1]

[1]Laboratorio de Biotecnología Vegetal, Instituto Tecnológico de Tuxtla-Gutiérrez, Tuxtla-Gutiérrez, Chiapas, México; [2]Centro de Biociencias, Universidad Autónoma de Chiapas, Carretera a Puerto Madero km 2.0, Tapachula, Chiapas (México), Zip Code 30700, México; [3]Laboratory of Soil Ecology, Dept. Biotechnology and Bioengineering, Cinvestav, Av. I.P.N. 2508, C.P. 07360 México D. F., México.
biotecveg@hotmail.com

ABSTRACT

The inoculation of several species of micropropagated plantlets with native diazotrophic bacteria and arbuscular mycorrhizal fungi has been reported to increase growth and survival percentage compared to plantlets without inoculation. The survival of *in vitro* developed potato (*Solanum tuberosum* L. cv. Alfa) plantlets co-inoculated with *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd & Trappe, *G. claroideum* (Schenck & Smith emend. Walker & Vestberg) and a native diazotrophic bacteria was evaluated at room temperature (16-35°C) or in a growth chamber (20-22ºC). Obtained plantlets for micropropagation were placed in a peat moss/agrolite (2:1 v/v) mixture, inoculated with arbuscular mycorrhizal (AM) fungi (2250 spores plant^{-1}) and native diazotrophic bacteria (3×10^8 cell plant^{-1}) and grown in the greenhouse at 18±2ºC for 4 weeks and at 20-26ºC for another 4 weeks. Plantlets were then transferred to near-commercial greenhouse and plant growth and minitubers yield were determined 15 weeks after *ex vitro* growth. Survival of the plantlets at room temperature doubled when inoculated with the two AM fungi and also biomass and minituber yield of plants compared to untreated plants. It was found that the added microorganisms increased survival of potato plantlets and AM fungi improved potato plant growth and minituber production.

Keywords: *Glomus claroideum*, *Glomus fasciculatum,* minituber production, potato micropropagation.

RESUMEN

La inoculación de varias especies de plantas micropropagadas con bacterias diazotróficas nativas y hongos micorrízicos arbusculares se ha reportado que incrementa el crecimiento y el porcentaje de sobrevivencia comparado con plantas sin inocular. Se evaluó la sobrevivencia de plántulas de papa (*Solanum tuberosum* L. cv. Alfa) desarrolladas *in vitro*, co-inoculadas con *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd & Trappe, *G. claroideum* (Schenck & Smith emend. Walker & Vestberg) y una bacteria diazotrófica nativa, a temperatura ambiente (16-35°C) o en una cámara de cultivo (20-22ºC). Las plántulas obtenidas por micropropagación se colocaron en una mezcla de turba/agrolita (2:1 v/v), se inocularon con los hongos micorrízicos arbusculares (HMA) (2250 esporas planta^{-1}) y la bacteria diazotrófica nativa (3×10^8 células ml^{-1}) y se cultivaron en el invernadero a 18±2ºC por 4 semanas y a 20-26ºC por otras 4 semanas. Posteriormente las plántulas se transfirieron a un invernadero semicomercial en donde se les determinó el crecimiento y el rendimiento de los minitubérculos a las 15 semanas después de crecimiento *ex vitro*. A temperatura ambiente, la sobrevivencia de las plántulas, la biomasa y el rendimiento de los minitubérculos se duplicaron cuando las plántulas se inocularon con los dos HMA en comparación con las plantas sin inoculación. Se encontró que los microorganismos adicionados incrementaron la sobrevivencia de las plántulas de papa y los HMA mejoraron el crecimiento de las plantas de papa y el rendimiento de los minitubérculos.

Palabras clave: *Glomus claroideum*, *Glomus fasciculatum,* producción de minitubérculos, micropropagación de papa.

INTRODUCTION

The production of potato (*Solanum tuberosum* L.) is important in Mexico with a total production of 1.2 million tons per year (Romero-Lima *et al*. 2000). The potato plant is highly efficient in the use of available nutrients (Struik 2006). Additionally, the potato tuber is rich in vitamins, minerals, proteins, essential amino acids and carbohydrates (Buckenhüskes 2005, Van Gijssel 2005).

Some microorganisms in the rhizosphere, such as diazotrophic bacteria and mycorrhizal fungus, establish beneficial interactions with plant roots (Jeffries *et al*. 2003, Kennedy *et al*. 2004). The plant-microbe interaction in the rhizosphere is important for plant development and disease control (Jeffries *et al*. 2003, Avis *et al*. 2008). Plant growth promoting microorganisms not only increase productivity but can also control disease, while biological control agents, such as *Trichoderma* and *Pseudomonas* spp., can also stimulate plant growth (Avis *et al*. 2008). Yao *et al*. (2002) inoculated the potato cultivar Goldrush with *Glomus etunicatum* Becker et Gerdemann and found a significant increase in shoot fresh weight, root dry weight and the number of minitubers produced per plant. Srinath *et al*. (2003) found that *Ficus benjamina* L. plantlets, obtained from micropropagation that were inoculated with *G. mosseae* (Nicol. et Gerd.) Gerdemann et Trappe, *Trichoderma harzianum* Rifai and *Bacillus coagulans* ATCC 7050, had significantly higher shoot and root dry weight and total phosphorus contents in shoots and roots than plantlets that were not inoculated.

Diazotrophic bacteria can fix atmospheric nitrogen and convert it to ammonium thereby stimulating plant growth (Postgate 1998). The inoculation of *Alpinia purpurata* K.Schum. plantlets obtained through micropropagation with native diazotrophic bacteria induced larger stem diameter, root dry mass, number of shoots and increased their survival percentage from 77 to 100% compared to plantlets without inoculation (Ovando-Medina *et al*. 2007).

Micropropagation can improve microplant quality *in vitro*, help acclimatization *ex vitro*, and increase yield and seed quality of minitubers in potato (Kowalski *et al*. 2006). The use of minitubers as experimental research tools has potential in the areas of plant metabolism, germplasm selection and evaluation, genetic transformation, and somatic hybridization (Coleman *et al*. 2001). Temperature is a main factor controlling dry matter production in potato plants. High temperature reduces total dry matter production and alters dry matter distribution in favor of vines at the expense of tubers (Donnelly *et al*. 2003).

The objective of this work was to evaluate the survival percentage of potato plantlets inoculated with *Glomus fasciculatum* Schenck et Smith, *G. claroideum* (Thaxter sensu Gerd.) Gerd. et Trappe and native diazotrophic bacteria at two temperatures. Additionally, growth, total P content in root, and minituber yield of the potato (*Solanum tuberosum* L. cv. Alfa) plantlets obtained from micropropagation was determined.

MATERIALS AND METHODS

Microorganism processing

Glomus claroideum and *G. fasciculatum* were obtained from the microbial collection at Cinvestav-Irapuato (Mexico) and maintained in sand:clayey soil (1:1 v/v) as substrate and Rhodes grass as host (material support). Diazotrophic bacteria isolated from banana plants were obtained from the Centro de Biociencias at UNACH (Tapachula, Chiapas, México). The bacteria were grown in nitrogen-free semisolid malate (NFb) enrichment medium added with Congo-red aqueous solution. The composition of NFb medium was (g L^{-1} of distilled water): K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 0.5; $FeCl_3.6H_2O$, 0.015; DL-malic acid, 5; KOH, 4.8. The pH was adjusted to 7.0 with 0.1 M KOH, and the medium was autoclaved at 121ºC for 20 min. Fifteen mL 1:400 aqueous solution of Congo red autoclaved previously was aseptically added to NFb medium before use.

Microorganisms were incubated at room temperature (16-35ºC) for 24 hours and then they were used as inoculum of potato plantlets micropropagated.

Co-inoculation effect on survival of plantlets cultivated at two temperatures

Potato plants (*Solanum tuberosum* cv. Alfa) were obtained from micropropagated apical meristems as explants in MS (Murashige & Skoog 1962) medium amended with Phytagel as gelling agent using with a 16/8 h light/dark photoperiod. *In vitro* potato plantlets

were removed from the culture medium, rinsed with distilled water to remove all culture medium and planted one plantlet per bag in cylindrical black plastic bags (length 50 cm, ø 10 cm) filled 2/3 with a peat moss/agrolite mixture (2:1 v/v). Plantlets were transplanted when they reached a height of 50 mm. In this step, plantlets were inoculated with 1 g of material support (250 spores g^{-1} of AM fungi) or 1 mL of a cell suspension (3 x 10^8 cells of native diazotrophic bacteria) directly into steam base, in accordance with a factorial design 2^3 (Table I). All treatments were carried out by triplicate and control plantlets were added with 1 mL NFb medium.

Thirty plantlets from each treatment were placed at random in a nursery without temperature control (16-35ºC) at a relative humidity ranging from 48% to 87%. Another group of thirty plantlets from each treatment were placed at random in a growth chamber in a laboratory (20-22ºC). All treatments were drip irrigated with tap water ranging from 1 to 2 dm^3 per plant per day depending on climate conditions and crop maturity. For all treatments, plantlets were fertilized with 6 mg L^{-1} KH_2PO_4 each 15 days and amended with 0.11 mg L^{-1} KNO_3 after 23 days.

PLANTLET GROWTH AND MINITUBERS YIELD

Survival, fresh and dry plant weight, root dry weight, total phosphorus in the roots, and weight of minitubers were determined 15 weeks after transplanting on the plantlets cultivated under controled conditions. Total root P measurements were obtained from dry root tissue using the micro-Kjeldahl method with samples digested for 1 h following addition of 1ml of H_2O_2 and 2ml of an H_2SO_4/Se catalyst. Phosphorus concentrations on digested P material were determined using Hanna kit HI 38073 (Hanna Instruments, Mexico). For mycorrhizal colonization (MC), roots from eight plants at 15 weeks after transplanting in each replicate were sampled and stained according to Phillips Hayman (1970). Mycorrhizal colonization was evaluated under a binocular microscope at the magnification of 120 X by grid-line intersect method according to Giovannetti & Mosse (1980).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES

Survival rate of potato plantlets was done with survival analysis method using SAS software (Allison 1995). Results of fresh and dry plant weight, root dry weight, and total root phosphorus and mycorrhizal colonization and yield of minitubers were subjected to a one-way analysis of variance to test for significant difference between the treatments (Proc GLM, SAS Institute 1989).

RESULTS AND DISCUSSION

SURVIVAL PERCENTAGE AND PLANTLET GROWTH AT TWO TEMPERATURES

After 12 days of *ex vitro* conditions, there were differences in survival of potato plantlets between treatments, but only when kept at room temperature (16-35ºC) and not under controlled conditions in the growth chamber (20-22ºC). The survival at room temperature fluctuated between 24% in the treatment without inoculation and 76% in the treatment with *G. fasciculatum* plus diazotrophic bacteria (Table I). Survival percentage in growth chamber varied between 90 and 100%. A similar pattern was observed at day 16 and 21, although the survival percentage decreased. The survival at room temperature was lower and more variable than in the growth chamber. This showed that temperature control is the major factor to increase survival percentage. It has been reported that an increase in temperature has a negative effect on root development, but rhizosphere bacteria improve *ex vitro* performance of a heat tolerant cultivar indicating that they may play a role in clonal adaptation of potato to heat stress (Bensalim *et al*. 1998).

EFFECT OF INOCULANTS ON GROWTH AND TUBER YIELD

Plant height was significantly different between the treatments and ranged from 81 to 174 mm (Table II). Treatments with *G. fasciculatum* plus *G. claroideum* resulted in the tallest potato plants while the smallest were found when inoculated with diazotrophic bacteria. Earlier studies into *in vivo* mycorrhization of potato plants with the selected inoculants showed that plant height, node number, fresh and dry weights of the plants were significantly greater than in uninoculated controls (Duffy *et al*. 1999). The increase in plant growth by mycorrhizal association is largely due to increased absorption of nutrients. It has often been reported that nutrient uptake by mycorrhizal plants is faster than that by nonmycorrhizal roots (Bolan 1991).

Stem diameter ranged from 1.3 to 2.8 mm (Table II). The thickest potato stems were found in the potato plants inoculated with *G. fasciculatum* plus *G. claroideum* and *G. fasciculatum* plus diazotrophic bacteria and lowest in those inoculated with *G. claroideum* plus diazotrophic bacteria.

TABLE I. Effects of inoculants on survival rate of potato plantlets (*Solanum tuberosum* cv. Alfa) obtained by micropropagation and determined after 12, 16 and 21 days in the nursery. Plants were cultivated at room temperature (18-41ºC) or under temperature controlled conditions (20-22ºC).

TABLA I. Efectos de los inoculantes sobre la tasa de sobrevivencia de las plántulas de papa (*Solanum tuberosum* cv. Alfa) obtenidas por micropropagación y determinadas después de 12, 16 y 21 días en el vivero. Las plántulas fueron cultivadas a temperatura ambiente (18-41ºC) o bajo condiciones de temperatura controlada (20-22ºC).

Treatments	After 12 days		After 16 days		After 21 days	
	Room temperature	Climatic chamber	Room temperature	Climatic chamber	Room temperature	Climatic chamber
	(%)					
Control non-inoculated	24 b [a]	90 a	14 c	90 a	8 cd	90 a
Glomus claroideum	60 a	90 a	29 b	90 a	13 c	83 a
G. fasciculatum	40 b	96 a	11 c	93 a	6 d	83 a
Diazotrophic bacteria	60 a	97 a	24 b	93 a	8 cd	93 a
G. claroideum+Diazotrophic bacteria	67 a	97 a	41 a	90 a	20 b	83 a
G. fasciculatum+Diazotrophic bacteria	76 a	93 a	40 a	93 a	27 a	93 a
G. fasciculatum+*G. claroideum*	68 a	90 a	40 a	90 a	21 ab	87 a
G. fasciculatum+*G. claroideum*+Diazotrophic bacteria	64 a	100 a	45 a	90 a	21 ab	90 a
LSD (0.05)	16	12	7	11	6	10

[a] Means followed by the same letter within a column were not significantly different at $p<0.05$ level using LSD test / Las medias seguidas por la misma letra dentro de una columna no fueron diferentes con un nivel de significancia a $p<0,05$ usando la prueba de diferencia mínima significativa (DMS).

Table II. Effects of inoculants on characteristics of potato (*Solanum tuberosum* L. cv Alfa) plantlets obtained by micropropagation. Evaluations were done after 15 weeks in *ex vitro* growth.

Tabla II. Efectos de los inoculantes sobre las características de las plántulas de papa (*Solanum tuberosum* L. cv Alfa) obtenidas por micropropagación. Las evaluaciones se realizaron después de 15 semanas en crecimiento *ex vitro*.

Treatment	Leaf number	Stem diameter (mm)	Plant height (mm)	Plant weight		Root	P	MC [b]	Yield
				Fresh	Dry (g)	Dry weight	(mg g dw^{-1})	(%)	(g plant^{-1})
Control non-inoculated	10.2 e [a]	2.0 de	155 ab	23 c	0.87 d	0.10 cd	0.3 c	0 c	0.12 d
Glomus claroideum	12.4 d	2.0 de	109 cd	25 bc	1.27 c	0.13 c	0.2 c	25 b	0.26 b
G. fasciculatum	14.1 c	2.3 cd	163 ab	31 ab	1.36 bc	0.20 b	1.8 a	20 b	0.26 b
Diazotrophic bacteria	10.9 e	2.4 bc	81 e	26 bc	1.00 cd	0.19 b	0.2 c	0 c	0.20 c
G. claroideum+Diazotrophic bacteria	7.1 f	1.3 f	92 de	16 d	1.30 bc	0.07 d	0.3 c	25 b	0.14 d
G. fasciculatum+Diazotrophic bacteria	16.7 b	2.8 a	148 b	29 abc	1.61 b	0.29 a	0.3 c	22 b	0.17 cd
G. fasciculatum+*G. claroideum*	18.0 a	2.7 ab	174 a	34 a	2.42 a	0.14 c	1.7 a	35 a	0.34 a
G. fasciculatum + *G. claroideum* + Diazotrophic bacteria	18.0 a	1.9 e	118 c	24 c	0.90 d	0.11 cd	0.2 c	34 a	0.35 a
LSD (0.05)	0.9	0.3	25	6	0.33	0.05	0.3	9	0.07

[a] Means followed by the same letter within a column were not significantly different at $p<0.05$ level using LSD test / Las medias seguidas por la misma letra dentro de una columna no fueron diferentes con un nivel de significancia a $p<0,05$ usando la prueba de diferencia mínima significativa (DMS).
[b] MC: Mycorrhizal colonization / Colonización micorrízica.

Plant fresh weight increased significantly with 11 g when plants were inoculated with the two AM fungi compared to those left untreated (Table II). The lowest plant dry weight was found in untreated plants while it increased significantly with 1.55 g when plants were inoculated with the two AM fungi. Inoculating potato plants with *G. fasciculatum* plus diazotrophic bacteria increased root dry weight significantly 0.19 g compared to those left untreated. Treating potatoes with *G. fasciculatum* plus *G. claroideum* increased total phosphorus content with 1.4 mg P g^{-1} root dry weight compared to those that were not inoculated (Table II).

Mycorrhizal colonization (MC) and minitubers yield were largest in plantlets inoculated with both AM fungi, with or without the diazotrophic bacteria (Table II). This indicated that co-inoculation of potato plantlets with the two AM fungi had a synergistic effect on the host (Duffy *et al*. 1999).

The minitubers should be suitable for tuber seed production and for germplasm exchange (Vecchio *et al*. 2000). Inoculation with two AM fungi improved plantlet growth parameters and minituber yield and they could thus be used as commercial inoculants for potato culture (Duffy & Cassells 2000). A well-developed mycorrhizal symbiosis enhances the survival of plants by increasing nutrient and water uptake, pathogenic resistance and phytohormone production (Jeffries *et al*. 2003). The increase in the yield of potato minitubers might be due to phytohormone production induced by the two AM fungi (Castro *et al*. 2000). Tuberization is under hormonal control and can be the result of a single compound or a balance of compounds with hormonal activity (Nowak *et al*. 1999). The results presented in this work are encouraging, however it is necessary to test other potato varieties, because they have proved that potato variety thus had a large effect on tuber number (Haverkort *et al*. 1991).

CONCLUSION

It was found that inoculation with AM fungi *Glomus claroideum* and *G. fasciculatum* increased plantlet survival, but only when cultivated under oscillating temperature conditions. Native diazotrophic bacteria did not improve plantlet growth and tuber yield, but application of two AM fungi improved growth and resulted in higher yields.

ACKNOWLEDGMENTS

The research was funded by the DGEST Project UR.513.06 “Inoculación de micorrizas con organismos promotores del crecimiento vegetal para incrementar la sobrevivencia de plantas micropropagadas” and FOMIX CONACYT-Gobierno del Estado de Chiapas. The conducted experiments comply with the current Mexican laws.

REFERENCES

Allison, P.D. 1995. Survival analysis using SAS system: A practical guide. SAS Institute. Cary. 292 pp.

Avis, T., V. Gravel, H. Antoun & R.J. Tweddell. 2008. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biology and Biochemistry 40: 1733-1740.

Bensalim, S., J. Nowak & S.K. Asiedu. 1998. A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. American Journal of Potato Research 75: 145-152.

Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134: 189-207.

Buckenhüskes, H.J. 2005. Nutritionally relevant aspects of potatoes and potato constituents. In: A.J. Haverkort & P.C. Struik (eds.) Potato in progress. Science meets practice, p. 17-26. Wageningen Academic Publishers, Wageningen, The Netherlands.

Castro, G., G. Abdala, C. Agoero & R. Tizio. 2000. Interaction between jasmonic and gibberellic acids on *in vitro* tuberization of potato plantlets. Potato Research 43: 83-88.

Coleman, W.K., D.J. Donnelly & S.E. Coleman. 2001. Potato microtubers as research tools: A review. American Journal of Potato Research 78: 47-55.

Donnelly, D., W.K. Coleman & S.E. Coleman. 2003. Potato microtuber production and performance: a review. American Journal of Potato Research 80:103-115.

Duffy, E.M. & A.C. Cassells. 2000. The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Applied Soil Ecology 15: 137-144.

Duffy, E.M., E.M. Hurley & A.C. Cassells. 1999. Weaning performance of potato microplants following bacterization and mycorrhization. Potato Research 42: 521-527.

Giovannetti, M. & B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytology 84: 489-500.

Haverkort, A.J., M.Van de Waart & J. Marinus. 1991. Field performance of potato microtubers

as propagation material. Potato Research 34: 353-364.

Jeffries, P., S. Gianinazzi, S. Perotto, K.K. Turnau & J.M. Barea. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils 37: 1-16.

Kennedy, I.R., A.T.M.A. Choudhury & L. Kecskés. 2004. Non-symbiotic bacteria diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploided? Soil Biology and Biochemistry 36: 1229-1244.

Kowalski, B., F. Jiménez-Terry, L. Herrera & D. Agramante-Peñalver. 2006. Application of soluble chitosan *in vitro* and in the greenhouse to increase yield and seed quality of potato minitubers. Potato Research 49: 167-176.

Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Nowak, J., S. Bensalim, C.D. Smith, C. Dunbar, S.K. Asiedu, A. Madani, G. Lazarovits, D. Northcott & A.V. Sturz. 1999. Behaviour of plant material issued from *in vitro* tuberization. Potato Research 42: 505-519.

Ovando-Medina I., L. Adriano-Anaya, A. Chávez-Aguilar, M.A. Oliva-Llaven, T. Ayora-Talavera, L. Dendooven, F. Gutiérrez-Miceli & M. Salvador-Figueroa. 2007. *Ex vitro* survival and early growth of *Alpinia purpurata* plantlets inoculated with *Azotobacter* and *Azospirillum*. Pakistan Journal of Biological Sciences 10: 3454-3457.

Phillips, J.M. & D.S. Hayman. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society. 55: 158-161.

Postgate, J. 1998. Nitrogen fixation, 3rd edition. Cambridge University Press, Cambridge UK.

Romero-Lima, M.R., A. Trinidad-Santos, R. García-Espinosa & R. Ferrera-Cerrato. 2000. Producción de papa y biomasa microbiana en suelo con abonos orgánicos y minerales. Agrociencia 34: 261-269.

SAS. 1989. Institute Inc, Statistic Guide for Personal Computers. Version 6.04 (eds.). SAS Institute, Cary.

Srinath, J., D.J. Bagyraj & B.N. Satyanarayana. 2003. Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. World Journal of Microbiology and Biotechnology 19: 69-72.

Struik, P.C. 2006. Trends in agricultural science with special reference to research and development in the potato sector. Potato Research 49: 5-18.

Van Gijssel, J. 2005. The potential of potatoes for attractive convenience food: focus on product quality and nutritional value. In A.J. Haverkort, P.C. Struik (eds.). Potato in progress. Science meets practice, p. 27-32. Wageningen Academic Publishers, Wageningen, The Netherlands.

Vecchio, V., S. Benedettelli, L. Andrenelli, E. Palchetti & L. Espen. 2000. Inductive and noninductive conditions on *in vitro* tuberisation and microtuber dormancy in potato (*Solanum tuberosum* subspecies *tuberosum* and subspecies *andigena*). Potato Research 43: 115-123.

Yao, M., R. Tweddell & H. Désilets. 2002. Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. Mycorrhiza 12: 235-242.

Recibido: 24.02.09
Aceptado: 04.05.09