The effect of *in vitro* mycorrhization on growth characteristics, changes in endogenous hormones and performance of microplants in potato (*Solanum tuberosum L.*)

Khosro PARVIZI[1]* and Farshad DASHTI[2]

[1]Assistant professor, Agricultural and Natural Resources Research Center, Faculty of Agriculture, Hamedan, Iran
*correspondence: kparvizi@yahoo.com, Tel: +98 811 442 4090 ; Fax :+98 811 4424012

[2]Assistant professor, Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

Abstract

Potato (*Solanum tuberosum L.*) plantlets were inoculated *in vitro* with an arbuscular mycorrhizal fungus (*Glomus intraradices*) and their growth response, performance and endogenous hormonal status evaluated. A factorial experiment based on a completely randomized design consisting of two potato cultivars (Agria and Sante) and four culture media was conducted with four replications. The culture media included non-inoculated Murashige and Skoog (MS) medium (control), inoculated MS medium, inoculated half-strength MS medium and inoculated MS medium without vitamins and sugar. To do inoculation, germinated spores of fungus were transferred to the root zone of plantlets. Colonization percentage, total chlorophyll content, internodes and stolon length, shoot diameter, shoot and root fresh and dry weight, leaf area and the level of three endogenous hormones (total auxins, gibberellins and cytokinins) were determined. In addition, mini-tuber production was assayed quantitatively and qualitatively. Results demonstrated that the effect of two factors on all characteristics including mini-tuber production was different significantly. Interaction between cultivar and inoculation systems was significantly associated with endogen auxins as well as all range of mini-tuber production. The inoculated MS medium showed the better results, but it did not have significant difference to half-strength MS in terms of plantlet performance and growing parameters. Responses of both cultivars to inoculation were very conspicuous in the production of endogen hormones. Higher endogen hormone levels were associated with elevated growth parameters, greater biomass production and better plantlet performance.

Keywords: growth responses, micro-plants, mycorrhizal inoculum, potato

Introduction

Micropropagation of plants under high moisture and low lighting conditions often causes low lignification and decreased functionality of root systems resulting in low survival rate. Plantlets have also non-functional stomata and poorly developed cuticles that leads to substantial desiccation (Preece and Sutler, 1991). Hyper-juvenility is another problem that prevents plantlets to develop disease resistance properly. High humidity and low light intensity that are necessary during weaning also make an environment favorable to growth of fungi where weak saprophytes may become pathogenic causing plantlets damping-off. As a result, significant losses occur during acclimatization due to poor physiological status of plantlets (Grunewaldt-Stoecker, 1997; Williamson et al., 1997).

Generally, there are some physical and chemical methods such as the application of plant growth retardants, light and CO_2 to increase acclimatization, hardening and performance of potato plantlets during *in vitro* culture and/or *ex vitro* transfer (George, 1993). However, these methods are often costly and defective having side effects on sanitation especially when growth retardants and chemicals are used.

Arbuscular mycorrhizal fungi (AMF) form symbioses with the roots of about 80% of all plant species including potato (Bonfante and Perotto, 1995). Mycorrhizal fungi have shown to reduce drought stress (Nelson and Safir, 1982) and increase disease resistance (Dehne, 1982; Adrien et al., 2001; Guillon et al., 2002).

In vivo mycorrhization of micropropagated plants has increased plant survival, shoot biomass and enhanced shoot phosphorus concentration significantly in five plant species including *Kummerowia striata* (Thunb.) Schindl, *Ixeris denticulate* L., *Lolium perenne* L., *Trifolium repens*L. and *Echinochloa crusgalli* var. mitis (Chen et al., 2005), and similarly, enhanced growth and viability of plants during *ex vitro* weaning (Nowak, 1998; Maier et al., 1999).

Bio-control of root diseases and growth-promoting effects of AMF in *in vivo* condition have extensively been documented (Niemira et al., 1995; Graham et al., 1996; Nowak et al., 1997; Elizabeth et al., 2000). In addition, *in vitro* inoculation of many horticultural crops offers potential advantage of allowing establishment of host plants without competition of the other beneficial soil microorganisms (Lazarovits and Nowak, 1997; Mark et al., 1997; Slezack et al., 1999). A change in the levels and the accumulation of cytokinin, abscisic acid and gibberellin-like substances in some host plants through mycorrhizal colonization have been reported (Allen et al., 1980 and 1982; Barea and Azcon-Aguilar, 1982). Duffy et al. (1999) demonstrated that *in vitro* and *in vivo* mycorrhization of potato plantlets without or by *Pseudomonas flourescens* isolates (CHA0 and IP10) would be accomplished successfully. Furethermore, their analysis has been shown that mycorrhization promotes microplant growth during and after weaning. Liesbeth et al. (2005) developed an autotrophic culture system (tripartite system) for *in vitro* mycorrhization of potato plantlets by *G. intraradices*. In their system, roots of micropropagated plantlets cultured in the modified MS were inoculated, while shoots developed under open-air conditions. Several thousand spores, an extensive extra radical mycelium and abundant root colonization were obtained. Spores were able to colonize new plantlets under the same conditions.

In potato, there are only a few example of *in vitro* inoculation. Most experiments have either carried out in *in vivo* conditions or merely focused on the biological aspects of mycorrhiza and spore production. The effects of *in vitro* inoculation of potato on growth characteristics, hormonal reaction and plant performance have therefore been much neglected. Hence, the main objectives of this study were to evaluate the growth responses, endogenous hormonal level and performance of the potato plantlets inoculated with a mycorrhizal fungus *in vitro*. Glomus intraradices is an arbuscular mycorrhizal fungus used as soil inoculants in agriculture and horticulture. This arbuscular mycorrhizal fungus also forms a compatible symbiosis with potato (Liesbeth et al., 2005) and for this reason; we used this species in the present study.

Material and methods

Experimental design and culture media

This study was conducted in a factorial experiment based on completely randomized design with four replications. All replication comprised of two jars each containing five single node stems prepared through potato meristem culture. A total number of 320 micro-plants were used. The factors included two potato cultivars (Agria and Sante) and four culture media [non-inoculated Murashige and Skoog (MS1) medium as control, inoculated MS with *Glomus intraradices* fungus “MS2”, inoculated half-concentration of MS “MS3” and inoculated MS without vitamins and sugar “MS4”] (table.1). Single nodes were cultured on MS medium and kept in a growth room at 16 h photoperiod, 50 µmol $m^{-2}*sec^{-1}$ light intensity with the temperature of 24±1°C. They were inoculated three weeks later when the roots formed and shoot growth started. Rooted cuttings were then transferred to the four culture media and inoculated with sterilized-germinated spores of *G. intraradices*.

Table 1. Definition of culture media and their codes as used in in vitro mycorrhization of potato plantlets.

Culture medium code	Definition
MS1	Non-inoculated Murashige and Skoog (MS)
MS2	Inoculated MS with Glomus intraradices fungus
MS3	Inoculated half-concentration MS with *Glomus intraradices* fungus
MS4	Inoculated vitamins and sugar free MS with *Glomus intraradices* fungus

In vitro mycorrhization

Spores of *G. intraradices* were prepared through the wet sieving, decanting method (Gerdemann and Nicolson, 1963) and centrifuged in a sucrose gradient (Walker et al. 1982). Surface sterilization was performed according to the method of Duffy et al. (1999). Spores were washed twice for one min. by shaking in 0.05 (w/v) aqueous Tween 20 followed by insertion in 2% (w/v) Chloramine T for 10 min. Spores were

then transferred into an antibiotic solution of 200 ppm streptomycin plus 100 ppm gentamycin and left for 24 h. In order to suppress germination it was essential to keep spores chilled during this procedure. Spores were aseptically transferred onto sterile nylon membrane and placed in glass petri dishes containing sterile sand. They were then incubated in the dark for two weeks at 24±2°C (Mark et al., 1997). The presence of germination tubes and hyphae was examined under the light microscope. Ten germinated spores were aseptically transferred to the root zone of micro-plants in a new culture medium in each treatment. Five micro-plants per jar and 40 plants per treatment were used. Non-inoculated controls were grown under the same conditions. Plantlets were finally transferred to the greenhouse four weeks after culture.

Growth response in the greenhouse

A peat-based planting medium consisting of a mixture of peat and perlite [3:1 v/v] was prepared and the plantlets were cultivated into the cube trays containing the medium with 8cm×6cm spacing. A complete commercial liquid fertilizer (Fusamku, BV. Heerlen, The Netherlands) was used to feed the micro-plants (by concentration of 3 cc per one liter of water) and repeated every two weeks. Plantlets were finally grown in a glasshouse at ambient temperature ranging from 18 to 27°C, 16 h photoperiod supplemented by high-pressure sodium lamps (400 watts, 290/240 volts; Thermoforce Ltd., CamplexPlantcare Division, Essex, UK).

Root colonization assessment

Root segments harvested from micro-plants during transplantation, were washed thoroughly in running tap water, cut into 1.0 cm pieces and treated with 10% KOH solution for 30 min. at room temperature. Thereafter, the roots were washed 3–4 times with sterilized distilled water and treated with 1% HCl for 3–4 min followed by staining with 0.05 trypan blue in lactophenol (Phillips and Hayman, 1970). The stained root segments were examined microscopically (×40 magnification) (Figure 1). The method described by Giovannetti and Mosse (1980) were used for root colonization assessment. A total of 25 root segments selected randomly from the stained samples were observed under an Olympus KH binocular microscope (×40 magnification). The colonization percentage was calculated as follows:

$$\text{Colonization Percentage} = \frac{\text{Number of colonized root segments}}{\text{Total number of examined segments}} \times 100$$

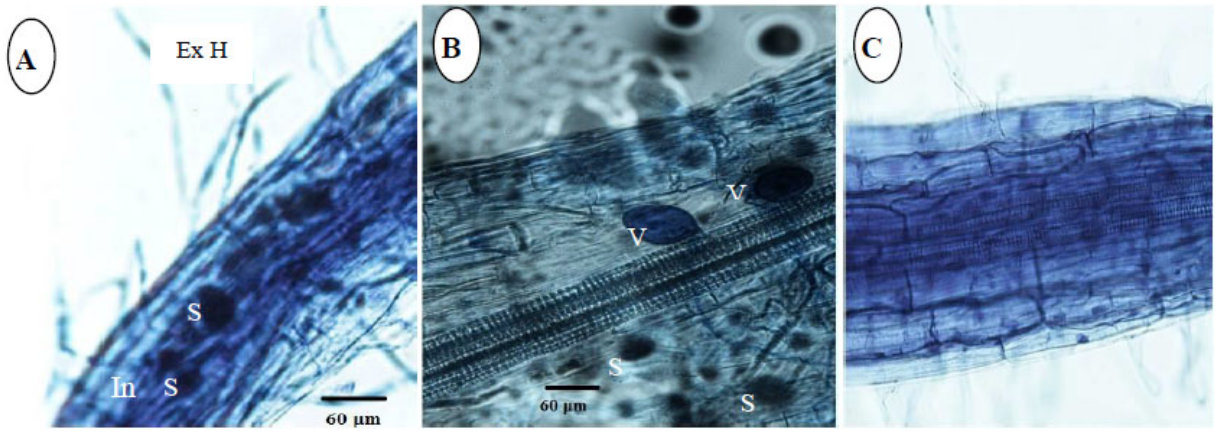

Figure 1. Colonization of root cells by *G. intraradices*. All images were obtained from stained potato plantlets root samples that were assessed for percentage root colonization. A and B show vesicles (V) and spors (S) of different sizes, extra and intraradical of hyphaes (Ex H and In H). C shows non-inoculated root (control).

Measurement of endogenous hormones

Total auxins, gibbrellins and cytokinins were extracted with 70% ethanol from the leaves and whole shoots grown over 21 days after transplanting using the method described by Jeffrey (2004). Hormones were analyzed using an HPLC (Jusco 900, Japan) connected to a UV detector (220 nm). Hormonal molecular metabolites were separated with a Waters C18 column (size 250 × 4.6 mm) using an isocratic gradient of water–acetonitrile–acetic acid (83:17:0.2, v/v/v). The elution rate was 0.9 ml*min^{-1} at a column temperature of 33°C.

Growth Parameters and mini-tuber production

Four weeks after transplanting, internode and stolon length, stem diameter, shoot and root fresh and dry weight and leaf area were measured. In order to measure leaf area, two plantlets from each replication were randomly selected and cut just at the bed. The area of each leaf was then measured directly using the Area Measurement System (Delta-T Devices Ltd, Cambridge, UK). Chlorophyll content was determined through the extraction procedure of Arnon (1949) followed by the calculation method stated by Gross (1991). Mini-tuber obtained from all plots were weighed, arranged in four groups including less than one gram (1), between one to three grams (2), between three to five grams (3) and more than five grams (4). Average tuber number per plant was determined and analyzed statistically. To assess mini-tuber's dry matter of all replication, three mini-tubers were randomly selected and sliced to one mm thick. The samples were weighed first, placed in drying oven for 48 hours at a temperature of 85ºC. The dried samples were weighed again and mini-tuber's dry matter percentage was calculated using the following formula:

$$\text{Mini-tuber dry matter percentage} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100$$

Tow-way analysis of variance (ANOVA) of the data was carried out using SAS software (v. 8.02, SAS Institute, Cary, NC) and the means were compared by the Duncan's Multiple Range Test.

Results

Root Colonization by *G. intraradices*

The result of the two-way ANOVA (Table. 2) showed that root mycorrhization by *G. intraradices* had significant effect (P≤0.1) on the plants' endogenous hormonal levels as well as colonization percentage. Root colonization was occurred at high level in both cultivars in MS2 and MS3 treatments as shown in Figure 2. However, there were no significant differences between the two culture media for any cultivar. Colonization percentage was lower in MS without vitamins and sugar (MS4) for both cultivars showing significant difference with the other two media. Highest colonization reached in case of Sante cultivar in all treatments (Figure 2).

Table 2. Significance level of effects of treatments and their interaction on colonization and endogenous hormones level of potato plantlets based on a two-way analysis of variance (ANOVA)

		Mean of Squares			
Source of variation	Degree of freedom	Colonization	Auxins	Cytokinins	Gibberellins
Potato cultivar[1] (PC)	1	780.12**	2891.80**	608.13**	5209.65**
Tissue culture medium (TCM)	3	5178.79**	1000.67**	573.29**	10294.16**
PC× TCM	3	2.50^{ns}	347.68**	22.22^{ns}	131.23^{ns}
Error	24	39.29	61.93	29.79	164.51
Corrected total	31				
Coefficient of variation		19.39	12.68	13.07	8.49

Significance levels: ns, P > 0.05; *, P ≤ 0.05 and **, P ≤ 0.01
1= Agria and Sante

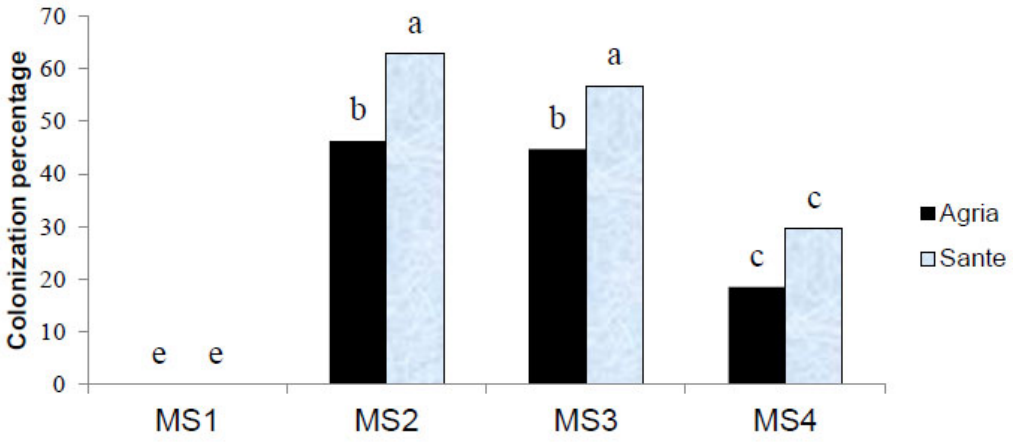

Figure 2. The effect of *in vitro* mycorrhization on root colonization in potato plantlets in different culture media

MS1 = Non-inoculated Murashige and Skoog (MS)
MS2 = Inoculated MS with Glomus intraradices fungus
MS3 = Inoculated half-concentration MS with Glomus intraradices fungus
MS4 = Inoculated vitamins and sugar free MS with Glomus intraradices fungus
Differences between treatments have been determined by Duncan's Multiple Range Test ($P \leq 0.05$)

Changes in endogenous hormones

Inoculation per se significantly affected three important endogenous hormone groups (Auxins, Cytokinins and Gibberellins) as shown in Table 2. The culture media also changed the amount of these hormones in inoculated plantlets significantly. Interaction between the two potato cultivars and inoculation systems was significant in association with the auxins only.

The results of *in vitro* mycorrhization (Figure 3 and 4) clearly indicated that inoculated plantlets in both completed and half concentration of MS media produced higher auxins, cytokinins and gibberellins compared to non-inoculated ones, but inoculated plantlets in MS without sugar and vitamin did not show the same differences with the control treatment. There was no significant difference between MS4 treatment and the control in terms of gibberellins and auxins synthesis but cytokinins level was different in the two media and cultivars. Inoculated cultivars showed different reactions in terms of auxin synthesis in completed and half MS concentration compared to non-inoculated plantlets. Inoculated Agria produced more auxin in completed and half MS concentration significantly compared to the control. In contrast, Sante did not show the same reaction to the two media (Figure 4).

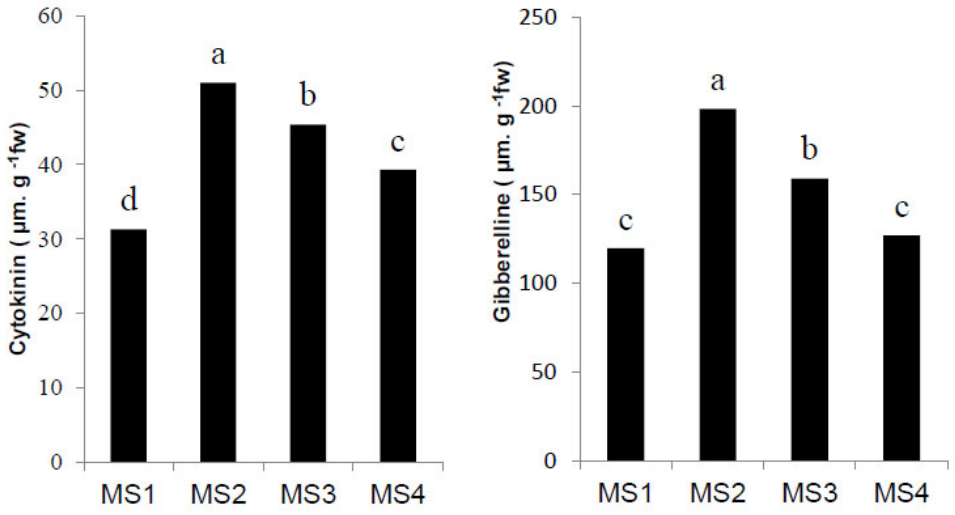

Figure 3. The effect of in vitro mycorrhization on endogenous plant growth regulators (Cytokinin and Gibberelline) in potato plantlets in different culture media

MS1= Non-inoculated Murashige and Skoog (MS)
MS2= Inoculated MS with *Glomus intraradices* fungus
MS3= Inoculated half-concentration MS with *Glomus intraradices* fungus
MS4= Inoculated vitamins and sugar free MS with *Glomus intraradices* fungus
Differences between treatments have been determined by Duncan's Multiple Range Test ($P \leq 0.05$)

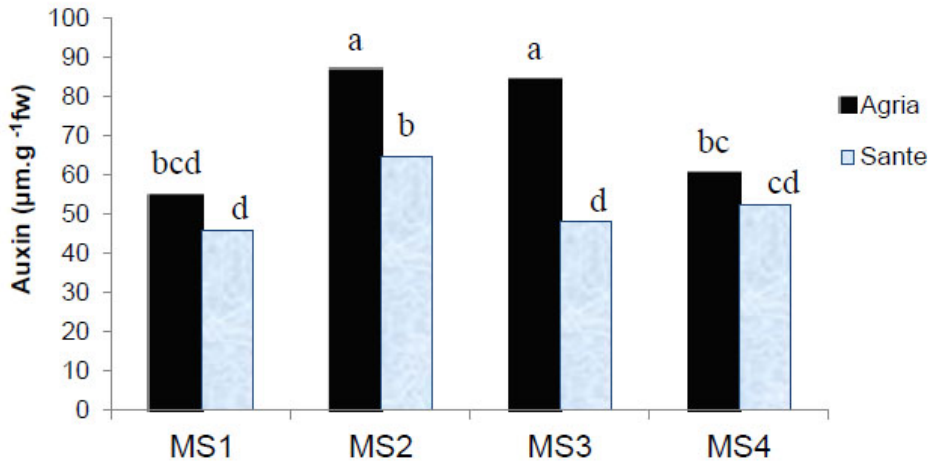

Figure 4.Interaction effect of cultivar and culture medium composition on Auxin levels in potato plantlets

MS1= Non-inoculated Murashige and Skoog (MS)
MS2= Inoculated MS with Glomus intraradices fungus
MS3= Inoculated half-concentration MS with Glomus intraradices fungus
MS4= Inoculated vitamins and sugar free MS with Glomus intraradices fungus
Differences between treatments have been determined by Duncan's Multiple Range Test ($P \leq 0.05$)

Microplants Growth Parameters

Results of the ANOVA of the data for growth parameters (Table 3) revealed that there were significant differences between the culture media and the cultivars for all measured parameters. However, no interaction effects were found between the cultivars and the culture media.

Inoculation of the plantlets with *G. intraradices* had significant effect on all growth parameters in the two culture media (MS2 and MS3) compared to the control. These results, however, did not show similar pattern with MS4 treatment. Only three parameters including root fresh and dry weight and leaf area had significant difference compared to the control (Figure 5; G, H and I). Chlorophyll content, internode and stolon length, shoot fresh and dry weight as well as stem diameter were shown to be not significantly different (Figure 5; A, B, C, D, E and F).

The highest amount of chlorophyll content (2.19 $mg*g^{-1}$) was obtained from the plantlets cultured in MS2 medium. Agria had higher chlorophyll content in all inoculated culture media and control treatment compared to Sante (Figure 5, A).

Stem internode length was higher in plants cultured in MS2 medium but there was no significant difference when compared to the MS3 medium. Two cultivars responded differently in this regard so that Agria had longer internode length in all culture media (Figure 5, B). In the MS2 medium culture, stem diameter increased conspicuously compared to the control. Plantlets cultured in MS4 did not have any significant difference with the control treatment in terms of stem diameter (Figure 5, C). Plantlets produced shorter stolons in MS2 medium when inoculated with G. intraradices. In addition, there were no significant interactions between the cultivars and culture medium systems in this regard. In general, Agria had longer stolon length than Sante in all cultures media (Figure 5, D). Total shoot fresh weight of Agria in MS3, MS4 and control media were not significantly different. Sante showed significant difference in shoot fresh weight in MS2 as compared to the other culture media. There was no significant difference between MS4 and the control in terms of producing fresh shoot weight by the two cultivars (Figure 5, E).

Both cultivars produced the highest amount of shoot dry weight in MS2 medium. Although plantlets cultured in MS2 and MS3 media showed no significant difference, they differed significantly with MS4 and the control treatments. Plantlets of both cultivars in MS4 medium were not significantly different in terms of shoot dry weight compared to the control treatment (Figure 5, F). They also showed similar trend in root fresh and dry weight when inoculated. Both cultivars produced the highest shoot fresh and dry weight when cultured in MS2. However, this treatment did not have significant difference to MS3 regarding root fresh weight, but showed differences in root dry weight compared to MS4 and the control treatment. Interaction between the cultivars and culture media was not significant (Figure 5, G). Plantlets had the highest leaf area in MS2 and MS3 media, but their difference was not significant. However, they were significantly different when cultured in MS4 and the control media. Plantlets in MS4 medium had significantly ($P \leq 0.05$) bigger leaf area in compared to those cultured in MS1 medium. Generally, Agria produced bigger leaf area in all treatments (Figure 5, I).

Table 3. Significance level of effects of treatments and their interaction on variables based on a two-way analysis of variance (ANOVA)

		Mean of Square								
Source of variation	Degree of freedom	Chlorophyll content ($mg*g^{-1}$ fw)	Internode length (cm)	Stem diameter (mm)	Stolon length (cm)	Shoot Fresh weight (g)	Shoot dry weight (g)	Root Fresh weight (g)	Root dry weight (g)	Leaf area (cm^2)
Potato cultivar[1] (PC)	1	1.56**	0.32**	2.89**	3.25**	2.25**	0. 01**	0.01**	0.0005ns	702.55**
Tissue culture medium (TCM)	3	0.14**	0.31**	0.46**	0.62**	1.40**	0.016**	0.02**	0.0019**	923.93**
PC×TCM	3	0.02^{ns}	0.058^{ns}	0.016^{ns}	0.14^{ns}	0.13^{ns}	0.006^{ns}	0.01^{ns}	0.00013^{ns}	36.42^{ns}
Error	24	0.01	0.036	0.034	0.106	0.08	0.0007	0.001	0.000012	23.67
Corrected total	31									
Coefficient of variation		4.90	8.10	4.87	7.49	8.36	8.28	12.52	11.04	4.50

Significance levels: ns, P > 0.05; *, P ≤ 0.05 and **, p ≤ 0.01
1= Agria and Sante

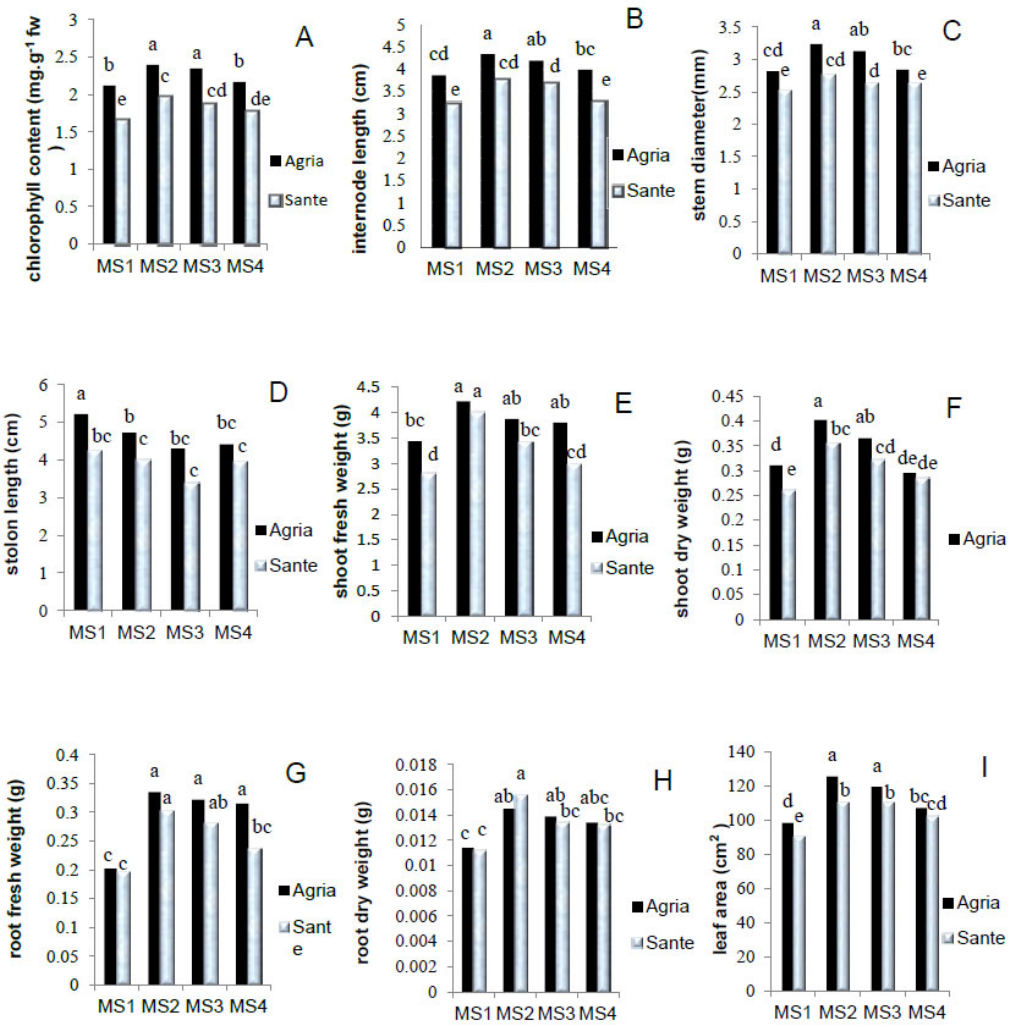

Figure 5. The effect of *in vitro* mycorrhization on growth parameters of potato plantlets in different culture media

(A=chlorophyll content, B=internode length, C=stem diameter, D= stolon length, E= shoot fresh weigh, f= shoot dry weight, G= root fresh weight, H= root dry weight and I= leaf area)

MS1= Non-inoculated Murashige and Skoog (MS)
MS2= Inoculated MS with *Glomus intraradices* fungus
MS3= Inoculated half-concentration MS with *Glomus intraradices* fungus
MS4= Inoculated vitamins and sugar free MS with *Glomus intraradices* fungus
Differences between treatments have been determined by Duncan's Multiple Range Test (P≤0.05)

Mini-tuber yield and dry matter

Significant difference (P≤0.05) was found in the interaction between the cultivars and culture media in all sizes and total number of mini-tubers. The two cultivars had different reactions and do not show the same response in inoculated systems and control treatment. Higher mini-tuber number was recorded in inoculated treatments compared to the control. Agria cultivar inoculated in complete and half-strength MS media had more effective response of producing larger mini-tubers (upper than 3

grams) compared to those inoculated in MS without vitamins and sugar. In contrast, Sante cultivar had no similar response when the above media were used. The highest number of smaller mini-tuber size (less than 1 gram) was produced by Agria inoculated in MS without vitamins and sugar, while Sante behaved similar when inoculated in completed MS, which was significantly ($p \leq 0.5$) different with the other inoculated media and control treatment.The uppermost number of mini-tubers ranging between 1-3 grams in Agria was belonged to the inoculation in MS without sugar and vitamins, whereas Sante produced the highest number of mini-tuber in this size when inoculated in half concentration of MS, which did not have significant difference with the inoculation in MS (Table 4).

In Agria, there was no significant difference when inoculated in complete and half concentration of MS in relation to the production of mini-tubers ranging between 3-5 grams (group 3). In contrast, Sante showed significant difference between the two media in terms of producing group 3 mini-tubers. Among all the treatments, highest number of larg mini-tubers (group 4) was recorded in Agria and plantlets inoculated in MS medium (Table 4).

In terms of total number of mini-tuber, Agria produced highest number in MS without sugar and vitamins but it had no significant difference with inoculation in MS medium. Sante produced highest number of mini-tuber when inoculated in MS, but it showed significant difference to the other tested media and control treatments (Table 4).

Interaction between the cultivars and media in terms of mini-tuber dry matter was not significant. As a result, two cultivars had the same response in different culture media and dependently had higher mini-tuber dry matter compared to the control treatment (Figure 6).

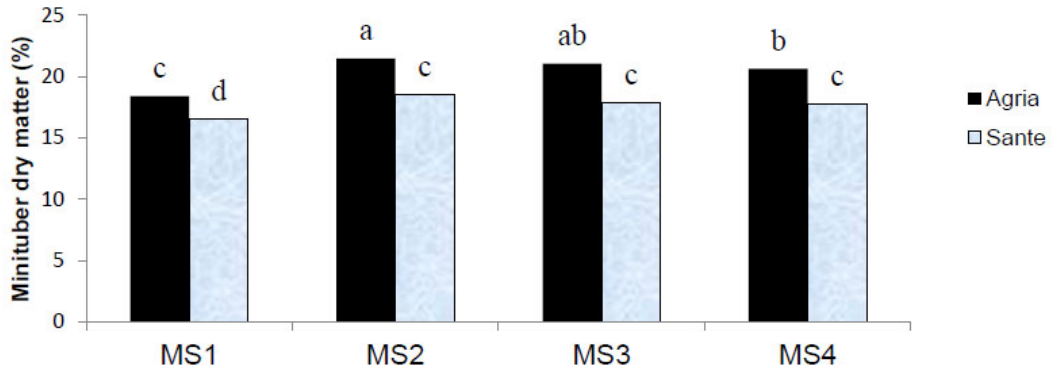

Figure 6. The effect of *in vitro* mycorrhization on mini-tuber dry matter percentage of potato plantlets in different culture media

Table 4. Interaction effect of cultivar and culture medium on mini-tuber production of potato plantlets. Each value is the mean of four replicates (Duncan's multiple range test, P ≤ 0.05)

Treatment	No minituber/plantlet (≤1 g)	No minituber/plantlet (1-3 g)	No minituber/plantlet (3-5 g)	No minituber/plantlet (>5 g)	Total number of minituber (No/plant)
Agria× MS1	1.02 g	1.60 e	0.95 c	1.32 c	4.90 f
Agria× MS2	2.02 e	3.55 c	1.70 a	2.80 a	10.07 d
Agria× MS3	1.65 f	2.27 d	1.67 a	1.60 b	7.20 e
Agria× MS4	2.70 d	5.37 b	1.45 b	1.27 c	10.80 d
Sante× MS1	5.45 c	3.30 c	1.05 c	0.47 d	10.27 d
Sante× MS2	6.72 a	6.72 a	1.72 a	1.25 c	16.42 a
Sante× MS3	6.10 b	6.95 a	0.87 c	0.57 d	14.50 b
Sante× MS4	5.90 b	5.25 b	1.32 b	0.47 d	12.95 c

MS1= Non-inoculated Murashige and Skoog (MS)
MS2= Inoculated MS with *Glomus intraradices* fungus
MS3= Inoculated half-concentration MS with *Glomus intraradices* fungus
MS4= Inoculated vitamins and sugar free MS with *Glomus intraradices* fungus

Discussion

The results of this study demonstrated that *in vitro*-cultured potato plantlets can be colonized successfully with mycorrhizal fungus, *Glomus intraradices*. These findings are also supported by previuos studies (Duffy et al., 1999, Liesbeth et al., 2005).

The results showed that *in vitro* mycorrhization of potato can not only be taken place properly, but also the inoculated plantlets have good physiological responses to this symbiotic partner. Furthermore, it was indicated that inoculation of potato plantlets can promote their vigor helping them to grow better and acclimatize well to *in vivo* condition.

To date there is no report on the effect of *in vitro* mycorrhization on endogenous hormonal levels in potato plant. The results showed that three important endogenous hormone groups were basically affected by the inoculation. These findings are also in agreement with the results of Jentschel et al. (2007) who stated that auxins play a significant role in mycorrhizal processes of *Tropaeolum majus*. In addition, there are some examples such as maize (Kaldorf and Ludwig, 2000) and soybean (Meixner et al., 2005) showing an increase in the endogenous auxins after inoculation of roots with Arbuscular Mycorrhiza fungi.

The amount of gibberellins was higher in all treatments compared to the two other hormone groups, as the level of hormones were lower after four weeks of transferring plantlets into the greenhouse (the time that stolon improvement was at the peak phase). The higher level of gibberellins at this time is inevitable since tuberization has not yet been occurred. It has been demonstrated that tuber induction is physiologically related to reduced gibberellin levels (Jackson, 1999). However, mycorrhization in three culture media could increase the amount of gibberellins as compared to the control treatment. It has been conceived that increasing hormonal levels has had promotion effects on the vigor of plantlets as those associated with the growing parameters in the plantlets.

The result also demonstrated that changes in the composition of the media could alter the colonization status and consequently affect the growing responses. The establishment of mycorrhiza had a good response to MS medium by itself. In addition, diluted MS by half concentration showed the similar result, but the elimination of sugar and vitamins from MS medium was not as suitable as the other two media. This result is different from the experiment conducted by Liesbeth et al. (2005) who showed that elimination of sugar from MS medium is more suitable for colonization and the establishment of mycorrhiza. This difference could be due to the different growth stage and physiological status of the plantlets since well-advanced plantlets were used in their experiment, while the plantlets of our experiment were stem nodal sections being primarily at the rooting stage. It is anticipated that if these nodal sections are cultivated into the MS medium without any sugars and vitamins, they would be stunted and consequently loss of plantlets would be occurred in at a high level. In addition, it had been shown that decreasing sugar and some agent metabolite materials have deficit effects on explants proliferation and cause vitrification disorder in tissue culture (Ziv, 1991). It is conceived that this condition is also not suitable for root development in nodal sections of potato. As a result, decreases in root development plays a basic role in physiological community in processes of fungal establishment. Therefore, it is a biotic factor that can affects the

response to inoculation with VAM fungi in the lack of vitamins and sugar in MS medium as compared to the two other media. We concluded that inoculation of single node stems in MS without vitamins and sugar could be quite efficient compared to the control treatment and somehow beneficially compensated the lack of sugar and vitamins in the culture media.

Mycorrhization of plants cultured in both completed and half concentration of MS showed the same response in terms of some growing indices, hormonal levels and performance of plantlets. Therefore, it is recommended to use half-strenght MS medium for *in vitro* mycorrhization projects of potato in order to reduce expenses. Furethermore, this finding could be useful in some studies intending to evaluate the biological aspects of mycorrhiza in the above culture media since such results have not previously ben reported in potato. Cassells et al. (1996) also described beneficial effect of using low-salt medium for inoculation of strawberry plantlets.

Inoculums of VAM fungus, *G. intraradices* increased minituber number in two potato cultivars but this effect was significantly different in Agria and Sante cultivars. Notable difference in dry matter of minitubers in inoculated and non-inoculated plantlets was observed in this study. This result could be related to the role of mycorrhiza in carbon assimilation. Allen et al. (1981) found that inoculated plants have much higher photosynthesis rate. In addition, Snellgrove et al. (1981) and Tester et al. (1986) obtained the same results.

Conclusions

The results demonstrated thet mycorrhization under in vitro condition increased the growth parameters of potato plantlets and those were in consistency with the morphological development of mycorrhizal structure. Moreover, mycorrhization increased the synthesis of three important endogenous hormone groups in potato plantlets. The increase of hormonal levels was contributed to the increase in growing parameters, plant performance and yield of potato. Therefore, micropropagation and mycorrhization of potato plantlets can be combined as a useful tool to improve viability, performance and survival capacities of potato plants.

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