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# Efficiency of plant growth-promoting rhizobacteria and fungi on enhancement of growth and minituber production of tissue-cultured potato cultivars (*Solanum tuberosum*)

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## ABSTRACT

The effect of plant growth promoting rhizobacteria (PGPR) and arbucular mychorizal fungi (AMF) on the growth and minituber yield of micropropagated potato cultivars (Agria, Arinda and Marfona) were investigated under organic conditions. The experiment design was completely randomized design with 5 replicates. Three PGPR strains (*Pseudomonas* CHAO-4, *Azotobacter* DSM-281 and *Bacillus* PTCC-1020) and AFM (*Glomus intraradice*) as a commercial fertilizer were tested alone or in combination on organically grown potato cultivars in term of growth and minituber yield. The results showed that all of the biological treatments stimulated plant growth and resulted in significant yield increase. Among different groups of inoculation, treatment of plants with triad inoculation in general and combination of *Azotobacter* + *Pseudomonas* + *Glomus* in particular produced the highest plant height and shoot dry weight in all cultivars. In addition, the highest minituber yield in all cultivars was observed in plants treated with *Azotobacter* + *Bacillus* + *Glomus*. Furthermore, the results of path analysis indicated that minituber size and number of minitubers had positive and high direct effects on minituber yield of potato cultivars. The results of this study suggest that PGPR and AMF have the potential to increase growth and minituber yield of potato cultivars under organic growing conditions.

**KEYWORDS:** Azotobacter, Bacillus, biological fertilizer, minituber, potato, Pseudomonas

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## Introduction

Intensive farming practices require extensive use of inputs such as synthetic compounds and chemical fertilizers to achieve high yield and yield quality. However, overuse of fertilizers is costly and causes unanticipated environmental impacts. Therefore, recently interest in environmental friendly, sustainable and organic farming is increasing (Esitken et al. 2005).

Organic agriculture is an eco-friendly production system, which avoids or largely inhibits the use of synthetically compounded fertilizers and sustain the health of soils, ecosystem and people (Lind et al. 2003). Uses of biofertilizers including beneficial microorganisms as an alternative of synthetic compounds are known to increase plant growth via supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). To date, considerable number of bacterial and fungal species largely associated with the plant rhizosphere and the interior of the plant, have been tested and found to exert beneficial effects on plant growth and yield as well as crop quality (Khalid et al. 2004; Egamberdiyeva 2007). They have been called 'Plant growth Promoting Rhizobacteria (PGPR)' and 'Arbuscular Mycorrhizal Fungi (AMF)' including the strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholdria*, *Enterobacter*, *Erwinia*, *Flavo bacterium*, *Rhizobium*, *Serrotia* and *Glumus* (Sturz and Nowak 2000; Sudhakar et al. 2000; Glick et al. 2007). Among these bacterial and fungal species fluorescent *Pseudomonas, Azotobacter*, *Bacillus* and *Glumus* have high efficiency in host root colonization and plant growth metabolites production in many plants and are known to colonize the rhizosphere of potato, wheat, maize, grasses, pea and cucumber (Brown and Rovira 1976; Howie and Echandi 1983; Khalid et al. 2004).

The mechanism by which PGPR and AMF promote plant growth are not fully understood, but are thought to include: (1) synthesizing or changing the concentration of plant growth regulators (Bjorkman et al. 1998), (2) facilitating the uptake and availability of nutrients through atmospheric $N_2$ fixation, solubilization of phosphorus and synthesis of siderophores for iron sequestration (Ehrlich, 1990), (3) suppressing plant pathogens (Brierley 1985), and (4) reducing ethylene production, allowing plants to develop longer roots and better establish during early stages of growth (Glick et al. 1998). Plant growth promoting microorganisms can also enhance resistance to some environmental stresses such as flooding (Grichko and Glick 2001), drought (Mayak et al. 2004a), and salinity (Mayak et al. 2004b).

In a traditional potato production system, the potato is mainly propagated via seed tubers. This method has disadvantages in term of poor seed health and low rate of multiplication (Beukema and Van der Zaag1990; Struik and Wiersema 1999). Nowadays, micropropagation is a widely adopted alternative to conventional propagation of potatoes. In this method, potato can be rapidly multiplied using nodal cutting produced *in vitro*. By this technique, a large number of disease free *in vitro* plantlets with high quality and maximum health can be produced in a short period in a small facility year round (Struik and Lommen 1990). Recently, minituber production has become popular worldwide. This system includes two stages: (a) *in vitro* multiplication and production of *in vitro* plantlets, (b) production of minitubers in the greenhouse (Struik and Wiersema 1999). Many factors operating during the second stage, including variety, size of pots, growth regulators and plant density can affect minituber yield (Hagman1990). In addition, environmental conditions can alter the quality of the transplants, used to produce minitubers in the greenhouse. PGPRs during the second stage can enhance survival, growth and nutrition uptake and consequently increase minituber production of plantlets (Fortuna et al. 1996; Borkowska 2002).

Previous studies showed that PGPR and AFM stimulated growth and increased tuber yield in potato under field condition (Graham et al. 1976; McArthur and Knowles 1992, 1993;Vosátka and Gryndler 1999; Douds et al. 2007). Furthermore, many authors reported that inoculation of micropropagated potato plants with AMF during the transfer from in vitro condition might improve the viability of potato and their

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physiological state (Niemira et al. 1995; Yao et al. 2002). However, in our literature search, we found few reports covering the use of PGPR and AMF in potato minituber production. Therefore, in the present study an attempt is made to investigate the effects of single, dual and triad inoculation with different PGPR and AMF on three cultivars of micropropagated potatoes under greenhouse conditions.

## Materials and methods

The experimentwas conducted in a growth chamber and controlled greenhouse conditions at Agricultural Biotechnology Research Institute Central region of Iran (ABRICI) during January-July 2011.

### *Culture media and potato micropropagation*

Plant propagation material was taken from stock plants of potato cultivars Agria, Arinda and Marfona available in the gene bank of ABRICI. The plantlets were micro-propagated using single-node cutting. Nodal segments (1-1.5 cm) of each cultivar were cultured in sterilized culture vessels containing 50 ml of MS medium. The culture vessels were closed with caps, sealed with household plastic foil and placed in a growth chamber with a regime of 16 h light and 8 h darkness and a temperature of 25 ± 2°C. Light was provided by white fluorescent lamps at 3000-4000 lux.

### *Hardening of plantlets*

After 5 weeks, *in vitro* rooted plantlets were acclimatized. Roots of *in vitro* plantlets were rinsed with distilled water. The *in vitro* plantlets were then planted in pots containing sterilized peat mass and vermiculite (3:1, v:v). The pots were covered with a clear beaker with a few holes and were frequently watered to maintain a high humidity; they were kept in a phytotron for 2 weeks.

### *Bacterial strain, culture conditions and media*

Strains of bacteria, *Azotobacter chrooccum*, DSM-281 (N2- fixing bacterium), *Bacillus polymyxa,* PTCC1020 and *Pseudomonas putida*, CHAO (phosphate solubilizing bacterium), were obtained from Isfahan University, Department of Microbiology. The arbuscular mycorrhiza fungi (*Glomus intraradice*) were purchased as a commercial fertilizer from Biologic Manure Company, Hamedan, Iran. Bacteria were grown on nutrient agar (NA) for routine use, and maintained in nutrient broth (NB) with 15% glycerol at – 80°C for long-term storage. For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 500 ml flasks containing NB and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 28 ± 1°C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of $3 \times 10^8$ colony-forming unit (CFU) $ml^{-1}$, and the resulting suspensions were used to treat micropropagated plantlets.

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### *Set-up of greenhouse experiment and treatments*

Experiment was carried out in a temperature-regulated greenhouse set at a day/night temperature of 20/15°C, a relative humidity of 65% and a day length of 12 h. Seedlings were transplanted after hardening into pots containing 4 kg of a mixture sterilized field soil and sand (3:1, v/v). For preparation of sterile soil, field soil was autoclaved twice for 20 min at 120°C with a 24 h interval. Bacterial applications of *Azotobacter*, *Bacillus*, *Pseudomonas* and their combination were performed using dipping method in which plant roots were inoculated with the bacterial suspensions at the concentration of $3 \times 10^8$ CFU $ml^{-1}$. For treatments 5 ml of bacteria suspension were applied into 8 cm deep holes made in pots for transplanting. In AMF treatments, 250 g mycelium of *G. intraradices*, grown on sandy-loam soil, was placed into the same dibble hole as the bacterial suspensions placed into pots. Plants were irrigated as needed and adequate soil moisture was maintained through daily watering.

### *Harvesting and data collection*

The plants were harvested 3 months after the initiation of inoculation with PGPR and AMF. Growth promoting effects of bacterial and fungal treatments were evaluated by determining total minituber yield (g $m^{-2}$), average minituber size (g), number of minituber per plantlet, number of lateral shoots per plant, plants length (cm) and shoot dry weight (g plant $^{-1}$). Shoot dry weights of plants were determined after drying at 70°C for 72 h to constant weight.

### *Experimental design and statistical analysis*

Data were subjected to analysis of variance (ANOVA) based on a completely randomized design with 5 replications using SAS 9.2 (SAS Institute, Cary, NC, USA). The means were separated using Fisher's protected least significant differences (LSD) at 0.05 level of probability when the F-value was significant. Orthogonal independent comparisons were conducted within and between groups (i.e. single, dual and triad inoculation) and for their interaction with cultivars as well. Normality test of the data was performed with SPSS. The data demonstrated to be normal. Simple correlations were calculated and stepwise regression was followed to determine interrelationship between independent and dependent variables and further to reveal the first- and second -paths of predictor variables. The independent variables were grouped according to their contribution in yield considering minimal multicollinearity effect. Sequential path analysis was followed according to Samonte et al. (1998) to reveal the cause-effect relationship. The first order independent variables were the traits with highest regression coefficients in the stepwise regression. Consecutively, these independent traits were considered as dependent variables to the remaining traits and second stepwise regressions were performed to reveal the second order independent traits.

## Results and discussion

### *Plant height*

Plant height significantly affected by treatments, cultivar, cultivar × treatment and different groups of inoculation (Table 1). Although, inoculation of potato cultivars with PGPR and AMF increased plant height of all cultivars compared to control treatments, the highest increase was observed in cultivar Agria treated with *Azotobacter* + *Pseudomonas* + *Glomus*, which increased plant height by 85.5% compared

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to control treatment (Table 2). Results of the present study clearly indicated that in all cultivars, the highest increase in plant height was observed as a response to triad inoculation compared to uninoculated controls or to plants inoculated with single and dual inoculation (Table 2). There was also significant difference within each group (Table 1). The highest plant height in single inoculation in all cultivars was observed in plants treated with *Azotobacter.* In dual inoculation, combination of *Azotobacter* + *Glomus* and *Azotobacter* + *Pseudomonas* produced the highest plant height in all cultivars studied. The responses of cultivars to triad inoculation were also same and the highest plant height was observed in plants treated with *Azotobacter* + *Pseudomonas* + *Glomus*. On the other hand, in all cultivars the lowest plant height was recorded in plants treated with *Bacillus*, which is not significantly different from uninoculated controls. In accordance to our results, Shaalan (2005) reported that *Azotobacter* and *Pseudomonas* through increase in nutrient uptake could increase plant height of *Nigella sativa*. Brown and Burlingham (1968) also reported that height of wheat and barley increased in plants treated with *Azotocabter*. It seems that, inoculation of plant with PGPR through improvement of soil physical and chemical properties such as soil organic matter content and nitrogen availability could increase plant height. [Table 1 near here]

### *Shoot dry weight*

Shoot dry weight was significantly affected by treatments, cultivar, cultivar × treatment and different groups of inoculation (Table 1). Although, single, dual and triad inoculation increased shoot dry weight of all cultivars compared to control treatments, the highest increase was observed in cultivar Agria treated with *Azotobacter* + *Pseudomonas* + *Glomus*, which increased shoot dry weight by 287% compared to uninoculated controls. The similar trend was observed in cultivars Arinda and Marfona, which the highest shoot dry weight was observed in triad inoculation in general and in plants treated with *Azotobacter* + *Pseudomonas* + *Glomus* in particular. In dual inoculation the highest shoot dry weights in all cultivars was observed in plants treated with *Azotobacter* + *Pseudomonas*. The responses of cultivars to single inoculation were also similar and higher shoot dry weights was observed in plants treated with *Azotobacter* and *Pseudomonas*. Fayez et al. (1985) reported that *Azotobacter* through $N_2$ fixation as well as by enhancing the production of plant growth regulators such as gibberelines, cytokinins and auxins increased root development and consequently promoted nutrient uptake and plant biomass accumulation. The increase in biomass accumulation in plants treated with *Azotobacter* has been previously observed by many authors in different crops (Carletti et al. 1994; Sumana and Bagyaraj 2002; Yasari and Patwardhan 2007).

### *Number of lateral shoot*

Number of lateral shoot was significantly affected by treatments, cultivar, cultivar × treatment and different groups of inoculation (Table 1). The highest number of lateral shoot was observed in cultivar Arinda treated with *Azotobacter* + *Pseudomonas* + *Glomus*. In cultivars Arinda and Agria single inoculation could not significantly increase number of lateral shoot, while in cultivar Marfona except for *Azotobacter* all treatments significantly increased number of lateral shoots. In cultivar Ardina dual inoculation could not significantly increased number of lateral shoots compared to single inoculation or uninoculation control, while in cultivars Agria, *Azotobacter* + *Pseudomonas* and *Pseudomonas* + *Glomus* and in cultivar Marfona, *Azotobacter* + *Glomus* followed by *Azotobacter* + *Pseudomonas* significantly increased number of lateral shoots. There was a significant difference within triad group. In all cultivars studied, the highest number of lateral shoot was recorded in plants treated with *Azotobacter* + *Pseudomonas* + *Glomus*. Similar to our results, Singh and Kapoor (1998) report that the highest increase in lateral shoot of *Cicer arietinum* obtained in plantlets treated with combination of *Azotobacter* and *Pseudomonas*. Yasari and Patwardhan (2007) also observed that inoculation



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of Colza with *Azotobacter* and *Pseudomonas* increased number of lateral shoot by 12% compared to control plants.

### *Number of minitubers*

Analysis variance results indicated that number of minituber significantly affected by treatments, cultivar and different groups of inoculation (Table 1). In single inoculation, the highest minitubers number of potato cultivars was observed in plants treated with *Azotobacter*. The responses of cultivars to dual inoculation were also similar. The efficient inoculation in dual group was observed in plants treated with *Azotobacter* + *Pseudomonas* followed by *Pseudomonas* + *Glomus*. The highest number of minitubers in potato cultivars was observed in triad inoculation, which increased number of minituber by 121.4, 56.1 and 8.3% compared to control, single and dual inoculation, respectively. Similar to our results, Douds et al. (2007) observed that inoculation of potato with *Azotobacter* significantly increased number of potato tubers. Yao et al. (2002) report that potato plants treated with *Glumus* produced higher number of minituber than control.

### *Average size of minituber*

Minituber size was significantly affected by treatments, cultivar and different groups of inoculation (Table 1). The highest size of minituber in all cultivars was observed in triad inoculation in general and in plants treated with *Azotobacter* + *Bacillus* + *Glomus* in particular. Triad inoculation increased average minituber size of potato cultivars by 89, 38.1 and 10.2% compared to control, single and dual inoculation, respectively. There was significant difference within single and dual groups of inoculation, and the responses of cultivars were similar. In all cultivars, the highest minituber size in single and dual inoculation was observed in *Pseudomonas* and *Azotobacter* + *Pseudomonas*, respectively. In single inoculation, application of *Glomus* did not have a considerable effect on minituber size of potato cultivars. Similar to our results, Yao et al. (2002) reported that inoculation of potato with *Glomus* did not have significant effect on tuber weight.

### *Minituber yield*

Minituber yield was significantly affected by treatments, cultivar and different groups of inoculation (Table 1). In all cultivars studied the highest minituber yield was observed in triad inoculation, which increased minituber yield of potato cultivars by 205.4, 75.5 and 35.6% compared to control, single and dual inoculation, respectively. There was a significant different within each groups of inoculation. In single inoculation the highest minituber yield in cultivars Agria and Arinda was recorded in plants treated with Pseudomonas, while in cultivar Marfone the highest minituber yield was observed in Azotobacter treatment. The responses of cultivars to dual and triad inoculation were the same in all cultivars. The highest minituber yield in dual and triad inoculation was observed in plants treated with Azotobacter + Pseudomonas and Azotobacter + Bacillus + Glomus, respectively. It seems that inoculation of potato plantlets with plant growth promoting rhizobacteria through production of growth regulators as well as development of the root system and increase water and nutrients availability could increase minituber yield (Grichko and Glick 2001). It is also possible that biological treatments via some mechanisms such as dissolving of phosphate and siderophore production increased plant performance (Bjorkman et al. 1998). Celik et al. (2004) reported that application of PGPR improved chemical and physical characteristics of soil and thereby increased plant performance. They also revealed that combination application of biological treatments due to synergic effect of fungi and bacteria stimulate growth and ultimately increase plant



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performance. The positive effect of plant growth promoting rhizobacteria and fungi on plant performance has earlier been observed by many authors (Graham et al. 1976; Niemira et al. 1995; Vosátka and Gryndler 1999). [Table 2 near here]

### *Correlation between Traits*

The overall correlations among the traits are presented in Table 3. Minituber yield was highly and positively correlated with all of the measured traits. The highest value was observed between minituber yield and number of minituber ($0.91^{**}$) and minituber yield and minituber size ($0.87^{**}$). Therefore, it can be stated that the cultivars, which have more average tuber weight and number of minituber, have higher minituber yield too. Similar to our results, Galarreta et al (2006) determined significant correlation between tuber yield with tuber number and tuber yield. Yildirim et al (1997) observed that both tuber number and tuber weight were associated with tuber yield, but they indicated that tuber numbers were more important than average tuber weight. Plant height had a positive and significant correlation with the number of minituber ($0.62^{**}$) and shoot dry weight ($0.74^{**}$). Similarly, Lopez et al (1987) reported that plant height has a positive and significant correlation with number of tubers and tuber yield. [Table 3 near here]

### *Stepwise regression and sequential path analysis*

Processing of the data by the sequential path coefficient analysis enabled the partitioning of the direct and indirect effects of minituber yields components and identification of minituber yields attributes as selection criteria. For this purpose, Minituber yield was considered as the dependent variable against the rest of the traits and stepwise regression was performed (Tables 4, 5). [Tables 4 and 5 near here]. Minituber size, minituber weight and number of lateral shoots were kept in the model ($R^2$ =0.95) and path analysis was followed (Figure 1 and Table 6). All studied traits, could be organized based on their contribution to minituber yield (Figure. 1). The traits including number of minituber, minituber size and number of lateral shoot positively influenced the minituber yield and were established as first-order variables. Among these traits, number of minituber produced the highest direct effect (0.59) compared to other traits (Figure 1). [Figure 1 near here].

In accordance to our results, Maris (1988) found that tuber number and average tuber weight had equal effects on total tuber yield. Yildirim et al (1997) stated that average tuber weight and number of tubers had positive and high direct effects on tuber yield. [Table 6 near here]

Considering the path diagram (Figure. 1), shoot dry weight and plant height were established as second-order variables. Thus, shoot dry weight and plant height may be considered as the second variable in relation to minituber yield in potato. Shoot dry weight positively influenced number of minituber (0.43), minituber size (0.30) and number of lateral shoot (0.09). Plant height also had positive direct effects on number of minituber (0.16), minituber size (0.18) and number of lateral shoot (0.09). Among first order variables, there were some indirect effects for number of miniyuber through minituber size (0.34) and number of lateral shoot (0.34) on minituber yield (Table 6). These results show that number of minituber is one of the most important agronomic traits to predict minituber yield in potato. Middling second-order variables, indirect effects of plant height through shoot dry weight on number of minituber (0.28), minituber size (0.23) and number of lateral shoot (0.17) were positive (Table 6).

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Table 1. A synopsis of analysis of variance (ANOVA) plant height (PH), shoot dry weight (SDW), number of lateral shoots (NLS), number of minituber (NM), minituber size (MS), minituber weight (MW) and mibituber yield (MY) of three *S.tuberosum* cultivars when micropropagated seedlings were allowed to grow for 6-weeks under normal or biological treatments

| **Source of variation** | **d.f** | **Mean square** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **PH** | **SDW** | **NLS** | **NM** | **MS** | **MY** |
| Treatments | 11 | 500.74$^{***}$ | 8.62$^{***}$ | 8.16$^{***}$ | 29.00$^{***}$ | 23.00$^{***}$ | 356254.83$^{**}$ |
| Single inoculation | 3 | 446.99$^{***}$ | 2.17$^{***}$ | 0.08$^{ns}$ | 6.06$^{***}$ | 13.73$^{***}$ | 43038.88$^{***}$ |
| Dual inoculation | 4 | 267.58$^{***}$ | 4.63$^{***}$ | 3.11$^{***}$ | 1.42$^{*}$ | 2.61$^{***}$ | 34930.22$^{***}$ |
| Triad inoculation | 1 | 154.13$^{*}$ | 7.98$^{***}$ | 3.33$^{*}$ | 4.80$^{*}$ | 0.90$^{ns}$ | 29776.50$^{*}$ |
| Between Groups | 3 | 980.92$^{***}$ | 20.61$^{***}$ | 24.57$^{***}$ | 96.77$^{***}$ | 67.08$^{***}$ | 1206729.72$^{}$ |
| Cultivars | 2 | 313.37$^{***}$ | 0.82$^{*}$ | 1.07$^{*}$ | 2.31$^{*}$ | 0.32$^{ns}$ | 37717.10$^{***}$ |
| Cultivars × Treatment | 22 | 60.90$^{***}$ | 0.64$^{***}$ | 0.78$^{*}$ | 0.55$^{ns}$ | 0.87$^{ns}$ | 4880.97$^{ns}$ |
| Cultivar × Single inoculation | 6 | 72.99$^{*}$ | 0.16$^{ns}$ | 0.2$^{ns}$ | 0.58$^{ns}$ | 0.53$^{ns}$ | 4987.59$^{ns}$ |
| Cultivar × Dual inoculation | 8 | 39.55$^{*}$ | 0.56$^{*}$ | 1.24$^{*}$ | 0.44$^{ns}$ | 0.84$^{ns}$ | 4931.98$^{ns}$ |
| Cultivar × Triad inoculation | 2 | 177.73$^{*}$ | 1.40$^{*}$ | 1.23$^{ns}$ | 1.90$^{ns}$ | 2.57$^{*}$ | 7452.46$^{ns}$ |
| Cultivar× Between Groups | 6 | 38.33$^{ns}$ | 0.99$^{*}$ | 0.59$^{ns}$ | 0.22$^{ns}$ | 0.68$^{ns}$ | 3849.20$^{ns}$ |
| Error | 144 | 23.01 | 0.26 | 0.46 | 0.66 | 0.69 | 5583.01 |

*$p$≤0.05,**$p$≤0.01,***$p$≤0.001, ns = non-significant

Table2- Effect of different biological treatments on plant height (PH), shoot dry weight (SDM), number of lateral shoots (NLS), number of minituber (NM), minituber size (MS) and minituber yield (MY**)** of *S.tuberosum* cultivars. Data are the mean of five replications.

| **Cultivars** | **Treatments** | | **Parameters** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **PH (cm)** | **SDW (g plant$^{-1}$)** | **NLS** | **NM** | **MS (g)** | **MY (g m$^{-2}$)** |
| Agria | Control | | 27.60 | 1.14 | 1.80 | 3.80 | 4.39 | 308.57 |
| | Single | *Glomus* | 38.80 | 1.84 | 1.80 | 4.60 | 4.69 | 375.34 |
| | | *Azotobacter* | 42.40 | 2.42 | 1.60 | 5.80 | 6.33 | 427.48 |
| | | *Pseudomonas* | 39.40 | 2.49 | 2.00 | 5.00 | 6.39 | 530.60 |
| | | *Bacillus* | 29.00 | 1.99 | 2.00 | 4.40 | 4.41 | 464.51 |
| | Dual | *Azotobacter* + *Bacillus* | 35.40 | 2.61 | 2.60 | 5.00 | 7.09 | 498.77 |
| | | *Azotobacter* + *Pseudomonas* | 43.40 | 4.06 | 3.00 | 6.60 | 7.97 | 657.57 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Azotobacter + Glomus* | 44.20 | 3.34 | 1.80 | 5.80 | 7.14 | 588.62 |
| | | *Bacillus + Glomus* | 31.60 | 2.05 | 1.60 | 5.80 | 5.93 | 556.94 |
| | | *Pseudomonas + Glomus* | 37.60 | 2.88 | 3.00 | 6.00 | 7.16 | 619.54 |
| | Triad | *Azotobacter + Pseudomonas + Glomus* | 51.20 | 4.41 | 3.80 | 8.20 | 8.01 | 717.40 |
| | | *Azotobacter + Bacillus + Glomus* | 42.00 | 2.96 | 3.40 | 8.40 | 7.15 | 843.00 |
| Arinda | Control | | 24.40 | 1.22 | 1.60 | 3.40 | 3.76 | 262.70 |
| | Single | *Glomus* | 28.20 | 1.82 | 1.60 | 5.00 | 4.39 | 380.75 |
| | | *Azotobacter* | 40.60 | 2.60 | 2.00 | 5.60 | 6.33 | 445.77 |
| | | *Pseudomonas* | 39.80 | 2.30 | 1.80 | 5.00 | 6.34 | 521.34 |
| | | *Bacillus* | 26.00 | 2.15 | 1.60 | 4.00 | 5.04 | 425.80 |
| | Dual | *Azotobacter + Bacillus* | 33.60 | 2.64 | 2.40 | 5.40 | 6.89 | 530.74 |
| | | *Azotobacter + Pseudomonas* | 37.40 | 3.65 | 2.40 | 6.20 | 7.62 | 669.34 |
| | | *Azotobacter + Glomus* | 40.40 | 2.31 | 2.20 | 5.80 | 6.51 | 624.37 |
| | | *Bacillus + Glomus* | 28.80 | 2.08 | 2.00 | 5.50 | 7.10 | 588.14 |
| | | *Pseudomonas + Glomus* | 29.80 | 2.37 | 2.40 | 6.10 | 7.57 | 535.77 |
| | Triad | *Azotobacter + Pseudomonas + Glomus* | 44.20 | 3.90 | 4.20 | 7.40 | 8.04 | 767.22 |
| | | *Azotobacter + Bacillus + Glomus* | 34.60 | 2.43 | 2.80 | 8.60 | 7.29 | 792.40 |
| Marfona | Control | | 25.60 | 1.74 | 1.00 | 4.00 | 4.16 | 306.17 |
| | Single | *Glomus* | 31.20 | 1.85 | 2.00 | 5.40 | 4.69 | 430.99 |
| | | *Azotobacter* | 41.20 | 3.01 | 1.80 | 6.00 | 6.22 | 547.34 |
| | | *Pseudomonas* | 32.20 | 2.46 | 2.00 | 4.40 | 6.95 | 522.51 |
| | | *Bacillus* | 29.80 | 1.90 | 2.20 | 4.40 | 5.63 | 460.11 |
| | Dual | *Azotobacter + Bacillus* | 39.80 | 2.59 | 2.40 | 6.10 | 6.57 | 561.82 |
| | | *Azotobacter + Pseudomonas* | 43.60 | 3.23 | 3.40 | 6.70 | 7.43 | 660.08 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Azotobacter + Glomus* | 41.60 | 2.27 | 3.60 | 5.90 | 6.72 | 594.25 |
| | *Bacillus + Glomus* | 34.80 | 2.42 | 1.60 | 6.00 | 6.82 | 639.74 |
| | *Pseudomonas + Glomus* | 34.60 | 2.04 | 2.80 | 6.60 | 7.05 | 622.17 |
| Triad | *Azotobacter + Pseudomonas + Glomus* | 44.00 | 4.05 | 4.00 | 7.80 | 8.34 | 848.48 |
| | *Azotobacter + Bacillus + Glomus* | 38.80 | 3.88 | 3.40 | 9.20 | 7.70 | 886.74 |
| LSD (P≤0.05) | | 5.99 | 0.64 | 0.85 | 1.01 | 1.04 | 93.40 |
| CV | | 13.2 | 20.03 | 28.72 | 13.93 | 12.92 | 13.31 |

Table 3. Pearson correlation coefficients between six traits of three potato cultivars

| **Traits** | **PH** | **SDW** | **NLS** | **NM** | **MS** | **MY** |
|---|---|---|---|---|---|---|
| PH | 1 | | | | | |
| SDM | 0.74*** | 1 | | | | |
| NLS | 0.58*** | 0.69*** | 1 | | | |
| NM | 0.62*** | 0.72*** | 0.75*** | 1 | | |
| MS | 0.41* | 0.54*** | 0.61*** | 0.63*** | 1 | |
| MY | 0.54*** | 0.68*** | 0.75*** | 0.91*** | 0.87*** | 1 |

Where PH, Plant height; SDW, Shoot dry weight; NLS, Number of lateral shoots; NM, Number of minituber; MS, Minituber size; MY, Minituber yield.

Table 4- The results of regression and stepwise regression

| **Regression** | | **Stepwise regression** | | | | | |
|---|---|---|---|---|---|---|---|
| Variables of model | **t- value** | **Variables added to model** | | **Coefficients** | **SE** | **C(p)** | **$R^2$** | **F** |
| Intercept | -220.39 | + | | -230.22 | 13.53 | - | - | 289.17*** |
| NM | 72.43*** | + | | 71.29 | 2.10 | 945.19 | 71.19 | 439.91*** |
| MS | 62.51*** | + | | 62.05 | 2.04 | 9.34 | 95.29 | 905.40.76*** |
| NLS | -7.11ns | + | | -9.40 | 3.58 | 4.45 | 95.47 | 6.87*** |
| PH | -0.525ns | - | MSE | 1263.65 | | | | |
| SDW | -2.62ns | - | Error df | 176 | | | | |
| $R^2$ | 0.95 | MY= - 230.22 + 71.29 NM + 62.05 MS - 9.45 NLS | | | | | | |

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Where PH, Plant height; SDW, Shoot dry weight; NLS, Number of lateral shoots; NM, Number of minituber; MS, Minituber size

Table 5.The results of stepwise regression NM, MS and NLS in contrast to PH and SDW

| | | NM | | |
|---|---|---|---|---|
| Variables | **Model parameter** | **C(p)** | $R^2$ | **F** |
| PH | 0.111 | 44.01 | 93.96 | 227.69*** |
| SDW | 0.708 | 2 | 95.16 | 44.01*** |
| | | MS | | |
| Variables | Model parameter | C(p) | $R^2$ | F |
| PH | 0.129 | 26.59 | 93.47 | 218.43*** |
| SDW | 0.652 | 2 | 94.32 | 26.59*** |
| | | NLS | | |
| Variables | Model parameter | C(p) | $R^2$ | F |
| PH | 0.055 | 15.78 | 92.28 | 188.03*** |
| SDW | 0.233 | 2 | 92.91 | 15.78*** |

Where PH, Plant height; SDW, Shoot dry weight; NLS, Number of lateral shoots; NM, Number of minituber; MS, Minituber size.

Table 6- Indirect effects for the predictor variables grouped into first, second order variable

| | | MY | | | | NM | |
|---|---|---|---|---|---|---|---|
| | **NM** | **MS** | **NLS** | | | **SDW** | **PH** |
| **NM** | - | 0.277 | 0.027 | | **SDW** | - | 0.114 |
| **MS** | 0.347 | - | 0.026 | | **PH** | 0.283 | - |
| **NLS** | 0.341 | 0.260 | - | | | | |
| | | MS | | | | **NLS** | |
| | **SDW** | **PH** | | | | **SDW** | **PH** |
| **SDW** | - | 0.133 | | | **SDW** | - | 0.106 |
| **PH** | 0.230 | - | | | **PH** | 0.171 | - |

Where MY, Minituber yield ; NM, Number of minituber; MS, Minituber size; NLS, Number of lateral shoots; SDW, Shoot dry weight; PH, Plant height.

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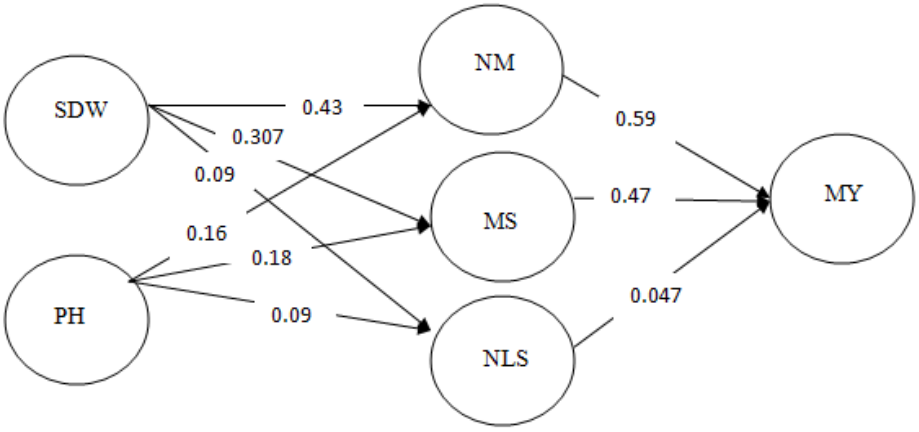


Figure 1.Sequential path analysis diagram depicting interrelationships between various traits contributing to minituber yield of potato. Where MY, minituber yield; Nm, number of minituber; MS, minituber size; NLS, Number of lateral shoot; SDW, Shoot dry weight; PH, Plant height.

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