

Potato plants micropropagated and grown from mini tubers: nutritional efficiency to phosphorus

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Abstract

One of the main concerns for the establishment of sustainable agriculture is the development of cultivars that absorb and use phosphorus (P) better. It is possible to produce quality seed potatoes from sprouts, microtubers, minitubers and minicuttings in closed off-soil growing systems. However, little is known if the origin of the propagating material interferes with the nutritional efficiency of phosphorus (P) by plants. Thus, the objective of this work was to compare the nutritional efficiency of P between plants produced from minitubers and micropropagation. For this, two experiments were carried out, one with plants produced from mini-tubers and the other from plants from micropropagation. Both experiments were carried out in a greenhouse and in a growing system without soil, using clones Asterix, Atlantic, SMIC 148-A and SMINIA 793101-3 and two levels of P in the nutrient solution (2.32 and 23.2 mg P L⁻¹). The propagative origin of potato plants changes with the biomass ratio between root and shoot and plants from minitubers have a higher harvest rate, higher utilization efficiency and a higher P response rate in P biomass production than plants from micropropagation. Therefore, we recommend that selection of PUE clones should occur with plants from mini-tubers, since the responses to PUE by plants from micropropagation are not representative, under the conditions tested in this work.

Introduction

One of the main concerns for the establishment of sustainable agriculture is the development of cultivars that can better absorb and use phosphorus (P) from the soil and maintain the current production of the crops (Wang et al., 2010). This fact is even more essential for the potato crop (*Solanum tuberosum* L.), which has a small root system (Pereira, 2003), high productive potential, relatively short cycle in addition to excellent market acceptance (Devaux et al., 2014). This causes high doses of phosphate fertilizers to be applied to achieve satisfactory yields, making production more expensive (Silva et al., 2015).

The multiplication systems used for seed potato production have received more attention. The substitution of conventional methods of obtaining seed potatoes in soil with closed off-soil growing systems has provided the production of mini-tubers with higher phytosanitary quality, standardization and increased the multiplication rate (Corrêa et al., 2008; Factor et al., 2007). In addition, they allow for staggering the harvest and better control of plant nutrition with appropriate nutrient solutions.

Recently, several propagation techniques have helped to obtain healthy seed potatoes (Bisognin and Dellai, 2015). In *in vitro* culture, it is possible to produce micropropagated plants and microtubers, which can be planted in a greenhouse or directly in the field (Kawakami and Iwama, 2012). Tubers harvested from these materials are called mini-tubers (Kawakami and Iwama, 2012) and are commonly used in pre-basic potato seed production programs (Bisognin and Dellai, 2015; Radouani and Lauer, 2015).

There are studies comparing the productivity of plants produced from seed potatoes, sprouts, microtubers, minitubers and mini-cuttings (Bisognin et al., 2015; Bisognin and Dellai, 2015; Kawakami et al., 2005; Kawakami and Iwama, 2012; Radouani and Lauer, 2015; Rykaczewska, 2015; Silva et al., 2006). However, if the origin of the propagating material interferes with the nutritional efficiency of P by plants, little is known. If the PUE in potato clones from micropropagated plants and cultivated in a semi-hydroponic system is representative of the PUE of plants from mini-tubers, the selection process for the most

efficient clones would be optimized. Thus, the objective of this work was to compare the nutritional efficiency to P between plants grown from minitubers and micropropagation in a closed off-soil growing system using sand as a substrate.

Results and discussion

Environments with P restriction induce plants to develop strategies that aim to maintain normal growth until the supply of P is restored (Espindula et al., 2009). One of these adaptive strategies is the increase in the redistribution of P from the shoot to the roots, with a consequent increase in the distribution of biomass between the roots and the shoot. Under a low level of P, it was observed that the Asterix and SMIC 148-A clones propagated from minitubers showed an increase of 82 and 139% in this relationship (Figure 1a). However, the origin of the propagating material seemed to influence the strategy that the plant uses in conditions of P restriction, since, in plants originating from micropropagation, the Asterix and SMIC 148-A clones showed no difference between the P levels while the clones Atlantic and SMINIA 793101-3 showed a higher root-to-shoot ratio when submitted to a high level of P (Figure 1b). Possibly because the demand for P for growth and development must have been low and were supplied by the P reserves of continuous in plant tissues. This proportion between the dry mass of roots and the shoot, as well as the proportion of P distributed between these organs, are important factors and deserve attention because they interfere in the efficiency of P use.

The efficiency of nutrient use corresponds to the ability of a plant to produce biomass through a specific concentration of the absorbed nutrient (Maia et al., 2011). With regard to the P utilization efficiency (PUE) of plants from minitubers, the clones Asterix, Atlantic and SMIC 148-A showed a difference between the levels of P, and in the low level of P, the PUE was 120, 830 and 307% higher, respectively than that at the high level of P, while the SMINIA 793101-3 clone decreases the PUE under low level of P by 78.5% (Figure 1c). When the plants came from micropropagation, the Asterix clone showed no difference in the PUE with the variation of the P supply and the Atlantic clone showed a 64% higher PUE at the high level of P compared to the low one (Figure 1d). On the other hand, SMIC 148-A and SMINIA 793101-3 clones showed higher PUE at low level, being 84.5 and 150% respectively. In general, it is expected that as the concentration of P in the nutrient solution increases, the PUE index will decrease for the production of dry mass according to the luxury consumption of the nutrient (Gill et al., 1992). However, such contrasting results in the PUE for the same clone were not expected due to the propagative origin (Figure 1c, d).

The greater the use of the nutrient in order to maximize the efficiency of use and, thus, optimize the productivity of the crop, the greater the savings in resources and the sustainability of the productive system can be (Veneklass et al., 2012). When the plants came from minitubers, at the low level of P, the Atlantic clone was the most efficient ($6.8\text{g}^2\text{mg}^{-1}$) and at the high level of the P, SMINIA 793101-3 clone ($12.3\text{g}^2\text{mg}^{-1}$) (Figure 1c). When the plants came from micropropagation, at the low level of P the clone SMIC 148-A was the most efficient ($1.3\text{g}^2\text{mg}^{-1}$) and at the high level of P was the clone Atlantic ($1.2\text{g}^2\text{mg}^{-1}$) (Figure 1d). Therefore, potato plants made better use of P when they came from

mini tubers. Thus, the origin of the propagating material must be considered when selecting the clones with best PUE in closed off-soil growing.

The harvest index (HI) consists of the biomass fraction of the tubers produced in relation to the total dry mass of the plant, that is, a measure of the efficiency of the transport of photoassimilates to the tubers. When the plants came from minitubers, only the SMIC 148-A clone showed a difference in HI between the levels of P, going from 0.6 in the low to 0.5 in the high level of P (Figure 1e). In the low level of P, the highest HI was found for Atlantic (0.7) and SMIC 148-A (0.6) clones. At the high level of P, the Atlantic clone was the most efficient in converting photoassimilates into material of economic importance (0.7). However, when the plants came from micropropagation, the HI for all clones was change by the P level (Figure 1f). The Asterix, Atlantic and SMIC 148-A clones showed higher HI at the low level of P, respectively, increased from 0.2, 0.4 and 0.4 to 0.1, 0.3 and 0.3 at the high level of P, while the SMINIA 793101-3 clone, which at the low level of P had a HI of 0.4, went to 0.5 at the high level of P. This shows that the origin of the propagating material also influences the HI, and the clones present higher HI when coming from minitubers. These responses are due to the different initial vigor presented by the plants due to the origin of the propagating material (Figure 2). Plants from minitubers have a higher initial vigor, which provides a quick establishment of the root system and thus better conditions for plant growth and development.

When considering the choice of potato clones that produce more biomass per unit of absorbed P or that produce more biomass with less consumption of P, we will be contributing to the sustainability of the potato production chain. Thus, it is preferable to choose the Asterix and Atlantic clones that obtained the highest P response rates in the production of fresh tuber mass (RITFM) when the plants originated from minitubers, producing 0.780 and $0.552\text{g mg tubers}^{-1}\text{ P}$, respectively (Table 1). Even when coming from minitubers, the Asterix clone was the clone that showed the highest P response rate in the production of total dry mass (RIDMTtotal), $0.246\text{ g of dry mass mg}^{-1}\text{ P}$. When the plants came from micropropagation, the SMIC 148-A clone showed the highest production of tuber fresh mass and total dry mass per unit of absorbed P, 0.298 and $0.139\text{g mg}^{-1}\text{ P}$, respectively. These results confirm that the propagative origin of potato plants interferes in the optimization of the use of phosphate fertilizers and should be considered in the selection of clones aiming at nutritional efficiency for P.

Materials and methods

Plant materials

Two experiments were carried out simultaneously in a greenhouse in the city of Santa Maria - RS ($29^{\circ} 42' 56''\text{S}$, $53^{\circ} 43' 13''\text{W}$ and 95m altitude), during the 2014 spring growing (09/12 to 11/05). The clones SMIC 148-A, SMINIA 793101-3 and the cultivars Asterix and Atlantic (which for simplification purposes will also be referred to as clones) were evaluated.

A part of the plant material was obtained by mini tubers, which were planted in 200ml plastic cups containing sand, remaining in this system for 30 days when the plants were detached from the parent tubers. Another part was obtained by micropropagated potato plants and inoculated in standard MS culture medium (Murashige and Skoog, 1962), kept in a growth room with a temperature of $25 \pm 2^{\circ}\text{C}$ and a

Table 1. Response index to P in the tuber fresh mass and in the total dry mass of the plant in potato clones from mini-tubers and micropropagation in off-soil growing. Santa Maria, RS, 2017.

Propagative Origin	Mini-tuber		Micropropagation	
Clone	Response Index to P in the TFM (RITFM) (g TFM mg P ⁻¹)			
Asterix	0,780	a	-0,066	b
Atlantic	0,552	a	-0,023	b
SMIC 148-A	0,101	b	0,298	a
SMINIA 793101-3	0,183	b	0,053	b
Average	0,404		0,065	
CV (%)	58,26		127,6	
	Response Index to P in the TDM (RIDMTotal) (g TDM mg P ⁻¹)			
Asterix	0,246	a	-0,032	d
Atlantic	0,056	c	0,076	b
SMIC 148-A	0,132	b	0,139	a
SMINIA 793101-3	0,036	c	0,023	c
Average	0,118		0,051	
CV (%)	19,25		22,27	

^aValues followed by equal lower-case letters in the columns for each propagation source do not differ significantly by P≤0.05 by the Scott-Knott test.

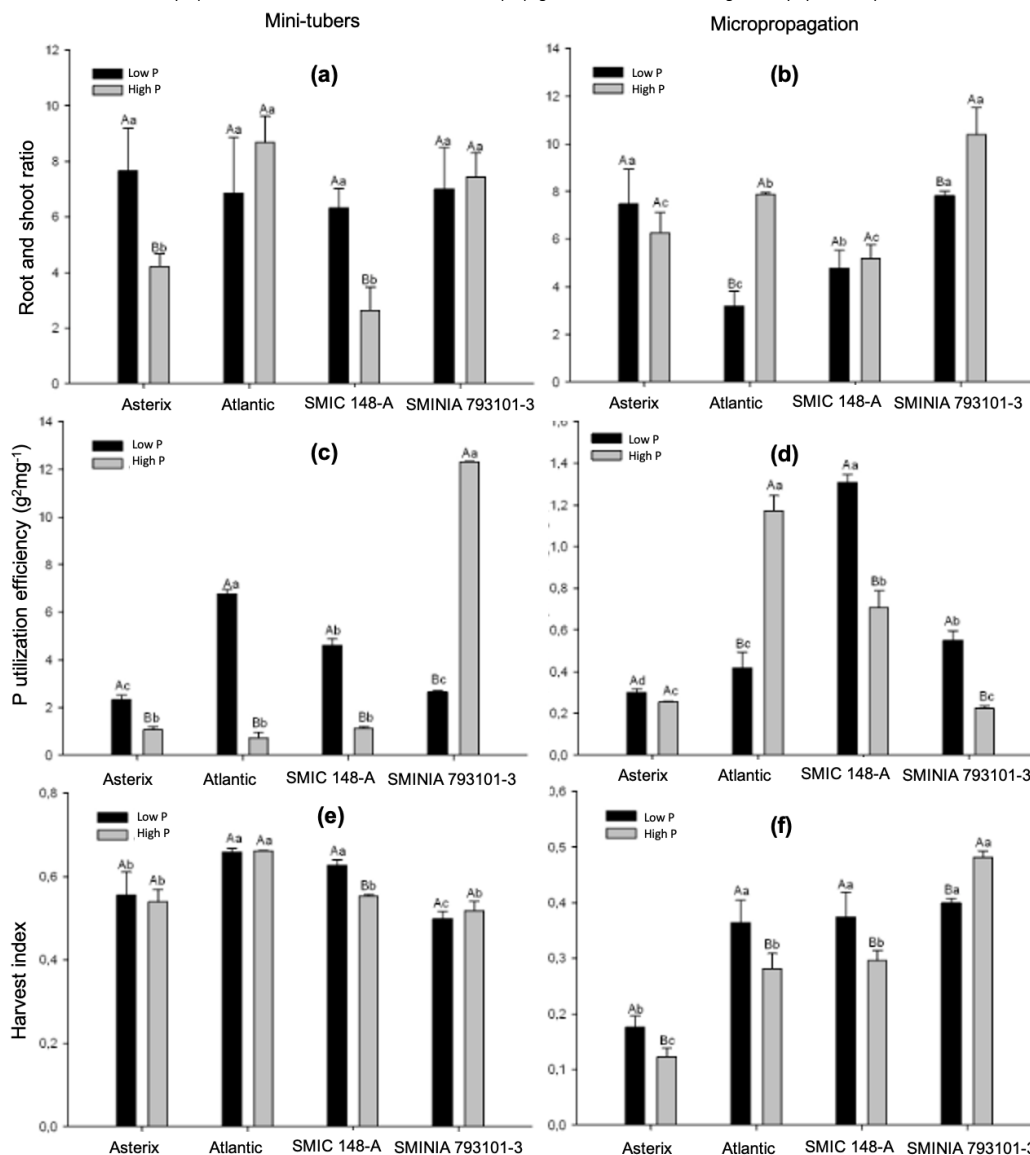


Fig 1. Effect of P on the biomass ratio between roots and shoots, on the P utilization efficiency and on the harvest index in potato clones from mini-tubers (A, C, E) and micropropagation (B, D, F) in off-soil growing. Santa Maria, RS, 2017. ^aUppercase letters indicate comparison between levels of P for each clone and lowercase letters indicate comparison between clones at each level of P by the Scott-Knott test at 5% probability of error.



Fig 2. Micropropagated potato plants (a) and plants from minitubers (b) at the time of transplanting to the growing bed. Santa Maria, RS, 2017.

photoperiod of 16h for 14 days and later acclimatized for another 10 days in a semi-hydroponic system and were ready to be transplanted to the definitive system. The plants produced from minitubers and micropropagation at the time of transplantation to the growing bed had between 4 and 6 leaves with average shoot length varying between 3 and 8 cm pl^{-1} (Figure 2).

Experimental design and conduction of study

The off-soil growing system using sand as a substrate (Bandinelli et al., 2013), consisted of trays and sand, previously washed once with sodium hypochlorite and three times with tap water. During the day, three irrigations were carried out with a nutritive solution, each lasting 15 minutes, with the aid of a digital programmer and a low-flow pump so that the entire substrate was saturated with solution. The excess solution was drained through a hole located at the base of the tray. After that period, the plants were transplanted to a sand growing system similar to the one used for acclimatization, where twelve plants remained in each tray in a 10 by 10 cm spacing.

The phosphorus treatments consisted of 5 and 50% of the standard concentration of P in the nutrient solution described by Bisognin et al. (2015) for potato off-soil growing, called low ($2.32mg P L^{-1}$) and high ($23.2mg P L^{-1}$) P levels in this work. To maintain the potassium content of the standard solution, KCl was used. The electrical conductivity (EC) was maintained at $2dS m^{-1} \pm 0.2$ (water was used to reduce the EC when necessary) and the pH at 5.7 adjusted every two days by adding HCl. Each experiment was carried out in a greenhouse, in a bifactorial (4 potato clones and 2 levels of P), in a randomized block design, with three replications. The experimental unit consisted of three plants.

Characters measured

At 42 days after transplanting (DAT) of plants from minitubers and 30 DAT of plants from micropropagation (10/22/2014), the tubers fresh mass (TFM) and the total dry

mass of the plant (TDM) were evaluated after drying the material for 15 days in an oven at 65 °C. Samples of the dry mass were weighed, macerated to perform the analysis of the total P content in the tissues according to Tedesco et al. (1995). After, 0.2 g of tissue was digested with 0.7 g of digestion mixture (100 g of Na_2SO_4 , 10 g of $CuSO_4 \cdot 5H_2O$ and 1 g of selenium) in sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2) remaining in block digestion for one hour at 350 °C. The determination of P in the plant extract was performed by colorimetry, according to Murphy and Riley (1962).

The accumulation of P was obtained by the product between the content of P in the tissue and the TDM. Once these data were obtained, the P utilization efficiency index (PUE) was estimated: (total dry mass of the plant) $2/P$ accumulation in the entire plant, according to Siddiqi and Glass (1981); the response index to tuber fresh mass (IRMFT): (difference between tuber productivity at the two levels of P)/(difference between the levels of P used), according to Fox (1978); and the response index to the total dry mass of the plant (RIDMTotal): (difference between the total dry mass of the plant at the two levels of P)/(difference between the levels of P used), according to Fox (1978).

Statistical analysis

The statistical analysis of the data for each experiment was performed using the Sisvar 5.3 software (Ferreira, 2011). Analysis of variance and comparison of means were performed using the Skott-Knott test (Scott and Knott, 1974) at 5% probability.

Conclusion

The propagative origin of potato plants interferes with the biomass ratio between root and shoot and plants from minitubers have a higher harvest index, greater P utilization efficiency and a higher rate of response to P in biomass production than plants from micropropagation. Therefore,

we recommend that selection of PUE clones should occur with plants from mini-tubers, since the responses to PUE by plants from micropropagation are not representative, under the conditions tested in this work.

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References

- Bandinelli MG, Bisognin DA, Gnolato FS, Mambrin RB, Sausen D, Nicoloso FT (2013) Concentração dos sais e da sacarose do meio MS na multiplicação *in vitro* e na aclimatização de batata. *Hortic Bras.* 31:242-247.
- Bisognin DA, Dellai J (2015) Shoot growth restriction in dry matter partitioning and minituber production of potato plants. *Ciênc Rural.* 45:1917-1924.
- Bisognin DA, Bandinelli MG, Kielse P, Fischer H (2015) Rooting potential of mini-cuttings for the production of potato plantlets. *A J Plant Sci.* 6:366-371.
- Corrêa RM, Pinto JEBP, Pinto CABP, Faquin V, Reis ES, Monteiro AB, Dyerd WE (2008) Acomparação de potato seed tuber yields in beds, pots and hydroponic systems. *Scien Hortic.* 116:17-20.
- Devaux A, Kromann P, Ortiz O (2014) Potatoes for sustainable global food security. *Potato Res.* 57:185-199.
- Espindula LF, Minella E, Delatorre CA (2009) Low-P tolerance mechanisms and differential gene expression in contrasting wheat genotypes. *Pesq Agropec Bras.* 44:1100-1105.
- Factor TL, Araujo JAC, Kawakami FPC, Iunck V (2007) Produção de minitubérculos básicos de batata em três sistemas hidropônicos. *Hortic Bras.* 25:082-087.
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Ciênc Agrotec.* 35:1039-1042.
- Fox RH (1978) Selection for phosphorus efficiency in corn. *Commun Soil Sci Plant Analysis.* 9:13-37.
- Gill MA, Salim RM, Zia MS (1992) Maize growth and uptake of phosphate and copper at different ambient phosphate concentrations. *Soil Sci Plant Nutri.* 8:631-636.
- Kawakami J, Iwama K, Jitsuyama Y (2005) Effects of planting date on the growth and yield of two potato cultivars grown from microtubers and conventional seed tubers. *Plant Prod Sci.* 8:74-78.
- Kawakami J, Iwama K (2012) Effect of potato microtuber size on the growth and yield performance of field grown plants. *Plant Prod Sci.* 15:144-148.
- Maia C, DoVale JC, Fritsche-Neto R, Cavatte PC, Miranda GV (2011) The difference between breeding for nutrient use efficiency and for nutrient stress tolerance. *Crop Breeding Applied Biotech.* 11:270-275.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Phys Plantarum.* 15:473-497.
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Analy Chimica A.* 27:31-36.
- Pereira AS (2003) Melhoramento genético. In: Pereira AS, Daniels J (ed.) O cultivo da batata na Região Sul do Brasil. Brasília. Embrapa. 105-124.
- Radouani A, Lauer FI (2015) Field performance of cultivars Nicola and Russet Burbank micro and minitubers. *A J Potato Resear.* 92:298-302.
- Rykaczewska K (2015) The effect of high temperature occurring in subsequent stages of plant development on potato yield and tuber physiological defects. *A J Potato Resear.* 92:339-349.
- Scott AJ, Knott M (1974) A cluster analysis method for grouping means in the analysis of variance. *Biometrics.* 30:507-512.
- Siddiqi MY, Glass ADM (1981) Utilization index: a modified approach to the estimation and comparison of nutrient efficiency in plants. *J Plant Nutr.* 4:289-302.
- Silva EC, Giusto AB, Dias JACS (2006) Produção de minitubérculos a partir de brotos de cultivares de batata em diferentes combinações de substratos. *Hortic. Bras.* 24:241-244.
- Silva GO, Pereira AS, Nazareno NRX, Ponijaleki R (2015) Desempenho de clones elite de batata para caracteres agrônômicos e de qualidade industrial. *R Ceres.* 62:71-77.
- Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Wolkweiss SJ (1995) Análise de solo, plantas e outros materiais. Porto Alegre. 174.
- Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible W, Shane MW, White PJ, Raven JA (2012) Opportunities for improving phosphorus-use efficiency in crop plants. *New Phyt.* 195:306-320.
- Wang J, Shen J, Liao H (2010) Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Sci.* 179:302-306.