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Physiological marker of phosphorus nutritional status in potato

Marcador fisiológico do estado nutricional do fósforo em batata

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A B S T R A C T

The development of more efficient plants in the use of nutrients will be fundamental for agriculture in the coming years. The objective of this work was to define a physiological marker of the nutritional status of P for potato clones cultivated soilless and *in vitro*, in order to facilitate the selection process of more efficient clones in the use of P. For this, two experiments were carried out with seven potato clones (SMIC 148-A, Dakota Rose, SMINIA 793101-3, SMIB 106-7, SMIF 212-3, SMIJ 319-1 and P 150) grown at two levels of P (low and high) in the culture systems in vitro (1.935 and 19.346mg P L^{-1}) and in soilless (2.32 and 23.2mg P L^{-1}) using sand as substrate. The inorganic P content and the total accumulation of P in shoot and roots seem to be good indicators of the nutritional status of P in potato plants, both in soilless and *in vitro* cultivation. The activity of the acidic phosphatase enzymes (APases) in the tissues of the potato depends very much on the culture system, the clone and the organ of the analyzed plant, making it difficult to choose it as a physiological marker for restriction of P in potatoes. The dry mass of the potato plant may be a good indicator of the nutritional status of P in clones grown soilless.

Keywords: acid phosphatases, *in vitro* cultivation, mineral nutrition, soilless cultivation, *Solanum tuberosum* L.

R E S U M O

O desenvolvimento de plantas mais eficientes no uso de nutrientes será fundamental para a agricultura nos próximos anos. O objetivo deste trabalho foi definir um marcador fisiológico do status nutricional do P para clones de batata cultivados fora do solo e *in vitro*, visando facilitar o processo de seleção de clones mais eficientes no uso de P. Para tanto, foram realizados dois experimentos com sete clones de batata (SMIC 148-A, Dakota Rose, SMINIA 793101-3, SMIB 106-7, SMIF 212-3, SMIJ 319-1 e P 150) cultivados em dois níveis de P (baixo e alto) no sistema de cultivo in vitro (1,935 e 19,346mg P L^{-1}) e no cultivo fora do solo (2,32 e 23,2mg P L^{-1}) usando areia como substrato. O teor de P inorgânico e a acumulação total de P na parte aérea e nas raízes parecem ser bons indicadores da condição nutricional de P em plantas de batata, tanto no cultivo fora do solo quanto no in vitro. A atividade das enzimas fosfatases ácidas (APases) nos tecidos da batata depende muito do sistema de cultivo, do clone e do órgão da planta analisado, dificultando a escolha deste como um marcador fisiológico para restrição de P em batata. A massa seca da planta de batata pode ser um bom indicador do status nutricional do P em clones cultivados fora do solo.

Palavras-chave: cultivo fora do solo, cultivo *in vitro*, fosfatases ácidas, nutrição mineral, *Solanum tuberosum* L.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the main commodities produced in Brazil. 100,000 hectares of potatoes are grown annually, which are destined for the fresh market (65%), the chip industry (15%), the pre-fried industry (12%) and seed potatoes (8%) (Abba, 2021). For the ideal growth of the potato plant and for high tuber productivity to be achieved, P must be available in the soil and the plant must be able to absorb and use this element (Fernandes and Sorotto, 2012).

Under conditions of limited phosphorus (P) availability, plants may promote: (i) morphological and architectural changes in the root system, such as reduction of main root extension, increase in number and extension of secondary, tertiary and root hair, aiming to increase the acquisition of P, associated with mycorrhizal fungi to increase the absorption of P; and/or (ii) biochemical and biological alterations such as increased synthesis of Pi transporters, exudation of organic acids and acid phosphatases (APases), which modify the chemistry of the rhizosphere, as well as (Lynch and Brown, 2008; Brown *et al*., 2013).

The increase in activity (Duff *et al*., 1994) and the expression (Ma *et al*., 2009) of APases is an adaptive response of plants, known to be induced in response to stress in the restriction condition of phosphorus (P). These proteins are important in the production, transport and recycling of soluble inorganic P (Pi). Thus, APases may have different metabolic functions and be found in the cell wall and / or be secreted by the roots in the rhizosphere, as well as in the intracellular environment, especially in the vacuole (Tran *et al*., 2010). Intracellular APases usually control internal Pi homeostasis, whereas secreted APases control the external acquisition of Pi (Duff *et al*., 1994). Thus, APases play an important role in the supply and metabolism of Pi for the maintenance of cellular metabolism (Tejera García *et al*., 2004; Tabaldi *et al*., 2007).

The production of dry matter and the accumulation of P distributed between roots and shoot of a plant have been studied and considered as factors in determining the efficiency of absorption and utilization of P (Machado *et al*., 2001). The nutritional limitation causes a greater reduction in shoot than in root growth (Hermans *et al*., 2006). This fact demonstrates that roots become preferential drains of photoassimilates, as observed in several species cultivated in soil and in nutrient solution (Vance *et al*., 2003). Thus, in order to select genotypes that are most efficient in the uptake and / or utilization of P, this work aimed to define a physiological marker of the nutritional status of P for clones of potatoes cultivated soilless and *in vitro*, in order to facilitate the selection process of potato clones that are more efficient in the use of P.

MATERIAL AND METHODS

The potato clones used in the experiments were from the Potato Genetics and Breeding Program of the Federal University of Santa Maria: SMIC 148-A, Dakota Rose, SMINIA 793101-3, SMIB 106-7, SMIF 212-3, SMIJ 319- 1 and P 150 and were derived from micropropagated seedlings in standard Murashige - Skoog (MS) culture medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose, 0.1 g L^{-1} myo-inositol and 6 g L^{-1} agar and maintained in a growth room under ideal conditions of growth (Sausen *et al*., 2020a), for 14 days.

In the first experiment, nodal segments of 1.0 cm long seedlings were innoculated in MS medium where they received P treatments consisting of 5 and 50% of the standard P concentration of the MS medium, called in this work of below (1,935 mg P L^{-1}) and high (19.35 mg P L^{-1}) levels of P, the source of P being KH_2PO_4, and to maintain the potassium (K) concentration, KCl was used. Thus, the concentrations of other nutrients were kept the same for both treatments. The experimental unit consisted of ten test tubes, each containing 10.0 mL of culture medium and three nodal segments with one gem and leaf (called explants), and four replicates were used in a completely randomized design (seven clones and two P levels). In other words, the average of 30 plants (experimental unit) in each repetition was evaluated, with four repetitions per treatment (120 plants), in each of the 14 treatments tested. The test tubes containing the explants were kept in a growth room with a temperature of 25 ± 2°C and photoperiod of 16 h (Sausen *et al*., 2020a). The plants were analyzed at 40

days after innoculated. At the end of the cultivation time, plant material was collected to analyze the soluble Pi concentration and the activity of the APases, as well as the total P concentration of the tissues and the dry mass of shoots and roots were evaluated. The material used to determine the dry mass was dried in an oven at 65°C until it reached constant weight and then the dry mass was determined.

In order to simulate a closer cultivation condition to that found in a potato crop, second experiment was conducted in a greenhouse in the city of Santa Maria in the state of Rio Grande do Sul - Brazil (29º 42 '56''S, 53º 43' 13''W and 95 m altitude), during the spring. In the period of the experiment, the average air temperature was 18.1ºC, the relative humidity 74.1% and the average wind speed 2.6 $m.s^{-1}$. Pre-micropropagated seedlings were acclimatized for 14 days in a soilless culture system, where the roots of the plants receive a nutrient solution containing all the nutrients essential for their growth and development, without waste (Sausen *et al*., 2020b), kept under shade cloth (60% light extinction) for 5 days. Afterwards, the plants were transplanted to a culture system composed of polyethylene and sand trays as substrate (Bandinelli *et al*., 2013), in which three irrigations were carried out with nutrient solution during the day, with a duration of 15 min each, with the aid of a digital programmer and a low - flow pump, so that the entire substrate was saturated with solution, maintaining the P of the treatment the plants. The excess solution was drained through a hole in the bottom of the tray, stored in a reservoir to return to the system at the next irrigation. Each tray had twelve plants spaced 10 by 10 cm apart. The treatments of P consisted of 5 and 50% of the standard concentration of P in the nutrient solution for the cultivation of soilless potatoes described by Bisognin *et al*. (2015), referred to in this work as low (2.32 mg P L^{-1}) and high (23.2 mg P L^{-1}) levels of P. To maintain the K concentration of the standard solution, KCl was used (Sausen *et al*., 2021). The electrical conductivity (EC) was maintained at 2 dS m^{-1} (water was used to reduce EC when necessary) and pH at 5.7 was adjusted every two days by the addition of HCl. The experiment was conducted in a two - factor scheme (seven clones and two P levels) in the randomized block design, using six replicates, the experimental unit consisted of the average of three plants. In other words, the average of 3 plants (experimental unit) in each repetition was evaluated, with six repetitions per treatment (18 plants), in each of the 14 treatments tested. At 40 days after planting (DAP), the activity of the APases and the concentration of Pi were analyzed. At 62 DAP, some clones showed visible signs of senescence beginning when cultivated at the low P level, and the evaluation of the total P concentration in leaves and roots tissues and the production of dry mass of leaves, stems, tubers and roots were carried out, after reaching constant weight in an oven at 65°C.

For the determination of APase activity in both experiments, the roots and leaves were frozen in liquid N_2, macerated and homogenized in 100 mM Tris-HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% albumin in a ratio of 1:3 and then centrifuged at 20,000 g for 30 min. The supernatant resulting from the centrifugation was used for the enzymatic assay. The acid phosphatase activity was determined according to Tabaldi *et al*. (2007) in a reaction medium containing 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffering, and pH 5.5 to a final volume of 200 µL. A 20 µL aliquot of the enzyme preparation was added to the reaction mixture, except for the control, and preincubated for 10 min at 35°C. The reaction was started by addition of substrate (3 mM PPi) and terminated by the addition of 200 µl of 10% TCA. The mean value of protein used in the calculations to determine APase activity was approximately 0.30 mg g^{-1} of fresh mass, because, after previous testing, there was no variation in protein concentration in the tissues between the doses of P tested. The concentration of total protein was quantified by the method of Bradford (1976).

The same material used in the APase assay was used to quantify the Pi concentration, using a standard curve constructed with KH_2PO_4. An aliquot of the diluted sample (800 mL) was incubated at 45 °C for 45 min in medium containing 2.5 N sulfuric acid, 4.8 mM ammonium molybdate and 35 mM ascorbic acid in a total volume of 1 mL. After cooling, the samples were read at 650 nm in a SF325NM spectrophotometer (Bel Engineering, Italy).

For the determination of total phosphorus concentration, three samples dry mass of shoots (*in vitro* cultivation), leaves (soilless cultivation) and roots of each treatment, of the two cultivation systems were macerate and subjected to acid digestion procedure in an open system with digestion block (Velp Scientifica, Model DK, Italy). Samples were preweighed and transferred to the decomposition vials. In each vial containing the samples were added 5mL of HNO_3 14 mol L^{-1}. The vials were capped and held at 140 °C for 2 h. After the digestion step, the samples were transferred to polypropylene vials and vented at 25 mL. The determination of P was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) on a Perkin Elmer spectrometer (Optima 4300 DV, USA) equipped with Gencone® nebulizer, cyclone type nebulizer chamber and quartz torch with injector of alumina with 2.0 mm of internal diameter.

The data of the two experiments was submitted to analysis of variance using the software Sisvar 5.3 (Ferreira, 2011). The means between clones and between levels of P were compared by the Scott - Knott test (Scott e Knott, 1974) at 5% probability.

RESULTS AND DISCUSSION

The variation of the soluble phosphorus concentration (Pi) in the tissues of the plant can express the nutritional status of the plant, considering that an extra accumulation would occur only when the requirements for growth have been reached. In *in vitro* and soilless cultivations, all potato clones presented a reduction in the concentration of Pi present in roots and shoots under low P levels, except for clone SMIC 148-A, which shows an increase of Pi in roots in *in vitro* culture (Table 1). When changes occur in the supply of P to plants, the concentration of Pi in the cytoplasm is regulated by its content in the vacuole (Bieleski and Ferguson, 1983). Vacuolar Pi has a reserve function, and if the amount of absorbed P is less than its demand for the cell it returns to the cytoplasm, taking into account the metabolic requirement of the cell. On the other hand, if there is less growth or development of cells and the supply of P in the external environment is abundant, the excess P uptake will be stored in the vacuole. Apparently, there is coordination in the mobilization of P between the different tissues in order to synchronize the growth and development of the plants and to avoid that these processes are paralyzed (Lin e*t al*., 2009).

Among the clones, the concentration of Pi in the shoot, independent of the P level, was higher for the clone P 150 (2.8 μmol Pi g $tissue^{-1}$) in the *in vitro* culture (Table 1) and for the clone SMINIA 793101-3 (2.8 μmol Pi g $tissue^{-1}$) in soilless cultivation (Table 2). In the roots, the concentration of Pi in the *in vitro* culture under low P levels was higher for the Dakota Rose clone without differing from the SMIC 148-A (1.25 μmol Pi g $tissue^{-1}$), and for the high level of P the highest Pi was determined in SMIB 106-7 (4.7 μmol Pi g $tissue^{-1}$) (Table 1). While in the soilless cultivation at high P, SMIC 148-A was the clone that concentrated more Pi in the roots (5.6 μmol Pi g $tissue^{-1}$), but at the low P level, it was not possible to differentiate clones (Table 2). In spite of this, the concentration of Pi in shoot and root appears to be a good indicator of the nutritional status of P in potato plants in both soilless and *in vitro* cultivation and can probably used to distinguish clones efficient in the use of P.

In conditions of restriction of P, the plants develop several biochemical and morphological adaptations. One of these adaptations is the increase in the synthesis or activity of the acidic phosphatase enzymes (APases), which aid in the release of Pi for plant metabolism (Tejera García *et al*., 2004). *In vitro* culture, the clones showed an increase in the activity of the APases in the shoots when cultivated in low P levels, except for the clone P 150 that had the highest activity of the enzyme in high level of P (Table 1). However, when the activity of APases in roots was analyzed, only the clones SMIC 148-A and SMIJ 319-1 maintained the greatest activity of the APases in the low level of P. Therefore, only for these clones the activity of APases is linked to a Pi remobilization strategy to maintain normal plant growth. In the soilless cultivation, the highest APase activity in leaves at low P levels occurred for SMIJ 319-1 clone, while at the high level of P, the Dakota Rose clone showed the highest APases activity in the leaves (Table 2).

Assuming the mean effect of P-rates, the APase activity in the *in vitro* culture was higher in

Table 1 - Effects of P levels on the inorganic P concentration in the shoot (Pi Shoot) and in the roots (Pi Root), in the APase activity in the shoot (APase Shoot) and in the roots (APase Root), in the total concentration of P in shoot dry mass (T Conc. S) and in root dry mass (T Conc. R), in the shoot dry mass (SDM) and root dry mass (RDM), of the potato clones evaluated at 40 days after inoculation in the *in vitro* culture with low (1.935 mg P L^{-1}) and high (19.346mg P L^{-1}) P in the culture medium

	Pi Shoot (μmol Pi g $tissue^{-1}$)			Pi Root (μmol Pi g $tissue^{-1}$)		
P levels	**Low**	**High**	**Means**	**Low**	**High**	**Means**
SMIC 148-A	0.506 bB	1.502 dA	1.004	1.182 aA	0.502 fB	0.842
Dakota Rose	0.230 cB	2.146 bA	1.188	1.304 aB	2.620 bA	1.962
SMINIA 793103-3	0.645 bB	1.775 cA	1.210	0.425 cB	1.562 dA	0.994
SMIB 106-7	0.404 bB	2.156 bA	1.280	0.859 bB	4.702 aA	2.781
SMIF 212-3	0.423 bB	1.841 cA	1.132	0.268 cB	0.951 eA	0.610
SMIJ 319-1	0.480 bB	1.970 bA	1.225	0.431 cB	2.342 cA	1.387
P 150	1.565 aB	2.776 aA	2.171	0.566 cB	2.324 cA	1.445
Means	0.608	2.024		0.719	2.143	
	APase Shoot (U.mg^{-1} protein)			**APase Root (U.mg^{-1} protein)**		
SMIC 148-A	663.01 cA	489.74 cB	576.4	853.75 aA	55.46 dB	454.61
Dakota Rose	587.28 dA	477.15 cB	532.2	217.45 cB	400.06 bA	308.76
SMINIA 793103-3	681.31 cA	530.11 cB	605.7	208.49 cB	574.13 aA	391.31
SMIB 106-7	836.43 bA	377.30 dB	606.9	589.14 bA	379.05 bB	484.10
SMIF 212-3	897.04 aA	636.52 aB	766.8	120.91 dB	252.37 cA	186.64
SMIJ 319-1	786.92 bA	587.36 bB	687.1	802.10 aA	285.10 cB	543.60
P 150	448.03 eB	648.95 aA	548.5	191.37 cB	381.22 bA	286.30
Means	700.00	535.30		426.17	332.48	
	T Conc. S (mg P SDM^{-1})			**T Conc. R (mg P RDM^{-1})**		
SMIC 148-A	2.02 bB	4.33 cA	3.17	2.34 aB	3.18 dA	2.76
Dakota Rose	1.94 bB	4.44 cA	3.19	2.41 aB	3.85 bA	3.13
SMINIA 793103-3	2.15 bB	4.60 cA	3.38	0.98 dB	3.50 cA	2.24
SMIB 106-7	1.98 bB	5.27 aA	3.63	2.06 bB	3.65 aA	2.86
SMIF 212-3	3.43 aB	4.95 bA	3.44	2.05 bB	3.83 bA	2.94
SMIJ 319-1	2.19 bB	4.29 cA	3.24	1.65 cB	3.55 cA	2.60
P 150	2.17 bB	4.98 bA	3.57	2.51 aB	4.05 aA	3.28
Means	2.27	4.69		2.00	3.66	
	Shoot Dry Mass (mg pl^{-1})			**Root Dry Mass (mg pl^{-1})**		
SMIC 148-A	3.73 aB	7.29 aA	5.51	1.54 aA	2.36 aA	1.95
Dakota Rose	1.79 aA	2.99 bA	2.39	1.02 aB	2.30 aA	1.66
SMINIA 793103-3	3.03 aB	6.13 aA	4.58	0.63 aB	1.73 aA	1.18
SMIB 106-7	2.87 aA	3.35 bA	3.11	0.49 aB	1.62 aA	1.06
SMIF 212-3	2.53 aB	5.77 aA	4.05	1.15 aA	1.85 aA	1.50
SMIJ 319-1	3.28 aB	5.31 aA	4.30	1.05 aA	1.49 aA	1.27
P 150	3.32 aA	4.18 bA	3.75	0.44 aA	1.13 aA	0.79
Means	2.93	4.97		1.56	1.13	

[a]For each variable, means followed by the same lowercase letter in the column do not differ significantly from each other by the Scott-Knott test, and means followed by the same capital letter in the line within each variable do not differ significantly from each other by the F-test at 5% probability.

shoot than in roots (Table 1), but in the soilless cultivation the opposite was observed (Table 2). This divergence may have occurred because P in soilless cultivation was less available for plants, compared to *in vitro* cultivation, in which there are no solid particles to support the roots, which demanding a greater action of APases in the roots to try to uptake more phosphorus from the substrate in soilless cultivation. Both extracellular and intracellular APases are regulated by the presence of P in the medium and the level of APases activity increases the greater the P deficiency (Ciereszko et al., 2017). In the *in vitro* culture, APases activity may be higher in the shoot than in the roots due to the remobilization processes of P internally, between cells and tissues of older sources for the younger (Zebrowska *et al.*, 2012; Ciereszko *et al.*, 2017).

Table 2 - Effects of P levels on the inorganic P concentration in the shoot (Pi Shoot) and in the roots (Pi Root), in the activity of the APases in the leaves (APase Leaf) and in the roots (APase Root), in the total concentration of P in the leaves dry mass (T. Conc. Leaf) and in the root dry mass (T. Conc. Root), in the dry mass of leaves (DM Leaf), stems (DM Stem), roots (DM Root), and tubers (DM Tuber), of the potato clones evaluated at 62 days after planting in the soilless cultivation with low (2.32 mg P L^{-1})

	Pi Shoot (μmol Pi g $tissue^{-1}$)			Pi Root (μmol Pi g $tissue^{-1}$)		
P Levels	**Low**	**High**	**Means**	**Low**	**High**	**Means**
SMIC 148- A	1.34 bB	2.37 bA	1.855	0.28 aB	5.59 aA	2.935
Dakota Rose	1.06 cB	1.99 cA	1.525	0.52 aB	4.38 bA	2.450
SMINIA 793103-3	1.60 aB	2.79 aA	2.195	0.60 aB	2.52 cA	1.560
SMIB 106-7	0.95 cB	2.30 bA	1.625	0.42 aB	4.77 bA	2.595
SMIF 212-3	1.03 cB	2.28 bA	1.655	0.38 aB	4.28 bA	2.330
SMIJ 319-1	1.41 bB	2.16 cA	1.785	0.32 aB	2.80 cA	1.560
P 150	1.27 bB	2.14 cA	1.705	0.25 aB	2.08 cA	1.165
Means	1.23	2.29		0.22	3.77	
	APase Leaf (U.mg^{-1}protein)			**APase Root (U.mg^{-1} protein)**		
SMIC 148- A	127.74 cA	116.97 cA	122.4	584.99 aB	656.28 aA	620.6
Dakota Rose	192.57 bB	289.08 aA	240.8	379.67 cA	424.07 bA	401.9
SMINIA 793103-3	168.10 bA	153.64 cA	160.9	333.66 cA	339.42 cA	336.5
SMIB 106-7	165.04 bA	116.48 cB	140.8	524.94 aA	483.49 bA	504.2
SMIF 212-3	149.00 cA	138.64 cA	143.8	456.98 bA	417.47 bA	437.2
SMIJ 319-1	251.27 aA	122.37 cB	186.8	474.31 bA	404.16 bB	439.2
P 150	128.99 cB	197.28 bA	163.1	340.52 cB	444.41 bA	392.5
Means	168.96	162.07		442.15	452.86	
	T. Conc. Leaf (mg P g LDM^{-1})			**T. Conc. Root (mg P g RDM^{-1})**		
SMIC 148- A	6.72 aB	90.57 bA	48.64	0.21 cB	1.90 bA	1.05
Dakota Rose	18.50 aB	51.98 cA	35.24	0.50 cB	1.58 bA	1.04
SMINIA 793103-3	7.21 aB	29.43 dA	18.32	0.22 cB	0.72 cA	0.47
SMIB 106-7	4.20 aB	47.69 cA	25.94	0.21 cB	1.10 cA	0.65
SMIF 212-3	3.13 aB	23.10 dA	13.11	0.11 cA	0.21 cA	0.16
SMIJ 319-1	11.36 aB	116.24 aA	63.80	3.71 aA	3.16 aB	3.43
P 150	5.02 aB	78.87 bA	41.94	1.88 bA	1.95 bA	1.91
Means	8.02	62.55		0.97	1.52	
	DM Leaf (g pl^{-1})			**DM Stem (g pl^{-1})**		
SMIC 148- A	7.39 cB	23.77 bA	15.58	1.19 cB	4.56 bA	2.87
Dakota Rose	9.44 bB	14.06 dA	11.75	1.72 bB	3.04 cA	2.38
SMINIA 793103-3	4.67 cB	9.38 eA	07.02	0.63 cA	1.28 dA	0.95
SMIB 106-7	4.80 cB	17.12 cA	10.96	0.78 cB	3.02 cA	1.90
SMIF 212-3	3.23 cB	7.17 eA	05.20	0.59 cA	1.07 dA	0.83
SMIJ 319-1	14.04 aB	40.88 aA	27.46	2.71 aB	14.55 aA	8.63
P 150	7.19 cB	22.25 bA	14.72	0.94 cB	4.25 bA	2.59
Means	7.25	19.23		1.22	4.54	
	DM Root (g pl^{-1})			**DM Tuber (g pl^{-1})**		
SMIC 148- A	0.30 dB	0.71 bA	0.505	5.02 bB	12.20 aA	08.61
Dakota Rose	0.76 bA	0.52 bB	0.640	9.03 aB	13.59 aA	11.31
SMINIA 793103-3	0.35 dA	0.25 cA	0.300	3.77 bB	8.02 bA	05.89
SMIB 106-7	0.30 dB	0.55 bA	0.425	5.25 bB	12.48 aA	08.86
SMIF 212-3	0.20 dA	0.31 cA	0.255	5.32 bA	6.54 bA	05.93
SMIJ 319-1	1.10aB	1.51 aA	1.305	10.28 aB	15.11 aA	12.69
P 150	0.51 cA	0.67 bA	0.590	6.83 bB	16.67 aA	11.75
Means	0.46	0.65		6.50	12.09	

[a]For each variable, means followed by the same lowercase letter in the column do not differ significantly from each other by the Scott-Knott test, and means followed by the same capital letter in the line within each variable do not differ significantly from each other by the F-test at 5% probability.

The total P concentration was lower at the low P level, independent of the part of the plant and the culture system analyzed (Tables 1 and 2). This reduction varied in leaves from 31 to 62% in *in vitro* cultivation and from 64 to 94% in soilless cultivation. In the roots, the total concentration of P ranged from 26 to 72% in *in vitro* cultivation and from 48 to 89% in soilless cultivation. The exception was clone SMIJ 319-1 that for roots in soilless cultivation increased the total concentration of P by 17% when cultivated at the low P level and P 150 and SMIF 212-3 which did not show a difference between the levels of P. Other authors also observed a reduction of the total P concentration in roots (Soratto *et al*., 2015), in the leaves (Balemi, 2009; Balemi and Schenk, 2009) and in the shoots (Balemi, 2011; Soratto *et al*. 2015) of potato plants grown under low P availability due to low P concentration in the substrate.

The reduction of growth due to P deficiency is usually greater in the shoot than in the roots, thus allowing root growth to be maintained in order to explore a larger surface in search of P (Vance *et al*., 2003). In the *in vitro* culture, clones SMIC 148-A, SMINIA 793101-3, SMIF 212-3 and SMIJ 319-1 showed reductions of 49, 51, 38 and 54% in shoot dry mass when cultivated at low P when compared to production in the high P (Table 1). However, no clone increased root growth at low P levels, as an adaptive strategy, to expand the exploration area in search of P. In contrast, the Dakota Rose, SMINIA 793101-3 and SMIB 106-7 clones showed reductions of 56, 64 and 70% in dry root mass production at the low P level. In addition, in dry matter partition between shoot and root under low P level, it was not possible to differentiate the clones, which makes it difficult to use this parameter as a physiological marker of the nutritional status to P in potato plants grown *in vitro*.

In the soilless cultivation, the clones presented an overall reduction in the dry mass of leaves, stems and tubers when cultivated at low P levels, with the exception of SMIF 212-3 clone, which showed no difference between P levels in dry mass production of stem, roots and tuders, and SMINIA 793101-3 in the dry mass of stems and roots (Table 2). Although a reduction of dry mass of leaves, stems and tubers was observed for the Dakota Rose clone, it was the only one that presented increase in root dry mass (46%) as an adaptive modification to the low level of P, strategy observed in plants of various species (Lynch and Brown, 2008). By analyzing the biomass altogether (data not shown), SMIC 148-A, SMIB 106-7, SMIJ 319-1 and P 150 clones showed the highest biomass reductions with the restriction of P. As the clones demonstrated, in general, a reduction in the dry mass of all parts of the plant, can be used as a parameter for a physiological marker of the nutritional status of P in potato plants in soilless cultivation.

CONCLUSION

The concentration of soluble P (Pi) and total P in shoots and roots seem to be good indicators of the nutritional status of P in potato plants. On the other hand, the enzymatic activity in the tissues of the potato plants depends highly on the cultivation system, clone type and plant organ, making difficult to choose it as a physiological marker for restriction of P in potatoes. Meanwhile, the dry mass of the potato plant may be a good indicator of the nutritional status of P in clones of potatoes in soilless cultivation.

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