

# Physiological and antioxidant responses of cape gooseberry (*Physalis peruviana* L.) seedlings to phosphorus deficiency

## Respuestas fisiológicas y antioxidantes en plántulas de uchuva (*Physalis peruviana* L.) a la deficiencia de fósforo

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### ABSTRACT

The main objective of present study was to understand the physiological effects of phosphorus (P) deficiency and the antioxidant response in cape gooseberry (*Physalis peruviana* L.) seedlings. Seedlings were grown in soil with five P levels: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>. The plant growth, gas exchange, chlorophyll content, membrane integrity and the antioxidant response in cape gooseberry were evaluated. In the P0, P6, P12 treatments, the seedlings showed a reduction in total biomass, the number of leaves, leaf area, root length density, shoot/root ratio, photosynthesis, transpiration, stomatal conductance, and chlorophyll content, as well as an increase in the electrolyte leakage, the proline content and the activity of catalase and peroxidase compared with the P50 treatment. The P25 treatment was not different compared to P50 in terms of photosynthesis, chlorophyll content and total biomass after 30 d of treatment, the number of leaves and root length density at 90 d of treatment, and in electrolyte leakage and peroxidase activity at 60 and 90 d of treatment. Doses below 25 mg of P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> cause P deficiency in cape gooseberry seedlings, inducing antioxidant and protection response mechanisms to cope with stress.

**Key words:** catalase, electrolyte leakage, mineral nutrition, proline, root length density.

### RESUMEN

El presente estudio tuvo como objetivo comprender los efectos fisiológicos de la deficiencia de fósforo (P) y la respuesta antioxidante de las plántulas de uchuva (*Physalis peruviana* L.). Las plántulas se cultivaron en suelo con 5 niveles de P: 0 (P0), 6 (P6), 12 (P12), 25 (P25) y 50 (P50) mg de P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>. Se evaluaron el crecimiento, el intercambio gaseoso, el contenido de clorofila, la integridad de la membrana y la respuesta antioxidante en las plántulas de uchuva. En los tratamientos P0, P6, P12, las plántulas mostraron una reducción en la biomasa total, el número de hojas, el área foliar, la densidad de la longitud de las raíces, la relación entre las raíces y la parte aérea, la fotosíntesis, la transpiración, la conductancia estomática y el contenido de clorofila, así como un incremento en la pérdida de electrolitos, el contenido de prolina y la actividad de la catalasa y la peroxidasa en comparación con el tratamiento con P50. El tratamiento con P25 no fue diferente con respecto a P50 en términos de fotosíntesis, contenido de clorofila y biomasa total después de 30 días de tratamiento, y en cuanto al número de hojas y la densidad de longitud de raíces a los 90 días de tratamiento y en la pérdida de electrolitos y actividad peroxidasa a 60 y 90 días de tratamiento. Dosis menores de 25 mg de P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> causan deficiencia de P en las plántulas de la uchuva, induciendo la producción de antioxidantes y mecanismos de respuesta de protección para atenuar los efectos del estrés.

**Palabras clave:** catalasa, pérdida de electrolitos, nutrición mineral, prolina, densidad de longitud de raíces.

## Introduction

*Physalis peruviana* L. is a plant of Andean origin cultivated in South America, from Venezuela to Chile. Nowadays, it is grown in other parts of the world, such as California, South Africa, India, New Zealand, Australia and Great Britain (Ramadan and Mörsel, 2003; El-Tohamy *et al.*, 2012). *P. peruviana* L. fruits are round, bright orange and have a pleasant taste related to their tropical origin (Fischer *et al.*, 2007). This species is very important due to its high nutritional value and potential health benefits,

derived from the high content of ascorbic acid, vitamins and antioxidants (Puente *et al.*, 2011; Briones-Labarca *et al.*, 2013). The high prices paid for cape gooseberries in local and international markets have made this plant attractive to producers (Fischer *et al.*, 2007; Ramírez *et al.*, 2013).

One of the main limiting factors of agricultural production, especially in the tropics and subtropics, is the phosphorus (P) deficiency (Sánchez, 1976; Ramaekers *et al.*, 2010). This element has an important role in various processes, such as nucleic acid synthesis, energy production,

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photosynthesis, respiration, synthesis and stability of the membrane, enzyme activation and signaling (Vance *et al.*, 2003; Hawkesford *et al.*, 2012; Manschadi *et al.*, 2014). It has been reported that P deficiency reduces the production of biomass in leaves, affecting plant growth (Kirschbaum and Tompkins, 1990; Plénet *et al.*, 2000; De Groot *et al.*, 2001; De Groot *et al.*, 2003; Chaudhary *et al.*, 2008; Maathuis, 2009; Reich *et al.*, 2009). A change in the root/shoot ratio due to an increase in the density of root hairs, an adaptive response of plants to P deficiency, has also been reported (Mollier and Pellerin, 1999; Hermans *et al.*, 2006; Yao *et al.*, 2007; Zhang *et al.*, 2013).

The decrease in CO<sub>2</sub> assimilation due to P deficiency is mainly associated to a decline in the regeneration of Rubisco (Jacob and Lawlor, 1992; Rao and Terry, 1995; Campbell and Sage, 2006; Singh *et al.*, 2013) and stomatal closure caused by the accumulation of CO<sub>2</sub> in intercellular spaces (Thomas *et al.*, 2006). This effect has also been observed in corn (Tewari *et al.*, 2004) and rice (Guo *et al.*, 2012). Phosphorus deficiency decreases the chlorophyll content, thus affecting photosynthesis. The imbalance in photosynthesis phases generated by P deficiency can cause an increase of reactive oxygen species (ROS), which alter plant functions by damaging lipids, proteins, enzymes, nucleic acids and photosynthetic pigments. Moreover, an increase in free radical production has been reported in bean (*Phaseolus vulgaris* L.) roots in plants with phosphate deficiency (Malusa *et al.*, 2002). ROS damage cell membranes, causing changes in permeability and resulting in electrolyte leakage (EL) and alterations in cellular metabolism (Blokhina *et al.*, 2003).

In order to reduce oxidative damage, plants have developed different response mechanisms, especially the production of antioxidants and protective molecules (Cruz de Carvalho, 2008). P deficiency in maize (Tewari *et al.*, 2004) and rice (Guo *et al.*, 2012) has been observed to cause a significant increase in the activity of antioxidant enzymes, such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), and an increase in the content of proline (Pro). In tomato plants, which are tolerant to low P availability, increased peroxidase activity and anthocyanin accumulation have been observed (Khavari-Nejad *et al.*, 2009). Also, an increase in the proline content of roots and stems (Sarker and Karmoker, 2011) has been found in lentil plants (*Lens culinaris* Medik).

*P. peruviana* L. is grown from 1500 m to 3000 m a.s.l. in the Andes, often in volcanic soil with an acidic pH and low phosphorus availability due to the high binding capacity of

phosphate anions in the soil solution (Fischer *et al.*, 2007; Ramirez *et al.*, 2013). These P limitations affect both the yield and quality of cape gooseberry plants (Garzón-Acosta *et al.*, 2014). However, there have not been any studies that describe the effects of phosphorus deficiency on the physiology of the plant at different stages of development. The purpose of this research was to determine the response mechanisms of cape gooseberry (*P. peruviana* L.) seedlings in the early stages of development under conditions of phosphorus deficiency through the analysis of morphological, physiological and biochemical responses.

## Materials and methods

### Plant material and experimental design

Cape gooseberry (*P. peruviana* L.) seeds ecotype Colombia were germinated in peat moss in a growth chamber with a photosynthetic photon flux density (PPFD) of 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 70% relative humidity, with 12 h of light at 22°C and 12 h of darkness at 10°C. Twenty days after germination, the seedlings were transplanted to 50-cell trays with an inert substrate (2:1:1 mixture of river sand, quartz sand and fine granite). A 0.8 strength Hoagland solution and the designated P doses were applied. The seedlings grew in a greenhouse with a PPFD range between 450–900  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (photosynthetic active radiation), 70% relative humidity, 12 h of light at 25°C and 12 h of darkness at 8°C. A completely randomized block design with five treatments, four replications and an experimental unit of eight plants was used. The treatments had five levels of P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> in the substrate: 0 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (P0), 6 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (P6), 12 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (P12), 25 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (P25) and 50 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> (P50). The variables were measured at 15 d of treatment (dt), 30 dt, 60 dt and 90 dt.

### Growth parameters

The plants were individually divided into roots, stems and leaves and the number of leaves (NL) and the leaf area (LA) were measured using a portable area meter (CI-202, CID Inc, USA). Then, the plant material was dried at 70°C until a constant weight was achieved. The root/shoot ratio was determined using these data and the root length density (RLD) was determined according to Newman and Ritz (1986).

### Gas exchange

The photosynthetic rate (A), stomatal conductance (gs) and transpiration (E) were registered at 90 dt using a photosynthesis measurement system (LCpro-SD, Portable BioScientific, UK). The measurements were taken from the fourth apical leaf on six plants per treatment, between 9:00

AM and 11:00 AM, with a CO<sub>2</sub> concentration of 360 µL L<sup>-1</sup> and a PPDF of 900 µmol m<sup>-2</sup> s<sup>-1</sup>.

### Chlorophyll content and leaf temperature

The pigments were extracted according to Cubas *et al.* (2008). The apical expanded leaves from six plants per treatment were homogenized in 80% acetone. The absorbance was determined at an optical density (OD) of 663 nm and 647 nm. The leaf temperature (LT) was determined with an infrared thermometer (HD550, Extech®, USA) on the fourth apical leaf.

### Membrane permeability

The membrane permeability was measured by electrolyte leakage (EL) according to Rodríguez *et al.* (2005). Eight 2.5 mm diameter leaf discs were placed in Falcon tubes with 2 ml of deionized water at 24°C. The electric conductivity (EC) was determined with an electrical conductivity meter (HI 9835 HANA, USA) at 24 h. The EC values are expressed as a percentage with respect to the highest value using the following equation:

$$PE = (EC1 * EC2 - 1) * 100 \quad (1)$$

where: PE = % of lost electrolytes, EC1 = electric conductivity at 24 h, and EC2 = electric conductivity after heating to 80°C. The measurements were carried out on the fourth apical leaf.

### Antioxidant enzymes, proline and protein content

A 0.2 g powdered leaf sample was treated with polyvinylpyrrolidone 40 (22% w/w) and with 1.5 ml cold 80% (v/v) acetone and centrifuged (8000xg for 30 min, 4°C) to eliminate pigments, according to Lichtenthaler (1987). Afterwards, samples were extracted in 3 ml of 110 mM sodium phosphate buffer (pH 7.2) containing 3.82% (w/v) polyvinylpyrrolidone 40. The homogenate was centrifuged (6000 x g, 1 h, 4°C) and the supernatant was used for the subsequent enzyme assays. The CAT activity was determined by the method of reduction of potassium permanganate (KMnO<sub>4</sub>) with hydrogen peroxide in sulfuric acid (Ulrich, 1974). The POD activity was estimated by the absorbance change at 436 nm caused by the oxidation of guaiacol according to Kireyko *et al.* (2006). The proline level was determined according to Bates *et al.* (1973) based on the proline-ninhydrin reaction. For the proline (Pro) determination, a 1:1:1 solution of proline, ninhydrin and glacial acetic was incubated at 97°C for 1 h. The reaction was stopped by placing the vials in an ice bath; the chromophore was extracted with toluene and its absorbance was measured at 520 nm. The soluble proteins were measured based on the

method of Bradford (1976), using bovine serum albumin as a reference.

### Data analysis

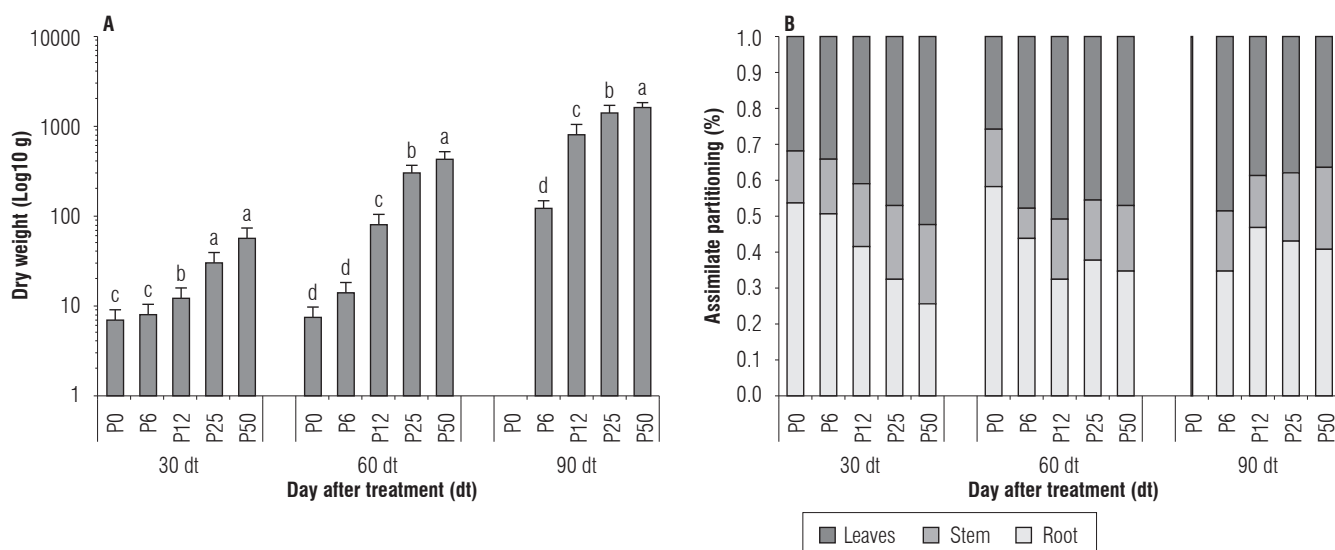
An analysis of variance (ANOVA) was carried out to determine the effect of the treatments in the analyzed variables. The comparison of the means was done with a Tukey multiple range test ( $P < 0.05$ ).

## Results and discussion

### Growth parameters

The seedling dry weight decreased by reducing the concentration of P in the nutrient solution (Fig. 1A). This decrease was significant between P0 and P6 and the other treatments. The highest values for the dry weight were observed in P50 at 30 dt (0.60 g), 60 dt (4.63 g) and 90 dt (18.13 g) and the lowest values were observed in P0 at 30 dt (0.07 g) and 60 dt (0.08 g); the P0 plants died before 90 dt. Although there were differences between P12, P25 and P50 at 90 dt, the values were closer to the values of 30 dt and 60 dt. The assimilate partitioning in P0 and P6 was higher towards the root, compared to the other treatments at 30 dt and 60 dt (P0: 0.038 g, 0.047 g and P6: 0.043 g, 0.064 g, respectively). At 90 dt, there were differences in the partition of assimilates to the root between P12 and P25 as compared to P50, where fewer assimilates were partitioned to roots (Fig. 1B).

The root/shoot ratio was higher at 30 dt and 60 dt for P0 (1.1; 1.5; nd) and P6 (1.0; 0.8; 0.7) compared to the others treatments. P12 and P25 did not show differences in the root/shoot ratio between each other, but were higher than P50 (Tab. 1). The NL, LA and RLD progressively decreased with a reduction in the concentration of phosphorous in the nutrient solution (Tab. 1). At 90 dt, no differences were observed in the NL and RLD between P25 and P50, but the LA was lower in P25 (168.3 cm<sup>2</sup>) than in P50 (196 cm<sup>2</sup>). Phosphorus is an essential element for the growth and development of plants; however, there are differences in requirements due to the efficient use some plants make of this element (Kirschbaum and Tompkins, 1990; Nielsen *et al.*, 2001; Chaudhary *et al.*, 2008; Maathuis, 2009; Reich *et al.*, 2009). In the cape gooseberry seedlings, there was a decrease in the dry weight, number of leaves and leaf area as a result of a decrease in the dose of phosphorus; similar data have been reported for different species (Radin and Eidenbock, 1984; Guo *et al.*, 2012). The seedlings in the P0 treatment, without phosphorus, achieved little growth, possibly by using seed reserves, and did not survive beyond 60 dt. In addition to the reduction of total biomass



**FIGURE 1.** Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of  $P_2O_5$   $kg^{-1}$  on the seedling dry weight, logarithmically transformed data (A) and assimilate partitioning (B) of the cape gooseberry seedlings (*Physalis peruviana* L.) at 30, 60 and 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at  $P < 0.05$  according to the Tukey test.

in plants, one of the indicators of phosphorus deficiency is an increase in the partitioning of photoassimilates to roots (Mollier and Pellerin, 1999; Hermans *et al.*, 2006; Yao *et al.*, 2007; Zhang *et al.*, 2013). It was observed that all of the treatments, except P50, had a higher proportion of biomass in the roots and a higher root/shoot ratio. This response has been described for many plants suffering P deficiency as a strategy to get through the depleted zone in the substrate and obtain more P (Mollier and Pellerin, 1999; Hermans *et al.*, 2006; Yao *et al.*, 2007). These data suggest that the cape gooseberry seedlings in P6, P12 and P25 had a P deficiency. Likewise, the decrease in the difference in the dry weight of the seedlings at 90 dt between P12, P25 and P50 suggests that the seedlings changed their metabolism to increase the efficiency of P use, as has been reported in many plants (Nakamura, 2013).

### Gas exchange and leaf temperature

The P25 and P50 treatments had higher A values ( $10 \mu mol m^{-2}s^{-1}$ ;  $10.54 \mu mol m^{-2}s^{-1}$ ) as compared to P12 and P6 ( $2.76 \mu mol m^{-2}s^{-1}$ ;  $1.67 \mu mol m^{-2}s^{-1}$ ) (Fig. 2A). E behaved similarly to A, with differences between P6 ( $0.26 mmol m^{-2}s^{-1}$ ) and P50 ( $2.81 mmol m^{-2}s^{-1}$ ) (Fig. 2B). P25 had the highest gs value ( $0.202 mmol m^{-2}s^{-1}$ ) and P6 and P12 had lower values ( $0.025 mmol m^{-2}s^{-1}$ ;  $0.100 mmol m^{-2}s^{-1}$ ) (Fig. 2C). The leaf temperature was higher in P0 ( $18.1^\circ C$ ) and P12 ( $16.1^\circ C$ ) compared to P25 ( $16.1^\circ C$ ) and P50 ( $14.8^\circ C$ ) (Fig. 2D). The temperature increased between  $3.3^\circ C$  and  $3.9^\circ C$  between the treatments with deficient P (P0 and P6) and the treatment with sufficient P (P50). Plant biomass production

**TABLE 1.** Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of  $P_2O_5$   $kg^{-1}$  on the Number of Leaves per plant (NL), Leaf area (LA), Root Length Density (RLD) and Root Shoot ratio (Root/Shoot) in cape gooseberry (*Physalis peruviana* L.) seedlings at 30, 60 and 90 d after treatment (dt). The data shown are the averages of sixteen replicates. Pi, Phosphorous levels; nd, data not available.

dt	Pi	NL	LA	RLD
	(mg $kg^{-1}$ )		( $cm^2$ )	( $mm^3 cm^{-3}$ )
30	P0	1.0 d	0.9 c	nd
30	P6	1.1 d	1.0 c	nd
30	P12	1.5 c	1.9 c	nd
30	P25	2.9 b	6.8 b	nd
30	P50	3.6 a	12.3 a	nd
	ANOVA	**	**	
60	P0	1.1 e	0.9 d	0.3 d
60	P6	2.3 d	2.0 d	0.7 d
60	P12	4.3 c	15.7 c	2.9 c
60	P25	5.9 b	43.9 b	12.2 b
60	P50	7.2 a	61.0 a	18.3 a
	ANOVA	**	**	**
90	P0	nd	nd	nd
90	P6	5.0 c	17.3 d	12.9 c
90	P12	7.6 b	94.3 c	51.5 b
90	P25	10.7 a	168.3 b	64.6 a
90	P50	11.1 a	196.0 a	70.5 a
	ANOVA	**	**	**

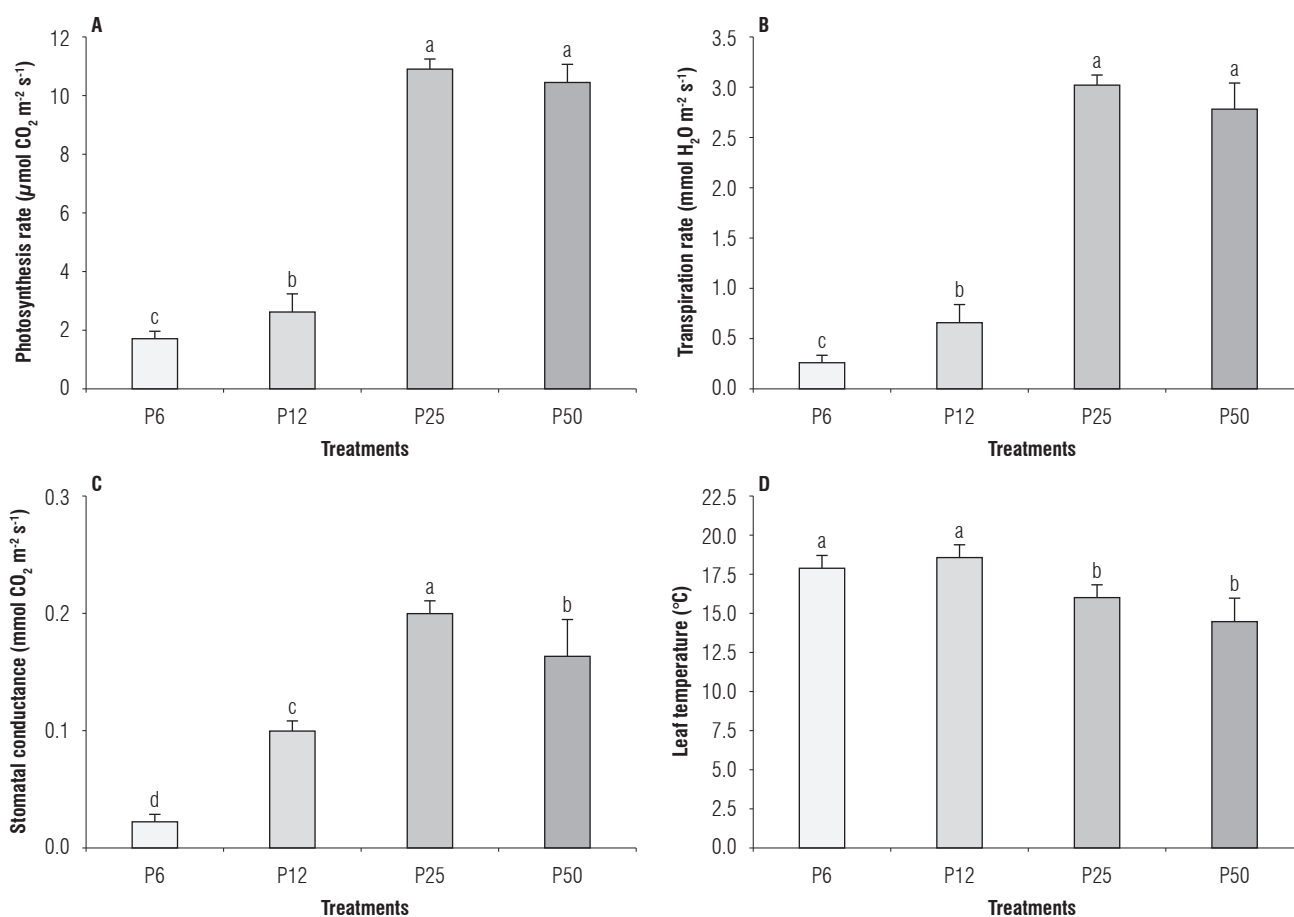
Means denoted by the same letter do not significantly differ at  $P < 0.05$  according to the Tukey test. Comparisons among treatments were analyzed by one-way ANOVA. Statistical significance for  $P < 0.05$  (\*),  $P < 0.01$  (\*\*).

depends directly on net photosynthesis, which is related to the plant ability to intercept light and the efficiency of photosynthetic metabolism (Singh *et al.*, 2013). Large decreases in *A* and *g<sub>s</sub>* were observed in P6 and P12, as compared to P50, possibly due to the decreased synthesis of ATP in the photosynthesis phase, which causes a limitation in the regeneration of the CO<sub>2</sub> acceptor ribulose-1,5-bisphosphate in the Calvin cycle. This slows carboxylation and increases the intercellular concentration of CO<sub>2</sub>, which causes stomatal closure (Singh *et al.*, 2013). Similar results have been reported in other species at P deficit conditions, such as *Arabidopsis*, sorghum, corn, beans and cotton (Radin and Eidenbock, 1984; Barrett and Gifford, 1995; Abel *et al.*, 2002; Tewari *et al.*, 2004; Yao *et al.*, 2007; Chaudhary *et al.*, 2008; Singh *et al.*, 2013). Similarly, the decrease in *g<sub>s</sub>* caused by an imbalance in the two phases of photosynthesis produced a reduction in *E* and an increase in *LT* due to a decrease in energy dissipation. Although the seedlings in P25 had a higher root/shoot ratio than P50 and a decrease in

dry weight accumulation, which indicates a deficiency of P, they did not show a decrease in *A*. This suggests that these plants exhibited a slight deficiency that affected cell division, but not the function of the photosynthetic apparatus.

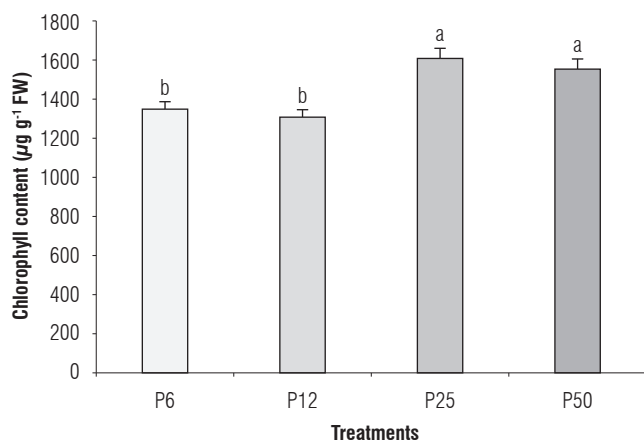
### Chlorophyll content

The chlorophyll content (Chl) decreased in P6 (1343 µg g<sup>-1</sup> DW) and P12 (1330 µg g<sup>-1</sup> DW) as compared to P25 (1615 µg g<sup>-1</sup> DW) and P50 (1568 µg g<sup>-1</sup> DW). The reduction in Chl in P6 compared to P50 was 14.3% (Fig. 3). A decrease in chlorophyll content due to P deficiency has been reported in plants such as maize (Tewari *et al.*, 2004). In the case of Solanaceae, such as tomatoes, a severe reduction has been reported in the chlorophyll content with combined N and P deficiency (Khavari-Nejad *et al.*, 2009). The reduction in the chlorophyll content can also be related to the degradation of these molecules by ROS (Misson *et al.*, 2005). The decrease in the *A* may also have been related to the decrease in the chlorophyll content observed in P6 and P12.



**FIGURE 2.** Effect of four phosphorous levels in the soil: 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> on the gas exchange. A, photosynthesis rate (A), B, transpiration rate (E), C, stomatal conductance (gs) and D, leaf temperature (LT) in cape gooseberry (*Physalis peruviana* L.) seedlings at 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at *P* < 0.05 according to the Tukey test.





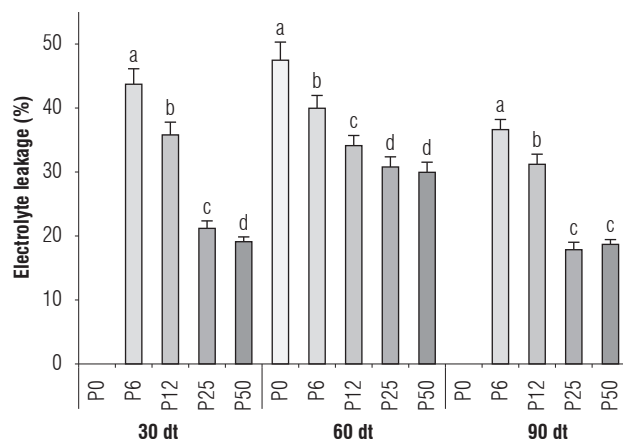
**FIGURE 3.** Effect of four phosphorous levels in the soil: 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of  $P_2O_5$   $kg^{-1}$  on the chlorophyll content in cape gooseberry (*Physalis peruviana* L.) seedlings at 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at  $P < 0.05$  according to the Tukey test.

### Membrane permeability

The P0, P6 and P12 treatments had higher percentages of EL (Fig. 4). The EL for P6 at 30 dt, 60 dt and 90 dt was 44%, 40% and 37%, respectively, while for P50 it was 19%, 30% and 19%, respectively (Fig. 4). The P25 and P50 treatments showed no significant differences between each other at 60 and 90 dt. Due to the imbalance in the two phases of photosynthesis caused by ATP deficiency, there is an increase in ROS production, which caused damage to macromolecules and structures as membranes (Misson *et al.*, 2005). Here, there was an increase in EL in P0, P6 and P12, suggesting that P deficiency causes damage to membranes, affecting the permeability degree, probably due to lipid peroxidation induced by ROS.

### Antioxidant enzymes, proline and protein content

The activity of the antioxidant enzyme CAT was determined in the leaves at 15 dt, 30 dt, 60 dt and 90 dt (Tab. 2). The CAT activity was higher for all of the treatments at 15 dt, when the activity in P0 (791 UA  $g^{-1}$  FW) was approximately 3.3 times higher than P50 (238 UA  $g^{-1}$  FW). The CAT activity gradually decreased over time in all of the treatments and, at 90 dt, presented lower values. Throughout the experiment, P50 had the lowest values of CAT activity (106.2 UA  $g^{-1}$  FW to 238.3 UA  $g^{-1}$  FW) (Tab. 2). The POD activity was low in all of the treatments, but had higher values in P6 (1.4 mg POD  $g^{-1}$  FW) and P12 (1.38  $g^{-1}$  FW) at 15 dt. The POD activity gradually reduced until 90 dt, when the lowest value was observed for all treatments.



**FIGURE 4.** Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of  $P_2O_5$   $kg^{-1}$  on electrolyte leakage in cape gooseberry (*Physalis peruviana* L.) seedlings at 30, 60 and 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at  $P < 0.05$  according to the Tukey test.

The contents of Pro throughout the experiment were higher in P0, P6 and P12 (Tab. 2). At 60 dt, P0 showed the highest value for the Pro content (771.7 mg  $g^{-1}$  FW), just before the plants died. At 90 dt, the Pro content in all of the treatments decreased between 101.8 mg  $g^{-1}$  FW (P6) and 29.4 mg  $g^{-1}$  FW (P50). The protein content was higher in all of the treatments at 90 dt, when the Pro content was lower (Tab. 2), with values between 2.5 mg  $g^{-1}$  FW (P25) and 2.7 mg  $g^{-1}$  FW (P6, P12). Higher antioxidant enzyme activity, such as peroxidase (POD) and superoxide dismutase (SOD), has been reported in rice seedlings (Guo *et al.*, 2012) and also in maize, with increase in other enzymes as CAT and ascorbate peroxidase (APX) under P deficiency (Tewari *et al.*, 2004). Additionally, in beans, P deficiency increases CAT and POD activities (Juszczuk *et al.*, 2001). In cape gooseberry seedlings, there was an increased CAT activity for the P0, P6, P12 and P25 treatments at 15 dt (Tab. 2). The increase was greater for the treatments with lower doses of P, showing that the expression of this enzyme is an early response in cape gooseberry seedlings to a deficiency of this element and may be related to the observed decrease in EL. Similarly, POD enzyme activity was low, but was higher in treatments with lower doses of P. It has also been reported that P deficiency can increase the Pro content in roots and stems in lentil plants (Sarker *et al.*, 2011) and in leaves and roots in beans that are deficient in P (Juszczuk *et al.*, 2001). The observed Pro increase in P0, P6 and P12, which was higher at 30 dt and 60 dt, could be another response of cape gooseberry seedlings to reduce ROS caused by an imbalance in photosynthesis (Hare *et*

**TABLE 2.** Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> on the content of Catalase (CAT), Peroxidase (POD), Proline (Pro) and total protein in cape gooseberry seedlings (*Physalis peruviana* L.) at 15 dt, 30 dt, 60 dt and 90 dt. Pi, Phosphorous level; FW, fresh weight; nd: data not available.

dt	Pi	CAT	POD	Pro	Total Protein
	(mg kg <sup>-1</sup> )	(UA g <sup>-1</sup> FW)	(UA g <sup>-1</sup> FW)	(mg g <sup>-1</sup> FW)	(mg g <sup>-1</sup> FW)
15	P0	791.0 a	1.0 b	43.0 c	1.6 a
15	P6	620.4 b	1.4 a	139.4 b	1.7 a
15	P12	546.8 c	1.4 a	178.8 a	1.5 c
15	P25	336.5 d	0.3 c	24.0 d	1.2 d
15	P50	238.3 d	0.2 c	16.4 e	1.3 c
	ANOVA	**	**	**	**
30	P0	392.4 a	0.7 a	94.0 c	0.1 d
30	P6	379.8 a	0.9 a	273.0 b	0.6 c
30	P12	344.7 a	1.1 a	332.1 a	0.6 c
30	P25	231.0 b	0.2 b	44.6 d	1.1 b
30	P50	163.7 c	0.2 c	30.4 e	1.3 a
	ANOVA	**	**	**	**
60	P0	420.8 a	0.6 b	771.7 a	0.2 e
60	P6	365.0 b	0.6 b	282.8 b	0.8 d
60	P12	339.5 b	0.6 b	220.2 d	0.8 c
60	P25	267.2 c	0.1 a	166.2 d	1.5 b
60	P50	134.9 d	0.1 a	161.2 d	1.7 b
	ANOVA	**	**	**	**
90	P0	nd	nd	nd	nd
90	P6	313.9 a	0.1 a	101.8 a	2.7 b
90	P12	310.0 a	0.1 a	97.2 a	2.7 a
90	P25	148.0 b	0.1 a	64.2 b	2.5 d
90	P50	106.2 c	0.1 a	29.4 a	2.6 c
	ANOVA	**	**	**	**

Means denoted by the same letter do not significantly differ at  $P < 0.05$  according to the Tukey test. Comparisons among treatments were analyzed by one-way ANOVA. Statistical significance at  $P < 0.05$  (\*),  $P < 0.01$  (\*\*).

*al.*, 1998). It has been reported that low molecular weight metabolites, such as Pro, are efficient at detoxifying the  $\cdot\text{OH}$  radical, and increased Pro synthesis has been reported in response to different abiotic stresses or by combining phosphorus deficiency simultaneously with other stresses, such as drought stress (Al-Karaki *et al.*, 1996) or salinity stress (Zribi *et al.*, 2015). Pro accumulation has also been reported in response to stress nutrients such as Al and Ca in beans (Yang and Chen, 2001), or Al and P (Ismail, 2005; Guo *et al.*, 2012) and Fe (Arias-Baldrich *et al.*, 2015), but not under conditions of phosphorus deficiency alone. The observed Pro accumulation may have been a stress response to P deficiency to protect macromolecules and structures from damage caused by ROS, due to its function as a compatible osmolyte (Schobert and Tschesche, 1978). The increase in

the defense and protective mechanisms in cape gooseberry seedlings was probably due to metabolic adjustment, which aims at reducing the effect of stress under moderate P deficiency, as noted here. The development of mechanisms such as increased antioxidant capacity and the synthesis of osmolytes has an energy cost that plants probably cannot assume with a severe phosphorus deficiency, as in P0 treatment, because the limitation imposed by deficiency is too high. Furthermore, the metabolic adjustment induced by P deficiency includes other mechanisms, such as the use of PPI-dependent enzymes in the glycolysis pathway and the replacement of membrane phospholipids with sulfolipids; plants have developed these mechanisms since P is a limiting element on the planet (Nakamura, 2013). These stress defense mechanisms, described here for the first time for *P. peruviana* L., may explain the acclimatization capability observed in cape gooseberry seedlings under moderate P deficiency.

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