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EFFECT OF SEED PRIMING AND FOLIAR APPLICATIONS OF P IN TOMATO SEEDLINGS

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ABSTRACT

The objective of this study was to evaluate the effect of different sources and forms of application of phosphate fertilizers on the initial growth of tomato (cv. Moctezuma F1) under greenhouse conditions. The sources consisted of commercial liquid fertilizer (FL), soluble monoammonium phosphate (MAP), and phosphoric acid (H_3PO_4), which were applied as pretreatment to seeds (seed priming) and foliar spray at a concentration of P_2O_5. of 240 mg L^{-1}. In the first case, the seeds were subjected for 24 h to solutions prepared with the different sources, while the foliar applications were made when the first true leaves appeared. The seeds were established in expanded polystyrene trays, using peat moss as substrate. For the statistical analyses, a completely randomized design with a 4 × 2 factorial arrangement (four sources of P and two forms of application) was considered, giving a final combination of eight treatments with five repetitions. The experimental unit consisted of 11 seedlings. The variables evaluated were the fresh and dry weight of the aerial part (leaf + stem) and root, stem diameter, SPAD units and the foliar concentration of N, P and K. The results indicate that there was no interaction between the factors studied in all the evaluated variables, except for fresh and dry root weight. In general, the applied treatments showed favorable results in the foliar application method. The results indicate that it is possible to favor the initial growth of tomato seedlings by applying P fertilizer sources, mainly through foliar applications of FL and MAP.

Keywords: *foliar fertilization, phosphorus, greenhouse, plant nutrition, Solanum lycopersicum.*

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INTRODUCTION

The constant increase in the population, as well as the loss of harvest surface due to soil erosion and contamination, forces us to look for alternatives to increase agricultural production, such as food, forages, fibers, and phytochemicals, seeking high yields per unit of space and better nutritional characteristics.

Phosphorus (P) is a macronutrient for all living organisms, and together with nitrogen (N), it is one of the most determining elements for agricultural production. P is a key and fundamental element of biomolecules such as ATP, nucleic acids, phospholipids, and proteins, so a deficiency of this element produces a negative impact on the growth and development of plants (Magalhaes *et al*., 2017).

In several regions of the world, there are problems of deficiency or low availability of P in the soil, mainly in areas of arid-semiarid climate, where predominate calcareous soils with high contents of $CaCO_3$ and pH values greater than 8-8.5, where this element is found in insoluble forms (Bai *et al*., 2017). Additionally, temperatures below 15 °C reduce the absorption of P by seedlings, where the deficiency of this element produces various physiological disorders.

The success in crop production depends largely on the establishment stage, where factors such as sowing method and seed quality influence. Recently, a set of seed pretreatment techniques have emerged that favor the germination and initial growth of seedlings, methodologies known as "seed conditioning" or "seed priming". In its origins, this concept included only the action of imbibing seeds and subsequent drying for sowing, increasing the vigor of the plants. In recent years, this concept has encompassed various techniques, such as the use of temperature (high and low), UV rays, phytohormones, microorganisms, and mineral salts (Waqas *et al*., 2019).

Currently, studies have been conducted on the positive effect of the imbibition of seeds in solutions with phosphate compounds (early germination, higher yield and increase in biomass) compared to the use of seeds without previous treatment (Baig. *et al*., 2020). On the other hand, Abdel-Aziz *et al*. (2019) compared foliar application and seed conditioning with NPK in *Phaseolus vulgaris*, where foliar treatment was more effective, increasing vegetative growth and grain yield. In the literature, there is little information about the use of different P sources for seed pretreatment or application in tomato seedlings.

Based on the above, the objective of this research was to evaluate the effect of three sources of phosphorus as seed priming and foliar applications on the initial growth of tomato seedlings.

MATERIALS AND METHODS

Location of the experiment and plant material

The experiment was carried out in the Department of Horticulture of the Universidad Autónoma Agraria Antonio Narro, located at 25° 21′ 12.8″ north latitude and 101° 1′ 51.9″ west longitude. The greenhouse used was a tunnel type, with an average temperature of 25 °C and a relative humidity of 60 %. The plant material was the hybrid "Moctezuma F1", from the Harris Moran seed house, a saladette tomato type with an indeterminate growth habit and resistance to Va, Ma, Mi, Mj, Fol (1,2,3), ToMV, Ff, TYLCV and TSWV, in addition to having a germination percentage greater than 98 %.

Treatments applied

The treatments consisted of different sources of P applied as seed pretreatment (seed conditioning) and by foliar spray. The fertilizer sources considered were commercial liquid fertilizer ProGerminator® (FL), with a composition of 9 % N, 24 % P and 2.8 % K; soluble monoammonium phosphate Ultrasol® SQM (MAP), with a composition of 12 % N and 61 % P; and phosphoric acid (H_3PO_4), with a content of 52 % P. The doses used for each fertilizer were 240 mg P_2O_5 L^{-1}: 1 mL FL L^{-1}, 0.4 g MAP L^{-1}, and 0.46 mL H_3PO_4 L^{-1}. In both forms of application, the application of distilled water was considered as a control.

Establishment of the experiment

For seed priming, the seeds were placed in each of the previously mentioned solutions for 24 h and then sown. The seeds of the rest of the treatments were sown directly. Sowing was performed in 72-cavity expanded polystyrene trays (110 cm^3 volume per cavity), using peat moss as substrate. When the first true leaves appeared, a base fertilization was started that consisted of a Steiner nutrient solution at a concentration of 10 %. This solution was used as irrigation water. From 15 days after sowing, foliar applications of the treatments were started, considering a weekly application for a period of four weeks.

Temperature monitoring

Air and substrate temperature were recorded with an Elitech® GSP-6 Data Logger (Elitech Technology, Inc., California, USA), placing a sensor at the height of the plant canopy and another at 5 cm inside the substrate. The data recording interval of both sensors was 15 min and was carried out from sowing until the end of the experiment (Figure 1).

Determination of agronomic variables

The agronomic variables evaluated were the fresh weight (FW) and dry weight (DW) of leaves, fresh and dry weight of roots, and stem diameter. For the determination of

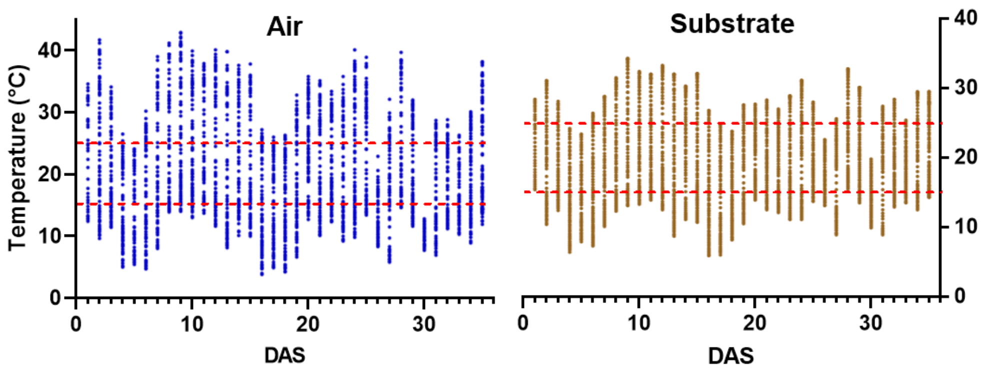

Figure 1. Variation in the daily temperature recorded in the air and substrate of tomato seedlings. DAS: Days after sowing. The red lines indicate the optimal ranges for the absorption of P by the root, ranging from 15 to 25 °C (Fan *et al*., 2020).

dry biomass, the samples were placed in a drying oven (Yamato DX602C, Yamato Scientific America, CA, USA) at 70 °C for 72 h, while the measurement was performed on an OHAUS® CS analytical balance with a precision of 0.001 g.

Relative chlorophyll content (SPAD)

At the end of the experiment, the relative chlorophyll content expressed in SPAD units was measured using the SPAD-502 portable equipment (Konica Minolta, Tokyo, Japan), performing the determination in the most recent mature leaf of the seedlings, obtaining readings in two leaflets to subsequently calculate the mean.

Determination of N, P and K by ICP–AES

The concentrations of N, P and K in the tissues were determined according to the methodology proposed by Alcántar-González and Sandoval-Villa (1999). The resulting dry matter material was crushed in a porcelain mortar, and then 0.5 g of powder was taken and subjected to acid digestion in a mixture of HNO_3:$HClO_4$ (2:1 mL) and 2 mL of H_2O_2 to 30 %.

N was quantified using the micro-Kjeldahl method, according to the methodology of Bremner (1965). The concentrations of P and K were determined using a coupled plasma induction atomic emission spectrometer model 725-ES (ICP–AES) (Agilent Technologies, Santa Clara, CA, USA). The results of N, P and K were expressed as mg kg^{-1} of DW.

Experimental design and statistical analysis

The experiment was established considering a completely random design with a 4 × 2 factorial arrangement, consisting of four treatments (control, FL, MAP and H_3PO_4) and two forms of application (seed priming and foliar spray), giving a total combination of eight treatments, for which five replicates were established, each with 11 plants as an experimental unit. The data were subjected to an analysis of variance, comparing means for the variables with statistically significant differences (*p*£0.05), and this comparison was carried out by the Tukey method. Additionally, to establish relationships between the variables evaluated, a Pearson correlation analysis was performed, considering a significance value of $p \leq 0.05$. The analyses were performed in the statistical package Infostat v. 2020.

RESULTS AND DISCUSSION

The results showed a statistically significant difference for the application method and source factors; however, a significant interaction was found only between the experimental factors for the fresh and dry root weight variables (Table 1).

The application of foliar treatments was more effective for all the variables studied. In the case of the fresh weight of leaves, the foliar method favored this variable by 17 % with respect to the seed priming technique. The applications of phosphate sources increased the fresh biomass of the seedlings, where the H_3PO_4 and the FL promoted increases of 54 and 57 %, respectively, while the MAP caused an increase of 105 %,

Table 1. Significance levels (p) and F values found in the analysis of variance for the application method and source factors and their interaction in the evaluated variables.

Factor	*p*/F	LFW	LDW	RFW	RDW	SPAD	SD	N	P	K
Method	*p* value	0.021	0.042	0.007	0.002	0.084	0.013	0.035	0.008	0.001
	F	5.86	4.5	8.32	10.41	3.14	6.8	4.99	8.33	25.76
Source	*p* value	0.000	0.000	0.016	0.000	0.001	0.001	0.004	0.006	0.016
	F	17.89	7.51	3.97	7.98	17.01	15.7	5.63	5.31	4.14
Method × Source	*p* value	0.088	0.09	0.001	0.005	0.083	0.689	0.22	0.13	0.301
	F	2.37	2.32	6.81	7.82	2.43	0.49	1.58	2.07	1.29

LFW: Leaf fresh weight; LDW: Leaf dry weight; RFH: Root fresh weight, RDW: Root dry weight; SPAD: Relative chlorophyll content; SD: Stem diameter.

the above with respect to the control treatment. A similar trend was observed in the dry biomass of leaves, where the foliar application method promoted an increase of 18 %, while the sources H_3PO_4 and FL showed increases of 40 and 45 %, respectively. On the other hand, the greatest increase was recorded in MAP, which increased the weight of the dry biomass of leaves by 71 % with respect to the control. On the other hand, the diameter of the stem of the seedlings increased by 8 % when performing foliar applications compared to the pretreatment of seeds. In turn, an increase of 28 % was observed with the application of FL, as well as an increase of 18 % when applying MAP, all of the above with respect to the control treatment (Table 2).

The relative chlorophyll content was also favored with the application of phosphate sources. Among the application methods, no significant differences were observed; however, all phosphoric sources positively affected the values of SPAD units in tomato leaves, as follows: FL>MAP>H_3PO_4, with increases of 20, 15, and 9 %, respectively, compared to the control treatment (Table 2). The same increase in SPAD units was reported by Makela *et al*. (2020) in foliar applications of various sources of P in *Fagopyrum esculentum* and *Brassica napus* plants.

Table 2. Fresh and dry weight of leaves, stem diameter and SPAD units in tomato seedlings.

Factor	LFW	LDW	SD	SPAD
		Method		
Seed priming	4834±1441 b	802.4±233 b	3.65±0.45 b	37.49±2.85 a
Foliar	5650±1861 a	945.1±303 a	3.94±0.54 a	39.49±4.15 a
		Source		
Control	3417±899 c	628.8±180 b	3.37±0.29 c	34.57±4.75 c
H_3PO_4	5283±1197 b	877.8±201 ab	3.52±0.33 c	37.88±1.90 b
FL	5369±824 b	913.8±225 a	3.97±0.48 b	39.94±3.66 ab
MAP	6998±1658 a	1074±308 a	4.32±0.32 a	41.57±1.93 a

LFW: Leaf fresh weight; LDW: Leaf dry weight; SD: Stem diameter; SPAD: Relative chlorophyll content. The values represent the mean ± standard deviation. Different letters in each column indicate significant difference (Tukey, $p \leq 0.05$).

The increases in the biomass of tomato seedlings are explained because P is an important constituent of the structure of nucleic acids and lipid membranes, in addition to being a component of adenosine triphosphate (ATP), for which it is involved in all energy transfer reactions in cells (Hawkesford *et al*., 2012). On the other hand, this element participates in photosynthesis, sugar translocation, nutrient movement in the plant and transfer of genetic information during cell division (Karthika *et al*., 2018). In addition, Li *et al*. (2007) discovered that there are at least 106 proteins that require P for their synthesis. These proteins are involved in various metabolic processes, such as phytohormone synthesis, carbon metabolism, signal transduction, cellular organization, and secondary metabolism. Due to these functions, a higher content of available P will favor the vegetative growth of plants.

Shah *et al*. (2011) reported that pretreatment with diammonium phosphate (DAP) and simple superphosphate (SSP) favored the percentage of germination, plant length and flowering in plants of *Abelmoschus esculentus*. On the other hand, the application of $KH_2 PO_4$ at doses of 0.1, 0.2 and 0.3 % (m/v) as pretreatment of wheat seeds also increased the dry matter gain in plants, as well as the number of spikes m^{-2}, spike length and days to physiological maturity (Khalil *et al*., 2010). The seed priming of *Zea mays* with P sources (KH_2PO_4, DAP and SSP) adjusted to obtain a concentration of 1 % P_2O_5 (m/v) increased the fresh and dry biomass, plant height and grain yield. Additionally, the foliar levels of P were higher when pretreatments of this element were applied (Miraj *et al*., 2013).

On the other hand, it has been shown that of the total P absorbed through foliar applications, 52 % is used mainly for metabolic processes, 17 % as part of nucleic acids, and 21 % as a structural component (mainly phospholipids of cell membranes), while 10 % of P is considered residual (undefined forms), which is not used by the plant. All of the above was reported by Mo *et al*. (2019) in *Clerodendrum cyrtophyllum*. The foliar application of nutrients is more efficient due to the rapid response of the plants since the ions are used directly in the required area (Alshaal and El-Ramady, 2017).

In the case of the fresh and dry weight of the roots, the statistical analysis showed an interaction between the application method and the source. The greatest increases in the dry and fresh weight of the roots were observed in the FL and MAP treatments applied via foliar application, where the fresh biomass of the roots increased by 57 and 60 %, respectively, with respect to the lowest value (control, seed conditioning). In turn, the treatment of H_3PO_4 to seeds favored an increase of 39 % with respect to the same control (Figure 2A). On the other hand, a similar effect was observed in the dry biomass of the root, where the foliar-MAP and foliar-FL combinations showed the greatest increases (78 and 76 %, respectively), while the H_3PO_4 by seed priming produced an increase of 35 % with respect to the control (Figure 2B).

P is an important element for the formation of the root system of plants (González *et al*., 2020), which explains the greater growth of roots in tomato seedlings. The increase found in the H_3PO_4 treatment applied via seed priming can be explained by the chemical properties of the acids, which possibly favored a thinning of the seed coat,

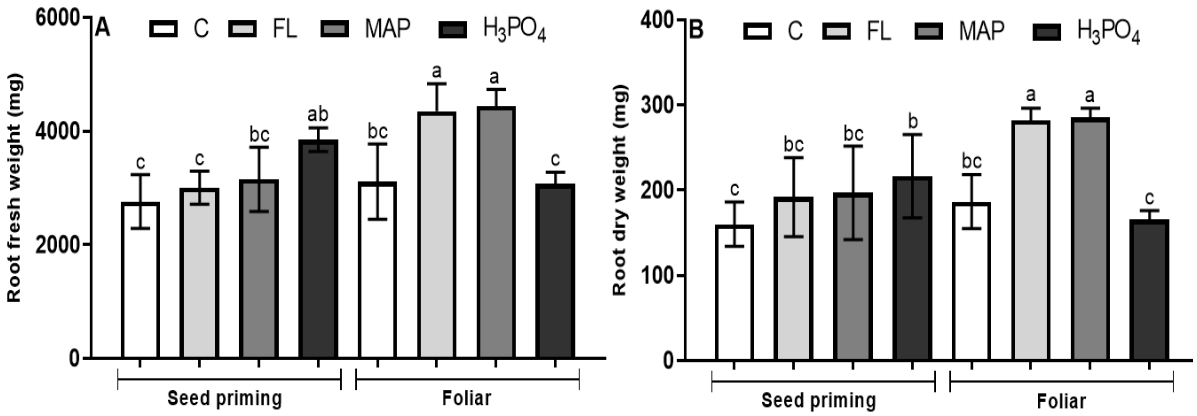

Figure 2. Root fresh weight (A) and root dry weight (B) of tomato seedlings. The bars represent the means of each treatment, and the lines above the bars indicate the standard deviation (n=5). Different letters on the bars indicate significant differences between treatments (Tukey, $p \leq 0.05$).

favoring the access of water and HPO_4^- ions, which produced greater root production (Lambers and Plaxton, 2015). The treatment of seeds with different acids is widely used to promote germination and the initial growth of seedlings (Bera *et al*., 2020).

In addition, the application of pretreatments with phosphate fertilizers favors a greater capacity to mobilize seed reserves, as well as a greater percentage of efficiency in the use of these reserves, promoting a greater initial growth of seedlings (Seyyedi *et al*., 2015.). P is also a constituent of nucleic acids (DNA and RNA) and phospholipids of cell membranes (White and Hammond, 2008).

The foliar concentrations of N, P and K also increased with the application of phosphate fertilizers. In the case of N, foliar applications increased the concentration of this element by 10 % with respect to the seed priming technique (Figure 3A). On the other hand, only the MAP and FL fertilizers increased the concentration of N, with increases of 25 and 23 %, respectively, compared to the control (Figure 3B). The same trend between the application methods was observed in the concentration of P, which was 9 % higher than the seed conditioning (Figure 3C), while all the treatments favored the concentration of P, for which the FL and H_3PO_4 produced increases of 14 %, while with MAP, there was an increase of 15 % with respect to the control (Figure 3D). On the other hand, the foliar K concentration increased by 20 % in the foliar application method compared to the seed priming method (Figure 3E). With respect to the phosphate sources, a similar trend was observed as that found in P, observing that all the fertilizers positively affected the levels of K in the leaves, as follows: H_3PO_4>FL>MAP, with increases. of 18, 17 and 13 %, respectively, compared to the control (Figure 3F).

The correlation analysis showed highly significant positive relationships ($p \leq 0.01$) for most of the variables studied, where the relationships between the concentration of nutrients and the relative content of chlorophylls are highlighted, as well as the latter with the variables of fresh and dry biomass of the seedlings (Figure 4).

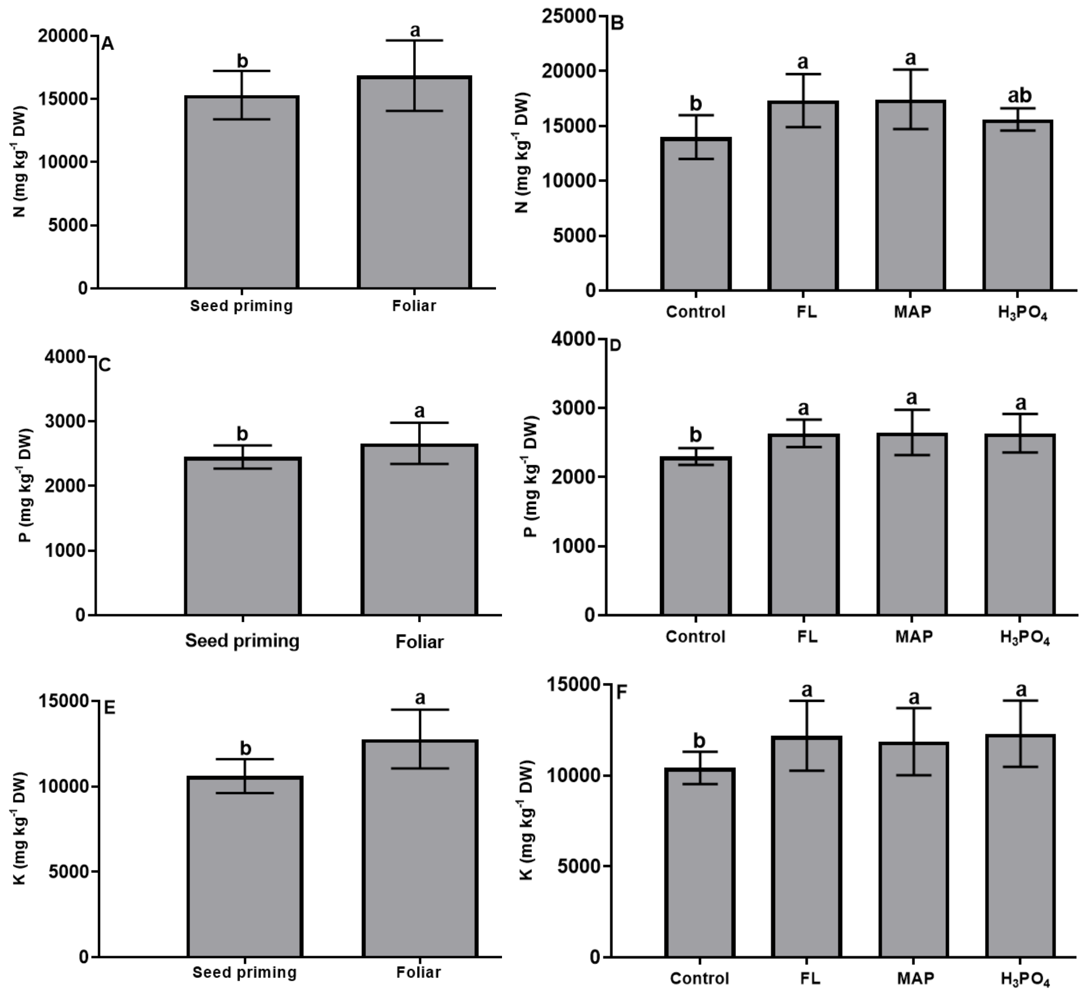

Figure 3. Concentrations of N (A, B), P (C, D), and K (E, F) in tomato leaves. The bars represent the means of each treatment, and the lines above the bars indicate the standard deviation (n=5). Different letters on the bars indicate significant differences between treatments (Tukey, $p \leq 0.05$).

According to Quintana-Blanco *et al*. (2017), in the face of an excess of P, the chlorophyll contents tend to be reduced due to the occurrence of an antagonism with anions such as NO_3^-, since chlorophyll and photosynthetic capacity are directly related to the content of P and N. in the plant (Singh and Reddy, 2015). Singh *et al*. (2017) suggested that under P deficiency, the decrease in chlorophyll content occurs as a mechanism to avoid excess light capture to protect PSII from light damage. On the other hand, P plays an important role in the linear flow of the electron chain in the stroma through the activity of ATP synthase (Carstensen *et al*., 2018), so this element is indispensable in the photosynthetic mechanism.

The increase in SPAD values is directly related to an increase in chlorophyll levels (Uddling *et al*., 2007). The higher content of N in the tissues explains the greater

Variables		LFW	LDW	RFW	RDW	SPAD	SD	N	P
LDW	r	.903**							
	p	.000							
RFW	r	.481**	.568**						
	p	.002	.000						
RDW	r	.620**	.663**	.741**					
	p	.000	.000	.000					
SPAD	r	.520**	.575**	.634**	.608**				
	p	.001	.000	.000	.000				
SD	r	.466**	.490**	.426**	.486**	.612**			
	p	.002	.001	.006	.001	.000			
N	r	.524**	.427*	.331	.424*	.488*	.427*		
	p	.002	.015	.064	.016	.011	.015		
P	r	.510**	.462**	.463**	.406*	.553**	.460**	.296	
	p	.003	.008	.008	.049	.001	.008	.100	
K	r	.339	.401*	.343	.429*	.433*	.423*	.235	.512**
	p	.058	.023	.055	.014	.013	.016	.196	.003

Figure 4. Correlation matrix between the variables evaluated. LFW: Leaf fresh weight; LDW: Leaf dry weight; RFH: Root fresh weight, RDW: Root dry weight; SPAD: Relative chlorophyll content; SD: Stem diameter; N; P; K. *Significant correlation ($p \leq 0.05$); **Highly significant correlation ($p \leq 0.01$); r: Pearson correlation coefficient; *p*: significance value.

production of chlorophylls due to the function of this element as a structural component of RuBisCO, the most abundant enzyme in the leaves (Hawkesford *et al*., 2012). On the other hand, N has high synergism with Mg absorption, so the increase in the latter also favors the increase in chlorophylls due to its importance as a central component of these molecules (Karthika *et al*., 2018). Krouk and Kiba (2020) mention that joint applications of N- and P-based fertilizers are more efficient than applying these elements separately. In this research, MAP and commercial fertilizers contained levels of N and P, which explains the results found.

On the other hand, the SPAD resulted in a positive relationship with the aerial biomass, root and stem diameter, which is explained because a higher content of chlorophylls in the leaves increases the photosynthetic rate (Rai-Kalal and Jajoo, 2021), resulting in greater vegetative growth. In turn, the foliar concentrations of N and P were related to the levels of SPAD, which was explained in the results previously presented. Likewise, N and P were positively correlated with most of the variables studied, which is explained by the functions of these elements in the metabolism of plants.

CONCLUSIONS

The application of different P fertilizer sources, such as seed pretreatments and foliar applications, favored the vegetative growth of tomato seedlings, as well as a higher relative content of chlorophyll and foliar concentrations of N, P and K. In general, the commercial liquid fertilizer and monoammonium phosphate applied via foliar application showed greater efficiency than seed pretreatment to increase the aforementioned variables. The results indicate that it is possible to increase the quality of the seedlings, which would potentially favor greater success in the establishment of this crop.

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