J. Agr. Sci. Tech. (2016) Vol. 18: 1079-1090

Physiological Responses of Chard and Lettuce to Phosphite Supply in Nutrient Solution

E. Estrada-Ortiz[1], L. I. Trejo-Téllez[1*], F.C. Gómez-Merino[2], H. V. Silva-Rojas[1], A. M. Castillo-González[3], and E. Avitia-García[3]

ABSTRACT

We evaluated the effect of different concentrations of Phosphite (Phi) (0, 0.25, and 0.50 mM) in nutrient solution on lettuce and chard. The fresh and dry biomass of lettuce shoots and heads, root volume, and P accumulation in roots showed no significant differences compared to the controls for different Phi concentrations in nutrient solution. In chard, no statistical differences were found among Phi concentrations for P concentrations in roots and shoots, total free amino-acids in leaves, chlorophyll-b, and soluble sugars. The phosphorus concentration in lettuce shoots was 15.6 and 50.6% higher in plants treated with 0.25 and 0.50 mM of Phi, respectively, compared with the controls. In lettuce, phosphorus levels in roots, total free amino-acids and soluble sugars in leaves were statistically greater for 0.25 mM of Phi in nutrient solution. The concentration of chlorophyll-a, b and total chlorophyll in lettuce leaves increased positively with Phi concentration in nutrient solution. The addition of more than 0.25 mM of Phi to the nutrient solution for chard negatively affected the fresh and dry biomass weight of shoots and roots, and P accumulation in roots and shoots. The concentration of chlorophyll-a, b and total chlorophyll in chard leaves was statistically higher with 0.25 mM of Phi in nutrient solution. We conclude that Phi has differential effects on lettuce and chard physiology, and positive plant responses may be observed when Phi is used up to 0.25 mM in sufficient P conditions.

Keywords: Biomass weight, Chlorophyll, Phosphorus, Total free amino-acids, Total soluble proteins.

INTRODUCTION

After nitrogen (N), phosphorus (P) is frequently the second most limiting macronutrient for plant growth. Phosphorus is an important plant macronutrient, representing up to 0.2% of plant dry biomass weight. It is a key component in molecules such as nucleic acids, phospholipids and ATP (Schachtman *et al*., 1998). Phosphorus is an essential element for plant growth, development and reproduction and is required in large quantities. Its functions cannot be performed by any other element, and without sufficient quantities the plant will not express its fullest potential yield because P plays an important role in energy storage and transfer in plant cells (Fageria, 2008), forms important parts of ribonucleic acids (RNA) and DeoxyriboNucleic Acid (DNA), is involved in protein synthesis and is a constituent of many essential compounds in plant metabolism (Alcántar *et*

[1] Graduate College Campus Montecillo, Department of Soil Science. Montecillo, Texcoco 56230, State of Mexico, Mexico.
[*] Corresponding author; e-mail: tlibia@colpos.mx
[2] Graduate College Campus Cordoba, Department of Plant Biotechnology. Manuel León, Amatlán de los Reyes 94946, Veracruz, Mexico.
[3] Chapingo Autonomous University, Department of Horticulture. Chapingo, Texcoco 56230, State of Mexico, Mexico.

[DOR: 20.1001.1.16807073.2016.18.4.5.9]

al., 2007). Phosphorus is also involved in the control of enzymatic reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). Phosphorus is absorbed and assimilated by the plant in the form of phosphate ($H_2PO_4^-$, Pi), a structural component of many organic compounds such as DNA, RNA, phospholipids and phosphorylated sugars (Berkowitz *et al*., 2013).

Phosphite ($H_2PO_3^-$, Phi) is a phosphate analog wherein a hydroxyl group is replaced by a hydrogen atom. Phosphite enters the cell *via* Pi transporters, so its absorption competes with Pi for mobility within the plant (Ouimette and Coffey, 1989; Danova-Alt *et al*., 2008). Phosphite has direct and indirect effects on plant growth and is considered a very valuable product in agricultural applications. Phosphite inhibits cell-wall synthesis and formation of mycelia and cytoskeletal functions in the fungus *Phytophthora cinnamomi* (King *et al*., 2010). In some species such as *Brassica napus,* it has been shown to have negative effects on growth (Carswell *et al*., 1997). Yet, the application of Phi to strawberry plants has different responses depending on phenological stage. For example, in fruit production stage, adding 30% of total P as Phi stimulated plant metabolism increased the concentrations of chlorophyll-a, b, total chlorophyll and amino-acids and proteins, while during blooming, positive effects were observed with the addition of 20% of P as Phi on total sugar concentration in leaves (Estrada-Ortiz *et al*., 2011). Also, the supplying of Phi at 30% or less in the nutrient solution does not significantly affect strawberry yield but does affect fruit quality and activates plant defense mechanisms by producing a higher concentration of anthocyanins (Estrada-Ortiz *et al.,* 2013). Just recently, Constán-Aguilar *et al*. (2014) found that the application of Phi as a P fertilizer at a rate of ≥ 0.50 mM would be an appropriate and effective strategy under suboptimal conditions of Pi in the growth medium, as it improves growth parameters, number of flowers, leaf area, nutritional state of P, incorporation of P in structural organs, and P-use efficiency in cucumber plants.

The objective of the present study was to evaluate the effect of different concentrations of phosphite in nutrient solution using growth and physiological indicators for lettuce (*Lactuca sativa* L. cv. Climax) and chard (*Beta vulgaris* L. var. *cicla* cv. Fordhook Giant).

MATERIALS AND METHODS

Experimental Conditions

The research was conducted during the summer of 2011, in an overhead-lighted greenhouse, located at 19° 29' N, 98° 53' W at an altitude of 2,250 m asl. The plant species used were lettuce (*Lactuca sativa* L. cv. Climax) and chard (*Beta vulgaris* L. var. *cicla* cv. Fordhook Giant) grown in a floating root hydroponic system with oxygenation. The maximum, minimum, and average temperatures during the experiment were 35.8, 5.2, and 18°C, respectively. Light intensity averaged 280 µmol m^{-2} s^{-1}.

Treatments and Experimental Design

We evaluated three different nutrient solutions differing only in the concentrations of Phi. The solutions were made with reference to Steiner's nutrient solution (Steiner, 1984) with 100% analytic reagents using the following mM concentrations: 4.49 $Ca(NO_3)_2$ $4H_2O$, 2.97 KNO_3, 1.03 KH_2PO_4, 1.99 $MgSO_4$ $7H_2O$ and 1.49 K_2SO_4. The nutrient solution was supplemented with micronutrients in the following µM concentrations: 29.12 Mn, 1.73 Cu, 79.56 B, 0.35 Zn and 0.50 Mo. Iron was supplied as Fe-EDTA at a concentration of 89.53 µM from a stock solution prepared following Steiner and van Winden (1970). The concentration of Phi in the solution was assessed at 0, 0.25 and 0.50 mM. Phosphite was obtained from analytical grade

[DOR: 20.1001.1.16807073.2016.18.4.5.9]

phosphorous acid (Sigma-Aldrich). The pH of the nutrient solution was maintained between 5.5 and 5.8 because it is considered optimal for Phi availability (Hanrahan *et al*., 2005) and was adjusted by adding 97% H_2SO_4 and 1N NaOH.

The experimental unit was represented by 6 plants in a floating root hydroponic system, placed in containers of 80×40×20 cm (length, width, height) and supported by a Styrofoam® plate, and with oxygenation. Each treatment had four replicates and a completely randomized design was used.

Variables Evaluated

Growth Parameters

To obtain fresh biomass weight of heads and shoots, plants were harvested early to avoid errors from dehydration and were immediately weighed on a balance (Adam Model CQT1501). Root volume was obtained by volumetric displacement of water in a 250 mL beaker.

Harvested plant material was dried in a forced air oven (Riossa Model HCF-125D) for 72 hours at 70°C, and then the dry weight of roots, shoots, and heads was determined using an analytical balance (Ohaus Model Adventurer Pro AV213C).

Phosphorus Concentration and Accumulation

The concentration of total phosphorus in roots and shoots was determined using wet digestion of dry plant material with a mixture of perchloric and nitric acids (Alcántar and Sandoval, 1999). The extracts were read using an Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) (Varian model 725-S).

The accumulations of P in roots and shoots were estimated from the corresponding dry biomass weights and the concentrations of this element in each part of the plant were analyzed. The difference between accumulation and concentration is that the former takes into consideration the dry biomass weight of the corresponding plant tissue and correlates it with the concentration of a determined element in such a tissue, whereas the latter does not.

Metabolites Concentration

The concentration of total soluble protein in leaves was determined using extractions performed according to Höfner *et al*. (1989). Quantification was performed using amido-black staining and bovine serum albumin as a standard protein. The extracts were read in a spectrophotometer (Thermo Fisher Scientific model Genesys 10 UV) at a wavelength of 640 nm.

In leaves, amino acids were extracted according to Geiger *et al*. (1998). Subsequently, amino acid concentration was determined using the ninhydrin method described by Moore and Stein (1954). Leucine was used to prepare the standard curve and concentrations were read by spectrophotometry at a wavelength of 570 nm.

Chlorophyll concentrations in leaves were determined following Harborne (1973) and samples were read by spectrophotometry at wavelengths of 663 and 645 nm. Chlorophyll-a, b and total chlorophyll were expressed as mg g^{-1} fresh biomass weight.

The soluble sugar concentration in leaves was determined by spectrophotometry according to the method described by Southgate (1976), at a wavelength of 620 nm. Sucrose was used to prepare the standard curve.

Statistical Analysis

The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to verify that the data followed a normal distribution, and the Levene, O'Brien and Bartlet tests were used to verify variance homogeneity. An Analysis Of Variance (PROC ANOVA) was then

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applied and means were compared using the Tukey test (α= 0.05) provided in the software Statistical Analytic System, version 9.3 (SAS Institute Inc., 2011).

RESULTS AND DISCUSSION

Growth Parameters

Root volume and the fresh biomass weight of lettuce heads and shoots were not significantly affected by the Phi treatments evaluated (Tukey: P= 0.58, P= 0.20 and P= 0.91, respectively). In chard, Phi concentrations above 0.25 mM reduced root volume and the administration of Phi decreased fresh shoot biomass as shown in Table 1. Studies assessing the phosphate-phosphite relationships in spinach, celery, Japanese spinach (Komatsuna) and lettuce have shown that as the concentration of Phi increases, plant growth decreases (Thao and Yamakawa, 2008; Thao *et al*., 2008a, b), which coincides with the results obtained in our experiment on chard. According to Constán-Aguilar *et al*. (2014), the validity of the foliar use of Phi as a P fertilizer in cucumber plants significantly depends on the Pi availability in the culture medium. Therefore, beneficial effects of Phi are evident especially when applied in the presence of sufficient Pi.

In lettuce, dry weight of roots and heads were not significantly affected by the addition of Phi to the nutrient solution (Table 2) (Tukey: P= 0.61 and P= 0.30, respectively). In contrast, adding more than 0.25 mM of Phi reduced dry biomass weight accumulation in chard roots and shoots (Tukey: P= 0.01 and P= 0.0009, respectively).

In the presence of sufficient Pi in the nutrient solution, the application of Phi to healthy lettuce plants has no positive effects on plant growth (Thao *et al*., 2009). Given a sufficient level of P in the cultivation of strawberries, there was no effect on the dry biomass weight of shoots by adding Phi (Estrada-Ortiz *et al*., 2012). Bertsch *et al*. (2009) found a synergistic effect of Phi on dry biomass weight when it was added in combination with Pi at 30 mg kg^{-1} of each P source in lettuce, bananas, and tomatoes. In contrast, a significant decrease in the growth and dry biomass weight of pumpkins has been reported when Phi was applied to the soil as a source of P (Ratjen and Gerendás, 2009).

Phosphorus Concentration and Accumulation

The concentrations of P in lettuce and chard roots were higher than those in shoots, regardless of treatment (Figure 1). In lettuce, Phi positively influenced the concentration of P in shoots; the addition of Phi to the nutrient solution at 0.25 and 0.50 mM resulted in increases in the concentration of P in shoots (15.6 and 50.6%, respectively) compared with the control. Moreover, the highest concentration of P in roots was

Table 1. Accumulation of fresh biomass and root volume for chard and lettuce in response to treatments with phosphite in nutrient solution.[a]

	Chard		Lettuce		
Phosphite (mM)	Root volume (cm^3 $plant^{-1}$)	Shoot weight (g $plant^{-1}$ FW)	Root volume (cm^3 $plant^{-1}$)	Head weight (g $plant^{-1}$ FW)	Shoot weight (g $plant^{-1}$ FW)
0.00	53.67 a	426.97 a	37.75 a	355.75 a	686.58 a
0.25	51.00 a	347.99 b	34.50 a	366.44 a	695.97 a
0.50	39.33 b	330.47 b	33.50 a	384.34 a	665.54 a
HSD	9.11	57.34	10.59	38.36	57.93
Pr> F	0.0064	0.0004	0.5800	0.1991	0.9082

[a] Values with different letters between columns are statistically different (Tukey, α = 0.05). *HSD*: Honestly

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Table 2. Accumulation of dry biomass for chard and lettuce in response to treatments with phosphite in nutrient solution.[a]

Phosphite (mM)	Chard		Lettuce	
	(g plant^{-1} DW)			
	Root	Shoot	Root	Head
0.00	5.38 a	42.58 a	1.83 a	20.23 a
0.25	5.15 a	44.83 a	1.78 a	19.40 a
0.50	4.35 b	31.15 b	1.53 a	14.08 a
HSD	0.80	7.00	0.87	11.27
Pr> F	0.0140	0.0009	0.6072	0.3027

[a] Values with different letters between columns are statistically different (Tukey, α = 0.05). *HSD*: Honestly Significant Difference, FW: Fresh Weight.

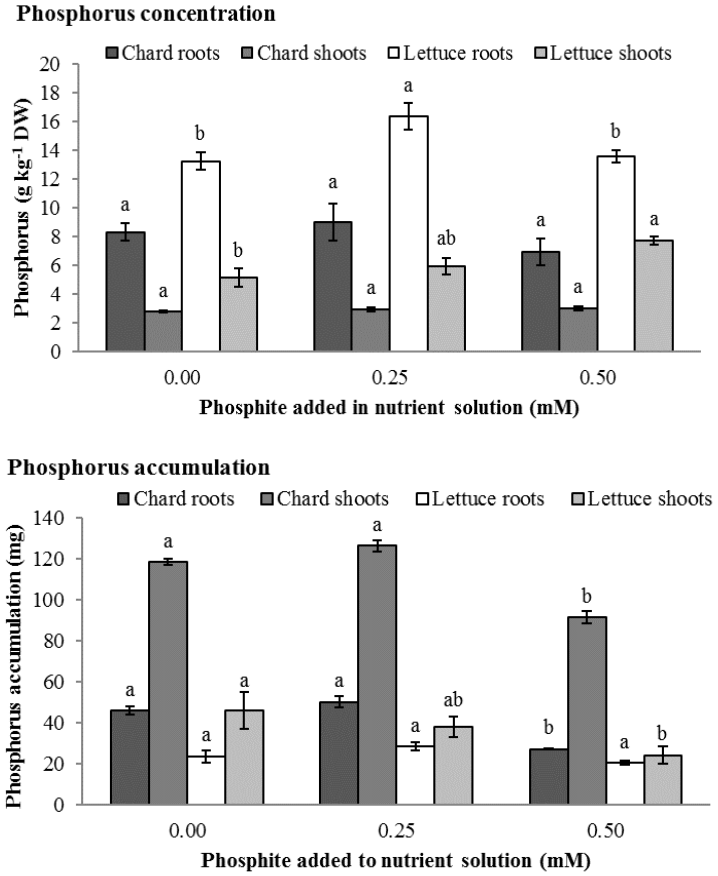

Figure 1. Phosphorus concentrations and phosphorus accumulation in chard and lettuce roots and shoots in response to different concentrations of phosphite in nutrient solution. Bars with different letters in each variable are significantly different (Tukey, Phosphorus concentration: Chard roots P= 0.37, chard shoots P= 0.660, lettuce roots P= 0.02 and lettuce shoots P= 0.02; Phosphorus accumulation: Chard roots P= 0.001, chard shoots P= 0.0001, lettuce roots P= 0.08 and lettuce shoots P= 0.056). DW= Dry Weight.

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observed in the treatment with 0.25 mM Phi and this was statistically higher than the other treatments (Figure 1). Reduction in the concentration of P in roots with 0.50 mM of Phi may be due to greater mobility of Phi within the plant which changed the distribution of P in the plant, since the highest concentration of P in shoots was observed in this treatment. In chard, no statistical differences were observed for P concentrations in roots and shoots with Phi added to the nutrient solution. Bertsch *et al*. (2009) found that the combination of Pi and Phi had a synergistic effect which led to a higher total absorption of P by the plants, especially in tomatoes, which agrees with the observations made on lettuce shoots in the present study.

The accumulation of this macronutrient was greater in shoots than in roots of lettuce and chard (Figure 1). In lettuce roots, no statistically significant effects from the different Phi concentrations in nutrient solution were observed on the accumulation of P, although in shoots there was a reduction of 17.7 and 47.2% with 0.25 and 0.50 mM of Phi, respectively. In chard, Phi applications exceeding 0.25 mM in nutrient solution significantly reduced P accumulation in shoots and roots (Figure 1, Tukey: P= 0.0001 and P= 0.001, respectively).

Ávila *et al*. (2013) found no statistically significant differences in P accumulation in beans by adding Phi to the nutrient solution for Pi-sufficient plants (800 µmol). As well, plants receiving higher concentrations of Phi (64, 128, 256 and 512 µmol) in nutrient solution in combination with a low concentration of Pi (80 µmol) did not produce seeds in their pods, and there was an increase in the total accumulation of P in shoots and roots as the concentration of Phi increased in nutrient solution.

Metabolites Concentration

The protein concentration in lettuce leaves was higher with 0.25 mM of Phi (Figure 2), but it was not statistically different from the control (Tukey, P= 0.01). On the other hand, a decrease in leaf protein concentration was observed in chard by adding 0.25 and 0.50 mM of Phi to the nutrient solution, resulting in reductions of 65.9 and 76.8%, respectively (Figure 2, Tukey, P= 0.0001).

Estrada-Ortiz *et al*. (2011) found that applying up to 30% of total P in the form of Phi in nutrient solution increased protein concentrations in strawberry leaves, while higher concentrations diminished such protein contents. These results are very

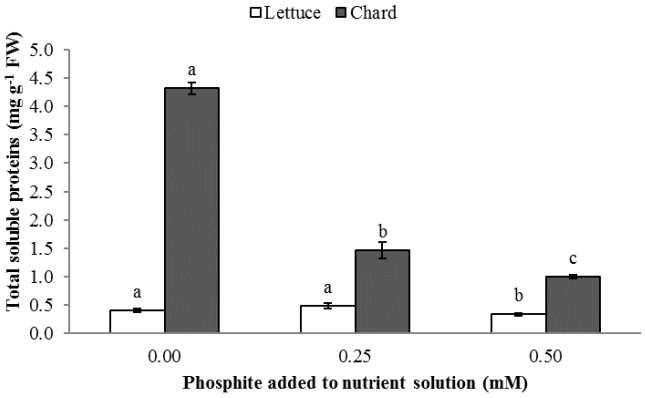

Figure 2. Total soluble protein concentrations in chard and lettuce leaves in response to different concentrations of phosphite in nutrient solution. Bars with different letters in each plant species are statistically different (Tukey, lettuce P= 0.01 and chard P= 0.0001). FW= Fresh Weight.

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similar to those in the present study, where the addition of 0.25 mM of Phi to the nutrient solution promoted a higher concentration of protein in lettuce, while the highest concentration of Phi evaluated (0.50 mM) resulted in a reduction in protein concentration.

Phosphite inhibits the phosphorylation of proteins when there is stress for P, a condition in which Phi suppresses nucleolytic enzyme activity and the expression of acid phosphatase and P transporter genes in *A. thaliana* (Ticconi *et al*., 2001), which may explain what happened in chard in our experiment, where the addition of Phi promoted competition in plant uptake between Pi and Phi, resulting in stress.

Amino-acid concentration in lettuce leaves followed a trend similar to that for the foliar concentration of total proteins in response to the administration of Phi. Adding 0.25 mM of Phi significantly increased the concentration of amino-acids (Figure 3) relative to the other treatments (Tukey, P= 0.0001), while no significant differences among treatments were observed for chard regarding the concentration of total free amino-acids (Figure 3, Tukey: P= 0.66).

Berkowitz *et al*. (2013) found that Phi reduced amino-acids such as asparagine, aspartate, glutamate and serine. A decrease of amino-acids under strong P limitation in *Arabidopsis* has also been reported by Morcuende *et al*. (2007). These results agree with those obtained in the present study when the concentration of Phi in nutrient solution was increased to 0.50 mM for lettuce. These results are important since recent studies have identified a primary function of metabolites such as amino-acids in the establishment of resistance against plant pathogens (Chanda *et al*., 2011; Hwang *et al*., 2011; Stuttmann *et al*., 2011; Voll *et al*., 2012). Hence, by altering the levels of specific metabolites in plant metabolic pathways, it is possible to induce resistance to pathogens. For example, variation in the concentration of amino-acids derived from the aspartate path promotes resistance to the oomycete *Hyaloperonospora arabidopsidis* and the bacterium *Pseudomonas syringae* (van Damme *et al*., 2009; Stuttmann *et al*., 2011; Navarova *et al*., 2012).

In lettuce and chard, different concentrations of Phi in the nutrient solution increased the concentrations of chlorophyll-a, b and total chlorophyll in leaves (Figure 4). By adding 0.25 mM of Phi to the nutrient solution for lettuce, there was an increase of 26.3, 60 and 33.3% in chlorophyll-a, b and

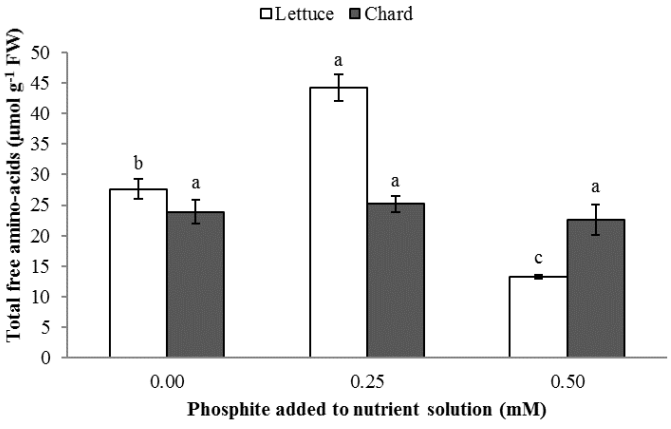

Figure 3. Total free amino-acid concentrations in chard and lettuce leaves in response to different concentrations of phosphite in nutrient solution. Bars with different letters in each plant species are statistically different (Tukey, lettuce P= 0.0001 and chard P= 0.0001). FW= Fresh Weight.

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total chlorophyll, respectively. On the other hand, by applying 0.50 mM of Phi to the nutrient solution for lettuce, chlorophyll-a, b and total chlorophyll increased by 71.1, 100 and 77.1%, respectively, relative to the control. In chard, the largest increases in chlorophyll-a, b and total chlorophyll (13.9, 2.8, and 9.9%, respectively) occurred when 0.25 mM of Phi was added to the nutrient solution, in comparison to the control (Figure 4).

Phosphorus is a nutrient that influences the stability of the chlorophyll molecule (Bojović and Stojanović, 2006). Phosphite signaling suppresses the need for P, which leads to changes in chloroplast photosynthetic membrane composition (Kobayashi *et al*., 2006). Accordingly, Estrada-Ortiz *et al*. (2011) observed an increase in the concentration of chlorophyll-a, b and total chlorophyll in strawberry leaves during the fruiting stage by adding 30% of P in the form of a Phi nutrient solution.

The soluble sugar concentrations were not affected by the addition of Phi in the nutrient solution for chard. Conversely, in lettuce plants, this variable was 72.5% higher when adding 0.25 mM de Phi, in comparison to the control plants. Interestingly, higher concentrations of Phi applied to lettuce had negative effects on soluble sugar concentrations (Table 3).

Total soluble sugars are positively

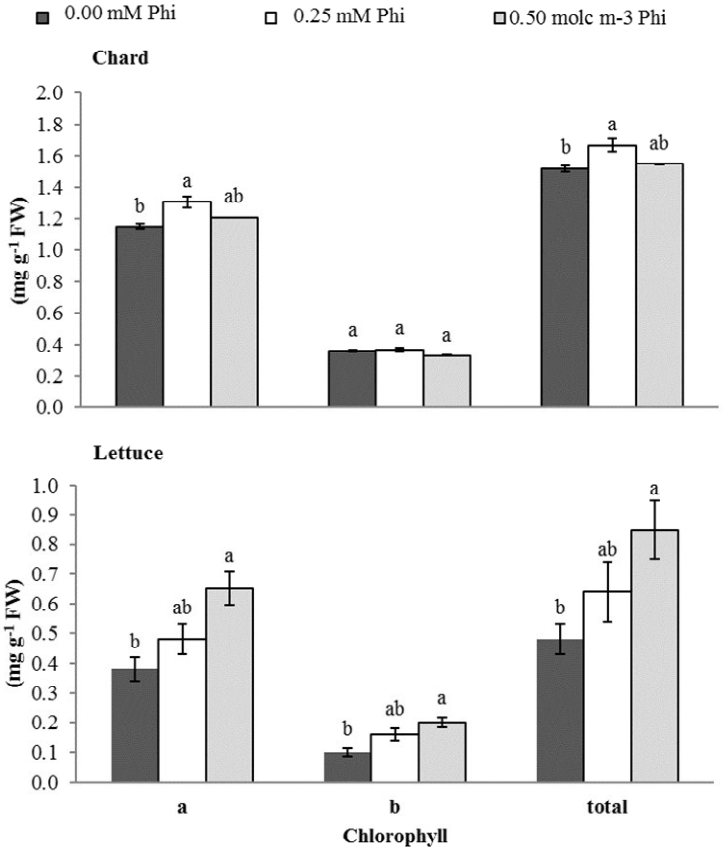

Figure 4. Concentration of chlorophyll-a, b and total chlorophyll in chard and lettuce leaves in response to different concentrations of phosphite in nutrient solution. Bars with different letters in each variable are statistically different (Tukey, chard chlorophyll-a P= 0.0137, chard chlorophyll-b P= 0.0457, chard total chlorophyll P= 0.0380, lettuce chlorophyll-a P= 0.0095, lettuce chlorophyll-b *P*=0.0095 and lettuce total chlorophyll P= 0.0082). FW= Fresh Weight.

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Table 3. Soluble sugars concentration in chard and lettuce leaves in response to treatments with phosphite in nutrient solution.[a]

Phosphite (mM)	Chard	Lettuce
	(mg g^{-1} FW)	
0.00	2.51 a	3.02 b
0.25	3.36 a	5.21 a
0.50	2.93 a	1.74 b
HSD	1.24	1.79
Pr>F	0.2173	0.0013

[a] Values with different letters between columns are statistically different (Tukey, α = 0.05). HSD: Honestly Significant Difference, FW: Fresh Weight.

correlated with plant P status and their biosynthesis depends on P availability in the cytosol (Ruiz *et al.,* 1996). In our study, P was always available in sufficient amounts for chard plants, and we were unable to observe any Phi effect on total soluble sugars in leaves. In lettuce, our results are comparable to those reported by Estrada-Ortiz (2010) in strawberry, as total sugars concentrations were the highest when applying 20% of P as Phi, while higher Phi concentrations had negative impacts on this variable. Sugars function not only as substrates to sustain plant growth, but are also important signaling molecules that regulate sink and source metabolism (Roitsch, 1999) and they have a pivotal role in crop yield.

CONCLUSIONS

Our results demonstrated that Phi may trigger different responses in plants depending on the genotypes and Phi concentrations used. In lettuce, Phi did not have significant effects neither on growth parameters nor on accumulation of P in roots compared to the control. Similarly, in chard, Phi did not affect concentrations of P, total free amino-acids in leaves and chlorophyll-b. However, in chard, applications of different Phi concentrations decreased the protein concentration in leaves compared with the control.

Interestingly, in lettuce plants, the application of 0.25 mM of Phi increased total P concentrations in roots, total free amino-acids, soluble sugars, and the concentration of chlorophylls in chard leaves.

The addition of more than 0.25 mM of Phi to the nutrient solution for chard negatively affected growth parameters and P accumulation in both roots and shoots. Conversely, additions of 0.50 mM of Phi increased the total concentration of P in lettuce shoots and the concentration of chlorophylls in lettuce leaves.

Our results suggest that the addition of 0.25 mM of Phi in the nutrient solution stimulates plant metabolism without detrimental effects on growth and yield. Therefore, to trigger positive effects on plants, Phi applications must be tightly regulated and used at low levels in the presence of sufficient Pi. The responses will finally depend on plant genotypes, Pi status and Phi concentrations applied.

ACKNOWLEDGEMENTS

We thank Mexico's National Science and Technology Council (CONACYT) for the PhD Fellowship granted to EEO. EEO also thanks the Programa de Fortalecimiento Académico del Posgrado de Alta Calidad 2013 supported by CONACYT. The authors are very grateful to the Colegio de

[DOR: 20.1001.1.16807073.2016.18.4.5.9]

Postgraduados, which provided laboratory space and infrastructure support through the Management and Investment Trust No. 167304 and to Priority Research Line No. 5 Microbiological, Plant and Animal Biotechnology.

REFERENCES

1. Alcántar, G. G. and Sandoval, V. M. 1999. *Manual de Análisis Químico de Tejido Vegetal. Guía de Muestreo, Preparación, Análisis e Interpretación*. Publicación Especial No. 10 de la Sociedad Mexicana de la Ciencia del Suelo A. C., Chapingo, México.
2. Alcántar, G. G., Sandoval, V. M. and Sánchez, G. P. 2007. Elementos Esenciales. Chapter 2. In: "*Nutrición de Cultivos*", (Eds.): Alcántar G., G. and Trejo-Téllez, L. I.. Colegio de Postgraduados, Editorial Mundi-Prensa, México, PP. 8–47
3. Ávila, F. W., Faquin, V., da Silva, L. A. K., Ávila, P. A., Marques, D. J., Silva G. E. M. and Yuen, T. D. K. 2013. Effect of Phosphite Supply in Nutrient Solution on Yield, Phosphorus Nutrition and Enzymatic Behavior in Common Bean (*Phaseolus vulgaris* L.) plants. *Aus. J. Crop Sci.*, **7:** 713–722.
4. Berkowitz, O., Jost, R., Kollehn, D. O., Fenske, R., Finnegan, P. M., O'Brien, P. A., Hardy, G. E. St. J. and Lambers, H. 2013. Acclimation Responses of *Arabidopsis thaliana* to Sustained Phosphite Treatments. *J. Exp. Bot*., **64:** 1731–1743.
5. Bertsch, F., Ramírez, F. and Henríquez, C. 2009. Evaluación del Fosfito Como Fuente Fertilizante de Fósforo vía Radical y Foliar. *Agronomía Costarricense*, **33**: 249–265.
6. Bojović, B. and Stojanović, J. 2006. Some Wheat Leaf Characteristics in Dependence of Fertilization. *Kragujevac J. Sci.*, **28**: 139–146.
7. Carswell, M. C., Grant, B. R. and Plaxton, W. C. 1997. Disruption of the Phosphate-starvation Response of Oilseed Rape Suspension Cells by the Fungicide Phosphonate. *Planta*, **203**: 67–74.
8. Chanda, B., Xia, Y., Mandal, M. K., Yu, K., Sekine, K. T., Gao, Q. M., Selote, D., Hu, Y., Stromberg, A., Navarre, D., Kachroo, A. and Kachroo, P. 2011. Glycerol-3-phosphate Is a Critical Mobile Inducer of Systemic Immunity in Plants. *Nature Gen.*, **43**: 421–427.
9. Constán-Aguilar, C., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., Camacho, M. A., Romero, L., Ruiz, J. M. and Blasco, B. 2014. Physiological and Nutritional Evaluation of the Application of Phosphite as a Phosphorus Source in Cucumber Plants. C*ommun. Soil Sci. Plant Anal.*, **45**: 204-222.
10. Danova-Alt, R., Dijkema, C., De Waard, P. and Kock, M. 2008. Transport and Compartmentation of Phosphite in Higher Plant Cells-kinetic and ^{31}P Nuclear Magnetic Resonance Studies. *Plant Cell Environ.,* **31:** 1510–1521.
11. Estrada-Ortiz, E. 2010. Fosfito en la Producción de Fresa. Tesis de Maestría en Ciencias, Posgrado en Edafología, Colegio de Postgraduados, Montecillo, Estado de México. 104 PP.
12. Estrada-Ortiz, E., Trejo-Téllez, L. I., Gómez-Merino, F. C., Núñez-Escobar, R. and Sandoval-Villa, M. 2011. Biochemical Responses in Strawberry Plants Supplying Phosphorus in the Form of Phosphite. *Rev. Chapingo Ser. Hortic.*, **17**: 129–138.
13. Estrada-Ortiz, E., Trejo-Téllez, L. I., Gómez-Merino, F. C., Núñez-Escobar, R. and Sandoval-Villa, M. 2012. Phosphite on Growth and Fruit Quality in Strawberry. *Acta Hortic*., **947:** 277–282.
14. Estrada-Ortiz, E., Trejo-Téllez, L. I., Gómez-Merino, F. C., Núñez-Escobar, R. and Sandoval-Villa, M. 2013. The Effects of Phosphite on Strawberry Yield and Fruit Quality. *J. Soil Sci. Plant Nutr*., **13:** 612–620.
15. Fageria, N. K. 2008. *The Use of Nutrients in Crop Plants.* CRC Press, Boca Raton, Florida, USA.
16. Geiger, M., Walch-Liu, P., Engels, C., Harnecker, J., Schulze, E. D., Ludewig, F., Sonnewald, U., Scheible, W. R. and Stitt, M. 1998. Enhanced Carbon Dioxide Leads to a Modified Diurnal Rhythm of Nitrate Reductase Activity and Higher Levels of Amino Acids in Young Tobacco Plants. *Plant Cell Environ*., **21**: 253–268.
17. Hanrahan, G., Salmassi, T. M., Khachikian, C. S. and Foster, K. L. 2005. Reduced Inorganic Phosphorus in the Natural Environment: Significance, Speciation and Determination. *Talanta,* **66:** 435–444.

[DOR: 20.1001.1.16807073.2016.18.4.5.9]

18. Harborne, J. B. 1973. Chlorophyll Extraction. In: "*Phytochemical Methods: Recommended Technique*", (Ed.): Harbone, J. B.. Chapman and Hall, London, PP. 205–207.
19. Höfner, R., Vásquez-Moreno, L., Abou-Mandour, A. A., Bohnert, H. J. and Schmitt, J. M. 1989. Two Isoforms of Phosphoenolpyruvate Carboxylase in the Facultative CAM Plant *Mesembryanthemum crystallinum*. *Plant Physiol. Biochem*., **27**: 803–810.
20. Hwang, I. S., An, S. H. and Hwang, B. K. 2011. Pepper Asparagine Synthetase 1 (CaAS1) Is Required for Plant Nitrogen Assimilation and Defense Responses to Microbial Pathogens. *Plant J.,* **67**: 749–762.
21. King, M., Reeve, W., Van der Hoek, M. B., Williams, N., McComb, J., O'Brien, P. A. and Hardy, G. E. St .J. 2010. Defining the Phosphite-regulated Transcriptome of the Plant Pathogen *Phytophthora cinnamomi*. *Mol. Gen. Genom.*, **284**: 425–435.
22. Kobayashi, K., Masuda, T., Takamiya, K. and Ohta, H. 2006. Membrane Lipid Alteration during Phosphate Starvation is Regulated by Phosphate Signaling and Auxin/Cytokinin Cross-talk. *Plant J.,* **47:** 238–248.
23. Moore, S. and Stein, W. H. 1954. A Modified Ninhydrin Reagent for the Photometric Determination of Amino Acids and Related Compounds. *J. Biol. Chem.,* **211**: 893-906.
24. Morcuende, R., Bari, R., Gibon, Y., Zheng, W., Pant, B. R. D., Sing, O. B., Usadel, B. R., Czechowski, T., Udvardi, M. K., Stitt, M. and Scheible, W. D. 2007. Genome-wide Reprogramming of Metabolism and Regulatory Networks of *Arabidopsis* in Response to Phosphorus. *Plant Cell Environ*., **30:** 85-112.
25. Navarova, H., Bernsdorff, F., Doring, A. C. and Zeier, J. 2012. Pipecolic Acid, an Endogenous Mediator of Defense Amplification and Priming, Is a Critical Regulator of Inducible Plant Immunity. *Plant Cell*, **24:** 5123-5141.
26. Ouimette, D. G. and Coffey, M. D. 1989. Phosphonate Levels in Avocado (*Persea americana*) Seedlings and Soil Following Treatment with Fosetyl-Al or Potassium Phosphonate. *Plant Dis*., **73**: 212-215.
27. Ratjen, A. M. and Gerendás, J. 2009. A Critical Assessment of the Suitability of Phosphite as a Source of Phosphorus. *J. Plant Nutr. Soil Sci*., **172:** 821:828.
28. Roitsch, T. 1999. Source-sink Regulation by Sugar and Stress. *Curr. Opinion Plant Biol.*, **2**: 198-206.
29. Ruiz, J. M., Belakbir, A. and Romero L. 1996. Foliar Level of Phosphorus and Its Bioindicators in *Cucumis melo* Grafted Plants: A Possible Effect of Rootstocks. *J. Plant Physiol.*, **149**: 400-404.
30. SAS Institute Inc. 2011. *SAS/STAT Users Guide, Version 9.3*. SAS Institute Inc., Cary, North Carolina, USA.
31. Schachtman, D. P., Reid, R. J. and Ayling, S. M. 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiol*., **116**: 447-453.
32. Southgate, D. A. T. 1976. Determination of Food Carbohydrates. Applied Science Publishers, Ltd., London, UK.
33. Steiner, A. 1984. The Universal Nutrient Solution, In: "*ISOSC Proceedings 6th International Congress on Soilless Culture",* The Netherlands, PP. 633-649.
34. Steiner, A. and van Winden, H. 1970. Recipe for Ferric Salts of Ethylenediaminetetraacetic Acid. *Plant Physiol*., **46:** 862-863.
35. Stuttmann, J., Hubberten, H. M., Rietz, S., Kaur, J., Muskett, P., Guerois, R., Bednarek, P., Hoefgen, R. and Parker, J. E. 2011. Perturbation of *Arabidopsis* Amino Acid Metabolism Causes Incompatibility with the Adapted Biotrophic Pathogen *Hyaloperonospora arabidopsidis*. *Plant Cell*, **23:** 2788-2803.
36. Thao, H. T. B. and Yamakawa, T. 2008. Growth of Celery (*Apium graveolens* var. *dulce*) as Influenced by Phosphite. *J. Fac. Agr. Kyushu U.,* **53:** 375-378.
37. Thao, H. T. B., Yamakawa, T., Sarr, P. S. and Myint, A. K. 2008a. Effects of Phosphite, a Reduced Form of Phosphate, on the Growth and Phosphorus Nutrition of Spinach (*Spinacia oleracea* L.). *Soil Sci. Plant Nutr*., **54:** 761-768.
38. Thao, H. T. B., Yamakawa, T., Shibata, K., Sarr, P. S. and Myint, A. K. 2008b. Growth Response of Komatsuna (*Brassica rapa* var. *Peruviridis*) to Root and Foliar Applications of Phosphite. *Plant Soil*, **308**: 1-10.
39. Thao, H. T. B., Yamakawa, T. and Shibata, K. 2009. Effect of Phosphite-phosphate Interaction on Growth and Quality of

[DOR: 20.1001.1.16807073.2016.18.4.5.9]

Hydroponic Lettuce (*Lactuca sativa*). *J. Plant Nutr. Soil Sci.*, **172**: 378-384.
40. Theodorou, M. E. and Plaxton, W. C. 1993. Metabolic Adaptations of Plant Respiration to Nutritional Phosphate Deprivation. *Plant Physiol.,* **101**: 339-334.
41. Ticconi, C. A., Delatorre, C. A. and Abel, S. 2001: Attenuation of Phosphate Starvation Responses by Phosphite in *Arabidopsis*. *Plant Physiol*., **127**: 963-972.
42. van Damme, M., Zeilmaker, T., Elberse, J., Andel, A., de Sain-van, der Velden, M. and van den Ackerveken, G. 2009. Downy Mildew Resistance in *Arabidopsis* by Mutation of Homoserine Kinase. *Plant Cell*, **21**: 2179-2189.
43. Voll, L. M., Zell, M. B., Engelsdorf, T., Saur, A., Wheeler, M. G., Drincovich, M. F., Weber, A. P. and Maurino, V. G. 2012. Loss of Cytosolic NADP-malic Enzyme 2 in *Arabidopsis thaliana* Is Associated with Enhanced Susceptibility to *Colletotrichum higginsianum*. *New Phytol.*, **195**: 189-202.

واکنش های فیزیولوژیکی چغندر برگی و کاهو به تامین فسفیت در محلول غذایی

ا. استرادا-اورتیز، ل. ی. ترجو-تلز، ف. س. گومز-مرینو، ه. و. سیلوا- روجاز، ا. م. کاستیلو-گونزالز، و ا. اویتیا-گارسیا

چکیده

در این پژوهش، اثر فسفیت (Phi) با غلظت های مختلف (۰، ۰/۲۵، و ۰/۵۰ میلی مول) در محلول غذایی روی کاهو و چغندربرگی (Chard) ارزیابی شد. در غلظت های مختلف فسفیت در محلول غذایی، زیست توده تر و خشک شاخساره و کله کاهو، حجم ریشه، و انباشت فسفر در ریشه هیچگونه اختلاف معنی داری در مقایسه با تیمار شاهد نشان ندادند. در چغندر برگی، بین غلظت های فسفیت تفاوت معنی داری در غلظت فسفر در ریشه و شاخساره، در کل آمینو اسید های آزاد برگ، و در کلروفیل ب و قندهای محلول وجود نداشت. در کاهوی تیمار شده با ۰/۲۵ و ۰/۵۰ میلی مول فسفیت،غلظت فسفر در شاخساره به ترتیب ۱۵/۶٪ و ۵۰/۶٪ بیشتر از شاهد بود. در بوته های کاهو، غلظت فسفر در ریشه ، کل آمینو اسید های آزاد و قندهای محلول در برگ به طور معنی داری در تیمار ۰/۲۵ میلی مول فسفیت در محلول غذایی بیشتر بود. غلظت کلروفیل آ و ب و کلروفیل کل در برگ کاهو به طور مثبتی با زیاد شدن غلظت فسفیت در محلول غذایی افزایش یافت. در مورد چغندر برگی، کار برد فسفیت بیشتر از ۰/۲۵ میلی مول در محلول غذایی اثر منفی روی وزن زیست توده تر و خشک شاخساره و ریشه و انباشت فسفر در ریشه و شاخساره داشت. غلظت کلروفیل آ و ب و کلروفیل کل در برگ چغندر برگی در تیمار ۰/۲۵ میلی مول فسفیت به طور معنی داری بیشتر بود. چنین نتیجه گرفته شد که فسفیت اثرات متفاوتی روی فیزیولوژی کاهو و چغندر برگی دارد و واکنش های مثبت گیاه زمانی مشاهده می شود که در شرایط وجود فسفر کافی، غلظت فسفیت از ۰/۲۵ میلی مول تجاوز نکند.

[DOR: 20.1001.1.16807073.2016.18.4.5.9]