Phosphite supply affects phosphorus nutrition and biochemical responses in maize plants

Fabrício William Ávila*, Valdemar Faquin¹, Josinaldo Lopes Araújo², Douglas José Marques¹, Pedro Martins Ribeiro Júnior³, Allan Klynger da Silva Lobato⁴, Silvio Junio Ramos¹, Danielle Pereira Baliza⁵

¹Departamento de Ciência do Solo, Universidade Federal de Lavras, Lavras, Brazil
²Centro de Ciências e Tecnologia Agroalimentar, Universidade Federal de Campinas Grande, Pombal, Brazil
³Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, Brazil
⁴Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia, Paragominas, Brazil
⁵Departamento de Agricultura, Universidade Federal de Lavras, Lavras, Brazil

*Corresponding author: F.W. Ávila: fabriciowilliamavila@yahoo.com.br

Abstract

Phosphate (Pi) is the major phosphorus (P) form used for plant nutrition, whereas phosphite (Phi) is effective in controlling important plant diseases caused by Oomycetes pathogens. However, Phi-based products also have been widely marketed as either P fertilizer or biostimulant, such as elicitor of biochemical responses to abiotic and biotic stress agents, although these effects are not as yet well understood. This investigation has aimed to evaluate the effect of Phi supply as part of the P fertilization, and its influence on the guaiacol peroxidase activity and contents of total phenolics and lignin in maize plants. This study was conducted in an experimental design completely randomized, with 2 P concentrations (52 µM = low P concentration, and 644 µM = adequate P concentration) and 2 P forms (100% phosphate, and 75/25% as Pi/Phi, respectively). Based on studies of uptake kinetics of the ^32P, it was shown Phi inhibits Pi uptake competitively in maize, regardless of the plant Pi status. Replacement of 1/4 of Pi by Phi decreased the biomass production of the plants grown under low Pi supply, but no effect was observed in the plants grown under adequate Pi supply, with the advantage of eliciting biochemical responses to stress agents, such as stimulation of the guaiacol peroxidase activity and lignin biosynthesis.

Keywords: Acid phosphatases, Dry mass, Elicitor, Lignin, Peroxidases, Phosphate, Total phenolics, Uptake kinetics, Zea mays.

Abbreviations: Cmin - concentration of the nutrient in solution below which there is no inflow into the root system, Km - affinity between the nutrient and its transporter, P - phosphorus, Phi - phosphite, Pi - phosphate, Vmax - maximum uptake rate of the nutrient.

Introduction

Phosphate (Pi) is the major phosphorus (P) form used by plants for their adequate growth and development. However, another P form known as phosphite (Phi) has been marketed as fungicide, biostimulant, and as a superior P source for plant nutrition (McDonald et al., 2001; Thao and Yamakawa, 2009; Deliopoulos et al., 2010). Previous research conclusively indicates Phi as effective in controlling some plant diseases, especially those caused by organisms taxonomically classified in the phylum Oomycota, such as Phytophthora sp. Action of Phi is based on two mechanisms: the first is a direct toxic action on the pathogen and the second in indirect action, as Phi induces biochemical responses to abiotic and biotic stress agents, such as Oomycetes pathogens (McDonald et al., 2001; Wilkinson et al., 2001; Shearer and Fairman, 2007; Orbović et al., 2008; Cook et al., 2009). However, Phi effects on these biochemical responses in plants are not well understood. Moor et al. (2009) found Phi supply increased both ascorbic acid and anthocyanin content in strawberry fruit, and Lovatt and Mikkelsen (2006) reported Phi may stimulate shikimic acid pathway. Since this pathway is responsible for the biosynthesis of many aromatic compounds, there is the hypothesis that Phi improves peroxidase activity and biosynthesis of total phenolics and lignin. In addition, some studies have shown positive effects of the both peroxidase activity and lignin content on induction of plant defense mechanisms to abiotic and biotic stress agents (Cavalcanti et al., 2007; Cavalcanti et al., 2008; Moussa and Abdel-Aziz, 2008; Nagesh Babu and Devaraj, 2008). In terms of plant nutrition, different Phi-based products have been widely marketed as liquid fertilizers for foliar application and fertigation. Phi anion is recommended as fertilizer because it contains P or perhaps due to the difficulties for the industry to register the product as a fungicide. There is no evidence that Phi can enter the plant metabolism and perform the same functions as Pi. Although scarce, there are studies for some crops that show that the root or foliar Phi supply cannot replace Pi as sole P source in plant nutrition (Varadarajan et al., 2002; Thao et al., 2008). Instead, there are indications that Phi is not metabolized by plants, causing growth depression when grown under low P supply in the form of Pi (McDonald et al., 2001; Schroetter et al., 2006; Thao et al., 2009). In this case, it appears that Phi inhibits the gene expression related to the activation of mechanisms for overcoming P deficiency, such as increased phosphatase activity, biosynthesis of high affinity transporters for P and elongation of the root system (Ticconi et al., 2001; Varadarajan et al., 2002; Lee et al., 2005). In this context, this
Treatments applied during the maize growth.

<table>
<thead>
<tr>
<th>Total P concentration (µM)</th>
<th>P source</th>
<th>Concentration of each P source (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>Only Pi</td>
<td>52Pi</td>
</tr>
<tr>
<td>52</td>
<td>3/4 Pi + 1/4 Phi</td>
<td>39Pi+13Phi</td>
</tr>
<tr>
<td>644</td>
<td>Only Pi</td>
<td>644Pi</td>
</tr>
<tr>
<td>644</td>
<td>3/4 Pi + 1/4 Phi</td>
<td>483Pi+161Phi</td>
</tr>
</tbody>
</table>

Table 1. Treatments applied during the maize growth.

Uptake solution Growth solution (µM) Abbreviation

<table>
<thead>
<tr>
<th>Uptake solution</th>
<th>Growth solution (µM)</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>52Pi</td>
<td>-Phi — 52Pi</td>
<td></td>
</tr>
<tr>
<td>-Phi (0 µM)</td>
<td>39Pi+13Phi</td>
<td>-Phi — 39Pi+13Phi</td>
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<tr>
<td>644Pi</td>
<td>-Phi — 644Pi</td>
<td></td>
</tr>
<tr>
<td>483Pi+161Phi</td>
<td>-Phi — 483Pi+161Phi</td>
<td></td>
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<tr>
<td>+Phi (25 µM)</td>
<td>39Pi+13Phi</td>
<td>+Phi — 39Pi+13Phi</td>
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<tr>
<td>644Pi</td>
<td>+Phi — 644Pi</td>
<td></td>
</tr>
<tr>
<td>483Pi+161Phi</td>
<td>+Phi — 483Pi+161Phi</td>
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</tbody>
</table>

Table 2. Treatments applied for the test of Pi uptake kinetics.

This study aimed to evaluate the effect of Phi supply as part of the P fertilization, and its influence on the guaiacol peroxidase activity and contents of total phenolics and lignin in maize plants.

Materials and methods

Experiment localization, plant material, nutrient solution and plant conduction

Study was conducted in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil (21°14' S; 45°00' W; 915 m asl). The plants remained in greenhouse environment under natural conditions day/night (air temperature minimum/maximum and relative humidity of 16.1/28.2°C and 52/79%, respectively). The photoperiod medium was of 12 h of light and photosynthesis radiation active maximum of 730 mol m⁻² s⁻¹ (at 12:00 h). Maize seeds (Zea mays L.) of the single cross hybrids GNZ 2004 were germinated on paper towel moistened with distilled water in a germination chamber. Five days after emergence (DAE), seedlings were transferred to plastic trays containing 36 L of nutrient solution modified to contain 1/5 of the total ionic strength. The nutrient solution in total ionic strength, without treatments, had the following composition: 0.5 mM N-NH₄, 7 mM N-NO₃, 0.64 mM P, 3.0 mM K, 2 mM Ca, 1 mM Mg, 1 mM S, 70 µM Fe-EDTA, 46.30 µM B, 9.11 µM Mn, 2 µM Zn, 0.5 µM Cu and 0.1 µM Mo. Seedlings were selected for regular leaf size and area transferred to plastic pots containing 3 L of nutrient solution modified to contain 2/5 of the total ionic strength, with the necessary modifications for the P concentrations according to the treatments. Phosphate used in the experiment was obtained by the reaction of phosphorous acid with potassium hydroxide (both pa grade), resulting in potassium phosphate. The nutrient solution was replaced twice a week. At each substitution, the ionic strength of the nutrient solution was increased by 1/5 until reaching total ionic strength. The solution volume in the pots was supplemented daily with deionized water and the pH adjusted to 5.5 (± 0.2), by adding 0.5 M NaOH or HCl. The nutrient solution was constantly aerated.

Treatments and experimental design

This study was conducted in an experimental design completely randomized, with 2 P concentrations (52 µM = low P concentration, and 644 µM = adequate P concentration) and 2 P forms (100% Pi, and 75/25% as Pi/Phi, respectively), being applied these treatments during the maize growth (Table 1). Each experimental unit consisted of one plant per pot.

Biomass production and P nutrition

Prior to plant harvest, 21 DAE, the leaf blade of the last fully expanded leaf was collected in 6 replications of each treatment applied during the maize growth to evaluate the in vivo acid phosphatase (EC 3.1.3.4.1) activity, according to Besford (1980) with minor modifications (Silva and Basso, 1993). Next, the leaf blade area of these plants was assessed using a leaf area meter (LI-3000A, attached to a transparent belt conveyor LI-3500A, LI-COR Inc., Lincoln, USA), and the dry mass weight of root and shoot determined, after drying in a forced air oven at 70°C. Subsequently, the P concentration was analyzed by colorimetry (Murphy and Riley, 1962) after nitric-perchloric digestion of the plant material (Johnson and Ulrich, 1959), and the P accumulation in root and shoot dry mass was verified. It is important to mention that the P accumulation in this paper refers to the total P contained in plant tissues, regardless of its form (Pi, Phi and organic P).

Parameters of phosphate uptake kinetics

Fourteen DAE, 6 replications of each treatment (applied during the maize growth) were transferred to a growth chamber under the following conditions: 16 h photoperiod; day and night temperature, respectively, 26 and 18°C; 60-80% RH; and 540 E m⁻² s⁻¹ irradiation. After 5 days, test of ³¹P (stable P isotope) uptake kinetics in the form of Pi (H₃²¹PO₄ and H¹⁰¹³PO₄⁻) were performed according to Claassen and Barber (1974). Plants remained 46 h in the P-free nutrient solution and with 1/10 of the total ionic strength, to increase the capacity of Pi uptake into the root system (Jungk and Barber, 1975), and subsequently they were exposed for 2 h to 20 µM of Pi, so that the system reached the steady state conditions for Pi uptake (Epstein and Hagen, 1952). At the end of this period, plants were transferred to the uptake solution (i.e. nutrient solution containing the Pi uptake kinetic treatments). From then on (time 0), 10 mL aliquots of uptake solution were sampled for 14 h, at intervals of 1 h, resulting in 15 samples per pot. Uptake kinetic treatments of Pi were composed of a combination of 2 P forms supplied in the

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Parameters of Pi uptake kinetics in relation to the treatments applied in uptake solution and during the maize growth.

All analyses were performed using the method of Spanos and Wrolstad (1990), with the use of the statistical package Sisvar (Ferreira, 2008). In cases of significant effects of treatments, means were compared by the Tukey test (p ≤ 0.05).

### Data analysis

Experimental data were subjected to analysis of variance by the F test (p ≤ 0.05) using software Sisvar (Ferreira, 2008).

### Results

**Biomass production and P nutrition**

The effect of Phi on maize biomass production was only significant (p ≤ 0.05) in plants exposed to the low P concentration (52 µM). At this concentration, analysis of variance showed that the replacement of 1/4 Pi by Phi caused considerable reductions in the root and shoot dry mass weight and total leaf blade area per plant (Fig. 1a and Fig. 1b). On average, these reductions were approximately 19% compared with plants treated only by Pi as P source, showing that Phi could not replace Pi in P nutrition of the plants. However, for plants grown in 644 µM P, the supply of 1/4 P in the form of Phi did not significantly (p > 0.05) influence dry mass weight and leaf blade area when compared to plants grown with Pi supply only. It is noteworthy that the values of the root to shoot ratio were highest in plants grown under 52 µM P, although these values were not influenced by the Phi proportion (Fig. 1c). The concentration and accumulation of P in shoot and root were highest in the treatments with 52 and 644 µM P, respectively (Fig. 2). The replacement of 1/4 of Pi by Phi did not influence P concentration in shoot significantly (p > 0.05), whereas P concentration increased in root treated with 644 µM P in the presence of Phi (Fig. 2a). On the other hand, P accumulation was reduced in shoot in the presence of Phi at both P concentrations in the nutrient solution, and the same was observed in root of the plants treated with 52 µM P (Fig. 2b). The acid phosphatase activity was much higher in the treatments with 52 µM P. Also, at this P concentration, the replacement of 1/4 Pi by Phi raised the activity of these enzymes considerably, which was not the case in the treatments with 644 µM P (Fig. 2c).

### Table 3. Parameters of Pi uptake kinetics in relation to the treatments applied in uptake solution and during the maize growth.

<table>
<thead>
<tr>
<th>Uptake solution</th>
<th>Treatments applied during the maize growth (µM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>52Pi</td>
</tr>
<tr>
<td></td>
<td>39Pi+13Phi</td>
</tr>
<tr>
<td></td>
<td>644Pi</td>
</tr>
<tr>
<td></td>
<td>483Pi+161Pi</td>
</tr>
<tr>
<td>Vmax</td>
<td>µmol h⁻¹ g⁻¹</td>
</tr>
<tr>
<td>-Phi</td>
<td>0.88 aA(2)</td>
</tr>
<tr>
<td>+Phi</td>
<td>0.67 bA</td>
</tr>
<tr>
<td>Km</td>
<td>µM</td>
</tr>
<tr>
<td>-Phi</td>
<td>5.02 bA</td>
</tr>
<tr>
<td>+Phi</td>
<td>6.54 aA</td>
</tr>
<tr>
<td>Cmin</td>
<td>µM</td>
</tr>
<tr>
<td>-Phi</td>
<td>0.50 aA</td>
</tr>
<tr>
<td>+Phi</td>
<td>0.42 aA</td>
</tr>
</tbody>
</table>

Vmax per unit of root fresh weight. (2) For each kinetic parameter, means followed by the same lowercase letters in columns and capital letters in rows did not differ from each other (Tukey, p ≤0.05).

uptake solution [25 µM Pi alone (treatment -Phi), and mixture of Pi and Phi at equal concentrations; 25 µM Pi + 25 µM Phi (treatment +Phi)], and 4 treatments applied during the maize growth (Table 2). This trial was arranged in a completely randomized design with three replications. Concentration of Pi in the aliquots was quantified by colorimetry (Murphy and Riley, 1962). Preliminary tests with this method were performed, with emphasis on precision and accuracy. It should be noted that the methodology allows the quantification of Pi, but cannot detect the presence of Phi. The values of Vmax, Km and Cmin were calculated according to Machado and Furlani (2004). The root net inflow of Pi was calculated by the modified Michaelis-Menten equation, where Cmin was introduced into the original equation: net inflow = [Vmax (C - Cmin)] / [Km + (C - Cmin)], where C corresponds to the Pi concentration from the uptake solution. The values of Vmax and net inflow were expressed per unit weight of fresh root (µmol Pi h⁻¹ g⁻¹).

**Guaiacol peroxidase activity, total phenolics and lignin**

Twenty-one DAE, in 6 replicates of each treatment (applied during the maize growth), the leaf blade of the last fully expanded leaf were removed, after freezing in liquid nitrogen, and stored in a freezer at -80°C. Posteriorly, the samples of the plant tissues were frozen in liquid nitrogen and then ground with mortar and pestle to obtain a fine powder. Then, approximately 1 g of powder was placed in a tube containing 10 mL of 50 mM sodium acetate buffer (pH 5.2), containing 0.1 mM EDTA and stirred for 10 s. The suspension was centrifuged at 12,000 g for 15 min at 2°C and the supernatant was used as source of enzyme extract. The total protein concentration of enzyme extract was measured according to the method proposed by Bradford (1976), using a standard curve of bovine serum albumin. The guaiacol peroxidase (EC 1.11.1.7) activity was determined following Urbanek et al. (1991) method. To analyze the lignin and total phenolics, plant tissue samples were ground in liquid nitrogen with mortar and pestle to obtain a fine powder, and lyophilized for 12 h. An aliquot of 30 mg of lyophilized material was transferred to 2 mL microtubes, homogenized with 1.5 mL of 80% methanol and maintained under stirring for 15 h on a rotary shaker protected from light, at room temperature. The suspension was centrifuged at 12,000 g for 10 min at 2°C. The supernatant (methanol extract) was used for determination of total phenolic concentration using the Spanos and Wrolstad (1990) method, while the solid residue was used to determine the concentration of lignin as described by Doster and Bostock (1988). All analyses were performed in triplicate.
Root and shoot dry mass weight (a), total leaf blade area per plant (b), and the root to shoot ratio (c) in maize, as related to concentrations (52 and 644 µM) and forms (only Pi and 3/4Pi+1/4Phi) of P supplied in the growth solution. Means followed by same letters did not differ from each other (Tukey, p ≤ 0.05).

**Parameters of phosphate uptake kinetics**

It was found that, regardless of Phi presence in the uptake solution, Pi depletion from the uptake solution was much faster with low P supply (52 µM P), as expected. For the same P concentration, it was generally observed that the presence of Phi, both in the uptake solution and during plant growth, decelerated Pi depletion in the uptake solution. However, an additional supply of 25 µM of Phi to the uptake solution affected the behavior of the curves more markedly than the replacement of 1/4 Pi by Phi for plant growth (Fig. 3). Table 3 shows the values for the parameters of Pi uptake kinetics (Vmax, Km and Cmin) in maize. The Vmax parameter was increased approximately by 4-fold in the plants grown in 52 µM P, in comparison to the plants grown in 644 µM P. However, the effect of Phi on Vmax was only significant for plants grown under 52 µM P. At this concentration, the additional supply of 25 µM of Phi in the uptake solution (treatment +Phi), independently of P supply in the plant growth, reduced Vmax by around 23%. In treatments without additional Phi in the uptake solution (treatment -Phi), Vmax was ca 11% lower for 39Pi+13Phi compared with treatment 52Pi. The same trend was observed in treatment +Phi, where in spite of not significant (p > 0.05), Vmax decreased by 9% in treatment 39Pi+13Phi, compared to 52Pi. The Km parameter was not affected by treatments applied to growing plants nor by their interaction with treatments of uptake kinetics. However, regardless of the applied treatments in the growing solution, the addition of Phi (25 µM) in the uptake solution significantly increased Km values on an average 40%. The Cmin parameter was only influenced by different P concentrations in the growing solution of plants, being on average, 70% lower for the treatment of 52 µM P. Phi supply, both in uptake solution and during maize growth, did not influence this variable. Fig. 4 shows the rate of net inflow of Pi per unit of root fresh weight. The net inflow includes all kinetic parameters involved in Pi uptake as influenced by low or adequate P supply in the growing solution of plants, in the presence or absence of Phi in the uptake solution. It was observed that, for the same P concentration in the uptake solution, net inflows of Pi were considerably higher under P deficiency (treatments 39Pi+13Pi and 52Pi). Within these treatments, the presence of Phi, both in growth (39Pi+13Pi) and in uptake solution (+Phi), reduced the net inflow of Pi. For the plants grown under adequate P supply (483Pi+161Pi and 644Pi), net inflows of Pi were much lower, due solely to the low Vmax values observed, since Km values were not influenced by the P concentration in growth solution (Table 3). Within these treatments, the presence of Phi reduced the net inflow of Pi only when present in the uptake solution (+Phi), due to a higher Km since Vmax was not influenced in this case (Table 3). However, analyzing the growth treatments within the same treatment applied to the uptake solution, it was observed that, independent of Phi effects in the uptake solution, the net inflow was slightly higher in the treatment 483Pi+161Pi, compared to treatment 644Pi. This was due to a slight rise in the value of Vmax which, although not significant (p > 0.05), was sufficient to cause this increase in Pi net inflow (Table 3). The Cmin can be clearly seen in Fig. 4 at the intersection of the curves with the abscissa axis. It was observed that the Cmin values for plants under low P supply are lower than those for plants under adequate P supply, as mentioned above in the presentation of Cmin (Table 3).

**Biochemical response to defense mechanisms**

Guaiacol peroxidase activity in the plants grown in 644 µM P was twice as high with the replacement of 1/4 of Pi by Phi. In the case of plants subjected to a low P concentration (52 µM), the Phi treatment tended to increase the activity of these enzymes, but, not significantly (p > 0.05) (Fig. 5a). Lignin concentration in the leaf tissue was not affected by P concentrations of the growth solution (52 and 644 µM), however, at both P concentrations the lignin values were significantly higher in treatments where Phi replaced 1/4 of Pi (Fig. 5b). Total phenolic concentration were higher in treatments with 52 µM P than with 644 µM P and without Phi. However, there was no significant effect (p > 0.05) of substitution of 1/4 of Pi by Phi, under low and adequate P supply (Fig. 5c).
Concentration (a) and accumulation (b) of P in root and shoot dry mass, and in vivo acid phosphatase activity (c) in the leaf blade of the last fully expanded leaf, due to concentrations (52 and 644 µM) and forms (only Pi and 3/4Pi+1/4Phi) of P. Phosphatase activity = \[\frac{(\mu M\ of\ p-nitrophenol)}{(g\ fresh\ weight\ of\ plant\ tissue\ h^{-1})}\]. Means followed by the same letters did not differ from each other (Tukey, \(p \leq 0.05\)).

**Discussion**

The negative effects of replacing 1/4 Pi by Phi on biomass production and P nutrition of the maize plants under low P supply can be confirmed to the increase in acid phosphatase activity and decreases in leaf blade area, dry mass weight root and shoot, and P accumulation in shoot. This conclusion was also supported by the visual aspect of these plants, in which the reduced shoot and root growth and intensive purple leaf color (anthocyanin accumulation) were evident. These results corroborate those of Schröetter et al. (2006) for maize. The same was observed for other species, such as *Ulva lactuca* and *Brassica rapa* grown in soil and nutrient solution (Lee et al., 2005; Thao et al., 2008). These studies reported that Phi
The influence of Pi supply. The parameters of Pi affinity.

bio

The mechanisms responsible for signaling P deficiency investigation study, Cavalcanti et al. (2007) described that Phi is a competitor of Pi for the same biosynthesis of other compounds promoted by guaiacol peroxidase. It is noteworthy that Phi masks the increase in Pi uptake activity and not substitute Pi in P nutrition of plants. Moreover, these findings showed that the induction of plant defense mechanisms in the cell synthesis of phosphatases, phosphodiesterases, acid phosphatase activity and increased lignin biosynthesis in the leaves. The peroxidases are part of a select group of proteins related to the induction of plant defense mechanisms, called PR-proteins. In this context, the main function of peroxidases is to catalyze the oxidation of hydroxycinnamic alcohols, resulting in phenolic precursors of lignin biosynthesis, which are important plant defense responses to external agents (Cavalcanti et al., 2006).

Some studies have shown positive effects of peroxidase activity on the induction of plant defense mechanisms to abiotic and biotic stress agents (Cavalcanti et al., 2007; Cavalcanti et al., 2008; Challaraj Emmanuel1 et al., 2010; Pourtaghi et al., 2011). On the other hand, studies that relate Phi effects on peroxidase activity are however rare. The stimulating effect of Phi on lignification of maize leaves was evident in this study, unlike for total phenolic concentration. This opposite behavior was expected, since most of the phenolic compounds are precursors of cell wall lignification. Furthermore, in support of this study, Cavalcanti et al. (2007) found that the induction of defense mechanisms in tomato (Lycopersicon esculentum) against Xanthomonas vesicatoria was simultaneous increasing lignin concentration and a decreasing in total phenolic concentration. According to the authors, the higher demand for phenolic intermediates during the biosynthesis of other compounds promoted by elicitor may mask the increase in biosynthesis of phenolics. Aside from the peroxidase activity during lignin biosynthesis, the activities of other enzymes that also use phenolic intermediates as substrate are intensified due to the presence of an elicitor.

did not substitute Pi in P nutrition of plants. Moreover, these authors reported that the use of Phi as sole P source generally caused a significant reduction in plant growth when compared to treatments with either null or insufficient Pi fertilization. They therefore suggested a potential harmful effect of Phi on plants grown under low Pi supply. The causes of this effect are not well understood. The most plausible hypothesis to date is that the plants do not metabolize Phi, which, after uptake, remains stable in the cell compartments. Also, Phi inhibits some mechanisms involved in overcoming P deficiency, such as intensified root growth and biosynthesis of phosphatases, phosphodiesterases, nucleases, and high-affinity P transporters. Most likely, the molecular mechanisms responsible for signaling P deficiency do not discriminate Pi from Phi. Thus, there is no expression of genes responsible for transcription of proteins related to response mechanisms to P deficiency (Ticconi et al., 2001; Varadarajan et al., 2002; Lee et al., 2005). In this study, acid phosphatase activity corroborated the hypothesis that Phi anion was not utilized as a nutrient source by the P-starved maize, since the activity of these enzymes upon replacement of 1/4 of Pi by Phi at low P concentration was high. It should also be noted that the root to shoot ratio was not affected by Phi proportions applied in the growing solution. These results disagree with reports of other studies, as under low P supply, Phi addition tended to inhibit mechanisms of overcoming P deficiency, such as acid phosphatase activity and root to shoot ratio, as discussed above. In this investigation, Pi concentrations in the grown solution was higher than of Phi, showing that this proportion of Phi was not harmful to P-deficient plants, but simply did not contribute to their P nutrition. It was explicitly stated that the presence of Phi in nutrient solution had little effect on the values of Vmax and Cmin of Pi in maize. It was found that Cmin and Vmax for plants grown at low P concentration, regardless of the form (Pi or Phi), were considerably higher. Probably, P deficiency induced the activation of genes related to biosynthesis of high-affinity P transporters (Franco-Zorrilla et al., 2004). Therefore, more Pi transporters were active, increasing values of Vmax. On the other hand, the affinity of each transporter with Pi was not altered so values of Km remained unchanged (Segel 1975; Fernandes and Souza, 2006). The influence of the Phi supply in supply nutrient solution on parameters of Pi uptake kinetics in maize was detected by substantial increases in values of Km. Ionic interactions that promote Km increases of the respective ions are known for competitive inhibition (Segel, 1975; Fernandes and Souza, 2006). It is therefore probable that Phi is a competitor of Pi for the same transporter site in maize. In this case, the total number of effective transporters is not changed, but there is a reduction of the total available sites for Pi transport. In general, under the conditions of this research, the results showed that the replacement of 1/4 of Pi by Phi did not significantly affect the biomass production of maize when grown under adequate P supply. In addition, Phi replacement had the advantage of stimulating biochemical responses to stress agents, as reflected by the intensified guaiacol peroxidase activity and increased lignin biosynthesis in the leaves. The peroxidases are part of a select group of proteins related to the induction of plant defense mechanisms, called PR-proteins. In this context, the main function of peroxidases is to catalyze the oxidation of hydroxycinnamic alcohols, resulting in phenolic precursors of lignin biosynthesis, which are important plant defense responses to external agents (Cavalcanti et al., 2006). Some studies have shown positive effects of peroxidase activity on the induction of plant defense mechanisms to abiotic and biotic stress agents (Cavalcanti et al., 2007; Cavalcanti et al., 2008; Challaraj Emmanuel1 et al., 2010; Pourtaghi et al., 2011). On the other hand, studies that relate Phi effects on peroxidase activity are however rare. The stimulating effect of Phi on lignification of maize leaves was evident in this study, unlike for total phenolic concentration. This opposite behavior was expected, since most of the phenolic compounds are precursors of cell wall lignification. Furthermore, in support of this study, Cavalcanti et al. (2007) found that the induction of defense mechanisms in tomato (Lycopersicon esculentum) against Xanthomonas vesicatoria was simultaneous increasing lignin concentration and a decreasing in total phenolic concentration. According to the authors, the higher demand for phenolic intermediates during the biosynthesis of other compounds promoted by elicitor may mask the increase in biosynthesis of phenolics. Aside from the peroxidase activity during lignin biosynthesis, the activities of other enzymes that also use phenolic intermediates as substrate are intensified due to the presence of an elicitor.
Conclusion

Phosphite inhibits phosphate uptake competitively in maize, regardless of the plant phosphorus status. Replacement of 1/4 of Pi by Phi decreased the biomass production of the plants grown under low Pi supply, but no effect was observed in the plants grown under adequate Pi supply, with the advantage of eliciting biochemical responses to stress agents, such as stimulation of the guaiacol peroxidase activity and lignin biosynthesis.

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