Kinetics of Phosphorus Uptake through Roots of Habanero Pepper (Capsicum chinense Jacq. cv. Mayapán)

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Abstract: The application of enzymatic kinetics theory on the nutrition of horticultural species is scarce. $I_{\text{max}}$ and $K_m$ describe the kinetics of nutrient absorption by the plant. $I_{\text{max}}$ and $K_m$ are necessary to predict phosphorus (P) uptake from soil using mathematical models, and their estimation gives information about the efficient use of P in plants. $I_{\text{max}}$ and $K_m$ for habanero pepper (Capsicum chinense Jacq. cv. Mayapán) were determined using the modified exhaustion method. Depletion of P by the roots was obtained with 0.01, 0.125, 0.25, 0.50, and 1.00 mM P L$^{-1}$. P-depletion data over time were fitted to an exponential-regression model to obtain the initial P-uptake rates by the roots. Initial P-uptake rates were significantly different ($p < 0.001$) depending on the levels of P in the solution. $I_{\text{max}}$ and $K_m$ were predicted by iteratively fitting the initial P-absorption rates in terms of the concentration of P to the Michaelis–Menten model. The average $I_{\text{max}}$ was $3.49 \times 10^{-7}$ mM cm$^{-2} s^{-1}$ and $K_m$ was $2.59 \times 10^{-2}$ mM P L$^{-1}$. These results show that the habanero pepper root can uptake $1.08 \times 10^{-5}$ mg P L$^{-1}$ per cm$^2$ in the soil solution per second and P transporters are saturated with $2.59 \times 10^{-2}$ mM P L$^{-1}$.

Keywords: habanero pepper; phosphorous; Michaelis–Menten

1. Introduction

Phosphorus is a key macronutrient in root development, flowering, and fruit production [1]. Phosphoric rock is a major source of phosphate fertilizer production; however, it is in a process of exhaustion [2]. Therefore, the search for new alternatives to improve the efficiency of P use in agriculture is a global priority. P is absorbed by plant roots from soil solutions as monobasic (H$_2$PO$_4^-$) or di-basic (HPO$_4^{2-}$) phosphate [3]. Mineral uptake through plant roots involves metabolic processes characterized by a large level of selectivity [4]. Plant P transporters, which are H$_2$PO$_4^-$ /H$^+$ symporters, move P through the symplastic pathway [5]. The family of genes known as PHT1 are expressed in root epidermal cells, and the encoded transporters are located on the plasma membrane [6,7].

The uptake of minerals by plant roots is a function of the concentration of ions in the soil solution and the ability of the roots to absorb them [8]. The rise in nutrient concentration in soil solution increases root mineral uptake rate; however, when the maximum rate of mineral absorption is achieved, the increment of nutrient concentration in the soil no longer affects the absorption rate [9]. The similarity between the reaction rate of the enzymatic processes, the substrate–enzyme, and the mechanism of phosphate absorption by the roots, as a function of their concentration and the availability of membrane transporters, gave rise to the application of the theory of enzymatic kinetics of Michaelis–Menten to study the mechanisms of nutrient absorption by plants [10].
The Michaelis–Menten equation, changed by Claassen and Barber [11], describes the kinetics of nutrient uptake by roots through the parameters $I_{\text{max}}$ (maximum rate of ion uptake) and $K_m$ (Michaelis–Menten constant). When all the sites of the mineral transporters of the plasma membrane are saturated, the plants achieve $I_{\text{max}}$ of the nutrients [12,13]. The $K_m$ is equal to the concentration of the ion in the medium when the $I_{\text{max}}$ of the absorption of the element is at 50%. $K_m$ describes the affinity of the transporters for the ion [14]. When the value of $K_m$ is high, the affinity of the ions for the transporters decreases. This means that the lower the $K_m$, the greater the sensitivity of the nutrient absorption by the plant [15]. Plant nutrient uptake has low- and high-affinity systems [15,16]. Low-affinity systems operate under conditions with high nutrient availability, while high-affinity systems originate under conditions of P deficiency [17].

Research reports indicate that the application of enzyme kinetics theory to the study of phosphorus and other nutrient uptake in grass, forestry, and other plant species has been extensive in comparison to horticultural plant species such as habanero peppers. [14,18–20].

The Mexican Institute of Property granted the denomination of origin of the habanero pepper to the Yucatan Peninsula, Mexico, in 2008, which has highlighted the gastronomic and economic importance of the crop in the region [21,22]. However, there are few studies related to the nutrition and the efficient use of nutrients for its production [23].

The estimation of $I_{\text{max}}$ and $K_m$ in habanero pepper crops are parameters necessary to predict P requirements for production purposes. Therefore, the aim of this study was to determine these parameters of the kinetics of P absorption in habanero pepper plants in a hydroponic system.

2. Materials and Methods

2.1. Experimental Conditions

The present study was carried out in the facilities of the water–soil–plant laboratory of the Tecnológico Nacional de México-Campus Tizimín (21°09′29″ N 88°10′21″ W) in the municipality of Tizimín, Yucatán, Mexico. The temperature inside the laboratory was kept at 25 °C, and the relative humidity was at 55%. Experimental treatments consisted of five phosphorous levels: 0.01, 0.125, 0.25, 0.50, and 1.00 mM P L$^{-1}$, using a completely randomized design. The treatments had three repetitions and fifteen experimental units. Each experimental unit was comprised 13 seedlings per container. The nutrient solutions for each treatment were elaborated using the modified Steiner’s nutritive solution [24,25] (Table 1).

<table>
<thead>
<tr>
<th>Treatment mM L$^{-1}$</th>
<th>NO$_3$</th>
<th>SO$_4^{2-}$</th>
<th>H$_2$PO$_4^-$</th>
<th>K$^+$</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0.125</td>
<td>15</td>
<td>2</td>
<td>0.125</td>
<td>7.125</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0.25</td>
<td>15</td>
<td>2</td>
<td>0.25</td>
<td>7.25</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0.50</td>
<td>15</td>
<td>2</td>
<td>0.5</td>
<td>7.5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1.00</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Nutrient solutions were prepared with potassium nitrate, calcium nitrate, magnesium sulfate, and potassium phosphate. The micronutrients were formulated with commercial products comprising a mixture of EDTA-Fe, EDTA-Mn, EDTA-Zn, EDTA-Cu, molybdenum oxide, and boron. Deionized water was used as the solvent, and the pH of the solutions was kept at 5.5.

2.2. Experimental Procedure

The parameters of the kinetics of P uptake by habanero pepper (Capsicum chinense Jacq. cv. ‘Mayapán’) roots were determined by the modified depletion technique [11].
Seeds were sown in polystyrene trays with 200 cavities and filled with peat moss. The surface of the seeds was disinfected with 10% sodium hypochlorite before sowing. After the emergence of the first true leaves, seedlings were maintained inside a greenhouse. The seedlings were fertilized with a modified Steiner’s nutritive solution at three-day intervals up to 45 days of age.

The 45-day-old seedlings were removed from the trays; the substrate was removed from the roots with purified water using a spray pump. Finally, the roots were rinsed with distilled water. The seedlings were grouped into clusters of 13 seedlings and placed in plastic containers with 100 mL of P-free Steiner’s nutritive solution for 48 h (Table 1). The solution was changed at 24 h intervals.

After the P-starvation period, each cluster of seedlings was resuspended in the containers with 100 mL of solutions with concentrations of 0.01, 0.125, 0.25, 0.50, and 1.00 mM P L$^{-1}$. All solutions were oxygenated with an air pump. One mL of solution was taken manually from each container, at 30 min intervals, up to 300 min. The volume of the solution was maintained in each container by adding deionized water manually. The samples were stored in 3 mL vials under refrigeration until analysis.

P content of the samples was analyzed with the colorimetric method using a UV spectrophotometer (HACH-brand ultraviolet light, model DR5000) [26]. The roots of the seedlings were separated from the shoot. Root length, total surface area, and diameter were determined by image analysis using the ARIA_v2.0 (Automatic Root Image Analysis) program [27]. The images of the roots were obtained using an Epson® perfection V550 brand scanner equipped with a film transparency unit. The roots were placed on a transparent acrylic tray with deionized water. The trays were placed upon a scanner to obtain images of the roots. The scanner parameters were adjusted to an 8-bit grayscale. The images were saved in JPEG format at 400 dpi and 100% scale [28].

Next, the roots and shoots of the plant were dried in a ThermoFisher Scientific forced-air oven at 65 °C for 72 h. Dry roots and shoots were weighed with an analytical balance OHAUS® Pioneer. The total dry biomass of the seedlings was ground using a Willey mill. Total P of seedlings was extracted and measured by acid digestion and colorimetry at 660 nm, respectively [26].

The initial rate of P uptake of each treatment was calculated by fitting the depletion data as a function of time to the exponential regression model [29]. The equation of the exponential model is described as follows:

$$y = c \times e^{-ax},$$

where $y$ is the concentration of P in the solution (mM L$^{-1}$), $x$ is the absorption time (min), $c$ is the concentration of P in the solution when time approaches 0 (abscissa), and $a$ is the slope and represents the initial rate of absorption of P (mM min$^{-1}$). The initial rates of P uptake were converted into mM cm$^2$ s$^{-1}$ by dividing the value of the slope of the regression by the surface area of the root and then multiplied by 60.

The parameters of absorption kinetics, $I_{max}$ and $K_m$, were determined by iterative curve fitting of the rates of P uptake by the roots (mM cm$^2$ s$^{-1}$), which were expressed as a function of the concentration of the anion (mM L$^{-1}$) in the solutions, to the Michaelis–Menten model ($p < 0.001$), described as:

$$I = \frac{I_{max} \times C}{K_m + C},$$

where $I$ is the total ion absorbed by the root (mM cm$^2$ s$^{-1}$), $I_{max}$ is the maximum rate of ion uptake (mM cm$^2$ s$^{-1}$), $C$ is the concentration of the ion in the solution (mM L$^{-1}$), and $K_m$ is the Michaelis–Menten constant (mM L$^{-1}$).

The Shapiro–Wilk test was conducted to check the normality of data of root and biomass characteristics of habanero pepper seedlings. Confidence intervals of the seedling parameters were calculated with the $t$-test at a 95% significance level. The differences in
the initial root P-uptake rates between treatments were determined by a one-way analysis of variance. The differences between means were compared by Tukey’s test ($p < 0.05$). All data were analyzed using R, version 4.1.2 [30].

3. Results
3.1. Characteristics of the Habanero Pepper Seedlings

The means and confidence intervals of the roots’ morphological characteristics and the biomass of habanero pepper seedlings are shown in Table 2. This information made it possible to estimate the confidence intervals inside which the true values of the means of the parameters are found.

Table 2. Root and biomass characteristics of habanero pepper seedlings. Means were estimated on thirteen seedlings per container ($n = 15$). The confidence interval of means was calculated at a 95% significance level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length (cm)</td>
<td>250.33</td>
<td>233.44</td>
<td>267.23</td>
</tr>
<tr>
<td>Root surface area (cm²)</td>
<td>7.06</td>
<td>6.25</td>
<td>7.87</td>
</tr>
<tr>
<td>Root diameter (mm)</td>
<td>1.05</td>
<td>0.99</td>
<td>1.13</td>
</tr>
<tr>
<td>Root dry weight (mg plant⁻¹)</td>
<td>38.44</td>
<td>36.03</td>
<td>41.18</td>
</tr>
<tr>
<td>Shoot dry weight (mg plant⁻¹)</td>
<td>261.95</td>
<td>245.49</td>
<td>280.2</td>
</tr>
<tr>
<td>P concentration (mg plant⁻¹)</td>
<td>1.39</td>
<td>1.28</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Additional information was obtained by calculating the root/shoot ratio of the seedlings, resulting in a value of 0.15.

3.2. Curves of P Depletion by Habanero Pepper Roots

Figure 1 shows the fitted time-dependent exponential models of P uptake by roots for the different levels of P treatments ($p < 0.001$). The chemical analysis of the solution with 0 mM P L⁻¹ resulted in an average quantity of 0.013 mM P L⁻¹ at the beginning of the study; this is because some chemical products used to prepare the nutrient solutions contained a minimal amount of P even though the products are reagent-grade (Table 3).

![Figure 1](image-url)  
Figure 1. Means of P uptake by habanero pepper roots in a period of 300 min. Individual values represent the mean of three samples collected at 30 min intervals.
Table 3. Initial and final P concentration in the nutritive solution, and the regression model used to adjust the means of phosphorus removed (y) by the roots of habanero pepper at different times (x) in a hydroponic system.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( C_{\text{max}} ) (mM L(^{-1}))</th>
<th>( C_{\text{min}} ) (mM L(^{-1}))</th>
<th>Model</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.013</td>
<td>0.010</td>
<td>( y = 0.013e^{-0.00065x} )</td>
<td>0.994</td>
</tr>
<tr>
<td>0.125</td>
<td>0.131</td>
<td>0.081</td>
<td>( y = 0.128e^{-0.001752x} )</td>
<td>0.956</td>
</tr>
<tr>
<td>0.25</td>
<td>0.264</td>
<td>0.149</td>
<td>( y = 0.262e^{-0.00182x} )</td>
<td>0.994</td>
</tr>
<tr>
<td>0.50</td>
<td>0.514</td>
<td>0.266</td>
<td>( y = 0.53e^{-0.00171x} )</td>
<td>0.899</td>
</tr>
<tr>
<td>1.00</td>
<td>1.04</td>
<td>0.75</td>
<td>( y = 1.04e^{-0.00109x} )</td>
<td>0.996</td>
</tr>
</tbody>
</table>

\( C_{\text{max}} \) (initial P concentration); \( C_{\text{min}} \) (final P concentration); \( R^2 \) (determination coefficient); x (time in minutes). The data of the exhaustion curves were fitted to the model with a value of \( p < 0.001 \).

3.3. P-Uptake Rates by Roots

The gradual increase in P concentration in the solution resulted in a significant (\( p < 0.01 \)) increment in the rate of P uptake by habanero pepper roots, from \( 8.47 \times 10^{-8} \) to \( 3.66 \times 10^{-7} \) mM cm\(^2\) s\(^{-1}\) (Table 4). The supply of the initial P concentration, 0.53 mM L\(^{-1}\), gave rise to an increase of 331.76% in the rate of P uptake by roots in relation to the initial P amount of 0.01 mM L\(^{-1}\) (Table 4). The highest rate of P uptake, \( 3.66 \times 10^{-7} \) mM cm\(^2\) s\(^{-1}\), was obtained with an initial P concentration of 0.53 mM L\(^{-1}\); this value was similar (\( p < 0.05 \)) to all rates of P root uptake (\( 2.78 \times 10^{-7} \)–\( 3.41 \times 10^{-7} \) mM cm\(^2\) s\(^{-1}\)) recorded with the initial P concentrations of 0.13, 0.26, and 1.04 mM L\(^{-1}\) (Table 4).

Table 4. P-uptake rates by roots of habanero pepper seedlings at different P initial levels in the solution.

<table>
<thead>
<tr>
<th>Initial Concentration of P (mM L(^{-1}))</th>
<th>P-Uptake Rate (mM cm(^2) s(^{-1}))</th>
<th>VC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>( 8.47 \times 10^{-8} ) b</td>
<td>2.65</td>
</tr>
<tr>
<td>0.13</td>
<td>( 3.41 \times 10^{-7} ) a</td>
<td>11.64</td>
</tr>
<tr>
<td>0.26</td>
<td>( 3.16 \times 10^{-7} ) a</td>
<td>2.97</td>
</tr>
<tr>
<td>0.53</td>
<td>( 3.66 \times 10^{-7} ) a</td>
<td>6.93</td>
</tr>
<tr>
<td>1.04</td>
<td>( 2.78 \times 10^{-7} ) a</td>
<td>7.37</td>
</tr>
</tbody>
</table>

Means of rates followed by different literals are significantly different at \( p < 0.01 \) (Tukey); VC (variation coefficient).

3.4. Parameters of the P-Uptake Kinetic of Habanero Pepper Roots

The fitted Michaelis–Menten regression model gave an estimate of \( 3.49 \times 10^{-7} \) mM cm\(^2\) s\(^{-1}\) for \( I_{\text{max}} \). The affinity of the transporters towards the ion, represented by the \( K_m \), was reached with a concentration of \( 2.59 \times 10^{-2} \) mM P L\(^{-1}\) (Figure 2).

Figure 2. P-uptake kinetics of habanero pepper roots (Capsicum chinense Jacq.) at different P concentrations. Error bars represent standard deviations of the means (\( n = 3 \)).
4. Discussion

The means of the parameters of the architecture of the root and dry biomass of the habanero pepper seedlings were within the ranges of the confidence intervals. Thus far, there have been no reports on the characterization of the root morphology of habanero pepper seedlings. Literature reports have shown that the supply of nitrogen and P regulates the root/shoot biomass ratio [5]. There are no specific reports related to the biomass ratio of the root/shoot in habanero pepper plants. However, in some species, such as wheat plants with deficient P levels, the root/shoot biomass ratio is larger (0.6) in comparison to plants treated with larger quantities of the element (0.26). In addition, the wheat plants exhibited an increase in the total surface area and total root length, as well as the concentration of P in the plant tissue [31]. Sattelmacher et al. [32] reported similar results with nitrogen in potato crops. According to the above, it can be considered that the conditions of the habanero pepper plants used in the study were produced under adequate nutritional conditions.

The temporary restriction of P to plants generates an accelerated increase in uptake when the element is supplied anew to the roots [33,34]. The restriction of essential minerals in plant nutrition leads to a large decrease in their concentrations within the cells, and when the minerals are supplied again, the concentration gradient drives, to a large extent, the increase in the absorption of ions by the cells [14]. This is possible as long as the environmental conditions are suitable for the metabolic function of plants; for example, low or high temperatures affect the processes of photosynthesis and cellular respiration, as well as the absorption of P [35,36].

The results of the current study on P absorption rates show that the equilibrium of the absorption rates of the phosphate by the roots started with 0.13 mM P L$^{-1}$, that is, 130 $\mu$M P L$^{-1}$. Increasing the concentration of the element in the nutrient solution also increases the absorption rates of the roots. However, it is known that at some point, the plants reach an equilibrium point (the maximum absorption rate of the ion), where increasing the availability of nutrients in soil does not lead to an increase in root uptake rates [37,38]. The balance of the rates of P absorbed by the habanero pepper roots indicates that the ion concentrations used in this study were sufficient to completely saturate the phosphate ion transport sites.

The P concentrations used in most studies of the kinetics of ion uptake by plants are based on the extremely low amounts of phosphate in the soil solution under natural conditions; nevertheless, P scarcity limits plant growth and development [39]. However, it is important to mention that the production of agricultural food requires the supply of sufficient amounts of P in the soil to achieve optimal yields [40]. Kelly and Kelly [29] indicate that the rates of P uptake by maple roots (obtained from a depletion curve at an initial concentration of 0.025 $\mu$M P L$^{-1}$) as a function of ion concentration did not fit the non-linear model of Michaelis–Menten. These authors mention that the low initial P concentration was probably not enough to completely saturate the ion transport sites. Based on these previous studies, higher P concentrations were used in this work, as indicated in the experimental protocol. The upper concentration limit used in this study was 1 mM P L$^{-1}$ based on Steiner’s nutritive solution [24,25], which indicates that this is an adequate concentration of phosphate for hydroponic crops in general.

The initial rates of P absorbed by habanero pepper plants against the initial concentrations of the element were able to fit the Michaelis–Menten equation (Figure 2), and the maximum rate of ion uptake ($I_{\text{max}}$) by the roots was estimated as $3.49 \times 10^{-7}$ mM cm$^2$ s$^{-1}$. This means that the habanero pepper plants could absorb $1.08 \times 10^{-5}$ mg P L$^{-1}$ from the soil solution per cm$^2$ of root per second.

According to Wright [41], the classification of the reaction rates according to the rate indicates that the $I_{\text{max}}$ of the absorption of P by habanero pepper is fast. The average of $I_{\text{max}}$ in this study suggests that habanero pepper probably contains a high concentration of P in the absorption sites per root unit.

P uptake by plant roots is classified into low-affinity and high-affinity systems [15,16]. Transporters with high-affinity systems have $K_m$ values that range from 1 to 27 $\mu$M P L$^{-1}$ [42,43].
while those belonging to low-affinity systems have $K_m$ values above 47 to 400 µM P L$^{-1}$ [44,45]. According to the value ($2.59 \times 10^{-2}$ mM P L$^{-1}$ or 25.9 µM P L$^{-1}$) of $K_m$ recorded in this study, the habanero pepper probably belongs to a high-affinity system (Figure 1); however, further studies are required to characterize the affinity systems of P absorption by the crop. The $K_m$ value of P absorption of habanero pepper shows that it is necessary to maintain a concentration of $2.59 \times 10^{-2}$ mM P L$^{-1}$, that is, 0.79 mg P L$^{-1}$, in the soil solution to achieve saturation of all transporter sites and to reach 50% of the $I_{\text{max}}$ of P uptake by the roots. The estimated average $K_m$ for the present study indicates that habanero pepper is efficient in absorbing P from the soil solution.

5. Conclusions

The rate of P uptake by habanero pepper roots increased as concentrations of phosphate in the solutions increased. The value of $I_{\text{max}}$ in this study was around $3.49 \times 10^{-7}$ mM P cm$^{-2}$ s$^{-1}$. Therefore, the maximum P uptake capacity of habanero pepper roots in the soil solution is $1.08 \times 10^{-5}$ mg P L$^{-1}$ in one cm$^2$ of root per second. The $K_m$ had an approximate average of $2.59 \times 10^{-2}$ mM P L$^{-1}$; therefore, to reach $\frac{1}{2} I_{\text{max}}$ phosphate uptake by habanero pepper seedlings, it is necessary to maintain 0.79 mg P L$^{-1}$ in the soil solution. The maximum rate of ion uptake ($I_{\text{max}}$) estimated in this study and the low value of $K_m$ suggest that the roots of the habanero pepper are efficient in the absorption of P. The values of $I_{\text{max}}$ and $K_m$ are key to mathematically predicting the P absorption process of habanero pepper crop in the soil and contribute to the efficient use of the element in agriculture.


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Conflicts of Interest: The authors declare that they do not have any conflicts of interest.

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