



Morphological and kinetic parameters of the uptake of nitrogen forms in clonal peach rootstocks



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ABSTRACT

Peach (*Prunus persica* L.) rootstock cultivars are typically selected for scion compatibility, ease of propagation, vigor, development, flowering season, yield, low need for cold temperatures, resistance to diseases, effects on the physical-chemical characteristics of the fruit, plant longevity and adaptation to adverse edaphoclimatic conditions. However, kinetic parameters related to nutrient uptake efficiency are usually not considered, such as those of nitrate (NO_3^-) and ammonium (NH_4^+). N is the nutrient that most impacts growth and yield. The objective of this study was to show the importance of the kinetic parameters of NO_3^- and NH_4^+ uptake as additional criteria for selecting peach rootstocks. The experiment was conducted in a greenhouse. Three rootstock ('Aldrighi', 'Tsukuba1' and 'Clone 15') were grown for 30 days in a pot containing 0.1 mol L^{-1} CaSO_4 solution to reduce internal reserves of N. Afterwards, the plants were placed in Hoagland nutrient solution, where periodic collections of the nutrient solution were carried out for three days and the concentrations of NO_3^- and NH_4^+ were determined. After the third day of collecting the solution, the plants were collected and then separated into leaves, roots and stems. Dry matter and total N content were assessed. The kinetic parameters related to NO_3^- and NH_4^+ uptake (maximum uptake rate - V_{max} , affinity constant - K_m , Minimum concentration - C_{min} , Influx - I) were calculated using Cinética software. The most efficient rootstock for NO_3^- and NH_4^+ uptake was 'Tsukuba1', as it showed the lowest values of C_{min} and K_m and the highest values of V_{max} and I_{max} for NO_3^- and NH_4^+ . NO_3^- uptake in 'Tsukuba1' and 'Aldrighi' showed a two-phase uptake pattern, suggesting the presence of low and high affinity transport systems. On the other hand, NH_4^+ uptake in the three cultivars apparently followed a one-phase uptake pattern, suggesting the presence of a high affinity transport system. The kinetic parameters of NO_3^- and NH_4^+ uptake are additional criteria that can be used in selecting peach rootstocks, as they directly influence shoot and root dry matter production and N accumulation in leaves.

1. Introduction

Peach (*Prunus persica* L.) rootstock cultivars are commonly selected for scion compatibility, ease of propagation, vigor, development, flowering season, yield, low cold requirement, resistance to diseases, effects on the physicochemical characteristics of the fruits, plant longevity and adaptation to adverse climatic conditions (Martins et al.,

2014; Picolotto et al., 2012; Warschefsky et al., 2016). However, kinetic parameters related to nutrient uptake efficiency are not typically considered, such as nitrogen (N) forms nitrate (NO_3^-) and ammonium (NH_4^+), as N is the nutrient that most affects growth, yield and fruit composition (Karavin et al., 2016; Zhang et al., 2016).

The kinetic parameters of nutrient uptake are represented by the maximum uptake rate (V_{max}), Michaelis-Menten constant (K_m),

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minimum concentration (C_{\min}) and influx (I) (Martinez et al., 2015; Yang et al., 2007). They allow the prediction of higher or lesser ion uptake efficiency by the plant roots (of NO_3^- and NH_4^+ , for instance) at a given concentration in the medium. V_{\max} refers to the amount of nutrient taken up when all the transporter sites present in the membranes of the root cells are saturated. K_m indicates the concentration of nutrient/ion in solution in which half of the maximum uptake rate is reached, and the lower its value, the higher the affinity of the ion with the uptake sites. Minimum concentration (C_{\min}) corresponds at which the roots can extract a nutrient from the solution, which is given by the amount of nutrient absorbed per unit mass of roots per unit of time. Thus, the ideal peach rootstock cultivar is one with lower values of C_{\min} and K_m , and higher values of V_{\max} and I (Martinez et al., 2015). Kinetic parameters can aid in breeding and evaluating rootstocks to identify the most suitable edaphoclimatic conditions for species and cultivars. Rootstock cultivars more efficient in nutrient uptake could be grown in soils with low fertility, while rootstock cultivars less efficient in nutrient uptake, but with other characteristics important to the market, could be used in soils with high fertility, thus optimizing the financial resources and efficiency of the scion/rootstock combination in the environment (Raseira et al., 2003; Scariotto et al., 2013).

Studies on kinetic parameters related to nutrient uptake in plants typically use the methodology proposed by Claassen and Barber (1974). The plant is acclimatized in Hoagland nutrient solution (Jones, 1983) for a certain period, and the plants are then placed in a container with distilled water for 24 h to reduce internal nutrient reserves. Afterwards, the plant is expected to be able to use all its uptake capacity to absorb the nutrient from the solution adjusted for the depletion period. Aliquot parts of the nutrient solution containing the plant are collected periodically over time and then prepared for nutrient analysis. However, this methodology (Claassen and Barber, 1974) was proposed for corn crop, using small plants with lower root volume and rapid growth. This justifies the 24 h suggested for the plant to reach its period of ideal nutritional depletion, which represents the period of decreased nutrient reserves inside the plant, but without symptoms of deficiency. However, in fruit trees such as the peach tree, the accumulation of N in the reserve organs (e.g., roots and stems) is expected to be greater than in annual crops, even in early stages (Brunetto et al., 2016; Jordan, 2015; Jordan et al., 2014). Thus, it is very likely that the period of nitrogen depletion in the plant is longer, and as a result the methodology proposed by Claassen and Barber (1974) may require adjustments.

'Aldrighi' was selected in 1940 because of its adaptability and fruit quality for industrialization. On the other hand, 'Clone 15' is resistant to *Meloidogyne javanica* and *M. incognita*, ease of cloning ability by herbaceous cuttings, graft compatibility with peach cv. Aurora-1 and increased fruit size (Mayer et al., 2006). 'Tsukuba1' is tolerant to excess water in the soil and resistant to some nematode species (Piccolotto et al., 2009; Souza et al., 2014). However, the kinetic parameters related to the uptake efficiency of N forms are not yet known in these peach cultivars and other commonly grown rootstocks. Therefore, the study aimed to show the importance of kinetic parameters of NO_3^- and NH_4^+ uptake as additional criteria in selecting young peach rootstocks.

2. Material and methods

2.1. Rootstocks

Herbaceous branches of peach 'Aldrighi', 'Tsukuba1' and 'Clone 15' were collected in November 2012 from six-year-old adult parent plants for the preparation of the cuttings. The adult plants belonged to the *Prunus* rootstock collection of Embrapa Clima Temperado, located in the city of Pelotas, state of Rio Grande do Sul, southern Brazil. The branches were 12 cm long and had three to five upper buds with whole leaves. The base of the branches was immersed for 5 min in a hydroalcoholic solution of indolebutyric acid at 3000 mg L⁻¹. The branches were immediately placed in pots containing vermiculite. The branches

remained 60 days in an intermittent mist chamber with an average temperature of 25 °C and 70% average humidity. The branches with roots were transplanted into perforated plastic bags (30 × 18 cm) containing commercial substrate (30% husk; 70% peat). The clonal rootstocks were cultivated for 18 months, conducted on a single stem and were not grafted.

'Aldrighi', 'Clone 15' and 'Tsukuba1' clonal rootstocks (aged 18 months) were removed from the substrate contained in the bags. Each plant was conditioned in an 8 L pot containing half-strength Hoagland nutrient solution (Jones, 1983). The full-strength Hoagland nutrient solution consisted of (in mg L⁻¹) N- NO_3^- = 196, N- NH_4^+ = 14, P = 31, K = 234, Ca = 160, Mg = 48.6, S = 70, Fe-EDTA = 5, Cu = 0.02, Zn = 0.15, Mn = 0.5, B = 0.5 and Mo = 0.01. A styrofoam sheet was placed on the surface of each pot with a hole in the middle to allow the plant to pass through. The styrofoam sheet allowed the fastening of the plant and the reducing of the evaporation of the solution. The pots containing the nutrient solution and the plants were placed on a metal table, in a greenhouse, with an average temperature of 25 °C and an average relative humidity of 60%. The plants remained in the solution for 15 days, and the solution was changed every three days. The aeration of the solution in each pot was performed using PVC tubes connected to an air compressor. The tubes were inserted into the solution through the styrofoam sheet of each pot. After 15 days, the half-strength Hoagland nutrient solution was replaced by the full-strength solution. Plants were grown in pots for 7 days. The pH of each solution was adjusted daily to obtain values of 6.0 ± 0.2 , and 1.0 mol L⁻¹ HCl or 1.0 mol L⁻¹ NaOH was added whenever necessary.

2.2. Collection of the solution to determine the kinetics of NO_3^- and NH_4^+ uptake, and chemical analyses

After the acclimation period of 21 days, the kinetic parameters of NO_3^- and NH_4^+ uptake in 'Aldrighi', 'Clone 15' and 'Tsukuba1' were determined by the methodology proposed by Claassen and Barber (1974), adapted to completely drain the internal reserves of N in the plants. The adaptations were obtained in preliminary experimental trials. Thus, rootstock plants were cultivated in 8 L pots containing distilled water and 0.1 mol L⁻¹ of CaSO_4 for 30 days. After this period, the solution containing CaSO_4 was removed from each pot. The half-strength Hoagland nutrient solution was added to each pot for 1 h in order for the system to reach the steady state of uptake required for the application of the kinetic model. After 1 h, the half-strength Hoagland nutrient solution was replaced with a new solution containing the same concentration. At this point, 50 mL of solution was collected every 1 h, up to 24 h. After 24 h, 50 mL of solution was collected every 3 h, up to 48 h. From 48 to 60 h, 50 mL of solution was collected every 6 h. After 60 h of evaluation, the plants were removed from the pots and separated into leaves, stems and roots.

Root length and stem diameter were evaluated using a digital caliper. Root and shoot fresh mass was evaluated in digital scale. The volume of nutrient solution remaining in each pot was measured using a graduated cylinder. The organs were dried in an oven with forced air at 65 °C until constant weight. The organs were then milled, prepared and subjected to sulfuric acid digestion. Subsequently, the sample was distilled by a semi-micro Kjeldahl steam distillation apparatus (Tedesco et al., 1995).

NO_3^- and NH_4^+ contents in the solution collected over time were analyzed according to methodology proposed by Tedesco et al. (1995). Therefore, 20 mL of the collected sample was added into digestion tubes with 0.7 g of MgO and distilled by a Kjeldahl steam distillation unit. After distillation, the extract (± 35 mL) was collected in 5 mL of boric acid and immediately titrated using 0.0025 mol L⁻¹ H_2SO_4 , which allowed us to determine NH_4^+ concentration. In the sample with cooled distilled MgO, 0.7 g of Devarda's alloy was added and it was then subjected to distillation again. After distillation, the extract (± 35 mL) was collected in 5 mL of boric acid and immediately titrated using

0.0025 mol L⁻¹ H₂SO₄, which allowed us to determine NO₃⁻ concentration.

2.3. Calculations and statistical analysis

Based on the NO₃⁻ and NH₄⁺ concentrations in the solution, on the initial and final solution volumes in the pots, and on the fresh matter of the roots, I_{max} and K_m were calculated using Cinética 2.0 software (Ruiz, 1985). For the C_{min} value, the concentration of NO₃⁻ and NH₄⁺ in the depletion solution at 60 h was considered. The influx (I) was calculated according to Eq. (1), proposed by Michaelis-Menten and modified by Nielsen and Barber (1978).

$$I = [V_{\max} \times (C - C_{\min})] / [K_m + (C - C_{\min})] \quad (1)$$

Where: V_{max} refers to the maximum uptake rate of the membrane transporters; C refers to the concentration present in the solution at a given moment; C_{min} refers to the minimum concentration at 60 h and K_m refers to the affinity coefficient. The experimental design was completely randomized with five replicates per treatment. Each replicate consisted of one plant.

The results obtained were submitted to analysis of variance and when significant the means of C_{min}, K_m and I in each rootstock cultivar were compared by the Tukey test at 5% of error probability.

3. Results and discussion

3.1. Morphological parameters

'Tsukuba1' rootstock had the highest dry matter production of roots and shoots, and the highest concentration and accumulation of N in leaves and roots (Table 1). This may have happened because the organs served as sinks for nutrients (such as N), because they had intense division and cellular elongation, which is reflected in increased dry matter (Lee et al., 2015; Zufferey et al., 2015). 'Aldrichi' rootstock presented the highest concentration of N in stems and roots, and together with 'Tsukuba1' presented increased root length and N accumulation in stems. 'Clone 15' rootstock exhibited larger stem diameter in comparison to the other rootstocks.

The results of the morphological parameters suggest that 'Tsukuba1' and 'Aldrichi' rootstocks are the ones with the highest number of characteristics favorable to nutrient uptake. This possibly happened because some cultivars may develop different mechanisms such as the emission of roots and modifications in root architecture to increase access to resources (nutrients and water), and tolerance to abiotic stress

Table 1

The morphological parameters, accumulation and total N content in organs of 'Aldrichi', 'Clone 15' and 'Tsukuba1' peach rootstocks grown in Hoagland nutrient solution after 30 days of reduction of internal nutrient reserves.

Parameters	'Aldrichi'	'Clone 15'	'Tsukuba1'	CV (%)
Root dry matter (g)	1.84b ⁽¹⁾	1.73c	2.24a	4.77
Shoot dry matter (g)	4.57b	4.34b	5.56a	4.75
Root/shoot ratio (g)	0.40ns	0.40	0.40	1.28
Root length (cm)	34.20a	26.30b	34.30a	4.71
Height (cm)	52.30b	54.40a	56.10a	3.48
Stem diameter (cm)	0.70b	0.90a	0.76b	9.13
Total N in leaves (%)	2.24b	2.13b	3.58a	5.75
Total N in stems (%)	1.96a	1.63b	1.58c	10.72
Total N in roots (%)	2.95a	2.80b	2.76b	2.87
N accumulated in leaves (g organ ⁻¹)	0.051b	0.046b	0.099a	5.02
N accumulated in stems (g organ ⁻¹)	0.045a	0.035c	0.043a	8.25
N accumulated in roots (g organ ⁻¹)	0.054b	0.048c	0.061a	4.06

⁽¹⁾ Means followed by the same letter (within a row) do not differ by the Tukey test at 5% probability; Ns = not significant.

Table 2

Kinetic parameters of NO₃⁻ and NH₄⁺ uptake of 'Aldrichi', 'Clone 15' and 'Tsukuba1' peach rootstocks grown in Hoagland nutrient solution after 30 days of reduction of internal nutrient reserves.

Parameters	'Aldrichi'	'Clone 15'	'Tsukuba1'	CV (%)	
NO ₃ ⁻	V _{max} (μmol g ⁻¹ h ⁻¹)	0.73b ⁽¹⁾	0.38c	0.83a	8.27
	K _m (μmol L ⁻¹)	12.36b	18.98a	5.53c	5.49
	C _{min}	2.85c	6.45a	1.34b	5.25
NH ₄ ⁺	V _{max} (μmol g ⁻¹ h ⁻¹)	0.0104 ^{ns}	0.0090	0.0112	11.01
	K _m (μmol L ⁻¹)	0.37b	0.39a	0.34c	3.89
	C _{min}	0.34b	0.37a	0.28c	2.83

⁽¹⁾ Means followed by the same letter (within a row) do not differ by the Tukey test at 5% probability; Ns = not significant.

(George et al., 2014; Sorgonà et al., 2005; Topp et al., 2016; White et al., 2013). Species or cultivars with higher dry matter yield and increased root length may explore a larger volume of soil, which facilitates the approximation of nutrients or their forms (such as NO₃⁻ and NH₄⁺) to the roots (Craine et al., 2015). In addition, the root is a carbohydrate reserve organ, which can be used to withstand stress conditions or be redistributed along with nutrients (e.g., N) to growing organs (Centinari et al., 2016; Hoekstra et al., 2015; Klodd et al., 2016).

3.2. Kinetic parameters of NO₃⁻ and NH₄⁺ uptake

The importance of 'Tsukuba1' in regards to the morphological parameters can be justified by the fact that it presented the highest V_{max} and the lowest K_m (Table 2). The results of V_{max} suggest that the plants of 'Tsukuba1' have a higher concentration of NO₃⁻ uptake sites per root unit compared to the other rootstock cultivars (Batista et al., 2016; Cerezo et al., 2007; Tomasi et al., 2015; Yang et al., 2007). On the other hand, the lower values of K_m show that its uptake sites have higher affinity for NO₃⁻, and are therefore more efficient in absorbing it in the solution (Cerezo et al., 2007; Yang et al., 2007). In addition, 'Tsukuba1' and 'Aldrichi' rootstocks showed the lowest values of C_{min}. This indicates that these rootstocks can be grown in solution or soil with lower nutrient availability, which is desired to reduce the amount of nitrogen fertilizer needed, thereby saving the grower input costs and reducing the risk of surface and groundwater contamination (Ahmad et al., 2014; Bednorz et al., 2016; Bindraban et al., 2015).

'Tsukuba1' and 'Aldrichi' absorbed NO₃⁻ in a more linear fashion at low concentrations of NO₃⁻ in solution (< 6 μmol L⁻¹) (Fig. 1a), because these two cultivars had the lowest values of K_m (Table 2). The two cultivars possibly possess two distinct NO₃⁻ uptake mechanisms (Fig. 1a), indicating the possible performance of a high affinity transport system (HATS) and a low affinity transport system (LATS) (Pii et al., 2014; Zamboni et al., 2016). The operation of these systems is dependent on the concentration of the ion in the solution (Dechorgnat et al., 2011). This result suggests that a cultivar that requires more N, due to its greater growth capacity, is more frequently deficient and therefore needs to develop a more efficient mechanism to supply its nutrient demand (Batista et al., 2016; Cerezo et al., 2007). The molecular basis of these systems has been identified, mainly in *Arabidopsis*, demonstrating the participation of transporters belonging to the NRT1 and NRT2 transporter families, respectively, for LATS and HATS, with the exception of NRT1.1, which is a dual affinity transporter. In addition, the NAXT1 protein, which also belongs to the large NRT1 family, has been identified and characterized as being involved in the efflux of NO₃⁻ to the outer cell membrane (Dechorgnat et al., 2011). Such mechanisms were also mentioned by Tomasi et al. (2015), who reported that two transport systems (low and high affinity) for NO₃⁻ were found in grapevine rootstocks, which are induced by exposure of the roots to NO₃⁻ after a period of N deprivation.

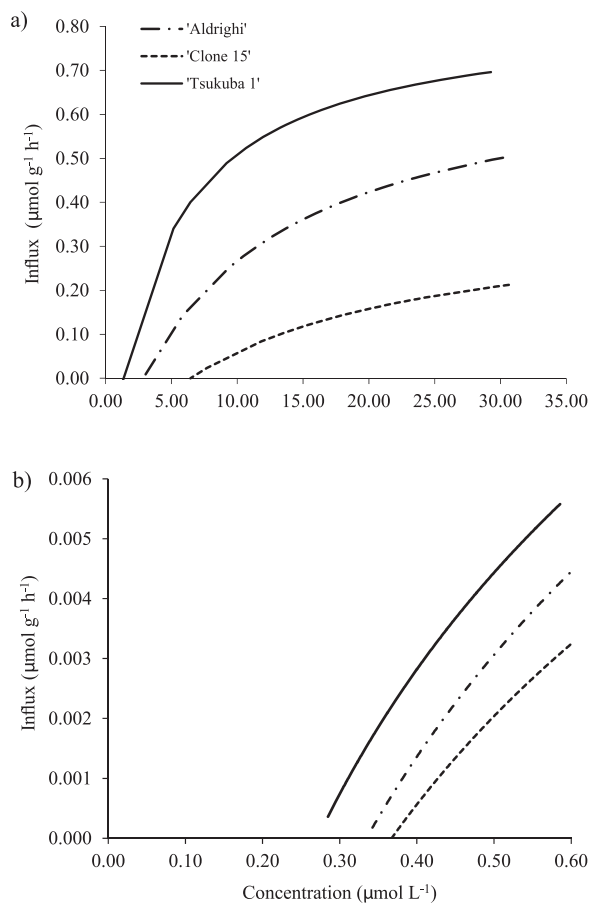


Fig. 1. Influx rates of NO_3^- (a) and NH_4^+ (b) in 'Aldrichi', 'Clone 15' and 'Tsukuba1' peach rootstocks grown in Hoagland nutrient solution after 30 days of reduction of internal nutrient reserves.

As there is a great predominance of N in the soil solution in the form of NO_3^- , the fact that 'Clone 15' rootstock exhibited the lowest value of V_{\max} and the highest values of K_m and C_{\min} explains why this rootstock did not stand out in regards to the morphological parameters.

'Tsukuba1' also excelled in absorbing NH_4^+ , presenting the lowest values of K_m and C_{\min} (Table 2). These results show that 'Tsukuba1' also has a great affinity for NH_4^+ compared to the other two cultivars, which is desired. Thus, 'Tsukuba1' will be efficient in absorbing NH_4^+ from a solution or soil that has low concentration of this N form. The highest value of I for 'Tsukuba1' can be explained because of the lowest value of K_m (Table 2). 'Tsukuba1' showed the highest I of NH_4^+ (Fig. 1b), whereas the lowest was found in 'Clone 15', suggesting that it has a slower growth rate in comparison to 'Tsukuba1'. The highest influx contributed to the outstanding values of shoot and root dry matter of 'Tsukuba1', while Clone 15 exhibited the lowest values of the same variables. Considering that NH_4^+ is an N form whose supply mechanism is through mass flow (where the water moves towards the roots and carries nutrients dissolved therein) and it is absorbed and assimilated with less energy expenditure (Zhou et al., 2015), the higher the dry matter production of shoots and roots, the greater may be its need to increase the influx of nutrients (Craine et al., 2015; Topp et al., 2016). In addition, it should be noted that the highest dry matter production of the shoots may have increased the transpiration of the plants, generating a higher water gradient between the solution and the plant, which stimulates the approach of N forms to the roots and therefore its absorption and transport, justifying a higher percentage and accumulation of N in plant organs (Table 1) (El-Jendoubi et al., 2013; Jordan et al., 2014; Rivera et al., 2016).

Unlike NO_3^- uptake, the NH_4^+ uptake pattern over time (Fig. 1b)

was similar among the rootstock cultivars, suggesting the same N uptake mechanism. These results possibly suggest the presence of a high affinity transport system, allowing the rootstock to absorb NH_4^+ at very low concentrations (Couturier et al., 2007; Li et al., 2012). Molecular studies have identified the participation of a family of genes that encodes proteins for the transport of NH_4^+ (AMT - ammonia transporters) and operates on the plasma membrane of plants. Members of this AMT1 family are responsible for the high affinity transport system (HATS) and AMT2 for the low affinity transport system (LATS) (Loqué and Wirén, 2004)

4. Conclusions

The kinetic parameters of NO_3^- and NH_4^+ uptake are additional criteria that can be used to select peach rootstocks, as they directly influence shoot and root dry matter production and N accumulation in leaves.

The most efficient rootstock for NO_3^- and NH_4^+ uptake was 'Tsukuba1', because it exhibited the lowest values of C_{\min} and K_m , and the highest values of V_{\max} and I_{\max} for NO_3^- and NH_4^+ .

NO_3^- uptake in 'Tsukuba1' and 'Aldrichi' showed a two-phase uptake pattern, which suggests the presence of low and high affinity uptake transport systems. On the other hand, NH_4^+ uptake in the three cultivars apparently followed a one-phase uptake pattern, suggesting the presence of a high affinity uptake transport system.

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