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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} CaSO4 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 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 (Picolotto 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

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