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Kinetic parameters related to nitrogen uptake efficiency of pear trees *(Pyrus communis)*

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ABSTRACT

Genetic improvement programs for pear trees in Brazil are characterized by rootstock (hypobiotic) selection mainly considering physiological attributes such as vigor, breakage of dormancy, propagation easiness, and sanitary characteristics, such as resistance to pests and diseases. However, kinetic parameters that determine nutrient uptake efficiency are not usually considered as, for example, nitrogen (N) in forms of NO_3^- and NH_4^+. The objective of this study was to evaluate the kinetic parameters related to N uptake in pear selection '54′ and '971′ as additional criteria for rootstock selection. The plants were acclimatized in a half-strength Hoagland solution and tested to assess the depletion of the internal reserves at 15 and 30 days, in $CaSO_4$ solution to evaluate the depletion period during 65 h. The selection '971′ showed more significant NO_3^- and NH_4^+ uptake efficiency, because presented higher V_{max} values and Influx. The internal reserve depletion (IRD) period for the evaluated selections was 30 days in $CaSO_4$ solution. The evaluation period of the depletion period to reach the C_{min} for selections '971′ and '54′ was 64 and 65 h for NO_3^- respectively, and 65 h for NH_4^+. The selection '971′ had a higher affinity with the NO_3^- ion when correlated with the physiological parameters of minimum fluorescence (Fo) and electron transport rate (ETRm).

1. Introduction

The world's breeding programs for pear trees utilize about 20 species from Europe and Asia, and the most used species are *Pyrus communis* L. (European), *Pyrus pirifolia* (Burm. F.) Nakai (Japanese) and the interspecific hybrid specie *Pyrus bretschneideri* Rehd. (Chinese) (Kumar et al., 2017; Janick, 2002). In Brazil, the most used rootstocks are *Pyrus calleryana* Decne and quinces from the *Cydonia* sp. gender (Machado et al., 2015; Pasa et al., 2011). The *P. Calleryana* is a tall tree highly vigorous and adaptable with a large treetop projection area. Such characteristics may hinder the conduction of this plant on modern orchards due to its high planting density (Machado et al., 2015; Pasa et al., 2011). The *Cydonia* sp. is less a vigorous rootstock, which enables a higher planting density and early fruiting. However, it may present treetop compatibility problems and lower edaphoclimatic adaptation (Pasa et al., 2011, 2010).

Pear trees orchards in Brazil are located in subtropical regions of colder and higher altitude, where predominate low pH soils with a lot of organic matter, which usually requires liming and mineral or organic fertilization, for fruit production (Brunetto et al., 2016, 2015; Neto et al., 2008, 2011). Therefore, the study of kinetic parameters of nutrient uptake by pear trees may be an alternative to select germplasm more adapted to soils with different fertilities and nutrient demands.

The kinetic parameters of nutrient uptake are defined by the maximum rate of absorption (V_{max}), which refers to the number of nutrients absorbed when all the sites transporters situated in the membranes of the root cells are saturated. The Michaelis-Menten constant (K_m) that indicates the concentration of nutrient/ion solution, where half of the maximum rate of absorption is reached. The minimum concentration (C_{min}), which specifies the minimum concentration amount that roots can uptake nutrients from the solution. Also, the Influx (I) that is the amount of nutrient absorbed per unit mass of roots per unit of time (Martinez et al., 2015; Yang et al., 2007). Thus, selections with lower values of C_{min} and K_m and higher values of V_{max} and I, have a greater

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chance to adapt to soils with low N availability.

To determine the kinetic parameters of N uptake in annual crops, the plants must be acclimatized in half-strength Hoagland nutrient solution for about 15 days (Sanes et al., 2013). After this period, the solution is replaced by distilled water for 24 h to reach the internal nutrient reserves depletion. It is expected that after this period, the plant will be able to max out its nutrient uptake capacity. Then, the plants are re- acclimatized in half-strength Hoagland's solution, to establish the depletion N rate. To achieve it, solution aliquots are collected every 1 h for 24 h (Claassen and Barber, 1974). However, for the evaluation of kinetic parameters with fruit species, such as in pear trees selection, the methodology needs to be adjusted since the plants can maintain more abundant internal N reserves, mainly allocated in perennial organs such as roots and stems (Dar et al., 2015; Neto et al., 2008; Rubio-Covarrubias et al., 2009).

The objective of this study was to evaluate N kinetic parameters of selection '54′ and '971′, as additional criteria for rootstock selection.

2. Material and methods

2.1. Location and treatments

The study was carried out in a greenhouse (latitude 29° 42′ 57.4″S and longitude 53° 43′ 12.2″W of Greenwich, average altitude of 115 m), at the Federal University of Santa Maria (UFSM), in Santa Maria city, state of Rio Grande do Sul, southern region of Brazil. The average relative air humidity in the greenhouse was 60 % and the average air temperature was 25 °C.

The selection '54′ and '971′ used in this study were two rootstock (hypobiotic) from open pollination of the germplasm bank of agricultural research (Embrapa) - Embrapa Uva e Vinho Unit, situated in the city of Vacaria (RS), in partnership with the State University of Santa Catarina (UDESC), located in Lages (SC). The selections are still in the process of genetic improvement and not available in the market.

The young plants after acclimation were grown on commercial substrate for about 12 months, and the most uniform specimens were used in the current study. In sequence, they were removed from the vessels to be washed carefully in running water until complete removal of the substrate. The plants with clean roots were conditioned in 5 l pots containing quarter-strength Hoagland nutrient solution (Jones Junior, 1983), composed by (in mg L): N-NO_3^- = 196, N-NH_4^+ = 14, P = 31, K = 234, Ca = 160, Mg = 48.6, S = EDTA = 5, Cu = 0.02, Zn = 0.15, Mn = 0.5, B = 0.5 and Mo = 0.01, where they remained for 7 days until the completion of the first acclimation step.

The pots were then arranged on metal tables inside the greenhouse in a completely randomized design, using ten plants of each selection, having an equal division for each nutrient reserves depletion period. On the surface of each pot, it was added a 3 mm thick styrofoam blade to prevent evaporation of the nutrient solution and assist plants fixation. The styrofoam blade had a central hole to allow pear tree stem to grow and a second hole for a Polyvinyl chloride tube (PVC), which was connected to an oil-free air compressor for continuous aeration in each pot (Fig. 1).

For 7 days, the young pear trees rested in quarter-strength Hoagland solution to be replaced to a half-strength solution. The pear trees remained another 21 days in this solution concluding the second period of acclimation. The solution pH was read daily and maintained between 6.0 ± 0.2, adding 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH whenever necessary. After the acclimation period, pear trees were induced to the depletion of internal nutrient reserves in a 0.01 mol L^{-1} $CaSO_4$ solution (de Paula et al., 2018), which provided only Ca and S needed to maintain the electrochemical potential of the cell membrane and preserve cell wall integrity.

The plants were then separated into two groups of 15 days and 30 days, both kept in a solution of $CaSO_4$ (0.01 mol L^{-1}), to evaluate the depletion of pear tree nutrient reserves. Thus, the experiment was conducted in experimental design of two levels factor (selection x period of reserve depletion) with 5 repetitions per treatment.

2.2. Fluorescence of chlorophyll ‘a’

The chlorophyll fluorescence at night time was determined using a JUNIOR-PAM (Walz, Germany) pulse amplitude modulated fluorometer (PAM), in the last day of each evaluation period at 15 and 30 days. The initial fluorescence (Fo) was determined and the leaf sample was subjected to a pulse of saturating light (10,000 µmol m^{-2} s^{-1}) for 0.6 s to establish the maximum fluorescence (Fm).

2.3. Depletion period evaluation

After each depletion period, the plants returned to the half-strength Hoagland nutrient solution for one hour, in order to reach steady-state uptake condition required for the application of the kinetic model proposed by Claassen and Barber (1974). The solution was again replaced by the same nutrient concentrations of the original half-strength Hoagland solution, to start the collection period of the solution aliquots. Ten milliliters from each 5 l pot was collected at time zero and every 6 h in the first 24 h, every 3 h between 24 and 48 h, and every 1 h between 48 and 65 h. At the end of the 65 h evaluation period, the plants were removed from the pots and separated into leaves, stems and roots. The stem height was identified using a metric measuring tape and the diameter was determined using a digital caliper.

The fresh mass (FM) of leaves, stem and roots were determined on a digital scale (Bel Engineering, Brasil). The volume of nutrient solution that remained in each pot was measured with the aid of a graduated cylinder.

2.4. Solution and leaves nutrient analysis

The plants organs were dried in a forced air circulation oven at 65 °C until maintain a constant weight to determine its dry mass (DM). Sequentially, the dried leaf fractions were milled, prepared and subjected to sulfuric acid digestion (Tedesco et al., 1995). For this purpose, 0.200 g dry mass of each fraction was added to a digestion tube (25 × 250 mm) with 1 ml de H_2O_2, 2 ml of H_2SO_4 and 0.7 g of digestion mixture (90.9 % Na_2SO_4 + 9.1 % $CuSO_4$ $5H_2O$). The tubes were then heated in a digester block at 150 °C, in which the temperature was raised gradually, 50 °C every 30 min up to 350 °C. After the complete digestion of the leaves, the tubes remained in the digester block for another 60 min at 350 °C.

After the digestion, the volume of the sample was adjusted by adding 50 ml of distilled water. In sequence, ten milliliters of the extract was collected and pipetted in another 100 ml capacity digestion tube along with 10 ml of NaOH NaOH 10 mol L^{-1}. The sample was then distilled with a semi-micro Kjeldahl steam distillation unit (Tecnal TE-0364, Brazil), until the accumulation of 35 ml in Erlenmeyer flask containing 5 ml of indicator-boric acid mixture. The extract was then titrated with standardized H_2SO_4 0005 mol L^{-1} to calculate the total N content (Tedesco et al., 1995). The NH_4^+ and NO_3- content in the solution collected during depletion period were determined by molecular absorption spectrophotometry in an automatic segmented flow analyzer (Skalar San^{++}, 1074 sampler, The Netherlands).

2.5. Determination of kinetic parameters

The parameters of V_{max}, C_{min} and K_m were calculated using the software Cinetica (RUIZ et al., 1985), using NO_3^- and NH_4^+ aliquot concentrations in function of time, the fresh mass of the roots, and the initial and final solution pot volumes. C_{min} was determined by considering the concentration of N present in the 65 -h depletion period, whereas the Inflow (I) was calculated according to Eq. 1, proposed by Michaelis-Menten and modified by Nielsen and Barber (1978).

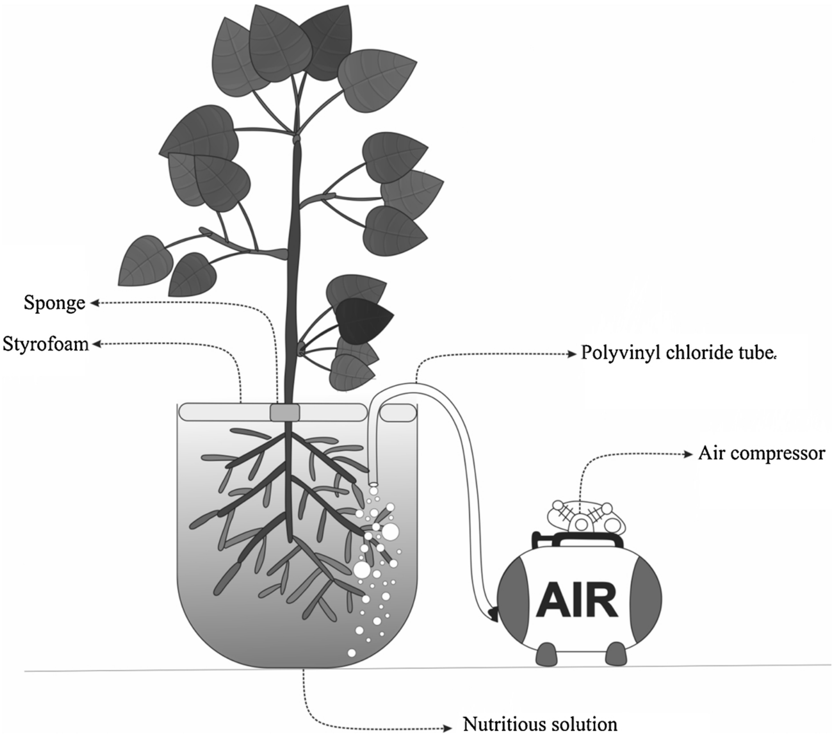

Fig. 1. Illustrative schematic of N-absorption kinetic experiment assembly in a pear tree.

$$IL = \left[\frac{V_{max} \times (C - C_{min})}{K_m + (C - C_{min})} \right] \tag{1}$$

Where: I = Nutrient Influx Rate, C = Nutrient concentration collected in each period, V_{max} = maximum uptake rate, K_m = Michaelis-Menten constant, and C_{min} = minimum concentration.

2.6. Statistical analysis

The results obtained from the morphological and physiological parameters were submitted to homogeneity and normality test (Biostat 5.0 2008), and later, the analysis of variance in the statistical environment R (R Core Team 2018). When the effects of the treatments were considered significant, the means of the results of V_{max}, C_{min}, K_m and I for each selection were compared by the Tukey test ($p < 0.05$); and the concentration difference of N-NH_4^+ and N N-NO_3^- over 65 h for each selection were compared by the Scott Knott test ($p < 0.05$).

The principal component analysis (PCA) was performed using the software Canoco version 4.5 (Ter Braak & Similauer, 2002). It was calculated from the mean of depletion response variables in the 30 days of evaluation in calcium sulfate, considering the kinetic parameter variables (Dry mass of roots, stem and leaves, height and stem diameter, N in leaves, stem and roots) and physiological parameters (minimum fluorescence - F_o, maximum fluorescence - F_m, F_v / F_m relation and the maximum electron transport rate - ETRm).

3. Results and discussion

3.1. Morphological parameters and fluorescence of chlorophyll a

The selection '54′ and '971′ presented high dry mass values of root and stem, having the stem diameter measured after 30 days of IRD (Table 1). The increase of the morphological parameter indicates that the plants continued to grow even after the end of the study. The '54′ selection had a higher N content in the roots when compared to the selection '971′ (Table 1) after 15 days of nutrient reserves depletion. This fact is due to a higher production of selection '54′ dry mass form the roots, in which N accumulation is favored mainly in the form of proteins (Moriwaki et al., 2019). Another essential factor is that the dry mass production in the leaves being smaller than in the roots and stem, decreased the need for redistribution of N forms from perennial organs to growing annual organs, in other words, from roots to leaves (Brunetto et al., 2016; Harrison-Kirk et al., 2014; Neto et al., 2008). The dry leaf mass, tree height, and total N content in the leaves and stem did not present any statistical divergence during the conduction of the experiment (Table 1).

Table 1
Morphological parameters of selection '54′ and '971′ in Hoagland nutrient solution for 15 and 30 days, after the internal reserves depletion of (IRD) in $CaSO_4$ solution (0.01 mol L^{-1}).

Morphological parameters	'54′		'971′		*P* (anova)
	15 IRD	30 IRD	15 DRI	30 IRD	
Root dry matter (g)	2.44 aB (1)	3.99 aA	1.60 aB	3.72 aA	0.00
Steam dry matter (g)	3.62 aB	6.07 aA	3.46 aB	5.21 aA	0.00
Leaf dry matter (g)	0.94 aA	1.49 aA	1.35 aA	1.43 aA	0.00
Heigh (cm)	42.93 aA	53.46 aA	49.23 aA	51.36 aA	0.00
Stem diameter (cm)	0.47 aB	0.57 aA	0.43 aB	0.53 aA	0.00
Total N in leaves (%)	2.59 aA	2.66 aA	2.77 aA	2.52 aA	0.00
Total N in stems (%)	1.75 aA	1.79 aA	1.76 aA	1.67 aA	0.00
Total N in roots (%)	3.40 aA	3.38 aA	2.85 bA	3.33 aA	0.00

(1) Averages followed by lowercase letters differ from the selections between periods and averages followed by the upper case letters differ the periods within each selection using the Tukey test ($p < 0.05$ %).

The Fv/Fm relationship for both selections was obtained by the difference between the maximum fluorescence (Fm) and minimum fluorescence (Fo) read at daybreak. The ratio is represented by a 0–1 scale, where the number closer to 1 means higher energy absorption efficiency by the plant. The fluorescence results showed that both selections lost low levels of energy, having an equal ratio Fv/Fm of 0.83 at the 30 days of IRD (Table 2). It means that the energy was transfer more efficiently from the light collecting system to the reaction center (Mascia et al., 2017). When compared to the 15 days of IRD in $CaSO_4$

Table 2

Physiological parameters of selection '54′ and '971′ in Hoagland nutrient solution for 15 and 30 days after the internal reserves depletion of (IRD) in $CaSO_4$ solution (0.01 mol L^{-1}).

Physiological parameters	'54′		'971′		*P* *(anova)*
	15 IRD	30 IRD	15 IRD	30 IRD	
Minimum fluorescence (Fo)	135.66 aA(1)	123.66 aA	129.33 aA	126.33 aA	0.00
Maximum fluorescence (Fm)	677.00 aA	732.66 aA	665.00 aA	688.00 aA	0.00
Fv/Fm ratio*	0.79 aB	0.83 aA	0.80 aB	0.83 aA	0.00
Electron transport rate (ETRm)	80.03bA	59.66 bB	102.16aA	85.46 aA	0.00

(1) Averages followed by lowercase letters differ from the selections between periods and averages followed by the upper case letters differ the periods within each selection using the Tukey test ($p < 0.05$ %). *Fv/Fm ratio: Fv = variable fluorescence and Fm = maximum fluorescence.

solution, it is evident that the photosynthetic process of the selections was not impaired.

The physiological parameters of minimum fluorescence (Fo) and maximum fluorescence (Fm), did not differ statistically for 15 and 30 days of IRD in both selections analyzed (Table 2). Yet, selection '971′ presented the highest rate of maximum electron transport (ETRm) at 15 and 30 days IRD (Table 2). This portrait that this selection is possibly better adapted to low N concentrations since it was able to absorb more of electrons in the chlorophyll '*a*' antenna system. As a consequence, more solar energy was converted into photochemical energy, contributing effectively for the reduction of $NADP^+$ to NADPH. Therefore, is expected from selection '971′ a higher demand of N and a greater uptake of the same N, because it is capable of converting more solar energy into chemical energy.

3.2. *N reserve depletion period*

At 15 and 30 days of internal nutrient reserves depletion, the selection '971′ presented the highest V_{max} values from NO_3^- absorption than selection '54′ (Fig. 2). However, the values of K_m and C_{min} related to NO_3^- absorption were statistically equal for both selections and higher after 15 days of IRD (Table 2). The V_{max} value was statistically equal between 15 and 30 days of IRD for both selections. The selections '54′ and '971′ have presented lower C_{min} values of NH_4^+ after 30 days of IRD (Fig. 3). The V_{max} and K_m values analyzed for the NH_4^+ were statistically equal for both IRD periods (15 and 30 days). These results show that after 15 days IRD, both selection still had internal reserves of NH_4^+ and, for this reason, the plants continued to absorb NH_4^+. The NH_4^+ C_{min} results suggest that the selection '54′ and' 971 after 30 days IRD, demonstrated greater ability in NH_4^+ uptake, absorbing even at low concentrations in the solution. The NH_4^+ C_{min} results suggest that the selection '54′ and' 971 after 30 days IRD, have a greater ability in NH_4^+ uptake by absorbing even at low concentrations in the solution, due to a larger number of absorption sites on the roots.

The influx rates of NO_3^- and NH_4^+ were higher in selection '971′ (Fig. 2 and 3), possibly because they had a higher demand for N and, as an adaptation mechanism, acquired a more considerable amount of NO_3^- and NH_4^+ in the root uptake sites (Mota et al., 2011). Moreover, the selection '54′ presented physiological parameters (Fv/Fm ratio and ETRm), that indicates a higher energy loss by fluorescence and a lower NO_3^- and NH_4^+ uptake. The highest rates of NO_3^- and NH_4^+ uptake by roots of '971′ selection cannot be justified by morphological characteristics since there is no difference in dry mass content in any assessed organs. However, there is a clear difference in the way that photosynthetic selection apparatus captures the solar energy, which may have generated a higher demand for N, supplied by a higher effectiveness of root's uptake. This possible adaptation of selection '971′ is very positive since a vast amount of N is required in the adult phase of the plant due to its export increase via fruit.

The uptake drop in NO_3^- and NH_4^+ by the roots of selection '54′ may be related to a solution concentration decrease or plant activity reduction (transport rate or reaction) of NRT1 and NRT2 absorption proteins, since such proteins are involved in nutrients uptake affecting negatively the enzymes engaged in NO_3^- and NH_4^+ assimilation (Giri et al., 2017).

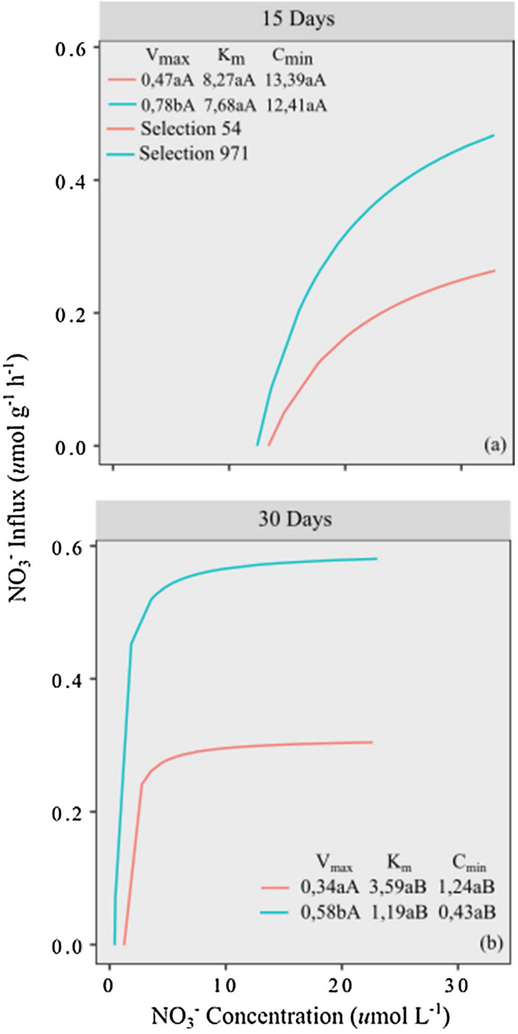

Fig. 2. Influence Rate, C_{min}, K_m and V_{max} of NO_3^- in selection '54′ and '971′, cultivated in Hoagland solution after 15 (a) and 30 days (b) of internal nutrient reserves depletion in a $CaSO_4$ solution (0.01 mol L^{-1}). Averages followed by lowercase letters differ from the selections between periods and averages followed by the upper case letters differ the periods within each selection using the Tukey test ($p < 0.05$ %).

3.3. *The best depletion period period to evaluate N uptake*

The selection '54′ absorbed NO_3^- more intensively and longer than selection '971′ reaching its C_{min} at the end of the assessment period

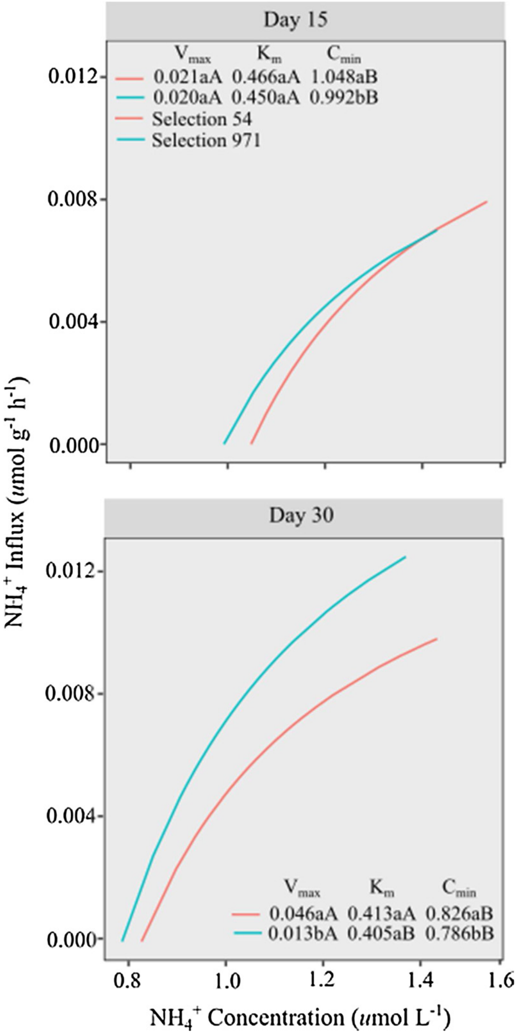

Fig. 3. Influence Rate, C_{min}, K_m and V_{max} of NH_4^+ in selection '54' '971', cultivated in Hoagland solution after 15 (a) and 30 days (b) of internal nutrient reserves depletion in a $CaSO_4$ solution (0.01 mol L^{-1}). Averages followed by lowercase letters differ from the selections between periods and averages followed by the upper case letters differ the periods within each selection using the Tukey test ($p < 0.05$ %).

(Fig. 4a and 4b). The C_{min} identified for both selection '54' and '971' were similar, therefore is recommended that in future experiments the extension of collections period for at least 70 h (Martinez et al., 2015; de Paula et al., 2018). It portraits that access '54' and '971' have similar morphological characteristics which provide similar NO_3- like uptake mechanisms.

The access reached C_{min} for NH_4^+ after 65 h from the beginning of the experiment, demonstrating the same depletion period (Fig. 5 a). The results of C_{min} parameters obtained with fruit trees differ from the studies carried out with annual crops, where the recommended collection period can be 15 h, having 60 min intervals between each collection (Li et al., 2017).

The same depletion period for both selection, can occur when NH_4^+ uptake is carried out by several NH_4^+ transporting agents (Mota et al., 2011). There are at least five NH_4^+ transporters that belong to AMT1 (CoAMT_1 to CoAMT1_5) subfamily contained in pear tree roots, which can partake in NH_4^+ uptake during the vegetative cycle (Mota et al., 2011). The results presented by these authors (Batista et al., 2016; Mota et al., 2011; de Paula et al. (2018); Pii et al., 2014; Tomasi et al., 2015) reinforce the understanding that N forms in perennial trees is quite different from annuals, and that kinetic uptake parameters of N forms may contribute to a better understanding of nitrogen fertilization in pear orchards.

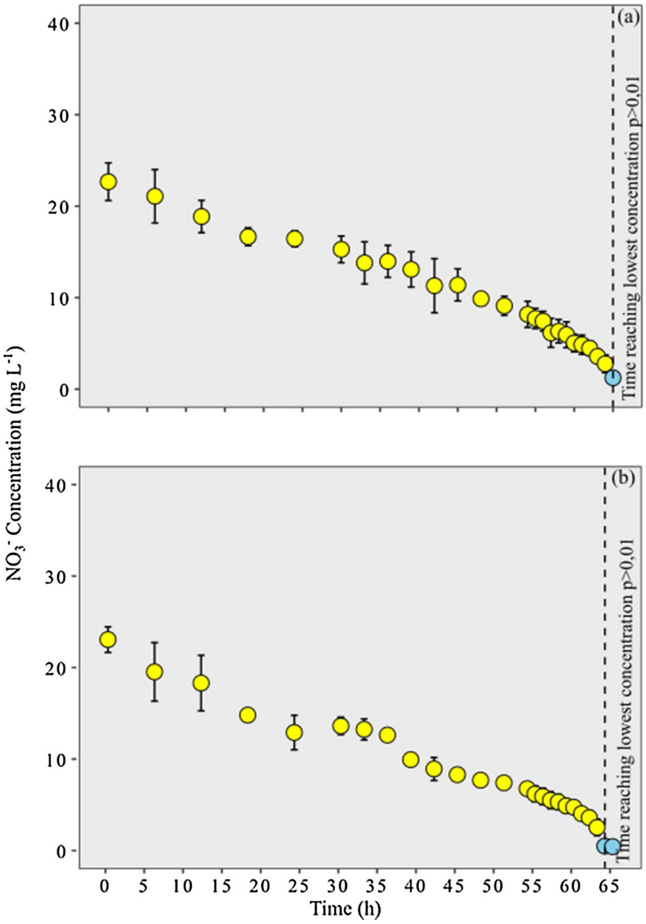

Fig. 4. NO_3^- concentrations in selection '54' (a) and '971' (b) after 30 days of internal nutrient reserves depletion. Average NO_3^- concentrations in blue differ significantly from averages of concentrations in yellow $\alpha = 0.05$ (Scott Knot's test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In previous studies by de Paula et al. (2018), kinetic parameters related to N uptake in rootstocks (hypobiotes) of peach trees Tsukuba 1, Aldrigh and Clone 15 were evaluated. Both the peach trees and selection pear trees '54' and '971' from the current study, found that the best period for N internal reserves depletion was at 30 days.

3.4. Principal component analysis (PCA)

The principal component analysis (PCA) from selection '54' and '971' explained 57.42 % of data variability (Fig. 6). The first component (PC1) explained 35.28 % of data variation and exposed the difference from kinetic parameter NO_3^- V_{max} uptake, which has an inverse correlation with C_{min} and K_m parameters. The higher influx of selection '971' (Fig. 2b) makes evident its affinity for NO_3- and its high interaction with the physiological parameter ETRm, which has an inverse correlation with stem N concentration. However, selection '971' absorbed a considerable amount of N in photosynthetic processes and did not accumulate N in reserve organs like the stem, which is usually expected in fruit trees since it is a passage flow of nutrients (Brunetto et al., 2016).

The highest ETRm values and its correlation with '971' selection revealed a higher demand for N by the plant, illustrated by the rise in NO_3^- and NH_4^+ inflows on both periods of IRD (Fig. 2,3). This shows that a more significant amount of energy was harnessed by selection

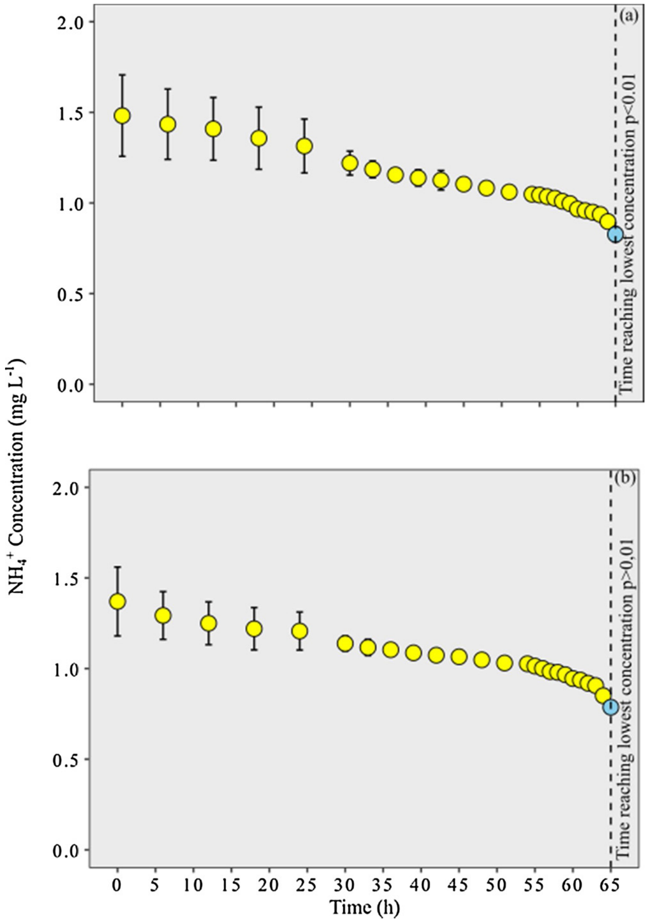

Fig. 5. NH_4^+ concentrations in selection ‘54′(a) and ‘971′ (b), after 30 days of internal nutrient reserves depletion. Average NH_4^+ concentrations in blue differ significantly from averages of concentrations in yellow α = 0.05 (Scott Knot's test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

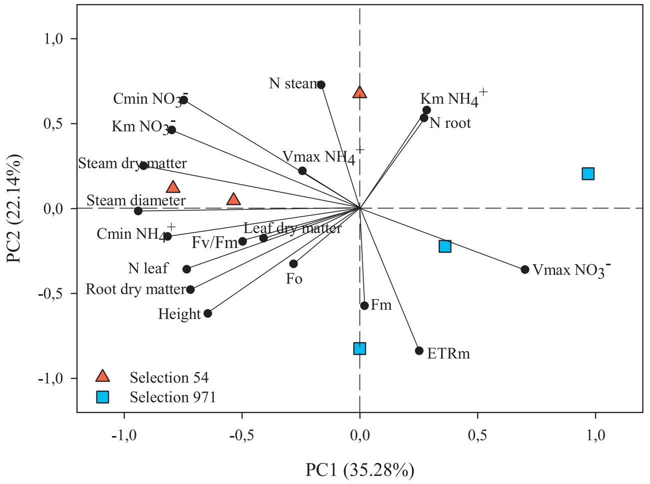

Fig. 6. Relationship between main component 1 (PC1) and main component 2 (PC2) for the variables of kinetic parameters (V_{max}, K_m and C_{min}), morphological parameters (dry matter of roots, stem and leaves, height, stem diameter; N ratio in leaves, stem and roots) and physiological parameters (minimum fluorescence - Fo, maximum fluorescence - Fm, Fv / Fm ratio and electron transport rate - ETRm).

'971′ and, consequently, more energy was absorbed and used in photochemical processes of photosynthesis. Also, selection '971′ presented a cluster of NH_4^+ K_m and N results from the roots of component 1, correlating it inversely with the minimum fluorescence (Fo), which explains a rise in the selection nutrient demand. Possibly, the plant suffered stress due to the initial nutrient demand at the beginning of the depletion period and stored more N in its roots due to genetic characteristics, which generated a higher affinity with the NO_3- ion.

4. Conclusion

The selection '971′ demonstrated a higher efficiency in NO_3^- and NH_4^+ uptake, as it presented higher values of V_{max} and influx for both nutrients. The internal N reserves depletion period for both selection assessed was 30 days in $CaSO_4$ solution. The depletion period to reach C_{min} for selection '971′ and '54′ was 64 and 65 h for NO_3- respectively and 65 h for NH_4^+. The selection '971′ had a higher affinity with the NO_3- ion when correlated with the physiological parameters of minimum fluorescence (Fo) and maximum electron transport rate (ETRm).

CRediT authorship contribution statement

Paula Beatriz Sete: Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Betania Vahl de Paula:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Matheus Severo de Souza Kulmann:** Investigation, Resources, Writing - review & editing. **Andrea de Rossi:** Resources, Writing - review & editing. **Danilo Eduardo Rozane:** Writing - review & editing. **Jacson Hindersmann:** Investigation, Resources, Supervision. **Amanda Veridiana Krug:** Investigation, Resources, Supervision. **Gustavo Brunetto:** Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The manuscript is original, has not been published before, and is not being considered for publication elsewhere in its final form neither in printed nor in electronic format and does not present any kind of conflict of interests.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2020.109530.

References

Batista, R.O., Furtini Neto, A.E., Deccetti, S.F.C., Viana, C.S., 2016. Morfologia radicular e cinética de absorção de nutrientes por clones de cedro-australiano. Rev. Caatinga 29, 153–162. https://doi.org/10.1590/1983-21252016v29n118rc.

Brunetto, G., De Melo, G.W.B., Toselli, M., Quartieri, M., Tagliavini, M., 2015. The role of mineral nutrition on yields and fruit quality in grapevine, pear and apple. Rev. Bras. Frutic. 37, 1089–1104. https://doi.org/10.1590/0100-2945-103/15.

Brunetto, G., Ceretta, C.A., de Melo, G.W.B., Girotto, E., Ferreira, P.A.A., Lourenzi, C.R., da Rosa Couto, R., Tassinari, A., Hammerschmitt, R.K., da Silva, L.O.S., Lazzaretti, B.P., de Souza Kulmann, M.S., Carranca, C., 2016. Contribution of nitrogen from urea applied at different rates and times on grapevine nutrition. Sci. Hortic. (Amsterdam). 207, 1–6. https://doi.org/10.1016/j.scienta.2016.05.002.

Claassen, N., Barber, S.A., 1974. A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. Plant Physiol. 54, 564–568. https://doi.org/10.1104/pp.54.4.564.

Dar, M.A., Wani, J.A., Raina, S.K., Bhat, M.Y., Malik, M.A., 2015. Relationship of leaf nutrient content with fruit yield and quality of pear. J. Environ. Biol. 36, 649–653.

de Paula, B.V., Marques, A.C.R., Rodrigues, L.A.T., de Souza, R.O.S., de S Kulmann, M.S., Kaminski, J., Ceretta, C.A., de Melo, G.W.B., Mayer, N.A., Antunes, L.E., Ricachenevsky, F.K., Nicoloso, F.T., Brunetto, G., 2018. Morphological and kinetic parameters of the uptake of nitrogen forms in clonal peach rootstocks. Sci. Hortic. (Amsterdam). 239, 205–209. https://doi.org/10.1016/j.scienta.2018.05.038.

Giri, A., Heckathorn, S., Mishra, S., Krause, C., 2017. Heat stress decreases levels of nutrient uptake and assimilation proteins in tomato roots. Plants 6, 6. https://doi.org/10.3390/plants6010006.

Harrison-Kirk, T., Beare, M.H., Meenken, E.D., Condron, L.M., 2014. Soil organic matter and texture affect responses to dry/wet cycles: Changes in soil organic matter fractions and relationships with C and N mineralisation. Soil Biol. Biochem. 74, 50–60. https://doi.org/10.1016/j.soilbio.2014.02.021.

Janick, J., 2002. The pear in history, literature, popular culture, and art. Acta Hortic. 596, 41–52. https://doi.org/10.17660/actahortic.2002.596.1.

Kumar, S., Kirk, C., Deng, C., Wiedow, C., Knaebel, M., Brewer, L., 2017. Genotyping-by-sequencing of pear (Pyrus spp.) accessions unravels novel patterns of genetic diversity and selection footprints. Hortic. Res. 4, 17015. https://doi.org/10.1038/hortres.2017.15.

Li, Q., Wu, Y., Chen, W., Jin, R., Kon, F., Ke, Y., Schi, H., Yuan, J., 2017. Cultivar differences in root nitrogen uptake ability of maize hybrids. Front. Plant Sci. 8, 1060 https://doi.org;10.3389;fpls.2017.01060.

Machado, B.D., Magro, M., Rufato, L., Bogo, A., Kreztschmar, A.A., Simões, F., 2015. Compatibilidade fenotípica entre cultivares de pereiras européias e porta-enxertos de marmeleiro. Ciã¯â¿â½ncia Rural. 45, 1551–1556. https://doi.org/10.1590/0103-8478cr20141128.

Martinez, H.E.P., Olivos, A., Brown, P.H., Clemente, J.M., Bruckner, C.H., Jifon, J.L., 2015. Short-term water stress affecting NO_3^- absorption by almond plants. Sci. Hortic. (Amsterdam). 197, 50–56. https://doi.org/10.1016/j.scienta.2015.10.040.

Mascia, F., Girolomoni, L., Alcocer, M.J.P., Bargigia, I., Perozeni, F., Cazzaniga, S., Cerullo, G., D'Andrea, C., Ballottari, M., 2017. Functional analysis of photosynthetic pigment binding complexes in the green alga Haematococcus pluvialis reveals distribution of astaxanthin in Photosystems. Sci. Rep. 7, 1–14. https://doi.org/10.1038/s41598-017-16641-6.

Moriwaki, T., Falcioni, R., Tanaka, F.A.O., Cardoso, K.A.K., Souza, L.A., Benedito, E., Nanni, M.R., Bonato, C.M., Antunes, W.C., 2019. Nitrogen-improved photosynthesis quantum yield is driven by increased thylakoid density, enhancing green light absorption. Plant Sci. 278, 1–11. https://doi.org/10.1016/j.plantsci.2018.10.012.

Mota, M., Neto, C.B., Monteiro, A.A., Oliveira, C.M., 2011. Preferential ammonium uptake during growth cycle and identification of ammonium transporter genes in young pear trees. J. Plant Nutr. 34, 798–814. https://doi.org/10.1080/01904167.2011.544350.

Neto, C., Carranca, C., Clemente, J., de Varennes, A., 2008. Nitrogen distribution, remobilization and re-cycling in young orchard of non-bearing 'Rocha' pear trees. Sci. Hortic. (Amsterdam). 118, 299–307. https://doi.org/10.1016/j.scienta.2008.06.023.

Neto, C.B., Carranca, C., Clemente, J., de Varennes, A., 2011. Assessing the nitrogen nutritional status of young non-bearing "Rocha" pear trees grown in a mediterranean region by using a chlorophyll meter. J. Plant Nutr. 34, 627–639. https://doi.org/10.1080/01904167.2011.540620.

Pasa, M.D.S., Fachinello, J.C., Schmitz, J.D., Kulkamp de Souza, A.L., de Franceschi, É, 2010. Desenvolvimento, produtividade e qualidade de peras sobre porta-enxertos de marmeleiro e Pyrus calleryana. Rev. Bras. Frutic. 34, 873–880.

Pasa, M., da, S., Fachinello, J.C., Schmitz, J.D., de Souza, A.L.K., Herter, F.G., 2011. Hábito de frutiicação e produção de pereiras sobre diferentes porta-enxertos. Pesqui. Agropecu. Bras. 46, 998–1005.

Pii, Y., Alessandrini, M., Guardini, K., Zamboni, A., Varanini, Z., 2014. Induction of high-affinity NO3- uptake in grapevine roots is an active process correlated to the expression of specific members of the NRT2 and plasma membrane H+-ATPase gene families. Funct. Plant Biol. 41, 353–365. https://doi.org/10.1071/FP13227.

Rubio-Covarrubias, Oa., Brown, P.H., Weinbaum, Sa., Johnson, R.S., Cabrera, R.I., 2009. Evaluating foliar nitrogen compounds as indicators of nitrogen status in Prunus persica trees. Sci. Hortic. (Amsterdam). 120, 27–33. https://doi.org/10.1016/j.scienta.2008.09.007.

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., 1995. Análises De Solo, Planta E Outros Materiais, 2nd ed. UFRG, Porto Alegre.

Tomasi, N., Monte, R., Varanini, Z., Cesco, S., Pinton, R., 2015. Induction of nitrate uptake in Sauvignon Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. Aust. J. Grape Wine Res. 21, 331–338. https://doi.org/10.1111/ajgw.12137.

Yang, T., Zhu, L., Wang, S., Gu, W., Huang, D., Xu, W., Jiang, A., Li, S., 2007. Nitrate uptake kinetics of grapevine under root restriction. Sci. Hortic. (Amsterdam). 111, 358–364. https://doi.org/10.1016/j.scienta.2006.11.005.