Plant Physiology and Biochemistry 115 (2017) 249–258

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Prognosis of physiological disorders in physic nut to N, P, and K deficiency during initial growth

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form
4 April 2017
Accepted 4 April 2017
Available online 6 April 2017

Keywords:
Jatropha curcas L.
Nitrate reductase
Phosphatase acid
Polyamines
Nutrient deficiency

ABSTRACT

The description of physiological disorders in physic nut plants deficient in nitrogen (N), phosphorus (P) and potassium (K) may help to predict nutritional imbalances before the appearance of visual symptoms and to guide strategies for early nutrient supply. The aim of this study was to evaluate the growth of physic nuts (*Jatropha curcas* L.) during initial development by analyzing the gas exchange parameters, nutrient uptake and use efficiency, as well as the nitrate reductase and acid phosphatase activities and polyamine content. Plants were grown in a complete nutrient solution and solutions from which N, P or K was omitted. The nitrate reductase activity, phosphatase acid activity, polyamine content and gas exchange parameters from leaves of N, P and K-deficient plants indicates earlier imbalances before the appearance of visual symptoms. Nutrient deficiencies resulted in reduced plant growth, although P- and K-deficient plants retained normal net photosynthesis (A), stomatal conductance (g_s) and instantaneous carboxylation efficiency (k) during the first evaluation periods, as modulated by the P and K use efficiencies. Increased phosphatase acid activity in P-deficient plants may also contribute to the P use efficiency and to A and gs during the first evaluations. Early physiological and biochemical evaluations of N-, P- and K-starved plants may rely on reliable, useful methods to predict early nutritional imbalances.

1. Introduction

Biodiesel is a renewable, biodegradable alternative fuel source (Aransiola et al., 2014). Physic nut (*Jatropha curcas* L.) has become a subject of interest as a raw material for biodiesel production because of its high oil production and consequent potential profitability. In addition to its economic potential, this species has shown adequate growth under adverse soil-climatic conditions. This suggests tolerance to abiotic stress, with the possibility of cultivation in areas with limited plant development (e.g., arid and semi-arid regions) and the ability to use nutrients efficiently (Silva et al., 2013; Garrone et al., 2016; Rodrigues et al., 2016). These characteristics make physic nut an excellent model to evaluate physiological responses regarding the tolerance of the species to abiotic stresses, such as low nutrient availability. Low nutrient availability reduced the yield and quality of biodiesel from physic nut (Yong et al., 2010), and thus nutritional deficiency also impairs biodiesel quality due to physiological disorders (Openshaw, 2000).

Nutrients represent approximately 1.5% of the dry weight of plants; nitrogen (N), phosphorus (P) and potassium (K) represent approximately 1.5, 0.2 and 0.9% of the total dry weight, respectively (Marschner, 2012). However, these nutrients are most commonly used in fertilization of agricultural crops. Responses to fertilization depend on the nutritional requirements of the plant and the soil physicochemical conditions. In highly weathered soils with a predominance of 1:1 clay structure and Fe- Al-sesquioxides, P availability is low due to a high degree of adsorption (Rodrigues et al., 2015), whereas K availability may be reduced due to leaching (Rosolem and Calonego, 2013). Nitrogen availability, in turn, is frequently reduced due to low soil organic matter content, low addition of nitrogen fertilizers, NO_3^- leaching, and losses caused by

Abbreviations: A, net photosynthesis; C_i, capacity for internal carbon use; DAN, days after nutrient withdrawal; E, transpiration; gs, stomatal conductance; k, instantaneous carboxylation efficiency; K, potassium; N, nitrogen; NRA, nitrate reductase activity; P, phosphorus; PAa, phosphatase acid activity; Put, putrescine; Spd, spermidine; Spm, spermine; UpE, uptake efficiency; UtE, utilization efficiency.

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http://dx.doi.org/10.1016/j.plaphy.2017.04.001
0981-9428/

immobilization and denitrification (Mariano et al., 2015). Although physic nuts may display productivity increases with N, P and K fertilization, there are reports of similar production even when these nutrients are present at low levels (Yong et al., 2010; Souza et al., 2011; Matos et al., 2014). For this reason, it is important to describe physiological alterations in physic nut and the possible strategies involved, to elucidate the tolerance of this species when grown under low N, P and K availability.

Alterations in plant growth and development caused by N, P and K deficiency directly influence the photosynthetic rate. In the primary stage of photosynthesis, K is transported to the lumen of chloroplasts and induced by light to promote load balancing in the lumen (Zhao et al., 2001). In the biochemical stage, the inorganic P concentration in the cytosol controls the transport of triose-phosphate to the cytosol (De Groot et al., 2003). The photosynthetic process is also influenced by nutrients participating in integral components of the photosynthetic apparatus (*e.g.*, N in the pyrrole ring of the chlorophyll molecule). This is in addition to the change in photosynthetic capacity as a function of the alteration of structural components caused by deficiencies in other nutrients, such as P (enzymes related to carbon fixation) and K (stomatal opening and closure) (Marschner, 2012). In this context, nutrient deficiency results in low photosynthetic efficiency. Nitrogen-deficient wheat plants display lower photosynthetic rates than well-nourished plants. Nitrogen-deficient plants have a limited ability to synthesize the available luminous energy for photosynthetic reactions, dissipating it in the form of heat (De Groot et al., 2003). Similar changes were also reported for P-deficient *Zizania latifolia* (Yan et al., 2015) and K-deficient rice plants (Weng et al., 2007).

The photosynthetic process can also be indirectly influenced by nutrient availability, resulting from effects on growth and source-sink relationships (Marschner, 2012). Plants deficient in N, P and K during the reproductive stage may accumulate carbohydrates in their leaves and roots (Marschner, 2012). Thus, the low photosynthetic efficiency of leaves—the main photoassimilate-producing plant tissue—observed in plants deficient in these nutrients is explained in part by the lower carbohydrate requirement for stronger drainage (reproductive organs) (Pieters et al., 2001).

Although photosynthetic responses have been studied under stress conditions stemming from drought and temperature (Silva et al., 2013; Khan et al., 2016; Rodrigues et al., 2016), little is known about the regulation of this physiological process in the growth of plants tolerant to nutritional deficiency, such as the physic nut. The description of physiological disorders in deficient plants may indicate the nutritional strategies of physic nuts to tolerate nutritional deficiency before the appearance of visual symptoms. Nutritional disorders can be used to prevent nutritional deficiencies before visual symptoms emerge. Moreover, the adaptation of physic nuts to low N, P and K availability is likely a result of physiological responses that ensure the metabolic availability of nutrients for an adequate photosynthetic rate. This study was conducted to evaluate the growth of physic nuts (*Jatropha curcas* L.) during initial development by determining photosynthetic parameters (leaf gas exchanges) and correlating them with the physiological availability of N, P and K as measured by chemical tests, and with N, P and K use efficiencies.

2. Materials and methods

2.1. Plant material and experimental conditions

The study was performed in a greenhouse with a galvanized structure (width, 8.0 m; length, 18.00 m; ceiling height, 4.00 m) with a ridge zenithal opening and covered with low-density polyethylene film (50 μm) that is also a light diffuser. The greenhouse was located at the University of São Paulo (USP), Piracicaba, São Paulo, Brazil (22°43′12″S and 47°38′54 "W, with 580 m of altitude). The greenhouse mean air temperature ranged between 24.7 °C (minimum) and 35.2 °C (maximum) and averaged 30.3 °C. The average air relative humidity was 65%, and the maximum photosynthetic photon flux density (sunlight) was approximately 700 μmol m^{-2} s^{-1}, with a photoperiod of 12 h. The experiment was performed for 120 days.

Physic nut (*Jatropha curcas* L.) seeds with the same size (±3.0 cm) and weight (±0.6 g) were selected, germinated in sand and irrigated with deionized water. These seeds were obtained from the germplasm bank of the Brazilian Agricultural Research Corporation (EMBRAPA). Seedlings 5 cm in height were transferred to a plastic tray (40 L) containing a diluted nutrient solution (25% of the usual concentration) (Hoagland and Arnon, 1950). After one week, seedlings of similar size (±5 cm) were transferred to individual pots (one plant per pot), where they were grown in nutrient solution at 100% concentration. The growth solutions remained under constant aeration and were monitored daily, with pH adjusted to 6.0 ± 0.5 with NaOH (1 mol L^{-1}) and HCl (1 mol L^{-1}) whenever necessary (Santos et al., 2013).

The plants were grown in four nutrient solutions: complete (control), without N, without P and without K, resulting in healthy plants (complete nutrient solution) and plants deficient in N, P or K, respectively (Table 1).

2.2. Measured physiological parameters

Nitrogen, phosphorus and potassium deficiencies were evaluated in the third and fourth recently expanded leaves, using non-destructive gas exchange measurements performed at 20, 30 and 40 days after nutrient withdrawal (DAN) and at the end of the experiment (120 DAN), when deficient plants stopped growing. Additionally, nitrate reductase activity, phosphatase acid activity and polyamine concentration were measured in the same leaves to evaluate the physiological availability of N, P and K. Evaluations were performed in the middle lobe of the third newly expanded leaves (Santos et al., 2013), and one leaf per plant was analyzed. All analyses were performed in quadruplicate (n = 4), using one plant per replicate.

Table 1
Composition of nutrient solution adapted from Hoagland and Arnon (1950) and volumes (mL L^{-1}) pipetted from the standard solution used to induce N, P and K deficiencies in physic nut (*Jatropha curcas* L.).

Standard Solution	Treatments			
	Complete	without N	without P	without K
KNO_3 (1 mol L^{-1})	6.0	–	4.0	–
$NH_4H_2PO_4$ (1 mol L^{-1})	2.0	–	–	2.0
NH_4NO_3 (1 mol L^{-1})	4.0	–	6.0	4.0
$MgSO_4.7H_2O$ (1 mol L^{-1})	2.0	2.0	2.0	2.0
$CaCl_2$ (1 mol L^{-1})	4.0	4.0	4.0	1.0
$Ca(NO_3)_2.4H_2O$ (1 mol L^{-1})	–	–	–	3.0
KCl (1 mol L^{-1})	–	4.0	2.0	–
KH_2PO_4 (1 mol L^{-1})	–	2.0	–	–
Micronutrients[a]	1.0	1.0	1.0	1.0
Fe-EDTA[b]	1.0	1.0	1.0	1.0

[a] g per 1 L: KCl (3.728) [50 μmol L^{-1}]; H_3BO_3 (1.546) [25 μmol L^{-1}]; $MnSO_4$. H_2O (0.338) [2 μmol L^{-1}]; $ZnSO_4.7H_2O$ (0.575) [2 μmol L^{-1}]; $CuSO_4.5H_2O$ (0.125) [0.5 μmol L^{-1}]; H_2MoO_4 (85% MoO_3) (0.081) [0.5 μmol L^{-1}].

[b] Dissolved 33.2 g of EDTA-2Na in 200 mL of deionized H_2O. Mixed 89 mL of NaOH (1 mol L^{-1}); Mixed 24.9 g of $FeSO_4.7$ H_2O in 200 mL of deionized H_2O, added EDTA solution [53.7 μmol L^{-1}].

2.3. Leaf gas exchange

Leaf gas exchange was measured from 09h00–12h00 using an infrared gas analyzer (LI-6400; LI-COR Inc., Lincoln, NE, USA) under a PAR of 1200 μmol m^{-2} s^{-1}, air CO_2 concentration of 380 μmol mol^{-1} and leaf temperature between 20 and 25 °C (Fukuazawa et al., 2012; Santos et al., 2013). Leaf net photosynthesis (A - μmol CO_2 m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_S - mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i - μmol mol^{-1}) were measured in the middle lobe of the third newly expanded leaves and instantaneous carboxylation efficiency ($k = A/C_i$) was calculated.

2.4. Nitrate reductase activity (NRa)

The *in vivo* nitrate reductase activity was determined using the procedure by Radin (1974). Leaf samples from the third and fourth recently expanded leaves were collected at 12h00. Freshly collected leaf tissue was stored in plastic bags, transported to the laboratory on ice and rinsed with deionized water. Next, 200 mg of fresh tissue cut into discs was transferred to assay tubes containing 5 mL of phosphate buffer solution, pH 7.4 (50 mM Na-phosphate buffer + 200 mM KNO_3). The assay tubes (wrapped in aluminum foil to protect from light) were incubated in a 37 °C water bath for 30 min. The reaction was stopped by adding 1 mL 1% sulfanilamide in 2 M HCl, followed by 1 mL 0.05% naphtylenediamine solution. The nitrite (NO_2^-) produced was measured in a spectrophotometer at 540 nm using a nitrite standard calibration curve. The enzyme activity was directly related to the amount of NO_2^-, and results were expressed in μmol NO_2^- g^{-1} h^{-1} fresh weight (FW).

2.5. Phosphatase acid activity (PAa)

In vivo phosphatase acid activity was determined according to the procedure of Raposo et al. (2004). One hundred milligrams of the third and fourth recently expanded leaves were incubated with 8 mL p-nitrophenyl phosphate (p-NNP) at 250 μmol L^{-1}, in 0.1 mol L^{-1} sodium acetate buffer at pH 4.0, and kept at 30 °C in a water bath for 30 min. Subsequently, 2 mL of 2 mol L^{-1} sodium hydroxide (NaOH) was added to 5 mL supernatant, and absorbance was read in a spectrophotometer at 490 nm using a standard phosphate calibration curve. The enzyme activity was expressed as μmoles of hydrolyzed p-nitrophenol phosphate (p-NPP) substrate per hour, per FW (μmol h^{-1} g^{-1}).

2.6. Concentration of polyamines

Polyamines (PA) were determined using thin-layer chromatography following the method described by Flores and Galston (1982). The fresh material was homogenized for 1 min in 5% (v/v) cold perchloric acid. After centrifugation for 20 min at 4 °C, dansyl chloride (95%) and saturated sodium carbonate were added to the supernatant. Proline (100 mg L^{-1}) was added to the supernatant. Proline (100 mg L^{-1}) was added after 1 h to stop the reaction at 60 °C. The solution was kept in the dark for 30 min at room temperature. Toluene was used to extract dansylated Pas, and aliquots were applied onto thin-layer chromatography plates (glass plates coated with 60G silica Gel – Merch; 20 × 20). Separation was performed in laboratory bowls containing chloroform:triethylamine (10:1). Putrescine, spermidine and spermine standards were subjected to the same process. The entire procedure was monitored under UV light (254 nm). Polyamines were quantified by comparison with standards, which were also applied to the plates, using fluorescence emission spectroscopy (excitation at 350 nm and emission at 495 nm), in a Video Documentation System, using Image Master® software version 2.0. The concentration of polyamines was expressed in nmol g^{-1}.

2.7. Dry matter yield and chemical analyses of plant tissues

After plants were harvested (120 DAT), plant height and stem diameter were measured. The plant material was identified and separated into leaves, stems and roots. Leaf area was measured using a leaf area meter (LI 3100, LI-COR, Lincoln, NE, USA). Immediately afterwards, the plant material was placed in paper bags and oven-dried at 65 °C (±0.5) for 72 h. Lastly, the material was weighed and crushed in a Wiley mill (sieved through a 1-mm mesh) to determine concentrations of N, P and K in the plant tissues. The extraction to determine N concentration was obtained via sulfuric digestion and total N was determined using the analytical micro-Kjeldahl method (Jones, 1987). The analysis of P and K was performed following perchloric-nitric acid digestion; the amount of P was determined using the ammonium metavanadate colorimetric assay, and the amount of K was determined by flame photometry (Mills and Jones, 1996). Nutrient content (mg) in plant tissues was calculated by multiplying their concentration (g kg^{-1}) by the dry matter (g) of each plant part (leaves, stem and root). Based on these results, the following variables were calculated: i) uptake efficiency (UpE, g^2 mg) = [(nutrient accumulated in total plant, mg)/(root dry weight), in g] (Swiader et al., 1994); and ii) use efficiency (UE, mg g^{-1}) = (Leaves + Stem dry weight, g)^2/(nutrient accumulated in shoot, in mg) (Siddiqi and Glass, 1981).

2.8. Statistical analysis

The experiment was conducted using a randomized block design with four replicates. Statistical analyses were performed using Statistical Analysis System (SAS) software for Windows 6.11. Data were subjected to analysis of variance (ANOVA) using the F test ($p < 0.05$). The means of treatments were compared using Tukey's test ($p < 0.05$), when more than two groups were analyzed. When two groups were compared, the treatments were compared using contrast of means based on Student's test. Graphs were made using SigmaPlot software.

3. Results

3.1. Physic nut growth under N, P and K deficiency

Physic nut plants showed growth reduction due to N, P or K omission in the nutrient solution at the end of the experiment (Fig. 1). Plants deficient in N, P or K were reduced in total dry matter by 69, 42, and 51%, respectively, compared to those grown in complete solution. The order of reduction of the measured plant parts (leaves, stem and root) was similar to that observed for total dry matter (Fig. 1A) (70, 41 and 50%, respectively). A similar result was observed for leaf area (Fig. 1B), plant height (Fig. 1C) and diameter (Fig. 1D).

Plants deficient in N, P and K showed 89, 49, and 72% decreases in leaf area, respectively, compared to plants grown in complete solution. Plants deficient in N, P and K also showed reductions of 53, 38 and 43% in height and 38, 10 and 23%, in diameter, respectively, compared to control plants. Thus, among the evaluated omissions, physic nut plants were less tolerant of total omission of N at 120 DAN than P and K omission.

Plants showed symptoms of nutritional deficiency at various times. The first manifestation occurred in plants kept in solution with N omission at 50 DAN, followed by those under omission of K at 80 DAN. No visual manifestations of P deficiency were detected in the physic nut leaves. However, plants grown in this condition (P

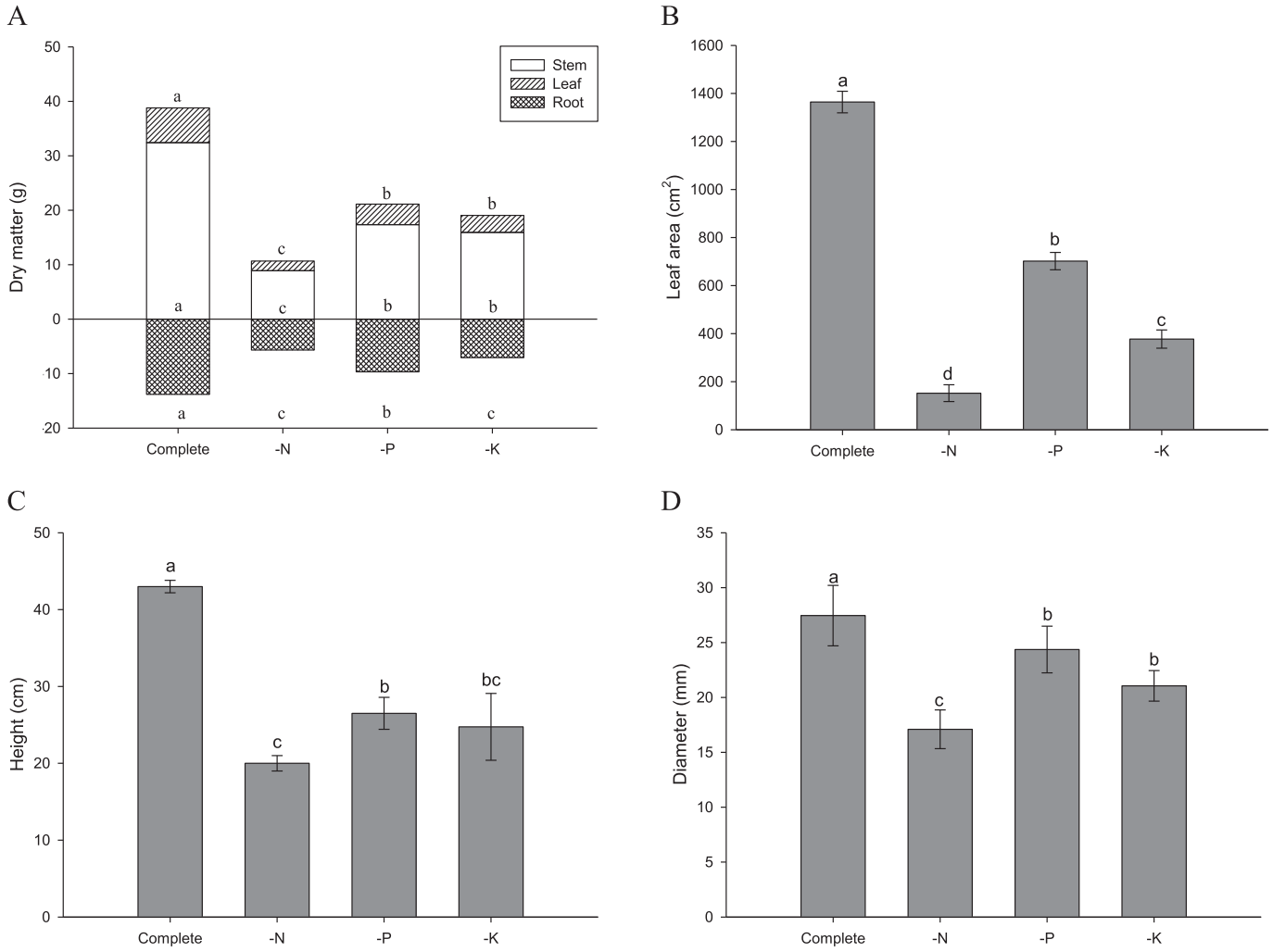

Fig. 1. Leaf, stem and root dry matter (A), leaf area (B), height (C) and diameter (D) of physic nut growth on a control nutrient solution containing all nutrients (complete) or N, P or K deficient nutrient solution (-N, -P or -K), at plant harvest (120 days after nutrient withdrawal). Different lowercase letters are significantly different by Tukey's test ($p < 0.05$). Bars errors indicate standard error (n = 4).

deficiency) showed lower growth than those grown in complete nutrient solution (Fig. 1).

3.2. N, P and K accumulation in plant tissue

Accumulation of N, P, and K in the plant tissues—leaves, stem, and root—increased as these nutrients were added to the nutrient solution (Table 2). The stem was the plant part that showed the highest N and P accumulation under both conditions (complete solution and omission of analyzed nutrient). The amount of N taken up by the stem corresponded to 54% of the total absorbed by plants grown in nutrient solutions with and without N. Phosphorus accumulation in the stem corresponded to 66% of the total absorbed by the physic nut plants grown in both the complete solution and the solution with omission of P. Unlike accumulation observed for N and P, the highest K accumulations were detected in the stem only in plants grown in complete solution. Potassium-deficient plants accumulated more K in the leaves, indicating an alteration in the source-sink relationship as a function of K deficiency. In potassium-deficient plants, the leaves presented 35% of the accumulated K total. In K well-nourished plants, leaves presented 16% of accumulated K.

Table 2
N, P and K accumulation (mg/plant) in the plant parts of physic nuts grown in nutrient solution containing all nutrients (complete) or with N, P or K omitted (-N, -P or -K), at plant harvest (120 days after nutrient withdrawal).

Treatment	Leaves	Stem	Root
N accumulation (mg/plant)			
Complete	199.61Ac	707.56Aa	367.71Ab
-N	31.99Bb	64.72Ba	23.39Bc
CV(%)	3.72	14.87	19.58
P accumulation (mg/plant)			
Complete	11.31Ac	77.33Aa	27.96Ab
-P	2.78Bc	24.58Ba	9.68Bb
CV(%)	8.07	11.07	11.95
K accumulation (mg/plant)			
Complete	23.39Ac	66.29Aa	52.19Ab
-K	3.77Ba	1.54Bb	5.42Bc
CV(%)	3.3	9.3	11.89

Different uppercase letter in columns are significantly different by Student's test ($p < 0.05$) (n = 4). Different lowercase letter in rows are significantly different by Tukey's test and ($p < 0.05$) (n = 4).

3.3. N, P and K uptake and use efficiency

Uptake efficiency was higher in plants grown in a complete

nutrient solution than those with individual omissions of N, P and K (Table 3). Therefore, the supply of each nutrient influenced their own uptake by the physic nut, and individual omission of each nutrient led to significant reductions in dry matter yield (Fig. 1).

Physic nut plants displayed the same N and P use efficiencies when grown in complete nutrient solution and in solution with individual omissions (Table 3). However, the individual omission of these elements resulted in reduced early development in physic nut plants (Fig. 1). It is also noteworthy that omission of N caused a 69% decrease in dry matter yield compared to plants grown in a complete nutrient solution. Therefore, the results observed for N use efficiency are explained by the slower development of N-deficient plants, which, in turn, required less N per unit of dry matter produced. In this way, the relationship between N accumulation and dry matter production from the shoots was similar between plants grown in a complete nutrient solution and with omission of N. Phosphorus-deficient plants showed a lower decrease in total dry matter and leaf area, among the evaluated deficiencies, compared to those grown in a complete nutrient solution. These results, along with the evaluation of leaf gas exchange (Fig. 2), suggest physiological characteristics of physic nuts that increase the tolerance of this species to P deficiency.

The results for K use efficiency differed from those observed for NUE and PUE (Table 3). The higher KUE of the K-deficient physic nut compared to healthy plants indicates the efficiency of this species in using K. In other words, physic nut plants produce proportionally more biomass per unit of absorbed K under K deficiency. However, this K use ability was not sufficient to ensure development similar to well-nourished plants (Fig. 1).

3.4. Leaf gas exchange

N, P and K deficiencies altered net photosynthesis (A), stomatal conductance (g_s), transpiration (E), and instant carboxylation efficiency (k) in the plants in the different treatments and evaluation periods (Fig. 2). Moreover, the lowest results for A, g_s E and k were found at the last time point (120 DAN). Plants deficient in N and K, as well as those grown in complete nutrient solution, displayed a reduction in photosynthetic rate from the first time point as they developed. The decrease in photosynthetic rate in N-deficient plants was already seen in the first evaluation. Potassium-deficient plants showed a lower A, g_s and k than those grown in complete nutrient solution, but only after 40 DAN. Omission of P in the growth solution, unlike other treatments, increased A, gs, E and k at 40 DAN (Fig. 2). However, at the last time point the gas exchange parameters in P-deficient plants were lower than in healthy plants.

In the first evaluation, the A of plants deficient in N, P and K was 54, 51, and 13% lower than in plants grown in complete nutrient solution, respectively (Fig. 2A). At the end of the experiment, plants deficient in N, P and K showed A decreases of 55, 61, and 63%, respectively, relative to plants cultivated in complete nutrient solution. A similar result was observed for g_s (Fig. 2B) and E (Fig. 2C). At 20 DAN, plants deficient in N, P, and K showed g_s decreases of 30, 75 and 33%, and E reductions of 16, 63, and 26%, respectively. At 120 DAN, plants deficient in N, P and K showed g_s reduced by 67, 70 and 72%, and E reduced by 50, 52 and 63%, respectively. For the k of plants grown under individual omission of N, P and K, however, the carboxylation capacity decreased by 67, 44 and 33% in relation to plants grown in complete nutrient solution. At the end of the experiment, plants showed decreases of 67, 17 and 50% in k compared to those cultivated in control nutrient solution (Fig. 2D).

3.5. Nitrate reductase activity (NRa), phosphatase acid activity (PAa) and polyamine concentration

Physic nut plants deficient in N displayed a lower NRa at all time points compared to the other treatments (Fig. 3A). In contrast, phosphorus-deficient plants, showed an increase in NRa, with a subsequent decline. At 40 DAN, NRa was 22% higher in P-deficient plants than in the complete treatment. Except for 40 and 120 DAN, plants grown in nutrient solution with omission of P showed a similar NRa to plants grown in complete nutrient solution.

Potassium-deficient plants showed differences in NRa compared to those with a complete nutrient solution, except at 120 DAN. It should be noted that at the last time point, the plants from all treatments showed a low NRa, including those grown in a complete nutrient solution. However, the N, P and K deficiencies demonstrate the decrease in the activity of this enzyme during this evaluation period.

Nitrogen- and potassium-deficient plants did not differ in terms of phosphatase acid activity compared to those grown in complete nutrient solution at all evaluation times (Fig. 3B). Phosphorus-deficient plants revealed increased activity of this enzyme compared to other treatments for all evaluation times, as observed for NRa (Fig. 3A). Phosphorus-deficient plants displayed two-fold higher PAa than those grown in a complete solution, except at the end of the experiment. At the last time point, the PAa of all plants was similar for all nutrient supply conditions.

Plants deficient in N and P did not show differences in polyamine (putrescine, spermidine or spermine) levels compared to well-nourished plants at any evaluation time (Fig. 4A). Putrescine concentrations in K-deficient plants were higher than in plants grown under adequate K supply at all evaluation times. The appearance of necrotic spots in the leaves of K-deficient plants at 80 DAN is a result of putrescine accumulation at a toxic level, as quantified in the present study.

Spermidine (Fig. 4B) and spermine (Fig. 4C) concentrations were lower in K-deficient plants at all evaluation times, unlike putrescine. Spermidine concentration rose as a function of the development of physic nuts, with leaf concentrations increasing from 5 to 61 nmol g^{-1} in plants without K deficiency. The opposite response was observed for spermine content, in which leaf concentrations decreased from 40 DAN, from 54 nmol g^{-1} at 20 DAN and to 19 nmol g^{-1} at 120 DAN, in K-nourished plants. It can be inferred that spermidine had a higher correlation with the development of physic nut plants at the end of the growth cycle in the present study; the opposite trend was observed for spermine. Potassium-deficient plants were also the only ones to present a high putrescine/spermidine + spermine ratio (Fig. 4D). The lowest results for NRa, PAa, and polyamine concentration were found at 120 DAN.

Table 3
– Uptake efficiency (UpE) and use efficiency (UE) of N, P and K of physic nuts grown in nutrient solution containing all nutrients (complete) and those from which N, P or K was omitted (-N, -P or -K), at plant harvest (120 days after nutrient withdrawal).

Treatment	UpE	UE
	g^2 mg	mg g^{-1}
Complete	92.4A	3.0A
-N	21.0B	2.8A
CV(%)	8.0	15.0
Complete	8.5A	15.82A
-P	4.0B	16.25A
CV(%)	2.50	14.5
Complete	10.2A	89.3B
-K	1.0B	111.4A
CV(%)	5.41	11.5

Different letter in columns are significantly different by Student's test ($p < 0.05$) (n = 4).

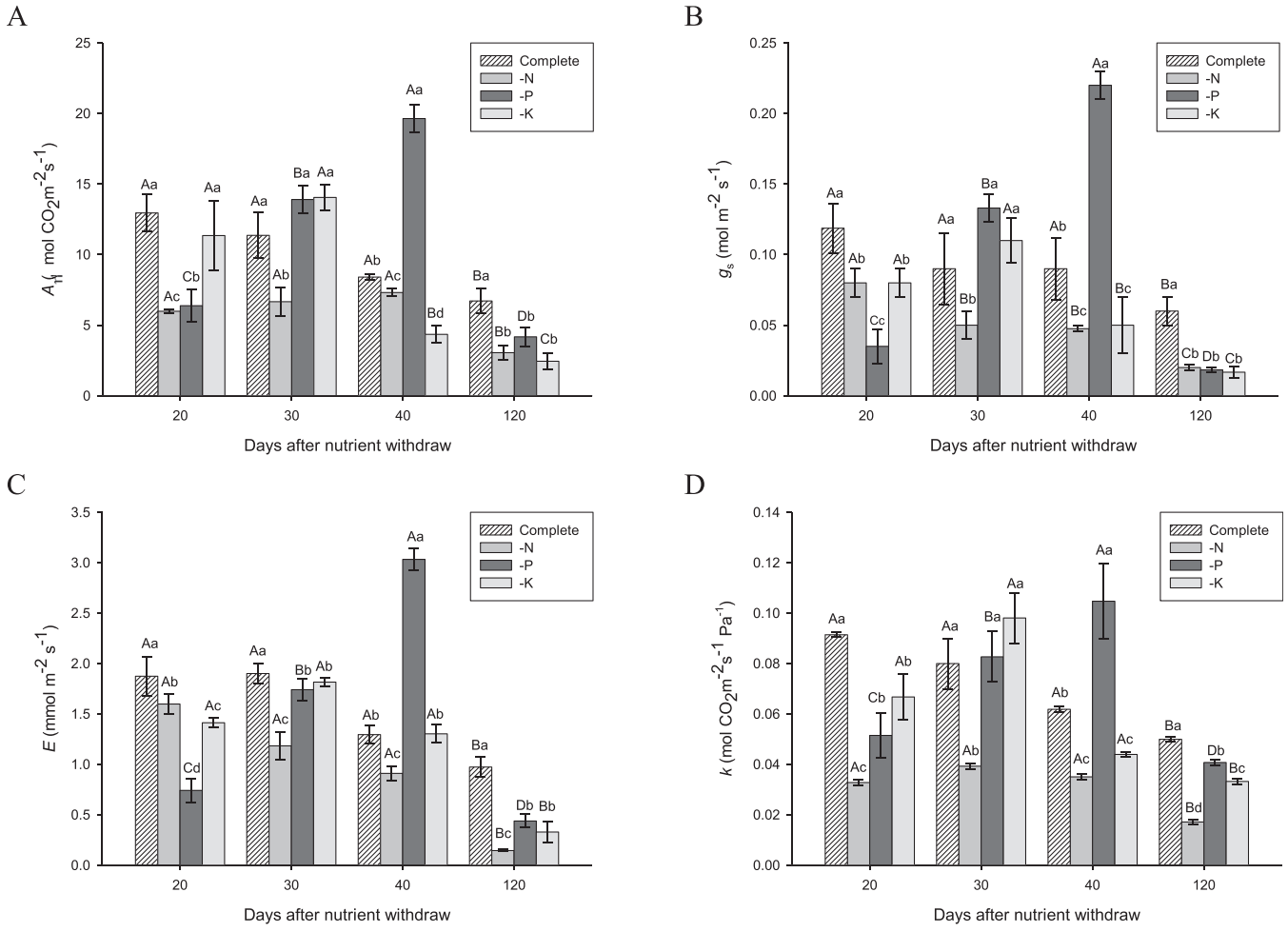

Fig. 2. Net photosynthesis (*A*) (A), transpiration (*E*) (B), stomatal conductance (g_S) (C) and instantaneous carboxylation efficiency ($k = A/Ci$) (D) of physic nut growth on a control nutrient solution containing all nutrients (complete) or N, P or K deficient nutrient solution (-N, -P or -K), according to evaluation time course. Different lowercase letters are significantly different by Tukey's test ($p < 0.05$), comparing the treatments with the same evaluation time. Different uppercase letter are significantly different by Tukey's test ($p < 0.05$), comparing the time evaluation to each related treatment (complete, -N, -P or -K). Bars indicate standard error (n = 4).

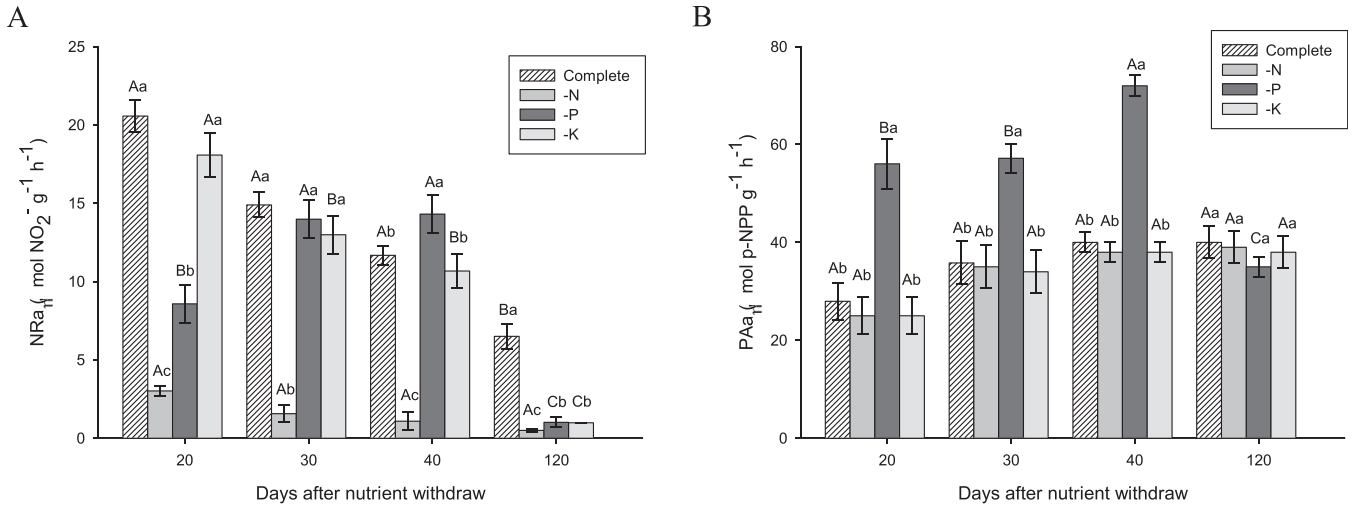

Fig. 3. Nitrate reductase activity (NRa) (A) and phosphate acid activity (PAa) (B) of physic nut growth on a control nutrient solution containing all nutrients (complete) or a deficient N, P or K nutrient solution (-N, -P or -K), according to the evaluation time course. Different lowercase letters are significantly different by Tukey's test ($p < 0.05$), comparing the treatments with the same evaluation time. Different uppercase letters are significantly different by Tukey's test ($p < 0.05$), comparing the evaluation time for each related treatment (complete, -N, -P or -K). Bars indicate standard error (n = 4).

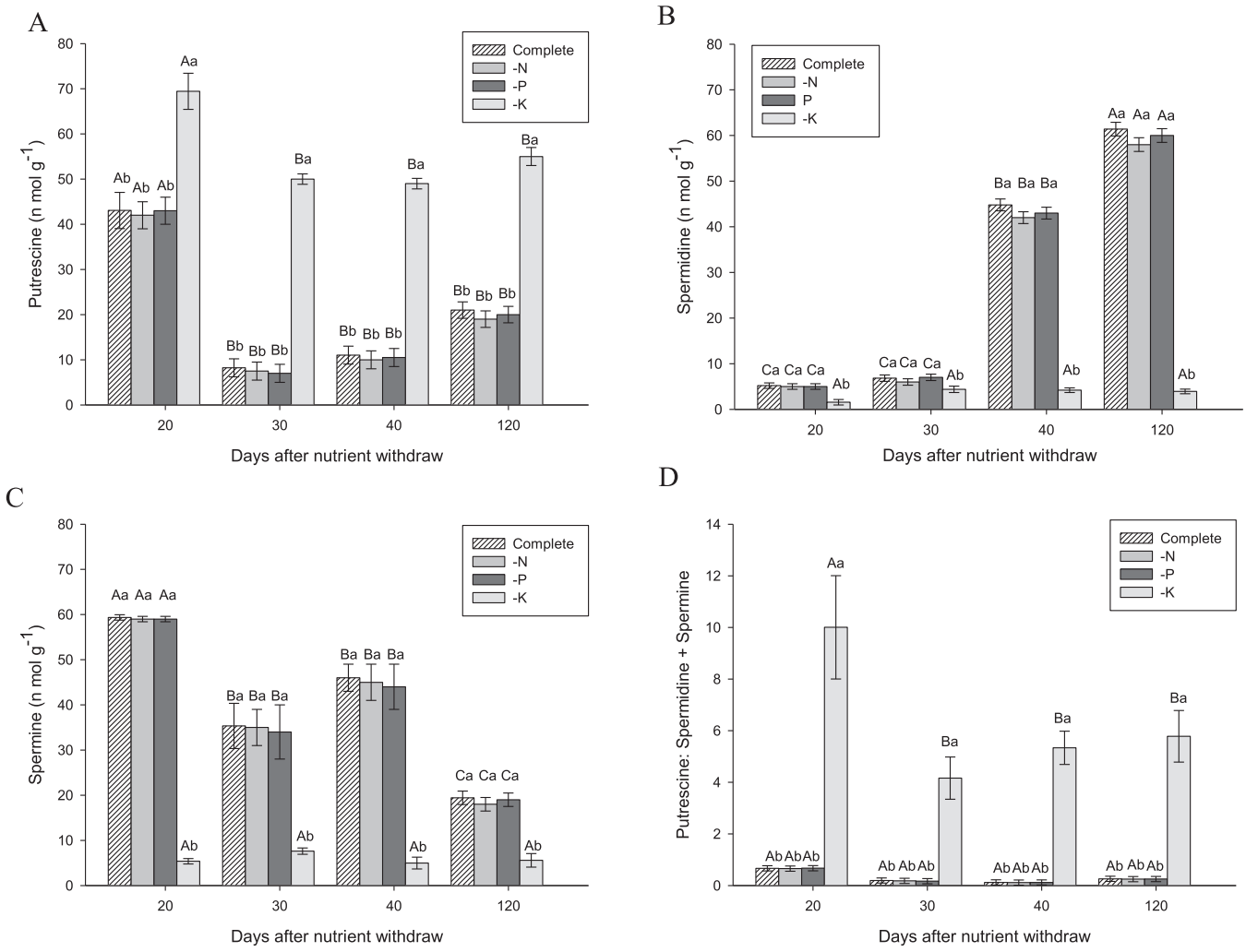

Fig. 4. Concentration of putrescine (A), spermidine (B), spermine (C) and ratio of putrescine: (spermidine + spermine) (D) of physic nut growth in a control nutrient solution containing all nutrients (complete) or solutions deficient in N, P or K nutrient (-N, -P or -K), according to the evaluation time course. Different lowercase letters are significantly different by Tukey's test ($p < 0.05$), comparing treatments at the same time. Different uppercase letters are significantly different by Tukey's test ($p < 0.05$), comparing the time evaluation to each related treatment (complete, -N, -P or -K). Bars indicate standard error (n = 4).

4. Discussion

Results of the present study show that physic nut has an efficient mechanism of adaptation to P and K deficiency conditions, maintaining and adequate photosynthetic rate as the physiological use of these nutrients increased at the first evaluation times. Furthermore, N, P, and K deficiencies affected plant growth differently as a function of growing time. All deficiencies resulted in reduced growth, and consequently lower biomass production.

The higher uptake efficiency of plants grown in complete nutrient solution compared to those with individual omission of N, P, and K indicated that the tolerance of the species to deficiencies of has no relationship to the development of plant characteristics that elevate the absorption capacity, such as alterations in root architecture. Santos et al. (2015) reported that species and/or genotypes with higher nutrient uptake efficiency have a greater ability to obtain the element under conditions of low availability. In the present study, we only found differences in nutrient use efficiency (Table 3).

Nitrogen-deficient physic nut plants showed the lowest growth (Fig. 1), and the lowest photosynthetic rate was detected in the first evaluation (20 DAN) (Fig. 2). Garrone et al. (2016) also noted a high correlation between the initial development of physic nuts and the availability of this nutrient. Nitrogen-deficient plants have a reduced photosynthetic rate, mainly due to their lower ability to use luminous energy and compromised amino acid metabolism (De Groot et al., 2003). Lower NRa—a key enzyme in the metabolic pathway of N—in N-deficient plants, however, indicated a lower physiological availability of N in leaves for photosynthesis. Reduced NRa in the leaves is associated with lower availability of N to leaves in cases of N deficiency in various plant species (Reis et al., 2009; Lavres Junior et al., 2010; Fiasconaro et al., 2013).

In the present study, physic nut plants showed tolerance to N deficiency at the beginning of the experiment. This is because, even with their N metabolism compromised, these plants displayed visual symptoms of N deficiency only at 50 DAN, namely aggravation of N deficiency. At 20 DAN, N-deficient plants had similar development to those grown in complete nutrient solution. Interestingly, N-deficient plants in the first physiological evaluation did not show symptoms of N deficiency, but had low NRa *A*, *E*, g_S and *k*, which were similar to those found in the last evaluation. Some species that were tolerant to N deficiency increased the redistribution of the nutrient to the leaves to ensure adequate photosynthesis (Makino, 2011). This was not observed for physic nuts (Fig. 3A).

Physic nut plants showed symptoms of N deficiency only at 50 DAN, according to the regulation of dry matter accumulation, i.e., lower use of units of available N for lower dry matter production. The similar NUE in plants with and without N deficiency confirms this hypothesis (Table 3). Fageria et al. (2013) reported that some rice genotypes also reduced their growth to optimize nitrogen use. Rajaona et al. (2013) evaluated gas-exchange characteristics in physic nuts as a function of N supply and reported efficient use of N by the species for net photosynthesis. The results observed in the current study for N-deficient physic nut plants indicate that the adaptation of the species to soils with low N is mainly a result of adjustments in development to the N supply. Thus, the productivity of physic nut plants in soils with low N content may be compromised without the appearance of visual symptoms of N, the so-called "hidden hunger." Souza et al. (2011) reported reduced productivity of physic nut biomass in response to low N supply in the initial vegetative stage of growth, although it was not observed as symptoms of N-deficiency.

Plants deficient in P showed the lowest decreases in photosynthetic rate (Fig. 2), resulting in smaller growth reduction compared to those grown in complete nutrient solution (Fig. 1). These findings indicate tolerance of physic nuts to P deficiency. Notably, the remobilized quantities of P from seeds to seedlings, as well as the adaptation period of plants in diluted solutions (prior to the beginning of treatments), might have partially contributed to this result. However, this is a characteristic of high P use efficiency, which was not observed in N-deficient plants in this study. The results observed for PUE by physic nut plants deficient in P are explained by the positive association between physiological P use ability and high phosphatase acid activity (PAa) (Zambrosi et al., 2015). It should be stressed that only plants grown in nutrient solutions without P did not display visual symptoms in the leaves; only a reduction in growth was observed. Therefore, physic nut plants produced the same amount of dry matter per unit of absorbed P, irrespective of the availability of this element in the growth solution (Table 3).

In the present study, P-deficient plants were grown with total omission of a nutrient from the solution; thus, they used the P assimilated in the organic compounds, i.e., from the carbon skeletons. The high PAa in P-deficient plants confirms the efficient P use in this growth condition. Thus, PAa served as an adaptive tool against P deficiency stress in these plants, explaining the PUE rates found in the present study (Table 3). High phosphatase acid enzymatic activity has been observed as an adaptive response to low P availability in many species (Zebrowska et al., 2011; Zambrosi et al., 2015). Under low P availability, plants use phosphatase acid to dephosphorylate organic compounds, providing inorganic P for the maintenance of cell metabolism, especially photosynthesis (Tran et al., 2010). Phosphorus deficiency in physic nut plants has been reported to interfere little with the development of this species (Souza et al., 2011), indicating efficient P use during deficiency of this element. This result is explained mainly by the participation of phosphatase acid in physic nut plants during early development, as found in the present study (Fig. 2B).

Responses observed for A, E, g_S and k (Fig. 2), as well as NRa (Fig. 3A), in P-deficient plants were similar to that observed for PAa (Fig. 3B) in the same plants throughout the experiment. It can be inferred that the high organic P mobilization (redistribution) capacity of physic nuts enabled a higher photosynthetic rate and assimilation of N observed when P was omitted, resulting in high tolerance to P deficiency. Under P deficiency, the guarantee of available P ensures adequate photosynthetic (Yan et al., 2015; Zambrosi et al., 2015), while a low concentration of physiological P inhibits plant growth due to the decreased RuBisCo carboxylation and regeneration ability, in addition to reduced cell-division (De Groot et al., 2003). In this study, decreased RuBisCo carboxylation (k) was observed, as well as lower g and E at 120 DAN. The high PAa ensured adequate photosynthesis in physic nut plants at the first evaluation times except in the last evaluation (Fig. 2).

Potassium-deficient plants showed the same KUE compared to those cultivated in complete nutrient solution (Table 3). This finding indicates that physic nut plants deficient in K efficiently produced a certain amount of dry matter per unit of absorbed K. The higher KUE of K-deficient plants was mainly due to the greater accumulation of K in leaves than stems and roots (Table 2). Plants that were not deficient in K displayed a greater accumulation of this nutrient in the stems compared with the other plant parts. The physic nut is an arboreal species, and most nutrient absorption occurs in the stem, to ensure the establishment of the species (Santos et al., 2013). Accumulation of N and P was also greater in the stem in plants that were deficient and non-deficient in these nutrients. The change in the source-sink relationship in deficient plants represented an adaptive strategy for K deficiency (Zhao et al., 2001). Potassium accumulation in the leaves ensured an adequate photosynthetic rate, similarly to the photosynthetic rate of plants grown in complete nutrient solution. Overall, in addition to being used mainly for the stomatal opening and closure processes, the K used in the leaves also ensured adequate NRa, since the activity of this enzyme was similar between K-deficient and non-deficient plants, except at the end of the experiment (Fig. 3A).

Potassium participates in the activation of nitrate reductase synthesis. The activity of this enzyme was low under K deficiency conditions in different species, indicating the importance of this element in the enzymatic activation of the process (Lavres Junior et al., 2010; Víllora et al., 2003). Similar NRa activities were observed in K-deficient and non-deficient plants in the present study (Fig. 3A), in addition to the greater accumulation of this nutrient in leaves in relation to stems and roots (Table 2). This can be explained by the efficient use of the nutrient contained in the plant reserves, originating from the adaptation solution and the reserves from the seed itself. Thus, the low level of K contained in the plant was sufficient for the efficient use of the enzyme in the early development of physic nut plants, in addition to its use in photosynthetic. However, K-deficient plants showed reduced growth (Fig. 1).

The decreased growth of K-deficient plants resulted from the reduction in spermidine and spermine concentrations (Fig. 4). The K that was physiologically available in the leaf was used to ensure N metabolism and the photosynthetic process. In this way, plants deficient in K displayed a high concentration of putrescine and low levels of spermidine and spermine. Potassium is a regulator (inhibitor) of enzyme activity that converts putrescine into spermidine and spermine (Fariduddin et al., 2013), which explains the results observed here.

Potassium deficiency changes the internal balance between inorganic cations and anions; cell juice acidity is thus expected to increase, but this was not observed (Pottosin and Shabala, 2014). Putrescine accumulation functions as a mechanism to maintain pH at a physiologically adequate value. However, at high concentrations, as observed in this study, putrescine has a toxic effect on plant tissue, in addition to generating energy expenditure for its degradation (Merlin et al., 2012). Thus, the high accumulation of putrescine in early development was an adaptive strategy, minimizing the effect of K deficiency on cell juice acidity and ensuring available K for adequate photosynthesis.

Plant growth and the higher K requirement in plant metabolism compromises the development of physic nut plants, culminating in visual symptoms. In addition to the negative effect of putrescine on the development of K-deficient plants, the lower concentrations of spermidine and spermine—plant growth-regulating

polyamines—also explain the reduced growth of K-deficient physic nut plants. The putrescine/spermidine + spermine ratio is usually correlated with cell elongation, and the transformation of putrescine into spermidine/spermine is important in the control of cell division (Merlin et al., 2012). Therefore, a high ratio may indicate lower plant growth (high concentration of putrescine and low spermidine + spermine). In the present study, this ratio increased in plants deficient in K, which led to reduced growth in physic nut plants.

Reduced accumulation of putrescine with plant growth has been reported for other species. This polyamine is a precursor of the ethylene hormone, which in turn is responsible for the leaf senescence characteristic of this species (Fariduddin et al., 2013), as observed at 120 DAN in physic nut plants. Decreases in nitrate reductase and phosphatase acid activities were also observed (Fig. 3).

The lower NRa observed in the last evaluation was a result of the allocation of nutrients and photoassimilates for the development of new tissues. This characteristic has already been reported for physic nut plants (Lima et al., 2015). According to Matos et al. (2012), with the development of physic nut plants, a large fraction of nitrate is reduced and allocated to the production of photosynthetic pigments, especially chlorophylls *a* and *b*. Thus, the large amount of nitrate already used by the plant metabolism contributes to greater allocation of nitrate only for the points of growth (leaf under expansion is a strong drain), explaining the low nitrate reductase activity in leaves at 120 DAN. Similar to nitrate, organic P was also allocated to the development of young tissues, explaining the similar activity of P-deficient and non-deficient plants at 120 DAN.

A, *E*, g_S and *k* decreased throughout the experiment in all treatments. The decreased photosynthetic rate in the physic nut development cycle can be explained by leaf senescence, which is typical of this species (Yong et al., 2010; Matos et al., 2012; Lima et al., 2015). Rajaona et al. (2013) reported that the development of physic nut plants reduces the photosynthetic capacity of leaves of this arboreal species, and this decline is a typical occurrence. Leaf senescence causes a reduction in Calvin cycle reactions, mainly due to the degradation of Ribulose 1,5-bisphosphate carboxylase (RuBisCo). In this process, no components of the photosynthetic apparatus, such as chlorophyll or chloroplasts, formed, leading to a low ability to convert luminous energy into organic compounds (Lu et al., 2002). However, it must be stressed that, in the complete treatments, plants displayed the lowest reduction in photosynthetic capacity at 120 DAN, demonstrating the negative effect of macronutrient deficiencies in physic nuts.

The evaluation of gas exchange, NRa, PAa and polyamines allowed us to predict the N, P, and K deficiencies in physic nut plants before the symptoms of deficiencies of these nutrients were visible. Gas exchange and NRa were effective parameters to predict N deficiency in physic nut plants. At the first evaluation time, NRa and gas exchange were lower in N-deficient plants than in control plants (Figs. 2 and 3A). Symptoms of N deficiency only occurred at 50 DAN, indicating that longer periods that can be used to predict N deficiency in the early development of physic nut plants. For P deficiency, however, only the PAa evaluated at 30 and 40 DAN (Fig. 3B) and gas exchange at 20 DAN (Fig. 2) efficiently predicted P deficiency in this plant species. Polyamines at all evaluation times, *E*, G_S and *k* efficiently predicted K deficiency in the initial development of physic nut.

5. Conclusion

Physic nut plants showed tolerance to N deficiency in their early development cycle, due to regulation of their growth rate. Their strong ability to dephosphorylate organic P to provide P for photosynthesis is the main characteristic of physic nut plants in their adaptation to limiting P conditions during growth. The higher K use efficiency by K-deficient *J. curcas* plants compared to those well-nourished in K suggests high tolerance of the species to deficiency of this nutrient during its initial establishment and development. In conclusion, early biochemical and physiological appraisals can be feasible, reliable techniques to predict nutrient imbalance before it occurs through visual symptoms.

Author contribution

All authors contributed in the same way to the preparation of all the parts of this manuscript.

Acknowledgments

This work was supported by FAPESP (São Paulo Research Foundation; 2011/15208-0).

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