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

Elcio Ferreira Santos [a], Fernando Giovannetti Macedo [a], Bruno José Zanchim [a], Giuseppina Pace Pereira Lima [b], José Lavres [a, *]

[a] USP – University of São Paulo, Center for Nuclear Energy in Agriculture, 13416-000 Piracicaba, SP, Brazil
[b] UNESP – São Paulo State University, 18600-000 Botucatu, SP, Brazil

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.

* Corresponding author.
E-mail addresses: elciokw@yahoo.com.br (E.F. Santos), giovannetti_agro@yahoo.com.br (F.G. Macedo), bruno.zanchim@yahoo.com.br (B.J. Zanchim), gpplima@ibb.unesp.br (G.P.P. Lima), jlavres@usp.br (J. Lavres).

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}].