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Simultaneous selection of physic nut genotypes (*Jatropha curcas* L.) for efficient absorption and utilization of N and P

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Abstract

The aim of this study was to select genotypes of physic nut for the efficiencies of absorption and utilization of nitrogen and phosphorus in contrasting environments with high and low availability of N and P in soil. The experiment was arranged in a factorial scheme 10×2, with four replications. The factors were 10 genotypes of physic nut and two levels of fertilization with nitrogen (0% and 100% of the recommended does for cultivation in greenhouse) and phosphorus (0% and 100% of the recommended does for cultivation in greenhouse). The plants were grown for 100 days in pots without restrictions and their dry matter and contents of N and P were evaluated (total, roots and aerial part, separately). Subsequently, the efficiency ratios for absorption and utilization for each nutrient were calculated. The results showed a significant variation between group of genotypes regarding their efficiency for absorption and utilization of N and P in the different scenarios of availability of nutrients in soil. In total, the minimum supply of N promoted higher efficiency of absorption in the genotypes. The genotypes CNPAE-167-II and CNPAE-180-I presented higher efficiency of absorption and utilization of N, independent of environment, while the genotypes CNPAE 110-II and CNPAE 275-I showed reduced efficiency of absorption and utilization of N. For phosphorus (P), the efficiency of absorption was the major limitation in the simultaneous study of genotypes for both nutritional efficiencies. Also, it was strongly affected by the limitation of available P in soil. The genotype CNPAE 167-II was highlighted for presenting simultaneously higher absorption efficiency of N and P and higher efficiency of utilization of P in both contrasting environments.

Keywords: physic nut, mineral nutrition, fertilization, crop breeding.
Abbreviations: EAN_efficiency of absorption of nitrogen, EUN_efficiency of utilization of nitrogen, EAP_efficiency of absorption of phosphorus, EUP_efficiency of utilization of phosphorus.

Introduction

The research for alternative energy sources, aiming to reduce the depletion of non-renewable resources, has been intensifying the search for renewable energy matrices, especially the ones based on the production of vegetable oils to manufacture biofuels. The physic nut (*Jatropha curcas* L.) is an interesting and viable option, presenting desirable agricultural traits associated with the high oil content (Martins et al., 2010, 2013a, Amaral et al., 2012; Nunes et al., 2013; Bhering et al., 2013).

Several plant species are able to develop mechanisms to adapt to conditions and stress, which may be due to characteristics and changes inherent of the genetic material of the species. In the mineral nutrition of plants, the genetic variability within a species allows the selection of genotypes with characteristics that predispose greater nutritional efficiency, which can represent a major factor in the selection process for breeding programs (Miranda et al., 2008; Souza et al., 2008; Vale et al., 2013; Martins et al., 2013a; Kumar et al., 2014). Nutritional studies can be done at early stages with disposal of unpromising materials and selecting genotypes at juvenile stage using a set of correlated traits. Studies on variability of physic nut genotypes are very important for breeders. It is known that Brazilian genotypes of physic nut have high genetic variability. The knowledge on Brazilian genotypes of physic nut is still insufficient (Laviola et al., 2010, 2011; Martins et al., 2013a).

The nutritional efficiency is related to the rate in which the plant can acquire a nutrient through absorption per unit of length or mass of root and its ability to use and convert the absorbed nutrients in dry matter (Baligar and Fageria, 1998). Therefore, it is important to identify genotypes that have different potentials to convert nutrients into biomass, which gives them distinct and desirable nutritional efficiency (Vose, 1987). Studies suggest a genetic control of many variables in the physiological processes that result in the nutritional efficiency, such as absorption of a given element, translocation of minerals in the plant tissues and plant uptake (Sacramento and Roselem, 1998).

For physic nut, nitrogen (N) is the nutrient required in greatest quantity (Laviola and Dias, 2008). It is essential for the photosynthetic process and in the formation of new plant organs, acting in the composition of amino acids, proteins, enzymes, RNA, DNA, ATP and other molecules (Marschner, 2012; Taiz and Zeiger, 2013). Nitrogen participates in formation and development of flower buds and fruits. It also promotes vegetative growth and production of proteins (Malavolta, 2006). Around 50 to 70% of total N in leaves get involved with different enzymes (Chapman and Barreto, 1997) that are associated with chloroplasts (Stoking and Ongun, 1962), corresponding to chlorophyll molecules.

Phosphorus (P) is also one of the nutrients that is required in larger quantities by physic nut plants in early stages (Laviola and Dias, 2008), participating in composition of membranes (phospholipids), RNA, DNA, ATP and esters of carbohydrates. Beside, P contributes in several key molecules in plant metabolism. This nutrient also accelerates the formation of roots and fruit maturation, increasing the levels of carbohydrates, oils, lipids and proteins (Malavolta, 2006). The deficiency of phosphorus causes, in most plants, accumulation of starch in the chloroplasts, reduction of transport of carbohydrates and decrease the activity of all enzymes that are dependent of phosphorylation, particularly those involved in the active processes of absorption of nutrients (Marschner, 2012).

Given the importance of N and P in the metabolism and plant development, as well as the importance of nutritional efficiency of genotypes, it is essential to study the correlation between the efficiencies of absorption and utilization and the biomass production. This will lead us to select genotypes that are more efficient in both parameters. It is also noteworthy that a higher nutritional efficiency for N and P can be achieved by increasing the efficiency, in which the nutrients are acquired and used in the plant metabolism (Chen et al., 2009; Presterl et al., 2002).

Therefore, this study was conducted to select the genotypes of physic nut with higher efficiency for absorption and utilization of nitrogen and phosphorus in contrasting environments with different availability of nutrients.

Results

Analysis of variance

There were significant interactions for different parameters of nutritional efficiencies for both N and P between genotypes of physic nut in both scenarios of nutrient supply in soil (Table 1). This fact shows that the rates of absorption and utilization of N and P are influenced by the genetic material as well as by the supply of N and P in the soil, which may allow separation of genotypes between distinct groups for efficiencies in each environment.

Nutritional efficiency for N of genotypes in contrasting environment

Fig 1. shows the distribution of the genotypes of physic nut as function of the efficiencies of absorption (EAN) and utilization (EUN) of nitrogen in environment with low supply of this nutrient (control level), allowing their separation into four groups. The Group 1, consisting of genotypes with higher efficiencies for both absorption and utilization of N (CNPAE 167-II, CNPAE 180-I and CNPAE 192-I); Group 2, with lower efficiency of absorption and higher efficiency of utilization of this nutrient (CNPAE 127-I, CNPAE 161-II, CNPAE 191-I and CNPAE 210-II); Group 3, with higher efficiency of absorption and reduced efficiency of utilization of this nutrient in the production of dry matter (CNPAE 255-I); and Group 4, with low efficiencies for both parameters (CNPAE 110-II and CNPAE 275-I). In Fig 2, the distribution of the genotypes of physic nut showed the differentiation of the same genotypes in an environment with adequate fertilization for nitrogen (100% N). The genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 191-I and CNPAE 255-I showed higher efficiencies for absorption and utilization of N (Group 1), the genotype CNPAE 210-II was allocated in Group 2, characterized for reduced efficiency of absorption but higher utilization of this nutrient, the genotype CNPAE 192-I presented inverse behavior (Group 3), and the remaining genotypes (CNPAE 110-II, CNPAE 127-I and CNPAE 275-I) presented lower efficiencies (Group 4).

Nutritional efficiency for P in contrasting environment

Fig 3. shows the distribution of the genotypes of physic nut regarding with efficiencies for phosphorus in environment with low supply. The results showed that the genotype Paraíso has higher efficiencies for P (Group 1), the genotypes CNPAE 200-I, CNPAE 210-II, CNPAE 315-I, CNPAE G2-I and Jales were placed in Group 2, characterized by lower efficiency of absorption and higher efficiency of utilization of phosphorus; the genotypes CNPAE 150-II, CNPAE 167-II; CNPAE 210-II and CNPAE 8001-I formed the Group 3, with higher efficiency to absorb and lower to utilize P to produce dry matter.

Fig 4. shows the diagram for distribution of genotypes in an adequate supply of phosphorus in soil (100% P), where they formed groups as: Group 1, with the genotypes CNPAE G2 and CNPAE 8001-I; Group 2, with the genotypes CNPAE 200-I; CNPAE- 315-I, Jales and Paraíso; Group 3, with the genotypes CNPAE 167-II, CNPAE 210-II and CNPAE G2-I; and Group 4, formed by the genotype CNPAE 1501-I only.

Discussion

Contrasting environments for N

The Fig 1. shows that the genotypes CNPAE 167-II, CNPAE 180-I and CNPAE 192-I (Group 1) have higher rates of efficiency (EAN and EUN) in this environment; therefore, these genotypes have potential in breeding programs in regions with marginal levels of N in the soil or rustic systems of cultivation. Currently, there is a need to identify appropriate genetic materials that meet the specifications

Table 1. Mean squares, coefficients of variation (CV) and overall means for efficiency of absorption of nitrogen and phosphorus (EAP and EAN, respectively) and for efficiency of utilization of nitrogen and phosphorus (EUN and EUP, respectively) for genotypes of physic nut grown in environments with discriminating levels of N and P in the soil[1].

Variation source	Degrees of freedom	EAN	EUN	EAP	EUP
Genotypes (G)	9	18595.77*	140608.45*	2.31*	172.01*
Fertilization (A)	1	662764.02*	1683125.79*	63.01*	556.51*
Interaction G*A	9	3842.77*	22247.87*	1.09*	151.34*
Residue	61	19.78	26.27	0.20	5.51
CV (%)		1.50	0.56	8.23	5.89
Overall mean		296.42	923.40	5.48	39.86

*Significant at 5% probability by F test. [1]Analyzes of variance were performed for each assay.

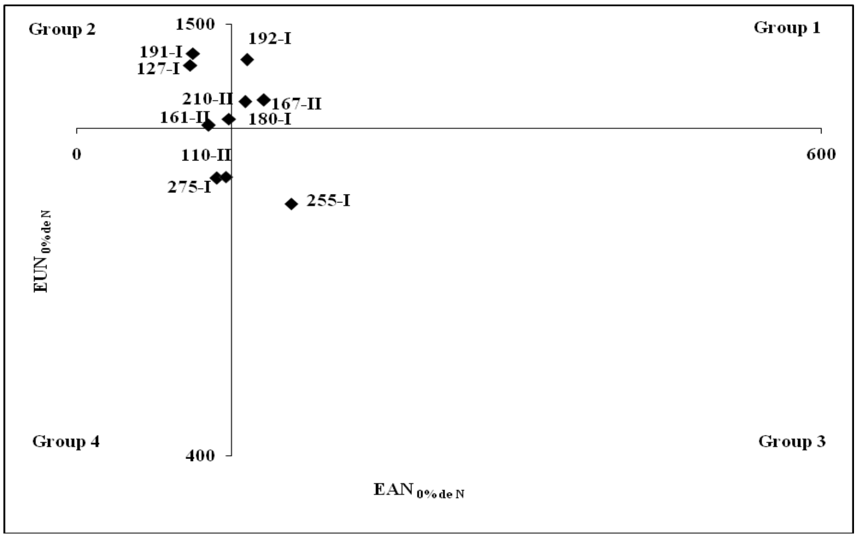

Fig 1. Distribution of genotypes of physic nut as function of the efficiencies of absorption (EAN) and utilization (EUN) of nitrogen in environment with low supply of this nutrient (control level – 0% N).

for low-income farmers, within the scope of family farming and also enabling its cultivation on marginal soils, leaving fertile soils for the sustainable agriculture.

In the environment with adequate supply of soil N (Fig 2), the genotypes CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 191-I and CNPAE 255-I presented higher efficiencies of absorption and utilization of N (Group 1). Therefore, those genotypes are indicated for selections aiming to standardize cultivars for intense cultivation and large scale productions, in systems with adequate fertilization supply or in soils with high natural fertility.

Comparing the environments, with and without nitrogen supply, the genotypes CNPAE 167-II and CNPAE 180-I were classified in Group 1 for both conditions (Fig 1 and 2), maintaining higher EAN and EUN. This fact indicates a broader capacity to adapt to environment conditions, regarding the limitation of nitrogen availability in soil, and also a capacity to respond to the fertilization with N, which supports their classification as efficient and responsive to nitrogen fertilization. This has been described in others studies (Christo et al., 2014). This finding indicate that those genotypes may be a promising option for breeding programs that can explore this desirable trait in their germplasm collections.

The fertilization with nitrogen caused the genotypes CNPAE 161-II and CNPAE 191-I to be reallocated from Group 2 (Fig 1) to Group 1 (Fig 2), indicating that a reduced supply of N enables a maximization of their EAN. In the same context, the genotype CNPAE I-255 was reallocated from Group 3 (Fig 1) to Group 1 (Fig 2), showing that his genotype has limitations for use in environments with low nitrogen levels. However, the nitrogen fertilization maximizes the efficiency of utilization of this nutrient. Various studies have reported that the efficiency of absorption and utilization of N is controlled by genotypic factors (Lee et al., 2005; Miranda et al., 2008; Souza et al., 2008; Vale et al., 2013). In plants of physic nut, there is high genetic variability among genotypes (Laviola et al., 2010), which induces a wide phenotypic variability (Laviola et al., 2011) and makes it possible to identify different responses causes by a similar nutritional stress (Martins et al., 2010, 2013a). These are in accordance with the results of this study.

In both scenarios (Fig 1 and 2), the genotypes CNPAE 110-II and CNPAE 275-I showed reduced efficiencies of absorption and utilization of N (Group 4). Aiming at the sustainability and advanced production of physic nut, cultivation of genotypes with low nutritional efficiency for N is not recommended. However, other agronomic traits may be explored from those genotypes in breeding programs.

Contrasting environment for P

The scenario with low P supply (Fig 3) revealed that the efficiency of absorption of P by genotypes was severely affected by the limitation of this nutrient in the soil as a larger number of genotypes were allocated in Group 2 such as

Table 2. Physical and chemical attributes of the soil (depth of 10 to 40 cm) used in the study.

Attributes	Values
Sand (g kg^{-1})[1]	553.00
Silt (g kg^{-1}) [1]	43.60
Clay (g kg^{-1}) [1]	403.40
Soil density (kg dm^{-3}) [2]	1.21
pH[3]	6.00
P (mg dm^{-3}) [4]	3.00
K (mg dm^{-3}) [5]	59.00
Ca ($cmol_c$ dm^{-3}) [6]	1.40
Mg ($cmol_c$ dm^{-3}) [6]	1.00
Al ($cmol_c$ dm^{-3}) [6]	0.00
H+Al ($cmol_c$ dm^{-3}) [6]	1.70
Sum of bases ($cmol_c$ dm^{-3})	2.51
CTC potential ($cmol_c$ dm^{-3})	4.18
CTC effective ($cmol_c$ dm^{-3})	2.51
Base saturation (%)	60.10

[1.] Pipette method (slow agitation); [2.] Beaker method; [3.] pH in water (1:2.5 ratio); [4.] Extracted by Mehlich 1 and determined by colorimetry; [5.] Extracted by Mehlich 1 and determined by flame photometry; [6.] Extracted with potassium chloride 1 mol L^{-1} and determined by titration.

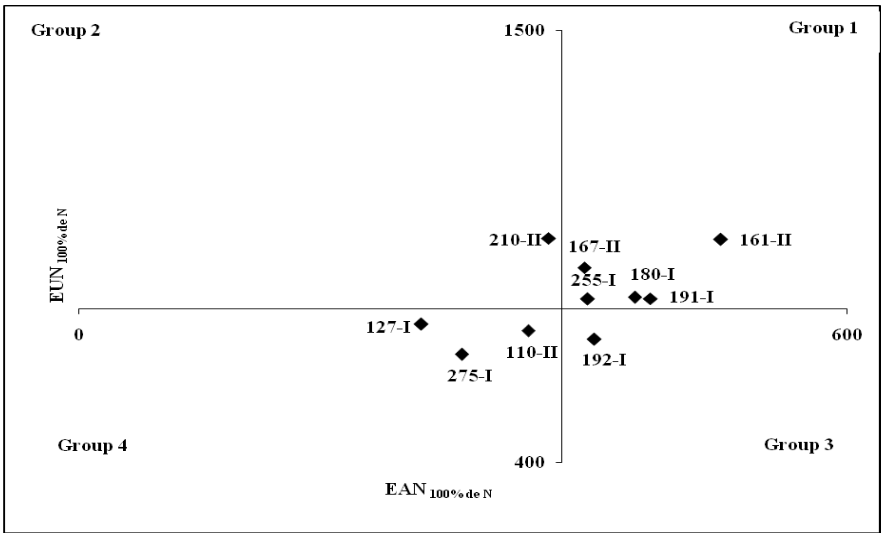

Fig 2. Distribution of genotypes of physic nut as function of the efficiencies of absorption (EAN) and utilization (EUN) of nitrogen in environment with adequate supply of this nutrient (100% N).

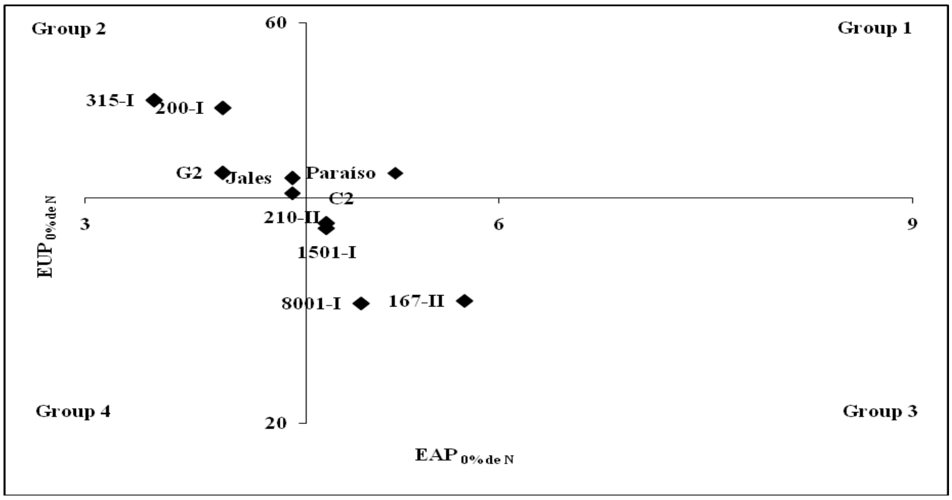

Fig 3. Distribution of genotypes of physic nut as function of the efficiencies of absorption (EAP) and utilization (EUP) of phosphorus in environment with low supply of this nutrient (control level– 0% P).

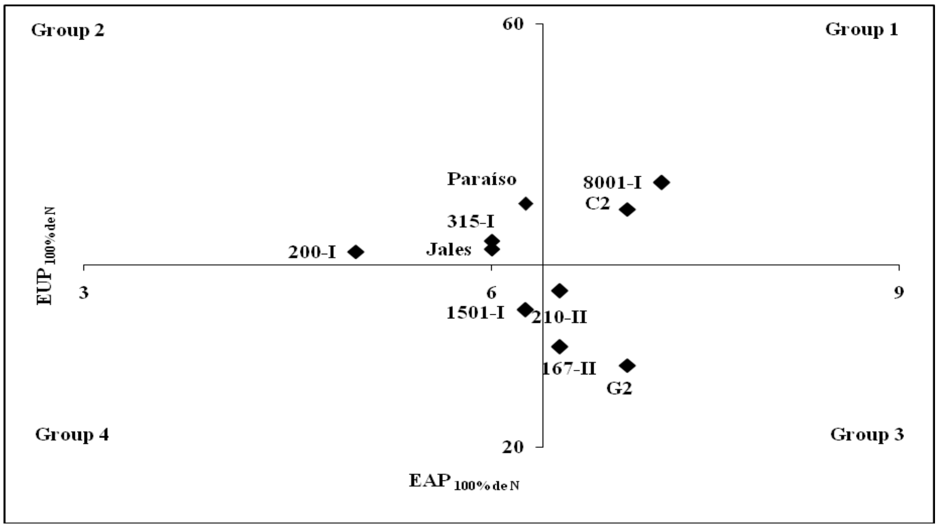

Fig 4. Distribution of genotypes of physic nut as function of the efficiencies of absorption (EAP) and utilization (EUP) of phosphorus in environment with adequate supply of this nutrient (100% P).

(CNPAE 200-I, CNPAE 210-II, CNPAE 315-I, CNPAE G2-I and Jales).

There are two main hypotheses that can explain the low rates of absorption in soils with low availability of P. The first is based on the different genetic mechanisms that govern the process of absorption of this nutrient. The absorption capacity is a multigene trait and can vary among species and genotypes of the same species, showing a high environmental influence (Fohse et al., 1998). The second hypothesis is based on the development of roots. In soils with low availability of P, plants may exhibit an increased root system that is developed due the necessity to explore a larger volume of soil to find the nutrients (Grant et al., 2001). It is possible that the roots of the genotypes had developed more to exploit larger volume of soil, resulting in higher values of dry matter of roots. Since the EAP is a ratio between the total P content in the plant and the dry matter accumulated in the roots, and there is no relative increase of the P content, the larger development of roots caused a reduction in the EAP (Amaral et al., 2012).

In the scenario with restriction of P supply, only the genotype Paraíso presented high efficiency of absorption and utilization of P (Group 1). There was no discrimination of genotypes with negative interaction for both efficiencies simultaneously (Group 4), which emphasizes the hypothesis that the efficiency of absorption of P is the major limitation in environments with low supply of P.

In tropical regions, the plantations of physic nut have to be expanded to areas with dystrophic soils, generally acids, weathered and with high capacity to fix P. Therefore, the fertilization with phosphorus becomes one of the most important factors to evade the limitation of plant growth. In this sense, genotypes that are more efficient in the absorption and utilization of this element are important to contribute to a more rational use of fertilizers and to reduce the production costs without compromising the crop yield (Amaral et al., 2012; Martins et al., 2013b).

In the environment with P supply, the genotypes CNPAE 8001-I and CNPAE C2 were classified as efficient in the absorption and utilization of P (Fig 4), indicating that the increase in the availability of P in the soil increased the efficiency of absorption of these genotypes (Fig 3). The same genotypes were also classified as efficient and responsive to phosphorus fertilization in other studies (Amaral et al., 2012). Conversely, increasing levels of P in soil made the genotype Paraíso (which were allocated in Group 1 in the environment with low supply of P) decreases the EAP, and in turn, reallocation to Group 2.

Several studies indicate that genetic variability among cultivars was essential for differentiation of rates of utilization of nutrients, including P (Fageria, 1998; Baligar et al., 2001). The large genetic variability found in accesses of physic nut (Laviola et al., 2010; Alves et al., 2013; Bhering et al., 2013) can also be used to explain physiological and biochemical differences inherent of each genotype that may contribute for different nutritional efficiencies.

Another determining factor for the differences is that this species does not yet have a standardized cultivar in Brazil. So, the morphological characteristics of roots (specific surface, area, length, size and volume) are variable, providing different effects over the ability to absorb and utilize the P from the soil.

Materials and Methods

Local conditions and plant material

The experiment was conducted under greenhouse condition in the experimental area of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES), in Alegre, ES, Brazil, with coordinates of 20°45' S latitude and 41°33' W longitude, and an average altitude of 277.41 meters, from December 2012 to March 2013. The mean air temperature was 27.3 ° C, relative humidity above 70% and photosynthetic photon flux 759 µmol m^{-2} s^{-1}.

The soil was collected at a depth of 10 to 40 cm, with the first 10 cm of the soil being discarded to reduce the effect of the organic matter present on the surface layer. A soil sample was sent to laboratory for chemistry and physic characterization, according to the methodology descrilbed by Embrapa (2013) (Table 2). The soil was characterized as a clayey red-yellow latosol.

After the characterization, the soil was dried under shade and homogenized in a 2.0 mm mesh sieve. Subsequently, the soil was separated into samples of 10 dm^3, standardized by weighing on a precision balance and placed in sealed plastic pots (with a capacity for 12 dm^3).

Along the assays, the pots were irrigated daily, maintaining the soil moisture at 60% of the total pore volume, obtained by particle density and soil density determination using the cylinder method, according to Embrapa (2013).

The physic nut seeds were provided by Embrapa Agroenergia (harvested in 2011), and processed by removing the immature and damaged seeds. The seeds were packaged and stored in the refrigerator (3°C) until use. Their water content maintained between 10-12%. All genotypes had the same growing season and agronomic characteristics.

Experimental design for the nutrition with N

The experiment was arranged in a factorial scheme 10×2, with fours replications. The factors were 10 genotypes of physic nut (CNPAE 110-II, CNPAE 127-I, CNPAE 161-II, CNPAE 167-II, CNPAE 180-I, CNPAE 191-I, CNPAE 192-I, CNPAE 210-II, CNPAE 255-I and CNPAE 275-I) and two levels of fertilization with nitrogen [0% and 100% of the level recommended by Novais et al. (1991)], following a completely randomized design. Four seeds were sown per pot, performing the thinning to one plant per pot on the 10^{th} day after emergence. Therefore, the experimental plot consisted of one plant per pot.

The nitrogen fertilization was performed with NH_2CONH_2 P.A., diluted in distilled water and applied over the soil surface, distant 10 cm of the plant collar, following levels consistent with the treatments of 0% and 100% of nitrogen supply (respectively 0.00 and 2.15 g dm^{-3} of N). The fertilization was divided into four applications, performed at 20, 40, 60 and 80 days after sowing. The fertilization with phosphorus and potassium was provided to all parcels in a single application before sowing using through the KH_2PO_4 P.A. diluted in water and applied in the entire volume of soil, according to the recommendation for studies in controlled environment (Novais et al., 1991).

Experimental design for the nutrition with P

The experiment was arranged in a factorial scheme 10×2, with fours replications. The factors were 10 genotypes of physic nut (Paraíso, Jales, CNPAE – C2, CNPAE-G2, CNPAE-167 II, CNPAE-200, CNPAE-210 II, CNPAE-315, CNPAE-1501 and CNPAE-08001) and two levels of fertilization with phosphorus [0% and 100% of the level recommended by Novais et al. (1991)], following a completely randomized design. Four seeds were sown per pot, performing the thinning to one plant per pot on the 10^{th} day after emergence. Therefore, the experimental plot consisted of one plant per pot.

Fertilization was performed according to recommendation for controlled environmental studies (Novais et al., 1991). Fertilization with nitrogen and potassium was performed in four cover applications, the first at 20 days after sowing, and the others, at an interval of 20 days. In all fertilizations, the nutrients were supplied via p.a. salts (KNO_3, KH_2PO_4 and NH_2CONH_2), seeking to establish the nutritional balance of the soil. The P_2O_5 levels corresponding to each experimental plot were applied in the form of p.a. salts (KH_2PO_4), diluted in distilled water and homogenized completely the volume of soil in the pot. The applied levels, referring to 0% and 100% of the P_2O_5 recommended for culture, according to Novais et al. (1991), consisted of 0.22 and 1.75 g dm^{-3} of P_2O_5.

Evaluation of the study and calculate indices

After 100 days of cultivation, the plant materials (leaves, stems and roots) were collected and separated in paper bags, which were then dried in oven, with forced air circulation at 60.0 °C (STF SP-102/2000 CIR) until reached a constant weight. After drying, the plant materials were weighed on analytical balance (SHIMADZU AUW-220D; precision: 0.00001 g) and sieved (CIENLAB EC-430, 8 blades, 1725 rpm, 20 mesh) to obtain a homogeneous powder.

To quantify the nitrogen content, 0.5 g (±0.001g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm × 200 mm) and submitted to the stages of sulfuric digestion (H_2SO_4), distillation (NaOH 40%) and titration (NaOH 0.02 mol L^{-1}) of nitrogen in "Kjeldahl" distillers (Marconi MA-036), according to the Kjeldahl method (Ma and Zuazaga, 1942).

To quantify phosphorus content, 0.5 g (±0.001g) of the prepared material, in triplicate samples, were transferred to Taylor tubes (25 mm × 200 mm) and submitted to the stages of nitropercloric digestion (HNO_3 e $HClO_4$) in a digestion block (Tecnal, TE-007D) at 180-190 °C for 3 hours; afterwards, 3 mL of ascorbic acid (C6H8O6, 0.87M) were added and the determination was done by spectrophotometry at 725 nm (Femto, 700 Plus) (Defelipo and Ribeiro, 1996).

The following indices were calculated: absorption efficiency = (total nutrient content in the plant)/(root dry matter), according to Swiader et al. (1994) and use efficiency = (total dry matter)^2/(total nutrient content in the plant), according to Siddiqi and Glass (1981).

For each nutrient (P and N), the genotypes were classified in 4 groups according to the efficiencies of absorption (horizontal axis) and utilization (vertical axis). The grouping was made into two different scenarios of nutrient supply (for N and P, separately), creating a set of contrasting environments. Therefore, there was cluster analyses in scenarios with low supply of N (Fig 1) and P (Fig 3) (0% of the recommended for studies in controlled environments by Novais et al, 1991) and cluster analyses in scenarios with standard supply of N (Fig 2) and P (Fig 4) [100% recommended for studies in controlled environments by Novais et al. (1991)]. The grouping, in each scenario, was performed with the intersection of the axes defined with the overall means for each variable.

Statistical analysis

The homogeneity of variances and normality of errors were verified according to Storck et al. (2011). The data were subjected to analysis of variance ($p \leq 0.05$) and the analyses were performed using the statistical software "GENES" (Cruz, 2013).

Conclusions

Scenarios of low N supply indicate maximization of EAN of many genotypes in these conditions. The genotypes CNPAE 167-II and CNPAE 180-I had high efficiencies of absorption and utilization of N, independent from the environment, while the genotypes CNPAE 110-II and CNPAE 275-I showed reduced efficiencies of absorption and utilization of N. For phosphorus, the efficiency of absorption is the major limitation in the simultaneous study of genotypes for both nutritional efficiencies, which is also strongly affected by the limitation of the P available in the soil. The genotype CNPAE 167-II is highlighted for presenting simultaneously higher absorption efficiency of N and P and higher efficiency of utilization of P in both contrasting environments.

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