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Phosphate application influences the initial growth and accumulation of phosphorus and nitrogen in UMBU (*Spondias tuberosa* Arruda Camara)

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Abstract

The objective of the current work is to evaluate the influence of phosphorus (P) on the accumulation of dry matter as well as the nitrogen (N) and P uptake by the umbu. The experiment was conducted under greenhouse conditions and seeds were placed in a 3-liter pot containing Yellow Latosol soil. The experimental design was entirely randomized, employing different P levels (0; 8; 16; 32 and 64 mg kg^{-1} of soil). The experiment was concluded 108 days post planting, and the characteristics of growth as well as the N and P uptake were evaluated. The P was found to be significant as it promoted an increase in stem diameter, which could improve grafting and establishment of the plants in the field. The least value of Total Dry Mass (TDM) was obtained when the plants were cultivated in the absence of P. Up to the dose of 32 mg of P the increase in the accumulation of Dry Mass by Absorbing Root (DMAR) was slow and gradual; however, between the doses of 32 and 64 mg of P, a substantial accumulation of dry mass in this plant region was observed. The deficiency of P significantly reduced the uptake of P and N, although it did not change the N: P ratio. Phosphorus fertilization allowed greater growth of the xylopodium, local where water and nutrients storage ocurrs; therefore, P fertilization may contribute to increased drought tolerance of this specie. The concept of P-fertilization is highly significant for the growth of the umbu.

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Introduction

The umbu tree (*Spondias tuberosa* - Anacardiaceae) is endemic to the Brazilian Semiarid region, whose fruits are used in the human feeding (Giulietti *et al.*, 2002; Lorenzi, 2002). Therefore, it is extremely important, both economically and socially, for the sustenance of some of the small-sized and poor farmers located in that area of Brazil. Moreover, the umbu is a highly drought tolerant species (Epstein, 1998). Thus, it is highly plausible to consider the umbu tree as a viable alternative to counteract the negative effects of the climate changes that will occur in various parts of the world. Despite this significance, studies related to the fertilization of the umbu tree, using mainly phosphorus (P) and nitrogen (N), are still very scarce. Studies of this nature are extremely valuable because these two nutrients systematically limit plant growth in the various regions of the tropical environment (Elser *et al.*, 2007). In some situations, the response to P application was found to be more effective in promoting plant growth than the application of N (Graciano *et al.*, 2006). Moreover, the increase in N levels in the soil may even decrease the plant growth if there is no corresponding increase in the availability of P (Schumacher *et al.*, 2013). These results highlight the strong relationship between the availability of N and P and plant growth. The low availability of P causes a reduction in leaf area and the CO_2 assimilation rate, consequently resulting in a reduced availability of photoassimilates for whole-plant growth (Thomas *et al.*, 2006); therefore, a drop in the accumulation of Total Dry Matter (TDM) is a major feature of plants cultivated under conditions of P deficiency. The reduction in the leaf area of plants grown under conditions of P deficiency is related to fewer numbers of leaves and/or decreased growth of leaves.

Phosphorus deficiency, besides reducing plant growth, may also affect the distribution of dry matter among the various plant organs. Results have shown that under conditions of P deficiency the growth of the shoot is more affected than that of the root system resulting in an increase in the root: shoot ratio of the plant (Vance *et al.*, 2003; Lambers *et al.*, 2006). This increased distribution of the photoassimilates to the roots, in plants grown under conditions of P deficiency, may stimulate greater exploration of the soil and enhance the extraction of more nutrients (Batterman *et al.*, 2013). Indirectly, the effect of P deficiency on plant growth also may be related to the reduced absorption of the essential nutrients vital for various important biochemical and physiological processes (Jayalakshmi *et al.*, 2012). In Eucalyptus, for example, a reduction in the P availability reduced the N uptake from the soil, helping to explain the slower growth of this species (Graciano *et al.*, 2006). For some species, the annuals in particular, these ratios of growth under conditions of P deficiency have been well established. However, similar studies are rather scarce for the umbu tree, despite its importance and potential for use in the world semiarid regions. Therefore, the aim of this work is to evaluate the influence of the phosphorus doses on plant growth and the phosphorus and nitrogen uptake by the umbu tree.

Material and methods

Experimental conditions and plant culture

The study was conducted in the greenhouse of the National Cassava and Fruits Research Center, located in Cruz das Almas, BA. Umbu seeds originated from Embrapa Semiarid, located in the municipality of Petrolina - PE were used for planting. The medium texture Yellow Latosol soil was used and showed the following chemical characteristics: pH (in water) = 4.8; P = 4.3 mg dm^{-3}; Ca^{2+} = 0.2 $cmol_c$ dm^{-3}; Mg^{2+} = 0.7 $cmol_c$ dm^{-3}; K^+ = 0.03 $cmol_c$ dm^{-3}; H^+ + Al^{3+} = 2.42 $cmol_c$ dm^{-3}; S = 0.20 $cmol_c$ dm^{-3}; CTC = 3.65 $cmol_c$ dm^{-3}, organic matter = 21.6 g kg^{-1}; V = 20%. Granulometric analysis showed the following composition: fine + coarse sand = 600 g kg^{-1}; silt = 160 g kg^{-1}; clay = 240 g kg^{-1}. Field capacity was 18.3%.

After processing, 3.0 kg of air-dried soil was placed in each pot and finely ground dolomitic limestone (100% sifted through a 0.25-mm sieve) was applied in sufficient quantity to increase the saturation of the bases to 75%. After a 30-day incubation period, with humidity of approximately 80-85% of field capacity, the treatments were begun. Treatments included five doses: 0; 8; 16; 32 and 64 mg of P kg^{-1} soil, corresponding to 0; 36.7; 73.3; 146.6 and 293.3 kg ha^{-1} of P_2O_5, respectively. Triple superphosphate powder was used as the phosphorus source. A few days prior to planting, the soil analysis showed that the P levels lay very close to the values of the treatments desired; however, the calcium levels did not differ between treatments.

Before sowing, to facilitate germination and seedling emergence, treatment to break dormancy was performed. This consisted of a diagonal cut on the distal part of the seed. Planting was performed by placing five seeds per pot to a depth of 2.5 cm. Throughout the experimental period, irrigation was performed daily, using water from a natural source, to maintain the soil moisture to around 80-85% of the field capacity, according to the methodology suggested by Figueroa *et al.* (2004).

Thirty days after sowing, thinning was done, leaving only those plants having the best vigor. During the course of the experiment, three topdressing fertilizations were performed with potassium sulfate and ammonium nitrate, totaling, in mg kg^{-1} soil, to 200 of N, 150 of K_2O and 61 of S. The topdressing fertilization using micronutrients (Fe, Zn, Cu, B, Mo and Mn) was performed 50 days post sowing.

Measurements

One hundred and eight days post planting, the experiment was terminated and the variables including plant height, stem base diameter, number of leaves and number of leaflets were evaluated. To determine the leaf area, the gravimetric method was used, in which the leaflets were printed on paper, cut out and weighed on a precision scale; later, their mass was related to the previously established mass of an area of the same paper used for printing. The length and diameter of the xylopodium were also measured. Subsequently, the stem plus the petiole, leaf, absorbing roots and xylopodium thus harvested were oven dried with forced air circulation for 96 h at 65 ^{o}C. Then, the dry masses were determined. The chemical analysis for phosphorus and nitrogen were carried out on five plants and limited only to the plant tissues of 8, 16 and 64 mg of P kg^{-1} of soil treatments, according to the analytical procedures described by Malavolta *et al.* (1989).

Statistical design

The experimental design was entirely randomized and conducted with 10 replicates. Each plant was considered a replicate. Regression equations were estimated between the variables and the P doses applied, opting for the model that had the highest coefficient of determination, the greater significance of the coefficients and those that were best explained from the biological viewpoint. The nitrogen and phosphorus uptakes were subjected to the analysis of variance by F test and compared by Tukey test at 5% probability level. The computer program SISVAR - System Analysis of Variance for Balanced Data (Ferreira, 2000) was used to perform all the analyses.

Results and discussion

Plant height

Plant height was significantly affected by the phosphorus application, with data revealing a quadratic relationship (Fig. 1A). In the absence of P, the average plant height was 19.9 cm, while at the maximum P dose this rate was increased to 42.4 cm. On the contrary, in the study done by Melo *et al.* (2005) no effect of P was observed on plant height, even when doses as high as 150 kg ha^{-1} of P_2O_5 were used. The beneficial effect of phosphorus was also observed on the diameter of the stem base (Fig. 1B), a characteristic that indicates whether the umbu plant is in a state to be grafted. In the treatment corresponding to 0 to 64 mg of P, the diameters were 4.0 mm and 5.2 mm, respectively. These results show that phosphorus may promote an increase in the stem diameter of the umbu tree and contribute towards enabling the plants to reach the ideal point for grafting, in a shorter time period, resulting in a better establishment of the plants in the field.

Total dry mass accumulation

The lower accumulation of total dry mass (shoot + absorbing roots + xylopodium) was observed to occur in plants grown with no P being applied (Fig. 2); this treatment yielded an increase in the production of dry matter, with data showing a linear relationship with the amounts of P applied (R^2 = 0.99**). Total Dry Mass Production (TDM) obtained by plants grown with 8 mg of P was 2.31 g, whereas for the higher dose of P the value was 5.96 g, resulting in an increase of 158%. The reduction in the accumulation of dry matter in the plants grown under conditions of application of lower amounts of P was expected, as this is one of the main problems related to the deficiency of this nutrient (Garrish *et al.*, 2010). The importance of the necessity of phosphorus for performing the processes associated with photosynthesis may explain, even partially, the lesser accumulation of the dry matter (Reich *et al.*, 2009).

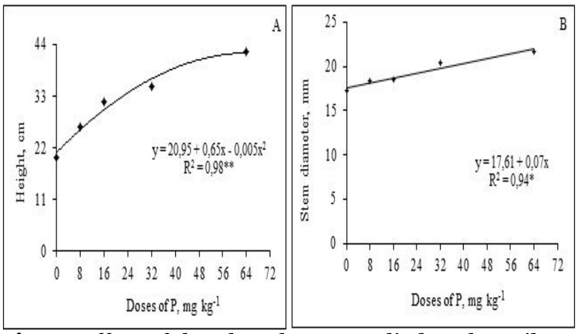
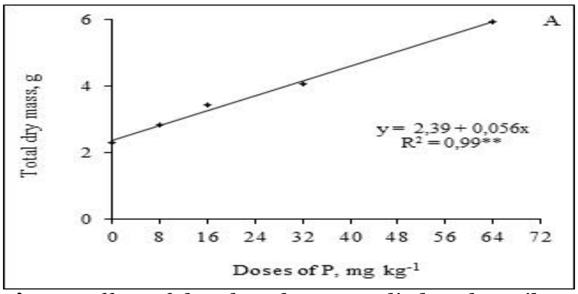


Fig. 1. Effect of the phosphorus applied to the soil on the height (A) and stem diameter (B) of the umbu tree seedlings (for all figures below * and ** indicate, respectively, significant at 5% and 1%).

P concentration and accumulation

The P concentration in the leaf lamina, the site of the occurrence of the important physiological processes, was not significantly altered by the doses of P applied (Fig. 3A). For plants grown under severe stress (8 mg of P) the value was 1.35 g kg^{-1}, while for the highest P supply this value was of 1.38 g kg^{-1}. No differences were observed between the treatments for P concentration in the stems + petioles, absorbing roots and xylopodium (data not shown). The results obtained for the leaves do not concur with those observed by Neves *et al.* (2008) which revealed a linear relationship between the application of P and the P concentration in the leaves of the umbu tree. However, in Eucalyptus, Xu *et al.* (2002) also observed that the variation in the P application of 13 kg ha^{-1} to 312 kg ha^{-1} did not result in a significant change in the leaf concentration of this element. The occurrence of the nutrient dilution due to higher accumulation of the dry matter in plants grown with the higher availability of P (Faustino *et al.*, 2013) can be used to justify the lack of variation in the concentration of this nutrient in the plant tissues in our study. As a result of the higher accumulation of dry matter the total P accumulation was higher for those plants grown with 64 mg of P (Fig. 3B). The lack of variation in the foliar P concentration, corresponding to the increasing values of the total dry mass for the umbu tree indicates that these two characteristics are not correlated.



Fig, 2. Effect of the phosphorus applied to the soil on the total accumulation of dry mass by the umbu tree seedlings.

Above-ground growth and dry mass accumulation

The increase in the P doses also exerted a pronounced effect on the Shoot Dry Matter Accumulation (SDMA) of the umbu plants (Fig. 4A). Without any application of the P, the value was 0.92 g, whereas after the treatment with 64 mg of P, the accumulation of dry matter reached the value of 2.91 g, representing an increase of 216%. Likewise, linear relationships were noted between the P levels and the accumulation of dry matter in the stem + petiole (Fig. 4B) and the leaves (Fig. 4C). The increase determined by the higher dose of P, relative to the non-application of this nutrient, was 150% for the stem + petiole and 323% for the leaves, clearly indicating that in this growth phase and under the conditions of good availability of the P, the distribution of the photoassimilates for the shoots is primarily directed to the leaves. The linear relationships obtained for the dry matter content of all the plant parts, even using doses up to 64 mg of P, show the importance of this nutrient to allow the umbu tree to achieve high growth rates.

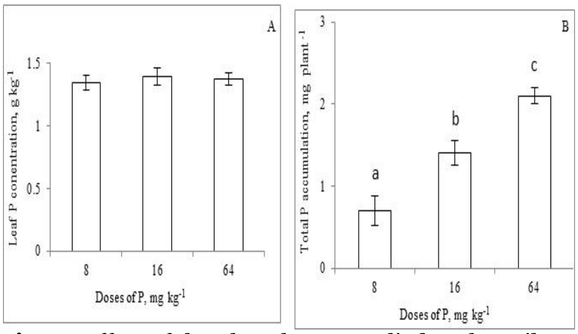


Fig. 3. Effect of the phosphorus applied to the soil on leaf P concentration (A) and P accumulation of the total dry mass (B) by umbu tree seedlings. Values given are the means ± standard error and the means followed by the same letter are not significantly different at $p < 0.05$.

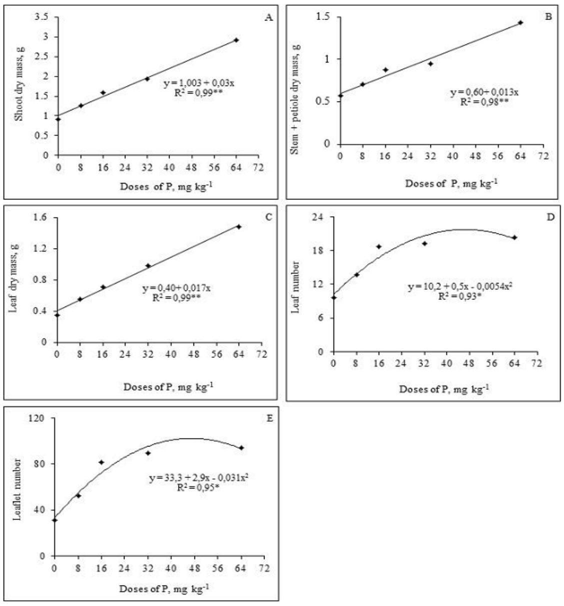


Fig. 4. Effect of the phosphorus applied to the soil on the dry mass of the shoots (A), stem + petiole (B), and leaves (C) and the number of leaves (D) and leaflets (E) of the umbu tree seedlings.

The average number of leaves (Fig, 4D) and leaflets (Fig. 4E) responded positively to the application of P. However, a trend towards stabilization of the values of these variables was observed in the dose next to the 40 mg P kg^{-1}. For the treatment in which P was not applied, the number of leaves was 9.6, while for the higher dose of P this value was 20.2 (111% increase). As a direct consequence of the increase in the number and size of the leaves and leaflets significant increases in the leaf area of those plants grown under the highest P supplies were observed (Fig. 5). For the lowest dose applied, the leaf area was only 149.2 cm^2, while at the dose of 64 mg of P the value was 534.8 cm^2, which represents an increase of 258%. The lower leaf area probably resulted in a reduced capacity of the plants to fix CO_2, contributing to the lowest value of accumulation of dry matter observed in the treatments under P stress. Comparatively, the effect of P on the leaf area was more pronounced than on the number of leaves (111%) and accumulation of dry matter in the shoot (216%). These results demonstrate that: (i) the effect of the P on the leaf area is less related to the number of leaves than to leaf size, and this observation is reinforced by the fact that the number of leaves for treatments with 32 and 64 mg of P were similar, whereas the leaf area was significantly higher for those plants grown under the highest dose of P; (ii) when the accumulation of dry matter was inhibited to a lesser extent than the leaf area, it became possible to suggest that the limitations in the supply of the photoassimilates was not the primary cause for the reduction in the rate of leaf expansion. Singh *et al.* (2006) have also indicated that the smaller size of the leaves of the plants grown under inadequate P supply is not related to the limited availability of carbon for growth. The reduction in the cell division and the increased wall stiffness of the cells formed have been used to justify the lower expansion of the leaves of those plants grown under conditions of deficiency of this macronutrient (Chiera *et al.*, 2002).

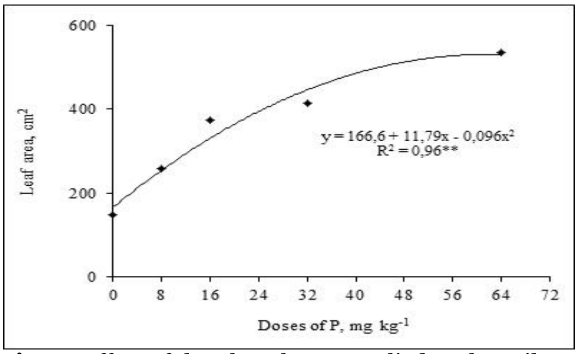


Fig. 5. Effect of the phosphorus applied to the soil on the leaf area of the umbu tree seedlings.

Below-ground growth and dry mass accumulation

The accumulation of dry matter in the absorbing roots (DMAR) showed a quadratic relationship with the doses of P applied (R^2 = 0.99**; Fig. 6A). From the absence of P up to the dose of 32 mg, the increase in the accumulation of DMAR was quite slow (from 0.178 g to 0.223 g). However, between the doses of 32 and 64 mg of P a substantial accumulation of dry matter in this plant region was recorded, reaching a value of 0.61g, which represents an increase of 173% compared with the 32 mg P kg^{-1} treatment. It must be remembered that for the treatments between 32 and 64 mg of P, the increase in the accumulation of TDM was only 46.7% (from 4.07 g to 5.96 g). The high amount of accumulation of dry matter in the roots, from the dose of 32 mg of P could suggest the existence of difficulties faced by the other plant organs in receiving the photoassimilates produced in this range of P availability, because under the high rates of availability of this nutrient the shoot should be preferred in the distribution of the photoassimilates (Zambrosi *et al.*, 2012). Up to a dose of 32 mg of P, the DMAR: TDM ratio was greater for those plants grown under the highest stress levels of this nutrient (0 and 8 mg of P; Fig. 6B). This result was expected as the plants grown under conditions of P deficiency tend to express a greater ratio between the DMAR and TDM (Garrish *et al.*, 2010; Gomes *et al.*, 2004), because under these circumstances the roots tend to become a stronger drain. This internal adjustment in the distribution of the assimilates helps enable the plant to maintain the growth of the absorbing root system to exploit a greater soil volume. However, the substantial accumulation of dry matter in the roots of plants grown with 64 mg of P determined that for this treatment the DMAR: TDM ratio was greater than for plants grown under stress. This result is contrary to the information that high doses of P reduce the DMAR: TDM ratio. Such behavior is not usually observed because, as mentioned prior, under conditions of good availability of P in the substrate, the absorbing roots do not appear as preferred drains. Probably, the inability of the shoots to absorb all the photoassimilates produced determined this significant increase in the accumulation of dry matter in the absorbing roots.

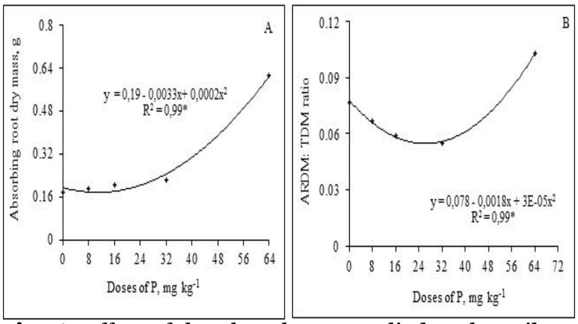


Fig. 6. Effect of the phosphorus applied to the soil on the absorbing roots dry mass (A) and its relation to the total dry mass (B) of the umbu tree seedlings.

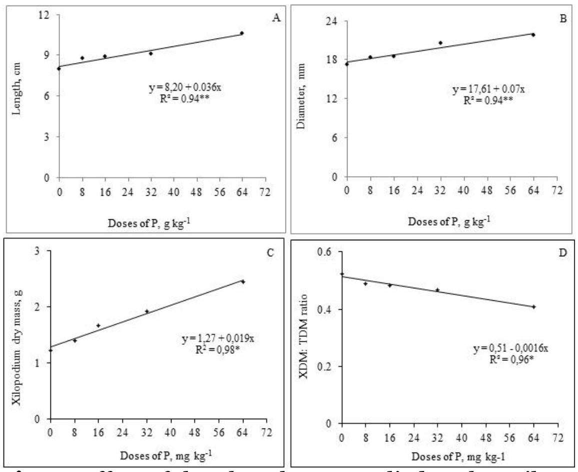


Fig. 7. Effect of the phosphorus applied to the soil on the length (A), diameter (B) and dry mass of the xylopodium (C) and its relation to the total dry mass (D) in the umbu tree seedlings.

Phosphorus deficiency affected the length (Fig. 7A), diameter (Fig. 7B) and dry matter accumulation in the xylopodium (Fig. 7C), with all the data revealing a positive linear relationship. On comparing the lower and higher doses of P, it was found that the length ranged from 8.0 cm to 10.6 cm, representing an increase of 32.5%, whereas the diameter varied from 17.24 mm to 21.76 mm (a 26% increase); for accumulation of dry matter the variation ranged from 1.21 g to 2.43 g (a 100% increase). This significant contribution of P towards growth and accumulation of Dry Matter in the Xylopodium (XDM) is important because this part of the plant could serve as a reservoir for water, nutrients and carbon to meet the demands of the plant under adverse weather conditions (DUQUE, 1980; MENDES, 1990); this is a very common situation that arises in the semiarid regions where the umbu tree is widely distributed. The relationship between XDM and TDM (Fig. 8D) was linear and higher for those plants grown under conditions of P deficiency ($R^2 = 0.95^*$). Plants grown under states of extreme P deficiency distributed around 52% of their total mass for the growth of the xylopodium, as against the higher dose of P distributed to only 41%. This indicates that under conditions of P deficiency the xylopodium becomes one of the preferred drains for receiving the few assimilates produced.

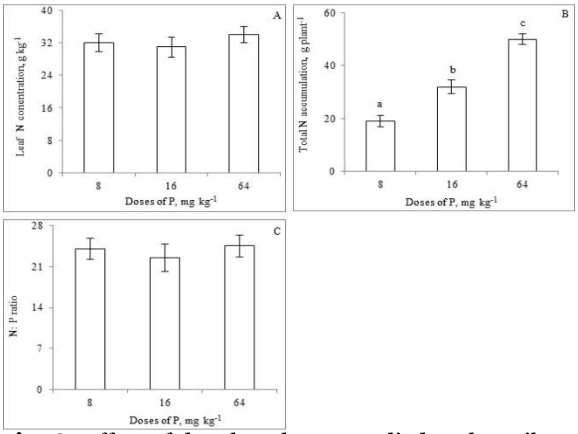


Fig. 8. Effect of the phosphorus applied to the soil on the concentration of leaf nitrogen (A), nitrogen accumulation (B) and N : P ratio (C) in the umbu tree seedlings. Values given are the means ± standard error and means followed by the same letter are not significantly different at $p < 0.05$.

N: P ratio

The N concentration of the leaves from those plants cultivated using 8 mg of P was not statistically different from those obtained from plants grown with 16 and 64 mg of P (Fig. 8A); however, the N accumulation was greater in the plants of the last two treatments (Fig. 8B), indicating that the phosphorus fertilization definitely increased the N uptake. The N: P ratio, which may suggest an imbalance in the absorption of these nutrients, did not vary between treatments, attaining values around 24:1 (Fig. 8C). It has been suggested that the optimal value of this ratio should hover at around 10:1 (Lambers *et al.*, 1998), although values as high as 22:1 have been found for eucalyptus plants (Graciano *et al.*, 2006). The high values obtained in this study suggest that there may have been an N uptake beyond the capacity of plants to use it and that in those circumstances, the phosphorus, and not the N was the most limiting factor for these plants to achieve a higher accumulation of dry matter. This observation is consistent with the non-achievement of the stabilization of accumulation of dry matter even when using the highest dose of P (Fig. 2A).

Conclusions

Phosphorus fertilization positively influenced all the growth characteristics evaluated. In this sense, the umbu tree proved to be a plant having a high demand for phosphorus during its early growth stages.

Phosphorus fertilization positively influenced the Nitrogen accumulation.

P was important in the promotion of increase in stem diameter, which can improve grafting and plant establishment in the field.

The umbu tree presented a high N: P ratio for leaf tissues.

The ratio of absorbing root to total dry matter was higher in the treatment where the higher dose of P was used; by contrast, P deficiency induced a higher percentage of accumulation of dry matter in the xylopodium.

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