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Ecophysiology of *Moringa oleifera* Lam in function of different rainfall conditions

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ABSTRACT

Drought conditions cause behavioral changes in plants, which proportions vary according to genotype, duration, severity and stage of development. One of their defense strategies is the accumulation of organic solutes, decreasing their osmotic potential to absorb water. This study aimed to compare the levels of photosynthetic pigments and soluble proteins in *Moringa oleifera* Lam plants grown in different rainfall conditions. Moringa leaves were collected in Recife-PE, with 227 mm.month^{-1} as average month rainfall during plant growth and in Pirauá, with 87 mm.month^{-1} in the same period, located at Natuba-PB. Solutes were quantified from leaf extraction in 80% acetone, followed by analysis at wavelengths 447, 595, 645 and 663 nm. Data were submitted to ANOVA and means were compared by Tukey test ($p < 0.05$). Moringa extracts from Pirauá presented the lowest chlorophyll b content; therefore carotenoid and soluble proteins concentration was higher than the extracts from Recife. *Moringa oleifera* plants grown in sites with lowest rainfall regime may have their higher soluble proteins and carotenoids accumulation as a physiological adaptation to prolonged water scarcity.

Keywords: chlrophyll, carotenoids, protein

Introduction

The moringaceae family belongs to the order Papaverales and it has only the Moringa genus, which includes 14 species, being *Moringa oleifera* the best-known specie (RAMOS et al., 2010). Native of the northeast India, its cultivation has become increasingly widespread in the arid and semi-arid areas of the Brazilian Northeast since its introduction in 1950 (AMAYA et al., 1992) due to its high adaptability to arid conditions (OLSON; FAHEY, 2011).

The moringa species can be found, native or introduced, in tropical or subtropical regions around the world, commonly subjected to environmental pressures of water deficit or high radiation (LUQMAN et al., 2012; RIVAS et al., 2013; ARAÚJO et al., 2016). This species can tolerate temperatures between 19 and 35 °C (KARMAKAR et al., 2010; THURBER; FABEY, 2010), rainfall indices ranging from 250 to more than 3,000 mm annually (MUHAMMAD et al., 2016) and can be cultivated up to 1,400 meters altitude in almost all types of soils, except in extremely humid soils (JESUS et al., 2013).

From the seed to the leaves, the moringa plant presents properties that can be exploited in several sectors. In addition to flocculant, coagulant and adsorbent properties (PEREIRA et al., 2011; MARQUES et al., 2012) as well as its oleic potential for biodiesel production (SILVA et al., 2013), its seed presents Lectin, a protein that acts as a sustainable and environmentally correct alternative for the control of *Aedes. aegypti*, since it has ovicidal and larvicidal properties (COELHO et al., 2009; SANTOS et al., 2012). Its leaves are not only alternatives to combat malnutrition (ANWAR et al., 2007; GUIGUER et al., 2016), but are also a potential source of biological control of nematode reproduction, acting as antihelminthic potential against intestinal parasitism (RAUL et al., 2012; MURSLAIN et al., 2013).

Plant growth is limited by habitat conditions and resources. These abiotic factors include temperature, humidity, solar radiation, water

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resources, minerals and carbon dioxide availability (RICKLEFS; RELYEA, 2016). The objective of this study was to compare the content of photosynthetic pigments and soluble proteins accumulated in leaves of *Moringa oleifera* grown in locations with different rainfall regimes.

Material and Methods

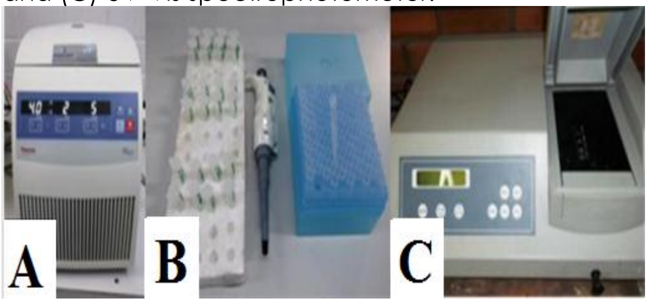
Moringa leaves were colected from plants grown at the outside of the Laboratório de Fisiologia Vegetal near by its greenhouse (8 ° 00'48 ''S, 34 ° 56'59'' W), Centro de Graduação Obra-Escola (8 ° 01'02 '' S, 34 ° 57' W) both at Universidade Federal Rural de Pernambuco (UFRPE), Centro de Ciências Biológicas (8 ° 3'5'' S, 34 ° 56'54'' W) at Universidade Federal de Pernambuco (UFPE) and Pirauá municipality from Natuba (7 ° 30' 05'' S, 35 ° 33' 26 ''W) in Paraíba. The plant accesses from Recife were grown at the average month rainfall of 227 mm.month^{-1} between January and June, 2016, while plant accesses from Pirauá (Figure 1) were grown at 87 mm.month^{-1} in the same period. Pirauá is located in the transition of Zona da Mata and Agreste (Figure 5), where precipitation rate fits the historical dry situation in which the Brazilian Northeast has been inserted (MARENGO et al., 2013. MARENGO et al., 2016).

Figure 1 - Pirauá Moringa plantation in July, 2016.

Soon after collecting the leaves, they were properly stored under refrigeration and in dark condition, and transported to the Laboratory of Plant Biochemistry at the UFRPE. The leaf extracts were prepared using 80% acetone as solvent and analyzed on the same day in wich leaves were collected, in a dark room, to avoid the photoxidation of the leaves.

The content of photosynthetic pigments was determined by destructive method taking the absorption values at wavelengths of 470, 645 and 663 nm (LICHTENTHALER; WELLBURN, 1983). The soluble protein content was obtained by the method of Bradford (1976), modified by Bezerra Neto and Barreto (2011), using *coomassie brilliant blue* and reading its absorbance at 595 nm in a UV-Vis spectrophotometer (Figure 2). The soluble protein was quantified based on the regression of BSA standard solutions versus its respective absorbances.

Figure 2 - Material and equipments used in photosynthetic pigments and soluble proteins quantification: (A) centrifuge, (B) precision pipette and (C) UV-Vis spectrophotometer.

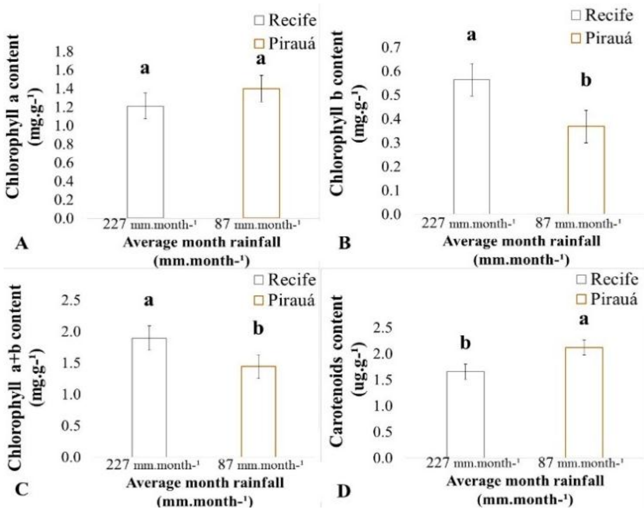


The experimental design was completely randomized, with two, one with 30 (227 mm.month^{-1}) and other with ten (87 mm.month^{-1}) replications, totaling 40 experimental units. The resulting data were submitted to analysis of variance and the means compared by the Tukey test at 5% probability using the statistical program Assistat 7.7 (SILVA; AZEVEDO, 2016).

Results and Discussion

The statistical analysis showed significant effect ($P < 0.05$) for chlorophyll *b* content (Figure 3B), the sum of chlorophyll *a* plus chlorophyll *b* (Figure 3C) and for carotenoids content (Figure 3D), while the content of chlorophyll *a* (Figure 3A) was not significantly affected by the treatments.

Figure 3 - Concentrations of chlorophyll *a* (A), chlorophyll *b* (B), sum of chlorophyll *a* plus chlorophyll *b* (C) and carotenoids (D) in leaves of *Moringa oleifera* grown in different rainfall conditios. Values with equal letters do not differ statistically ($P < 0.05$) by the Tukey test.



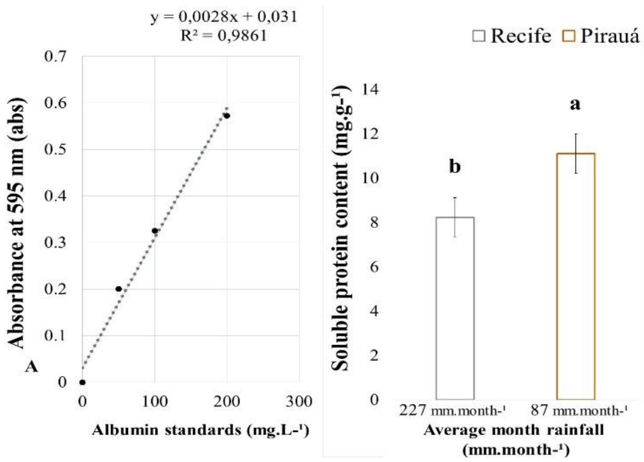
Moringa plants grown in place with lower rainfall, presented less chlorophyll *b* content than plants grown in place with higher rainfall (Figure 3B). There was no significant difference in chlorophyll *a* content between the places where plants were grown (Figure 3A). Carotenoids content were higher in plants grown in place with low rainfall (Figure 3D). These pigments act in the photoprotection of chlorophylls, intensifying their production when subjected to stress, however, if not enough protection, pigment reduction occurs (SILVA, 2013). Those defensive mechanisms are important to protect the photosynthetic apparatus and it is an important strategy to guarantee the photosynthetic metabolism in these conditions (LOGGINI et al., 1999; MENDONÇA et al., 2011).

Carotenoids are also necessary to maintain animals normal health and behavior, and no one animal species synthesizes these compounds therefore rely on their diet for these food (NISAR et al., 2015). Moreover, provitamin A carotenoids play essential roles in animals as precursors for the synthesis of retinoid, retinol, retinal, a main visual pigment, and retinoic acid, which controls morphogenesis (FRASER; BRAMLEY, 2004; KRINSKY; JOHNSON, 2005). In addition, carotenoids serve as antioxidants, reducing age-related macular degeneration of human eyes, the leading cause of blindness in the elderly worldwide (JOHNSON; KRINSKY, 2009; FIEDOR; BURDA, 2014).

According to Guiguer et al. (2016), properties of Moringa leaves may cause reduction of visceral fat, total cholesterol, triglycerides, LDL-c, and VLDL-c and increase in the HDL-c levels and there is no adverse effect, indicating it might be used by food-processing companies. Related to animal breeding, Macambira (2016) found Moringa have a great potential in poultry meal, once the plant is nutritive and increases the amount of fat in broilers carcasses, as well as promotes a meat-differentiated pigmentation due to its Carotenoids content. González et al. (2015) also studied the effect of Moringa flour in goats feeding and concluded it is an option for these animals diet.

Olson et al. (2016) compared protein and mineral nutrient concentrations of eleven Moringa genus species and observed *M. oleifera* had the highest protein content. In the present study was found a significant difference (P<0.05) in the soluble proteins content in leaves of *Moringa oleifera* grown in sites with different rainfall rates (Figure 4).

Figure 4 - Results of BSA standards regression (A), soluble protein content (B) in function of the average month rainfall during *Moringa oleifera* plants growth. Values with different letters differ significantly (P < 0.05) by the Tukey test.



The accumulation of proteins in the access submitted to the lowest average month rainfall (Figure 4B) was about 40% in relation to the other, being able to characterize a change in the osmotic potential of the cell to avoid the loss of water to the environment. According to Efeoğlu, Ekmekçi and Çiçek (2009), the synthesis of these osmolytes is widely used by plants to stabilize membranes and maintain protein conformation under low water potential.

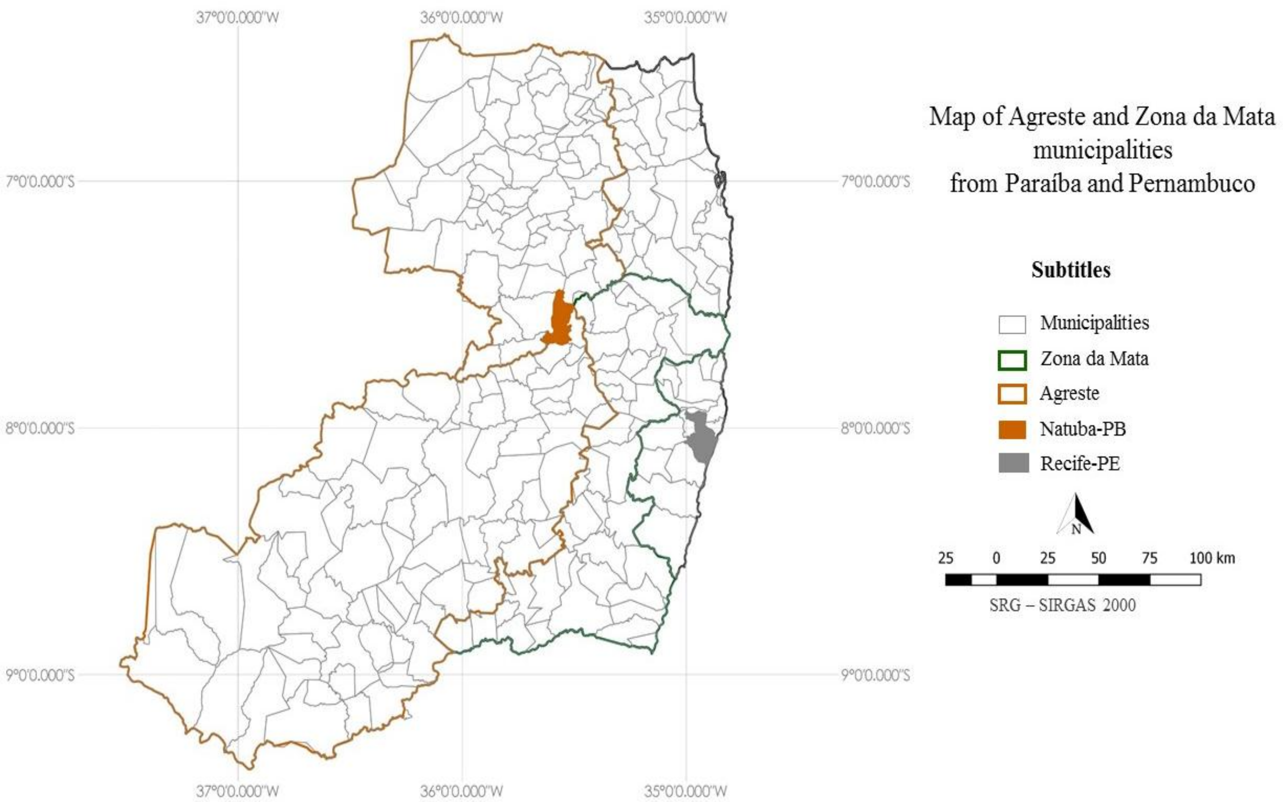
Rivas et al. (2013) also observed a increase in organic solutes of Moringa leaves submitted to water stress, suggesting a drought tolerance when young plants. It was observed by Silva (2013) since Moringa plants were not damaged in ten days after water suppression, even reaching values close to zero for photosynthesis, recovering after 24 hours of rehydration.

According to Marengo et al. (2016), the drought from 2010 to 2015 in the semi-arid Brazilian Northeast had an intensity and impact not seen in several decades, impairing extensive areas of agricultural land, affecting hundreds of cities and towns throughout the region. This water shortage implies changes in plant behavior, the proportions of which vary according to genotype, duration, severity and stage of development of the plant (LEVITT, 1980). The reduction of the water potential of the atmosphere causes the evaporation of cell walls water, decreasing the osmotic potential of the plants and accumulating organic solutes (WARREN et al., 2012).

Adaptive strategies are activated by plants in response to abiotic stresses such as temperature fluctuations, dehydration, and osmotic pressure. These adaptive mechanisms include changes in physiological and biochemical processes, since it is associated with metabolic adjustments that lead to the accumulation of several organic solutes such as sugars, polyols, phenols, and proline (TESFAY et al., 2011).

Problems related to rainfall distribution tend to be intensified with the global warming, which results in economic losses, especially in semi-arid regions (MISRA, 2014). *Moringa oleifera* can be an alternative to increase the Human Development Index in these regions and to mitigate economic damages caused by drought, since it presents tolerance to the water stress, high temperatures and the salinity (SILVA, 2013) and numerous properties which can be used by food-processing companies (ANWAR et al., 2007; GUARUER et al., 2011), to control biologicaly parasits (COELHO et al., 2009; SANTOS et al., 2012; RAUL et al., 2012; MURSLAIN et al., 2013), to produce pharmacus (LUQMAN et al., 2012), in the biodiesel industries (SILVA et al., 2013), in non-ruminant breeding (GONZÁLEZ et al., 2015, MACAMBIRA, 2016) and aquiculture (MCGRAW et al., 2006 ; BARON et al., 2008)..

Figure 5 - Map of Agreste and Zona da Mata municipalities from Paraíba and Pernambuco states.



Conclusions

Moringa oleifera grown in place with low rainfall presents higher soluble proteins and carotenoids accumulation as a physiological adaptation to the prolonged drought conditions, implying the viability of their planting in arid and semi-arid regions.

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