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Environmental seasonality influences on reproductive attributes of *Moringa oleifera*

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Abstract - *Moringa oleifera* Lam (Moringaceae) is a species tolerant to arid and semi-arid environments, such as the Brazilian Northeast. In this region climatic conditions make water a scarce resource. Water limitation affects plant growth and development where its impact will depend on its duration, intensity and the plant's development stage. During the reproductive period water deficit can be critical since there is high water demand in this phase. The present study had as objective to verify how *M. oleifera* reproductive attributes respond to the Brazilian semiarid (Brazilian tropical dry forest) seasonality. We hypothesize attributes such as fruit size, seed number and physiological quality are different between dry and rainy seasons, with lower values for the dry season. The biometric characteristics of the fruits and *M. oleifera* seed germination produced in the two seasons were analyzed. It was observed differences in fruit weight, length and seed number, as well as for speed and germination percentage, with greater values during the rainy season. Seasonality affected important reproductive aspects for the successful recruitment and establishment of the specie, showing that, even tolerating drought, water deficit may limit *M. oleifera* reproductive success.

Influência da sazonalidade ambiental em atributos reprodutivos de *Moringa oleifera*

Resumo - *Moringa oleifera* Lam. é uma espécie tolerante a ambientes áridos e semi-áridos, como o nordeste brasileiro. Nessa região as condições climáticas fazem da água um recurso escasso. A limitação hídrica afeta o crescimento e desenvolvimento vegetal e seu impacto dependerá da sua duração, intensidade e estádio de desenvolvimento da planta. Durante o período reprodutivo o déficit hídrico pode ser crítico, pois nesta fase há alta demanda hídrica. O presente trabalho teve o objetivo de verificar como os atributos reprodutivos de *M. oleifera* respondem à sazonalidade do semi-árido brasileiro. Partimos da hipótese que atributos como tamanho dos frutos, número e qualidade fisiológica das sementes são diferentes entre a estação seca e chuvosa, apresentando valores inferiores para a estação seca. Foram analisadas as características biométricas dos frutos e a germinação de sementes de *M. oleifera* produzidos nas duas estações. Observaram-se diferenças no peso, comprimento dos frutos e número de sementes, assim como velocidade e porcentagem de germinação entre as estações, sendo os valores superiores para a estação chuvosa. A sazonalidade afetou aspectos reprodutivos importantes para o sucesso de recrutamento e estabelecimento dessa espécie, evidenciando que, mesmo tolerando à seca, o déficit hídrico pode limitar o sucesso reprodutivo de *M. oleifera*.

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Introduction

Moringa oleifera, also known as drumstick tree, is a perennial species from Moringaceae family. It is native to India; however it is widespread in tropical regions. In Brazil, it was introduced around 1950 as an ornamental tree and currently has a wide distribution across the Country (Rivas et al., 2013). This species is tolerant to arid and semi-arid conditions (Olsen & Fahey 2011), such as the Brazilian Northeast (Brazilian tropical dry forest), where this characteristic in addition to its diverse usage potential by the population, make this plant a valuable crop alternative in the region (Gómez & Angulo, 2014).

The main climatic characteristics of this region are the dry periods which are caused mainly by low precipitation in combination with high temperatures, which make water a scarce resource (Prado, 2003). Water limitation is one of the main factors affecting plant growth and development, since several vital processes are influenced by water availability (Silva et al., 2016).

Moringa oleifera is a drought-tolerant species (Olson & Fahey, 2011); however, factors such as the intensity and duration of water deficit, as well as the plant's development stage determine the degree of impact of these conditions on plants (Machado et al., 2009). During the reproductive period, for example, water limitation can be critical (Prado et al., 2007), especially during the fruiting phase where the demand for photoassimilates is increased to supply the growth and development of diaspores. Water deficit, in addition to negatively influencing photosynthetic rates, may also alter the transport and distribution of photoassimilates, thus affecting fruit development (Méndez et al., 2012; López et al., 2009).

Thus, this study sought to understand how the reproductive aspects of *M. oleifera*, such as the production of diaspores and characteristics inherent to the recruitment of seedlings are affected by the variation of water availability due to seasonality of the semiarid. The initial hypothesis states attributes such as biometric fruit parameters and seed vigor present differences between the dry and rainy seasons, with the fruits produced during the dry season being: (i) inferior with respect to weight, diameter and length, and (ii), in addition to fewer seeds, they also present less vigor. To test this hypothesis, *M. oleifera* fruits and seeds produced in the two seasons were evaluated.

Material and methods

The study was carried out at the Centro de Formação de Professores/CFP in Campina Grande Federal University (UFCG) in the city of Cajazeiras, Paraíba State (6°52'19,97" S, 38°33'30,83" W) between May and August 2017. The study area is located in a semiarid climate region where temperatures are high, averaging between 24 °C and 27 °C, with low rainfall levels with an annual average of 800 mm (Araújo, 2011).

A total of 100 pods from 10 *Moringa oleifera* individuals present in the Campus landscape were used, with 10 fruits per individual being collected. The same individuals were sampled in both seasons. The fruit collection followed the availability of mature pods, prioritizing to contemplate different branches in the individual and to obtain a generalization of the position and location of the pods in the plant. As this species presents two reproductive events during the year, one from February to May and another from September to November (Jyothi, 1990), it was possible to obtain the fruits corresponding to drought and rainy periods. Fruits which had developed during the dry season reproductive event (September to November of 2016) were collected in May, while fruits corresponding to the rainy season (February to May of 2017) were collected in July.

According to data from the Executive Agency for Water Management (Aesa, 2017), between September and November 2016, rainfall indices recorded for the municipality of Cajazeiras were only 0.5 mm of precipitation, and data from the National Institute of Meteorology (INMET), showed a water deficit of -150 to - 200 mm with a 36 ºC average maximum temperature for the region in this period (Inmet, 2017). From February to May 2017, an accumulated 535.9 mm of precipitation were recorded with 36 ºC average maximum temperature and a water deficit reduced to -10 to -100 mm (Figure 1).

Analysis and measurements

Following collection, the fruits were taken to the laboratory for biometric analysis, taking into consideration the following parameters: weight (g), length (cm), diameter (mm) and seed number per fruit. The data obtained in the two seasons were evaluated through descriptive analysis.

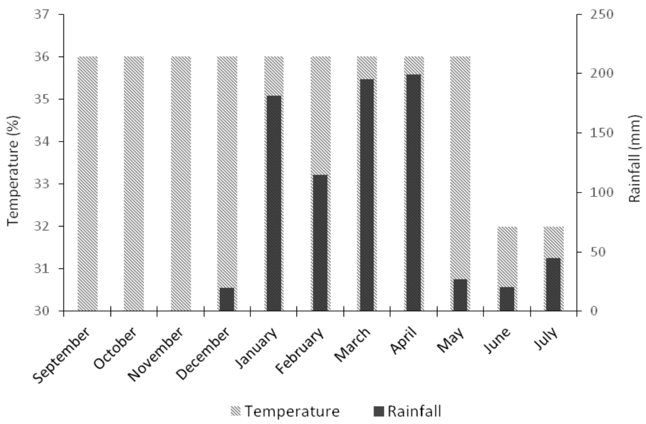

Figure 1. Monthly precipitation and temperature averages for the period from September 2016 to July 2017.

Germination experiment

The germination experiments were carried out following the fruit biometric analysis. We used 500 seeds, divided into 20 replicates with 25 seeds each, from fruits collected in the two seasons (n = 1000). A qualitative filter paper moistened with distilled water using a 2.5 times the dry paper weight proportion was used as the substrate.

For the germination analysis, the seeds were germinated following the paper roll (PR) method established by the Brazilian seed analysis guidelines-RAS (Brasil, 2009). After the wings were removed, the seeds were placed on two filter paper sheets with a third one coating them, wrapped in roller forms, packed in transparent plastic bags and taken to the germination chamber (M/FANEM MOD. 347 CDG) at 25 ºC with a 12 h photoperiod.

Seeds with all essential embryo structures were considered germinated, these were namely: root system and aerial part, following the RAS criteria (Brasil, 2009). Germination was monitored until the 14th day after sowing. The number of germinated seeds was obtained every two days to derive the germination speed index (GSI) using the formula proposed by Maguire (1962).

For the final germination percentage (%G), the total number of seeds germinated at the 14th day after sowing were taken into account. In addition to the germination tests, the length of the aerial part (PAcm) and root (Rcm), the shoot dry mass (PAg) and the root dry mass (Rg) were measured. The dry mass was obtained by drying the material in an oven at 80 ºC for 24 h, the shoot and root being sectioned with the aid of slides and placed separately in paper bags. Subsequently, the dry material was weighed using an analytical balance.

Statistical analysis

The data was evaluated by Lilliefors test, showing differences regarding normality of distribution. Therefore, the data of pods weight and the number of seeds were analyzed by Kruskal-Wallis non-parametric test with significant values being analyzed by the Dunn test ($p < 0.05$). The other variables which adhered to normality were analyzed by ANOVA followed by Student's t-test.

Germination percentage data were transformed using arcsine and they were submitted to a Kruskal-Wallis non-parametric analysis. The GSI was evaluated by ANOVA. The aerial and root length values were submitted to ANOVA and Kruskal-Wallis, respectively.

All statistical analyzes were performed using BIOESTAT 5.0 software (Ayres et al., 2007).

Data concerning the number of normal seedlings, as well as aerial and roots dry mass were obtained considering the total data of the germination tests and not by repetition, considering only the amplitude of the values, to verify the superiority of these parameters during the two seasons.

Results

The mean values for all evaluated parameters of *Moringa oleifera* fruits, except for diameter, were greater for fruits collected in the rainy season, with values of 13.5 g for weight, 36.6 cm for length and 17.7 seeds per fruit (Table 1). A significant difference between weight, length and seed number per fruit was observed when comparing the two seasons, with superiority for fruits produced during the rainy season, as shown in Table 2. No significant difference was observed for the diameter of fruits produced during the two seasons. The correlation test was positive for seeds number and weight, as well as for fruits length and diameter between the two seasons (Table 3).

Table 1. Descriptive summary of the biometry of *Moringa oleifera* fruits collected (N = 100) in dry and rainy seasons in Cajazeiras, Paraíba State.

	Dry season			Rainy season		
Parameters	Average	Sd	Max – Min	Average	Sd	Max – Min
Weight (g)	10.816	3.5	19.738 – 4.782	13.448	3.5	21.886 – 4.109
Length (cm)	34.3	4.6	46.5 – 24.0	36.6	4.7	46.8 – 21.0
Diameter (mm)	19.99	2.8	26.41 – 13.00	19.50	2.5	25.91 – 11.86
Number of seeds	16.51	3.5	23 – 5	17.69	3.5	25 – 2

Sd = Standard deviation; max = maximum value; min = minimum value

Table 2. Analysis of variance of biometric means of *Moringa oleifera* fruits collected (N = 100) in dry and rainy seasons in Cajazeiras, Paraíba State.

Parameters	Teste Kruskal-Wallis (H) e Anova (F)	p
Weight (g)	H = 25.7424	< 0.01
Length (cm)	F = 3.5965	< 0.01
Diameter (mm)	F= 1.6100	> 0.05
Number of seeds	H = 5.6884	< 0.05

ANOVA (F) for variables with normal distribution: variance compared by Student's t-test ($p < 0.05$).

Table 3. Spearman correlations ($p < 0.05$) between the biometric parameters of the fruits collected (N = 100) in the dry and rainy seasons in Cajazeiras, Paraíba State.

Spearman coefficient	Weight x length	Weight x diameter	Weight x number of seeds
Dry season	0.7477*	0.7565*	0.4905*
Rainy season	0.7206*	0.7360*	0.5583*

Germination and seedling analysis

M. oleifera seeds germinated on the 9th day with primary root emergence from the 5th day. Seeds produced during the rainy season had higher seed germination speed index, as well as higher percentage of germination and length of aerial part. The greatest normal seedling number at the last count, as well as the greatest dry mass of shoot, also corresponded to the rainy season (Table 4). The only parameter which presented significant variance with superiority for the dry season was the dry mass of roots.

Table 4. Data of germination and seedling analysis in the dry and rainy seasons in Cajazeiras, Paraíba State.

	Dry season	Rainy season		
Parameters	Average	Average	Analysis of variance	p
%G	81.2	91.4	H = 16.1752	0.05
IVG	6.9520	7.7385	F = 11. 8356	< 0.01
PAcm	5.13	5.3507	F = 5.5915	< 0.01
Rcm	9.0499	7.9769	H = 12.4878	< 0.05
PN	335	359	-	-
PAg	3.830	4.330	-	-
Rg	3.718	3.533	-	-

H = Kruskal-Wallis test; F = ANOVA, %G = percentage of germination, IVG = germination speed index, PAcm = length of aerial part; Rcm = root length; PN = normal seedlings; PAg = dry mass of shoot; Rg = dry mass of roots.

Discussion

When comparing the biometric analysis results (Tables 1 to 4) with studies carried out with this species in other areas, it was observed that *Moringa oleifera* presents variation in biometric traits. Ramos et al. (2010) recorded averages of 9.91 g, 28.50 cm and 12, for the weight, length and seed number, respectively, in *M. oleifera* fruits in the State of São Paulo. These values were lower than those found in this study, even when compared to data from the dry season where the fruits presented the lowest averages (Table 1).

On the other hand, Kshirsagar et al. (2016), found fruits with averages of 47.6 cm in length, 52 mm in diameter and 15 seeds in *M. oleifera* in India. In Nigeria, Ndubuaku (2014) recorded fruits with up to 35 seeds. The biometric information data corroborates with the existence of intraspecific variability of these traits, as pointed out by Papoola et al. (2016). Despite these being determined characteristics, such biometric parameters are influenced by the environment (Santos et al., 2009).

According to Zuffo et al. (2014), the biometric variations observed in the different areas in which a species occur may be explained by the genotype/environment interaction effect in the determination of these characteristics, that is, variations in environmental conditions exert a differential effect on genotype expression leading to phenotypic differences.

In the present study, differences in the biometric parameters of fruits in the same *M. oleifera* individuals in the same area, across two seasons, were observed. This highlights that, similarly to spatial variations, temporal variations of environmental conditions can also exert an effect on biometric characteristics. Ndubuaku (2014) observed that fruits from individuals which grew in drier regions obtained lower values for length and seed number than those which grew in an area with higher precipitation, affirming the hypothesis that water availability influences the determination of these parameters. Such influence was also pointed out by Zuffo et al. (2014) in *Dipteryx alata* Vog. (Fabaceae) fruits collected in years with different rainfall indices.

These statements are supported by the fact that the reproductive phase, for most plants, is one of the most sensitive to water deficiency stress since plants display a higher demand for this resource (Silva et al., 2016). Water restriction during this phase affects fruit and seed growth and development, mainly due to a reduction in photoassimilate allocation to these organs, as a consequence of a decrease in photosynthetic rate caused by stress (Silva et al., 2011). At this stage, fruits and seeds which are in formation become the main drains, with roughly 80% of carbohydrates being destined to these organs (Prado et al., 2007). Photoassimilate availability may therefore have influenced the *M. oleifera* fruit biometric differences in the two seasons.

Photosynthesis limitation due to water deficit and consequent photoassimilate reduction, destined for the composition of seed reserves, may have contributed to their lower vigor, since in the present study seeds produced during the dry season presented lower percentage and germination speed. This water limitation influence on seed physiological quality was verified by Silva et al. (2016) in *Sesamum indicum* L. (Pedaliaceae) exposed to water deficits during the flowering and fruiting phase, which resulted in a production of less vigorous seeds. According to Rabbani et al. (2012), seed germination speed is an important factor for the successful establishment of seedlings in the field, since a delay in this process may increase the seed exposure to unfavorable conditions, such as temperature and pathogens.

In addition to germinating faster, seeds originated from the rainy season generated seedlings with greater aerial development, which according to Hassan & Ibrahin (2013) is determinant in initial seedling establishment in field conditions, since it can confer a competitive advantage against other species.

Seedlings originating from seeds acquired in the dry season showed longer roots, a characteristic that is common in species occurring in regions where water is limiting, such as the Brazilian semiarid. A long primary root is important in initial seedling establishment as it allows access to water in deeper soil layers when this resource is scarce (Hassan & Ibrahin et al., 2013). According to the same authors, longer roots constitute an adaptive trait, since it is expressed even in conditions of greater water availability. However, in this study, root growth was observed in detriment of aerial growth in seeds from the dry season, showing there may have been conflict in seed reserve distribution due to its limitation.

We observed a correlation between the fruit and seed number biometric parameters. Papoola et al. (2016) also observed, for this same species, that the longer the fruit, the greater the number of seeds. According to Macedo et al. (2009) plants adjust the number of seeds according to their capacity to supply the seeds assimilated to them. Thus, under unfavorable environmental conditions, such as a dry season, photoassimilate availability may limit the number of seeds per fruit, an adjustment which allows the plant to complete its reproductive cycle even under adverse conditions.

Moringa oleifera is tolerant to semiarid conditions, being able to face, according to Dao et al. (2017), up to six months of dry season without great damage to their survival. Several mechanisms, among those cited by Rivas et al. (2013), are used to promote high water use efficiency by the plants even in low availability conditions of this resource in the soil. However, these authors observed that under water deficit, young *M. oleifera* individuals presented a reduction in photosynthetic rates, inducing stomatal closure as a first defense line. Araújo et al. (2016) suggest that the decrease in photosynthetic rate in this species may be more dependent on biochemical limitation than on stomatal closure. In stress conditions, the authors observed that *M. oleifera* individuals presented soluble sugar accumulation in their leaves, which are responsible for RuBisCo enzymatic activity limitation and consequently photosynthesis.

Even though *M. oleifera* is a drought-tolerant species with characteristics that allow it to survive in adverse periods, lower water availability conditions in the soil influence reproductive aspects that are important for recruitment and establishment of the species, such as seed number and quality.

Conclusions

Most of the available literature on *Moringa oleifera* focuses on its potentials and applications, where ecophysiological approaches turn to the response and tolerance mechanisms of this species to abiotic stresses. However, few so far have reported their influence on reproductive aspects under natural conditions.

The present study shows that reduced water availability interferes in reproductive aspects, these affecting seed vigor and, as a consequence, reproductive success. New information regarding environmental influence on *M. oleifera* is provided, which will serve as subsidies for later studies addressing the implication of these variations in the specie's potentiation, especially those of greater interest to the semiarid region, such as in water treatment and its nutritional value. Since water deficit interferes with seed physiological quality, as a result of a reduction in reserve deposition, their effectiveness for phytoremediation may also be altered.

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