Australian Journal of Crop Science
AJCS
AJCS 16(06):833-837 (2022) ISSN:1835-2707
doi: 10.21475/ajcs.22.16.06.p3629

Influence of precipitation on reproductive phenophases in *Moringa oleifera* Lam.

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Abstract

Phenology works as an indicator of the response of plants to local climatic and edaphic conditions, enabling a better understanding of the dynamics of the species. The seasonal climate also influences the occurrence of periodic rhythms of growth and reproduction (phenophases). *Moringa oleifera* Lam., belong to the Moringaceae family, is widely distributed in tropical countries and easily adapts to semiarid conditions, having medicinal value as a forage, flavoring, in the cosmetics, honey, fuel and in the treatment of water purification through the coagulant solution made from the seeds. The objectives of the present work were to study the phenological behavior of *M. oleifera* and to relate the variations in phenophases with climatic factors, precipitation and temperature. For collection of phenological data, ten trees were selected, and the records were carried out between the first half of August 2020 and the first half of July 2021. The observations were carried out at fortnightly intervals, recording the presence and absence of the flowering and fruiting phenophases including flowering – period in which the tree is in full flowering; fructification – which starts from the moment that small fruits are visible after the fertilization of the flowers and ends with the dispersal of seeds. The Fournier intensity index was determined using a semiquantitative interval scale of five categories (0 to 4), with an interval of 25% between each category. The phenological data of intensity of each phenophase (flowering and fruiting) were related to precipitation and average temperature fortnightly using Spearman's correlation. The flowering and fruiting phenophases of *M. oleifera* intensified in August, September and December 2020, showing Fournier intensity peaks of 47.5 and 77.5, 72.5 and 92.5, and 35 and 55 %, respectively, and April and June 2021, in the monthly intervals of greater rainfall, with percentage rates of 82.5 and 95, 77.5 and 80%, respectively. In general, we found that *M. oleifera* produced seeds between the first half of August and the first half of September, and the second half of December 2020, and the second half of April and the first half of June 2021. Thus, it can be stated that the production of fruits by *M. oleifera* is interrupted in the months with less rainfall (October to November 2020, and January to February 2021). The intensity and duration of the flowering and fruiting phenophases in *M. oleifera* are synchronized with the temporal distribution of the precipitation pulses in that habitat.

Keywords: Moringaceae. reproductive phenology. seed production. semiarid conditions.
Abbreviations: G_germination; PC_first count of normal seedlings; IVG_Germination Speed Index; U_germination uniformity (U).

Introduction

Phenology describes repetitive biological events, enabling to relate these events to biotic and abiotic factors at the population or community level (Veit et al., 2019). Therefore, the characterization of plant phenophases works as an indicator of the response of plants to local climatic and edaphic conditions. In addition, it enables a better understanding of the species' dynamics and provides scientific support on aspects of biology in its natural habitat (Beyerlein et al., 2019).
According to Silva et al. (2019), the seasonal climate also influences the occurrence of periodic rhythms of growth and reproduction (phenophases). Therefore, phenology would be one of the tools to identify the factors that influence the reproduction and survival of the species, while it is an important line of research to understand the functioning of forest ecosystems, their conservation and management (Bassaco and Nogueira, 2019).
Moringa oleifera Lam. belongs to the Moringaceae family, which is widely distributed in tropical countries. It has attacted many scientific researches due to its easy adaptation to semiarid conditions. Its wide geographic distribution is a consequence of the various forms of cultivation and its multiple uses and good adaptability (Medeiros et al., 2019). The species also has multiple uses, mainly due to its medicinal value as forage, flavoring in the cosmetics industry, honey, fuel, and in the treatment of water purification through the coagulant solution derived from seeds. This coagulant solution derived from seeds can remove waste precipitates, after contact with water from dams and ponds, providing good quality water. This water

cleaning function is realized worldwide especialy by the use of aluminum sulfate. However, moringa seeds have this function, replacing the use of this natural product instead of chemicals (Noronha et al., 2019).
Studies on Brazilian species was intensified in mid-2009, driven by its probable use in the Biodiesel Program. Dias et al. (2009) reported satisfactory results in the process of producing biodiesel from the oil extracted from moringa seeds. This is an atractive option of raw material mainly for the Northeastern states of Brazil, which do not have a tradition in soybean production.
For the production and development of this species, the temperature, seed type, saline stress (Benedito et al., 2008) and substrate (Neves et al., 2007) have already been researched. These factors can affect seedling germination and development as basic conditions to support the plant and supply nutrients, water and oxygen (Carvalho and Nakagawa 2012). In Brazil there is an effort to spread it as a vegetable rich in vitamin A, since its leaves has around 23.000 IU of vitamin A. This stands out among the well-known vegetables such as broccoli, carrots, cabbage, spinach and lettuce, which have, respectively, 5.000, 3.700, 2.200, 1.900, 1000 IU of vitamin A (Silva and Kerr, 1999).
Based on the above, this work aimed to (a) study the phenological behavior of *M. oleifera*; (b) relate the variations in phenophases with climatic factors such as precipitation and temperature; and (c) provide information for the development of recovery programs for their natural populations, through knowledge of the best seed collection time in the region and the maximization of seedling production.

Results and discussion

Recording of phenological data

The flowering and fruiting phenophases of *M. oleifera* was intensified in August, September and December 2020, showing Fournier intensity peaks of 47.5 and 77.5, 72.5 and 92.5, and 35 and 55 %, respectively, and April and June 2021, in the monthly intervals of greater rainfall, with percentage rates of 82.5 and 95, 77.5 and 80%, respectively (Figure 1). The phenophases events were occurred as soon as the precipitation pulses started in the rainy season.
Andrade et al. (2006) mentioned that the timing and magnitude of precipitation pulses were essential for ecological processes, especially with regard to soil water availability for plants and soil microbiological activity.
According to Noy-Meir (1973), the responses of physiological processes in the plant are highly dependent on this pulse.
In general, it was found that *M. oleifera* produced seeds between the first half of August and the first half of September, and in the second half of December 2020, and in the second half of April and the first half of June 2021 (Figure 1). On the other hand, only fortnightly rainfall affected fruit maturation.
As shown in Figure 2, in August/2020, at the beginning of the work, the trees were already in the reproductive phase (floral buds, anthesis and fruiting), with a high percentage of flower and fruit activity (90 and 100%, respectively), demonstrating the same phenological behavior during April/2021. However, for the flowering phenophase, the activity peak occurred in September/2020, reaching 100%.
Variations in precipitation controlled the intensity and activity indices of each phenophase, because flowering and fruiting were positively correlated with precipitation and negatively correlated with temperature (Table 1). Thus, it can be said that fruit production by *M. oleifera* is interrupted in the months of lower rainfall (October to November 2020, and January to February 2021). These results corroborate with the influence of precipitation interpulses in the reproductive phase, regulating the production intensity of the phenophases.
Seasonality related to plant phenophase is usually seen in places with a seasonal rainy climate, considering the rain pulse as an important factor in triggering phenophase (Corrêa et al., 2018).
The flowering of *Aniba rosaeodora* was occurred in the wet season. However, it was influenced by climatic variables in relation to the phenophases of the species in areas with mild seasonal factors and uniform climates (Felsemburgh et al., 2016). Such results were verified in the study by Neves et al. (2010), with three species of Jatropha being more evident in *J. mollissima* and *J. mutabilis*, where temperature strongly influenced the phenophases.

Evaluation of the physiological potential of seeds
Table 2, shows no significant differences in germination (G), first count (PC) of normal seedlings, Germination Speed Index (IVG) and germination uniformity (U), of seeds collected on 15/August, 15/September and 31/December of year 2020.
In 2021, first count (PC) of seeds collected on 30/April and 15/June showed the highest germination (82 and 80%, respectively). In 2020, the seeds collected on 15/August, 15/September and 31/December were of intermediate quality, in which 75, 78 and 77%, of the seeds germination at five days was observed, respectively (Table 2).
Thus, probably, the physiological quality of seeds in *M. oleifera* also depended on precipitation.

Materials and methods

The experiment was conducted at the Plant Propagation Laboratory, Campus of Engineering and Agricultural Sciences (CECA), Federal University of Alagoas, Rio Largo, AL, Brazil.

Recording of phenological data

For phenological monitoring, ten trees with similar size with approximately straight trunks, abundant crowns and good phytosanitary conditions were selected (apparent absence of diseases and parasite infestations) (Nunes et al., 2008).
The records of phenological data were carried out between the first half of August 2020 and the first half of July 2021.
The observations were carried out at fortnightly intervals, recording the presence and absence of flowering and fruiting phenophases. These phases were defined as follows: flowering – period in which the tree is in full bloom; fructification - starts from the moment of observation of small fruits after the fertilization of the flowers and ends with the dispersal of seeds (Lima et al., 2018). To determine the Fournier intensity index (1974), phenological data were obtained in the field, using a semiquantitative interval scale of five categories (0 to 4), with an interval of 25% between each category.

Table 1. Spearman correlation between the phenological data of intensity and activity of each phenophase of *M. oleifera* and climatic factors from August/2020 to July/2021, in the experimental area of CECA.

Phenophase	Index	Climate factor	r	P
Flowering	Intensity	Temperature	0.152	0.238
		Precipitation	-0.515	0.000*
	Activity	Temperature	0.179	0.138
		Precipitation	-0.502	0.000*
Fruiting	Intensity	Temperature	0.251	0.093
		Precipitation	-0.534	0.000*
	Activity	Temperature	0.238	0.077
		Precipitation	-0.621	0.000*

r = Spearman correlation; P = Probability; * Significant at 5% probability.

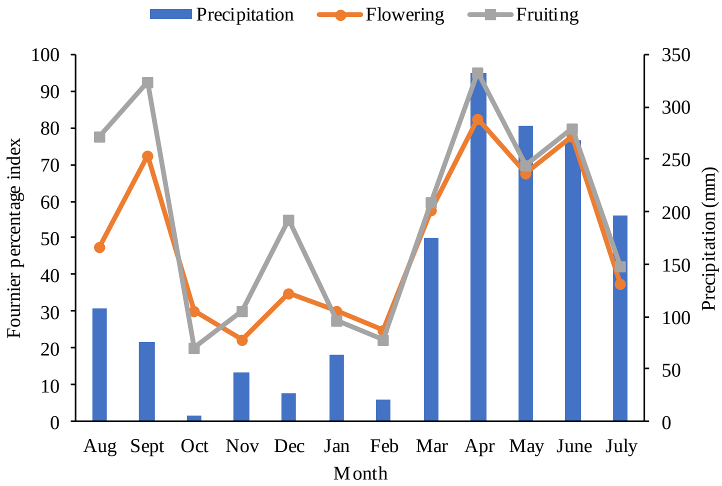

Figure 1. Phenological intensity presented by *M. oleifera* and monthly pluvial precipitation, in the experimental area of CECA (2020-2021).

Table 2. Evaluation of germination (G), first count (PC) of normal seedlings, Germination Speed Index (IVG) and germination uniformity (U), of *M. oleifera* seeds harvested at different times

Year	Month	G (%)	PC (%)	IVG	U (bit)
2020	August	84 b	75 d	23.2 c	1.3769 bc
	September	87 b	78 bc	22.4 c	1.5981 c
	December	86 b	77 cd	24.1 bc	1.1557 ab
2021	April	93 a	82 a	36.4 a	0.9315 a
	June	91 a	80 ab	27.1 b	1.0224 ab

The means followed by the same letter, in the column, do not differ statistically from each other by the Tukey test at the 5% significance level.

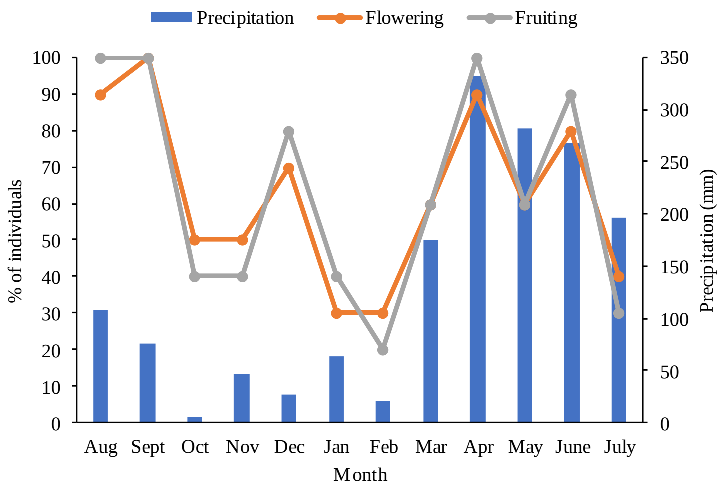

Figure 2. Phenological activity presented by *M. oleifera* and monthly pluvial precipitation, in the experimental area of CECA (2020-2021).

Every two weeks, the values obtained from all individuals were added and divided by the maximum possible value (number of individuals multiplied by four). This value corresponds to a proportion that was then multiplied by 100 to turn it into a percentage value (D´eça-Neves and Morellato, 2004).

To determine the presence or absence of phenophase and estimate the synchrony between individuals in the population, the activity index was used (Morellato et al., 1990). Every 15 days, all individuals exhibiting phenophase were summed. This number was multiplied by 100 and divided by the maximum possible value (number of individuals), and the result was obtained in percentage values.

The climatological data (August/2020 to July/2021) were provided by the Irrigation and Agrometeorology Laboratory, through the Automatic Agrometeorological Station, located in CECA, at 09º28´29,1´´S, 35º49´43.6´´W and at 127 meters of altitude.

Plant materials

To obtain the seeds, fruits were harvested from ten *Moringa oleifera* Lam. (Moringaceae) trees located in the experimental area of the CECA, located at 09º27´57´´S, 34º50´01´´W and at 127 meters of altitude. The climate type is humid coastal tropical, according to the Köppen climate classification (Cardim, 2003).

The fruits were harvested with a telescopic extension pruner at the end of the ripening period, characterized by their dark brown color, before spontaneous opening, and then kept in artificial shade (shelter protected from the sun and rain) for a few days to facilitate the extraction of seeds (Agustini et al., 2015).

The seeds were manually extracted and processed, discarding the malformed ones attacked by insects or fungi, and then placed in glass flasks and stored in a dry chamber at a temperature of ± 20 ºC and relative humidity between 50 and 55%, until the experiments are carried out (Pereira et al., 2015).

Evaluation of the physiological potential of seeds

The seeds were previously disinfected with 2% sodium hypochlorite and washed with distilled water, removing excess moisture with a paper towel (Noronha, Medeiros and Pereira, 2018).

For the germination test, the seeds were placed to germinate between two sheets of paper towels moistened with a volume of water equivalent to 2.5 times the weight of the substrate, wrapped in rolls (Brasil, 2009). The rolls were placed inside plastic bags, then kept in a Biochemical Oxygen Demand (B.O.D.) type chamber at 25 ºC, with the first count (PC) of normal seedlings performed at five and the final count at ten days, for germination evaluation (G) (Bezerra et al., 2004).

Seeds that grew to normal seedlings, with all their essential structures, were considered germinated, showing the potential to continue their development and rise to normal plants (Brasil, 2009).

The first count test was conducted in conjunction with the germination test.

For the Germination Speed Index (IVG), the count was daily until the end of the test and calculated according to the formula $IVG = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn)$ (Maguire, 1962), were: G1, G2, G3, ..., Gn = number of normal seedlings obtained in the first, second, third and last counts;

N1, N2, N3, ..., Nn = number of days between sowing and first, second, third and last counts.

To obtain the germination uniformity (U), the formula $U = -\Sigma Fr \log_2 Fr$ was used, where Fr is the germination frequency and log2 – logarithm in base 2 (Labouriau and Valadares, 1976; Labouriau, 1983).

Experimental design and statistical analysis

The phenological data of intensity and activity of each phenophase (flowering and fruiting) were related to the fortnightly precipitation and average temperature through the Spearman correlation (Zar, 1996).

The physiological potential was evaluated using the Completely Randomized Experimental Design (DIC) with four replications of 50 seeds. The PC, G, IVG and U data were submitted to the Analysis of Variance (ANOVA), and the averages were compared using the Tukey test ($p \leq 5\%$), using the Sisvar program.

Conclusions

The intensity and duration of flowering and fruiting phenophases in *M. oleifera* are synchronized with the temporal distribution of pluvial precipitation pulses in that habitat. The reproductive phenology of *M. oleifera* depends on the length and frequency of rainfall pulses during the rainy season. *M. oleifera*, in the experimental area of CECA, presents intricate ecological relationships with this environment of marked seasonality.

Acknowledgments

Thanks to the Research Coordination – CPq/PROPEP and the Institutional Program for Scientific Initiation Scholarships, in the PIBIC CNPq quota.

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