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THE PHYSIOLOGY OF CIRCADIAN RHYTMS OF PLANTS

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ABSTRACT

Studying circadian rhythms in plants is essential for understanding how they coordinate their biological processes according to the daily cycles of light and darkness. This field of research is of particular importance from several points of view. It ensures the optimization of photosynthesis and growth, since, as is known, circadian rhythms control the opening and closing of stomata, regulating gas exchange and water loss. They synchronize the activity of enzymes involved in photosynthesis with the time of day, maximizing the energy efficiency of the plant.The biological rhythms can be affected by external factors, but are not determined exclusively by them. Light, temperature and humidity can adjust the internal clock, but they do not generate it - it is endogenous. Desynchronization of biological rhythms negatively affects plant development. If the biological clock is disrupted, plants become less efficient in metabolic processes and have reduced growth.

INTRODUCTION

Plants, although they do not have a nervous system, possess complex internal regulatory mechanisms that allow them to respond to and adapt to periodic variations in their environment. These mechanisms are manifested by biological rhythms, also known as biorhythms or biological oscillations. They represent rhythmic changes in physiological, biochemical, and behavioral processes essential for survival, reproduction, and metabolic efficiency. Plant biological rhythms are largely endogenous, but can be synchronized by environmental factors such as light, temperature, or humidity.

Biological rhythms in plants constitute a fascinating field that combines physiology, genetics and ecology. Understanding these mechanisms has not only theoretical value, but also applied value, with direct implications in agriculture, forestry and biodiversity conservation. Their research can contribute to the development of biotechnologies that allow the artificial regulation of biological rhythms to optimize plant production.

Regulation of flowering and life cycle is also cyclical, with plants using the circadian clock to "measure" the length of the day (photoperiod) and decide the optimal time for flowering. This mechanism is crucial for plant adaptation to different latitudes and climatic conditions.

Stress resistance and protection against diseases can also be influenced by biological rhythms, with circadian rhythms regulating the immune responses of plants, making them more capable of fighting pathogens at certain times of the day.

They also influence protection mechanisms against abiotic stress (drought, extreme temperatures, salinity).

The classification of biological rhythms in plants can be done based on the duration of the rhythmic cycle and the factors that control them. Biological rhythms (or biorhythms) are periodic manifestations of physiological, biochemical or morphological activity that reflect the adaptation of plants to environmental cycles. Their classification is substantiated by several sources in specialized literature.

According to the duration of the rhythmic cycle (chronobiological), the most widely used classification at international level is according to Kronfeld-Schor & Dayan 2008, Taiz et al. 2015:

- Circadian ~24 hours, for example leaflet movements
- Ultradian < 20 hours - occur in rapid processes, such as root growth pulses or oscillations of calcium concentration in the cytoplasm
- Infradian > 28 hours - include monthly, seasonal or annual rhythms, such as flowering or leaf loss.
- Circannual ~1 year - takes place over the course of a year and are responsible for phenomena such as budding, flowering and fruiting of perennial plants, so they are related to the phenophases of plants
- Circalunar ~29.5 days - sensitivity of marine plants to lunar tides
- Tidal, 12.4 hours (tidal cycle) - behavior of marine plants in coastal areas

According to the nature and origin of synchronization, according to plant chronobiology studies (Bünning 1935, Halberg 2003):

- Endogenous - internally generated rhythms, independent of stimulus (circadian leaf movements, phytohormone synthesis)
- Exogenous - determined by external stimulus (light, temperature, etc.): stomatal opening depending on light

Many apparently exogenous rhythms are endogenous synchronized by external stimulus. These are called synchronized (entrained) circadian rhythms.

Arabidopsis thaliana is a model plant for the study of circadian rhythms. It has been shown that nearly 30 % of the genome is expressed in a circadian manner (Covington et al. 2008).

In soybean and wheat crops, flowering is strictly controlled by photo periods, which are circadian rhythms synchronized with day length (Dodd et al. 2005).

Knowledge of biological rhythms is vital for choosing the optimal sowing time and controlling growth (Harmer 2009).

In nyctinastic movements, leaves open during the day to capture sunlight and fold at night, resembling a "sleep" function. Meanwhile, thigmonastic movements involve more immediate reactions, such as the folding of sensitive plant leaflets when touched, which can help deter herbivores. The physiological mechanism behind these movements often involves changes in turgor pressure within specialized motor cells, allowing plants to rapidly adjust their structure in response to environmental cues. Understanding nastic movements provides insight into the adaptive strategies plants use for survival and interaction with their surroundings. (https://www.ebsco.com/research-starters/anatomy-and-physiology/nastic-movements).

All leaf movements are controlled by turgorin, a new class of phytohormones that regulate plant turgor. However, turgorin is not a genuine leaf movement factor, and there are several substances involved in this type of movement. It has also been revealed that leaf opening substances exist along with leaf closing substances in every nyctinastic plant. The leaf opening substances differ in certain aspects from indole-3-acetic acid (IAA) which is believed to induce leaf opening in nyctinastic plants. The bioactivities of the leaf opening substances were much stronger than that of IAA (Schildcknecht 1983).

An interesting circadian rhythm is observed in many orchid species, which emit fragrance only during the day or only at night. In the orchids *Phalaenopsis violacea* and *Phalaenopsis bellina* the emission of monoterpenes such as linalool and geraniol is predominant during the day. Exposure to light significantly influences this emission, and in the absence of light (in constant darkness), the emission decreases considerably, indicating an internal regulation related to the circadian rhythm and light signals (Chuang 2017).

In the orchid *Gymnadenia conopsea* the scent emission varies between day and night, correlating with the activity of pollinators. In populations in Sweden, where nocturnal pollinators are predominant, the scent emission is more intense at night. In contrast, in Norway, where diurnal pollinators are more active, the scent emission is more pronounced during the day. These differences suggest an ecological adaptation of the orchids to the types of pollinators prevalent in each region (https://academic.oup.com/aob/article/121/4/711/4816904).

Recent studies highlight that fragrance emission in orchids is a regulated process of complex interactions between genetic factors, circadian rhythm and environmental conditions. This rhythmicity ensures the efficient attraction of specific pollinators, thus contributing to the reproductive success of the plants (https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-022-03850-z).

MATERIAL AND METHODS

The intensity of the photosynthesis and transpiration process was determined with the Lci portable device that measures the respective parameter with great precision and also has the advantage that the leaves of the analyzed plants can be kept on the plant, so that at intervals of time, new determinations can be made on the same leaves. In this way, graphs can be made regarding the diurnal dynamics, but also the seasonal dynamics of this physiological process. In addition, the device also measures the temperature in the assimilation chamber, as well as the amount of water vapor and light intensity, factors that influence the vital processes of the plants.

The plants used in the experiments were: *Oxalis tetraphylla, Mimosa pudica* and *Brasavolla nodosa.*

Oxalis tetraphylla from the *Oxalidaceae* family is a bulbous perennial herbaceous plant native to Mexico. It grows up to 15 cm tall when in flower and has green clover-like leaves with dark brown spots at the base of each heart-shaped leaflet. The pink funnel-shaped flowers appear from late spring to late summer.

Mimosa pudica is part of the *Fabaceae* family and is a perennial plant that performs nyctinastic and seismonastic movements.

Brasavolla nodosa - *Orchidaceae* family, grows spontaneously in Mexico, Guatemala, Belize, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Caymans, Aruba, Curacao, Bonaire, French Guiana, Suriname, Guyana, Venezuela, Colombia and Brazil, from sea level to about 500 meters.

It is a medium-sized epiphytic or lithophytic species, with stiff, furrowed leaves. It has a pleasant citrus scent at night, and the white flowers are about 7-8 cm long and are arranged on a bracted inflorescence about 20 cm long (https://www.orchidspecies.com/brassavolanodosa.htm).

In all the studied plants, the periodicity of photosynthesis and transpiration processes was determined with the portable Lci device. In *Mimosa pudica* and *Oxalis tetraphylla*, the periodicity of nyctinastic movements was determined, and in *Brasavolla nodosa,* the emission of perfume during the night was determined.

RESULTS AND DISCUSSIONS

Rhythmicity of the photosynthesis process

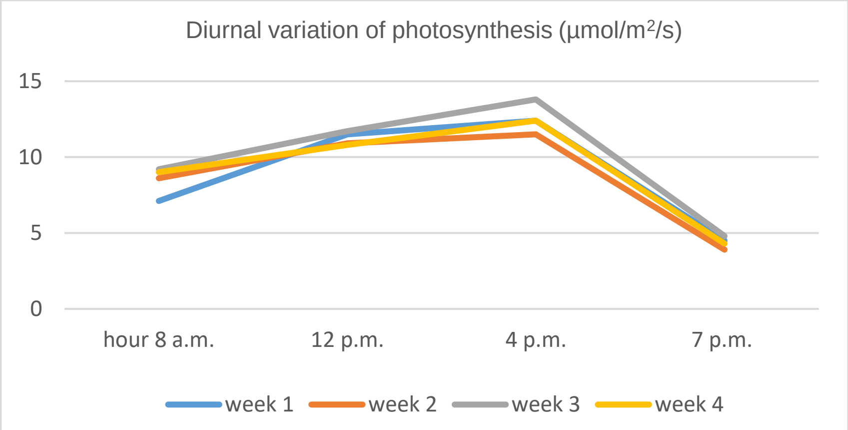
To determine the periodicity of the photosynthesis process, weekly determinations were made over a period of 4 weeks. To graphically realize the diurnal dynamics of the process, measurements were made at 8 a.m., 12 p.m., 4 p.m., 7 p.m. hours.

The photosynthesis process is influenced by several external factors, the main one being light, but the data presented also indicate periodicity determined by internal (endogenous) factors.

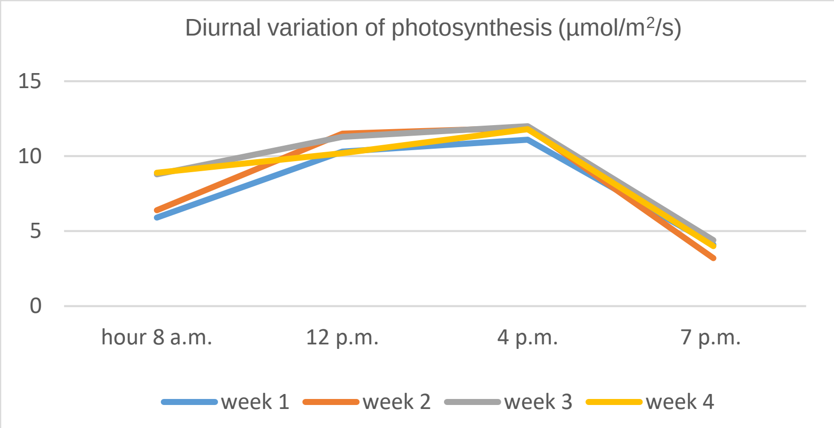
Thus, it was found that although the light intensity was maintained constant (30000 lx), starting with 7 p.m. the process significantly reduced in the case of the two species that perform nyctinastic movements. The folding of the leaves reduced the assimilation surface and thus decreased the intensity of the process until it completely ceased.

In the species *Oxalis tetraphylla* the maximum value of photosynthesis was 13.8 μmol/m^2/s in week 3 at 4 p.m. and the minimum value was 3.9 μmol/m^2/s in week 2, at 7 p.m. At 7 p.m., throughout the period, photosynthesis did not exceed 4.8 μmol/m^2/s (Graph 1).

For *Mimosa pudica*, the data were similar, but the recorded values were lower throughout the determination period (Graph 2).

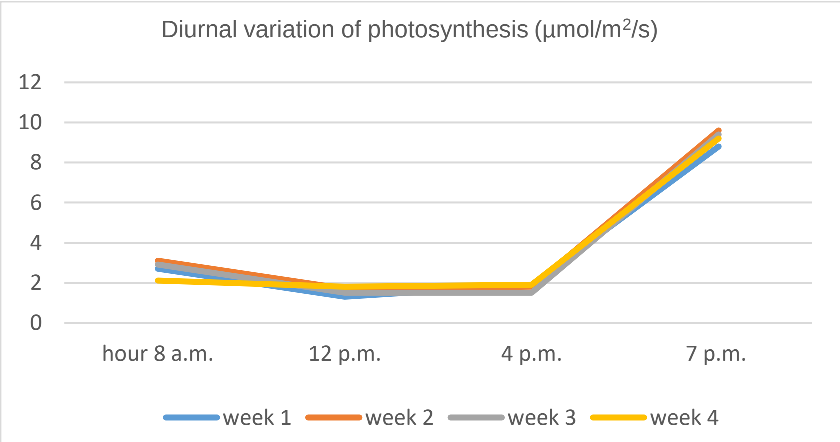


Graph 1. Diurnal variation of photosynthesis in *Oxalis tetraphylla*



Graph 2. Diurnal variation of photosynthesis in *Mimosa pudica*

In *Brasavolla nodosa*, being a plant with a CAM photosynthetic type, the stomata remain closed during the day and open towards evening, for this reason photosynthesis registers a significant reduction during the day (Graph 3). Considering that the light was present with the same intensity throughout the day, it is understood that there is a very pronounced endogenous rhythmicity that manifests itself independently of external factors.



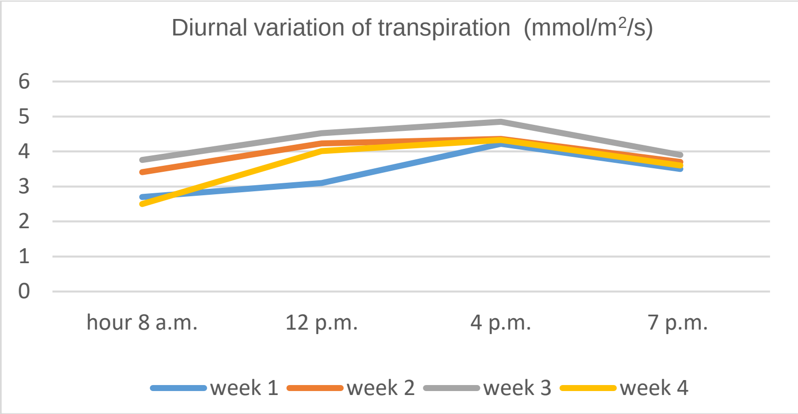
Graph 3. Diurnal variation of photosynthesis in *Brasavolla nodosa*

Rhythmicity of transpiration

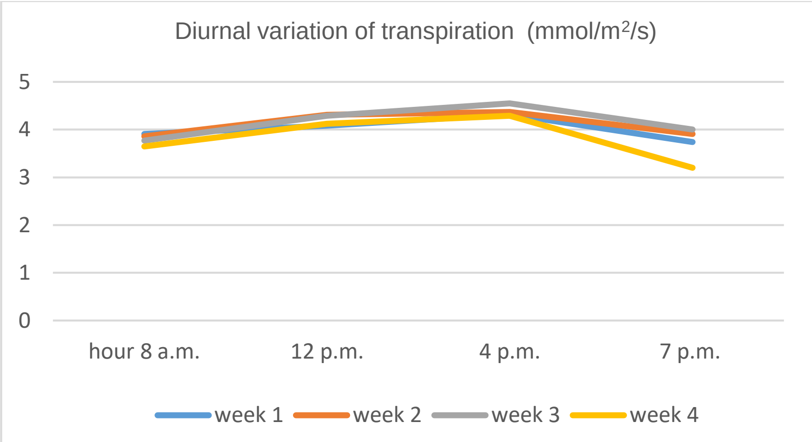
The transpiration process, being influenced mostly by the degree of stomata opening, presented a variation like the variation of the photosynthesis process in all three plant species studied.

In *Oxalis tetraphylla,* the maximum transpiration had a value of 4.85 mmol/m^2/s and was recorded around 4 p.m. (Graph 4).

In *Mimosa pudica*, with small variations during the determination period, the maximum transpiration had an average value of 4.55 mmol/m^2/s (Graph 5).



Graph 4. Diurnal variation of transpiration in *Oxalis tetraphylla*



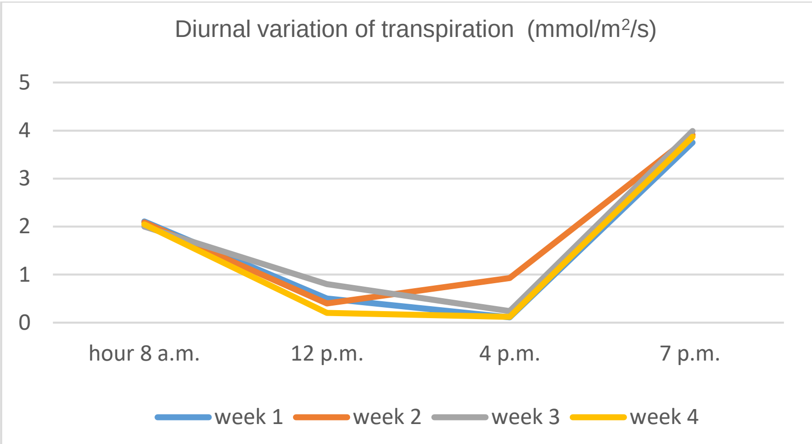
Graph 5. Diurnal variation of transpiration in *Mimosa pudica*

Brasavolla nodosa, keeping its stomata closed during the day, had the maximum value of the process at 7 p.m., which was 3.99 mmol/m^2/s. The last determination was made at this time, but it is possible that the process continued to increase in intensity during the night, to decrease towards the morning, so that at 8 a.m. it had an average value of 2.51 mmol/m^2/s (Graph 6).

The endogenous rhythmicity of transpiration is determined by the cyclical variations in water loss that are determined by the plant's internal biological clock, independent of variations in external factors.

Stomata cells rhythmically change their osmotic pressure, which causes the opening or closing of the stomata. These changes are internally coordinated, and not just by light or moisture.

Plant transpiration exhibits a clear endogenous rhythm, determined by the internal biological clock and regulated by hormonal and physiological factors. This rhythm persists even in the absence of external variations, demonstrating that plants have well-established internal mechanisms for regulating water loss.



Graph 6. Diurnal variation of transpiration in *Brasavolla nodosa*

Rhythmicity of nictinastic movements and scent emission

The nyctinastic movement of leaflet folding in the species *Mimosa pudica* was not determined by external factors, because throughout the period of determination, it was performed around 7 p.m. hours independently of light intensity. Opening was performed before 8 a.m. hours each morning, demonstrating that the movement is determined by endogenous, genetic or hormonal factors.

The same phenomenon was observed in *Oxalis tetraphylla*, with differences in leaf closure being about 30 minutes, in the evening around 7.30 p.m. and in the morning around 7 a.m. Given that the light intensity was relatively constant, it can be concluded that rhythmicity also has an endogenous determinism.

Regarding the emission of the perfume at *Brasavolla nodosa*, it began every day at 6 p.m. and ended completely around 7 a.m., even though the light source was not interrupted during the night.

CONCLUSIONS

Rhythmicity in plants is determined mainly by endogenous factors and to a lesser extent by exogenous ones, represented by environmental factors - light and temperature.

The fact that endogenous factors are determinants is demonstrated by observations made on plants that grow in the same habitat and in which the transition from the vegetative to the generative stage is carried out at the same time.

Under the same temperature and light conditions, the opening of flowers occurs in different species at different times. This demonstrates that there is an internal "clock" of each species, formed during its evolution over time

Temperature and light can exert a moderate influence on this rhythmicity, in the sense that variations in the time interval in which it occurs may occur, but they cannot suppress or amplify rhythmic movements.

REFERENCES

Bünning E. 1935. The Endogenous Daily Rhythms and their Significance for Physiology. Zeitschrift für Botanik.

Chuang Yu-Chen, Lee Ming-Chi, Chang Ya-Lan, Chen Wen-Huei, Chen Hong-Hwa. 2017. Diurnal regulation of the floral scent emission by light and circadian rhythm in the Phalaenopsis orchids. https://pubmed.ncbi.nlm.nih.gov/29143225

Covington M. F., Maloof J. N., Straume M., Kay S. A., Harmer S. L. 2008. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. Genome Biology, 9(8).

Dodd A. N., Salathia N., Hall A., Kevei E., Tóth R., Nagy F., Webb A. A. 2005. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science, 309(5734), 630-633.

Halberg F. 2003. Chronobiology: Principles and Applications to Shifts in Schedules. In: Handbook of Chronobiology. https://www.researchgate.net/publication/281196685_General_Principles_of_Chronobiology

Harmer S. L. 2009. The circadian system in higher plants. Annual Review of Plant Biology, 60, 357-377. https://doi.org/10.1146/annurev.arplant.043008.092054

Kronfeld-Schor N., Dayan T. 2008. Activity patterns of rodents: Circadian rhythms and the influence of season and habitat. Biological Rhythm Research. https://www.researchgate.net/publication/233434751_Activity_patterns_of_rodents_The_physiological_ecology_of_biological_rhythms

Schildcknecht H. Angew. 1983. Chem. Int. Ed. Engl., 22, 695-710. https://link.springer.com/chapter/10.1007/978-3-642-71425-2_19

Taiz L., Zeiger E., Møller I. M., Murphy A. 2015. Plant Physiology and Development. Sinauer Associates. https: //www.researchgate.net/publication/325735135 Plant Physiology and Development

https://www.ebsco.com/research-starters/anatomy-and-physiology/nastic-movements

https://academic.oup.com/aob/article/121/4/711/4816904

https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-022-03850-z