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A study examining the effects of diethyl ether on the photosynthetic activity of *Dionaea muscipula*

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Abstract
Research on photosynthetic activity in plants exposed to chemical stress is gaining importance in plant physiology. Studying how plants respond to anaesthetics has practical implications for comparative biology, plant neurobiology, and experimental medicine. This study assessed changes in photosystem II (PSII) efficiency using fluorescence measurements taken before and during exposure to diethyl ether. The methodology was used to identify any physiological effects of diethyl ether on photosynthetic processes, based on the hypothesis that anaesthetics could influence both plant and animal signal transmission mechanisms. In sensitive plants such as *Dionaea muscipula*, mechanical stimuli trigger action potentials that travel through the plant and cause the trap to close. Exposure to diethyl ether inhibits the transmission of these electrical signals, preventing the plant from responding to touch. This process results in a temporary suppression of the plant's signalling pathways. The present study aimed to examine the impact of diethyl ether on the photosynthetic activity of *Dionaea muscipula*, a plant species distinguished by its sensitivity and complex responses to external stimuli. Throughout the experiment, physiological and behavioural changes resulting from exposure to this volatile compound were evaluated to elucidate how plants respond to acute chemical stress. The findings clearly demonstrate that diethyl ether functions as a specific inhibitory agent, producing discernible and measurable effects on both the photosynthetic apparatus and plant communication systems in *Dionaea muscipula*. Because *Dionaea muscipula* exhibits a rapid response comparable to systems found certain animal species, it is relevant to examine the effects of a volatile anaesthetic like diethyl ether on these processes.

Keywords: photosynthesis, carnivorous plant, fluorescence, stress

Introduction

Model organisms are non-human species that scientists use in the laboratory to investigate and understand biological processes. The choice of *Dionaea muscipula* as a model organism in the study of the reduction of the quantum yield of photosystem II (ΦPSII) is justified by several physiological and adaptive aspects specific to this plant. Since *Dionaea muscipula* lives in oligotrophic habitats, the efficiency of photosystem II becomes essential for survival.

Recent studies have shown that biotic stress (such as prey digestion) or abiotic stress (such as the application of anaesthetic substances) can induce a transient inhibition of the quantum yield of PSII in the trap leaves, suggesting a high sensitivity of this photosynthetic system [13,14,15,17,18,19]. Thus, *Dionaea muscipula* represents an appropriate model system for investigating the interaction between physiological stress and photosystem II efficiency.

Dionaea muscipula belongs to the *Kingdom Plantae*, *Phylum Magnoliophyta* (Angiosperms), Class *Magnoliopsida* (Dicotyledons), Order *Caryophyllales*, Family *Droseraceae*, and Genus *Dionaea*. It is the only species in its genus, making it taxonomically unique. *Dionaea muscipula*, commonly known as the *Venus flytrap*, is one of the most recognized and studied carnivorous plants, a truly remarkable species in the plant kingdom. This species is endemic to the wetlands of the southeastern United States, particularly in North and South Carolina. Its evolutionary adaptation to a harsh environment has led to the development of specialized structures for capturing insects [4,5,16]. These responses are based on electrical impulses (action potentials), similar to those found in animal nerve cells, a phenomenon known as plant excitability [2,3].

Plant excitability represents the ability of certain plant cells or tissues to generate, conduct, and respond to stimuli through electrochemical impulses called action potentials. This phenomenon occurs especially in plants sensitive to mechanical stimuli, light, or temperature variations, involving rapid changes in the cell membrane potential similar to those observed in animal nerve cells [6].

Carnivorous plants such as *Dionaea muscipula* exhibit particularly pronounced plant excitability, using electrical impulses to trigger rapid movements, such as trap closure. These action potentials are generated by the opening and closing of ion channels for Ca^{2+}, K^+, and Cl^- in response to the touch of sensitive trigger hairs [7].

This action potential is generated by ion fluxes controlled through specific membrane channels, particularly those for calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-). Initially, the influx of calcium ions into the cytoplasm causes depolarization of the cell membrane. This is followed by the opening of potassium and chloride channels, which facilitate membrane repolarization. These rapid electrochemical changes induce a physiological reaction in the pulvinus tissue at the base of the trap, resulting in the instant closure of the two lobes and the capture of the prey [8].

The choice of *Dionaea muscipula* as a model organism in this study is based on a series of morphological, physiological, and bioelectrical characteristics that clearly distinguish it from other plant species. This plant integrates complex functions - photosynthetic, sensory, and motor - into a single structure, making it a unique model for investigating the relationship between bioelectrical signaling and the functioning of photosystem II.

The absorption and distribution of volatile substances in plant organisms, such as diethyl ether, occurs predominantly through passive mechanisms, among which gas diffusion plays a central role. This form of transport does not require energy consumption by the plant, as it relies on the spontaneous movement of gas molecules from an area of higher partial pressure to one of lower partial pressure. The internal structure of plant tissues contributes significantly to the efficiency of this process, through the presence of intercellular spaces (aerenchyma), which act as channel networks for gas movement.

These air spaces are located mainly in the mesophyll of leaves and in the cortex of underground organs, with an essential role in the dynamics of gas exchange between plants and the environment. Physiologically, they allow the entry of carbon dioxide (CO_2) needed for photosynthesis and the diffusion of oxygen (O_2) produced as a result, as well as the elimination of water vapor through transpiration. In the presence of volatile anaesthetic substances, such as diethyl ether, these spaces facilitate the penetration of the compound into the tissues, where it can reach the vicinity of cellular organelles, including chloroplasts [9].

In the case of *Dionaea muscipula*, diethyl ether directly affects the excitable tissues, which are essential for triggering the rapid movements of the trap. These spectacular movements are the result of a complex mechanism of electrical excitation and rapid changes in turgor pressure in the involved cells.

The main goal of this work is to investigate how substances with anaesthetic potential, such as diethyl ether, influence photosynthetic activity and excitability in carnivorous plants, especially *Dionaea muscipula.* This species is recognized for its unique ability to respond rapidly to mechanical stimuli, a behaviour rarely found in the plant kingdom but extremely relevant for the study of plant excitability.

Material and Methods

For an accurate assessment of the effect of ether on the photosynthetic system, an essential step is the analysis of chlorophyll fluorescence indicators. These indicators faithfully reflect the general physiological state of the plant and allow the identification of functional changes in PSII as a result of exposure to diethyl ether [10].

Assessment of fluorescence indicators in the context of diethyl ether application:

a) Fs represents the steady-state level of chlorophyll fluorescence measured under constant illumination, before the application of a saturating light pulse. This parameter reflects the operating state of photosystem II under continuous light conditions and is influenced by photochemical and non-photochemical processes within the photosynthetic apparatus.
b) Fm’ is the maximum value of chlorophyll fluorescence under illuminated conditions, obtained immediately after applying a saturating light pulse in the presence of background light. This indicates the temporary closure level of the photosystem II reaction centers and allows for

the estimation of the maximum photochemical conversion capacity at the moment of measurement.

c) The effective yield of PSII under light conditions (ΦPSII) is calculated according to the formula:

$$\Phi PSII = \frac{(Fm' - Fs)}{Fm'}$$

This index expresses the proportion of photons absorbed by chlorophyll that is actually used for the photochemical reactions of photosystem II under light conditions. Its value provides direct information about the efficiency of the photochemical processes and the degree to which the photosynthetic apparatus utilizes light energy [1].

The protocol was implemented under controlled laboratory conditions, with rigorous adherence to the preparation, exposure, and analysis stages of the plants. The use of fluorimetry provided a non-invasive and precise means of monitoring the functional status of the photosystems, thus contributing to a deeper understanding of how *Dionaea muscipula* responds to chemicals that affect cellular excitability.

For the experiment, two healthy specimens of the *Dionaea muscipula* species were used, obtained commercially and previously acclimated under controlled conditions. The plants were placed in a room with diffuse natural light, a constant temperature of 22 ± 1°C, and relative humidity of approximately 60%, for a minimum period of two hours. This adaptation period was essential for stabilizing photosynthetic processes, especially the activity of photosystem II, thus avoiding sudden fluctuations in fluorescence caused by abiotic stress.

After completion of the adaptation stage, initial measurements were taken using a portable field fluorimeter with pulse modulation for chlorophyll (FMS2+ - Field Portable Pulse Modulated Chlorophyll Fluorometer) {Hansatech Instruments} a high-precision device designed for the non-invasive analysis of photosystem II (ΦPSII) activity. This equipment uses Pulse-Amplitude-Modulated (PAM) technology to accurately quantify photosynthetic parameters, allowing for the assessment of the functional state of chlorophyll in the plant's leaves. The producer is Hansatech Instruments Ltd., based at Narborough Road, Pentney, King's Lynn, Norfolk PE32 1JL, England (UK) [20].

The parameters evaluated were:

- ΦPSII (the effective quantum yield of photosystem II)
- Fm' (maximum fluorescence, after a saturating light pulse)
- Fs (steady-state fluorescence of the leaf)

The plants were then divided into two groups: Plant A (control) and Plant B (experimental variant). Plant A was kept under the same environmental conditions, without exposure to any chemical substances, so that the collected data would reflect the baseline physiological conditions of the *Dionaea muscipula* species. To ensure a faithful representation of the plant's general photosynthetic state, the analysis was carried out at eighteen distinct points on the surface of a fully developed trap leaf, avoiding damaged or incompletely formed areas. At the same time, Plant B was also measured to demonstrate PSII efficiency before diethyl ether administration. This method allowed for the capture of local variations, and the obtained values were subsequently averaged to reduce sampling errors and ensure a robust estimation of the photosystem parameters.

For each measuring point, data were collected for (Fs) and (Fm'), obtained using a saturating light pulse, as well as the effective quantum yield of photosystem II (ΦPSII). The procedure involved the firm placement of the fluorimeter's optical sensor perpendicular to the leaf surface, maintaining a constant distance to avoid signal intensity fluctuations. Measurements were performed under standardized lighting conditions, using the diffuse natural light available in the room, without artificial interference.

Plant B was designated for treatment with diethyl ether, a volatile agent known for its ability to disrupt physiological activity, including at the photosynthesis level.

The recordings were automatically saved by the instrument's software, and the calculated average values served as a reference point for comparison with subsequent measurements, after the application of the diethyl ether treatment to Plant B.

For the treatment, a watch glass with a 12 cm diameter was used, on which Plant B was placed. In its center, in a small glass container (porcelain capsule), 5 ml of diethyl ether (≥99.7%) were added. Immediately after administering the substance, the setup was hermetically covered with a glass bell jar 20 cm height, 15 cm diameter), to ensure a microclimate saturated with ether vapors. Thus, the plant was indirectly exposed to the action of diethyl ether by inhaling the vapours, without direct contact with the liquid.

After 30 minutes of exposure, the bell jar was removed and measurements with the fluorimeter were resumed on Plant B, using the same parameters and methodology as in the initial stage. In parallel, Plant A was measured again, to verify the stability of the parameters in the absence of treatment.

The data obtained before and after the treatment application were analysed comparatively. The differences in ΦPSII, Fs, and Fm’ values between the control and treated plants were interpreted in the context of the anaesthetic effect of diethyl ether on plant excitability and the temporary inhibition of photosynthetic electron transport.

Results and Discussion

The results obtained from measurements taken at approximately the same points on the plants, before the application of diethyl ether, were carefully analysed.

Before any intervention, Plant A demonstrated an optimal physiological state, validated by the chlorophyll fluorescence parameters. The average value of the effective quantum yield of photosystem II (ΦPSII), a key indicator of photosynthetic efficiency, was 0.66 - a high and stable value, perfectly aligned with the standards for healthy plants under light conditions [11].

Additionally, the steady-state fluorescence (Fs) had an average value of 656.11 relative units, while the maximum fluorescence in light (Fm′) reached 2120.56 relative units. This combination of parameters confirms an efficient use of light energy and the structural integrity of the photosynthetic apparatus, with minimal energy dissipation through non-photochemical processes.

The values in Table 1 indicate that, prior to the administration of diethyl ether, Plant B was also in an optimal physiological state. This conclusion is supported by the average values of the chlorophyll fluorescence parameters: the effective quantum yield of photosystem II (ΦPSII) was 0.65, a high value specific to a healthy plant. Furthermore, the steady-state fluorescence (Fs) was 658.33 relative units, and the maximum fluorescence in light (Fm′) was 2121.11 relative units, confirming high efficiency in the use of light energy.

Table 1. Results regarding the effective quantum yield of photosystem II (ΦPSII), Fs and Fm`, before and after exposure to organic solvents (relative units)

Dionaea muscipula	ΦPSII ($\bar{X} \pm S_{\bar{x}}$)	Fs ($\bar{X} \pm S_{\bar{x}}$)	Fm` ($\bar{X} \pm S_{\bar{x}}$)
Plant A (control)	0.66 ± 0.01	656.11 ± 15.33	2120.56 ± 32.05
Plant A` (water-based solvent as treatment)	0.63 ± 0.02	662.22 ± 14.26	2084.44 ± 34.19
Plant B (control)	0.65 ± 0.02	658.33 ± 16.41	2121.11 ± 30.16
Plant B` (after treatment with diethyl ether)	0.37 ± 0.02	1003.89 ± 56.78	1500 ± 94.34

The results obtained after testing the PA’ specimen, following its exposure to the water-based organic solvent, demonstrated the maintenance of an optimal physiological state, with minor fluctuations considered normal. The average data shows a value for the quantum yield of photosystem II (ΦPSII) of 0.63 relative units which, although slightly lower than the initial value (0.66), falls within the normal range for a healthy plant. The steady-state fluorescence (Fs) was 662.22 relative units, while the maximum fluorescence in light (Fm′) reached 2084.44 relative units, confirming that the water did not induce significant stress but rather helped maintain the plant’s internal balance (Fig. 1, 2).

By stabilizing plant, A as a control, in which the photosynthetic parameters ΦPSII, Fs, and Fm’ remained almost unchanged throughout the observation period, it was possible to rule out the possibility that any observed changes were caused by nonspecific factors.

Results obtained from testing the PB’ specimen, after its exposure to the organic solvent (diethyl ether), indicate a severe negative impact on the photosynthetic process. The average value of the effective quantum yield of photosystem II (ΦPSII) dropped dramatically to 0.37 relative units, indicating profound inhibition of electron transport. At the same time, steady-state fluorescence (Fs) increased massively to an average of 1003.89 relative units. Additionally, maximum fluorescence in light (Fm′) decreased significantly to 1500 relative units, suggesting structural deterioration of PSII and an intensification of energy dissipation mechanisms as a response to the stress induced by diethyl ether.

Plants A and B initially exhibited almost identical physiological characteristics, with the fluorescence index values suggesting maximal and equivalent photosynthetic functionality.

A comparative analysis of the data for plant B highlights a severe decrease in photosynthetic activity. Compared to the initial values of ΦPSII (0.65 relative units) and Fs (658.33 relative units), there is a pronounced reduction in the effective yield of photosystem II, dropping to an average of 0.37 for ΦPSII, concurrently with a significant increase in steady-state fluorescence, reaching 1003.89 relative units (Fig. 3).

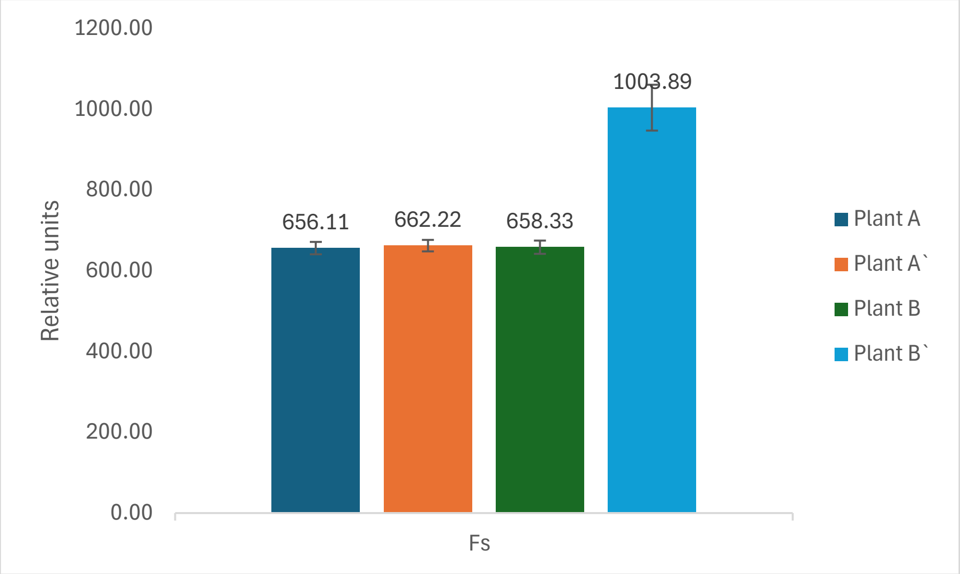

Figure 1. Results regarding steady-state fluorescence (Fs) of the leaf in *Dionaea muscipula* (relative units)

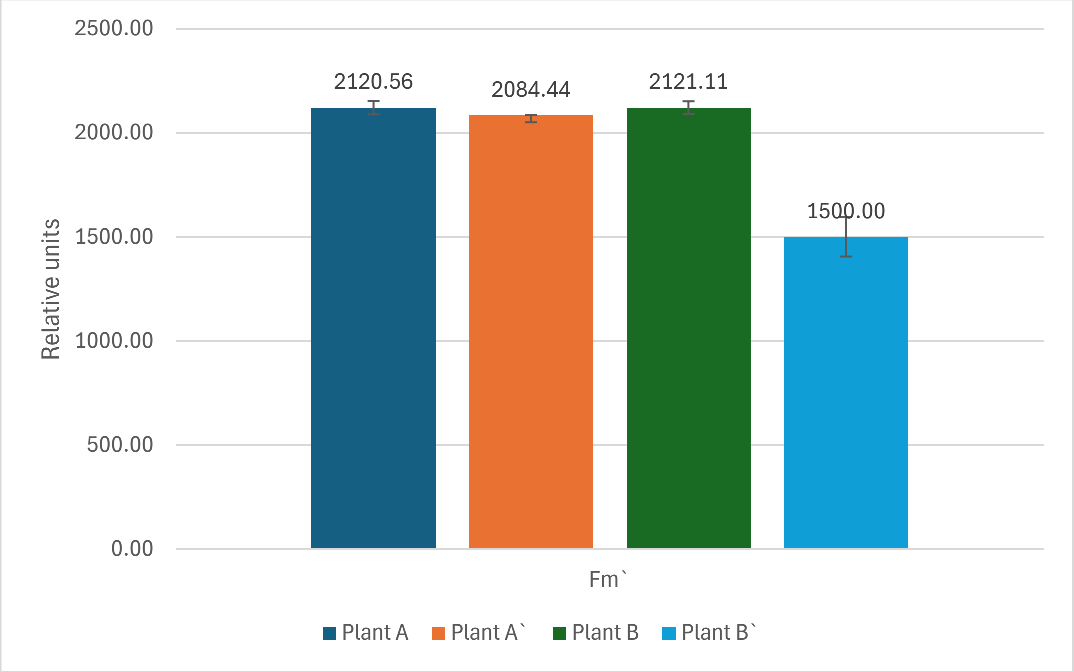

Figure 2. Results regarding maximum fluorescence after a saturated light pulse (Fm`) in *Dionaea muscipula* (relative units)

This direct relationship between diethyl ether exposure and the changes in measured parameters indicates that the anaesthetic substance profoundly affected electron flow, inhibiting photochemical processes and causing the excess light energy to be dissipated as fluorescence and heat - a protective mechanism typical of a stress response, which confirms the compound's inhibitory effect. Overall, these changes underscore a considerable impact of ether on photosystem II functionality in plant B.

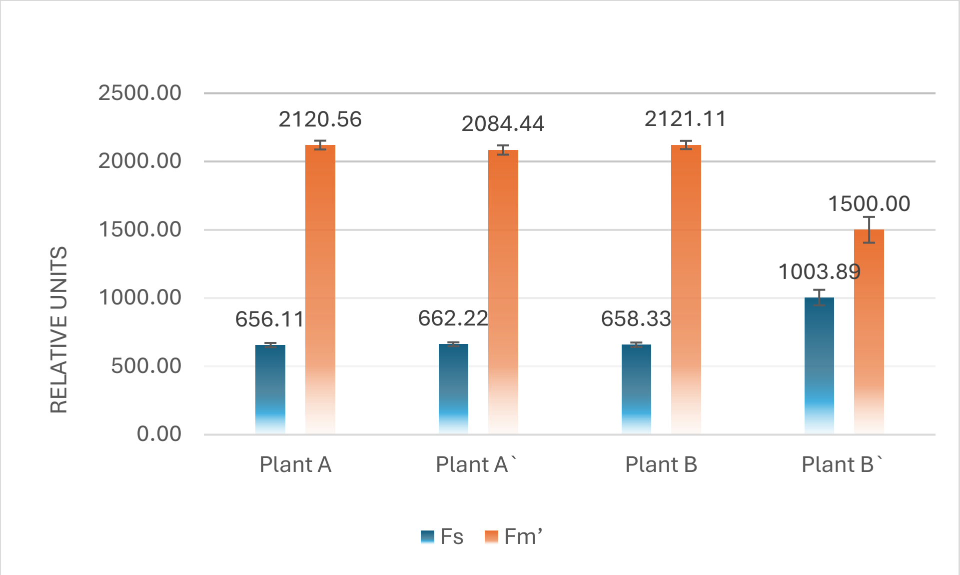

Figure 3. Experimental results regarding fluorescence before and after a saturated light pulse (Fs and Fm`) in *Dionaea muscipula*

The reduction in ΦPSII suggests a decreased efficiency of photophosphorylation and conversion of light energy into chemical energy, while the increase in Fs and decrease in Fm reflect an accumulation of unused energy, which is dissipated as fluorescence and heat. These changes constitute a physiological stress response, demonstrating that diethyl ether acts as a potent inhibitory agent of photosynthesis, disrupting photoprotective mechanisms and affecting the normal functioning of photosystem II.

These variations cannot be explained by natural fluctuations or environmental influences, but rather reflect the plant’s direct reaction to diethyl ether exposure. Plant A remains a physiological reference point, while the changes observed in Plant B provide clear evidence of the effect exerted by ether on the photosynthetic process.

The average values obtained are consistent with the data in the literature for *Dionaea muscipula* under optimal physiological conditions [12]. The effective quantum yield of photosystem II, denoted as ΦPSII, for a healthy *Dionaea muscipula* plant generally falls within the range of 0.40 – 0.70 relative units, when measured under optimal lighting conditions. This value indicates the proportion of absorbed light energy that is effectively used in the electron transport process during the light phase of photosynthesis.

In addition to fluorescence parameters, leaf temperature values were also recorded at the time of measurement using an infrared thermometer: the average surface temperature of the trap was 21.7 °C. This value confirms that the plant was not subjected to thermal stress and that the light energy did not cause local overheating, which could have altered chlorophyll fluorescence.

All measurements were conducted in the absence of wind, air currents, or other disruptive environmental factors. Furthermore, the leaves were gently cleaned with a sterile tweezer before sensor placement to remove any dust particles or other impurities that could have affected the accuracy of the recorded data.

The measurements performed prior to diethyl ether administration indicate robust photosynthetic activity, with no evident signs of abiotic stress or physiological deterioration. These results provide a solid reference point for analysing changes induced by subsequent treatment, while also ensuring reproducibility and validity of experimental interpretation.

Fig. 4 highlights the changes recorded both before and immediately after treatment application, thereby facilitating a clear visual interpretation of the influence of the investigated compound. The presented data support the hypothesis that diethyl ether has a significant impact on photosynthesis, disrupting Photosystem II function.

In the case of Plant A, after the application of the water-based solvent as treatment, photosynthetic efficiency (ΦPSII = 0.63 relative units) remained virtually unchanged, indicating physiological stability and confirming that treatment with a neutral solvent does not affect photosystem II function.

In contrast, Plant B, exposed to diethyl ether, showed a severe inhibition of photosynthesis, evident in the marked decrease of ΦPSII to 0.37 relative units. This significant difference suggests that ether acts as a specific inhibitor of Photosystem II, disrupting the electron flow in the transport chain and reducing the plant's ability to convert light energy into chemical energy.

The values obtained, 0.66 for Plant A and 0.63 relative units for Plant A', indicate high and relatively constant photosynthetic efficiency between the two samples. The minimal difference between the two values suggests that the plants remained within normal physiological parameters, without being subjected to significant stress that could affect the processes of capturing and converting light energy.

From a biological perspective, the maintenance of similar values reflects the functional stability of PSII and a good adaptation of the plant organism to the experimental conditions. Thus, it can be concluded that plants in this category retain their physiological integrity, and the mechanisms for utilizing light energy remain active and efficient.

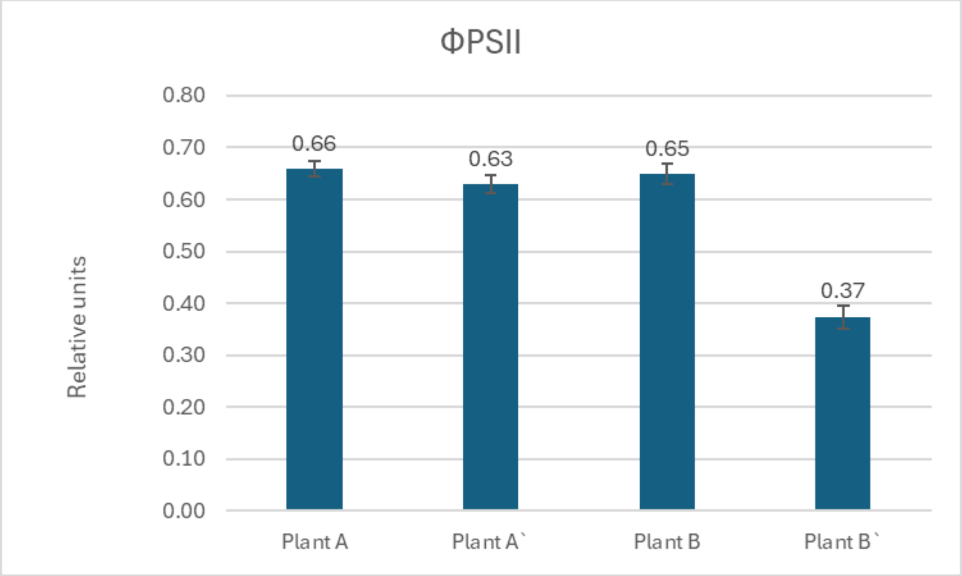

Figure 4. Experimental results regarding the quantum yield of photosystem II (ΦPSII), before and after treatment with organic solvents (relative units)

Conclusions

The obtained data highlight how experimentally induced chemical stress influences plant signalling processes and provide a useful reference point for comparing excitability mechanisms found in plant and animal organisms.

Investigations into the impact of diethyl ether on photosynthetic processes in *Dionaea muscipula* have clearly shown that this volatile substance acts as a selective inhibitor, producing measurable changes both at the level of the photosynthetic apparatus and in the dynamics of the plant's internal signalling systems.

The significant reduction in fluorescence values, alongside the complete blockage of the trap-closing mechanism, indicates a profound inhibition of the plant's essential physiological functions, analogous to the effects of anaesthetics on animal neural networks. Under normal physiological conditions, the value of this parameter (ΦPSII) remains stable in healthy specimens, reflecting an optimal balance between light absorption and its conversion into usable chemical energy.

Diethyl ether clearly influences two essential components of plant functioning: metabolic activity, which is reflected in photosynthetic efficiency and can be evaluated by the Fs and Fm' parameters, and motor behaviour, evident in the trap's ability to close, determined by the initiation and propagation of action potentials.

Thus, the possibility that the recorded effects are caused by nonspecific factors, such as experimental stress or environmental fluctuations, is ruled out, since both the control specimens and those treated with the solvent maintained complete functionality throughout the duration of the experiment.

Therefore, the plant's response to diethyl ether goes beyond a simple, localized inhibition, representing a systemic suppression of vital functions that is functionally similar to the effects of general anaesthesia observed in animal organisms.

Support for this hypothesis is based on both experimental observations, as indicated by the ΦPSII, Fs, and Fm' values and the absence of response to stimuli, and on the specialized literature, which increasingly emphasizes plants' ability to generate, transmit, and process signals in a manner comparable to the functioning of primitive neural networks.

This study makes a substantial contribution to clarifying how chemical stress factors influence the vital functions of plants, highlighting the interdependence between physiological processes and behavioral responses. By analyzing the effects of diethyl ether on mechanical excitability and photosynthetic activity in *Dionaea muscipula*, the research provides an integrative perspective on the complexity of plant signaling and adaptation mechanisms, emphasizing the sophistication of internal systems for perception, transmission, and coordination of responses to chemical stress factors.

This research makes a significant contribution to the understanding of the interaction between chemical stress and essential plant functions, offering a new perspective on the complexity of physiological and behavioral responses in the plant world.

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