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*Corresponding author

Pimentel, Carlos, Department of Crop Science, Federal Rural University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

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Key Words

Chlorophyll; Fluorescence; Visible region; Discriminate; Environmental stress; Light energy; Water

Abbreviations

PSI: Photosystem; ETR: Electron Transport Rate; LSPC: Leaf Soluble Protein Content

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Mini-Review

A New Application of Fluorescence Measurements to Discriminate the Behavior of Plants under Environmental Stress

Pimentel, Carlos*

Department of Crop Science, Federal Rural University of Rio de Janeiro, Brazil

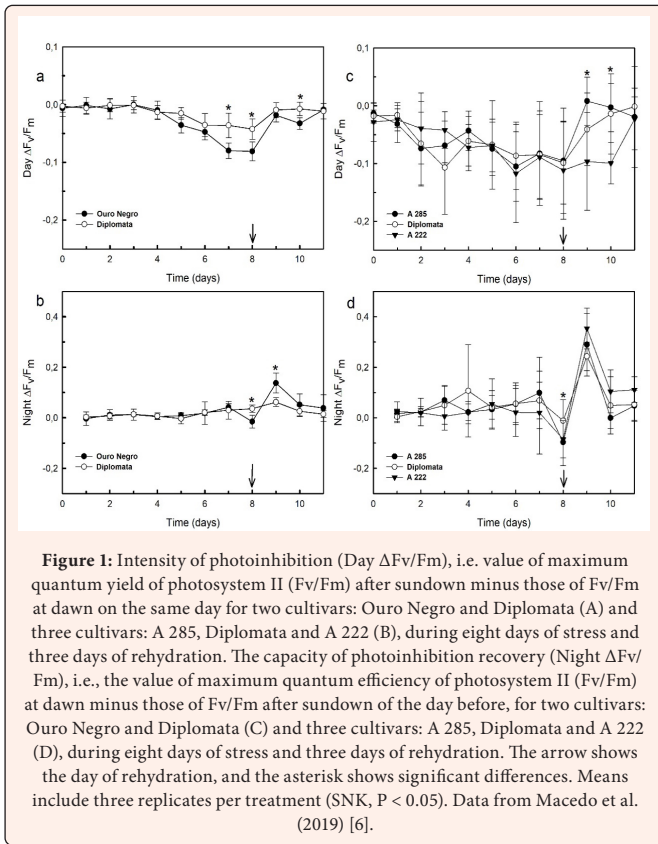
Introduction

Chlorophyll a fluorescence is the emission of radiation in the visible region (red and far red) from the return of the excited state of chlorophyll a molecules to their basal state after receiving light energy ($\lambda = 0,690 \mu\text{m}$ for photosystem II (PS II) and $\lambda = 0,740 \mu\text{m}$ for Photosystem I [PSI]) [1]. The light energy absorbed by chlorophyll molecules can be used for photosynthesis, dissipated as heat, or re-emitted as fluorescence, and these processes compete with each other [2]. In this way, variations in fluorescence function are valuable tools for exploring the amount of energy absorbed to excite PSII, which is proportional to the amount of photochemical energy generated to assimilate CO_2 [1]. Various studies have shown that chlorophyll a fluorescence analysis is an excellent tool for better understanding photosynthetic metabolism, even when plants are subjected to stress, such as high temperatures, water deficit, and other biotic and abiotic factors [3]. The chlorophyll a fluorescence analysis technique has been widely used in plant physiology and breeding studies because it is non-invasive, simple, and quick to measure [4]. The potential of fluorescence analysis data lies in its relationship with photosynthesis. The light absorbed by plants that do not drive carbohydrate production is dissipated as heat or re-emitted as light in the form of fluorescence. Plant physiologists and plant breeders have tried to relate fluorescence measurements to the specific responses of each genotype to stress [3].

Research using the chlorophyll a fluorescence analysis results in a range of variables generated by the apparatus, which enables a greater understanding of the plant's photosynthetic activity. Some of these variables stand out, such as Fv/Fm, which is obtained by adapting the plant to the dark so that all the reaction centers remain open (oxidized). It is the most frequently used indicator of photoinhibition or damage to PSII due to environmental stresses [1]. It also quantifies the maximum capacity of PSII's reaction centers, with a value between 0.75 and 0.85 found for most healthy plant species [2]. However, it is worth noting that this variable is less sensitive to variations in instantaneous environmental conditions, unlike the Electron Transport Rate (ETR), which measures the photosystems' actual photochemical activity under the actual conditions of the measurement, which varies too much, especially depending on the light intensity of the moment of the measurement. It is not of the maximum quantum yield, as with Fv/Fm [1]. Fv/Fm', on the other hand, is a variable used to measure the proportion of the light absorbed by the chlorophyll associated with PSII, which is also used in photosynthesis at the moment of the measurement. A decrease in these values is related to the closure of the reaction centers and the processes of energy extinction in the form of heat [2]. According to Zlatev (2009) [5], when observing the behavior of wheat plants under drought, he concluded that photoinhibitory damage to PSII may be a secondary effect of drought [6]. This same study revealed that the Katia wheat variety showed greater tolerance to drought regarding photosynthetic activity since the Fv/Fm parameter remained high during the drought. Of all the variables mentioned above, the maximum quantum yield of photosystem II (Fv/Fm) is the parameter most often used to indicate photoinhibition or any other type of damage caused to PSII complexes [1]. It quantifies the maximum quantum yield (capacity) of PSII by the open reaction centers. It is noticeably reduced for stressed and damaged plants, as in the case of drought [3]. Numerous studies have shown that the fluorescence a technique is sensitive enough to record the effects of drought stress and can be used to discriminate a large number of genotypes, different from the measurement of the gas exchange using an infra-red gas analyzer, which takes at least 30 minutes to have a measure [3]. According to Maxwell and Johnson (2000) [2], Fv/Fm is related to the proportion of energy absorbed by the chlorophyll molecules associated with PS II that is used in the photochemical stage, with a reduction in values being related to the closure of reaction centers and energy dissipation processes in the form of heat. Simulations of decreased maximum quantum efficiencies of CO_2 via decreased Fv/Fm predict significant effects on whole plant carbon gain [3].

Therefore, a first study was conducted with the common bean genotypes Ouro Negro, a genotype with high yield even under drought [7], commonly cultivated in Brazil, and Diplomata, a genotype that shows temperature tolerance with high yield under this condition [8]. In a second experiment, genotypes A 285 and A 222, with high stomatal control [7], were used to compare with the Diplomata genotype. They used measurements of water potential (Ψ_w), chlorophyll a fluorescence, and Leaf Soluble Protein Content (LSPC), which can be made rapidly. However, the main objective was to use the differences in dark-adapted measurements of Fv/Fm performed after sundown [9] and just before dawn to evaluate the intensity of and capacity for recovery from photoinhibition during drought, to discriminate drought tolerance between genotypes, which can be done in a large number of naturally dark-adapted plants [9].

Then, the difference between Fv/Fm after sunset minus Fv/Fm at dawn of the same day (day $\Delta\text{Fv/Fm}$) and the difference between Fv/Fm at dawn minus Fv/Fm after sunset on the previous day (night $\Delta\text{Fv/Fm}$) were evaluated [6]. The day $\Delta\text{Fv/Fm}$ was used to assess the effect of the photoinhibition during the day, and the night $\Delta\text{Fv/Fm}$ showed the capacity of recovery from the photoinhibition of the day before. Both day $\Delta\text{Fv/Fm}$ and night $\Delta\text{Fv/Fm}$ were significantly higher for the Diplomata cultivar under water stress than the other cultivars in both experiments (Figure 1).



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