Quantifying abiotic stress of plants - advantages and disadvantages of chlorophyll fluorescence

Quantificação do estresse abiótico em plantas - vantagens e desvantagens da fluorescência da clorofila

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Abstract

Fluorescence analysis has become a powerful and widely used technique among plant physiologists and ecophysiologists because its measurement bears a relationship to photosynthesis. Therefore, chlorophyll fluorescence (CF) measurements have become a method to study the functioning of the photosynthetic apparatus in response to environmental stress. Light absorbed by plants that does not drive the production of carbohydrates is dissipated as heat or re-emitted as light in the form of fluorescence. Every environmental factor deviating from the optimum constitutes a stress to plants. Consequently, any stress that affects the function of photosystem II and associated de-excitation pathways will have an effect on chlorophyll fluorescence. Measuring CF assesses the efficiency of both photochemistry and non-photochemical processes. This review uses published examples from the literature to discuss advantages and limitations of leaf chlorophyll fluorescence of photosystem II measurements to quantify and to discriminate the effects of various abiotic stresses upon plant growth and development.

Key words: plant response; environmental stressors; non-destructive plant measurement.

Resumo

A análise da fluorescência tornou-se uma técnica poderosa e amplamente utilizada entre os fisiologistas e ecofisiologistas vegetais, tendo em vista a sua relação com a fotossíntese. Assim, mensurações da fluorescência da clorofila tornou-se uma metodologia

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amplamente reconhecida para estudar o aparelho fotossintético em resposta ao estresse do ambiente. A luz absorvida e não utilizada pelas plantas na produção de fotoassimilados é dissipada na forma de calor ou reemitida na forma de fluorescência. Qualquer fator do ambiente que se desvie do ótimo constitue-se em um estresse para as plantas. Consequentemente, qualquer estresse que altere o funcionamento do fotossistema II e as rotas de excitação associadas afetará a fluorescência da clorofila. Medir a fluorescência da clorofila avalia a eficiência dos processos fotoquímico e não-fotoquímico. Esta revisão utiliza exemplos publicados na literatura para discutir vantagens e limitações das medições da fluorescência da clorofila utilizadas para quantificar e discriminar os efeitos de vários estresses abióticos sobre o crescimento e desenvolvimento das plantas.

Palavras-chave: resposta vegetal; estressores ambientais; mensuração não-destrutiva.

Introduction

Predicted increases in the atmosphere temperature and changes in the precipitation pattern (MEEHL et al., 2007) are likely to lead to novel combinations of stresses in several ecosystems worldwide that have not yet faced such stress combinations in the past in spite of the periodical changes on the environmental conditions over geological ages. Consequently, further and detailed scientific efforts are needed to anticipated plant responses to those perturbations (i.e. stress).

The classical stress concept states that every environmental factor deviating from the optimum constitutes a stress to plants. Every factor, especially those of abiotic origin, can be individually controlled under laboratory conditions while abiotic and biotic factors fluctuate simultaneously and quite often synergistically under field conditions.

Plants in nature may be exposed during their ontogeny to a wide variety of biotic and abiotic factors favorable or unfavorable. Therefore, physiological status of an organism can be indicative of its productivity and adaptability to stress (CHAPIN, 1991; COLOMBO and PARKER, 1999 apud ZARCO-TEJADA et al., 2002). The environmental factors that cause plant stress may occur within different time-scales. Therefore, non-destructive methods for initial detection of plant stress have assumed practical importance and academic interest.

Particularly, fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (MAXWELL; JOHNSON, 2000).

Plant growth depends on photosynthesis, which is affected by environmental factors such as salinity, drought, temperature and light. Stress may be apparent in morphological and physiological characteristics, which represent integrated responses to multiple environmental factors. Early detection of stress could therefore identify plant physiological condition at both spatial and temporal scales before visible effects are apparent (FLEXAS; MEDRANO, 2002).

Functioning of photosystem II (PSII) can be considered the most sensitive indicator of environmental stress in plants (BALL et al., 1994). Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus in response to environmental stress (MASSACCI et al., 2008).

Light absorbed by a leaf excites electrons at PSII which can be converted to chemical energy. If photochemistry is inefficient, energy must be dissipated as heat or emitted as chlorophyll fluorescence to avoid leaf damage. Therefore, fluorescence is highest when there is little photochemistry and heat dissipation. Thus, measuring chlorophyll fluorescence allows one to understand the efficiency of both photochemistry and non-photochemical processes (FALBEL et al., 1994).

Fluorescence as an indicator of plant stress

In order to use chlorophyll fluorescence to analyze photosynthetic effectiveness of plants is necessary to distinguish photochemical quenching from non-photochemical (or heat quenching). Such distinction can be achieved by reducing photochemical quenching to negligible levels by applying a short and high intensity flash of light to the leaf resulting in closing all PSII reaction centers. Non-photochemical quenching is not affected by the short burst of light. During the flash, maximum fluorescence (F_m) is reached because of the absence of any photochemical quenching (MULLER et al., 2001).

The efficiency of photochemical quenching (qP) can be estimated by comparing maximum fluorescence (F_m) to the steady yield of fluorescence in the presence of light (F_t) and to the yield of fluorescence in the absence of photosynthetic light (F_0). The efficiency of non-photochemical quenching is altered by various internal and external factors. Alterations in heat dissipation result in changes of maximum fluorescence (F_m). Heat dissipation cannot be totally stopped; consequently, the yield of chlorophyll fluorescence in the absence of non-photochemical quenching cannot be measured. Therefore, it is recommended to use a dark-adapted leaf to obtain estimations of non-photochemical quenching. Care must be taken with short term dark adaptation of leaves not to interfere with gas exchange between the leaf surface and the environment. The maximum efficiency of PSII (Fv/Fm) and the operating efficiency of PSII (ϕPSII) represent the capacity for photon energy absorbed by PSII to be utilized in photochemistry under dark- and light-adapted conditions, respectively (BUSCHMANN, 1995).

Any stress that affects the function of PSII and associated de-excitation pathways will have an effect on chlorophyll fluorescence because fluorescence signal is assumed to originate primarily from PSII (KRAUSE; WEIS, 1991). According to these authors, changes in chlorophyll function which occur before changes in chlorophyll content is observed results in changes in the fluorescence signal and before apparent signs are visible.

Nowadays, the increasing demands of industrial, municipal and agricultural consumption on dwindling water supplies (JOHNSON et al., 2001) have instigated the development of sustainable farming practices. The success of such practices depends in part on advancement of the current understanding of plant responses to drought stress, and the mechanisms to minimized loss of yield involved in drought-induced (SOMERVILLE; BRISCOE, 2001).

Decreases in Fv/fm are frequently observed when plants are exposed to abiotic and biotic stresses. This is such a widespread phenomenon that Fv/Fm measurements provide a simple and rapid way of stress monitoring stress. Unfortunately, the reasons for decreases in Fv/Fm stress-induced are often complex. Stressing photosynthetic tissues in the light can result in increases in non-photochemical quenching processes, which decrease Fm. According Mellis (1999), in many situations of stress, increases in non-photochemical quenching can often be accompanied by photo-inactivation of PSII reaction centers, which dissipate the excitation energy as heat rather than photochemical energy.

Possibly the most straightforward and relevant application of drought stress is through experiments where water is withheld from soil grown plants. Soil-drying techniques are generally regarded as the most practical means of approximating field drought conditions for laboratory-based research. However, difficulties from factors such as variation in leaf water content or rates of soil water loss in response to differences in plant size and soil composition may necessitate frequent monitoring and adjustment of soil water content (WHITMORE; WHALLEY, 2009).

The simplest assessment of response to drought is the plant capacity to grow and remain alive under progressively increasing water deficit conditions using survival assays and destructive analyses. These approaches may influence future measurements to compare drought performance of different plant species, genotypes, or lines and may not accurately represent the plant as a whole. In order for soil-drying experiments to yield quantifiable comparisons it is crucial that a suitable, replicable and non destructive method of assessment be used.

Measurements of stomata conductance, leaf or soil water potential, or plant relative water content provides meaningful quantitative data necessary in a detailed physiological analysis of plant drought response. However, determination of leaf water potential or leaf water content involves destructive analyses that influence future measurements. Furthermore, physical disturbance is also typically unavoidable during analysis of transpiration and soil water content (WOO et al., 2008).

The extent to which photosynthetic capability is maintained during periods of water stress may play an important role in plant adaptation to drought environment. The negative impact of drought on photosynthesis is well-documented. A decrease in photosynthetic rate is usually observed in plants grown under water stress conditions (LI et al., 2004) attributed to either a decrease in stomatal conductance and/or non-stomatal limitations (JONES, 1992; CORNIC, 2000; CORNIC and MASSACCI, 1996). According to Bolhàr-Nordenkampf et al. (1989), Bolhàr-Nordenkampf and Öquist (1993) and Baker (1993) changes in the photochemical efficiency of plants under drought conditions may be assessed by the analysis of chlorophyll *a* fluorescence efficiency associated with PSII. Results from Percival and Sheriffs (2002) with 30 woody plants following 24 hours of dehydration indicated that screening of detached leaves in vitro using chlorophyll fluorescence can provide an indication of drought tolerance.

Water stress may damage oxygen-evolving complex and reaction centers of PSII (SUBRAHMANYAM et al., 2006). In the literature, there exist contradictory reports of the direct effects of water stress on PSII functionality (GENTY et al., 1987;

COLOM and VAZZANA, 2003). However, a trend involving the progressive decline in carbon assimilation with increasing water deficit as result of limitations on stomata (FLEXAS; MEDRANO, 2002) and metabolism (CORNIC, 2000) seems to be well accepted.

Photosynthetic tolerance to high temperature, often associated with drought, may involve either preventing breakdown of PSII or allowing PSII repair processes to continue at high temperature (MURATA et al., 2007; ALLAKHVERDIEV et al., 2008; TAKAHASHI et al., 2009). Heat stress relevant to photosynthesis often occurs for short periods (SHARKEY and SCHRADER, 2006). During moderate heat stress the operating efficiency of PSII (ϕPSII) oscillates. Heat stress relevant to photosynthesis will occur almost exclusively in the presence of light (SHARKEY; ZHANG, 2010).

Furthermore, light-adapted leaves are more tolerant to heat than dark-adapted leaves according to Weis (1982) but even a brief period of darkness during leaf heating can exacerbate heat damage (SCHRADER et al., 2004). Sharkey and Zhang (2010) concluded that photosynthesis tolerance of heat is the energization of the thylakoid membrane caused by electron and proton transport.

Light is perhaps the most influential factor involved in the survival, growth and reproduction of tropical species. Plants growing in exposed sites often experience an imbalance between light absorption and photosynthetic light utilization. Photochemical reactions of photosynthesis are sensible to high irradiance condition, being affected by decreased potential and effective quantum efficiency of PSII (OSMOND, 1994).

The absorption of excessive light has the potential to lead to photo-oxidative damage (LOGAN et al., 1998) which can result in decreased photochemical efficiency of PSII and photo-inhibition (DEMMIG-ADAMS, 2003; VALLADARES et al., 2005). Under high light-conditions, a depression on PSII efficiency and an increase on antheraxanthin + zeaxanthin occurs because the amount of light absorbed can be in excess of that able to be utilized by photosynthesis (WENG et al., 2006).

Soil moisture deficit has been reported to be one of the key factors limiting plant growth and ecosystem productivity worldwide (CHAVES et al., 2003; DUURSMA et al., 2008) and is a key determinant of vegetation type, including relative abundance of grasses and woody species. Studying the fluorescence emission of tropical tree species growing in a gap of a semi-deciduous forest in Rio Claro (SP), Ribeiro et al. (2004) concluded that effective quantum efficiency of PSII (ϕPSII) was the most significant parameter to distinguish among a pioneer (*Croton floribundus* Spreng.), a secondary (*Astronium graveolens* Jacq.), and a late successional species (*Esenbeckia febrifuga* A. Juss).

Many tropical soils are millions of years old and have been exposed to continuous weathering. As a consequence, tropical soils are exposed to high levels of leaching which results in creating acidic soils. Soil acidity is usually associated with areas of high rainfall and good drainage and has different effects upon plant growth. In a pot experiment to study the adaptability of *Pinus resinosa* seedlings to soil pH levels in China, Liu et al. (2009) concluded that the highest

Fv/Fm and ϕ PSII values were measured in plants grown under soil of pH 5.5.

Changes in fluorescence measurements may be experimentally assessed on clipped branches and leaves. Conifers are better able to minimize water loss because of the xeromorphic leaf structure. Nevertheless, broadleaf wood species also can if the material is kept cool and moist after cutting (RICHARDSON; BERLYN, 2002).

Chlorophyll fluorescence of *Picea rubens* Sarg. and *Abies balsamea* (L.) Mill. trees growing on two watersheds one of which was fertilized with $CaSiO_3$ to replace the soil Ca losses of the past 50 years showed that dark-adapted ratios of variable to maximum fluorescence (F_v/F_m) were significantly greater ($p = 0.05$) in the Ca-treated watershed for both species (BOYCE, 2007).

The maximum quantum yield of primary photochemistry in dark adapted leaves is the most widely used parameter to express the physiological condition of a plant, as assessed by fluorescence. The consistency of the Fv/Fm parameter in dark adapted leaves increases the ease with which a threshold level can be defined and allows rapid screening of a large number of plants. However, the quantum efficiency of PSII (ϕPSII) open centers in light-adapted samples at 180 $\mu mol\ m^{-2}\ s^{-1}$ irradiance yielded better discrimination than with dark-adapted leaves of *Triticum aestivum* L. cultivar *Öthalom* and a landrace *Kobomugi* subjected to osmotic stress (GALLÉ et al., 2002). Naumann et al. (2007) reported significant changes in the efficiency of PSII (ϕPSII) using light adapted leaves of *Myrica cerifera* (L.) and *Phragmites australis* (Cav.) Trin. ex Steud at a salinity level of 10 $g\ l^{-1}$ prior to visible signs of salt stress.

Philip and Azlin (2005) concluded that maximum quantum efficiency of PSII (ϕPSII) offered a rapid screening technique for assessing soil compaction tolerance of *Lagestromia speciosa* a popular tree in Malaysia´s street. Additionally, measurements of fluorescence into light-adapted is necessary as dark adaptation is not practical at scales beyond leaf level.

Limitations

Although chlorophyll fluorescence (CF) is a powerful technique it is also limited. CF is characterized as an intriguing technique that can reveal information on plant responses to stressors via rapid and non-intrusive measurements. However, if CF limitations are not recognized, measurements can be misleading and/or erroneous (ADAMS; DEMMIG-ADAMS, 2004).

Absence of leaves during dormancy has limited use of CF to examine seedling physiological activity and dormancy status with temperate deciduous hardwood species (WILSON and JACOBS, 2006). However, seedlings of many hardwoods have photosynthetically active chlorophyll in stems suggesting potential for CF evaluation of this tissue (LENNARTSSON and ϕGREN, 2002; 2003; DAMESIN, 2003).

The use of fluorescence measurements to discriminate effects of environment stresses in plants may not always yield clear results. Chlorophyll fluorescence is affected in *Castanea dentata* (Marsh.) Borkh. seedlings without irrigation for 48 hours. However, the abruptness of the F_v/F_m transitions from turgor to permanent wilting point suggested a lack of resolution according to Woolery et al. (2010). In addition, a review of seventy eight experimental cases where F_V/F_M values were used to evaluate the effects of ozone in woody plants by Bussoti et al. (2011) showed that about 48% of them with no significant differences between treated and control plants where deciduous broadleaved trees represented the largest number of the cases.

Soil waterlogging is an important factor affecting the growth, development and survival o numerous plant species not only in natural ecosystems but also in agricultural and horticultural systems. The effects of soil flooding for period between 14 and 63 days on the photosynthesis of *Genipa Americana* L. seedlings under glasshouse conditions by Mielke et al. (2003) revealed no differences for the ratio between variable to initial fluorescence (F_v/F_0) as well as for the maximum quantum efficiency of PSII (ϕPSII).

Studies have shown that fluorescence measurements might be considered an efficient technique to distinguish among species. However, fluorescence measurements do not distinguish populations or progenies of native tree species when growing under a non-stressful environment. For instance, in a study that monitored photosynthesis performance during two consecutive summers of four co-occurring evergreen Mediterranean tree species growing on a south-facing rocky slope, *Pinus halepensis* exhibited higher values of photochemical efficiency of PSII (ϕPSII) than *Quercus* and *Juniperus* at midday according to Martínez-Ferri et al. (2000). Likewise, Lemos Filho et al. (2004) reported that non-significant differences were found among thirteen progenies of *Cassia ferruginea* nor among three populations of *Stryphnodendron adstringens* from the Brazilian Atlantic Rain Forest. More recently, Corcuera et al. (2011) examined variation in photosystem II activity by chlorophyll fluorescence to select genotypes for resistance to low winter temperatures of maritime pine (*Pinus pinaster* Ait.). The authors reported that variations in Fv/Fm, ϕPSII, photochemical quenching (qP) and non-photochemical quenching (NPQ) in response to winter stress were primarily due to the differences between winter conditions and sites, and secondarily due to the differences among families and their interactions with the environment.

Conclusion

Chlorophyll fluorescence has several advantages over traditional photosynthetic measurements of CO_2 assimilation and gas exchange. Using a fluorometer, assessment of CF is a rapid process, requiring only a few seconds per measurement. Therefore, not only simple evaluations of photosynthesis can be made, but also the relationship between photosynthetic efficiency, heat dissipation, and fluorescence can be assessed.

Quantification of PSII efficiency either in darkness or during exposure to light can be assessed instantaneously, as well as computed or interpreted even in the absence of a control from non-stressed plants. However, reduction in F_v/F_m (in dark adapted leaves) or F_v'/F_m' (in light adapted leaves) could be the result of an increase in photo-protective energy dissipation or a decreases in photo-chemistry. Therefore, without additional accompanying measures it is not possible to discern between these two processes.

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