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ПРОМЕНИ В ХЛОРОФИЛНАТА ФЛУОРЕСЦЕНЦИЯ НА МЛАДИ РАСТЕНИЯ ОТ ФАСУЛ ПРИ ЗАСУШАВАНЕ
DROUGHT-INDUCED CHANGES IN CHLOROPHYLL FLUORESCENCE OF YOUNG BEAN PLANTS

Златко Златев[1]*, Андон Василев[1], Василий Голцев[2], Георги Попов[1]
Zlatko Zlatev[1]*, Andon Vassilev[1], Vasilii Goltsev[2], Georgi Popov[1]

[1]Аграрен университет – Пловдив
[2] Софийски университет „Св. Климент Охридски", Биологически факултет, София
[1]Agricultural University - Plovdiv
[2]St. Kliment Ohridski University of Sofia, Faculty of Biology

***E-mail: zl_zlatev@abv.bg**

Резюме

Проучено е влиянието на почвеното засушаване върху параметрите на хлорофилната флуоресценция на фотосистема II (ФСII) в млади растения от фасул (*Phaseolus vulgaris* L.) от два сорта – „Чер Старозагорски" и „Лоди". Засушаването е приложено върху двуседмични растения чрез прекратяване на поливането за 10 дни. Установено е, че засушаването предизвиква повишаване на базовата (нулевата) (F_0) флуоресценция и понижаване на максималната(F_m) и вариабилната (F_v) флуоресценция, както и на параметъра F_v/F_m в тъмнинно адаптирани листа. В светлинно адаптирани листа е установено значително понижение на квантовия добив (Y), фотохимичното гасене (qP) и скоростта на електронния транспорт (ETR) във ФСII. Въз основа на получените експериментални резултати е установено, че в сорт „Чер Старозагорски" фотосинтетичният апарат е по-толерантен към засушаване, а в сорт „Лоди" – по-чувствителен.

Abstract

The effects of drought on chlorophyll fluorescence characteristics of photosystem II (PSII) in young bean plants (*Phaseolus vulgaris* L.) – cv. Lodi and cv. Cher Starozagorski, were studied. Drought conditions were imposed on 2-week-old plants by withholding water for 10 days. It was found that drought stress increases ground (F_0) fluorescence and decreases maximal (F_m), and variable (F_v) fluorescence, as well as F_v/F_m parameter in dark adapted leaves. In light adapted leaves a significant decrease in quantum yield (Y), photochemical quenching (qP) and electron transport rate (ETR) of PSII was occurred. In conclusion, it is considered that in cv. *Cher Starozagorski* photosynthetic apparatus is more tolerant and in cv. *Lodi* photosynthetic apparatus is more sensitive to drought.

Ключови думи: фасул, хлорофилна флуоресценция, засушаване, фотохимично гасене.
Key words: bean, chlorophyll fluorescence, drought, photochemical quenching.

INTRODUCTION

In the field plants are often exposed to various environmental stresses. Drought stress is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al., 2003). Under this stress usually a water deficit in plant tissues develops, thus leading to a significant inhibition of photosynthesis. The ability to maintain the functionality of the photosynthetic machinery under water stress, therefore, is of major importance in drought tolerance. The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration (Cornic, 1994), (Lawlor, 1995). Despite of fact that photosystem II (PSII) is highly drought resistant under conditions of water stress photosynthetic electron transport through PS II is inhibited (Chakir and Jensen, 1999), (Chen and Hsu, 1995). Several *in vivo* studies demonstrated that water deficit resulted in damages to the oxygen evolving complex of PSII (Lu and Zhang, 1999), (Skotnica et al., 2000) and to the PSII reaction centers associated with the degradation of D1 protein (Cornic, 1994; He et al.,1995).

In the last years effects of water deficit were studied on different levels: from ecophysiology to cell metabolism (Shinozaki and Yamaguchi-Shinogzaki, 1997), (Dekov et al., 2000). The range and importance of these effects depend on the genetically determined plant capacity and sensitivity, as well as on the intensity and duration of the stress, when applied alone or in combination (Bhadula et al., 1998).

The aim of this study was to determine the effects of drought stress on chlorophyll fluorescence parameters in leaves of two bean (*Phaseolus vulgaris* L.) cultivars–cv. Cher Starozagorski and cv. Lodi.

MATERIAL AND METHODS

Plant material and growth conditions

For this study young bean plants (*Phaseolus vulgaris* L.) - cv. Lodi and cv. Cher Starozagorski were used. Plants were grown as soil culture in the plastic pots, according the method described previously (Zlatev and Yordanov, 2005). The measurements were made at the end of stress period on the first trifoliate leaf, which was fully matured.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured using a pulse amplitude modulation chlorophyll fluorometer MINI-PAM (Walz, Effeltrich, Germany). Minimal fluorescence, F_0, was measured in 60 min dark-adapted leaves using weak modulated light of < 0.15 μmol m^{-2} s^{-1} and maximal fluorescence, F_m, was measured after 0.8 s saturating white light pulse (>5500 μmol m^{-2} s^{-1}) in the same leaves. Maximal variable fluorescence ($F_v=F_m–F_0$) and the photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves were calculated. In light adapted leaves steady state fluorescence yield (F_s), maximal fluorescence (F'_m) after 0.8 s saturating white light pulse (> 5500 μmol m^{-2} s^{-1}) and minimal fluorescence (F'_0) measured when actinic light was turned off, were determined. Photochemical (qP) and non-photochemical (qN) quenching parameters were calculated according to Schreiber et al. (1986), using the nomenclature of van Kooten and Snel (1990). The efficiency of electron transport as a measure of the total photochemical efficiency of PSII (Y) and the rate of electron transport (ETR) were calculated according to Genty et al. (1989).

Statistical analysis

Values are the mean ± SE from three consecutive experiments, each including at least five replications of each variant. The Student's *t*-test was used to evaluate the differences between control and stressed plants.

RESULTS AND DISCUSSION

Drought stress induces an increase in F_0 accompanied by a decrease in F_m and F_v in the first trifoliate leaf of the studied cultivars, being cv. Cher Starozagorski less affected (Table 1). An increase in F_0 is characteristic of PSII inactivation, whereas a decline in F_m and F_v may indicate the increase in a non-photochemical quenching process at or close to the reaction center (Baker and Horton, 1987).

The F_v/F_m ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves, was changed significantly in Lodi and only showed a slight tendency to a decrease in Cher Starozagorski .

Cv. Lodi presented a decrease of 43% in the proportion of energy driven to the photosynthetic pathway (qP) in the firsr trifoliate leaf, while in cv. Cher Starozagorski qP decreased with 17%. Accordingly, in cv. Lodi Y decreased strongly with 32%, while in cv. Cher Starozagorski Y was less affected (Table 2).

By the end of drought period a significant increase was observed in non-photochemical quenching (qN) in the leaves of studied cultivars, and thus denoting an increase in the energy dissipation through non-photochemical processes.

Concerning electron transport rate (ETR) the plants from studied cultivars were significantly affected and presented reduction of 29% (cv. Lodi) and 20% (cv. Cher Starozagorski).

As Baker and Horton (1987) mentioned, two distinct phenomena at least, are involved in producing the changes in the fluorescence parameters under unfavorable environmental conditions. One phenomenon results in an

Таблица 1. Параметри на хлорофилна флуоресценция в тъмнинно адаптирани листа при контролни и засушени растения от фасул

Table 1. Parameters of chlorophyll fluorescence in dark adapted leaves of control and drought stressed bean plants

	F_0	F_m	F_v	F_v/F_m
cv. Lodi				
Control	439±21	2096±93	1657±79	0.791±0.036
Droughted	553±23* (126)	1614±75* (77)	1061±51** (64)	0.657±0.031* (83)
cv. Cher Starozagorski				
Control	463±22	2168±96	1705±78	0.786±0.035
Droughted	509±24 (110)	1995±91 (92)	1486±70* (87)	0.746±0.033 (95)

* P<0.5; ** P<0.1

Таблица 2. Параметри на хлорофилна флуоресценция в светлинно адаптирани листа при контролни и засушени растения от фасул

Table 2. Parameters of chlorophyll fluorescence in light adapted leaves of control and drought stressed bean plants

	Y	**qP**	**qN**	**ETR**
cv. Lodi				
Control	0.526±0.023	0.817±0.036	0.339±0.014	126.2±5.3
Droughted	0.358±0.016** (68)	0.464±0.022** (57)	0.494±0.023** (146)	89.4±4.2** (71)
cv. Cher Starozagorski				
Control	0.495±0.024	0.838±0.038	0.355±0.017	140.7±6.6
Droughted	0.399±0.017* (81)	0.695±0.034* (83)	0.426±0.021* (120)	112.3±5.2* (80)

* P<0.5; ** P<0.1

increase in F_0, possibly due to the reduced plastoquinone acceptor (Q_A^-), being unable to be oxidized completely because of retardation of the electron flow through PSII (Yordanov et al., 2003), or to the separation of light-harvesting Chl a/b protein complexes of PSII from the PSII core complex (Cona et al., 1995). The second one is responsible for the quenching both F_v and F_m. Preferential quenching of F_v would indicate more extensive damage to the reaction centers, such that charge recombination is prevented. The drop of F_m may be associated with processes related to a decrease in the activity of the water-splitting enzyme complex and perhaps a concomitant cyclic electron transport within or around PSII (Aro et al., 1993). Gilmore and Bjцrkman (1995) have pointed out that increased non-radiative energy dissipation would be expected to be accompanied by a quenching of F_m.

In the present work the increase of F_0 and decrease of F_m under drought stress occurred concomitantly to a decrease in F_v/F_m (Table 1) in the studied cultivars. That seems to indicate, to some extent, the occurrence of chronic photoinhibition due to photoinactivation of PSII centers, possibly attributable to D1 protein damage (Campos, 1998). Photoinhibitory impact over PSII might be occurred in bean droughted leaves since a given light intensity (even at low PPFD) is potentially in greater excess under stress conditions, which usually limit photosynthetic activity. Indeed, during illumination of *Zea mays* wilted leaves, a strong inhibition of PSII efficiency was observed even under moderate PPFD (Saccardy et al., 1998). Low relative leaf water content clearly predisposes the leaves to photoinhibitory damage, and the inhibition of photosynthetic activity could in fact reflect an inactivation of PSII activity and the concomitant uncoupling of non-cyclic photophosphorylation, as shown in *Nerium oleander* (Bjцrkman and Powles, 1984). Down-regulation at the PSII level with inactivation of PSII RCs, progressive disconnection of the two photosystems with no effect on the capability of P700 to get oxidized is established under severe drought stress in *H. Rhodopensis* (Strasser et al., 2010). F_v/F_m reflects the maximal efficiency of excitation energy capture by "open" PSII reaction centers. A decrease in this parameter indicates down regulation of photosynthesis or photoinhibition. First trifoliate leaves showed a slight decrease in this parameter (Table 1). This is the result of a large proportion of absorbed light energy not being used by the plants in the photosynthesis process, as shown by the increase in qN (Table 2).

In the studied cultivars the occurrence of photoinhibition was further highlighted by the significant decline of quantum yield of electron transport (Y), which is a measure of the total photochemical efficiency of PSII under photosynthetic steady-state conditions. Photochemical quenching (qP) presented a similar behaviour to Y (Table 2). This means that under our experimental conditions, Y is mainly dependent on the proportion of reaction centers which are photochemically "open" (expressed by qP), rather than on the efficiency with which an absorbed photon can reach a reaction centre. Decreases in Y are associated with increases in excitation energy quenching in the PSII antennae and are generally considered indicative of "down-regulation" of electron transport (Horton et al., 1996). Consequently, the decreases in Y exhibited during drought in all the species can be taken as indicative of a physiological regulation of electron transport by increasing excitation energy quenching process in the PSII antennae.

Despite the decreases in the photochemical efficiency of PSII, cv. Cher Starozagorski presented highest qP and Y, as well as the lowest energy dissipation (qN) values. Cv. Lodi showed stronger decrease in photosynthetic capacity under water stress. These decreases may be due to a direct dehydration effect on Rubisco (Kaiser, 1987), reflecting an increase in Rubisco hydrolysis, since the amount of Rubisco largely determines photosynthesis, and/or a decline in its catalytic ability. In fact, changes in the ATP pool size (Seeman, 1989), or the tight binding of inhibitors and failure of the Rubisco activase to operate in stressed leaves (Lawlor, 2002) will decrease enzyme affinity for the substrate, and hence, influence its activity. Decreases in qP are attributable to either decreases in the rate of consumption of reductants and ATP produced from non-cyclic electron transport relative to the rate of excitation of open PSII reaction centres or damage to PSII reaction centres. The large drought-induced decreases in qP in cv. Lodi could to be due to a combination of both of these factors.

CONCLUSIONS

This study supports the contention that photodamage to PSII reaction centres is not a primary factor in the depression of CO_2 assimilation of the bean leaves induced by the water stress. However, photoinhibitory damage to PSII may be a secondary effect of drought. Our data are in accordance with the statement of Baker and Horton (1987) that the bulk of quenching in the stressed leaves is due to reversible qN processes, since Q_A was maintained in a highly reduced state throughout the quenching. PSII activity in cv. Cher Starozagorski was more efficiently protected than in the cv. Lodi, as indicated by fluorescence measurements. In conclusion, cv. Cher Starozagorski showed a higher drought tolerance in what concerns photosynthetic activity since F_v/F_m was maintained, Y and qP were significantly less affected than in the other cultivar, and it presented a lower increase in qN.

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Рецензент – доц. д-р Невена Стоева
E-mail: stoeva_au_bg@yahoo.ca