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# Monitoring of the Drought Tolerance of Various Cotton Genotypes Using Chlorophyll Fluorescence

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Additional information is available at the end of the chapter

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## Abstract

In this chapter, chlorophyll fluorescence in plant leaves of three genotypes of cotton cultivated in Uzbekistan and characterized at different degrees of drought tolerance is studied. The light and CO<sub>2</sub> responses of the chlorophyll fluorescence and the photosynthesis and possible mechanisms of adaptation of plants to moderate long-term drought are described. The chlorophyll fluorescence and various morpho-physiological indicators of well-watered and moderately drought-stressed cotton plants have been measured simultaneously over a long period of plant ontogenesis to establish direct correlations between them to estimate the magnitude of drought effect using fluorescence parameters. It is shown that determination of such correlations and their calibration by photoacoustic signals generated in plant leaves at application of low-frequency-modulated light may be used for monitoring of the drought tolerance of crops in the field.

**Keywords:** photosynthesis, chlorophyll fluorescence, cotton genotypes, drought effect

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## 1. Introduction

Drought is an important environmental stress exerting a critical effect on plants that can reduce their productivity, on average, up to 50% [1]. Approximately one third of Earth's arable land all over the world suffers from chronic water deficiency for agriculture and by various estimations; in 2050s, this area can be doubled [2]. Particularly, in Central Asia, located mostly in desert zones, the first-limiting factor of crop yield is water deficit and the agriculture can be practiced only with additional irrigation. However, the irrational use of water resources of the region

for cotton production in the past has lead to an excessive soil salinization and to the exhaustion of its largest water resource—the Aral Sea. Therefore, revealing the adaptation potential of local agricultural crops to water deficit and creating their drought-tolerant genotypes are an important task: this would allow, in particular, to obtain higher cotton yield and quality in conditions of limited water resources and to improve local environment by stopping desertification of the region.

Creating drought-tolerant genotypes of agricultural crops is complicated because the lack of systematic knowledge on physiological parameters reflecting the genetic potential for improved productivity under conditions of water deficiency. The effect of drought stress on the photosynthetic performance and drought-induced morpho-physiological, biochemical and biophysical changes in various plant species have been extensively studied; stomatal and non-stomatal limitations to photosynthesis, their role and possible mechanisms have been suggested [3]. These studies have shown that photosynthetic performance is very informative and sensitive indicator of stress effects of drought in plants.

Nowadays, the methods of chlorophyll fluorescence control along with the classical measurements of photosynthesis based on gas-exchange analysis are widely used by agronomists in monitoring of crops and their response to environmental stresses [4]. Revealing physical characteristics of chlorophyll fluorescence in plant leaves and employing achievements in laser physics, optoelectronics and computer technologies allowed developing a variety of efficient experimental methods and easy to use devices for measuring such key fluorescence parameters, as a maximal (saturated) and a minimal (dark) fluorescence, a prompt and a delayed fluorescence, a kinetics of induction of chlorophyll fluorescence and their relationship with quantitative indicators of photosynthesis in plants [5, 6]. These methods are fast, noninvasive and estimate the photosynthetic performance of plants even under mid-day solar radiation, and portable devices commercially manufactured on their basis determine the parameters of plant photosynthetic performance with multiple replication of measurements and recording the results in a memory for subsequent statistical processing using relevant computer programs [7, 8].

Here, the results of long-term effect's study of drought on the chlorophyll fluorescence and morpho-physiological indicators of cotton plants grown under field conditions are described. Literature on researches concerning to mechanisms of stress effect of drought on photosynthesis in plants are analyzed. The long-term effect of drought on cotton plants has been studied during the key period of their ontogenesis — in flowering and maturing stages from last July to last September by simultaneously measuring indicated parameters in well-watered and moderately drought-stressed plants. Correlations between the chlorophyll fluorescence and morpho-physiological indicators (leaf blade area and thickness, relative water content and transpiration) have been defined in three genotypes of cotton with different degrees of drought tolerance.

Comparative measurements of the operating quantum efficiency of photochemistry in Photosystem II,  $\Phi_{PSII}$ , and its changes during the day time in well-watered and moderately drought-stressed plants have shown that in contrary to the widely accepted idea on tight links between  $\Phi_{PSII}$  and the quantum efficiency of  $CO_2$  uptake [9], and decline of photosynthesis in

plants under drought stress [10, 11], the sustainable higher values of  $\Phi_{\text{PSII}}$  in drought-stressed plants have been registered [12, 13]. It was also defined considerable changes in morpho-physiological parameters under drought stress.

For better understanding of mechanisms of such an unexpected increase in the quantum efficiency of primary photochemistry, the chlorophyll fluorescence was measured simultaneously with the gas-exchange analysis at different light intensities and  $\text{CO}_2$  concentrations [14]. Drought-stressed plants displayed elevated rates of photorespiration playing a protective role in conditions of water deficit, when plants can gradually adapt to such a stress, regulating various phases of photosynthetic reactions.

The measurement of photoacoustic waves generated in plant leaves on application of a modulated light simultaneously with the chlorophyll fluorescence allowed us to determine quantitatively the magnitude of photosynthetic oxygen evolution. This has an especial importance in the case of elevated photorespiration, when tight links between  $\Phi_{\text{PSII}}$  and the quantum efficiency of the  $\text{CO}_2$  uptake is broken. Photobaric component of the photoacoustic waves at low-modulation frequencies (~10 Hz) originated in the photosynthetic oxygen evolution process [15, 16], as quantitative indicator of the photosynthetic performance of plants, may be used for the calibration of the values  $\Phi_{\text{PSII}}$  determined in chlorophyll fluorescence measurements.

In this way, the chlorophyll fluorescence parameters measured simultaneously with morpho-physiological indicators of plants proposed for monitoring of the drought tolerance of various cotton genotypes in the field that can be applied in the practice of a plant breeding.

## 2. Materials and methods

Three local genotypes of cotton (*Gossypium hirsutum* L.), Navbakhor, Liniya-49, and Gulsara were grown on the two levels of irrigation: under well-watered and moderately drought-stressed conditions [17] at the experimental cotton station of the Institute of Genetics and Plant Experimental Biology, Uzbekistan Academy of Sciences, Tashkent (41°10'N, 69°07'E, 400 m above sea level), in 2013–2014. All plants were sown on 10th April with the scheme of 90 cm (distance between rows) × 20 cm (distance between plants) × 1 (amount of plants per hole). Thousand plants of each genotype and water treatment were grown in 4 rows, 250 plants each. During the entire period of ontogenesis, well-watered plants were irrigated 5 times: 1–before flowering, 3–during flowering-maturing, and 1–in maturing stages, and the drought-stressed plants–3 times: in the scheme 1–2–0. Thus, moderate drought stress was induced in the most sensitive stage of cotton plants—in mass flowering-maturing period. During this period, rainfall did not occur. All other growth conditions, including content of nutrients in soil, were the same.

The chlorophyll fluorescence was measured in attached leaves by using portable chlorophyll fluorometer Mini-PAM (Walz, Effeltrich, Germany) allowing up to 3000 measurements in the field without battery recharging [7]. The Mini-PAM fluorometer measures the chlorophyll

fluorescence parameters even under mid-day solar radiation by means of simultaneous application of a CW measuring light and saturating light flashes. Measurements were carried out in the early morning, from 7.00 to 8.00, on the third, matured leaves with 10-fold replication. In most of the experiments, the operating quantum efficiency of primary photochemistry,  $\Phi_{PSII} = F_V' / F_M' = (F_M' - F_S') / F_M'$  ( $F_M'$ —is a maximum and  $F_S'$ —a steady state levels of fluorescence at any arbitrary moment of a leaf illumination [18]), was determined as an indicator of the photosynthetic performance. For calculation of this parameter, measurements of a dark fluorescence and, consequently, dark adaptation of leaves were not a need [19, 20], which essentially simplified field experiments. The maximum fluorescence was measured at application of saturating light flashes with duration 0.8 s and photosynthetic photon flux density (PPFD)  $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . However, in some experiments, the photochemical quenching factor  $q_p = (F_M' - F') / (F_M' - F_0')$  and the non-photochemical quenching  $NPQ = F_M' / F_M - 1$ , characterizing efficiencies of photochemical utilization and non-photochemical losses of the absorbed light energy accordingly, were also determined. The electron transport rate  $ETR = \Phi_{PSII} \times PAR \times 0.5 \times \alpha$  was controlled as an indicator of activity of the photosynthetic electron transport chain; here photosynthetic active radiation (PAR) is the solar radiation intensity in spectral range 400–750 nm expressed as PPFD in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and  $\alpha$  is leaf absorption. In general, it is assumed that  $\alpha = 0.85$  and a ratio  $PSII : PSI = 1 : 1$ . PAR intensities were controlled by portable luxometer Yu-116 with a dielectric multilayer filter filtering out PAR from the whole solar radiation.

The gas-exchange measurements were carried out using photosynthesis analyzer LI-6400 (Licor, USA) at temperature  $24^\circ\text{C}$  [21]. The curves of  $\text{CO}_2$  response were measured in leaves of both water treatments by means of gradual lowering of the external  $\text{CO}_2$  concentration, from  $400 \mu\text{mol mol}^{-1}$  to  $0 \mu\text{mol mol}^{-1}$  at PPFD  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and the light response curves—at ambient  $\text{CO}_2$  concentration with step-by-step increasing of PPFD from  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The light and  $\text{CO}_2$  responses of the chlorophyll fluorescence and the photosynthesis were measured after adaptation of leaves to each value of PPFD and  $\text{CO}_2$  concentration during 15 min. The operating values of the minimum fluorescence under continuous illumination during the measurements,  $F_0'$ , were calculated according to [22] using the equation  $F_0' = F_0 / (F_V / F_M + F_0 / F_M)$ .

Relative water content and transpiration of plant leaves were determined by their weighting [23]. In addition, a leaf thickness and a leaf blade area were also measured in each cotton genotype. For estimation of the magnitude and diurnal variations of photoinhibition, the values of  $\Phi_{PSII}$  have been consistently measured simultaneously in both well-watered and drought-stressed plants every hour during 24 h.

Photoacoustic spectrometer of special design with  $\sim 1 \text{ cm}^3$  sample chamber having higher sensitivity at low (10–250 Hz) frequencies of light modulation [24] has been used for measuring photoacoustic characteristics of plant leaves. The sources of a CW measuring light and saturating light flashes of the spectrometer were a semiconductor LED (650 nm, 20 mW) and a halogen lamp (400–700 nm, 20 W) with a mechanical chopper, respectively. Intensity of the measuring light was supported as  $50\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$  and intensity of the saturating flashes



did not exceed  $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The photobaric component was selected from the total photoacoustic waves generated in a plant leaf at application of low-frequency (10 Hz)-modulated light by recording quadrature signal from a lock-in amplifier [25].

### 3. Effect of drought to photosynthesis

Drought stress is primarily affected to photosynthetic performance of plants. The long-term drought effect is expressed as reducing/delaying of a plant growth and development, premature leaf senescence, and related reduction in a crop productivity [26, 27]. The dispute, what, mainly, limits photosynthesis under conditions of water deficiency: stomata closure or impairment of the metabolism is long enough [28, 29], but in the past decade, closure of stomata was perceived by experts as the predominant factor in mild and moderate drought stress [30].

The first response of a plant to onset of drought stress is the stomata closure and associated reduction of the relative water content of leaves and intracellular  $\text{CO}_2$  concentration,  $C_i$  [3, 31]. This, in turn, causes decrease in a leaf turgor and a water potential [32]. In such a condition, gas-exchange analysis in plant leaves would be an informative technique for assessment of stomatal limitation to  $\text{CO}_2$  assimilation.

Non-stomatal mechanisms of the photosynthesis limitation under long-term or severe drought in the soil include changes in chlorophyll synthesis [33], structural changes in photosynthetic apparatus and depressing the Calvin cycle enzymes activities, which reduces crop yield [34] and decline in Rubisco activity [35, 36].

Short-term or mild drought-induced non-stomatal limitations to photosynthesis have smaller magnitude than stomatal ones. Closure of stomata and limited access of  $\text{CO}_2$  bring about reduced utilization of the energy of electron transport, and, accordingly, over-excitation of the plant photosynthetic apparatus. This, accordingly, increases the susceptibility of the system to photo-damage. Accumulation of singlet oxygen or superoxide radicals, when a dynamic balance between producing of such reactive substances and functioning of the plant antioxidant defense system is broken, may cause destruction of photosynthetic proteins and membrane lipids [37, 38].

Reduced rate of transpiration, especially at higher ambient temperatures, increases the heat accumulation and relevant increase in leaf temperature. The latter can also cause decline of the plant photosynthetic performance under drought [30].

A number of experiments have shown that the closure of stomata is controlled, mostly, by reducing soil water content, but not leaf water status. This suggests response of stomata to a chemical signal from roots, i.e. presence of abscisic acid produced by dehydrating roots, while a leaf water status is constant [39, 40]. The same time it means that the efficient way to control the stomatal conductance is to change the soil water content even preserving constant level of leaf water status.

Activity of the photosynthetic electron transport chain is rigidly regulated by the availability of  $\text{CO}_2$  in the chloroplast, limited by closure of stomata under drought stress [41]. Leaf

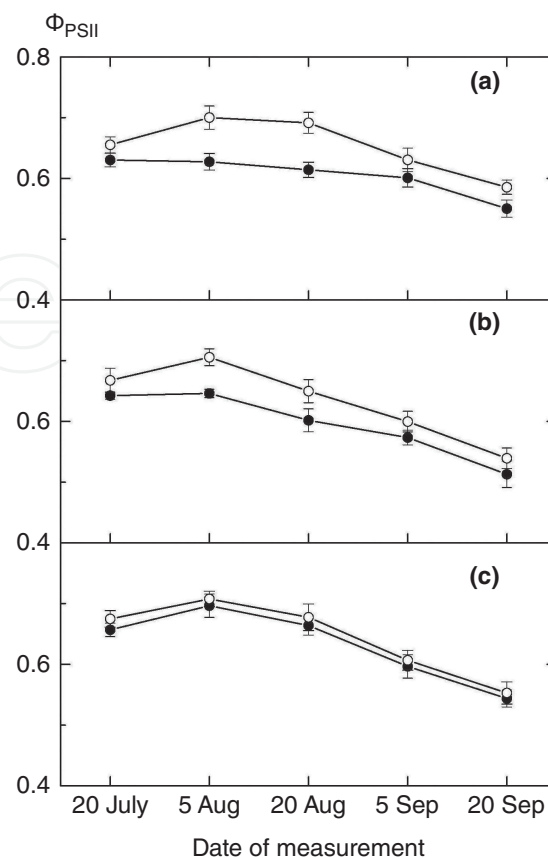
dehydration leads to shrinking of cells and accordingly reducing of their volume. This causes an increase in the internal viscosity of the cell contents, and interaction between proteins and, consequently, their aggregation and denaturation [42].

Comparison of the results from different studies is quite difficult due to the essential variations in responses of the stomatal conductance and photosynthesis to changes of leaf water potential and relative water content in different genotypes [3]. It is considered as well established that drought-induced stomata closure declines the net photosynthesis in all plant species, though, with different magnitudes. That is why comparative studies of the photosynthetic parameters in different plant genotypes under drought stress may provide an important information concerning to the photosynthetic performance and adaptation potential of plants to moderate long-term drought.

Analysis of the chlorophyll fluorescence and photosynthesis in plant leaves has revealed that in conditions favorable for photosynthesis, i.e. lack of environment stresses, at low light intensities, etc. when alternative mechanisms of light energy utilization did not required, the quantum efficiency of photochemistry is tightly linked with quantum efficiency of CO<sub>2</sub> fixation [9], and the photosynthesis rate is not sensitive to mild under drought stress [10, 43]. In this condition, photorespiration increases and its magnitude depends on the light intensity [44]. In a number of researches, the reduction in  $\Phi_{\text{PSII}}$  has been observed under long-term drought, which has been attributed, mostly, to reducing of photochemistry and, in less extent, to dissipative processes in the plant photosynthetic apparatus. However, in some other researches, the increasing  $\Phi_{\text{PSII}}$  has been observed in plants exposed to moderate long-term drought [12, 13]. Such contradiction in behavior of  $\Phi_{\text{PSII}}$  may be explained by a heterogeneity of the photosynthetic performance across the leaf blade [14, 45]. Thus, simultaneous analysis of chlorophyll fluorescence and photosynthesis in plant leaves may reveal mechanisms and magnitude of protective changes in plants under drought stress, and correlations between changes in chlorophyll fluorescence parameters and morpho-physiological indicators, traditionally used for estimation of drought tolerance of plants, may be used as an effective instrument for monitoring of plants in the field.

#### 4. Comparative measurements of $\Phi_{\text{PSII}}$ in different cotton genotypes

The operating quantum efficiency of photochemistry,  $\Phi_{\text{PSII}}$ , has been determined simultaneously in well-watered and moderately drought-stressed plants of three genotypes of cotton cultivated in Uzbekistan with the aim of estimating the magnitude of the effect of drought on the photosynthetic performance and monitoring its changes during a key period of the ontogenesis—in flowering and maturing stages from last July to last September [12, 46]. Figure 1 shows the results of this experiment. The dates of measurements are shown on the X-axis. Stressed plants of all cotton genotypes display higher values of  $\Phi_{\text{PSII}}$  in comparison with well-watered plants. Moreover, in the drought-tolerant plants of Navbakhor, this increase was maximal (up to 15% over the most period of measurements), while in Gulsara characterized by lower drought tolerance, it was minimal (approximately 2%). And, in Liniya-49 having an



**Figure 1.** The changes in  $\Phi_{PSII}$  in leaves of three genotypes of cotton: Navbakhor (a), Liniya-49—(b) and Gulsara—(c) growing in well-watered (●) and moderately drought-stressed (○) conditions during a long period of their ontogenesis.

intermediate degree of drought tolerance had intermediate values for differences in  $\Phi_{PSII}$ . Irrigation of the drought-stressed plants on 10th September shortened this difference, though, with different extent in different genotypes.

Measurements of morpho-physiological indicators in plants of all genotypes have demonstrated considerable reduction in leaf relative water content and of leaf blade expansion and increase in leaf thickness under long-term drought stress. These changes are presented in Table 1. It is seen that in the most drought-tolerant cotton genotype Navbakhor, these changes are maximal, and in Gulsara having lower drought tolerance, these are minimal. Correlations between  $\Phi_{PSII}$  and these morpho-physiological indicators have been defined in all three genotypes, but with different extent. The last may be attributed to the possibility of other protective reactions in plants affected to long-term drought stress [47].

Leaf transpiration was lower in drought-stressed plants than in well-watered plants of all genotypes for 5–15% (not shown), which may be considered as typical for the field-grown cotton plants [48]. However, diurnal changes in transpiration of plants were much more than differences between two treatments, therefore reliable correlations between changes in the

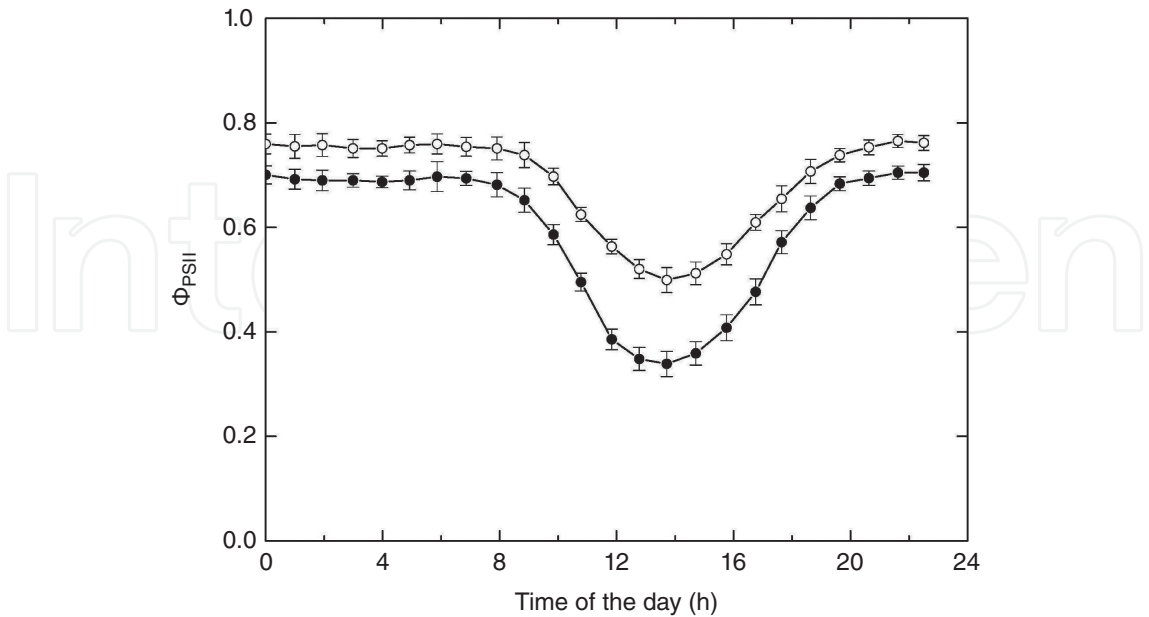


Morpho-physiological indicators	Water treatment	Cotton genotypes		
		Navbahor	Liniya-49	Gulsara
Relative water content, %	Well-watered	79.4	78.8	77.4
	Drought-stressed	72.5	74.4	74.3
	Percentage of the difference	8.7%	5.6%	5.0%
Leaf blade area, m <sup>2</sup>	Well-watered	71.1	77.7	80.9
	Drought-stressed	63.1	73.0	77.1
	Percentage of the difference	11.3%	6.1%	4.7%
Relative leaf thickness, g m <sup>-2</sup>	Well-watered	0.853	0.974	0.987
	Drought-stressed	0.981	1.09	1.052
	Percentage of the difference	15.0%	11.9%	6.6%

**Table 1.** Morpho-physiological indicators of the well-watered and moderately drought-stressed cotton genotypes.

transpiration and the chlorophyll fluorescence parameters under drought stress were not established.

For determination of changes in the photosynthetic performance of plants under drought stress and kinetics of photoinhibition over the day, the quantum efficiency of photochemistry has been measured hourly during 24 h. Figure 2 shows such dependencies measured in well-watered and drought-stressed plants of Navbakhor. As shown in previous figure, in the drought-stressed plants,  $\Phi_{PSII}$  is higher than in well-watered plants during all the day, including a night time. In addition, decline of  $\Phi_{PSII}$  in mid-day in the drought-stressed plant is smaller but occurs for longer time [12]. Such a photoinhibitory depression of the primary photochemistry under high-intensity solar radiation is characterized by various components with different relaxation periods [49, 50]. Obviously, adaptive changes in the structure and func-



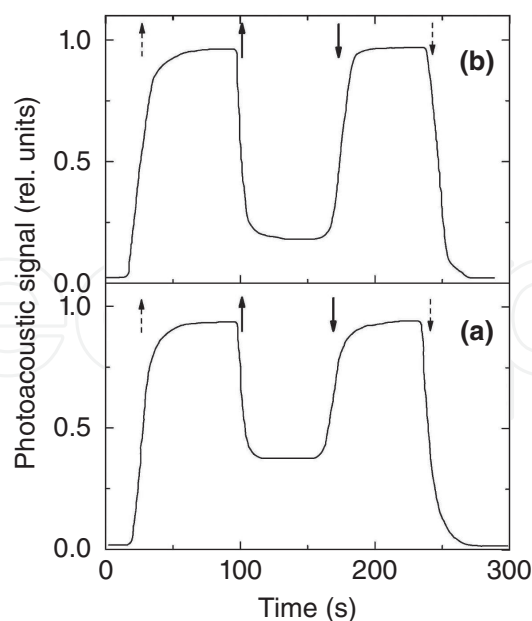
**Figure 2.** Diurnal changes in  $\Phi_{PSII}$  measured in leaves of the cotton genotype Navbakhor grown in well-watered (●) and moderately drought-stressed (○) conditions in the field.

tioning of the plant photosynthetic apparatus under moderate long-term drought may bring about depressing, mainly short-period, components of photoinhibition and its long-period components will dominate in drought-stressed plants [51]. Such changes in the proportion of different components of photoinhibition results in decreasing of the amplitude and reshaping of the form of diurnal changes  $\Phi_{PSII}$  as it is shown in Figure 2. It should be noted that difference in values of  $\Phi_{PSII}$  measured in well-watered (0.34) and drought-stressed (0.48) plants at mid-day, 0.14, is considerably higher than those in other periods of the day. This fact may be considered as enhancing of photorespiration that may contribute in  $\Phi_{PSII}$  only as a prompt component.

Therefore, protective response of cotton plants to drought stress expressed in photosynthetic indicators is the increase in quantum efficiency of primary photochemistry, in morphology is the increase of leaf thickness with decreasing leaf blade expansion and in physiology is the reduce in transpiration. If reduce in the leaf blade expansion and transpiration may be explained logically by considerations of minimizing the moisture loss [47], increase of  $\Phi_{PSII}$  looks as somehow contradictory with the literature data: at the onset of drought stress, the plant should response by reducing photosynthesis to protect the photosynthetic apparatus [52]. At constant values of efficiency of alternative ways of energy utilization, this has to bring about lower quantum efficiency of photochemistry. Then, the excessive energy of absorbed light may be utilized by enhancing the activity of an alternative channel—photorespiration. Lastly, in C3 plants could be significant, particularly in cotton, which typical growth conditions are associated with higher temperatures and water deficiency. At present, protective role of photorespiration under environmental stresses are poorly studied and published researches on this matter is very minor [53].

Thus, cotton genotypes with different degrees of drought-tolerance studied displayed specific changes in the chlorophyll fluorescence parameters, as well as in morpho-physiological indicators under long-term drought stress. Diurnal curves of  $\Phi_{PSII}$  variations in well-watered and moderately drought-stressed plants provide information on the magnitude and different time components of photoinhibition developed under high-intensity solar radiation.

Photoacoustic waves generated in plant leaves at application of modulated light have been studied for precise control of the photosynthetic performance and quantitative estimation of the photosynthetic oxygen evolution. Photobaric component of the photoacoustic waves related to photosynthetic evolution of oxygen has been measured in the photoacoustic cell of special design with a small measuring chamber ( $\sim 1 \text{ cm}^3$ ) in lock-in amplifier by selecting quadrature signal at low frequencies [15, 54]. Figure 3 shows kinetics of changes of the photoacoustic signal from the well-watered (relative water content 100%) and short-term dehydrated (relative water content 65%) leaves of the cotton genotype Navbakhor, generated at application of low frequency (10 Hz) measuring light. It is shown from the figure that the steady-state photoacoustic signal considerably declines at application of additional CW light of high intensity ( $\sim 2500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) to plant leaf, which saturates photosynthetic oxygen evolution process and, accordingly, excludes periodic changes of pressure in the measuring chamber, which is the photobaric wave. Therefore, relative change in the photoacoustic signal (ratio of amplitude of change to the total photoacoustic signal) may be used as a measure of

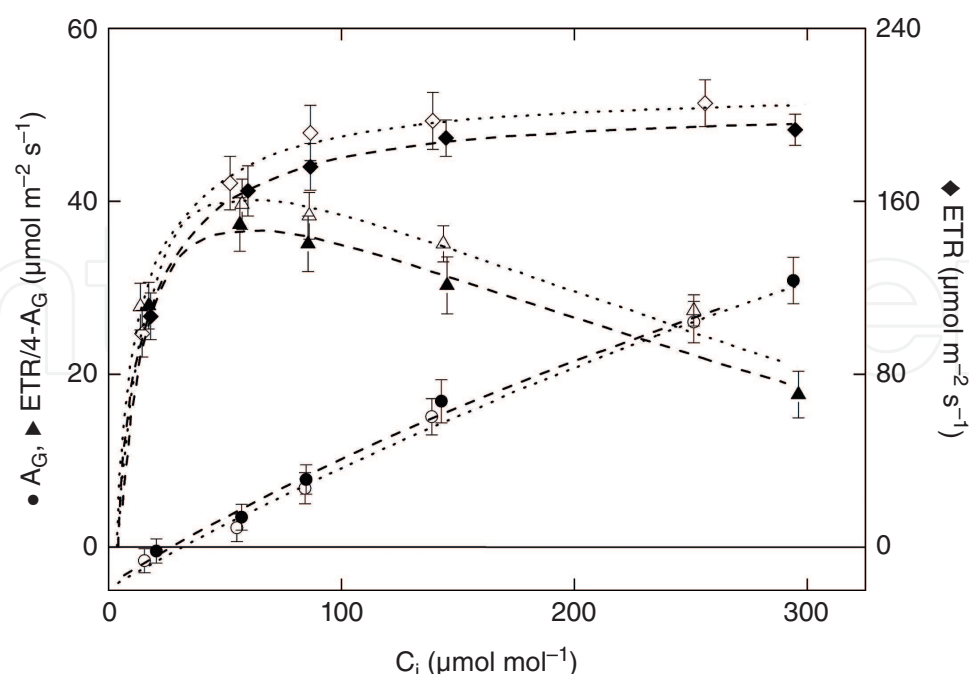


**Figure 3.** Induction curves of the photoacoustic signal generated in leaves of the cotton genotype Navbakhor with relative water content 100% (a) and 65% (b). Arrows up and down show switching on and off, respectively, the measuring (dashed arrows) and saturating (bolt arrows) lights.

the photosynthetic oxygen evolution. In experiments, before measuring photoacoustic signals, the plant leaves were adapted to dark for 10 min. After reaching the steady-state photoacoustic signal, the saturating CW light was applied, which causes decrease in the photoacoustic signal for 0.82 (Figure 3a) in the well-watered leaf and for 0.50 (Figure 3b) in the dehydrated leaf. Thus, the photoacoustic measurements have shown that photosynthetic oxygen evolution in plant leaves depresses in short time water deficiency: decrease in the relative water content for 45% causes decrease of photosynthetic activity 1.5 times. Simultaneous measurements of  $\Phi_{\text{PSII}}$  in these two samples displayed decline of the operative quantum efficiencies of photochemistry in the same ratio (0.75:0.51). However, the advantage of photoacoustic measurements is evident in the case of significant level of photorespiration in plant leaves, when direct correlation between  $\Phi_{\text{PSII}}$  and the net photosynthesis is disturbed (see the next section).

## 5. Simultaneous measurements of ETR and photosynthesis in well-watered and moderately drought-stressed cotton plants

Electron transport rate (ETR) and photosynthesis in cotton plants of both water treatments have been measured simultaneously for revealing the role and magnitude of alternative channels for utilization of the energy of electron transport and obtaining new insights into mechanisms of adaptation of the plant photosynthetic apparatus to long-term drought stress. Indicated photosynthesis parameters have been determined at  $\text{CO}_2$  concentrations 0–400  $\mu\text{mol mol}^{-1}$  under constant PPFD of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and under PPFD of 0–2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at ambient  $\text{CO}_2$  concentration in plants of genotype Navbakhor (Figure 4). It is seen that the rate



**Figure 4.** Response of the photosynthesis,  $A_G$ , electron transport rate, ETR, and photorespiration, estimated as  $\text{ETR}/4-A_G$ , to  $\text{CO}_2$  concentration in leaves of the cotton genotype Navbakhor grown in well-watered (closed symbols) and moderately drought-stressed (open symbols) conditions in the field.

of  $\text{CO}_2$  assimilation ( $A_G$ ) increases linearly with increase of intracellular  $\text{CO}_2$  concentration,  $C_i$ , while the dependence ETR *versus*  $C_i$  is non-monotonic: sharp increase of ETR with increase of  $\text{CO}_2$  concentration at  $C_i < 100 \mu\text{mol mol}^{-1}$ , further saturates on the level of  $\text{ETR} \sim 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The measurements were carried out in the field, early morning, from 7.00 to 8.00 at temperature 22–24°C.

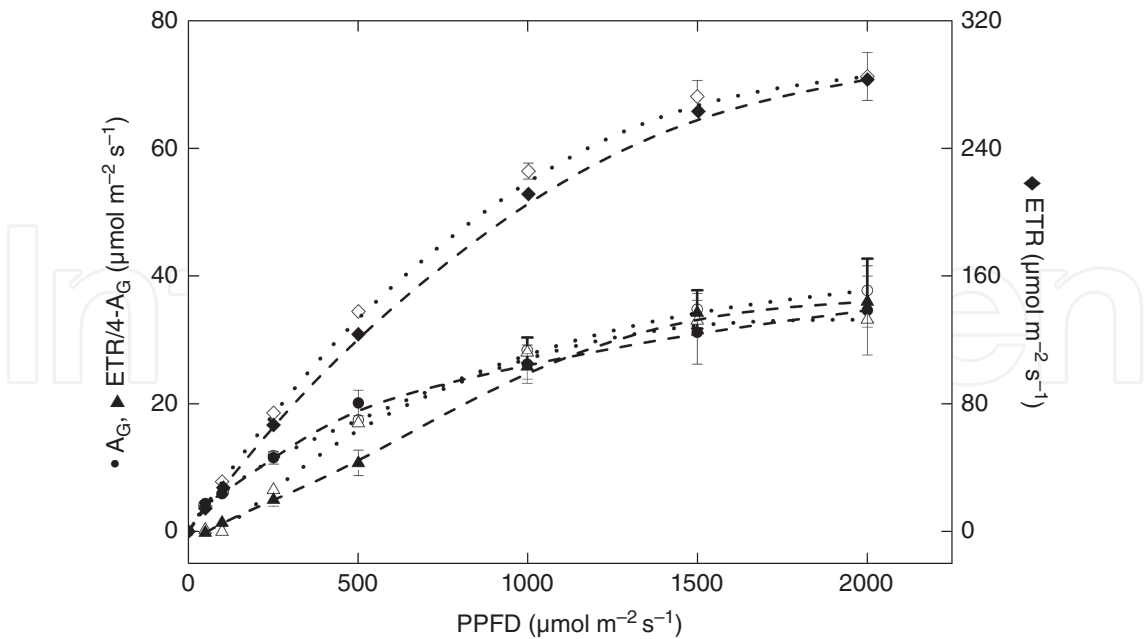
At higher light intensities and/or low  $\text{CO}_2$  concentrations, the plant photosynthetic apparatus cannot cope with the coming light energy and a portion of this energy has to be utilized through alternative channels; photorespiration or some other processes, including Mehler reaction, may play a role of a sink for electrons transported through the photosynthetic electron transport chain [55]. In most of the cases, excluding severe drought stress, the photorespiration considered as prevailing mechanism of utilization of such an excessive light energy [56]. The magnitude of this energy utilization may be estimated by comparing ETR and photosynthesis. Assuming that assimilation of one molecule  $\text{CO}_2$  requires four electrons transported through the chain, the amount of photorespiration may be defined by dividing ETR by four and subtracting the photosynthesis [55]. By calculating this way, values of the photorespiration rate are also presented in Figure 4: photorespiration increases sharply at low concentrations up to  $100 \mu\text{mol mol}^{-1}$ , and further slowly drops with increase of  $\text{CO}_2$  concentration. The fact seems reasonable, because  $\text{CO}_2$  is a product of photorespiration. Figure 4 shows that drought stress noticeably increases ETR and slightly decreases the photosynthesis in cotton plant leaves. As a result, the photorespiration in drought-stressed leaves calculated as above is considerably higher than in well-watered plants, especially at higher  $\text{CO}_2$  concentrations. In

Water treatment	$R_D$	$\Phi_{PSII}$
Drought-stressed	$3.8 \pm 0.5$	$0.67 \pm 0.023$
Well-watered	$5.2 \pm 0.6$	$0.62 \pm 0.021$

**Table 2.** “Dark” respiration,  $R_D$ , and operating quantum efficiency of primary photochemistry in Photosystem II,  $\Phi_{PSII}$ , measured in leaves of well-watered and moderately drought-stressed cotton genotype Navbakhor.

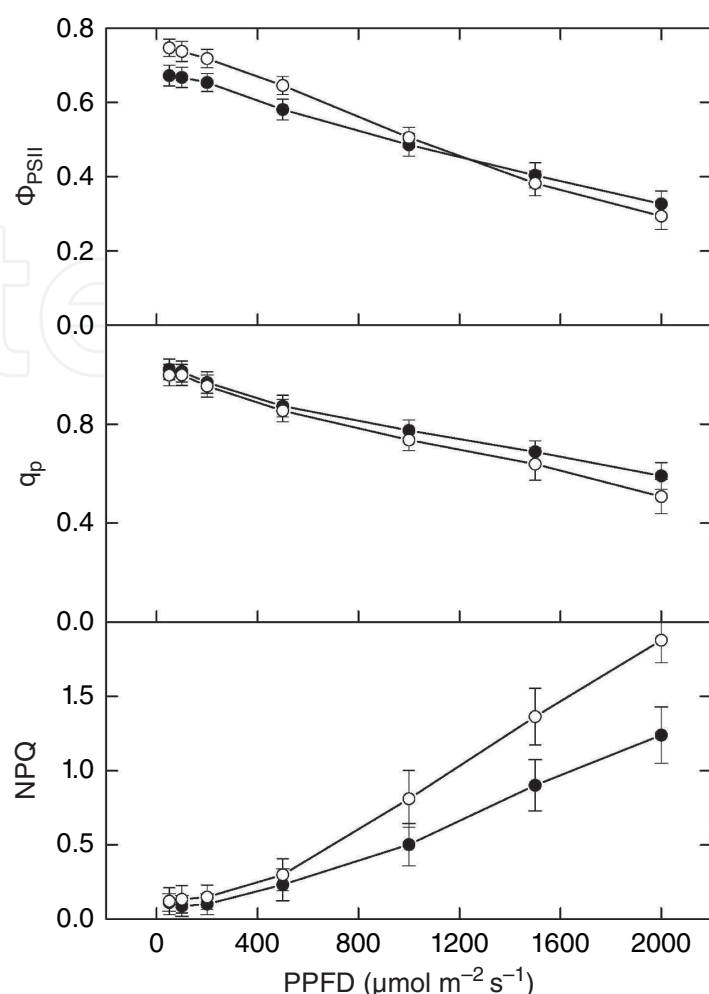
addition, the effect of drought stress to “dark” respiration has been measured in plants of both water treatments simultaneously with the quantum efficiency of primary photochemistry (Table 2). The “dark” respiration, as an additional source of bioenergy necessary for supporting vital biochemical reactions in plants, was considerably higher in drought-stressed plants. The same occurred with the quantum efficiency of photochemistry, but with less magnitude.

The light response of ETR and photosynthesis measured in plants of Navbakhor of the two treatments was similar to the  $CO_2$  response (Figure 5). At low light intensities, most of the energy from the electron transport is utilized in photochemical reactions, and with increasing of light intensity, more and more portion of this energy is spent for photorespiration. However, the increase in ETR induced by drought stress in light response was less expressed than that in  $CO_2$  response, particularly at higher intensities. Considerable variations of photosynthesis in different replications comparable with its difference between the treatments may be attributed to diurnal changes of stomatal conductance,  $g_s$ , which can induce relevant changes in photosynthesis [57]. In view of tightly links between stomatal conductance and photosynthesis, and efficiency of primary reactions of photosynthesis remains constant, the changes in stomatal conductivity during the day may bring about considerable changes in photosynthesis



**Figure 5.** Response of the photosynthesis,  $A_G$ , electron transport rate, ETR, and photorespiration, estimated as  $ETR/4-A_G$ , to light intensity (PPFD) in leaves of the cotton genotype Navbakhor grown in well-watered (closed symbols) and moderately drought-stressed (open symbols) conditions in the field.





**Figure 6.** Light response of the chlorophyll fluorescence parameters: quantum efficiency of photochemistry in Photosystem II,  $\Phi_{\text{PSII}}$ , photochemical quenching factor,  $q_p$ , and non-photochemical quenching, NPQ, in leaves of the cotton genotype Navbakhor grown in well-watered (closed symbols) and moderately drought-stressed (open symbols) conditions in the field.

[58]. In this case, the sum of photosynthesis and photorespiration, as measured using the ETR/4, is not constant, but varies during the day.

In the Figure 6 are shown the light response of the three key fluorescence parameters, operating quantum efficiency of photochemistry,  $\Phi_{\text{PSII}}$ , photochemical quenching factor,  $q_p$ , and non-photochemical quenching, NPQ, determined in leaves of well-watered and moderately drought-stressed cotton genotype Navbakhor. As shown from the figure, at low and moderate light intensities,  $\text{PPFD} < 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $\Phi_{\text{PSII}}$  in drought-stressed plants was higher than in well-watered plants, whereas  $q_p$  was the same and near to its maximum. However, with increase of light intensity,  $\Phi_{\text{PSII}}$  and  $q_p$  decrease with increments, which are higher in drought-stressed plants. And finally, at  $\text{PPFD} > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ , both  $\Phi_{\text{PSII}}$  and  $q_p$  become lower in drought-stressed plants in comparison with well-watered plants. What concerns to NPQ, it is negligibly low at low intensities in both treatments but increases rapidly at moderate and high

intensities and under drought stress. So, increasing light intensity activates photosynthetic performance of plants. At low and moderate intensities, when the plant photosynthetic apparatus copes with coming light energy, the efficiency of photosynthetic conversion of light energy is very high, when photochemical quenching factor is near to its maximum and non-photochemical quenching is negligibly low. Long-term drought stress due to stomatal and non-stomatal limitations to photosynthesis induces enhancement of photorespiration as an alternative sink for transported electrons in reaction centers of photosynthesis. However, further increase of light intensity increases non-photochemical quenching, and in drought-stressed plants, it is higher than in well-watered ones. This causes faster decrease of  $\Phi_{\text{PSII}}$  and  $q_p$  in drought-stressed plants.

Experiments with the measurement of chlorophyll fluorescence and the gas-exchange in different cotton genotypes showed that under drought stress,  $\text{CO}_2$  uptake slightly decreases, while ETR increases considerably. Simultaneously measuring these two parameters of photosynthesis allowed us to estimate the magnitude of photorespiration in the plant leaves, assuming that changes in the  $\text{ETR}/4-A_G$  reflect the changes in photorespiration. Photorespiration increases with increasing light intensity and decreasing  $\text{CO}_2$  concentration. Moderate drought stress noticeably increases the rate of photorespiration, which can be considered as a characteristic response of C3 plants to a drought [44].

Leaves of drought-stressed cotton plants displayed higher  $\Phi_{\text{PSII}}$  and photorespiration at low and moderate light intensities, and non-photochemical quenching, NPQ, was stronger in drought-stressed plant than that in well-watered one. Obviously, higher levels of photorespiration in plant leaves during the drought stress exerts the “pressure” to the rate of electron flow and makes Photosystem II to operate with higher efficiency.

## 6. Conclusion

The photosynthetic apparatus of plants supports higher performance of electron transport chain through enhancement of quantum efficiency of photochemistry in Photosystem II under drought stress. The accumulated energy in this state of over-excitation may be utilized in enhanced photorespiration. This protective reaction of the plant photosynthetic apparatus to drought stress has different magnitude depending on its drought tolerance. Field measurements of the chlorophyll fluorescence parameters simultaneously with morpho-physiological indicators of the cotton genotypes studied have displayed direct correlations between these parameters under drought stress. These correlations together with possible calibration of chlorophyll fluorescence parameters by photoacoustic characteristics determined at application of low-frequency-modulated light to plant leaves give new opportunities in monitoring of drought tolerance of various cotton genotypes in the field.

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