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REGULAR ARTICLE

Drought-induced changes and recovery of photosynthesis in two bean cultivars (*Phaseolus vulgaris* L.)

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Abstract

The effects of soil drought on photosynthesis and chlorophyll fluorescence in the leaves of two common bean (*Phaseolus vulgaris* L.) cultivars – Zarya and Tangra were studied, as well as recovery of photosynthesis after re-watering. Drought was imposed 14 days after the emergency by withholding water for 10 days in which soil water potential reached -0.9 MPa. Water stress led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} in the primary leaf of the studied cultivars. The most marked reduction in leaf gas exchange was observed in cv. Tangra. α was reduced more than three folds and A_{max} - more than six folds. Exposure of bean plants to soil drought and provoked leaf water deficit resulted in a dramatic reduction (with an 84.17%) of A_n at normal ambient CO_2 concentration (C_a=370 μmol mol^{-1}). The lower reduction in leaf gas exchange parameters were observed in cv. Zarya. Drought stress induces an increase of F_0 accompanied by a decrease of F_m in the studied cultivars, being cv. Zarya less affected. The F_v/F_m ratio was significantly decreased in cv. Tangra and only showed a slight tendency to a decrease in cv. Zarya. Cv. Tangra presented a decrease of 56% in qP in the primary leaf, while in cv. Zarya qP decreased with 32%. Accordingly, Y strongly decreased in cv. Tangra, while in cv. Zarya Y was less affected. 3 days after re-watering photosynthesis of cv. Zarya was about 87% from the control plants, while in cv. Tangra photosynthesis was only 68% from control. Chlorophyll fluorescence parameters were recovered to the greater extent. On the basis of the data obtained we could arrange photosynthetic apparatus of cv. Zarya as relatively drought tolerant and that of cv. Tangra as drought sensitive.

Key words: Drought, Photosynthesis, Chlorophyll fluorescence, *Phaseolus vulgaris*, Recovery

Introduction

Maintaining growth and crop productivity under adverse stress environmental conditions is presumably the major challenge facing modern agriculture. To meet this challenge, it is necessary to understand the physiological and biochemical bases of plant acclimation to growth in stressed conditions, and the relationship between them and environment. 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). Global climatic changes will probably make water shortage an even greater limitation to plant productivity across an increasing amount of land (Chaves et al., 2009). The limitation of plant growth imposed by low water availability is mainly due to reductions of plant carbon balance, which is largely dependent on leaf photosynthesis. For this reason, photosynthesis responses to water stress have been the subject of study and debate for decades, in particular, concerning which are the most limiting factors for photosynthesis under water stress (Flexas and Medrano, 2002; Lawlor and Cornic, 2002; Grassi and Magnani, 2005; Souza et al., 2004).

The ability to maintain the functionality of the photosynthetic machinery under water stress and extent of recovery, 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). As a consequence, the diffusion of CO_2 into the leaf is restricted (Chaves et al., 2003). The decrease in net photosynthetic rate under drought stress observed in many studies is often explained by a lowered internal CO_2 concentration that results in a limitation of photosynthesis at the acceptor site of ribulose-1,5-bisphospate carboxylase/oxygenase

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(Rubisco) (Cornic et al., 1992; Chaves et al., 2003) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthase (Nogués and Baker, 2000).

Despite of fact that photosystem II (PSII) is highly drought resistant (Yordanov et al., 2003) under conditions of water stress photosynthetic electron transport through PS II is inhibited (Chakir and Jensen, 1999, Zlatev et al., 2010). 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; Rivero et al., 2010). The mechanism by which the water deficit inhibits this electron transport is still unclear.

The aim of this study was to determine the effects of soil drought stress on leaf gas exchange and chlorophyll fluorescence parameters in leaves of two common bean (*Phaseolus vulgaris* L.) cultivars. Analyses of the response of net CO_2 assimilation to intercellular CO_2 concentration and chlorophyll fluorescence measurements allow evaluation of the relative limitations to leaf photosynthesis imposed by changes in stomatal conductance, carboxylation efficiency, capacity for regeneration of RuBP and PSII electron transport efficiency. The degree of recovery of photosynthesis is also analyzed.

Materials and Methods

Plant material and growth conditions

For this study two common bean (*Phaseolus vulgaris* L.) cultivars were used: cv. Zarya and cv. Tangra. Seeds were washed in distilled water, after that surface sterilized with a 2% sodiumhypochloride (NaOCl) solution for 5 min, and germinated on moist filter paper, in Petri dishes, maintained at 25°C, in the dark, for 3 days. After germination seedlings with well-developed roots and having approximately the same morphological aspect were selected and cultivated in pots as soil culture in a growth chamber. Dissolved nutrients were added to the soil 15 days before planting: 270 mg $Ca(NO_3)_2$ kg^{-1} dry soil, 190 mg KNO_3 kg^{-1} dry soil and 210 mg $NH_4H_2PO_4$ kg^{-1} dry soil. One seedling was maintained in each pot. The environmental conditions in the growth chamber were: photosynthetic photon flux density (PPFD) of 320 μmol m^{-2} s^{-1}, day/night temperature 25±2/15±2ºC, photoperiod of 14 h, and relative air humidity between 60-65%. Pots were watered daily to maintain control soil water content of 41% (0.410 g H_2O g^{-1} dry soil) corresponding to soil water potential (Ψ_{soil}) of -20 kPa. It is considered that soil is well watered and there is no water stress if Ψ_{soil} is above -30 kPa (Ali et al., 1999). Water stress was progressively induced in 14-day old plants by withholding water supply for 10 days in which soil water content reached 23% (0.230 g H_2O g^{-1} dry soil) corresponding to soil water potential of -0.9 MPa. After imposition of stress, re-watering period for 3 days was applied. The measurements were made at the end of stress period, and at the end of re-watering period, 2 h after the start of photoperiod on primary leaf, which was fully matured.

Gas exchange measurements

Gas exchange measurements were performed with a portable photosynthetic system LCA-4 (Analytical Development Company, Hoddesdon, UK) equipped with a PLCB-4 chamber. PPFD was 650 μmol m^{-2} s^{-1} generated by a metal halide lamp, leaf temperature was 27±2ºC and ambient CO_2 concentration (C_a) was 370 μmol mol^{-1}.

Maximal carboxylation efficiency (α) was calculated by the initial slope of the CO_2 curve representing the net CO_2 assimilation (A_n) versus intercellular CO_2 concentration (C_i), according to von Caemmerer and Farquhar (1981).

The following function was fitted to the experimental data:

$$A_n = a + b\, e^{(-Ci/c)}, \qquad [1]$$

where a is maximal CO_2 assimilation (A_{max}) at saturated zone; b is parameter which is used for the calculation of CO_2 evolved during the dark respiration (R) at A_{max} (R = a + b) (Nacheva et al., 2002); c is constant.

CO_2-compensation point (Γ) was calculated from [1] at y=0 as follow:

$$\Gamma = -c \ln(-a/b)\ [\mu mol\ mol^{-1}] \qquad [2]$$

The stomatal limitations of photosynthesis (SL) were calculated according Farquhar and Sharkey (1982) as: $SL = (A_{Ci} - A_{Ca})/A_{Ci}$, where A_{Ci} is the net photosynthetic rate at C_i = 370 μmol mol^{-1} and A_{Ca} is the net photosynthetic rate at ambient CO_2 concentration (C_a), C_a= 370 μmol mol^{-1}.

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) was calculated according to Genty et al. (1989).

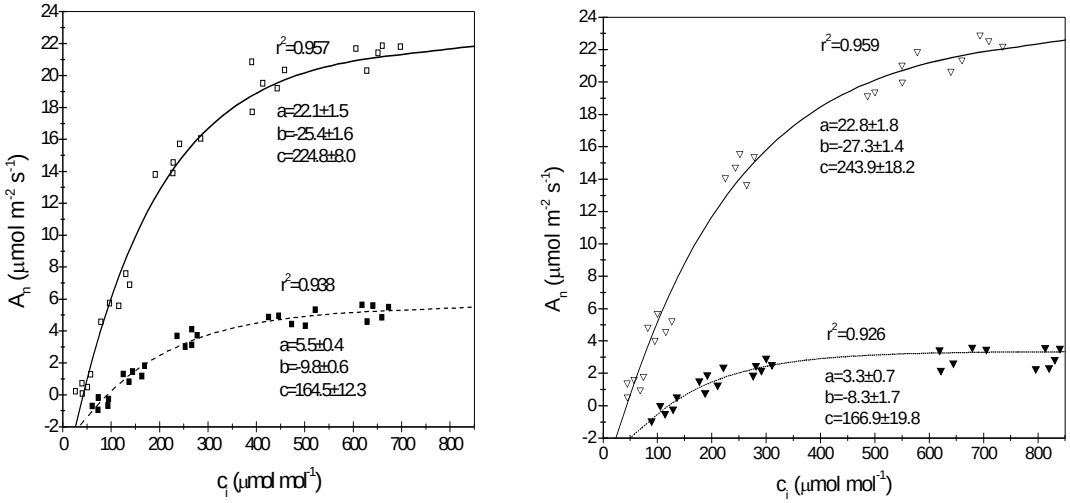
Statistical analysis

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 variants.

Results

Effects of drought on photosynthetic rate at different intercellular CO_2 concentrations

The changes in net photosynthetic rate of bean leaves as a function of the intercellular CO_2 concentration were used to determine the role of stomatal limitations (SL) on A_n under drought stress. Leaf water deficit led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} in the primary leaf of the studied cultivars (Fig. 1). A decline in the initial slope indicates a decreased Rubisco activity, while a low level of A_{max} at saturating CO_2 implicates a suppressed capacity for RuBP regeneration (von Caemmerer and Farquhar, 1981). The most marked reduction in leaf gas exchange was observed in cv. Tangra (Table 1). α was reduced more than three folds and A_{max} - more than six folds. Exposure of bean plants to soil drought and provoked leaf water deficit resulted in a dramatic reduction (with an 84.7%) of A_n at normal C_a (370 μmol mol^{-1}). CO_2 compensation point (Γ) increased more than three folds. SL increased slightly which suggests a stronger influence of non-stomatal (biochemical) factors. Lower reduction in leaf gas exchange parameters were observed in cv. Zarya. It is noteworthy that SL increased significantly in this cultivar, which suggests a stronger influence of stomatal factors.



A B

Figure 1. Changes of net photosynthetic rate to intercellular CO_2 concentration in primary leaf of control and drought stressed bean plants. **A** – cv. Zarya, control (□)) and drought stressed plants (■); **B** – cv. Tangra, control (∇) and drought stressed plants (▼). The function $A_n = a + be^{(-Ci/c)}$ was fitted to experimental data. The values of parameters a, b and c with their standard errors are given in the figure and they were used for calculation of the photosynthetic characteristics in Table 1.

Table 1. Effect of soil drought on leaf gas exchange parameters in primary leaf of control and drought stressed bean plants. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, net photosynthetic rate at saturating CO_2; Ac_a=370, net photosynthetic rate at 370 μmol mol^{-1} ambient CO_2 concentration; A_{Ci}=370, net photosynthetic rate at 370 μmol mol^{-1} intercellular CO_2 concentration; SL, stomatal limitation of photosynthesis.

Variant	α (μmol m^{-2} s^{-1} mol^{-1})	Γ (μmol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	Ac_a=370 (μmol m^{-2} s^{-1})	A_{Ci}=370 (μmol m^{-2} s^{-1})	SL (%)
Control						
Zarya	0.105	31.3	22.1	14.13	18.76	24.7
Tangra	0.103	43.9	22.8	13.84	17.24	19.7
Drought stressed						
Zarya	0.045	95.0	5.5	2.61	4.62	43.5
Tangra	0.033	153.9	3.3	2.11	2.73	22.7

Chlorophyll fluorescence

Data in Table 2 show that drought stress induces an increase of F_0 accompanied by a decrease of F_m in primary leaf of the studied cultivars, being cv. Zarya less affected (Table 2). According Baker and Horton (1987) an increase in F_0 is characteristic of PSII inactivation, whereas a decline in F_v may indicate the increase in a non-photochemical quenching process at or close to the reaction center.

The F_v/F_m ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves was decreased significantly in cv. Tangra, and only showed a slight tendency to a decrease in cv. Zarya.

Cv. Zarya presented a decrease of 18.85% in the proportion of energy driven to the photosynthetic pathway (qP) in the primary leaf, while in cv. Tangra qP decreased with 48.47%. Accordingly, Y strongly decreased - in cv. Tangra with 46.74% and in cv. Zarya with 27.83%.

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

Recovery effects

Data in Table 3 show photosynthetic parameters after 3 day period of recovery. The lowered levels of all parameters under soil water deficit had a tendency to recover. The plants reached levels of α, Γ, and photosynthesis were similar to those in the control. SL was slightly reduced in studied cultivars. The photosynthetic parameters of cv. Zarya were recovered in greater extent.

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

Variant	F_0	F_m	F_v/F_m	Y	qP	qN
Control						
Zarya	387±18	2014±82	0.808±0.039	0.539±0.031	0.732±0.038	0.493±0.025
Tangra	373±21	2010±79	0.814±0.041	0.522±0.029	0.751±0.036	0.474±0.031
Stressed						
Zarya	426±20 *	1716±64 *	0.752±0.037	0.389±0.023 *	0.594±0.029 *	0.732±0.041**
Tangra	448±25 *	1593±71 *	0.719±0.028 *	0.278±0.016 **	0.387±0.026 **	0.945±0.052***

* P<0.05; ** P<0.01; *** P<0.001

Table 3. Leaf gas exchange parameters in primary bean leaves after 3-day period of recovery. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, net photosynthetic rate at saturating CO_2; Ac_a=370, net photosynthetic rate at 370 μmol mol^{-1} ambient CO_2 concentration; A_{Ci}=370, net photosynthetic rate at 370 μmol mol^{-1} intercellular CO_2 concentration; SL, stomatal limitation of photosynthesis.

	α (μmol m^{-2} s^{-1} mol^{-1})	Γ (μmol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	Ac_a=370 (μmol m^{-2} s^{-1})	A_{Ci}=370 (μmol m^{-2} s^{-1})	SL (%)
Control						
Zarya	0.117	41.5	21.9	13.78	17.89	23.0
Tangra	0.111	49.9	22.5	12.65	17.38	27.4
Recovery						
Zarya	0.097	58.2	18.3	12.53	15.30	18.1
Tangra	0.084	88.6	16.7	10.86	14.60	25.6

Table 4. Parameters of chlorophyll fluorescence in bean leaves after 3-day period of recovery.

Variant	F_0	F_m	F_v/F_m	Y	qP	qN
Control						
Zarya	411±19	1974±92	0.791±0.034	0.517±0.028	0.756±0.041	0.503±0.032
Tangra	402±21	2003±85	0.799±0.035	0.528±0.024	0.729±0.043	0.485±0.030
Recovery						
Zarya	437±22	1920±71	0.772±0.036	0.489±0.021	0.686±0.032	0.623±0.041*
Tangra	440±23	1825±91 *	0.759±0.032	0.476±0.023 *	0.673±0.029	0.682±0.044**

* P<0.05; ** P<0.01

Parameters of chlorophyll fluorescence are recovered to greater extent (Table 4). F_0 of stressed plants still remain higher than control plant, and F_m of primary leaf was decreased, being cv. Zarya recovered in a greater extent. F_v/F_m and qP show only tendency to decreasing, and Y was significantly lower in cv. Tangra. Only qN was significantly higher in leaves of studied cultivars.

Discussion

Soil drought and leaf water deficit lead to a permanent depression of photosynthtetic carbon assimilation (Yordanov et al., 2003; Chaves et al., 2009). Decreased photosynthetic rate is result of stomatal and non-stomatal (biochemical) limitations (Yordanov et al., 2000; Zlatev and Lidon, 2012).

There are many reports, which underline the stomatal limitation of photosynthesis as a primary event, which is then followed by the adequate changes of photosynthetic reactions (Chaves, 1991; Yordanov et al., 2000; Zlatev and Lidon, 2012). Today, there is a consensus that a decrease of photosynthesis due to water stress has been attributed to both stomatal and non-stomatal limitations (Shangguan et al., 1999). Non-stomatal limitation of photosynthesis has been attributed to reduced carboxylation efficiency (Jia and Gray, 2004), reduced ribulose-1,5-bisphospate (PuBP) regeneration (Tezara and Lawlor, 1995), reduced amount of functional Rubisco (Kanechi et al., 1995; Medrano et al., 2002), or to inhibited functional activity of PSII. Concomitantly inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor and Cornic, 2002; Subrahmanyam et al,. 2006). Since maximal CO_2 assimilation (A_{max}) reflex the result of those mesophyllic impairments, its determination under severe water stress allows us to evaluate non-stomatal limitations of photosynthesis and hence, the degree of drought tolerance of the photosynthetic machinery.

According to von Caemmerer and Farquhar (1981), the initial slope of the CO_2 curve is defined as the maximal carboxylation efficiency of Rubisco, whereas the rate of photosynthesis at high C_i reflects the capacity of the leaves to regenerate RuBP, which is connected with electron transport activity. In our study drought treatment led to a reduction of both Rubisco carboxylation activity and RuBP regeneration capacity, as indicated by the lowering of the initial slope and the plateau of saturation. This dependence is strongly expressed in leaves of cv. Tangra (Figure 1A). Thus, photosynthesis could be adjusted through a balance between Rubisco carboxylation capacity, RuBP utilization and its regeneration. It may be suggested that some of the reactions of Calvin cycle taking part in RuBP regeneration are inhibited. RuBP regeneration could be limited either by an inability to supply reductants and ATP from electron transport or by an inactivation or loss of Calvin cycle enzymes other than Rubisco (Baker et al., 1997; Nogués and Baker, 2000). The large depression in A_{max} occurring at the end of drought period was accompanied by such large changes in the relative quantum efficiency of electron flux through PSII-Y (Table 2). This suggests that decrease in the ability to regenerate RuBP can be attributed to a reduction in non-cyclic electron transport and the ability to produce ATP and reductants, as is the situation in sunflower where inhibition of RuBP regeneration induced by water stress has been attributed to decrease in ATP supply resulting from a loss of ATP synthase (Tezara et al., 1999). Decrease in α is likely to result from loss or inactivation of Rubisco (Allen et al., 1997).

Despite of significant stomatal limitation of photosynthesis in cv. Zarya determined by SL parameter (Table 1), this was not accompanied with reduction of C_i. One of the reasons for the slight decrease in C_i could be the increased mesophyllic resistance for CO_2 transport. Another reason could be the intensified respiratory processes that are implied by the enhanced value of the CO_2 compensation point. Our results are consistent with those presented by Stolf-Moreira et al. (2010), and

are connected also with a significant influence of non-stomatal factors. Restricted diffusion of CO_2 into the leaf might not be the only reason for decreased A_n under drought stress, because high external CO_2 concentrations (1500 μmol mol^{-1}) fail to restore A_n to values of control plant. Direct inhibition of biochemical processes by altered ionic or osmotic conditions, which affect, e.g. ATP synthase and Rubisco activity, might be another reason for decreased A_n under drought (Tezara et al., 1999; Haupt-Herting and Fock, 2000). The suggestion that biochemical factors are involved in the response of photosynthesis to drought stress is supported by the reduced rate of A_{max}, the occurrence of increasing CO_2 compensation points (Γ), and reduced α.

At the end of drought period the value of SL in the primary leaf of cv, Zarya is significantly higher than the control plants, suggesting enhanced stomatal limitation. In cv. Tangra SL show only slight tendency to increase; therefore, mainly non-stomatal factors determine the response of photosynthesis to drought.

The rise of F_0 under unfavorable environmental conditions is usually due to the reduced plastoquinone acceptor (Q_A^-), being unable to be oxidized completely because of retardation of the electron flow through PSII (Velikova et al., 1999), or to the separation of light-harvesting Chl a/b protein complexes of PSII from the PSII core complex (Komura et al., 2010). The decrease 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 (Rochaix, 2011). Gilmore and Björkman (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 significantly decrease in F_v/F_m (Table 2) in cv. Tangra. 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, as previously noted by Verhoeven et al. (1997), a given light intensity (even at low PPFD) is potentially in greater excess under stress conditions, which usually limit photosynthetic activity.

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.

Cv. Tangra showed a greater decrease in the proportion of energy driven to the photosynthetic pathway (qP), what agrees with the most probable overreduction of the electron transport chain caused by the strong loss of PSI activity also, as shown in vigna plants (Campos, 1998).

Despite the decreases in the photochemical efficiency of PSII, cv. Zarya presented highest qP and Y, as well as the lowest energy dissipation (qN) values, what agrees with the higher photosynthetic capacity and carboxylation efficiency (Table 1). Similar effects on these Chl fluorescence parameters have been observed in different species and under various stress conditions. Velikova et al. (1999) established significant decrease in F_v/F_m, Y and qP in bean plants after simulated acid rain. Therefore, any factor that reduces the utilization of photosynthetic energy in carbon metabolism and affects high-energy-state-related qN, e.g. drought and water stress, will modify the rate of electron transport through PSII.

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 (Hu et al., 2010). Primary leaves showed a slight decrease in this parameter (Table 2). 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). Photochemical quenching (qP) presented a similar behaviour to Y. 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.

High values of qP are related to the presence of Q_A in the oxidized state. In this situation, non-photochemical quenching (qN) values are low and, if light intensity increases to values close to light saturation, qN increases rapidly corresponding to high rates of energy dissipation (Plesnicar and Pancovic, 1991)

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 can be taken as indicative of a physiological regulation of electron transport by increasing excitation energy quenching process in

the PSII antennae. In leaves of the studied cultivars the capacity for CO_2 assimilation decreases significantly (Table 1). However, in the cv. Tangra Y decreases with 46.74% (Table 2). This suggests that a considerable greater rate of non-cyclic electron transport is occurring than is required to maintain CO_2 assimilatory. An alternative sink to CO_2 assimilation for electrons would be oxygen reduction by photorespiration and/or a Mehler reaction, although in drought bean leaves it has been shown that photorespiration does not act to protect the photosynthetic apparatus from photodamage (Nogués and Baker, 2000).

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 Tangra could to be due to a combination of both of these factors. The very large decreases in the gas exchange parameters that occur in young been plants under drought and relatively smaller decreases in F_v/F_m suggests that demand for reductants and ATP has decreased dramatically and this is a major factor in the closure of PSII reaction centres. The larges decreases in Y in leaves of Tangra indicating that either PSII reaction centres had been damaged or slowly relaxing quenching had been induced. Clearly, negligible photodamage to PSII occurs during drought in leaves of cv. Zarya since no significant changes are found in F_v/F_m. Consequently, the drought induced decreases in Y that occur in Zarya are attributable to "down-regulation" of electron transport. 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 leaves induced by the water stress. However, photoinhibitory damage to PSII may be a secondary effect of drought in Tangra.

Photosynthetic parameters were almost recovered to the well-watered control level in both cultivars after 3 days of re-watering. Net photosynthetic rate of cv. Zarya returned near the well watered control level in greater extent than that for cv. Tangra, which could reflect the less severe damage in photosynthetic system during drought stress or less recovery required returning to non-stressed values.

At 3 days of re-watering, SL values of cv. Zarya decreased significantly below the levels at 10 days of drought, which may be the result of stomatal opening and resumption of metabolic activities from rehydration. Cv. Tangra had significantly higher SL than Zarya at 3 days of re-watering. The drought-induced decrease in photosynthetic capacity (A_{max},) was rapidly reversed following re-watering in cv. Zarya, indicating rapid recovery in Rubisco carboxylation and light saturated electron transport rates or less damages in both carbon fixation and light reaction of photosynthesis under drought stress in this cultivar. Our results are consistent with those presented by Hu et al. (2010) for *Poa pratensis* L. Decreased photochemical efficiency (F_v/F_m) after 10 days of drought and fast restoring to the control level also supporting the conception that the photosynthetic apparatus in leaves of cv. Zarya was maintained in a relatively high photoprotected state under drought stress and during recovery.

Conclusions

This study pointed out that drought produced significant increase of stomatal limitation in the primary leaf of cv. Zarya. This is accompanied by the decrease in all photosynthetic parameters and, consequently, stomatal closure would appear to be a more important factor contributing to the depressed CO_2 assimilation. In primary leaf of cv. Tangra non-stomatal limitations seem to assume a more important role in drought response. PSII activity in cv. Zarya was more efficiently protected than in cv. Tangra, as indicated by fluorescence measurements. After 3-days period of re-watering photosynthetic performances are recovered in greater extent in cv. Zarya.

In conclusion, cv. Zarya 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 genotype, and it presented a lower increase in qN. Photosynthetic apparatus in cv. Tangra can be considered as drought sensitive.

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