EFFECTS OF SOIL DROUGHT ON PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE IN BEAN PLANTS

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Abstract. The effects of soil drought on photosynthesis and chlorophyll fluorescence in the leaves of three bean (*Phaseolus vulgaris* L.) genotypes were studied. Drought was imposed 14 days after plants growing up. In the primary leaf of all the cultivars, water stress led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max}. The most strongly marked reduction in leaf CO_2 exchange was observed in cv. Dobrudjanski ran. Maximal carboxilation efficiency (α) and CO_2 assimilation (A_{max}) was reduced over five folds. At normal ambient CO_2 concentration (C_a 350 μmol mol^{-1}), leaf water deficit resulted in a dramatic reduction (92.2%) of A_n. CO_2 compensation point (Γ) increased with 127.5%. Stomatal limitation of photosynthesis (SL) increased significantly (131.5%), which suggests a stronger influence of stomatal factors. Lowest reduction in leaf gas exchange parameters were observed in cv. Prelom. Cv. Plovdiv 10 showed moderate behavior. In the primary and in the first trifoliate leaf of all genotypes studied, drought stress induced an increase in the minimal chlorophyll fluorescence (F_0), accompanied by a decrease in the maximal one (F_m). Cv. Prelom was less affected. The F_v/F_m ratio practically was not changed and showed a slight tendency to decrease in all genotypes. Cv. Dobrudjanski ran presented the highest decrease (52% and 43%) in photochemical quenching (qP), in contrast to cv. Prelom (29% and an 18%) in primary and first trifoliate leaves, respectively. The quantum yield of electron transport (Y) strongly decreased in cvs. Dobrudjanski ran and Plovdiv 10, while in cv. Prelom Y it was less affected. At the end of the drought period, in the primary and first trifoliate

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leaf, a significant increase was observed in the non-photochemical quenching (qN) of all genotypes, except for Prelom, thus denoting an increase in the energy dissipation through non-photochemical processes. Data obtained suggest that cv. Prelom is drought tolerant and cv. Dobrudjanski ran is drought sensitive. Plovdiv 10 showed moderate behavior.

***Keywords**:* drought, photosynthesis, chlorophyll fluorescence, *Phaseolus vulgaris* L.

***Abbreviations**:* A_{max} – maximal CO_2 assimilation; A_n – net CO_2 assimilation; C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; F_0 – minimal chlorophyll fluorescence in dark adapted leaves; F_m – maximal chlorophyll fluorescence in dark adapted leaves; F_v/F_m – maximal photochemical efficiency of PSII; PPFD- photosynthetic photon flux density; qN – non-photochemical fluorescence quenching; qP – photochemical fluorescence quenching; Rubisco – ribulose-1,5-bisphosphate carboxilase/oxienase; RuBP – ribulose-1,5-bisphosphate; SL – stomatal limitation of photosynthesis; Y – quantum yield of electron transport; α – maximal carboxylation efficiency; Γ – CO_2 compensation point; Ψ_{soil} – soil water potential.

INTRODUCTION

Drought stress is one of the major causes for crop loss worldwide, reducing average yields with 50% and over (Wang et al., 2003). Under such stress, water deficit in plant tissue develops, thus leading to a significant inhibition of photosynthesis. The ability to maintain the photosynthetic machinery functionality under water stress, therefore, is of major importance for drought tolerance. Plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration (Cornic, 1994). As a consequence, CO_2 diffusion into the leaf is restricted (Chaves, 1991). The decrease in net photosynthetic rate under drought stress observed in many studies is often explained by the lowered internal CO_2 concentration, which results in a limitation of photosynthesis at the acceptor site of ribulose-1,5-bisphospate carboxylase/oxygenase (Rubisco) (Cornic et al., 1992) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthase (Tezara et al., 1999; Nogués and Baker, 2000).

Despite of the fact that photosystem II (PSII) is highly drought resistant (Yordanov et al., 2003) under water stress, photosynthetic electron transport through PS II is inhibited (Chakir and Jensen, 1999). Several *in vivo* studies demonstrated that water deficit results in damages of the PSII oxygen-evolving complex (Lu and Zhang, 1999; Skotnica et al., 2000) and of the PSII reaction centers associated with the degradation of D1 protein (Cornic, 1994; He et al., 1995). Yet, the mechanism by which water deficit inhibits this electron transport is unclear.

However, many other studies have shown that the decreased photosynthesis rate under water stress can be attributed to the perturbations of the biochemical processes (Lauer and Boyer, 1992). There are several reports, which mark the photosynthesis stomatal limitation as a primary event, followed by respective changes of the photosynthetic reactions (Chaves, 1991). Today, there is a consensus that a decrease of the photosynthesis rate under water stress can be attributed to both stomatal and non-stomatal limitations (Shangguan et al., 1999). Non-stomatal photosynthesis limitation has been attributed to the reduced carboxylation efficiency (Jia and Gray, 2004), reduced ribulose-1,5-bisphospate (PuBP) regeneration, reduced amount of functional Rubisco (Kanechi et al., 1995), or to the inhibited functional activity of PSII. Inhibition or damages in the primary photochemical and biochemical processes may occur simultaneously (Lawlor, 2002). Since CO_2 maximal assimilation (A_{max}) reflexes the result of the mesophyllic impairments, its determination under severe water stress allows to evaluate the non-stomatal photosynthesis limitations and hence, the degree of drought tolerance of the photosynthetic machinery.

The present study aims to determine drought stress effects on leaf gas exchange and chlorophyll fluorescence parameters in leaves of three bean (*Phaseolus vulgaris* L.) genotypes. Analyses of the response of net CO_2 assimilation to intercellular CO_2 concentration, along with chlorophyll fluorescence measurements, allow the evaluation of the relative limitations of leaf photosynthesis imposed to changes in the stomatal conductance, carboxylation efficiency, capacity for regeneration of RuBP and PSII electron transport efficiency.

MATERIALS AND METHODS

Plant material and growth conditions

For the purposes of the present study, three genotypes of bean (*Phaseolus vulgaris* L.) were used: cv. Plovdiv 10, cv. Dobrudjanski ran and cv. Prelom. Seeds were washed in distilled water, surface sterilized and germinated on moist filter paper, in Petri dishes at 28 ^{0}C, in the dark, for 3 days. After germination, seedlings having well developed roots and being morphologically similar were selected and cultivated in pots as soil culture in a growth chamber. In order to eliminate the nutrient deficiency, dissolved salts were added to the soil 15 days before planting: 280 mg $Ca(NO_3)_2$ kg^{-1} dry soil, 180 mg KNO_3 kg^{-1} dry soil and 220 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 150 µmol m^{-2} s^{-1}, day/night temperature 25±2/17±2 ^{0}C, day/night photoperiod of 14/10 h**,** relative air humidity between 65-70 %. Pots were watered daily to maintain the 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 until soil water content reached 23% (0.230 g H_2O g^{-1} dry soil) corresponding to soil water potential of -0.9 MPa. In all genotypes studied, relative water content in the primary leaf was less than 65% and in the first trifoliate leaf - less than 75%. Measurements were taken at the end of the stress period on fully matured primary and first trifoliate leaves.

Gas exchange measurements

Gas exchange measurements were performed by a portable photosynthetic system LCA-4 (Analytical Development Company, Hoddesdon, UK) equipped with a PLCB-4 chamber. PPFD was 750 μmol m^{-2} s^{-1}, provided by a 500 W incandescent lamp with a reflector and a water filter. Leaf temperature was 27±2 ^{0}C, and ambient CO_2 concentration (C_a) was 350 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 used: $A_n = a + b\, e^{(-Ci/d)}$, 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); *d* is constant.

Photosynthesis stomatal limitations (SL) were calculated according to Farquhar and Sharkey (1982): $SL = (A_{Ci} - A_{Ca})/A_{Ci}$, where A_{Ci} is the net photosynthetic rate at C_i = 350 μmol mol^{-1} and A_{Ca} is the net photosynthetic rate at C_a = 350 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} photosynthetic photon flux density (PPFD) and maximal fluorescence, F_m, was measured after 0.8 s saturating white light pulse (>5500 μmol m^{-2} s^{-1} PPFD) in the same leaves. Maximal variable fluorescence (F_v=F_m–F_0) and PSII photochemical efficiency (F_v/F_m) of 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) were determined when actinic light was turned off. 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 one including at least five replications of each variant. The Student's *t*-test was used to evaluate the differences between the control and the stressed variants.

RESULTS

Drought effects on photosynthetic rate at different intercellular CO_2 concentrations

Net phototsynthetic rate changes in primary and first trifoliate bean leaves, as a function of the intercellular CO_2 concentration, were used to determine the role of stomatal limitations (SL) of A_n under drought stress. In the primary leaf of all the cultivars, leaf water deficit led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} (Fig. 1). A decline in the initial slope indicated a decreased RuBP carboxylase activity, while a low level of A_{max} at saturating CO_2 implicated a suppressed capacity for RuBP regeneration (von Caemmerer and Farquhar, 1981). The most strongly marked reduction of leaf gas exchange was observed in cv. Dobrudjanski ran (Table 1). α and A_{max} were reduced more than five folds. Exposure of bean plants to soil drought and leaf water deficit resulted in a dramatic reduction (with 92.2%) of A_n at normal C_a (350 μmol mol^{-1}). CO_2 compensation point (Γ) increased with 127.5%. SL increased significantly (131.5%), which suggests a stronger influence of stomatal factors. The lowest reduction in leaf gas exchange parameters was observed in cv. Prelom. There were no changes in SL, wich suggests a stronger influence of non-stomatal (biochemical) factors. Cv. Plovdiv 10 showed moderate behaviours.

Net phototsynthetic rate changes in first trifoliate bean leaves, as a function of the intercellular CO_2 concentration under drought stress, are shown in Fig. 2 and Table 2. In all genotypes studied, leaf water deficit led to a noticeable decrease in both the initial slope of the A_n/C_i curve and A_{max} (Fig. 2).

Highest reduction in leaf gas exchange was observed again in cv. Dobrudjanski ran (Table 2). α was reduced more than three folds and A_{max} was reduced more than six folds. Exposure of bean plants to soil drought resulted in a dramatic reduction (with a 83.5%) of A_n at normal C_a (350 μmol mol^{-1}). Γ increased with 193.5%. There were no changes in SL, which suggests an influence of stomatal as well as of biochemical factors. The lowest reduction in leaf gas exchange parameters was observed in cv. Prelom. SL increased with *ca.* 14%. Cv. Plovdiv 10 showed moderate behaviours. Stomatal limitation increased with 67%, thus suggesting an influence of stomatal factors.

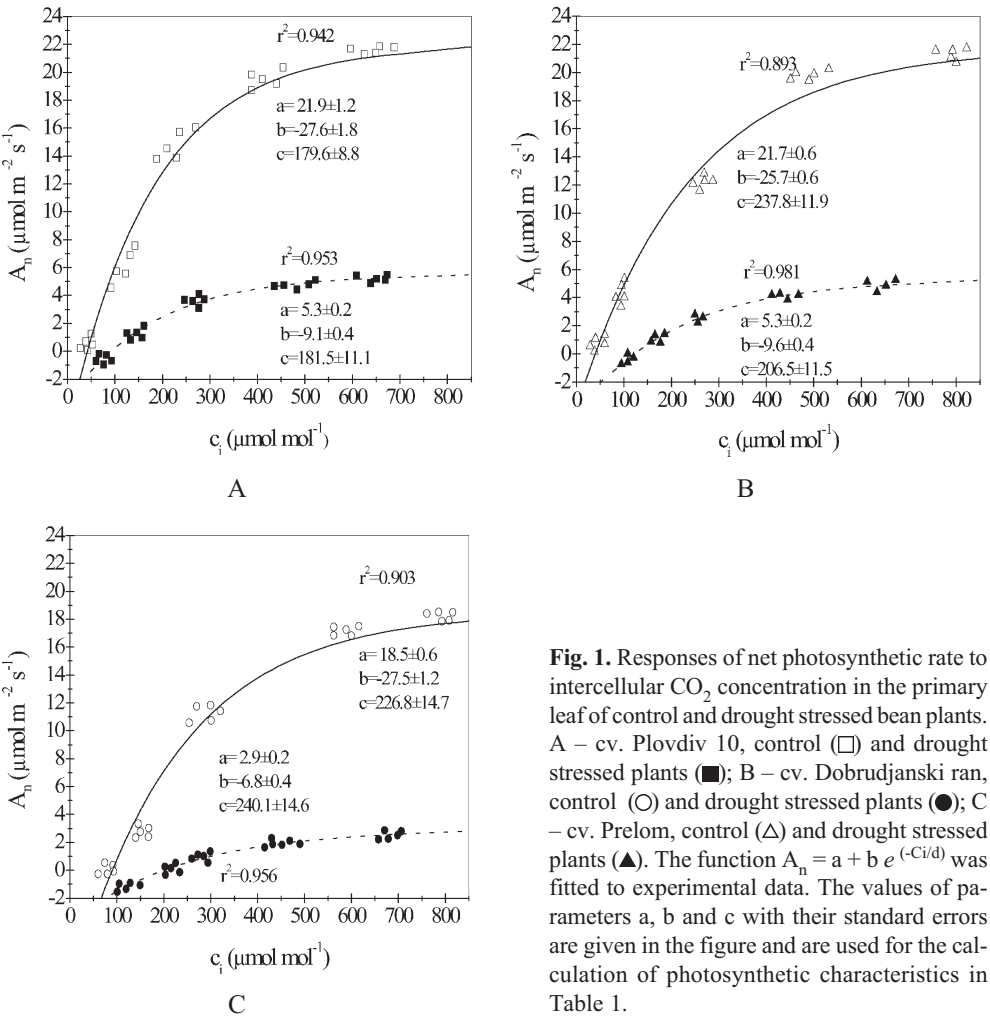


Fig. 1. Responses of net photosynthetic rate to intercellular CO_2 concentration in the primary leaf of control and drought stressed bean plants. A – cv. Plovdiv 10, control (□) and drought stressed plants (■); B – cv. Dobrudjanski ran, control (○) and drought stressed plants (●); C – cv. Prelom, control (△) and drought stressed plants (▲). The function $A_n = a + b\, e^{(-Ci/d)}$ was fitted to experimental data. The values of parameters a, b and c with their standard errors are given in the figure and are used for the calculation of photosynthetic characteristics in Table 1.

Chlorophyll fluorescence

In all genotypes studied, drought stress induced an increase in F_0 accompanied by a decrease in F_m in the primary, as well as the first trifoliate leaf. Cv. Prelom was less affected (Table 3). 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 (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, practically was not changed, except for the primary leaf of cv. Dobrudjanski ran, and in all genotypes showed a slight tendency to decrease.

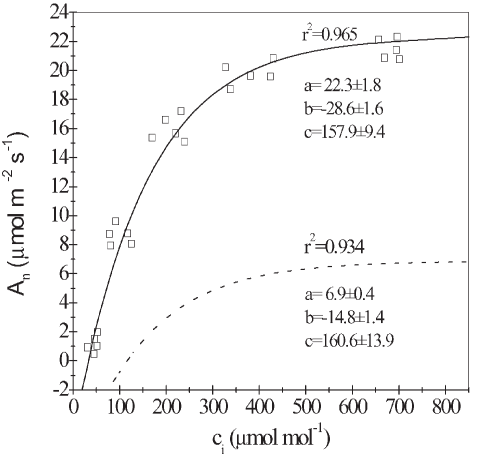
Table 1. Effect of soil drought on leaf gas exchange in primary leaf of control and drought stressed bean plants. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, maximal CO_2 assimilation at saturating CO_2; $A_{ca=350}$, net CO_2 assimilation at 350 μmol mol^{-1} ambient CO_2 concentration; $C_{i(Ca=350)}$, intercellular CO_2 concentration at 350 μmol mol^{-1} ambient CO_2 concentration; SL, stomatal limitation of photosynthesis.

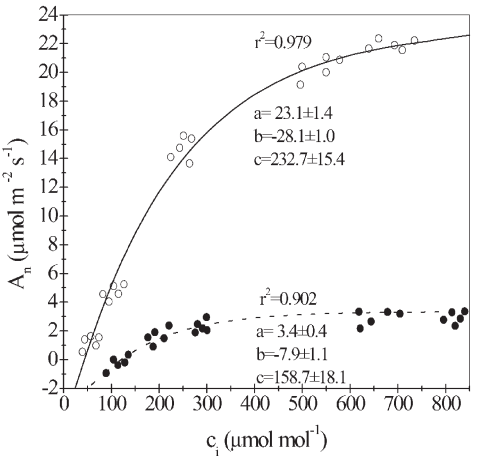
	α (μmol m^{-2} s^{-1} mol^{-1})	Γ (μmol mol^{-1})	A_{max} (μmol m^{-2} s^{-1})	$A_{Ca=350}$ (μmol m^{-2} s^{-1})	$C_{i(Ca=350)}$ (μmol mol^{-1})	SL (%)
			Control			
Plovdiv 10	0.137	41.5	21.9	13.29	254	18.4
Dobrudjanski ran	0.100	89.9	18.5	10.61	262	16.5
Prelom	0.099	40.2	21.7	14.31	233	20.4
			Drought stressed			
Plovdiv 10	0.038	98.1	5.3	2.51	253	28.5
Dobrudjanski ran	0.019	204.6	2.9	0.84	284	38.2
Prelom	0.035	122.7	5.3	3.42	270	19.3

Cv. Dobrudjanski ran presented a decrease of 52% and 43% in the proportion of energy driven to the photosynthetic pathway (qP) in the primary and first trifoliate leaves, respectively, while in cv. Plovdiv 10 qP decreased with 36% and 28%, respectively. Cv. Prelom showed a 29% and an 18% decrease in qP. Y strongly decreased in cvs. Dobrudjanski ran and Plovdiv 10, while in cv. Prelom was less affected (Table 3).

By the end of the drought period, in the primary and first trifoliate leaf, significant increase was observed in the non-photochemical quenching (qN) of all genotypes, except for cv. Prelom. This denoted an increase in the energy dissipation through non-photochemical processes.

The differences between control and droughted plants were greatest in the effective quantum yield, i.e. Genty parameter, Y (Genty et al., 1989) and in qP and qN parameters, as well. Cv. Dobrudjanski ran, droughted for 10 days, showed a decrease in Y of 4-fold and 2.5-fold for primary and first trifoliate leaves, respectively. Under the same conditions, the inhibition of cv. Plovdiv 10 was a little higher, 50% in both measured leaves. On the other hand, in cv. Prelom the inhibition was only about 20% for primary and trifoliate leaves. The qP and qN were less informative (Table 3). Significant decrease in PS2 efficiency (Fv/Fm) was observed only in cv. Dobrujanski ran. Hence, data obtained showed that the inhibition of photosynthesis in droughted plants is caused not only by injury of both thylakoid membrane electron transport and Calvin cycle reactions, but also by other factors. The decrease of electron transport efficiency might be a result of Calvin cycle disturbances, which delays reoxidation of Q_A- and induced PS2 down regulation, causing considerable decrease of linear electron transport. The contribution to the drastic reduction of maximal effectiveness of





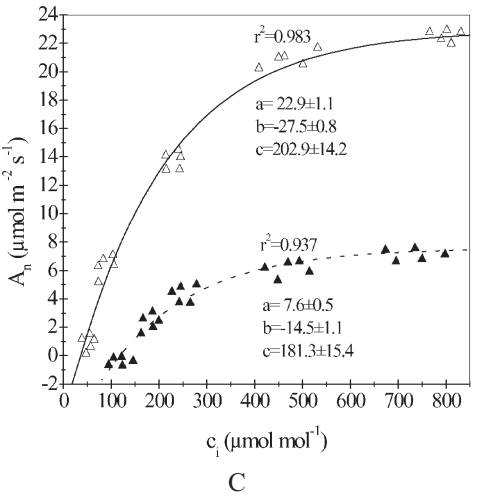


Fig. 2. Responses of net photosynthetic rate to intercellular CO_2 concentration in the first trifoliate leaf of control and drought stressed bean plants. A – cv. Plovdiv 10, control (□) and drought stressed plants (■); B – cv. Dobrudjanski ran, control (○) and drought stressed plants (●); C – cv. Prelom, control (△) and drought stressed plants (▲). The function $A_n = a + b\ e^{(-Ci/d)}$ was fitted to experimental data. The values of parameters a, b and c with their standard errors are given in the figure and are used for the calculation of photosynthetic characteristics in Table 2.

carboxylation (α) in vivo may probably have also acidified chloroplast stroma, which slowed the substrate affinity of Rubisco (Chaves, 1991). Regulation occured between the two photosystems – in contrast to PS2, PS1 became more oxidised and rate constant for P700 re-reduction decreased. In addition, fructose-1,6-bisphosphatase and seduheptulose-1,7-bisphosphatase are rather sensitive to drought and this is a result of their subsequent damages by reactive oxygen species formed. It is also probably a result from a deviation of electrons to Mehler reaction and/or PS2 cyclic electron flow, generation of ROS and overreduced PQ pool, followed by injury of D1 protein of PS2 reaction center.

Table 2. Effect of soil drought on leaf gas exchange in first trifoliate leaves of control and drought stressed bean plants. α, maximal carboxylation efficiency; Γ, CO_2 compensation point; A_{max}, maximal CO_2 assimilation at saturating CO_2; $A_{ca=350}$, net CO_2 assimilation at 350 μmol mol^{-1} ambient CO_2 concentration; $C_{i(Ca=350)}$, intercellular CO_2 concentration at 350 μmol mol^{-1} ambient 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})	$A_{Ca=350}$ (μmol m^{-2} s^{-1})	$C_{i(Ca=350)}$ (μmol mol^{-1})	SL (%)
			Control			
Plovdiv 10	0.160	39.3	22.3	14.93	204	23.9
Dobrudjanski ran	0.110	45.6	23.1	13.80	245	20.4
Prelom	0.124	37.1	22.9	14.30	228	22.3
			Drought stressed			
Plovdiv 10	0.064	122.6	6.9	3.31	223	40.0
Dobrudjanski ran	0.033	133.8	3.4	2.28	286	19.4
Prelom	0.059	117.1	7.6	3.90	248	30.5

DISCUSSION

Soil drought and leaf water deficit lead to a progressive suppression of photosynthtetic carbon assimilation (Chaves, 1991; Yordanov et al., 2000). Decreased photosynthetic rate is a result from stomatal and non-stomatal (biochemical) limitations (Yordanov et al., 2003).

Our results showed that drought reduces gas exchange and maximal carboxylation efficiency, and increases the CO_2 compensation point of young been plants. This treatment changes photosynthesis CO_2 curves shape. As compared to the control plants, plants subjected to drought exhibited a noticeable decrease in both the initial slope and the plateau of these curves (Figs. 1 and 2). According to von Caemmerer and Farquhar (1981), the initial slope of the CO_2 curve is defined by 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 associated with the electron transport activity. Drought treatment led to a reduction of both Rubisco carboxylation activity and RuBP regeneration capacity, indicated by the initial slope lowering and the CO_2 plateau saturation. According to Lawlor and Cornic (2002), decreased A_{max} under low relative water content is caused by an impaired metabolism (storage of ATP, limiting RuBP synthesis without or with less inhibition of photosynthetic enzymes including Rubisco). This dependence is strongly expressed in leaves of cv. Dobrudjanski ran (Fig. 1B and 2B). Thus, photosynthesis could be adjusted through a balance between Rubisco carboxylation capacity, RuBP utilization and its regeneration. 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 (Nogués and Baker, 2000). A_{max} depres-

Table 3. Parameters of chlorophyll fluorescence in leaves of control and drought stressed bean plants

Genotype	Variant	F_0	F_m	F_v/F_m	Y	qP	qN
				Control			
Plovdiv 10	Primary leaf	425±16	2083±82	0.796±0.028	0.485±0.021	0.773±0.031	0.573±0.028
	I trifoliate leaf	361±13	1900±77	0.810±0.031	0.514±0.026	0.811±0.039	0.569±0.027
Dobrudjanski ran	Primary leaf	484±19	2343±79	0.793±0.026	0.424±0.020	0.742±0.032	0.644±0.034
	I trifoliate leaf	385±13	2047±70	0.812±0.033	0.497±0.023	0.801±0.041	0.681±0.036
Prelom	Primary leaf	407±18	2157±74	0.811±0.035	0.491±0.028	0.788±0.035	0.572±0.032
	I trifoliate leaf	382±13	1900±66	0.799±0.029	0.534±0.031	0.816±0.043	0.546±0.027
				Drought treated			
Plovdiv 10	Primary leaf	484±19 *	1820±64 *	0.734±0.025	0.262±0.013 ***	0.495±0.026 ***	0.802±0.042***
	I trifoliate leaf	398±15	1780±74	0.776±0.027	0.324±0.017 ***	0.584±0.037 **	0.745±0.038**
Dobrudjanski ran	Primary leaf	570±24 *	1915±71 *	0.702±0.021 *	0.107±0.011 ***	0.356±0.022 ***	0.969±0.051***
	I trifoliate leaf	433±15 *	1721±58 *	0.748±0.024	0.204±0.014 ***	0.457±0.028 ***	0.984±0.053***
Prelom	Primary leaf	451±19	1914±68 *	0.765±0.023	0.397±0.019 *	0.559±0.036 **	0.670±0.041
	I trifoliate leaf	403±14	1850±67	0.782±0.028	0.465±0.024 *	0.668±0.039 *	0.607±0.033

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

sion occurring at the end of drought period was accompanied by changes in the relative quantum efficiency of electron flux through PSII (Y). Similar changes were observed 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 the significant photosynthesis stomatal limitation determined by SL parameter, it was not accompanied with reduction of C_i (Tables 1 and 2). In fact, there was a slight increase (10 – 14%) in C_i at C_a=350 μmol mol^{-1} in primary and first trifoliate leaves of the genotypes studied. One of the reasons for the slight increase 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. 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, e.g. ATP synthase and Rubisco activity, might be another reason for the 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 and reduced α.

At least two distinct phenomena are involved in the changes of the fluorescence parameters under unfavorable environmental conditions (Baker and Horton, 1987). The first phenomenon results in an increased F_0, possibly due to the reduced plastoquinone acceptor (Q_A^-), unable to be oxidized completely because of the electron flow retardation through PSII (Krause and Weis, 1991; Velikova et al., 1999), 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 phenomenon is responsible for the quenching of both F_v and F_m. Preferential quenching of F_v would indicate more extensive damage to the reaction centers, so that charge recombination is prevented. F_m decrease may be related to the 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 Björkman (1995) have pointed out that increased non-radiative energy dissipation would be accompanied by a quenching of F_m.

In all the genotypes, the increase of F_0 and decrease of F_m under drought stress occurred concomitantly to the decrease in F_v/F_m (Table 3). This suggests the occurrence of chronic photoinhibition due to photoinactivation of PSII centers, possibly attributable to D1 protein damage (Rintamäki et al., 1994; Campos, 1998). In bean droughted leaves, photoinhibitory impact on PSII could occur due to the increase of light intensity (even at low PPFD) under stress conditions, which usually limits photosynthetic activity (Verhoeven et al., 1997). 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 (Björkman and Powles, 1984), and the inhibition of photosynthetic activity could reflect the inactivation of PSII activity and the concomitant uncoupling of non-cyclic photophosphorylation, as was observed in soybean (Younis et al., 1979) and *Nerium oleander* (Björkman and Powles, 1984).

In all the cultivars, the occurrence of down regulation was reinforced by the decline of electron transport quantum yield (Y). Cv. Dobrudjanski ran showed a greater decrease in qP, in accordance with the most probable overreduction of the electron transport chain caused by the strong loss of PSI activity as shown in vigna plants (Campos, 1998).

Despite the decreases in the photochemical efficiency of PSII, cv. Prelom presented highest qP and Y, as well as the lowest energy dissipation (qN) values, in accordance with the higher photosynthetic capacity and carboxylation efficiency (Tables 1 and 2). Cv. Dobrudjanski ran showed stronger decrease in photosynthetic capacity and carboxylation efficiency than cvs Plovdiv 10 and Prelom. These decreases could be due to a direct dehydration effect on Rubisco (Kaiser, 1987), an increase in Rubisco hydrolysis (Evans, 1989), 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.

Similar effects on these Chl fluorescence parameters have been observed in different species and under various stress conditions. Vassilev and Manolov (1999) demonstrated a significant decrease of Y and qP accompanied by an increase of qN in cadmium treated plants. 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 which reduces the utilization of photosynthetic energy in carbon metabolism and affects high-energy-state-related qN, e.g. 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 (Öquist et al., 1992). Primary and first trifoliate leaves showed a slight decrease in this parameter (Table 3). 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 3). Photochemical quenching (qP) presented a similar behavior to Y. This suggests that Y is dependent mainly on the proportion of the reaction centers, which are photochemically "open" (expressed by qP), rather than on the efficiency of the absorbed photons in reaching a reaction center.

Y decreases are associated with excitation energy quenching increases in the PSII antennae and are generally considered indicative of "down regulation" of elec-

tron transport (Horton et al., 1996). In the leaves of all three species, the capacity for CO_2 assimilation decreased significantly (Table. 1). However, in the cv. Prelom Y decreased with only 19% and 13% for primary and first trifoliate leaf, respectively (Table 3). This suggests that a considerably greater rate of non-cyclic electron transport is occurring compared to the required for the maintaining of CO_2 assimilation. An alternative sink to CO_2 assimilation for electrons would be the oxygen reduction by photorespiration and/or a Mehler reaction.

The high decreases observed in the gas exchange parameters that occur in young been plants under drought and relatively smaller decreases in F_v/F_m, suggest the demand for reductants and ATP has decreased dramatically. All this is a major factor in the closure of PSII reaction centres. Y decreases in the leaves of Dobrudjanski ran indicating that either PSII reaction centers had been damaged or slowly relaxing quenching had been induced. This study supports the contention that photodamage to PSII reaction centers is not a primary factor in the depression of CO_2 assimilation of the leaves induced by water stress. Photoinhibitory damage of PSII may be a secondary effect of drought in Dobrudjanski ran. Data obtained 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.

Drought produced increase in stomatal limitation in the primary leaves of cvs. Plovdiv and Dobrudjanski ran and in the first trifoliate leaves of cv. Plovdiv 10 and Prelom. Increases in stomatal limitation accompanied the decreases in all photosynthetic parameters and, consequently, stomatal closure is found to be an important factor contributing to the depressed CO_2 assimilation. PSII activity in cv. Prelom was more efficiently protected than in the other genotypes, as indicated by the fluorescence measurements.

In conclusion, cv. Prelom and cv. Plovdiv 10 can be qualified as drought tolerant, while cv. Dobrudjanski ran can be considered as drought sensitive.

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