Research Article

Impact of soil drought stress on photochemical efficiency of photosystem II and antioxidant enzyme activities of *Phaseolus vulgaris* cultivars

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Abstract: Common bean (*Phaseolus vulgaris* L.) cultivars (cvs) (Göynük 98, Karacaşehir 90, Şehirali 90, ES 855, and Yunus 90) were subjected to drought stress in order to assess the levels of drought tolerance through the analysis of growth parameters, leaf water potential (Ψ_{leaf}), stomatal conductance (g_s), chlorophyll (chl) content, and lipid peroxidation. Significant differences were recorded among cultivars in most traits. Yunus 90 was identified as the most tolerant, and Karacaşehir 90 was the most sensitive. Furthermore, the changes in antioxidant enzyme activities, H_2O_2 content, and Photosystem II were investigated under drought stress, where Antioxidant enzyme activities increased while H_2O_2 contents decreased. In Yunus 90, increases in catalase (CAT) and ascorbate peroxidase (APX) activities were higher than those of the other cvs. Increase in GPX activity was higher in Karacaşehir 90 compared to Yunus 90. Drought stress reduced quantum yield of PS II photochemistry (Φ_{PSII}) and photochemical quenching (qP) in all cultivars. The reduction was more pronounced in Karacaşehir 90. These results showed that different common bean cvs had different photochemical efficiencies and used different antioxidant enzymes in order to scavenge reactive oxygen species.

Key words: Ascorbate peroxidase, catalase, chlorophyll fluorescence kinetics, drought tolerance, *Phaseolus vulgaris*

Topraktaki kuraklık stresinin *Phaseolus vulgaris* kültivarlarında fotosistem II fotokimyasal etkinliği ve antioksidan enzim aktiviteleri üzerindeki etkisi

Özet: Fasulye kültivarları (Göynük 98, Karacaşehir 90, Şehirali 90, ES 855 ve Yunus 90), büyüme parametreleri, yaprak su potansiyeli (Ψ_{yaprak}), stoma iletkenliği (g_s), klorofil (chl) içeriği ve lipid peroksidasyonu ile kuraklık tolerans seviyelerinin belirlenmesi amacı ile kuraklık stresine maruz bırakıldı. Kültivarlar arasında çoğu özellikler açısından önemli farklılıklar belirlendi. Yunus 90 en toleranslı, Karacaşehir 90 en hassas, diğer kültivarlar ise ara formlar olarak belirlendi. Ayrıca, antioksidan enzim aktivitelerindeki değişimler, H_2O_2 içeriği ve fotosistem II kuraklık koşulları altında çalışıldı. Kuraklık koşulları altında H_2O_2 içeriği azalırken, antioksidan enzim aktiviteleri arttı. Yunus 90'da, katalaz (CAT) ve askorbat peroksidaz (APX) aktivitelerindeki artış diğer kultivarlar ile kıyaslandığında daha yüksekti. Karacasehir 90' da GPX aktivitesindeki artış, Yunus 90'ınkinden daha fazlaydı. Kuraklık stresi, bütün kültivarlarda PS II kuantum verimi (Φ_{PSII}) ve fotokimyasal söndürme (qP)' i indirgedi. İndirgenme, Karacaşehir 90 için daha fazla idi. Sonuçlar, farklı fasulye kultivarlarının farklı fotokimyasal etkinlik gösterdiğini ve reaktif oksijen türlerini temizlemek için farklı antioksidan enzimleri tercih ettiklerini gösterdi.

Anahtar sözcükler: Askorbat peroksidaz, katalaz, klorofil floresans kinetiği, kuraklık toleransı, *Phaseolus vulgaris*

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Abbreviations: APX: ascorbate peroxidase, CAT: catalase, chl: chlorophyll, F_0: minimum fluorescence yield in the dark adapted state, F_m: maximum fluorescence yield in the dark adapted state, F_m': maximum fluorescence yield in the light adapted state, F_s: steady-state fluorescence yield, F_v/F_m: maximum quantum yield of photosystem II photochemistry, GPX: guaiacol peroxidase, g_s: stomatal conductance, MBTH: 3-methyl-2-benzothiazolinone hydrazone, MDA: malondialdehyde, NAR: net assimilation rate, PS: photosystem, *NPQ*: non-photochemical quenching, *qP*: photochemical quenching, Φ_{PSII}: quantum yield of PS II photochemistry, RGR: relative growth rate, SLA: specific leaf area, SOD: superoxide dismutase.

Introduction

Drought is a major abiotic stress that severely affects agricultural systems and food production. Common bean (*Phaseolus vulgaris* L.) is an important crop from the Fabaceae family that is cultivated worldwide for human consumption. Its sensitivity to water deficit is average when compared to other grain legumes (Cruz de Carvalho et al., 1998). A water deficiency during any growth stages of bean species often results in a loss of yield. Therefore, it is important to elucidate the drought tolerance mechanisms of these species in order to improve its agronomic performances and to obtain more resistant cultivars (Subbarao et al., 1995).

Various tests and selection procedures are often used for identifying drought tolerant genotypes. Tests usually involve seedling growth or survival under conditions of induced water deficit (O'Toole et al., 1978). One of the factors associated with many critical aspects of plant growth and survival is specific leaf area that is the ratio of fresh foliage surface area to unit dry foliage mass or projected leaf area per dry mass (Shipley & Vu, 2002). Specific leaf area is often positively correlated with seedling potential relative growth rate (Muller & Garnier, 1990) and leaf net assimilation rate (Shipley & Lechowicz, 2000) and is reduced under drought conditions (Marcelis et al., 1998). Few traits such as osmotic adjustment, stomatal conductance and lipid peroxidation are also considered to be important for drought resistance (McWilliam, 1989; Van Rensburg & Kruger, 1994).

On the other hand, understanding the physiological and biochemical mechanisms providing these common bean cvs with drought tolerance is very important in terms of developing selection and breeding strategies. There are some studies on the responses of plants to drought suggesting that different mechanisms may result in improved drought tolerance (Ristic & Cass, 1991; Grzesiak et al., 1997). Modulation in the activities of antioxidant enzymes may be one of the important factors in tolerance of various plants to environmental stress. The close relationship between antioxidant activity and drought stress tolerance has been observed in several crops (Bowler et al., 1992; Perl et al., 1993; Behnamnia et al., 2009).

Another important factor in tolerance of plants to stress is probably the regulation of photosynthesis. Generally, water stress may damage oxygen-evolving complex of photosystem II and PS II reaction centres (Subrahmanyam et al., 2006). In the literature, there are contradictory reports of the direct effects of water stress on PS II functionality (Genty et al., 1987, Colom & Vazzana, 2003). It has been reported that the reduction in photosynthesis and other related traits was more pronounced in relatively susceptible cultivars than in the relatively tolerant ones (Subrahmanyam et al., 2006). Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and are a powerful tool to study the plant's response to environmental stress (Massacci et al., 2008).

There are some reports on photochemical efficiency of PS II (Loggini et al., 1999; Nar et al., 2009) and antioxidant mechanisms under drought stress in tolerant and susceptible cvs of crop species (Sairam et al., 1997b; Shao et al., 2005). However, to the best of our knowledge, antioxidant defence mechanism together with efficiency of PS II was not studied in *Phaseolus vulgaris* cvs with different tolerance levels under drought. The first objective of the present study was to assess the drought tolerance based on variation of physiological characteristics and lipid peroxidation within different common bean (*Phaseolus vulgaris*) cvs. The other objective was to determine efficiency of PS II and changes in enzyme activities of APX, GPX, CAT, and superoxide dismutase (SOD) and H_2O_2 level in these cvs differing in drought tolerance.

Materials and methods

Plant material, growth, stress conditions

Seeds of *Phaseolus vulgaris* cultivars (Göynük 98, Karacaşehir 90, Şehirali 90, ES 855, and Yunus 90) were provided by the Anatolian Agricultural Research Institute, Eskişehir, Turkey. Plants were grown in plastic pots (16 cm height, and 18 cm top and 12 cm bottom diameter) containing peat and sand (5:1) in a greenhouse (temperature: 25 °C ± 2 and relative humidity: 60% ± 5) for 21 days. Plants were maintained well watered by daily irrigation or subjected to drought stress by withholding irrigation for 10 days similar to França et al. (2000). First trifoliate leaves were harvested on the 10th day of drought and the following analyses were performed.

Water potential

Leaf water potential was measured with a C52 thermocouple psychrometer (Wescor, Inc., Logan, UT, USA). Discs about 6 mm in diameter were cut from leaves and sealed in a C-52 psychrometer chamber. Samples were equilibrated for 45 min before the readings were recorded by a Wescor PSYPRO water potential data logger in the psychrometric mode. Values of leaf water potential were measured as MPa.

Stomatal conductance

Stomatal conductance was monitored using a dynamic diffusion porometer (AP4, Delta-T Devices, Burwell, Cambridge, UK) after calibrating with a standard calibration plate following the manufacturer's instructions. Values of stomatal conductance were measured as mmol m^{-2} s^{-1}.

Plant growth characters

The oven dry weight of the plant and the size of the assimilating system in terms of leaf area were utilized for plant growth analysis. The parameters for the growth analysis, the net assimilation rate (NAR), relative growth rate (RGR), and specific leaf area (SLA) were calculated for each interval between 2 samplings by the formulae described by Watson (1952) and Radford (1967).

Chlorophyll contents

Total chlorophyll and chl a, and b contents were determined following the method of Arnon (1949).

Fresh leaf samples were selected randomly and homogenized in a mortar in 80% acetone. The extract was centrifuged at 5000 ×g for 5 min. Absorbance of the supernatant was recorded at 663, 645, and 450 nm by spectrophotometer (Nicolet evolution 100, Thermo Scientific, USA).

Lipid peroxidation

Lipid peroxidation was measured in the term of malondialdehyde content (e = 155 mM^{-1} cm^{-1}), a product of lipid peroxidation following the method of Heath & Packer (1968). Leaf samples were homogenized in 0.1% (w/v) trichloroacetic acid. To 1 mL aliquot of supernatant, 4 mL of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid was added. The mixture was heated at 95 °C for 30 min, quickly cooled, and centrifuged at 10,000 ×g for 10 min. The absorbance of the supernatant was recorded at 532 and 600 nm. The MDA content was measured as nmol g^{-1} FW.

Antioxidant enzyme assays

Frozen leaf segments (0.5 g) were crushed into fine powder in a mortar and pestle under liquid N_2. The leaf powder was homogenized in 50 mM potassium phosphate (K_2HPO_4) buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpolypyrrolidone, with the addition of 5 mM ascorbic acid in the case of the APX assay. The homogenate was centrifuged at 20,000 ×g for 20 min at 4 °C and the supernatant was used for the following enzyme assay.

Superoxide dismutase (EC 1.15.1.1) activity was based on the method of Beauchamp and Fridovich (1971) as modified by Dhindsa and Matowe (1981). The reaction product was measured at 560 nm. The volume of supernatant corresponding to 50% inhibition of the reaction was assigned a value of 1 enzyme unit.

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed by the method of Nakano and Asada (1987). The rate of decrease in the absorbance of ascorbic acid at 290 nm (e = 2.8 mM^{-1} cm^{-1}) was measured in 1 mL of a reaction mixture containing an aliquot of supernatant, 0.25 mM ascorbic acid, and 5 mM hydrogen peroxide in 50 mM phosphate buffer (pH 7).

Guaiacol peroxidase (EC 1.11.1.7) was determined by a previously described method (Mika & Lüthje, 2003). At a wavelength of 470 nm, the increase in absorbance of tetraguaiacol was assayed over a period of 3 min. The extinction coefficient of tetraguaiacol at 470 nm is 26.6 mM^{-1} cm^{-1}.

Catalase (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (e = 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min (Aebi, 1983). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 10 µL of enzyme extract in a 1 mL volume.

Assay of H_2O_2

Leaves (1 g) were ground in 5% trichloroacetic acid with activated charcoal at 0 °C. The homogenate was filtered with cheesecloth, centrifuged, and the supernatant was adjusted to pH 3.5 with 4 N KOH. H_2O_2 content was measured according to the method of Capaldi and Taylor (1983). The sample (200 µL) was added to 100 µL of reagent solution containing 3.4 mM MBTH and 3.32 mM formaldehyde. The reaction was initiated by adding 500 µL of the solution of horseradish peroxidase (0.5 U) in 0.2 M sodium acetate buffer (pH 3.5), and after 2 min it was quenched with 1400 µL of 1N HCl. The absorbance at 630 nm was measured 15 min after quenching. H_2O_2 content was measured as µmol g^{-1} FW.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed by OS1-FL, a pulse modulated fluorometer (OptiScience Corporation, Tyngsboro, MA) according to Zhang et al. (2005). Fifteen typical leaves were selected and dark-adapted for 20 min before measuring the chlorophyll fluorescence. The minimal fluorescence yield (F_0) was determined under weak modulated λ_{660}-irradiation (<0.1 µmol m^{-2} s^{-1}). Maximal fluorescence yield (F_m) was reached by exposing leaves to saturating λ_{690} pulse (0.8 s) of white light (8000 µmol m^{-2} s^{-1}). After dark measurements, the leaves were exposed to an actinic light (5.5 W halogen lamp). Steady state fluorescence (F_s) was achieved after exposure to the actinic light for 10 min. Intensity of the actinic light was 120 µmol photons m^{-2} s^{-1}. Saturating pulses (0.8 s) of white light (8000 µmol photons m^{-2} s^{-1}) were applied to determine,

maximum fluorescence in the light. Definitions of fluorescence parameters (qP, NPQ, F_v/F_m and Φ_{PSII}) were used as described by Van Kooten and Snel (1990). Fv/Fm and Φ_{PSII} are indicators of the maximum and effective quantum yield of the PSII, respectively. The photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated according to Dall'Osto et al. (2007) and Bilger and Björkman (1990), respectively. Maximum quantum yield of photosystem II photochemistry (F_v/F_m) and quantum yield of PSII photochemistry (Φ_{PSII}) were calculated by fluorometer according to equations ($F_v/F_m = (F_m-F_0)/F_m$, $\Phi_{PSII} = (F_m' - F_s)/F_m'$) of Genty et al. (1989). All parameters were quantified at module 4 of the OS1-FL.

Statistical analysis

Analysis of variance (ANOVA) of means of 6 replicates for plant growth parameters, water potential, stomatal conductance, photosynthetic pigment contents, and lipid peroxidation was performed with Duncan Multiple Comparison test using SPSS for Microsoft Windows (Ver. 10.0, SPSS Inc., USA) and statistical significance was determined at P < 0.05 level.

Results

Leaf water status and stomatal conductance

The decrease in leaf water potential was found to be lowest in Yunus 90, average in Göynük 98, Şehirali 90, and ES 855, and highest in Karacaşehir 90 (Table). Similarly, stomatal conductance declined during drought stress in the leaves. Yunus 90 had the lowest decrease while Karacaşehir 90 and Şehirali 90 had higher decreases in g$_s$ (Table).

Effects on plant growth

Drought stress resulted in marked drop in RGR, NAR, and SLA. Different responses in plant growth characters were determined among the cultivars. The decreases in RGR and NAR were lowest in Yunus 90, average in Şehirali 90, and highest in Karacaşehir 90. The lowest and highest decreases in SLA were observed in Göynük 98 and ES 855, respectively, while the decreases in SLA were average in Yunus 90, Şehirali 90, and Karacaşehir 90 (Table).

Table. The effect of drought period on water potential (Ψ_{leaf}), stomatal conductance (g_s), growth analysis, chlorophyll contents (chl a, chl b, and total chl), and lipid peroxidation (MDA) on common bean cultivars. Values are expressed as Ψ_{leaf}: (MPa), g_s: (mmol m^{-2}s^{-1}), RGR: (mg day^{-1}), NAR: (g cm^{-2} day^{-1}), SLA: (cm^2 mg^{-1}), chl a, chl b, and total chl: (mg g^{-1} DW), MDA: (nmolg^{-1} FW). Means followed by different letters in each column are significant at P < 0.05 (n = 6).

Cultivars	Ψ_{leaf}	g_s	RGR	NAR	SLA	Chl a	Chl b	Total chl	MDA
				Control					
Şehirali 90	-0.25 a	152 h	0.66 ef	0.039 f	33.4 f	5.2 d	1.8 d	7.0 f	24.3 a
Yunus 90	-0.22 a	70 f	0.70 f	0.027 cd	35.6 g	5.9 g	1.9 d	7.8 i	22.0 a
ES 855	-0.24 a	107 g	0.63 ef	0.035 ef	37.3 h	5.6 f	1.8 d	7.4 h	21.6 a
Göynük 98	-0.23 a	161 i	0.60 e	0.017 a	46.2 j	5.3 de	1.9 d	7.2 g	24.1 a
Karacaşehir 90	-0.26 a	303 j	0.50 d	0.032 de	28.1 d	5.5 ef	1.9 d	7.4 h	33.3 cd
				Stress					
Şehirali 90	-0.91 d	4.8 a	0.31 b	0.023 bc	26.4 c	3.2 b	0.9 ab	4.1 c	35.8 d
Yunus 90	-0.63 b	8.2 c	0.65 ef	0.027 cd	29.5 e	4.4 c	1.0 bc	5.4 e	30.3 bc
ES 855	-0.96 e	6.0 b	0.29 b	0.019 ab	25.8 b	3.0 ab	0.9 b	3.9 b	32.8 cd
Göynük 98	-0.70 c	8.7 d	0.41 c	0.013 a	41.3 i	3.2 b	1.1 c	4.3 d	28.3 b
Karacaşehir 90	-1.03 f	12.5 e	0.20 a	0.016 a	22.0 a	2.8 a	0.8 a	3.6 a	65.0 e

Effect on chlorophyll contents

Chlorophyll contents were also affected by drought stress. There were significant decreases in chlorophyll a (chl a), chlorophyll b (chl b), and total chlorophyll contents under drought stress in all cultivars. Yunus 90, Şehirali 90, and Göynük 98 had lower decreases in chl a contents compared to ES 855 and Karacaşehir 90 in leaves under drought stress. In addition, Göynük 98, Yunus 90, and ES 855 had lower decreases in chl b contents compared to Şehirali 90 and Karacaşehir 90. Yunus 90, Göynük 98, and Şehirali 90 also had lower decreases in total chl contents compared to ES 855 and Karacaşehir 90 in leaves (Table).

Lipid peroxidation

The increases in the lipid peroxidation were lowest in Göynük 98, average in Yunus 90, Şehirali 90, and ES 855, and highest in Karacaşehir 90, respectively (Table).

Antioxidant enzyme activities and H_2O_2 content

Drought stress affected antioxidant enzyme activities and H_2O_2 contents of all cultivars with different responses to drought. The activities of APX, GPX, SOD, CAT, and H_2O_2 contents are presented in Figures 1-3. The largest increase in APX was recorded in Şehirali 90 while increase was lowest in Karacaşehir 90. However, increase in GPX activity was higher in Karacaşehir 90 compared to Yunus 90. The greatest increase in CAT activity was found in Yunus 90 while the lowest increase was in Göynük 98 (Figure 2). SOD activity did not change significantly in Karaceşehir 90 although it increased in the other cvs. The increase in SOD activity of Yunus 90 was found to be lower than the average cvs (ES 855, Göynük 98, and Şehirali 90).

H_2O_2 contents decreased under drought while antioxidant enzyme activities increased in all cvs. Decrease in H_2O_2 content was low in Karacaşehir 90 (45.3%) but high in Göynük 98 (61.2%) and Yunus 90 (66.9%) (Figure 3).

Effect of drought stress on chlorophyll fluorescence parameters

The results of chlorophyll fluorescence parameters are given in Figure 4. Water stress had no significant effect on F_v/F_m but reduced the quantum yield of electron transport (Φ_{PSII}) in comparison with their controls. The reduction was more pronounced in the susceptible (Karacaşehir 90) and average cvs, such as Şehirali 90. Decrease in Φ_{PSII} was lowest in Yunus 90. *NPQ* increased by 85% and 80% in Şehirali 90 and Karacaşehir 90 by drought stress, respectively, but it did not change significantly in Yunus 90, Göynük 98, and ES 855. In addition, *qP* decreased in all cvs. The highest decrease in *qP* was observed in Karacaşehir 90.
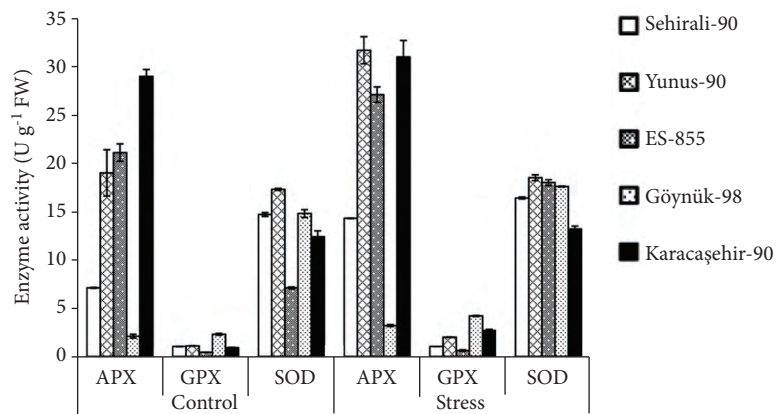
Figure 1. APX, GPX, and SOD activities of common bean cultivars. Vertical bars represent standard deviation of 6 replicates.
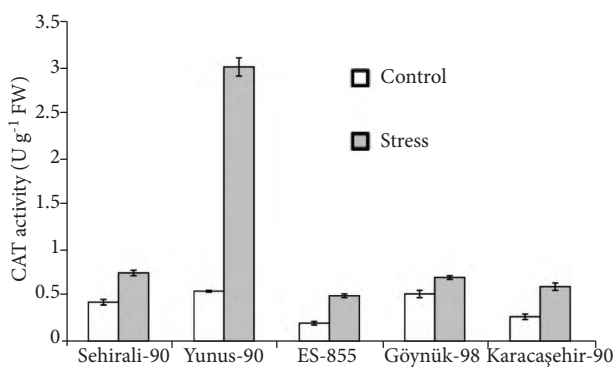

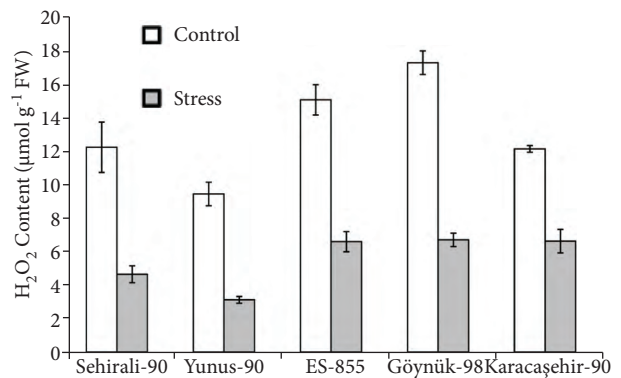Figure 2. CAT activities of common bean cultivars.

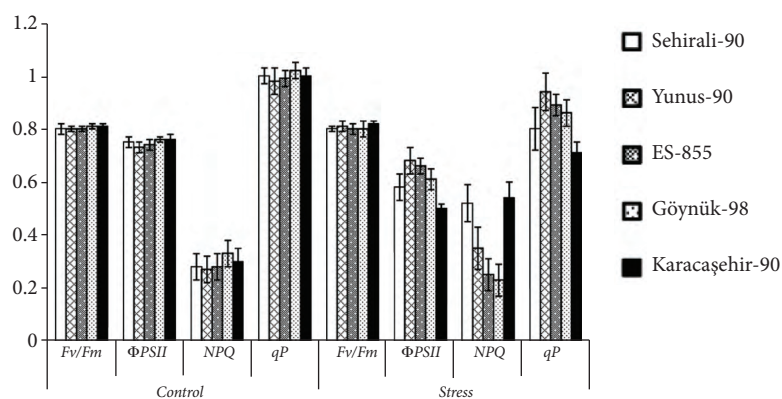Figure 3. H_2O_2 content of common bean cultivars.

Figure 4. Chlorophyll fluorescence of common bean cultivars.

Discussion

In this study, common bean cultivars were compared regarding their tolerance to drought stress during pre-flowering stage, which was used in previous drought studies. For instance, França et al. (2000) suggested that differences exist in drought tolerance among *P. vulgaris* cvs and that such differences were expressed in the vegetative phase of growth. Present results showed that drought stress affected the leaf water status, stomatal conductance, and lipid peroxidation in bean cvs. Karacaşehir 90 and ES 855 lost their leaf water content more severely than the other cvs under drought stress. Yunus 90 also kept its leaf water content better than the other cvs in leaves under drought. Loggini et al. (1999) recorded that drought stress induced a slightly larger decrease in Ψ_{leaf} in sensitive wheat cv compared to tolerant cv. As for our Ψ_{leaf} results, we can conclude that the most tolerant and the most sensitive cvs were Yunus 90 and Karacaşehir 90, respectively.

Karacaşehir 90 generally showed larger drops in RGR and NAR than the other cvs while Yunus 90 had the lowest decrease as compared to the other cvs. Higher RGR under drought condition may be associated with higher assimilation rate. Similarly, decrease in SLA was relatively high in Karacaşehir 90 and ES 855 but it was low in Göynük 98 and Yunus 90. Different decrease in SLA may be due to the different sensitivity of photosynthesis and leaf area expansion to soil drying (Tardieu et al., 1999).

Yunus 90 slowly closed its stomata, and presented the least reduction in RGR and NAR in unifoliate and trifoliate leaves. Karacaşehir 90 also closed its stomata rapidly and had high reductions in RGR, NAR, and SLA. Stomatal closure may be a common drought avoidance response allowing plants to keep water in their tissues (Ludlow, 1980). Our results evidenced the close relationship between stomatal conductance and the reduction of NAR, RGR, and SLA. As it is well known, photosynthetic assimilation rate and stomatal conductance measured before and after water stress treatment are reliable physiological parameters to be used in early screening for tolerant bean cultivar. *P. vulgaris* cultivar, being the more sensitive to drought, has a more rapid stomata closure and a decrease in assimilation rate during drought when compared to the other cvs, therefore, photosynthesis sustained longer (Cruz de Carvalho et al., 1998).

There were significant decreases in chl a, chl b, and total chl contents under drought stress in all cvs. Considerable reduction in chlorophyll content due to water deficit was reported in most crop species (Ashraf et al., 1994; Garg et al., 1998). The decrease in chlorophyll under drought stress is mainly because of the damage to chloroplasts by active oxygen species (Smirnoff, 1995). Our results showed that Yunus 90 and Göynük 98 could maintain high chlorophyll content but not Karacaşehir 90. Indeed, it has been reported that in tolerant genotypes, decrease in chlorophyll content under stress was lower than those of susceptible genotypes (Kraus et al., 1995; Sairam et al., 1997a, b).

MDA accumulation is often used as an indicator of lipid peroxidation (Smirnoff, 1995). Lipid peroxidation was low in Yunus 90 and Göynük 98 while it was high in Karacaşehir 90. Similarly, various authors have suggested that tolerant genotypes showed a low lipid peroxidation (Pastori & Trippi, 1992; Kraus et al., 1995; Sairam et al., 1997b). Based on the results of the leaf water status, stomatal conductance, growth parameters, photosynthetic pigments, and lipid peroxidation, Yunus 90 was evaluated as the most tolerant to drought stress, while Karacaşehir 90 was the most sensitive.

Activities of antioxidant enzymes generally enhanced under drought stress in all cultivars while H_2O_2 content decreased. Our results showed that various common bean cultivars clearly responded to drought stress differently in terms of antioxidant enzyme activities. This result showed that CAT and GPX were more effective in scavenging reactive oxygen species in the tolerant and sensitive common bean cvs, respectively. In addition, we recorded that APX and SOD activities exhibited different trends in all cvs. This implied that different bean cvs had discrete water stress threshold and, therefore, they had different physiological adaptive mechanisms to regulate their levels of reactive oxygen species. On the other hand, high activities of antioxidant enzymes during drought are related to diminished lipid peroxidation under drought condition (Bowler et al., 1992).

In our study, decrease in H_2O_2 content may be resulted from the increase in antioxidant enzyme activities. APX and other antioxidant enzymes play a role in maintaining low levels of H_2O_2 in tolerant wheat cv under drought stress (Zhang & Kirkham, 1994). The increase in the capacities of antioxidant enzymes coincides with the decrease in the H_2O_2 level in the primary leaves of *P. vulgaris* as a consequence of metal intoxication (Weckx & Clijsters, 1996). In addition, Sharma & Dubey (2005) reported that the concentration of H_2O_2 declined with imposition of drought stress while SOD and APX activities were increasing in rice seedlings. Similar to our comments, they attributed the sharp decline in H_2O_2 level to the efficient removal of H_2O_2 by increased activity of antioxidant enzymes as well as certain non-enzymatic reactions working efficiently in the stressed plants (Sharma & Dubey, 2005).

In the present study, we also investigated photochemical efficiency of photosystem II to clarify the involvement of regulation of photosynthesis in tolerance strategies to drought. Soil drought did not influence the maximum quantum yield of photosystem II photochemistry (F_v/F_m) measured in dark-adapted leaves. There are examples of studies showing resistance of PSII to water deficit in leaf tissues (Cornic & Fresneau, 2002). Water stress resulted in reduced Φ_{PSII} in all cultivars. Generally decreases in PSII quantum yield can result from the photo-protective increase in *NPQ* (Demming-Adams & Adams, 1996). The results showed that Yunus 90 and Göynük 98 had a little decrease in Φ_{PSII}, but Karacaşehir 90 exhibited a high decrease in Φ_{PSII}. Similarly, it has been reported that the reduction of Φ_{PSII} was more pronounced in susceptible cultivars of wheat (Subrahmanyam et al., 2006) and *Eragrostis curvula* (Colom & Vazzana, 2003). In our

experiments, *NPQ* increased in Şehirali 90, Yunus 90, and Karacaşehir 90 but did not change significantly in ES 855 and Göynük 98. Since drought stress did not affect *NPQ* in these cvs, the decrease in Φ_{PSII} may be due to reduced *qP* during stress. The decrease in *qP* was more pronounced in Karaceşehir 90 compared to the less susceptible cvs in the present study. Although conflicting results regarding the effect of water stress on *qP* were reported in the literature, there were many reports about a decrease in *qP* under water stress in plants (Loreto et al., 1995; Lu & Zhang, 1999).

In conclusion, the results of the present study pointed out the necessity of using several traits, such as water potential, stomatal conductance, photosynthesis, growth, chlorophyll content, and lipid peroxidation, to evaluate drought resistance of *Phaseolus vulgaris* cvs during the vegetative phase. In addition, decrease in Φ_{PSII} in Karacaşehir 90 was much more noticeable than Yunus 90. Moreover, the increase in *NPQ* was much higher in Karacaşehir 90 than tolerant Yunus 90. Thus, photosynthetic apparatus was more protected in the tolerant cv than susceptible cv under drought. Tolerant cv had much more increment in APX and CAT activities than susceptible cv. Conversely Karacaşehir 90 had more increment of GPX activity than Yunus 90. Inducing antioxidant mechanism at the cell allows the tolerant cv to resist better than the others.

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