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ORIGINAL PAPER

Study of the defensive mechanism against drought in French bean (*Phaseolus vulgaris* L.) varieties

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Abstract The response to drought of six green- and yellow-podded varieties of green bean was investigated in a phytotron. These varieties were also grown without irrigation in field experiments and analysis of leaf samples was carried out three times; before and under flowering and pod ripening, respectively. At first, the carotene and raffinose contents of leaves increase during mild drought (30/15°C). High temperature (35/25°C) combined with water deficiency resulted in a considerable decrease in leaf weight, chlorophyll *b*, maltose content, as well as the level of water-soluble antioxidants of the leaves, while the content of lipid-soluble antioxidants and raffinose content increased when compared with the control. The yellow-podded varieties of green beans responded to drought much more sensitively than the green-podded ones. The results suggested that a selection method based on the activity of antioxidants in the leaves can be used for testing the adaptability of numerous bean genotypes to drought.

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Introduction

The irregular occurrence of drought periods accompanied by high temperature due to climatic changes results in large variations in yield in French bean production. Both the quality and the yield of beans are negatively affected by brief periods of water shortage (Ramirez-Vallejo and Kelly 1998). However, climatic effects can be reduced not only by irrigation, but also by the water use and drought tolerance of green bean varieties as these two factors substantially determine yield stability. The responses of plants to stresses depended on many factors, such as the phenological stage and the time and strength of stresses (Torres et al. 2006). High yield is guaranteed by satisfactory water supplies and optimal temperature; 25/10°C (day/night) during the flowering of French beans. During this period, drought (water deficit of the soil associated with high temperature) decreased the pod number per plant and the number of seeds in the pods (Boutraa and Sanders 2001; Bonfil et al. 2007; Lesznyák et al. 2008). Water deficiency after flowering hampers the development of seeds and causes a great number of seeds to shrivel during seed production (Nemeskéri 1994; Isik et al. 2005). Green pods with seed abortion due to low fertility are also not suitable for food processing, as their quality is considered to be substandard. Liu et al. (2004) reported that the decrease in the hexose to sucrose ratio in pods after anthesis contributed to early pod abortion in drought-stressed soybeans which resulted in a decrease in the number of developed pods.

The strategy of avoiding water deficit in plants during short-term drought periods is based on restraining of transpiration by paraheliotropic leaf movement (Pastenes et al. 2005), stomatal closure and the deep root system. This system enables the extraction of water from the soil to reduce the dehydration of cells (Heszky 2007). In common beans, the mechanisms of drought avoidance principally include the development of an extensive root system (Sponchiado et al. 1989; Nemeskéri 2001; Mohamed et al. 2002; Micklas et al. 2006) and efficient stomata closure (Barradas et al. 1994; Costa Franca et al. 2000; Miyashita et al. 2005). While protecting itself against longer or severe drought periods, a plant undergoes different biochemical and molecular genetic changes to maintain osmotic adjustment and the structure of cell membranes in order to avoid cell dehydration. Decreases in sugars and oligosaccharides in *Arabidopsis* have been shown (Anderson and Kohorn 2001) while in others, such as maize and rice, sugars accumulated during drought (Pelleschi et al. 1997; Vue et al. 1998). The accumulation or absence of these compounds was determined by overexpression of regulated genes or down-regulated genes (Liu et al. 2007; Micheletto et al. 2007; Meglic et al. 2008; Torres-Franklin et al. 2008).

Oxidative damage of the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant mechanisms. The water deficit increases the lipid peroxidation (membrane injury index, H_2O_2 and OH’ production) in the leaves of 14-day-old bean plants; however, the activity of catalase and superoxide-dismutase were high in the drought tolerant bean varieties (Zlatev et al. 2006). The differences in water deficiency tolerance observed between bean varieties could be attributed to differences in the activity of superoxide-dismutase and catalase enzymes (Türkan 2005). During simultaneous drought and light stress, gamma-tocopherolquinone, which is an oxidation product of gamma-tocopherol, accumulates in the young leaves of runner bean (Szymanska and Kruk 2008).

Few reliable methods have been developed to evaluate the defensive mechanisms against drought in bean plants under field conditions. In this study, the responses of green beans to drought and heat stress were investigated, in order to develop a selection method for testing the adaptability of bean genotypes to drought.

Materials and methods

Experiments in phytotron

The drought and heat responses of six green bean varieties with different genetic backgrounds and pod types (yellow-podded varieties: Hungold, Maxidor and Debreceni sárga; green-podded: Buvet, Zsófi and Masai) were investigated in a controlled environment in a phytotron chamber. The varieties were sown in pots at 18 cm height and 19 cm of upper diameter and 9 cm of the bottom diameter in a greenhouse. A total 1.8 kg of soil mixture of peat, clay, humus and sand (pH 6.5–7.0) was loaded into the each pot. Each treatment consisted of six pots, each containing two plants. In the greenhouse, all plants were given satisfactory supplies of water until the appearance of the green buds. During this experiment, each pot was watered uniformly every 2 days to 70% of the field water capacity (FWC), which was controlled by weighing. At 29 days the plants were transferred to Conviron PGR-15 climatic chambers in the phytotron. In the three chambers, the treatments of temperature were 25/15°C, 30/15°C and 35/25°C (day/night), respectively. Each chamber had a 1.4 square meter plant growing plate and 600 μmol/m^2 s PPFD (Photosynthetic Photon Flux Density) of light density was provided in the climatic program (Tischner et al. 1997). During the 14 days in the phytotron, the control pots received the same quantity of water (100 ml per pot) as in the greenhouse (FWC 70%) and were maintained at daytime/nighttime temperatures of 25/15°C with high humidity (RH 75%). Those pots which were drought stressed received only half of quantity of water of the control plants. The drought-stressed plants were kept at high temperature (30/15°C) under water deficiency (50% water of control) for a week before flowering. Next the water-stressed plants were divided into two groups; the first group was exposed to severe drought stress at 35/25°C and the second was maintained under mild drought stress at 30/15°C while the irrigated water was 50% of control and RH 60% for 7 days during flowering period. The increase in the temperature and decrease in humidity (RH 60%) simulated the air drought that often occurs during the flowering of beans grown in field conditions in our country (Table 1). For leaf analysis, the middle leaf at the third node was removed from both plants in each plot (representing two replications). The leaf samples were weighed and stored in liquid nitrogen prior to chemical analysis. Samples were taken in a similar manner at flowering, except that all three leaves were removed from the fourth node, due to the smaller leaf size.

Field experiments

The bean varieties were planted in field experiments with two repetitions randomized, without irrigation, in breeding place of Agrona Ltd. The experiments were carried out in chernozem soil with 3.0% humus content. The plot size was 10.3 m^2 with 50 cm of row distance and 5 cm of plant distance. For leaf analysis, the middle leaf was removed from ten of healthy plants in each plot in four replications. The leaf sampling was carried out three times in 7 days periods of before and during flowering and green pod ripening, respectively, as in the phytotron drought model trials.

Table 1 Meteorological data for field experiments under growth phases

Periods	Growth phases	*T*min (°C)	Average *T* (°C)	*T*max (°C)	Precipitation (mm)	RH (%)
14 June–21 June	Before flowering	15.06	21.53	28.00	1.8	60.3
22 June–30 June	During flowering	18.76	24.20	29.57	20.9	63.0
1 July–13 July	Green pod ripening	15.34	21.9	28.45	1.0	53.5

The data presented the average of 2006 and 2007 years

Tmin minimum temperature, *Tmax* maximum temperature, *RH%* air humidity

Chemical analysis

An analysis was made of the chlorophyll and carotene contents of the lyophilized leaves as for their dry matter in mg/g (Hendry and Price 1993).

Determination of antioxidants

The photochemiluminescence method (PCL) was carried out with the procedure described by Popov and Lewin (1999) in order to determine the quantity of antioxidant compounds in the bean leaves. The PCL is based on photo-induced autoxidation inhibition of luminol by antioxidants, mediated from the radical anion superoxide and is suitable for measuring the radical scavenging properties of single antioxidants, as well as more complex systems, in the nanomolar range. In the water-soluble fraction, antioxidants such as flavonoids, ascorbic acid and amino acids are detected, while in the lipid solubles, fraction tocopherols and tocotrienols, carotenoids are measured, among others (Besco et al. 2007). The 10 mg of lyophilized leaf samples were powdered and extracted using 1 ml of bidestilled water at 25°C. The extract was centrifuged for 5 min at 10,000 rpm. The measurement of antioxidant capacity of water (ACW) and lipid-soluble (ACL) substances was performed by using PHOTOCHEM (Analytic Jena Ag. Germany) chemiluminometer equipment. Water-soluble ACW, corresponding to the activity expressed as μmol equivalents of ascorbic acid for each gram of tested product. The preparation of leaf samples and the measurements of ACL are similar to that of ACW but the extraction of ACL compounds of lyophilized leaf samples was made by 1 ml of methanol at 25°C as described by Besco et al. (2007). The ACL antioxidant capacity, corresponding to the activity is expressed as μmol equivalents of Trolox for each gram of tested product. The quantity of both antioxidant compounds was given as μg/g for dry matter.

Determination of carbohydrates

The methods used for measurement of soluble carbohydrates (monosaccharides and sucrose) as described by Sárdi et al. (1999). Earlier results showed the quantity and changes of these carbohydrates affected by stresses related to the stress tolerance (Sárdi et al. 1996). The advantage of the methods is able to compare simultaneously the components of carbohydrates in 10–15 of samples under the same experimental conditions. The measurement and identification of carbohydrates were performed by standards with known concentrations. These were xylose, fructose, glucose, sucrose, maltose and raffinose. The fresh leaves were frozen with liquid nitrogen, powdered and extracted with methanol: $H_2O = 80:20$, V/V. This suspension was centrifuged at 1,500 *g* for 10 min at −1°C. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Co., Ltd, Budapest, Hungary). For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), $\lambda = 540$ nm was used (Sárdi et al. 1999).

Statistical analysis

All data from the phytotron and field experiments were evaluated by one-way analysis of variance (ANOVA) using the SPSS 13.0 for Windows software. The means of each treatment were compared by Duncan's Multiple Range test at $P < 0.05$. Regression analysis was used to reveal the relationship between the temperature and antioxidant compounds in the leaf.

Results

The model of drought in the phytotron

A short period (7 days) of drought and heat stress (30/15°C days/night) prior to flowering resulted in a reduction in the leaf mass, but the chlorophyll and carotene contents rose significantly. However, the quantity of antioxidants did not change when compared with the control. A combination of high temperature (35/25°C) and water deficiency during flowering led to a decline in leaf mass. Chlorophyll *b* and ACW contents decreased, while ACL contents of leaves increased significantly when compared with the control. Under the same conditions, the maltose concentration of the leaves decreased significantly, but raffinose content increased in comparison to the control. Contrary to

Table 2 Effect of drought stress on the leaves of French beans during flowering

Properties	Temperature (°C day/night)		
	25/15[a]	30/15	35/25
Average leaf weight (g)	0.37 a	0.15 c	0.19 bc
Chlorophyll *a* (mg/g)	34.13 a	35.20 a	31.60 a
Chlorophyll *b* (mg/g)	5.19 a	5.89 a	4.57 b
Chlorophyll *a*/*b*	6.58 ab	5.98 b	6.92 a
Carotene (mg/g)	28.23 b	39.70 a	25.80 b
Chlorophyll/carotene	1.39 a	1.06 b	1.40 a
ACW (μg/mg)	10.29 a	7.26 ab	5.37 b
ACL (μg/mg)	3.11 b	4.33 b	6.52 a
Glucose (μg/g)	2,770.333 a	3,090.260 a	3,718.520 a
Fructose (μg/g)	2,077.552 a	2,259.938 a	1,900.390 a
Sucrose (μg/g)	3,187.857 ab	2,658.155 b	4,998.964 a
Maltose (μg/g)	845.340 a	601.870 ab	345.620 b
Raffinose (μg/g)	659.777 b	1,246.975 b	2,585.464 a
Xylose (μg/g)	1,072.237 a	1,447.185 a	1,658.166 a

[a] Control with optimum water supplies. Values in each row having different letters are significantly different at the $P < 0.05$ level using Duncan's multiple range test

expectations, the glucose and fructose levels in French bean leaves did not change significantly in response to drought and heat stress (Table 2).

Under longer periods of water deficiency (14 days), the leaves of plants maintained at 30/15°C started to turn yellow and brown and, while the flowers became fertile, only a few pods developed. Under these conditions, the loss of leaves and flowers was much more intensive in large-leaved varieties than in those with smaller leaves. The plants responded to water deficiency and high temperature (35/25°C) during flowering with a reduction in leaf size. The decrease of leaf mass depending on varieties was 43–67%, in comparison with the control (Table 3).

The lowest contents of chlorophyll *a*, chlorophyll *b* and carotene were recorded for the Debreceni sárga variety, which had the most sensitive response to a rise in temperature; at 30/15°C there was a significant reduction in the chlorophyll *a* component when compared with the control (Table 3). At this temperature, the highest differences were among the varieties in chlorophyll *b* content, but with further increase of temperature (35/25°C), the differences were eliminated. At this high temperature, a substantial decrease when compared with the control was only recorded for Maxidor. There was no change in the chlorophyll *a*/*b* ratio among the varieties; however, this ratio was the lowest in the green-podded variety Masai, due to the large chlorophyll *b* content of leaves at 30/15°C. At high temperature (35/25°C), the chlorophyll *a*/*b* ratio did not change in comparison with the control except for the Zsófi green-podded variety. The protection against mild drought (30/15°C) is represented by the rise of carotene content in the leaves of all varieties, but the differences were only significant for yellow-podded variety Hungold and the green-podded variety Masai in comparison with the control. A further increase in temperature (35/25°C) considerably reduced the carotene content in the leaves to approximately the control value. There was no difference in the ratio of chlorophyll to carotene of control plants among the varieties but this ratio was considerable low in the yellow-podded varieties Maxidor and Debreceni sárga and in the Masai with green pods exposed to mild drought stress (30/15°C). Under severe drought stress (35/25°C) the ratio of total chlorophyll to carotene resembled to the control value of these varieties.

In the dark green leaves of green-podded varieties, ACW was lower than in the yellow-podded varieties and did not change as a result of drought and heat stress (Table 3). Water deficiency during flowering resulted in a significant decrease in the level of ACW antioxidant in the yellow-podded varieties Hungold and Debreceni sárga at 30/15°C. This indicates that yellow-podded bean varieties respond to mild drought stress more sensitively than green-podded ones. When the temperature was increased further, the ACW level in the leaves only remained significantly lower than the control level in the varieties Hungold and Debreceni sárga, while no change was detected for the green-podded varieties.

The largest content of ACL was recorded in the leaves of variety Maxidor and did not change at 30/15°C. The smallest content of ACL antioxidants was in the leaves of the green-podded variety Buvet, which increased poorly at 30/15°C. The results showed that mild heat stress (30/15°C) did not result remarkable changes in the level of ACL antioxidants in comparison to the control. At high temperature (35/25°C), the level of ACL antioxidants in the leaves of yellow-podded varieties significantly increased in comparison to the control, but did not change in green-podded ones. At this high temperature, there was the largest activity of ACL in the leaves of the variety Debreceni sárga, indicating an intensive response to heat stress. The range of heat tolerance based on the increasing level of ACL of leaves was Debreceni sárga > Maxidor > Hungold varieties (Table 3).

Since the yellow-podded French bean varieties had the most intensive reaction to drought and heat stresses while flowering, the changes of carbohydrates in the leaves have only been investigated for these varieties. The sucrose content of the leaves independent of heat stress was the largest in both the Debreceni sárga and Maxidor varieties (Fig. 1). The changes in the production of maltose already show the differences between the varieties under mild drought stress (30/15°C); a significantly large decrease in

Table 3 Reactions of French bean cultivars for drought stresses during flowering in the phytotron

Cultivars:		Yellow-podded cultivars			Green-podded cultivars		
Properties	Temperature (°C day/night)	Hungold	Maxidor	Debreceni sárga	Buvet	Zsófi	Masai
Average leaf weight (g)	25/15 (Co)	0.36 b	0.36 b	0.29 c	0.49 a	0.33 b	0.42 a
	30/15	0.22 c*	0.20 cd*	0.16 d*	0.22 c*	0.12 d*	0.19 c*
	35/25	0.12 d*	0.15 d*	0.10 d*	0.20 c*	0.16 d*	0.24 b*
Chlorophyll *a* (mg/g)	25/15 (Co)	29.22 ab	39.47 a	22.33 b	34.40 ab	33.83 ab	45.54 a
	30/15	41.22 a	35.69 a	16.66 c*	40.47 a	35.84 a	41.33 a
	35/25	27.50 ab	24.85 ab	25.55 bc	37.05 ab	33.90 ab	40.77 a
Chlorophyll *b* (mg/g)	25/15 (Co)	4.26 c	5.70 ab	3.20 c	5.59 ab	5.13 bc	7.24 a
	30/15	6.40 b*	5.62 ab	2.44 c	6.47 ab	5.79 bc	8.64 a
	35/25	4.15 c	3.59 c*	3.81 c	5.54 bc	4.14 c	6.22 b
Carotene (mg/g)	25/15 (Co)	23.85 cb	31.90 abc	19.70 c	29.15 bc	27.75 bc	37.00 b
	30/15	42.05 a*	42.55 a	22.30 c	41.68 ab	31.58 bc	52.06 a*
	35/25	22.50 cb	21.80 cb	22.70 cb	31.30 b	24.30 bc	32.20 b
Chlorophyll *a*/*b*	25/15 (Co)	6.86 a	6.92 a	6.98 a	6.15 a	6.59 a	6.29 a
	30/15	6.44 a	6.35 a	6.82 a	6.26 a	6.19 a	4.78 b*
	35/25	6.62 a	6.92 a	6.70 a	6.69 a	8.19 b*	6.55 a
Chlorophyll/carotene	25/15 (Co)	1.40 a	1.42 a	1.30 a	1.37 a	1.40 a	1.43 a
	30/15	1.13 ab	0.97 b*	0.86 b*	1.13 ab	1.32 a	0.96 b*
	35/25	1.41 a	1.31 a	1.29 a	1.36 a	1.57 a	1.46 a
ACW (μg/mg)	25/15 (Co)	20.65 a	11.95 b	18.60 a	1.61 cd	3.01 c	5.91 c
	30/15	10.55 b*	11.06 b	7.81 bc*	3.86 cd	4.03 cd	6.21 c
	35/25	9.90 b*	7.67 b	6.76 b*	1.91 cd	3.12 cd	2.89 cd
ACL (μg/mg)	25/15 (Co)	2.60 c	5.27 b	2.96 bc	1.53 c	2.59 bc	3.72 bc
	30/15	4.26 bc	4.07 bc	5.11 bc	3.27 c	2.95 c	6.33 b
	35/25	5.81 b*	8.55 a*	10.89 a*	3.21 bc	4.73 b	5.98 b

Values in each row and column having different letters are significantly different at the $P < 0.05$ level using Duncan's multiple range test

Co control

* Significantly different from the control at the $P < 0.05$ level

the content of maltose in the leaves of the Debreceni sárga variety, and a smaller one in the leaves of the variety Maxidor were recorded, but no change was detected in that of the variety Hungold when compared with the control. Under severe drought stress (35/25°C), the decrease in the maltose content of the leaves was intensified in the varieties Debreceni sárga and Maxidor, but no change was found in the Hungold variety. Severe drought stress (35/25°C) resulted in a significant increase in the content of raffinose in the leaves of the Hungold and Maxidor varieties when compared with the control, but this did not change considerable in the variety Debreceni sárga (Fig. 1).

Field experiment

The study of bean varieties corresponding with drought was also made under field conditions with natural distribution of precipitation. In 2006 and 2007, the drought was less serious than that which was induced in the phytotron. However, during the flowering period, the temperature (as minimum and maximum values) was similar to the treatment of 30/15°C (day/night) in the phytotron (Table 1). Although the biochemical parameters of the leaf were larger in the field conditions during flowering than in the phytotron at 30/15°C (Tables 3, 4), the response of bean varieties to drought could be compared. The content of chlorophyll *b* in the leaves was significantly larger in green-podded varieties in the field than in yellow-podded ones, but no difference could be noted in the chlorophyll *a*/*b* ratio whether between varieties or on the basis of growing conditions. Under field conditions during flowering, the ratio of chlorophyll to carotene in Maxidor, Debreceni sárga and Masai varieties was the same to that of plants studied on the phytotron at 30/15°C (Tables 3, 4).

The level of ACW in the leaves, particularly in green-podded varieties, was high during flowering in the field conditions. Under flowering the extent of ACW activity in

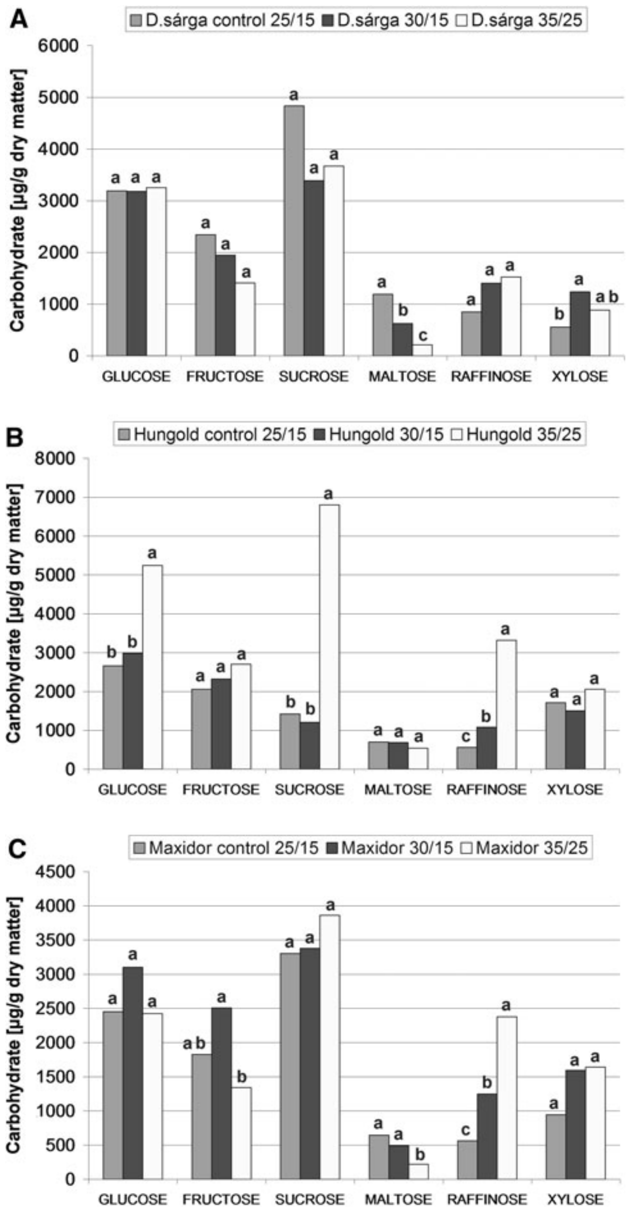

Fig. 1 Carbohydrates of the leaves of Debreceni sárga (**a**) Hungold (**b**) and Maxidor (**c**) French bean varieties under irrigation (25/15°C day/night) and water deficit with heat stress (30/15 and 35/25°C day/night) during flowering in phytotron. Significant differences between treatments are indicated by different letters at the $P < 0.05$ level

the leaves of varieties grown in the field was the same as that in the phytotron at 30/15°C; the lowest level of ACW in the leaves was measured in the variety Buvet and the largest in the Hungold and Masai varieties (Table 4). Although there was no difference in ACL activity in the leaves between the varieties in the phytotron at 30/15°C, the largest content of ACL antioxidants in the leaves was recorded in the Debreceni sárga and Masai varieties in the field conditions. High temperature increased the content of ACL antioxidants in the leaves during flowering under water deficiency in phytotron, but there was no correlation between the temperature and the content of ACW in the leaves of green beans in phytotron. We found a positive significant correlation ($r = 0.662$) between the temperature and the level of ACL antioxidants in the leaves of bean in the phytotron that corresponded to the correlation ($r = 0.64$) between the content of ACL in the leaves measured during flowering and the seed yield of bean in the field experiment. These results suggested that the heat tolerance of the green bean varieties, based on the production of ACL antioxidants in the leaves, can also be evaluated under dry conditions in the field. The defense against drought had already begun prior to flowering when the correlation between the content of ACL in the leaves and seed yield ($r = 0.779$) is so significant, that it determined 61% of seed yield (unpublished data). While the level of ACL in the leaves decreased in all varieties during flowering, only those varieties have high tolerance to heat stress in which the extent of the decrease is small or capable of re-increasing the production of ACL antioxidants during pod ripening. The yellow-podded varieties of Debreceni sárga and Hungold show this response, which proves their tolerance to heat stress in field conditions (Fig. 2). The content of ACW antioxidants in the leaves decreased similarly to ACL during flowering period. The increase in activity of ACW antioxidants indicates the efficiency of protection against water deficiency, which was great in the variety Hungold and small in the Zsófi and Debreceni sárga varieties. The Buvet green-podded variety is sensitive to water deficit and high temperature as confirmed by the low content of ACW and ACL antioxidants in its leaves. The Zsófi variety originated by the crossing the Masai and Buvet varieties is sensitive to high temperature stress as proved by the low content of ACL in the leaves. Yet, it is moderately tolerant to water deficiency, as evident in the increasing production of ACW (Fig. 2).

As both the phytotron and field experiments demonstrated the Debreceni sárga and Maxidor of the varieties are sensitive to water deficiency is a finding based on change of carbohydrates; however, their reaction was different under severe drought conditions (35/25°C). As the carotene content of the leaves was very low in the Debreceni sárga variety, this seemed to be an intensive response to heat stress due to the high activity of ACL. The accumulation of carbohydrates and the activity of antioxidants confirmed that the Maxidor variety is sensitive to water deficiency due to the high sucrose content of the leaves, but the changes of ACL level proved to be a sign of moderate tolerance to heat stress. The defense of the Maxidor variety against water deficit is due to the larger increase in raffinose content in the leaves, and was more favorable than what was seen in the Debreceni sárga variety.

Table 4 Character of leaves of French bean cultivars during the flowering period in dry field conditions

Cultivars:	Yellow-podded cultivars			Green-podded cultivars		
Properties	Hungold	Maxidor	D. sárga	Buvet	Zsófi	Masai
Average leaf weight (g)	0.51 a	0.68 a	0.73 a	0.66 a	0.68 a	0.68 a
Chlorophyll *a* (mg/g)	35.56 ab	35.99 ab	32.61 b	55.62 a	57.78 a	41.22 ab
Chlorophyll *b* (mg/g)	5.05 bc	4.49 c	4.49 c	8.04 a	8.27 a	5.79 ab
Carotene (mg/g)	35.59 b	38.90 ab	38.73 ab	58.84 ab	61.28 a	45.30 ab
Chlorophyll *a*/*b*	7.04 bc	8.02 a	7.26 bc	6.92 c	6.99 c	7.12 bc
Chlorophyll/carotene	1.14 a	1.04 b	0.96 b	1.08 ab	1.08 ab	1.04 b
ACW (μg/mg)	24.66 a	16.03 b	14.00 bc	10.63 c	14.12 bc	29.13 a
ACL (μg/mg)	5.07 bc	5.87 bc	9.06 a	6.53 b	8.45 ab	9.17 a

Values in each row having different letters are significantly different at the $P < 0.05$ level using Duncan's multiple range test

D.sárga Debreceni sárga

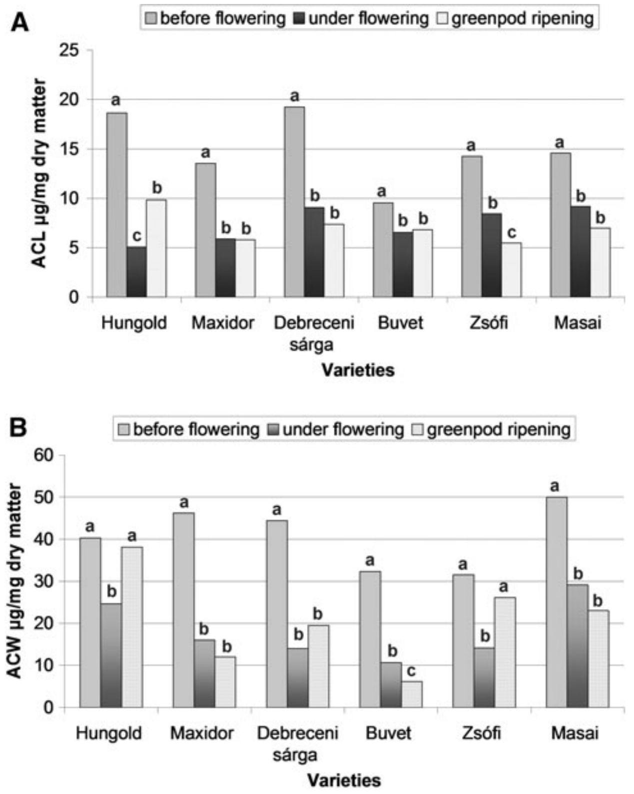

Fig. 2 Changes in the level of lipid-soluble antioxidants (ACL) (**a**) and water-soluble antioxidants (ACW) (**b**) of bean leaves under dry field conditions. Significant differences between phases of development are indicated by different letters at the $P < 0.05$ level

Discussion

The defense mechanisms in genotypes with tolerance to drought are due to several physiological and biochemical processes operating simultaneously or consecutively. Bean varieties that prove to be more sensitive to drought could be summarized as followed: the reduction in water content of the leaves is faster, the level of lipid peroxidation as an indicator of membrane damage by abiotic stress is found to be higher, and much higher reduction is in the individual yield of the plant (number of pods per plant and number of seed per pod) under water deficiency conditions, than drought tolerant bean variety (Rainey and Griffiths 2005; Lizana et al. 2006). Plants are capable of adapting to different light environments by changing their photosynthetic pigment compositions. During continuous irradiation, the rapid transformation of chlorophyll *b* to chlorophyll *a* occurred in the leaves of light sensitive varieties and carotene, as non-enzymatic antioxidants, ensures the great protection against photodamage (Procházková and Wilhelmová 2004). Wentworth et al. (2006) also found that high light and temperature tolerant varieties are characterized by an increase in leaf thickness and β-carotene content, but the ratio of chlorophyll *a*/*b* does not change. The findings showed that the ratio of chlorophyll to carotene was decreased even in mild drought stress (30/15°C) in some varieties but that for all varieties raised again to control levels at higher temperature (35/25°C), due to the degradation of chlorophyll the leaves yellowed. Under mild drought stress (30/15°C) during flowering, the ratio of chlorophyll to carotene was low for yellow-podded Maxidor, Debreceni sárga and green-podded Masai varieties, in both phytotron and field experiments. Others (Tsai and Hsu 2009) demonstrated that the thermotolerance of *Phaseolus* species grown at 35°C was different, but after acclimating to 35°C, this enhanced to about the same level due to the induction of acquired thermotolerance in the chloroplast. Photochemical sensitivity of the common bean leaves to heat stress is altered by a previous transient water deficit; however, this adaptive change is genotype-dependent (Ribeiro et al. 2008). Nevertheless, our results show that the differences in adaptability to mild drought for bean varieties during flowering had already been detected by the changes of leaf pigment at 30/15°C, which often occurs under field conditions.

When water deficiency associated with high temperature occurs for longer periods, the specific biochemical mechanisms at the cellular level contribute to an efficient adaptation and allow the plants to resist drought better. Sugars act as osmotic compounds in protecting against drought and contribute to the stabilization of cell membrane structures. A strong correlation between sugar accumulation and osmotic stress tolerance has been reported (Streeter et al. 2001; El-Tajeb 2006). A starch reduction in the leaf is a common observation in water-stressed plants (Cabuslay et al. 2002; Chao et al. 2006) and changes in starch content due to the degradation under stress period give useful information about the drought tolerance (Ramos et al. 1999; Lloyd et al. 2005), but carbohydrates seem to better indicators of drought tolerance (Borókay and Sárdi 1999). However, the accumulation and components of carbohydrates differ according to the individual responses of plant species. The sucrose content of leaves increases due to the decrease in sucrose phosphate synthase enzyme (SPS) activity in the leaves of plants subjected to drought/mild water stress (Castrillo 1992). The large level of sucrose in the leaves of the varieties Maxidor and Debreceni sárga under water deficiency, independent of the temperature, presume a low activity of SPS and therefore the sensitivity to drought of these varieties. The Hungold variety has some tolerance to water deficiency because the sucrose content of its leaves increased only under severe drought (35/25°C) (Fig. 1). However, others (Borókay and Sárdi 1999) found that sucrose content in the leaves of drought tolerant varieties was high in the unstressed stage. The differences in drought tolerance of yellow-podded French bean varieties can be detected reliably by examining the increase of raffinose level in the leaves at high temperature (35/25°C) associated with water deficit. This corresponds to the report of Gupta and Kaur (2005), whereas the accumulation of galactinol and raffinose under controlled conditions improved drought tolerance.

Numerous other as yet undetected water-soluble antioxidant compounds may be responsible for the adaptation of plants to environmental stress factors. These compounds react with free radicals and neutralize them, thus overcoming the damage caused by stress. The alleviation of oxidative damage and increasing resistance to environmental stresses is often correlated with an efficient antioxidative system (Kranner et al. 2002). In the phytotron the level of ACW antioxidants in the leaves of plants kept on water deficiency at 30/15°C under flowering decreased by 30% for control plants and the further rise in temperature did not change this level. Under the same climatic conditions in the field experiments, the decrease in the content of the ACW antioxidants of the leaves during the flowering period was also 30% of that prior to flowering. We assumed the change in ACW of the leaves rather expressed the degree of tolerance to water deficit than the heat tolerance. This was also confirmed by the absence of any correlation between rising temperature and ACW level. In addition, the ACW antioxidant contents in the leaves can be influenced by the stomata closure, because this is related by the ascorbic acid redox state of guard cells that control the stomata movement (Chen and Gallie 2004). The close positive correlation between the high temperature stress and the content of ACL in the leaves in the drought model trial in the phytotron proved that the high level of ACL antioxidants in the leaves contributes to defense against lipid peroxidation and cell damage.

The drought tolerance and heat stress of plant varieties is difficult to evaluate separately in the field growing conditions. The change in the content of photosynthetic pigments rather depends on climatic factors and reveals the tolerance of varieties only under mild drought conditions. During longer dry periods (14 days) the production of all compounds with antioxidant effects more reliably shows the differences in heat tolerance of the varieties than the changes of photosynthetic pigments. The most sensitive phase of development in French beans is flowering, when drought and heat stress determine the yield. The yellow-podded green bean varieties response to drought is more sensitive that of green-podded ones (Nemeskéri et al. 2008), so the stages of their defensive mechanisms can be easily investigated. The experiments proved that the tolerance of French beans to climatic stresses involved many stages where not only a single enzyme or compound is responsible for the overexpression or accumulation of antioxidants. The recovery ability after desiccation relates to the maintenance or elevation of level of antioxidants (Torres-Franklin et al. 2008). The rapid recovery of plants after shorter or prolonged stress periods was affected by their protective mechanisms. During flowering and pod ripening, the content of ACL antioxidants in the leaves of the beans contributes for the prevention of yield decrease. However, during flowering, the level of ACL in the leaves decreased in all varieties in comparison with the time prior to flowering. Some varieties, such as Hungold and Debreceni sárga varieties are able to increase or maintain it during pod ripening ensuring their heat tolerance in the field (Fig. 2). The level of ACW in the leaves decreased as did ACL antioxidants during flowering, but only Hungold with yellow pods enhanced the production of ACW in the leaves during green pod ripening thereby confirming it has a tolerance to water deficit.

As both the phytotron and field experiments illustrated the defense of a plant against damage from climatic stresses during flowering is occurs in many stages in bean varieties with tolerance to drought and high temperature. At first, the carotene and raffinose contents of leaves increase during mild drought (30/15°C). With rises in temperature (35/25°C), additional protective mechanisms are activated e.g. the production of raffinose, glucose and sucrose are increased and the level of ACL antioxidants in the leaves rises. These processes occur in the Hungold variety, which is capable of recovering from a drought due to the rise in the production of antioxidants (Fig. 2). The defensive reaction of green-podded varieties to drought could be evaluated on the basis of examination of all the antioxidants found in their leaves more than on the basis of the change of photosynthetic pigments during flowering.

Breeding for resistance to drought is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create a repeatable water stress conditions where large populations can be evaluated efficiently. The findings demonstrate that tolerance to water deficiency is related to the content of ACW antioxidants in the leaves and the tolerance to high temperature by the ACL antioxidants. These results suggested that a selection method based on the activity of antioxidants in the leaves can be used for testing the adaptability of numerous bean genotypes to drought.

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