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DROUGHT TOLERANCE OF BULGARIAN COMMON BEAN GENOTYPES, CHARACTERISED BY SOME BIOCHEMICAL MARKERS FOR OXIDATIVE STRESS

СУХОУСТОЙЧИВОСТ НА БЪЛГАРСКИ ГЕНОТИПИ ФАСУЛ, ХАРАКТЕРИЗИРАНИ ЧРЕЗ НЯКОИ БИОХИМИЧНИ МАРКЕРИ ЗА ОКСИДАТИВЕН СТРЕС

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Abstract

The aim of this study was to characterize drought tolerance of 20 common bean genotypes using some biochemical markers for oxidative stress. 10 common bean cultivars (9 Bulgarian and a Mexican - BAT 477) and 10 mutant lines $M_{(19–20)}$, previously obtained by us after the treatment of seeds from *Dobroudjanski* 2 and *Dobroudjanski* 7 cultivars with ethyl methan sulphonate (EMS) and N-nithroso-N-ethyl urea (NEU) were used in this investigation. BAT 477 was chosen as a control and it was presented in unique cluster group. Three biochemical markers – malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline were analyzed. The results were statistically elaborated by mono-, bifactorial ANOVA and cluster analyses. Our preliminary results demonstrated that to obtain more valuable information, concerning drought tolerance of both common bean cultivars and mutant lines, MDA, H_2O_2 and proline should be used as early warning markers. Genotypes studied could be of interest in future investigations being a geneplasme source of common bean drought tolerance.

Keywords: biochemical markers, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), *Phaseolus vulgaris* L., proline

Резюме

Целта на настоящото проучване е да се характеризира сухоустойчивостта на 20 генотипа фасул, използвайки някои биохимични маркери за оксидативен стрес. В това проучване са използвани 10 сорта фасул (9 български и мексиканският - BAT 477) и 10 мутантни линии $M_{(19-20)}$ получени преди това от нас след третиране на

семена от сортовете Добруджански 2 и Добруджански 7 с етилметан сулфонат (EMC) и N -нитрозо-N-етил карбамид (НЕК). BAT 477 бе избран като контрола и той бе представена в самостоятелна клъстерна група. Анализирани са три биохимични маркери - малондиалдехид (МДА), водороден прекис (H_2O_2) и пролин. Резултатите са обработени статистически чрез едно-, двуфакторен ANOWA и клъстър анализ. Нашите предварителните резултати показват, че за да се получи по-ценна информация, относно сухоустойчивостта на сортовете и мутантните линии фасул, МДА, H_2O_2 и пролинът, трябва да се използват като ранни маркери. Изследваните генотипи представляват интерес за бъдещи проучвания, като източник на генплазма за сухоустойчивост при фасула.

Ключови думи: биохимични маркери, водороден пероксид (H_2O_2), малондиалдехид (МДА), пролин, *Phaseolus vulgaris* L.

Разширено резюме

Засушаването е един от основните видове абиотичен стрес, който засяга селскостопанските култури и производството на хранителни продукти от тях. То индуцира различни физиологични, биохимични и молекулярни отговори при растенията, включително и при фасула. Малондиалдехидът (МДА) е цитотоксичен продукт на липидната пероксидация и индикатор за произвеждането на свободни радикали, които предизвикват сериозно увреждане на клетките. Установено е, че екзогенното прилагане на водороден прекис в ниски концентрации стимулира и засилва устойчивостта на растенията на засушаване. Пролинът пречиства хидроксилните радикали и увеличава кислорода, така предпазва растителните клетки от нарушения. В настоящото изследване е проучена сухоустойчивостта на 20 генотипа фасул, използвайки някои биохимични маркери за оксидативен стрес - малондиалдехид (МДА), водороден прекис (H_2O_2) и пролин. Резултатите са обработени статистически чрез едно-, двуфакторен ANOWA и клъстър анализ. Нашите предварителните резултати показват, че за да се получи по-ценна информация, относно сухоустойчивостта на сортовете и мутантните линии фасул, МДА, H_2O_2 и пролинът, трябва да се използват като ранни маркери. Изследваните генотипи представляват интерес за бъдещи проучвания, като източник на генплазма за сухоустойчивост при фасула.

Introduction

Drought is considered as one of the general abiotic stressess affected agricultural systems and food production and also introduces main physiological, biochemical and molecular responses in several crop plants as bean (Abass and Mohamed, 2011; Foyer and Noctor, 2002; Graham and Ranalli, 1997; Torres et al., 2008; Xoconostle-Cázares et al., 2011). During last decade new alternative strategies have been developed and introduced in modern sustainable agriculture, for example creation of cultivars with increased tolerance to drought (Khush, 1999; Xoconostle-Cázares, 2011)

Such strategies are based either on accelerating the selection of natural varieties or/and inserting genes from other plant varieties or species with the capacity to provide drought tolerance (CIAT, 2001; CIMMYT, 2003; Xoconostle-Cázares, 2011). Drought tolerance is an increasingly important trait in common bean (*Phaseolus vulgaris* L.) due to the reduction in water resources, a shift in production areas and increasing input costs. Common bean (*Phaseolus vlugaris* L.) is the major food legume for human nutrition in the world, and a major source of calories and proteins, particularly in many Latin American, African (Asfaw et al., 2012; Brougthon et al., 2003; Rao, 2001) and European countries.

Phaseolus vlugaris L. generally, is known to be drought-sensitive crop (Beebe et al., 2008). Inspite this fact about 50-60% of bean production in the developing countries occurs under conditions of significant drought stress (Graham et al., 1997). That is why the mechanisms involved in the formation of drought tolerance are of great importance with regard further improvement of common bean agronomic performances and obtaining of more resistant cultivars (Beebe et al. 2010; Subbaro et al. 1995).

Having in mind that *Phaseolus vlugaris* L. is one of the most important agricultural crops in Bulgaria we addressed the question whether Bulgarian common bean cultivars and some mutant lines $M_{(19–20)}$ obtained by us would differ in their response to oxidative stress induced by drought.

The aim of this study was to characterize drought tolerance of Bulgarian common bean genotypes using some biochemical markers for oxidative stress.

Materials and Methods

Experiments were conducted in the field of Agricultural University in Plovdiv, Bulgaria. A standard method for cultivation in 5 replicates was applied.

Plant material

Table 1. Investigated common bean genotypes
Таблица 1. Проучвани генотипи фасул

№	Mutant lines	Selection	№	Cultivars	Selection
1.	D_2-0,0062 M EMS	1, BG	**11.**	Plovdiv 11 M	1, BG
2.	D_2-0,0031 M NEU	1, BG	**12.**	Plovdiv 10	1, BG
3.	D_2-0,0062 M EMS	1, BG	**13.**	Abritus	2, BG
4.	D_2-0,0125 M EMS	1, BG	**14.**	Plovdiv 2	1, BG
5.	D_2-0,0062 M EMS	1, BG	**15.**	Doubrudjanski ran	2, BG
6.	D_2-0,0125 M EMS	1, BG	**16.**	Doubrudjanski 7	2, BG
7.	D_2-0,0062 M EMS	1, BG	**17.**	Plovdiv 15 M	1, BG
8.	D_7-0,0125 M EMS	1, BG	**18.**	Plovdiv 564	1, BG
9.	D_2-0,0125 M EMS	1, BG	**19.**	Doubrudjanski 2	2, BG
10.	D_2-0,0031 M NEU	1, BG	**20.**	BAT 477	CIAT, Colombia

Note: The mutant lines and cultivars are selected in: 1 - Agricultural University, Plovdiv, 2 - Dobrudja Agricultural Institute, near the town General Toshevo.

10 common bean (*Phaseolus vulgaris* L.) mutant lines and 10 cultivars, grown under rainfed and irrigated conditions (Table 1) were tested. The numbers in parentheses after each genotype, as described in the text, are taken from Table 1. Cultivar BAT 477 is obtained by exchanging germoplasme between Dobrudja Agricultural Institute, General Toshevo and CIAT, Colombia.
Mutant lines are stable (M_{18}-generation). They are mainly derived from a cultivar Dobroudjanski 2. Exception line D_7-0, 0125 M EMS, which is obtained from a cultivar Dobrudjanski 7. Mutagenic factors etilmethan sulfonate (EMS) and N-nitroso-N'-ethyl urea (NEU) were used. Concentrations are listed at the end of the name of the mutant line. All studied genotypes are with Mesoamerican origin.

Biochemical analysis

Three different markers for oxidative stress were used: malondialdehyde, intracellular hydroden peroxide and proline. The third leaf was research. Experience was conducted in triplicate.

Measurements of lipid peroxidation (MDA)

Lipid peroxidation was determined by measuring MDA (Dhindsa et al., 1981). Fresh tissues (0,2 g) were homogenized with liquid nitrogen in 3 ml 0.1 % trichloracetic acid (TCA). After centrifugation at 12,000rpm for 20 min, 0,5 ml supernatant was added to 0,5 ml of the phosphate buffer and 1 ml of 0,5% thiobarbituric acid (TBA). The mixture was heated at 100°C for 30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was read at 532 nm and correction for unspecific turbidity was done by subtracting the apsorbance at 600 nm. The MDA content was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen Peroxide Determination

Hydrogen peroxide concentration was evaluated as described by Heath and Packer (1968). Fresh tissues (0,2 g) were homogenized with liquid nitrogen in 3 ml 0.1 % trichloracetic acid (TCA). After the centrifugation at 12,000rpm for 20 min, 0,5 ml supernatant was added to 0,5 ml of the 1M pH 7,5 phosphate buffer [(1.) prepared as followes: 1M KH_2PO_4; (2.) 1M $Na_2HPO_4.12H_2O$); (3.) mixed 1+2 and added dH_2O] and 1 ml of potassium iodide (KI). The mixture was kept 45 min in the dark. The absorbance of the supernatant was read at 390 nm.

Proline content determination

Proline analysis was performed according to Bates et al.(1973). Bean leaves (0.5g) were immediately homogenized in 5ml of 3% sulfosalicylic acid. After centrifugation at 10,000rpm for 20min, 2ml supernatant was added to 2ml acetic acid and 2 ml of ninhydrin. The mixture was kept at 100 ^oC for 60min, and then the reaction was stopped quickly by an ice bath. Toluene (2ml) was added to the mixture, the organic phase was extracted and monitored at 520 nm by the spectrophotometer.

Statistical analysis

Mono- and bi-factorial ANOVA was conducted (Sokal and Rohlf, 1969).
Data from biochemical analyses were analyzed using NTSYS-pc program version 2,01 b (1986-1997, Applied Biostatistic Inc.). DIST coefficient was used to group genotypes

using the SAHN procedure that uses the program UPGMA (Rolf, 1989). Dendrogrammes for convergence between the genotypes are obtained using the TREE DISPLAY subroutine.

Result and Discussion

As it was described above, sampling of common bean leaves was performed on two dates - 07.06.2011 and 14.07.2011 and contents of MDA, H2O2, and proline was measured (Table 2). All tested genotypes showed statistically significant lower contents of MDA ($P_{0,1\%}$) in comparison with BAT 477, used as a control.
However, it should be mentioned, that in both dates of sampling mutant lines D_2-0,0125 M EMS (№ 6), D_2-0,0125 M EMS (№ 9) and D_2-0,0031M NEU (№ 10), as well as cultivars Abritus (№ 13) and Dobroudjanski 2 (№ 19) stand out with higher levels of MDA. A clear tendency of increasing of MDA content was revealed for all genotypes, when second sampling was carried out.
Regarding the content of H_2O_2 it was shown that mutant lines D_2-0,0062 M EMS (№ 1), D_2-0,0062 M EMS (№ 3), D2-0,0062 M EMS (№ 5) and cultivars Plovdiv 11M (№ 11), Dobroudjanski ran (№ 15) and Plovdiv 564 (№ 18) differ in statistically significant way ($P_{1\%}$).
In the second date of sampling all studied genotypes show approximately the same levels of H_2O_2 ,very close to those in the control (BAT 477). Although the differences were not statistically significant (n.s.), it should be noted that mutant lines D_2-0,0062 M EMS (№ 1), D_2-0,0125 M EMS (№ 4), D_2-0,0125 M EMS (№ 9) and cultivars Plovdiv 15 M (№ 17) and Dobroudjanski 2 (№ 19) responded with higher levels of H_2O_2.
Content of proline, measured in leaves collected at the first date of sampling (06.07.2011), was highest in mutant lines D_2-0,0062M EMS (№ 7), D_2-0,0031M NEU (№ 10) and cultivars Dobroudjanski ran (№ 15) and Dobroudjanski 2 (№ 19). All differences were statistically significant in comparison with the control, $P_{5\%}$ and $P_{0,01\%}$.
On the second date of sampling (07.14.2011), higher content of proline was measured for mutant lines D_2-0,0031 M NEU (№ 2) and D_2-0,0125M EMS (№ 6), statistically significant ($P_{1\%}$). In the other studied genotypes, the values were statistically close to the level of control - BAT 477.
However, it was noted, that cultivars Plovdiv 11M (№ 11), Plovdiv 15M (№ 17), Dobroudjanski ran (№ 15) and Dobroudjanski 2 (№ 19) were distinguished with higher values.
Analysis of variance (Table 3), conducted by ANOVA, was performed in order to establish the influence of biochemical markers. Variation in the content of biochemical markers, studied genotypes and their interaction in the first reporting date were almost two times greater than that at the latter date. Found experimental values of the F-criterion of Fisher (Sokal and Rohlf, 1969) were higher than the reported table values for $P_{5\%}$, which defines significant influence of the determined variation causes.
Summarized results obtained by ANOVA are presented in a Table 4. They are very informative in clarifying how the three biochemical markers MDA, H_2O_2, and proline depend both on the environmental conditions (two dates of reporting) and the genotypes.
From the analysis of variance, tracking obtained data showed that the content of H_2O_2 was influenced most strongly by both environmental conditions and individual reaction of

Таблица 2. Биохимичен анализ на листа от фасул, взети на 07.06. и 14.07.2011 година

№	GENOTYPES	MDA/mmol/g		H_2O_2/mmol/g.W		Proline/micromol/g	
		07.06.2011	14.07.2011	07.06.2011	14.07.2011	07.06.2011	14.07.2011
1.	D_2-0,0062 M EMS	0.091 ---	0.145 ---	46.64 ++	35.89 n.s.	2.40 n.s.	3.68 n.s.
2.	D_2-0,0031 M NEU	0.107 ---	0.161 ---	37.65 n.s.	30.73 n.s.	2.92 n.s.	5.02 ++
3.	D_2-0,0062 M EMS	0.096 ---	0.158 ---	41.25 ++	29.90 n.s.	2.23 n.s.	3.62 n.s.
4.	D_2-0,0125 M EMS	0.085 ---	0.249 n.s.	34.33 n.s.	37.05 n.s.	2.80 n.s.	3.87 n.s.
5.	D_2-0,0062 M EMS	0.079 ---	0.153 ---	41.19 ++	19.59 n.s.	2.65 n.s.	2.78 n.s.
6.	D_2-0,0125 M EMS	0.126 ---	0.207 -	19.99 -	27.29 n.s.	2.62 n.s.	5.13 ++
7.	D_2-0,0062 M EMS	0.090 ---	0.241 n.s.	29.45 n.s.	29.45 n.s.	4.24 +++	3.89 n.s.
8.	D_7-0,0125 M EMS	0.065 ---	0.152 ---	20.43 -	25.35 n.s.	2.65 n.s.	2.85 n.s.
9.	D_2-0,0125 M EMS	0.121 ---	0.197 --	26.95 n.s.	33.86 n.s.	2.57 n.s.	4.06 n.s.
10.	D_2-0,0031 M NEU	0.133 ---	0.204 -	24.14 n.s.	31.38 n.s.	3.40 +	2.84 n.s.
11.	Plovdiv 11 M	0.078 ---	0.145 ---	44.00 ++	19.55 n.s.	3.06 n.s.	4.10 n.s.
12.	Plovdiv 10	0.091 ---	0.129 ---	42.11 ++	25.07 n.s.	2.61 n.s.	3.06 n.s.
13.	Abritus	0.127 ---	0.193 --	23.02 n.s.	22.26 n.s.	1.72 n.s.	2.34 n.s.
14.	Plovdiv 2	0.121 ---	0.131 ---	41.31 ++	18.17 -	2.58 n.s.	2.48 n.s.
15.	Dobroudjanski ran	0.097 ---	0.163 ---	45.80 ++	23.05 n.s.	3.91 +++	3.47 n.s.
16.	Dobroudjanski 7	0.073 ---	0.163 ---	42.52 ++	22.59 n.s.	2.27 n.s.	1.92 n.s.
17.	Plovdiv 15 M	0.068 ---	0.205 -	34.22 n.s.	40.40 n.s.	2.27 n.s.	3.77 n.s.
18.	Plovdiv 564	0.159 ---	0.104 ---	45.74 ++	19.68 n.s.	1.84 n.s.	3.28 n.s.
19.	Dobroudjanski 2	0.130 ---	0.172 ---	36.25 n.s.	32.64 n.s.	3.84 ++	3.49 n.s.
20.	**BAT 477 (control)**	**0.227**	**0.276**	**30.59**	**33.02**	**2.41**	**3,02**
	GD $_{P5\%}$ =	**0.034**	**0.057**	**10.07**	**12.27**	**0.83**	**1.21**
	GD $_{P1\%}$ =	**0.045**	**0.077**	**13.76**	**16.75**	**1.14**	**1.65**
	GD $_{P0,01\%}$ =	**0.062**	**0.104**	**18.67**	**22.75**	**1.55**	**2.35**

Table 3. Bi-factorial dispersion analysis to establish the relationship between biochemical markers and studied genotypes, depending on various environmental conditions
Таблица 3. Двуфакторен дисперсионен анализ за установяване взаимовръзката между биохимичните маркери и изследваните генотипи, в зависимост от различните условия на средата

Source of variation	S^2	*F exp.*	*F crit*
	7.06.2011		
Biochemical markers	15393.84	1977.21	3.15
Genotypes	52.02	6.68	1.76
	14.07.2011		
Biochemical markers	9148.10	790.25	3.15
Genotypes	32.15	2.78	1.76

the studied genotypes.
When reporting the contents of MDA and proline, the environmental conditions were found to have the most profound influence on variability. The impact (role) of genotypes and the interaction between genotype x environmental conditions was approximately equivalent.

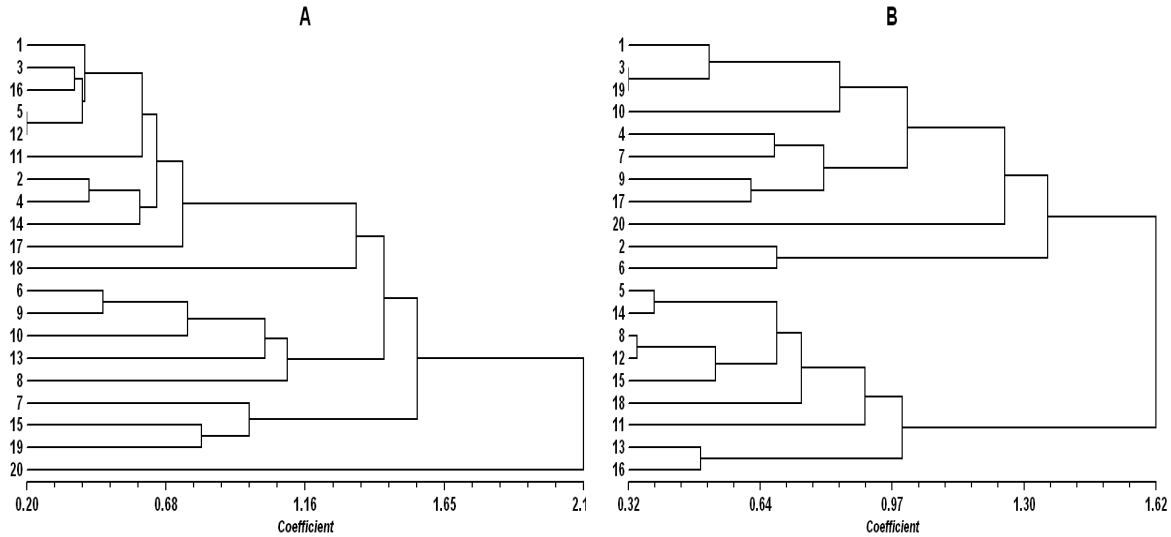

Figure 1. Dendrogram presented the variation of 20 common bean genotypes in terms of MDA, H_2O_2 and proline contents in their leaves, sampled at 07.06.2011 (**A,** *r = 0,846*) and 14.07.2011 (**B,** *r = 0,715*)
Фигура 1. Дендрограма, представяща варирането на 20 генотипа фасул по отношение на съдържанието на МДА, H_2O_2 и пролин в техните листа, събрани на 07.06.2011 (**A,** *r = 0,846*) и 14.07.2011 (**B,** *r = 0,715*)

Concerning obtained results by Neto et al. (2004) proline accumulation is not linear and temperature dependant.

In our investigations, dendrogrames obtained after performed cluster analysis (Fig. 1, A and B) reflect the clustering of genotypes according to the mean values of content of biochemical markers.

According to their stress response genotypes, at the first date of sampling (Fig. 1 A), could separated into two large groups. Some individual expression was obtained for mutant line D_2-0,0062 M EMS (№ 1) and cultivars - Plovdiv 564 (№ 18) and BAT 477 (№ 20).

Good correspondence was found between data obtained by both approaches - biochemical markers of stress and dendrogram performance. Based on this finding genotypes could be separated into two groups – some individual expression was found for mutant line D_2-0,0062 M EMS (№ 1) and cultivars - Plovdiv 564 (№ 18) and BAT 477 (№ 20), (Fig. 1, A).

Genotypes sampled at the second date (Fig. 1, B), were grouped into two large and one smaller intermediate clusters, including lines D_2-0,0031 M NEU (№ 2) and D_2-0,0125 M EMS (№ 6) .

There are different data in the literature in accordance or not with our results. According to Al-Karaki et al. (1996), for example, there were no differences in proline accumulation among bean species. However, Andrade et al. (1995) showed that in *P. vulgaris* L. there were peculiarities in this character between different cultivars among the four bean growing types.

Table 4. Bi-factorial dispersion analysis for evaluation of the influence of environmental conditions and genotypes on studied biochemical markers

Таблица 4. Двуфакторен дисперсионен анализ за установяване влиянието на условията на средата и генотипите върху проучваните биохимични маркери

Source of variation	**S^2**	***F exp.***	***F crit***
	MDA/mmol/g		
Environmental conditions	0.096	185.32	4.08
Genotypes	0.004	8.54	1.85
Interaction	0.002	4.39	1.85
	H_2O_2/mmol/g.W		
Environmental conditions	1119.98	38.90	4.08
Genotypes	90.74	3.15	1.85
Interaction	150.86	5.24	1.85
	Proline/micromol/g		
Environmental conditions	9.37	37.74	4.08
Genotypes	1.39	5.58	1.85
Interaction	0.82	3.30	1.85

Only mutant lines D_2-0,0062M EMS (№ 1) and D_2-0,0062M EMS (№ 3) occupied the same position in both dendrogrames. Genotypes D_2-0,0031M NEU (№ 10), Plovdiv 11M (№ 11), Plovdiv 564 (№ 18) and BAT 477 (№ 20) were separated into different clusters. This allows us to speculate that in this case different stress response to specific environment could be considered as genotypically determined.
It could be seen at the presented dendrogrames that other studied genotypes changed their position. Some explanations of this could be found in different environmental conditions recorded for the two dates of sampling.
The data from weather forecast for this period show that temperature, relative humidity and soil temperature at a depth of 35 cm have changed significantly (Fig. 2 and 3). These changes could be considered as one of the reasons that provoke the differences in stress response – content of MDA, H_2O_2 and proline in common bean leaves, sampled at two dates.
For the second period of sampling of leaves (07.14.2011) an increasing of air and soil temperatures (6.8 °C and 3.9 °C respectively) and 9% reducing of humidity were recorded.
Thus our results show a statistically significant influence of environmental changes that lead to change of biochemical response (Table 4).
Young and well shaped three-part leaves were sampled at both dates, in our analyses. Regardless of this fact, it should be noted, that plants have been at different physiological phases of development. Thus differences in physiological status of plants could be regarded as another reason for the different biochemical response.
Because it is well known that adverse environmental conditions increase the rate of reactivated oxygen species (ROS) in this work we analyze some indicators of oxidative stress such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline. Malondialdehyde is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Meloni et al., 2003; Munné-Bosch et al., 2001; Ohkawa et al., 1979).
Hydrogen peroxide can be produced under various abiotic and biotic stresses (Jubany-Marı et al., 2009; Liao et al., 2011). It is relatively stable and diffuses through membranes (Vranova *et al.* 2002), thus exogenous application of hydrogen peroxide at low concentrations stimulated and enhanced resistance to drought (He *et al.* 2009; Kocsy et al., 2005).
Proline has been demonstrated to scavenge hydroxyl radicals and singlet oxygen, thus providing protection against ROS-induced cell damage (Reddy et al. 2004).
BAT 477 (№ 20), was used as a control for drought tolerance in our investigations. This cultivar shows clear divergence compared with studied Bulgarian genotypes. This is evidenced by its positioning on its own cluster. (Fig. 1A and B). It should be noted, however, that initial soil moisture is a very important factor for seed's germination of BAT 477 (№ 20). Bulgarian genotypes of common bean have not such requirement - mutant lines and cultivars can germinate and grow normally without the presence of such moisture.
Two major conclusions could be drawn for our future work concerning drought tolerance: in addition to field experiments, laboratory experiments under controlled conditions must

be conducted; biochemical markers should be supplemented with other physiological and/or molecular markers for drought tolerance.

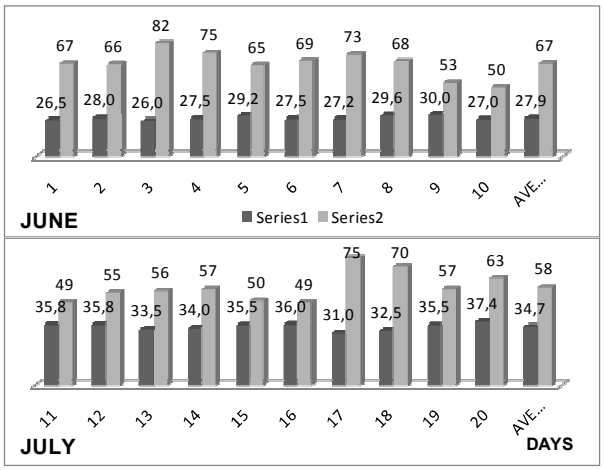

Figure 2. Temperature (series 1) and relative air humidity (series 2) in both periods 1-20 June and 11-20 July
Фигура 2. Температура (series 1) и относителна влажност на въздуха (series 2) през периода 1-20 юни и 11-20 юли

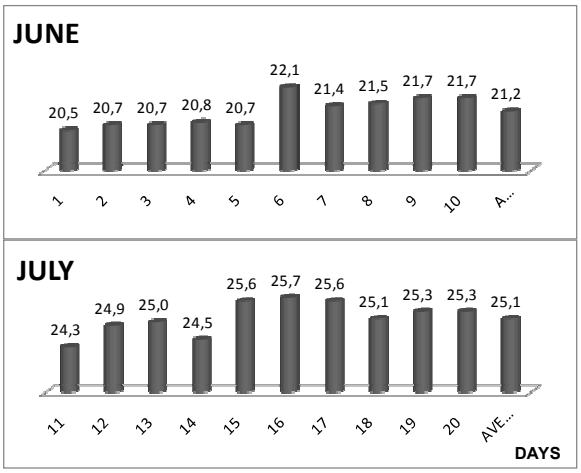

Figure 3. Soil temperature in depth of 35 cm in both periods 1-20 June and 11-20 July
Фигура 3. Температура на почвата на дълбочина 35 cm през периода 1-20 юни и 11-20 юли

Conclusions

As a result of our study the following conclusions could be drawn:
1. Our preliminary results demonstrated that to obtain more valuable information, concerning drought tolerance of both common bean cultivars and mutant lines, malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline should be used as early warning markers.
2. Content of MDA, H_2O_2 and proline was influenced most strongly by both environmental conditions and the genotypes. The interaction between these factors was also statistically significant.
3. Studied genotypes could be of interest in future investigations being a source of geneplasme of plants drought tolerance.

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