

DROUGHT TOLERANCE OF BULGARIAN COMMON BEAN GENOTYPES, CHARACTERIZED BY SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

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Abstract

This study aims to characterize sensitivity to the drought of some Bulgarian common bean genotypes, studying physiological (leaf water potential and leaf gas exchange) and biochemical (proline content) parameters to introduce them in farther breeding programs for creation of new cultivars with better drought tolerance. Fourteen common bean genotypes - five mutant lines and nine cultivars (8 Bulgarian and a Mexican one - BAT 477), were tested in three years investigations conducted in the field of the department Genetic and Plant Breeding at Agricultural University, Plovdiv. Mutant lines (M₁₉-generation) were previously obtained by treatment of seeds from the cultivar Dobroudjanka 2 with ethylmethan sulphonate (EMS) and N-nitroso-N-ethyl urea (NEU). BAT 477 was used as a control variant. The obtained results in that study highlight the fact that water deficit influenced leaf water relations in young common bean plants. Changes in water potential (Ψ_w) were the highest in mutant lines D₂-6.2⁻³ M EMS (№ 3), D₂-3.1⁻³ M NEU (№ 5) and cultivars - Plovdiv 15 M (№ 12) and Dobrudjanka 2 (№ 13). Leaf gas exchange parameters, comparing to the control variant (BAT 477), were higher in Bulgarian common bean genotypes – D₂-3.1⁻³ M NEU (№ 5) and Dobrudjanka 2 (№ 13). During rainfed conditions the following Bulgarian genotypes have a bigger amount of proline: mutant lines D₂-6.2⁻³ M EMS (№ 3), D₂-3.1⁻³ M NEU (№ 5) and cultivars Dobrudjanski ran (№ 10) and Dobrudjanka 2 (№ 13) in comparison to the other studied genotypes. Based on water relations and leaf gas exchange parameters, mutant line D₂-3.1⁻³ M NEU (№ 5) and cultivar Dobrudjanka 2 (№ 13), studied for stress tolerance, can be included as donors for drought tolerance in further breeding programs. All data presented in the tables and the figure are averaged on the basis of the results obtained from the three-years investigations.

Keywords: Leaf gas exchange, leaf water potential, *Phaseolus vulgaris* L., proline.

INTRODUCTION

Drought is one of the most important environmental stress factors affecting the growth and development of agricultural plants. Considering global climate change, drought is likely to increase in the coming years (Zhou et al., 2017). It is considered that an effective approach to raise crop productivity and food production can be based on the improvement of crop's drought tolerance (Ayranci et al., 2014, Morosan et al., 2017).

Plants are more sensitive to drought at some stages. For example, drought stress on reproductive stages of common bean can be a problem for reduction of production because it affects flowering and pod-filling processes which are highly drought-sensitive (Dipp et al., 2017).

Drought resistance is a complex quantitative trait, involving interactions of many metabolic pathways related to stress-resistant genes. Identification of a standard evaluation assay has

been the most pressing problem for the selection of drought-resistant genotypes and ultimately for elucidating the internal genetic mechanisms (Zadehbagheri, 2014).

Selection based on phenotype is complicated by associated physiological, biochemical, anatomical, cellular and molecular changes. The adaptive plant strategies in the common bean are mostly genetically determined (Asfaw and Blair, 2012). Marker-assisted selection can be applied to breed new drought-tolerant common bean cultivars combined a range of tolerance mechanisms (Asfaw et al., 2012; Beaver et al., 2003; Ghanbari et al., 2013).

Villordo-Pineda et al. (2015) used Single Nucleotide Polymorphisms (SNPs) to link them to specific gene functions and discovered that SNP 18 is related to proline biosynthesis, well-known osmotic protector.

Mukeshimana et al. (2014) also used single nucleotide polymorphism (SNP) markers to identify quantitative trait loci (QTL) associated with traits

related to drought tolerance in common bean.

Drought is one of the most important constraints for plant production, but the improvement of drought tolerance is a very complicated process because of the set of mechanisms involved in.

Under the influence of this type of stress, usually, a water deficit is developed in plant tissue, thus leading to significant inhibition of photosynthesis. Even moderate drought can reduce the net photosynthetic rate (P_N) in water stress-sensitive plants, such as common beans (Santos et al., 2006). The ability to maintain the functionality of the photosynthesis under water stress can be of significant importance for the plant's drought tolerance. The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration. As a consequence, the diffusion of CO_2 into the leaf is restricted (Chaves, 1991).

The decrease in net photosynthetic rate (P_N) under drought stress, observed in many studies, is often explained by a lowered intercellular CO_2 concentration (c_i) that results in a limitation of photosynthesis at the acceptor site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Cornic, 1994) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthetase (Nogués and Baker, 2000).

However, many other studies have shown that decreased photosynthesis under water stress can be attributed to the perturbations of the biochemical processes (Lauer and Boyer, 1992). There are several reports, which underline the stomatal limitation of photosynthesis as a primary event, which is then followed by the adequate changes of photosynthetic reactions (Chaves, 1991).

Today, there is a consensus that a decrease of photosynthesis, due to water stress, has been attributed to both - stomatal and non-stomatal limitations (Shangguan et al., 1999; Stoilova et al., 2014). Non-stomatal limitation of photosynthesis has been attributed to reduced - carboxylation efficiency (Jia and Gray, 2004), ribulose-1,5-bisphosphate (PuBP) regeneration, amount of functional Rubisco, or to the inhibited functional activity of PSII. Concomitantly inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor, 2002). Since maximal CO_2 assimilation (A_{max}) reflects the result of those mesophyll cells impairments, its determination under severe water stress allows us to evaluate non-stomatal limitations of photosynthesis and hence, the degree of drought tolerance of the photosynthetic apparatus.

Some authors (Zdražnik et al., 2013) revealed that the levels of proteins involved in various cellular pathways are affected during drought

stress in common bean.

Abid et al. (2017) discovered that water deficit increases proline and soluble sugars in faba beans. According to Beebe et al. (2008) and Ghaffari et al. (2012) proline accumulation may associate with osmotic adjustment resulting inhibition of protein synthesis.

Proline acts as a compatible osmolyte and can be a way to store carbon and nitrogen. It has been proposed it function as a molecular chaperone that stabilize the structure of proteins and that proline accumulation can provide a way to buffer cytosolic pH and to balance cell redox status (Verbruggen and Hermans, 2008). Finally, proline accumulation may be a part of the stress signal, influencing adaptive responses (Maggio et al. 2002).

Proline has also been demonstrated to scavenge hydroxyl radicals and singlet oxygen, thus protecting against induced cell damages (Reddy et al. 2004).

The main goal in our investigations was to characterize sensitivity to the drought of some Bulgarian common bean genotypes, studying physiological (leaf water potential and leaf gas exchange) and biochemical (proline content) parameters to introduce them in farther breeding programs for creation of new cultivars with better drought tolerance.

MATERIALS AND METHODS

All obtained results are on the base of three years of investigations.

Plant material

Fourteen common bean (*Phaseolus vulgaris* L.) genotypes - five mutant lines and nine cultivars (8 Bulgarian and a Mexican one - BAT 477), were tested in three years investigations conducted in the field of Agricultural University, Plovdiv (Table 1). Mutant lines (M_{19} -generation) were previously obtained by treatment of seeds from the cultivar Dobroudjanka 2 with ethylmethan sulphonate (EMS) and N-nitroso-N-ethyl urea (NEU). Concentrations are listed at the end of the name of the mutant line.

The numbers of each genotype, as described in the text of the article, are taken from Table 1. BAT 477 is obtained by exchanging germoplasm between Dobrudja Agricultural Institute, General Toshevo and CIAT, Colombia. All studied genotypes are of Mesoamerican origin.

Experimental conditions

Seeds were sterilized with diluted sodium hypochlorite (commercial bleach solution), rinsed thoroughly with water and sown on a mixture of peat, perlite, and vermiculite (2:1:1) moistened with half-

strength Hoagland nutrient solution (Hoagland and Arnon, 1950). Water stress treatments (rainfed conditions) were initiated when the first trifoliolate leaves appeared (14 days after sowing). Normally grown plants were watered twice a week with half-strength Hoagland solution. Plants development was

carried out in a controlled environment chamber under long-day photoperiod (16 h light, 8 h darkness), temperature 25±1 °C/18±1 °C (day/night), air humidity – 65-70%.

Table 1. Investigated common bean genotypes

No	Mutant lines	Selection	No	Cultivars	Selection
1.	D ₂ -3.1 ⁻³ M NEU	1, BG	8.	Abritus	2, BG
2.	D ₂ -6.2 ⁻³ M EMS	1, BG	9.	Plovdiv 2	1, BG
3.	D ₂ -6.2 ⁻³ M EMS	1, BG	10.	Dobrudjanski ran	2, BG
4.	D ₂ -1.25 ⁻² M EMS	1, BG	11.	Dobrudjanski 7	2, BG
5.	D ₂ -3.1 ⁻³ M NEU	1, BG	12.	Plovdiv 15 M	1, BG
6.	Plovdiv 11 M	1, BG	13.	Dobrudjanka 2	2, BG
7.	Plovdiv 10	1, BG	14.	BAT 477 (Control variant)	CIAT, Colombia

Note: *The mutant lines and cultivars are selected in: 1 - AU, Plovdiv, Bulgaria; 2 - Dobrudja Agricultural Institute, near the town General Toshevo, Bulgaria. ** NEU - N-nitroso-N-ethyl urea; EMS - ethylmethan sulphonate

Leaf water potential

Leaf water potential (Ψ_w) was measured on the first trifoliolate leaves of 10 plants per genotype using the middle parts (excluding leaf nerves) of fully developed trifoliolate leaves by a pressure chamber EL 540-305 (ELE-International Ltd., Hemel Hempstead, England), according to Turner (1988).

Leaf gas exchange

The leaf gas exchange parameters – net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO₂ concentration (c_i) were determined with a portable photosynthetic system LCA-4 (Analytical Development Company Ltd., Hoddesdon, England) on the same plants as for measuring leaf water potential. Water use efficiency in photosynthesis (P_N/E) was calculated.

The measurements were made under a light intensity of 1200-1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, at a temperature of 25 °C, an external CO₂ concentration of 390 $\mu\text{mol mol}^{-1}$ and relative air humidity of 65-70%.

Proline content determination

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

520 nm by the spectrophotometer. After that, it was calculated as $\mu\text{mol/g}^{-1}$ FW against standard proline.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared by the Student test when significance ($P < 0.05$) was detected (Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

Studying the physiological parameter water potential (Ψ_w) was evaluated that genotypes have different reactions when they are grown in irrigated or rainfed conditions (Table 2).

The conducted analysis showed that the highest value of water potential (Ψ_w), studied in the first trifoliolate leaves of the plants grown in irrigated conditions, has the mutant line № 5. Control variant - BAT 477 occupies 11th place in the hierarchical order of the reported average values.

With lower values, compared to the control variant BAT 477, are cultivars Dobrudjanski 7, Plovdiv 2 and the mutant line № 1.

Studied genotypes represented different reaction concerning their water potential (Ψ_w) when they were grown in rainfed conditions.

Genotypes: mutant lines № 5, № 3 and cultivars Dobrudjanka 2 and Abritus stand with proven highest water potential in the range I. Regardless to the arrangement relative to the control variant - BAT 477, the differences are statistically significant at the level $P_{0.1\%}$. The cultivar Plovdiv 11 M

is the only one in range II, which differs from control variant at significant level $P_{1\%}$.

The highest value has the mutant line № 3. Control variant - BAT 477 occupies 9th position in the hierarchical order of the reported average values.

Some authors (Beebe et al., 2013) explain the better adaptation to water deficits of BAT 477 by attribution to drought avoidance through greater root length density and deeper soil moisture extraction of the plants.

Table 2. Water potential (Ψ_w) values found in the first trifoliolate leaves of plants growth in different conditions and warranted differences between common bean genotypes, compared to the control variant BAT 477

Genotypes	$\frac{(\Psi_w)}{\bar{X}}$	D	Warranted	Range	Genotypes	$\frac{(\Psi_w)}{\bar{X}}$	D	Warranted	Range
Irrigated conditions					Rainfed conditions				
D ₂ -3.1 ⁻³ M NEU	3.93	0.86	+++	I	D ₂ -6.2 ⁻³ M EMS	2.92	0.76	+++	I
Dobrudjanka 2	3.90	0.83	+++	I	Dobrudjanka 2	2.85	0.72	+++	I
D ₂ -6.2 ⁻³ M EMS	3.77	0.70	+++	I	D ₂ -3.1 ⁻³ M NEU	2.84	0.68	+++	I
Abritus	3.73	0.66	+++	I	Plovdiv 15 M	2.70	0.54	+++	I
Plovdiv 11 M	3.43	0.36	++	II	Plovdiv 11 M	2.49	0.33	+	III
D ₂ -6.2 ⁻³ M EMS	3.30	0.23	n.s.	IV	Plovdiv 2	2.41	0.25	n.s.	IV
D ₂ -1.25 ⁻² M EMS	3.30	0.23	n.s.	IV	Dobrudjanski ran	2.36	0.20	n.s.	IV
Dobrudjanski ran	3.27	0.20	n.s.	IV	D ₂ -1.25 ⁻² M EMS	2.31	0.15	n.s.	IV
Plovdiv 10	3.23	0.16	n.s.	IV	BAT 477 (Control)	2.16	-	-	IV
Plovdiv 15 M	3.13	0.06	n.s.	IV	Plovdiv 10	2.11	-0.05	n.s.	IV
BAT 477 (Control)	3.07	-	-	IV	Dobrudjanski 7	2.07	-0.09	n.s.	IV
Dobrudjanski 7	3.05	-0.02	n.s.	IV	Abritus	1.98	-0.18	n.s.	IV
Plovdiv 2	3.00	-0.07	n.s.	IV	D ₂ -6.2 ⁻³ M EMS	1.93	-0.23	n.s.	IV
D ₂ -3.1 ⁻³ M NEU	2.77	-0.30	n.s.	IV	D ₂ -3.1 ⁻³ M NEU	1.88	-0.28	n.s.	IV
GD P_{5%} = 0.32; GD P_{1%} = 0.43; GD P_{0,1%} = 0.56					GD P_{5%} = 0.28; GD P_{1%} = 0.39; GD P_{0,1%} = 0.51				

Compared to the control variant - BAT 477, with lower values are cultivars Plovdiv 10, Dobrudjanski 7 and Abritus, as well as mutant lines - № 2 and № 1.

Mutant lines № 3, № 5 and cultivars Dobrudjanka 2 and Plovdiv 15 M stand with proven highest water potential in the range I. Regardless of the arrangement relative on the control variant - BAT 477, the differences are statistically significant at the level $P_{0.1\%}$. The cultivar Plovdiv 11 M is the only one in range III, which differs from control variant at significant level $P_{5\%}$.

Eight genotypes with unproven differences, relative to the control variant level, are indicated as range IV.

Concerning data in table 3, it is possible to see that the plants of the mutant line № 3 and cultivar Dobrudjanka 2, in irrigated and rainfed conditions, have higher photosynthetic (P_N) and transpiration (E) rates, compared to the control cultivar BAT 477. On the opposite, those parameters are lower for the mutant line № 2 and cultivar Abritus.

The regime of farming (irrigated and rainfed conditions) are not significantly affected the

parameters P_N and E, only in two cultivars – Plovdiv 15 M and Dobrudjanski ran. In all other genotypes, their reaction has been proved with different levels of significance.

Water use efficiency in photosynthesis (P_N/E), compared to the control variant BAT 477, is highly reduced in the mutant line № 1.

Mutant line № 4, cultivar Dobrudjanski ran, and Dobrudjanski 7 also showed low results. Similar data were established by Zlatev (2005) and Zlatev et al. (2012).

It is important to mention that, compared to BAT 477, P_N/E for cultivars - Plovdiv 10 and Abritus, is the highest (Table 3).

Comparing to the control variant BAT 477, mutant lines № 4, № 5 and cultivars Plovdiv 2, Dobrudjanski ran, and Dobrudjanka 2 showed higher values for the stomatal conductance (g_s) and intercellular CO_2 concentration (c_i) in both regimes of plant cultivation. In the opposite, Abritus has lower values.

With unproven differences, concerning the values of the parameter g_s , between the two regimes of cultivation (irrigated and rainfed) are cultivars

Plovdiv 11 M and Dobrudjanski ran, while on the base of ci, the situation is the same for half of the studied genotypes.

In the other genotypes, the regime of cultivation has importance, and the differences are statistically significant at level P_{5%} and P_{1%} (Table 4).

It is possible to see in tables 3 and 4, that all studied leaf gas exchange parameters have low values when plants are developed in rainfed, than in irrigated conditions.

Content of proline (fig. 1), measured in leaves collected from grown plants at rainfed conditions, was highest in mutant lines № 3, № 5 and cultivars Dobroudjanski ran, Dobroudjanski 7 and Dobroudjanka 2 in comparison to the content determined in grown plants at irrigated conditions, All differences were statistically significant, in comparison to the control variant – BAT 477 (P_{5%} and P_{0,01%}).

Table 3. Leaf gas exchange parameters (P_N, E and P_N/ E), measured under a light intensity of 1200-1900 μmol m⁻² s⁻¹ PAR, T - 25 °C and warranted differences between common bean genotypes, compared to the results found for plants growth in irrigated and rainfed conditions

Genotypes	Condi-tions	P _N (μmol CO ₂ m ⁻² s ⁻¹)			E (mmol H ₂ O m ⁻² s ⁻¹)			P _N / E (mmol/ mol ⁻¹)
		\bar{X}	D	t	\bar{X}	D	t	
D ₂ -3.1 ⁻³ M NEU	Irrigated	21.40±0.52	6.11	8.04 **	5.56±0.17	1.08	6.00 **	3.85
	Rainfed	15.29±0.55			4.48±0.08			3.41
D ₂ -6.2 ⁻³ M EMS	Irrigated	19.36±0.15	2.96	8.70 ***	4.85±0.08	0.89	7.41 **	3.99
	Rainfed	16.40±0.31			3.96±0.09			4.14
D ₂ -6.2 ⁻³ M EMS	Irrigated	24.06±0.43	3.29	6.85 **	5.77±0.12	1.03	7.35 **	4.17
	Rainfed	20.77±0.23			4.74±0.09			4.38
D ₂ -1.25 ⁻² M EMS	Irrigated	21.69±0.66	6.56	9.37 ***	5.15±0.14	0.61	2.90 *	4.21
	Rainfed	15.13±0.23			4.54±0.16			3.34
D ₂ -3.1 ⁻³ M NEU	Irrigated	20.86±0.21	1.65	3.05 *	5.11±0.14	0.95	4.13 *	4.08
	Rainfed	19.21±0.5			4.16±0.19			4.63
Plovdiv 11 M	Irrigated	22.00±0.28	1.71	4.07 *	5.40±0.20	1.18	4.53 *	4.16
	Rainfed	20.29±0.32			4.22±0.17			4.82
Plovdiv 10	Irrigated	22.31±0.25	2.25	7.89 **	4.65±0.16	0.95	6.78 **	4.81
	Rainfed	20.06±0.17			3.7±0.01			5.40
Abritus	Irrigated	20.47±0.51	1.24	1.96 n.s.	3.82±0.08	0.78	8.04 **	5.35
	Rainfed	19.23±0.37			3.04±0.06			6.31
Plovdiv 2	Irrigated	22.39±0.27	3.92	6.87 **	5.62±0.10	1.09	8.38 **	3.98
	Rainfed	18.47±0.51			4.53±0.09			4.07
Dobrudjanski ran	Irrigated	19.74±0.75	2.47	3.12 *	4.93±0.16	0.39	1.69 n.s.	4.01
	Rainfed	17.27±0.28			4.54±0.19			3.80
Dobrudjanski 7	Irrigated	24.05±0.47	5.65	10.08 ***	5.22±0.20	0.86	3.90 *	4.61
	Rainfed	18.40±0.32			4.36±0.10			4.32
Plovdiv 15 M	Irrigated	20.90±0.32	0.69	1.81 n.s.	5.15±0.14	1.01	4.39 *	4.06
	Rainfed	20.21±0.22			4.14±0.19			4.89
Dobrudjanka 2	Irrigated	22.64±0.15	1.37	4.56 *	5.41±0.03	1.07	7.13 **	4.18
	Rainfed	21.27±0.27			4.34±0.15			4.90
BAT 477 (Control variant)	Irrigated	22.25±0.24	2.75	4.82 **	4.70±0.17	0.74	4.93 **	4.67
	Rainfed	19.50±0.53			3.96±0.06			4.94
t P_{5%} = 2.776; t P_{1%} = 4.604; t P_{0,1%} = 8.610								

No statistically significant difference between the content of proline in the plants grown in rainfed

and irrigated conditions was calculated only for the cultivar Plovdiv 2.

Compared to the control cultivar BAT 477, the content of proline was lower in the leaves of mutant lines № 2, № 5 and cultivars Abritus, Plovdiv 2 and Plovdiv 15 M, grown in rainfed conditions.

Since water deficiency can cause strong photosynthesis impairment in *Phaseolus vulgaris* even under mild water deficit (Santos et al., 2006), the maintenance of shoot hydration (given by high Ψ_w) may alleviate the harmful effects of drought on photosynthesis (Santos et al., 2009).

Bulgarian genotypes that were included in our studies, also react in a different way to the water

deficit (Table 2).

Drought tolerance is defined as the ability of the crop to withstand water deficit with low tissue water potential. It is achieved through maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in the cell), increase in cell elasticity and decrease in cell size, and desiccation tolerance by protoplasmic resistance (Beebe et al., 2013).

Among the physiological mechanisms that act to maintain leaf turgor pressure, decreased osmotic potential resulting either from a decrease in osmotic

Table 4. Leaf gas exchange parameters (g_s and c_i), measured under a light intensity of 1200-1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, T - 25 °C and warranted differences between common bean genotypes, compared to the results found for plants growth in irrigated and rainfed conditions

Genotypes	Condi- tions	g_s (mol m ⁻² s ⁻¹)			c_i ($\mu\text{mol mol}^{-1}$)		
		\bar{X}	D	t	\bar{X}	D	t
D ₂ -3.1 ⁻³ M NEU	Irrigated	0.43±0.02	0.11	5.50 ++	190.00±6.20	0.70	0.09 n.s.
	Rainfed	0.32±0.02			189.30±3.80		
D ₂ -6.2 ⁻³ M EMS	Irrigated	0.41±0.02	0.13	4.60 +	203.00±6.06	34.00	3.86 +
	Rainfed	0.28±0.02			169.00±6.40		
D ₂ -6.2 ⁻³ M EMS	Irrigated	0.47±0.02	0.11	3.90 +	184.00±4.40	14.0	2.30 n.s.
	Rainfed	0.36±0.02			170.00±4.20		
D ₂ -1.25 ⁻² M EMS	Irrigated	0.48±0.02	0.13	6.50 ++	209.00±1.20	22.70	5.04 ++
	Rainfed	0.35±0.01			186.30±4.40		
D ₂ -3.1 ⁻³ M NEU	Irrigated	0.47±0.02	0.19	9.5 ⁰⁺⁺⁺	213.30±2.80	14.30	3.19 +
	Rainfed	0.28±0.01			199.00±3.50		
Plovdiv 11 M	Irrigated	0.54±0.04	0.10	2.20 n.s.	213.00±4.30	12.00	2.03 n.s.
	Rainfed	0.44±0.01			201.00±4.10		
Plovdiv 10	Irrigated	0.41±0.04	0.15	3.75 +	187.00±4.80	14.00	2.15 n.s.
	Rainfed	0.26±0.02			173.00±4.40		
Abritus	Irrigated	0.37±0.02	0.10	3.57 +	181.0±5.60	38.00	4.92 ++
	Rainfed	0.27±0.02			143.0±5.30		
Plovdiv 2	Irrigated	0.57±0.08	0.24	3.00 +	218.0±5.09	44.00	5.23 ++
	Rainfed	0.33±0.03			174.0±6.70		
Dobrudjanski ran	Irrigated	0.57±0.06	0.15	2.50 n.s.	218.0±1.80	46.00	8.15 ++
	Rainfed	0.42±0.03			172.0±5.10		
Dobrudjanski 7	Irrigated	0.47±0.03	0.13	3.25 +	186.0±4.40	22.40	2.88 +
	Rainfed	0.34±0.03			163.6±6.40		
Plovdiv 15 M	Irrigated	0.46±0.01	0.11	7.85 ++	207.3±1.40	10.70	2.41 n.s.
	Rainfed	0.35±0.01			196.6±4.20		
Dobrudjanka 2	Irrigated	0.54±0.02	0.11	5.00 ++	209.6±5.70	16.60	2.25 n.s.
	Rainfed	0.43±0.01			193.0±4.70		
BAT 477 (Control variant)	Irrigated	0.39±0.03	0.15	5.00 ++	191.3±3.40	10.30	1.85 n.s.
	Rainfed	0.24±0.01			181.0±4.41		
t P_{5%} = 2.776; t P_{1%} = 4.604; t P_{0.1%} = 8.610							

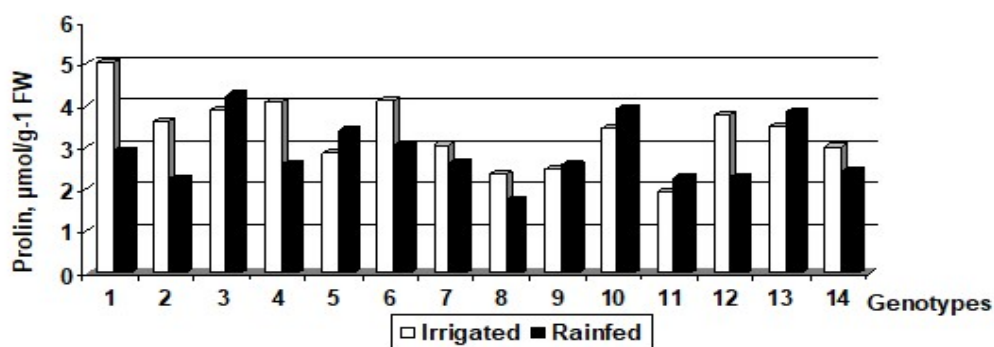


Fig. 1. Proline content ($\mu\text{mol g}^{-1}\text{FW}$) in leaves of common bean plants grown in irrigated and rainfed conditions. Genotype's names are presented in table 1.

water fraction or from an osmotic adjustment (net accumulation of solutes in the symplast) was pointed out (Zlatev, 2004, 2005). Changes in tissue elasticity in response to drought, which modify the relationship between turgor pressure and cell volume, might contribute to drought tolerance, as observed in black spruce (Blake et al., 1991) and sunflower (Maury et al., 2000). Leaf water relations data may provide a useful indication of the capacity of species to maintain functional activity under drought (White et al., 2000).

During the period of water deficit, water potential and relative water content decreased with an associated decrease of net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) (Yordanov et al., 2001). These authors have found a significant correlation among the components of leaf water status and the measured photosynthetic parameters.

Plants assimilate gases from the environment by their leaves. They require oxygen for respiration and carbon dioxide for photosynthesis. The gases diffuse into the intercellular spaces of the leaf through pores, which usually are on the underside of the leaf - stomata.

Stomata role in maintaining the functional activity of the photosynthetic apparatus during periods of drought

differ in the plants (Chaves, 1991; Stoilova et al., 2014). In some cases, the stomata control is of great importance, and these plants are characterised by increasing water use efficiency (P_N/E). In others, when the plants keep stomata relatively open, due to the possibility to compensate for water losses or to a loss of stomata control, the water use efficiency could remain unchanged or insignificantly reduced.

Our results showed that the young bean plants of cultivars Abritus, Plovdiv 10 and the control variant BAT 477, related to the first group and those like mutant line № 1 - to the second one.

It is known that crop plants have developed many mechanisms to survive water deficit, including escape, tolerance, and avoidance of tissue and cell dehydration (Turner, 1986). Avoidance of stress includes rapid phenological changes - increased stomatal and cuticular resistance, changes in leaf area, orientation and anatomy, among others (Jones and Corlett, 1992). Plants tolerate drought by maintaining sufficient cell turgor and allow metabolism to continue under increasing water deficits. Tolerance to stress involves at least two mechanisms - osmotic adjustment and changes in the elastic properties of tissues (Savé et al., 1993).

Osmotic adjustment is generally thought to be the major mechanism to maintain cell turgor in many species as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity (Gunasekera and Berkowitz, 1992). Lowering of the osmotic potential of the cells accumulating solutes is considered to be due to osmotic adjustment if the buildup of compounds is not merely the result of tissue dehydration (Zlatev, 2005).

In our investigations, all studied Bulgarian genotypes, cultivated in rainfed conditions, had higher values of stomatal conductance (g_s) than control variant BAT 477. Good correlation between stomatal conductance (g_s) and intercellular CO_2 concentration (c_i) was found for the Bulgarian genotypes - № 2, № 4, № 5, Plovdiv 2, Dobrudjanski ran and Dobrudjanka 2.

The decrease of photosynthesis caused by drought has been attributed to both stomatal (restricted CO_2 availability) and non-stomatal limitations (Shangguan et al., 1999; Yordanov et al., 2000). Stomatal closure was the most prominent determinant for the increased WUE. In addition, our results support observations that transpiration efficiency differed significantly between cultivars

with different drought acclimation capabilities. Metabolic acclimation via the accumulation of compatible solutes is also regarded as a basic strategy for the protection and survival of plants in extreme conditions (Yordanov et al., 2001). The synthesis of proline in leaves can enhance protection to drought. The major role of metabolites like proline, sugar alcohols, amino acids and their derivatives is to serve as organic osmolytes with compatible properties at high concentrations. Such osmolytes increase the ability of cells to retain water without disturbing normal cellular function (Yordanov et al., 2001). Differences between cultivars can also be due to the operation of additional mechanisms functioning in some cultivars but not in others.

In our investigations, mutant lines № 3, № 5 and cultivars Dobroudjanski ran and Dobroudjanka 2 seems to have good adaptation to drought because they showed higher proline content, while mutant lines № 2, № 4 and cultivars Abritus, Plovdiv 2, Plovdiv 15 M, have lowest proline levels in rainfed conditions.

Concerning opinion of Jimenez-Bremont et al. (2006) proline accumulation is believed to play adaptive roles in plant stress tolerance and thus can be considered as an important component in the spectra of salicylic acid in response to water stress (Sadeghipour and Aghaei, 2012).

Proline is a reliable marker of the level of stress affecting bean plants but is not directly involved in tolerance mechanisms (Morosan et al., 2017).

Kusvuran and Dasgan (2017) also indicated that an antioxidant defence system, proline and secondary metabolites play important roles in common bean during drought stress.

Other authors, such as Mathobo et al. (2017) have revealed that drought stress resulted in a reduction in photosynthetic rate, intercellular carbon dioxide concentration, stomatal conductance, transpiration and chlorophyll fluorescence.

CONCLUSIONS

Changes in leaf water potential (Ψ_w) were the highest in mutant lines D₂-6.2⁻³ M EMS (№ 3), D₂-3.1⁻³ M NEU (№ 5) and cultivars Plovdiv 15 M (№ 12), Dobrudjanka 2 (№ 13).

Leaf gas exchange parameters, comparing to the control variant (BAT 477), were higher in the Bulgarian common bean genotypes – D₂-3.1⁻³ M NEU (№ 5) and Dobrudjanka 2 (№ 13).

During rainfed conditions next Bulgarian genotypes have a bigger amount of proline – mutant lines D₂-6.2⁻³ M EMS (№ 3), D₂-3.1⁻³ M NEU (№ 5) and cultivars Dobrudjanski ran (№ 10) and

Dobrudjanka 2 (№ 13) in comparison to the other studied genotypes.

Based on water relations and leaf gas exchange parameters, studied for stress tolerance, mutant line D₂-3.1⁻³ M NEU (№ 5) and cultivar Dobrudjanka 2 (№ 13) can be included as donors for drought tolerance in further breeding programs.

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