

## GENETIC VARIABILITY OF FREE ENERGY IN A FUNCTION OF DROUGHT TOLERANCE IN COMMON BEAN ACCESSIONS

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### ABSTRACT

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Characterisation of bean genotypes, particularly local landraces is important for ongoing breeding programs, especially for drought tolerance. Susceptibility to drought is emphasized when bean is grown as a stubble crop and sown at the middle of summer. The aim of this study was to compare variability of ten bean genotypes to optimal (25 °C) and higher (30 °C) temperatures in combination with optimal (80%) and reduced (40%) field water capacity (FWC), from the point of growth (root and shoot length and fresh matter accumulation) and thermodynamic parameters of free energy (calculated parameter after drying at 60 °C, 105 °C and 130 °C) during the early seedlings stage. Significant and positive correlation between root length and fresh matter with free energy at symplast and chemically bound water occurred under temperature stress (i.e. 30 °C). Root growth and elongation were affected by drought stress, i.e. under a combination of high temperature (30 °C) and water deficit (40% FWC). Based on higher energy consumption, the ability of shoot to continue a growth in stressful conditions could be possible to achieve, as was confirm through significant and positive correlation between evaluated growth parameters in shoot and free energy of free water.

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It could be concluded that most of the examined dry bean accessions expressed some sensitivity to stress applied. Among genotypes tested, local landrace zecak expressed lesser susceptibility to stresses applied. Accession Maksa was more tolerant to changes at cytoplasmic level, while Medijana and Sataja 425 expressed root i.e. shoot stress tolerance. Those accessions could be considered as potentially drought tolerant genotypes.

*Keywords:* field water capacity, free energy, genotype, temperature, water deficit, *Phaseolus vulgaris* L.

## INTRODUCTION

Common bean is an important crop, with high nutritional value due to high content of proteins, dietary fibers, vitamins and minerals. In undeveloped countries, it is an important source of proteins (BROUGHTON *et al.*, 2003), while in developed countries it is respectable as food source with low content of fats and polyunsaturated fatty acids. Bean is considered as national food in many countries worldwide, including Balkan countries, where owing to edaphic and climatic conditions, significant divergence of *Phaseolus vulgaris* L. species was evolved (VASIĆ, 2004). Characterisation of bean genotypes, particularly local landraces is important for ongoing breeding programs. Being the original biological material created by the process of natural selection and adapted to local growing conditions, landraces present sources of desirable traits for different breeding purposes (e.g. drought tolerance), thus contributing to yield increase (SAVIĆ *et al.*, 2014; MARAS *et al.*, 2015; JEČMENICA *et al.*, 2016).

Bean crop could be grown over a wide range of habitats, but its production is mainly limited by fluctuations in air temperatures and soil moisture availability present at the different time during a season. Susceptibility to drought is emphasized when it is grown as a stubble crop and sown at the middle of summer. Water deficit induces changes in plant metabolism, altering root to shoot ratio, while on the leaf level, dissipation of excitation energy through photorespiration is one of the important defence mechanisms. Some plant species accumulate reserves, reflected in increased stem biomass and/or root length (CHAVES *et al.*, 2002). Common bean genotypes have evolved different mechanisms to maintain plant water status. These strategies imply maximization of root water uptake, while shoot optimizes the usage of absorbed water for grain production (BEEBE *et al.*, 2013). SINGH (2007) reported many bean varieties tolerant to drought developed from combinations with Durango. On the other hand, susceptible genotypes react to drought by shrinkage of cell volume, resulting in aggregation and denaturation of proteins, delaying normal functioning of enzymes involved in photosynthesis. These, together with limited CO<sub>2</sub> influx induced by stomatal closure, increases photo-respiratory losses and production of reactive oxygen species (IMPA *et al.*, 2012), causing oxidative damage by lipid peroxidation, protein degradation and DNA fragmentation (ANJUM *et al.*, 2011).

Studies with nuclear magnetic resonance (NMR) spectroscopy revealed different types of water present in hydrated tissue. KRISHNAN *et al.* (2004) observed three-component water proton system (bound, bulk and free water) in germinating soybean. Bound water plays an important role in dehydration tolerance. RASCIO *et al.* (2005) found that increased tissue affinity for strongly bound water implied a simultaneous increase in the affinity for weakly bound water (bulk), what is associated with the solute potential plots of differential energies of water sorption. From this point, free energy presents the work necessary to make the sorption sites available, and so the higher the moisture content is, the number of available sites are lower (NKOLO MEZE'E *et*

*al.*, 2008; OLIVEIRA *et al.*, 2010). The status of free energy defines also trend of cellular metabolism, i.e. if energy is consuming, endergonic reactions are present (indicating biosynthesis significant for proliferation and growth), and if energy is releasing from exergonic reactions, respiration takes over biosynthetic processes. From this point, alterations of free energy could be useful indicator for stress presence, i.e., stress tolerance (SPASOJEVIĆ *et al.*, 2014; DRAGIČEVIĆ, 2015).

The aim of this study was to compare reaction, i.e. variability of ten bean genotypes to optimal (25°C) and higher (30°C) temperatures in combination with optimal (80%) and reduced (40%) field water capacity, from the point of growth (root and shoot length and fresh matter accumulation) and free energy as a thermodynamic parameter .

#### MATERIALS AND METHODS

A set of ten bean accessions from Institute of Field and Vegetable Crops Novi Sad (IFVCNS) dry bean working collection was the objective of the present study. Some of the data for the evaluated accessions were given in Table 1.

*Table 1. The origin, status and basic characteristics of chosen dry bean accessions*

Genotype	Name	Origin	Status	Grain color	Grain shape
G1	Balkan	IFVCNS <sup>1</sup>	cultivar	white	ellipsoid
G2	Zlatko	IFVCNS	cultivar	golden-yellow	cylindrical
G3	Medijana	IVCSP <sup>2</sup>	cultivar	white	ellipsoid
G4	Sataja 425	U S A	cultivar	black	ellipsoid
G5	Dvadesetica	IFVCNS	cultivar	white	kidney
G6	Sremac	IFVCNS	cultivar	greenish-yellow	cylindrical
G7	Slavonski zutozeleni	CRO	landrace	greenish-yellow	cylindrical
G8	Maksa	IFVCNS	cultivar	white	cylindrical
G9	Belko	IFVCNS	cultivar	white	ellipsoid
G10	zacak	SRB	landrace	greenish-yellow	cylindrical

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For the laboratory experiment, slightly calcareous chernozem soil from the local field (without application of any fertilizer or other agrochemicals) was used. For seedlings growth, field water capacity (FWC) was determined thermo-gravimetrically: air dried soil sample was weighed, then water was added up to the saturation, weighted again and dried at 105 °C. After final weighting, FWC was calculated. Soil used (1.5 kg) was added into the plastic boxes with dimensions: 17.5 x 23.5 x 7 cm. For each genotype, ten seeds per box were sown in two rows, with intra-row spacing of 5 cm. Experiment was performed in five replications for each treatment. Plants were grown in germination room under a 12-h of photoperiod, with irradiance of 40 Wm<sup>-1</sup> and relative humidity of 70%. For the first seven days in all treatments, seedlings

were grown at 25 °C and 80% FWC (optimal conditions). For the last seven days of growing period, treatments applied included the following combinations of temperature and FWC: 25 °C + 80% FWC (T1 treatment - control, i.e. optimal growing condition); 25 °C + 40% FWC (T2 treatment - water deficit stress); 30 °C + 80% FWC (T3 treatment - temperature stress); 30 °C + 40% FWC (T4 treatment - drought stress). After that, fourteen-day old seedlings were taken out of the soil and rinsed quickly from the soil particles. Each plant was uprooted carefully and length and fresh matter of both root and shoot were measured. Dry matter was determined after drying the samples at 60°C, 105°C and 130°C (each phase continued for 24 h), in order to calculate the contents of free water (subtraction between fresh and dry weight after drying at 60°C), bulk water (subtraction between weight determined at 60°C and after drying at 105°C) and chemically bound water (subtraction between weight determined at 105°C and after drying at 130°C). The values obtained were used for calculation of thermodynamic parameters by using the sorption isotherm, as suggested by SUN (2002):

$$\Delta G = RT \ln(a_w)$$

where,  $a_w$  is the relative water content achieved after drying at T (60, 105 and 130°C); R is the gas constant (8.3145 J mol<sup>-1</sup> K<sup>-1</sup>);  $\Delta G$  is differential free energy, which represents the amount of work that the system can perform. Its decrease signifies a domination of exergonic (spontaneous) processes which release free energy, and its increase signifies endergonic (non-spontaneous) processes which consume energy.

All the analyses were performed in five measurements (n = 5) and the obtained data of root to shoot ratio for length and fresh matter were statistically analyzed and presented as mean values. Significant differences between genotypes means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) using three-factorial completely randomized block design. Differences between means values with P values  $\leq 0.05$  were considered significant. Free energy of all three water types was presented as mean  $\pm$  standard deviation (SD). Correlation analysis between seedlings growth parameters (i.e. root and shoot length and fresh matter) and thermodynamic parameter of free energy was performed using Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

According to the results present in Table 2, both factors (genotype and temperature), as well as interactions between all the factors (genotype, temperature and FWC), had statistically significant influence on the root to shoot ratio for length in examined bean genotypes. Moreover, single effect of each factor observed (G, T and FWC,  $P \leq 0.001$ , respectively), as well as the effects of each interaction (G x T, G x FWC, G x T x FWC,  $P \leq 0.001$ ; T x FWC,  $P \leq 0.01$ , respectively) on root to shoot ratio for fresh matter were highly significant. This means that the seedlings length, as a parameter of growth, is less sensitive to temperature increase and FWC decrease when compared to fresh matter, having a tendency of elongation in stressful conditions (RACHMILEVITCH *et al.*, 2005). Significantly higher average values of root to shoot ratio for both traits were obtained by Balkan (G1), Zlatko (G2), Medijana (G3) and Belko (G9), respectively. Among the evaluated common bean accessions, the highest average root to shoot ratio for length was recorded in Zlatko (accession G2) at 25 °C and in Medijana (accession G3) at 30 °C, particularly pronounced at 40% FWC. That could refer to root elongation as an adaptive response to water deficit, contributing to improved water stress tolerance (CHAVES *et al.*, 2002;

DRAGIČEVIĆ, 2015). The lowest root to shoot value under both temperatures was observed in Dvadesetica (accession G5), indicating promoted shoot growth.

Table 2. Root to shoot ratio for seedlings length and fresh matter in ten bean genotypes under different treatments applied

Genot.	25 °C			30 °C			TA
	FWC		APG	FWC		APG	
	80%	40%		80%	40%		
	Length						
G1	0.503	0.691	0.597	0.513	0.698	0.605	0.601
G2	0.662	0.779	0.720	0.590	0.659	0.625	0.672
G3	0.643	0.742	0.692	0.781	0.709	0.745	0.719
G4	0.437	0.481	0.459	0.530	0.536	0.533	0.496
G5	0.379	0.352	0.365	0.343	0.359	0.351	0.358
G6	0.402	0.418	0.410	0.840	0.515	0.678	0.544
G8	0.445	0.403	0.424	0.408	0.468	0.438	0.431
G9	0.583	0.720	0.651	0.628	0.619	0.623	0.637
G10	0.598	0.580	0.589	0.708	0.570	0.639	0.614
APT	0.523	0.589	0.556	0.600	0.578	0.589	
<i>LSD</i> <sub>0.05</sub>	Genotype	Temp.	FWC	G x T	G x FWC	T x FWC	G x T x FWC
	0.0653***	0.1640**	0.1640 <sup>ns</sup>	0.5187***	0.0924***	0.2320***	0.1306*
	Fresh matter						
G1	0.315	0.411	0.363	0.391	0.491	0.441	0.402
G2	0.318	0.324	0.321	0.467	0.504	0.486	0.403
G3	0.360	0.472	0.416	0.488	0.371	0.429	0.423
G4	0.448	0.346	0.397	0.402	0.356	0.379	0.388
G5	0.284	0.386	0.335	0.345	0.334	0.339	0.337
G6	0.288	0.332	0.310	0.456	0.400	0.428	0.369
G7	0.374	0.330	0.352	0.420	0.252	0.336	0.344
G8	0.282	0.270	0.276	0.368	0.397	0.383	0.329
G9	0.490	0.500	0.495	0.418	0.476	0.447	0.471
G10	0.378	0.414	0.396	0.326	0.333	0.329	0.363
APT	0.354	0.378	0.366	0.408	0.391	0.400	
<i>LSD</i> <sub>0.05</sub>	Genotype	Temp.	FWC	G x T	G x FWC	T x FWC	G x T x FWC
	0.0292***	0.0733***	0.0734***	0.2320***	0.0413***	0.1037**	0.0584***

FWC - field water capacity; APG - Average per genotype; APT - Average per treatment; TA - total average;

\*, \*\*, \*\*\* - significant values at the 0.05, 0.01 and 0.001 level, respectively

Considering the interactions between all three factors regarding root to shoot ratio for lengths, the highest values were observed in Zlatko (accession G2) at 25°C and 40% FWC (treatment T2 - water deficit stress) and in Sremac (accession G6) at 30°C and 80% FWC (treatment T3 - temperature stress), respectively. In most of the genotypes evaluated, average values of root to shoot ratio for fresh matter were higher at 30°C, being the highest in Zlatko, indicating the existence of tolerance to higher temperature in this accession, as was similar to the results obtained on wheat (BENLLOCH-GONZALEZ *et al.*, 2014). The interaction G x T x FWC underlined Belko (G9), as the genotype with the highest value (0.500) at 25°C and 40% FWC, as well as Zlatko, as the genotype with the highest value (0.504), at 30°C and 40% FWC. This could indicate that observed genotypes expressed higher root growth rate under diminished water availability (CHAVES *et al.*, 2002), although at the different temperatures.

Free energy of free water ( $\Delta G$  60°C) expressed lower average values for root and shoot (0.23 J mol<sup>-1</sup> and 0.24 J mol<sup>-1</sup>, respectively) at 80% FWC at both temperatures (Figure 1). This is linked with fitted hydration level, since free energy presents the work necessary to make the sorption sites available, and so the higher the moisture content is, the number of available sites are lower (NKOLO MEZE'E *et al.*, 2008; OLIVEIRA *et al.*, 2010). The highest variability between genotypes was observed under T2 treatment (i.e. 25°C + 40% FWC), related to water deficit, i.e. the ability for maintaining leaf water status (OMAE *et al.*, 2012). In the noted treatment, Zlatko (accession G2) obtained the highest values of  $\Delta G$  60°C in both root and shoot, while Sataja (G4) in root. Also, the highest values of this trait were observed at the root level in genotypes G2 - Zlatko (25°C + 80% FWC - T1 treatment), G4 - Sataja (30°C + 80% FWC - T3 treatment) and G1 - Balkan (30°C + 40% FWC - T4 treatment), respectively. At the shoot level, the highest values were noticed in genotypes Medijana (G3) and zecak (G10) (25°C + 80% FWC), as well as in G3 - Medijana under both T3 and T4 treatments (30°C + 40% FWC, 30°C + 80% FWC), indicating higher energy consumption, i.e. increase in endergonic processes.

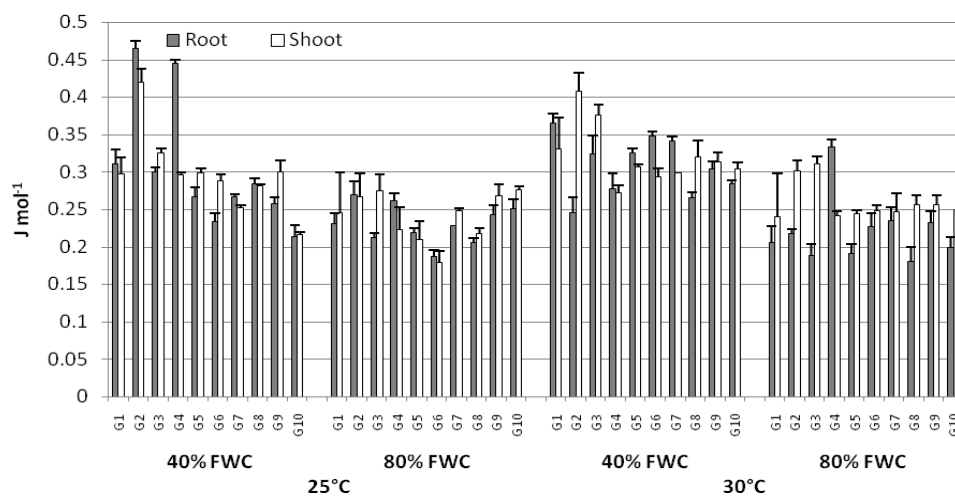


Figure 1. Free energy of free water ( $\Delta G$  60 °C) in roots and shoots for bean seedlings under different treatments applied

The higher fluctuations between applied treatments of values for  $\Delta G$  105°C and  $\Delta G$  130°C (Figure 2 and 3), in relation to  $\Delta G$  60°C (Figure 1) were present, indicating increased variability in economising with cytoplasmic and chemically bound water. At the root level, lower average values of  $\Delta G$  105°C and  $\Delta G$  130°C (11.33 J mol<sup>-1</sup> and 11.92 J mol<sup>-1</sup>, respectively) were obtained under T3 treatment (30°C + 80 FWC), as well as under T4 treatment (30°C + 40 FWC, 9.93 J mol<sup>-1</sup>) at the shoot level. Average value of 10.44 J mol<sup>-1</sup> for free energy of chemically bound water ( $\Delta G$  130°C) was obtained under T2 treatment (25°C + 40% FWC), indicating lower energy consumption, that could be, at the shoot level, linked with increased photorespiration, as a mechanism of energy dissipation (FLEXAS and MEDRANO, 2002). Among the genotypes tested, the highest  $\Delta G$  105°C (Figure 2) was achieved in roots of G1 under T1 (25°C + 80% FWC) and T3 treatments (30°C + 80% FWC) and G5 under T2 (25°C + 40% FWC) and T4 (30°C + 40% FWC) treatments, indicating higher energy consumption at symplast level. In shoots, the highest values of  $\Delta G$  105°C exhibited genotypes G6 and G7 (25°C + 40% FWC), G4 (30°C + 80% FWC) and G9 (30°C + 40% FWC), pointed to higher energy consumption for maintenance of cellular - symplast water level (OMAE *et al.*, 2012; DRAGIČEVIĆ, 2015).

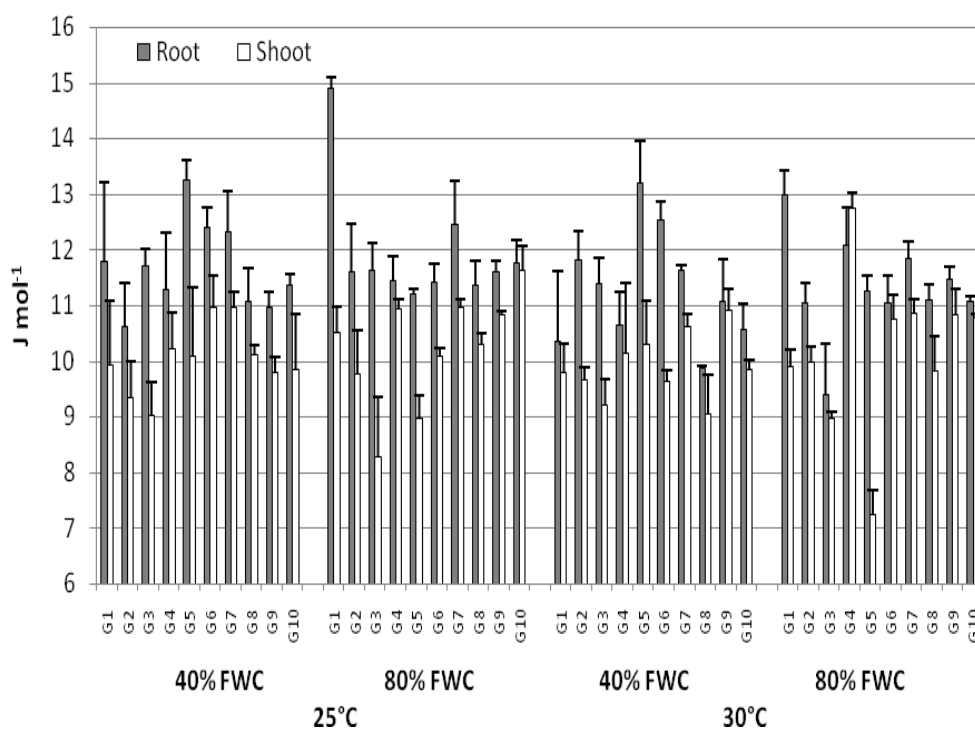


Figure 2. Free energy of bulk water ( $\Delta G$  105 °C) in roots and shoots for bean seedlings under different treatments applied

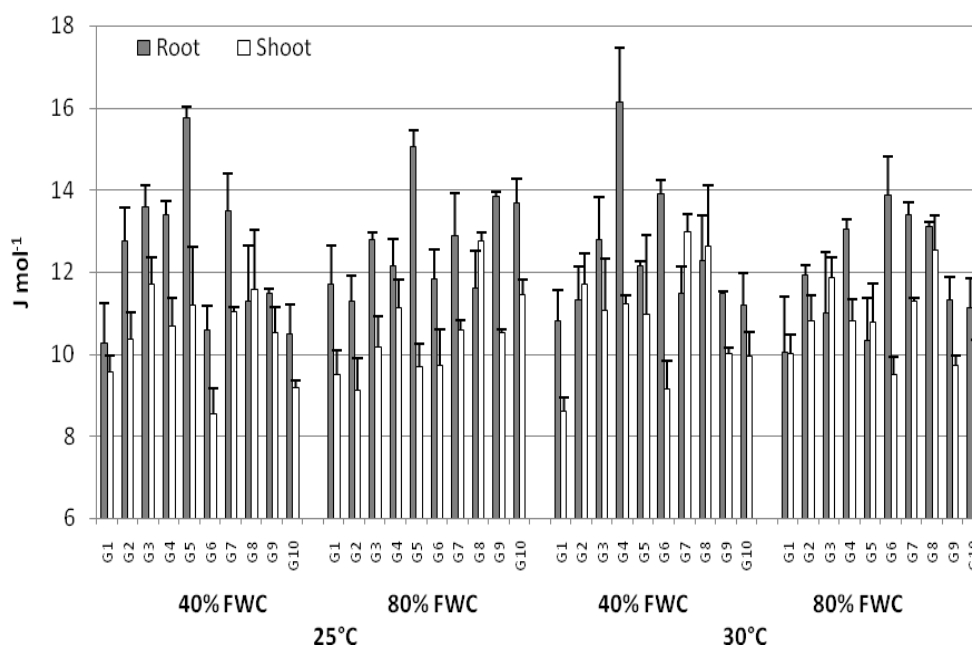


Figure 3. Free energy of bound water ( $\Delta G$  130 °C) in roots and shoots for bean seedlings under different treatments applied

The highest variations among genotypes tested, as well as between root and shoot, was found for  $\Delta G$  130°C, with average value of 2.22 J mol<sup>-1</sup> obtained under 25°C + 80% FWC (T1 treatment). Accordingly, some of the genotypes are able to avoid conformational changes of proteins and other cellular structures caused by temperature and water stress and their combination, indicating the existence of drought tolerance in them (ANJUM *et al.*, 2011; IMPA *et al.*, 2012). When genotypes were compared, the highest  $\Delta G$  130°C (Figure 3) was in roots of G5 - Dvadesetica (under T1 and T2 treatment), G4 - Sataja (under T4 treatment) and the accession G6 - Sremac (under T3 treatment). In shoots, the highest values of  $\Delta G$  130°C were found in G8 - Maksa (under all treatments), as well as in G7 - Slavonski zutozeleni (under T4 treatment). This could indicate their susceptibility to stressful conditions, reflected in increased energy consumption for maintaining the stability of cellular structures (ANJUM *et al.*, 2011; IMPA *et al.*, 2012).

It must be underlined that under the T4 treatment (drought stress), the lowest values of  $\Delta G$  60°C for seedlings roots and shoots were found in G2 - Zlatko and G4 - Sataja, respectively; being the lowest in G8 - Maksa (for  $\Delta G$  105°C) and in G1 - Balkan seedlings (for  $\Delta G$  130°C), respectively, pointed to less available work, i.e. reduced sorption sites (NKOLO MEZE' E *et al.*, 2008; OLIVEIRA *et al.*, 2010) for apoplast water (G2 - Zlatko and G4 - Sataja), symplast water (G8 - Maksa) and for chemically bound water (G1 - Balkan). Based on the results, it could be concluded that G1 - Balkan has a good potential to cope with conformational changes caused by



stress (ANJUM *et al.*, 2011; IMPA *et al.*, 2012), G8 - Maksa could retain cellular water level essential for normal metabolism functioning (OMAE *et al.*, 2012), while G2 - Zlatko and G4 - Sataja could be able to uphold free water to a certain extent under unfavourable conditions.

In 2015, the variability of the chosen genotypes on existing agro-ecological conditions was additionally evaluated in field trials, conducted at three locations (Novi Sad 45°20'N, 19°51'E, 80 m asl; Smederevska Palanka 44°22'N, 20°57'E, 121 m asl. and Omoljica near Pančevo 44°53'N, 20°40'E, 71 m asl., respectively). Yield components (number of pods per plant, number of grain per pod, number of grain per plant, 1000 grain weight) and grain yield per plant were measured. Very small amount of rainfall in July (when stubble crop was grown) characterised the locations: Novi Sad (2.0 mm), Smederevska Palanka (1.7 mm) and Omoljica (4.4 mm), respectively. At all three locations, genotype Balkan - G1 achieved stable, although a lower average yield (126.2 kg ha<sup>-1</sup>), while, genotype zecak - G10 achieved both stable and higher average yield (215.3 kg ha<sup>-1</sup>). These genotypes could be considered as favourable source in breeding for drought tolerance.

Under T1 treatment (25 °C + 80% FWC - optimal conditions), significant and negative correlation between  $\Delta G$  105 °C and shoot length was found (-0.847,  $P \leq 0.01$ ), being significant and positive with root fresh matter (0.791,  $P \leq 0.01$ ), as presented in Table 3. On the other hand, under T2 treatment (25 °C + 40% FWC - water deficit), significant and positive correlations between  $\Delta G$  105 °C and shoot length and fresh matter (i.e. 0.861 and 0.844,  $P \leq 0.01$ ) were observed, being significant and negative between  $\Delta G$  60 °C and root fresh matter (-0.735,  $P \leq 0.05$ ). It could be suggested that increased shoot growth (elongation and accumulation of fresh matter) was achieved by higher energy consumption on symplast level, driven by the lack of extracellular water. However, at the root level, fresh matter accumulation was based mainly on energy production and higher extracellular water amounts - reduced sorption sites (NKOLO MEZE'É *et al.*, 2008; OLIVEIRA *et al.*, 2010), as was supported by higher values of root to shoot ratio for length and fresh matter (Table 2). This could indicate situation of stimulated root growth over the shoot growth (CHAVES *et al.*, 2002; BENLLOCH-GONZALEZ *et al.*, 2014).

Table 3. Correlations between growth parameters and free energy of different water types measured in seedlings roots and shoots

Treatment	25 °C - 80% FWC		25 °C - 40% FWC		30 °C - 80% FWC		30 °C - 40% FWC	
	L	FM	L	FM	L	FM	L	FM
Root								
$\Delta G$ 60 °C	0.498	0.548	0.291	-0.735*	-0.186	-0.713*	0.774**	-0.382
$\Delta G$ 105 °C	0.502	0.791**	0.403	0.605	0.690*	0.704*	-0.456	-0.674*
$\Delta G$ 130 °C	-0.275	0.417	0.314	0.206	0.517	-0.651*	0.766**	-0.375
Shoot								
$\Delta G$ 60 °C	-0.596	-0.304	-0.300	-0.312	-0.409	-0.505	0.781**	0.783**
$\Delta G$ 105 °C	-0.847**	-0.209	0.861**	0.844**	-0.575	-0.427	0.328	0.421
$\Delta G$ 130 °C	0.411	0.155	0.259	-0.109	0.313	0.073	0.369	0.325

L - Length; FM - Fresh Matter; ( $\Delta G$  60°C) - free water; ( $\Delta G$  105°C) - bulk water; ( $\Delta G$  130°C) - bound water; \*, \*\* - the significant values at the 0.05 and 0.01 level, respectively;

Under temperature stress (25°C + 80% FWC -T3 treatment), significant and positive correlation between root length and fresh matter with  $\Delta G$  105°C (0.690 and 0.704,  $P \leq 0.05$ , respectively) was found (Table 3). In addition, significant and negative correlations between root fresh matter with  $\Delta G$  60°C (-0.713,  $P \leq 0.05$ ) and  $\Delta G$  130°C (-0.651,  $P \leq 0.05$ ) were observed, signifying the importance of symplast water and the higher energy production at the apoplast level and chemically bound water. Such a situation could indicate alterations in the metabolism at the cellular level, with conformational changes of biomolecules, induced by higher temperature (IMPA *et al.*, 2012). As was shown in Table 2, root growth and elongation were affected by drought stress, i.e. a combination of high temperature (30°C) and water deficit (40% FWC). Under T4 treatment, significant and positive correlation between root length and both  $\Delta G$  60°C (0.774,  $P \leq 0.01$ ) and  $\Delta G$  130°C (0.766,  $P \leq 0.01$ ) were found, (Table 3), caused by a severe lacking in extracellular water and by domination of endergonic reactions. In parallel, significant and negative correlation between evaluated root growth parameters and  $\Delta G$  105°C (-0.744 for root length, i.e. -0.674 for root fresh matter, respectively;  $P \leq 0.05$ ) indicate a trend of decrease in sorption sites available for intracellular water (NKOLO MEZE' E *et al.*, 2008; OLIVEIRA *et al.*, 2010), i.e. drift in energy production, as a driving force for root elongation. Since growth is generally an energy requiring process, genotypes with less energy consumption for growth at early developmental stage, particularly for root growth, could yield better under drought stress (KRAVIĆ *et al.*, 2013; NIKOLIĆ *et al.*, 2013). However, based on significant and positive correlation found between evaluated growth parameters in shoot and  $\Delta G$  60°C (0.781 for shoot length, i.e. 0.783 for shoot fresh matter, respectively;  $P \leq 0.001$ ), it could be supposed that ability of shoot to continue a growth in such unfavourable conditions, could be achieved only through higher energy consumption.

#### CONCLUSIONS

Based on the results obtained, it could be concluded that most of the examined dry bean accessions expressed some sensitivity to stress applied. Genotypes G2 - Zlatko and G6 - Sremac exhibited sensitivity to water deficit and Sremac to higher temperature in both seedlings roots and shoots, with increased energy consumption and water sorption sites for all three water types. Accession G1 - Balkan expressed increased root susceptibility to temperature, while G4 - Sataja and G5 - Dvadesetica had higher root susceptibility to water deficit, possibly reflected through conformational changes of biomolecules and alteration of cellular metabolism. Genotypes G7 - Slavonski zutozeleni and G8 - Maksa expressed shoot susceptibility to combined stress (drought), mainly by conformational changes, while G9 - Belko expressed alterations on symplast level in shoots. Among genotypes tested, G10 - zecak expressed lesser susceptibility to stresses applied. Accession Maksa was more tolerant to changes on cytoplasmic level, while Medijana and Sataja expressed root i.e. shoot stress tolerance. Those accessions could be considered as potentially drought tolerant genotypes.

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## REFERENCES

- ANJUM, S.A., X.Y.XIE, L.C.WANG, M.F. SALEEM, C. MAN, W. LEI (2011): Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.*, 6: 2026-2032.
- BEEBE, S.E., I.M. RAO, M.W. BLAIR, J.A. ACOSTA-GALLEGOS (2013): Phenotyping common beans for adaptation to drought. *Front. Physiol.*, <http://dx.doi.org/10.3389/fphys.2013.00035>
- BENLLOCH-GONZALEZ, M., R. BOCHICCHIO, J. BERGER, H. BRAMLEY, J.A. PALTA (2014): High temperature reduces the positive effect of elevated CO<sub>2</sub> on wheat root system growth. *Field Crops Res.*, 165: 71-79.
- BROUGHTON, W.J., G. HERNANDEZ, M. BLAIR, S. BEEBE, P. GEPTS, J. VANDERLEYDEN (2003): Beans (*Phaseolus* spp.) - model food legumes. *Plant Soil*, 252: 55-128.
- CHAVES, M.M., J.S. PEREIRA, J. MAROCO, M.L. RODRIGUES, C.P.P. RICARDO, M.L. OSÓRIO, I. CARVALHO, T. FARIA AND C. PINHEIRO (2002): How plants cope with water stress in the field? Photosynthesis and growth. *Ann. Bot.* 89: 907-916.
- DRAGIČEVIĆ, V. (2015): Thermodynamics of abiotic stress and stress tolerance of cultivated plants. In: Recent Advances in Thermo and Fluid Dynamics. Gorji-Bandpy M. (eds.), INTECH, Rijeka, Croatia, pp. 195-221.
- FLEXAS, J. and H. MEDRANO (2002): Energy dissipation in C<sub>3</sub> plants under drought. *Functional Plant Biol.* 29: 1209-1215.
- IMPA, S.M., S. NADARADJAN, S.V.K. JAGADISH (2012): Drought Stress Induced Reactive Oxygen Species and Anti-oxidants in Plants. In: Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability, Ahmad P., Prasad M.N.V. (Ed.), Springer Science+Business Media, pp. 131-147.
- JEČMENICA, M., Z. GIREK, S. OLJAČA, T. ŽIVANOVIĆ, A. SAVIĆ, N. KRAVIĆ, M. VASIĆ (2016): Components of bean plant in different growing conditions. In: Ivetić V., Mladenović-Drinić S. (eds.) Book of abstracts: V Symposium of the Section of the breeding of organisms of the Serbian Genetic Society, Kladovo, Serbia, 27-31 May 2016, pp. 113.
- KRAVIĆ, N., K. MARKOVIĆ, V. ANĐELKOVIĆ, V. HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V. BABIĆ, M. VULETIĆ (2013): Growth, proline accumulation and peroxidase activity in maize seedlings under osmotic stress. *Acta Physiol. Plant.*, 35: 233-239.
- KRISHNAN, P., D.K., JOSHI, S. NAGARAJAN, A.V. MOHARIR (2004): Characterization of germinating and non-viable soybean seeds by nuclear magnetic resonance (NMR) spectroscopy. *Seed Sci. Res.*, 14: 355-362.
- MARAS, M., B. PIPAN, J.ŠUŠTAR-VOZLIČ, V.TODOROVIĆ, G.ĐURIĆ, M.VASIĆ, S.KRATOVALIEVA, A.IBUSOSKA, R.AGIĆ, Z. MATOTAN, T. ČUPIĆ, V.MEGLIĆ (2015): Examination of Genetic Diversity of Common Bean from the Western Balkans. *J. Amer. Soc. Hort. Sci.*, 140: 308-316.
- NIKOLIĆ A., V. ANDJELKOVIĆ, D. DODIG, S. MLADENOVIĆ DRINIĆ, N. KRAVIĆ, D. IGNJATOVIĆ-MIČIĆ (2013): Identification of QTL-s for drought tolerance in maize, II: yield and yield components. *Genetika*, 45: 341-350.
- NKOLO MEZE'E, Y.N., J.N. NOAH GAMVENG, S. BARDET (2008): Effect of enthalpy-entropy compensation during sorption of water vapour in tropical woods: the case of Bubinga (*Guibourtia Tessmanii* J. Léonard; *G. Pellegriniana* J. L.). *Thermochim. Acta*, 468: 1-5.
- OLIVEIRA, D.E.C., O. RESENDE, T.A. SOUZA SMANIOTTO, K.A. SOUSA, R.C. CAMPOS (2013): Thermodynamic properties of maize grains for different equilibrium moisture contents. *Pesquisa Agropecuária Tropical*, 43: 50-56.
- OMAE, H., A. KUMAR, M. SHONO (2012): Adaptation to high temperature and water deficit in the common bean (*Phaseolus vulgaris* L.) during the reproductive period. *J. Bot.* Article ID 803413, 6 pages, doi:10.1155/2012/803413
- RACHMILEVITCH, S., H. LAMBERS, B. HUANG (2006): Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type *Agrostis* species. *J. Exp. Bot.*, 57: 623-631.
- RASCIO, A., G. NICASTRO, E. CARLINO, N. DI FONZO (2005): Differences for bound water content as estimated by pressure-volume and adsorption isotherm curves. *Plant Sci.*, 169: 395-401.

- SAVIĆ, A., G. PETROVIĆ, M. MILOŠEVIĆ, Z. NIKOLIĆ, A. STOJANOVIĆ, J. GVOZDANOVIĆ-VARGA, V. TODOROVIĆ, M. VASIĆ (2014): Morpho-chemical characterization of dry and snap bean (*Phaseolus vulgaris*L.) landraces collected on Fruška Gora Mt. *Genetika*, 46: 303-313.
- SINGH, S.P. (2007): Drought resistance in the race Durango dry bean landraces and cultivars. *Agron. J.* 99: 1219-1225.
- SPASOJEVIĆ, I., V. DRAGIČEVIĆ, M. SIMIĆ, D. KOVAČEVIĆ, M. BRANKOV (2014): Effects of different cropping systems and weed management on free energy and content of pigments in maize. *Pestic. Phytomed*, 29: 45-54.
- SUN, W.Q. (2002): Methods for the Study of Water Relations under Desiccation Stress, In: *Desiccation and Survival in Plants: Drying Without Dying*, Black M. & Pritchard H. W. (Ed.), CABI Publishing, New York, USA, pp. 47-91.
- VASIĆ, M. (2004): Genetic divergence in a bean collection. Andrejević Endowment, Belgrade, Serbia. ISBN 86-7244-411-6
- VASIĆ, M., J. GVOZDANOVIĆ-VARGA, M. ZORIĆ, M. KRALJEVIĆ BALALIĆ, J. ČERVENSKI (2010): Analysis of grain size in bean (*Phaseolus vulgaris* L.) by linear and bilinear models. *Genetika*, 42: 535-544.

**VARIJABILNOST SLOBODNE ENERGIJE U FUNKCIJI TOLERANTNOSTI NA SUŠU  
KOD UZORAKA PASULJA**

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**Izvod**

Karakterizacija genotipova pasulja, na prvom mestu domaćih populacija, je neophodna za savremene programe oplemenjivanja, posebno programe u vezi sa tolerantnošću na sušu. Posedovanje svojstva tolerantnosti na sušu je veoma važno za genotipove pasulja koji se seju sredinom leta i gaje kao postrni usev. Cilj ovog rada je bio da se uporedi varijabilnost deset genotipova pasulja pri optimalnim (25 °C) i višim (30 °C) temperaturama u kombinaciji sa optimalnim (80%) i smanjenim (40%) poljskim vodnim kapacitetom (PVK), sa stanovišta rasta (dužine i akumulacije sveže materije korena i izdanka) i termodinamičkih parametara slobodne energije (parametara izračunatih nakon sušenja na 60 °C, 105 °C i 130 °C) u ranoj fazi klijanca. U uslovima temperaturnog stresa (tj. 30 °C), utvrđena je statistički značajna i pozitivna korelacija između parametara rasta (dužine i sveže materije) korena i slobodne energije na nivou simplasta i hemijski vezane vode. Stres suše, odnosno kombinacija visoke temperature (30 °C) i vodnog deficita (40% PVK), imale su negativan uticaj na rast i izduživanje korena. Utvrđena veća potrošnja energije ukazuje na sposobnost izdanka da nastavi sa rastom u uslovima stresa, što je potvrđeno postojanjem statistički značajne i pozitivne korelacije između praćenih parametara rasta izdanka i slobodne energije slobodne vode. Može se zaključiti da je većina ispitivanih genotipova pasulja ispoljila netolerantnost na primenjeni stres. U odnosu na ostale netolerantne genotipove, domaća populacija zečak ispoljila je najmanji stepen netolerantnosti. Domaća sorta Maksa ispoljila je tolerantnost prema promenama na nivou citoplazme, dok su sorte Medijana i Sataja 425 ispoljile tolerantnost na nivou korena, odnosno izdanka. Ovi genotipovi bi se mogli smatrati potencijalnim izvorima tolerantnosti na sušu.

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