

## MORPHOLOGICAL CHARACTERIZATION OF SWEET SORGHUM GENOTYPES ACROSS ENVIRONMENTS

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### ABSTRACT

Sweet sorghum being a C4 crop accumulates more sugar in its stalks, also suitable for biofuel production and has high degree of tolerance to biotic and abiotic stresses. Morphological characteristics i.e., plant height, plant biomass, leaves on the stem, panicle length and yield of crude biomass of sweet sorghum genotypes were studied across different environments. Environments and genotype by environment interaction (GEI) had a significant effect on the yield of crude biomass. The smallest yield of crude biomass was achieved in 2016 (42.54 t ha<sup>-1</sup>), which was found significantly lower as compared to that in 2014 and 2015. The average plant height had a significant and positive correlation with the number of leaves (0.54) and number of leaves had highly a significant and positive correlation with the mass of stem (0.46) and panicle length (0.61). Biomass yield was positively and significantly correlated with precipitation (0.72) and negatively significantly correlated with temperature (-0.57). In breeding of sweet sorghum, the highest attention should be given to biomass yield as it manage the whole variation and controlled by polygenes.

**Key words:** Sweet sorghum, yield componenets, genotype by environment interaction, correlation.

### INTRODUCTION

Sorghum is the world's fifth most important cereal crop after rice, wheat, maize and barley. Sweet sorghum (*Sorghum bicolor* L. Moench) is cultivated for its grain (used as feed and food by animals and humans, respectively), and for production of ethanol and biofuels. Several factors are decisive in increasing yields: the cultivar, cultural practices, local climatic and soil characteristics, mineral nutrition and adequate plant protection against diseases, pests and weeds (Đekić *et al.*, 2014; Glamočlija *et al.*, 2015; Sikora *et al.*, 2015; Kendal and Sayar, 2016; Khan and Mohammad, 2016; Dražić *et al.*, 2016). The desirable cultivars for high grain yield and quality traits needs to express genetic potential under different environments (Đekić *et al.*, 2014; Kendal and Sayar, 2016; Popović *et al.*, 2016a, b). Sweet sorghum thrives better under drier and warmer conditions than other crop plants and is grown primarily for forage, silage, syrup and biofuels production (Dillon *et al.*, 2007; Regassa and Charles, 2014).

*Sorghum bicolor* (L.) Moench is grown throughout the arid and semi-arid tropics and areas with low rainfall. *Sorghum* originated in northern Africa, and is now cultivated widely in tropical and subtropical regions. Sweet sorghum is well-adapted to marginal growing conditions such as water deficits, water logging, salinity, alkalinity, and other constraints. The drought stress occurrence is a major constraint to sorghum

production globally. Post-anthesis drought stress can result in significant yield loss due to small grain size, premature plant death and susceptibility to diseases (Dillon *et al.*, 2007).

In addition to the economic benefits, it is estimated that the production of energy by cultivation of appropriate bio-energy crops will significantly contribute to the multi-functionality of agriculture (Dželetović and Glamočlija 2015), and the main motive for the future use of these crops in Serbia is the possibility of achieving a high level of energy autonomy of agricultural holdings, Dželetović *et al.* (2009). The produced biomass of plants are used for energy purposes in several ways: as solid fuel, whole or in the form of briquettes. Since biomass is rich in non-dangerous extractive substances (BEM), it serves to produce bio-ethanol. Compared to other one-year-old types, the sugar syrup gives per unit area the highest yield of biomass suitable for processing into sugar (Mask and Morris 1991), as well as into solid and liquid fuels. This species can be the most important energy crop for a large geographical area, from tropical to temperate continental climate, especially as it does not occupy a significant place in world's food production of plant origin.

Continuous monitoring of the farm microclimate includes first of all measuring of precipitation, soil properties and the supply of soil nutrients. Depending on the current precipitation situation, the crop fertilization system may be adjusted in order to achieve the expected optimal grain yield. If natural precipitation surpasses the

water required for maximum yield, nutrient supply must be reduced, because in this case surplus of fertilizers could lead to economic losses and ecological damage due to nutrients flushing. GEI - genotype by environment interaction effect, may be defined as the differential genotypic expression across environments, and reduces the association between phenotypic and genotypic values. Level of GEI is a major element in determining many key aspects of a breeding programme including, whether to aim for wide or specific adaptation, and will affect the choice of locations for selection (Chipeta *et al.*, 2017).

In breeding of sweet sorghum. Sweet sorghum was first introduced into the United States in 1852. Isaac Hedges called it the "Northern Sugar Plant" because of its high sugar content in the stalks. Sweet sorghum is the same species (*Sorghum bicolor* L. Moench) as grain sorghum. Although several sweet sorghum breeding programs have been initiated in United States, most of the varieties in cultivation were developed at the U.S. Sugar Crops Field Station at Meridian, Mississippi. This breeding program produced four important varieties namely: Theis, Keller, Dale and M81E. Although sweet sorghum is primarily grown to produce sorghum syrup, it can also be used as a feedstock and for bio-fuels. In favorable environment, sweet sorghum varieties can grow 14 feet tall and produce 20 to 50 t of biomass (fresh weight) per acre. It is more drought tolerant than corn and requires less nitrogen fertilizer (Gene 2014). The highest attention should be given to biomass yield which is controlled by large number of genes and recorded with greater variation among the genotypes and genotype-environment interaction (Ikanović *et al.*, 2010, 2013; Dražić *et al.*, 2016; Popović and Dražić 2016; Popović *et al.*, 2015). Correlation coefficient analysis may be utilized as a vital tool to gather the information about right reason and effective association between yield and related components (Khan *et al.*, 2003; Ahmad *et al.*, 2016; Popovic *et al.*, 2013).

The description of important agronomic characteristics is a useful prerequisite for breeding programs. Sorghum, as a drought tolerant plant, is interesting for growing in semi arid climate of Serbia region. Sorghum, thanks to its large biomass production, can be used as an alternative "agricultural" plant species, but also as a valuable renewable resource in the energy sector. From the aspect of environmental protection, it is great advantage of sorghum cultivation (Ikanovic *et al.*, 2010; 2013; Dražić *et al.*, 2016; Popović *et al.*, 2016a, b). The yield is the most important and polygenic trait in sweet sorghum breeding which largely depends on genetic resources and agro-ecological factors (Glamočlija *et al.*, 2015). Therefore, the present study was carried out to investigate the productivity of sweet sorghum genotypes under different environmental conditions.

## MATERIALS AND METHODS

**Experimental design:** This study was conducted over a three years of period (2014, 2015 and 2016) in Stara Pazova, in the region of Srem, in Republic of Serbia (44° 59' 04" N, 20° 09' 23" E, 76 m a.s.l.), at location 76 m a.s.l. (Figure 1), on a chernozem soil type. The trials were set up as randomized complete block (RCB) design with two sweet sorghum varieties and five replications. That area has a moderate continental climate, characterized by warm and dry summers and moderate cold winters.



**Figure 1. Region of Srem in Serbia.**

The size of the basic plots was 10 m<sup>2</sup> (5 x 2 m<sup>2</sup>). Sowing was done in the third decade of April in all three years of examination. Standard technological practices for sorghum cultivation were applied. Taking plants was carried out in the beginning of tasseling phase, in mid-July. For the analysis of morphological characteristics: PH - plant height, MS - mass of the stem, NL - number of leaves on the stem and the PL - panicle length, samples were collected from crude biomass. The yield of crude biomass - YCB was determined also.

**Plant materials:** The trials were set up with two sweet sorghum varieties: *Dale* and *NS Šećerac*. Genotype *Dale* is sweet sorghum variety that was developed at the U.S., in Sugar Crops Field Station, Meridian, Mississippi, while the sorghum variety *NS Šećerac*, was selected at the Institute of Field and Vegetable Crops in Novi Sad, Serbia.

**Environmental conditions:** In agro-climatic conditions of Serbia, the weather conditions in the years were different and they significantly affected the growth, development and yield (Popović *et al.*, 2011; 2016a; Dražić *et al.* 2016). Temperature and precipitation were recorded throughout the entire experiment by a

meteorological station (Stara Pazova, Republic of Serbia), which is located next to the experimental field. In first year, 2014, the average temperature was 21.67°C and total precipitation was 406 mm. During the second year, 2015, average temperatures was 20.83°C while the total precipitation was 408 mm. During the third year, 2016, of the average temperature amounted to 22.17°C while the total precipitation was 310 mm (Figure 1a and b, Figure 4 b).

**Statistical analysis:** Data reported for yield parameters of sweet sorghum were assessed by analyses of variance (ANOVA) and Fisher's LSD test was used for any significant differences at the  $p < 0.05$  level between the means. All the analyses were conducted using software package Statistics 12 (StatSoft Inc. (2012) USA). Significance of differences between the calculated mean values of the analysed factors (Environment and Genotype) was tested by two-factorial analysis of variance (Maletic 2005). Relative dependence was defined by correlation analysis method (Pearson's correlation coefficients) and obtained coefficients were tested by the T-test (Maletic 2005), for significance level of 0.05 % and 0.01 %.

## RESULTS AND DISCUSSION

**Plant height:** The analysis of variance indicated significant effect of environment (E), genotype (G), and their interaction (E x G). The effects of G, E and G x E interaction caused by variation from calculated variance components over three environments of five characteristics were presented in Table 1.

Plant height is an important parameter of *Sorghum* breeding process. The average plant height for all genotypes was 2.43 m. In 2016, on average, for all tested genotypes, higher plant height were recorded (2.52 m), compared with 2014 and 2015 (2.41 m and 2.36 m). GEI - Environment x Genotype interaction had statistically significant effects on the plant height. Genotype Dale had the highest average plant height in 2016 (2.66 m), which is significantly higher than its average in 2015 (2.32 m) and higher than average of genotype NS Šećerac (2.32 m) in 2014 (Tables 1, 2, Figure 2a).

**Number of leaves:** The average number of leaves for all genotypes was 9.43. In 2016, average number of leaves (9.9) for all tested genotypes was not significantly higher, compared with averages in 2014 and 2015 (9.2), (Tables 1, 3, Figure 2b).

**Mass of stem:** The average mass of stem of all examined genotypes was 178.90 g. Genotype and GEI had statistically significant effects on the mass of stem. Genotype Dale had, on an average, the highest the mass

of stem (184.87 g), significantly higher than genotype NS Šećerac, 172.93 g,  $p < 0.05$ , Table 1 and 4, Figure 3a.

**Panicle length:** The average panicle length for all genotypes was 0.18m. Looking at the average of the panicle length of the examined sorghum genotypes in 2014, 2015 and 2016, it is evidently that average of panicle length, among years, were statistically highly significantly different ( $p < 0.05$ ). The Environment had statistically significant effect on the panicle length level. In 2016, on average, for all tested genotypes, highly statistically significantly higher results for panicle length were recorded (0.19m), compared with 2014 and 2015 (0.17m and 0.18m), ( $p < 0.05$ ), Table 1 and 5, Graph. 3b. GEI had a statistically significant effect on the panicle length. The genotype Dale in 2016 had a significantly higher panicle length than genotype Dale in 2015 and genotype NS Šećerac in 2014.

**Yield of crude biomass:** Environmental factors and GEI had a statistically significant effect on the yield of crude biomass,  $p < 0.01$ . The tested genotypes brought very high crude biomass yields, average 44.86 t ha<sup>-1</sup>. The highest yield of crude biomass per plant was determined for genotype Dale (45.15 t ha<sup>-1</sup>) compared to the genotype NS Šećerac (44.57 t ha<sup>-1</sup>) but not statistically significantly difference.

Meteorological conditions recorded high variability during the year (Popovic *et al.* (2015); Đekić *et al.* (2014). In 2016, on average, for all tested genotypes, highly statistically significantly lower results for yield of crude biomass were recorded (42.54 t ha<sup>-1</sup>), compared with 2014 and 2015 (45.99 t ha<sup>-1</sup> and 46.06 t ha<sup>-1</sup>), Table 1 and 6, Graph. 3b.

GEI had a statistically significant effect on the yield of crude biomass. The genotype NS Šećerac in 2016 had a significantly lower yield of crude biomass than genotypes: Gale in 2014, 2015 and 2016 and NS Šećerac in 2014 and 2015, Table 1 and 6, Graph. 3b

The stabilities of Sweet sorghum cultivars under various environments have always been a concern to breeders. Breeding for improving various biofuel-related characteristics (i.e. fiber, sugar, juice, and biomass) is becoming an important breeding objective for sweet sorghum breeders to meet the rapidly increased demand for biofuel production worldwide. It is well known that progress in plant breeding depends on the extent of genetic variability existed in a population (Zou *et al.*, 2011). The highest attention in breeding of sweet sorghum is given to a biomass yield, because it is a characteristic that records a large variations, which depend upon a great number of genes, as well as on the agro-ecological conditions, the genotype and GEI.

Murray *et al.* (2008) found that the variances of three traits within the RI population (176 lines, F4:5) from BTx623 and Rio were 11.3 for Brix, 157 cm for plant height and 23.5 for days flowering time,

respectively. Srinivas *et al.* (2009) studied agronomically important traits in a RI population (168 lines, F7 ) from 296B and IS18551. The variances were 124 cm for plant height, 25 days for days to anthesis, 5 no. plant<sup>-1</sup> for total number of leaves and 24 cm for panicle length, respectively. And, Shiringani *et al.* (2010) investigated another RI population (188 lines, F5:6) from SS79 and M71, the variances were 7.9 for Brix, 182 cm for plant height, 51 days for heading time and 0.93 cm for stem diameter, respectively.

Sweet sorghum yields vary considerably depending on the cultivars/hybrids used, the location (soil, water, climate, pests, and diseases), inputs, and production practices, Vermerris *et al.* 2009.

**Correlations between studied features:** Correlations are important indicators in breeding programs. The significant correlations between the examined factors suggest mutual synergies between their effects ( $p < 0.05$ ) (Popovic *et al.*, 2013; Popović and Dražić, 2016). The results of relative dependence of examined indicators of Sweet sorghum genotype were presented by Pearson's correlation coefficients and shown in Table 7. The average plant height was in a positive and statistically highly significant correlation with the number of leaves (0.54). The average yield of crude biomass in the tested period was negative non significant correlation with panicle length (-0.15), and the yield of crude biomass had a positive but not significant correlation with plant height (0.01), number of leaves (0.04), and mass of stem (0.25), Table 7.

Sweet sorghum yield of crude biomass varied according to temperature and quantities and distribution of precipitation. Yield was positively highly significantly correlated with precipitation (0.72) and negatively significantly correlated with temperature (-0.57). Based on the obtained value, number of leaves was in a positive statistically highly significant correlation with the mass of stem and in very highly statistically significant correlation with panicle length (0.46, 0.61), Table 7.

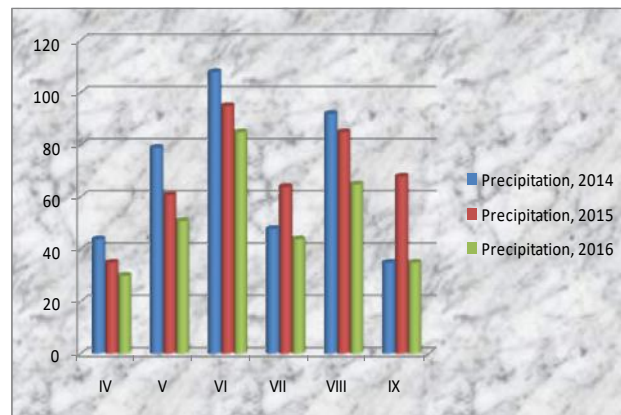
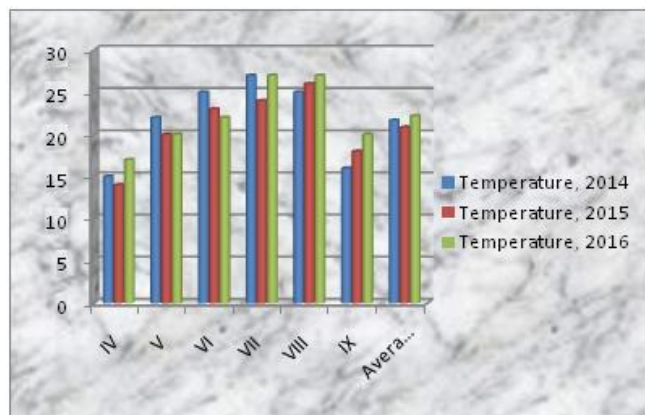
Positive correlation between grain yield and stress conditions has been established Khan and Mohammad (2016) and Drazic *et al.*, (2016).

Sweet sorghum [*Sorghum bicolor* (L.) Moench] has been suggested as a biofuel feedstock due to its high productivity, low N requirements. Yields were highest when sweet sorghum was planted in 20 cm row widths in late May (26.3 Mg ha<sup>-1</sup>) or early June (29.0 Mg ha<sup>-1</sup>), Bonin *et al.*, 2016. Application of mineral elements has a significant effect on this grain yield of cereal crops (Jamil *et al.*, 2017; Terzić *et al.*, 2018). Plant height increased significantly ( $P \leq 0.05$ ) with increasing fertility levels. Test genotype SPV 2075 had the tallest plant (290 cm). Application of 80:40:40 kg NPK ha<sup>-1</sup> increased the grain yield by 153 % over control and the test variety CSV 2074 produced 108 % higher grain yield than the check CSV 19SS. Fresh stalk yield, juice and potential ethanol yields were also increased with application of RDF (80:40:40 kg NPK ha<sup>-1</sup>). Juice yield was significantly ( $P \leq 0.01$ ) and positively correlated with plant height and fresh stalk yield (Mishra *et al.*, 2015).

**Table 1. Productivity parameters for sweet sorghum genotype.**

Genotype / Year	Parameter					
	2014	2015	2016	Average	Std.Dev	Std.Err
	<b>Plant height (m)</b>					
NS Šećerac	2.32	2.41	2.37	2.37	0.132	0.034
Dale	2.49	2.32	2.66	2.49	0.339	0.087
Average	2.41	2.36	2.52	2.43	0.260	0.048
	<b>No of leaves</b>					
NS Šećerac	8.80	9.20	9.20	9.07	0.961	0.248
Dale	9.60	9.20	10.60	9.80	1.082	0.279
Average	9.20	9.20	9.90	9.43	1.073	0.196
	<b>Mass of stem, g</b>					
NS Šećerac	170.18	175.59	173.00	172.93	10.843	2.799
Dale	196.24	174.72	183.65	184.87	11.979	3.092
Average	183.21	175.16	178.32	178.90	12.763	2.330
	<b>Panicle length, m</b>					
NS Šećerac	0.16	0.19	0.19	0.18	0.023	0.006
Dale	0.19	0.18	0.20	0.19	0.019	0.005
Average	0.17	0.18	0.19	0.18	0.022	0.004
	<b>Yield of crude biomass, t ha<sup>-1</sup></b>					
NS Šećerac	45.70	46.80	41.22	44.57	2.933	0.757
Dale	46.28	45.32	43.86	45.15	1.566	0.404
Average	45.99	46.06	42.54	44.86	2.329	0.425

	LSD	Genotype	Year	G x Y
Plant height	0.5	0.190	0.233	0.329
	0.1	0.257	0.315	0.446
No of leaves	0.5	0.744	0.233	0.330
	0.1	1.007	0.315	0.125
Mass of stem	0.5	7.525	10.039	13.035
	0.1	10.179	13.579	17.631
Panicle length	0.5	0.015	0.018	0.025
	0.1	0.020	0.024	0.034
Yield of crude biomass	0.5	1.112	1.361	1.924
	0.1	1.502	1.840	2.602



a.

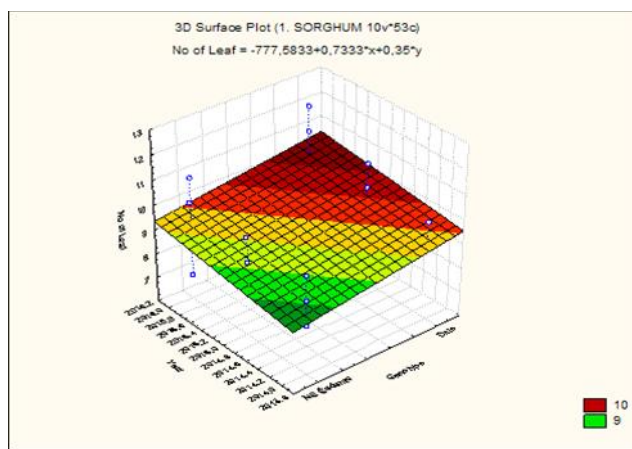
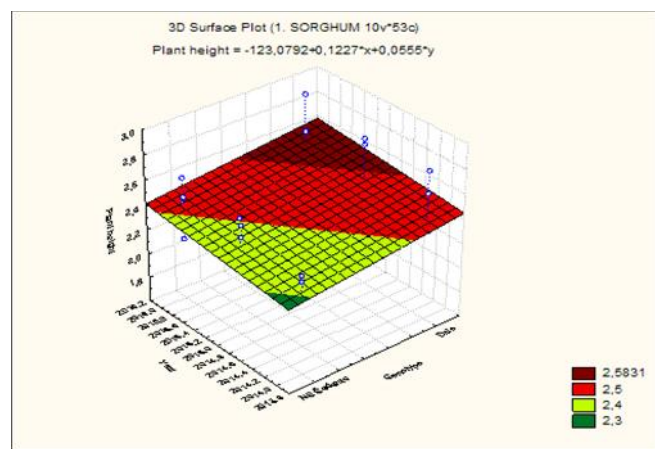
b.

Figure 1. Monthly air temperature (°C), a, and total precipitation (mm), b, S. Pazova, Serbia.

Table 2. The analysis of variance for plant height, Serbia.

Effect	df	Mean sq Effect	Mean sq Error	F	p-level
Intercept	1	177.244*	177.244*	2761.172	0.00000**
Genotype, (G)	1	0.1129	0.1129	1.7580	1.19734
Environment, (E)	1	0.1266	0.0631	0.9841	0.38851
Genotype x Environment, (GxE)	2	1.5406	0.0918	1.4301	0.25887
Error	24	1.5406	0.0642		

<sup>n</sup>non significant; \* significant at 0.05; \*\* signiftant at 0.01;



a.

b.

Figure 2. Plant height, a, and number of leaves, b, for Sorghum genotype, Serbia



**Table 3. The analysis of variance for number of leaves, Serbia.**

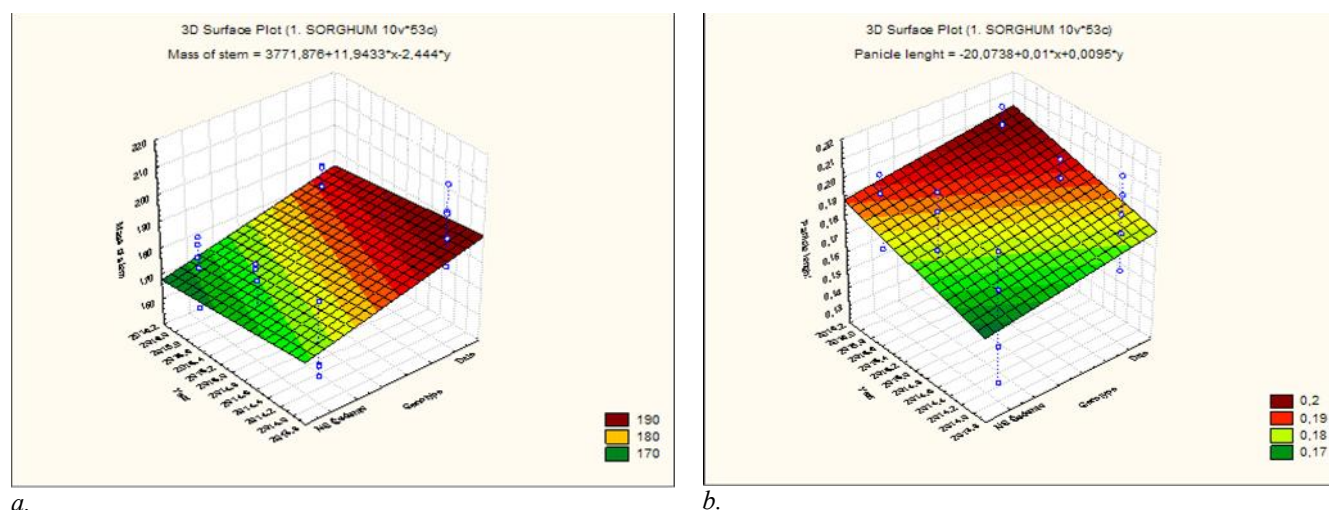
Effect	df	Mean sqr Effect	Mean sqr Error	F	p-level
Intercept	1	2669.635*	2669.635*	2714.881*	0.000000**
Genotype, (G)	1	4.033	4.003	4.102	0.054101
Environment, (E)	1	3.267	1.633	1.661	0.21104
Genotype x Environment, (GxE)	2	2.467	1.233	1.254	0.30333
Error	24	23.600	0.983		

\*significant at 0.05; \*\*significant at 0.01;

**Table 4. The analysis of variance for mass of stem, Serbia.**

Effect	df	Mean sqr Effect	Mean sqr Error	F	p-level
Intercept	1	960131.3*	960131.3*	9554.93**	0.000000**
Genotype, (G)	1	1069.81**	1069.81	10.646	0.003297**
Environment, (E)	1	329.41	164.71	1.639	0.215212
Genotype x Environment, (GxE)	2	912.8*	456.41	4.542**	0.021238**
Error	24	2411.7	100.5		

\*significant at 0.05; \*\*significant at 0.01;

**Figure 3. Mass of steam, a, and panicle length, b, for Sorghum genotype, 2014-2016, Serbia.****Table 5. The analysis of variance for panicle length, Serbia.**

Effect	df	Mean sqr Effect	Mean sqr Error	F	p-level
Intercept	1	1.01200*	1.01200*	2651.53**	0.000000**
Genotype, (G)	1	0.00075	0.00075	1.965	0.17377
Environment, (E)	1	0.00184	0.00092	2.419	0.11037
Genotype x Environment, (GxE)	2	0.00194	0.00097	2.541	0.09974
Error	24	0.00916	0.00038		

\*significant at 0.05; \*\*significant at 0.01;

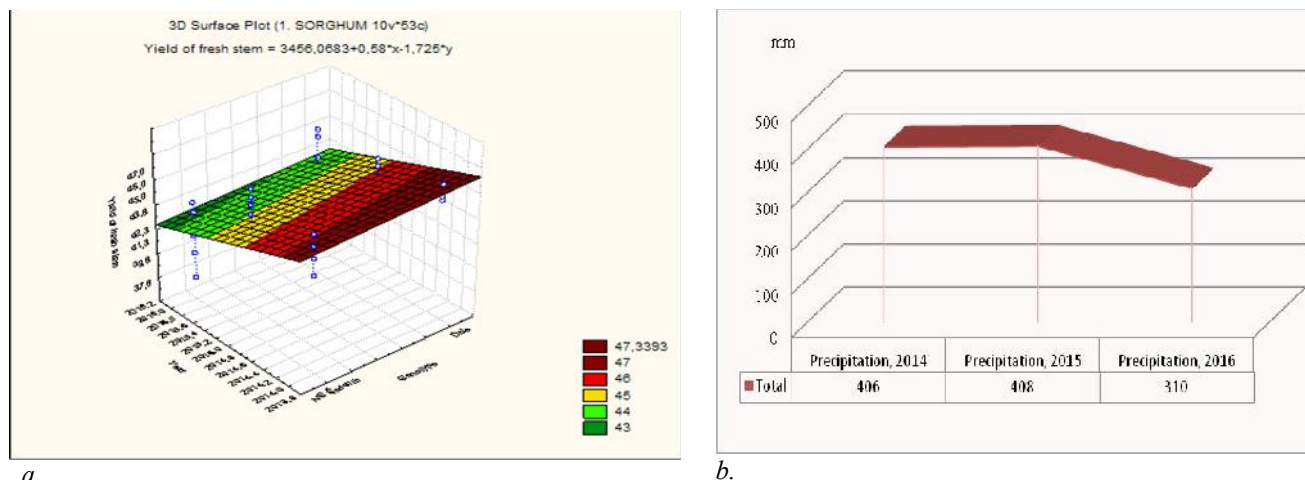


Figure 4. Average yield of crude biomass,  $t\ ha^{-1}$ , a, and total precipitation, b, 2014-2016.

Table 6. The analysis of variance for yield of crude biomass, Serbia.

Effect	df	Mean sq SS	Mean sq MS	F	p-level
Intercept	1	60381.54*	60381.54*	27584.08*	0.00000**
Genotype, (G)	1	2.52	2.52	1.15	0.293686
Environment, (E)	2	80.99**	40.50*	18.50*	0.000014**
Genotype x Environment, (GxE)	2	21.22**	10.60*	4.85*	0.017063**
Error	24	52.54	2.19		

<sup>n</sup>non significant; \*significant at 0.05; \*\*significant at 0.01;

Table 7. The correlation of yield of crude biomass and yield components.

Parameters	Yield of crude biomass	Plant height	Number of leaves	Mass of stem	Panicle length	Tempe rature	Precipitation
Yield of crude biomass	1.00	0.01	0.04	0.25	-0.15	-0.57*	0.72**
Plant height	-	1.00	0.54*	0.09	0.06	0.23	-0.25
Number of leaves	-	-	1.00	0.46*	0.61**	0.25	-0.31
Mass of stem	-	-	-	1.00	0.35	0.14	0.03
Panicle length	-	-	-	-	1.00	0.19	-0.34

\* and \*\* significant at probability level of 0.05 and 0.01, respectively.

**Conclusions:** Because of genotype by environment interaction (G×E), the morphological values of a Sweet sorghum are different when grown under different environmental conditions. These differences can be very significant. The stability and adaptability of a Sweet sorghum cultivar during growth periods is determined in part by the G×E interaction, and both stability and adaptability appear to decrease as the effects of these interactions become larger.

The tested genotypes had brought very high biomass yields. The studied characteristics of Sweet sorghums varied significantly depending on genotype and year. Genotype had a statistically significant effects on the PH and MS and environment had statistically

significant effects on the PH, PL, and YCB. GEI - interaction genotype x environment had a statistically significant effect on the PH, NL, MS, PL and YCB.

The average plant height was in positive statistically highly significant correlation with the number of leaves (0.54). Based on the obtained value, number of leaves was in a positive, statistically highly significant correlation with the mass of stem and in a positive, statistically very highly significant correlation with panicle length (0.46, 0.61).

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