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EVALUATION OF SUGAR BEET GENOTYPES FOR ROOT TRAITS BY PRINCIPAL COMPONENT ANALYSIS AND CLUSTER ANALYSIS

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Sugar beet is the most important crop for sugar production in Europe. Wide genetic variability is essential in sugar beet breeding programs. The aim of this study is to evaluate variability for the main root traits and differences between monogerm and multigerm sugar beet genotypes from the breeding collection at the Institute of Field and Vegetable Crops. The following traits were analyzed: root weight (g), dry matter content (%), root head weight (g), root/head ratio (%), number of cambial rings, root length (cm) and root diameter (cm). Mean values for two years per genotype were standardized and used for analysis. Principal Component Analysis (PCA) and Cluster Analysis (CA) were used to examine the level of diversity for 20 genotypes and to rank the contributions of the variables. According to CA genotypes could be placed into five main groups, where a large number of multigerm genotypes were put in one group. On average multigerm genotypes were characterized by higher mean values for root weight, length, diameter and lower root head ratio. Multigerm genotypes had higher coefficients of variation for nearly all measured root traits.

Key words: multivariate analysis, root,sugar beet

INTRODUCTION

Sugar beet is a relatively young crop, which began to be cultivated for sugar production about 200 years ago. The main source of germplasm for early varieties was probably fodder beet called "White Slesian" (FISHER, 1989). The most important progress in increase of sugar content in root started when Vilmorin 1850th introduced a new method of selection, (progeny test), which included an analysis individual root for sugar content, i.e. checking progeny in next generations.

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Thus were developed varieties with a sugar content from 13 to 17%, which are considered to be a starting breeding material for all breeding programs, why is the genetic base of sugar beet is considered narrower than in other open pollinated crops (BOSEMARK, 1989). According to MCGRATH *et al.* (2000) a reduction in genetic diversity of sugar beet is a great problem because:

a) sugar beet has been selected from a narrow population,

b) selection pressure in order to achieve an increased sugar content has been intense, and

c) a limited number of varieties from Europe formed germplasm breeding base worldwide.

From the beginning of sugar beet breeding program to the registration of non-commercial genotypes it is required eight to fifteen years (PANELLA and LEWELLEN 2007). This long term process with a narrow genetic basis significantly complicates breeding of this plant species. In order to develop varieties with many favorable traits, sugar beet breeders must have starting material, with wide genetic variability. Working with a large number of genotypes requires knowledge of their most important traits.

The sugar beet root weight usually consists of 73-77% water and 16-22% dry matter, of which 80% is sucrose (BICHEL, 1988; BOHN *et al.*, 1998). It is highly variable and influenced by environmental factors such as: soil fertility, climatic conditions, presence of disease, pests and number of plants per unit area (CAMPBELL, 2002, KHAN *et al.*, 2005). Root weight, beside sugar content, is the main indicator of the value of sugar beet hybrids. Sugar content and dry matter content of beet root have very high positive correlation (0.70-0.80) (THEURER, 1979). In contrast, a negative correlation between sugar content and root yield is a consistent problem in sugar beet breeding, because increase of sugar content affects the reduction of water content, which results in the reduction of yield (CAMPBELL, 1989).

Sugar beet root head is the upper part of the root which carries the leaves and that reaches the lowest leaf buds (JUS E. B1. 080, 2002). Root head is in fact a stem and makes about 6-17% of the root weight (MILFORD and HOUGHTON, 1999). Root with a smaller proportion of the root head is desirable in the sugar industry, because the root head has lower sugar content than taproot and the concentration of impurities is about 70% higher than in the taproots (ZIELKE and SNYDER, 1974; COLE and SEILER, 1976). The density of the cambial rings (number of rings in relation to the root diameter) is in positive correlation with sugar content, since the beets with the highest sugar content are those with the largest number of rings (VIVIEN, 1920). Larger cells in the root and expanding cambial rings are positively correlated with higher yield, while the smaller cells and narrower rings are positively correlated with a higher sugar content. According to WYSE (1979), research should be focused on finding and producing plants with large and multiple root cambial rings.

Root length was important in the sugar beet breeding and it is measured from the root neck to the root apex with 2 cm in a diameter. CAMPBELL and COLE (1986) have founded a negative correlation between root length with root yield, while a positive correlation between these two traits found ĐORĐEVIĆ (1972). If the roots are too long, the losses of root yields may increase due to the breakage of root apex. Root diameter is positively correlated with root yield and the weight of the root head. Because of that it can be used as a selection criterion for high root yield (CAMPBELL and COLE, 1986).

Tolerance to different pathogens is desirable trait, since it is the most efficient way of protecting plants from various pests. The most important and destructive sugar beet disease is rhizomania, caused by beet necrotic yellow vein virus (BNYVV), transmitted by soil fungi

Polymyxa beta (SCHOLTEN and LANGE, 2000). Yield losses that may arise due to rhizomania are up to 100% on plots with a high prevalence of this virus (HJERDIN-PANAGOPOULOS, 2003). So far, several types of resistance to rhizomania were described: Alba, Rhizor, Holly and WB42 (SCHOLTEN and LANGE, 2000; KOVAČEV *et al*., 2005, STEVANATO *et al*., 2015), among them the most widely used are Holly and Rhizor type. For this reason, the entire breeding material in this research had Holly or Rhizor type of resistance.

The aim of this study was to evaluate diferences between randomly chosen ten monogerm and ten multigerm sugar beet genotypes from the collection at the Institute of Field and Vegetable Crops and evaluate variability of the main root traits: root weight, dry matter content, root head weight, root/head ratio, number of cambial rings, root length and root diameter.

MATERIALS AND METHODS

Ten monogerm (mm) inbred lines (cytoplasmic male sterility maintainer - "O" types) with a high degree of self-pollination and ten multigerm (MM) population with a narrow genetic base were evaluated (Table 1).

Table 1. Plant material, type, presence of self-sterility and self-fertility genes, type of rhizomania resistance and origin

Genotype	Characteristics	Rhizomania type of resistance	Origin
1	mm, inbreed line, S^f, S_5	Holly	Serbia
2	mm, inbreed line, S^f, S_5,	Holly	Serbia
3	mm, inbreed line, S^f, S_5,	Holly	Serbia
4	mm, inbreed line, S^f, S_5,	Holly	Serbia
5	mm, inbreed line, S^f, S_5,	Holly	Serbia
6	mm, inbreed line, S^f, S_5,	Holly	Serbia
7	mm, inbreed line, S^f, S_5,	Holly	Serbia
8	mm, inbreed line, S^f, S_4,	Holly	Serbia
9	mm, inbreed line, S^f, S_4,	Holly	Serbia
10	mm, inbreed line, S^f, S_5,	Holly	Serbia
11	MM, population, S^s	Rhizor	Denmark
12	MM, population, S^s	Holly	USA
13	MM, population, S^s	Holly	Greece
14	MM, population, S^s	Rhizor	Germany
15	MM, population, S^s	Holly	USA
16	MM, population, S^s	Rhizor	West Europe
17	MM, population, S^s	Rhizor	Denmark
18	MM, population, S^s	Rhizor	Denmark
19	MM, population, S^f	Holly	USA
20	MM, population, S^s	Rhizor	Denmark

mm-monogerm, MM-multigerm
S^f– autofertility, S^s - autosterility

S_4- 4 generation of inbreeding,S_5 - 5 generation of inbreeding

The field trials were conducted for two years at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad (Serbia). The genotypes were sown in the first week of March in randomized complete block design with three replications. The plot size was 16 m^2 (8 m length and 2 m wide), with four rows. Density of plants after manual thinning was 50 x 20 cm. Throughout the growing season were applied regular cultural practices for sugar beet (inter-row cultivation, chemical treatment against leaf spot). Roots were harvested in October manually, by fork, to minimize root damage.

The following traits were analyzed: root weight (g), dry matter content (%), root head weight (g), root/head ratio (%), number of cambial rings, root length (cm) and root diameter (cm). The basic sample consisted of 30 plants per year, 10 plants per replication. Measurements were done according to Yugoslav standards for sugar beet (JUS E. B1. 080, 2002). Number of cambial rings was determined by counting the rings without optical aids. Root analysis were done in the laboratory for quality in Sugar beet Department at the Institute of Field and Vegetable Crops, Novi Sad. Dry matter content was determined by using a digital universal refractometer (DUR-W, SCHMIDT + HAENSCH GmbH & Co.).

Software package Statistica for Windows ver. 12, (StatSoft. Inc. 2013) was used for one factorial ANOVA, The significance of differences between genotypes was tested by LSD test. Variation in evaluated traits were described by Coefficient of Variation (CV). Principal Component Analysis (PCA) and Cluster Analysis (CA) were used to evaluate the level of diversity for 20 sugar beet genotypes from collection and to rank the contributions of the variables. Mean values for two years per genotype were standardized and used for analysis. For the construction of dendrograms were used Wards method and squared Euclidean dinstances. Since the significant proportion of variance was explained by first few principal components, only they were retained and interpreted. The most frequently used method is to eliminate the linear combinations whose variance is less than 1 (eigenvalue <1) (JAĆIMOVIĆ, 2012).

RESULTS AND DISCUSSION

The highest root weight was recorded in MM genotype 14 (1260.0 g), while the lowest root weight was observed in mm genotype 6 (347.7 g) (Table 2). Mean values of root head weight had wider range in mm genotypes (37.7-286.8 g) compared to MM genotypes (84.8-211.2 g). The results were similar to root/head ratio, which was more variable in mm (8.0-37.3%) than in MM genotypes (13,4-17.8%). Multigerm genotypes were characterized with higher root weight, root length and root diameter compared to the monogerm genotypes. MM genotypes had, on the average, lower root/head ratio, while the dry matter content, root/head ratio and the number of rings did not showed differences between those two groups of genotypes.

According to STANČIĆ *et al.*, (1999) higher mean values for root yield, sugar content and sugar yield were obtained in multigerm pollinators in relation to monogerm male sterile lines. High coefficients of variation for root weight were confirmed in this study, particularly in multigerm genotypes, because the root weight is a trait that is highly influenced by the environment and shows great variability (STOJAKOVIĆ *et al.*, 1992; SKLENAR, 1997; VESELINOVIĆ *et al.*, 1997). Besides environmental factors, root weight is also significantly affected by certain characteristics of the root, although their impact varies depending on the origin of the studied population (SKLENAR *et al.*, 1997). The dry matter content is not as often studied as sugar content, although it is important for selection, because the proportion of sugar in the dry matter of roots is about 80% (BOHN *et al*., 1998). The values for dry matter content in this study varied significantly depending on the genotype, which is in accordance with results of previous studies (STOJAKOVIĆ *et al*., 1992; ĆURČIĆ, 2007). The highest dry matter content was similar in several genotypes (4, 5, 15, 20 and 7), where the mean value ranged from 21.6-21.1%.

Table 2. Mean values and coefficients of variation (CV) of evaluated sugar beet root traits

Genotype	Type	Root weight (g)		Dry matter content (%)		Root head weight (g)		Root/head ratio (%)		Number of rings		Root length (cm)		Root diameter (cm)	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	mm	712.4	18.5	20.4	5.9	258.8	29.7	36.0	19.2	6.3	8.1	20.5	11.0	9.1	8.9
2		847.4	14.6	18.2	9.0	123.5	27.9	14.6	24.5	5.6	12.2	25.4	7.2	8.4	8.7
3		826.9	22.7	20.1	4.4	216.2	28.0	26.2	15.7	5.4	8.6	19.9	8.9	8.9	8.5
4		607.5	14.1	21.6	3.0	65.7	20.6	10.8	14.9	6.1	8.8	24.4	9.6	6.3	8.3
5		477.4	16.3	21.6	4.0	37.7	28.9	8.0	26.7	5.2	6.2	23.4	12.0	5.6	7.8
6		347.7	17.5	20.6	4.7	66.5	27.2	19.0	17.7	5.5	12.3	17.2	8.0	5.9	13.4
7		626.5	17.6	21.1	6.0	86.7	19.1	13.9	14.8	4.7	9.8	24.4	7.4	7.3	7.6
8		668.9	17.2	20.4	5.7	92.9	26.5	13.9	21.1	5.6	9.0	23.4	9.9	7.3	9.2
9		764.4	14.2	19.0	5.9	286.3	21.4	37.3	13.8	4.9	9.4	20.7	8.3	7.8	8.8
10		798.0	14.7	17.4	6.1	241.9	21.2	30.4	14.6	5.1	10.3	16.4	9.4	9.5	5.7
Average mm		**667.7**	**16.7**	**20.0**	**5.5**	**147.6**	**25.1**	**21.0**	**18.3**	**5.4**	**9.5**	**21.6**	**9.2**	**7.6**	**8.7**
11	MM	1162.5	27.1	19.7	5.2	192.7	35.4	16.8	28.3	6.3	13.1	23.4	13.1	9.6	12.6
12		1083.5	33.5	19.4	5.7	151.8	34.3	14.4	25.4	5.4	12.8	24.2	12.4	9.3	13.8
13		999.7	30.7	20.3	4.5	145.9	40.2	14.9	29.3	5.0	13.3	23.4	13.1	8.8	10.1
14		1260.0	30.2	20.4	5.5	211.2	47.0	16.6	32.1	5.7	16.0	24.6	13.3	9.4	20.2
15		866.0	27.7	21.4	5.6	148.9	41.7	17.2	31.6	5.3	14.3	21.0	12.6	8.6	30.6
16		1024.4	30.5	20.6	4.7	138.2	49.7	13.4	29.8	5.5	13.7	23.7	12.7	8.8	16.4
17		462.2	39.9	19.5	7.1	84.8	56.5	17.8	26.2	7.0	9.5	22.1	16.0	5.8	18.1
18		893.3	26.5	20.0	5.7	149.6	36.9	16.8	26.9	4.8	16.1	22.3	12.0	8.6	12.1
19		706.8	23.3	18.5	7.7	112.5	46.9	15.9	38.5	4.6	12.8	23.9	8.8	6.7	10.9
20		950.1	31.6	21.4	5.5	130.9	59.9	13.6	41.3	4.8	15.4	23.3	14.2	8.8	12.3
Average MM		**940.9**	**30.1**	**20.1**	**5.7**	**146.7**	**44.9**	**15.7**	**30.9**	**5.4**	**13.7**	**23.2**	**12.8**	**8.4**	**15.7**
LSD 0.05		86.1		0.61		26.6		1.61		0.36		1.54		0.53	

The root head weight is in positive correlation with root yield and can be used as a selection criterion for sugar beet breeding (CAMPBELL and COLE, 1986). It is desirable that the varieties have a lower than 15% of the root/head ratio (STEHLIK, 1982). Selection can reduce the amount of root head for about 18% per selection cycle in diploid genotypes (MESKEN and DIELMAN, 1988). The average root/head ratio was lower in multigerm compared to monogerm genotypes.

The first principal component (PC) accounted for 42.83% of the total phenotypic variation expressed. Traits accounting for the most of variation expressed in the first PC were root head weight, root diameter and root/head ratio (Table 3). The second PC accounted for 27.03% of the variation with root weight and taproot length as the main traits in this component.

Table 3. Eigen values, proportion of total variability between the original variables and the first two principal components (PC)

Trait	PC1	PC2
root weight	0.550	**0.812**
dry matter content	**-0.577**	0.202
root head weight	**0.964**	-0.022
root/head ratio	**0.783**	-0.541
number of cambial rings	-0.078	-0.192
root lenght	-0.417	**0.766**
root diameter	**0.800**	0.524
Eigen value	2.998	1.892
% Total variance	42.832	27.027
Cumulative %	42.832	69.858

A plot of the first and second principal component was made to show the relative groupings of the genotypes (Figure 1). In the plot, genotypes were grouped into four groups. Multigerm genotypes with the exception of genotypes 17 and 19 were placed in the largest group which was in the middle of the plot. Genotypes 4, 5, 7, 8 were positioned from the left side of the largest group and they were characterized with a smaller root weight, root head weight and root/head ratio. The MM genotype 19 was also placed in this group, because of smaller root weight and root diameter than the other MM genotypes. According to previous results of DANOJEVIĆ *et al.* (2011), showed that the parents with the lowest root weight per se, produce the test hybrid with the greatest root weight. Therefore, the parents with a small root should not be discarded before testing of general combining abilities. Genotype 19 was autofertile which is probably the main reason for distinctness from the other MM genotypes. According to ĆURČIĆ (2014), autofertile pollinators compared with autosterile pollinators showed a positive heterosis in all hybrids. Hence, autofertile genotypes should have priority in the future sugar beet breeding programs. In the right part of the plot were grouped mm genotypes 1, 3, 9 and 10. They had higher taproot weight, root head weight and root/head ratio than other mm genotypes. The smallest group consisted of genotypes 6 and 17. These genotypes had the smallest root weight in both germinity type.

Cluster analysis placed genotypes into five groups (A, B, C, D and E) (Figure 2). The top group in the dendrogram (group A), consisted of four mm genotype 1, 3, 9 and 10. In the group C were almost all MM genotypes (8 from 10). In the group D there were mm genotypes 4, 5, 7, 8. In the bottom group on dendrogram (E) there are two genotype 6 and 17. The results of cluster analysis confirmed grouping genotypes by PCA with exception of group B in dendrogram. That group was consisted of mm genotype 2 and MM genotype 19. Those two genotypes had similar mean values but they were belonged to separate groups in PCA score plot. According to this differences, CA separated genotypes more clearly than PCA.

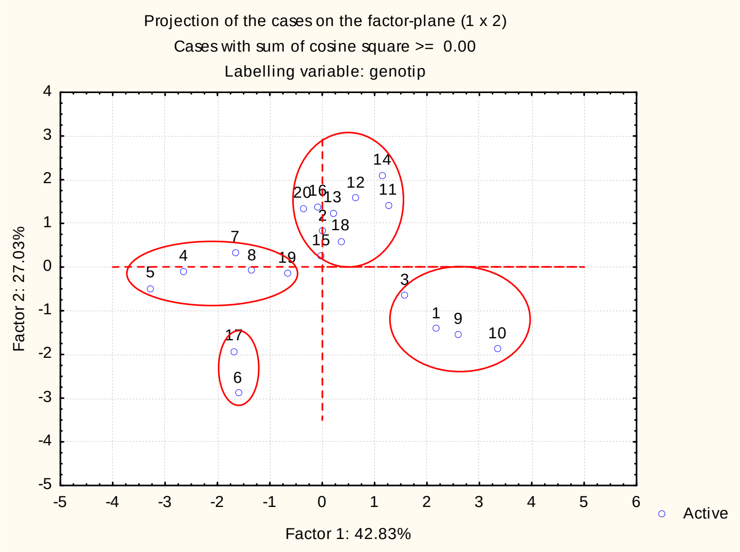

Figure 1. Principal component score plot of sugar beet genotypes based on evaluated root traits

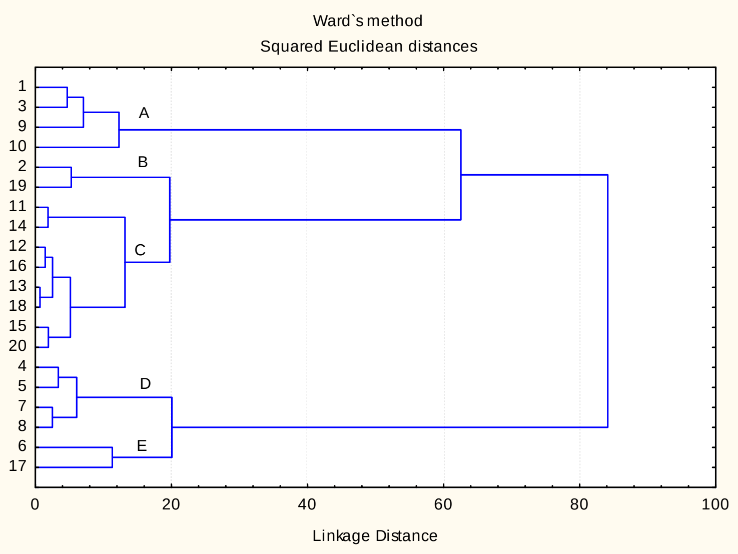

Figure 2. Dendrogram of sugar beet genotypes based on evaluated root traits

CONCLUSION

On average, multigerm genotypes had the higher mean values for root weight, length and diameter, higher yield and lower root/head ratio than monogerm genotypes, as well as higher coefficients of variation for nearly all evaluated root traits. One of the reasons is because all multigerm genotypes in this study, except genotype 19, did not have a self-fertility gene. The tested genotypes had large divergence which is an important basis for the success of existing breeding programs and starting new ones, with the main purpose for creating a new sugar beet hybrid variety. According to our results, genotypes 14, 11 and 12 will be used in breeding programs for high root weight, genotypes 4, 5, 15 and 20 for high dry matter content and genotypes 5, 4, 16 for low root/head ratio.

Multigerm genotypes, on average, showed better performance than monogerm having greater root weight, lower root head weight, lower root/head ratio, greater root length and root diameter. It would be useful to improve these traits in monogerm lines, by crossing with multigerm populations, because monogerm lines translated in the form of sterile cytoplasm are used as female hybrid parent. Improving these traits in monogerm genotypes would allow a further increasing in productivity of sugar beet hybrids. It is also important to import self fertility gene in multigerm material. This would lead to a reduction in root weight and deterioration of other good traits, but it would increase the uniformity and reduce variability within multigerm genotypes.

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PROUČAVANJE SVOJSTAVA KORENA RAZLIČITIH GENOTIPOVA ŠEĆERNE REPE METODAMA MULTIVARIJACIONE ANALIZE

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Izvod

Šećerna repa je najvažnija biljna vrsta za proizvodnju šećera u Evropi. Široka genetska varijabilnost je veoma važna u oplemenjivačkim programima šećerne repe. Cilj ovog istraživanja je da se ustanovi varijabilnost za glavna korenska svojstva i razlike između monogermnih i multigermnih genotipova šećerne repe koji se nalaze u kolekciji Instituta za ratarstvo i povrtarstvo u Novom Sadu. Analizirana su sledeća svojstva: masa korena (g), sadržaj suve materije (%), masa glave korena (g), udeo glave korena (%), broj kambijalnih prstenova, dužina korena (cm) i prečnik korena (cm). Primenjena je analiza glavnih komponenti (PCA) i klaster analiza (CA) kako bi se ustanovila raznovrsnost odabranih 20 genotipova i rangirao doprinos varijabli. Za analizu su korišćene standardizovane srednje vrednosti iz dve godine istraživanja. Prema CA, genotipovi su se grupisali u pet glavnih grupa, a većina multigermnih genotipova se svrstala u jednu grupu. Rezultati PCA su u skladu sa rezultatima CA. U proseku multigermni genotipovi su se odlikovali većim srednjim vrednostima mase, dužine i prečnika korena, a nižeg udela glave korena. U proseku multigermni genotipovi su imali veće koeficijente varijacije za skoro sva izmerena svojstva.

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