ratar. povrt.

original scientific paper
Ratar. Povrt. 2022, 59(1): 9-15
doi: 10.5937/ratpov59-35862
Received: 13 January 2022
Accepted: 18 March 2022

Morphological diversity and principal component analysis of sugarcane (*Saccharum officinarum* L.) genotypes at Finchaa Sugar Estate, Ethiopia

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Summary: This study was conducted to assess the extent of morphological variation, cluster the genotypes into relatively homogenous groups and to identify the major characters contributing to the overall diversity of 22 exotic sugarcane genotypes at Finchaa Sugar Estate. The experimental design used was RCBD laid in three replications. Quantitative traits such as number of internodes, millable stalk, plant height, stalk girth, single cane weight, cane yield, brix percent juice, pol percent, sugar recovery percentage and sugar yield were investigated. ANOVA indicated the existence of high phenotypic and genetic diversity between genotypes for all quantitative traits studied which could be utilized for further sugarcane improvement. The principal component analysis for the genotypes exhibited variance of 43.12%, 18.8% and 13.71% for the first three categories and accounts about 75.63% of the total variation and the juice quality traits showed greater loading for the variation in the first principal component category. Cluster analysis grouped genotypes in to five distinct classes with maximum number of genotypes (7) in cluster II and minimum (1) in cluster V. In the first cluster six sugarcane genotypes were included this accounted 27.27% of the investigated materials which had been categorized by high cane length and single stalk weight. The second cluster which accounts 31.82% of the materials were characterized by lower cane length and single stalk weight. Recoverable sugar percent had almost zero correlation with cane length, millable stalk count and sugar yield (r= 0.02, 0.02 and -0.01 respectively) indicating the possibility of simultaneous improvement of these traits.
Keywords: clustering, diversity, PCA, selection, sugarcane

Introduction

Sugarcane (*Saccharum officinarum* L.), is a strongly growing grass with a C4 carbon cycle photosynthetic pathway and a high chromosome number (Marshall, 2002). It is highly adapted to a wide range of tropical and subtropical climates, soils and cultural conditions and is propagated in over 100 countries situated between latitudes 37^0 N and 32^0 S, grown for different purposes especially for it sugar constituent (Singh, 2003). Today, sugarcane agriculture is a key economic activity particularly in developing countries as a provider of income and employment especially for under-skilled rural people of Ethiopia, where agriculture is a cornerstone of economic development (ESCRT, 2012).

Assessment of phenotypic and genetic diversity is also very important for the improvement of sugarcane because diverse parents could be crossed by breeders for producing elite varieties (Barreto et al., 2019). Several genetic variability studies have been conducted on different crop species based on quantitative and qualitative traits in order to select genetically distant parents for hybridization (Dai et al., 2012; Muhe & Alemayehu, 2011). Genetic and phenotypic diversity analysis and preservation is basic to develop varieties with high yield potential and resistance/tolerance to abiotic and biotic stresses, with acceptable end-use quality. It is the most viable and environment-friendly option to sustainably increase sugar yield. In Ethiopia, >1,000 sugarcane accessions were introduced and collected locally. Each sugarcane genotype should be evaluated in

Acknowledgment:
The author is thankful to the financial grant of the Research and Development Center of Ethiopian Sugar Corporation. The technical assistants and staff of Finchaa Research Station are duly acknowledged for their supports.

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Cite this article:
Alemu S., Fetene T., Tadesse F. (2022). Morphological diversity and principal component analysis of sugarcane (*Saccharum officinarum* L.) genotypes at Finchaa Sugar Estate, Ethiopia. Ratar. Povrt., 59 (1), 9-15.

various environments, allowing the identification and recommendation of factory specific superior varieties as well as broadly adapted varieties (Tena et al., 2016). Morphological characters must be recorded to select parents and their progenies, and they must always be used to describe and classify germplasm (Karimi et al., 2009).

Principal component analysis (PCA) and cluster analysis (CA) are widely used tools in many experimental procedures for better understanding of data as PCA aids to minimize the variables and demonstrates the relationship among them (*Mundaragi et al.,2017)*. Further, several studies indicate that multi variate statistical analysis, such as PCA and CA, are more effective in evaluating genetic diversity among sugarcane varieties (Arrey & Mih, 2016; Baloch, 2017). These techniques have been employed in assessment of genetic diversity among several other crops such as wheat, maize and chilli (*Yatung et al., 2014)*. In recent past, several studies have reported on the sugarcane genetic diversity assessment using the CA and PCA analyses (*Agrawal & Kumar, 2017)*. Therefore, this study was conducted to assess the extent of genetic diversity, cluster the genotypes into relatively homogenous groups and to identify the major characters contributing to the overall diversity of sugarcane genotypes at Finchaa Sugar Estate in Ethiopia.

Material and Methods

Study Area

The study was conducted at Finchaa Sugar Estate. It is located at about 330 km west of Addis Ababa, 9° 31' to 10°N latitudes and 37° 15' to 37° 30' E longitude with an elevation of between 1350 to 1650 m asl. The area receives about 1280 mm annual average rainfall with mean annual maximum and mean minimum temperature of 30.6°C and 14.5°C, respectively.

Materials and Experimental Design

Twenty-two sugarcane genotypes (Tables 1 and 2) were grown from December 2016 to June 2020 for plant cane, ratoon I and II. The experiment was laid in a randomized block design of three replications with 6m length of each rows spaced 1.45m. Twelve traits were investigated: number of internodes, number of tillers, millable stalk, stalk height (m), cane girth (cm), single cane weight (kg), cane yield, brix percentage, pol percent, purity percentage, sugar recovery (%) and sugar yield.

Table 1. Performances of introduced mid-way breeding sugarcane clones in France (Origin)

Variety	Origin	Characteristics in the country of origin			
		Cane Yield	Sugar Yield	Flowering	Resistance to diseases
FG 04 356	CIRAD		Medium	No	Smut (R)
FG 05 155	CIRAD		High	Heavy	Smut (R)
FG 05 319	CIRAD		High	Moderate	Smut (R)
FG 05 336	CIRAD		High	No	Smut (R)
FG 05 425	CIRAD		High	No	Smut (R)
FG 05 771	CIRAD		Medium	Heavy	Smut (R)
FG 06 114	CIRAD	High	High	No	Smut (R)
FG 06 117	CIRAD	High	High	No	Smut (R)
FG 06 119	CIRAD			No	Smut (MR)
FG 06 621	CIRAD		Medium	No	Smut (R)
FG 06 622	CIRAD		High	Heavy	Smut (R)
FG 06 659	CIRAD		Medium	Moderate	Smut (R)
FG 06 725	CIRAD		Medium	No	Smut (R)
FG 06 692	CIRAD			No	Smut (R)

NB: HR = Highly Resistant; R = Resistance; MR = Moderate Resistance and S = Susceptible

Table 2. Performances of introduced advanced breeding sugarcane clones in France (Origin)

Variety	Origin	Parents	Characteristics in the country of origin			
			Cane Yield	Sugar Yield	Flowering	Disease resistance
CP 96 1252	Canal Point, Florida	CP 90-1553 x CP 84 1198	High	High	High	Smut (R), Brown rust (MR), Leaf scald (MR), Mosaic (R), Ratoon stunt (R)
CP 00 2180	Canal Point, Florida	HoCP 91-55 x HoCP 91-552	High	Medium	Moderate (late)	Smut (R), Brown rust (MR), Orange rust (R), Leaf scald (MR), Mosaic (R), Ratoon stunt (R)
CPCL 02 926	Canal Point, Florida	CP 80-1743 x CL 92-0046	High	High	None	Smut (R), Brown rust (R), Orange rust (R), Mosaic (S), Ratoon stunt (MR)
VMC 96-89	Philippines	VMC 71-238 x ?	High	Medium	Moderate to profuse	Smut (HR), Yellow spot (R)
VMC 96-120	Philippines	VMC 76-105 x VMC 71-238	High	Medium	Low	Smut (R), Yellow spot (R), Orange rust (HR)
VMC 96-134	Philippines	VMC 81-131 x Poly cross	Medium	Medium	Low	Smut (R), Yellow spot (HR), Orange rust (HR)
MPT 96-035	Thailand	ZC 85-036 x Co 87-265	Medium	High	Low	White leaf (S)
MPT 96-273	Thailand	Co 88-011 x Co 87-025	Medium	High	Low	Red rot (S)

NB: HR = Highly Resistant; R = Resistance; MR = Moderate Resistance and S = Susceptible

Data Analysis

The quantitative data recorded was subjected to analysis of variance using statistical procedures described by Gomez & Gomez (1984) with the help of SAS. To reduce data dimensions for better visualization of genotypes and traits, principal component analysis (PCA) and clustering analysis were conducted for eleven traits and ten genotypes using statistical system software. PCA is a multivariate technique for examining relationships among several quantitative variables. PCA was performed using correlation matrix in order to evaluate the relationships among characters that are correlated among each other by converting into uncorrelated characters called principal components.

Results and discussion

Phenotypic Characterization

The analysis of variance revealed significant difference among the genotypes for all traits, indicating the presence of sufficient variability

Table 3. Combined ANOVA for twenty-two sugarcane genotypes of three crop cycles at Finchaa

Sources of Variation	DF	NIN	CL	CG	SSW	MSC	CY	Brix %	Pol %	Purity %	RSP	SY
Crop Cycles (C)	2	5487*	386624*	102.71*	15.94*	495316*	12510.8*	81.44*	73.2*	19.0ns	37.5*	435.5*
Genotypes (G)	21	31.88*	8170*	23.95*	0.41*	12732*	1375.7*	7.17*	8.75*	21.2*	5.5*	25.2*
Replication	2	22.97*	635ns	4.70ns	0.05ns	15108*	1608.8ns	1.55ns	0.188ns	21.6ns	0.6ns	33.2ns
G x C	42	15.83*	1057ns	4.70*	0.07*	5714*	628.8ns	2.99*	3.09*	10.8ns	1.87*	13.1ns
	Mean	24.34	262.14	24.60	1.53	276	134.9	20.21	17.93	88.76	12.15	16.49
	CV	10.18	11.01	6.98	13.88	14.74	18.36	5.64	7.26	3.29	8.81	22.19

Where: Significant (*) and non-significant (ns) at ($p \leq 0.05$)
DF= degree of freedom, NIN= number of internodes, CL= cane length, CG= cane girth, SSW= single stalk weight, MSC= millable stalk count, CY= cane yield, RSP=recoverable sugar percent and SY= sugar yield

Table 4. PCA of twelve quantitative characters in 22 sugarcane genotypes showing eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first five PCs

Traits	Eigenvectors				
	Prin1	Prin2	Prin3	Prin4	Prin5
Number of internodes	0.353365	-0.137226	0.068822	0.226002	0.040443
Stalk length	0.127179	0.129238	0.544078	0.386933	-0.450806
Cane girth	-0.322905	0.106454	-0.289498	0.211156	0.514990
Single stalk weight	-0.231421	0.316545	0.109709	0.532279	0.068533
Stalk count	0.257025	0.112000	0.142000	-0.584854	0.013970
Cane yield (t/ha)	-0.067139	0.620490	0.140094	-0.196610	-0.000092
Sugar yield (t/ha)	0.185786	0.592265	-0.013986	-0.089123	0.079198
Brix %	0.351296	0.003189	-0.394092	0.155958	-0.196727
Pol %	0.402274	0.047858	-0.245865	0.154548	-0.009800
Purity (t/ha)	0.350613	0.114204	0.171821	0.108325	0.538810
Sugar Recovery %	0.413119	0.050238	-0.190412	0.150437	0.084641
Eigenvalue	5.17	2.26	1.64	1.51	0.7
Individual %	43.12	18.8	13.71	12.56	5.84
Cumulative %	43.12	61.92	75.63	88.19	94.03

among the sugarcane genotypes for the traits under consideration. Javed et al. (2001) stated that presence of variability in crops is very important for further sugarcane crop improvement utilizations. Feyissa Tadesse et al. (2014) also reported considerable genetic variability for yield and its component characters in studied sugarcane genotypes in Ethiopia. Hence, selection could be effective for different quantitative characters or for inclusion in crossing program for creating variability. Javed et al. (2001) and Ftwi et al. (2016) also reported considerable genetic variability for cane yield and its component characters in studied sugarcane genotypes.

Principal Component Analysis (PCA)

The PCA categorized the eleven quantitative traits in to twelve components for the entire variability between genotypes.

Categories with an eigenvalue of less than 1 should be ignored (Chatfield & Collin1980)

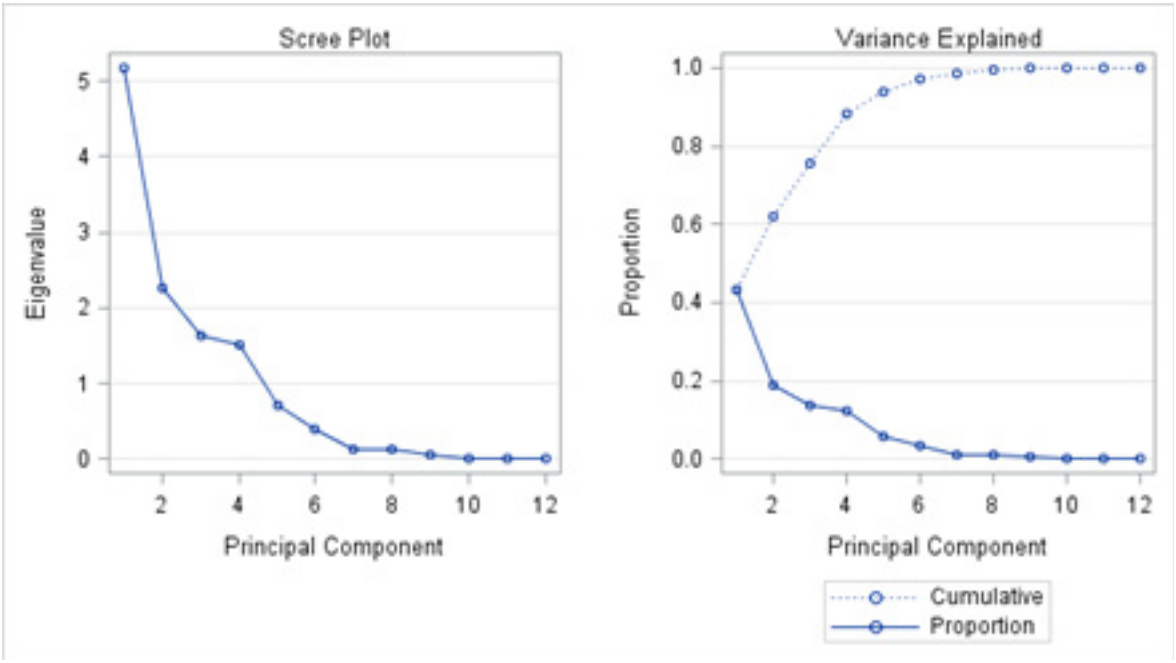

Figure 1. The variance plot for the twelve principal components for 22 sugarcane genotypes

and eigenvalues greater than one are considered significant and component loadings greater than ±0.3 were considered (Hair et al., 1998). The PCA for the genotypes exhibited variance of 43.12%, 18.8% and 13.71% for the first three categories and accounts about 75.63% of the total variation (Fig. 1).

The juice quality traits including sugar recovery percent, pol percent, and purity percentage and brix % juice showed greater loading for the variation in the first principal component category. On the other hand, sugar yield, cane yield and single stalk weights contributed major variation in the second principal components.

The variations in the third principal component were mainly due to stalk length and brix percent juice. The eigenvectors of the third principal component showed large positive loadings for the stalk length followed purity percentage and high negative loading for brix percent juice and followed by pol percentage (Table 4). In line with the present finding, Muhe & Alemayehu (2011) employed principal component analysis for detecting variation in 400 sugarcane germplasm of which the first six principal components contributed 79.26% of total variation.

The couples of traits most correlated positively were among the juice quality traits including recoverable sugar percent with pol percentage (r=0.99), pol percentage with brix percent juice (r=0.95) and recoverable percent juice with purity percentage (r=0.76). Poorly correlated traits with recoverable sugar percent were three characters; cane length, millable stalk count and sugar yield (r= 0.02, 0.02 and -0.01 respectively), indicating the possibility of simultaneous improvement of both traits (Table 5). It came out the phenotypic traits allowing to better explaining the diversity of the genotypes were by recoverable sugar percent from the first principal component (Table 4).

Cluster Analysis (CA)

CA was made to group the 22 exotic sugarcane materials into components based on their quantitative traits for the sake of understanding the share components contribute to major variation in the study. The dendrogram obtained from the cluster analysis grouped the 22 genotypes into 5 clusters. Number of genotypes per cluster varied from seven in cluster II to one in cluster five. In cluster I six sugarcane genotypes were included which accounted 27.27% of the investigated materials. This category had been categorized by genotypes high cane length and single stalk weight. On the other hand, the second cluster which accounts 31.82% of the materials and included seven genotypes were characterized by lower cane length and single stalk weight.

Six genotypes relatively with lower millable stalk count and shortest in cane length were grouped under cluster three which contribute 27.27% of the genotypes. The fourth cluster includes two genotypes which means 9.09 % of the investigated materials and was characterized by its highest performance in number of millable stalks, cane length and recoverable sugar percent. While one genotype was grouped in the fifth cluster and characterized by higher number of millable stalks and sugar recovery percent and lower cane length (Figure 2 and Table 6). This cluster analysis revealed that the exotic sugarcane genotypes originated from different sources.

Table 5. Correlation matrix for some important quantitative traits for 22 sugarcane genotypes

Traits	IN	CL	CD	SSW	MSC	CY	SY	BRX	POL	PUR	RSP
CL	0.361										
CD	-0.54	-0.46									
SSW	-0.33	0.32	0.60								
MSC	0.29	-0.001	-0.62	-0.63							
CY	-0.36	0.14	0.13	0.38	0.25						
SY	0.11	0.20	-0.17	0.12	0.44	0.80					
BRX	0.58	0.03	-0.42	-0.36	0.24	-0.25	0.33				
POL	0.68	0.16	-0.50	-0.36	0.35	-0.17	0.44	0.95			
PUR	0.69	0.32	-0.43	-0.20	0.45	0.02	0.49	0.44	0.67		
RSP	0.72	0.02	-0.52	-0.37	0.02	-0.16	-0.01	0.91	0.99	0.76	

NIN= number of internodes, CL= cane length, CG= cane girth, SSW= single stalk weights, MSC= millable stalk count, CY= cane yield, RSP=recoverable sugar percent and SY= sugar yield

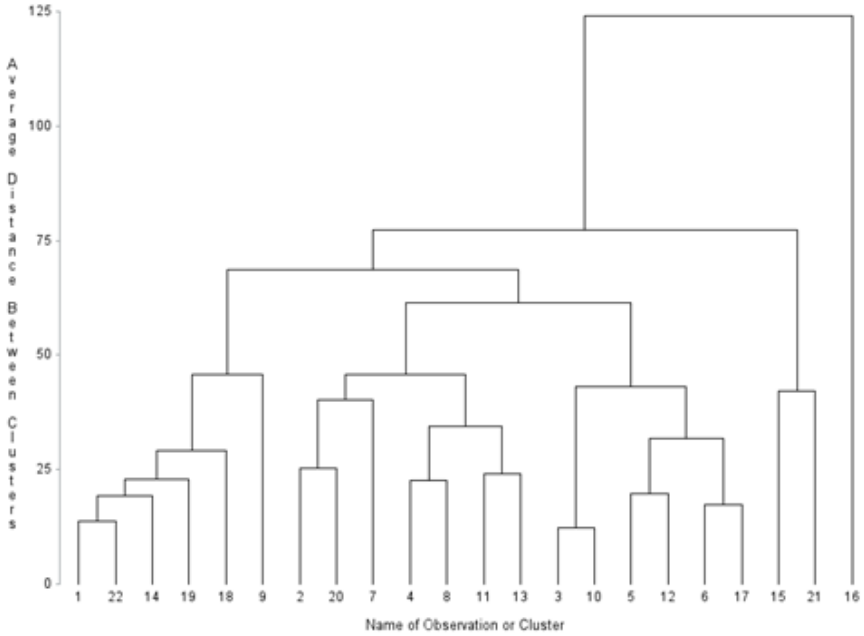

Figure 2. Dendrogram showing the clusters of 22 exotic sugarcane genotypes tested at Finchaa Sugar Estate

Table 6. Cluster-wise mean values of eleven characters and Distribution of 22 exotic sugarcane genotypes in different cluster groups at Finchaa Sugar Estate

Cluster-wise mean values of characters											
Cluster	NIN	CL	CD	SSW	STC	CY	SY	BRX	POL	PUR	RSP
I	24.72	293.13	24.37	1.70	251.67	135.32	16.43	20.16	17.87	88.64	12.10
II	24.38	246.20	24.23	1.38	295.19	134.31	16.31	20.21	17.86	88.28	12.08
III	22.93	237.96	26.51	1.66	245.70	135.33	16.16	19.86	17.50	88.29	11.79
IV	27.00	293.38	21.52	1.23	316.11	126.31	16.71	21.05	19.18	91.06	13.22
V	24.89	270.44	23.27	1.45	385.22	151.61	19.59	20.84	18.94	90.92	12.93

Distribution of 22 exotic sugarcane genotypes in different cluster groups		
Cluster	Number of genotypes	Genotype code
I	6	FG 06 695, VMC 96-273, FG 05 771, VMC 96-134, VMC 96-89 and FG 05 425
II	7	FG 06 114, VMC 96-120, FG 05 336, FG 06 119, FG 04 356, FG 06 622 and FG 06 692
III	6	FG 06 117, FG 06 621, FG 05 155, FG 06 659, FG 05 319 and MPT 96 035
IV	2	FG 06725 and FG CPCL 02 926
V	1	CP 96 1252

Where; NIN= number of internodes, CL= cane length, CG= cane girth, SSW= single stalk weights, MSC= millable stalk count, CY= cane yield, RSP=recoverable sugar percent and SY= sugar yield

Conclusion

All quantitative traits revealed high variability for twenty-two sugarcane genotypes which could be utilized for further sugarcane crop improvement. The principal component analysis for the genotypes exhibited variance of 43.12%, 18.8% and 13.71% for the first three categories and accounts about 75.63% of the total variation. The juice quality traits including sugar recovery percent, pol percent, and purity percentage and brix % juice showed greater loading for the variation in the first principal component category. The dendrogram obtained from the cluster analysis grouped the 22 genotypes into five clusters. In the first cluster six sugarcane genotypes were included which accounted 27.27%

of the investigated materials which had been categorized by high cane length and single stalk weight. The second cluster which accounts 31.82% of the materials were characterized by lower cane length and single stalk weight. Recoverable sugar percent had almost zero correlation with cane length, millable stalk count and sugar yield (r= 0.02, 0.02 and -0.01 respectively) indicating the possibility of simultaneous improvement of these traits.

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Morfološki diverzitet i PCA genotipova šećerne trske (Saccharum officinarum L.) u Finchaa Sugar Estate, Etiopija

Shitahun Alemu · Tesfaw Fetene · Feyissa Tadesse

Sažetak: Istraživanje je sprovedeno u cilju ocene opsega morfoloških varijacija, grupisanja genotipova u relativno homogene grupe i identifikacije glavnih osobina koje doprinose ukupnom diverzitetu 22 egzotična genotipa šećerne trske u Finchaa Sugar Estate u Etiopiji. Analizirane su sledeće kvantitativne osobine: broj internodija, stabljike pogodne za mlevenje, visina biljke, obim stabljike, masa jedne trske, prinos trske, Brix procenat soka, procenat saharoze, procenat fermentabilnog šećerai prinos šećera. ANOVA je pokazala postojanje visokog fenotipskog i genetičkog diverziteta među genotipovima za sve analizirane kvantitativne osobine koji se može iskoristiti za buduće poboljšanje šećerne trske.PCA je pokazala varijansu od 43,12%, 18,8% i 13,71% za prve tri kategorije i objasnila 75,63% ukupne varijacije. CA je grupisala genotipove u pet različitih klasa sa najvećim brojem genotipova (7) u grupi II i najmanjim (1) u grupi V. Prva grupa sadrži 6 genotipova šećerne trske (27,27% analiziranog materijala) i karakteriše je veća dužina trske i masa jedne stabljike. Drugu grupu (31,82% analiziranog materijala) karakteriše manja dužina trske i masa jedne stabljike.Procenat fermentabilnog šećeraje imao skoro nultu korelaciju sa dužinom trske, brojem stabljika pogodnih za mlevenjei prinosom šećera (r= 0.02, 0.02 i -0.01, redom) što ukazuje na mogućnost istovremenog poboljšanja ovih osobina.

Ključne reči: grupisanje, diverzitet, PCA, selekcija,šećerna trska