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GENETIC VARIABILITY AND ASSOCIATION PATTERN AMONG QUANTITATIVE NUTRITIONAL TRAITS IN SWISS CHARD (*Beta vulgaris* subsp. L. var. *cicla*) ACCESSIONS AND ITS IMPLICATION FOR BREEDING

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In addition to improving agronomic traits, enriched cultivar such as nutritional elements and health promoting compounds are new demands for today's and the future's perspectives of crop breeding. In this respect, among leafy vegetables Swiss chard is a good source of nutritional elements and supplies large amounts of health promoting compounds. The existing knowledge of genetic variability for mineral composition both at the phenotype and genotype level, heritability of characters and also relationships among investigated minerals is fundamental for variety selection in Swiss chard. This also applies for the assurance of desirable agronomic traits with optimum mineral concentrations. This research analysis of variance indicated highly significant differences among Swiss chard accessions for all investigated mineral concentrations and the accessions display higher phenotype coefficient variation than genotype coefficient variation for all traits. The results revealed that phosphorus, magnesium, sodium, iron, copper, zinc, manganese, nitrate and nitrite exhibited high genetic advance accompanied with high heritability (>60%). The remaining mineral content demonstrated high heritability with moderate genetic advance. Genotype correlations were higher than the phenotype correlation for significant mineral concentrations. Genotype and phenotype correlations followed similar trends in all significant cases indicating the high heritable nature of the characters and the results showed

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that Swiss chard accessions should allow for the selection of individuals for enriched mineral concentration in edible parts of the plant.

Key words: Association analysis, mineral concentration, selection parameters, Swiss chard, variability

INTRODUCTION

Fruit and vegetables not only provide essential nutrients and vitamins but also contribute to other compounds for health promotion and disease prevention (LIU, 2003). Leafy vegetables or green vegetables are good sources for amino acids, fibre and antioxidants and it is known for the cheapest and largest amount of protein sources (ALETOR *et al.,* 2002). Most leafy vegetables can grow all year round in temperate regions and contain significant amounts of the most essential nutrient elements, amino acids and minerals such as iron and zinc. Among the leafy vegetables, Swiss chard (*Beta vulgaris* L. var. subsp. *cicla*) is considered a good source of minerals and nutritional elements and it has been grown in Europe for centuries, and leaf beet has been cultivated for about 2500 years in China (SHUN *et. al.,* 2000). The leaves of Swiss chard are widely used as a vegetable either in cooked or raw form in salads and also the stems are chopped or cooked like celery. It is available throughout the year (PYO *et al.,* 2004). Petiole and stalks are edible parts of the plant, the leaves may either be smooth or curly and the stalk observes white, red, yellow and orange colors depending on the genotype.

As well as Swiss chard successfully grown under various soil and climatic conditions, under tolerant moderately saline conditions it is a good source for several vitamins (SANTAMARIA *et al.,* 1999) and contains a high amount of phosphorous, potassium, calcium and magnesium (DZIDA and PITURA 2008; POKLUDA and KUBEN 2002).

In recent years, there is growing scientific concern regarding mineral concentration and the nutritional value of vegetables. Besides improving agronomic traits, enriched cultivar in terms of nutritional elements and health promoting compounds with breeding, these are new demands for plant breeders. In recent years, breeding strategies have focused on increasing vitamin and mineral nutrition, and improving cultivars particularly in essential elements may reduce malnutrition in a diet which is a significant problem for human health (GRUSAK and DELLAPENNA 1999), especially in developing countries. The intake of nutritionally enriched vegetables such as Swiss chard could be expected to contribute to a large amount of mineral requirements in the body and to maintain mineral deficiency in diets.

Although Swiss chard has diverse uses and contributes to significant nutrition sources in a diet, the knowledge on the extent and magnitude of genetic variability for mineral composition is limited and also no scientific reports are available on genetic variability, heritability and character associations among Swiss chard for mineral elements. The existing knowledge of genetic variability for mineral composition both at the phenotype and genotype levels, heritability of characters and also relationships among investigated minerals is fundamental for variety selection and for assurance desirable agronomic traits with optimum mineral concentrations.

Thus, the present research was examined to assess genetic variability, heritability, genetic advance and character associations for mineral concentration of Swiss chard accessions for further selection and breeding programs.

MATERIALS AND METHODS

In the present study a total of fifty-four Swiss chard accessions, which have fifty-two local populations and two cultivars as a reference; one local and one foreign cultivar gathered from Turkey and Germany, were examined. Evaluated accessions represent whole Swiss chard genetic resources from collections in Turkey received from the national public gene bank of AARI (Aegean Agricultural Research Institute), Izmir, Turkey.

The experiment was carried out in the autumn of 2007 and the winter of 2008 at the experimental field of Ege University, Agriculture Faculty, Department of Horticulture, Izmir, Turkey. The seeds were sewn in October in a randomized block design with 3 replications and the plot size of each accession was 4.5 m^2 and contained 20 plants. The soil of the experimental site was sandy loam soil with the following characteristics: pH 7.58, organic matter content of 1.10%, $CaCO_3$ = 1.45%. Mineral content of soil N = 0.110%, P = 5.67 mg kg^{-1}, K= 319.8 mg kg^{-1}, Ca = 4300 mg kg^{-1}, Mg = 174.2 mg kg^{-1}, Na = 56.4 mg kg^{-1}, Fe = 19.61 mg kg^{-1}, Cu = 2.18 mg kg^{-1}, Zn = 0.90 mg kg^{-1}, Mn = 4.36 mg kg^{-1}.

The seeds were sewn by hand and usual cultural practices were followed. The furrow irrigation method was employed regularly every week until the beginning of the rainfall season. No chemical fertilizers, fungicides or insecticides were applied during cultivation, and the weeds were controlled by hand until the plant reached harvest maturity. Plant samples were harvested by hand until the leaves fully matured and were ready for edible use.

The edible parts of the leaves (both leaf lamina and petioles) were washed with distilled water and dried at room temperature to remove external moisture and then placed in a paper bag and oven dried at 65^0C for 24 hours. The dried plant samples were powdered in a blender for mineral composition determination. The total amounts of N in the leaf samples were determined by the modified Kjeldahl method, phosphorus (P) in wet digested samples with colorimetry, potassium (K), calcium (Ca) and sodium (Na), with flame photometry (Eppendorf, Germany) and magnesium (Mg), iron (Fe), Zinc (Zn), copper (Cu) and manganese (Mn) using atomic absorption spectrometry (SpectrAA-220 FS, Varian, Australia). Appropriate calibration controls (calibration curve method with commercial certified ICP multi-element standard solution Merck) were applied to each set of measurements. N, P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn concentrations were given basis on dry weights, and nitrate and nitrite concentrations were determined colorimetrically basis on fresh weight.

To estimate the extent of magnitude variation among examined traits, all the data was subjected to analysis of variance. The mean, standard error and range were calculated, and components of variance $\sigma^2 g$ = genotype variance, $\sigma^2 p$ = phenotype variance and $\sigma^2 e$ = error variance were estimated using the following formula (NWOFIA and OKWU 2012);

Genotypic coefficient of variations $$GCV = \frac{\sqrt{\sigma^2 g}}{\bar{X}} x100$$

Phenotypic coefficient of variations $$PCV = \frac{\sqrt{\sigma^2 p}}{\bar{X}} x100$$

where, σ_p, σ_g, and X are the phenotype and genotype standard deviation and grand mean of the traits respectively. Heritability in the broad sense (h^2) was estimated using the genotype mean described by ALLARD (1999) as:

Heritability broad sense $$\left(h^2B\right) = \frac{\sigma^2 g}{\sigma^2 p}$$

Expected genetic advance (GA) and GA as a percentage of the mean was calculated according to SHUKLA *et al.* (2006). Expected genetic advance $(GA) = i\sigma p h^2$

Genetic advance (% of mean) $$= \frac{Kx\sigma x h^2}{X} \times 100$$

where, *i:* standardized selection differential, a constant (2.06), σp: phenotype standard deviation. Genotype (r_g) and phenotype (r_F) correlation coefficients between x and y traits were calculated based on the procedure described reported by (YIMRAM *et al.,* 2009);

Genotype correlations $$r_g(x, y) = \frac{COV_g(x, y)}{\sqrt{\sigma^2{}_g(x)}\sqrt{\sigma^2{}_g(y)}}$$

Phenotype correlations $$r_p(x, y) = \frac{COV_p(x, y)}{\sqrt{\sigma^2{}_p(x)}\sqrt{\sigma^2{}_p(y)}}$$

where, Cov_{xy}(g) and $Cov_{xy}(p)$ are genotype and phenotype covariance between x and y characters, $\sigma^2 g$ = genotype variance, $\sigma^2 p$ = phenotype variance

RESULTS AND DISCUSSION

The ANOVA showed significant differences among accessions for all examined mineral concentration at the level of $P<0.01$ probability (Table 1). Mineral concentration displayed a wide range which provides opportunity for selection of higher nutrient content among the examined nutritional elements. Some accessions collected from nearby provinces showed a wide range of mineral concentration and the mean performance of 54 Swiss chard accessions are given in table 2. There were substantial differences among accessions and the largest variability obtained from magnesium, iron, copper, zinc and manganese concentrations among investigated mineral compositions and Mg content ranged from 3.33-9.43 g kg^{-1}, Fe ranged between 77.22-159.65 mg kg^{-1}, Cu content ranged from 7.60 to 16.96 mg kg^{-1} and Zn ranged between 22.31-65.52 mg kg^{-1}.

Table 1. Analysis of variance of examined nutritive elements

Source of variations	df	Mean square											
		N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn	NO_3	NO_2
Genotype	53	0.296**	0.015**	0.014**	0.003**	0.034**	0.009**	1306.50**	8.932**	184.120**	4837.890**	7452.700**	0.00007**
Replication	1	0.305	0.009	0.013	0.008	0.020	0.011	28.665	9.705	35.880	115.217	1381.057	0.00003
Error	107	0.013	0.000	0.001	0.000	0.001	0.000	4.560	0.358	1.577	18.271	70.386	0.00000

** Significant at 1% probability level, respectively

Estimates of variance components ($\sigma^2 g$, $\sigma^2 p$, $\sigma^2 e$), phenotype (PCV) and genotype (GCV) coefficients of variation, broad sense heritability (h^2B) and genetic advance (GA) (%) for the examined twelve mineral elements are summarized in table 3. The phenotype coefficient of variation (PCV) was higher than the genotype coefficient variation (GCV) for all mineral concentrations. Estimates of PCV were highest for Fe (100.25%) and Mn (100.22%) concentrations, whereas the lowest value of PCV was received from Ca (94.58%) content. Broad sense heritability (h^2B) ranged from 93.52% for Ca to 99.60% for Mn content. Genetic advance (GA) in percentage was the highest for phosphorus (40.07%), the lowest in calcium (17.80%), and the remaining mineral elements showed moderate genetic advance in terms of percentage.

Table 2. Range, mean, standard error (SE), variance components ($\sigma^2 g$, $\sigma^2 p$, $\sigma^2 e$), phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability ($h^2 B$) and genetic advance (GA)

Traits	Range			Mean	SE	$\sigma^2 p$	$\sigma^2 g$	$\sigma^2 e$	PCV (%)	GCV (%)	$h^2 B$	GA	GA (%)
N	44.30	±	28.81	35.52	0.026	0.099	0.094	0.005	95.64	3.10	94.75	0.61	17.28
P	5.99	±	2.38	3.55	0.006	0.005	0.005	0.000	98.45	7.37	97.81	0.14	40.07
K	49.4	±	28.6	36.9	0.006	0.005	0.004	0.000	96.07	3.76	95.24	0.14	18.25
Ca	4.74	±	2.66	3.51	0.003	0.001	0.001	0.000	94.58	1.46	93.52	0.06	17.86
Mg	9.43	±	3.33	5.42	0.009	0.011	0.011	0.000	98.00	6.69	97.33	0.21	39.66
Na	5.72	±	2.40	4.0	0.004	0.003	0.003	0.000	96.14	3.87	95.32	0.10	26.42
Fe	159.65	±	77.22	105.74	1.635	435.522	433.927	1.594	00.25	0.07	99.63	42.83	41.29
Cu	16.96	±	7.60	12.03	0.141	2.977	2.829	0.148	95.88	3.46	95.02	3.38	28.07
Zn	65.52	±	22.31	38.55	0.618	61.373	60.742	0.632	99.59	9.07	98.97	15.97	41.43
Mn	33.89	±	12.57	20.80	3.148	512.632	506.243	6.389	00.22	0.02	99.60	82.40	39.62
NO_3	496.55	±	262.48	358.79	3.935	484.234	456.727	27.507	99.51	8.96	98.89	01.54	28.30
NO_2	0.047	±	0.024	0.03402	0.00038	0.000	0.000	0.000	99.08	8.32	98.46	0.01	28.59

(%) for various nutrient traits in Swiss chard.

Correlations and relationships among various traits are both necessary in order to improve selection efficiency in the breeding program. In the present research, simple correlation coefficients among mineral elements are given in table 3. Association analysis revealed that ammonium showed significant positive correlation with P, NO_2 and NO_3 at $P<0.01$ level. Although Phosphorus had a positive correlation with K, Cu, Zn and NO_3, it had a negative association with Ca and Na. A significant positive correlation was found between potassium and Ca, and Fe as well, whilst a negative relationship was shown with Zn. Calcium had a positive association with Mg, Na, Fe, and Mn. Sodium and zinc negatively correlated with NO_3, and NO_2, with Cu, and Mn. Nitrate correlated highly with NO_2 as expected.

Correlation analysis measures relationships between examined characters in order to assess which characters could affect the improvement of traits through selection**.** Genotype and phenotype correlation coefficients among various traits of mineral elements are given in table 4. The genotype correlation coefficient revealed a higher magnitude of corresponding values for phenotype correlation coefficients for all mineral concentrations.

Table 3. Simple correlation coefficients among the thirteen nutrition traits of Swiss chard

	N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn	NO_3
P	0.255**										
K	-0.045	0.257**									
Ca	-0.038	-0.197*	0.380**								
Mg	0.121	-0.063	0.035	53**							
Na	-0.067	-0.279**	0.060	75**	0.386**						
Fe	-0.081	-0.012	0.303**	0.178*	0.053	0.023					
Cu	0.144	0.385**	0.072	0.032	-0.029	0.092	-0.276**				
Zn	0.109	0.193*	-0.196*	-0.142	-0.186*	-0.011	-0.194*	0.570**			
Mn	0.004	-0.088	-0.143	0.343**	0.060	0.046	0.102	-0.210**	-0.108		
NO_3	0.514**	0.143	-0.007	-0.101	-0.079	-0.305**	-0.006	0.141	0.213**	0.121	
NO_2	0.517**	0.157*	-0.018	-0.117	-0.077	-0.307**	-0.015	0.155*	0.216**	0.110	0.996**

*, ** Significant at 5% and 1% probability level, respectively

Local landraces are primary sources and essential for successful crop improvement programs as they conserve large variability in terms of agronomic traits and nutritional elements. In order to evaluate germplasm for better selection estimation of the extent of genetic variability existing in the germplasm and characters association are required**.** JALATA *et al*. (2013) underlined that genetic variability and selection for desirable agronomic traits is primarily an activity for any plant breeder and effective selection not only depends on genetic variability but also heritable variation is essential for permanent genetic improvement (SINGH, 2000). Up until now most of the vegetable breeding program had more focus on improving agronomic traits such as size, weight, color, texture, appearance of edible parts of the plant and yield. The selection of agronomic characters over the period of plant breeding resulted in a narrow genetic base in most crops (MCGRATH *et al.,* 1999). Furthermore, the application of breeding strategies to improve the yield and quality properties of the plant resulted in a decline of 5% to 40% in minerals, vitamins and protein content of most foods, particularly in vegetables (DAVIS, 2009). Thus, improving nutrient composition and enriching the health benefit compound of today's and future perspectives of crop breeding. Researchers aim to reduce mineral deficiency and malnutrition which is a major problem in the human diet, particularly in the developing part of the world by increasing consumption of fruit and vegetables. The improvement of nutritional composition of new cultivars with better yield by breeding can be another strategy for improving public health (WELCH and GRAHAM 2004). In this respect, leafy vegetables are good sources for nutritional elements and supply large amounts of health promoting compounds. Furthermore, it is easily grown in various climatic conditions and could participate to low input farming.

Table 4. Genotypic (G) and phenotypic (P) correlation coefficients among traits of thirteen of Swiss chard

Traits		N	P	K	C	Mg	Na	Fe	Cu	Zn	Mn	NO_3
P	G	0.280**										
	P	0.090**										
K	G	-0.049**	0.214**									
	P	-0.015*	0.069**									
Ca	G	0.000	0.000	0.475**								
	P	0.000	0.000	0.145**								
Mg	G	0.124**	0.000	0.000	0.301**							
	P	0.040**	0.000	0.000	0.096**							
Na	G	-0.063**	-0.275**	0.000	0.612**	0.364**						
	P	-0.020**	-0.089**	0.000	0.193**	0.117**						
Fe	G	-0.087**	-0.012*	0.323**	0.196**	0.054**	0.024**					
	P	-0.028**	-0.004	0.105**	0.063**	0.018**	0.008					
Cu	G	0.165**	0.425**	0.080**	0.038**	-0.034**	0.103**	-0.294**				
	P	0.052**	0.137**	0.025**	0.012	-0.011	0.033**	-0.096**				
Zn	G	0.118**	0.200**	-0.211**	-0.155**	-0.194**	-0.012	-0.196**	0.615**			
	P	0.038**	0.066**	-0.068**	-0.050**	-0.064**	-0.004	-0.065**	0.078**			
Mn	G	0.004	-0.090**	-0.153**	0.375**	0.062**	0.049**	0.102**	-0.225**	-0.109		
	P	0.001	-0.030**	-0.045**	0.121**	0.020**	0.016**	0.034**	0.039**	-0.036**		
NO_3	G	0.558**	0.148**	-0.008	-0.112**	-0.083**	-0.328**	-0.006	0.153**	0.218**	0.122**	
	P	0.180**	0.049**	-0.003	-0.036**	-0.027**	-0.106**	-0.002	-0.059**	0.072**	0.040**	
NO_2	G	0.686**	0.000	0.000	0.000	0.000	0.000	-0.020**	0.125**	0.216**	0.110**	1.020**
	P	0.442**	0.000	0.000	0.000	0.000	0.000	-0.007	0.040**	0.071**	0.036**	0.336**

*, ** Significant at 5% and 1% probability level, respectively

Keeping this in mind, Swiss chard is potentially an important species among leafy vegetables and rich in mineral concentration, whereas little scientific concern was expressed on nutritional composition of the plant. In addition, no comprehensive explanation was found for the extent of genetic variability, heritability and association analysis which is an essential prerequisite for the mineral concentration improvement program. Examined studies evaluated genetic variability and their association in terms of mineral concentration and showed great diversity among investigated minerals, and two or three fold times differences received among accessions. A large genetic variability due to genotype differences for mineral element concentration was also determined in several vegetables such as wild Jerusalem artichoke (*Helianthus tuberosus* L.) (SEILER and CAMPBELL, 2004), the traditional tomato (*Lycopersicon esculentum*) cultivars of Southern Spain (RUIZ *et al.,* 2005), seeds of bean cultivars (*Phaseolus vulgaris* L.) (MORAGHAN and GRAFTON, 2001), potatoes (*Solanum tuberosum* L.) (DI GIACOMO *et al.,* 2007), wild (*Diplotaxis tenuifolia*) and cultivated rocket plant (*Eruca sativa* L.) (BOZOKALFA *et al.,* 2009; BOZOKALFA *et al.* 2011,). Besides the genetic architecture of the genotype, environment and soil composition mainly influenced nutritional value and chemical composition of the plant. In addition, cultivation and fertilization may greatly be affected (DAVEY *et al.,* 2009; DZIDA and PITURA, 2008). The growing site and growing conditions are other factors that have a great effect on nutrient uptake (KUZNOVA, 2007). Genotype variation which is reflected in the genotype differences was reported for drought tolerance in sugar beet (*Beta vulgaris*) (OBER and LUTERBACHER, 2002). Significant variability was reported among kale and collards genotype for Ca, Mg, K, Fe, and Zn concentration (KOPSELL *et al.,* 2004). Mineral concentration differences were reported in nutrient composition between cultivars and non-hybrid. Furthermore intensive varietals improvements and breeding reduced nutrient composition of vegetables (DAVIS 2009). DIZIDA and PITURA (2008) showed that the concentration of nutrients in edible parts of Swiss chard depend on nitrogen rates and fertilizer reported lower ammonium (0.49-1.27 g kg^{-1}) and calcium (1.01-1.39 g kg^{-1}), higher phosphorus (0.53-1.19 g kg^{-1}) and potassium (6.45-9.23 g kg^{-1}). Close values were obtained in magnesium (0.64-0.94 mg kg^{-1}) concentration compared to our nutritional composition of Swiss chard germplasm.

Although phenotype coefficients of variation observed higher magnitude than genotype coefficients of variation for all investigated minerals, there are small differences calculated between two variations which imply examined traits are decreasingly influenced by the environment (JALATA *et al.,* 2011). Understanding heritability in examining traits provides sufficient information for planning selection, also the results of the study reveal that all examined mineral concentrations showed high heritability (>60%) which indicates a variation in these traits mostly due to genotype effects (YIMRAM *et al.,* 2009). The heritability percentage mainly can be categorized as an 0-30% low, 30-60% moderate, 60% and above high. A high level of heritability was calculated for all concentrations. However, heritability is not enough for improvement through selection unless accompanied with higher genetic advance, and further high heritability coupled with high genetic advance in traits can be very amenable for selection. The genetic advance in terms of percentage may be categorized as suggested by JOHNSON *et al.* (1955) who indicated 0-10% low, 10-20% moderate, 20% and above high genetic advance. In this regard, the results reveal that phosphorus (40.07%), magnesium (39.66%), sodium (26.42%), iron (41.29%), copper (28.07%), zinc (41.43%), manganese (39.62%), nitrate (28.30%) and nitrite (28.59%) showed high genetic advance accompanied with high heritability (>60%), the remaining mineral content observed high heritability with moderate genetic advance. EID (2009) showed that high heritability is not accompanied with high genetic advance in all circumstances and to maintain selection based on high heritability accompanied by the genetic advance shows better genetic gain in the selection procedure (EŞIYOK *et al.,* 2011).

Genotype correlation coefficients were higher than their corresponding phenotype correlation for all mineral concentrations which imply association between traits at genetic level and suggest that the genetic improvements for these mineral elements can improve by selection.

The objective of the current work was to show genetic variability, heritability and association analysis in mineral concentration of Swiss chard accessions for selection for the further breeding program. The high magnitude of variation and heritability coupled with genetic advance achieved for all examined mineral concentrations except ammonium, potassium and calcium. Furthermore, genotype and phenotype correlations followed similar trends for all significant cases indicating the high heritable nature of the characters. Genetic variation of the Swiss chard accessions should allow for the selection of individuals for enriched mineral concentration in edible parts of the plant (SEILER and CAMPBELL, 2004). Consequently, improvement or selection based on these minerals may predict the performance of progenies.

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GENETIČKA VARIJABILNOST I ASOCIJACIJA KVANTATIVNIH OSOBINA ŠEĆERNE REPE (*Beta vulgaris* subsp. L. var. *cicla*) I PRIMENA U OPLEMENJIVANJU

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Izvod

Šećerna repa Swiss chard (*Beta vulgaris* subsp. L. var. *cicla*) je dobar izvor hranljivih elemenata od značaja za zdravlje. Analiza varijanse 54 genotipa šećerne repe ukazuje na visoko značajne razlike ispitivanih genotipova u koncentreaciji minerala. Utvrđen je viši koeficijent variranja na nivou fenotipa u poređenju sa koeficijentom variranja na nivou genotipa za sve osobine. Utvrđeni su viši koeficijenti korelacije na nivou genotipa za značajne koncentracije minerala u poređenju sa koncetracijom na nivou fenotipa. Korelacije na nivou genotipa i fenotipa pokazuju isti trend u svim značajnijim slučajevima ispitivanja što ukazuje na visok nivo nasleđivanja ispitivanih osobina. Ovo opravdava individualnu selekciju na bogat mineralni sastav jestivog dela šećerne repe.

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