

Evaluation of Genetic Diversity of Sugar Beet (*Beta vulgaris* L.) Crossing Parents Using Agro-morphological Traits and Molecular Markers

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ABSTRACT

Eight pollinators (as pollen parents) and four CMS lines (as seed parents) differing in salinity and drought tolerance were evaluated using agro-morphological traits and microsatellite markers. Root yield, leaf weight, sugar content, sodium content (Na⁺), potassium content (K⁺), α -amino nitrogen content, alkalinity coefficient, molasses sugar, white sugar content, extraction coefficient of sugar, dry matter, sugar yield, and white sugar yield were evaluated. The genetic diversity of 14 individual plants within each parent (a total of 168 genotypes) was also assessed using 18 microsatellite (SSR) markers. The results showed that 43 and 32.6% of the total variation in agro-morphological traits could be explained by the first 2 principal components, which could discriminate salinity and drought tolerant parents. Based on SSR analysis, a total number of 104 alleles were detected with an average number of 5.7 alleles per primer pair and an average polymorphism information content (PIC) of 0.64 with the highest PIC belonging to EST-SSR *FDSB502*. Cluster analysis based on SSR markers clearly discriminated 112 plants belonging to pollen parents from 56 plants of seed parents. In conclusion, the SSR markers have been shown to be efficient and reliable for assessing genetic diversity in sugar beet crossing parents.

Keywords: Genetic variation, Microsatellite, Root-sugar yield.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) supplies about a quarter of the world's sugar demand (Draycott, 2006). Assuming its origin from the indigenous Mediterranean *B. maritima*, sugar beet is a relatively young crop possessing a narrow genetic base (van Geyt *et al.*, 1990). It is a salt-tolerant crop that has been grown in parts of central Iran where either or both saline soil and saline groundwater restrict cultivation of crops sensitive to salinity. Its tolerance to osmotic stress and potential for producing economically efficient yield in moisture deficit (Jones *et al.*, 2003) along with its deep roots to tap moisture stored well below

soil surface have made sugar beet adaptable to grow under marginal environments. As water resources for agronomic uses become more limiting, the deployment of water-stress tolerant crops gains more importance and breeding programs should be explicitly directed towards the development of higher drought-tolerant cultivars in such crops (Ober *et al.*, 2004).

Knowledge of genetic diversity and heritability of traits can guide to the appropriate selection schemes in plant breeding programs (Izadi-Darbandi *et al.*, 2013). Overall genetic diversity of *Beta* species including sugar beet, other cultivated beet crops, and their wild relatives is relatively high (Fievet *et al.*, 2007). In sugar

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beet, over time, selection appears to have reduced genetic variability in the improved cultivars (McGrath *et al.*, 1999). An array of approaches using morphological and molecular markers has been employed to analyze diversity and to support the management of genetic resources. Molecular markers are commonly used to characterize genetic variation within and between populations and provide an efficient means for linking phenotypic and genotypic variations. Despite the advantages and drawbacks of both kinds of markers, their combined utilization is recommended for enhancing the resolving power of genetic diversity analyses (Singh *et al.*, 1991).

Different types of biochemical and molecular markers have been developed and used in sugar beet (*Beta vulgaris* L.). Amplified fragment length polymorphisms (AFLPs) (Barzen *et al.*, 1995; Schondelmaier *et al.*, 1996; Schumacher *et al.*, 1997) and cleaved amplified polymorphic site (CAPS) (Paran and Michelmore, 1993) markers have also been used for mapping and fingerprinting. Among the variety of molecular markers, SSR markers have gained widespread application in plant genetics and breeding owing to many desirable attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage (including organellar genomes) and chromosome specific location (Kandemir *et al.*, 2010; Parida *et al.*, 2009). The co-dominant nature of SSR markers allows the allelic relationships among genotypes to be estimated, a property that makes this technique particularly well-suited for application to sugar beet as a self-incompatible and cross-pollinated crop (Rae *et al.*, 2000). In sugar beet, a few hundred SSR markers have been developed and genetic maps based on SSR markers are publicly available (Laurent *et al.*, 2007; McGrath *et al.*, 2007). SSR markers have been found to be a powerful tool for the evaluation of genetic diversity in both wild and domestic sugar beets (Richards *et al.*,

2004). Moreover, they have been widely used for population genetic analyses in different beet species (Andersen *et al.*, 2005; Arnaud *et al.*, 2003; Viard *et al.*, 2004). In this regard, the following subjects have been investigated: (i) gene flow and introgression from cultivated sugar beet to their wild relatives (Viard *et al.*, 2002), (ii) genetic diversity and population structure of wild beets (Arnaud *et al.*, 2003), and (iii) phylogenetic relationships (Arnaud *et al.*, 2009; Arnaud *et al.*, 2003; Fénart *et al.*, 2008). However, reports on the genetic diversity of elite sugar beet germplasm using SSRs are scant (Li *et al.*, 2010). Furthermore, few studies have been devoted to the analysis of genetic diversity in sugar beet using agro-morphological traits.

Hybrids are produced in sugar beet through crosses of diploid male sterile (CMS) lines with tetraploid, or increasingly, diploid pollinator lines, resulting in triploid or diploid cultivars, respectively (Fénart *et al.*, 2008). The objectives of the present study were to: (i) investigate genetic diversity among and within sugar beet crossing parents differing in salinity and drought tolerance, and (ii) compare morphological and molecular methods to assess genetic relationships in sugar beet. The assessment of genetic diversity was conducted using agro-morphological traits as well as SSR and EST-SSR markers. The suitability of microsatellite markers for characterization of sugar beet germplasm including pollinator families (pollen parents) and CMS lines (seed parents) was also assessed.

MATERIALS AND METHODS

Plant Materials and Experimental Conditions

In this study, 8 diploid pollinators (S_0 and S_2) and 4 diploid CMS lines were used, some of which were selected for either salinity or drought tolerance (Table 1). They

Table 1. Description of the 8 diploid multigerm pollen parents and 4 diploid monogerm seed parents of sugar beet used in this study.

ID code	Pollen parents	Type	Germplasm description
1	181	Salt sensitive	S ₀
2	7233-P.29	Salt tolerant	S ₀
3	BP-Mashhad	Drought tolerant	S ₀
4	M249	Drought sensitive	S ₂ family selected from BP-Mashhad population
5	191	Salt sensitive	S ₀
6	M224	Drought tolerant	S ₂ family selected from BP-Mashhad population
7	29823-P.5	Salt sensitive	S ₂ family selected from 7233-P.29 population
8	29819-P.17	Salt tolerant	S ₂ family selected from 7233-P.29 population
Seed parents			
9	26039	Salt tolerant	CMS line
10	26051	Salt tolerant	CMS line
11	26564	Salt sensitive	CMS line
12	25944	Salt sensitive	CMS line

were chosen to represent parent materials employed in hybrid production breeding programs. Two entries, namely, 7233-P.29 and the breeding population-Mashhad (BP-Mashhad) as genetically broad open pollinated populations, were considered as the base populations (Table 1). The BP-Mashhad population was improved after 3 cycles of simple recurrent selections using selected roots for drought tolerance under early season drought stresses at Sugar Beet Seed Institute (SBSI) of Iran. The selected roots were planted in one seed-plot, and the bulked seed was coded as SBSI24367 BP-Mashhad. The population of 7233-P.29 was improved after some cycles of simple recurrent selections using selected roots for salinity tolerance under saline field conditions. Two S₂ families M249 (as drought sensitive) and M224 (as drought tolerant) were selected from the BP-Mashhad population under drought conditions. Two S₂ families: 29823-P.5 (as salt sensitive) and 29819-P.17 (as salt tolerant) were selected from the 7233-P.29 base population under saline conditions. Populations "181" and "191" were S₀ diploid pollinators and originated from eastern Europe (Sadeghian and Johansson, 1993). Four CMS lines were selected under saline

conditions (~16 dS m⁻¹) in a greenhouse experiment (Table 1).

All the entries (8 pollinators and 4 CMS lines) were grown under field conditions using a randomized complete block design with 4 replications in 2010 and 3 replications in 2011. Identical plot sizes of 3 rows of 8 m long with a row spacing of 60 cm were used in both years. The experiment in 2010 was conducted at the research farm of Isfahan University of Technology located at Lavark, Iran (40 km south west of Isfahan, 32° 32' N and 51° 23' E, 1630 m asl). The experiment in 2011 was conducted at the Agricultural Research Experiment Station located at Rodasht (65 km east of Isfahan, 328290 N and 528100 E, 1560 m asl).

Agro-morphological Traits

Leaf weight (including crowns, the uppermost part of the taproot where leaves emerge) and root yield (RY) were determined after harvest. Pulps prepared of the taproot materials were quick-frozen and stored at -26°C until analysis. Dry matter (DM) concentration of taproot was determined from subsamples after oven drying at 105°C for 24 hours.



Quality-related traits in the two experimental years (2010 and 2011) were analyzed using Betalysar (ICUMSA, 2003). The instrument measures Na⁺ and K⁺ via its flame photometer, α-amino-N by double beam filter photometry using the blue number method, and sugar content (SC) through its polarimeter.

Molasses sugar (MS) and extraction coefficient of sugar (ECS) were calculated using the following formula (Reinefeld *et al.*, 1974):

$$MS = 0.343 (K^+ + Na^+) + 0.094 (\alpha\text{-amino-N}) - 0.31$$

$$ECS = (WSC/SC) \times 100$$

White sugar content (WSC), sugar yield (SY) and white sugar yield (WSY) were calculated according to the following formula:

$$WSC = SC - MS, SY = RY \times SC \text{ and } WSY =$$

RY × WSC.

Alkaline level content (ALC) was calculated using the following formula (Reinefeld *et al.*, 1974):

$$ALC = (K^+ + Na^+) / \alpha\text{-amino-N}$$

SSR Marker Analysis

Genomic DNA was extracted from 14 randomly selected plants from shoots of 3-week-old seedlings of each population/line that was sown in the greenhouse and following the protocol of Murray and Thompson (1980). This made total DNA samples of 168 plant genotypes. Twenty-six SSR, EST-SSR and STS markers selected from several sources covering the whole sugar beet genome were used (Table 2). At

Table 2. List of 26 markers used with marker type, chromosome location, allele size(bp), average number of alleles per locus, polymorphism information content (PIC), observed heterozygosity (Ho) and the expected heterozygosity (He) using 168 plant samples.

Marker locus	Marker type	Chromosome location	Reference ^a	Allele size (bp)	No. of Alleles	PIC	Ho	He
2KWS	SSR	2	8	234-266	8	0.744	0.476	0.773
<i>AiNHX3</i> ^b	STS	-	6	-	-	-	-	-
<i>badh</i>	SSR	5	5	800	1	-	-	-
<i>Bmb3</i>	SSR	-	2	262-282	6	0.724	0.417	0.760
<i>BQ583448</i> ^b	SSR	1	1	-	-	-	-	-
<i>BQ588629</i>	SSR	1	1	164-196	6	0.721	0.399	0.753
<i>Bvm3</i>	SSR	9	4	115-143	8	0.692	0.351	0.722
<i>Cmo</i> ^b	SSR	6	5	-	-	-	-	-
<i>FDSB1007</i>	SSR	8	4	280-296	6	0.739	0.113	0.774
<i>FDSB1027</i>	SSR	3	4	207-235	4	0.706	0.571	0.748
<i>FDSB1250</i>	EST_SSR	7	4	176-184	4	0.468	0.202	0.516
<i>FDSB1300</i>	EST_SSR	2	4	130-146	3	0.548	0.464	0.624
<i>FDSB1427</i> ^b	EST_SSR	9	4	-	-	-	-	-
<i>FDSB502</i>	EST_SSR	7	4	272-329	10	0.836	0.214	0.850
<i>FDSB568</i>	EST_SSR	6	4	222-248	5	0.631	0.417	0.683
<i>FDSB957</i>	EST_SSR	3	4	126-158	9	0.645	0.179	0.684
<i>GTT1</i>	SSR	6	1	134-138	2	0.361	0.226	0.402
<i>SB04</i>	SSR	5	3	192-208	6	0.797	0.554	0.822
<i>SB06</i>	SSR	4	3	163-181	4	0.566	0.339	0.636
<i>SB07</i>	SSR	4	3	272-290	7	0.721	0.232	0.756
<i>SB13</i>	SSR	3	3	140-148	4	0.480	0.244	0.543
<i>SB15</i>	SSR	5	3	154-184	9	0.736	0.441	0.766
<i>USDA29</i>	SSR	8	1	152-160	3	0.415	0.476	0.521
<i>BvGer165</i>	STS	-	7	515	1	-	-	-
<i>BvGer171</i>	STS	-	7	830	1	-	-	-
<i>BvGer172</i>	STS	-	7	850	1	-	-	-

^a 1 (McGrath *et al.*, 2007); ² (Cureton *et al.*, 2002); ³ (Richards *et al.*, 2004); ⁴ (Laurent *et al.*, 2007); ⁵ (Schneider *et al.*, 1999); ⁶ (Liu *et al.*, 2008); ⁷ (De los Reyes and McGrath, 2003), and ⁸ kindly provided for research purpose by KWS Saat AG, Einbeck, Germany, ^b Not amplified.

least 2 markers were located on each linkage group. Amplification reactions were carried out in 10 μ L mixture containing 30 ng template DNA, 0.1 mM of each dNTPs, 0.3 μ M of reverse primer, and 0.08 μ M of M13-tailed forward primer at the 5' end, 0.05 μ M of the universal fluorescent-labeled (IRD700/800) M13 primer (5'-tgtaaacgacggcagcag-3'), 1.5 mM of MgCl₂ and 0.5 U *Taq* DNA polymerase (Schuelke, 2000). The PCR amplification was performed using a PTC 200 thermal cycler (Bio-Rad Laboratories Inc.). The PCR reaction used the following profile: initial denaturation for 3 minutes at 95°C followed by 35 cycles with 30 seconds denaturation at 94°C, 45 seconds at primer-specific annealing temperature, 45 seconds at 72°C, and a final extension at 72°C for 10 minutes. The reaction was terminated with a continuous cycle at 6°C. The labeled PCR products from multiplex amplification with IRD 700 and IRD 800 were bulked in equal proportion before loading and fragments were separated in a 6.5% denaturing polyacrylamide gel with the DNA analyzer LI-COR 4300 (LI-COR Inc., Lincoln, NE, USA.). This part of the research was performed in Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops in Germany.

Statistical Analysis

Morphological Data

A two-way analysis of variance (ANOVA) was carried out to estimate the variance components of genotype, year and genotype \times year interaction for each of the studied traits using PROC GLM of SAS 9.1 (SAS Institute, 2001). Broad-sense heritability (h^2_b) of the studied traits was estimated based on the mean of plot according to the following equation (Burton and De Vane, 1953):

$$h^2_b = \sigma^2_g / (\sigma^2_g + (\sigma^2_e/r))$$

Where, σ^2_g and σ^2_e are genotypic and residual variances, respectively, and r is the number of replications. Phenotypic correlation coefficients between traits were estimated using the following equation (Burton and De Vane, 1953):

$$r(xy) = \text{Cov}(x,y) / [V(x) \times V(y)]^{1/2}$$

Where, $r(xy)$ is the correlation coefficient between x and y characters; $\text{Cov}(xy)$ is the covariance between x and y characters; $V(x)$ is the variance of x character; and $V(y)$ is the variance of y character. The genotypic coefficient of variation (CV_g) and phenotypic coefficient of variation (CV_p) were calculated as: $CV_g = (\sigma_g / \mu) \times 100$ and $CV_p = (\sigma_p / \mu) \times 100$ where, σ_g and σ_p are the standard deviations of the genotypic and phenotypic effects, respectively, and μ is the phenotypic mean (Burton and De Vane, 1953).

A genotype-by-trait table on yield and quality-related traits (across two years) was derived from the raw data and displayed in a bi-plot to visualize the genetic correlations among the various traits and to determine the traits most effective in discriminating the entries. These calculations were performed using STATISTICA 6.0 (Stat Soft Inc., Tulsa, OK, USA).

SSR Data

For each marker locus, the average number of alleles per locus, the expected heterozygosity (H_e), the observed heterozygosity (H_o), and polymorphism information content (PIC) for assessing the informativeness of each marker were calculated using SAS 9.3. Cluster analysis among the 168 genotypes used was performed *via* the unweighted pair group method using arithmetic averages (UPGMA) based on Jaccard's similarity coefficients. To obtain a visual representation of genetic relationships among the genotypes, a principal coordinate analysis (PCo) of the 168 individual genotypes at 104 loci was



conducted using Jaccard's similarity coefficients and the NTSYSpc 2.0 program.

A binary matrix was used to calculate the analysis of molecular variance (AMOVA) using the program Arlequin version 3.1 (Excoffier *et al.*, 2005). The degree of inbreeding within groups (F_{SC}), the degree of relatedness between markers within groups (F_{CT}), and the fixation index (F_{ST}) were computed according to Weir and Cockerham (1984).

RESULTS

Morphological Traits

The results of combined analysis of variance showed that the genotypes differed significantly ($P < 0.01$) for most of the studied traits including root yield, sugar content, white sugar content, white sugar yield, extraction coefficient of sugar, and sugar yield (results not shown). The effect of year was highly significant for all traits with the exception of Na^+ and alkalinity coefficient. There was a genotype \times year interaction for root yield, sugar yield, and white sugar yield.

Means, ranges, coefficient of variation, and broad-sense heritability of the studied traits in the 2010 and 2011 experiments are presented in Table 3. Root yield ranged from 16.4 to 54.5 t ha⁻¹ in 2010 and 12.8 to 69.2 t ha⁻¹ in 2011. Leaf weight (LW) and alkalinity coefficient possessed the highest phenotypic and genotypic coefficient of variation in 2010. The Broad-sense heritability estimated for economically important traits, root yield and white sugar yield, were fairly high (85 and 89%, respectively) in 2010. In both years, the lowest h^2_b (0.41 and 0.16, respectively) belonged to α -N.

In 2010 and 2011, positive and highly significant correlations ($r = 0.97^{**}$ and $r = 0.98^{**}$, respectively) were observed between root yield and sugar yield (Table 4). Root yield was also positively associated

with leaf weight and white sugar yield while there was no significant association between sugar content and root yield. In both years, sugar content was positively correlated with extraction coefficient of sugar and dry matter while it was negatively associated with Na^+ and molasses sugar (Table 4).

A genotype-trait type biplot visualized the genetic associations between various traits (Figure 1). The first 2 principal components (PC) explained 43.01 and 32.65% of the total variability. The cosine of the angle between the vectors of the 2 traits approximates the genetic correlation coefficients between them. A 90° angle means a zero correlation (completely independent), a 0° angle means a correlation of +1, and an 180° angle means a correlation of -1. An acute angle indicates a positive correlation, while an obtuse angle indicates a negative correlation. The biplot showed that yield-related traits (i.e., root yield, sugar yield, and white sugar yield) had the same discriminating values for the genotypes as did extraction coefficient of sugar, white sugar content, sugar content, and dry matter. Traits with short vectors were less variable among genotypes. For example, α -N (amino nitrogen content) has the shortest vector and, hence, variation among genotypes for amino nitrogen content (α -N) should be very small. It is interesting to note that the PC analysis could almost discriminate between salinity and drought genotypes (see Figure 1; genotype numbers 3, 4, 6 vs. 2, 5, 11, 12). The very small angle between sugar yield and root yield shows their strong positive correlations, which supports their highly significant correlation presented in Table 4.

SSR Markers

Twenty six microsatellites were used to test the genetic diversity of 168 genotypes comprising 56 CMS lines as seed parents and 112 S₀ and S₂ families as pollen parents (Table 2). Eighteen SSR markers generated polymorphism in the germplasm studied.

Table 3. Means, ranges, coefficients of variation and broad sense heritability calculated for 13 morphological traits evaluated in 2010 and 2011.

Trait	Mean		Min-Max		CVp ^a (%)		CVg ^b (%)		h ² b ^c		h ² b ^c	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
RY (t ha ⁻¹) ^e	34.71	39.12	16.40-54.50	12.8-69.2	8.64	27.71	7.98	17.04	0.85	0.38	0.51	0.51
LW (t ha ⁻¹) ^f	13.41	15.31	5.60-23.40	4.23-26.60	12.81	21.32	11.17	12.21	0.76	0.37	0.43	0.43
SC (%) ^g	15.32	19.34	13.05-18.15	16.55-21.2	5.26	4.45	5.10	2.15	0.94	0.23	0.49	0.49
Na ⁺ (mmol kg ⁻¹) ^h	4.22	4.25	2.53-6.39	2.21-6.8	10.17	25.22	8.94	12.30	0.77	0.24	0.36	0.36
K ⁺ (mmol kg ⁻¹) ⁱ	7.15	4.17	5.57-8.99	2.8-5.98	5.58	12.26	5.07	5.39	0.83	0.19	0.23	0.23
α-N (mmol kg ⁻¹) ^j	3.23	2.66	2.05-4.90	1.01-4.88	5.55	26.03	3.54	10.53	0.41	0.16	0.24	0.24
ALC	3.69	3.67	2.17-6.15	1.66-8.42	13.58	38.39	12.69	23.03	0.87	0.36	0.57	0.57
MS (%) ^l	3.89	2.83	2.92-4.95	2.07-3.59	5.90	12.91	5.90	9.47	0.84	0.54	0.49	0.49
WSC (%) ^m	10.83	15.92	8.49-14.58	12.73-18.53	9.58	7.07	9.33	4.08	0.95	0.33	0.51	0.51
ECS (%) ⁿ	70.33	82.15	62.02-80.34	76.35-87.4	4.37	3.02	4.21	2.04	0.93	0.46	0.59	0.59
DM (%) ^o	24.44	28.54	5.00-22.23	26.16-31.91	3.39	3.43	3.29	0.45	0.94	0.20	0.37	0.37
SY (t ha ⁻¹) ^p	5.305	7.53	2.25-8.15	2.49-13.81	6.60	26.72	5.97	15.13	0.82	0.32	0.31	0.31
WSY (t ha ⁻¹) ^q	3.73	6.21	1.46-6.00	2-11.92	6.87	27.35	6.49	14.67	0.89	0.29	0.40	0.40

^a CVp= Genotypic coefficient of variation; ^b CVg= Phenotypic coefficient of variation, and ^c h² b^c= Broad-sense heritability. ^e Root Yield; ^f Leaf Weight; ^g Sugar Content; ^h Sodium content; ⁱ Potassium content; ^j α-amino nitrogen content; ^k Alkalinity Coefficient; ^l Molasses sugar; ^m White Sugar Content; ⁿ Extraction Coefficient of Sugar; ^o Dry Matter; ^p Sugar Yield, and ^q White Sugar Yield;

Table 4. Correlation coefficients between yield and quality-related traits in sugar beet populations grown in 2010 (above diagonal) and 2011 (below diagonal).

Traits	RY ^a	LW ^b	SC ^c	Na ⁺ ^d	K ⁺ ^e	α-N ^f	α-N ^f	AIC ^g	WSC ^h	SY ⁱ	WSY ^j	MS ^k	ECS ^k	DM ⁿ
RY	0.81**													
LW	0.75**	0.81**												
SC	-0.25	-0.26	0.81**											
Na ⁺	-0.39*	-0.57**	-0.59**	0.81**										
K ⁺	0.28	0.06	0.04	-0.49*	0.81**									
α-N	-0.04	0.21	0.15	-0.22	0.55*	0.81**								
ALC	-0.23	0.15	-0.23	0.49*	-0.48*	-0.83**	0.81**							
WSC	-0.11	0.24	0.97**	-0.70**	-0.02	0.03	-0.21	0.81**						
SY	0.98**	0.70**	-0.08	-0.51**	0.27	-0.03	-0.27	0.07	0.81**					
WSY	0.96**	0.71**	0.02	-0.58**	0.24	-0.05	-0.28	0.17	0.99**	0.81**				
MS	-0.28	0.35*	-0.59**	0.77**	0.16	0.29	0.09	-0.77**	-0.41*	-0.51**	0.81**			
ECS	0.09	-0.19	0.81**	-0.78**	-0.10	-0.16	-0.13	0.93**	0.26	0.37	-0.95**	0.81**		
DM	-0.37	0.09	0.84**	-0.48*	0.13	0.43*	-0.44*	0.78*	-0.24	-0.16	0.59**	0.72**	0.81**	

^a Root Yield; ^b Leaf Weight; ^c Sugar Content; ^d Sodium content; ^e Potassium content; ^f α-amino nitrogen content; ^g Alkalinity Coefficient; ^h White Sugar Content; ⁱ Sugar Yield; ^j White Sugar Yield; ^k Molasses sugar; ^l Extraction Coefficient of Sugar; ^m Dry Matter; * and ** significant at P<0.05 and P<0.01, respectively.

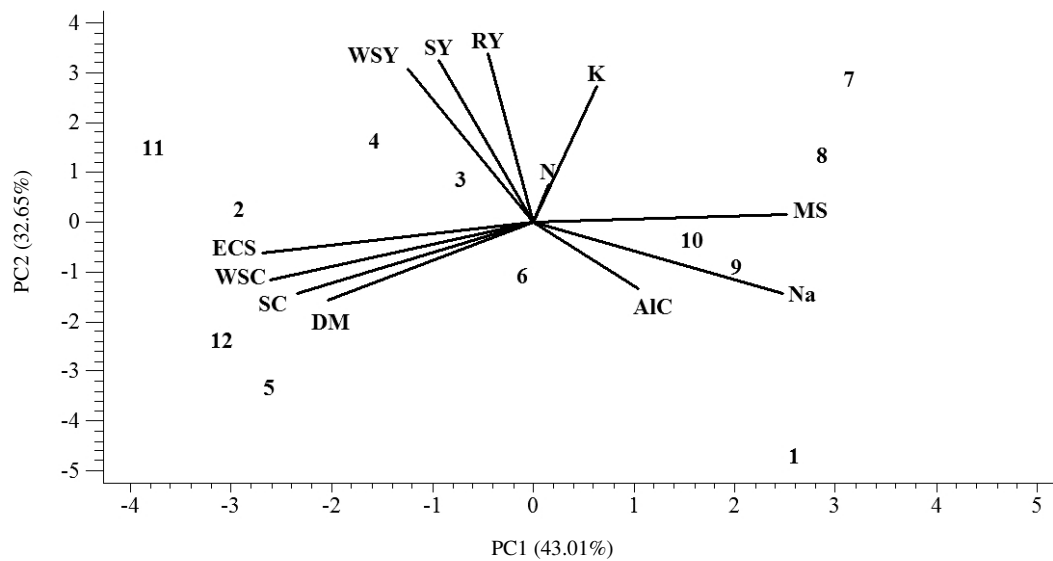


Figure 1. A genotype-by-trait bi-plot showing the genetic relations between agro-morphological traits. PC1 and PC2 refer to the first and second principal component, respectively. The details of the genotypes' codes represented here by numbers presented in Table 1. RY: Root Yield; SC: Sugar Content; Na: Sodium content; K: Potassium content; ALC: ALkalinity Coefficient; MS: Molasses Sugar; WSC: White Sugar Content; ECS: Extraction Coefficient of Sugar; DM: Dry Matter; SY: Sugar Yield, and WSY: White Sugar Yield.

Four markers (*AtNHX3*, *BQ583448*, *cmo* and *FDSB1427*) did not amplify and 4 markers (*badh*, *BvGer165*, *BvGer171*, and *BvGer172*) produced monomorphic bands (Table 2). A total of 104 alleles were detected for 18 SSR loci. The number of alleles per locus varied from 2 (primer pair *GTT1*) to 10 (primer pair *FDSB502*), with a mean of 5.7 alleles per locus (Table 2). The EST-SSR *FDSB502* generated a PIC value of 0.84 and 5 uncommon alleles (0.06, 0.04, 0.02, 0.02, 0.06), indicating that this SSR primer was particularly informative for this study. An example of DNA bands amplified

with *FDSB502* in 42 sugar beet genotypes is presented in Figure 2. The size of the polymorphic alleles ranged from 115 to 329 bp. PIC had a mean value of 0.64 and ranged from 0.36 for *GTT1* to 0.84 for *FDSB502*, demonstrating the good discriminatory power of the markers (Table 2).

Cluster and PCo Analyses

Scoring of the markers allowed the construction of a 104×168 binary array, which was subsequently applied to Jaccard's similarity coefficients for all pairs of

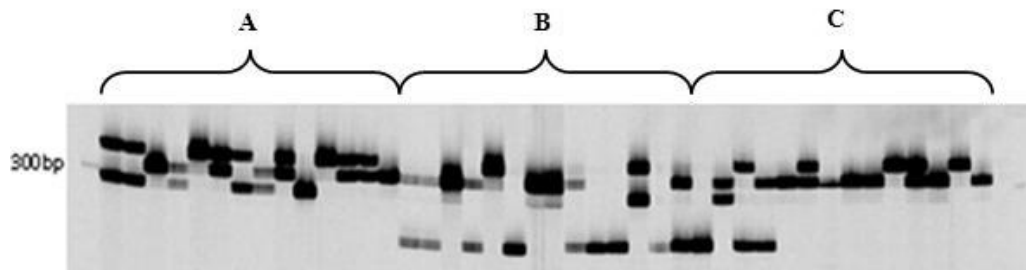


Figure 2. PCR amplification profile of 14 plants of each (A) 26039 CMS; (B) 26051 CMS, and (C) 26564 CMS lines in *Beta vulgaris* using SSR marker *FDSB502*.

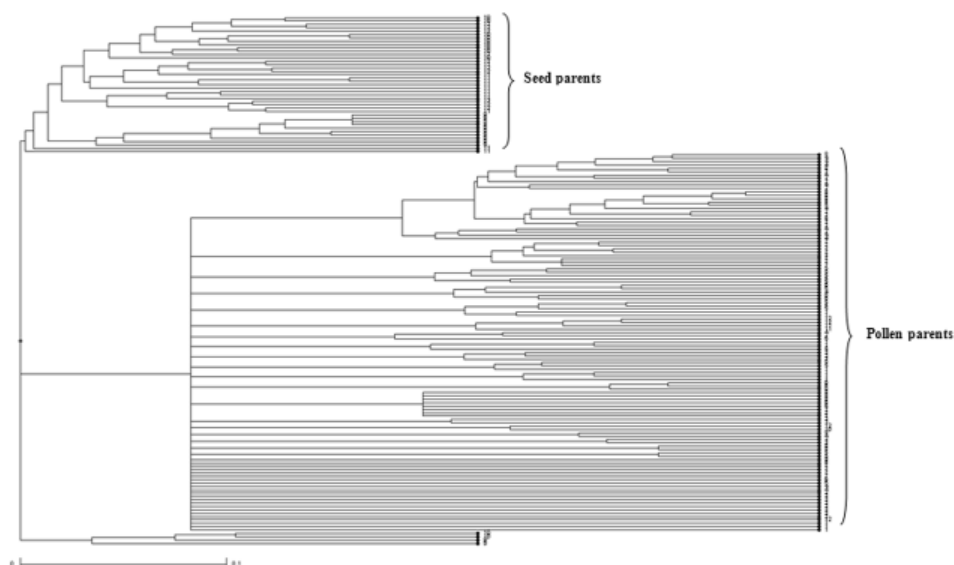


Figure 3. Dendrogram resulting from an UPGMA clustering of 168 genotypes of sugar beet based on 18 SSR markers

accessions studied. The mean genetic distance values ranged from 0.26 for 26039 (CMS line) and 25944 (CMS line) to 1 for M224 and 29823-P.5 (Results not shown). In the dendrogram generated from the data set, the sugar beet genotypes belonging to the pollen parents and seed parents were clearly discriminated, which had a quite good fit to their breeding history (Figure 3).

The PCo analysis explained 43.45% of the

total variation observed in the first 2 principal coordinates (Figure 4). Four groups could be distinguished by a 2-dimensional representation of the dendrogram. It is interesting to note that SSR markers could largely distinguish S_0 populations from S_2 families. Moreover, salt-tolerant populations 2 and 8 and salt sensitive populations 1 and 5 were grouped into different clusters (Figure 4).

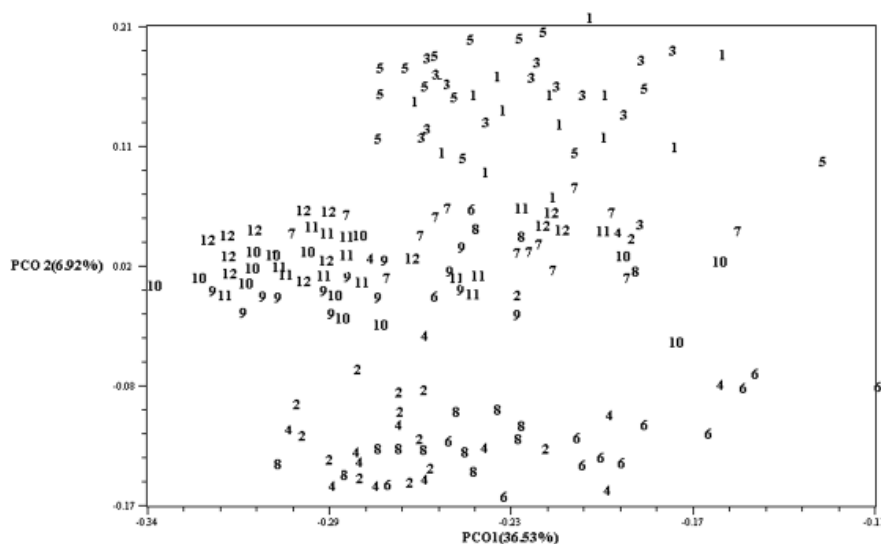


Figure 4. Principal coordinate analysis (PCo) of 168 sugar beet genotypes based on 18 SSR markers. The details of the genotypes' codes represented here by numbers presented in Table 1.



Analysis of Molecular Variance (AMOVA)

To conduct AMOVA, the data were organized into 2 main groups (pollen and seed parents) as suggested by the dendrogram obtained by grouping the 168 genotypes based on SSR markers (Figure 3). The results showed significant variations between groups, among populations within groups, and among individuals within populations (Table 5). However, the major variation, i.e. 68% of the total variance, was attributed to within-population variations. F_{ST} designating an estimation of genetic differentiation between subpopulations exhibited an average value of 0.32. Values of F_{ST} (fixation index) ranged from 0 (completely undifferentiated) to 1 (completely differentiated).

DISCUSSION

Hybrids are going to be the dominant cultivars grown in sugar beets worldwide. Therefore, essential breeding components of a hybrid development program geared towards improving *O*-type lines and a pollinator program geared towards breeding for desirable traits are being undertaken. Sugar beet (*Beta vulgaris* L.) is a sucrose-rich product with an extensive resistance to water stress, salinity, and cold that has found many industrial applications.

The negative correlation between root yield and sugar content observed in this study and many earlier studies conducted on sugar beet (Ahmadi *et al.*, 2011; Biancardi *et al.*, 2010; Hoffmann, 2010; Schneider *et al.*, 2002) shows that selection on both traits in an originally undifferentiated population could lead to differentiated populations, i.e. *E*-types with a large root yield and *Z*-types with a high sugar concentration. The explanation is consistent with the observation of Schneider *et al.* (2002) who reported that genes related to sugar content and root yield were mapped in the divergent genomic regions.

In this study, the heritability estimates obtained for Rodasht site (2011) were smaller than those obtained for Lavark site (2010). This result can be explained by the differences between the two sites for the environmental conditions and, in particular, due to the saline soil at the Rodasht site. This result is consistent with that of Ober and Rajabi (2010) who emphasized that the stress conditions caused heritability decreases in yield of sugar beet. In general, the genetic parameters such as genetic variance and heritability of a trait are smaller in stressed environments than under optimum growing conditions (Fernandez, 1993).

In the present study, SSR and EST-derived SSR amplified 2-10 alleles per locus with an average of 5.7 alleles. This is comparable to the 2-11 alleles found by Richards *et al.* (2004) for their microsatellite markers in a set of sugar beet and sea beet plants. Desplanque *et al.* (1999) and Viard *et al.* (2002) found up to 10 alleles per locus in wild sugar beet. Smulders *et al.* (2010) reported that microsatellite markers amplified 3-21 haplotypes in 40 diploid and triploid cultivars of sugar beet. Nevertheless, breeding systems that manage separate gene pools for paternal and maternal parents increase the gene diversity of individual plants, and the habit of working with pools of parental plants containing a large amount of genetic diversity (Viard *et al.*, 2002) may support the claim that the majority of genetic variation in the crop is present within crossing parents (De Riek *et al.*, 2007).

A high level of polymorphism recorded in the present study using 18 SSRs on 168 genotypes (PIC= 0.361–0.836, Mean= 0.64) indicated both the highly informative nature of the microsatellites and the diversity of the genotypes used. The degree of DNA sequence polymorphism detected in a species depends largely on the type of the germplasm used, the number of lines chosen, and the type of loci selected for analysis. Richards *et al.* (2004) characterized 8 microsatellite loci in 2 cultivated beet

accessions and 1 accession of the wild progenitor of sugar beet to obtain high PIC values. The genomic-SSRs and EST-derived SSRs showed similar levels of PIC values (PIC= 0.600 for SSR vs. PIC= 0.626 for EST-SSR) on the 168 sugar beet genotypes evaluated.

The 18 SSR markers were able to distinguish clearly between seed parents and pollen parents (Figure 3), which was in accordance with their breeding history. This finding is in good agreement with that of Li *et al.* (2010) who detected 2 distinct subgroups in sugar beet germplasm corresponding very well to the seed and pollen parent heterotic pools which had been genotyped with 23 SSR markers. In our study, 56 CMS genotypes were found to have low genetic variation and, thus, clustered closely together with short branch lengths between populations. This finding might be explained by the permanent selection for monogermity in the seed parent pool, which has the potential to lead to more homogeneous populations. These results indicate that the genetic diversity estimates between and within genotypes depends not only on the markers chosen for the particular study but also on the choice of the collections and individual genotypes analyzed (Viard *et al.*, 2002). It has been repeatedly reported that it is difficult to reliably resolve the relationships between closely related lines, even when a large number of markers are employed (Kraft *et al.*, 2000).

In the present study, several markers associated with quantity and quality-related traits, some of which related to more than two traits. *SB06* (c-180 bp) marker was significantly associated with such quality-related traits as Na⁺, K⁺ and N⁺ in root. Furthermore, two markers *SB07* (c-278bp) and *SB15* (e-166) related to root yield, sugar yield, and white sugar yield. This finding is agreement with that of Schneider *et al.* (2002) who reported that genes related to white sugar yield and root yield overlapped at a particular map position. In this study, *FDSB502* with high PIC value and

discriminative feature significantly associated with most of the quality-related traits such as, sugar content, white sugar content, and extraction coefficient of sugar. This marker, hence, appears to be an appropriate marker for employing in the sugar beet breeding programs aimed at improving the quality of sugar beet.

The results of this study indicate that the grouping of genotypes based on molecular data either by UPGMA clustering or PCo analyses leads to clear discrimination between seed and pollen parent, indicating, in turn, that both clustering methods are equally appropriate for assigning genotypes to subgroups. Comparisons between seed and pollen parents for all genetic measures showed that genetic distances between pollen parents were on average higher than those observed between seed parents. These findings might be explained by the higher selection intensity applied during the breeding of seed parents compared to pollen parents, possibly leading to the narrow genetic base of the seed parents.

The large variation residing at the within-population level (67.78% of the total variation in AMOVA) suggests that there would be enough variation at the population level to improve desirable traits. Similarly, other studies of sugar beet (De Riek *et al.*, 2001; De Riek *et al.*, 2007) have found an extensive within-population genetic diversity when using different types of molecular markers. De Riek *et al.* (2007) conducted AMOVA on 8 sugar beet cultivars using AFLP, CAPS, and SSR markers and attributed 94.4, 90.9 and 84.3%, respectively, of the total variations to within-population variations.

CONCLUSIONS

The high value of F_{ST} (0.32) could indicate that it is feasible to develop well-characterized populations to select parents with definitive characters and, in particular, with drought or salinity stress tolerance and high sugar yield. In the long term, though,



monitoring the levels of genetic diversity available would be advisable in order to refresh the variability existing at the within-population level, to introgress valuable alleles from wild populations, and to prevent the loss of complementary gene interactions due to inbreeding. It is also concluded that SSRs are sufficiently polymorphic in sugar beet so that they offer a reliable and effective means of assessing genetic diversity and are capable of identifying breeding materials based on their breeding history. Moreover, by choosing SSR markers that are evenly distributed across the genome rather than the randomly distributed ones, it is possible to reduce their number while also achieving the same level of precision in assigning genotypes to groups and subgroups. Therefore, it would be possible to establish a small set of highly polymorphic SSRs to facilitate germplasm management and breeding strategies in cultivated sugar beet with due consideration to the principal obstacle of self-incompatibility in this crop.

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بررسی ژنتیکی والدین چغندر قند (*Beta vulgaris L*) با استفاده از صفات زراعی - مورفولوژیک و نشانگرهای مولکولی

ز. عباسی، ا. ارزانی و م.م. مجیدی

چکیده

در این پژوهش هشت گرده افشان (بعنوان والد پدری) و چهار لاین نرعقیم (بعنوان والد مادری) چغندر قند که از نظر تحمل به شوری و خشکی متفاوت بودند، با استفاده از صفات مورفولوژیک در دو سال زراعی وهم‌چنین با استفاده از نشانگرهای ریزماهواره ای مورد ارزیابی قرار گرفتند. صفات مورد بررسی شامل: عملکرد ریشه، وزن برگ، درصد قند، میزان سدیم، پتاسیم و α -آمینو ازت ریشه، ضریب آلکالوئیدی، میزان قند ملاس، درصد قند سفید، ضریب استحصال شکر، ماده خشک، عملکرد شکر و عملکرد شکر سفید بودند. تنوع ژنتیکی با استفاده از ۱۸ نشانگر ریزماهواره در چهارده بوته از هر والد (جمعاً ۱۶۸ ژنوتیپ) مورد مطالعه قرار گرفت. نتایج نشان داد که ۴۳٪ و ۳۲/۶٪ از کل تنوع موجود در صفات زراعی-مورفولوژیک توسط دو مولفه اصلی اول بیان شدند که این مولفه‌ها توانستند والدین متحمل به شوری و خشکی را از یکدیگر تفکیک کنند. براساس آنالیز مولکولی، ۱۸ نشانگر ریزماهواره پلی مورفیسم در این آزمایش در مجموع ۱۰۴ آلل با میانگین ۵/۷ آلل در هر جایگاه و با میانگین محتوای اطلاعات چند شکلی ۰/۶۴ ایجاد نمودند که بالاترین محتوای اطلاعات چند شکلی متعلق به نشانگر *EST-SSR FDSB502* بود. تجزیه خوشه‌ای بر اساس نشانگرهای *SSR* توانست به وضوح ۱۱۲ ژنوتیپ گرده‌افشان متعلق به والدین پدری را از ۵۶ لاین نرعقیم مادری تفکیک نماید. در نهایت، نتایج این پژوهش نشان داد که نشانگرهای *SSR* ابزاری کارآمد و قابل اعتماد برای ارزیابی تنوع ژنتیکی در والدین تلاقی چغندر قند می‌باشند.