SSR analysis of sugar beet parental forms of different origin for predicting possible heterotic effects

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Background

Pre-breeding is a first step in the use of genetic diversity from wild relatives and other unimproved germplasm. For cultivated sugar beet, many valuable for breeding improvement traits imported from wild beets are known – monogermity, cytoplasmic male sterility, genetic male sterility, salt tolerance, resistance to beet cyst nematode *Heterodera schahtii*, beet necrotic yellow vein virus BNYVV, *Rhizoctonia solani, Cercospora beticola*, frost tolerance, resistance to beet curly top virus BTCV(1). Beet accessions with such traits/genes are conserved in regional genebanks.

Sugar beet is an obligate cross-pollinated species with its breeding based on crosses between diploid male sterile (CMS) lines and mainly diploid pollinator populations. The most labor and time-taking stage in a hybrid production program exploiting the phenomenon of heterosis, is the identification of parental forms. Information on the genetic diversity and distance between the breeding lines, and the correlation between genetic distance and hybrid performance are important for classifying the parental lines, heterotic groups and predicting hybrid performance.
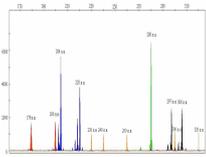
SSR alleles profile

Objective

The objective was to assess the capability of SSR molecular markers set to identify genetic variation among and within pedigree parental populations for further crosses output forecast.

In pursuance of the heterotic pattern concept as a specific pair of heterotic groups (defined as populations/lines of related/unrelated genotypes from the same/different populations which display a similar combining ability when crossed with genotypes from other germplasm groups) that express in their crosses high heterosis and high hybrid performance (2).

Plant materials

In this SSR analysis study we took 150 sugar beet plants representing 4 monogerm CMS lines with resistance to Rhizoctonia root rot and Cercospora Leaf Spot disease from Washington State University Regional Plant Introduction Station (pool # 1) and 220 sugar beet plants representing 5 populations of multigerm pollinators – three of which came from European breeding programs, including one gynogenetic DH line created by ourselves (pool # 2) and two others – from US breeding programs (pool#3). Description of the plant material is given in Table 1.

Table 1. Description of the 4 monogerm seed parents and 5 multigerm pollen parents used in this study

N	Name	Germplasm description	Type
1	CMS-1	Colorado, United States. Biennual. Breeding material. Diploid, monogerm. Cytoplasmic male-sterile. Resistant to root and crown rot (Rhizoctonia solani); relatively homogenous, easy bolting, moderately tolerant to the curly top virus. Moderate resistance to Cercospora leaf spot (Cercospora beticola)	Seed parents
2	CMS-2	Colorado, United States. Biennual. Breeding material. Diploid, monogerm. Cytoplasmic male-sterile. As the female in experimental hybrids has shown good combining ability for sucrose content as well as Rhizoctonia root rot resistance.	
3	CMS-3	California, United States. Biennial. Breeding material. Diploid, monogerm. Near cytoplasmic male sterile. Reproductive uniformity: Population.	
4	CMS-4	California, United States. Biennual. Breeding material. Pedigree. Diploid, monogerm. Curly top resistant male-sterile line.	
5	Janasz DH	Minsk, Belarus. Doubled haploid line of gynogenetic origin developed in vitro from unfertilized ovules of diploid Yanash A 3 cultivar with high % sugar content (Poland) donor plants. Fertile. Diploid. Multigerm, with good agronomic performance and specific combining ability.	Pollen parents
6	OP 5050	Krasnodar, Russian Federation. Fertile. Diploid, multigerm, with good agronomic performance. Moderate resistance to Cercospora leaf spot (Cercospora beticola). With good specific combining ability.	
7	OP12099	Krasnodar, Russian Federation. Fertile. Diploid, multigerm, with good agronomic performance. Moderate resistance to Cercospora leaf spot (Cercospora beticola). With good specific combining ability.	
8	PI 663871	California, United States. Annual/Biennial and/or Perennial. Breeding material. Homozygous resistant to root knot nematode caused by species of Meloidogyne, segregating for resistance to Rhizomania (Rz1) caused by Beet necrotic yellow vein virus, moderate resistance to curly top, virus yellow, powdery mildew, bolting, and Erwinia root rot. Mostly self-sterile, multigerm, with good agronomic performance. Pedigree.	
9	PI 663872	California, United States. Annual/Biennial and/or Perennial. Breeding material. Self-fertile, multigerm population that segregates for genetic male sterility and for resistance to Rhizomania (Rz1) caused by Beet necrotic yellow vein virus. A source for combined disease resistance in a high sugar background, about 25% of which comes from very high % sugar accessions from Poland.	

Molecular and Statistical Analysis

DNA was extracted (3) from leaves of 40+ individual 1-month old seedlings for each pollen parental populations and from leaves of 40+ individual 2-year vegetation plants for each seed parental populations. All plants were characterized using in one probe 6 SSR markers Gtt1, FDSB 1007, FDSB1011, FDSB1027, FDSB1033, Sb6 (4-6) located on 6 different linkage groups in order to assess multiallelism and heterozygosity in parental forms. For each marker locus, the average number of alleles, the expected heterozygosity (He), the observed heterozygosity (Ho) and polymorphism information content (PIC) were calculated by GenAlex version 6.1: genetic analysis in Excel. For the purpose of estimating genetic diversity, TREECON for Windows version 1.2 with UPGMA method of clustering and PCA analysis were carried out. The SSR loci alleles identification and size assessment were carried out using Genetic Analyzer 3500 (Applied Biosystems).

Results

In 370 sugar beet plants a total of 25 alleles representing 6 SSR markers were detected with an average allele number of 3.58 per locus. The number of alleles was higher in pollen parents as compared to seed parents. PIC values ranged between 0.04 and 0.78 demonstrating high SSR marker diversity within 9 sugar beet populations under investigation. Heterozygosity (Ho) rate in both parental groups was relatively low and varied from 0.07 – 0.39. The results for the AMOVA for 9 entries revealed that the within entry variation (49%) was a bit less than the between entry variation (51%). PCA analysis of SSR data ranked sugar beet germplasm into pools of Amercan origin and European origin (Fig. 1) and set apart seed parents from pollen parents (Fig.2)
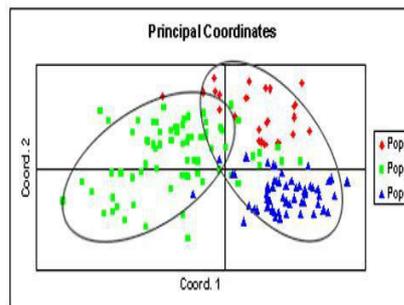
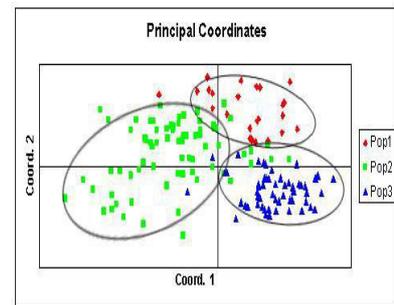Fig.1 PCA of sugar beet genotypes (Pop1+Pop 3) of **American origin** and of **European origin** (Pop 2) based on 6 SSR markers.

Fig.2 PCA of sugar beet **seed parents** of American origin (Pop1) and **pollen parents** of American origin (Pop 3) and of European origin (Pop 2) based on 6 SSR markers.

Conclusions

Based on SSR-markering results and pedigree information from breeders, predictions for parental combinations were done. It is planned to make crosses in the field between entries 1 (CMS-1) and 2 (CMS-2) with entries 5 and 6 (Janasz DH and OP 5050) from European breeding schemes as well as with entries 8 and 9 PI 663871 and PI 663872 from US. Hybridization will take place this year. Final results on yield, sucrose content and diseases display in crosses will be known in 2014. They might give us certain chance for identification of heterotic group -pairs of low inbreeding inbreds with good combining ability leading to formation of hybrid cultivar with high mean performance and genetic variance.

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