

Original Research Paper

SSR Analysis of the Chloroplast and Mitochondrial Genomes of Cultivar Lines and Wild Types of the Sunflower (*Helianthus L.*)

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Abstract: SSR analysis of chloroplast and mitochondrial genomes of cultivar lines and wild species of sunflower (*Helianthus L.*) were completed. The polymorphism of 18 microsatellite loci of the chloroplast and mitochondrial genomes was investigated. Based on the obtained data was constructed dendrogram showing the genetic diversity of chloroplast and mitochondrial genomes of sunflowers. The studied species were clustered into annual and perennial. This set of novel polymorphic SSR markers can be useful for breeders and molecular biologists for the genetic diversity analysis, lines identification and improve breeding.

Keywords: Chloroplast Genome, Mitochondrial Genome, SSR Markers, Polymorphism, Sunflower

Introduction

Sunflower is a valuable crop cultivated all over the world. To intensify the crops selection, carry out investigation of DNA polymorphism inbred lines and wild sources plants (Usatov *et al.*, 2014; Kaur *et al.*, 2019). Modern research methods allow the most effective study of the genetic diversity of organisms and use it in population and breeding studies (Song *et al.*, 2018). Molecular markers are among the pre-requisites to accelerate the crop breeding program through genomics assisted breeding (Bohra *et al.*, 2017). Furthermore, plant breeding still rely heavily on the inner genetic resources, which are essential to accurately identify genotypes and to delineate the various genetic relationships between available accessions in germplasm collections. These resources can be utilized effectively to preserve and develop the species and to enhance its applications (Mornkham *et al.*, 2016).

SSR markers are widespread tool in crop selection and plant systematics area (Zong *et al.*, 2015). Plastids and mitochondria are usually uniparentally inherited, typically maternally. As genomes of chloroplasts and mitochondria do not undergo sexual recombination are a

good tool for studying accumulated differences at them. The contribution of the cytoplasm to the adaptive potential of plants to environmental factors has a greater impact than previously thought (Mashkina *et al.*, 2010; Usatov *et al.*, 2016; Sobanski *et al.*, 2019). Generally, for cultivar lines characterized lower level of polymorphism of the chloroplast genome relative wild type forms (Wills *et al.*, 2005; Singh *et al.*, 2015).

The objective of the current research was to estimate the genetic diversity and Polymorphic Information Contents (PIC) among in the Sunflowers (*Helianthus L.*) species using PCR-based SSR markers.

Materials and Methods

Plant Materials

The study was carried out on the sunflower (*Helianthus L.*) from the collection of the All-Russian plant genetic resources named N.I. Vavilov. The samples was represented by *H. decapetalus*, *H. divaricatus*, *H. grosseserratus*, *H. hirsutus*, *H. microcephalus*, *H. occidentalis*, *H. rigidus*, *H. salicifolius*, *H. strumosus*, *H. tuberosus*, *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H.*

neglectus, *H. praecox*, *H. petiolaris*, *H. annuus* (15 different numbers of introductions) and 16 cultivar lines.

DNA Isolation

The total DNA was isolated from the first pair of true leaves of sunflower seedlings according to Boom *et al.* (1990) with our modification.

DNA Amplification

SSR analysis was carried out using 18 primer pairs, the forward of which (F) was labeled 5(6)-carboxyfluorescein (FAM) by the 5'-terminal. Design of primers based on the reference plastid genome of the HA383 line (NC_007977.1) and the mitochondrial genome of *H. annuus* (NC_023337.1) from the NCBI database. A few important properties of these primers are provided in Table 1. The PCR protocol was conducted in 25 µL of mixture according to manufacturer's instructions

(Eurogen, Moscow, Russia). The PCR cycles with the following specification: initial denaturation for 5 min at 95°C, 30 cycles of 15 s at 95°C (denaturation), 25 s at 58°C, 1 min 72°C and a final elongation for 10 min at 72°C. Amplification was performed in a thermocycler C1000 (Bio-Rad, California, USA).

Data Analysis

The level of cpSSR and mtSSR loci polymorphism was assessed by the Polymorphism Information Content (PIC) index, which was calculated as follows: $PIC = 1 - \sum p_i^2$, where p_i is frequency of i -th allele detected in the analyzed sampling (Nei, 1973).

A binary matrix was constructed by converting the data of allele sizes into “1” (present) or “0” (absence). UPGMA cluster analysis was completed with the treecon 1.3 b software.

Table 1: Primers used in the SSR analysis

Genome	№	Locus name	Sequence of flanking primers 5'-3'	Number of alleles	Size of amplifications fragments, bp	PIC
Chloroplast	1	<i>clpP</i> , intron	F: TTCAGCAATCCCAAAGTGGC R: AGTCCAGTTCAGCGTCACAA	8	120-139	0,959
	2	<i>atpB</i> – <i>rbcL</i>	F: GACGGCTCATCGGGATAGATG R: ATTTTACGGATTCTTTCCTATCCT	6	149-155	0,977
	3	<i>clpP</i> , intron	F: TGAGGGGAAGATACAAAGAAAGAT R: TCTGAAAGGAAGGGTGGTAAGT	3	186-188	0,937
	4	<i>trnR</i> – <i>trnG</i>	F: CGCTTTCATTGTGATTTTGGGT R: CCTCCAAGCTAACGATGCG	7	223-247	0,979
	5	<i>trnM</i> – <i>atpE</i>	F: GGCGGGAGTCATTGGTTCAA R: GCTAGGACACGAGTAGAGGC	10	276-291	0,989
	6	<i>psbK</i> – <i>psbI</i>	F: TGTCCGGTATGAAAGTGATTTGTGG R: TCACGTCCAGGATTACGTCC	5	302-306	0,972
	7	<i>psbC</i> – <i>trnS</i>	F: CGTCCGGTCAAAACCAATCC R: GTACATGGAGTCGGGGGAGA	12	322-336	0,995
	8	<i>clpP</i> , intron	F: CGCCTTTTGACGCATACGGT R: TGTGCAAATCAAAAATCGGGCG	13	350-378	0,994
	9	<i>rpoCI</i> , intron	F: AGCCGGATGAAAGGAAACTT R: TGCTTAGCCAATTCCATGCG	5	386-390	0,977
Mitochondrial	10	<i>trnE</i> – <i>coxI</i>	F: CGAACGAGATAAGGTTGTCAAATG R: GGAAGAAATGGATCCGAACGAA	3	128-130	0,883
	11	<i>trnS</i> – <i>cob</i>	F: GGACGCGAGAACCTAAGAAA R: GAGCGCTCCTGCACTATAC	10	158-169	0,991
	12	<i>rpl 16</i> – <i>matR</i>	F: GTCATCACCCAGGTCCAATAG R: GTTCGGGACGGCTGTATTT	6	176-181	0,950
	13	<i>atp1</i> – <i>ccmFn</i>	F: GCAATACGGAGAGAGGTAGAAAAG R: CCATGCCTTCTAATCGGCTAAT	4	277-283	0,912
	14	<i>atp1</i> – <i>ccmFn</i>	F: CTCGTAGCTCCCGTTTGATAC R: TGTCCCTTGATGACCAACCATAA	5	334-338	0,964
	15	<i>trnS</i> – <i>cob</i>	F: CTTGTTCCGTGCTATTGAGAGA R: AATCGTAGGTCCCCTCGTC	8	373-384	0,976
	16	<i>atp6</i> – <i>trnK</i>	F: GCTCGGCTAAATAGGCTCAAT R: CCCCTTCTCTGCTCCTTTCC	4	394-397	0,958
	17	<i>rpl5</i> – <i>trnD</i>	F: AGTGGGAAGTAATGAGGCTAGT R: CCCATGACTTTCCGGACCAA	4	432-435	0,964
	18	<i>trnD</i> – <i>trnK</i>	F: GCTGTACACCTGGGATTGTAG R: CGCTGACCGAATTCTGCTTC	4	460-464	0,921

Notation. F – forward primer, R – reverse primer, Polymorphism Information Content (PIC) – locus polymorphic information content index

Results and Discussion

In the present work, the polymorphism of 18 microsatellite loci of the chloroplast and mitochondrial genomes was investigated. The number of detectable alleles with use of the primers system ranged from 3 to 13 and averaged 6,5 for investigated samples. The greatest polymorphism was shown for 5, 7, 8, 11 primer pairs and was represented by 10, 12, 13, 10 allelic variants, respectively. Sizes of amplified fragments ranged from 120 to 465 bp. Loci are characterized by high heterogeneity, which is confirmed by the high values of PIC which was from 0,883 to 0,995 Table 1. Most lines have their own unique patterns that different from each other.

Based on the obtained data was constructed UPGMA-dendrogram showing the genetic diversity of chloroplast, mitochondrial genomes and cytoplasms in generally of sunflowers.

The dendrogram constructed for the chloroplast genomes includes three major clusters Fig. 1. The first cluster includes cultivar lines of sunflower. The second cluster is represented by annual sunflower species: *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H. neglectus*, *H. praecox*, *H. petiolaris* and 15 different numbers of introductions of *H. annuus*. The third cluster consists of perennial sunflower species: *H. decapetalus*, *H. divaricatus*, *H. grosseserratus*, *H. hirsutus*, *H. microcephalus*, *H. occidentalis*, *H. rigidus*, *H. salicifolius*, *H. strumosus*, *H. tuberosus*.

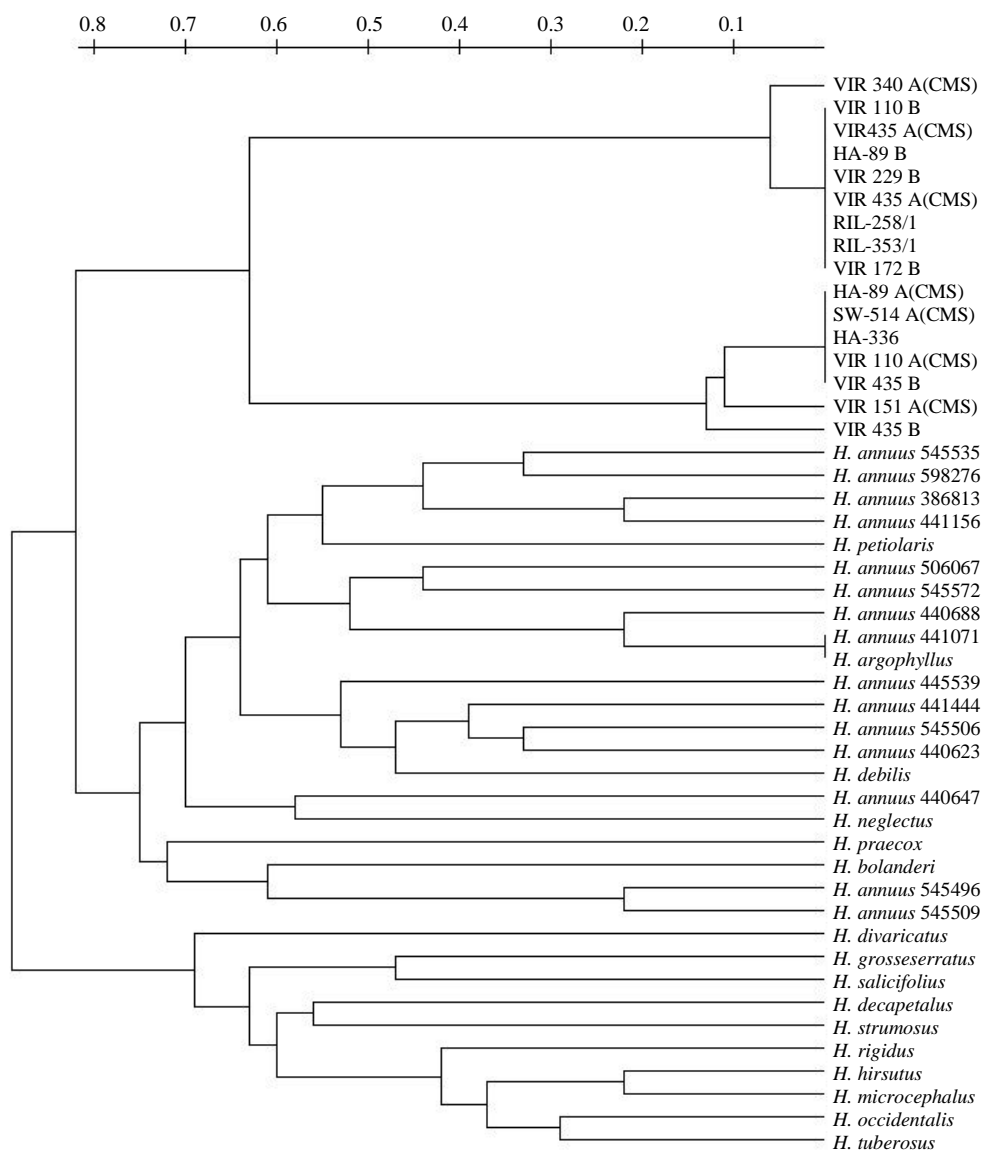


Fig. 1: The dendrogram of genetic diversity of sunflower, based on the results of SSR-analysis of cpDNA

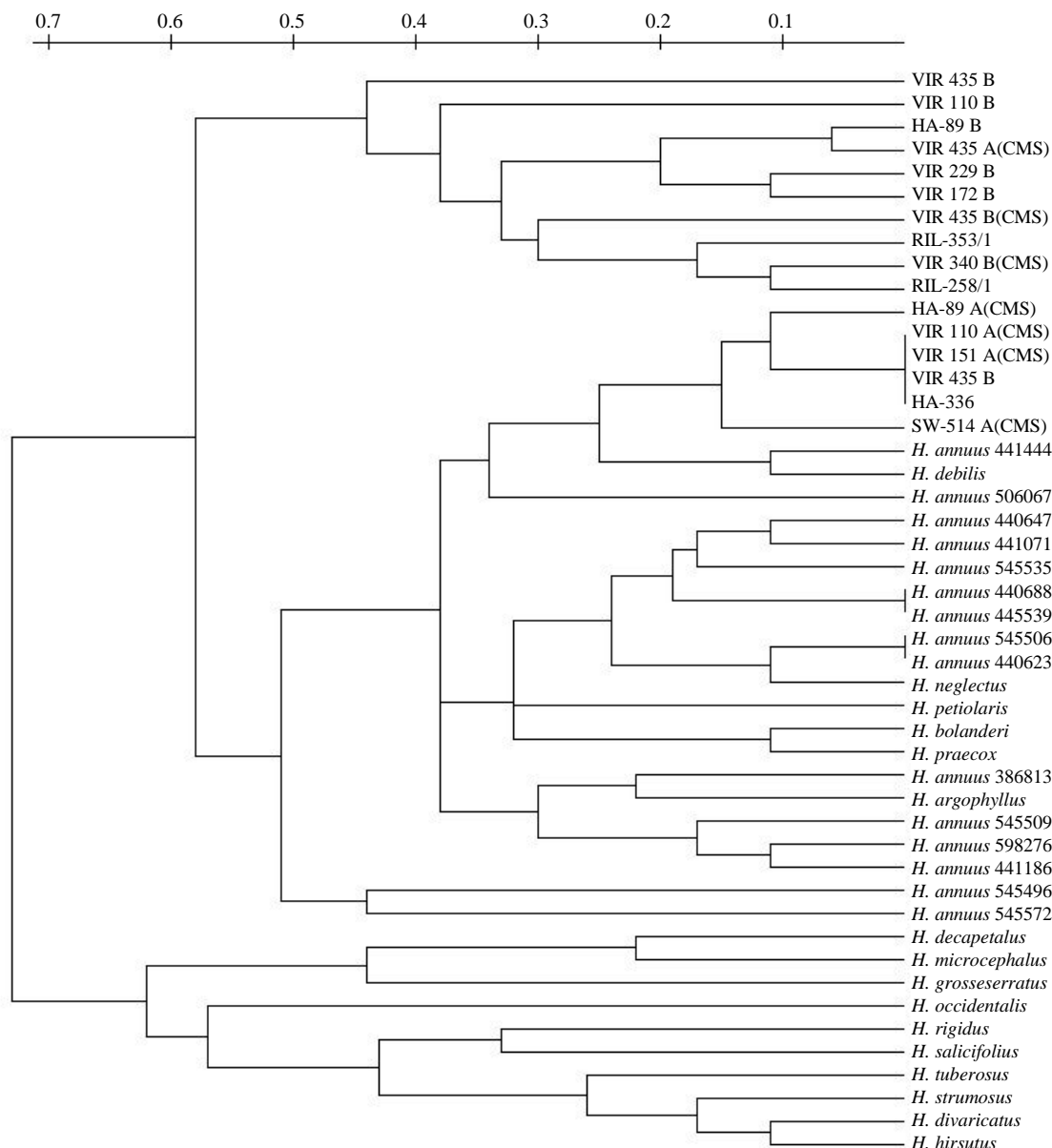


Fig. 2: The dendrogram of genetic diversity of sunflower, based on the results of SSR-analysis of mtDNA

The dendrogram constructed for the mitochondrial genomes includes three major clusters too Fig. 2. However, the cluster of wild annual forms sunflower includes cultivar lines such as HA-89 A (CMS), VIR 110 A (CMS), VIR 151 A (CMS), VIR 435 B, HA-336, SW-514 A (CMS).

The complete dendrogram constructed for the chloroplast and mitochondrial genomes includes three main clusters too but shows better detalization than individual ones Fig. 3.

Differentiation into annual and perennial species is probably due to the accumulation of mutations in the genome over time. These mutations are localized in

intergenic regions and do not receive the pressure of natural selection (Singh *et al.*, 2015). It is known that the selection process leads to the appearance of many homozygotes and, as a result, to a reduction in genetic diversity (Markin *et al.*, 2015). This explains the separation of cultural lines from annual wild species. The investigated markers are suitable for certification of a linear material and searching of genetic raw materials for selection.

Molecular markers can be used for genetic variation analysis if it has high polymorphism, codominance and specific characteristics and wide distribution (Aristya *et al.*, 2019).

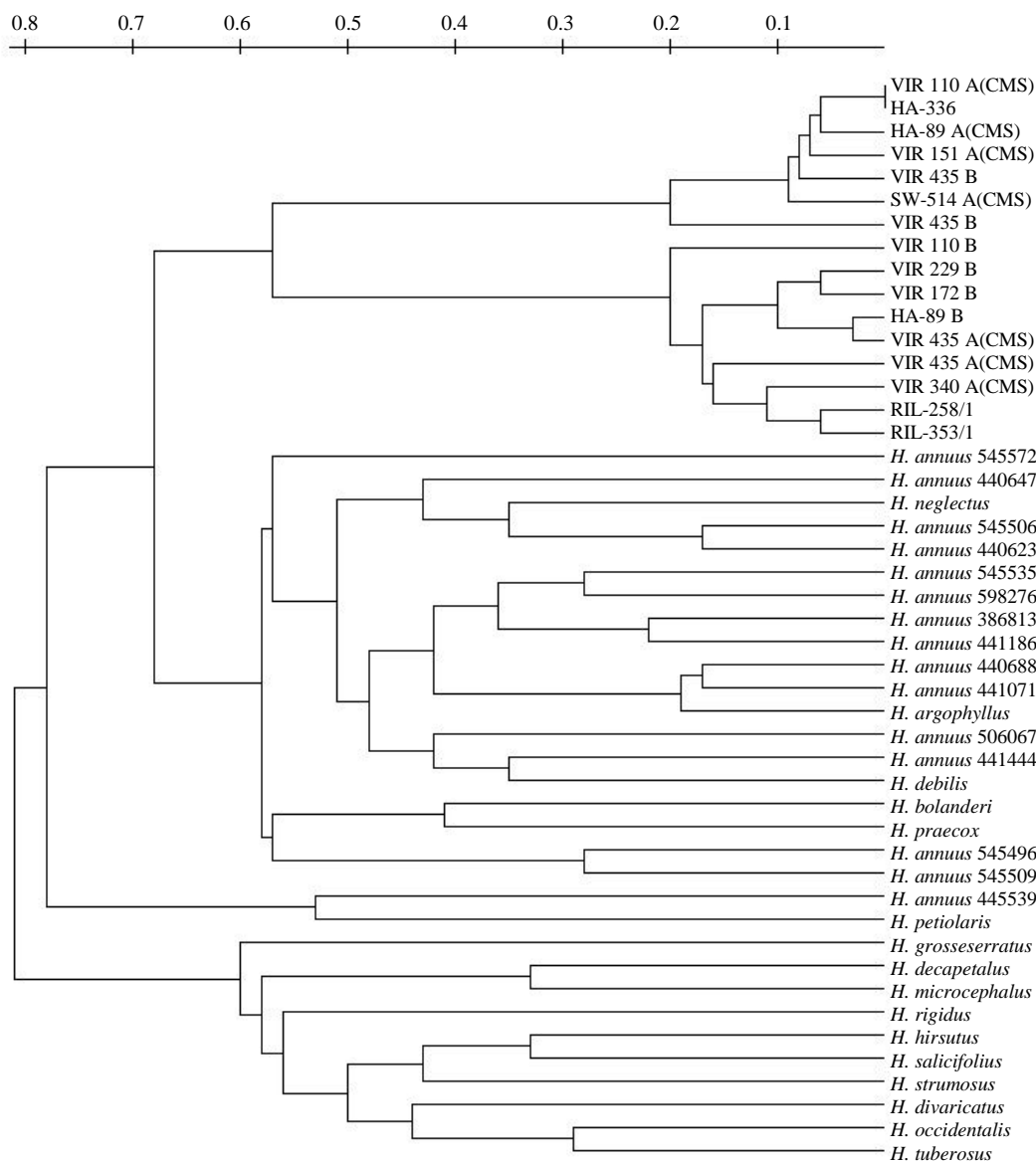


Fig. 3: The dendrogram of genetic diversity of sunflower, based on the results of SSR-analysis of cpDNA and mtDNA

Conclusion

The 18 polymorphic SSR markers developed here proved useful in the evaluation of the genetic diversity of sunflowers. All 18 SSR markers showed high polymorphic information content index for annual and perennial species. Based on the obtained data was constructed UPGMA-dendrogram showing the genetic diversity of chloroplast and mitochondrial genomes of sunflowers. This set of novel polymorphic SSR markers can be useful for breeders and molecular biologists for the genetic diversity analysis, lines identification and improve breeding.

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Author's Contributions

Vladimir Arturovich Khachumov: Wrote the first draft of the manuscript, designed experiments participated

in the laboratory study, participated in the collected and data analysis, read and approved the final manuscript.

Alexander Vyacheslavovich Usatov: Designed experiments, read and approved the final manuscript.

Kirill Vitalievich Azarin: Designed experiments, data analysis. Author read and approved the final manuscript.

Nikolay Victorovich Markin: Participated in the laboratory study, data analysis, read and approved the final manuscript.

Vera Alekseevna Gavrilova: Collected and analyzed data. Author read and approved the final manuscript.

Oleg Fedorovich Gorbachenko and Fedor Ivanovich Gorbachenko: Designed experiments, read and approved the final manuscript.

Ethics

The authors state that this article conforms to the ethical standards specified by the American Journal of Biochemistry and Biotechnology.

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