MORPHOLOGICAL AND MOLECULAR VARIABILITY OF
HELIANTHUS GIGANTEUS L. AND HELIANTHUS MAXIMILIANI SCH.
SPECIES

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Genus Helianthus consists of 49 species. Two species H. gigan-
etus L. and H. maximiliani Sch., distributed and collected in North
America, were investigated. In order to determine morphological vari-
bility in/between these two species, fifteen populations of each species
were used. Thirty traits were measured on five plants per species, grown
in the same conditions in the wild species nursery at Rmski Šančevi. Ac-
cording to the investigated morphological traits, three species of H. gi-
ganteus were closer to H. maximiliani populations, which possibly indi-
cates the existence of a new intraspecies taxon in H. giganteus. In order to
test this hypothesis molecular variability of the same populations-species,
was also investigated. The polymorphism of genomic DNA, that was
isolated from frozen leaves, was investigated by microsatellites, recently
shown to be the most powerfull for the analysis of molecular genetic vari-
bility in genus Helianthus. Obtained results confirm the high variability
between examined populations. Dendrograms constructed by cluster
analysis of examined morphological traits and molecular markers are dis-

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INTRODUCTION

One of major approaches in broadening the genetic base of domesticated sunflower is the use of wild Helianthus spp. for interspecific hybridization. Wild perennial Helianthus spp. are generally regarded as important sources for disease resistance (ŠKORIĆ, 1992). H. giganteus and H. maximiliani are considered as sources of resistance to white rot and wilt (HENN et al., 1997; CERBONCINI et al., 2002). Since only some of populations and clones have exhibited resistance to white rot, the investigation of morphological and genetic variability in/between these two species has already attracted attention (MILJANOVIĆ et al., 2000; VASIĆ et al., 2003).

So far RAPD markers were used for the investigation of Helianthus genomes (SOSSEY-ALAOUI et al., 1998), introgression of crop genes into wild sunflower populations (LINDEY et al., 1998), to detect markers for drought and disease tolerance in sunflower (PANOVIĆ et al. 2000; PANOVIĆ et al., 2004) and interspecies hybrids of sunflower (ATLAGIĆ et al., 2003), but as often nonreproducible, RAPDs are nowadays substituted by SSRs. Several hundred microsatellite markers have been developed recently (TANG et al., 2002) and proved to have the highest sensitivity in discriminating between elite inbred lines of sunflower (YU et al., 2002) and land races and wild populations of sunflower (TANG and KNAPP, 2003).

MILJANOVIĆ et al. (2000) have analysed morphological variability of H. giganteus and H. maximiliani populations and found that three H. giganteus populations differed morphologically in relation to the typical populations. The goal of this study was to compare morphological and molecular variabilities of fifteen populations of each H. giganteus L. and H. maximiliani in order to examine the raised question if the examined two species are monotypical or they can be separated into intraspecific taxa (varieties or forms).

MATERIALS AND METHODS

Wild perennial species H. giganteus and H. maximiliani originating from North America were grown in wild species nursery of the Institute of Field and Vegetable Crops at Rimski Šančevi. Fifteen populations of each H. giganteus (78, 1605, 1617, 1889, 1890, 1896, 1897, 2014, 2015, 2016, 2017, 2018, 2020, 2021, 2029) and H. maximiliani (28, 30, 31, 32, 40, 41, 1645, 2007, 2098, 2100, 2115, 2214, 2219, 2226, 2230) were analysed. Morphological traits were evaluated as described in MILJANOVIĆ et al. (2000). All morphological traits were analyzed by CLUSTER program modules of SYSTAT to construct united dendrogram for populations of two examined species.

Genomic DNA was isolated from frozen leaves of examined populations according to the modified CTAB method. SSRs were analyzed as in TANG and
KNAPP (2003). Polymorphic fragments were used for the calculation of genetic distances between each pair of examined populations as in PANKOVIĆ et al. (2004). The pairwise distance matrix of genetic distances was used for cluster analysis by UPGMA (Statistica for Windows, v.5.0, StatSoft, USA).

RESULTS

The united dendrogram of *H. giganteus* and *H. maximiliani* populations constructed on the basis of variability of measured morphological traits is presented in Fig. 1. All *H. maximiliani* (B1) and majority *H. giganteus* populations (A1) clustered in separate clusters. Three *H. giganteus* populations (78, 2014 and 2018) were more related to *H. maximiliani* populations. MILJANOVIĆ et al. (2000) have shown that *H. giganteus* populations 78, 2014 and 2018 differed from other *H. giganteus* populations in: number of leaves, bract length and length of ray flowers; stem colour and leaf margin dentation; and in leaf colour and width and angle of lateral venation, respectively.

Fig. 1. United dendrogram based on the analysis of 30 measured morphological traits for examined populations of *H. giganteus* and *H. maximiliani*. 
The screening of genomic DNA, isolated from the same populations, was done with 15 SSR primers. The size of synthesized fragments varied from 150 bp to 300 bp as in Yu et al. (2002). Obtained markers were more polymorphic than in sunflower inbred lines so it was possible to separate them on 2% agarose gels (Fig. 2). Forty-one polymorphic fragments were screened for presence/absence in each pair of examined populations. Simple matching coefficients were determined and used for the calculation of genetic distances (Pankovic et al., 2004). Genetic distances between examined populations varied from 0% (populations M31 and M30) to 46% (populations G2018 and M2230), with the mean value 22.6% (Table 1).


The pairwise distance matrix of genetic distances was used for cluster analysis by UPGMA. Obtained dendrogram is presented in Fig. 3. Generally two main clusters separated: A1 with the majority of *H. giganteus* populations and B1 with all *H. maximiliani* and the following *H. giganteus* populations: 2018, 1890, 2014, 78 and 1896.

DISCUSSION

The two species investigated in this study *H. giganteus* nad *H. maximiliani* belong to the section Divaricati (Schilling and Heiser, 1981). Some authors have described a number of intraspecific taxa within the species *H. giganteus* (Long, 1954), while Heiser (1969) treated these intraspecific taxa as synonyms.
Our results based on the cluster analysis of all measured morphological traits confirm that all *H. maximiliani* (B1) and majority *H. giganteus* populations (A1) are clustered in separate clusters (Fig 1.). Three *H. giganteus* populations (78, 2014 and 2018) were more related to *H. maximiliani* than to *H. giganteus* populations.

Populations 2014 and 2018 originated from Wisconsin and population 78 from Minnesota, which is the westernmost part of the distributional range of the species. MILJANOVIĆ et al. (2000) considered that the higher morphological variability of these populations is connected with their distribution in the peripheral part of the species distributional range. This was probably true for the initial difference of the variability between populations when they were introduced in the nursery. Meanwhile populations have been maintained in the nursery for 15 years, and thus exposed to different ecological conditions, and selection pressure.
Cluster analysis of genetic distances on the DNA level reveals almost identical relations of *H. giganteus* populations in cluster A1 (Fig. 3). Similarly as in Fig. 1, the genetic distances between *H. giganteus* populations 78, 2014 and 2018 and *H. maximiliani* populations are lower than with the rest of *H. giganteus* populations. Moreover two other *H. giganteus* populations (1890, 1896) clustered with *H. maximiliani* populations in cluster B1.

Due to the relatively low number of used SSR primers we were not able to discriminate between two *H. maximiliani* populations: M31 and M30 (Table 1; Fig. 3.). The same populations grouped to neighboring clusters that were connected at the same linkage distance in the dendrogram on Fig. 1. Nevertheless, the applied SSR primers were sufficient to reveal DNA polymorphism between examined populations of two species, and basically confirm the results obtained by analysis of 30 morphological traits. The high sensitivity of SSR markers for the analysis of molecular genetic diversity of sunflower was recently demonstrated. While allozyme and RAPD polymorphism were insufficient to distinguish between closely or distantly related germplasm accessions (RIESEBERG and SEILER, 1990; ARIAS and RIESEBERG, 1995), TANG and KNAPP (2003) have uncovered extraordinary diversity in native American land races and wild populations of cultivated sunflower with SSR markers. Also, their results obtained with microsatellites even uncovered the possibility of multiple domestication origins of sunflower.

Our data based on both morphological and DNA markers indicate that some *H. giganteus* populations are more related to *H. maximiliani* populations. One possible explanation of the origin of common alleles between these populations is speciation through hybrid recombination. This theoretical genetic model was already confirmed by comparative linkage mapping with DNA markers on the model system *H. anomalus* (the hybrid of *H. annuus* and *H. petiolaris*) (RIESEBERG et al., 1995). Opinions on the possible crossings between *H. maximiliani* and *H. giganteus* are contradictory. HEISER et al. (1969) reported on existence of natural and artificial hybrids between these two species. On the other hand Georgieva-Todorova (1990) stated that crossings between *H. maximiliani* and *H. giganteus* does not occur, while crossings between *H. annuus* and *H. giganteus* or *H. maximiliani* occur only in determined conditions. However, more authors agree that crossings between *H. giganteus* and *H. maximiliani* with wild annual species are frequent (WHELAN and DORRELL, 1980; WHELAN, 1981; ROGERS et al.; 1982, JAN, 1997). Hybrids between cultivated and wild sunflowers are also frequently reported. For example ATLAGIĆ et al. (1995) have successfully crossed cultivated sunflower with *H. maximiliani* by conventional breeding. Moreover, LINDER et al. (1998) have shown that the average overall frequency of cultivar markers in wild species surrounding the cultivar field was greater than 35%.
In conclusion, both morphological and DNA markers indicate that *H. giganteus* is probably not a monotypic species. Whether the origin of common alleles between *H. giganteus* and *H. maximiliani* populations is direct hybrid recombination or hybridization mediated by wild or cultivated *H. annuus* remains to be determined.

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MORFOLOŠKA I MOLEKULARNA VARIJABILNOST VRSTA
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