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Original scientific paper

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

Dejana SAFTIĆ-PANKOVIĆ, Jovanka ATLAGIĆ, Tomka MILJANOVIĆ, and Nataša RADOVANOVIĆ

Institute of Field and Vegetable Crops, 21000 Novi Sad, Maksima Gorkog 30, Serbia and Montenegro

Saftić-Panković D., J. Atlagić, T. Miljanović, and N. Radovanović (2005): *Morphological and molecular variability of Helianthus giganteus L. and Helianthus maximiliani Sch. species*. – Genetika, Vol. 37, No. 1, 121-130.

Genus *Helianthus* consists of 49 species. Two species *H. giganteus* L. and *H. maximiliani* Sch., distributed and collected in North America, were investigated. In order to determine morphological variability 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 Rimski Šančevi. According to the investigated morphological traits, three species of *H. giganteus* were closer to *H. maximiliani* populations, which possibly indicates 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 variability 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 discussed.

Corresponding author: Dejana Saftić-Panković, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia and Montenegro
Phone: 381 21 4898 416; fax 381 21 413 833; e-mail: dejanapa@ifvcns.ns.ac.yu

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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 (LINDER *et al*., 1998), to detect markers for drought and disease tolerance in sunflower (PANKOVIĆ *et al*. 2000; PANKOVIĆ *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* pupulations (78, 2014 and 2018) were more related to *H. maximiliani* populations. MILJANOVIĆ *et al*. (2000) have shown that *H. giganteus* populations 78, 2018 and 2014 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.

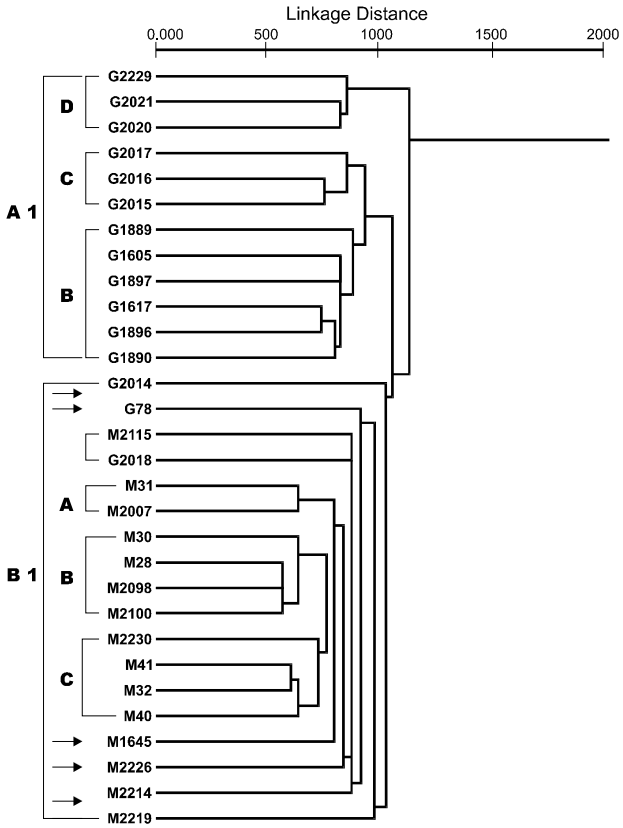

Fig. 1. United dendrogram based on the analysis of 30 measured morphological traits for examined populations of *H. giganteus* and *H. maximiliani*.

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). Fortyone 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 (PANKOVIĆ *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).

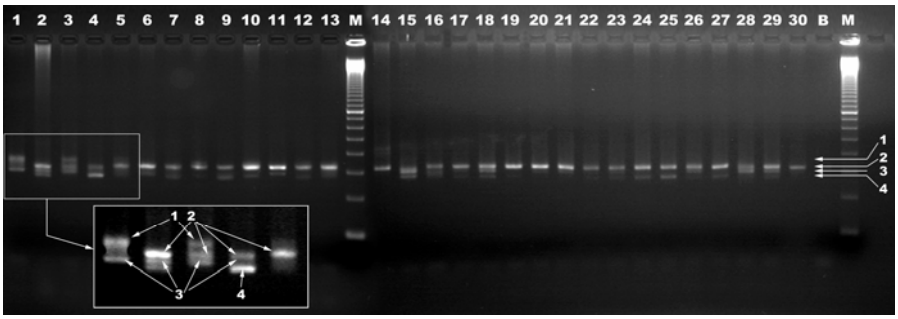

Fig. 2. Polimorphism of genomic DNA isolated from the leaves of examined populations revealed with primer ORS5. The arrows indicate polimorphic fragments, and M stands for 100 bp ladder MW standard. Numbers refer for the following populations: 1. G2029; 2. G2021; 3. G2020; 4. G2017; 5. G2016; 6. G2015; 7. G1889; 8. G1605; 9. G1897; 10. G1617; 11. G1896; 12. G1890; 13. G2014; 14. G78; 15. G2018; 16. M2115; 17. M31; 18. M2007; 19. M30; 20. M28; 21. M2098; 22. M2100; 23. M2230; 24. M41; 25. M32; 26. M40; 27. M1645; 28. M2226; 29. M2214; 30. M2219.

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.

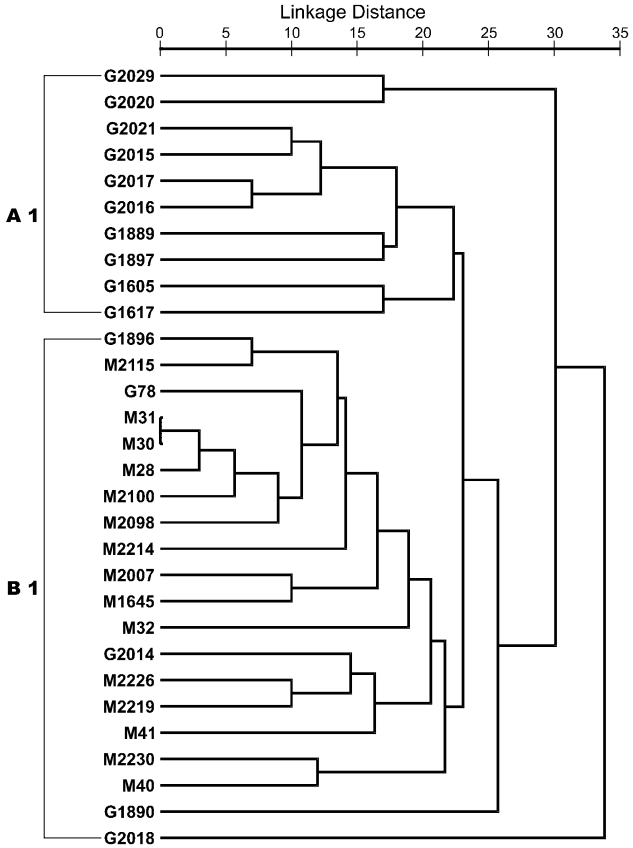

Fig. 3. Dendrogam of genetic distances between examined populations, based on polimorphic microsatellite markers

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* pupulations (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 exsposed 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 poymorphism 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 insufficinet 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 exsistance 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 succesfully 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 sorrounding 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 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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REFERENCES

ARIAS D.M. and L.H. RIESEBERG (1995): Genetic relationships among domesticated and wild sunflowers (*Helianthus annuus* L. Asteraceae). Econ. Bot., *49*, 239-248.

ATLAGIĆ J., B. DOZET, and D. ŠKORIĆ (1995): Meiosis and pollen grain viability in *Helianthus mollis*, *Helianthus salicifolius*, *Helianthus maximiliani* and their F_1 hybrids with cultivated sunflower. Euphytica, *81*, 259-263.

ATLAGIĆ J., D. PANKOVIĆ, and A. PEKANOVIĆ (2003): Backcrosses in interspecific hybridization in sunflower. Genetika, *35* (3), 187-197.

CERBONCINI C., G. BEINE, P.C. BINSFELD, B. DRESEN, H. PEISKER, A. ZERWAS, and H. SCHNABL (2002): Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in the natural *Helianthus* gene pool. Helia, *25*, 167-176.

GEORGIEVA-TODOROVA J. (1990): Genetic and cytogenetic investigations of the genus *Helianthus* L. (in Bulgarian), Bulgarian Academy of Science, Sofia, 1-129.

HEISER C.B., D. SMITH, and W. MARTIN (1969): The North American sunflower (Helianthus). Mem. Torrey Bot. Club, *22* (3), 1-218.

HENN H.J., U. STEINER, R. WINGENDER, and H. SCHNABL (1997): Wild type sunflower clones: source for resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary stem infection. Angew. Bot., *71*, 5-9.

JAN C.C. (1997): Cytology and interspecific hybridization. *In:* Sunflower Technology and Production, *Ed.* A:A: Schneiter, United Publishers, Madison, Wisconsin USA, pp 497-558.

LINDER C.R., I. TAHA, G.J. SEILER, A.A. SNOW, and L.H. RIESEBERG (1998): Long-term introgression of crop genes into wild sunflower populations. Theoretical and Applied Genetics, *96*, 339-347.

LONG W.R. (1954): Synopsis of *Helianthus giganteus* L. and related species. Rhodora, *56*, 198-203.

MILJANOVIĆ T., P. BOŽA, J. ATLAGIĆ, and D. ŠKORIĆ (2000): Morphological variability of *H. giganteus* L., and *H. maximiliani* Sch. populations. Helia, *23* (32), 45-52.

PANKOVIĆ D., S. JOCIĆ, N. LAČOK, Z. SAKAČ, and D. ŠKORIĆ (2004): The use of PCR-based markers in the evaluation of resistance to downy mildew in NS-breeding material. Helia, *27* (40), 149-158.

PANKOVIĆ D., Z. SAKAČ, M. PLESNIČAR, and D. ŠKORIĆ (2000): Identification of RAPD markers linked to drought tolerance by bulked segregant analysis. Proc. of the 15th Internat. Sunflower Conference, Toulouse, France, 12-15 June M38-M44.

RIESEBERG L.H., C. VAN FOSSEN, and A.M. DESROCHERS (1995): Hybrid speciation accompanied by genomic reorganization in wild sunflowers. Nature, *375*, 313-316.

RIESEBERG L.H. and G.J. SEILER (1990): Molecular evidence and the origin and development of the domesticated sunflower (*Helianthus annuus*, Asteraceae). Econ. Bot., *44*, 79-91.

ROGERS C.E., T.E. THOMPSON, and G.J. SEILER (1982): Sunflower species of the United States, pp 1-75.

SCHILLING E.E. and C.B. HEISER (1981): Infrageneric classification of *Helianthus* (*Compositae*). Taxon, *30* (2), 393-403.

ŠKORIĆ D. (1992): Achievements and future directions of sunflower breeding. Field Crops Res, *30*, 231-270.

SOSSEY-ALAOUI K., H. SERIEYS, M. TERSAC, P. LAMBERT, E. SCHILLING, Y. GRIVEAU, F. KAAN, and A. BERVILLE (1998): Evidence for several genomes in *Helianthus*. Theoretical and Applied Genetics, *97*, 422-430.

TANG S., J.-K. YU, M.B., SLABAUGH, D.K. SHINTANI, and S.J. KNAPP (2002): Simple sequence repeat map of the sunflower genome. Theoretical and Applied Genetics, *105*, 1124-1136.

TANG S. and S.J. KNAPP (2003): Microsatellites uncover extraordinary diversity in native american land races and wild populations of cultivated sunflower. Theoretical and Applied Genetics, *106*, 990-1003.

VASIĆ D., J. MILADINOVIĆ, A. BERVILLE, and D. ŠKORIĆ (2003): Variability of *Helianthus maximiliani* Schrader revealed by RAPD analysis. Plant Genetic Resources Newsletter, *133*, 13-15.

WHELAN E.D.P. (1981): Cytoplasmic male sterility in *Helianthus giganteus* L. x *H. annuus* L. interspecific hybrids. Crop Science, *21*, 855-858.

WHELAN E.D.P. and D.G.DORREL (1980): Interspecific hybrids between *Helianthus maximiliani* Schrad. and *H. annuus* L.: Effect of backcrossing on meiosis, anther morphology, and seed characteristics. Crop Science, *20*, 29-34.

YU J.K., J. MANGOR, L. THOMPSON, K.J.EDWARDS, M.B. SLABAUGH, and S.J. KNAPP (2002): Allelic diversity of simple sequence repeat markers among elite inbred lines in cultivated sunflower. Genome, *45*, 652-660.

MORFOLOŠKA I MOLEKULARNA VARIJABILNOST VRSTA *HELIANTHUS GIGANTEUS* L. I *HELIANTHUS MAXIMILIANI* SCH.

Dejana SAFTIĆ-PANKOVIĆ, Jovanka ATLAGIĆ, Tomka MILJANOVIĆ i Nataša RADOVANOVIĆ

Naučni Institut za ratarstvo i povrtarstvo, Maksima Gorkog 30, Novi Sad, Srbija i Crna Gora

Izvod

Rod Helianthus se sastoji od 49 vrsta. U ovom radu ispitivane su dve vrste *H. giganteus* L. i *H. maximiliani* Sch., koje su rasprostranjene i sakupljene u severnoj Americi. Da bi se utvrdila morfološka varijabilnost unutar/između ove dve vrste ispitivano je po petanest populacija od svake vrste. Analizirano je trideset morfoloških osobina na pet biljaka od svake populacije, koje su gajene u istim uslovima u kolekciji divljih vrsta na Rimskim Šančevima. Tri populacije iz vrste *H. giganteus* su na osnovu morfoloških osobina pokazale veću sličnost sa populacijama iz vrste *H. maximiliani*, što možda ukazuje na postojanje novog intraspecies taksona u vrsti *H. giganteus*. Da bi se proverila ova hipoteza ispitivana je i molekularna varijabilnost na istim populacijama-vrstama. Izolovana je genomska DNK iz zamrznutih listova navedenih populacija. Za ispitivanje polimorfizma genomske DNK korišteni su markeri za mikrosatelite, za koje je nedavno pokazana dosad najveća molekularna genetička varijabilnost u rodu Helianthus. Dobijeni rezultati potvrđuju visok stepen varijabilnosti između ispitivanih populacija, a u radu su upoređeni dendrogrami dobijeni klaster analizom morfoloških osobina i molekularnih markera.

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