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# Molecular traits to elucidate the ancestry of *Helianthus* x *multiflorus*

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ABSTRACT

The triploid perennial *Helianthus* x *multiflorus* L. is a beloved garden ornamental, but its phylogenetic origin has long been a source of discussion. Sequence comparison of genes involved in the biosynthesis of the sesquiterpene lactones was used to identify the common sunflower *Helianthus annuus* L. and the diploid taxon *Helianthus decapetalus* L. as the most likely parental combination in the origin of the triploid hybrid species. A pair of deletions (22 and 26 bp in length) in an intron part of the germacrene A synthase 1 gene differentiated the diploid from the tetraploid karyotype of *H. decapetalus*. The same deletion was found in one of the two alleles (299 bp in length) in *H.* x *multiflorus*, whereas a second allele (350 bp in length) showed a very high similarity with the sequence found in *H. annuus*. Quantification of the two amplification products in *H.* x *multiflorus* showed a higher concentration of the larger amplicon thus suggesting that *H. annuus* has contributed an unreduced genome to the hybrid. Comparison of variable regions of the cpDNA showed highest identity of *H.* x *multiflorus* with cpDNA sequences of the diploid *H. decapetalus*. This suggests that *H. decapetalus* served as the maternal parent in a cross with *H. annuus* to produce *H.* x *multiflorus*.



## 1. Introduction

Attempts to reconstruct the phylogeny of *Helianthus* have shown, that the sunflower genus includes a very complex network of diploid to hexaploid taxa with auto- or alloploid origins and numerous ancient and recent cases of hybridization (Rieseberg, 2006; Timme et al., 2007a; Bock et al., 2014). The frequency of hybridization in the genus has already been noted by Heiser et al. (1969). This process not only accounts for natural speciation and introgression as reviewed by Rieseberg (2006), but also is a useful feature for sunflower breeders searching for new sources of resistance or stress tolerant phenotypes to utilize in altering the economically important oil crop sunflower, *Helianthus annuus*. *Helianthus* x *multiflorus* L. is one of the taxa for which a hybrid nature had already been suspected centuries ago (Heiser and Smith., 1960). The taxon exists in single and double flowering types and has been known as an ornamental since the late 16th century, but it has never been found in any natural habitats. Heiser and Smith (1960) intensively studied the perennial plant which was never found to set viable seeds. They found low pollen viability and an unusual karyotype showing 17 bivalents and 17 univalents in the metaphase stage. The triploid nature explained why the taxon is infertile and only propagates through rhizome fragments. Various parental combinations were suggested to have contributed to the origin of *H.* x *multiflorus* and amongst them *H.*

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*annuus* L. and *Helianthus decapetalus* L. were viewed as the most likely parents. Heiser and Smith generated F1 progenies from artificial crossings of *H. annuus* with the tetraploid karyotype of *H. decapetalus* and obtained several plants that were phenotypically similar to *H.* x *multiflorus*. Moreover, this combination appeared reasonable from the karyotype, but the authors did not rule out that the triploid nature could also have resulted from the crossing of diploids when one gamete contained an unreduced chromosome set. Subsequent chemical analysis of the glandular trichome metabolome supported this alternative hypothesis (Spring and Schilling, 1990). The sesquiterpene lactone (STL) profile of *H.* x *multiflorus* contained desacetyleupasserrin, the major compound of the diploid *H. decapetalus*, but a compound that was not observed in tetraploid material of this species. Moreover, the chemical profile of *H.* x *multiflorus* had less than 25% of the STL in common with the artificial hybrids of Heiser's crossings between the tetraploid *H. decapetalus* with the annual sunflower. Comparisons of the STL profile of *H.* x *multiflorus* showed ca. 77% similarity to the additive pattern of the putative parental combination of *H. annuus* and diploid *H. decapetalus*, whereas there was only 35% similarity to the additive pattern of *H. annuus* and tetraploid *H. decapetalus* (Spring and Schilling, 1990). Subsequently, key enzymes of the STL biosynthesis in *Helianthus* that are responsible for the transformation of farnesyldiphosphate into germacrene A (Fig. 1) and subsequent oxidation to germacrene A acid and its hydroxylated derivatives, have been identified and characterized from the transcriptome of glandular trichomes in the secretory stage (Göpfert et al., 2005, 2009; Nguyen et al., 2010; Ikezawa et al., 2011).

This has now made it possible for the first time to test the conclusions from the chemical studies on *H.* x *multiflorus* with genomic sequences of genes participating in the STL synthesis of the taxa putatively involved in the origin of the hybrid. This study compares partial sequences of the germacrene A synthase 1 (GAS1) of *H.* x *multiflorus* with those of the putative parents *H. annuus*, and the diploid and tetraploid karyotypes, respectively, of *H. decapetalus*. Moreover, sequence characteristics of the cpDNA will be shown to provide additional information on the maternal parentage of *H.* x *multiflorus*.

## 2. Materials and methods

### 2.1. Plant material used for DNA extraction

*H. annuus* L. cv. HA300 seed material was obtained from the Landessaatzuchtanstalt University of Hohenheim; *H. decapetalus* 2n (cultivated from seeds of a population collected at Tennessee, Knox Co.; OS001); *H. decapetalus* 4n (cultivated from seeds of a population collected at Indiana, Monroe Co.; OS045); *H.* x *multiflorus* variety Meteor (cultivated plant from the Botanical Garden, University of Tübingen, Germany). The classification of the *H. decapetalus* plants followed the description of Heiser et al. (1969) and Heiser and Smith (1960) which characterized the tetraploid plants as more robust and with broader leaves than the diploid plants. In addition, guard cell measurements showed larger cells in the tertaploid. Finally, the STL profile classified the two plants according to the presence and absence of desacetyleupasserrin in the diploid and tetraploid variety, respectively (Spring and Schilling, 1991).

### 2.2. DNA extraction, amplification, and sequencing

DNA extraction of fresh leaf material was carried out using a protocol from Ristaino et al. (2001), with twice the extraction volume and the following modifications: The leaf samples were collected in a 2 ml reaction tube with three iron beads and disrupted in a mixer mill (9 Hz, 3 min) prior to DNA extraction, in the DNA precipitation step ethanol was replaced by isopropanol, and the final DNA pellet was dissolved in 100 µl sterile water. PCR was carried out in a peqSTAR 96 Universal Gradient thermo cycler (Peqlab Biotechnologie GmbH, Erlangen). The amplification protocol for the partial GAS1 gene was: 0.2 U/µl Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 µg/µl BSA and 1 µM primer in a total volume of 12.5 µl. PCR was carried out as follows: 95 °C, 4 min; 36× (95 °C, 0:40 min; 62 °C, 0:40 min; 72 °C, 0:40 min); 72 °C, 4 min. For peak area quantification of the smaller and the larger GAS1 amplification product, MultiNA

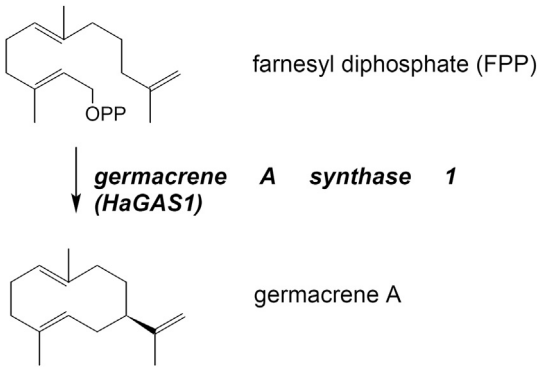


**Fig. 1.** Germacrene A synthase 1 reaction.