

## INTERGENERIC HYBRID BETWEEN CULTIVATED SUNFLOWER (*Helianthus annuus* L.) AND *Tithonia* *rotundifolia* (Mill.) Blake

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### SUMMARY

In this paper we describe an intergeneric hybrid between *H. annuus* (HA 89) and *T. rotundifolia* obtained by normal pollination of a male-sterile cultivated sunflower. The hybrid had a combination of morphological traits from both parents; it was unbranched and exhibited male-sterility. The plant did not produce viable achenes when pollinated with HA 89. DNA fingerprinting through the AFLP methodology exhibited 240 polymorphisms and confirmed the hybrid nature of the experimental plant. The genetic distance analysis discounted the phenomenon of partial hybridization.

**Key words:** *Helianthus annuus*, *Tithonia rotundifolia*, hybridization, AFLP

### INTRODUCTION

Sunflower improvement is a prime example of breeding through hybridization between domestic and wild germplasm. Sunflower breeders successfully use a wide range of germplasms as sources of resistance to diseases and other pests, employing a cytoplasmic male-sterility hybrid system derived from wild annual sunflower *Helianthus petiolaris*. Additionally, variations in fertility restoration and fatty acids and salt and drought tolerance are traits being sought by means of interspecific hybridization (Laferrière, 1986; Seiler, 1992; Fick and Miller, 1997; Jan, 1997). Furthermore, interspecific hybridization in sunflowers has been performed to shed light on the mechanisms of plant evolution (Rieseberg *et al.*, 1995) and the intriguing partial hybridization (Faure *et al.*, 2002).

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Cultivated sunflower can be readily crossed with several other annual species of *Helianthus*; difficulties arise with polyploids and perennials of the same genus (Laferrière, 1986; Georgieva-Todorova, 1983). The use of colchicine for chromosome doubling and embryo rescue techniques provide means to overcome these difficulties, offering the possibility of crossing with members of other genera (Friedt, 1992; Seiler and Rieseberg, 1997).

The genera *Helianthus* and *Tithonia* are members of the *Viguiera* assemblage, a group of *Asteraceae* that share, among other traits, the basic chromosome number  $x = 17$  (Seiler and Rieseberg, 1997). At least one species of *Tithonia*, namely *T. diversifolia*, has been the target of some research due to its putative medical effects in the treatment of painful inflammatory conditions (Owoyele *et al.*, 2004). On the other hand, the genus *Tithonia* has been reported to be resistant to mildew, *Phomopsis* and *Sclerotinia*, common diseases in cultivated sunflower (Christov and Panayotov, 1991). A total of 19 species of *Tithonia* have been identified in Mexico, with the main distribution from medium to high lands of the Sierra Madre Occidental and Sierra Madre Oriental (Gómez-Sánchez and González-Elizondo, 1994).

*Tithonia rotundifolia* is a wild species probably originated in Central America. Its bright, orange-yellow flowers have attracted the attention of gardeners as a potential ornamental. The achenes show dormancy, and require 3 months after ripening to start germination (Upfold and Vanstaden, 1990). In Mexico, *T. rotundifolia* occurs in the states of Coahuila, Nayarit, Colima, Jalisco, Puebla, Michoacán, Estado de México, Guerrero and Oaxaca (Gómez-Sánchez and González-Elizondo, 1994).

Wide crossing between *T. rotundifolia* and cultivated *H. annuus* was reported by Cristov and Panayotov (1991). They used the open-pollinated variety Peredovik and the lines 3004 and HA 89 as the cultivated parents. Hybrid plants were obtained only when these parents were used as females. From the crosses Peredovik  $\times$  *T. rotundifolia* and 3004 (*cms* of *H. petiolaris*)  $\times$  *T. rotundifolia* they obtained plants with a single inflorescence. However, from the cross HA 89 (*cms* of *H. argophyllus*)  $\times$  *T. rotundifolia*, they obtained very branched plants, with inflorescence numbers ranging from 24 to 92. Only one seed was obtained from the cross with the line 3004, whereas 1.31% and 2.2% seed set was obtained with HA 89 and Peredovik, respectively. They reported fertility, either for self-pollination or free-pollination, in the hybrids. Although *T. speciosa* and *T. tagetiflora* were included in their research, no hybrids were obtained using these species.

In this study we report a hybrid plant obtained from the cross HA 89 (*cms* of *H. petiolaris*)  $\times$  *T. rotundifolia*, and its morphological and DNA analysis through AFLP (amplified fragment length polymorphism) fingerprinting (Vos *et al.*, 1995).

## MATERIAL AND METHODS

The female parent was the cultivated sunflower line *cms* HA 89, with the classical French male-sterile cytoplasm PET1 originated from *H. petiolaris* (Leclercq, 1969). The male parent was a member of a population of *T. rotundifolia* sampled in Iguala, Guerrero (Mexico) in 1997. Fresh pollen from *T. rotundifolia* was applied to the inflorescences of 20 HA 89 plants in 2001. Pollen was applied twice at two-day interval, starting from the beginning of anthesis. Pollination was performed with a flannel applicator. At physiological maturity, the HA 89 heads were screened for mature, fully developed achenes.

The single hybrid plant obtained was morphologically analyzed for traits normally used in taxonomy of flowering plants. After morphological analysis, two lower leaflets from the hybrid plant were removed to isolate DNA for AFLP analysis. Also, DNA from one HA 89 plant and one *T. rotundifolia* individual was extracted. These were not the true parents but members from the same line (HA 89) and the same population sample (*T. rotundifolia*). Nuclear DNA was isolated by the method of Doyle and Doyle (1990). The AFLP analysis (Vos *et al.*, 1995) was carried out through the protocol "IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis LI-COR<sup>®</sup>," that uses two fluorescent dyes (700 and 800 nm). The procedure involved DNA digestion, adaptor ligation, and preselective and selective PCR amplification. Electrophoresis of the PCR products was performed with an automatic LI-COR<sup>®</sup> sequencer machine. The rare-cutter restriction enzyme was *Eco* RI, and the frequent cutter was *Truq* 1, an analogue to *Mse*I. The primer-dye combinations were: E-AAG700/M-CAA; E-AGC800/M-CAA; E-ACC700/M-CTA; E-AGG800/M-CTA; E-ACA700/MCTT; E-ACG800/MCTT. The gel images were analyzed with the software LI-COR SAGA MX version 2.1 for gel analysis.

Binary data were used for paternity testing, band sharing and squared Euclidean distances in the *Mathematica*<sup>®</sup> system. The distances were calculated with data coded with 0 (band present) and 1 (band absent), according with the following formula (Johnson and Wichern, 1992):

$$d_{XY} = \sum_{i=1}^k (x_i - y_i)^2$$

where  $x_i$  and  $y_i$  are the binary values for individuals  $X$  and  $Y$ , respectively, and  $k$  is the number of polymorphic bands. Since this measure provides a number of mismatches, additivity is expected in the distances for a set of two pure-bred lines and the hybrid. Thus, it provides a test for partial hybridization. Standard errors of the distances estimates were obtained by bootstrapping.

## RESULTS AND DISCUSSION

Only three achenes were obtained from the 20 male-sterile HA 89 plants pollinated with *T. rotundifolia*. The three achenes were sown in spring of 2003 with only one single-headed plant developing to maturity. The other two died prematurely, although one produced a single bud that initiated flowering.

The following taxonomical description was based on the plant that survived until maturity (Figure 1).

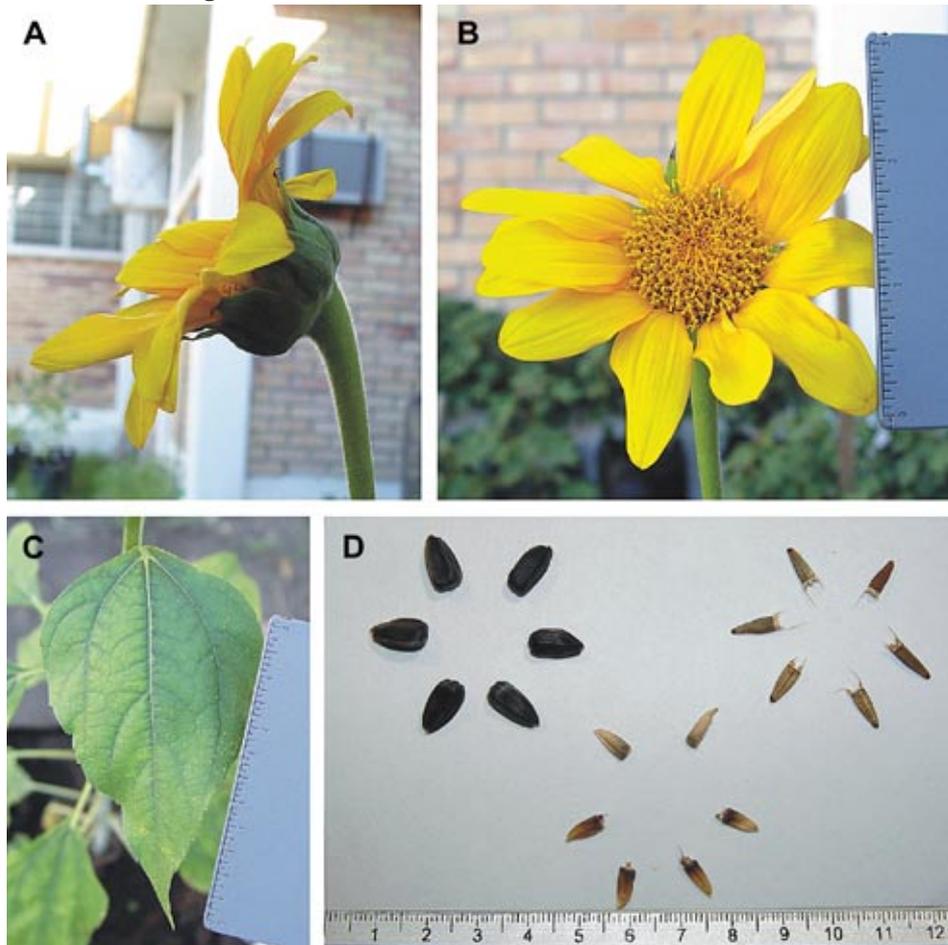


Figure 1: **A.** Lateral view of hybrid inflorescence. **B.** Hybrid inflorescence showing male-sterility. The scale is 7.62 cm. **C.** Hybrid leaf. The scale is 7.62 cm. **D.** Achenes from *H. annuus* (upper left), *T. rotundifolia* (upper right) and the hybrid (lower middle). The scale units are centimeters.

Annual herb, 94 cm tall, pilose to glabrescent with age; leaves alternate, petioles 3-6 cm long, blades rhombic to triangular-ovate, 9-12 cm long, 6-7 cm wide, the base acute, the apex acuminate, the margin crenate, pilose, dark green; head single,

8-11 cm broad, peduncles fistulate, 8-12 cm long, pilose, phyllaries in 2 rows, oblong, 2-2.5 cm long, 8-10 mm wide, pilose; receptacle convex, 2.5-3.5 cm broad; ray flowers 26-48 mm long, yellow orange; disk flowers 30-60, corolla 5-7 mm long, yellow orange; achenes oblong, cuneate at the base, 6-8 mm long, 2.5-3.5 mm broad, velutinous; pappus of 2 subequal awned scales, 3-4.5 mm long and 4(5) ovate scales, slightly lacerate, 0.4-2 mm long.

The inflorescence of this plant resembled the *T. rotundifolia* type, although the flower color was more yellow than orange (Figures 1A and 1B). However, the plant was single-headed like the domestic *H. annuus*. The shape of the leaves resembled more the *H. annuus* type since they were not lobed (Figure 1C), although they were intermediately pilose. A taxonomic comparison between the three entities is summarized in Table 1.

Table 1: Morphological comparison between *H. annuus* (HA 89), *T. rotundifolia* and the hybrid plant

	<i>H. annuus</i>	Hybrid	<i>T. rotundifolia</i>
Pubescence	Hispid	Pilose	Densely pilose
Leaves	Triangular-ovate	Rhombic	Triangular-ovate, lobed
Achenes	9-11 mm × 5-7 mm, brownish, velutinous	6-8 mm × 2.5-3.5 mm, brownish, velutinous	6-8 mm × 2.5-3.5 mm, brownish, velutinous
Pappus	2 lanceolate scales or none	1-2 awned scales and 4(-5) scales	2 awns and 12-14 scales
Inflorescence	Single	Single	Multiple

The single-headed hybrid described in this study contrasts with the multiple headed plants obtained by Christov and Panayotov (1991) from the cross HA 89 × *T. rotundifolia*. However, they used a line HA 89 with cytoplasmic male-sterility derived from *H. argophyllus*, whereas we used the line with the classical French *cms* cytoplasm PET1 from *H. petiolaris* (Leclercq, 1969). This is the same cytoplasm used in the *cms* line 3004 used by Christov and Panayotov (1991), from which they obtained an unbranched hybrid plant. Also, the hybrids they obtained with the variety Peredovik were unbranched. Therefore, the multiple headed plants obtained by these authors may have come from *H. argophyllus* as a recessive branching gene was still present in their line HA 89.

The hybrid plant obtained in this study was male sterile (Figure 1B). This condition may have resulted either from the presence of the PET1 cytoplasm and absence of fertility restorer genes in the *T. rotundifolia* plant, or from some kind of incompatibility between the *H. annuus* and *T. rotundifolia* genomes that precluded pollen development. Fertilization with HA 89 pollen was attempted, but only empty achenes were obtained. The achenes morphologically resembled those of *T. rotundifolia* (Figure 1D).

The AFLP-based DNA fingerprinting revealed 240 polymorphic bands and confirmed the hybrid nature of this plant. Figure 2 shows the result of one of the AFLP combinations. A sample of band matches between *H. annuus* and the hybrid are marked with +, whereas a sample of matches with *T. rotundifolia* are marked

with\*. A total of 109 bands unique to the line HA 89 and 108 bands unique to *T. rotundifolia* were present in the hybrid. There were only 19 bands present in the hybrid and absent in both HA 89 and *T. rotundifolia* samples; however, a few band mismatches were expected by the fact that the sampled plants were not the true individual parents of the hybrid.

The estimated Euclidean distances plus bootstrap standard errors were: *H. annuus* –*T. rotundifolia*, 231(2.92); *H. annuus* – hybrid, 117(7.77); and *T. rotundifolia* – hybrid, 124(7.48). As can be seen, the parental genomes were almost equidistant to the hybrid, and the sum of both distances, 241(10.98), is not far from the distance between both parents. This symmetry in DNA fingerprints discounts partial hybridization, a phenomenon reported by Faure *et al.* (2002) in wide crosses between *H. annuus* and the perennial species *H. mollis* and *H. orgyallis*, where band sharing in the hybrids occurred predominantly with the female parent.

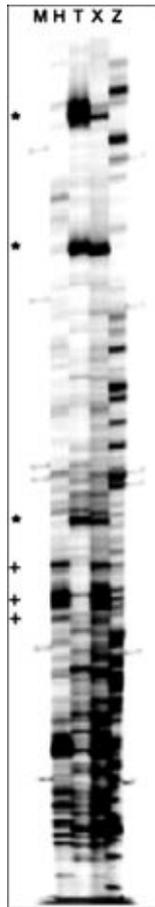


Figure 2: AFLP fingerprints. M is the size marker, H is *H. annuus*, T is *T. rotundifolia*, X is the intergeneric hybrid, and Z is a *Zea mays* control. A sample of positive matches of the hybrid with bands unique to *H. annuus* and unique to *T. rotundifolia* are marked with + and \*, respectively.

### CONCLUSIONS

The cultivated sunflower, *H. annuus*, can be crossed as a female parent with *T. rotundifolia*, but more than 20 plants had to be pollinated to have a high likelihood of obtaining at least one hybrid. The F<sub>1</sub> hybrid obtained in this study was infertile and had a combination of morphological traits from both parents. The absence of multiple heads in the hybrid was inherited from the cultivated line HA 89. The AFLP methodology for DNA fingerprinting allowed the testing the hybrid nature of the plant, and discounted partial hybridization.

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**HÍBRIDO INTERGENÉRICO ENTRE EL GIRASOL CULTIVADO (*Helianthus annuus* L.) Y LA ESPECIE *Tithonia rotundifolia* (Mill.) Blake**

RESUMEN

Describimos un híbrido intergenérico entre *H. annuus* (HA 89) y *T. rotundifolia* obtenido a través de polinización normal de un girasol cultivado androestéril. El híbrido presentó una combinación de caracteres de ambos progenitores, sin ramificación y con androesterilidad. La planta no produjo aquenios viables cuando se polinizó con HA 89. La huella de ADN a través de la metodología AFLP exhibió 240 polimorfismos y confirmó la naturaleza híbrida de la planta experimental. El análisis de distancia genética descartó el fenómeno de hibridación parcial.

**HYBRIDE INTER GÉNÉRIQUE ENTRE LE TOURNESOL  
CULTIVÉ (*Helianthus annuus* L.) ET LE *Tithonia*  
*rotundifolia* (Mill.) Blake**

RÉSUMÉ

Nous décrivons une plante hybride intergénérique faite par la combinaison de *H. annuus* (HA 89) et *T. rotundifolia* qui a été obtenu au moyen de pollinisation normale d'un tournesol cultivée male-sterile. Cette plante a peu combiner les caractères morphologiques des parents mâle et femelle; elle n'a pas été ramifié et a manifesté stérilité mâle. Elle n'a pu produit des akènes vivantes quand elle été fécondée par le pollen de HA 89. Le dactylographie d'ADN par AFLPs a montré 240 polymorphismes et a confirmé la nature hybride de la plante expérimentale. L'analyse de la distance génétique a rejetée le phénomène de la hybridation partielle.