Gene transfer from wild *Helianthus* to sunflower: topicalities and limits

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**Abstract:** Sunflower (2n=17) belongs to the *Helianthus* genus (Asteraceae). Wild *Helianthus* species display morphological variation for branching and stem number, for architecture and seed size, and for resistance to abiotic and biotic stresses due to which they thrive in different environments in North America. The genus is divided into botanical sections, two for annual as sunflower, and two for perennial species as Jerusalem artichoke that produces rhizomes (tubers). We explain the difficulties and successes obtained by crossing sunflower with these species to improve the agronomic traits of the sunflower crop. It is easier to cross the annual species than the perennials’ with sunflower. Several traits such as Cytoplasmic male sterility and restorer RF-PET1 genes, Downy mildew resistance, Phomopsis resistance, Sclerotinia resistance, Rust resistance, and Orobanche resistance have already been introduced from annual species into sunflower crop, but the complex genomic organization of these species compared to sunflower limits their important potential. Perennial species are much more diverse, and their genomes display 2n, 4n, or 6n chromosomes for n 17. The realities of inter-specific hybridization are relatively disappointing due to the introgression lines that have low oil and low seed yield. We report here several attempts to introgress agronomic traits from these species to sunflower, and we present as a case study, an introgressed progenies from *H. mollis*, a diploid species with sessile small leaves. We constructed a preliminary genetic map with AFLP markers in 21 BC1 plants, and we then showed that some progenies display 6 to 44% of introgression from *H. mollis*. Although this study is promising due to the novel compact architecture of the progenies, we cannot estimate the transferability from *H. mollis* to other perennial Helianthus to improve sunflower.

**Key words:** architecture, Helianthus mollis, introgression, sessile leaves, sunflower crop

**Introduction**

*Helianthus* is a much-diversified genus in various environments (Rogers et al., 1982). The uses of wild species to improve crops had many successes. The advantages are due to classical crosses, which are more or less sustained by embryo rescues and screening in greenhouses or nurseries for the traits under transfer that deal with abiotic and biotic stress resistance, and quality of the products (Serieys, 1987). Many examples exist for most crops and in particular, wheat (Ordon et al., 2009). The classical scheme of these breeding programs is to produce first-generation hybrids and then to backcross the hybrid with the crop in order to eliminate most of the wild genome except the region carrying the useful traits. If the scheme is simple and clear, in practice, breeders face many difficulties. Some crosses fail, which are as much important, as the wild parent is distant from the crop. Breeders would have the presentation based on agronomic traits to improve sunflower with the list of wild putative donor species. However, we do not focus on traits, but merely on the methods to transfer genes or traits from wild to crop. Consequently, it is logical to examine the potential of wild *Helianthus* following the phylogeny of the genus (table 1).

Based on the numerical taxonomy, the genus *Helianthus* (table 1) is divided into four sections (Schilling and Heiser, 1981). Annual species are grouped in Sect. *Annui* and Sect. *Agréstis*, which are composed of 13 and 1 species, respectively. Perennial species are grouped in Sect. *Ciliare* and Sect. *Atrorubentes*, which are composed of 7 and 30 species, respectively. Their spreading areas are shown in (figure 1). Many basic works on the origins of *Helianthus* have been published, thus this aspect is still under debate, and is not addressed in this review. For recent review on these aspects see Strasburg and Riesenberg (2008).

Crosses between wild *Helianthus* species and sunflower are more or less possible and may be helped by embryo rescue (Asad et al., 1986; Denat et al., 1991; Serieys 1992). The basic chromosome number in Helianthus genus fits with x=17, if most of species are diploid 2n=34 such as *H. annuus*, *H. mollis*... some polyploids are observed in perennial sections with 68 (*H. hirsutus*, *H. ciliaris*) or 102 chromosomes (*H. tuberosus*, *H. resinosus*...). One therefore expects hybrids with 34, 51, or 68 chromosomes with sunflower. Polyploidy in these species may have occurred by different mechanisms schematized in figures 2A-B. The problems to cross and...
backcross hybrid with sunflower crop come from the differences in chromosome number and structure due to chromosome rearrangements in most of *Helianthus* in comparison with sunflower (Rieseberg et al., 1995) due to pollen viability, (Quillet et al., 1995) and the self-incompatibility system, which is not unraveled yet in *Helianthus* (Strasburg and Rieseberg, 2008; Gandhi et al., 2005; Hiscock and Allen, 2008). The structural differences between homologous chromosomes often lead to a reduction of the gametes for the hybrids, consequently hybrids, leading to a chromosomal sterility or semi-sterility (Rieseberg et al., 1995). Usually, this leads to the effect of reducing the chromosome length and disrupts or inhibits introgression, which is within or adjacent to the rearranged segment. This is attributed to the chromosomal rearrangement during the meiotic pairing. The chromosome segment rearrangement occurs and leads to the introgression. However, the chromosomal effect is still unclear in the hybrids’ fertility process. The impact of the chromosomal effect on introgression for species who share the same number of chromosomes is confusing. Physically, the recombination between wild and crop, introgression from a wild species of an alien DNA fragment into the genome of a crop broadly has several consequences. Besides the specific mechanisms that may lead to mix genomes between wild and crop, introgression from a wild species of an alien DNA fragment into the genome of a crop broadly has several consequences. Physically, the recombination between the introgressed fragment and
homologous regions – if any – of this fragment in the genome is practically blocked, and if it occurs, it is at very low rates. The recombination for the chromosome carrying the introgression with its homologue without the introgression has decreased considerably, but it may occur at a very low rate. Consequently, two types of introgressed progenies and lines will exist: Type-1 will carry small introgression region that are homozygous and behave without many troubles to cross with the crop. Type-2 will probably carry larger introgression fragment that will stay in the heterozygous state.

For Type-1, plenty of sunflower lines carry introgressed regions and an intense study would be required to determine the introgressed regions. Two of the best examples in sunflower is provided by lines derived from *H. argophyllus* carrying Plag downy resistance gene(s) (Dussle et al., 2004) and Phomopsis resistance genes (Besnard et al., 1997). The male fertility is conserved, but the chromosomal rearrangement disappears.

For Type-2, the best example of such a line is provided by the HIR34 line (Leclercq et al., 1970; Rahim et al., 2001). The first introgression from *H. tuberosus* leads to Downy mildew resistance. Obviously, such lines cannot be involved in commercial hybrids due to their heterozygosity and some instability since they lead to nonhomogeneous progenies. Such materials are of interest for breeding purposes when they display any useful trait for breeding purposes when they display any useful trait for breeding purposes, which is probably located in the introgression (Serieux, 1997; Pinochet et al., 2001 Serieux, 2009). They are also of interest to map traits and to evaluate the introgression fragment content for useful and flawed traits to ensure that there is no linkage between the useful and flawed traits.

In this review, we described the main tools used for introgression (or to introgress) between wild species and sunflower crop. We examined the species, topicalities, and limits of their use to breed sunflower through literature, and experiences in our laboratory unit. The latter part of this review is based on our research. Recent literature reveals renewed interest for handling introgression as a common tool for breeding sunflower (Jan et al., 2007; Heesacker et al., 2009; Tu et al., 2009) in order to use the diversity existing in the natural resources and to improve the different agronomic of the crop.

**Main rearrangements, tools, and techniques to examine and exploit introgression**

The main chromosomal rearrangements are due to the physical changes in the DNA sequences: mutation, insertion–deletion or indel, nonreciprocal translocation, reciprocal translocation, and inversion are the most important chromosomal rearrangements. Besides these chromosomal changes, duplication may change the pattern of gene expression. Allele transmission is in segregation distortion when the two alleles are not transmitted to progenies in the same frequencies. Rearrangements may cause distortion, particularly when the introgression is in the heterozygous state (Type-2). Further, some of the wild alleles may be linked to lethal locus and thus may be underrepresented in the progenies. Due to the hitchhiking or the selective sweep process, the portion where the wild region is localized is not retained, and consequently underrepresented in the progenies. The physiological mechanisms may be various and they are usually not known. Conversely, when a distortion is found, it is corrected by adding a lethal allele at the distance explaining the distortion (Lorieux et al., 1995; Luro et al., 1995).

**Reciprocal translocation**

This affects two different chromosome pairs and at meiotic process to match homologous fragments the two chromosome pairs twist showing quadivalent (figure 3A). This rearrangement is common between close-relative species. The main consequence of such pairing is that half of the gametes will be unbalanced and consequently are not viable. Thus, the male fertility will be around 50% of the total fertility. The female gametes are less affected by reciprocal translocation for reasons that are not known yet. However, this does not induce segregation distortion (figure 3B).

**Insertion-deletion or indel**

They correspond to short-base deletion or insertion, modifying the length of a sequence. In most cases, indel occurs in noncoding sequences and thus they are relatively frequent and useful for obtaining length polymorphisms enabling one to map the markers. Indel, in phylogenetic analysis is not informative, unless there are several under verifiable hypotheses (figures 3C-I). In coding regions of the genome, unless the length of an indel is a multiple of three, they produce a frameshift mutation. Indels can be contrasted with a point mutation, where an indel inserts and deletes nucleotides from a sequence, a point mutation is a form of substitution that replaces one of the nucleotides. Indels can also be contrasted with Tandem Base Mutations (TBM), which may result from fundamentally different mechanisms. A TBM is defined as a substitution at adjacent nucleotides (primarily substitutions at two adjacent nucleotides, but substitutions at three adjacent nucleotides have also been observed).

**Nonreciprocal translocation**

It affects four or more chromosomal pairs since one chromosome of one species has been split, and one of the fragments has fused with the initial chromosome, whereas the other fragment has fused with the next chromosome. Thus, chromosome pairing (hexavalent, quadivalent) is highly disturbed at meiosis, but it may appear that chromosome pairing as cross figures that cause nonreciprocal translocation are difficult to predict from the cytological data.

**Inversion**

It affects one chromosome (figures 3DG). One segment of a chromosome has been cut, followed by a 180 °C rotation of this fragment and...
**A**  
*Autopolyploidisation: Hybridization then diplogamete production*

- **Diploid individual**  
  Species A (2n = 2x = 4)

  **Hybridization:**  
  Diploid zygote (2n = 2x = 5)

  **Anomaly meiosis:**  
  No possible matching between the 2 parents chromosomes

  **Deadlock**

- **Diploid individual**  
  Species A (2n = 2x = 6)

  **Deadlock**

**B**  
*Autopolyploidisation: Hybridization and triploid bridge*

- **Diploid individual**  
  Species A (2n = 2x = 4)

  **Abnormal meiosis**

  **Diplogametes**  
  (2x = 4)

- **Diploid individual**  
  Species A (2n = 2x = 6)

  **Normal meiosis**

  **Hybridization:**  
  Triploid zygote (2n = 3x = 7)

  **Gametes**  
  (x = 3)

  **Out tretraploid zygote**  
  (2n = 4x = 10)

Figure 2. Autopolyploidization effect occurs during the interspecies crosses. A) hybridization then diplogamete production, B) hybridization and triploid bridge.
Figure 3. Drawings of chromosome pairing and rearrangements. A. pairing in reciprocal translocation, B. chromosomal composition and abortion (/) in a backcross. C: indel deletion/insertion; D inversion of a central segment; E deletion; F duplication; G inversion; H reciprocal translocation; I mutation consequences. I. Different chromosomal rearrangements lead to five consequences. Case 1: The nucleotide change C to T does not modify anything in the amino acid sequence, it is a synonymous change. Case 2: A to G mutation is a transition, but no amino acid change. Case 3: A to C mutation is a transversion, with amino acid change, leucine becomes valine. Case 4: C insertion changes other amino acid because of a frame shift; Case 5: Mutation G to A introduces a stop codon, consequently the amino acid chain is interrupted.
rearrangement associated with the phenotype is usually invisible; indeed no genetic information is available. When the region includes the centromere, the duplication is “pericentric” vs the “paracentric duplication”. Thus, chromosome pairing displays a loop and an inversion at meiosis, and the rearrangement decreases the recombination rate for the chromosome pair in inter-specific crosses near the extremities of the inversion fragment drastically. One of the two structures is inherited in the chromosome pair in inter-specific crosses near the extremities of the inversion fragment drastically. One of the two structures is inherited in the progenies depending on the parent used in the backcross.

**Chromosome duplication**

Duplication represents a doubled chromosome fragment. The duplication event involves four copies of the gene in the genome. Therefore, transcript is over-expressed, and could be a source of lethality. It has affected one or a few chromosomes. It is well-documented in most genomes of maize (Gaut, 2001) Arabidopsis (Heijnen et al., 1999), and Helianthus (Cavallini et al., 1986). It may induce segregation distortion due to repeated loci. Such duplication may cause troubles in diversity analyses, and are revealed by mapping.

**Tools and techniques**

When the goal of the work is to transfer genes from the wild to the crop, the best design to proceed is to use a cytoplasmic male sterile (CMS) to avoid self-fertilization of the sunflower line or (CMS-Y x B-X, B- for maintainer line) hybrid as the female with the wild accession as the pollen donor (table 2). In most cases, the wild species carries restorer alleles (Rf) and this warrants when the progenies are male fertile, or when seeds harvested on the female are probably hybrids. However, the number of seeds and the examination of the plantlets for morphological traits must be done at the seed germination stage. The best way is to check putative hybrid plants with co-dominant molecular markers. However, it has been shown that partial hybrids plants should occur, and they should be differentiated from true hybrids with molecular markers (Faure et al., 2002a, b, c). Putative hybrid plants should be controlled for pollen viability using Alexander staining solution, which is one of the best criteria to detect true hybrid with very low pollen viability, and partial hybrid plants with normal pollen viability. The CMS parent line and the wild species have to be planted as controls to verify the first-generation progenies. The maintainer (B) line has to be planted (three shifted, sowing for 2 weeks) as pollen donor for the first backcross. If the putative hybrid plant has several heads, one can be left open pollinated to ensure progenies in case of failure of backcrosses (table 2).

The backcross method is known to be efficient to transfer any single trait, or complex traits that segregate at one locus. The advanced backcross - Quantitative Trait locus (QTL) method is efficient for such traits (Tanksley and Nelson, 1996; Bernacchi et al., 1998) and for traits spreading on several dispersed QTLs. Mapping Type-1 and 2 introgression requires different strategies. In Type-1 introgression line, the introgression is homozygous and has to be mapped in F2 or RIL populations since all F1 are identical, as done for an introgression line derived from H. argophyllus (Besnard et al., 1997). In Type-2 introgression lines, the introgression is heterozygous and thus can be mapped in F1 plants in cross with another line with advantage of clear polymorphisms in the considered region (Heesacker et al., 2009).

**Transfer from annual species to sunflower**

**Sect.Agrestes**

H. agrestis has not been crossed with sunflower yet.

**Sect.Annui**

Annual Helianthus species thrive in different environments that make them putative sources of abiotic stress resistance traits (drought, high temperature, salt soil, and cold temperature). Moreover, these species have been evaluated for different biotic stresses (Downy Mildew, Phomopsis, Sclerotinia, Rust, and Botrytis as examples [Serjeys, 1997, Pinochet et al., 2001]), and some carry favorable traits. Most of the kernel components (proteins and oil composition) have also been evaluated, and average kernel composition as the range of variation for seed proteins and fatty acid constituents are more or less known (Helianthins, Globulins, and oleic acid) (Anisimova, 2002; Seiler et al., 2006; Seiler et al., 2007). However, crosses of sunflower in both directions with these species are easy, and the homologies between sunflower and each of these species are not known except for H. petiolaris Nutt, H. anomalus Blake, H. deserticola Heiser, and H. paradoxus Heiser have been comparatively mapped using the series of SSR markers developed in S. Knapp’ laboratory (Tang et al., 2002, 2003; Yu et al., 2002, 2003; Burke et al., 2004; Lai et al., 2005).

Only 4 of the 17 linkage groups are collinear between these species, and 11 of the linkage groups are non collinear in pair-wise comparison (Heesacker et al., 2009). Nevertheless, we have no way to predict which part of the wild genome is introgressed, unless by constructing the map. Consequently, many introgression lines have to be produced and screened for detecting introgression traits. Moreover, the location of each agronomic trait (QTLs) is not known in these species making any forecast illusive for efficient gene transfer from the wild to crop sunflower. Consequently, the only way to proceed is to develop introgression lines from the species, and to check whether the trait is transmitted. Other species such as H. argophyllus T. and G. induce vigor, drought, and tolerance (Aspiroz et al., 1988), Phomopsis, mildew resistance (Duselle et al., 2004), and Sclerotinia, resistance (Röncke et al., 2004). H. debils Nutt. induces drought tolerance, broomrape (Orobanche cumana) resistance (Labrousse et al., 2001) H. argophyllus Phomopsis (Besnard et al., 1997), H. paeceox Engelm. and A.Gray, H. niveus Benth.) Brandgeee, H. neglectus Heiser, H. bolanderi Gray, H. exilis A. Gray induce drought tolerance.

<table>
<thead>
<tr>
<th>Crop lines: line status</th>
<th>Male sterile</th>
<th>CMS-83HR4</th>
<th>GMS-RHA801</th>
<th>PEF1-RHA274</th>
<th>PET1-92A6</th>
<th>PET1-D34</th>
<th>PET1-HAB9</th>
<th>(mixed pollen of 6 cultivated lines)</th>
<th>(mixed pollen from 6 wild H. annuus)</th>
<th>H. annuuswild</th>
</tr>
</thead>
</table>

Table 2. List of lines used.
Several introgression genetic pools derived by INRA-Montpellier from annual species have been released to sunflower breeders since about 10 years (Serieux et al., 2000). In most cases, the introgression regions have not been mapped, but several programs (Sunyfuel, Oleosem P. Vincent 2008) of genotyping include these materials, and probably new data will be obtained soon but not yet available. A detailed mapping of H. argophyllus by Heesacker et al. is in press (Heesacker et al., 2009) and this work will provide tools to unravel introgression in most introgression lines from this species. The genomic differences between H. argophyllus and sunflower are higher than expected, as observed from phylogenetic studies (Sossey-Alaoui et al., 1998, Timme et al., 2007).

It appears that though the maintenance of the annual Helianthus genetic resources is a intense work, it is very important to breed sunflower, since selection pressure for one trait under study will enhance the probability to recover the trait. In all the cases quoted earlier, no selection pressure had been applied to produce introgression lines. Indeed, when the desired introgressed fragment is not in a favored region, it is probably rapidly eliminated. Thus, applying selection pressure to favor the region will improve the introgression rate until the region becomes homozygous. Deep evaluation to estimate the potential of Helianthus annual species remains to be carried out in different environments.

Transfer from perennial species to sunflower

We rapidly examine the potential of these species grouped in the botanical series. Perennial species have high potential to breed sunflower for many traits, but progenies of sunflower line x perennial have usually low oil content that requires several years of improvement. 

**Sect. Atrorubentes**

Series Corona-Solis: H. decapetalus L.; H. divaricatus L.; H. occidentalis Riddell (Atlagic, 1996); H. nuttallii T. and G. (Atlagic, 1996); H. maximilianii Schrad. Whelan and Dorrell (1980) have inter-specific hybrids H. maximilianii x H. annuus: studied effect of backcrossing on meiosis, anther morphology, and seed characteristics. Jan et al. (2007) examined these species for white moldon different organs (Sclerotinia). In this series is H. tuberosus L. or Jerusalem artichoke were also cultivated for its tubers. This species has been widely used to improve disease resistance in sunflower (Phomopsis, Mildew, rust). Many studies deal with cytological aspects (Atlagic et al., 1993) and DNA content (Cavallini et al., 1986). H. rigidus (Cass.) Desf. or H. paullonii S.Nutt. is also source of resistance to Sclerotinia and to parasitic broomrape (Seiler 1992a, b; Seiler et al., 2006; Gavrilova et al., 2005).

H. mollis Lam. was chosen as a model species (Faure et al., 2002a, b, c; Cazaux et al., 1996; Sossey-Alaoui et al., 1998) to study interspecific hybridization (true and partial hybrids) since it is difficult to cross with sunflower (Serieux, 1987) based on the work by Georgieva-Todorova (1990), the cytogenetic of the hybrids H. mollis x H. annuus is known. The species is diploid with sessile leaves that could be favorable to the sunflower plant to reduce its low use of space in the field, but the head is apparently normal. Introgression lines from H. mollis (HM-) were studied at INRA-Montpellier since 1996. All lines were derived from BC2S2 progenies of 48 plants because it displayed compact plants with more or less erected leaves. After further fixation, the HM-derived lines showed several families with the more or less the same architecture. One family (HM374) was retained and was used to produce F2 progenies in order to map the introgression (Serieux, 2009). Cytogenetical data obtained by GISH (Genomic In Situ Hybridization) (Kahane-Weinachter et al., 2008) have shown that the line HM374 carries almost one chromosome with H. mollis sequences.

A case study dealing with H. mollis is under publication (Breton et al. submitted). Genetic studies were made on a BC1S2 progenies from H. mollis (Breton et al., submitted) and a derived line HM374 was used in a cross with crop to build a F2 population for mapping H. mollis introgression (Breton et al., in prep). Several progenies derived from a unique BCI plants were genotyped with AFLP markers to evaluate the introgression from H. mollis. Twenty-one BC2 plants revealed 6 to 44% of introgressed genome from H. mollis. Plants with compact architecture and shrewed leaves carry a common introgressed fragment spending onto one linkage group of 62cM, whereas a small sunflower linkage group of 10 cM directed QTLs for domestication (branching) and agronomic traits (head diameter).

Sect. Ciliares, Series Ciliares, H. arizonensis R.C. Jackson; H. ciliaris DC. and Series pumili, H. gracilentus A. Gray are creeping plants with robust rhizomes making the plant invasive in collection. These species are very difficult to cross with sunflower, although they could be sources of biotic and abiotic resistance factors. Helianthus perennial species probably offer huge potential to improve sunflower, but many difficulties limit their potential for breeding. They are also used as a source of new alleles and many sequences are available in molecular databases (Compositae genome database http://compgenomics.ucdavis.edu/).

Realities

During the spring and summer of 2007, we crossed sixteen wild species and six crop lines either CMS or GMS (Genic Male Sterility) by pollen samples from five plants of each accession. We realized five crosses for each combination wild species x crop sunflower leading to 1250 crosses. The six lines, two bulks, and one wild sunflower are described (table 2).

Using the Duncan test (it is a rank test) to compare the average seed number harvested on single head, we concluded that the aptitude for obtaining hybrids is higher with any of the polyploid accesses, whatever the direction of the crosses, and further, the size of the progenies was enhanced with the polyploid accession as female (tables 3-4).

Moreover, we found that with any of the polyploid accesses as female pollination with pollen bulks, pollen from wild sunflower was more efficient than using pollen from any of the crop lines. With sunflower crop lines as female, we computed the line that displayed the higher aptitude in inter-specific crosses. We also observed that the three perennial polyploid accesses are efficient as male in inter-specific crosses. We computed the effect of the pollen donor or receptor on the seed size samples (tables 3-6; figure 1AB). We found that wild HIR-672 and RIG-236 produced compatible hybridizations whatever the direction of crosses, but for other accesses it is better to use wild parent as the pollinator (table 7; figures 4AB). Crop lines present advantage to be used as the female parent under the CMS form avoiding castration. We noticed that the line HA89 had one of the best behavior in cross with perennial species.

On the whole, we obtained 5384 putative inter-specific seeds from 16 inter-specific combinations in both directions with the sunflower crop (table 3). Obviously, all these seeds should be examined to verify whether hybridization occurred both by (1) phenotyping, checking both male and female sterility of each plant to determine self-incompatibility and to perform cytogenetic study on the first-generation hybrid plants; (2) genotyping plants with different available molecular markers to determine their hybrid status.

For those plants that were validated as first-generation hybrids, we should have pollen samples to ensure the first backcross generation.
Troubles and limits

We determined priorities to check the putative hybrid seeds. We first targeted putative hybrid seeds from *H. nuttallii* (NUT-103) and *H. rigidus* (RIG-101), which were tolerant to *Sclerotinia* based upon tests on the stem, leaf, and head [41].

Our earlier experiences in such putative first-generation hybrid enabled us to predict that progenies from diploid accessions (Nut-103) will be more sterile than progenies from tetraploid and hexaploid accessions (RIG-101) (Faure et al., 2002a, b, c; Cazaux et al., 1996). There are two strategies to overcome the barrier of sterility in such hybrid plants. The first is to pollinate with sunflower pollen, thousand heads of the progenies to harvest a few (1-3) seeds for ten thousand heads, which are pollinated. In a preceding work with *H. mollis*, pollinated with sunflower, we obtained several first-generation hybrid seeds studied with Random Amplified Polymorphic DNA (RAPD) markers (Cazaux et al., 2002). Pollination of ten thousand heads using bulks of crop pollen has led to three BC1 seeds. Thus, this work is tedious and poorly efficient, and we cannot merely forecast chromosome number of the crop (2n=34). Apparently, aneuploidy seems remnant in such progenies.

The second strategy, which is widely used for other crops and sunflower, is to double the chromosome set of the F1 plants with hybridization. (Jackson and Murray, 1983) This method has been

Table 3. Cumulative seed size from crosses made in 2007 at INRA Montpellier (wild.*sunflower and sunflower *wild).

<table>
<thead>
<tr>
<th>Species Croplines</th>
<th>2n =</th>
<th>83HR4</th>
<th>92B6</th>
<th>HA89</th>
<th>RHA274</th>
<th>D34</th>
<th>RHA801</th>
<th>HA734</th>
<th>BU_HAC</th>
<th>BU_HAS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. giganteus-553</td>
<td>34</td>
<td>113</td>
<td>21</td>
<td>2</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>H. giganteus-554</td>
<td>34</td>
<td>6</td>
<td>101</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
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<td>H. hirsutus-260</td>
<td>68</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>H. hirsutus-672</td>
<td>68</td>
<td>15</td>
<td>35</td>
<td>469</td>
<td>300</td>
<td>260</td>
<td>19</td>
<td>113</td>
<td>189</td>
<td>144</td>
<td>1544</td>
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<td>34</td>
<td>1</td>
<td></td>
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<td>H. maximiliani-1014</td>
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<td>27</td>
<td>9</td>
<td>92</td>
<td>84</td>
<td>85</td>
<td>5</td>
<td>122</td>
<td>424</td>
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<tr>
<td>H. maximiliani-1050</td>
<td>34</td>
<td>1</td>
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<tr>
<td>H. nuttallii-103</td>
<td>34</td>
<td>19</td>
<td>2</td>
<td>28</td>
<td>17</td>
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<td>2</td>
<td>19</td>
<td>93</td>
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<td>H. nuttallii-934</td>
<td>34</td>
<td>18</td>
<td>6</td>
<td>43</td>
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<tr>
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<td>125</td>
<td>2</td>
<td>850</td>
<td>56</td>
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<td>75</td>
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<td>4</td>
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<td>H. rigidus-101</td>
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<td>1</td>
<td></td>
<td></td>
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<td></td>
<td>18</td>
<td>26</td>
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<tr>
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<td>259</td>
<td>10</td>
<td></td>
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<tr>
<td>H. strumosus-1224</td>
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<td>25</td>
<td>1</td>
<td></td>
<td></td>
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<td>65</td>
<td>20</td>
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<td>H. strumosus-1506</td>
<td>102</td>
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<td>14</td>
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<tr>
<td>H. tuberosus-572</td>
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<td>47</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1</td>
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<tr>
<td>H. tuberosus-732</td>
<td>102</td>
<td>41</td>
<td>38</td>
<td>257</td>
<td>56</td>
<td>96</td>
<td>74</td>
<td>39</td>
<td>601</td>
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<tr>
<td>Total</td>
<td>346</td>
<td>93</td>
<td>2297</td>
<td>542</td>
<td>708</td>
<td>219</td>
<td>390</td>
<td>384</td>
<td>405</td>
<td>5384</td>
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</tr>
</tbody>
</table>

Table 4. Female parent’s effect on seed size per head and total number of seeds with wild as female.

<table>
<thead>
<tr>
<th>Female</th>
<th>Seed size/head</th>
<th>Number of crosses</th>
<th>Duncan</th>
<th>Female</th>
<th>Seed size/head</th>
<th>Number of crosses</th>
<th>Duncan</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIG-236</td>
<td>26.625</td>
<td>12</td>
<td>A</td>
<td>NUT-934</td>
<td>0.75</td>
<td>71</td>
<td>D</td>
</tr>
<tr>
<td>HIR-672</td>
<td>13.29</td>
<td>71</td>
<td>B</td>
<td>GIG-553</td>
<td>0.688</td>
<td>71</td>
<td>D</td>
</tr>
<tr>
<td>STR-1224</td>
<td>6.271</td>
<td>16</td>
<td>C</td>
<td>MAX-1050</td>
<td>0.573</td>
<td>58</td>
<td>D</td>
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<tr>
<td>TUB-732</td>
<td>5.237</td>
<td>59</td>
<td>C</td>
<td>GIG-554</td>
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<td>60</td>
<td>D</td>
</tr>
<tr>
<td>MAX-104</td>
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<td>40</td>
<td>DC</td>
<td>STR-1506</td>
<td>0.409</td>
<td>55</td>
<td>D</td>
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<tr>
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<td>10</td>
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<td>HIR-260</td>
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<td>D</td>
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<tr>
<td>NUT-103</td>
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<td>73</td>
<td>D</td>
<td>TUB-572</td>
<td>0.161</td>
<td>56</td>
<td>D</td>
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<td>PAU-1033</td>
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<td>40</td>
<td>D</td>
<td>MAX-1019</td>
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<td>16</td>
<td>D</td>
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</tbody>
</table>

We determined priorities to check the putative hybrid seeds. We first targeted putative hybrid seeds from *H. nuttallii* (NUT-103) and *H. rigidus* (RIG-101), which were tolerant to *Sclerotinia* based upon tests on the stem, leaf, and head [41].

Our earlier experiences in such putative first-generation hybrid enabled us to predict that progenies from diploid accessions (Nut-103) will be more sterile than progenies from tetraploid and hexaploid accessions (RIG-101) (Faure et al., 2002a, b, c; Cazaux et al., 1996). There are two strategies to overcome the barrier of sterility in such hybrid plants. The first is to pollinate with sunflower pollen, thousand heads of the progenies to harvest a few (1-3) seeds for ten thousand heads, which are pollinated. In a preceding work with *H. mollis*, pollinated with sunflower, we obtained several first-generation hybrid seeds studied with Random Amplified Polymorphic DNA (RAPD) markers (Cazaux et al., 2002). Pollination of ten thousand heads using bulks of crop pollen has led to three BC1 seeds. Thus, this work is tedious and poorly efficient, and we cannot merely forecast chromosome number of the crop (2n=34). Apparently, aneuploidy seems remnant in such progenies.

The second strategy, which is widely used for other crops and sunflower, is to double the chromosome set of the F1 plants with hybridization. (Jackson and Murray, 1983) This method has been
used routinely by Jan and Chandler (1989) to recover male fertility in inter-specific hybrid plants and more recently by Jan et al. (2002), to recover orobanche resistance in progenies of inter-specific crosses. However, the disadvantage of this method is to return to the diploid state of the crop without aneuploidy in progenies (Jan et al., 2002).

We have not yet explored all the potential of partial hybridization to improve sunflower. Plenty of seeds from “sunflower CMS line × H. mollis” have been produced and stored awaiting favorable projects. Based on preceding results by Faure et al. (2002a,b,c) and Tu et al. (2009) it seems reasonable to think that each should carry different introgression fragment from the wild species, and thus each seed should be evaluated separately.

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