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*. Agrestis, which are composed of 13 and 1 species, respectively. Perennial species are grouped in *Sect*. Ciliares 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 Rieserberg (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. Polyploidization in these species may have occurred by different mechanisms schematized in *figures 2A-B*. The problems to cross and

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Table 1. *Sections, series, and subspecies for the Helianthus with chromosome numbers.*

Section	Series	Species	Subspecies	(2n=)
I.AGRESTIS		agrestis		34
II. ANNUUI		niveus	niveus, tephrodes, canescens	34
		debilis	debilis, vestitus, tardilorus, silves-tris, cucume-rifolius	34
		praecox	praecox, runyonii, hirtus	34
		petiolaris	petiolaris, fallax	34
		neglectus, annuus, argophyllus, bolanderi, anomalus, paradoxus		34
III. CILIARES	1. Pumili	gracilenthus, pumilus, cusickii		34
	2. Ciliares	arizonensis, laciniatus		34
		ciliaris		68
IV. ATRORUBENTES	1. Divaricati	mollis, divaricatus, decapetalus		34
		occidentalis	occidentalis, plantagineus	34
		hirsutus, strumosus		68
		eggertii, tuberosus, strumosus		102
		rigidus	rigidus, sub-rhomboideus	102
	2. Gigantei	giganteus, grosseserratus		34
		nuttallii	nuttallii, parishii, rydbergii	34
		maximiliani, salicifolius		34
		resinosus, schweinitzii, cali-fornicus		102
	3.Microcephali	microcephalus, glaucophyllus, smithii, longifolius		34
		laevigatus		68
	4. Angustifolius	angustifolius, simulans, floridanus		34
	5. Atrorubentes	silphioides, atrorubens, heterophyllus, radula, carnosus		34

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. Basically, the impact of the chromosomal difference in introgression will be reduced if they do not decrease the hybrid fertility (Rieseberg *et al.*, 1995). Moreover, meiotic abnormalities are frequently observed in karyotype of hybrids, indicating that the differences in genes can lead to production of meiotic irregularities like those observed with chromosomal rearrangement. In nature, the confusion can be observed in chromosomal divergent species for the first-hybrid generation, which is the narrow tension zone (Rieseberg *et al.*, 1995). Therefore, it is really difficult to explain the role of chromosomal rearrangement in the reproductive barriers. Recently, partial hybridization between perennial species and sunflower has been reported, which means that the hybrid plants carry much more DNA of the crop genome than of the wild species, but its chromosome number is n = 34. One can read the recent reviews in *Brassica*, *Helianthus*, and *Oryza* (Tu *et al.*, 2009). The progenies of such materials are fertile, and there is no trouble to maintain it for sunflower (Faure *et al.* 2002a, b, c) *(figures 2A,B)*.

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

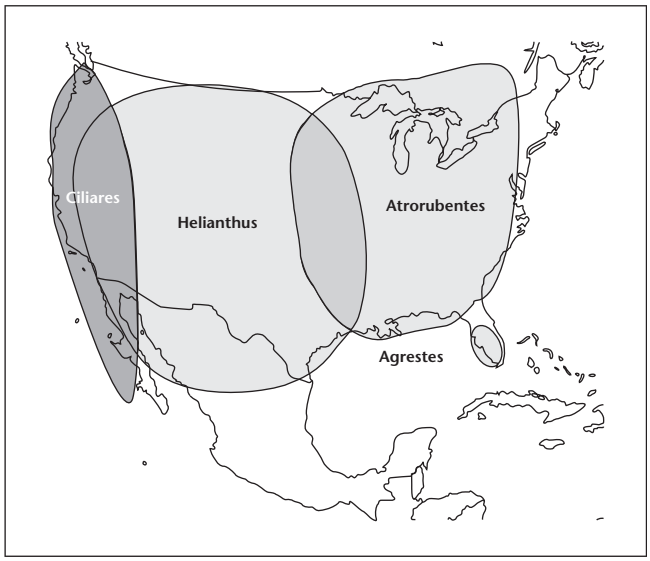

Figure 1. *Map for the location of* Helianthus *species (Sections) in their natural habitat.*

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 *Plarg* 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, which is probably located in the introgression (Serieys, 1997; Pinochet *et al.*, 2001 Serieys, 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 quadrivalent *(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 unverifiable 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, quadrivalent) 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

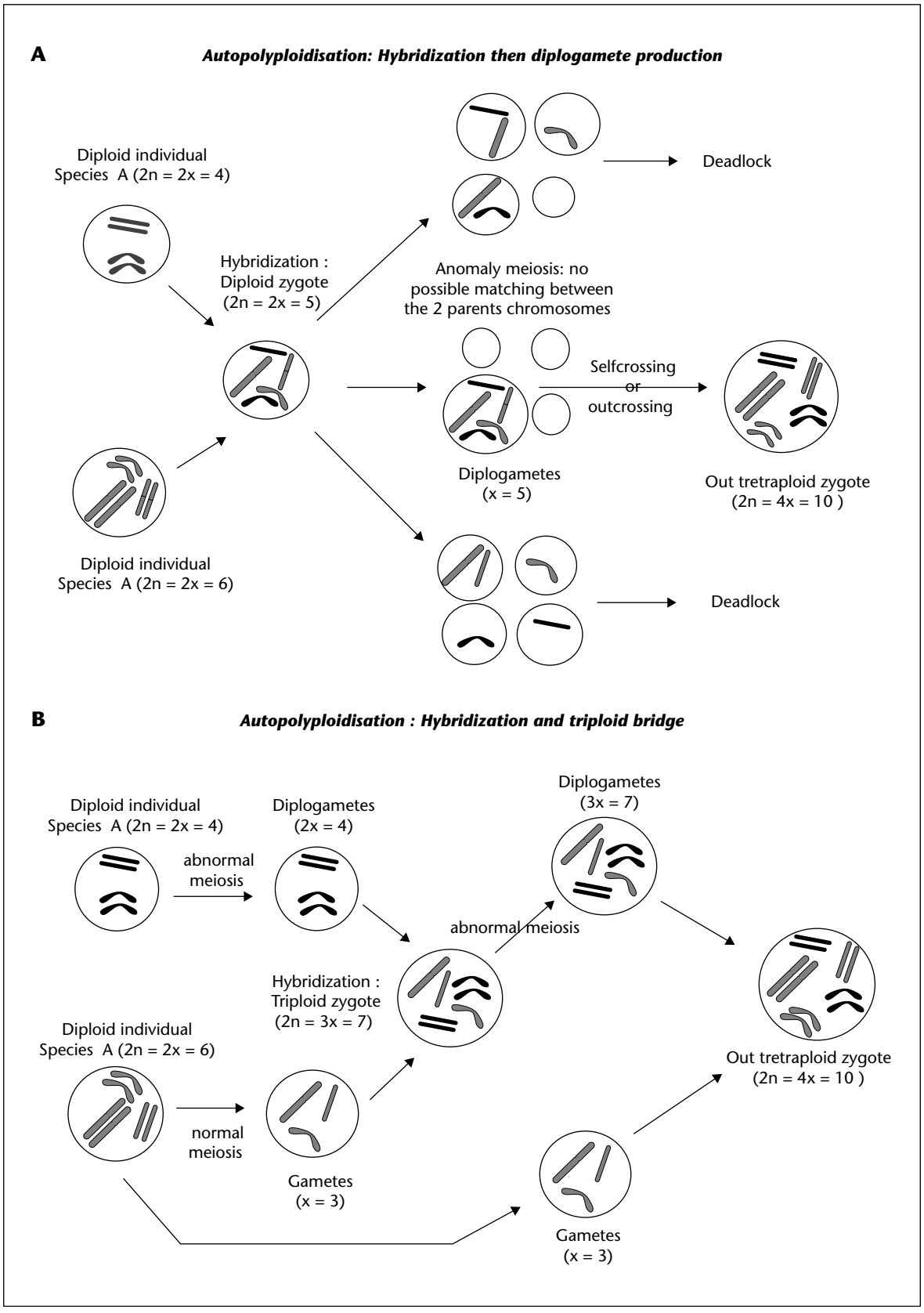

Figure 2. *Autopolyploidization effect occurs during the interspecies crosses. A) hybridization then diplogamete production, B) hybridization and triploid bridge.*

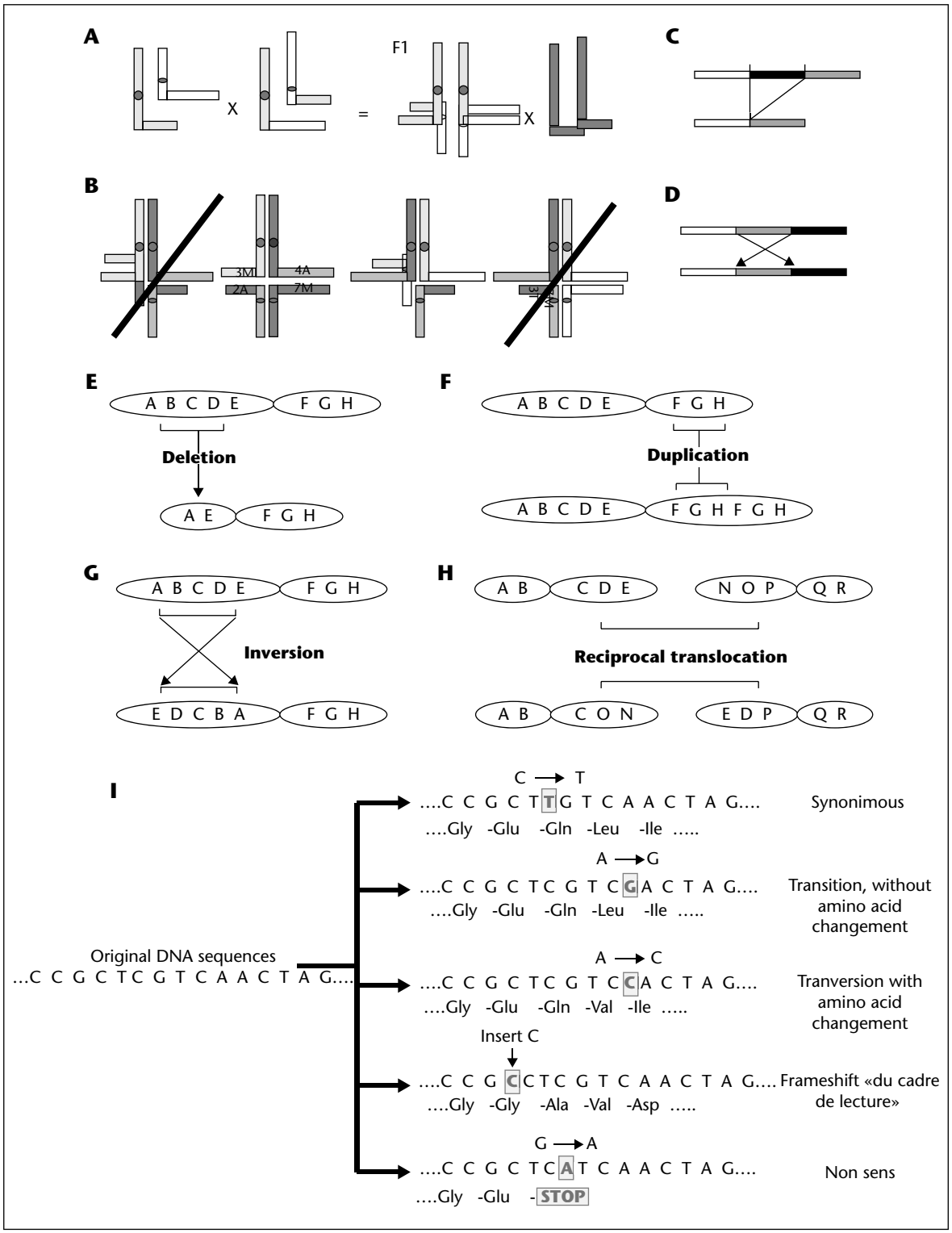

Figure 3. *Drawings of chromosome pairing and rearrangements. A. pairing in reciprocal translocation, B. chromosomal composition and abortion (/) in a backcross. C: indel deletion/insertion; D inversion of a central segment; E deletion; F duplication; G inversion; H reciprocal translocation; I mutation 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 tranversion, 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.*

reinserted in the other direction between the same ends. This type of 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 and fixed 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 *Helianth*us (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 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 hyrbrids 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)*.

Table 2. *List of lines used.*

Crop lines: line status	
Fertile	Male sterile
83HR4	GMS-83HR4
RHA801	GMS-RHA801
RHA274	PEF1-RHA274
92B6	PET1-92A6
D34	PET1-D34
HA89	PET1-HA89
BULK_HAC (mixed pollen of 6 cultivated lines)	
BULK_HAS (mixed pollen from 6 wild H. annuus)	
HA734 H. annuuswild	

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 soiled, and cold temperature). Moreover, these pecies have been evaluated for different biotic stresses (Downy Mildew, Phomopsis, Sclerotinia, Rust, and Botrytis as examples [Serieys, 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 (Helianthinins, 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 not 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 (Dussle *et al.*, 2004), and Sclerotinia, resistance (Rönicke *et al.*, 2004). *H. debilis* Nutt. induces drought tolerance, broomrape (*Orobanche cumana*) resistance (Labrousse *et al.*, 2001) *H. argophyllus* Phomopsis (Besnard *et al.*, 1997), *H. praecox* Engelm. and A.Gray, *H. niveus* Benth.) Brandegee, *H. neglectus* Heiser, *H. bolanderi* Gray, *H. exilis* A. Gray induce drought tolerance.

Several introgression genetic pools derived by INRA-Montpellier from annual species have been released to sunflower breeders since about 10 years (Serieys *et al.*, 2000). In most cases, the introgression regions have not been mapped, but several programs (Sunyfuel, Oleosem P. Vincourt 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. nuttalii* T. and G. (Atlagic, 1996); *H maximiliani*Schrad. Whelan and Dorrell (1980) have inter-specific hybrids *H.maximilianixH. 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 Jerusalemn 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. paucifloru 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 interpecific hybridization (true and partial hybrids) since it is difficult to cross with sunflower (Serieys, 1987) based on the work by Georgieva-Todorova (1990), the cytogenetic of the hybrids *H. mollis* × *H. annuus* is known. The species is diploïd 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 (Serieys, 2009). Cytogeneticaldata 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 is under publication (Breton *et al.* submitted). Genetic studies were made on a BC_2S_2 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 BC1 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 shriveled 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 accessions, 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 accessions 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 accessions are efficient as male in inter-specific crosses. We computed the effect of the pollen donor or receptor on the seed size samples *(tables 5-6; figure 4AB)*. We found that wild HIR-672 and RIG-236 produced compatible hybridizations whatever the direction of crosses, but for other accessions 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.

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

Species Croplines	2n =	83HR4	92B6	HA89	RHA274	D34	RHA801	HA734	BU_HAC	BU_HAS	Total
H. giganteus-553	34	113		21	2	9	7	9	5	4	170
H. giganteus-554	34	6		101	2	4	7	3	1	7	131
H. hirsutus-260	68					1				6	7
H. hirsutus-672	68	15	35	469	300	260	19	113	189	144	1544
H. maximiliani-1019	34			1							1
H. maximiliani-104	34	27	9	92	84	85	5		122		424
H. maximiliani-1050	34		1	42					7	3	53
H. nuttallii-103	34			19	2	28	17	6	2	19	93
H. nuttallii-934	34	18	6	43	12	27	7				113
H. pauciflorus-1033	102	125	2	850	56	198	75	8	4	1	1319
H. rigidus-101	102			1				7		18	26
H. rigidus-236	102			259	10			179	14	177	639
H. strumosus-1224	102			25	1			65		20	111
H. strumosus-1506	102		2	70	14		2			5	93
H. tuberosus-572	102	1		47	3		6		1	1	59
H. tuberosus-732	102	41	38	257	56	96	74		39		601
Total		346	93	2297	542	708	219	390	384	405	5384

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

Female	Seed size/head	Number of crosses	Duncan	Female	Seed size/head	Number of crosses	Duncan
RIG-236	26.625	12	A	NUT-934	0.75	71	D
HIR-672	13.29	71	B	GIG-553	0.688	71	D
STR-1224	6.271	16	C	MAX-1050	0.573	58	D
TUB-732	5.237	59	C	GIG-554	0.442	60	D
MAX-104	4.496	40	DC	STR-1506	0.409	55	D
RIG-101	2.5	10	DC	HIR-260	0.389	18	D
NUT-103	0.969	73	D	TUB-572	0.161	56	D
PAU-1033	0.804	40	D	MAX-1019	0	16	D

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 tetraploïd and hexaploïd 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 5. *Male parent's effect on seed size per head and total number of seeds with wild as female. (N = Number of crossed heads)*

Male	Nseeds/heads	N	Duncan
HA734	4.821	73	A
BULK_HAC	4.623	75	BA
BULK_HAS	4.066	83	BAC
D34	3.949	102	BAC
HA89	2.211	116	BDAC
RHA274	2.158	95	BDAC
RHA801	1.913	67	BDC
92B6	1.782	44	DC
83HR4	1.035	71	D

Table 6. *Effect of female parent on seed size per head with crop sunflower as female and total number of seeds. (N = Number of crossed heads)*

Female	N	Nseeds/heads	Duncan
PET1-HA89	119	17.05	A
GMS-83HR4	62	4.532	B
PET1-D34	82	3.159	B
PEF1-RHA274	84	3.119	B
GMS-RHA801	45	1.578	B
PET1-92A6	34	0.294	B

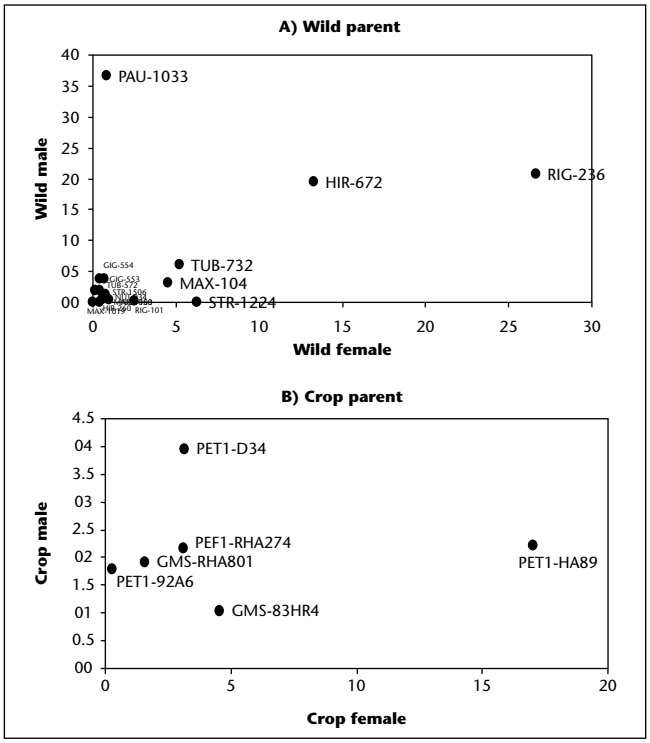

Figure 4. *Effect of the pollen contribution A) « Donnor » or B) « Receptor » from the wild parent, on pollen viability of the progenies. Wild parent: compatibles hybridizations (HIR-672, RIG-236), independence of the direction of the cross, thus it is an advantage to use the wild parent as the pollinator. Crop parent: advantage to use the crop parent as the female (CMS). PET1-HA89 is the best parent.*

Table 7. *Male parent's effect on seed size per head and total number of seeds with crop sunflower as female. (N = Number of crossed heads)*

Male	N	Nseeds/heads	Duncan	Male	N	Nseeds/heads	Duncan
PAU-1033	35	36.71	A	NUT-934	38	1.24	B
RIG-236	13	20.69	BA	MAX-1050	45	0.51	B
HIR-672	29	19.52	BA	NUT-103	34	0.38	B
TUB-732	42	6.1	B	RIG-101	8	0.13	B
GIG-554	27	3.78	B	MAX-1019	10	0.1	B
GIG-553	32	3.69	B	HIR-260	11	0	B
MAX-104	35	3.23	B	STR-527	2	0	B
STR-1506	35	1.94	B	STR-1224	2	0	B
TUB-572	27	1.85	B				

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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REFERENCES

Asad A, Jonard R, Seireys H. La culture d'embryons isolés in vitro dans un programme d'amélioration du Tournesol. *Comptes Rendus Academie des Sciences Paris* 1986; 302: 161-4.

Atlagic J. Cytogenetic study of interspecific hybrids (*H. annuus* L x *H. nuttalii* T and G. Eucarpia. *Proc oil and protein crops* 1996; 27-31.

Atlagic J, Dozet B, Skoric D. Meiosis and pollen vialbility in Helianthustuberosus L. and its hybrid with cultivated sunflower. *Plant Breeding* 1993; 111: 318-24.

Azpiroz HS, Vincourt P, Serieys H. Utilisation of "in vitro" test as an early screening technics for drought stress evaluation in sunflower. In: *Proc.12th Sunflower conf. Int., Novi Sad* Yugoslavia, 1988 : 207-13.

Anisimova I. Identification of Sunflower Genetic and Selection Material by Seed Proteins. *J AgrarnayaRossia* 2002: N52-N59.

Bernacchi D, Beck Bunn T, Eshed Y, *et al.* Advanced backcross QTL analysis of tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersiconhirsutum*. *Theor Appl Genet* 1998; 97: 381-97.

Besnard G, Griveau Y, Quillet MC, *et al.* Specifying of introgressed regions from *H.argophyllus* in cultivated sunflower (*Helianthus annuus L.*) to mark Phomopsis resistance genes. *Theor Appl Genet* 1997; 94: 131-8.

Burke JM, Lai Z, Salmaso M, *et al.* Comparative mapping and rapid karyotypic evolution in the genus *Helianthus*. *Genetics* 2004; 167: 449-57.

Cavallini A, Zolfino C, Cionini G, *et al.* Nuclear DNA changes within *Helianthus annuus* L.: cytophotometric, karyological and biochemical analyses. *Theor Appl Genet* 1986; 73: 20-6.

Cazaux E, Serieys H, Lambert P, Sossey-Alaoui K, Tersac M, Bervillé A. Phenotypic and molecular analyses of « sunflower x *Helianthusmollis* » interspecific crosses.Pekin 14th *International Sunflower Conference* 1996; Proc 2 : 1093-98.

Denat JF, Coumans M, Serieys H, Jonard R. Définition d'un milieu de culture permettant le développement d'embryons immatures de tournesol de 3 jours. *Comptes Rendus Academie des Sciences Paris* 1991;313 (série III): 45-51.

Dussle CM, Hahn V, Knapp SJ, Bauer E. Plargfrom Helianthus argophyllusis unlinked to other known downy mildew resistance genes in sunflower. *TheorAppl Genet* 2004; 109: 1083-6.

Faure N, Serieys H, Cazaux E, Kaan F, Bervillé A. Partial Hybridization in wide crosses between sunflower and *H. mollis* or *H. orgyalis*: effect of in vitro culture compared to natural crossing. *Plant Cell Report* 2002a; 20: 943-7.

Faure N, Serieys H, Cazaux E, Kaan F, Bervillé A. Partial hybridisation in wide crosses between cultivated sunflower and the perennial Helianthus species *H. mollis* and *H. orgyalis*. *Ann Bot* 2002b; 89: 31-9.

Faure N, Serieys H, Cazaux E, Bervillé A, Kaan F. Occurrence of partial hybrids in wide crosses between sunflower (Helianthus annuus) and perennial *H. mollis* and *H. orgyalis*. *TheorAppl Genet* 2002c; 104: 652-60.

Gandhi SD, Heesacker A, Freeman CA, Argyris J, Bradford K, Knapp SJ. The selfincompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. *Theor Appl Genet* 2005; 111: 619-29.

Gaut BS. Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. *Genome Res* 2001; 11: 55-66.

Gavrilova V, Savchenko NA. 2005 *Studies of Hybrids Between Cultivated Sunflower and Wild Perennial Helianthus Species*, www.vir.nw.ru/sunflow/sunflow.htm.

Georgieva-Todorova J. The Wild Growing *Helianthus*-Spp as Genetic Potential for Improvement of the Cultivated Sunflower. *Genetika i Selektsiya* 1990; 23: 276-82.

Heesacker AF, Bachlava E, Brunick RL, Burke JM, Rieseberg LH, Knapp SJ. Karyotypic Evolution of the Common and Silverleaf Sunflower Genomes. *The Plant Genome* 2009; 2: 233-46.

Heijnen TN, De Keyser L, Van Asseldonck A, *et al.* Evidence for an ancient chromosomal duplication in Arabidopsis thaliana by sequencing and analyzing a 400-kb contig at the APETALA2 locus on chromosome 4. *FEBS Lett* 1999; 445: 237-45.

Hiscock SJ, Allen AM. Diverse cell signalling pathways regulate pollen-stigma interactions: the search for consensus. *New Phytol* 2008; 179: 286-317.

Jackson RC, Murray BG. Colchicine induced quadrivalent formations in the *Helianthus:* evidence of ancient polyploidy. *Theor Appl Genet* 1983; 64: 219-22.

Jan CC, Chandler JM. Sunflower Interspecific Hybrids and Amphiploids of Helianthus annuus x H. Bolanderi. *Crop Sci* 1989; 29: 643-6.

Jan CC, Fernández-Martínez JM, Ruso J, Muñoz-Ruz J. Registration of Four Sunflower Germplasms With Resistance to *Orobanchecumana* Race F. *Crop Sci* 2002; 42: 2217-8.

Jan CC, Feng J, Seiler GJ, Rashid KY. Development of Sclerotinia head rot resistant germplasm utilizing H. maximiliani and H. nuttallii. *29th Sunflower Research Workshop*, 2007, Fargo, ND. Available: http://www.sunflowernsa.com/research/research-workshop/documents/Jan_etal_Germplasm_2007.pdf.

Kahane-Weinachter C, Serieys H, Bervillé A. *Rapport Promosol. Carrefour de la sélection*. Cahors, 2008.

Labrousse P, Arnaud MC, Serieys H, Bervillé A, Thalouarn P. Several mechanisms are involved in Helianthus resistance to *Orobanchecumana* Wallr. *Ann Bot* 2001; 88: 859-68.

Lai Z, Nakazato T, Salmaso M, *et al.* Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. *Genetics* 2005; 171: 291-303.

Leclercq P, Cauderon Y, Dauge M. Sélection pour la resistance au mildiou du tournesol a partir d'hybrides Topinambour x Tournesol. *Ann Amél Plantes* 1970; 20: 363-73.

Lorieux M, Goffinet B, Perrier X, Gonzalez de Leon D, Lanaud C. Maximum-likelihoodmodels for mappinggenetic markers showingsegregationdistortion. 1. Backcross populations. *Theor Appl Genet* 1995; 90: 73-80.

Luro F, Lorieux M, Laigret F, Bove JM, Ollitrault P. Cartographie du génome des agrumes à l'aide des marqueurs moléculaires et distorsions de ségrégation. In: Bervillé A, Tersac M, eds. *Techniques et utilisations des marqueurs moléculaires. Colloques de l'INRA n° 72*. Versailles, France: INRA, 1995: 69-82.

Ordon F, Habekuss A, Kastirr U, Rabenstein F, Kuhne T. Virus Resistance in Cereals: Sources of Resistance, Genetics and Breeding. *J Phytopathol* 2009: 3-11.

Pinochet X, Serieys H. Selection du tournesol: les promesses des formes sauvages d'*Helianthus. Oleoscope* 2001; 64: 4-7.

Quillet MC, Madjidian N, Griveau Y, *et al.* Mapping of genetic factors controlling interspecific pollen viability in an interspecific cross in *Helianthus* sect. *Helianthus*. *Theor Appl Genet* 1995; 91: 1195-202.

Rahim M, Jan CC, Gulya TJ. Inheritance of resistance to sun¯ower downy mildew races 1, 2 and 3 in cultivated sunflower Downy Mildew Race 2 in Sunflower. *J Biol Sci* 2001; 1: 821-4 [*Helianthus annuus* L].

Rieseberg LH, Linder CR, Seiler GJ. Chromosomal and genic barriers to introgression in *Helianthus*. *Genetics* 1995; 141: 1163-71.

Rogers CE, Tompson TE, Seiler GJ. *Sunflower species of the United States*. National Sunflower Association (ed), Bismarck, USA 1982; 75 pp.

Rönicke S, Hahn V, Horn R, *et al.* Interspecific hybrids of sunflower as a source of Sclerotinia resistance. *Plant Breeding* 2004; 123: 152-7.

Schilling EE, Heiser CB. Infrageneric classification of *Helianthus* (Compositae). *Taxon* 1981; 30: 393-403.

Seiler GJ, Gulya TJ, Marek FL. Exploration for wild *Helianthus* species from the desert southwesternusa for Potential drought tolerance. *HELIA* 2006; 29: 1-10.

Seiler GJ, Jan C, Gulya TJ. Sunflower Germplasm Diversification and Characterization Utilizing Wild Sunflower Species. *Cytogenetics, and Applied Genomics* 2007; http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=231245.

Seiler GJ. Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research* 1992; 30: 195-230.

Serieys H. Genetic Evaluation and Use of Helianthus Wild Species and their Use in Breeding Programs, In: Skoric D. (Editor), FAO, Rome, Italy, FAO Subnetwork report 1984-1986. 1987; 1-23.

Serieys H. In vitro culture of zygotic embryos: its use in Soya and Sunflower improvement. *Reproductive Biology and Plant Breeding*. Springer-Verlag 1992 ; 235-46.

Serieys H. Interêt des Helianthus sauvages pour l'amélioration de la resistance aux maladies du tournesol. Carrefour de la sélection Montpellier. 1992.

Serieys H. L'apport des espèces sauvages à l'amélioration du tournesol. *CETIOM-Oléoscope* 1997; 42: 14-7.

Serieys H, Tagmount A, Kaan F, *et al.* Evaluation of an interspecific sunflower population issued from the perennial species *H. occidentalis* ssp. *plantagineus* for resistance to *Diaporthehelianthi* and *Sclerotiniasclerotiorum* in relation with phenotypic and molecular traits. *Proc 15th Int. Sunflower Conf* Toulouse, France, 2000; II pp O25-O30.

Serieys H. Le programme introgression *H. mollis* CR PROMOSOL 2009. *Carrefour de la sélection*, Albi.

Sossey-Alaoui K, Serieys H, Tersac M, *et al.* Evidence for several genomes in *Helianthus*. *Theorw Appl Genet* 1998; 97: 422-30.

Strasburg JL, Rieseberg LH. Molecular demographic history of the annual sunflowers *Helianthus annuus*and *H. petiolaris*– Large effective population sizes and rates of long-term gene flow. *Evolution* 2008; 62: 1936-50.

Tang S, Yu JK, Slabaugh MB, Shintani DK, Knapp SJ. Simple sequence repeat map of the sunflower genome. *Theor Appl Genet* 2002; 105: 1124-36.

Tang S, Knapp SJ. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. *Theor Appl Genet* 2003; 106: 990-1003.

Tanksley SD, Nelson JC. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadaptedgermplasm into elite breeding lines. *Theor Appl Genet* 1996; 92: 191-203.

Timme RE, Simpson BB, Linder CR. 2007 high-resolution phylogeny for helianthus (Asteraceae) using the 18s-26s ribosomal DNA. *Amer J Bot* 2007; 94: 1837-52.

Tu Y, Sun J, Ge X, Li Z. Chromosome elimination, addition and introgression in intertribal partial hybrids between *Brassicarapa* and *Isatisindigotica*. *Ann Bot* 2009; 103: 1039-48.

Whelan RJ, Dorrell DG. Interspecific hybrids *H. maximiliani*Schrad. X H. annuusL: effect of backcrossing on meisosis, anther morphology and seed characteristics. *Crop Sci* 1980; 20: 29-34.

Yu JK, Mangor J, Thompson L, Edwards KJ, Slabaugh MB, Knapp SJ. Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. *Genome* 2002; 45: 652-60.

Yu JK, Tang S, Slabaugh MB, *et al.* Towards a saturated molecular genetic linkage map for cultivated sunflower. *Crop Sci* 2003; 43: 367-87.