Developing Unique Interspecific Germplasm for Sunflower Improvement

C. C. JAN
USDA-Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND 58105

Abstract

The narrow genetic base of cultivated sunflower limits future improvement and increases the crop's vulnerability. Among the 49 *Helianthus* species, the 11 annual species are relatively easy to utilize via conventional breeding. However, because of postzygotic abortion of the hybrid embryos and F_1 sterility, most perennial wild species have not been available for breeding purposes. A modified B_5 culture medium effectively rescued immature interspecific embryos, and a subsequent colchicine treatment successfully doubled the chromosome number of the F_1 hybrids, leading to the production of 10 amphiploids, including hybrids of *H. mollis*, *H. strumosus*, *H. gracilentus*, *H. cusickii*, *H. pumilus*, *H. grosseserratus*, *H. maximiliani*, *H. nuttalii*, and *H. hirsutus* crossed with cultivated line P21. These amphiploids contain complete genetic diversity of the wild species parents, have restored fertility, and will produce sufficient seeds in continuing backcrosses for adequate selection in a breeding program. Without amphiploidization, the BC_1F_1 seed set was often low, and most seedlings were too weak to produce early generation germplasms with sufficient genetic variability. These hexaploid and tetraploid amphiploids represent the earliest generation germplasm with maximum genetic diversity from wild species in a form easily utilizable for conventional breeding.

Key Words: interspecific hybridization, embryo rescue, chromosome doubling, amphiploids, germplasm

Introduction

The narrow genetic base of cultivated sunflower (*Helianthus annuus* L.) resulting from the widespread use of the early Russian germplasm sources and the lack of sizable addition of new genetic variability has been a major concern of the sunflower industry for many years. This awareness has promoted utilization of wild *Helianthus* species in recent years through conventional interspecific hybridization and backcrossing. Over 40 cytoplasmic male sterility sources currently are available (SERIEYS 1994) and 40 interspecific germplasm lines were released by Seiler (1991 a, b, c; 1993). Interspecific gene transfer from wild annual *Helianthus* species into cultivated lines has been successful for resistance to powdery mildew (JAN & CHANDLER 1985), rust (QURESH & JAN 1993;

QURESH et al. 1993), and downy mildew (JAN et al. 1992). However, crossing wild perennial *Helianthus* species with cultivated lines often results in embryo abortion and F_1 sterility, and thus limits its utilization for sunflower improvement.

Interspecific crosses between wild diploid or tetraploid perennial *Helianthus* species and cultivated line P21 have been in progress for over 10 years. Basic embryo rescue techniques of CHANDLER and BEARD (1983) were followed for culturing immature interspecific embryos. Modifications included the addition of the MES buffer to culture media to maintain a constant pH of 5.5, and a change in the germination medium from liquid to solid with 0.7% agar and 2% sucrose. Apical meristems of F_1 seedlings were treated with a solution of 0.15% colchicine in 2% DMSO (dimethyl sulfoxide) for 5 h in the dark (JAN 1988). Chromosome doubling of each head was verified by pollen grain size and stainability (JAN 1988; ALEXANDER 1969). Chromosomally doubled heads of each cross combination were sib-pollinated to produce amphiploid seeds. This report examines the tetraploid and hexaploid amphiploid plants for chromosome number, seed set from self-pollination, sib-pollination and backcrosses with cultivated sunflower, and their potential use for sunflower improvement.

Materials and Methods

Seeds resulting from sib-pollination of chromosomally doubled heads of each of 10 F_1 cross combinations, including hybrids of *H. mollis, H. strumosus, H. gracilentus, H. cusickii, H. pumilus, H. grosseserratus, H. maximiliani, H. nutalii,* and *H. hirsutus* crossed with P21, were germinated in petri dishes following the procedure of Chandler and Jan (1985), transferred to 3.5-cm diameter jiffy pellets, and then to 11-cm diameter clay pots for root-tip sampling. Root-tips were sampled one month after germination. Preparation of the root-tips for chromosome count followed the procedure of Jan Dvorak (personal communication) with slight modification. Root tips were cold treated at 2°C in distilled water for 19 h, fixed in 3 parts of 95% ethanol and 1 part of glacial acetic acid for a minimum of 2 h, in 1 N HCl at 37°C for 15 min, Feulgen solution for 50 min, enzyme solution of 0.2% pectinase and 0.2% cellulase for 23 min, and stored in tap water for 24 h before examination. Amphiploid plants were grown in the greenhouse in 1993 and 1994, and were self-pollinated, sib-pollinated, and backcrossed with pollen of inbred line HA89. Selected BC_1F_1 families of the amphiploids were again self-pollinated, sib-pollinated and further backcrossed with HA89.

Results, Discussion and Conclusion

Plant survival, chromosome number and the seed set of amphiploids are presented in Table 1. The overall germination was acceptable and most seedlings survived to maturity. There was variation in the $2n$ chromosome numbers for both tetraploid (4x) and the hexaploid (6x) amphiploids. All the amphiploids were self-

incompatible, indicating a strong dominance effect of the wild species self-incompatible genes. Good seed set was obtained from both sib-pollination and backcrosses for most amphiploids, except for the crosses involving *H. pumilus*, *H. cusickii*, and *H. hirsutus* 1537. The fact that the amphiploid of *H. hirsutus* 1126 had good BC seed set and that of *H. hirsutus* 1537 had nearly zero BC seed set indicated intraspecific variations useful for conducting interspecific gene transfer by geneticists and breeders.

Descriptions of the BC_1F_1 families are presented in Table 2. All the BC_1F_1 plants were from direct seed germination, except for those involving *H. hirsutus* 1537, which were obtained only through embryo rescue. The BC_1F_1 families had improved seed germination, and almost all the plants grew to maturity and produced abundant BC_2F_1 seeds, regardless of whether they were 3x or 4x plants. The triploid plants in the family of *H. arizonensis* x P21 were from crosses of chromosomally doubled F_1 heads, instead of amphiploids, with HA89.

In the BC_1F_1 generation, large variations in chromosome number and the resulting unbalanced genetic constitution of individual plants affected plant survival (Table 3). For 4x amphiploids, plants with chromosome numbers closer to either 34 or 51 survived better than plants with intermediate chromosome numbers. For the 6x amphiploids, their BC_2F_1's had $2n$=50-52 and survived well.

In order to demonstrate interspecific gene transfer, rust resistance to the four North American (NA) races was used for two selected crosses. In a separate study (JAN & ZHANG 1995), $2n$=35 plants resistant to NA rust races 1 and 2 were obtained among BC_3F_1 progenies of *H. hirsutus* 1537 x P21 amphiploids. Further backcrosses of those plants resulted in $2n$=34 plants resistant to NA rust races 1 and 2, indicating successful gene transfer. However, after crosses of $2n$=43 BC_2F_1 plants of (*H. arizonensis* x P21, D) $HA89^2$, immune to the four NA rust races, with HA89, none of the resulting plants had resistance. The fact that over 90% of the plants had $2n$=34 and half of them were self incompatible indicated that the quick elimination of *H. azrizonensis* chromosomes and the rust resistance did not affect the transfer of self-incompatible genes into the cultivated background. Future use of molecular markers, in addition to conventional markers, will greatly facilitate our understanding of interspecific gene transfer.

Our preliminary evaluations of these amphiploids indicated total immunity to the four NA rust races for crosses involving *H. strumosus*, *H. grosseserratus*, *H. nuttallii*, *H. maximiliani*, and *H. hirsutus*. Evaluations by Dr. J. Fernandez-Martinez (personal communication) indicated that perennial *Helianthus* accessions were nearly totally immune to all the races of *Orobanche*, including newly evolved virulent race(s) attacking all the resistance genes in cultivated sunflower. Backcross of difficult interspecific hybrids without chromosome doubling resulted in very low seed set (JAN 1988). The BC_1F_1 plants are often too weak to survive

and to produce BC seeds. With dramatically improved seed set fertility and genetic variability from wild species parents, these amphiploids represent a unique source of germplasm for future sunflower improvement. All the amphiploids will be released to the public when sufficient seed is produced, begining in 1996.

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Table 1. Plant survival, chromosome number, and seed set of amphiploids.

Parentage	Seed	Seedling	Matured plant	2*n* chromosome	Seed set (seeds/head)†		
					BC	Sib	SP
	No.						
H. gracilentis 1442 x P21	33	25	22	66-69	38	67	---
H. pumilus 773 x P21	---	---	7	67-68	3	1	---
H. hirsutus 1126 x P21	6	6	5	96-102	50	17	0
H. mollis 1531 x P21	18	8	7	68-69	18	19	0
H. strumosus 30-002-1 x P21	19	10	7	99-103	22	19	0
H. grosseserratus x P21	13	9	9	66-68	50	6	0
H. maximiliani x P21	30	24	20	66-69	115	44	0
H. nuttallii 730 x P21	30	29	29	66-70	44	88	0
H. cusickii 17-002-1 x P21	3	3	3	67-68	2	1	0
H. hirsutus 1537 x P21	---	---	---	94-102	0.03	5.3	0.06

Table 2. Plant survival, chromosome number, and seed set of amphiploids x HA89, BC_1F_1.

Parentage	Seed	Seedling	Matured plant	2*n* chromosome	Seed set (seeds/head)		
					BC	Sib	SP
	No.						
(*H. grac.* x P21, AP) x HA89	---	---	31	49-53	25	0	---
(*H. pum.* x P21, AP) x HA89	---	---	29	50-51	31	0	---
(*H. hir.* x P21, AP) x HA89	2	2	2	68	215	---	---
(*H. str.* x P21, AP) x HA89	6	5	5	67-68	581	263	38
(*H. gro.* x P21, AP) x HA89	11	5	5	50-51	176	---	0
(*H. max.* x P21, AP) x HA89	12	11	9	50-52	118	---	0
(*H. cus.* x P21, AP) x HA89	12	10	9	48-51	66	---	0
(*H. ari.* x P21, D) x HA89	17	16	13	51	93	---	0
(*H. hir.* x P21, AP) x HA89	---	---	---	66-68	64	28	0.3

† BC=backrossed; Sib=sib-pollinated; SP=self-pollinated.

Table 3. Plant survival, chromosome number and backcross seed set of BC_2F_1 generation.

Parentage	Seed	Seedling	Matured plant	2*n* chromosome	BC seed/ head
	No.				
(*H. gracilentus* x P21, AP) HA89[2]	39	35	0	35,43	—
(*H. pumilus* x P21, AP) HA89[2]	78	74	8	34-51	70
(*H. arizonensis* x P21, AP) HA89[2]	78	74	15	34-50	105
(*H. hirsutus* x P21, AP) HA89[2]	40	39	38	50-52	15

Table 4. Plant survival, chromosome number, and rust reactions of BC_3F_1 plants, (*H. arizonensis* x P21, AP) HA89[3].

Parentage[†]	Seed	Seedling	Matured plant	2*n* chromosome	Rust race 1	2	3	4
	No.							
(*H. arizonensis* x P21, D) x HA89[3]	80	32	1	38-46	–	–	–	–
HA89 x [(*H. arizonensis* x P21, D) x HA89[2]]	270	259	25	34-37	–	–	–	–

[†]AP=amphiploid; D=chromosomally doubled head.