

Using interspecific hybrids with *Helianthus tuberosus* L. to transfer genes for quantitative traits into cultivated sunflower, *H. annuus* L.

Brent S. Hulke¹, Donald L. Wyse²

¹ USDA-ARS, Northern Crop Science Laboratory, 1307 18th St. N., Fargo, ND 58105 USA,
E-mail: brent.hulke@ars.usda.gov

² Department of Agronomy and Plant Genetics, University of Minnesota, 411 Borlaug Hall, 1991 Upper
Buford Circle, Saint Paul, MN 55108 USA, E-mail: wysex001@umn.edu

ABSTRACT

Interspecific hybrids in sunflower have been used historically as sources of disease resistance. Backcrossing for several generations is required to introgress genes from hexaploid species to annual sunflower (*Helianthus annuus* L.). A result of this repeated backcrossing is the rapid loss of genes from the donor parent. This article describes ongoing work to improve the efficiency and efficacy of introgressing genes from the hexaploid perennial *H. tuberosus* L. for quantitative traits. Of particular interest are genes which control host-plant resistance to diseases and insect pests, and perennial habit.

Key words: disease resistance – *Helianthus annuus* L. – *Helianthus tuberosus* L. – insect resistance – interspecific hybrid – perennial sunflower.

INTRODUCTION

Interspecies hybrids are commonly developed to introduce traits of interest, most notably disease resistance, into the domesticated annual sunflower (*Helianthus annuus* L.). Of the perennial sunflowers, the Jerusalem artichoke (*H. tuberosus* L.) has been a commonly utilized donor species historically. This is most likely due to its world-wide presence as a naturalized species and its ability to form hybrids easily with *H. annuus*. There are several notable discoveries of disease resistance introgressed from *H. tuberosus*. Resistance to downy mildew (caused by *Plasmopara halstedii*) has been transferred from *H. tuberosus* to *H. annuus* by Pustovoit et al. (1976). This germplasm was the source of the *PI5* downy mildew resistance gene (Miller and Gulya, 1987). Resistance to Phomopsis stem canker (caused by *Phomopsis helianthi*) has also been discovered in *H. tuberosus*, and germplasms have been developed in Europe that have a high degree of resistance to Phomopsis (Škorić, 1985). Resistance to Phomopsis is important to American sunflower breeders as this disease has caused notable losses in recent years in the USA. Resistance to Sclerotinia head rot (caused by *Sclerotinia sclerotiorum* (Lib.) De Bary) was transferred from *H. tuberosus* into interspecific populations in Russia (Pustovoit et al., 1976), and has been an important source of resistance. Additionally, *H. tuberosus* has been shown in trials to produce antixenosis to oviposition and antibiosis to larva of the banded sunflower moth (*Cochylis hospes* Walsingham), an insect which damages the heads of sunflower (Charlet and Brewer, 1995). This insect can cause significant damage, and resistance is of particular interest to sunflower breeders in the northern production area in the USA.

There is also interest by some U.S. researchers to develop perennial crops for use in a permaculture system, particularly in areas where soil erosion and leaching or runoff of fertilizers has caused environmental damage. Perennial crops provide living ground cover for longer periods during the growing year because the plants often emerge from dormancy long before annual crops are planted. This characteristic of perennial crops has been linked to reduced tile drainage flow and concurrent leaching of nitrogen from the soil (Randall et al., 1997). Perennialization of domesticated sunflower was first proposed by Ščibria (1936), who noted that populations of *H. tuberosus* X *H. annuus* could be candidates for development of a perennial sunflower. The investigator also noted the tendency for the F₁ hybrids to show hybrid vigor or heterosis. While hybrid populations have been formed by breeders in the past, no one has published specifically on the mode of inheritance of perennial habit in such populations. It is currently assumed to be under the control of many genes, and as such, may act as a quantitative trait. There has been mention of rhizome/tuber production in some populations, while in other populations there was no production of rhizomes or tubers (Cedeno et al., 1985; Kräuter et al., 1991). The populations with tuber production tend to have *H. tuberosus* as the female, indicating that perennial habit may have, at least, partial cytoplasmic inheritance.

Previous investigators have found that the F_1 hybrid plants are tetraploid ($2n=4x=68$) and the BC_1F_1 plants are triploid ($2n=3x=51$) (Cedeno et al., 1985). Low fertility was found in the BC_1F_1 plants, which was likely due to the triploid condition. Further backcrossing in populations like these is expected to result in progeny with greater meiotic stability through progressive loss of chromosomes.

The objective of this work is to determine the feasibility of introducing quantitative traits into domesticated sunflower using *H. tuberosus* as the genetic source of those traits. Traits of interest include resistance to diseases and insect pests, as well as perennial habit and traits of agronomic interest.

MATERIALS AND METHODS

Germplasm. Tuber stock of 18 *H. tuberosus* plants were collected in 2001 from UMORE Park, Rosemount, MN, USA. Nine of the plants were collected from cultivated areas and were named JA1 to JA9. Nine more plants were collected from undisturbed areas approximately 8 km from the first site of collection. These were named JA10 to JA18. All plants were transplanted to plots at the St. Paul Agricultural Experiment Station, St. Paul, MN, USA, where they still exist as a living plant collection. All plants are believed to be native to the area in which they were collected in east central Minnesota. In addition, seed of *H. annuus* inbred lines RHA 265, RHA 274, CMS HA 89, HA 89, CMS HA 434, and HA 434 were obtained from Gerald Seiler and Jerry Miller, USDA-ARS, Fargo, ND, USA, over the course of the project.

Experiment 1. This experiment was initiated to generate and study a maintainer (B-line) interspecific population. During the summer of 2003, seed of RHA 265, RHA 274, and CMS HA 89 were grown in the field in St. Paul, MN. Crosses were made in the following manner: JAx/RHA 265, JAx/RHA 274, and CMS HA 89/ JAx , where 'x' = 1 to 18. Each pairwise cross was maintained as a separate pedigree, and grown in a greenhouse during the winter of 2003-2004. Sib-mating among the plants within each full-sib family was attempted. During the summer of 2004, the F_1 plants of the crosses JAx/RHA 265 were transplanted to the field and pollen from HA 434 was used to pollinate the F_1 plants. Backcrosses were made with HA 434 as the pollen parent for three more generations, resulting in the equivalent of a BC_4F_1 population. This population was grown at the St. Paul station during the summer of 2006, and plants were self-pollinated.

Experiment 2. This experiment was initiated to generate and study a fertility-restorer (R-line) interspecific population. The F_1 progenies of the crosses JAx/RHA 265 were backcrossed in the greenhouse during the winter of 2004-2005 with RHA 265 as the pollen source. A second backcross was made on the BC_1F_1 plants during the summer of 2005. The BC_2F_1 plants were grown in the greenhouse during the winter of 2005-2006 and backcrossed again with RHA 265 as the pollen parent. The BC_3F_1 plants were grown in the field during the summer of 2006 for observation.

Experiment 3. This experiment was initiated to generate and study two sets of reciprocal maintainer populations of sunflower. The initial crosses were as follows: JAx/HA 89, CMS HA 89/ JAx , JAx/HA 434, and CMS HA 434/ JAx . The CMS lines contribute the French male-sterile cytoplasm of Leclercq (1969). The JA lines contribute *H. tuberosus* cytoplasm. At the same time, tetraploid derivatives of HA 89 and HA 434 were obtained by treating seedlings of the two inbred lines with colchicine, according to the protocol of Jan and Chandler (1989). Successfully doubled plants were pollen donors for a backcross with the F_1 plants. This procedure was adopted to avoid a sterility barrier observed in the BC_1F_1 plants in the previous experiments, believed to be due in part to the triploid nature of the BC_1F_1 plants. By crossing tetraploid F_1 plants with tetraploid inbred lines, we could obtain tetraploid BC_1F_1 plants, which we distinguished from standard BC_1F_1 plants using the notation $BC_1F_1^{(4x)}$. The $BC_1F_1^{(4x)}$ plants were grown in the field in 2006, and attempts were made at self pollination, sib-mating within full-sib families, and backcrossing with tetraploid HA 89 or HA 434 as the pollen donor.

RESULTS

Experiment 1. F_1 hybrids between the JA plants and the inbred lines were highly successful, as previously reported. Pollen production was observed on some of the F_1 plants. Sib-mated F_1 plants did not produce many seeds, and the seeds that were obtained produced plants with little vigor. Despite the lack of vigor, the progeny were perennial as determined by regrowth in the field after the winter of 2005-2006.

Backcrosses with the inbred line HA 434 as the pollen parent resulted in viable offspring. These BC₁F₁ plants were triploid and male sterile in appearance. Recurrent backcrossing was performed on the BC₁, BC₂, and BC₃ plants because the plants were male sterile and possessed considerable female sterility. A single BC₄F₁ plant survived to maturity in the field in 2006. The head was bagged and selfed seed was produced, indicating that the plant produced viable pollen. Most of the harvested BC₄F₂ seed was not filled, indicating that some sterility due to cytogenetic imbalance still exists in the population. The restoration of male fertility in the BC₄F₁ generation is common in hexaploid X diploid *Helianthus* populations (C.C. Jan, personal communication). None of the backcross generations beyond the F₁ appeared to produce perennial organs (rhizomes or tubers), and the BC₁F₁ and BC₂F₁ plants failed to recover from the winter of 2005-2006 in the field, indicating loss of perennial habit in the population. By comparison, the *H. tuberosus* parents and F₁ population had nearly 100% survival after every winter since 2004. The population will be maintained for phenotypic evaluation of other traits of interest.

Experiment 2. The BC₁F₁ plants of the R-line population were grown in the field in 2005. The plants were generally short in stature and lacked vigor. During the time in which backcrosses were being made, it was observed that some plants within the JA9/RHA 265//RHA 265 population produced pollen. These plants were sib-mated, but produced no viable seed. The BC₁F₁ plants did not survive the winter of 2005-2006, a result similar to that in Experiment 1, which indicates that the plants were not perennial. The BC₂F₁ and BC₃F₁ generations were obtained by backcrossing with RHA 265 pollen, and neither generation had pollen production. No additional work has been performed on this population since 2006, due to lack of different results. It is likely that fertility will be restored if the BC₄F₁ generation is produced.

Experiment 3. The F₁ plants of the reciprocal populations were grown in the greenhouse, and perennial organs were observed both in plants with the *H. tuberosus* cytoplasm and plants with the *H. petiolaris* (French) cytoplasm. Both sets of F₁ plants were transplanted to the field in 2006, and almost 100% of the plants survived the winter of 2006-2007. The BC₁F₁^(4x) generation was grown in the field in 2006. The estimated number of BC₁F₁^(4x) plants was around 1000, which was, by far, the largest BC₁F₁ nursery obtained in our experiments. The plants possessed considerable vigor, except for one pedigree JA13/HA 434//HA 434^(4x), which seemed to be shorter in stature and lacking vigor. The height of the plants of the other pedigrees was similar to that of the annual inbred lines (Fig. 1). Pollen production was observed on many of the plants. Self-pollination and sib-mating within full-sib families was attempted on most of the plants. A small subset of the plants had evidence of rhizome production, especially in the HA 89-derived populations. These plants were backcrossed using pollen from the tetraploid annual inbred line. Despite the vigor of these lines, very few seeds were produced. The viability of this seed is currently being investigated. Eight plants from these populations were perennial, because they were able to regrow after the winter of 2006-2007. These plants have been transplanted to a permanent collection.



Fig. 1. Appearance of $BC_1F_1^{(4x)}$ plants in the 2006 field nursery. The annual inbred parents appear on the left half of the photo and the $BC_1F_1^{(4x)}$ plants on the right half of the photo.

DISCUSSION

Experiments 1 and 2 represent a backcrossing procedure that has been used in the past to introgress genes of interest from perennial hexaploid species to annual breeding germplasm. This procedure has been used repeatedly worldwide to introgress genes from *H. tuberosus* and other perennial species into *H. annuus*. Most of the traits introgressed by this method are disease resistance traits, although Pustovoit et al. (1976) noted heterosis for yield in hybrids with *H. tuberosus* genes.

The more genes involved in producing a trait, the more difficult it becomes to transfer the trait to a finished line in a backcross program. Most of the disease resistance traits are controlled by one or a few genes or gene blocks. The genetics for perennial habit are likely to be more complex. Hu et al. (2003) discovered that two dominant genes caused production of rhizomes in a rice interspecific hybrid population, with 14 other QTL modifying the characteristics of the rhizomes, including the number, branching habit, length, and size. Because backcrossing rapidly causes loss of genes from the donor species (*H. tuberosus*, in our case) and we have not successfully carried perennial habit through even one standard backcross in Experiments 1 and 2, it is likely that the genetics for perennial habit are at least as complex as that of rice. Until we develop a population with a stable genome and segregation for perennial habit, it will be impossible to perform a similar study in sunflower. In the meantime, the population produced from Experiment 1 will be useful in finding at least partial resistance to diseases and insect pests common to the USA.

The findings of Experiment 3 were more promising for studying perennial habit. First, it appears that perennial cytoplasm is not required to produce perennial plants, because those plants with the French cytoplasm were also perennial. Second, using a tetraploid version of the annual inbreds as the pollen donor in a backcross resulted in a larger BC_1 population that was more vigorous and more closely resembled the annual parent in appearance. It also resulted in 8 out of approximately 1000 plants that were successful perennials, thus indicating that this modified backcross procedure helped to better retain the required genes for perennial habit. This could be due to the fact that a larger population was obtained from these crosses, thus providing more observations. It could also be due to the fact that the tetraploid genome was more stable than the triploid genome of a standard BC_1 population, resulting in less gene silencing. Gene silencing via DNA methylation in interspecific sunflower populations is a documented event (Natali et al., 1998). A discouraging finding is the lack of seed formation in the $BC_1F_1^{(4x)}$ populations. This may be explained by our method of sib-mating, which was to intermate only those plants within a full-sib family. Despite introducing genes for self fertility, it is possible that self-incompatibility genes are still active in almost all of the plants in these populations. If this is true, pollen from full-sib individuals in the population would have been rejected because they would share the same

self-incompatibility alleles. Intermating half-sibs with different *H. tuberosus* parents may improve seed production, and will be attempted on the perennial BC₁F₁^(4x) plants. The reason for failure of a second backcross with the tetraploid inbred lines is unknown, but is likely due to mispairing of the chromosomes in the BC₁F₁^(4x) plants. Based on chromosome pairing in F₁ interspecies hybrids, Kostoff (1939) determined that the genomic formula of *H. tuberosus* is At₁At₂Bt, where the Bt genome is closely related to the *H. annuus* genome. Thus, the F₁ would have nearly normal pairing, but the BC₁F₁^(4x) plants would have abnormal pairing because the At genome is only present in a haploid form. More attempts at backcrossing the BC₁F₁^(4x) plants will be made in the future, possibly in combination with embryo rescue.

Rieseberg et al. (1995) theorized that sib-mating interspecific sunflower populations before backcrossing may allow for introgression of more and smaller blocks of genes from the wild parent. In our situation, this may mean that more of the necessary perennial genes may be transferred with greater ease and less linkage drag if backcrossing is attempted after sib-mating the F₁ population for a few generations. More genes for disease, insect, and agronomic traits may be introduced into the *H. annuus* genome, as well. The process of sib-mating half-sib families has already begun, with the first generation of sib-mated progeny already produced. Information from our current and ongoing backcrossing experiments will allow us to perfect a backcross procedure for use after sib-mating to maintain high levels of genetic variability in the population.

ACKNOWLEDGEMENTS

The authors wish to thank Kevin Betts for his assistance in making crosses, maintaining field plots, and supervising student workers. This work has been made possible by graduate student grants from The Land Institute, Salina, KS, USA; and North Central Region – Sustainable Agriculture Research and Education (NCR-SARE).

REFERENCES

- Cedeno, R., M.S. McMullen, and J.F. Miller. 1985. Cytogenetic relationship between *Helianthus annuus* L. and *H. tuberosus*. p. 541-546. In: Proc. 11th Int. Sunflower Conf., Mar del Plata, Argentina.
- Charlet, L.D., and G.J. Brewer. 1995. Resistance of native sunflowers (Asterales: Asteraceae) to the banded sunflower moth (Lepidoptera: Cochylidae). Environ. Entomol. 24:1224-1228.
- Hu, F.Y., D.Y. Tao, E. Sacks, B.Y. Fu, P. Xu, J. Li, Y. Yang, K. McNally, G.S. Khush, A.H. Paterson, and Z.K. Li. 2003. Convergent evolution of perenniality in rice and sorghum. Proc. Natl. Acad. Sci. USA 100:4050-4054.
- Jan, C.-C., and J.M. Chandler. 1989. Sunflower interspecific hybrids and amphiploids of *Helianthus annuus* X *H. bolanderi*. Crop Sci. 29:643-646.
- Kostoff, D. 1939. Autosyndesis and structural hybridity in F₁-hybrid *Helianthus tuberosus* L. X *Helianthus annuus* L. and their sequences. Genetica 21:285-300.
- Kräuter, R., A. Steinmetz, and W. Friedt. 1991. Efficient interspecific hybridization in the genus *Helianthus* via “embryo rescue” and characterization of the hybrids. Theor. Appl. Genet. 82:521-525.
- Leclercq, P. 1969. Une stérilité male cytoplasmique chez le tournesol. Ann. Amélior. Plantes 19:99-106.
- Miller, J.F., and T. Gulya. 1987. Inheritance of resistance to race 3 downy mildew in sunflower. Crop Sci. 27:210-212.
- Natali, L., T. Giordani, E. Polizzi, C. Pugliesi, M. Fambrini, and A. Cavallini. 1998. Genomic alterations in the interspecific hybrid *Helianthus annuus* X *Helianthus tuberosus*. Theor. Appl. Genet. 97:1240-1247.
- Pustovoit, G.V., V.P. Ilatovsky, and E.L. Slyusar. 1976. Results and prospects of sunflower breeding for group immunity by interspecific hybridization. p. 193-204. In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.
- Randall, G.W., D.R. Huggins, M.P. Russelle, D.J. Fuchs, W.W. Nelson, and J.L. Anderson. 1997. Nitrate losses through subsurface tile drainage in Conservation Reserve Program, alfalfa, and row crop systems. J. Environ. Qual. 26:1240-1247.
- Rieseberg, L.H., C. R. Linder, and G.J. Seiler. 1995. Chromosomal and genic barriers to introgression in *Helianthus*. Genetics 141:1163-1171.
- Ščibria, N.A. 1936. Hybrids between the Jerusalem artichoke (*Helianthus tuberosus* L.) and the sunflower (*Helianthus annuus* L.) C.R. (Doklady) de l'Académie des Sci. de l'URSS 11:193-196.
- Škorić, D. 1985. Sunflower breeding for resistance to *Diaporthe/Phomopsis helianthi*. Helia 8:21-24.