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Evaluating an interspecific *Helianthus annuus* × *Helianthus tuberosus* population for use in a perennial sunflower breeding program☆

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ABSTRACT

Perennial crops show promise for sustainable agricultural production while providing ecosystem services (maintaining healthy soil, controlling erosion, improving water quality, and enhancing wildlife habitat). Perennial crops could also provide economically viable cropping option to farmers. Sunflower (*Helianthus annuus* L.) is an ideal crop for perennialization because of existing genetic resources and a wide variety of end-uses. The objective of this research was to evaluate interspecific hybrids between perennial *Helianthus tuberosus* L. ($2n = 6x = 102$) and annual *H. annuus* L. ($2n = 2x = 34$) for perenniality and agronomic traits; assessing their utility in developing a perennial seed crop. Field trials indicated that seed yield traits were positively correlated with head traits. Tuber traits, which are required for perenniality, and seed yield traits were not correlated, indicating that simultaneous selection may be able to target high yielding lines that also tuberize. The F_1 individuals were intermated for one generation and the intermated F_1 (IM_1F_1) showed increases in head size (up to 20%) compared to the best F_1 individual. The lack of correlation between tuber and seed traits coupled with phenotypic improvement after one generation of intermating suggest that the best improvement strategy for perennial sunflower is a recurrent selection program focusing on yield.

1. Introduction

Over the past century, agricultural research has contributed to dramatically increased crop yields and productivity, yet this increase in productivity has often come at the expense of long term environmental sustainability through greater use of fossil fuel-based fertilizers, the depletion of fresh water, and the reduction of arable land (Baulcombe et al., 2009; Tilman et al., 2002). Addressing environmental damage and enhancing ecosystem services such as climate regulation, water management, and soil fertility will be essential for the adequate production of food in the future (Baulcombe et al., 2009; Costanza et al., 1997; Tilman et al., 2002). Currently there are cultural practices such as zero tillage and cover cropping, which provide many ecosystem services without a yield reduction. Recently, the addition of perennial plants, particularly perennial crops, has been suggested as another tool for incorporating ecosystem services into the landscape while maintaining productivity (DeHaan et al., 2005; Baulcombe et al., 2009; Glover et al., 2010; Chia et al., 2012). The potential of perennial crops to reduce the environmental impact of agricultural systems through reduction in fall tillage, soil erosion, and nutrient runoff has long been ignored, but recently has regained popular interest (Glover et al., 2010). In addition, due to reduced input costs, perennial grains can be as profitable as annual counterparts over a three year life of the perennial crop if the market price is equal and the perennial yields at least 60% as much as the annual crop (Bell et al., 2008).

Recent research has shown that the genetics of perenniality (development of perennial organs) may not be as complex as previously thought, with several studies identifying only a few quantitative trait loci (QTL) necessary for perennial organ development (Wang et al., 2009; Sacks et al., 2007; Hu et al., 2003). Hu et al. (2003) identified two QTL that controlled production of rhizomes in rice (*Oryza longisteminata* × *Oryza sativa* hybrids) with the segregation matching a dominant two gene model with complementary gene action. Stolon presence:absence segregated in a 3:1 fashion in F_2 families of the interspecific cross *O. sativa* × *O. rufipogon* (Sacks et al., 2007). Further, Wang et al. (2009) identified a single gene, perpetual flowering 1 (*PEP1*), which regulates

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perennial flowering in *Arabis alpina*. These findings suggest it may be possible to introduce perennial habit into annual crops without introducing large portions of wild relatives of the species.

Domesticated sunflower (*Helianthus annuus* L., $2n = 2x = 34$) is an annual crop that produces a diverse range of products, including oilseed types (used to produce birdseed or high-quality vegetable oil) and confection seeds for direct human consumption. Sunflower is a compelling target for perennialization, as *Helianthus* includes 49 species, many of which are perennial (Kane et al., 2013). Breeders have used interspecific hybridization to introgress useful wild traits into *H. annuus* for disease resistance (Miller and Gulya, 1987), insect resistance (Charlet and Brewer, 1995), adaptation to distinct environments, abiotic stress (Rieseberg, 1997), and cytoplasmic male sterility (Kohler and Friedt, 1999). A similar approach could be used to introgress the perennial habit, as perennial *Helianthus* species can potentially be used as donor materials for transferring perennial habit into domesticated sunflower. Breeding for perennial seed crops poses a unique problem because perennials need to allocate photosynthetic resources to both the perennial organs used for carbon storage and the seed itself (DeHaan et al., 2005). It has been suggested that perennial plants can be selected for increased seed production while maintaining asexual reproduction (Cox et al., 2002; DeHaan et al., 2005). Perennial plants have a longer growing season to assimilate nutrients and breeding can influence photosynthate utilization to optimize seed production and perennial habit. In addition, there is historical precedent as farmers who initially domesticated rice selected for perennial habit during low intensity production (Hill, 2010).

Helianthus tuberosus, a tuber-bearing perennial species, is a prime candidate for the introduction of perenniality into domestic sunflower. It has been used to introgress traits into *H. annuus* for nearly a century and has a separate history as a specialty crop (Hulke and Wyse, 2008). *H. tuberosus* ($2n = 6x = 102$), is an autoallohexaploid with three sub-genomes. The three sub-genomes have been traditionally designated as A_1, A_2, and B_t (Kostoff, 1939). The B_t sub-genome is thought to be very similar to the *H. annuus* genome (Kostoff, 1934, 1939; Scibria, 1938), which may help stabilize meiotic chromosome pairing in interspecific hybrids between the two species. Through conventional hybridization it is possible to create large populations of interspecific *H. annuus* × *H. tuberosus* hybrids. The hybrids are perennial by way of tuber-sprouting and have good fitness. Commercial varieties have been released in Russia and Sweden for tuber production and forage purposes (Kays and Nottingham, 2008). *H. annuus* × *H. tuberosus* hybrids generally have a stable intermediate number of chromosomes ($2n = 4x = 68$), although meiotic abnormalities can reduce fertility and decrease stability in initial generations (Sujatha and Prabakaran, 2006; Chandler et al., 1986; Atlagic et al., 1993).

Three breeding strategies have been proposed to create perennial grain crops: direct domestication of perennial relatives of crop plants, transgenic modification of annual plants, and genetic introgression of perennial habit from wild relatives into domesticated crops through wide hybridization (Glover et al., 2010). There is doubt regarding the feasibility of direct domestication of perennial sunflower relatives as QTL mapping studies within annual sunflower suggest that a larger number of loci contribute to domestication-related phenotypes in sunflower than in other species (Burke et al., 2002; Wills and Burke, 2007; Doebley and Stec, 1991), Furthermore, the most important domestication trait in sunflower is suppression of axillary flowers (single headed state) (Chapman et al., 2008), a trait present at low frequencies in wild sunflower populations. Transgenic modification is not possible at present, as no known "perenniality" genes have been identified for sunflower, and only one has been identified so far in other species (Wang et al., 2009). Moreover, sunflower is recalcitrant to regeneration and transformation (Lewi et al., 2006; Piqueras et al., 2010), and gene flow issues with weedy conspecifics have halted regulatory acceptance of transgenic sunflower (Snow et al., 2003). Introgression of perennial habit from wild relatives through wide hybridization may be the most feasible approach. The main advantage of this approach is that a copy of the domesticated genome is present in a hybrid, enabling the selection of existing domesticated or elite loci that may not be present at high frequency in the wild germplasm. This approach can be implemented in at least two different ways: (1) selection on a population backcrossed to the domesticated parent or; (2) recurrent selection on populations derived from intermating the hybrid materials.

This study evaluates an interspecific population of *H. tuberosus* × *H. annuus* as a base for developing a perennial oil-seed sunflower. We validate the interspecific origin of hybrid populations, examine parental diversity, and then evaluate the potential for improving the perennial populations based on the interactions between perennial, agronomic, fertility and yield traits.

2. Materials and methods

2.1. Populations

Five populations were investigated. The first population was 18 *H. tuberosus* individuals collected from UMore Park in Rosemount, MN. The second was a set of 187 interspecific F_1 hybrids between *H. annuus* and *H. tuberosus*. The interspecific hybrids were developed during the years 2003–2006 (Hulke and Wyse, 2008) by crossing the 18 *H. tuberosus* (perennial) parents with three inbred *H. annuus* (annual) lines (CMS HA 89 [PET1], HA 89 (released by the USDA-ARS in 1971) and HA 434 (Miller et al., 2004)). HA 89 and HA 434 were used as male parents and CMS HA 89 was used as a female parent (Supplementary Table 1). The third population was a derivative of the second, as the F_1 hybrids were intermated to form an intermated F_1 population (designated as the IM_1F_1 population). This population was developed in 2007 by Hulke and Wyse (2008) (Supplementary Table 1). The fourth population was a backcross of the interspecific F_1 to the inbred lines HA 434 and HA 89 (designated as the BC_1F_1 population). This population was developed in 2006 by Hulke and Wyse (2008) (Supplementary Table 1). The fifth population was 31 *H. tuberosus* plants from the seed stocks of the United States Department of Agriculture Germplasm Resources Information Network (GRIN) that were collected from a diverse set of geographical locations (Supplementary Table 1) (USDA, 2012).

2.2. Flow cytometry

Individuals in the following populations were examined for genome size using flow cytometry: 187 interspecific F_1s, 170 IM_1F_1s, 120 BC_1F_1s, the 18 *H. tuberosus* parental lines and two of the *H. annuus* parental lines (HA 89 and HA 434).

Nuclear DNA content was assessed using a BD FACSCalibur (BD Biosciences, San Jose, CA) flow cytometer. Two technical replicates of the same clone were performed (on different days) for each plant on 42 F_1 individuals and the inbred annual lines. A single measurement was performed on the other individuals. Fully expanded leaf tissue sections of 0.55 cm^2 were finely chopped in 500 ml of extraction buffer (Partec, CyStain PI Absolut P), followed by filtration through a 50 micron nylon mesh. Filtered nuclei were stained with 2 ml of propidium iodide staining solution (Partec, CyStain PI Absolut P), stored at 4 °C, and examined within 12 h of preparation. A commercial standard of trout erythrocytes (Partec, DNA Control UV, 25 ml) as well as the internal standard from diploid HA 89 were used to calculate DNA content. A minimum of 1000 nuclei were examined for each sample. DNA content was calculated by taking the ratio of the peak intensity of each sample to that of the known standard and then multiplying the ratio by the picogram (pg) genome