

Progress in breeding perennial grains

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Abstract

Annual cereal, legume, and oilseed crops remain staples of the global food supply. Because most annual crops have less extensive, shorter-lived root systems than do perennial species, with a correspondingly lower capacity to manage nutrients and water, annual cropping systems tend to suffer higher levels of soil erosion and generate greater water contamination than do perennial systems. In an effort to reduce soil degradation and water contamination simultaneously – something that neither no-till nor organic cropping alone can accomplish -- researchers in the United States, Australia, and other countries have begun breeding perennial counterparts of annual grain and legume crops. Initial cycles of hybridization, propagation, and selection in wheat, wheatgrasses, sorghum, sunflower, and Illinois bundleflower have produced perennial progenies with phenotypes intermediate between wild and cultivated species, along with improved grain production. Further breeding cycles will be required to develop agronomically adapted perennial crops with high grain yields.

Key Words

Perennial grains, sustainable agriculture, plant breeding, wheat, sorghum, sunflower

Introduction

Before the introduction of agriculture, almost all of the world's landscapes were covered primarily by perennial plants growing in mixed stands; today, the bulk of global cropland is sown to monocultures of annual crops. The transformation is accelerating, with more land worldwide

having been converted from perennial to annual cover since 1950 than in the previous 150 years (Chiras and Reganold 2004). As the area occupied by annual species continues to expand, the threat of soil degradation looms larger. In a field experiment encompassing 100 years of data collection, annual crops were 50 times more susceptible to soil erosion than were perennial pasture crops (Gantzer et al. 1990).

“No-till” cropping, a method of curbing soil erosion using annual grain crops, reduces soil loss but requires chemical inputs for control of weeds and other pests. As it improves the soil permeability of previously tilled land, no-till cropping of annual species decreases nutrient runoff, but it does not address the increasingly serious problem of nutrients and water leaching from annual crop fields into groundwater and eventually into rivers and seas (Randall and Mulla 2001; Tilman et al. 2001). Nitrogen losses from annual crops may be 30 to 50 times higher than those from perennial crops (Randall and Mulla 2001). Organic farming of annual crops addresses the problem of pesticide contamination but not the physical erosion of soil. And organic systems do not compensate for the relative inefficiency of annual species in capturing water and nutrients.

Diverse, perennial grain-cropping systems have been proposed as a means of protecting against soil erosion, conserving water and nutrients, storing more carbon below ground, and building better pest tolerance (Glover et al. 2007). However, before such systems can be deployed and tested, new, perennial cereal, grain legume, and/or oilseed crops must be developed through breeding (Cox et al. 2002, 2006).

One of the globe’s most striking impacts of annual cropping has been the emergence of soil salinisation in parts of Australia. Citing a survey of lucerne research by Ward (2006), Bell et al. (2007) projected that escape of rainwater below the root zone (which can lead to rising water tables and salinisation) could be reduced 90 percent by replacing annual wheat with perennial wheat. Bell et al. (2007) concluded that “perennial wheat used for the dual purposes of grain and forage production could be developed as a profitable option for mixed crop/livestock producers” while helping prevent salt buildup in the soil.

The Land Institute in the United States, Australia’s Future Farm Industries Cooperative Research Centre, the Yunnan Academy of Agricultural Sciences in China, and other research groups are conducting or initiating breeding programs in intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey], perennial wheat (*Triticum* spp. / *Thinopyrum* spp.), perennial

sorghum [*Sorghum bicolor* (L.) Moench / *S. halepense* (L.) Pers.], Maximilian sunflower (*Helianthus maximiliani* Schrad.), perennial sunflower (complex hybrids of *Helianthus* spp.), Illinois bundleflower (*Desmanthus illinoensis* (Michaux) MacMillan), and perennial rice (hybrids of *Oryza* spp.) This paper summarises some of the results obtained so far at The Land Institute from two breeding approaches: direct domestication of wild perennial species, and hybridisation between annual crops and related perennial species followed by selection.

Materials and Methods

All hybridization, propagation, and experimental activities described below were conducted between 2002 and 2008, inclusive, on The Land Institute's research farm and in its glasshouses in Saline County, Kansas. Soil types on the research farm include Hord silt loam (fine-silty, mixed, superactive, mesic Cumulic Haplustolls) and Cozad silt loam (coarse-silty, mixed, superactive, mesic Typic Haplustolls). The location is 370 m above sea level on the western fringe of the tallgrass prairie region, near the geographical centre of the continental United States. Mean annual precipitation in the area is 737 mm, with an average of approximately one-tenth of that precipitation coming in the form of snow. Rainfall is concentrated in spring and fall, and extended droughts are common in summer. The mean daily high temperature in July is 34° C, the mean nightly low in January is -7° C, and rapid, wide swings in temperature are common in all seasons. Leading crops in the region are hard winter wheat (*Triticum aestivum* L.), grain sorghum, maize (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] Winter-killing of wheat is not common but can occur. Chief stresses for winter wheat in the area are created by a variety of pathogens and by heat during reproductive growth; for the warm-season crops, summer drought is the most common stress. In experimental perennial crops, additional stresses include late summer heat for wheat, prolonged periods of sub-freezing soil temperatures for sorghum and sunflower, and feeding by a range of insects in sunflower.

Intermediate wheatgrass

An intermediate wheatgrass population was developed by intermating germplasm from a wide range of sources, including a population acquired from an earlier selection program conducted by the Rodale Institute and US Department of Agriculture (Wagoner 1995). Each of 1000 plants was divided into 3 clonal replicates, and the population was evaluated in a randomised complete-block design in 2003-05. Plants were transplanted into a 92 cm grid pattern. Plant height, grain yield per

stem, and 1000-kernel weight were determined for all plants in the second summer of growth. A selection index was constructed, giving equal weight to yield per stem and kernel weight. Based on this index, the four plants with the highest values for the index, plus a fifth, extremely short plant, were intermated in isolation to establish the 'I5C1' population. Additionally, 50 plants (including the 5 I5C1 parents) were intermated to establish the 'I50C1' population. A total of 4000 plants from the two populations were evaluated as unreplicated spaced plants in 2005-08. The same traits were evaluated as in the first cycle, again in the second summer of growth. The proportion of kernels threshing free of hulls was estimated for each plant, and the yield and kernel-weight values were adjusted to estimate these values on a naked-kernel basis.

Perennial wheat

Among hundreds of hybrids produced between wheat and perennial grasses since 2002 was a hybrid between a tetraploid, carthlicum-type wheat (*T. turgidum* L.) accession PI 573182 with a *Thinopyrum intermedium* plant derived from a population that had undergone two cycles of selection for seed yield and grain quality. The male-sterile F₁ hybrid was pollinated with another *Th. intermedium* plant. Although several F₁ plants were obtained from that cross, only 2 produced selfed seed. The F₂ seeds from one F₁ individual were used for GISH analysis and grown in the glasshouse. Root tips and slides were prepared for genomic in situ hybridisation (GISH). Methods of DNA extraction, labelling, and hybridization were carried out as described by Zhang et al. (2001). Total genomic DNA extracted from *Th. intermedium* was used as probe. *T. aestivum* DNA was used as the competitor to block non-specific DNA hybridization with the probe.

Perennial sorghum

Perennial sorghum populations were initiated by fertilizing diploid (2n=20) sorghum plants with pollen from tetraploid, perennial plants (2n=40). Diploid parents included the genetic male-sterile inbred line N216 and the cytoplasmic-genetic male-sterile inbred lines ATx2921, ATx2925, ATx2926, and ATx2928. Tetraploid parents were obtained from an anonymous bulk population of perennial plants first created in the early 1980s at The Land Institute by hybridizing induced tetraploid sorghum plants with unidentified accessions of *S. halepense* (Piper and Kulakow 1994). The male sterile diploid parents, when pollinated by tetraploid plants, produced very small numbers of viable seed: approximately one seed per pollinated panicle, an outcome consistent with Hadley (1958). All hybrids thus produced were tetraploid. Out of approximately 20,000 F₃ plants harvested

in 2005, approximately 300 survived the winter and emerged in spring 2006. The surviving plants were descended from 28 diploid / tetraploid parental combinations; the number of plants descended from any one parental combination ranged from 1 to 37. Of the surviving plants, 38 plants representing 16 parental combinations were selected on the basis of phenotype, and 38 F₃-derived families derived from those plants via self-pollination were entered in a field experiment along with two cultivated checks (BTx2921 and a commercial hybrid, ‘Phillips 664’) in 2007. The experiment was sown in a randomised complete-block design with 4 replicates. The experimental unit (plot) was a single row 3 m long bordered by single rows of ‘Phillips 664’ on both sides. Spacing between rows was 92 cm. Each plot was assigned an agronomic index incorporating equally weighted, visual ratings of plant height, plant form, and panicle compactness. The range of the index was 0 to 9, with 9 assigned to the grain sorghum parent. Harvested grain was de-hulled and dried to uniform moisture before weighing. In April and May, 2008, re-emergence from rhizomes was scored in the experiment described above as well as in two sets of smaller, replicated, randomized plots located 2 km from the main plots. Observations of the proportion of plants surviving the winter in all field plots were combined into a single, unitless winter-hardiness index, ranging from 0 to 9, that was associated with each family. Families were allocated between two groups: “most winter-hardy”, which contained 13 families with winter-hardiness indices of 5 or greater, and “other”, which contained 25 families with indices below 5. Significance of differences between the two groups for traits other than winter-hardiness was tested via analysis of variance based on a generalized linear model using GenStat, Release 11.1 (VSN International Ltd., Hemel Hempstead, U.K.) Spearman rank correlation was used to test for significance of association between traits.

Perennial sunflower

Crowns from 300 wild *Helianthus maximiliani* plants grown from seeds collected across Kansas were divided and transplanted in a selection experiment with six clonal replicates of each plant. An index including second-year estimates of shattering resistance, seed size, and stalk yield was used to rank the plants; the 20 plants with highest index values were transplanted into an intermating nursery in 2006. One hundred and twelve progeny seedlings from each of the 20 intermated parents were transplanted to the field in a 92 cm grid pattern in spring 2007. At maturity, but before shattering could occur, five heads were harvested from each plant and dried. The heads were placed in a gentle electric mixer for 1 minute to encourage shattering, and the seed was weighed. Those seeds remaining in the heads were threshed manually or using a more aggressive electric mixer and

weighed. In 2008, head samples were placed in a plastic jar and the jars tumbled in an electric concrete mixer for 3 minutes to encourage shattering. (Domestic sunflower heads would not have fit in the jars used and were not used as checks.) Data were collected on number and diameter of stalks. After heads remaining in the field had been exposed to killing frosts, heads from each plant were examined and visual estimates of the percent seed lost through shattering were made. GenStat REML-based spatial analysis software was used to account for spatial variation and obtain adjusted values for each plant. Several subsets of the full spatial model for each trait were compared. Model selection followed the criteria described in the GenStat user guide. Adjusted values are reported.

Results

At The Land Institute, we are working toward direct domestication of 3 perennial species: intermediate wheatgrass, Illinois bundleflower, and Maximilian sunflower. That work does not involve interspecific hybridisation. We have used cycles of interspecific hybridisation and selection as well, in efforts to develop 3 other perennial crops: wheat, sorghum, and sunflower.

Intermediate wheatgrass and perennial wheat

Intermediate wheatgrass is an allogamous, perennial relative of wheat. Both species are allohexaploids with $2n=42$, and share no homologous genome. We are domesticating intermediate wheatgrass by selecting for increased grain yield and larger, free-threshing kernels. The evaluation phase of a second cycle of population improvement was completed in 2008. Each of the 4000 plants in the selection nursery was identified as having been produced by intermating either the top 5 selected plants (I5C1) or top 50 selected plants (I50C1) from the first cycle. The two sub-populations, which resulted from first-cycle selection intensities of 0.5% and 5.0%, respectively, both displayed significant positive skewness for the traits on which selection had been based in the first cycle: yield per head and kernel weight. All of the traits used in selecting the parents of the I5C1 population (yield per head, kernel weight, and reduced height) had substantially larger values for kurtosis and skewness (always in the direction of selection) than did the I50C1 population (Fig. 1; Table 1). If selection is having an impact on these statistics, the effect would be expected to be larger in populations undergoing higher selection intensity, and that is what occurred. Traits not subjected to selection, including fraction of naked kernels and yield per plant, had skewness and kurtosis in I5C1 equal to or lower than those in I50C1 (Table 1).

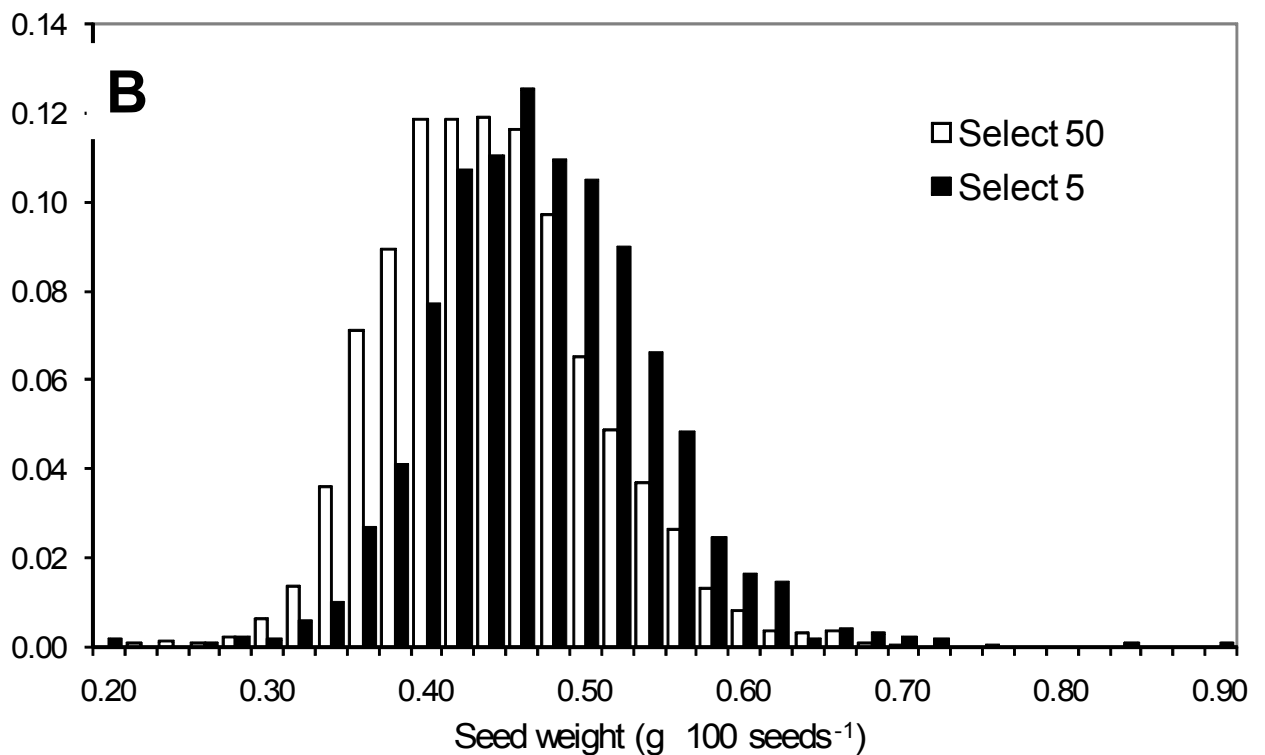
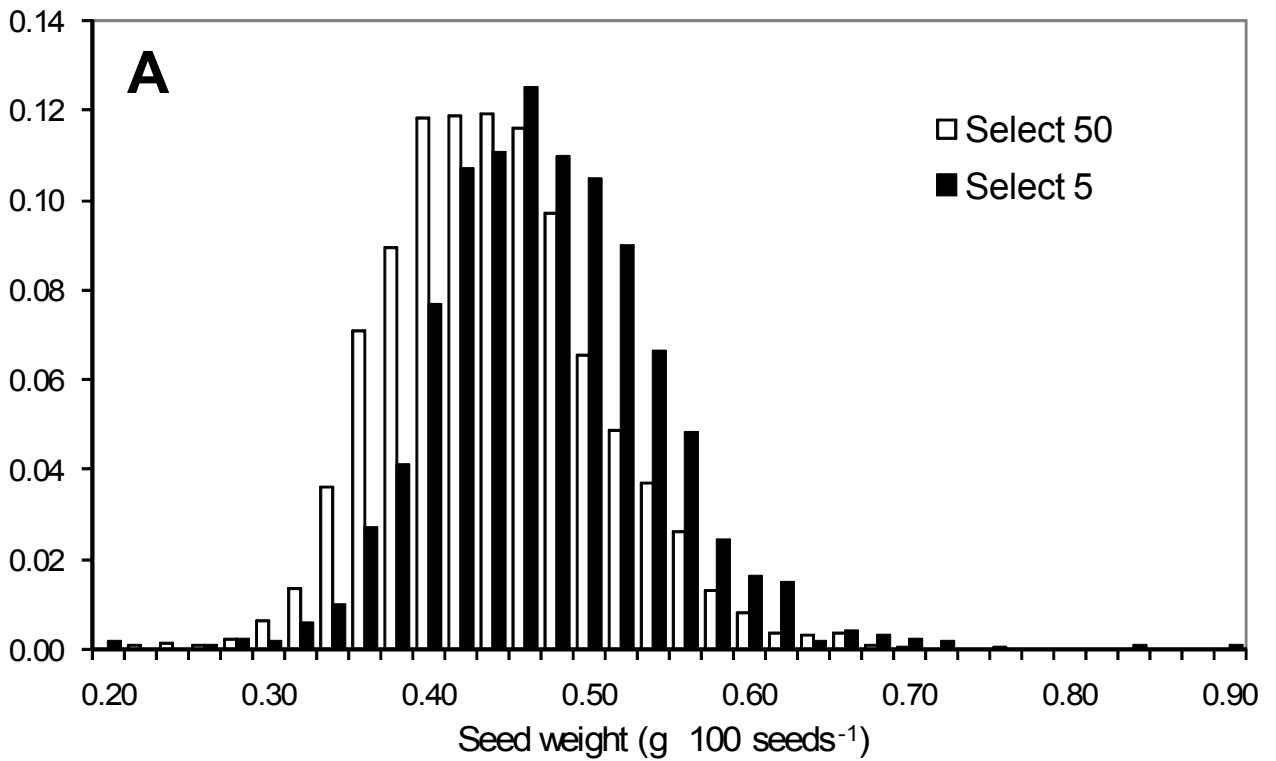


Figure 1. Distributions of average seed weight (A) and average yield per stem (B) for plants derived from selecting and intermating 50 versus 5 plants. One plant in the Select 5 group with a seed weight of 1.36 g 100 seeds⁻¹ is not shown on graph A. Not shown in graph B are three plants in the Select 5 group and one plant in the Select 50 group with seed yield per head greater than 0.5 g.

Table 1. Skewness and kurtosis (\pm standard error) of traits measured in the base population (C0) and populations derived from selecting 5 (I5C1) or 50 (I50C1) individuals from the base population and intermating the selected plants. Traits include fraction of free-threshing seed (fraction naked), total seed yield per plant (plant yield), average yield per stem (stem yield), kernel weight, and average height of heads at maturity (plant height). Skewness and kurtosis are unitless statistics, and have expected values of zero in a normal distribution. The range of standard errors is given for each row.

	Popu- lation	Fraction naked	Yield per plant	Yield per spike	Kernel weight	Plant height	Standard errors
Skewness	C0			0.52	0.28	-1.17	0.04 to 0.05
	I50C1	1.82	0.86	5.78	0.42	-0.46	0.04
	I5C1	1.72	0.85	18.67	1.88	-1.15	0.07 to 0.08
Kurtosis	C0			2.61	0.77	0.66	0.09
	I50C1	5.83	0.97	150.29	0.88	1.20	0.09
	I5C1	4.66	0.86	478.66	19.28	2.03	0.14 to 0.16

The most dramatic result of selection was an increasing frequency of short, stiff-stawed genotypes with erect leaves and large spikes. Some of those plants, along with others in the long positive tails of the skewed distributions for traits under selection, had very high values. Some 1000-kernel weights were 8 g and higher, compared with a population median less than 4.4 g. Yields per 100 spikes of the top 10 plants ranged from 41 to 320 g, compared with a median of 6 g. The planting arrangements of the selection experiments were not appropriate for estimating yield per unit land area.

Thinopyrum species can be hybridised with tetraploid and hexaploid wheat plants, with the objective of developing perennial, wheat-like plants. Chromosome doubling in the high-ploidy hybrids is extremely difficult, so few amphiploids have been made. Our backcrosses to the annual parent have produced thousands of plants with good fertility and kernel size. In the glasshouse, a large proportion of such plants continue to live after their mature seed has been harvested, but in The Land Institute's field plots, a very small minority of plants re-grow after summer harvest, and they invariably die in late summer.

To obtain larger numbers of perennial plants, we have crossed interspecific hybrid plants to *Thinopyrum* plants. Out of hundreds of such species-backcross hybrids, many were sterile, as expected. Crossing male-sterile hybrid plants with the few male-fertile plants produced a population of approximately 1100 progeny, almost all of which displayed some degree of fertility

along with improved kernel size and re-growth capacity. F₂ plants descended from a plant designated ‘B930’ (*T. turgidum*/*Th. intermedium*//*Th. intermedium*), for example, have a phenotype intermediate between the annual and perennial parent and strong re-growth in the glasshouse (Fig.2a and b). Fertility is low, with fewer than 5 kernels per spike, and kernels are intermediate in weight between those of the annual and perennial parents. Among 3 plants in family B930, chromosome numbers were 57, 59, and 60. Based on fluorescence patterns in GISH images (Fig. 2c), the genomes of two plants from B930 carried 42 chromosomes from *Th. intermedium* – the normal number for pure *Th. intermedium* plants – and 17 to 18 wheat chromosomes. Plant B930-36 had 43 *Th. intermedium* and 14 wheat chromosomes. The complete genome of *Th. intermedium* and varying portions of the wheat genome appear to be carried by these 3 plants. No spontaneous interspecific translocations were detected in these plants. We find such translocations only rarely; therefore, we cannot rely on them to separate known beneficial and deleterious genes that might be located on the same chromosome in the interspecific lines. To effect recombination between, for example, a gene conferring perenniality and genes found on the same *Thinopyrum* chromosome that confer undesirable traits, tools such as the *ph* alleles that permit nonhomologous pairing must be used (Gill et al., 2006).

Perennial grain sorghum

The Land Institute’s perennial sorghum breeding program, initiated in 2001 with rhizomatous tetraploid germplasm originally developed in the 1980s, has focused on improving winter survival of rhizomes and reducing height and tillering while increasing kernel size. *S. halepense* overwinters in temperate regions through survival of rhizomes. Genetic control of rhizome production and growth is highly complex (Paterson et al. 1995); despite that, increasing the grain yield and kernel weight of rhizomatous plants has proceeded without apparent hindrance. However, the majority of rhizomatous plants prove not to be winter-hardy in central Kansas.

In 2005-06 and 2006-07 yield trials, approximately 1.5% out of an estimated 20,000 F₃ and F₄ plants derived from annual / perennial crosses survived to re-emerge from rhizomes in spring. In smaller observation plots 2 km distant from the yield trials, mean survival was 20%. Selfed seeds from the 38 least “wild” plants that survived in 2005-06 were used to sow a 2007 yield trial. The mean yield across the 38 families was 60% of an inbred parental check’s yield and 36% of the yield of a locally adapted commercial grain sorghum, ‘Phillips 664’. Mean kernel size was 36% of the value of the grain sorghum entries, which were similar to each other. Taken together, the results

imply that numbers of seeds produced per unit of area were similar between the experimental families and grain sorghum checks, although seed numbers were not determined directly. The one-third of families with winter-hardiness indices of 5 and above had 11% lower yields and 5% lower kernel weights than did less winter-hardy families with indices of 0 to 4 (Table 2). Differences between means of the two groups were highly significant ($P < 0.01$), as were differences between progeny and parent means.

Grain production in current perennial sorghum germplasm is clearly lower than in modern annual cultivars. However, the yields and kernel weights shown in Table 2 are higher than those of most other perennial plant species currently under development as perennial grains (Cox et al. 2006). Studies since 2003 have demonstrated no strong associations between rhizome development or winter-hardiness on one hand and agronomic traits on the other. Despite the mean differences between groups in Table 2, there were no significant correlations between perenniality and productivity traits, with one exception: a weak rank correlation of -0.33 between the agronomic and winter-hardiness indices. Of the traits going into the agronomic index, the one having the strongest association with winter-hardiness was panicle compactness, with a rank correlation of -0.70 ($P < 0.01$). Studies are underway to determine whether the association between these complex traits is a result of linkage or pleiotropy.

Figure 2. a. Plant B930-16 (*T. turgidum*/*Th. intermedium*//*Th. intermedium*) approaching maturity in the glasshouse, 2009. b. Developing seed on B930-16. c. Genomic in situ hybridization (GISH) patterns in mitotic metaphase cell of a sibling plant, B930-36. The 14 red chromosomes are inherited from the wheat parent, and the 43 yellow-green chromosomes are from *Th. intermedium*.

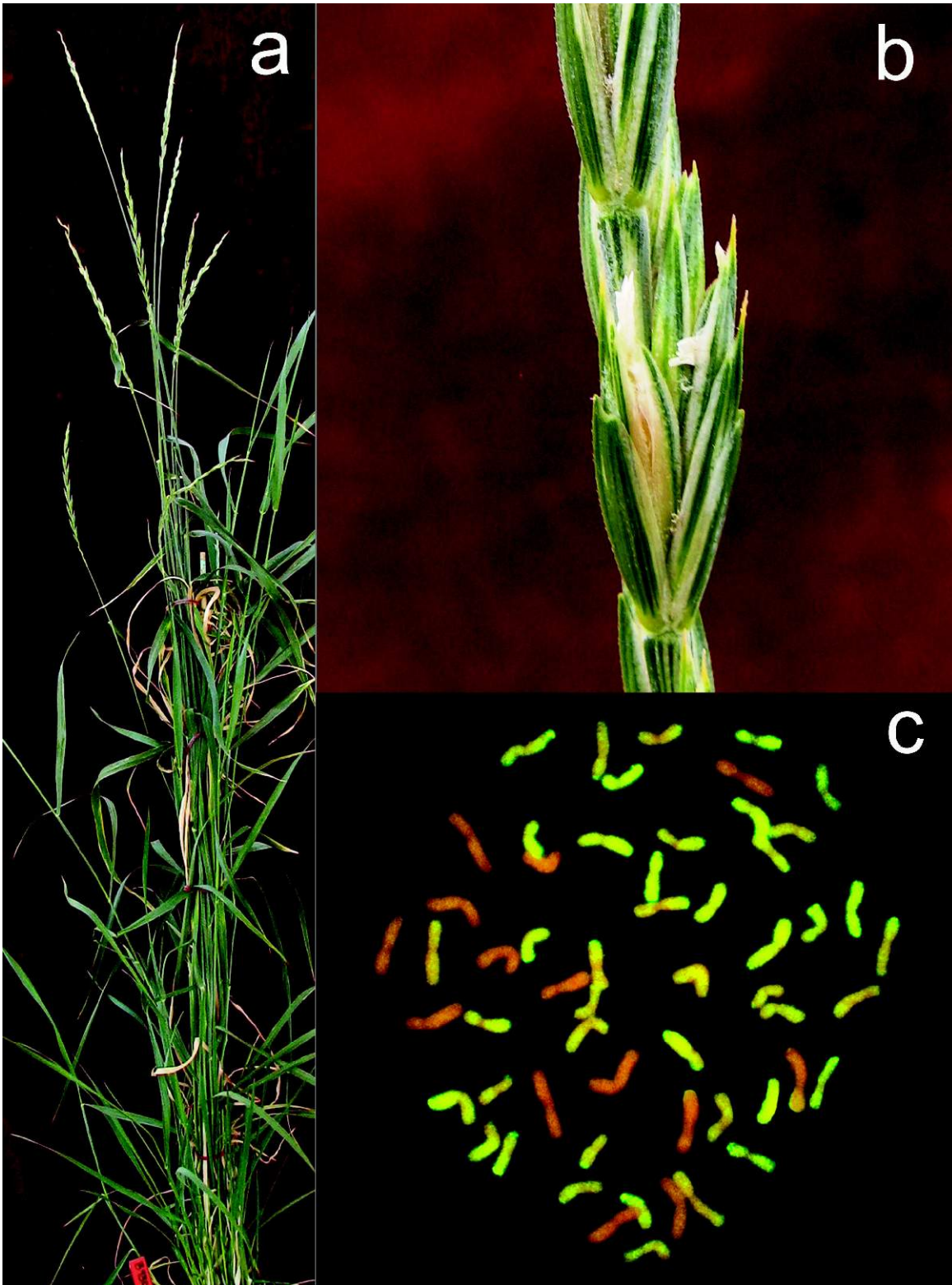


Table 2. Numbers of sorghum F3-derived F4 families from annual/perennial crosses classified as “winter-hardest” (with indices 5 or greater) or “other” (with indices below 5), along with their mean winter-hardiness and agronomic indices, grain yields, and kernel weights, as compared with means of ATx2922 (one of the inbred grain sorghum parents) and Phillips 664 (a commercial hybrid cultivar) in the same experiment.

Families	Number	Mean			
		Winter-hardiness index	Agronomic index	Grain yield (g/m ²)	Kernel weight (mg)
Winter-hardest families	13	6.9**	5.0**	78**	9.6**
Other families	25	2.8	6.2	88	10.1
Tx2922	1	0	9.0	141	27.4
Phillips 664	1	0	9.0	235	27.5

** Significantly different from mean of “other families” (P<0.01)

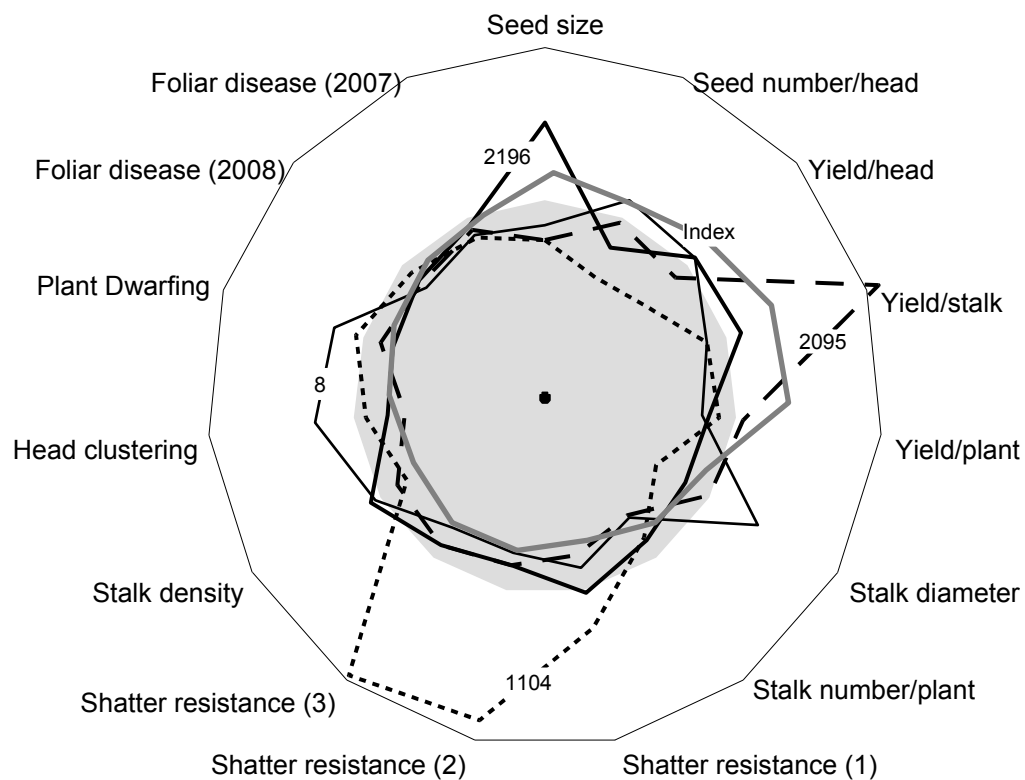
Perennial sunflower

Maximilian sunflower is a diploid perennial species (2n=34) native to the central United States. We are domesticating it for use as an oilseed crop, via methods similar to those described above for intermediate wheatgrass. Seed production is low, but phenotypic variation is extensive. In addition to the usual production traits, selection goals include elimination of shattering and dormancy, along with a more determinate growth habit, with small seed-heads fused into larger, more highly clustered ones.

Data collected from a population of 2240 plants in 2007-08 were used to select parents for a third cycle of intermating and selection. Forty plants were selected based on an index in which trait values, standardized to mean 0 and standard deviation 1, are summed: yield per plant + yield per head + yield per stalk – height – mean shattering. With second-year plants averaging 2 m tall, height was selected against as an indirect way to reduce lodging and increase harvest index. Based on selection intensities, the selection index would be expected to move the population in the direction of greater seed productivity without improving other traits (Fig. 3).

Figure 3. A radial representation of trait expression in 4 Maximilian sunflower plants, along with the population mean and the expression of plants selected according to an index of 5 traits, all standardised to mean 0 and standard deviation 1. The radial axis scale for each trait is the number of standard deviations above or below the mean of the full population. The perimeter of the filled-grey polygon represents the population mean, 0, of each trait. The centre of the diagram represents -10 standard-deviation units, and the outermost, open polygon represents +10 units. Trait means for the group of 40 plants selected using a 5-trait index are represented by an open polygon enclosed by a solid grey line, labelled “Index”. The remaining open polygons represent individual plants. Shattering resistance was estimated in the laboratory in (1) 2007 and (2) 2008 and (3) in the field in 2008. respectively.

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Meanwhile, rare individual plants in the population exhibited extreme trait expression for individual traits, including non-shattering, head clustering, stalk diameter, and single-seed size. In this work, we are domesticating a wild population in which no single plant would be rated acceptable for cultivation; therefore, it is not surprising that such plants, which warranted retention on the basis of superlative single-trait values, did not score high on more than one trait of interest

(Fig. 3). For example, we identified three plants with shattering scores in the lowest 20 percent of all plants in 1 natural and 2 mechanical shattering tests. None of the low-shattering plants, including plant 1104 (Fig. 3), was superior for any other trait. (For the 2040 samples evaluated for shattering resistance by the concrete-mixer method, percent shattering ranged from 36 to 100%, with a mean of 89%. As a screen for outliers with reduced shattering, this method was the most useful of those used. However, resolution was sacrificed at the middle and upper ranges of the distribution. Researchers seeking more uniform resolution would need to use a less vigorous method.)

Plant 2108's seed yield per stalk was 6 times the population mean. Although its yield per plant was only slightly above the mean, its lower stalk density may provide better adaptation to production conditions. Plant 273 was unusual in having unbranched stalks and a single head per stalk. Its yields per plant and stalk were very low; however, its heads were larger than those of any other plant in the population. Plant 2196's 1000-seed weight was 5.3 g, compared with a population mean of 3.1 g, but it had lower-than-average yield per plant and higher-than-average shattering and height. We identified 3 plants with height in the bottom 2.5 percentile of the population and compactness and stem diameter in the top 97.5 percentile – all desirable traits. One of them, plant 8, was among the top 20 plants for stalk diameter, and at 1.5 m, it was almost 0.5 m shorter than the population mean. Plants were not clonally replicated, so the effects of genotype, environment, and their interaction on that expression cannot be separated. However, plants falling far outside the usual range, and in a desired direction, were considered likely to produce useful progeny when used in plant-to-plant crosses.

The parallel route to perennial sunflower is through hybridisation. Domestic sunflower, a diploid, can be hybridised with several perennial species in its genus, including Maximilian sunflower and two hexaploids: rigid-leaf sunflower [*H. rigidus* (Cass.) Desf.] and Jerusalem artichoke (*H. tuberosus* L.). Hybrids between annual and Maximilian sunflower are highly sterile. The strategy we have followed in producing perennial, partially fertile plants involves crossing both annual and Maximilian sunflower to *H. rigidus* and *H. tuberosus* to produce tetraploid plants, which we then inter-pollinate. Large perennial populations thus produced are now undergoing selection for greater seed fertility. In 2007, 102 interspecific hybrid plants produced more than 100 seeds each, and 26 produced more than 500 each. Those are small seed numbers compared with the annual parent, but large compared with most such hybrids.

Illinois bundleflower

Bundleflower is a long-lived, widely adapted, native perennial. Plants produce unusually large seed for an herbaceous perennial species, and the seed has high protein concentration (Kulakow 1999). Current domestication efforts are focused on improving shattering resistance, synchronous maturity, seed yield, and seed size and quality. The germplasm base is a large collection of accessions from a wide geographical area (Kulakow 1999) that exhibits great allelic diversity (DeHaan et al. 2003). Making controlled hybrids is difficult, but methods have been developed to foster natural hybridisation and identify hybrids using morphological or molecular markers. Almost all Illinois bundleflower plants have means of seed dispersal, which are essential to fitness in the wild but make harvest difficult. Rare non-shattering families have been selected and used as initial parents. In addition, plants whose pods open in order to shed seed are being hybridised with other, rarer plants whose pods abscise and fall to the soil intact. The goal is to select recombinants whose pods neither open nor abscise before harvest.

Discussion

DeHaan et al. (2005) predicted that artificial selection in a properly managed agricultural environment could increase seed yield while maintaining perenniality. The experience of forage breeders in improving seed production can suggest strategies and techniques for the direct domestication of species such as intermediate wheatgrass, Maximilian sunflower, and Illinois bundleflower; however, without the need to select for forage yield or quality, grain production may be increased more quickly than is seed production in forages. The process may be complicated by high degrees of skewness and kurtosis in distributions for traits under selection, as were apparent in intermediate wheatgrass. In the absence of gene interaction, kurtosis is expected to be near zero or negative (Choo and Reinbergs 1982); positive skewness has been associated with complementary epistasis (Pooni et al. 1977), and higher selection intensities are expected to be more effective when interaction is complementary.

Results to date from the intermediate wheatgrass selection program are consistent with the above predictions. Phenotypes of the top-five selected plants – parents of the I5C1 population – and segregation in their progeny were consistent with existence of major genes for seed size, short stature, reduced stem number, nonshattering, or increased yield per head. The differences could generally be identified qualitatively due to gross morphological differences. When only the 5 plants

expressing those traits were intermated in isolation, we saw a few outstanding plants appearing in the I5C1 population. The I5C1 parents made up only 10% of the parentage of the I50C₁ population, so their major-effect genes, if they exist, were seldom brought together in the same plants. These results suggest that traditional population improvement through cycles of selection and random mating may not be an effective method for domesticating a self-incompatible perennial species. Inbreeding, though more complex and difficult in obligately allogamous species than in others, will be required to bring desired genes closer to fixation. Similarly in Maximilian sunflower, plants such as number 273, with its hitherto unfamiliar growth form, and seed dispersal mutants in Maximilian sunflower and Illinois bundleflower, should be handled in biparental crosses or very small populations as well as in the large main population.

During domestication, selection for yield begins at a low starting point. Yields of dehulled intermediate wheatgrass grain produced in small, solid-seeded plots during 2006-08 have been in the range of 30 g/m². Managed gene flow from modern crop species can, in theory, introduce genes conferring higher yields as well as those important for domestication (DeHaan et al. 2005). However, the useful genes acquired through hybridisation do not come without a genetic cost. When most crops – including wheat, rice, barley, rye, maize, sorghum, pearl millet, soybean, and sunflower – are crossed with perennial relatives, differences in chromosome number, lack of chromosome homology, or other factors can cause moderate to complete sterility and restrict genetic recombination in the progeny (Cox et al. 2002). The plant breeder working with such crosses must struggle with genomic disruptions in addition to selecting to improve multiple traits simultaneously.

Loss of *Thinopyrum* chromosomes in early generations of progeny derived directly from hybrids and amphiploids between *Triticum* and *Thinopyrum* species typically produces plants with approximately 42 *Triticum* and 14 *Thinopyrum* chromosomes (Banks et al. 1993). Such plants do not function as perennials in the Kansas environments in which we have tested them; therefore, we are examining the results of backcrossing to the *Thinopyrum* parent. Such crosses have produced plants whose relative chromosomes numbers -- 42 to 43 from *Thinopyrum* and 14 to 18 from *Triticum* (Fig. 2) – form a reverse image of what is typically obtained from progeny of amphiploids. We have generated other plants, not shown, that carry the entire *Thinopyrum* complement along with a single pair of wheat chromosomes. GISH cannot identify specific chromosomes, so we will employ other techniques such as C-banding and fluorescence in situ hybridisation (FISH) to determine which specific wheat chromosomes are being retained in

backcross plants. Determining chromosomal constitutions of key plants in such populations is important. Cox et al. (2002) predicted that perennial wheat breeders would need to subdivide their programs, assigning plants of similar chromosomal makeup to subpopulations within which hybridization would not lead to excessive aneuploidy and sterility. Furthermore, with further backcrossing, we hope to determine which individual pairs of wheat chromosomes might be added to wheatgrass to improve kernel weight or other traits.

In the taxa studied to date, plants derived from interspecific hybrids tend to be perennial only when the expected proportion of their total genome derived from a perennial parent is at least 50 percent (Cox et al. 2002). Therefore, one random backcross to the annual species tends to produce populations consisting largely of annual plants; with the autogamous species sorghum and rice at The Land Institute and at YAAS (Hu Fengyi, YAAS, pers. commun.; Sacks et al. 2003) respectively, we have found it most efficient to self-pollinate interspecific hybrids one or more generations, select the most strongly perennial progenies, and only then backcross to annual parents. Perenniality in sorghum and rice is under complex genetic control (Hu et al. 2003; Paterson et al. 1995) and interacts strongly with the environment. Therefore, it will not be fully achieved through manipulation of individual genes. On the other hand, marker-assisted selection could help accelerate the simultaneous improvement of perenniality, fertility, and grain production in rice, sorghum, and other species (Hu et al. 2003; Paterson et al. 1995). The Land Institute's sorghum program has chosen strong winter-hardiness as a breeding objective. But agronomically improved, rhizomatous plants with little or no winter-hardiness have been developed and maintained as breeding material for warm temperate or tropical environments. There, plants regrowing from rhizomes could have the potential for faster early growth and greater vigour than plants in a traditional ratoon crop re-growing from crowns would have.

The time scale needed to bring perennial grain crops to the farm will vary across species. Intermediate wheatgrass, for example, is ready for small-scale on-farm testing to work out agronomic practices, handling, and processing. More time will be required before perennial wheat is ready for field-scale testing. In the central plains of the United States, the ultimate goal of breeding perennial grains is to develop a system that serves the ecological functions that the original prairie did; herein, we have described progress toward that goal. In the Palouse region the northwestern United States, perennial wheat could make cropping on steeply sloping land more sustainable, and a breeding program aimed at that goal has made significant progress (Scheinost et al., 2001). In Western Australia, alternating strips of trees and yet-to-be-developed perennial wheat

could serve to obtain good production while preventing the rise in water tables that causes salinity problems. In other parts of Australia, perennial wheat could conserve soil and water; provide a new tool for dealing with persistent droughts; and improve the profitability of farms that incorporate both grain production and grazing (Bell et al., 2008). Parental germplasm to develop perennial wheats for Australia is undergoing preliminary evaluation there (Philip Larkin, CSIRO, and Len Wade, Charles Sturt University, pers. commun.) In Southeast Asia, systems involving perennial upland rice and food-producing trees could prevent erosion on lands that are highly susceptible (Sacks et al. 2003). Toward that goal, the perennial rice program at the Yunnan Academy of Agricultural Sciences (YAAS) in China has selected rhizomatous rice plants with high seed fertility from a cross between *O. sativa* and *O. longistaminata* (Hu Fengyi, YAAS, pers. commun.) Those all are long-term goals and programs, but it now appears that germplasm and strategies are in place to develop perennial cereals, oilseeds, and grain legumes.

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