IV. Breeding perennial grains: warm-season grasses

A. Sorghum

1. Hybridization with Sorghum propinquum

In tropical environments, grain sorghum (*S. bicolor,* 2n=20) is able to regrow from basal nodes to produce a rattoon crop. But breeding a sorghum that is winterhardy in temperate regions will require transfer of genes from related species.

A perennial native of southeast Asia, *S. propinquum* is rhizomatous and diploid, with chromosomes largely homologous to those of grain sorghum. Paterson et al. (1995) evaluated rhizome-related traits of 370 F_2 and 378 BC_1 plants from a cross between the two species. Surviving a mild winter in southern Texas, USA, with only three nights reaching temperatures of -3°C to -4°C, 92% of F_2 plants and 46% of BC_1 regrew in the spring. Plants regrew either from tillers or from rhizomes. Forty-eight F_2 plants representing the range of the population were selected for progeny testing.

From all F_2 plants and F_3 lines, Paterson et al. (1995) collected data on number of rhizomes producing above-ground shoots, distance between the center of the crown and the most distal shoot, a subterranean rhizome score, tillering, and regrowth. They mapped chromosomal segments affecting these traits in the F_2 plants and F_3 lines, using 78 RFLP loci.

Rhizomatousness was a complex trait, with nine different chromosomal regions on seven of sorghum's ten chromosomes having detectable effects on at least one of the rhizome traits. Individual segments accounted for between 5 and 13% of the total variation. All but one of the seven segments associated with regrowth was also associated with one or more rhizome traits, and all four segments associated with tillering were also associated with regrowth or rhizomatousness.

Because *S. propinquum* is a tropical species, rhizomatous progeny of crosses between *S. bicolor* and *S. propinquum* would probably not be winterhardy at middle or northern latitudes, without successful selection for deeper rhizome growth. But there is great potential for developing a perennial grain sorghum for the tropics or subtropics from such populations.

2. Hybridization with johnsongrass

Johnsongrass (*S. halapense*, 2n=40) is a tetraploid, probably an amphiploid that combines the genomes of *S. bicolor* and *S. propinquum* (Paterson et al., 1995). It is a very strong and aggressive perennial, and a notorious weed. Like *S. propinquum*, johnsongrass stores starch in its rhizomes. As a consequence, its rhizomes have no cold hardiness, unlike those of temperate grasses, which store fructosans (Monaghan, 1979). Natural selection for deeper-growing rhizomes has allowed johnsongrass to spread as a weed as far north as Ontario (Warwick et al., 1986). Although the most northerly biotype reproduces mainly by seed, regrowth from rhizomes occurs throughout the range of the species.

Early research on hybrids between diploid sorghum and johnsongrass (Hadley, 1953, 1958; Hadley and Mahan, 1956) produced two types of hybrids: 30-chromosome plants that were male-sterile but could be backcrossed to the diploid parent and fertile 40-chromosome plants derived from unreduced female gametes in the diploid parent. The 30-chromosome plants were more strongly rhizomatous. Hadley and Mahan (1956) identified seven 20-chromosome backcross plants that were rhizomatous, but most were chlorophyll mutants. Three years of selection failed to produce a single diploid line that was rhizomatous.

Hybrids between *S. halapense* and induced tetraploid lines of *S. bicolor* are easily made (Casaday and Anderson, 1952; Sanguden and Hanna, 1984; Piper and Kulakow, 1994). In an effort to produce a perennial grain sorghum at The Land Institute, Piper and Kulakow (1994) crossed *S. halapense* with tetraploid grain sorghum lines. In an interspecific F_3 population, approximately 40% of plants were rhizomatous. There was no significant negative correlation between rhizome production and grain yield in the F_3 generation, but a negative association arose with backcrossing. Yield was strongly related to plant biomass and root biomass, both with pheontypic correlations of 0.70. The F_3 population - selected for winterhardiness but not for yield - had a grain yield 62% as high as the mean non-irrigated sorghum yield in Saline County, Kansas, where the experiments were conducted. But rhizome production dropped to near zero in other populations derived by backcrossing to tetraploid *S. bicolor* in an effort to increase grain yield.

Land Institute breeders have selected for winter survival among the rare rhizomatous BC_2 plants and their selfed progeny. The phenotypes of winterhardy selections remain very distant from that of the cultivated parent, despite the latter's expected 87.5% genetic contribution to the BC_2. The selections are taller and later maturing than either parent, have open panicles and small seed, and produce many tillers, although not as many as johnsongrass. Rhizome mass is less than 10% that of johnsongrass □ sufficient for overwintering, but not enough to allow interspecific progenies to become aggressive weeds.

Both rhizomes and tillers originate from meristems at the base of the plant, and there appears to be considerable overlap in their genetic control (Paterson et al., 1995). It may not be possible, or even desirable, to select a low-tillering, sufficiently rhizomatous genotype. Indeed, selection for yield improvement may be more effective in highly tillering populations. If a grass plant is regarded as a population of largely autotrophic tillers (Jackson and Jackson, 1999), and each tiller supports both seeds and rhizomes, then the most direct route to increased yield is via additional tillers. Of course, this implies increased biomass.

Piper and Kulakow (1994) concluded that development of a winterhardy sorghum (i.e., one that produces 80g of rhizomes per plant) with a grain yield of over 4000 kg/ha is feasible, through selection for greater biomass and reallocation of photosynthate to seed production. The foundation germplasm for breeding a perennial sorghum may necessarily consist of high-biomass plants that produce more tillers than annual sorghum. With a sufficiently large genetic base, subsequent selection for improved harvest index and seed size could be successful.

Because there is some homology between the chromosomes of grain sorghum and johnsongrass, multivalent chromosome associations are common at meiosis in interspecific tetraploids.

Multivalents, in turn, cause poor seed set because of nondisjunction of chromosomes. Luo et al. (1992) demonstrated that selection for fertility can be effective in autotetraploid grain sorghum, which is generally plagued by low seed set. Breeders could cross perennials with the highly fertile tetraploid germplasm that Luo et al. (1992) have produced.

Broadening and improving the genetic base of tetraploid perennial sorghum will require introduction of more agronomically elite germplasm. One rapid method of incorporation would be to pollinate both diploid and induced-tetraploid strains of elite, large-seeded inbred lines with the best tetraploid perennials. From the diploid/tetraploid crosses, breeders can select 40-chromosome hybrids that arise from unreduced gametes (Hadley, 1953). The Land Institute is now taking this approach to develop genetically diverse breeding populations.

B. Pearl millet

Pearl millet (*Pennisetum glaucum*, 2n=14), like sorghum, is a tropical, annual diploid with a perennial, tetraploid relative. Napiergrass, *P. purpureum* (2n=28) has one genome homologous and one nonhomologous to that of pearl millet (Jauhar, 1981). Amphiploids resulting from colchicine treatment of hybrids between the species are male and female fertile (Gonzalez and Hanna, 1984). Hanna (1990) backcrossed these hexaploids to diploid and tetraploid pearl millet lines and produced perennial progeny; however, both types of backcross plants (tetraploid and pentaploid) were highly sterile and unable to survive the mild winters of south Georgia, USA. Napiergrass, the only known species in pearl millet's secondary gene pool, is not rhizomatous, so selection for winterhardiness would probably not be successful. A perennial millet for grain production in the tropics is a reasonable prospect.

Dujardin and Hanna (1990) interpollinated hybrids and their derivatives from crosses between tetraploid pearl millet and *P. squamulatum* (2n=54), a more distant, perennial, apomictic relative. Some progenies (2n=48) were both perennial and apomictic. Apomixis can be used to ensure grain production and genetic stability in highly heterozygous progenies of interspecific crosses (Hanna and Bashaw, 1987). *Pennisetum squamulatum* is also non-rhizomatous, and its progeny are not likely to be perennial outside of the tropics.

C. Maize

1. Hybridization with tetraploid perennial teosinte

Efforts to develop perennial maize (*Zea mays* ssp. *mays*, 2n=20) have been sporadic at best; as in other crops, hybridization between maize and perennial relatives has led primarily to improvement of the annual crop (Wagoner, 1990a). Shaver (1964) first attempted development of maize-like perennials from crosses between colchicine-induced tetraploids of maize and a wild, perennial, tetraploid relative, *Z. mays* ssp. *perennis* (2n=40). Selection within the resulting tetraploid populations and backcrosses to tetraploid maize effectively increased the frequency of perennial progeny. Crosses to diploid maize produced perennial triploids, but all diploid selections were annual.

Shaver (1967) combined a postulated gene (*pe*) for perenniality with recessive genes for indeterminacy (*id*) and grassy tillers (*gt*) in a diploid background, to produce perennial plants; however, the *idid* genotype prevented production of ears. Because Shaver (1967) had developed a separate *idid* population in a different genetic background that did produce ears, he suggested that perennial diploids could also be made fertile if the genetic background were manipulated.

2. Hybridization with diploid perennial teosinte

Little further attention was paid to perennial maize until the dramatic discovery of a diploid species of perennial teosinte, *Z. mays* ssp. *diploperennis* (Iltis et al., 1979). Initial studies showed that inheritance of perenniality was relatively simple in maize/*diploperennis* crosses, but perenniality was inferred from tillering habit, a potentially misleading technique (Shaver,1964). In subsequent, larger-scale experiments, inheritance of tillering in progeny of similar inter-subspecific crosses was more complex, and perennial maize types were not recovered even in large segregating populations (Srinivasan and Brewbaker, 1999).

Genetic mapping in maize/annual teosinte crosses show that most traits of domestication separating the species are oligogenic, and the loci tend to be clustered on the map, through either linkage or pleiotropy (Doebley and Stec, 1993). A similar study of these traits, plus perenniality, in crosses between maize and diploid perennial teosinte would be of great value to any breeding program attempting to combine perenniality with the agronomic phenotype of maize. This would require substantial effort to evaluate large segregating populations for tillering, rhizome production, and capacity to produce seed over multiple seasons. Once the genomic regions of interest are identified, marker-assisted selection can be used to incorporate them into a maize background and eliminate unwanted alleles such as those conditioning hard glumes and shattering.

One serious obstacle to adoption of any teosinte-derived perennial grains is the lack of winterhardiness of these tropical species. There are no winterhardy species of *Zea*. Because the bulk of maize production and breeding occurs in temperate areas, there has been little incentive to develop perennials from crosses with *Z. mays* ssp. *diploperennis.* One possible approach has not been suggested to date: selection for rhizome depth. As we have seen, johnsongrass rhizomes also are not winterhardy if near the soil surface, but dispersal of the species into higher latitudes has been made possible by selection for deeper rhizomes. Superimposing selection for this undoubtedly complex trait on selection for perenniality and traits of domestication, not to mention yield, may entail a much larger effort than any breeding program is willing to undertake.

3. Hybridization with eastern gamagrass

The closest winterhardy relatives of maize are in the genus *Tripsacum.* Eastern gamagrass (*T. dactyloides*), for example, is currently grown as a perennial forage grass as far north in the western hemisphere as Kansas and Massachusetts, and can be grown in the Corn Belt (Voigt et al., 1995). *T. dactyloides* has been hybridized many times with maize, beginning with the work of Manglesdorf and Reeves (1931). Plants of the diploid (2n=36) or tetraploid (2n=72) races may be crossed with maize. If *Tripsacum* is used to pollinate maize, embryo rescue is necessary (deWet et al., 1973; James, 1979), but if maize is used as the male, some hybrid seed may be obtained without rescue (deWet et al, 1973). In addition, several strains of popcorn, when pollinated with tetraploid *T. dactyloides,* produce large amounts of hybrid seed that does not require embryo rescue (Kindiger and Beckett, 1992). Some have good crossability with diploid *T. dactyloides* as well. Contrary to typical results, Eubanks (1995, 1997) reported that a putative 20-chromosome hybrid between *T. dactyloides* and *Z. diploperennis* showed 93 to 98% pollen fertility.

Natural introgression between *Tripsacum* and maize has not been observed, but morphological (deWet et al., 1983) and molecular (Talbert et al., 1990; Dewald and Kindiger, 1998) evidence supports the hypothesis that the species *T. andersonni* is an intergeneric hybrid containing three genomes (54 chromosomes) from *Tripsacum* and 10 chromosomes from *Zea* in *Tripsacum* cytoplasm. The uniformity of this ancient natural hybrid indicates that *T. andersonii* arose from a single hybridization. It has been able to spread across tropical Latin America because of its vigorous perenniality (deWet et al., 1983; Dewald and Kindiger, 1998).

In addition to being perennial, tetraploid *T. dactyloides* is a facultative apomict. Perennial hybrids result from artificial crosses between tetraploid *Tripsacum* and maize (Farquharson 1957), and some seed-set can result from apomixis. The hybrids, derived from parents with different basic chromosome numbers and chromosomes of different sizes (those of maize being larger), are male sterile, with cytological behavior that is anything but regular. Harlan and deWet (1977) summarized methods for utilizing such hybrids in maize improvement. Either 28-chromosome or 46-chromosome hybrids - derived from diploid and tetraploid *T. dactyloides* parents, respectively - can be backcrossed to maize. In either case, *Tripsacum* chromosomes are eliminated with backcrossing. Elimination occurs more gradually in progeny of 46-chromosome hybrids, and the 20 chromosomes of the resulting backcross plants can contain significant genetic material from *Tripsacum* (Harlan and deWet, 1977; Stalker et al., 1977a and b). All 20-chromosome backcross plants derived to date have been annual and non-apomictic. Kindiger et al. (1996) derived an annual, 39-chromosome line that carried 9 *Tripsacum* chromosomes and displayed an intermediate level of apomixis.

Most hybridization with *Tripsacum* has been for the purpose of either elucidating the evolution of maize or transferring resistance or other genes to annual maize. The latter purpose implies backcrossing to maize. But development of perennial populations may require interpollinating plants in early backcross generations that still carry many *Tripsacum* chromosomes (Harlan and deWet, 1977), or even backcrossing to *Tripsacum.* As Harlan and deWet (1977) commented, "Apparently, if one wishes to contaminate maize with *Tripsacum* one should first contaminate *Tripsacum* with maize."

New approaches to perennial maize are being explored. An anomalous fertile hybrid between diploid *T. dactyloides* and maize was discovered by one of the authors (BEZ) in 1997 near the mouth of the Big Nemaha river in Richardson County, Nebraska, USA. This derivative of natural introgression between *T. dactyloides* and a putative commercial hybrid is being hybridized with gynomonoecious *Tripsacum* - both diploid (Dewald and Dayton, 1985; see below) and tetraploid (Salon and Earle, 1998) - and with tassel-seed popcorn to develop a 56-chromosome perennial cultivar for production of grain, forage, fiber, and fuel.

The difficulties encountered in introgressing apomixis from *Tripsacum* into maize (Kindiger et al., 1996) should temper hopes for a rapid synthesis of perenniality with high grain yield. Whatever the initial population, and even with marker-assisted selection, the process of recovering perennial, winterhardy segregants with maize-like ears, and then breeding for yield and other agronomic traits will be long and arduous.

D. Direct domestication of warm-season grasses

1. Eastern gamagrass

Could *T. dactyloides* be domesticated directly, without introgression of genes from maize? To do so would be an accomplishment parallel to that of domesticating maize from annual teosinte □ a feat requiring thousands of years and producing genetic and physiological changes much greater than those involved in domestication of Asian cereals such as wheat and rice (Iltis, 2000; Beadle, 1980). To develop a crop from eastern gamagrass using the knowledge and techniques provided by 21st-century genetics, while leaving aside the important genes of domestication available in maize, would be an ambitious project.

Wagoner (1990a) described in detail the status of eastern gamagrass as a potential grain crop, and the species' most discouraging characteristic: very low seed yield. Interest had been stimulated by the discovery of a gynomonoecious, or pistillate, mutant (DeWald and Dayton, 1985) in which pistillate and perfect spikelets replace the staminate spikelets of the normal inflorescence. The result is an increase of up to 20-fold in the number of seeds produced per plant; however, the seeds are small, so that the weight of seed produced per plant is increased by only a factor of 3 (Jackson and Jackson, 1999).

Plants of eastern gamagrass are large, vigorous and widely adapted. The increase in sink size made possible by the pistillate mutant may provide an opportunity to increase seed yield dramatically through increased harvest index - the yield component usually found to have had the greatest effect on yield improvement in traditional grain crops (Evans, 1998). Furthermore, Jackson and Dewald (1994) found that the increased seed yield of pistillate plants did not come at the expense of plant vigor or longevity. Carbohydrate reserves were significantly higher in pistillate than in normal genotypes. For breeders, there is a huge pool of genetic variability available in the species (Newell and deWet, 1974; Wright et al., 1983). Although tetraploid *T. dactyloides* reproduces apomictically, parental combinations can be produced via BIII hybrids, in which an unreduced egg is fertilized by a haploid sperm (Kindiger and Dewald, 1994). Alternatively, obligately sexual tetraploid plants can be produced via colchcine treatment of diploid *T. dactyloides* plants, all of which are sexual. The genus *Tripsacum* contains many species that lack winterhardiness but have desirable traits that, potentially, could be transferred to eastern gamagrass: synchronous flowering, large spikelet number, higher seed yield, and other variations in plant morphology.

The maximum seed yield of eastern gamagrass in plots at The Land Institute has been 240 kg/ha, in the third year after sowing (Piper, 1999). It remains to be seen how rapidly yield can be improved through selection within pistillate populations. And there is another question: would the 10-, 20-, or 30-fold yield improvements required to make eastern gamagrass a viable grain crop have larger negative effects on plant vigor and persistence than did the three-fold yield boost brought about by the pistillate mutation? That increase was large relative to the grain yield of a normal plant but required diversion of only a small amount of photosynthate, relative to the plant's large biomass.

The food quality of Eastern gamagrass is excellent (Bargman, 1989). But, even if yield can be improved, other problems must be solved. One problem is disease. Infection by maize dwarf mosaic virus B has been very serious in plots at The Land Institute (Seifers et al., 1993). Also, the hard fruitcase of *Tripsacum* weighs almost three times as much as the seed itself and makes processing difficult. It was an extremely rare mutation in annual teosinte that freed the kernel from the fruitcase and allowed its use as a grain and the development of maize (Iltis, 2000). This may be a gene that breeders will be forced to transfer from maize.

2. Indian ricegrass

Grain is currently being harvested from a perennial grass for human food in northeastern Montana, USA. Indian ricegrass (*Oryzopsis hymenoides*), cultivar Rimrock (Jones et al., 1998), has reduced seed-shattering (Jones and Nielson, 1991) and produces gluten-free grain. The grain is being produced, milled, and marketed under the trade name Montina. Yields vary between 250 and 500 kg/ha, but improvement through breeding may be feasible. Germplasm collections exhibit great phenotypic diversity, and very large-seeded genotypes are known (T.A. Jones, USDA-ARS, Logan Utah, pers. commun.). Certainly, other perennial grasses native to the western USA could be considered for domestication as grain producers, but no attempts have been made.

V. Breeding perennial grain legumes: prospects

A. Soybean

The genus *Glycine* is divided into two subgenera, *Glycine* and *Soja.* The cultivated soybean, *Glycine max,* and its close relative and ancestor *Glycine soja,* both annual diploids (2n=40; genome GG), make up the subgenus *Soja.* The subgenus *Glycine* contains 16 perennial species (Singh and Hymowitz, 1999). Numerous attempts have been made to cross perennials with *G. max,* but hybrids have been produced only with *G. tomentella* (2n=80; genomes DDEE; Bodanese-Zanettini et al., 1996; Hymowitz and Singh, 1987; Hymowitz et al., 1998; Newell and Hymowitz, 1982; Shoemaker et al., 1990; Singh and Hymowitz, 1999). In most cases, successful hybridization has required the use of ovule culture (Newell and Hymowitz, 1982). All hybrids have been sterile, but when their chromosome number has been doubled with colchicine to produce amphiploids with the genome constitution DDEEGG, some fertility has been restored (Shoemaker et al., 1990; Singh et al., 1993).

Recently, Singh et al. (1998) produced 22 monosomic addition lines of soybean, each carrying a single chromosome from *G. tomentella.* During development of the lines, a slight tendency toward perenniality persisted up to the BC_2, but all monosomic lines had an annual growth habit

(R. Singh, Univ. of Illinois, personal communication). Little or no pairing occurs between chromosomes of the two species (Singh et al., 1998), so even if genes governing perenniality can be identified, their transfer to soybean will be difficult. Perhaps even more difficult would be the incorporation of winterhardiness, which does not exist in any species of *Glycine.* Winterhardiness would need to derive from another legume species such as alfalfa, via transgenesis.

B. Illinois bundleflower

To dispel doubts that herbaceous perennials can produce large amounts of seed, one need only point to a legume species of the North American prairie. Illinois bundleflower (*Desmanthus illinoensis*) has a wide geographic range, stretching well beyond the prairie, from Colorado to Minnesota to Florida to Texas (Latting, 1961). It is a good nitrogen-fixer, a preferred forage for livestock, and produces relatively high yields of large seeds with favorable nutritional profiles (Kulakow, 1999).

In evaluating a germplasm nursery of 141 highly diverse *D. illinoensis* accessions, Kulakow (1999) identified 15 in which seed shattering was reduced or eliminated, a prerequisite for grain crop. The highest-yielding 20 accessions in the first sowing (1988) produced a mean of 1500 kg/ha in their first year and 1180 kg/ha in their second year of growth. These accessions significantly exceeded the nursery's mean yield in a second experiment sown in 1990, averaging 1090 kg/ha. Severe drought occurred in their second year of growth, and their yields fell below 500 kg/ha. In related studies, yields have approached 2000 kg/ha (Piper, 1993).

The germplasm collection studied by Kulakow (1999), along with more extensive plant collection of *D. illinoensis* and its southern relative *D. leptobolus,* can provide the foundation for breeding Illinois bundleflower as a grain crop. The chief hurdle to be overcome is the palatability of the seed. Although the seed contains no toxic levels of oxalates, cyanides, nitrates, or alkaloids, and cooked seed has digestibility and a protein efficiency ratio similar to that of cooked oats (Kulakow et al., 1990), unprocessed bundleflower seeds have a foul odor and taste. Nothing is known about the compound(s) involved. The Land Institute is investigating induced mutagenesis, but some method of processing, as is necessary for utilization of soybeans as human food, for example, may solve the problem. Our results (DLV) indicate that roasting whole seeds may eliminate the undesirable taste.

C. Other legumes

Wild senna, *Cassia marilandica,* has one of the highest seed yields of any perennial species yet evaluated by The Land Institute (Piper, 1992, 1993), but yields decline after the first year. Perhaps most importantly, the species is not known to establish associations with *Rhizobium* for symbiotic nitrogen fixation; therefore, its usefulness would be limited in cropping systems that include little or no application of inorganic nitrogen fertilizers.

The chickpea (*Cicer arietinum*) is one of nine annual species in the genus *Cicer,* which also contains 34 perennial species (van der Maesen, 1987). The perennial species are little studied; accessions representing 12 species are maintained at the Western Region Plant Introduction

Station in Pullman, Washington. Two species, *C. anatolicum* and *C. songaricum,* have survived for 10 years in the field and continue to produce seed. One accession of *C. songaricum* is white-flowered, has some degree of shattering resistance, and produces seed similar to the commercial 'desi' type (F.J. Muehlbauer, WRPIS, personal communication). The presence of these traits suggests that *C. songaricum* was once cultivated in Asia.

Many species of the Leguminosae may be grown as perennials for seed production in the tropics. For example, second-crop seed yields of pigeonpea (*Cajanus cajan*) were higher than first-crop yields in two studies in India (Newaj et al., 1996; Nimbole, 1997).

VI. Breeding perennial composites: prospects

A. Sunflower

The relatives of the annual cultivated sunflower (*Helianthus annuus,* a diploid with 2n=34) are genetically diverse, consisting of 14 annual and 36 perennial species (Seiler and Riesenberg, 1997). If attempts to develop a perennial sunflower fail, it will not be because the available gene pool is too small. According to data compiled by Jan (1997), along with a recent study by Sukno et al. (1999), 20 perennial species have been hybridized with *H. annuus.* In approximately half of the crosses, F_1 plants were perennial; sometimes one accession of a species produced perennial hybrids, while another produced annuals. The majority of hybrids have enough fertility to be backcrossed.

Some hybrids were produced before the development of the first effective embryo-rescue technique for sunflower by Chandler and Beard (1983). Heiser and Smith (1964) crossed wild *H. annuus* with the *H. decapetalus*, *H. hirsutus,* and *H. strumosus* (all 2n=68), and Jerusalem artichoke (*H. tuberosus,* 2n=102). They found, as have subsequent researchers, that crosses were far more successful when the polyploid species was used as the female. Only the hybrids with *H. strumosus* and *H. tuberosus* were winterhardy. Whelan (1978) failed to produce interspecific crosses with cultivated sunflower, but his hybrids between wild *H. annuus* (as the male) and two diploid perennial species, *H. maximiliani* and *H. giganteus*, were fertile enough to backcross to a cultivar. Neither the hybrids nor the backcrosses were perennial. Whelan (1978) found that the perennials differed cytologically from the annual in three translocations and a paracentric inversion. Hybrids were highly sterile, but backcrossing to *H. annuus* rapidly restored male and female fertility (Whelan and Dorrell, 1980).

More recently, many crosses with perennial diploids, tetraploids, and hexaploids have been made - usually, but not always - via embryo rescue (Georgieva-Todorova, 1984; Krauter et al., 1991; Atlagic et al., 1995; Espinasse et al., 1995; Sukno et al., 1999). However, few backcrosses have been attempted. Seiler (1991, 1993) released BC_1-derived germplasms from crosses between cultivated sunflower and three perennial species: *H. hirsutus, H. resinosus* (2n=102), and *H. tuberosus.* All germplasms were annual, but hybridizing them with perennial parents could increase the probability of recovering perenniality in another round of backcrossing.

Protoplast fusion is an efficient method of producing interspecific amphiploids in *Helianthus.* Krasnyanski and Menczel (1995) produced *H. annuus* + *H. giganteus* hybrids that had good fertility but were not perennial. Henn et al. (1998) produced large numbers of *H. annuus* + *H. giganteus* and *H. annuus* + *H. maximiliani* hybrids that developed rhizomes from which shoots emerged. Colchicine-induced or protoplast fusion-derived amphiploids of different parentage could be intercrossed to develop populations for breeding perennial, grain-producing plants.

Little is known about the genetics of perenniality in sunflower, and there have been no efforts to develop a perennial crop. When cultivated *H. annuus* is used as the female parent, the F_1 is usually a weak, short-lived perennial, and perenniality is quickly lost upon backcrossing to the annual. When the wild perennial is used as the female, some hybrids are more strongly perennial and bear a closer resemblance to the wild parent. Recovery of full perenniality and winterhardiness may require backcrossing to the wild parent (G. Seiler, personal communication). As in sorghum, a "wilder" plant type, with a greater number of heads per plant, may not be incompatible with good grain yield, if maturation is relatively synchronous.

Breeders aiming to develop a perennial sunflower should select perennial parents based on past success in hybridization and occurrence of perennial progeny, as well as regions of adaptation. The Land Institute is investigating *H. maximiliani* as a parent because of its wide adaptation across much of the North American prairie, its vigorous growth and seed production, and its crossability with *H. annuus.*

B. Direct domestication of perennial Helianthus

Annual sunflower is the only species of *Helianthus* that has been domesticated as a seed crop. But *Helianthus* contains a wide diversity of perennial species (Seiler and Riesenberg, 1997), many of which are potential domesticates. The Land Institute has studied the common prairie species *H. maximiliani* as a candidate for direct domestication (Jackson and Jackson, 1999). It is a vigorous prairie plant with small but edible seed. Seed yield estimates have varied widely (Jackson and Jackson, 1999; Piper, 1999), and yields tend to decline after the first year of propagation in monoculture. Maximilian sunflower has strong allelopathic properties, making it potentially useful in suppressing annual weeds during the establishment of perennial polycultures (Piper, 1999). It spreads so aggressively that thinning or partial tilling to reduce stands can actually increase grain yield (Jackson and Jackson, 1999).

VII. Establishing gene pools

A. Widening genetic bottlenecks

Developing the first perennial grain genotype with acceptable yield will be a demanding process in itself, but that will be only the beginning; no new agricultural system can be based on only a handful of genotypes per species. Sown over large regions, even polycultures will be genetically vulnerable if they do not incorporate intraspecific as well as interspecific variation. Furthermore, breeding of perennial grains will quickly reach a dead end without sufficient genetic diversity.

During the domestication process, most crop species suffered a similar "founder effect" that restricted the gene pool on which early cultivators drew (Ladizinsky, 1985). In probably the most extreme example, all modern bread wheat may have descended from one or a few natural interspecific hybrids that occurred around 5000 years ago (Cox, 1998). If new perennial crops are to be developed on a shorter time scale, a founder effect must be avoided.

To ensure intraspecific diversity in the field and foster further genetic improvement, breeders will need to develop deep gene pools for each crop. In rye, rice, sorghum, intermediate wheatgrass, wildrye, lymegrass, eastern gamagrass, Indian ricegrass, Illinois bundleflower, or Maximilian sunflower, this will be a straightforward matter of incorporating new annual or perennial parents, manipulating ploidy or ensuring homozygosity of translocations, and alternate rounds of selection and recombination. It will involve considerable effort, but large gene pools exist to be drawn on.

Where the perennial crop has resulted from hybridization between species with nonhomologous chromosomes - as has been accomplished in a limited way in wheat and maize and remains to be done in oat, soybean, and sunflower - introducing sufficient diversity will be a more daunting task. In such a situation, new germplasm can be brought into the breeding pool only by repeated interspecific hybridization. The human-made species triticale is an example of an annual crop developed through interspecific hybridization that has always been hampered by a restricted gene pool (Skovmand et al., 1984).

Because of their more readily available gene pools, we may expect perennial versions of rye, rice, or sorghum to be the first to move beyond the experimental stage. Creating a gene pool of interspecific hybrids in wheat, oat, maize, or sunflower may take as long as or longer than domesticating wild species such as Illinois bundleflower, Maximilian sunflower, or those of the perennial Triticeae "from scratch".

B. Biotechnology

We have seen that sophisticated chromosomal manipulation will be necessary in developing perennial versions of some crops from interspecific hybrids. We have not discussed genetic engineering in its narrower sense: asexual insertion of individual genes. We can speculate on the potential for transforming annual into perennial plants by gene insertion, but with the state of knowledge today, we can go no further.

No research to date suggests that perenniality is governed by a single gene, or even two or three genes, in any crop or crop relative. Although some wheat amphiploids with as little as 25 to 30% of their genome derived from the perennial parent are themselves perennial (Cai et al., 2001), we have seen that in rye, triticale, sorghum, maize, soybean, and sunflower, dilution of the perennial-derived genome to below approximately 50% often eliminates perenniality. This, along with the lack of success by breeders of any crop to backcross a gene or chromosome conditioning perenniality into any annual genotype attests to, but does not prove, the complexity of the trait.

In a few taxa - for example, *Sorghum, Secale*, and *Oryza* - segregation of mostly homologous chromosomes can be observed in crosses between annual and perennial species. As we have seen in sorghum and rice, molecular-marker studies do not show that perenniality is simply inherited (Paterson, 1995; Kohm et al., 1997). The postulation of a single gene for perenniality on one of the three *S. montanum* chromosomes $4R^{mon}$, $6R^{mon}$, or $7R^{mon}$ (Dierks and Reimann-Philipp, 1966) has not been confirmed, and the relatively weak perenniality of the cultivar Permontra, which carries all three chromosomes, indicates that the trait is more complex.

Of course, it is not impossible that a gene could be isolated that conditions the perennial growth habit when transferred to an annual plant. But if a "perenniality gene" is identified in a particular species and cloned, its effect when transferred to any but very closely related species is entirely unpredictable. Anamthawat-Jonsson (1996) lists other obstacles to employing gene-transfer technology to improve physiologically complex traits in genetically complex species such as the polyploid Triticeae. Transgenic technology may be useful, once perennial grain crops have been developed, in improving their pest resistance, food quality, or other more simply inherited traits; however, other breeding and cultural methods will also be available in most cases.

VIII. Breeding perennial vs. annual grains

A. General considerations

Almost all crop breeding involves direct selection for plant productivity - grain yield in the present context - whether the plants are annual or perennial. Discussion of differences between breeding strategies for annuals and perennials have usually concentrated on allocation of photosynthetic resources between seeds and vegetative structures (Gardner, 1989). But perennials will also require arrays of adaptive traits very different from those usually addressed by breeders of annuals.

Breeders developing perennial grains for existing monoculture systems will be faced with finding genetic solutions to problems that are exacerbated when individual plants must survive and produce over a period of years rather than months in the same patch of soil. Breeding for resistance to diseases, insects, and adverse soil conditions will probably be even more important in developing perennial grains for monoculture than they are for annuals. But the majority of perennial grain breeders will not be selecting for adaptation to monoculture. Most efforts to breed perennial grains have environmental protection as an explicit goal. This will lead breeders to select genotypes adapted to systems like organic farming (Wagoner, 1990b; Scheinost, 2001) or natural systems agriculture (Jackson and Jackson, 1999) that receive lower subsidies of nonrenewable energy and synthetic chemicals. Such systems usually incorporate inter- and intraspecific diversity. The greater biodiversity and better soil conditions inherent in these systems is designed to reduce pressures on the breeder to select for resistance to pests and soil problems.

B. Differences in selection criteria between perennial and annual grains

Obviously, persistence and maintenance of grain yield over seasons has been difficult to achieve in the past and will occupy much of the attention of perennial-grain breeders. We will need to consider adopting some of the methodologies used by breeders of forages (Sleper, 1987) or tree crops (Libby, 1992). Breeding of perennials obviously entails longer selection cycles, but perenniality can also have a positive effect on genetic gain, through greater control over pollination (Knowles, 1977; Reimann-Philipp, 1995), the capacity for asexual propagation in breeding nurseries, and a longer time scale for evaluating traits and genetic markers.

Avoidance of tillage will leave crop debris on the soil surface, favoring pathogens that overwinter on plant parts. The potential for damage to a perennial crop rooted in place for several years in the midst of its own debris is obvious. Breeders may need to incorporate a higher level of genetic resistance to saprophytic pathogens in perennial grains, just as they must in some annual crops intended for no-till production (Bockus and Shroyer, 1998).

Unless they are grown in more ecologically sound farming systems than are most annual grains, perennial grains may need increased resistance or tolerance to soil-borne fungi, nematodes, and viruses. Annual crops are often vulnerable to these organisms, but an infection that is late or slow to develop may not cause serious damage, and a new, initially healthy crop can be sown the next year. Crop rotation can suppress such problems in annual systems. In contrast, perennial crop plants, once infected, could be subject to damage over a period of years or killed outright. Without ecological or genetic protection, replanting and rotation cycles would have to be shortened.

Breeding for perenniality through interspecific hybridization may bring some genetic protection against pests. The majority of disease-resistance genes in wheat, to take one important species, have been transferred from other species (Cox, 1998). The extensive hybridization with wheat's perennial relatives envisioned herein would bring in a bonus of new resistance genes. The same may occur in sunflower (Seiler, 1992) and other species.

The need for rapid stand establishment from seed, especially under cooler conditions, has become even more important for breeders of annual crops with the widespread adoption of no-till agriculture (Crosson, 1981). Breeders of perennials will have to devote some attention to seedling establishment, but they can divert much of that effort into selection for persistence over seasons. Because sowing would be necessary only once every few years, much more effort could be put into using cultural methods to ensure good establishment.

C. Problems rendered less severe by growing perennials in biologically diverse systems

Plant breeders continually face the effects of genotype-environment interactions, with "environment" including everything except the crop populations under selection. Breeding methodology traditionally attempts to control all environmental factors except the one or few under study. In more genetically diverse systems with lower external subsidies, genotype-environment and genotype-genotype interactions cannot be controlled or eliminated but must be relied upon to drive the system.

Biodiversity, which can be manifested at many different levels in agricultural systems (Cox and Wood, 1999), is the plant breeder's friend when it provides relief from problems that would otherwise be handled genetically. Breeders of annual crops for monoculture are being asked, more and more, to find genetic remedies for problems that do not exist in natural ecosystems. Natural systems agriculture seeks to mimic those ecosystems and eliminate many problems from the breeder's checklist: soil compaction, phosphorus deficiency, low-pH and/or aluminum toxicity, and other potential consequences of annual monoculture.

Breeding programs across the globe probably spend a larger proportion of their time and effort on incorporating genes for resistance to diseases and insects than on any other activity. But lower levels of genetic resistance to diseases should be acceptable in breeding perennials to be grown in mixtures. Species diversity can provide protection against pathogens (Browning, 1974) and insects (Altieri and Nicholls, 1999). To take a simple example, a bacterial leaf spot causes severe attacks in monocultures of Illinois bundleflower, but is insignificant in polyculture because a grassy understory cushions raindrops and reduces splashing (Jackson and Jackson, 1999). Perennial crop cultivars should also be designed as populations of genotypes rather than inbred lines or F_1 hybrids. Intraspecific diversity can retard development of virulent insect biotypes (Cox and Hatchett, 1986; Gould, 1986) and reduce infection by aerial, splash-borne, or insect-vectored pathogens (Garrett and Mundt, 1999, 2000; Zhu et al., 2000). On the other hand, diversity may not compensate for the increased vulnerability of perennial grains to soil-borne pathogens.

Perennial mixtures will require new breeding methodologies, as do annual intercropping systems (Francis, 1990). Other crop species, weeds, mycorrhizal fungi, nitrogen fixers, other soil microorganisms, dead organic material, and a wide variety of herbivores and their own predators or parasites will affect the growth and productivity of the crops under selection.

IX. Conclusion

We have described parental germplasm and possible methodologies for breeding at least 20 species of perennial grains. Prospects for success vary among species, but we can be certain that highly productive perennial grains will not be developed quickly or without immense effort. For any one of these species, the debate over feasibility of breeding a perennial grain crop could be the subject of an entire book or symposium; however, the only way to answer the question of feasibility is to carry out breeding programs with adequate resources and appropriate methodologies on a sufficient time scale. A massive program for breeding perennial grains could be funded by diversion of a relatively small fraction of the world's agricultural research budget.

[continued]