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Breeding perennial grain crops (part 1 of 3)

**by T. S. Cox, M. Bender, C. Picone, D. L. Van Tassel, J. B. Holland, E. C. Brummer,
B. E. Zoeller, A. H. Paterson, and W. Jackson**

Abstract: One-third of the planet's arable land has been lost to soil erosion in recent decades, and the pace of this degradation will increase as the limits of our food-production capacity are stretched. The persistent problem of worldwide soil erosion has rekindled interest in perennial grain crops. All of our current grain crops are annuals; therefore, developing an array of new perennial grains - grasses, legumes, and others - will require a long-term commitment. Fortunately, many perennial species can be hybridized with related annual crops, allowing us to incorporate genes of domestication much more quickly than did our ancestors who first selected the genes. Some grain crops - including rye, rice, and sorghum - can be hybridized with close perennial relatives to establish new gene pools. Others, such as wheat, oat, maize, soybean, and sunflower, must be hybridized with more distantly related perennial species and genera. Finally, some perennial species with relatively high grain yields - intermediate wheatgrass, wildrye, lymegrass, eastern gamagrass, Indian ricegrass, Illinois bundleflower, Maximilian sunflower, and probably others - are candidates for direct domestication without interspecific hybridization. To ensure diversity in the field and foster further genetic improvement, breeders will need to develop deep gene pools for each crop. Discussions of breeding strategies for perennial grains have concentrated on allocation of photosynthetic resources between seeds and vegetative structures. But perennials will likely be grown in more diverse agro-ecosystems and require arrays of traits very different from those usually addressed by breeders of annuals. The only way to address concerns about the feasibility of perennial grains is to carry out breeding programs with adequate resources 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.

Key terms: natural systems agriculture, interspecific hybridization, crop domestication, cereal, legume

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T. S. Cox, M. Bender, D.L. Van Tassel and W. Jackson are scientists at The Land Institute, Salina, KS, 67401. C. Picone, Biology, Warren Wilson College, Ashville, NC 28815; J.B. Holland, USDA-ARS, Dept. of Crop Science, North Carolina State University, Raleigh, N.C., 27695; E.C. Brummer, Dept. of Agronomy, Iowa State University, Ames, IA, 50011; B.E. Zoeller, Maiz.org, Hiawatha, KS 66434; A.H. Patterson, Plant Genome Mapping Laboratory, University of Georgia, Riverbend Research Center, Athens, GA, 30602. Corresponding author is T.S. Cox (cox@landinstitute.org).

I. The need for perennial grains

Annual grain crops have dominated the earth's agricultural landscape since the time of the earliest farmers 10,000 years ago. Soil erosion followed tillage agriculture as it spread across the earth's surface (Lowdermilk, 1953; Hillel, 1991). In the last few decades, one-third of the planet's arable land has been lost to soil erosion (Pimentel et al., 1995). The pace of this degradation will increase as the limits of our food-production capacity are stretched to feed a growing population of humans and domestic animals.

It may indeed be possible to expand food production to feed 10 billion people by the year 2050 (Evans, 1998); however, if grain continues to be produced in 2050 by methods that erode soil and waste other nonrenewable resources, the Earth may not have the capacity to sustain adequate food production into the 22nd century. Tillage agriculture on sloping land always brings the risk of soil erosion. The increasingly common practice of no-till production of annual crops, designed to control soil loss, has so far required increased use of herbicides. Furthermore, direct-seeded annual cropping systems have been shown to produce as high or higher nitrate emissions as tillage systems (Mummey et al., 1998). Research on wholly new agricultural systems may provide the means to produce food on otherwise marginal lands and arrest or even reverse losses of ecological capital associated with many current systems. Our best examples to follow in developing solar-powered, less-polluting, soil-conserving forms of agriculture are natural systems.

Natural systems agriculture, still in the experimental phase (Piper, 1999), is a new approach to crop production. The principles of natural systems agriculture can be applied to any food-producing landscape, but most research to date has been conducted in central Kansas, USA, aimed at developing a "domestic prairie", with herbaceous, perennial grain-producing crops grown in polyculture (Jackson and Jackson, 1999; Piper, 1999).

Other, less far-reaching recommendations for reducing soil erosion also involve perennial grains (Wagoner, 1990a; Scheinost et al., 2001). But all of our major grain crops are annuals, and no current perennial species produce sufficiently high yields. Plant breeders must develop an array of perennial grain crops - grasses, legumes, and plants representing other families - before natural systems agriculture or other methods of producing food without soil erosion can succeed.

Wagoner (1990a) published in this journal a comprehensive review of efforts to develop perennial grains in the grass family. Her article made a convincing argument for development of perennial grains, provided a thorough history of breeding programs worldwide up to 1990, and recommended approaches to be taken in further research on breeding and crop production. We will first re-examine the feasibility of perennial grain crops, building on Wagoner's discussion. Then we will review genetic research and efforts to breed perennial grains in the grass family since 1990 and examine some possibilities for breeding perennial grains other than grasses. Finally, we will undertake an expanded discussion of breeding methodology relevant to all perennial grains.

We will keep the discussion general, recognizing that the term "perennial" has very different meanings in different environments. We will be discussing tropical and temperate crops, crops of tropical origin grown in temperate zones, and temperate crops that can be grown in the tropics.

But we will discuss only those species on which research aimed at perennial grain production has been done or proposed, with concentration on the environment targeted by each research project.

II. Perennials to annuals, and now back to perennials

A. Prevalence of annual grain crops

Whyte (1977) noted that the rapid warming of the earth's climate at the end of the Pleistocene Ice Age 11,000 years ago created three large and three small "arid cores" on the Asian continent. On the fringes of these cores there formed concentric "isoexothermic zones", where the highest annual temperatures occurred during the dry season. The new climatic regime in those belts favored annual grasses and legumes, which could survive long hot, dry periods in the form of seeds. Their seeds were relatively large and could germinate and grow quickly with seasonal rains and moderating temperature. Annuals largely displaced perennial species in isoexothermic zones such as the Fertile Crescent of southwest Asia, becoming "suddenly and abundantly available" to human hunter-gatherers.

According to Whyte (1977), the wide availability of annuals in the various isoexothermic zones led humans, who previously had relied in part on seeds of perennial grasses for food, to initiate the agricultural revolution and carry it well beyond its regions of origin. Wagoner (1990a) summarized the subsequent events that led to the human species' almost complete reliance on annual species for use as grain crops, with tiny pockets of perennial grain production persisting into modern times. As she points out, "our ancestors took the easy route" by concentrating on annual grains.

A difficult road lies ahead for breeders intending to develop perennial grains. Breeding herbaceous perennials for adaptation in regions where the vegetation until recently was almost entirely made up of herbaceous perennials (e.g., the prairie of the central United States) should be eminently feasible, but simultaneous selection for persistence and grain yield will require intensive work. To domesticate wild perennials with no genetic input from other species would entail a genetic retreat of 10,000 years. Fortunately, it is possible to shorten this new round of crop development by orders of magnitude. Many perennial species can be hybridized with related annual crops, allowing us to incorporate genes of domestication much more quickly than did our ancestors who first selected the genes.

B. Resource allocation and negative correlations

Breeders have before them the genetic resources for breeding perennial grains, but just beyond lies the question of a "tradeoff" between grain yield and perenniality (Jackson and Jackson, 1999). In the words of Wagoner (1990a), "Yield from a perennial grain will probably never be as high as that from annuals because the life strategies of annuals and perennials are so different. ... The photosynthetic energy assimilated by a perennial plant over the course of a growing season must be divided among its perennating structures and seeds."

Indeed, high-yielding perennial grains do not exist today. Wagoner's (1990a) survey of 51 experimental studies in 27 species of perennial grasses showed that seed yields most often fell below 1000 kg/ha but could exceed that level. Piper (1999) reported similar yields in perennial grasses; however, two perennial legumes, Illinois bundleflower (*Desmanthus illinoensis*) and wild senna (*Cassia marilandica*), yielded up to 2000 kg/ha. Suneson et al. (1963) reported that their 25 years of work with perennial wheat (*Triticum aestivum* hybridized with perennial grasses) in California had produced lines whose first-year yields fell "within the range of the lowest yielding commercial wheat varieties" of the time, with a rapid yield decline in subsequent years. Recently, eight intergeneric wheat lines selected for regrowth ability in Washington state, USA yielded between 1600 and 5800 kg/ha, compared with almost 9000 kg/ha for the popular annual wheat cultivar 'Madsen' (Scheinost et al., 2001).

Is a tradeoff between perenniality and grain yield inevitable? The fundamental assumption of tradeoff theory - that the pool of carbon to be shared by reproductive and vegetative structures is fixed and cannot be increased by breeding - is open to question (Jackson and Jackson, 1999). Grain production can be sink-limited (Slafer and Savin, 1994); for example, shading during development of wheat inflorescences (i.e., during determination of sink size) depressed grain yield, whereas shading during grain-filling (once sink size was fixed) had much less effect (Evans, 1978). When yield is sink-limited, more or larger reproductive structures will induce greater production of photosynthate, resulting in little competition with perennating structures. Basal or rhizome-derived tillers on a grass plant are largely self-sufficient (Nyahoza et al., 1973; Jackson and Dewald, 1994), and their own inflorescences can supply much of the photosynthate for seed development (Blum, 1985). Perennials also may be able to maintain green tissue and continue to photosynthesize late in the growing season, after the photosynthetic tissue of annuals has senesced (Scheinost et al., 2001). For all of these reasons, there need not be a gram-for-gram tradeoff between grain and perennating structures.

Most experimental studies have addressed the question of grain yield per hectare vs. persistence over seasons only indirectly (see Jackson and Jackson, 1999). Jackson and DeWald (1994) compared half-sib populations from a population of *Tripsacum dactyloides* segregating for a 'pistillate' mutation that causes a large increase in seed production per inflorescence. The increased seed yield did not come at the expense of plant vigor or longevity. Piper and Kulakow (1994) found no correlation between seed and rhizome production in a population of unreplicated, winterhardy F₃ plants from an interspecific cross between tetraploid *Sorghum bicolor* and *S. halapense*; however, rhizome production was all but lost in backcrosses to *S. bicolor*, the cultivated species.

Any tradeoff between yield and perenniality should occur only during establishment of the first year's crop from seed. Once perennating structures are developed, they can serve as a source of carbon for plant establishment in subsequent years (Jackson and Jackson, 1999). By way of comparison, Wagoner et al. (1993) computed the energetic costs of sowing and establishing a hectare of annual wheat, including all energy required to produce the seed (Heichel, 1980), cultivate the land, and sow the seed. The resulting cost of "annuality" was equivalent to the usable energy contained in 715 kg of wheat grain, amounting to 32% of the crop's yield in that study. Wagoner (1995) provided a table of calculations by Watt (1989) showing that

intermediate wheatgrass yielding only 673 kg/ha over four years without resowing would have a break-even price of \$3.60 per bushel, similar to that of spring wheat at the time.

The negative relationship assumed to exist between perenniality and grain yield is largely based on life-history theory (e.g., Gadgil and Solbrig, 1972; Jackson and Jackson, 1999). Gardner (1989) has provided a physiological application of the theory to crop plants. But these theories are based on observation of existing species, which are products of natural and artificial selection in divergent directions (Fig. 1). High-seed-yielding herbaceous perennials are not found in nature - nor are triticale (*X Triticosecale*) or maize (*Zea mays*), and for the same reason. Artificial hybridization and selection produced triticale and maize, and they can, conceivably, generate productive perennial grains.

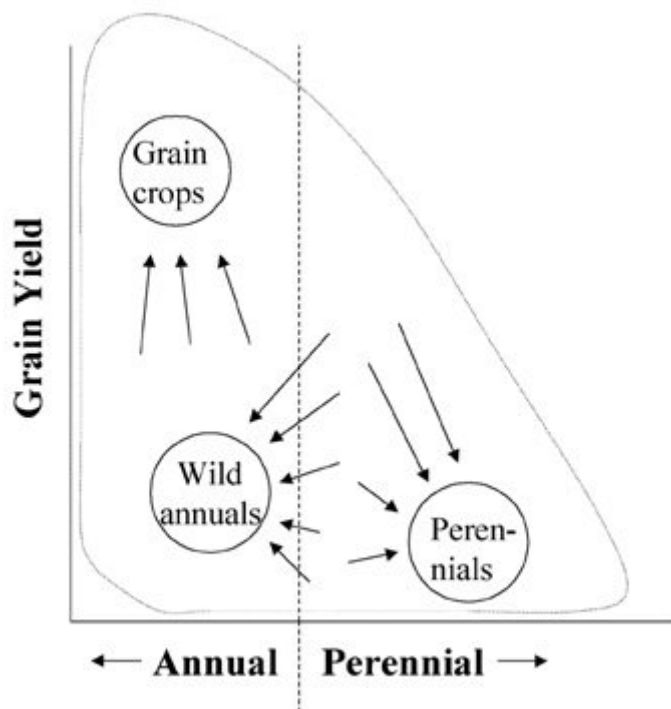


Figure 1. Divergent selection pressures restricting the variability of perennial plants, wild annual plants, and annual grain crops. The horizontal axis represents a gradient ranging from strong annuality to strong perenniality, with a threshold (dashed line) separating the growth habits. The vertical axis is grain yield per season. The dotted curve represents hypothetical genetic limits of the two-way distribution.

Even if there is a negative correlation between perenniality and grain yield, it does not preclude selection. Consider two sexually compatible gene pools (Fig. 2): an annual population with high grain yield (P_1) and a perennial one with low grain yield (P_2). If P_1 and P_2 are crossed, the hybrid population H_0 will tend to lie in an elongated distribution between them - a "recombination spindle" (Anderson, 1949). The long axis is oriented between the parents because of genetic linkages and pleiotropic trait associations. The latter includes any negative correlation between grain yield and perenniality that might result from a carbon tradeoff.

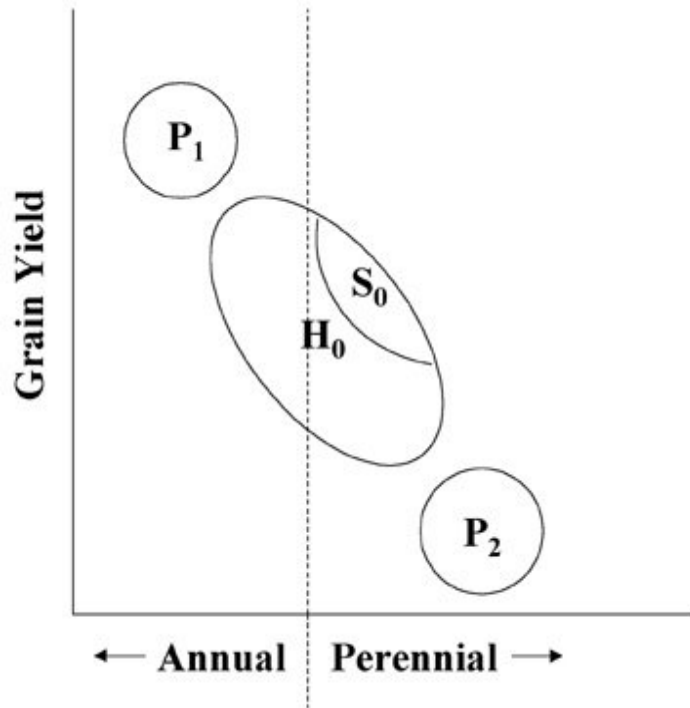


Figure 2. Distributions of an annual grain-crop population P_1 , a perennial population P_2 , a population H_0 derived by hybridizing P_1 with P_2 , and a segment of H_0 (S_0) selected for both perenniality and grain yield.

Selection in the hybrid population along the long axis of its distribution (i.e., toward P_1 or P_2) would result in a larger response per generation than selection perpendicular to the long axis. But even when selection is perpendicular to the long axis - in the direction of higher grain yield and stronger perenniality - it has the potential to approach the ancestral genetic limits (Fig.1) or even exceed them, via new introgression or mutation (Figs. 2 and 3). Direct selection within a perennial population for grain yield (Fig. 3) does not take advantage of genes from the annual crop and may be a longer-term project.

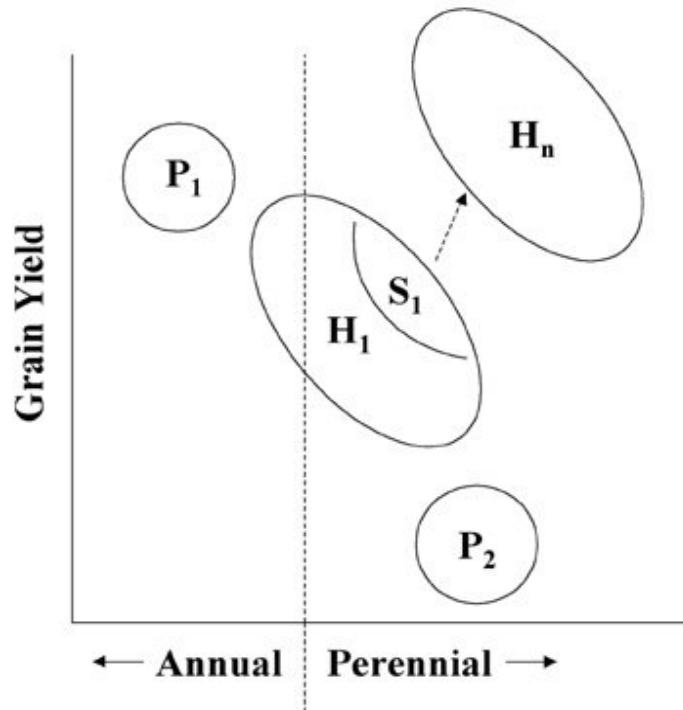


Figure 3. Distributions of two parental populations (P_1 and P_2), a population H_1 derived by interpollinating selected individuals (S_0 from Fig.1), a selected segment of H_1 (S_1), and a population H_n resulting from n cycles of selection for grain yield among selected individuals (dashed arrow). Direct selection within P_2 for grain yield (dotted arrow) could also produce a population such as H_n .

In any plant breeding program, negative correlations are a daily challenge. As Figs. 2 and 3 illustrate, simultaneous selection for negatively correlated traits can succeed if compromises in gains for individual traits are accepted. For example, selection for seed protein or oil concentration often has a negative effect on grain yield (Loffler et al., 1983; Brim and Burton, 1978; Burton and Brim, 1981); nevertheless, yield and grain quality have been improved simultaneously by breeding programs (Cox et al., 1989). Plant breeders routinely make some sacrifice in grain yield to improve pest resistance, and the cost in resources and effort is real, whether or not the resistance is genetically linked to lower yielding ability. Breeders of legumes could obtain almost immediate yield increases by eliminating symbiotic fixation of atmospheric nitrogen, which has a higher energetic cost to the plant than does absorption of nitrate from the soil (Finke et al., 1982; Salsac et al. 1984). But they forgo that yield increment because the biological nitrogen fixation is highly valued.

The relatively low yield of perennial grains recorded to date should not be attributed entirely to the carbon tradeoff. Perennial grains currently undergoing domestication are still essentially wild. In perennial grains being developed from interspecific hybridization, different genetic problems, each with its own array of possible solutions, have hampered breeding for yield. Among such obstacles are partial sterility caused by chromosomal differences between parental species; instability of chromosomal constitution; failure to eliminate genes for poor adaptation (unassociated with perenniality) from breeding populations; and lack of genetic diversity.

If breeding perennial grains is a difficult but attainable goal, it is important for research institutions to initiate cereal-, legume-, and oilseed-breeding programs aimed exclusively at

developing perennials. Today, while 33% of the world's cereal crop is fed to animals in the developed countries (Evans, 1998), we have some latitude to begin breeding crops that preserve the land while, initially at least, producing fewer total bushels than our current crops with their heavy subsidy of energy and chemicals. If we wait several decades to begin a breeding program, it may very well be too late.

We will now review recent results and prospects for perennial counterparts of major annual crop species and some perennial species that have not yet been domesticated. A realistic consideration of prospects, crop by crop, may give some insight into how research resources and efforts should be allocated among and within species in coming decades.

III. Breeding perennial grains: cool-season grasses

A. Wheat

1. Development of perennials up to 1990

Hexaploid wheat [*Triticum aestivum*, $2n=42$, genomes AABBDD] arose approximately 5000 years ago when the genomes of tetraploid wheat (*T. turgidum* or *T. carthlicum*, both $2n=28$, AABB) and an Asian goatgrass (*Aegilops tauschii*, $2n=14$, DD) were combined via amphiploidization (i.e., natural hybridization followed by spontaneous production of $2n$ gametes in the hybrid.) Tetraploid wheat itself is a much older natural amphiploid, incorporating the genomes of two diploid grasses. Both wheats are part of the large and diverse Triticeae tribe of the grass family (Gramineae), which also includes scores of perennial species, many of which can be hybridized with wheat (Dewey, 1984).

Wagoner (1990a) examined in detail the early history of efforts in the United States, Canada, Germany, and, most importantly, the USSR, to transfer genes for perenniality from alien grass species into bread wheat, citing more than 65 publications on the subject. None of these efforts produced a truly perennial grain cultivar, but they did spin off much valuable annual germplasm with genes for disease resistances and other traits. In the end, most of the effort in the perennial-wheat programs was diverted into producing improved annual cultivars, where progress was more easily achieved.

Of the few perennial, grain-producing genotypes developed from wide hybrids at the time of Wagoner's review, none was agronomically successful. Soviet-developed 'perennial' cultivars (Tsitsin, 1965) produced good grain harvests only in the year in which they were established from seed; in the end, they were used mainly as forage cultivars that provided no more than one grain harvest. The US germplasm 'MT-2', derived from a hybrid between *T. turgidum* and *Thinopyrum intermedium* ($2n=42$), released by Schulz-Schaeffer and Haller (1987) in Montana, had very low kernel weight and unreliable persistence. In Sweden, Fatih (1983) found that yields of perennial *T. aestivum*/*Th. intermedium* partial amphiploids ($2n=56$) were, on average, only 48% of the yields of 42-chromosome, annual, backcross-derived lines of similar parentage.

2. Production of new hybrids

In the decade since Wagoner's review, no perennial wheat cultivars have been released for production. But basic research on hybridization and cytogenetics has opened up new possibilities for geneticists and breeders interested in the problem.

Species of the genus *Thinopyrum* have been hybridized with wheat more often than have any other perennial species (Dewey, 1984) because of the ease of producing partially fertile hybrids, often without embryo rescue (Jauhar, 1995). *Thinopyrum intermedium* ($2n=42$) is rhizomatous, and two other commonly utilized species, *Th. ponticum* ($2n=70$) and *Th. elongatum* ($2n=14$) are caespitose. Researchers at Washington State University have launched a new program to develop perennial wheat from *Thinopyrum* crosses, with promising preliminary results (Scheinost et al., 2001; Cai et al., 2001). As we shall see (III.A.3.), recent cytological and molecular studies have explained why *Thinopyrum* crosses have often led to frustration, and in doing so, have suggested new approaches.

Other species in the genera *Thinopyrum*, *Elymus*, and *Leymus* have long been investigated as sources of perenniality (Wagoner, 1990a), and the perennial gene pool available to wheat geneticists is growing rapidly. In a review, Sharma and Gill (1983) listed only 16 perennial species that had been hybridized with hexaploid or tetraploid wheat, including only one species - *Elymus giganteus* - outside the *Agropyron-Thinopyrum* complex. A decade later, Jiang et al. (1994) added 38 additional species, including 17 in *Elymus* and 6 in *Leymus*, to the list of hybrids. Sharma (1995) reviewed the production of hybrids between wheat and more than 50 perennial species.

Our ability to make crosses and backcrosses between distantly related species has grown along with advances in embryo rescue, hormone treatments, intra-ovarian fertilization, bridge crosses, and protoplast fusion (Sharma, 1995). The effects of these techniques are magnified when they are used to exploit intraspecific variation for crossability, combining ability, or variation among species carrying the same or similar genomes. Especially important are reciprocal crosses followed by embryo rescue when the traditional method of using the species with higher chromosome number fails (Sharma, 1995).

Within each perennial species, accessions can vary in crossability, and choice of the annual wheat parent can also have a strong effect. For example, homozygosity for *kr* crossability alleles often makes hybridization possible, and may even improve early seed development; however, it may not affect results in extremely wide crosses (Sharma, 1995). Choice of the wheat parent may depend on other considerations. Hybrids of *Thinopyrum* and *Leymus* with Chinese Spring (*krkr*) could not survive winter temperatures, whereas hybrids with two Japanese spring wheats were winterhardy (Comeau et al., 1985). Fertility in the F_1 through production of unreduced gametes can be induced by crossing the perennial species with the tetraploid wheat *T. carthlicum* and maintaining low temperatures during pollination and embryo development (Anamthawat-Jonsson et al., 1997).

Many wheat/*Elymus* hybrids have been made in recent years (see Jiang et al., 1994; Sharma, 1995), and addition, substitution, and translocation lines have been developed (Jiang et al., 1993;

Gill et al., 1988; Morris et al., 1990). But to our knowledge, no explicit effort to transfer perenniality from *Elymus* to wheat is underway. Lu and von Bothmer (1991) produced hybrids between 12 *Elymus* species ($2n=28$ or 42) and wheat ($2n=42$). Hybridization was made possible by using *Elymus* as the female parent and rescuing hybrid embryos, as first demonstrated by Sharma and Gill (1983). All hybrids were perennial, but chromosome pairing was very low, as expected. The hybrids were not treated with colchicine to produce amphiploids.

Hybridization between wheat and the genus *Leymus* has a long history, but has not led to perennial cultivars (Wagoner, 1990a). Hybrids are most easily obtained with the larger-seeded, self-pollinated species of the genus: *L. arenarius* ($2n=56$), *L. racemosus* ($2n=28$), and *L. mollis* ($2n=28$) (Dewey, 1984), and these species also have the greatest agricultural potential (Anamthawat-Jonsson, 1996). Wheat has been crossed with eight *Leymus* species in all (see Jiang et al., 1994; Sharma, 1995). Most crosses require embryo rescue (e.g., Mujeeb-Kazi and Rodriguez, 1981; Plourde et al., 1989a and b; Anamthawat-Jonsson et al., 1997). Hormonal treatment has been used in producing F₁ (Anamthawat-Jonsson et al., 1997) and BC₁ (Mujeeb-Kazi and Rodriguez, 1981) seed.

Wheat/*Leymus* hybrids produced by the Soviet perennial-wheat breeding program were perennial (Bodrov, 1960; Petrova, 1960) but less winterhardy than wheat-*Thinopyrum* hybrids (Bodrov, 1960). In recent studies, hybrids produced by pollinating two wheat species (*T. aestivum* and the tetraploid *T. carthlicum*) with *L. arenarius* and *L. mollis* were perennial, producing short rhizomes (Anamthawat-Jonsson et al., 1997) and exhibiting some intergenomic pairing and a high enough level of fertility to permit backcrossing (Anamthawat-Jonsson and Bodvarsdottir, 1998). Hybrids between *T. aestivum* and two other species, *L. innovatus* and *L. multicaulis*, were non-rhizomatous (Plourde et al., 1989a and b).

3. Advances in chromosome identification

A revolution in cytogenetic techniques has led to a better understanding of the problems faced by perennial wheat breeders. MT-2 provides a good example. It was derived by selfing a 70-chromosome amphiploid containing the genomes of durum wheat (*T. turgidum*, $2n=28$, genomes AABB) and *Th. intermedium*, ($2n=42$, genomes StStEEEStESt). Schulz-Schaeffer and Haller (1987) predicted that MT-2 would stabilize at $2n=56$ through elimination of *Thinopyrum* chromosomes. In fact, according to Jones et al. (1999), individual plants of MT-2 vary in chromosome number, with most having $2n=56$. But genomic *in situ* hybridization (GISH) showed that 56-chromosome MT-2 plants contained numbers of wheat chromosomes varying between 24 and 28. Therefore, there had been loss of wheat chromosomes in some plants, and *Thinopyrum* chromosomes had been eliminated at random. Plants contained up to four St-E or St-ESt translocated chromosomes but no wheat-*Thinopyrum* translocations.

A wheat/*Thinopyrum* hybrid known as AT 3425, which has resistance to Cephalosporium stripe disease and perennial growth habit (Mathre et al., 1985), has $2n=56$; fluorescent genomic *in situ* hybridization (FGISH) and C-banding showed that AT 3425 has 36 wheat chromosomes, 14 *Thinopyrum* chromosomes, and 6 chromosomes resulting from translocations between the two species (Cai et al., 1998). The *Thinopyrum* chromatin in AT 3425 and another line with the same chromosome configuration, PI 550713, probably originated from *Th. ponticum*, and both lines

are cytologically stable (Cai et al., 2001). Another perennial, 56-chromosome line, AgCs, carries the combined genomes of hexaploid wheat and the diploid species *Th. elongatum* (Cai et al., 2001).

Banks et al. (1992) examined meiotic pairing in crosses among eight 56-chromosome partial amphiploids derived from crosses between hexaploid wheat (*T. aestivum*) and *Th. intermedium*. As is often found (Dvorak, 1970), the lines had all 42 wheat chromosomes as well as 14 from *Thinopyrum*. The sets of chromosomes originating from *Th. intermedium* differed in all but two lines. Unfortunately, all eight amphiploids studied meiotically by Banks et al. (1993) were annual; two Soviet-developed perennial wheats were examined only phenotypically.

Recently, partial amphiploids that had originated in the Soviet program (Bodrov, 1960; Petrova, 1960) have been found to be hexaploid, containing 30 chromosomes from tetraploid wheat and 12 from *L. mollis* (Anamthawat-Jonsson, 1999). Thus, as in wheat-*Thinopyrum* amphiploids, elimination of the perennial parent's chromosomes had occurred. In addition, one pair of wheat chromosomes had been substituted for a pair from *L. mollis*. The partial amphiploids were annual.

Thinopyrum elongatum ($2n=14$) is a diploid that is more difficult to hybridize with wheat than are *Th. intermedium* or *Th. ponticum*; however, the resulting amphiploids are vigorous, stable, and perennial. Because *Th. elongatum* contributes only a single genome, all plants should have the same 56-chromosome complement. For example, the wheat/*Th. elongatum* amphiploid AgCs is cytologically stable and perennial (Cai et al., 2001). Jauhar (1992) produced trigenic hybrids between a *Th. bessarabicum*-*Th. elongatum* amphiploid and tetraploid wheat (*T. turgidum*). The hybrids ($2n=28$, ABJE) were vigorous and perennial.

The maximum number of chromosomes that can be tolerated in amphiploids between wheat and *Thinopyrum* spp. appears to be 56 (Dvorak, 1976; Dewey, 1984), although 42-chromosome genotypes are more meiotically stable. Usually, partial amphiploids resulting from crosses with tetraploid wheat will contain approximately 28 chromosomes from the wheat parent and approximately 28 from *Thinopyrum*, whereas partial amphiploids with hexaploid wheat will have approximately 42 from wheat and 14 from *Thinopyrum*. Cauderon (1979) pointed out that partial amphiploids are automatically selected for "good balance" between wheat and perennial chromosomes through the elimination process; however, as we have seen, the same chromosomal complements are not consistently selected. Breeding populations based on collections of such lines would be plagued with sterility and lack of chromosome pairing unless a diploid perennial parent such as *Th. elongatum* is used.

The goal of a breeding program cannot be to develop a single perennial wheat cultivar. One partial amphiploid carefully selected to be cultivated as a perennial would have a unique chromosomal constitution. It would be a gene pool of one individual - a dead end in a breeding program. To launch a perennial wheat breeding program based on partial amphiploids would be an ambitious undertaking, involving the following steps for any polyploid perennial species targeted:

- Hybridize tetraploid and hexaploid wheats with the polyploid perennial species, making many parental combinations and sampling diversity of all parental species.
- Produce amphiploids and self-pollinate with mild selection for enough generations to achieve stable chromosome numbers.
- Use *in situ* hybridization, chromosome banding, genetic markers, and other techniques to identify chromosomes in a large population of selected plants representing many parental combinations.
- Assign partial amphiploids to groups of homogeneous chromosomal constitution.
- Compare groups for all phenotypic traits of interest and select one or a few on which to base further breeding. Develop foundation breeding pools from those groups. Experiment with intercrosses between groups, selecting for potentially superior chromosomal combinations.
- In creating new partial amphiploids to introduce into breeding pools, select strictly for appropriate chromosomal complements.

Such a plan would require a mammoth investment of resources, but the initial production of stable partial amphiploids is feasible on a large scale. Selfing and stabilization will occupy several years; by the time genotypes requiring chromosome identification can be produced, vastly more efficient cytological and molecular techniques are almost certain to be available, bringing the breeding program into the realm of the practical. But technological improvements do not guarantee the development of truly perennial grains, and parallel strategies are needed.

4. New strategies for perennial wheat

The perennial grasses of the tribe Triticae have long been used in wheat improvement, primarily as sources of individual resistance genes. A traditional strategy for transferring genes is to produce F₁ hybrids between wheat and the donor species; either double the chromosome number of the hybrid to produce an amphiploid or pollinate the F₁ directly; backcross to wheat genetic stocks to produce lines carrying the normal wheat complement of 42 chromosomes plus one or a pair of chromosomes from the donor parent; and - at some point in the process - attempt to induce a translocation that transfers a segment carrying the target gene to a wheat chromosome. There are many variations on this strategy, but the usual goal is to transfer a single gene, eliminating as much of the rest of the donor genome as possible.

Perenniality in wheat's relatives is more genetically complex than the single-gene traits transferred to date and may require a different approach. Amphiploids are not always perennial, and as we have seen, they are usually genetically unstable and agronomically undesirable. Backcrossing to wheat usually results in a return to the annual habit. Therefore, Ananthawat-Jonsson (1996) proposed backcrossing instead to the perennial parent - in their research, either *Leymus arenarius* or *L. mollis*. The objective then becomes to improve traits such as grain yield and kernel weight in the perennial species.

Ananthawat-Jonsson (1996) listed the traits to be improved by incorporating wheat germplasm into *Leymus* species: perenniality, grain quality, harvestability, threshability, kernel weight, synchronization of maturity, lodging and shattering resistance, meiotic stability, and, of course,

grain yield. She has backcrossed partial amphiploids to *L. mollis*, and the progeny were vigorous with long rhizomes.

In light of the many problems that have been encountered in transferring perenniality to wheat, the converse approach - using wheat to improve the perennial species - may have considerable merit. Backcrossing to the perennial will almost certainly produce breeding populations with low average grain yields and a high frequency of shattering, requiring the screening of large numbers of genotypes. Logic suggests that perennial allopolyploid species will be more tolerant of added or substituted wheat chromosomes. As we have seen, *L. arenarius* has been used as a grain crop in the past and efforts to improve it using wheat as a donor parent are underway (Anamthawat-Jonsson et al., 1997). *Th. ponticum* is easily crossed with wheat but its high ploidy level and lack of diploidization (Jauhar, 1995) probably would prevent its use in grain production. Wheat might be used as a donor parent for improving *Th. intermedium* or some hexaploid species of *Elymus* with which it can be crossed (Jiang et al., 1993, 1994).

B. Rye

1. Diploids

Rye (*Secale cereale*) appears to be at least as promising a candidate for perennialization as is wheat. Its chromosomes are homologous with those of its direct perennial ancestor, *S. montanum*. Both species are diploid and cross-pollinated. Rye is very winterhardy, well adapted for grazing, and useful in weed control because of its allelopathic properties (Anaya, 1999). The Soviet perennial-grains program included a large effort in rye (Derzhavin, 1960a), and they produced some weakly perennial genotypes that were used in limited production (Wagoner, 1990a). Later, a decades-long effort to breed perennial rye in Germany met with only partial success (Reimann-Philipp, 1995). Recently, a perennial rye cultivar, 'Perenne', was released in Hungary for grain and forage production (Hodosne-Kotvics et al., 1999).

Despite initial expectations, no perennial rye cultivar has been used in full-scale grain production. Breeders have been stymied by the tendency of plants in *S. cereale*/*S. montanum* populations to be either fertile and annual or highly sterile and perennial (Reimann-Philipp, 1995). A chain of translocations involving three of rye's seven pairs of chromosomes separates the two species (Stutz, 1957; Devos et al., 1993), and gene(s) from *S. montanum* governing perenniality are located on one or more of the translocated chromosomes. Because meiosis in plants heterozygous for one or more translocations produces many inviable gametes with duplications or deficiencies of chromosomal segments, plants in interspecific rye populations fall into one of three categories: homozygous for the *S. cereale* chromosomal arrangement (fertile, annual); heterozygous for one or more of the translocations (highly sterile); or homozygous for the *S. montanum* arrangement (perennial, fertile).

Plants in this last category would seem to answer the breeder's need; however, they are rare, and the large portion of their genomic content derived from *S. montanum* reduces their agronomic desirability and spike fertility. According to Reimann-Philipp (1995), seed-set in *S. montanum* itself is low - approximately 80%. Reimann-Philipp (1995) selected intensely for the *S. cereale* phenotype within an interspecific, perennial population. This population was presumed to be

homozygous for the three *S. montanum*-derived chromosomes involved in the translocations, identified as 4R^{mon}, 6R^{mon}, and 7R^{mon} by Koller and Zeller (1976) but referred to as 2R^{mon}, 6R^{mon}, and 7R^{mon} by Reimann-Philipp (1995). He was attempting to keep these chromosomes fixed while restoring completely the other four chromosome pairs from *S. cereale* through recombination and selection. But he could not achieve a kernel weight greater than 15 mg (compared with typical values of 40 mg for annual rye under those conditions.)

As an alternative, Reimann-Philipp (1995) proposed selection for perennial plants carrying chromosomes 2R, 6R, and 7R of *S. cereale*. Presumably, such plants would arise from recombination within the ring of six translocated chromosomes. Dierks and Reimann-Philipp (1966) had postulated that perenniality was governed by a single gene that lay approximately 10 crossover units from one of the breakpoints. Selection for perenniality would be routine, but selection for the *S. cereale* chromosomal constitution would require either a laborious testcross procedure or a cytological test. A morphological difference between chromosomes 6R and 6R^{mon} (Reimann-Philipp and Rohde, 1968) did not prove satisfactory for this purpose. Today, the extensive genetic map of rye (Melz et al., 1992) could allow marker-assisted selection for the *S. cereale* arrangement. A large initial experiment could provide much more detailed information on the genetic control of perenniality; as noted by Reimann-Philipp (1995), the trait is probably affected by more than one gene.

Yet another strategy was followed by L.F. Myers and R.J. Kirchner in the breeding of 'Black Mountain' perennial rye in Australia (Freer et al., 1997; Oram, 1996): backcrossing the interspecific hybrid twice to the *S. montanum* parent. Perennialism (and, presumably, the *S. montanum*-type chromosomal arrangement) was quickly restored by backcrossing. But this cultivar was intended primarily as a forage grass, with *S. cereale* donating genes for nonshattering rachis and improved seed production. Oram (1996) practiced six cycles of half-sib family selection for grain and forage yield in Black Mountain, achieving gains in both traits while maintaining a low level of shattering. With grazing, stands of 'Black Mountain' decline after 3 years; however, if shattering is permitted, stands can be continually replenished by volunteer seedlings (Oram, 1996). Without selection to develop a cultivar strictly for grain production, we cannot know whether backcrossing to perennial rye while selecting for alleles from annual rye can achieve sufficient yield improvement.

Reimann-Philipp (1995) warned of a hazard when growing diploid perennial rye with the *S. montanum* chromosomal arrangement on a field scale. If pollen from the perennial drifted into seed production fields or breeding nurseries of annual rye, the resulting translocation heterozygosity would seriously and irreversibly degrade fertility in subsequent generations.

2. Tetraploids

In an effort to improve kernel weight, Reimann-Philipp (1995) used colchicine to double the chromosome number of a perennial *S. cereale*/*S. montanum* population homozygous for the 4R^{mon}, 6R^{mon}, and 7R^{mon} chromosomes. The resulting tetraploid, named 'Permontra', had a kernel weight of approximately 30 mg (double that of the diploid), and first-year grain yields over 2000 kg/ha when grown in Germany. Yields declined in subsequent years (Reimann-Philipp, 1986). 'Permontra' achieved a similar grain yield in the Land Institute's plots in Kansas, but only 15 to

20% of the plants regrew in the next season (Piper, 1993). After a first-year harvest in the hot, dry summer of 2001, a stand of Permontra at The Land Institute died out completely by September.

Another problem with 'Permontra' - poor seed set - is also common in annual tetraploid rye, because the formation of multivalent chromosomal associations leads to production of gametes with extra or missing chromosomes (Bremer and Bremer-Reinders, 1954). Selection can improve seed-set in tetraploid rye (Bremer and Bremer-Reinders, 1954), and Reimann-Philipp (1995) pointed out that selection for seed set or meiotic stability can be practiced much more effectively in a perennial, by screening phenotypically or cytologically in one flowering cycle and intercrossing selected plants in the next. He found that phenotypic selection greatly improved seed-set in the perennial spring rye 'Soperta', which was derived from seven 'Permontra' plants that did not require vernalization in order to flower. Another approach would be to introduce the *Ph1* gene from wheat into a tetraploid hybrid between *S. cereale* and *S. montanum* to enforce diploid pairing and improve fertility. The *Ph1* gene was shown to operate when the chromosome carrying it was added to rye (Schlegel et al., 1991).

Tetraploid perennials may provide advantages beyond increased kernel weight. They are reproductively isolated from diploid annual seed production fields (Reimann-Philipp, 1995). In one study, 'Permontra' had greater heat and drought tolerance, and a much more extensive root system, than did diploid or tetraploid annuals (Gordon-Werner and Dorffling, 1988). But perennial rye, whether tetraploid or diploid, will not be grown widely as a grain crop until the problems of sterility, persistence, and maintenance of yield over seasons are solved.

C. Triticale

To date, the only species to be synthesized by artificial hybridization for use as a cereal crop is triticale (X *Triticosecale*), an amphiploid of durum wheat (*T. turgidum*) and *S. cereale*. (Octoploid triticale cultivars - *T. aestivum*/*S. cereale* - have also been produced but not widely used commercially.) Triticale has not become one of the world's leading cereals, but its modest success suggests the possibility of developing perennial *T. turgidum*/*S. montanum* triticales. Derzhavin (1960b) produced and intercrossed many amphiploids derived from crosses between durum wheat and perennial rye accessions. He augmented the gene pool by allowing the amphiploids to pollinate a large number of different wheat/rye F₁ hybrids. But the resulting populations were only weakly perennial. Three-way hybrids - from crosses between wheat/*Th. intermedium* hybrids and perennial rye - were more strongly perennial but had sterility, low yields, and small seeds (Derzhavin, 1960b; Lapchenko, 1960).

Robert Metzger (USDA-ARS retired, Corvallis, OR, personal communication) reports that a *S. montanum*-derived triticale that he has developed is not sufficiently perennial, but he recommends producing and screening more new amphiploids involving a wider range of *S. montanum* germplasm. It could be that no triticale will be fully perennial, having only one of three genomes derived from a perennial species. Intercrossing of diverse lines followed by selection could improve persistence over years. If improved hexaploid or tetraploid perennial wheats can be developed, they could be crossed with *S. montanum* to produce more strongly perennial triticales.

In attempting to develop perennial triticales, breeders can take lessons from development of the annual crop (Skovmand et al., 1984; Horlein and Valentine, 1995). Primary triticales, i.e., newly doubled wheat/rye hybrids, inevitably suffer from sterility, seed shrivelling, lodging, and low yield potential. Decades of intense selection and introgression have resulted in triticales that are cytologically stable and improved for all of these traits, but they stand on a narrow germplasm base. Improving the performance and genetic variability of the triticales gene pool can be accomplished by several means: production of new primary triticales; triticales/wheat crosses; triticales/rye crosses; and crosses between hexaploid and tetraploid triticales (Skovmand et al., 1984).

Primary triticales derived from the wild *S. montanum* are even more agronomically primitive than *S. cereale*-based primary triticales and will require even greater breeding effort with a wide range of parents. "Substituted" triticales in which one or more wheat chromosomes replace those of rye are often agronomically superior; chromosomes 2D and 6D appear to be selectively propagated by breeders in populations segregating for R- and D-genome chromosomes (Gustafson et al., 1989). But it must be kept in mind that until genes conditioning perenniality can be mapped, random substitution of wheat for *S. montanum* chromosomes will reduce the chances of selecting a strongly perennial triticales.

D. Direct domestication of the perennial Triticeae

1. Intermediate wheatgrass

From domestication of perennial grasses with wheat or rye as a donor parent (Anamthawat-Jonsson, 1996; Oram, 1996), it is a relatively short leap to domestication without any interspecific crossing. Three large-seeded perennial species that have been hybridized with wheat also have attracted attention as candidates for direct domestication. By far, the most work in this area has been done with *Th. intermedium* by Wagoner (1990a, 1995) at the Rodale Institute in Pennsylvania and her colleagues at the USDA-NRCS Big Flats Plant Materials Center in New York. Wagoner (1990a) described in detail the characteristics that make intermediate wheatgrass a good candidate for domestication as a perennial grain, while noting shortcomings that must be addressed. Becker et al. (1991) concluded that its grain has protein quality "superior to the cereal grains now commonly grown", with no significant amounts of antinutrients.

Recurrent selection is a logical breeding method for improving an only slightly domesticated, cross-pollinated species like *Th. intermedium*. Using mass selection without controlled pollination, Knowles (1977) increased seed yield in an intermediate wheatgrass population by 10% per cycle. In each cycle, 1000 plants were evaluated for spike fertility over three years, and the best 50 were selected. When selected plants were removed to the greenhouse over the winter to exclude pollination by non-selected plants, thereby doubling parental control, gain per cycle increased to 20% - a result perfectly consistent with selection theory (Fehr, 1987).

Gridded mass selection is used to exercise control over microenvironmental effects and increase selection response (Gardner, 1961). Wagoner (1990a, 1995) evaluated 300 accessions of intermediate wheatgrass, for grain yield, yield components, and end-use quality, selecting the 20 best accessions in 1989. The selections were transplanted into a polycross nursery, and 380

progeny resulting from pollination among the selections were evaluated, in a field divided into blocks of 25 plants each, between 1991 and 1994 (Wagoner, 1995; Wagoner et al., 1996). The best 11 plants resulting from within- and among-block selection, plus three selections resulting from further evaluation of other accessions, were put into a second-cycle polycross, and 400 individual progenies were evaluated in a second blocked nursery (Wagoner, 1995). Yield per plant in the 14 selections was approximately 25% higher than the population mean. Evaluation of the second-cycle population is underway, and selected plants will be intermated in 2002 to complete another breeding cycle (M. van der Grinten, USDA-NRCS, Big Flats, NY, pers. commun.) Better environmental control through selection within blocks may have produced the five-percentage-point improvement in selection response over that of Knowles (1977), but it remains to be seen if the small effective population size in the second cycle (14 plants) will restrict genetic gain in the future.

2. Wildrye

The Land Institute in Kansas has studied perennial cool-season grasses as potential grain crops for over 20 years (Jackson and Jackson, 1999). They evaluated almost 1500 accessions representing 85 species of *Agropyron*, *Thinopyrum*, *Elymus*, and *Leymus*, along with 2630 accessions of other species, between 1979 and 1987. The species selected as having the greatest potential for domestication was *L. racemosus*, known commonly as giant or mammoth wildrye. However, prospects for utilization of this species in the near future are unclear. Among 16 accessions evaluated over 2 years, yields did not exceed 830 kg/ha (Piper, 1993), and yield declined rapidly in the second and third years. Wildrye's great vigor, accompanied by large spikes but sparse seed-set, resulting in low harvest index (Wagoner, 1990a), may provide considerable scope for breeders to select for diversion of photosynthate toward grain production. But there is no current breeding program for grain yield in *L. racemosus*; until selection is undertaken, no conclusions can be drawn regarding its potential. *L. racemosus* is self-pollinated and would require a breeding approach different from that taken with *Th. intermedium*.

3. Lyme grass

Lyme grass or beach wildrye (*Leymus arenarius*) has been used as a food grain since the time of the Vikings (Griffin and Rowlett, 1981), and, as we have seen, is being studied as a potential grain crop in Iceland (Ananthawat-Jonsson, 1996). There is significant genetic variation among accessions of *L. arenarius* and *L. mollis* (Ananthawat-Jonsson et al., 1999), and it would be interesting to know which approach would result in more rapid genetic progress: direct selection within the species or an interspecific backcross program using wheat as a donor parent (Ananthawat-Jonsson, 1996). The latter strategy takes advantage of genes selected through millennia of wheat domestication and breeding, but introduces chromosomal instability.

4. Prospects for direct domestication

Is there sufficient genetic variation within these three, or other, cool-season grasses to support large improvements in yield, kernel weight, and other traits? The very existence of annual grain crops proves that selection over thousands of years can move the mean of a species far beyond its original phenotypic range. Gains of 20 to 25% per cycle (Wagoner, 1995; Knowles, 1977) are

much more rapid than typical gains in major annual crops, even considering the longer selection cycle of perennials. But to effect sufficient changes in a matter of decades rather than centuries - while possibly working "uphill" against the problem of resource allocation in perennials - will require much larger breeding efforts than have been undertaken to date. Relatively small efforts at domestication, which are within the capabilities of nonprofit organizations such as the Rodale Institute or Land Institute (or small-scale breeding programs within larger organizations such as USDA or universities), must be expanded to a much larger scale by university, government, or corporate breeding programs if wholly new perennial grain crops are to be developed.

The yield increases of 25% per cycle achieved by Wagoner et al. (1996) are remarkable, especially considering that they selected for other traits in addition to yield. But because response to recurrent selection tends either to follow a linear path or decelerate (Hallauer and Miranda, 1988), future gains per cycle will probably be no greater than a constant percentage of the base population's yield. If a hypothetical perennial grass population yielding 500 kg/ha of grain undergoes selection, with a yield increase of 125 kg/ha/cycle (25% of the base yield), 20 cycles will be required to reach 2500 kg/ha. Because selection in perennials must be based on evaluation over two or more seasons, a single cycle can occupy four or five years. Obviously, if it is going to take almost a century to develop a high-yielding perennial crop through direct selection, a long-term commitment is required; however, such a rate of progress is much greater than the rate at which our annual crops were domesticated and improved. Marker-assisted selection and/or some genetic input from wheat could speed up the process.

E. Oat

Perennial oats for grain production might be developed from crosses between the cultivated hexaploid oat (*Avena sativa*, $2n=42$, genomes AACCCDD) and a wild, perennial, autotetraploid relative, *A. macrostachya* ($2n=28$, CCCC). Such crosses require embryo rescue. The F_1 is highly sterile, but backcrosses to *A. sativa* have been made, and limited pairing between chromosomes from different parents does occur (Leggett, 1985). J. P. Murphy (North Carolina State Univ., pers. commun.) has produced a 70-chromosome amphiploid between the species. The objective of this cross is to improve winterhardiness in the annual crop; perenniality has not been evaluated. Because of the partial homology that exists between chromosomes of the parental species, the amphiploid is likely to suffer from the same chromosomal instability found in wheat amphiploids. But the amphiploid, like the hybrid, can be backcrossed to *A. sativa* (Murphy, pers. commun.) Ladizinsky (1995) domesticated accessions of two wild annual oat species by using the cultivated oat *A. sativa* as the donor of genes for nonshattering and other traits. Perhaps this approach could be tried with *A. macrostachya*.

More hybrid combinations and larger populations will be needed if genetic studies and selection for perenniality are to succeed in the backcross generations. A more diverse sample of *A. macrostachya* parents would be desirable, but the species is restricted to two mountain ranges in Algeria, limited germplasm collections exist in the United States, and there are very few accessions held in other countries (Guarino et al., 1991). Selection for perenniality in colder climates could be thwarted by the lack of winterhardiness in oats; winter annual oats are not generally sown above 35 degrees latitude in North America. At sites where *A. macrostachya* was

collected by Guarino et al. (1991), the mean minimum temperature of the coldest month ranged from -0.6 to -3.6°C.

F. Rice

Although tropically adapted, rice (*Oryza sativa*) has the C₃ carbon fixation pathway and is included here with the cool-season grasses. The perennial ancestor of *O. sativa* is *O. rufipogon*. Both species are diploid (2n=24) with homologous chromosomes and they can be hybridized easily. Indeed, natural hybridization and introgression occur in the field (Majumder et al., 1997).

From 1995 to 2001, the International Rice Research Institute (IRRI) had a program for development of perennial rice cultivars to reduce erosion on the steep slopes where upland rice is often grown (Bennett et al., 1998; Schmit et al., 1996). Populations from IRRI's breeding program, which was discontinued in 2001, have been distributed to cooperators in China, where perennial rice breeding efforts continue.

Sacks et al. (2000) found wide variation in second-year survival among 51 *O. sativa/O. rufipogon* F₁ hybrids. Sixteen percent of the hybrid combinations had greater than 50% survival, and 19% of all hybrid plants survived. In a cross between a rice cultivar with a regeneration score of 1.0 and an accession of *O. rufipogon* with a score of 3.8, the F₁ had a regeneration score of 4.0, and the scores of F₂ clones ranged from 0 to 5 (Xiong et al., 1999). Paradoxically, in three of the four chromosomal segments that affected regeneration ability, it was the annual parent's allele that had a positive effect. In contrast to mapped regrowth loci in *Sorghum bicolor/S. propinquum* populations (Paterson et al., 1995), none of the regeneration loci were associated with effects on tiller number.

Selection in interspecific populations may be aided by rice's detailed molecular map and the known locations of chromosomal segments affecting traits of domestication (Xiong et al., 1999; Kohm et al., 1997; Cai and Morishima, 2000). Many of the traits separating the annual and perennial species show polygenic inheritance. Surprisingly, *O. rufipogon* was the source of four chromosomal segments with positive effects on testcross grain yield in one set of backcrosses (Xiao et al., 1998); however, three of the four segments were adjacent to segments that either increased plant height or delayed maturity. With positive alleles affecting perenniality and productivity apparently being contributed by both parental species, prospects for breeding high-yielding perennial rice genotypes may be bright.

Oryza sativa can also be hybridized with *O. longistaminata*, the perennial ancestor of West African rice, *O. glaberrima*. The perenniality of *O. rufipogon* lies in its ability to regrow repeatedly through production of new tillers, whereas *O. longistaminata* regrows from rhizomes (Maekawa et al., 1998). In crosses between *O. sativa* and *O. longistaminata*, genes affecting rhizome production appear to be linked to genes for hybrid embryo abortion. Consequently, IRRI scientists backcrossed rare hybrids to both parental species and intercrossing the progeny in an effort to develop a rhizomatous, agronomically acceptable genotype (Bennett et al., 1998). Tao et al. (2001) recovered a single rhizomatous individual from among 162 plants produced by backcrossing an *O. sativa/O. longistaminata* hybrid to *O. sativa*.

A long-lived, three-species hybrid (*O. sativa/O. rufipogon/O. longistaminata*) has persisted through winters in China with monthly mean temperatures as low as 5°C (Li, 1998). Crossing the hybrid with *O. sativa* and intermating perennial progenies has eliminated shattering (Li, 2000). One possibility for breeding an even more cold-tolerant perennial rice exists. An ecotype of *O. rufipogon* known as 'Dongxiang' has the ability to regrow in regions of China where temperatures below -10°C are common (He et al., 1996). [continued]