

Breeding Intermediate Wheatgrass for Grain Production

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ABSTRACT

Intermediate wheatgrass [IWG; *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey] is a perennial grass with the unique distinction of having been, for more than 30 years, the target of active breeding for use as a grain crop for human consumption. Improving the grain production characteristics of a perennial forage grass to economically viable levels is a long-term endeavor that was undertaken because of the potential for profound benefits to farmers, human society, and the environment. Even before research as a perennial grain, IWG has had a history of improvement as a forage species, and as one of wheat's closest perennial relatives it has also been used to transfer desirable traits into annual wheat. Since initial work in the 1980s, long-term breeding programs have been initiated in Kansas, Minnesota, and Utah (United States), Manitoba (Canada), and Uppsala (Sweden). Coupling advances in molecular technologies, many of these programs have harnessed the power of genomic selection and other cutting-edge tools to rapidly improve IWG. This has resulted in estimated gains of up to 8% per year for spike yield, and across eight breeding cycles grain yield has increased 9% per cycle, yet another 23 breeding cycles may be required before IWG yields are comparable to annual wheat. In addition to improving key domestication and agronomic traits, molecular research has provided a wealth of information about the genomic regions controlling trait expression through linkage mapping and genome-wide association studies. These results suggest that leveraging new molecular and breeding tools could potentially lead to *de novo* domestication of new crops in approximately 40 years or less.

KEYWORDS: breeding, domestication, ecosystem services, forage, perennial grain, genomic selection, intermediate wheatgrass, mapping, quantitative trait locus, *Thinopyrum intermedium*

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ABBREVIATIONS

BLS	bacterial leaf streak
cM	centiMorgan
DH	doubled-haploid
FHB	Fusarium head blight
FSU	floret site utilization
GBS	genotyping-by-sequencing
GEV	genomic-estimated breeding value
GS	genomic selection
GWAS	genome-wide association study
GxE	genotype-by-environment
HMW-GS	high-molecular-weight glutenin subunits
IWG	intermediate wheatgrass
JGI	Joint Genome Institute
LD	linkage disequilibrium
LG	linkage group
LOD	logarithm of odds
MAS	marker-assisted selection
NAM	nested association mapping
QTL	quantitative trait locus
RIL	recombinant inbred line
RRC	Rodale Research Center
RRS	reduced-representation-sequencing
SI	self-incompatibility
SLU	Swedish University of Agricultural Sciences
SNP	single-nucleotide polymorphism
TILLING	Targeted Induced Local Lesions in Genomes
TLI	The Land Institute
UMB	University of Manitoba
UMN	University of Minnesota
USDA	US Department of Agriculture
WGR	whole-genome resequencing

I. INTRODUCTION

The soils that today grow our cereals, oilseeds, and pulses were formed under the grasslands, forests, savannahs, and other ecosystems that preceded agriculture. An interesting and important similarity across terrestrial ecosystems is that, with very few exceptions, their vegetation is made up of diverse perennial plant species, and these plant communities played a central role in pedogenesis, or soil formation

(Jenny 1980; Li et al. 2014). In stark contrast, the vegetation of arable lands is overwhelmingly composed of annual plant species, occupying over 90% of croplands, 78% of which are annual grains (Monfreda et al. 2008). To nurture annual crops from seed to harvest, it is necessary to suppress or kill competing vegetation, especially during crop germination and early growth stages. While there clearly were some compelling reasons for early agriculturalists to favor the domestication of annual species over perennial candidates (Van Tassel et al. 2010), we now know that the expansion of annual crop production to some 10% of ice-free land has had far-reaching negative consequences, especially with respect to soil health, evidence that the biophysical limits of the agricultural ecosystem appears to have been reached (Klitgaard 2020).

Soil erosion from landscapes under no-till management is estimated to be an order of magnitude greater than soil formation, while landscapes managed with conventional tillage erode at more than 100 times the rate of soil formation (IPCC 2019). When free of living vegetation, soils experience extended periods of time with minimal or no root activity. Without roots, mobile nutrients such as nitrate (N) can easily be lost to groundwater and proximate rivers or lakes. Between nitrate runoff and losses of N through denitrification, only about half of the N applied as fertilizer is actually utilized by crops (Ladha et al. 2005). Without dense vegetation covering the soil surface, phosphorus (P) is commonly lost in runoff, also ending up in streams or lakes. Once in aquatic ecosystems, N and P are responsible for widespread eutrophication in freshwater ecosystems in agricultural regions around the world (Rabalais et al. 2010). And when nitrate-enriched rivers enter marine ecosystems, hyperproductivity of algae commonly results in the formation of hypoxic or “dead” zones that persist for several months each year (Rabalais et al. 2010). While the biogeochemistry underlying the formation of hypoxic zones can be complicated, N fertilizer leached from agriculture is a major driver of more than 400 dead zones that have been reported at the mouths of major rivers around the world (Diaz and Rosenberg 2008).

Carbon is another element that soils predictably lose when continuously cropped with annuals. Soil organic matter, which is about 58% carbon, is the result of a dynamic balance between ongoing organic inputs into soils, especially roots (Schmidt et al. 2011), and the outputs of carbon, primarily from respiration of CO₂ by heterotrophic microbes that consume soil organic matter. When native perennial ecosystems are converted to croplands, the input of carbon in the form of roots decreases by 40–80% (Goudriaan et al. 2001; Saugier et al. 2001; Whalen and Sampe-drok 2009). In contrast, output of carbon via respiration increases as tillage breaks up stable aggregates, exposing what had previously been pools of soil organic matter protected from microbial mineralization (Grandy

and Robertson 2007). The combination of reduced organic inputs into soils and increased losses of carbon due to tillage resulted in an almost universal decline of between 20 and 70% of soil organic matter content in the years following conversion (Davidson and Ackerman 1993; Lal 2004).

Loss of organic matter, nutrients, and soil itself are three examples of how soil health is diminished under continuous annual cropping. In recent years, these and other shortcomings have been widely acknowledged, and US governmental agencies like the Natural Resources Conservation Service (NRCS) as well as private not-for-profit groups such as the Soil Health Institute and the Nobel Foundation have promoted similar versions of four general principles that promise to regenerate fertile and productive farmlands: maximize continuous living roots, minimize disturbance, increase soil cover, and maximize biodiversity. It is logical that these principles reflect central features of natural perennial plant communities that facilitated soil development in the first place. Considerable effort has gone into designing annual cropping systems that utilize cover and relay crops to implement some of these principles. In many cases, these efforts have resulted in measurable improvements in soil health indicators and related ecosystem services. Yet a large and expanding body of evidence suggests that perennial crops have the potential to substantially improve soil health and multiple ecosystem functions beyond what is possible with annual cropping systems (Robertson et al. 2011; McGowan et al. 2019; Sprunger et al. 2020).

Perennial grain crops present the unique opportunity to produce abundant human-edible food directly from easily harvested herbaceous perennial plants. While efforts are underway to introduce the perennial habit into existing grain crops such as wheat, rice, and sorghum (Crews and Cattani 2018), the work we will describe here focuses on the development of intermediate wheatgrass (IWG hereafter) as a perennial grain crop through direct *de novo* domestication. IWG, now being marketed as Kernza®, is in the initial stages of commercial adoption by farmers and yet there is a strong interest in the ecosystem services it might deliver given its prodigious root system. In a study that spanned three years and multiple fertility treatments in Michigan, United States, IWG was found to produce 3 to 12 times the root biomass of annual winter wheat (Sprunger 2015). Given the importance of root biomass in the formation of stable soil carbon (Schmidt et al. 2011; Sokol and Bradford 2019), IWG has the potential to promote greater C sequestration than comparable annual cereals (Duchene et al. 2020). An IWG field in Kansas, United States, that was monitored using eddy covariance was found to accumulate an average of 3.7 tons C ha⁻¹ yr⁻¹ over five years as carbon stored in IWG biomass and soil carbon increased (de Oliveira et al. 2018).

Improved nutrient retention is also derived from IWG's allocation to root biomass. For example, a mature stand of IWG in Michigan, United States,

was found to reduce nitrate leaching by 86–99% relative to annual winter wheat (Culman et al. 2013). In a similar study in Minnesota, United States, IWG reduced nitrate leaching by 96–99% relative to maize (Jungers et al. 2019). Duchene et al. (2020) linked IWG's ability to retain nutrients to its high rooting density and depth of soil exploitation. Greater and more consistent allocation of carbon below-ground relative to annual crops is also predicted to influence the composition and functions of the soil microbiome. In a comparison of soil fungal communities sampled from a 15-year experiment in Kansas, United States, that included stands of IWG, prairie restoration, and an annual rotation of wheat, soy, and sorghum, the overall fungal communities in general, and saprotroph and mutualist communities in particular, were similar in IWG and prairie restoration, whereas both were distinct from annual rotation fungal communities (McKenna et al. 2020). In France, an increase in soil fungal community biomass in topsoil layers was found in a comparison between IWG and two annual cereals (Duchene et al. 2020). Sprunger et al. (2019) reported that soils conditioned by IWG supported more highly structured and complex nematode and bacterial food webs relative to soils conditioned by winter wheat.

Our understanding of how soil health indices and ecosystem services might improve under IWG is just beginning to unfold. Early findings confirm that IWG's investment in roots is considerably greater than that of wheat, rye, and other annual small grains, and that this investment has the potential to result in greater soil carbon accumulation, nutrient retention, and an alternative and possibly higher functioning microbiome community. Other ecosystem services that may potentially improve with a shift from annual to perennial grains include water infiltration and utilization efficiency, weed suppression through competition, and a reduction in energy required for crop management (Crews et al. 2018). A greater reliance on ecological intensification as opposed to input intensification may also translate into lower fertilizer, herbicide, and fuel costs for farmers.

II. PLANT BIOLOGY AND BEHAVIOR

A. Geographical Distribution, Botanical Description, and Genetic Characteristics

Thinopyrum intermedium is native to shrub steppes and slopes up to the lower mountain belts of southern Europe, through the Middle East and southern former Soviet Union to western Pakistan (Tzvelev 1976) including parts of Afghanistan, Albania, Austria, Bulgaria, Central European Russia, Czechoslovakia, East Aegean Is., East European Russia, France, Germany, Greece, Hungary, Iran, Iraq, Italy, Kazakhstan, Kyrgyzstan, Krym, Lebanon-Syria, North Caucasus, Pakistan, Poland, Romania, South European Russia, Spain, Switzerland, Tajikistan,

Transcaucasus, Turkey, Turkey-in-Europe, Turkmenistan, Ukraine, Uzbekistan, and Yugoslavia (POWO 2021). Ten *Thinopyrum* taxa, including *Th. intermedium*, are included in the first priority list of crop wild relatives (CWRs) found in the Fertile Crescent (Zair et al. 2018). *Thinopyrum* was ranked fifth, among 54 genera, in terms of the number of species per genus with CWR priority species in the Fertile Crescent (Zair et al. 2018), but the distribution of *Th. intermedium* is not restricted to this region. It is also widely naturalized in western N. America where it has been used for erosion control, revegetation, pasture, and hay (Barkworth 2007; POWO 2021).

The US Department of Agriculture (USDA)'s National Plant Germplasm System (NPGS) includes 356 *Th. intermedium* accessions from potentially natural sources and another 54 accessions donated from breeding programs in N. America. Most of the potentially natural NPGS accessions come from Afghanistan (7), Iran (149), Kazakhstan (28), Russian Federation (79), and Turkey (63). Other potentially natural sources of NPGS accession include Austria (1), China (3), Denmark (1), France (3), Germany (2), Moldova (1), Morocco (1), Poland (2), Portugal (2), Romania (4), Serbia (1), Spain (4), Tajikistan (1), Turkmenistan (1), Ukraine (2), and Uzbekistan (1). The exact collection localities of many potentially natural NPGS accessions are unknown, but the approximate location of 142 collection sites (Figure 3.1) has been deduced (Jensen et al. 2016). Genetic studies showed that most of the USDA collections belong to one of two groups identified as European and Asian (Jensen et al. 2016), which are recognizable in the first principal component of molecular genetic diversity (Figure 3.2).

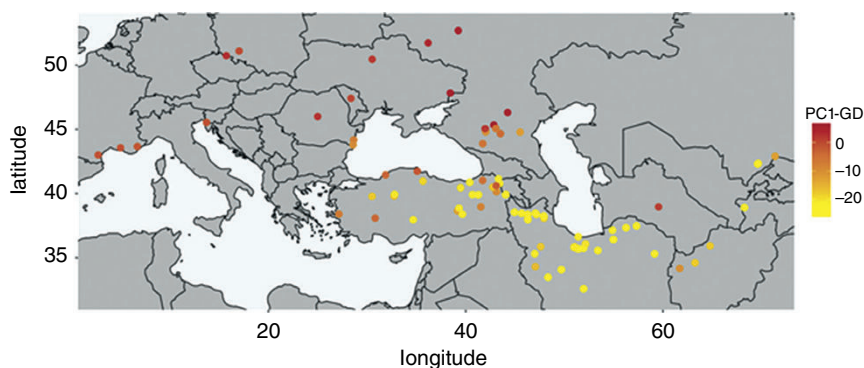


Fig. 3.1. Origin of 142 intermediate wheatgrass accessions deposited at the United States Department of Agriculture, National Plant Germplasm System. The yellow-brown shade scale is based on the first principal component of molecular genetic diversity.

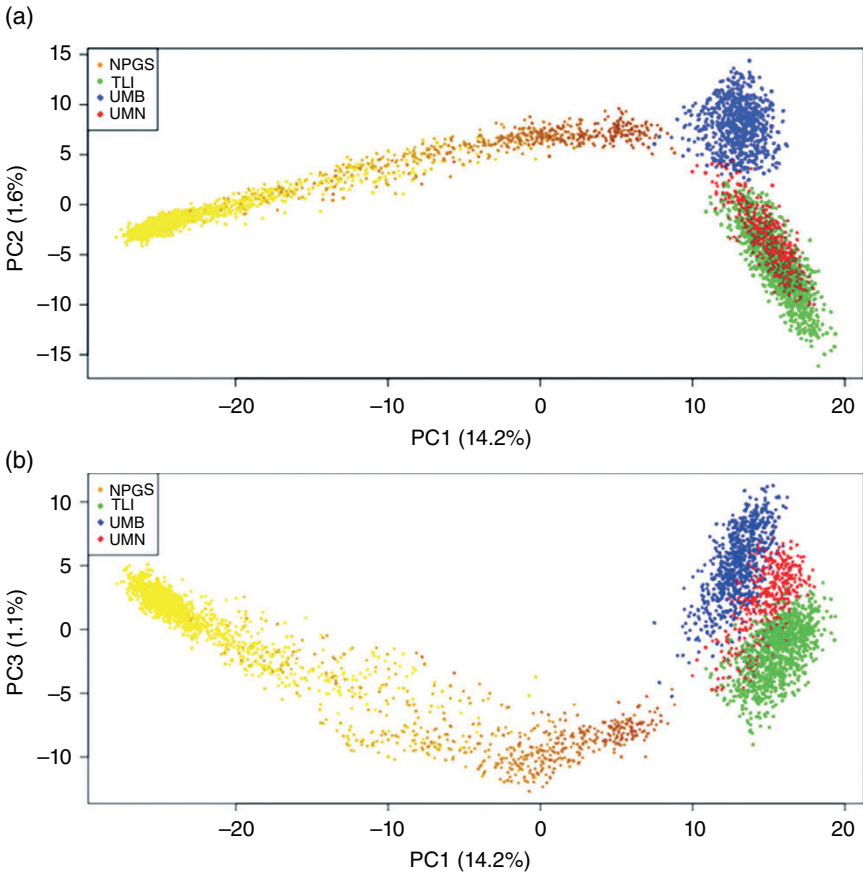


Fig. 3.2. Principal component analysis of 1,916 intermediate wheatgrass genotypes: 334 accessions were sampled from the United States Department of Agriculture, National Plant Germplasm System (NPGS), 1,078 genotypes from 14 half-sib families from The Land Institute (TLI), 765 plants from 10 half-sib families/10-clone polycross selected from Cycle 1 at the University of Manitoba (UMB), and 384 plants from 5 synthetic populations selected from Cycle 1 at the University of Minnesota (UMN). The shade of colors for NPGS genotypes is scaled from yellow to brown based on eigenvalues from principal component 1 (PC1). The percent variance explained by principal components 1 (PC1), 2 (PC2), and 3 (PC3) are 14.2, 1.6, and 1.1%, respectively.

IWG is an allohexaploid ($2n = 6x = 42$) perennial grass belonging to the Triticeae tribe. The Triticeae tribe encompasses 360–500 species or subspecies (Bernhardt 2015) including wheat, barley, rye, and a plethora of wild annual and perennial grasses. It has been estimated that 80% of Triticeae species are perennials (Ceoloni et al. 2015). Like

most species in the Triticeae taxa, *Th. intermedium* has spike inflorescence with sessile spikelets. In a taxonomic key of 14 Triticeae genera found in North America, the genus *Thinopyrum* is coupled with genera *Aegilops*, *Triticum*, *Pseudoroegneria*, and some *Elymus* taxa by having one spikelet per node and spikelets being less than three times the length of the middle rachis internodes (Barkworth 2007). However, the key differences between *Thinopyrum*, *Pseudoroegneria*, and *Elymus* and *Aegilops* and *Triticum* taxa are that the first three genera are perennial and their glumes do not have lateral teeth or awns like the annual genera *Aegilops* and *Triticum* (Barkworth 2007). The genus *Thinopyrum* generally differs from other Triticeae genera by having thick, stiff glumes and lemmas, at least several cells thick even between the veins (Barkworth 2007). Four *Thinopyrum* species occur in N. America including *Th. intermedium*, *Th. ponticum* (Barkworth & D. R. Dewey), *Th. pycnanthum* [(Godr.) Barkworth], and *Th. junceum* [(L.) Á. Löve] with *Th. intermedium* and *Th. ponticum* being more common than others. Tall wheatgrass (*Th. ponticum*) is distinguished from the other three *Thinopyrum* species by not having rhizomes and having truncate glumes with midveins equal in length and prominence to the lateral veins. *Th. intermedium* is considered rhizomatous, although rhizomatous spreading is limited in some plants and its glumes have midveins longer and more prominent than lateral veins. The culms of *Th. intermedium* reportedly range from 50 to 115 cm in the Flora of North America (Barkworth 2007), but culms up to 219 cm tall have been observed in cultivated nurseries (Larson et al. 2019; Figure 3.3).

Although taxonomic treatments of the Triticeae vary (Dewey 1984; Löve 1984; Barkworth and Dewey 1985; Kellogg 1989), these species have been organized into approximately 20–30 diploid or autopolyploid genera and several exclusively allopolyploid genera, including *Elymus*, *Leymus*, and *Pascopyrum* (Kellogg et al. 1996; Bernhardt 2015). The genus *Thinopyrum*, which contains approximately 10 species (Barkworth 2007), is an exception in that it contains diploid, autopolyploid, and allopolyploid taxa (Kellogg et al. 1996; Ceoloni et al. 2014). Only 80 Triticeae species are diploid, with many polyploid species originating from independent hybridization events (Mason-Gamer 2004, 2013; Bernhardt 2015; Brassac and Blattner 2015). To understand the evolution of *Th. intermedium* and other polyploid Triticeae species, such as bread wheat, it is necessary to identify their diploid ancestors. Early cytogenetic studies indicated that *Th. intermedium* was a strict allohexaploid (Stebbins and Pun 1953) or segmental autoallohexaploid (Dewey 1962). Low-frequency chromosome pairing in a polyhaploid *Th. intermedium* plant fit a model of a segmental autoallohexaploid, having two



Fig. 3.3. Intermediate wheatgrass used as a perennial grain crop: (a) seedlings growing in the field; (b) diseases of IWG from left to right: Fusarium head blight (scab), ergot, bacterial leaf streak, spot blotch; (c) IWG plants next to a 91.4 cm (36 in) long ruler; (d) spaced plants during heading in the field; (e) direct-combine harvest of IWG stands; (f) spaced plants during anthesis in the field; (g) threshed IWG grain; and (h) sourdough bread baked with 20% IWG flour.

similar and one distinct genome (Dewey 1962). Additional studies of chromosome pairing in the pollen mother cells of wide hybrids also supported the assertion that *Th. intermedium* contained two similar genomes and one distinct genome (Dvořák 1981) designated **J°J°J°StSt** where **J°** originates from diploid *Th. elongatum* (Host) D.R. Dewey and **St** originated from a diploid Eurasian *Pseudoroegneria* species (Liu and Wang 1993; Zhang et al. 1996) or somewhat equivalent as **J°J°J°StSt** where **J°** originates from diploid *Th. bessarabicum* (Savul. & Rayss) Á. Löve (Zhang et al. 1996; Chen et al. 1998; Tang et al. 2011). Recent linkage mapping demonstrated 21 linkage groups (LGs) indicative of disomic inheritance (Kantarski et al. 2017), which refutes the possibility that it is an autopolyploid or autoallopolyploid. Subsequent work based on DNA markers and sequencing implicate at least two or three of the diploid Triticeae genera as possible ancestors of allohexaploid *Th. intermedium*, including one diploid *Pseudoroegneria* ancestor, at least one diploid *Thinopyrum* ancestor, and possibly a third diploid *Aegilops*, *Dasypyrum villosum* (L.) Borb. or *Taeniatherum* ancestor (Chen et al. 1998; Mahelka et al. 2011, 2013; Wang et al. 2015; Divashuk et al. 2016). The consensus of these studies is that the diploid ancestors of *Th. intermedium* originated from *Pseudoroegneria* (**St** genome), *Thinopyrum* (**J** genome), and possibly *Dasypyrum* (**V** genome). Moreover, evidence also suggests that the maternally inherited chloroplast genome of *Th. intermedium* originates from a *Pseudoroegneria* ancestor such as *Pseudoroegneria stipifolia* (Trautv.) Á. Löve (Chen et al. 2020), which is native to Central European Russia, Krymia, North Caucasus, South European Russia, Transcaucasus, and Ukraine (POWO 2021). The **St** genome of *Pseudoroegneria* is present in many, if not most, polyploid perennial Triticeae species, often as the maternal ancestor (Ceoloni et al. 2015). In any case, *Pseudoroegneria*, *Thinopyrum*, and *Dasypyrum* are all closely related to each other and more related to *Aegilops*, *Triticum*, and *Secale* than other Triticeae genera including *Agropyron*, *Australopyrum*, *Eremopyrum*, *Hordeum*, or *Psathyrostachys* (Hsiao et al. 1995; Kellogg et al. 1996; Bernhardt et al. 2017; Chen et al. 2020).

The genus *Thinopyrum* includes the two most important perennial Triticeae species in the context of wheat (*Triticum aestivum*) improvement, *Th. intermedium* and decaploid ($2n = 10x = 70$) *Thinopyrum ponticum*, [(Podp.) Barkw. and D.R. Dewey (syn. *Agropyron elongatum*)] which readily hybridize with wheat (Tsitsin 1965; Dewey 1984; Li and Wang 2009) and other species in the secondary or tertiary gene pool of wheat (Harlan and de Wet 1971; Ceoloni et al. 2014, 2015; Chaudhary et al. 2014). It is possible that polyploid species of *Thinopyrum* hybridize with wheat easier than their diploid ancestors because they meet

endosperm genic balance number requirements (Johnston et al. 1980), a phenomenon observed in other polyploid Triticeae (Larson et al. 2017). The earliest efforts to utilize *Thinopyrum* for wheat improvement aimed to transfer perenniality genes into wheat (Tsitsin 1965). Transfer of perenniality from *Thinopyrum* to wheat has been challenging (Ceoloni et al. 2015), but substantial progress has been made (Cui et al. 2018; Hayes et al. 2018). Greater successes have been achieved using *Thinopyrum* as a source of disease resistance (Ceoloni et al. 2014, 2015; Chaudhary et al. 2014) including Fusarium head blight (FHB) (Oliver et al. 2005; Li and Wang 2009; Wang et al. 2020), barley yellow dwarf virus (BYDV) (Banks et al. 1995; Ayala-Navarrete et al. 2009), salt tolerance (Wang et al. 2003a; Colmer et al. 2006; Mullan et al. 2009), and even yield (Ceoloni et al. 2014; Kuzmanović et al. 2014). Interestingly, it was shown that one of the FHB genes (*Fhb7*) used in wheat breeding is thought to have originated by horizontal gene transfer from an endophytic *Epichloë* fungus to the genome of perennial *Th. elongatum* (Wang et al. 2020).

B. History of use as a Forage Crop

In North America, utilization of IWG began between 1935 and 1950 (Hitchcock 1935). Introduction through collections is noted to have taken place in 1932 (Musil 1948) with the first named releases of ‘Ree’ and ‘Greenar’ in 1945 (Hanson 1972). By 1956, IWG was the most frequently used grass for mixtures with alfalfa (*Medicago sativa* L.) in the northern half of the United States (Schwendiman 1956). This use in hay production was due to its later flowering, allowing for high biomass production coupled with relatively high forage quality due to later maturation relative to other perennial grasses (Morrison 1972). IWG is still a component of high-yielding hay mixtures with alfalfa (Jefferson et al. 2011) but has yet to be adapted to the Southern Plains of the United States (Gillen and Berg 2005).

Recommended use of IWG was initially for both hay and pasture (range) (Oregon State University 1963). In Canadian environments, IWG has generally demonstrated suboptimal regrowth potential under grazing, which has led to its recommendation for hay use only in Saskatchewan (Heinrichs and Clark 1961). ‘Oahe’, however, was recommended for hay, pasture, and green manure in Washington state (Slinkard 1964). In Canada, releases concentrated on winter hardiness, drought tolerance, and seed production with ‘Clarke’ (Lawrence 1981) and seedling vigor and forage production with ‘Greenleaf’ (Wilson and Smoliak 1977). The most recent breeding efforts with this

species have led to the release of a pasture-specific cultivar Beefmaker (Vogel et al. 2005a) and a hayfield-specific cultivar Haymaker (Vogel et al. 2005b).

Production agronomy research for forage use has focused on nitrogen fertilization (Crowle 1966; Lawrence et al. 1970) and the use of IWG in mixtures (Kilcher and Heinrichs 1958; Campbell 1961). Dry matter production of IWG is comparable to or higher than other grasses (Heinrichs and Clark 1961; Darwent et al. 1987) in Canada, with the newest US cultivars also yielding as high as or greater than other released cultivars (Vogel et al. 2005a, b).

C. Above- and Below-Ground Biomass

Compared to annual grasses, perennial grass species such as IWG produce more root and shoot biomass (Kering et al. 2012; Sainju et al. 2017). In a single growing season, IWG above-ground biomass (shoot biomass hereafter) yields can exceed 10 tons ha^{-1} (Tautges et al. 2018). Below-ground biomass (root biomass hereafter) of IWG can exceed 23 tons ha^{-1} (3-yr average of 18 tons ha^{-1}) per year, which is 12–15 times higher than that of spring wheat (Sainju et al. 2017). IWG was used as a forage crop in the US Midwest, Northern Plains, and Intermountain regions after its introduction to the United States in the early 1900s (Hitchcock 1935; Moore et al. 1995). High root biomass has been helpful in controlling soil erosion in regions with substantial amounts (360–460 mm) of annual rainfall (Hybner and Jacobs 2012; Favre et al. 2019). The root biomass of IWG is equally beneficial in agricultural systems in reducing nutrient runoff and nutrient leaching to underground water (Culman et al. 2013; Jungers et al. 2019). Contemporary IWG populations that are being developed as grain varieties still yield considerable biomass with relatively high feed values. Therefore, IWG is a promising dual-use crop that could generate revenue from both grain and forage production in the same season (Jungers et al. 2017).

Both root and shoot biomass of IWG are affected by various factors such as the age of the plant stand, soil depth, fertilizer application, and seeding rate. In a study by Fernandez et al. (2020), a high planting density (145 seeds m^{-2}) consistently led to more shoot biomass production compared to low planting density (36 seeds m^{-2}). Biomass was typically the highest in fertilized (N) and non-fertilized monoculture regimes, whereas IWG bicultured with alfalfa produced less biomass (Tautges et al. 2018). Biomass yields in several IWG varieties were invariably the highest with a higher rate of N application (Jungers et al. 2017; Fernandez et al. 2020). Significant interactions between shoot biomass,

location, and plant age (year) have also been reported (Frahm et al. 2018; Tautges et al. 2018; Fernandez et al. 2020). Duchene et al. (2020) suggest that the resource-conservative strategy of IWG is seen in the first growing season and leads to deeper, denser root systems through higher below-ground biomass allocation. Limited research has been done to understand the effect of fertilizer treatment on IWG root biomass. A study by Sainju et al. (2017) found that root biomass at different soil depths was not significantly affected by fertilization rate. The same study also reported that root biomass declined as soil depth increased.

Within breeding populations (spaced plant selection nurseries), significant variation has been observed for shoot biomass (DeHaan et al. 2014; Zhang et al. 2016). In the University of Minnesota's first two breeding cycles (Cycles 1–2), Zhang et al. (2016) observed high genetic correlations between biomass and grain yield (correlation coefficient, $r = 0.75$) and biomass and plant height ($r = 0.54$). The same study also reported a high heritability estimate (0.69) for biomass. In a separate population created by crossing two genotypes from The Land Institute (TLI hereafter), strong correlations were observed between biomass and plant height ($r = 0.73$) and biomass and number of tillers per plant crown ($r = 0.91$) (Mortenson et al. 2019). This study also reported a high heritability estimate (0.73) for biomass, indicating that genetic factors are largely responsible for biomass yield differences in widely spaced IWG plants.

In recent years, IWG breeding programs have prioritized rapid improvement of domestication traits such as increased grain size, grain yield, free threshing, and reduced shattering. However, the strong and significant correlations observed between biomass and other important traits that are under direct selection such as higher grain yield and optimal plant height should still ensure indirect improvement of biomass. For example, in TLI's first three breeding cycles (Cycles 0–2), biomass production remained steady despite a strong selection pressure for grain yield and seed production traits (DeHaan et al. 2014). However, as selection for domestication traits continues to intensify, it may be necessary to periodically reevaluate shoot biomass, forage quality traits, and root biomass to maintain IWG's functionality as a dual-purpose grain crop.

D. Self-Incompatibility

Self-incompatibility (SI) is a common feature in grass (Poaceae) plants (Baumann and Langridge 2008) that facilitates outcrossing rather than inbreeding (Newbigin et al. 1993). In many grasses, this system is controlled by a gametophytic, two loci (S and Z) system (Baumann and Langridge 2008). While the molecular mechanisms of SI have not been fully

elucidated (Thorogood et al. 2017), research has shown that the *S* locus is controlled by a *domain-of-unknown-function* (*DUF*) gene (Manzanares et al. 2016) and the *Z* loci is either controlled by another *DUF247* gene (Shinozuka et al. 2010) or a *ubiquitin-specific protease* (*USP*) gene (Hackauf and Wehling 2005). The *S* and *Z* system was first reported (Lundqvist 1954) in rye (*Secale cereale* L.) and established in perennial ryegrass (*Lolium perenne*) by Cornish et al. (1979). Although much work has been conducted to understand SI, most of the studied systems involve diploid plants, although SI can remain active in polyploids such as tetraploid rye (Lundqvist 1957; Canto et al. 2020). While there has been some speculation that increased ploidy levels would reduce SI activity, currently little evidence exists to support this hypothesis (Mable 2004).

In IWG, Dewey (1978) reported varying levels of SI. Of 247 selfed plants, an average of 5.7 seeds per spike was obtained, which was 16% of cross-pollinated fertility. Further work by Jensen et al. (1990) showed that IWG had variable levels of SI, with a mean of 4.7 seeds per spike from 128 selfed plants. Recent work by Altendorf et al. (2021c) found an average of 6.3% (74 of 1168) genets to be selfed progeny in a nested association mapping (NAM) population with self-pollination ranging from 0 to 15.9% within 10 different families. Genet refers to a plant with a single genetic composition (Zhang et al. 2016). One investigation of SI in IWG used paternity analysis and TLI breeding material to evaluate favorable crosses and found 3.9% (15 of 380) progeny from direct crosses as selfed progeny (Crain et al. 2020b). This work showed that observed progeny combinations were not random, and the most significant genome-wide association marker for this study was located within 5.7 MB of the *S-DUF247* gene located on chromosome 1 of IWG.

Though not studied directly, SI has been implicated in a variety of quantitative trait locus (QTL) and genetic studies. Kantarski et al. (2017) noted that the most severe segregation distortion of seven full-sib families occurred on chromosomes 1–6. These chromosomes are homeologous to perennial ryegrass chromosomes 1 and 2 that have putative SI genes (Shinozuka et al. 2010; Manzanares et al. 2016; Thorogood et al. 2017). In mapping studies, Zhang et al. (2017), Larson et al. (2019), and Crain et al. (2022) found markers associated with seed size and seed fertility traits located near *S* loci on IWG chromosome 1. A QTL affecting seed length on chromosome 1 was identified by Bajgain et al. (2019a) that was near the known *S* loci as well. Larson et al. (2019) also found significant QTL for seed size, fertility, and yield on chromosome 6 near a known *Z* locus. While these studies were not specifically evaluating SI, work by Studer et al. (2008) in perennial ryegrass showed that SI could impact seed size and fertility, suggesting that the IWG results are likely being influenced by active SI loci.

The mechanism of SI in IWG is not completely characterized and how SI may act within and among the three subgenomes is unknown. SI poses several challenges for breeding IWG. First, it may limit desired breeding combinations. Since IWG shows a range of SI activity, it may be possible to overcome this through targeted crosses, although it will be more resource-intensive than random mating (Crain et al. 2020b). Second, SI genes could influence many agronomic traits, and these interactions may increase the complexity and make breeding more challenging, given that either SI could influence these traits or are closely linked to these agronomic regions. As IWG breeding advances, understanding and utilizing SI will be essential to unlocking its potential as a perennial grain.

E. Heading, Anthesis, and Height

Reports of anthesis timing in IWG in the northern hemisphere suggest that it generally occurs between June 6 and July 2 (Altendorf et al. 2021c; Duchene et al. 2021). IWG has a vernalization requirement, and flowering time is driven by photoperiod sensitivity, length of vernalization, and growing degree days accumulated (Duchene et al. 2021). Modeling IWG growth stages, especially anthesis, is important for determining the timing of management practices and optimal harvesting (Jungers et al. 2018; Duchene et al. 2020). In the context of breeding, knowledge of the genetic control of flowering is important to optimize cultivar development for new environments. Evidence from correlation analyses suggests early flowering is sometimes associated with increases in yield and fertility, e.g. seeds per floret, seeds per spikelet, and seeds per inflorescence (Larson et al. 2019; Altendorf et al. 2021a). In an evaluation of spaced plants in MN and KS using structural equation modeling, maturity timing did not always significantly affect yield, as results depended on the environment and year. When it was significant, it played a somewhat limited role relative to other yield component traits (Altendorf et al. 2021a). One QTL mapping study evaluated the genetic control of flowering time (measured as Zadoks maturity) and determined flowering time was complex, with QTL across 10 different chromosomes (Larson et al. 2019). Another study found a similar result, where QTL were identified on 11 chromosomes, with effects that varied across environments and depended on how the trait was measured (as either spike emergence or anthesis) (Altendorf et al. 2021c). These mapping studies have identified several candidate orthologs from related species in IWG that influence flowering time, including *Ppd-H1*, several genes from the *Constans* family, and *PhyB*.

Plant height in IWG ranges from 90 to 160 cm in cultivated nurseries (Zhang et al. 2016; Jungers et al. 2017; Jungers et al. 2018; Larson et al. 2019; Mortenson et al. 2019; Bajgain et al. 2020b; Crain et al. 2021a). Although reduced plant height might be desirable for IWG grain varieties, studies indicate that plant height is positively correlated with grain yield per plant (Zhang et al. 2016; Cattani and Asselin 2018) and grain yield per spike (Larson et al. 2019; Altendorf et al. 2021a). In comparison with eight other populations, the first Kernza IWG perennial grain cultivar, MN-Clearwater, had equal or higher grain yield and equal or lower plant height (113 cm) and lodging (Bajgain et al. 2020b). Plant height variation shows relatively high heritability compared to other traits (Zhang et al. 2016; Larson et al. 2019; Altendorf et al. 2021a). Genetic studies have indicated that height in IWG is a complex trait with multiple small-effect QTL, some of which are shared with yield component traits. In a full-sib family of 266 F_1 progenies, eight QTL each were detected for height and seed yield per inflorescence, yet none of these QTL were overlapping (Larson et al. 2019). Genetic studies in a NAM population of 1,168 F_1 progenies derived from 10 donor parents and one common parent detected two plant height QTLs, two floret site utilization (FSU) QTL, and three thousand-grain-weight QTL; one of the plant height and FSU QTL was associated with the same marker (Altendorf et al. 2021a). Four to six QTL influencing the number spikes per plant were also detected in the biparental (Larson et al. 2019) and NAM (Altendorf et al. 2021a) populations, respectively. Two of the four QTL for spike number overlapped with plant height QTL in the biparental population and one of the six QTL for spike number was also associated with one of the same markers influencing plant height and FSU in the NAM population. The number of spikes was the most important yield component contributing to yield per plant in the NAM population (Altendorf et al. 2021a). Thus, there is some evidence that some plant height QTL may affect yield traits, despite this not being true for most of the plant height QTL. In any case, the optimal plant height in IWG may vary depending on other factors related to growing conditions, management practice, utilization of forage, lodging potential, and ecosystem services.

F. Seed Properties

As with other monocots, IWG seeds display a wide range of colors, from light amber to deep purple. Preliminary research and observations from food scientists and plant breeders indicate that IWG populations being selected for grain have soft endosperm (Tyl et al. 2019; Bajgain

et al. 2020b), although diversity for grain hardness is likely present in the species. IWG seeds currently have an average grain weight of 6 mg kernel⁻¹ in cultivars grown in dense stands (Bajgain et al. 2020b) and up to 15 mg kernel⁻¹ in space-planted single plants (Zhang et al. 2016; Bajgain et al. 2019a; Bajgain and Anderson 2021). These values are substantially smaller than mainstream domesticated cereal grains such as wheat, barley, and rye, each of which weigh approximately 35 mg kernel⁻¹ (Smith et al. 2013; Wilde et al. 2018; Anderson et al. 2019). Seed length in IWG often exceeds that of wheat or barley, yet width is smaller. Grain size and weight in IWG are often significantly positively correlated with other yield component traits such as spike and spikelet characteristics and grain yield (Larson et al. 2019; Bajgain et al. 2019a). Broad-sense heritability estimates for seed weight and size are typically high, with values observed up to 0.91 (Zhang et al. 2016; Larson et al. 2019; Bajgain et al. 2019a; Bajgain and Anderson 2021). This indicates that seed characteristics in IWG are largely under the influence of genetic factors.

Seed size was a key trait targeted during crop domestication (Fuller 2007; Meyer and Purugganan 2013). It is an important agronomic trait in most crops and is one of the determining factors of grain yield. IWG seed weight in the first breeding population at TLI was 4 mg on average (DeHaan et al. 2014) with rapid improvement since then. For example, the first University of Minnesota (UMN) IWG breeding population was derived from the third selection cycle at TLI and had seed weight measurements ranging from 3.3 to 13.3 mg with a population mean of 8.3 mg (Zhang et al. 2016). Cycle 4, the recently completed (2018–2020) breeding cycle at UMN, had seed weight ranging from 3.1 to 15.4 mg (Bajgain and Anderson 2021). Seed size in IWG, similar to other perennial forage grasses, is influenced by a seed's inflorescence position. A recent investigation by Heineck et al. (2022) found maximum spikelet yield, and the largest seed size was almost always observed in the basal portion of a spike where seeds were nearly 21% larger than those on the distal end. The same study also found that seed weight per spike in IWG is strongly influenced by the growing environment (in MN, WI, or NY).

G. Agronomic Performance

1. Agronomics of Seed Production

IWG has been a relatively under-researched crop, especially in its early years of production. The breeding and use of IWG have historically been as a forage crop and coupled with its relatively limited use, provided for little agronomic work on seed production. In Canada, research in

Saskatchewan accounts for the majority of the published literature and addresses the areas of breeding (Heinrichs 1953; Knowles 1977), forage productivity (Heinrichs and Clark 1961; Lawrence and Ashford 1969), and seed productivity (Crowle 1966; Lawrence and Ashford 1969). A literature review of seed production was carried out by the Saskatchewan Forage Council (Kruger 1997), and the following section will highlight more recent research contributions.

In the United States, the USDA-NRCS in Mandan, North Dakota, invested in breeding research (Berdahl et al. 1992, 1993; Berdahl and Baker 1997) and related seed production research (Berdahl and Frank 1998). With growing interest in the use of IWG as a perennial grain, both breeding (DeHaan et al. 2014; Zhang et al. 2016; Bajgain et al. 2020b) and agronomic-related investigations (Jungers et al. 2017; Duchene et al. 2021) have expanded. Implications of Duchene et al. (2021) are that agronomic recommendations for grain production will be defined by the growth environment in conjunction with germplasm developed for the various regions of potential production. Cultivar development (see Section IV) will provide a more stable genetic background upon which to develop agronomic practices for sustainable grain production.

2. Agronomic Research

A seeding depth from 2.5 to 5 cm (1–2 in) was shown to be effective for IWG establishment, and while emergence from deeper in the soil showed differences between lines, this was not related to seed size (Lawrence 1957). Row spacing was demonstrated to impact seed productivity in IWG, with 90-cm spacings averaging greater seed yields than 30-cm row spacings across five production years (Crowle 1966). A seed production trial in central Alberta, Canada (55° N), found that 16–60-cm row spacings produced statistically similar seed yields, with 16-cm producing significantly higher seed yields than 82- and 104-cm row spacings (Darwent et al. 1987). Recent work in Minnesota (United States) has shown 30- and 60-cm row spacings provided higher seed yields than 15-cm row spacings in some years (Hunter et al. 2020a), while straw yield at grain harvest was found to be more economically valuable than IWG harvested as hay, from the 15- and 30-cm row spacings, due in large part to the greater dry matter yields (Hunter et al. 2020b), thus adding value to seed production.

Fertilizer studies have shown that nitrogen applications can increase forage yields (Lawrence and Ashford 1969). A study in Lethbridge, Alberta, Canada, indicated that forage yield of IWG increased with nitrogen fertilizations, with highest dry matter productivity

reported between 440 and 580 kg N ha⁻¹ (Lutwick and Smith 1979). For seed production, irrigation with added nitrogen fertility (56 kg ha⁻¹ in the first two weeks of September) increased seed productivity in the final three years of irrigated production, while not impacting it in the first two reproductive years or under rain-fed production (Crowle 1966). The author noted that between-row cultivation was practiced on the wider row spacing, which may have influenced the fertility response. A similar response to spring-applied levels of nitrogen (45–67 kg N ha⁻¹) was seen by Black and Reitz (1969) in Montana, United States. Optimal timing of fertility applications is likely associated with the growing region (Duchene et al. 2021). More recent agronomic results have shown spring applications enhancing seed yield but with decreasing seed yields across years (Jungers et al. 2017). Postharvest regrowth period and length of winter may restrict fall regrowth time and influence the period available for vernalization. Selection under the agronomic conditions that will likely be employed by producers is critical to enhancing the success of selection for grain yield in each production region. Recent fertility work in Minnesota (United States) did not find fertilizer applications to greatly impact seed production and also found that trinexapac-ethyl, a growth regulator, was only effective in increasing seed yield by reducing lodging under conditions that were favorable to lodging (Frahm et al. 2018).

Intercropping with a legume has been investigated under organic production conditions to supply nitrogen to the IWG seed crop. Nitrogen benefit has been shown at a young growth stage (Li et al. 2020), although the competitiveness of the legume crop with IWG needs to be accounted for when designing a mixture of species with more aggressive species (e.g. *Medicago sativa*) lowering seed yields (Dick et al. 2018). Intercropping with red clover has been demonstrated to increase the forage quality of the IWG (Favre et al. 2019). It has been suggested that selection for bi- or polyculture use should take place under those conditions (Weiner et al. 2010).

Forage production is not indicated as being negatively impacted by selection for seed yield (Knowles 1977; Jungers et al. 2017). However, optimal N fertility levels for seed production will be lower than optimal N for forage production due to the negative effects of lodging (Jungers et al. 2017). Additionally, fertility may impact competitive interactions, with fertilization of IWG intercropped with cheatgrass (*Bromus tectorum* L.) leading to a reduction in IWG ground cover, with grazing further exacerbating reduction in IWG cover (Kay and Evans 1965). This was

likely influenced by the timing of reproductive tiller elongation in the spring (Duchene et al. 2021).

III. HISTORY OF IWG BREEDING

A. Breeding of Intermediate Wheatgrass for Forage and Reclamation

A collection trip to the native range of IWG in 1934 provided valuable materials for the development of IWG as a forage crop for the western portions of North America (Anonymous 1970). In 1953, improved germplasm was not yet widely available, but Heinrichs (1953) was investigating the ability of IWG to self-pollinate and the variability of progeny under different breeding systems.

Table 3.1 Cultivars released, germplasm registered, vegetatively propagated parental materials registered, and other lines mentioned of intermediate wheatgrass (including pubescent wheatgrass), their origin and breeding methods.

Name and location ^a	Year released	Origin	Breeding method	Source
Cultivars registered				
Ree – South Dakota	1945	PI 98568	No selection	Hanson (1972)
Greenar – Washington	1945	PI 98568	Selection out of PI 98,568	Hein (1957)
Amur – New Mexico	1952	PI 131532	Mass selection	Hanson (1972)
Topar – Washington, Idaho, Oregon	1953	PI 107330	Mass selection	Schwendiman (1972)
Oahe – South Dakota	1961	PI 98568	Polycross, recurrent selection, 4 clone synthetic	(Ross 1963)
Chief – Saskatchewan	1961	PI 98568 USSR introductions	Mass selection, progeny testing, 5 lines	Hanson (1972)
Luna – New Mexico	1963	USSR, Turkey	Mass selection	Niner (1967)
Greenleaf – Alberta	1966	North Dakota, Washington	Synthetic – 12 lines	Wilson and Smoliak (1977)
Tegmar – Idaho and Washington	1968	PI 109219	Open-pollinated selections	Hanson (1972)
Slate – Nebraska	1969	PI 98568 PI 131532	Clonal selection with progeny testing (57 clones)	Newell (1974)

Table 3.1 (Continued)

Name and location ^a	Year released	Origin	Breeding method	Source
Clarke – Saskatchewan	1980	Colorado, USSR, Saskatchewan	20 clone synthetic, recurrent selection	Lawrence (1981)
Rush	1994	Germany	Mass selection	USDA-SCS and Idaho Ag Experimental Station (1994)
Reliant – North Dakota	1991	Base population of 24 cv.'s and experimental strains	Initial selections were followed with half-sib progeny testing. Synthetic – six clones.	Berdahl et al. (1992)
Manska – North Dakota	1992	PI 116252 (Mandan 759)	Polycross, 116 line synthetic	Berdahl et al. (1993)
Beefmaker	2003	PI 345586 PI 273733 PI 273732 PI 315353 PI 315067 PI 155355	Polycross, modified restricted recurrent phenotypic selection, 120 clones	Vogel et al. (2005a)
Haymaker – Nebraska	2003	PI 440015 PI 440008 PI 440011 Slate	Synthetic, 54 lines	Vogel et al. (2005b)
Germplasm registered				
SC81E – South Carolina	No	50 PI's 3 cultivars	Mass selection, early flowering	Rice (1983)
SC81L – South Carolina	No	50 PI's 3 cultivars	Mass selection, late flowering	Rice (1983)
Trigo – California	No	PI 107328	Clonal selection	Hanson (1972)
Montana-1	No	<i>Triticum turgidum</i> x <i>Agropyron intermedium</i>	Male sterile line Original cross-followed by backcrossing, last backcross to Oahe	Schulz-Schaeffer (1978)
Vegetatively Propagated Parental Lines registered				
MT-6 Montana	No	PI 440018	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-7 Montana	No	PI 268118	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)

(Continued)

Table 3.1 (Continued)

Name and location ^a	Year released	Origin	Breeding method	Source
MT-8 Montana	No	PI 273732	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-9 Montana	No	PI 273733	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-10 Montana	No	PI 440004	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-11 Montana	No	PI 316122	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-12 Montana	No	PI 228274	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-13 Montana	No	PI 401017	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-14 Montana	No	PI 173630	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
MT-15 Montana	No	PI 369174	Clonal selection based on polycross progeny testing.	Schulz-Schaeffer and Ditterline (1991)
Other Materials Mentioned				
Nebraska 50	1950	PI 98568	No selection	Newell (1974)
Mandan 759 – North Dakota	No	PI 116252	Progeny tested	Hanson (1972)
Mandan 1284	No	PI 98568	No selection	Hanson (1972)
A-12496 – New Mexico and Utah	No	PI 98568	No selection	Hanson (1972)

^a Cultivar names in **bold** font were also selected for seed production.

The first widely noted IWG varietal release in North America, “Ree,” was released by South Dakota State University (SDSU) (Table 3.1), primarily for use in pastures due to its highly rhizomatous nature (Franzke 1945). The early materials tested in western Canada indicated

that IWG did not tolerate repeated cutting and lacked acceptable winter hardiness as compared to other potential forage grasses such as crested wheatgrass (*Agropyron cristatum* L.), streambank wheatgrass (*Agropyron riparium* Scribn. and Smith, currently *Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould ssp. *lanceolatus*), green speargrass (*Stipa viridula* Trin., currently *Nassella viridula* (Trin.) Barkworth), and Russian wild ryegrass (*Elymus junceus* Host Beauv., currently *Psathyrostachys juncea* (Fisch.) Nevski) (Heinrichs and Clark 1961). Introduction of these materials to N. America was in part for reclamation of farmlands and pastures that were depleted and abandoned due to drought (Lawrence 1981). The initial end uses were forage, pasture, and revegetation (Hitchcock 1935). Breeding for seed productivity was a recurrent theme in a number of the cultivars, in part due to demand for seed for reclamation use (Ross 1963; Wilson and Smoliak 1977; Lawrence 1981; Berdahl et al. 1993). Forage quality traits such as *in vitro* dry matter digestibility (IVDMD) can likely be improved through selection in IWG (Berdahl et al. 1994). Lignin content was shown to be different among lines (Sosulski et al. 1960). Many of the cultivars were selected for forage yield, e.g. Clarke (Lawrence 1981), Reliant (Berdahl et al. 1992), and Haymaker (Vogel et al. 2005b), with only Manska (Berdahl et al. 1993) and Beefmaker (Vogel et al. 2005a) mentioning forage quality traits. The use of extensive progeny testing was recommended for complex traits such as forage yield and quality (Berdahl and Baker 1997).

The breeding methods employed in the early development of IWG populations were largely mass selection. The development of 'Ree' was from a seed increase of PI 95628 harvested at Fort Collins, CO, obtained and planted in 1937 at Brookings, SD, where it was evaluated and then released as 'Ree' (Franzke 1945). 'Chief' IWG was bred by Agriculture and Agri-Food Canada (AAFC) and licensed in 1961 (Elliott and Bolton 1970). The breeding was through mass selection for plants with high seed yields and good seed quality, with five lines being selected after five years of testing (Elliott and Bolton 1970). Hanson (1972) describes Nebraska 50 arising out of PI 98568, similar to 'Ree,' while 'Amur' IWG arose from materials brought in from Manchuria, China, as PI 131532 and was selected for use in New Mexico (Hanson 1972; Wills et al. 1998).

'Oahe' (Ross 1963) was the first cultivar developed using a new, more elaborate breeding method for outcrossing species, the poly-cross method (Tysdal and Crandall 1948; Fransden 1952; Wellensiek 1952). 'Clark' IWG, a 20-clone synthetic cultivar, was developed through recurrent selection at the Canada Department of Agriculture (now AAFC) Swift Current, with selection for numerous characteristics including winter tolerance, forage yield, seed yield, and drought

tolerance and was recommended for use as both hay and pasture (Lawrence 1981).

Other plant materials have been developed and described, including germplasms (Hanson 1972; Rice 1983) and a male sterile line (Schulz-Schaeffer 1978). Vegetatively propagated parental lines have also been developed (Schulz-Schaeffer and Ditterline 1991), although their subsequent use is not clear.

IWG has been used as forage throughout its native range, with landraces and improved varieties developed in many countries. Detailed information about varieties developed outside of North America is not available in English journals to our knowledge. To assess the extent of variety development with the species, we surveyed records in the EURISCO Catalog (Weise et al. 2017, <http://eurisco.ecpgr.org>). Improved varieties or landraces were recorded from Bolivia, Italy, Japan, Kazakhstan, Russia, and Ukraine, with the largest number of varieties originating from Kazakhstan. In Russia, six varieties have been released between 1959 and 2021 (A. Morgunov, pers. comm. 2021).

B. Early Efforts to Breed Intermediate Wheatgrass for use as a Perennial Grain Crop

The first effort to develop IWG as a perennial grain was initiated at the Rodale Research Center (RRC) in Pennsylvania, United States (Wagoner 1990). The rationale for undertaking this effort was to protect soil from erosion, reduce mechanical operations to save fuel and labor, reduce costs to farmers for seed and planting, improve soil quality, and potentially enhance wildlife habitat. Beginning in 1983, nearly 100 perennial grass species were evaluated by the RRC (Wagoner 1990). The following criteria were used to identify promising species for domestication as a perennial grain: vigorous perenniality, easy threshing, larger seed, synchronous maturity, shatter resistance, lodging resistance, seed heads above foliage for easy harvest, and potential for mechanical harvest. Based on this evaluation, IWG was selected for domestication (Figure 3.3).

The RRC endeavored to assemble a diverse set of IWG germplasm, obtaining 250 accessions, with about half originating from the USDA's Plant Introduction Office in Pullman, WA (Wagoner 1990). Collections originated largely from the former Soviet Union, Iran, Turkey, and other countries of the eastern Mediterranean. Evaluation of the assembled germplasm began in Pennsylvania in 1987. Basic morphological and phenological differences among accessions were small, but seed

traits such as mass per seed, fertility, and threshability were substantial (Wagoner 1990). In total, 22 accessions were identified as exhibiting favorable characteristics and 19 of these originated from the former Soviet Union. These accessions had 100 seed weight ≥ 0.5 g, seed-set rating $\geq 45\%$, 10 spike yield ≥ 2 g, and free threshing ability $\geq 75\%$ (Wagoner 1989). The conclusion was that former Soviet Union germplasm might be most useful in breeding for grain production in Pennsylvania. After two growing seasons with evaluation of a large number of traits, twenty “accessions with favorable seed yield characteristics” were selected in the fall of 1989 and intermated in a greenhouse during 1989–1990 (Wagoner et al. 1996). Unfortunately, records of the particular accessions used in crosses are not available. Seed from the intermating was used to establish a space-planted nursery at the Big Flats Plant Materials Center (BFPMC) in New York.

The methods used in the BFPMC selection program were outlined by Wagoner et al. (1996) and additional details were received from a personal account of the methods from Dr. Martin van der Grinten who led the effort in collaboration with the RRC. What follows is a summary from these sources. The plants were evaluated for four growing seasons, 1991 to 1994. The 360-plant nursery was divided into 14 blocks (to reduce the impact of spatial heterogeneity). Initially, visual selection was used to identify the most promising 5 plants from each of the 14 blocks. The visually selected plants were harvested to measure seed production traits such as yield per spike, seeds per plant, seed mass, and fertility. Ultimately, 11 plants were selected, all of which were among the top performers in 3 out of the 4 years of evaluation. Three additional accessions – 937, 960, and 961 – were selected based on evaluations at the RRC. Unfortunately, these cannot be traced back to original collections as they were obtained as breeding lines from Dr. John Berdahl of the Northern Great Plains Research Center in Mandan, ND (Wagoner 1993). In the first polycross nursery established in 1995 at the BFPMC, 14 individual genets were intermated. As later breeding programs are derived almost exclusively from this population, we can conclude that the effective population size for modern IWG breeding programs for grain is about 14.

A second cycle of selection was initiated at the BFPMC when a 400 spaced plant Cycle 2 nursery was established in August 1997. The second cycle followed a protocol similar to the first, as the nursery was divided into 16 blocks of 25 plants. In each of the four years, the most promising five plants were selected from each block based on visual evaluation. Spikes were harvested from the selected plants and selections were made for yield traits such as yield per spike, seed set, and seed mass. Seed harvested from individuals that were selected based on

performance over four years was distributed to TLI, which included this material along with the BFPMC Cycle 1 seed in establishing their first cycle of selection.

IV. BREEDING METHODOLOGIES BY PROGRAM

A. The Land Institute

1. Breeding With Phenotypic Selection, TLI-Cycles 1-6

The initial breeding cycles in the IWG domestication program at TLI have been described in detail previously (DeHaan et al. 2018). Here, we will summarize methods used and outcomes observed. Methods changed substantially over the breeding cycles as resources available for the program grew and results pointed to enhanced efficiency with different methodologies.

TLI-Cycle 1 was initiated in fall 2003 using three ramets from each of 961 genets, established in a randomized complete block design at 0.91-m grid spacing (Figure 3.3). The source of these genets was from the BFPMC breeding program, cycles one and two. In the first year, the nursery was mowed after heading to prevent seed set and allow plants to mature for a second year. An index of seed mass and grain yield per spike in the second year was used to select 50 individuals for random intermating in an isolation plot the following season.

Analysis of TLI-Cycle 1 data suggested that multiple ramets of each genet were not an optimal use of resources due to high genetic variance and high heritability of relevant traits. Therefore, TLI-Cycle2 was established with 2,466 genets without replication. Plant spacing was again on a 0.91-m grid. Evaluation of reproductive traits was delayed until the second season of growth in 2008. Selections were made based on an index of total seed yield per plant, yield per spike, percent naked seed, seed mass, and foliar disease rating. Beginning in TLI-Cycle 2 and all later TLI cycles, selections were based on predicted values using all available pedigree data plus an $AR1 \times AR1$ spatial residual variance model using the software ASREML (Gilmour et al. 1995). The 50 selected genets were dug from the field, vernalized in a growth chamber, and intermated over the winter in a greenhouse.

TLI-Cycle 3 was established by transplanting 4,800 seedlings to the field on a 0.91-m grid in May 2009 at two locations (one irrigated and one unirrigated). Spring establishment was intended to reduce the breeding cycle from three years to two by allowing plants to mature and produce reliable data in the first summer of reproduction (2010). This method resulted in well-established plants and data was collected

in the first summer of flowering. In this cycle, selections were made manually on an array of traits, rather than on a simple index as in previous cycles. Traits measured and considered in selection included seed yield per spike, seed mass, percent naked seed, short stature, low shattering, and floret site utilization (FSU) (percent seed set). In total, 94 genets were selected, dug from the field, vernalized, and intermated in the greenhouse. For this cycle, controlled crossing was used in the greenhouse to produce pairwise crosses. Attempts were made to cross plants with complementary traits and to mate individuals with outstanding values for a single trait.

After the success of spring establishment in TLI-Cycle 3, TLI-Cycle 4 was transplanted to the field in spring 2011. However, a severe drought killed most of the plants during the summer. Therefore, the field was reestablished in the fall using remnant seed to obtain a population of 13,983 spaced plants. Beginning in this cycle, establishment of new breeding cycles was always in the fall, followed by measurement of traits and selections the following summer (in the first year of growth). Experimentation demonstrated that good heritability of yield per spike was possible in the first year, so long as care was taken to harvest only larger and earlier maturing spikes from each plant (DeHaan, unpublished data). Traits such as shatter resistance and free threshing were found to have consistent values across years. Based on percent naked seed, yield per spike, and seed mass, 71 plants were selected. As in TLI-Cycle 3, pairwise intermating was performed in the greenhouse.

TLI-Cycle 5 consisted of 11,042 spaced plants established in one location. Data collection was performed in the first summer of flowering, 2014. Selections were based on seed yield per spike, shatter resistance, seed mass, and percent naked seed. In total, 66 plants were selected, vernalized, and used for intermating and pairwise crossing in the greenhouse.

In fall of 2015, the TLI-Cycle 6 nursery was established with 20,360 spaced plants at one field location. Due to the large number of traits evaluated and the large number of plants in the nursery, an approach was developed to reduce the number of plants measured. The full set of traits was measured on a subset of plants representing all the full- and half-sib families in the nursery. After analyzing this data, additional measurements were only made on the most promising families in the entire nursery. Selections in this cycle were based on yield per spike, FSU, growth stage, seed mass, percent naked seed, erect stem angle, seed width, seed area, seed plumpness, short height, peduncle diameter, stem strength, and low shattering. In total, 86 plants were selected, vernalized, and used for intermating in the greenhouse.

The spaced plant environment with low competition is very different from the closely seeded stands typically used in production. Of primary concern is whether selection performed on yield per spike in spaced plants will translate into higher yield in dense stands. This question has been tested at several points using the TLI breeding materials. Initially, half-sib seed from TLI-Cycle 1 plants was used to establish seeded row plots. In this evaluation, strong correlations between the spaced plant and progeny row data (DeHaan, unpublished data) provided evidence for continuing the spaced plant approach. After two cycles of selection, remnant seed from TLI-Cycles 0, 1, and 2 was used to establish seeded sward experiments. In this evaluation, seed yield per area was found to have increased by 77% in two generations (DeHaan et al. 2014). By comparison, seed mass increased by only 23%, indicating that progress is expected to be slower for this trait.

In Section III.B, we noted that the foundational breeding program at the BFPMC, derived from only 14 genets, was the source material for the other programs described here. One exception to this limitation was an effort to enhance genetic diversity within TLI's program. From 2006 to 2009, 401 accessions from the USDA's National Genetic Resources Program were evaluated at TLI. Although all accessions were inferior to the populations developed by the BFPMC in terms of seed yield, promising variation was seen for seed size, early maturity, and free threshing ability. Individuals with outstanding performance for these traits were crossed with selected plants from TLI-Cycle 2. Because pedigrees were tracked in the breeding program where possible, looking at the pedigree records in the fifth selection cycle we could see that eight parents from the diverse set of accessions had progeny persisting in the breeding program. Therefore, within the TLI program after the fourth cycle, we expect the effective population size to be about 22 heterozygous individuals. Distribution of germplasm to the UMN and Manitoba programs began prior to the addition of these diverse materials (Figure 3.4).

2. Breeding With Genomic Selection, TLI-Cycles 7-10

Beginning in 2017, the TLI breeding program made a major shift in breeding strategy by fully adopting genomic selection (GS, Figure 3.5). The TLI-Cycle 6 material that was evaluated in 2016 and 2017 formed the initial training population. TLI-Cycle 7 was formed by randomly intermating phenotypically selected parents from TLI-Cycle 6. To develop a one-year breeding cycle, TLI-Cycle 7 genets were started in the greenhouse in late summer followed by genotyping and genomic prediction. This allowed the best genets, based on genomic estimated breeding values (GEBVs), to be moved to the greenhouse for intermating, and other genets to be transplanted in the field for phenotypic

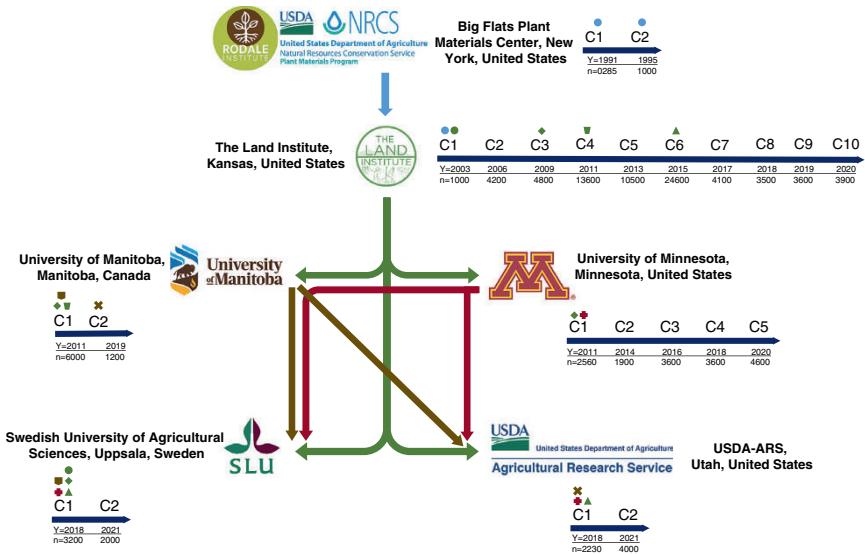


Fig. 3.4. Movement of intermediate wheatgrass elite germplasm among breeding programs. For each breeding program, “C1,” “C2,” etc., indicate the breeding/selection cycle. “Y” indicates the establishment year of the first population or selection cycle; succeeding years indicate the start of the next selection cycle. Population size per cycle is indicated by “n.” Colored arrow shows movement of IWG materials from institution A to institution B. Same-colored shapes/symbols indicate the origin materials received by institution B from institution A; e.g. the blue circle shows that Cycle 2 materials from the Big Flats Plant Materials Center became the starting (Cycle 1) materials at The Land Institute.

evaluation and model development (Crain et al. 2021b). This breeding scheme has been followed yearly since 2017, resulting in TLI-Cycle 10 selection in 2020 (Crain et al. 2021a). For each cycle, approximately 4,500 genets are started in the greenhouse in late summer. Tissue sampling for genotyping is conducted on approximately 4,000 genets each year with visual selection discarding poor performing seedlings. Genotyping-by-sequencing (GBS) using a two-enzyme system (Poland et al. 2012b) has been used to genotype and profile all genets followed by genomic prediction using the additive genomic best linear unbiased prediction. Approximately 100 genets are selected primarily based on predicted spike yield, free threshing, shattering, and seed mass to intermediate and form the subsequent generation, and these genets are referred to as the breeding population. Approximately 1,000 genets are selected to be field evaluated and are named the validation population. The validation population is transplanted in mid-October with phenotypic evaluation beginning in subsequent years (Crain et al. 2021a).

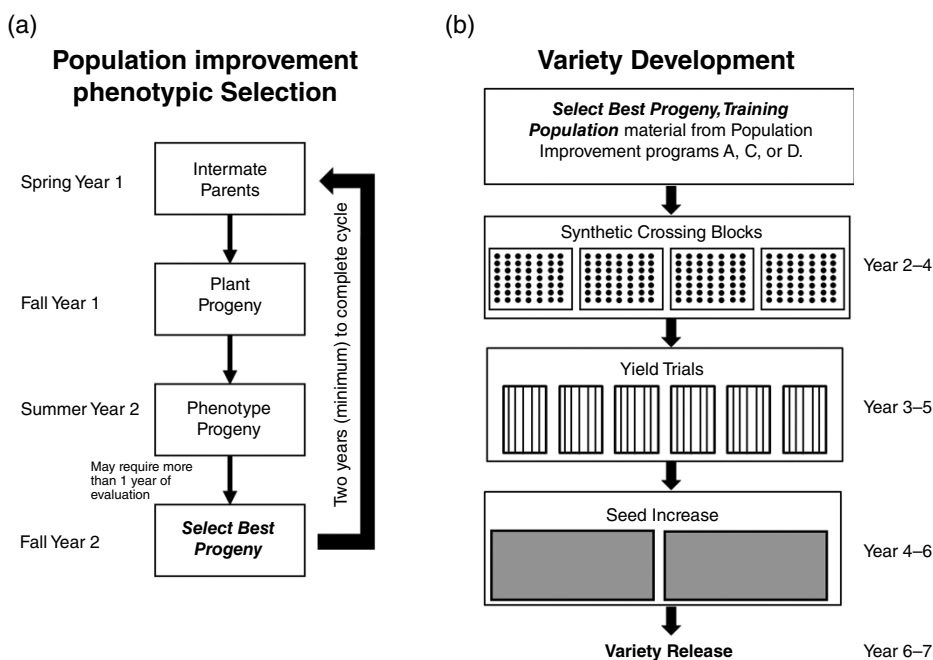


Fig. 3.5. Diagram of population improvement and cultivar development for intermediate wheatgrass (IWG). Panel (a) is a program using only phenotypic recurrent selection. Panel (b) shows a synthetic variety development processes using superior genotypes identified through population improvement (panels a, c, and d). Panel (c) summarizes The Land Institute single-year genomic selection (GS) breeding program (Crain et al. 2021b) and panel (d) shows the University of Minnesota GS breeding program Zhang et al. (2016). The major difference is prior generations represented by the circle are used to predict progeny in panel (c) for a one-year breeding cycle, whereas in panel (d), a combination of phenotypic and genomic selection allows two genetic recombinations (generations) to be completed in a two-year breeding cycle.

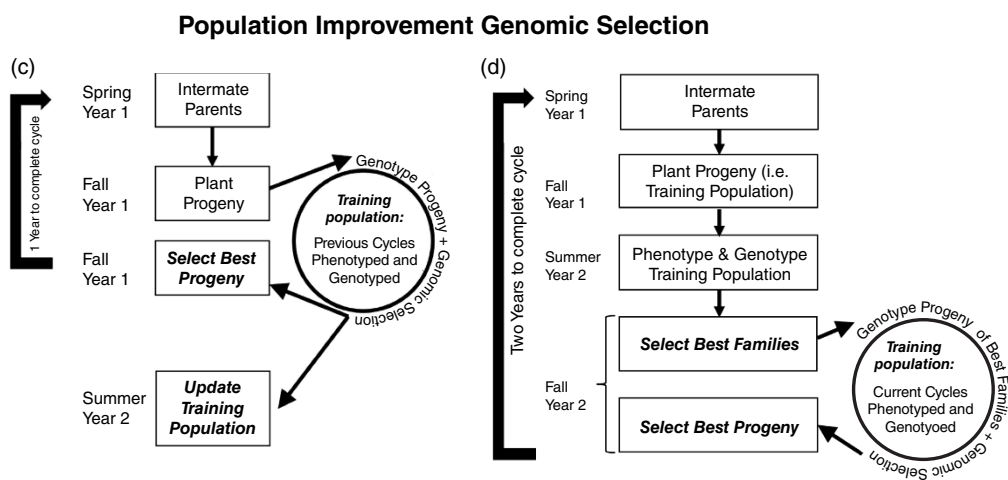


Fig. 3.5. (Continued)

The breeding population is grown in the greenhouse and cloned to form multiple ramets. Two groups of ramets are formed with vernalization of each group being about two weeks apart to allow for genetic recombinations of plants with different flowering times. Once vernalization is completed, the ramets are placed on greenhouse benches and during pollination the ramets are physically moved every few days. Oscillating fans are also used to aid in pollen dispersion in an effort to make as many random crosses as possible (Crain et al. 2020b). Unless a particular cross is desired, all crosses are made by random pollination, and work by Crain et al. (2020b) has demonstrated random crossing provides a greater diversity of crosses than can be achieved by targeted crosses. Seed is harvested from each plant, providing known maternal information, and the following cycle is initiated by starting 45–50 seedlings per maternal plant (Crain et al. 2021a). In an effort to prevent inbreeding and maintain wide genetic diversity, paternity is assigned using molecular markers at the same time that genomic predictions are made, allowing selection based on predicted trait value as well as pedigree information (Crain et al. 2020b).

In addition to forming the breeding population, much effort has been devoted to updating and evaluating the prediction model through the validation populations. Of the 1,000 genets that form the validation population, one half the genets are transplanted under irrigation and the other half placed in dryland conditions, providing potentially two different growing environments. All validation material is planted in single replication with no checks due to the challenge of cloning the large number of checks that would be needed. Genets are planted on a grid spacing at 0.91 m to allow for spatial correction of the data (Crain et al. 2021a, b). Phenotypic evaluation can be carried out for multiple years, even with genetic recombination (crossing) occurring each year. This has resulted in a validation population for GS that has overlapping generations being phenotyped each year with the goal that this will allow gains to be made on traits such as yield decline that may not reliably be estimated in the first year of evaluation (Crain et al. 2021a). Given the shift from phenotypic selection to GS, TLI has devoted much effort into evaluating breeding decisions to ensure that the results are desirable. One of the first analyses has been correlating the predicted phenotypes (on seedling) to measured phenotypic results (validation population). Across 46 traits, 37 traits had correlation $r > 0.3$ between predicted and observed values. For traits such as free threshing, shattering, and seed mass, these correlations ranged from 0.52 to 0.76 (Crain et al. 2021a). For spike yield, which is expected to have a large genotype-by-environment (G×E) interaction, correlations were

much lower, ranging from 0.02 to 0.27. These results achieved across TLI Cycles 7 and 8 indicated that GS models could accurately predict future plant performance.

Harnessing the perennial nature of IWG, the breeding population has been moved to the field and evaluated alongside the validation population after the first year of each cycle. This has allowed a determination of the selection differential, difference between the selected parents and population mean, that indicates breeding parents are indeed better for desired traits, with the selection differential often exceeding 10% (Crain et al. 2021a). Flexibility is a strong point of the GS program as the size, traits, and selection intensity can be altered to meet any current needs. For example, if a high performing genet is identified in the validation population, it can easily be brought into future breeding cycles while not limiting the number of genetic recombinations (generations) possible. Even though a pipelined breeding program has been developed, there are still many unknowns to the application of GS breeding. Currently, efforts are underway to develop the best ways to model data collected from multiple years on the same genet. While multiple cycles are evaluated during a single year, these cycles are not synchronous in stand-age and trials are planned to fully evaluate the genetic gain from TLI Cycles 7–10 in the future. While current breeding results are promising, accelerated efforts will be required to move Kernza into a mainstream crop. To this end, techniques to perform multiple breeding cycles in a single year are being explored.

Through the sixth breeding cycle, the TLI program involved evaluating large fields to make selections. After selections were made, these evaluation nurseries were maintained for several years and harvested to obtain seed for planting by farmers. The selection nursery from the sixth cycle was harvested repeatedly, and the seed has been widely grown by farmers, under the identifier “TLI-C5” because the seed is the result of five selection cycles by TLI. After moving to GS, the TLI program has transitioned to developing synthetic varieties using 5 to 10 clonally propagated parents. Four synthetic variety candidates are currently being evaluated in replicated trials, and seed increased from the most promising of these will soon be made available to producers.

Although to date, named varieties have not been released from the TLI breeding program, the Russian cultivar Sova was developed by selection out of TLI-Cycle 4 materials that were distributed to researchers in Omsk, Russia in 2014. Plants that were able to successfully overwinter in Western Siberia were used as parents of the new variety. Over three years, the variety yielded on average 910 kg ha⁻¹ grain and 7,100 kg ha⁻¹ hay per year (Shamanin et al. 2021). Furthermore, root length of the

variety was found to be 6.9 to 9.8 times greater than that of winter and spring wheat varieties.

B. University of Minnesota

The UMN began its IWG breeding program in 2011. A total of 2,560 seedlings from 66 mother plants (i.e. half-sib families) were obtained from TLI (Figure 3.4). In the last 10 years, the UMN IWG breeding program has completed four selection cycles and released their first Kernza perennial grain cultivar, MN-Clearwater (Bajgain et al. 2020b). Each breeding cycle is two years long and occasionally the breeding nursery is carried into year 3 or 4 for additional evaluations.

The IWG breeding scheme at UMN can broadly be divided into two main categories: (1) population improvement and (2) variety development. In population improvement, a GS training population of approximately 600 individuals is phenotyped at two MN locations for 2–3 years (Figure 3.5). Both locations resemble the growing environments where most current Kernza acreage is distributed in Minnesota. The training population usually comprises 70 families with 8–9 genets per family, as this was found to give the optimal genomic prediction estimates (Zhang et al. 2016). Genets are transplanted approximately 1 m apart in an augmented design with 3–5 checks. The same field layout is used in the second location. Phenotypic data from the training population is used to select the best ~40 families (phenotypic selection) and to develop new genomic prediction models. Approximately 4000 progeny from the best-performing families are genotyped, with these plants originating from the seed produced during the phenotypic evaluation of the training population. These individuals form a second recombination during the breeding cycle. Predictions are made with mixed-model equations that use additive genomic values (best linear unbiased estimates) and $G \times E$ interaction effects to select 70–100 genotypes with best predicted performance (GS). Strong selection pressure is applied on genotypes with larger seed size, higher threshability, and minimal shattering, followed by short plant height, lodging resistance, and uniform days to anthesis. The selected 70–100 genotypes are intermated in a greenhouse to obtain progeny that will constitute the training population of the next breeding cycle. Each season, several important agronomic and domestication traits are evaluated in spaced-planted selection nurseries (same as the training population). These traits include spring growth vigor (0–9 scale, 9 being the best), days to heading, days to anthesis, grain yield (total seed weight per genet in g), seed size (g), seed shatter (0–9 scale, 0 being the best), free threshing

(0–9 scale, 9 being the best), plant height (cm), lodging resistance (0–9 scale, 0 being the best), and resistance to diseases, including FHB (scab) and ergot (both on 0–9 scale, 9 being the worst). Disease incidence and severity are measured as they appear in the nurseries. The strongest selection emphasis is given to larger seed size, reduced shattering, and improved grain threshability. This is followed by higher grain yield per spaced plant (i.e. per genet) and uniform days to heading and anthesis. As MN experiences a higher rainfall amount with occasional strong winds, selecting genotypes with lodging resistance is also a high priority. An ideotype would be a relatively short plant with large grain yield or size, no shattering, 100% threshability, no lodging, with nearly all tillers flowering and maturing simultaneously.

New IWG varieties are established using the genets within the training population with the best field performance. These plants are cloned in 5–8 replications and transplanted into a crossing block as the parents of synthetic variety candidates. Each crossing block is arranged in a 7 row \times 7 column design with a total area of 49 m² and 1 m distance between the genets. Establishment and development of synthetic varieties in this manner has been completed in other cereal crops, including perennial forage grasses (Hill and Elgin 1981; Tamaki et al. 2007; Laidig et al. 2017). Typically, 5–8 variety candidates are established in each breeding cycle and are designated the “SYN0” generation. SYN1 grain harvested from SYN0 is increased in a 250 m² area to obtain SYN2 which is increased in approximately 1000 m² to obtain SYN3 generation seed. The SYN2 and SYN3 generations are evaluated in variety trials at 4–6 Minnesota locations for 2–3 years. The SYN2 generations of the top two candidates observed in the state-wide trials are planted in large plots (≥ 4000 m²) for seed increase (SYN3), after which the variety is released to the grower at SYN3 and SYN4 generation. Breeder’s seed is maintained at the SYN2 generation.

Along with developing improved germplasm, the breeding data is analyzed to identify genomic regions associated with traits of interest (Bajgain et al. 2019a). The multilocation and multiyear trait data obtained from the selection nursery (training population) provide an excellent opportunity to study the effect of G \times E interactions on trait expression and heritability. In the third-cycle breeding population that was evaluated at two MN locations for two years, Bajgain et al. (2019a) observed that yield component traits and total grain yield in spaced plants had high heritability estimates (range of 0.31–0.73). These traits often had significant moderate to strong correlations within and across locations. The UMN IWG breeding program pioneered a GS-based breeding approach in IWG. In 2016, Zhang et al. (2016) proposed an

optimized pathway to rapidly improve IWG populations using genomic prediction and since that time several new methods have been evaluated, for example the inclusion of significant QTL in the model and $G \times E$ interaction (Section VI.3, (Bajgain et al. 2019a, b)). These findings are encouraging for current and to-be-established IWG breeding programs as they evaluate their populations and develop a suitable strategy to breed for better IWG germplasm in their respective regions. Additionally, information obtained from these studies suggests that strategic synergistic approaches could be taken to improve key traits across all breeding programs.

C. University of Manitoba

The University of Manitoba (UMB) program began with a visit to TLI in the fall of 2010. During that visit, TLI offered materials from TLI-Cycle 3 for the initiation of a breeding program at UMB (Figure 3.4). These materials were planted out in 2011 along with Plant Introductions that were received from USDA GRIN in 2010. TLI-Cycle 4 materials were later received and planted in 2012 (Cattani 2017), and these made up the materials used in UMB-Cycle 1. A description of planting methods and field design can be found in Cattani (2017). The spring of 2012 resulted in selection against materials that had begun growth in mid-March, during an early spring, with approximately 60% of the materials suffering damage following a mid-April -9.2°C frost while a late May frosts in 2014 (-5°C) eliminated all TLI-Cycle 4 materials from consideration (Cattani 2017). Similar overwintering losses in IWG have been reported in trials in Saskatchewan in western Canada (Campbell 1961; Heinrichs and Clark 1961; Lawrence and Ashford 1969). The three Canadian bred cultivars, Chief (Hanson 1972), Greenleaf (Wilson and Smoliak 1977) and Clarke (Lawrence 1981) were all selected for winter survival, with Greenleaf and Clarke showing better winter cold tolerance than Chief (Lawrence 1981).

The eventual selections were almost exclusively from TLI-Cycle 3 due to the lack of consistent yield across years of the GRIN materials (Cattani 2017; Cattani and Asselin 2018). Selections were made in the program after three consecutive years of seed harvest. Some selected materials were then placed into isolated polycross blocks, and half-sib progeny plots were seeded into rows at two sites to evaluate parental clones for seed yield over three consecutive harvests. Using both clonal and progeny testing in selection for complex traits was influenced by the researchers that bred IWG in North Dakota (Berdahl and Baker 1997). Seed yield in herbaceous perennial grasses can be very complex and

dependent on growing season conditions (Cattani et al. 2004; Abel et al. 2017). In general, the selected materials were in the higher range for seed size and produced moderate sized crowns (Cattani and As-selin 2018). From these materials, a nine-clone synthetic cultivar has been developed with breeder's seed expected to be harvested in 2022.

A delay in implementation of UMB-Cycle 2 occurred until a determination of the phenological development of this species was completed in 2020 (Duchene et al. 2021). This information has provided the critical timings for developmental stages that can impact grain yield. This study will be used to determine the response to postharvest renovation, nutrients, and precipitation and may enhance our selection for fall regrowth, which is likely critical under a condensed growing season such as experienced in western Canada (Duchene et al. 2021).

The UMB-Cycle 2 materials were generated in large part from directed crosses between the selected UMB materials and will be evaluated in the field from 2021 to 2023 for seed yield and ease of threshing. These materials will form the basis of the next cycle of selection. The UMB-Cycle 1 materials, now in their second reproductive year, are being tested in a number of locations in Canada, the United States, and in northern Europe with both researchers and producers, and these results will help evaluate the selection method used. UMB-Cycle 2 materials have been started, cloned in the greenhouse, and transplanted to two locations. Molecular analysis and screening for FHB will be carried out on these materials.

D. Swedish University of Agricultural Sciences, Uppsala

Breeding and development of perennial cereal grains could facilitate a transition toward perennial agriculture also in Northern temperate regions such as in Sweden. As wheat and barley are the most cultivated grain crops in these regions, perennial wild relatives are interesting candidates. While domestication and breeding of perennial barley is at an early stage (Westerbergh et al. 2018), IWG is promising as the first perennial grain crop in Sweden and other parts of Europe. Small plots of IWG were established from 2013 to 2017 in central Sweden. IWG was also included in a comparison with hybrids of wheat and IWG (Hayes et al. 2018). Based on the good perennial growth of IWG, a breeding program was initiated in 2018.

Additional perennial crops in Sweden are needed to maintain soil cover through the main part of the year, in contrast to the annual crops currently cultivated on 47% of the arable agricultural land in Sweden (The Swedish Board of Agriculture 2021, www.jordbruksverket.se, accessed 05/01/2021). Perennials could mitigate emissions

of greenhouse gasses, and nutrient leaching that cause eutrophication of watersheds and the Baltic Sea (European Environment Agency (EEA) 2020). Since Swedish farmers are well experienced in cultivating perennial forage crops, new perennial grain crops, if available, could be included in their cropping systems and significantly contribute to mitigating these negative effects.

The conditions for plant growth in Sweden at a Northern latitude (55–69°N) differ in several respects from other regions where breeding of IWG is carried out. The day length – an environmental signal used by plants to control seasonal growth and dormancy – varies from 14 to 16 hours in spring, 17 to 24 hours in summer, and 9 to 10 hours in fall. The climate is cold temperate with an average temperature in the summer that ranges from +17°C in the south to +14°C in the north, and in the winter from +2°C to –6°C (Swedish Meteorological and Hydrological Institute 2021, www.smhi.se, accessed 05/01/2021). The vegetation period has no clear dry season. The average monthly precipitation ranges from 25 to 75 mm in spring and from 50 to 120 mm in summer, fall, and winter. The length of the vegetation period (daily average temperature > +5°C) is 167 days in northern Sweden and 212 days in southern Sweden. During the last 40 years, this has increased by about 2 weeks.

Since the growing conditions are different in Northern Europe compared to at other IWG breeding programs, the breeding and development in our program were initiated using broad genetic and phenotypic diversity. Initially, the starting plant material for evaluation and selection originated from several selection cycles and breeding programs conducted under different climates (Figure 3.4). In particular, material from early selection cycles that had not yet been strongly selected for adaptation to specific growing conditions was used. This plant material, that is regarded as Swedish University of Agricultural Sciences (SLU)-Cycle 1, included accessions obtained from (1) the TLI breeding program: TLI-Cycles 0, 3, and 5 (DeHaan et al. 2018); (2) the UMN breeding program: synthetic populations MN1504-SYN1 (Bajgain et al. 2020b), MN1505-SYN1, MN1604-SYN1, MN1606-SYN1, MN1607-SYN1, MN1608-SYN1, and half-sib families G02, G045, G047, G060, G108; and (3) from the UMB breeding program: 20 half-sib families from UMB-Cycle 1 and a bulked sample of UMB-Cycle 1 (Cattani 2017). The accessions from UMN and UMB were derived from TLI-Cycle 3. In spring of 2018, seedlings of all accessions were started in pots with potting soil in a net garden and transplanted into the field at the Torsåker farm, south of Uppsala, Sweden, during summer of 2018. Each of the accessions was planted as isolated populations separated at a distance

of about 50 meters to avoid cross-pollination between populations. Each population had a total of 156 plants planted at a distance of 45 cm within rows and 90 cm between rows. First, the phenotypic diversity for several agronomic traits within and among these populations was measured. These traits were yield component traits such as number of spikes per plant, seed yield per spike, and seed weight; growth traits such as tiller number, average, and uniformity in tiller height; and phenology traits such as re-growth in spring, days to heading and physiological maturity, and second growth of tillers in fall. Data from individual plants from these populations were collected during 2018 and continued during 2019 and 2020 to learn how the plants develop over multiple years and to investigate trait correlations across multiple years.

In early summer of 2020, the 100 best-performing plants in the isolated populations were selected based on data from 2018 and 2019, with strongest emphasis on yield components, followed by low production of secondary tillers in fall. These plants were in their third field season and included 14 accessions from TLI, 72 from UMN, and 14 from UMB. They were cloned, cultivated in planting soil outdoors, and transplanted into the field at three new locations at the Torsåker farm in late summer of 2020. Each location had four randomized blocks with one cloned genet in each block.

The 100 cloned genets at the three different locations are evaluated for several traits for the estimation of heritability and the effect of $G \times E$ interaction on the expression of traits. Also, since these plants were planted as crossing blocks, they will constitute the SLU-Cycle 2 parents of the next generation of progeny. The SLU-Cycle 2 progeny will be evaluated for further selections of SLU-Cycle 3 parents and for the development of training populations with the purpose to apply GS in the breeding program. In future trait evaluation, domestication and agronomic traits such as resistance to shattering and threshability need to be addressed. Additional advanced germplasm from later selection cycles at TLI is also planned to be included in the breeding program.

As a complement to the isolated populations, the accessions TLI-Cycle 3, TLI-Cycle 5, MN1504-SYN1, MN1505-SYN1, G023, G047, and the bulked sample of UMB-Cycle 1 were evaluated for yield components at the population level at another location at the Torsåker farm in 2019. Seedlings were started in pots and each accession was planted in three replicated plots randomized in the field. Each plot included 3 rows with 10 plants each at a spacing of 90 cm, surrounded by 2 rows of TLI-Cycle 5 border plants. Additionally, to develop ideas around a perennial polyculture cultivation system that includes IWG and

N-fixing legumes, perennial legumes were intercropped with TLI-Cycle 3 and TLI-Cycle 5 in 2019. IWG was planted at a spacing of 90 cm and perennial legumes were sown in rows between IWG. Plots with each of three perennial legumes (alfalfa, white clover, and bird's-foot trefoil), a mixture of white clover and bird's-foot trefoil and no legume, were replicated three times over a total field size that included 10,800 IWG plants. This was conducted to evaluate how IWG and perennial legumes that are cultivated in Sweden would grow together and also screen for rare IWG phenotypes in a large population. The main purpose of the evaluation was to identify a legume that could be co-cultivated with IWG during selection and breeding of improved progeny.

E. US Department of Agriculture, Utah

The USDA ARS Forage and Range Research (FRR) unit initiated genetic research on IWG grain production traits using a full-sib family derived from the TLI-Cycle 3 genet C3_3471, in approximately 2012 (Larson et al. 2019; Mortenson et al. 2019). Some previous work involving the perennial MT-2 hybrid of tetraploid durum wheat (*Triticum turgidum* L. var. *durum*) and IWG was conducted at FRR, but this was phased out because of its variable chromosome constitution (Jones et al. 1999). Other ongoing IWG research at FRR has focused on forage breeding (Jensen et al. 2016; Vogel and Jensen 2001) and genetics (Dewey 1962, 1984; Wang et al. 2015).

A new FRR USDA breeding project aimed at developing dual-purpose forage and grain varieties of IWG for semiarid regions of the western United States was initiated in 2017 using 29 accessions from TLI, UMN, and UMB (Figure 3.4). These 29 accessions included (1) a subset of 14 half-sib families from a crossing block of 89 TLI-Cycle 6 plants (Crain et al. 2021a), (2) a full set of 10 half-sib families from a crossing block of 10 parents from UMB-Cycle 1 (Cattani 2017), and (3) five synthetic populations (MN1501-SYN2 – MN1505-SYN2) developed from the UMN-Cycle 1 (Bajgain et al. 2020b). Accessions from UMB and UMN originated from TLI-Cycle 3, with one additional cycle of selection (Figure 3.4). Genetic differences among the three sources of these 29 accessions are discernible (Figure 3.2), but it should be recognized that these accessions do not represent all the diversity in the TLI, UMB, and UMN breeding programs. A total of 77 seedlings from each of the 29 accessions were raised in an FRR greenhouse and sampled for DNA analysis, in the 2017–18 winter, and then transplanted to a nursery at the Utah State University (USU) Evans Research Farm in the spring of 2018. Plants were spaced approximately 0.5 m apart

within rows and 1 m between rows, in a randomized complete block design with one plant from each of the 29 accessions in each of 77 blocks. Plants were evaluated for plant height, seed size, free threshing, number of florets per spike, seed yield per spike, and total seed yield per plant in 2019. Approximately 900 of the half-sib families harvested in 2019 were also seeded into replicated plots at the USU Richmond Research Farm to evaluate grain yield. Each plot was comprised of one 2 m row with 1 m between rows and 1 m between plots within rows to simulate a management system that may be feasible for IWG grain production (Hunter et al. 2020a), even though reduced spacing may be optimal for dual-purpose forage and grain production (Duchene et al. 2021).

A subset of 97 mother plants from the FRR-Cycle 1 were selected as parents for FRR-Cycle 2 based on several indices of seed shattering, seed size, and spike yield data recorded on spaced plants in 2019. These 97 selections included a total of 10 plants from 5 UMN accessions, 76 plants from 14 TLI accessions, and 11 plants from 9 UMB accessions. Accessions from the more advanced cycles of selection at TLI (TLI-Cycle 6) were generally favored with up to 13 and 15 selections from half-sib families of TLI-Cycle 6 K004201A and K005444, respectively. Paternity analysis based on DNA sequencing (Crain et al. 2020b) of the FRR-Cycle 1 population indicated that 76 selections derived from a total of 45 of the 89 TLI-Cycle 6 genets, including 14 females and 39 males. The 97 FRR-Cycle 1 crossing block genets were transplanted to a new location at the USU Evans Research Farm to form FFR-Cycle 2 seed in March of 2021. Genotypic data from FRR-Cycle 1 will be used to make genomic predictions for single-plant traits that were measured in 2019 and first-year yield data from half-sib families evaluated in 2021. These genomic predictions will be used to screen up to 5,000 FRR-Cycle 2 progeny following a similar strategy shown in Figure 3.5. At least 500 of the resulting FFR-Cycle 2 selections will be used for additional single-plant and half-sib evaluations. One or more subsets of the FRR-Cycle 2 plants with high genomic predictions will be isolated to increase seed for larger field evaluations. IWG is already recognized as a valuable forage and biomass crop for the western United States (Robins 2010; Robins et al. 2013, 2020; Jensen et al. 2016; Larson et al. 2017). Development of a dual-purpose IWG forage and grain crop will have many benefits for farmers and ranchers in this region, where supplemental feed for livestock through the winter is a cost-limiting factor in cow-calf production systems. Thus, major points of emphasis of the new FRR breeding program will be evaluation of grain yield based on half-sib families in irrigated and dryland management

systems for semiarid regions of the western United States. Future evaluations of half-sib families may also incorporate legume intercropping, as different cropping systems may benefit from specialized varieties of IWG in dual-purpose forage and grain production systems (Weißhuhn et al. 2017; Ryan et al. 2018; Mortenson et al. 2019; Tesdell et al. 2020).

V. BREEDING GOALS AND PROGRESS

A. Yield Components

Improvement of grain yield and seed size in IWG has been major targets of selection since the initiation of IWG domestication programs (Wagoner 1990). In an effort to improve selection methodologies for yield, breeders and geneticists have sought to better understand the relationships among yield, a highly complex trait, and its component traits to explore opportunities for indirect selection. The most common approach for assessing these relationships has been through correlation analyses and in evaluating shared marker–trait associations among traits. It is important to note that the majority of yield component evaluations in improved IWG populations have been conducted in the context of breeding programs on individuals grown in the spaced plant configuration.

It is well established in recent literature that in improved IWG germplasm, positive and often significant associations exist between seed dimensions (length, width, and area) and seed weight (Zhang et al. 2017; Larson et al. 2019; Bajgain et al. 2019a; Bajgain and Anderson 2021). This finding has been supported by marker–trait associations that are common among the traits (Zhang et al. 2017; Larson et al. 2019; Bajgain et al. 2019a; Bajgain and Anderson 2021), several of which were consistent across studies (Larson et al. 2019; Bajgain et al. 2019a; Bajgain and Anderson 2021; also as demonstrated in Figure 3.6). Likewise, multiple studies have also reported strong correlations between yield and biomass or biomass-related traits such as plant height, flag leaf area, reproductive tiller number, and spike length (Zhang et al. 2016; Cattani and Asselin 2018; DeHaan et al. 2018; Bajgain et al. 2019a; Altenendorf et al. 2021a; Bajgain and Anderson 2021). This relationship has been cited as mostly positive for the development of dual-purpose forage and grain IWG, yet there could be some negative trade-offs, e.g. increased likelihood of lodging associated with taller plants (DeHaan et al. 2018). In an analysis using structural equation modeling to parse out direct and indirect effects on yield, biomass traits were found to have relatively limited direct impacts on yield *per se* but were more

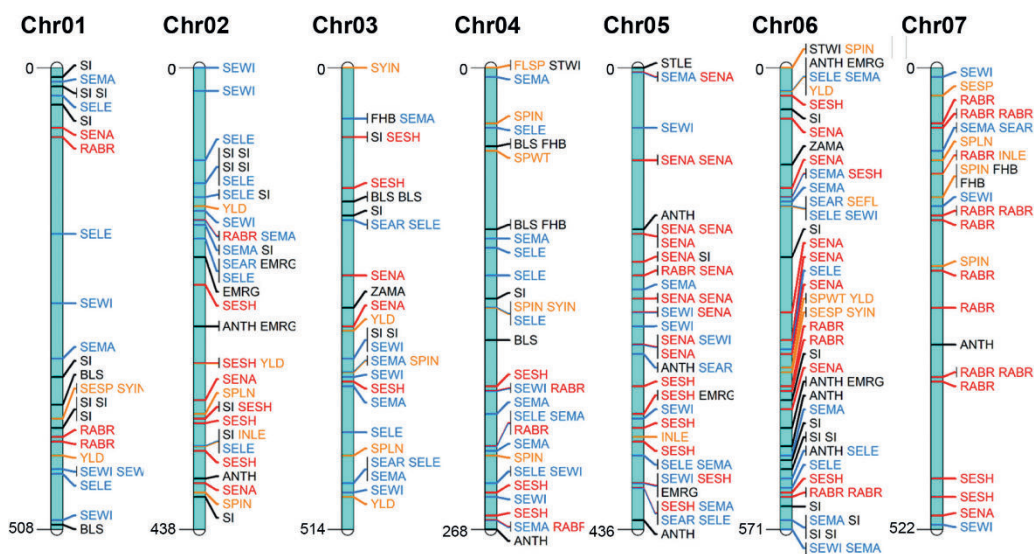


Fig. 3.6. Quantitative trait loci (QTL) associated with agronomic, domestication, and disease resistance traits of intermediate wheatgrass. Physical positions of the peak markers of all known QTL are shown on the v2.1 reference genome. Numbers on the left side of the chromosomes represent the physical length of each chromosome Mb. Traits are grouped into four main categories: agronomic traits (black font), domestication traits (red font), seed-related traits (blue font), and yield-component traits other than seed-related traits (green font). ANTH: Anthesis, BLS: bacterial leaf streak, CRCL: crown circumference, EMRG: spike emergence time, FHB: Fusarium head blight, FLSP: number florets per spikelet, INCR: number of inflorescences per crown, INLE: inflorescence length, RABR: Rachis breaks, SEAR: seed area, SEFL: number of seeds per floret, SELE: seed length, SEMA: seed mass, SENA: threshability/percentage of seeds threshed out naked, SESH: seed shattering, SESP: number of seeds per spikelet, SEWI: seed width, SI: self-incompatibility, SPIN: number of spikelets per inflorescence, SPLN: spike length, SPWT: spike weight, STLE: stem length, STWI: stem width, SYIN: total seed yield per inflorescence, YLD: grain yield per plant.

Fig. 3.6. (Continued)

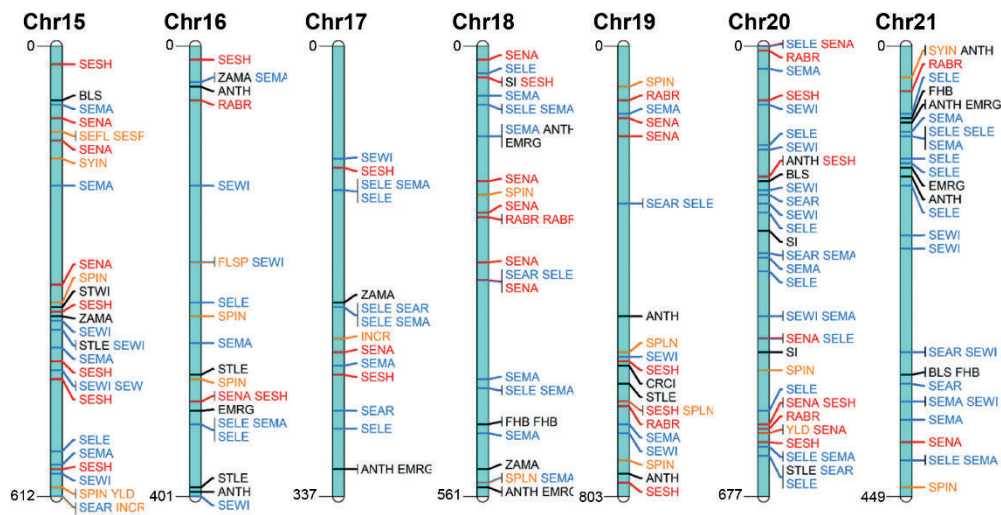


Fig. 3.6. (Continued)

associated with increases in seed size, and that yield component traits such as floret and spikelet number had greater direct effects on yield (Altendorf et al. 2021a). Additional research has also shown positive associations between spikelets per spike and grain yield, but negative associations between seed size and spikelets per spike or spikelet density (Bajgain et al. 2019a; Altendorf et al. 2021a).

Yield component analyses have also demonstrated the major influence that fertility traits, such as FSU or percent filled florets, have on yield (Larson et al. 2019; Altendorf et al. 2021a). IWG produces more florets than are utilized, resulting in low FSU estimates, which range from 12 to 60% (Larson et al. 2019; Altendorf et al. 2021a). The heritability of this trait is low, suggesting gains from phenotypic selection alone may be low. Other major findings are that yield components can be variable across locations, specifically between different breeding locations, but also within similar growing regions (Bajgain et al. 2019a), and should be selected accordingly in their target environments. Altendorf et al. (2021a) also demonstrated that the relative importance of yield component traits varies depending on the method of evaluation (i.e. whether it is based on yield per spike, or yield per plant). On a per plant basis, yield nearly doubled in two-year old plant stands, a finding supported by few studies (Zhang et al. 2016; Altendorf et al. 2021a). Altendorf et al. (2021a) noted that while most component traits such as seed size, spikelets per spike, florets per spikelet, and biomass traits such as flag leaf area, and stem diameter decreased in the second year of plant evaluation, reproductive tiller number nearly doubled. This poses a challenge for selection in spaced plant environments for grain yield as the phenotypic and genetic correlations between reproductive tiller number in swards and spaced plants were demonstrated to be relatively low (Mortenson et al. 2019). Hunter et al. (2020a) also found that with age, IWG swards produce more nonreproductive tillers, which further demonstrates the need to understand tillering dynamics and its impact on selection for grain yield. Future work is needed to verify how relationships between yield components differ in plot and spaced plant environments to further inform selection efforts to improve yield in IWG.

In addition to the work on assessing IWG's performance across different growing configurations, research is also needed to understand the extent to which $G \times E$ interactions affect yield. $G \times E$ interaction is a well-known aspect of plant breeding and agronomic production (for review see DeLacy et al. (1996)). Compared to annual wheat that has numerous evaluations within state breeding programs and regional evaluations (Graybosch 2017), IWG breeding is still in its infancy.

While the programs described here (Section 4) span regional areas, to date there has not been a sustained or targeted effort to dissect $G \times E$ interaction in IWG.

One of the few studies of IWG in multiple locations was a two-year phenotypic evaluation of an IWG-NAM population, which was cloned and replicated in spaced plant nurseries in both St. Paul, MN, and Salina, KS. This study revealed significant $G \times E$ interaction for families for all 13 yield components, biomass, and maturity traits evaluated. The interactions, as well as confounding factors of plant age, led to the analysis of each unique year by location interaction as a unique environment (Altendorf et al. 2021a). While not $G \times E$ interaction *per se*, it was evident that the population showed variable growth habits across locations, specifically in the first year. In KS, the plants were generally lower yielding per spike but achieved similar yield per plant via larger number of tillers, more spikelets, and greater numbers of florets per spikelet compared with MN. The plants in KS were also shorter in height and had lower spike density and larger flag leaf area. These findings suggest that adaptive traits in IWG are variable across environments and could have an important role in helping the crop cope with environmental factors.

Currently, there is collaborative research among TLI, UMN, and USDA FRR to evaluate genetic material in these regional locations. For example, early maturity is a target of selection in KS growing environments to avoid late-season heat and drought stress. In an analysis of IWG yield components in a year with severe drought stress in KS, plants that went into anthesis earlier tended to have greater FSU, a finding that supports this trait as a selection target (Altendorf et al. 2021a). As IWG production expands, breeding programs will continue work to understand and utilize $G \times E$ interactions for optimal yield not just at regional scale but also more local levels.

B. Tolerance to Biotic Stress

1. Fusarium Head Blight (scab)

FHB in IWG is caused by *Fusarium graminearum* (Oliver et al. 2005; Turner et al. 2013), which infects the developing grain if favorable conditions, 20–25 °C with high moisture or humidity (Manstretta and Rossi 2015), occur during pollination and grain fill (Trail 2009). *F. graminearum* can produce multiple mycotoxins, including deoxynivalenol (DON) which compromise the value and safety of the grain (Trail 2009). Infection may be visible on the spikes, resulting in a bleached appearance of spikelets prior to senescence. Pink-orange fungal mycelia can also be observed on spikelets, often near the attachment

point with the rachis. Scabby or bleached kernels, sometimes called “tombstone” kernels in other cereal grains, are rare in IWG and are not easily observed in grain prior to dehulling.

In inoculated field and greenhouse trials, most IWG spaced plants are resistant to *F. graminearum*, with few infected spikelets on inoculated heads and limited spread of infection among spikelets (Figure 3.3). But even at low infection levels, this fungal pathogen can produce DON in harvested grain at levels that exceed the thresholds for human consumption established by the Food and Drug Administration (Silver Spring, MD, USA) (Turner, unpublished data). Improving resistance to FHB and limiting the effects of toxin contamination through management and postharvest practices are very important to the development and widespread use of IWG as a grain crop. Current methods of breeding for resistance have included developing field inoculation procedures for FHB and evaluating a GS strategy to predict and eliminate highly susceptible plants from the breeding population. The underlying genetic mechanisms of FHB resistance are currently not well understood. To date, there is one published study reporting 15 QTL associated with FHB resistance (Bajgain et al. 2019b). When combined additively, 2–3 resistance loci combinations could reduce FHB disease severity by 15%. This mapping study was conducted in a single mapping population and by combining QTL from other populations, the predicted level of control could provide sufficient protection against FHB in IWG.

2. Bacterial Leaf Streak

Bacterial leaf streak (BLS) in IWG is primarily caused by the bacterium *Xanthomonas translucens* pv. *undulosa* (*Xtu*) – the same pathogen that causes BLS of wheat (Curland et al. 2021). Another pathovar of the same species, *X. translucens* pv. *cerealis*, is also known to cause BLS in IWG (Mohan et al. 2001). BLS is observed on leaves as elongated light brown to dark brown lesions (Figure 3.3) and may be accompanied by translucent to milky exudates if growth conditions are favorable to the pathogen (Duveiller et al. 1992; Duveiller 1994). Under severe conditions, the lesions become necrotic and glumes develop black stripes, also known as black chaff (Curland et al. 2018). In the field, BLS is typically present in negligible levels in IWG breeding nurseries and can often be difficult to identify due to other biotic and abiotic factors. For this reason, the UMN IWG breeding program rates leaf health as a whole near harvest time in an attempt to deselect genotypes that could potentially be susceptible to the disease. The direct effect of BLS on yield reduction has yet to be quantified. However, breeders’ observations indicate that BLS

is not considered to affect grain yield in IWG. A larger concern may be loss of forage quality of postharvest residue due to leaf death.

An in-depth understanding of BLS infection and host–pathogen interaction in IWG is currently lacking. To date, one study has characterized the genetic mechanism of host resistance in IWG to BLS (Bajgain et al. 2019b). In this study, Bajgain et al. used an F_1 mapping population developed from two parental genets in UMN-Cycle 1. These parents had contrasting reactions to BLS in the field (St. Paul, MN, United States) during 2011–2013. Eleven medium-effect QTL were identified in the population on 9 IWG chromosomes conferring resistance to BLS, some of which also provided resistance against FHB. The authors then combined the 2–3 largest QTL, i.e. QTL responsible for the highest amount of phenotypic distribution in the disease environments, to develop a “stacked QTL” model to estimate the % reduction in BLS severity. Results showed that these models reduced BLS severity by 8–17% in the population under study. Co-expression of few stacked resistance genes has been shown to be a difficult hurdle for the pathogen to overcome (Joshi and Nayak 2010). Increasing the frequency of resistance alleles via either phenotypic selection, marker-assisted selection (MAS), or GS (Jannink et al. 2010) could therefore be an effective strategy to fight BLS and other diseases in IWG breeding programs.

3. Ergot

Ergot is a fungal disease caused by several species of the genus *Claviceps* in cereal, grass, and forage crops. The pathogen that causes ergot in mainstream cereal crops such as wheat, barley, and rye is *Claviceps purpurea* (Fries ex Fries) Tulasne (Miedaner and Geiger 2015); the pathogen causing ergot in IWG is yet to be determined. Infection by the pathogen is primarily dependent on the host flowering mechanism, since the fungal spore mimics the pollination process and is also influenced by timing of anthesis, pollen availability, host resistance, and weather conditions (Parh et al. 2008; Miedaner et al. 2010). Upon successful infection of the ovaries, the fungus produces a dark gray and black colored seed-like mycelial body called sclerotium that can be several centimetres long. The fungus typically infects unfertilized ovaries, but ovaries that have already been fertilized can also be infected (Kirchhoff 1929). Sclerotia produce toxic alkaloids of three major groups: clavine alkaloids, d-lysergic acid and its derivatives, and ergopeptines (Hulvová et al. 2013). While ergot can cause yield reduction in cereals by up to 10% (Wegulo and Carlson, 2011), the bigger threat posed by this disease is on the health of humans and livestock because of the toxicity of these alkaloids. Wheat with >0.05% of ergot bodies

is considered unsuitable for food; the threshold is 0.1% for barley, oat, and triticale, and 0.3% for rye (Wegulo and Carlson, 2011; Coufal-Majewski et al. 2016).

A detailed understanding of host resistance to ergot is still lacking in cereal crops, yet the timing of flowering and pollen fertility are considered to be confounded with host resistance (Miedaner and Geiger 2015; Kodisch et al. 2020). Studies conducted to screen rye genotypes resistant to ergot showed that disease incidence was lower in germplasm with higher pollen shedding such as landraces and old cultivars, whereas it was higher in germplasm with lower pollen shedding such as newer synthetics and hybrid varieties (Mirdita and Miedaner 2008; Mirdita et al. 2008). In hexaploid winter wheat varieties, partial genetic control of sclerotia size was found to be co-located with semi-dwarfing alleles at the *Rht* loci *Rht-1B* and *Rht-1D* (Gordon et al. 2015). Another recent study by Gordon et al. (2020) studied gene expression in a durum wheat cultivar and found that resistance to ergot could be controlled by MYB (myeloblastosis) transcription factors, F-box and ankyrin repeat-containing proteins, and BTB/POZ-containing proteins. Studies aimed at characterizing the genetics of host resistance to ergot in IWG are yet to be carried out.

In IWG breeding nurseries, plants closer to the plot border, particularly on the upwind side, tend to exhibit higher ergot severity, presumably due to a smaller pollen load relative to plants inward from the border. In the UMN breeding program, some families exhibit susceptibility regardless of their location in the plot. Genotypes with higher disease levels are not advanced to the next breeding cycle. Synthetic crossing blocks, although established with the goal of obtaining congruency in anthesis among the parents, may occasionally exhibit high ergot levels due to asynchronous flowering among the parents. These synthetic blocks are also discarded from entering generational increase and variety trials. Producers of Kernza grain can currently manage ergot by not harvesting field edges if they are contaminated and being careful not to mix contaminated seed lots with clean lots. Contamination can be slightly reduced through conventional cleaning techniques such as sieves, length graders, and gravity tables. However, severe contamination, which is more common in humid production areas, has generally required the use of color sorting equipment.

4. Other Diseases

Several other leaf spotting diseases are regularly observed in IWG breeding nurseries and production fields but have minor effects on the grain yield of IWG. Some of these diseases include spot blotch, caused

by *Bipolaris sokoriana* (Sacc.) Shoemaker (telomorph: *Cochliobolus sativus* Ito & Kurib) (Figure 3.3), tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs., and Septoria blotch caused by *Septoria tritici* and *Leptosphaeria nodorum* E. Müller (Berdahl and Krupinsky 1987; Farr et al. 1989). These diseases are monitored routinely but currently are not targets of improvement in IWG breeding programs.

5. IWG as a Source of Disease Resistance Genes

IWG is immune or asymptomatic to many diseases of annual grains and, due its close relationship with Triticum, has been used for many decades to transfer resistance genes to annual wheat. It has been used as a source of disease resistance genes in wheat for: FHB (*Fusarium graminearum* Schwabe (telomorph: *Gibberella zeae* (Schw.) Petch) (Han et al. 2003; Oliver et al. 2005), BYDV (Brettell et al. 1988; Banks et al. 1993), eyespot (*Tapesia yallundae* Wallwork and Spooner and *Tapesia acuformis* Boerma, Pieters and Hamers Crous [anamorph *Pseudocercospora herpotrichoides* (Fron.) Deighton]) (Cox et al. 2005), stem rust (*Puccinia graminis* Pers.:Pers.) (Friebe et al. 1996; Li and Wang 2009), stripe rust (*P. striiformis* Westend.) (Luo et al. 2009), leaf rust (*Puccinia triticea* Eriks.) (Friebe et al. 1993) wheat streak mosaic virus (WSMV) and its vector wheat curl mite (WCM) (Wells et al. 1982; Li and Wang 2009), and powdery mildew (*Blumeria graminis* (DC.) E.O. Speer) (Li and Wang 2009). A few examples of introgressed disease resistance genes from IWG into wheat include the stem rust gene *Sr44* (Khan 2000), stripe rust gene *Yr50* (Liu et al. 2013), powdery mildew gene *Pm43* (He et al. 2009), BYDV *bdv2* (Anderson et al. 2021), and wheat streak mosaic gene *Wsm1* (Wells et al. 1982). Identifying resistance genes in IWG and characterizing closely linked genetic loci can benefit the improvement of wheatgrass and has had additional benefits for other small grain crops.

C. Tolerance to Abiotic Stress

Selection for adaptation to different growth environments will be critical to maximize productivity of IWG in challenging and diverse growth environments. The breeding programs in North America represent a wide range of climatic conditions with elevations ranging from sea level to 1500 m, temperature extremes from -57 to 50 °C, and a strong west to east precipitation gradient ranging from 38 to 127 cm. The international programs also have their own, unique climates. As climate change has the potential to increase drought and temperature stress (Lobell et al. 2011; Kukal and Irmak 2018), the range of conditions

that IWG may experience will be expanded. To date, IWG has been documented to be a source of abiotic resistance for wheat improvement (Fedak and Han 2005; Cui et al. 2018; Zhang et al. 2021), and IWG performance indicates large genetic variability for abiotic stresses, although testing outside of local breeding programs has been limited. Even this limited testing has led to attrition of plants that have been impacted by drought, heat, and cold. As IWG acreage increases, it is likely that more efforts will be devoted to conducting targeted environmental testing for specific abiotic stresses.

While IWG is anticipated to experience a full range of environmental stresses, particular emphasis has been placed on cold tolerance and winter survival as many production regions experience significant winter temperatures. As Sandve et al. (2011) notes, “Winter survival is a very complex trait determined by combinations of frost, desiccation, water logging, ice-encasement, anoxia, and snow cover.” Plant processes of cold acclimation (development of cold-induced dormancy) and de-acclimation (breaking of the dormancy) represent the beginning and end of the plant processes for winter survival (Ergon 2017). Light and temperature influence both of these processes (Sandve et al. 2011; Dalmanndottir et al. 2017; Ergon 2017), and there can be an interaction between day length and temperature with respect to the success of cold acclimation (Dalmanndottir et al. 2017). In *Triticum aestivum*, Chen et al. (2019) found seven QTL related to in-field winter survival, including the *Vrn-1* gene, while 15 QTL have been identified in barley (Muñoz-Amatriaín et al. 2020). At least seven genes have been indicated to be important in successful cold acclimation in the perennial grass meadow fescue (*Schedonorus pratensis* (Huds.) P. Beauv. (formerly *Festuca pratensis* Huds.)) (Rudi et al. 2011). Enhanced freezing tolerance was conferred to a back-cross-generated *Lolium* × *Festuca* hybrid with the inclusion of a *Festuca* chromosome coding for enhanced non-photochemical quenching in the cold acclimation process when compared to the *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot (formerly *L. multiflorum* Lam.) parent (Humphreys et al. 2007). Cell dehydration is also thought to be a key component of winter survival due to the co-localization of QTL with drought response in meadow fescue (Sandve et al. 2011). Recently, it has been demonstrated that older plants of IWG appear to be better able to repair damage caused by cold stress (Jaikumar et al. 2020), although loss of stand due to winter stress may take place throughout the life of an IWG stand (Heinrichs and Clark 1961).

Overwintering has been recognized as an issue in the culture of IWG, especially in Canada (Heinrichs and Clark 1961; Lawrence 1981;

Cattani 2017), and it is expected to be of concern in other continental or more northern climates (Hayes et al. 2018). Cultivar descriptions often include an emphasis on selection for winter hardiness or overwintering (Lawrence 1981) as does the more recent selection of IWG as a perennial grain (Cattani 2017). Overwintering issues have been found in other perennial grass species in Manitoba (Cattani et al. 2000) even where the LT_{50} (lethal temperature where 50% of the plants died) of the species was -39°C . Limin and Fowler (1988) found similar LT_{50} values for the IWG forage cultivars Chief and Clarke, -21.8 and -21.5°C , respectively, although Clarke had been reported to possess greater overwintering tolerance compared to Chief (Lawrence 1981). In fall rye (*Secale cereale* L.), LD_{50} 's (lethal duration of maintenance of cold tolerance) was found to better predict winter survival than LT_{50} 's (Willick et al. 2021). This is potentially indicative of factors other than only lethal temperatures influencing overwintering success, with possibly timing of regrowth in the spring being important (Cattani 2017). There is an increase in the optimum temperature for reactive oxygen species mitigating enzymes with the loss of winter hardening reducing the ability of dehardened plants to recover from a return to freezing temperatures (N.S. Jaikumar, personal communication).

As the growth environment moves further from the equator, an interaction between latitude and day length will become more important since reproductive induction timing moves closer toward the spring equinox as the latitude increases. Coupled with this is the apparent secondary induction (Heide 1994) requirement of 13–14 hour day lengths to begin reproductive tiller elongation (Duchene et al. 2021). As overwintering is associated with successful flowering, understanding flowering control and its components may allow for selection for earlier flowering (e.g. a 12-hour secondary induction) for growth environments where growth resumes before the spring equinox.

D. End-Use Traits

A central idea behind the domestication of IWG as a new perennial cereal species is the use of its grain as human food. Out of nearly 100 perennial grass species, IWG was found to be the best candidate for domestication because of its grain quality and other desirable agronomic traits (Wagoner 1990). The nutritional quality of IWG grain was promising and its use in food products was deemed acceptable at the earliest stages of domestication (Wagoner and Schauer 1990). Flour of early IWG cultivars was found suitable to make pancakes, muffins, cookies, and bread (Figure 3.3; Becker et al. 1991). Current IWG

breeding methods continue to preserve these culinary attributes of the grain while improving other agronomic traits.

IWG germplasm is periodically evaluated to quantify chemical and nutritional compositions. IWG has substantially higher bran content relative to wheat, likely owing to the small seed size (Becker et al. 1991). In addition, compositional analysis of different IWG breeding populations has shown differences in protein, fat, ash, fiber, starch, amylose, and antioxidant compounds among the different populations (Tyl and Ismail 2019). IWG grain is considerably richer in protein (20%) compared to wheat (13%) and fiber content (17% in IWG, 11% in wheat) yet has lower starch content (47%) compared to that in wheat (72%) (Marti et al. 2015). Despite an overall higher protein content, IWG flour is deficient in high-molecular-weight glutenin subunits (HMW-GS) compared to wheat (Zhang et al. 2015; Tyl and Ismail 2019). HMW-GS provides higher dough elasticity, and good bread-making flours have a higher ratio of HMW-GS to low-molecular-weight glutenin subunits (Jood et al. 2000). The deficiency of HMW-GS and low starch levels make IWG flour unsuitable as a standalone flour for baking bread with high loaf volume (Zhang et al. 2014; Rahardjo et al. 2018). Bread baked with either a mixture of 25 or 50% IWG flour with hard wheat flour or gluten supplement has been shown to be more successful (Becker et al. 1991; Marti et al. 2015; Tyl et al. 2020). However, genetic variation in traits influencing mixing quality is large, indicating that selection for bread baking quality would likely be successful (Zhang et al. 2015). It is possible to imagine breeding for different market classes targeting a range of end uses, as has been achieved in wheat. Food applications of IWG can also be affected by $G \times E$ interaction effects. A recent study by Banjade et al. (2019a) reported that differences in locations where IWG is grown affected bran and flour composition of the grain. Bread baked with IWG flours from different locations also resulted in different bread dimensions, volume, and crumb firmness, suggesting that growing environments can have substantial impact on end-use traits (Banjade et al. 2019b). As breeders select genotypes with larger seed size, reduction in the relative amount of bran and germ can be expected. Therefore, continual evaluation of newer IWG varieties and breeding materials for their use in food and beverage products will be necessary to identify and select the best material for these purposes. While there has been a recent surge in research involving chemical composition and functionality of IWG grain and its application in food products, additional research is needed to accurately characterize the effect of growing conditions, grain harvest and storage, milling, and additional processing steps on IWG's functional and flavor properties.

VI. MODERN BREEDING TOOLS

A. Use of Genomic Resources in IWG Breeding

1. IWG Genome Sequencing and Map Construction

Next-generation DNA sequencing and bioinformatic methods have ushered in a level of molecular methods that were previously unattainable in terms of number of individuals sequenced and sequence coverage compared to Sanger sequencing (Schuster 2008). High-throughput and multiplexed genotyping methods like GBS provided the opportunity to discover polymorphic markers and genotype populations even without reference genomes (Poland et al. 2012a, b). Within IWG, some of the first applications of GBS were to develop genetic maps of seven full-sib families, with these maps integrated into the first consensus map for IWG (Kantarski et al. 2017). These maps were derived from TLI-Cycle 3 and 4 germplasm with 6 biparental families from 12 unique parents, including M26 \times M35 and C3-2331 \times C3-2595, and 1 self-pollinated plant (Kantarski et al. 2017). The resulting consensus map had 10,029 GBS markers spanning 5,061 Haldane centiMorgan (cM) in 21 LGs including 3 sets of 7 LGs aligned to the 7 chromosome sequences of barley. In addition to developing genetic resources, GBS has been extensively used to dissect quantitative traits (i.e. genetic mapping) and GS within IWG plant breeding programs.

The IWG genome sequencing project was undertaken beginning in 2016 in conjunction with the Joint Genome Institute (JGI) (https://phytozome-next.jgi.doe.gov/info/Tintermedium_v2_1). A whole-genome shotgun-based strategy, similar to wheat as shown by Chapman et al. (2015), used several short insert paired-end libraries complemented with large insert size mate pair libraries. Using resources at JGI, a genome of approximately 11,917 Mb was assembled using NRGene's DeNovoMAGIC from a haploid plant compared favorably to the estimated genome size of 12.75 Gb (Vogel et al. 1999). The IWG genome is still being improved, since sequencing technology has advanced rapidly since the initial IWG genome sequencing effort, e.g. highly accurate, long reads using circular consensus sequencing (Wenger et al. 2019). Even though these methods will greatly improve upon the short-read sequencing draft genome, the draft genome has been useful to the IWG community. The initial genome has been used to identify single-nucleotide polymorphisms (SNPs) for GS within breeding programs and genetic studies to dissect quantitative traits through QTL mapping and genome-wide association analysis.

2. Genetic Mapping of Yield and Domestication Traits

Considerable effort has been put forth to construct linkage maps (Kantarski et al. 2017; Altendorf et al. 2021c) identifying QTL and possible genes controlling agronomic and domestication traits in IWG (Table 3.2, Figure 3.6). This includes seed-related traits of seed length, width, weight, and area; spike and yield-related traits like spike length, spike weight, number of spikelets per spike, and spike yield; domestication traits such as plant height, free grain threshing, and shattering; and other agronomic traits including flowering time and disease resistance. Linkage mapping studies of outcrossing species such as IWG are normally based on F_1 progeny of two uniquely heterozygous plants rather than F_2 progeny of inbred parents. Although it is impossible to develop healthy fully inbred plants in IWG due to SI, it is relatively easy to clonally replicate individual IWG plants compared to animals, trees, or even alfalfa. Thus, clonal replication of plants, or “ramets,” has been extensively used in genetic studies of IWG. As a perennial species, these genets can be repeatedly evaluated over multiple years, which provides another form of replication across environments (years).

The first QTL study in IWG (Zhang et al. 2017) examined seed mass, seed length, seed width, and seed area by association mapping using GBS markers in a heterogeneous breeding population, the first recurrent cycle at the UMN (UMN-Cycle 1). In addition, two clonally replicated biparental populations comprised 172 and 265 full-sib genets derived from crosses of M26×M35 and C3-2331×C3_2595 were evaluated as these families were used to construct the first IWG consensus linkage map (Kantarski et al. 2017). This study detected 33 QTL by association mapping and 38 QTL by interval mapping, with 23 common QTL (Figure 3.6). Fifteen QTL explained 37.6% of the variation for seed weight, whereas 14 QTL explained as little as 25.1% of the variation in seed length. The largest logarithm-of-odds (LOD) value was 5.2, for seed width, in the heterogeneous breeding population. Although 10,171 GBS markers were discovered in the breeding population, association mapping was done with a subset of 4,731 markers that were present in the GBS consensus map because a genome reference sequence assembly was not available at that time (Zhang et al. 2017).

A more comprehensive evaluation of domestication traits in the M26×M35 family was conducted in clonally replicated field experiments in KS and UT over three and two years, respectively (Larson et al. 2019). The M26 parent was an F_1 hybrid of C3-3471×C3-3941 where the C3-3471 genet was identified as one of the first predominantly free threshing and non-shattering plants in the TLI breeding

Table 3.2 Number of quantitative trait loci (QTL) discovered in different IWG populations for several important agronomic and domestication traits.

Trait category	Trait	Population							Total
		A77- 3 × C20-7	UMN-Cycle 1, M26 × M35, C3- 2331 × C3-2595	UMN-Cycle 3	UMN-Cycle 4	TLI Cycles 4, 5	IWG-NAM	M26 × M35	
Agronomic	ANTH	—	—	—	—	—	26	—	26
Agronomic	BLS	13	—	—	—	—	—	—	13
Agronomic	CRCI	—	—	—	—	—	—	3	3
Agronomic	EMRG	—	—	—	—	—	17	—	17
Agronomic	FHB	19	—	—	—	—	—	—	19
Agronomic	SI	—	—	—	—	39	—	—	39
Agronomic	STLE	—	—	—	5	—	—	8	13
Agronomic	STWI	—	—	—	—	—	—	4	4
Agronomic	ZAMA	—	—	—	—	—	—	10	10
Domestication	RABR	—	—	—	—	—	41	—	41
Domestication	SENA	—	—	—	6	—	37	9	52
Domestication	SESH	—	—	—	4	—	48	5	57
Seed-related	SEAR	—	30	—	—	—	—	6	36
Seed-related	SELE	—	22	45	8	—	—	7	82
Seed-related	SEMA	—	29	53	3	—	—	4	89
Seed-related	SEWI	—	27	30	5	—	—	8	70
Yield component	FLSP	—	—	—	—	—	—	4	4
Yield component	INCR	—	—	—	—	—	—	4	4
Yield component	INLE	—	—	—	—	—	—	7	7
Yield component	SEFL	—	—	—	—	—	—	7	7

(Continued)

Table 3.2 (Continued)

Trait category	Trait	Population							Total
		A77- 3 × C20-7	UMN-Cycle 1, M26 × M35, C3- 2331 × C3-2595	UMN-Cycle 3	UMN-Cycle 4	TLI Cycles 4, 5	IWG-NAM	M26 × M35	
Yield component	SESP	—	—	—	—	—	—	8	8
Yield component	SPIN	—	—	17	—	—	—	8	25
Yield component	SPLN	—	—	11	—	—	—	—	11
Yield component	SPWT	—	—	3	—	—	—	—	3
Yield component	SYIN	—	—	—	—	—	—	8	8
Yield component	YLD	—	—	14	—	—	—	—	14
	Total	32	108	173	31	39	169	110	662
	Reference	Bajgain et al. (2019b)	Zhang et al. (2017)	Bajgain et al. (2019a)	Bajgain and Anderson (2021)	Crain et al. (2020b)	Altendorf et al. (2021b, c)	Larson et al. (2019)	

ANTH: Anthesis, BLS: Bacterial leaf streak, CRCI: Crown circumference, EMRG: Spike emergence, FHB: Fusarium head blight, FLSP: Number florets per spikelet, INCR: Number of inflorescences per crown, INLE: Inflorescence length, RABR: Rachis breaks, SEAR: Seed area, SEFL: Number of seeds per floret, SELE: Seed length, SEMA: Seed mass, SENA: Threshability/Percentage of seeds threshed out naked, SESH: Seed shattering, SESP: Number of seeds per spikelet, SEWI: Seed width, SI: Self-incompatibility, SPIN: Number of spikelets per inflorescence, SPLN: Spike length, SPWT: Spike weight, STLE: Stem length, STWI: Stem width, SYIN: Total seed yield per inflorescence, YLD: Grain yield per plant.

program and also had exceptionally long and heavy seeds. Complimenting work by Zhang et al. (2017), the M26×M35 family was further evaluated for threshability, seed shattering, and critical spike yield traits including seeds per floret (best described as FSU, since there is a maximum of one seed per floret), florets per spikelet, spikelets per spike, and agronomic traits like earliness of spike emergence (Larson et al. 2019). A total of 111 QTL for 17 traits were detected with at least 2 QTL on each LG, including QTL with relatively high LOD scores for seed length (12.0) on LG6, seed shattering (15.0) on LG11, and spike length (13.0) on LG9. A total of nine significant markers explained up to 46.8% of the variation for threshability across five location-years. The highest broad-sense heritability ($H = 0.88$) and most QTL (10) was observed for the earliness of spike emergence, with a maximum of 49.6% of the variation explained. For seed size traits, seven QTL markers explained up to 37% of the variation in seed length and five markers explained 20.8% of the variation in seed mass. The M26×M35 QTL were also aligned to the newly available IWG version 2.1 draft genome sequence for comparison to possible domestication orthogenes (Larson et al. 2019).

As a self-incompatible species, IWG is ideally suited to genome-wide association studies (GWASs) because linkage disequilibrium (LD) is expected to be low, meaning that potentially high resolution of QTL locations is possible if a sufficient number of DNA markers and a genome reference sequence are available (Flint-Garcia et al. 2003; Gaut and Long 2003; Wright et al. 2008). The IWG draft genome sequence has been especially useful for GWAS (Bajgain et al. 2019a, b; Bajgain and Anderson 2021; Altendorf et al. 2021c, b), beyond what was achieved with the first GBS consensus map (Zhang et al. 2017; Larson et al. 2019). Using 8,899 GBS markers at four site years in Crookston and St. Paul, MN, a total of 154 loci associated with 7 yield traits including spike yield, thousand kernel weight (TKW), seed length, seed width, number of spikelets per spike, spike weight, and spike length were detected (Bajgain et al. 2019a). As many as 53 QTL were detected for TKW, with R^2 values of 2.0–4.4%. As few as three QTL were detected for spike weight, with R^2 values of 2.6–4.1%. The single highest R^2 and value for any one marker was 10.8%, LOD = 11.9, for seed width, but no other trait exceeded 4.9%, LOD = 5.9. In addition to identifying QTL, the draft genome allowed estimates of LD decay. In the UMN-Cycle 3 population, LD when $r^2 = 0.2$ was 0.7 Mbp or 4.38 cM when using genome sequence or consensus linkage map, respectively (Bajgain et al. 2019a). This compared favorably to 5 cM estimated in the UMN-Cycle 1 population that used the consensus linkage map

only (Zhang et al. 2017). This value in the UMN-Cycle 4 population is 0.23 Mbp (Bajgain and Anderson 2021).

A NAM population of 1,168 F_1 progeny derived from 10 unique donor parents to one common parent was used to identify QTL controlling the maturity, or “earliness,” at phenological stages of spike emergence and anthesis (Altendorf et al. 2021c) in addition to seed shattering and threshability traits (Altendorf et al. 2021b) based on clonally replicated field evaluations in MN and KS over two years. The NAM study used both association mapping and QTL interval mapping to identify loci associated with traits of interest. The NAM study also constructed a new consensus linkage map which contains 3,144 GBS markers spanning 3,385 cM (Haldane’s mapping units) in 21 LGs with high correlations ($r > 0.95$) to the linear map order of at least 17 LGs in the first IWG GBS consensus map (Kantarski et al. 2017). Association mapping in the NAM population identified 17 and 26 significant markers for spike emergence and anthesis, respectively, though only 2 markers, both for spike emergence, were significant across both locations and years. A total of 16 and 6 QTL were detected by linkage mapping for spike emergence and anthesis, with greater repeatability across locations and years. Interestingly, the maximum percent variation explained (PVE, R^2) by association mapping markers was about 4.1%, but the R^2 for QTL ranged from 9 to 66% in specific NAM families. Several QTL influenced both spike emergence and anthesis by association mapping and linkage mapping, respectively, with some makers closely associated with an IWG ortholog of the barley *Ppd-H1* gene. Spike emergence QTL aligned to a *Phytochrome B* (*PhyB*) orthogene on chromosome 11, and a *Vernalization 1* (*Vrn1*) orthogene on chromosome 13 in the IWG-NAM population and M26 x M35 family (Larson et al. 2019).

Association mapping detected a total of 75 markers, including 2 or more markers on 7, 14, and 3 chromosomes for brittle rachis, floret shattering, and free threshing traits, respectively, across 2 or more location-years in the IWG-NAM study (Altendorf et al. 2021b). Another 7, 3, and 9 chromosomes had only one significant marker per chromosome in 1 location-year for these traits, respectively. The PVE of these GWAS markers was less than 2%, but one marker explained 49% of the variation in floret shattering at one location. The only brittle rachis marker that was significant in all four location-years was located 216 bp from an ortholog of the *Brittle rachis 2* (*Btr2*) gene, providing compelling evidence of meaningful GWAS analyses in the IWG-NAM population. Many of these GWAS markers were also supported by linkage mapping, which detected 6, 7, and 6 QTL across 3 location-years for brittle rachis, floret

shattering, and free threshing traits, respectively. Another 1, 3, and 5 QTL were also detected in one environment for these traits, respectively.

A recent GWAS study by Bajgain and Anderson (2021) used multi-allelic haplotype markers as well as single markers to discover genomic regions controlling domestication traits in IWG. Specifically, genetic mapping was carried out for plant height, seed length, seed width, shattering, threshability, and seed mass using the fourth-cycle IWG breeding population at the UMN with a population size of 637 genets. The population was evaluated at two locations in MN, United States, in 2019 and 2020. The GWAS used 5,379 haplotype blocks and 25,909 single SNP markers. A total of 31 SNP markers and 17 haplotype blocks were significantly associated with the domestication traits which were distributed across 14 and 11 chromosomes, respectively. All marker–trait associations were found to be of small–medium effect as the PVE of significant markers ranged from 4 to 6%. One SNP marker located on Chromosome 8 was discovered in both single-marker analysis as well as haplotype analysis and was additionally associated with both seed length and seed mass. Differences in QTL detection between single markers and haplotype blocks can be attributed to differences in allelic frequencies and marker LD with the QTL (Slatkin 2008; Hamblin and Jannink 2011). This work by Bajgain and Anderson suggest that using both marker types in GWAS can provide a more comprehensive understanding of the genetic architecture of complex quantitative traits in IWG.

The effort invested in characterizing the genetic control of IWG traits has resulted in discovery of more than 650 QTL in several breeding populations across multiple research groups (Table 3.2, Figure 3.6). Essentially, all the IWG breeding and genetic research to date has focused on yield and yield-related traits as well as domestication traits such as seed size, seed shattering, and threshability. The research has overwhelmingly shown that the major traits targeted for improvement in IWG are highly polygenic even for domestication traits of non-shattering and free threshing. Results by Crain et al. (2022) suggest that non-shattering and free threshing phenotypes could be individually controlled by over 150 loci. This contrasts with work in many domesticated species where major domestication alleles are qualitative such as the *Q* gene for free threshing in wheat (Simons et al. 2006) and the brittle rachis (*Btr*) genes in barley (Pourkheirandish et al. 2015). While few large-effect loci have been discovered, these results have helped better understand the genetic architecture of important IWG traits. Discovery of QTL and associated markers can also help make informed breeding decisions, e.g. by applying the information in GS models as discussed in Section VI.A.3.

3. Genomic Selection

Molecular techniques such as GBS and access to a draft genome have allowed the IWG breeding community to exploit GS. In GS, markers are used to cover the entire genome, ideally where each gene is in LD with a marker, and the GEBV can be established for each individual (Meuwissen et al. 2001). Zhang et al. (2016) provided the first assessment of GS in IWG in the UMN breeding material, suggesting that GS could be a viable selection strategy (Figure 3.5). Since that initial study, several other studies have been conducted within the UMN and TLI breeding programs (Bajgain et al. 2019a, b, 2020a; Crain et al. 2020a, 2021a, b). While numerous studies have evaluated GS in *Triticeae* species (reviewed by (Rutkoski et al. 2017)), the application of GS in IWG has led researchers to evaluate many of the factors that can affect GS accuracy, including choice of statistical models, marker number, and training population design.

The choice of statistical model has been shown to affect GS accuracy. A wide variety of statistical models have been evaluated with GS, with all models being able to accommodate a larger number of markers than sampled individuals (Lorenz et al. 2011; Heslot et al. 2012). In two studies, Zhang et al. (2016) (UMN breeding program) and Crain et al. (2021b) (TLI breeding program) evaluated the choice of statistical model in GS accuracy. Zhang et al. (2016) found evidence that either a Gaussian kernel or random forest often produced optimum results, and other tested models including RR-BLUP, Bayes A, Bayes B, Bayes Cpi, Bayesian LASSO, Bayesian ridge regression, and Bayesian RKHS all performed similarly. Both random forest and a Gaussian kernel should be able to detect nonadditive effects, which could have led to the higher prediction accuracy as IWG is highly heterozygous. Within the TLI breeding program, Crain et al. (2021b) found that RR-BLUP generally performed the best across 46 different traits, and that RR-BLUP's simple implementation and minimal computational resources made it a preferred choice. Similar to Zhang et al. (2016), other tested models were similar in GS performance. One caveat in explaining these differences could be how the training population was analyzed before entering the GS model. Crain et al. (2021b) adjusted data with an interaction between parents, potentially removing dominance (nonadditive) interaction, where this potential interaction remained in the data by Zhang et al. (2016). Bajgain et al. (2020a) evaluated dominance interaction in GS models and found that inclusion of dominance effects in the model improved trait predictions by up to 3 percentage points. While current results suggest that GS can be effective, choice of GS model will remain an active area of research as accurate GS results will be imperative to driving genetic gains (Crossa et al. 2017).

Along with choosing an appropriate statistical model, marker number can play an important role in GS accuracy. Typically, as marker density increases GS accuracy increases (Solberg et al. 2008; Jannink et al. 2010). Both Zhang et al. (2016) and Crain et al. (2021b) showed that increasing marker number increased GS accuracy in different IWG populations. In both studies, there appeared to be diminishing returns as the marker number increased. However, in both studies even at 3,200 and 17,500 markers, GS accuracy was still increasing with marker number, although large differences in GS accuracy and increased variability occurred when using less than 2,500 markers. Thus, both studies concluded that several thousand markers were sufficient to obtain high GS accuracy. As GBS data often has high levels of missing data (Davey et al. 2011; Poland and Rife 2012), Crain et al. (2021b) evaluated the effect of missing data, finding that GS model accuracy was higher with more markers, even if missing data increased. This included markers with up to 70% missing data, suggesting that the number of markers was more important than optimizing the genotyping method or obtaining markers with minimal missing data. As genotyping technologies improve, potentially leading to whole-genome skim-sequencing approaches (Jensen et al. 2020; Pavan et al. 2020), it is likely that the number of markers available for GS will also increase.

Training population design has also been studied by Zhang et al. (2016) and Crain et al. (2021b). Work within the UMN breeding program by Zhang et al. (2016) showed that using 8–10 genets per each half-sib family was sufficient to make accurate predictions, although selection within a family was not recommended. Using the TLI breeding program, Crain et al. (2021b) showed that GS accuracy increases with increasing population size. Using over 2,500 genets per trait, accurate GS predictions could be made regardless of family (half-sib) structure of the training population. This suggests that increasing training population size is more imperative than optimizing a specific distribution of families within the training population. Following up on large training populations, Crain et al. (2021a) combined up to three breeding cycles (>3500 genets) into GS predictions. While some work in wheat has shown that including historical data can reduce GS accuracy (Rutkoski et al. 2015), current results in the TLI breeding program have suggested a benefit of retaining historical data in models.

The detailed studies of GS implementation in IWG at the UMN and TLI breeding program have provided a strong foundation for application and future research. The initial study by Zhang et al. (2016) implied that large field trials evaluating tens of thousands of genets could be reduced to monitoring a thousand genets while still making

genetic gains. This amounted to substantial labor and time-savings while maintaining a two-year selection cycle. Within the TLI breeding program, genetic gains were boosted by completing a breeding cycle each year, theoretically doubling gains (Crain et al. 2021b). Current results from the single-year cycle suggest that GS can result in gains of up to 8% per annum for spike yield and up to a 14% per annum change in free threshing and shattering (Crain et al. 2021a). In addition, the perennial nature of IWG allows multiyear training populations to be developed. Under phenotypic selection, multiple years of evaluation would directly lengthen the breeding cycle, three years to complete a breeding cycle with two years of phenotypic observation (DeHaan et al. 2018), yet in GS multiple years for each cycle can be evaluated while crossing plants each year (Crain et al. 2021a). This multiyear evaluation could provide a way to make progress for perennial traits such as yield decline which are not observed in the first year of data collection (Jungers et al. 2017; Hunter et al. 2020a).

In addition to performing foundational GS work for IWG, several studies have evaluated techniques to account for the $G \times E$ interactions that are common in crop breeding populations. Work by Crain et al. (2020a) combined data from the UMN and TLI breeding programs with GS models developed to predict yield, free threshing, and shattering. Interestingly, models for the key domestication traits of free threshing and shattering had high GS accuracy even when the training population was based on one program and the prediction was for the other program. This across-program prediction accuracy was not observed for spike yield, suggesting that for traits that experience less $G \times E$ interaction training models can be made with data from any program. Thus, only one breeding program could evaluate a trait, and progress could be made for all programs. For spike yield, with much more $G \times E$ interaction, genetic progress may be tied to program-specific or local models. Work by Bajgain et al. (2020a) incorporated $G \times E$ interaction, which improved model predictions by 23 percentage points for free threshing, 18 percentage points for grain yield, 15 percentage points for spike weight, and 20 percentage points for spike length. These results suggest that GS including $G \times E$ interaction effects could play an important role in driving genetic gain and identifying germplasm that is suited to diverse environments.

As breeding programs continue to search for better approaches to improve genetic gain, GS appears to be a practical tool (Figure 3.5). While the early results of GS research show promise in the development of accurate GS models (Zhang et al. 2016; Bajgain et al. 2019b, 2020a; Crain et al. 2020a, 2021a), GS will continue to be an area of active research. Enhancing GS models to provide the most accurate prediction

will directly drive genetic gain (Crossa et al. 2017), and this could occur through judicious use of training population, marker number or statistical model. For example, GWAS is also straightforward to implement with the data required for GS. Studies by Bajgain et al. (2019a, b) have harnessed GWAS results as fixed effects within the GS models resulting in up to a 14 percentage points increase in accuracy of GS model prediction depending on the trait. Using QTL identified for resistance to FHB, Bajgain et al. (2019b) found the predictions toward improved FHB resistance increased by 24–125%. In addition, new techniques like high-throughput phenotyping (Araus and Cairns 2014) can provide high dimensional data that can be linked to trait expression. Within wheat, canopy temperature and normalized difference vegetation index have been combined with GS models to increase model accuracy (Rutkoski et al. 2016; Crain et al. 2018). The choice of application for each breeding program should reflect the resources available and the estimated gains from each potential application method in an effort to optimally allocate resources (Heslot et al. 2015).

B. New Approaches for Trait Enhancement

1. Genome Editing/EcoTILLING (identifying useful genes in wild material)

With current grain yield and seed mass of IWG less than 20–25% of annual wheat, traditional breeding with or without GS have yet to produce IWG varieties that are profitable enough for farmers to produce at large scale without some other incentives to plant perennials. The challenge faced in IWG's domestication as a grain crop may be partially attributed to evolutionary adaptations resulting from ecological limitations associated with its perennial life history (Smaje 2015). However, ecological limitations and structure of natural ecosystems do not always apply to domesticated species and agricultural ecosystems (Denison et al. 2003; Crews and DeHaan 2015). Potentially useful domestication traits may arise from recessive loss-of-function mutations (Østerberg et al. 2017) or random modifications of genes and physical features that are already present (i.e. slow gain of new functions), without the need for fundamentally new genes or characteristics (Denison et al. 2003; Doebley 2006). As an allohexaploid, IWG has up to three independent sets of genes (from three subgenomes) and potentially two alleles totaling up to six possible alleles per gene per plant. This duplication of genes with high levels of heterozygosity might mask or obscure potentially useful alleles for traits such as large seed size and are likely to be rare in natural IWG populations.

Targeted Induced Local Lesions in Genomes (TILLING) and EcoTILLING (Ecotype TILLING) utilize rapidly improving DNA sequencing technologies to locate and detect induced mutations or natural mutations, respectively, in specific genomic regions of interest (Barkley and Wang 2008). A number of important domestication genes have been identified in cereal crop species for traits including seed size, shattering, and free threshing (Doebley 2006; Gross and Olsen 2010; Lenser and Theißen 2013; Swinnen et al. 2016; Haas et al. 2019; Purugganan 2019), and some of these traits have potential value in IWG (Larson et al. 2019; DeHaan et al. 2020). Along with targeted mutagenesis of specific genes or features, it is now possible in species such as wheat to use base editors that do not require double-strand breaks or exogenous DNA and DNA-free genome editing systems (Liang et al. 2017; Zong et al. 2018). These approaches of targeted mutagenesis have potential use for IWG, though they still require time-consuming and resource-intensive tissue culture steps. While an efficient plant regeneration system utilizing embryonic suspension cultures has been developed for IWG and related species (Wang et al. 2003b), other gene editing approaches that leverage viruses (Jackson and Li 2016; Hu et al. 2019) or nanoparticles (Cunningham et al. 2018; Demirer et al. 2019) to circumvent the need for tissue culture are being developed for plants including wheat. Methods to deliver plant regulators and gene editing reagents directly into somatic cells that can produce gene-edited shoots, without tissue culture, have also been developed for some dicots (Maher et al. 2020). All of these diverse approaches to targeted mutagenesis have potential use for IWG (DeHaan et al. 2020).

Gene editing, TILLING, and EcoTILLING are potentially useful approaches of generating and discovering new or rare DNA variants in IWG, yet the duplication of genes and high levels of heterozygosity could pose significant obstacles to uncovering such useful domestication alleles (DeHaan et al. 2020). Innovative approaches of next-generation EcoTILLING (Harfouche et al. 2012) and MAS (Wang et al. 2007) are needed to efficiently pyramid multiple genes present in IWG in order to overcome roadblocks to its successful domestication and improvement (DeHaan et al. 2020).

2. Genotyping Methods

Although GBS was initially designed as a reduced-representation-sequencing (RRS) strategy using restriction enzymes to reduce the genome complexity and decrease genotyping cost, improved sequencing methods and availability of crop genomes can enable a shift from

RRS to whole-genome sequencing (WGS). The WGS approach can be extremely cost-effective if the library preparation is multiplexed and if the overall genome coverage is reduced. Skim sequencing is a WGS approach that targets less than 1x coverage and can be used to genotype a large breeding population (Bayer et al. 2015). Compared to GBS, WGS provides high-resolution markers which can benefit linkage and QTL mapping, GWAS, genome assembly validation, and marker-assisted GS. Using high-density markers can increase the ability to identify causative SNPs or genes associated with traits of interest, which are difficult to identify using GBS methods. WGS can also provide benefits for genome assembly validation because of the high marker density and more precise anchoring.

While RRS methods (primarily GBS) have been widely used for GWAS and GS study of IWG (Zhang et al. 2016; Bajgain et al. 2019a; Crain et al. 2021a), an economical and higher throughput genotyping method based on WGS can afford screening larger breeding populations to provide higher density markers and increase efficiency of GS. However, to leverage this technology in heterozygous IWG, imputation of missing alleles is crucial to implementation. Recombinant inbred lines (RILs) or doubled-haploid (DH) populations are currently better suited for WGS strategies because they are completely homozygous and imputation of missing alleles is not as challenging of a problem. However, for an outcrossing population with high level of heterozygosity (IWG), imputation is not as accurate because many heterozygous alleles are miscalled as homozygous alleles due to low coverage of sequencing (Scheben et al. 2017). While there are several existing software packages used for imputation, such as Beagle (Browning and Browning 2009), STITCH (Davies et al. 2016), AlphaImpute (Hickey et al. 2011), AlphaPeel (Whalen et al. 2018), or magic impute (Zheng et al. 2018), none of them takes population structure into account for genotype calling and imputation. Current work is evaluating how imputation programs in conjunction with the reference genome can be used to create accurate imputations even at low sequence coverage levels. While WGS is poised to become the marker platform of choice, there is currently a trade-off in cost and accurate imputation, especially at low sequence coverage levels.

3. Phenomic Selection

In search of faster and more efficient methods to accelerate IWG's domestication timeline, multiple methods are being evaluated to generate predictive breeding value models. While GS has reduced breeding

cycles down to a single year and is making rapid progress, the cost of genotyping species like IWG, which has a large and complex genome, is still substantial at more than \$10 per plant. TLI, in collaboration with researchers from the Danforth Center, KSU, and USDA, is testing the feasibility of using phenomics-estimated breeding values (PEBVs) in selection to accelerate new crop improvement. Recent research suggests that with sufficient phenotypic dimensionality for traits like seedling growth and spectral signatures, breeding values of traits like yield can be predicted in the same way as with GS by using a kinship matrix (Segura et al. 2014; Rincent et al. 2018; Parmley et al. 2019; Lane et al. 2020; Moreira et al. 2020). Within the long life of a perennial species, phenomics could also enable accurate seedling selection for important later-stage traits. Once this approach is validated, phenomic relatedness could supplement or substitute genomic relatedness in breeding applications. This would reduce breeding cost by eliminating the need for genotyping breeding populations while enabling larger and more diverse populations, resulting in accelerated progress (Van Tassel et al. 2022).

4. Introgression from Wheat

Annual wheat is the most widely grown crop in the world and has many properties that are desirable to humans as a food source. Variation for many essential traits may be present in IWG, but it could take many years to identify the variants in the genome and breed to improve these traits. Several traits such as the kernel width or plumpness, large seed size, and semi-dwarf habit have not been observed in IWG. Since it is possible to make crosses between annual wheat and IWG, TLI is introgressing small segments from wheat chromosomes into IWG through wide crosses between wheat and IWG followed by backcrossing to IWG.

To introgress wheat chromosomes into IWG, durum wheat has been used as the female parent, and the resulting F_1 progeny were treated with colchicine to double the chromosome number and restore fertility. These durum wheat \times IWG full amphiploids were then used as the male parent in crosses to IWG. These crosses are difficult to achieve, as thousands of developed seeds yield only a few live plants through embryo rescue. Subsequently, these plants and their progeny have been backcrossed to IWG repeatedly. Currently, a set of putative monosomic addition lines, possessing the full genome of IWG with single durum wheat chromosome additions, are being identified using GBS and skim sequencing approaches. Field screening of large populations of these amphiploids is expected to identify desirable traits, and promising individuals will be genotyped to determine the precise length and locations of wheat introgression segments.

Genotyping of these plants has revealed that some progeny have now eliminated all alien durum wheat chromosomes. However, many of these plants show unique phenotypes, such as reduced height. Wide hybridization is recognized as a method to induce genetic variation through transpositions, mutations, deletions, methylation changes, and other genomic restructurings (Shaked et al. 2001; Bashir et al. 2018; Qiu et al. 2020). Therefore, wide hybridization of IWG to other species, followed by backcrossing to eliminate alien chromosomes, may be a viable technique to generate novel variation to accelerate domestication when low genetic variability limits progress.

5. Mutation Breeding

Although IWG germplasm contains abundant variation for most traits of interest for domestication as a grain crop, some variation is apparently lacking. Thus far, breeders have not identified dwarfing alleles that would substantially reduce height while benefiting grain production. Another trait of interest is seed plumpness. Wild plants have very slender seed, and a mutation producing more spherical seed would be a great improvement.

Induced mutations have contributed substantially to useful genetic variation in crop species (Mba 2013). However, detecting and using induced mutations presents unique challenges in an outcrossing polyploid species such as IWG. Ukai (2010) developed a model which would allow identification and use of mutated alleles in an outcrossing species. Chemical mutagenesis has been used successfully in wheat (Chen et al. 2012). At TLI, an ethyl methanesulfonate (EMS) protocol for IWG was developed (DeHaan, unpublished data). While a 0.8% solution of EMS has obtained a germination rate of about 40% in wheat, this concentration eliminated germination in IWG. The final methodology for IWG was to soak seed in a 0.18% EMS solution for 16 hours, followed by 4 hours of rinsing and three days at 13 °C. This protocol resulted in a germination rate of about 65%, compared to 93% for the untreated control.

About 2,400 EMS-treated seedlings were established at TLI in the fall of 2014. Because new recessive mutants would be masked by dominant alleles, mutagenized plants were screened for detectable dominant mutations. As some dwarfing genes in wheat are dominant (Sun et al. 2019), the objective was to search for short but vigorous semi-dwarf mutants. Unfortunately, there was no clear evidence of novel mutations controlling any traits of interest. All plants appeared to be well within the natural range of variation. Likely, a TILLING approach would be necessary to separate novel mutations from the

background variation (Chen et al. 2012). MAS could then be used to pyramid the necessary mutations in homologs across the three subgenomes of IWG.

VII. RATE OF INTERMEDIATE WHEATGRASS DOMESTICATION

We define domestication as the transformation of other species, through interactions with humans, such that the utility of the species to humans is increased. Non-shattering is a classic example of a clear domestication trait, since loss of shattering represents reduced natural dispersal for the plant species, dependence upon humans for dispersal, and facilitation of human harvest. Ancient domestication of crops such as wheat, barley, and rice is thought to have begun thousands of years after humans first began harvesting wild ancestors of these crops (Meyer et al. 2012). In these millennia, humans were harvesting wild grains, but the clear achievement of a domestic non-shattering phenotype had to wait upon the chance mutation of genes controlling shattering, human recognition of the novel phenotype, and then spread of the non-shattering phenotype throughout a growing region. Although in theory a highly effective allele that eliminates shattering could be selected and fixed within a population within about a century, in reality this process is found to have generally required millennia, as is documented in rice and wheat (Tanno and Willcox 2006; Allaby et al. 2017). Traits such as seed size appear to have changed very slowly, with seed size in wheat essentially unchanged for the first several thousand years of its cultivation (Tanno and Willcox 2006).

Since ancient domestications proceeded over millennia, the timeline for modern domestication efforts is an open question. Using modern methods to collect phenotypic data and perform statistically rigorous selection, how rapidly might a new grain crop be domesticated? In the case of IWG, we have the added consideration of the plant's perennial nature. Is it possible to obtain the necessary increases in seed size, grain yield, and other economic traits to develop a crop that can compete in productive capacity with annual grains? Some have argued that ecological limitations associated with life history and the inherent trade-off between longevity and reproductive effort will make the domestication of a perennial grain crop prohibitively difficult (Smaje 2015). Others contend that the perennial habit will not necessarily prevent domestication, and could perhaps allow greater yields in some environments (DeHaan et al. 2005; Van Tassel et al. 2010; Crews and DeHaan 2015). Here we will examine recent evidence of

progress in domesticating IWG to estimate the required timeline for domesticating this new crop and reexamine the feasibility of the effort based on current data.

In fall 2018, TLI established direct-seeded trials in Salina, KS, United States, to evaluate breeding progress using remnant seed across eight breeding cycles. Due to space and seed limitations, the design was partially replicated with not all populations evaluated in every environment. Performance in seven environments was tested, including the following variables: year of harvest (2019 or 2020), row spacing (19.1 versus 91.4 cm), and irrigated versus dryland field. Combine harvesting was used to obtain estimates of mechanically harvested yields. Prior to mechanical harvest, about 20 random spikes were harvested by hand from each plot. These were measured and threshed separately to determine shattering (a visual rating from 0 to 4), FSU, free threshing ability (as percent naked seed), and average yield of naked seed per spike. Harvested yield per area was divided by yield per spike to obtain an estimate of spike density within each plot. The Cycle 0 used in this evaluation was the population resulting from one cycle of selection at the BFPMC, as described in Cox et al. (2002). The Cycle 1 population was the starting population for selection at TLI, which included plants from the second cycle of selection at BFPMC. Cycles 2–8 in this experiment are the populations resulting from recurrent selection at TLI. Methods of selection in Cycles 2–7 are described in DeHaan et al. (2018), and methods for GS in Cycle 8 are described in Crain et al. (2021a). Notably, the Cycle 7 seed was not available for this study, and the Cycle 8 seed was subdivided into populations based on unique traits of the parents. The data points are presented from each subdivision, but the mean is representative of the Cycle 8 population.

Free threshing ability, estimated as percent naked seed in the sample after threshing, responded quickly to selection over eight generations, with naked seed percentage increasing by 394% (Table 3.3). The response rate was even more surprising, considering that in the early cycles free threshing was not directly targeted by selection (DeHaan et al. 2018). In the ancient domestication of wheat, free threshing ability is understood to have substantially reduced time necessary for threshing and was likely greatly preferred by farmers (Tzarfati et al. 2013). However, several thousand years of wheat cultivation were required before free threshing was identified and spread throughout wheat growing regions. In the case of IWG, free threshing was readily identified and is rapidly increasing with fewer than 10 generations of breeding. Modern producers attempting to grow Kernza grain are frequently requesting free threshing varieties, due to the inefficiencies they identify in harvesting,

Table 3.3 Response to eight cycles of selection for grain production traits in intermediate wheatgrass. Populations were evaluated in a partially replicated design across seven different environments with varied conditions: two years, irrigated, dryland, narrow rows, and wide rows. Varied environments were standardized by subtracting the mean performance of Cycle 0 at each location.

Trait	Average value of cycle 0 across sites	Change cycle ⁻¹ , slope of regression	Percentage change from cycle 0 to 8
Grain yield (kg Ha ⁻¹)	228	58.5	205
Seed mass (mg Seed ⁻¹)	5.0	0.47	75
Shatter rating (0–4)	3.1	–0.14	–36
Naked seed (%)	12.6	6.21	394
Single-spike yield (g)	0.276	0.027	78
Floret site utilization (%)	37.9	NS	NS
Spike density (Spikes m ⁻²)	47.4	4.0	68

transporting, and cleaning grains that remain within their hulls at harvest.

Grain yield on an area basis tripled in eight generations of selection (Table 3.3). The consistent progress observed for grain yield (Figure 3.7) is particularly important because this trait is likely the most critical to commercial success of IWG grown for grain. The rapid gain in yield per area observed is somewhat surprising, given that selection was only performed on widely spaced plants, while in the evaluation direct seeding was used to establish plants in commercially relevant densities. However, this result is consistent with progress obtained by Knowles (1977). In that long-term selection experiment with IWG, about 1,000 widely spaced plants were evaluated per generation, with selection in the second or third year of seed production. Five cycles of selection with these methods resulted in an approximate doubling of seed yield per area.

Knowles (1977) documented a 25% increase in FSU (floret site utilization). This increase was presumed to be one of the drivers of increased seed yield over five cycles of selection. However, selection at TLI did not produce a measurable change in FSU. Presumably, increased grain yield in this program is coming through other avenues, such as increased seed size, reduced seed shattering, and an increase in heads m⁻² (Table 3.3, Figure 3.7). One might expect that selecting on grain yield per spike would produce plants with fewer spikes, due to a likely trade-off between seed number and size. Instead, these results show selection increasing the number of heads produced on an area basis. Perhaps plants selected for high seed production are more likely to produce abundant heads when grown in a dense stand. Alternatively, because spike number was calculated rather than measured directly,

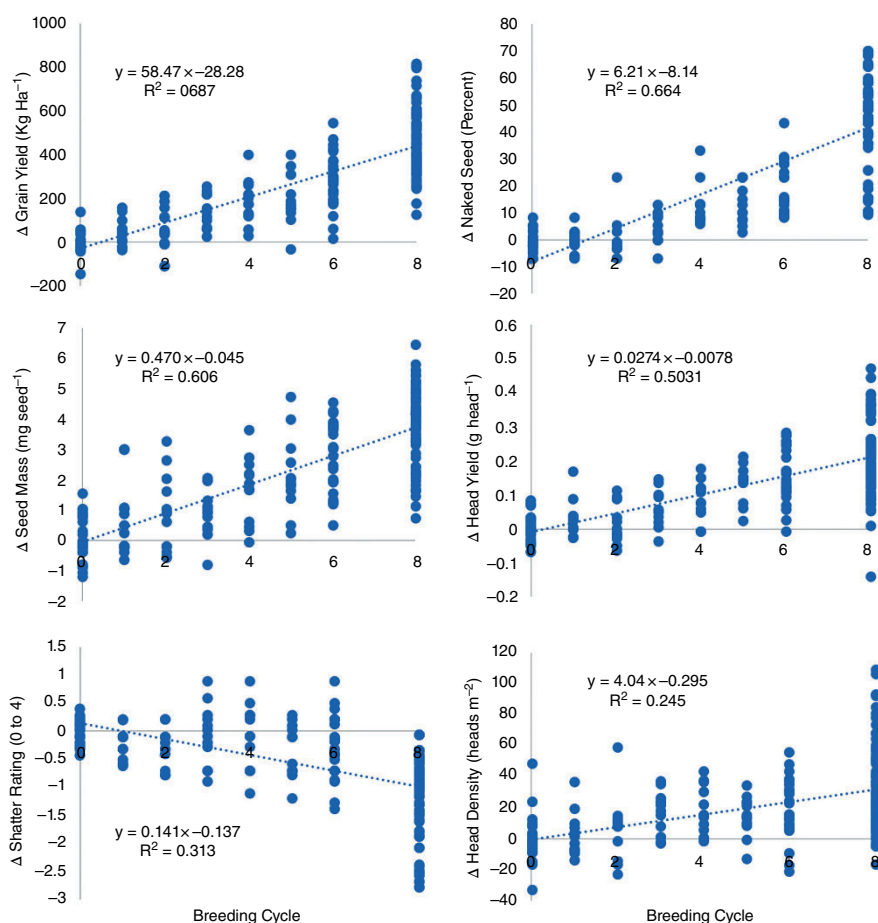


Fig. 3.7. Changes in six traits across eight cycles of intermediate wheatgrass breeding at The Land Institute. Data is presented as change relative to the starting population in each of seven different evaluation environments. Populations were partially replicated across environments with Cycles 0, 1, and 6 evaluated in all environments. Cycle 0 was the population resulting from one cycle of selection by Big Flats Plant Materials Center. Cycle eight was evaluated as a subdivided population, resulting in a large number of data points. Cycle 7 seed was unavailable for evaluation.

it is possible that reduced shattering and improved free threshing (Figure 3.7) inflated the spike number calculation.

Modern breeding approaches applied to the domestication of IWG appear on track to fix important domestication traits such as non-shattering and free threshing in decades rather than the centuries to

millennia required in ancient domestications. Extrapolating from the results (Table 3.3) and considering three more cycles have been completed, full shatter resistance should be fixed in the population within 11 more cycles. Note that many individuals in the population are fully shatter-resistant, although the trait is not fixed. Free threshing is approaching fixation more rapidly, with four more cycles predicted to attain a consistently free threshing population.

In the 10-year period of 1919 to 1928, yield of wheat in Kansas averaged 888 kg ha^{-1} (Kansas State Agricultural College 1929). Extrapolating from the linear model for yield (Figure 3.7), the current breeding cycle at TLI is expected to produce on average 872 kg ha^{-1} . For the sake of simplicity, we will assume that IWG in Kansas currently yields on par with wheat of the 1920s. From 2010 to 2019, average Kansas wheat yield was $2,825 \text{ kg ha}^{-1}$ (National Agricultural Statistics Service 2019). Therefore, in 91 years, wheat yields in Kansas increased by about 1937 kg ha^{-1} , or $21.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$. Over roughly the same period, genetic gains were estimated to increase wheat yield in this region by about $14.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Battenfield et al. 2013). Therefore, about 69% of the yield increase in this period can be attributed to breeding and genetics, and the remainder to improved agronomic practices.

The expected time required for IWG to achieve yields on par with recent Kansas wheat yields of $2,825 \text{ kg ha}^{-1}$ will depend on breeding progress and agronomic improvements in management of this new crop. If we assume that the situation is similar to wheat over the past 9 decades, then 69% of the yield increase must come from breeding. We will also assume for simplicity that with GS, one breeding cycle is completed per year and gain per cycle remains on the linear trend observed thus far. With these assumptions, we would predict that yields equivalent to current wheat production would require another 23 years. If management is already optimal, then all gains would have to come from breeding and we would expect more than 33 years would be required to match current wheat yields.

These projected timelines to wheat-like yields in Kansas are substantially longer than those presented in a previous analysis of two breeding cycles in the same location (DeHaan et al. 2014). In the previous study, populations were only evaluated in a single environment for one year, and yields were abnormally high, resulting in the prediction that only 12 more breeding cycles would be required to attain wheat-like yields. Projections regarding seed size were more similar to the current analysis, with another 55 cycles predicted to attain 30 mg seed^{-1} , while extrapolation from the current analysis (Table 3.3) predicts another 45 breeding cycles to reach 30 mg seed^{-1} .

If yields equivalent to current wheat production can be obtained from IWG with another 2 to 3 decades of research, as implied by the above analysis, we will be able to roughly estimate the timeline for *de novo* domestication of this new grain crop using modern methods. If accelerated breeding using GS and a single year per breeding cycle were used from the beginning, we might expect that a species could attain performance on par with comparable annual cereal crops within 35 cycles of selection. With each cycle taking a year and an initial five years to acquire germplasm and develop selection models, a timeline of less than 40 years might be realistic. Every species will present unique opportunities and challenges, but the experience with IWG allows us to roughly describe a reasonable timeline for modern domestication of a new perennial grain crop.

It is worth noting a few of the assumptions that would go into a 40-year timeline for domestication. First, we would assume that genetic diversity is not soon exhausted, so that progress can continue on a linear trend for several decades at least. Second, we must assume that major new challenges to productivity do not arise, such as a new pest or disease. Unforeseen obstacles of this nature could easily delay crop development by decades. Finally, we assume that domestication follows a linear pathway toward incremental improvement, rather than leaping ahead as domestication traits are fixed in a population. So far, the improvement in classic domestication traits such as non-shattering in IWG has been steady and incremental. However, accelerated domestication through gene editing, mutagenesis, or introgression remains possible (DeHaan et al. 2020). These approaches may reduce the timeline by a decade or more but are not expected to fully avoid the inherent time required to domesticate a new species (Van Tassel et al. 2020).

VIII. FUTURE DIRECTIONS

The past two decades of intensified breeding and genetic improvement of IWG have largely focused on important domestication and agronomic traits. While continual progress of these traits remains a top priority, improvement of additional traits will be needed to help the crop expand acreage in mainstream agriculture. One such trait is successful seedling establishment of drilled fields. Our current observations suggest de-hulled and smaller grains have less than optimal emergence rates, affecting proper establishment and growth of seedling stands which can have adverse effects on plant yield. Agronomists have evaluated different planting densities (Jungers et al. 2017; Fernandez et al. 2020), yet active selection for robust seedling establishment and vigorous growth is

not currently a primary focus for breeding programs. Also important is selection for sustained yield and seed mass over multiple cropping seasons. In some environments, IWG plant stands show strong yield decline after 1–2 years of production (Fernandez et al. 2020; Bajgain et al. 2020b). If IWG is to be used as a dual-purpose crop, maintaining a high level of grain yield for food use and biomass yield for forage is essential.

To further the appeal and utilization of IWG grain to food producers and consumers, it is also imperative that the grain provides high nutrition levels while being amenable to different processing techniques such as mixing, malting, and baking. Considerable research has been carried out to characterize the chemical composition and nutritional benefits of IWG flour obtained from IWG varieties (Tyl et al. 2019; Zhong et al. 2019; Banjade et al. 2019a). While the generated knowledge is useful to food processors and consumers, it is not directly applicable in making selection decisions because these studies have used varieties post-release or close to being released. An effective way to incorporate food science research into existing breeding pipelines would be to evaluate spaced-planted genets (training populations, in most cases) for quality traits and apply the information to advance the most promising genets (perhaps in GS).

The effective population size for most modern IWG breeding programs can be traced to just 14 parents. While this extreme genetic bottleneck was initially needed to filter the best-performing genets, a gradual increase in genetic diversity might be warranted to (1) preserve minor yet beneficial alleles of IWG and (2) safeguard the crop from any future biotic or abiotic stress factor that jeopardizes its success. One approach toward this goal is to intermate modern breeding germplasm with wild collections. For example, the IWG accessions deposited at the NPGS are genetically distant from those used in modern breeding programs (Figure 3.2). These genets could be evaluated in isolation away from the modern populations, and the best-performing ones can be crossed with modern genotypes to introduce new alleles. Genome editing is another tool that could be implemented to selectively introduce novel beneficial variation.

Breeding efforts to date have targeted above-ground traits. For the crop to continue providing ecosystem services at a high level, root phenotypes cannot be ignored. Root phenotyping should be carried out to identify potential impact on carbon sequestration, nutrient scavenging, recycling and utilization, and the diversity of root microbiomes. As arbuscular mycorrhizal fungi are known to help the plant cope with drought stress (Li et al. 2019; Mathur et al. 2019), a carefully implemented selection strategy to consider root-related traits while

advancing breeding germplasm could assist in IWG's survivability in hotter environments. On a similar note, selection of genotypes that perform well in waterlogged soil would help expand IWG's current geographical footprint. Combined, IWG genotypes and varieties with improved drought resistance and flood tolerance would safeguard the crop against unpredictable and adverse climatic shifts.

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