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Title: Retention of Wheat Alleles in Imidazolinone-Resistant Wheat x Jointed Goatgrass Recurrent Backcross Generations

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Carol A. Mallory-Smith

The retention of D genome wheat alleles in the progeny of imidazolinone-resistant wheat (R-wheat) and jointed goatgrass hybrids backcrossed to jointed goatgrass was documented in this study. Resistance to the imazamox application was used as a marker for the imidazolinone-resistance gene. Fourteen simple sequence repeat (SSR) markers, for the wheat D genome, were used as unselected markers. R-wheat x jointed goatgrass F_1 hybrids were backcrossed to jointed goatgrass. The resulting BC_1 seed was divided into 2 sub-populations, BC_1s and BC_1u. The BC_1s population was treated imazamox at 26.9 kg ai/ha. The BC_1s survivors (five out of 10 plants) and the BC_1u subpopulation were backcrossed to jointed goatgrass to produce the BC_2s and BC_2u generations. The BC_2s population was treated with imazamox, and three out of 14 plants were resistant. The BC_2u sub-population was either self-pollinated to produce BC_2S_1 plants, or backcrossed

to jointed goatgrass. The BC_2s plants produced no progeny. The BC_2S_1u and BC_3u plants were treated with imazamox. There were resistant individuals in the progeny groups of 10 of 12 BC_1s; 6 progeny groups would have been expected to contain resistant individuals. The D genome wheat SSR markers used in this study allowed polymorphism to be indentified between wheat and jointed goatgrass in 3% agarose gels stained with ethidium bromide. The SSR markers were inherited Mendelianly. The wheat SSRs, because they amplify polymorphic products from various loci in different sizes in wheat and jointed goatgrass will be a valuable tool to further examine gene flow between wheat and jointed goatgrass.

Retention of Wheat Alleles in Imidazolinone-Resistant Wheat x Jointed Goatgrass Recurrent Backcross Generations

by

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Lori Jennifer Kroiss, Author

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DEDICATION

This thesis is dedicated to my little sister, Sara Madelene Kroiss, R.N., who could have gone anywhere, but came here to be with me.

RETENTION OF WHEAT ALLELES IN IMIDAZOLINONE-RESISTANT WHEAT X JOINTED GOATGRASS RECURRENT BACKCROSS GENERATIONS

INTRODUCTION

BACKGROUND

The development of herbicide-resistant wheat, *Triticum aestivum* L., will allow for the control of weeds, such as jointed goatgrass, *Aegilops cylindrica* Host, for which there are no selective herbicides. These new varieties hold promise for the reclamation of jointed goatgrass infested fields and imidazolinone-resistant wheat is performing well in field trials in the Pacific Northwest (Ball et al., 1999). One concern with this technology is the potential for resistance genes to move from wheat to jointed goatgrass as a result of hybridization and subsequent recurrent backcrossing to jointed goatgrass (Zemetra et al., 1998).

Naturally occurring seed bearing hybrids were found in Idaho wheat fields (Mallory-Smith et al., 1996) and more recently in an imidazolinone-resistant wheat field trial (Seefeldt et al., 1998). Six of the seven BC_1 seedlings produced from seed on the hybrids from the field trial survived an application of an imidazolinone herbicide.

An assessment of the potential for herbicide resistance to be transferred from resistant-wheat to jointed goatgrass has begun. An evaluation of the effect

that selection pressure has on resistance gene frequency over generations of backcrossing to jointed goatgrass, and on the retention of wheat D-genome alleles must be included in the assessment. This information would complement recent gains made in the understanding of the fertility (Wang et al., 2000b; Zemetra et al., 1998; Snyder et al., 2000) and the chromosome constitution (Crémieux, 2000; Wang et al., 2000a) of the hybrid and backcross generations.

HERBICIDE-RESISTANT WHEAT ALLOWS FOR SELECTIVE CONTROL OF JOINTED GOATGRASS

Relationship of wheat and jointed goatgrass

Soft white winter wheat (*Triticum aestivum* L.), an allohexaploid, has three paired sets of seven chromosomes (2n = 6x = 42). Each set of paired chromosomes, referred to as a genome, was contributed by a different diploid parent: AA from *T. urartu* Tamanian *ex* Gandilyan, BB from the same ancestral donor as the *Siptosis* section species (Blake et al., 1999), and DD from *Ae. tauchii* Coss. (Cox, 1998; Dvorak et al., 1998).

Both monophyletic and polyphyletic theories have been proposed to explain the origin of domesticated hexaploid wheat. Currently, the predominant theory supports a monophyletic origin that wheat was generated less than 9000 years ago when the cultivated tetraploid *T. dicoccum*, AABB, crossed with *Ae. tauchii* DD. However, various polyphyletic theories have been introduced in the last decade.

One polyphyletic theory is that temporary amphiploids recurrently appeared in tetraploid and tetraploid-hexaploid combination wheat fields, providing a conduit for alleles to move from *Ae. tauchii* to hexaploid wheat (Dvorak et al., 1998). Another proposed scenario is that multiple independent hybridization events occurred over time, resulting in the AABBDD hexaploids.

Jointed goatgrass (*Aegilops cylindrica* Host.) is an allotetraploid, CCDD (2n = 4x = 28). The C genome was donated by *Ae. markgraffi* (= *Ae. caudata*) (Greuter) Hammer, the D genome parent is *Ae. tauchii*, as is the D genome donor in wheat (Dubcovsky and Dvorak, 1994; Linc et al., 1999). Hybridization between wheat and jointed goatgrass is thought to be facilitated by the shared D genome.

Jointed goatgrass within the wheat production system

In the Pacific Northwest (PNW), soft white winter wheat is the most common type of wheat grown. It is primarily exported to Asia, where it is used to make noodles. Jointed goatgrass cannot be selectively controlled in wheat; therefore, the use of clean equipment and the planting of certified seed, to prevent the spread of jointed goatgrass is stressed. Current recommendations for control of jointed goatgrass infestations in the PNW generally center on crop rotation. Advocacy of this management strategy has not been widely successful in combating the spread of jointed goatgrass for two reasons, economics and the possible lack of vernalization requirement for jointed goatgrass. Due to a lack of a

market for suggested rotation crops, the number of seasons between wheat plantings is often shorter then the 3 to 5 or more years that jointed goatgrass remains viable in the soil (List et al., 1988). Because of this, new jointed goatgrass seed is produced before jointed goatgrass seed produced in previous years can germinate or become non-viable. In this way, the quantity of jointed goatgrass seed in the soil seed bank is not depleted, and may even increase.

Secondly, the recommendation that longer crop rotations would suppress jointed goatgrass populations in winter wheat assumed that switching to spring-seeded crops does not allow the jointed goatgrass to complete its lifecycle. Some observations suggest that jointed goatgrass is a facultative winter annual, with limited vernalization requirements, and mature jointed goatgrass has been found in spring wheat and barley fields in Eastern Oregon (Crémieux and Morrison, personnel communication).

Development of imidazolinone resistant wheat

Imidazolinone herbicides interfere with amino acid synthesis. Like the sulfonylureas and triazolopyrimidines, imidazolinones inhibit acetolactate synthase (ALS), the first common enzyme in the synthesis pathway of branched-chain amino acids. Examples of the imidazolinones are imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin and imazethapyr. ALS inhibitors can be used in an array of crops, are effective against a variety of weeds, are highly effective at low

application rates, and are both less expensive and less toxic than many other herbicide options, and thus they are widely used (Saari et al., 1994).

Resistance to imidazolinone herbicides is widespread and well documented (Heap, 2001). Imidazoline resistance is usually due to mutation(s) in the ALS enzyme binding site resulting in less sensitivity to ALS inhibiting herbicides (Saari et al., 1994). Annual ryegrass (*Lolium multiflorum*) (Christopher, et al., 1994) and downy brome (*Bromus tectorum*) (Mallory-Smith et al., 1999) populations with resistance based on metabolism, have been identified.

Imidazolinone resistance was produced in wheat by mutation with sodium azide (Newhouse et al., 1992). The mutants were screened for resistance by selection of survivors to imazethapyr application. The mutation conferring imidazolinone resistance is thought to occur on a chromosome of the D genome. At this time, the specific location of the resistance gene is not public knowledge. If the resistance mutation had occurred on the A or B genome, the transfer of the resistance gene to jointed goatgrass might be less likely because A and B genome chromosomes cannot pair with the C and D genome chromosomes of the jointed goatgrass, and are therefore more likely to be lost (Zemetra et al., 1998). Efforts are underway to transfer the imidazolinone-resistance gene to the A or B genome, but as A/B chromosomes have been detected in the BC_2 generation (jointed goatgrass having served as recurrent parent), resistance could still potentially be transmitted to jointed goatgrass (Wang et al., 2000a).

One advantage of mutagenesis, such as that used to develop imidazolinone-resistant wheat, is that resulting crops are not genetically modified organisms (GMOs). As the public perceives GMOs to be potentially hazardous, non-GMO products are better received by the public at this time. A commodity to which the public is less opposed is easier to market and handle on its way to point of sale, as it does not need to be separated in the transport stream. Furthermore, herbicide-resistant crops generated though mutation are not subject to regulations prior to release. This may lead to earlier and wider acceptance of imidazolinone-resistant wheat in the PNW, because the Asian market for the soft white winter wheat is dominated by Japan, which currently bans the importation of GMOs.

FERTILE HYBRIDS

One risk associated with the release of herbicide-resistant wheat is the potential for the herbicide-resistance gene to move from wheat to jointed goatgrass via a hybrid bridge. A hybrid between wheat and jointed goatgrass (ABDCD) has 35 chromosomes (21 ABD + 14 CD). When the hybrid is backcrossed to either parent, plants with variable numbers of chromosomes can result (Crémieux, 2000; Wang et al., 2000b; Zemetra et al., 1998).

Historic recognition of hybridization between wheat and jointed goatgrass

Although wheat and jointed goatgrass, are both considered primarily self-pollinating species, they do exhibit facultative outcrossing. Outcrossing has been reported at 2 to3% for wheat (Poehlman and Sleeper, 1995). The rate of outcrossing is thought to be even higher when a plant is stressed. Jointed goatgrass outcrossing rates have not been reported. The assumption that the level of outcrossing of wheat and jointed goatgrass is negligible is one the factors that led to the fertility of wheat x jointed goatgrass hybrids being so long ignored.

Wheat x jointed goatgrass hybrids have been documented in wheat growing regions of the United States since 1921 (Johnson and Parker, 1929). Their appearance was not a cause for concern because the hybrids were reported to be sterile, as they had been reported to be earlier in Europe (Belea, 1968; Johnson and Parker, 1929).

The myth of hybrid sterility

Fertile wheat x jointed goatgrass hybrids were found in wheat fields of the Pacific Northwest (Mallory-Smith et al., 1996), and studies to document the fertility of the F_1 hybrids and successive generations of recurrent backcrosses to jointed goatgrass were initiated. In greenhouse studies, the F_1 was found to be male sterile, with a 2.2% female fertility rate when pollinated with jointed goatgrass (Zemetra et al., 1998). The BC_2 generation exhibited 20.9% mean self-

fertility, with a range of 0.0% to 73.2%, and a mean chromosome number of 33. The D genome chromosomes from wheat and jointed goatgrass paired in meiosis, forming 7 bivalents.

In a subsequent greenhouse study, also using jointed goatgrass as the recurrent parent, the F_1 was found to be male sterile, but to have a female fertility rate of 0.87% (Wang et al., 2000b). The fertility of the backcross generations was examined in the greenhouse. The BC_1 generation had an average of 1.8% male fertility, with a range of 0.0 to 9.5%. Female fertility for the BC_1 generation averaged 4.4%, and ranged from 0.0 to 13.2% fertility. The BC_1 generation was found to have limited mean self-fertility, 0.06%, with a range from 0.0 to 1.6%.

The fertility of the F_1 found in these studies is not completely contradictory to earlier findings. Belea (1968) transplanted synthetic F_1 hybrids between jointed goatgrass and various European wheat cultivars into the field. The number of crossed made per variety of wheat was small (2-11) and they produced an average of 0.0 to 1.5 seeds per plant. The difference between these earlier reports of fertility and more recent reports, is in how the level of fertility was perceived. This level of self-fertility was considered insignificant, and the hybrids labeled sterile, as they had been in earlier European reports. More recent reports of the fecundity of wheat x jointed goatgrass F_1 hybrids have labeled the limited fertility as partially female fertile. F_1 hybrids of tetraploid wheat and various *Aegilops* tetraploid species were also observed to be self-fertile, with a seed set percentage between 0.97 and 39.72% (Xu and Dong, 1991).

The BC_2 generation was markedly more fertile when crossed to jointed goatgrass than the BC_1 generation had been under greenhouse conditions. An average male fertility of 8.9%, an average female fertility of 18.0%, and an average self-fertility of 6.9% were reported (Wang et al., 2000b). The BC_2S_1 generation is partially self-fertile, and the BC_2S_2 had a range of self-fertility from 57.7 to 93.1%, in a greenhouse environment where jointed goatgrass had 93.3% self-fertility (Wang et al., 2000b).

In 1996 and 1997, F_1 wheat x jointed goatgrass hybrids were transplanted into plots of wheat or jointed goatgrass, and seed was produced in all plots (Snyder et al., 2000). Following both field seasons, these BC_1 were germinated, and under greenhouse conditions, the unbagged heads had 4% and 2.1% self-fertility, respectively.

GENE FLOW VIA A HYBRID BRIDGE

When hybridization between two populations is followed by backcrossing, genes may be transferred between the populations. If the hybrid is backcrossed to one of the parental lines, a certain percentage of the non-recurrent parent's genetic material is maintained. Through successive backcross events, the resultant individuals may become morphologically almost indistinguishable from the recurrent parent. However, some of the non-recurrent parent's genetic material will still be retained through each generation. In theory, the recurrent genotype is never completely restored by backcrossing. In the absence of selection pressure, the

frequency of individuals morphologically indistinguishable from the recurrent parent, but carrying the genetic material of the non-recurrent parent, may be very low.

Hybridization must occur for gene flow to be possible. Hybridization between cultivated plants and their weedy relatives includes the following examples: crookneck squash and wild squash (Wilson and Payne, 1994); canola (*Brassica napus* and *B. campestris*) and dog mustard (*Erucastrum gallicum* (Willd.) O.E. Schulz) and wild mustard (*Raphanus raphanistrum* L. ssp. *raphanistrum* (Lefol et al., 1997); sugarbeet and wild beet (Dietz-Pfeilstetter and Kirchner, 1998); cultivated rice and red rice (both *Oryza sativa*) (Sankula et al., 1998).

Hybridizations also occur between wild relatives. The *Helianthus* varieties *H. annuus* and *H. petiolaris* are the progenitors of *H. anomalus*. Introgression between *H. annuus* and *H. petiolaris* was found to be inhibited by both chromosomal and genic barriers (Reiseberg et al., 1995). A series of synthetic hybrids lineages were created to approximate the formation of *H. anomalus*. The fertility barriers that arose between the parents and the hybrid lines gave support to the author's theory that speciation develops as a consequence of fertility selections (Reiseberg, 2000). Previous research showed that almost 50% of the obstruction to introgression between *H. petiolaris* and *H. annuus* can be attributed to chromosomal rearrangements by following the inheritance of 85 RAPD markers (Reiseberg et al., 1999). Inferences were made from frequency of alleles about the

effects of introgressed blocks on hybrid fitness. Blocks that appeared more often than would be expected were assumed to contribute to hybrid fitness and blocks that appeared less often than would be expected were assumed to reduce hybrid fitness.

Introgressions have been documented with evidence taken from morphological observations, cytological studies, comparisons of allozymes, and molecular marker data. Whenever possible, a combination of documentation should be employed in hypothesizing direction of gene movement, as the different types of evidence often lead to different conclusions (Doebly, 1990). Introgressions can occur between different populations of the same species and between related species. Interspecies introgressions can be further divided into those between wild plants, between a weed and a wild relative, between a crop and wild relative (perhaps a progenitor), and between a crop and a weed.

Crop-weed introgressions have been studied for a number of reasons. Principally, there is concern over the loss of genetic diversity that may occur as the genes of introduced cultivars are introgressed into weedy or wild native species. The possible narrowing of the gene pool is thought to be undesirable because the non-cultivated relatives of cash crops are increasingly being looked at as potentially valuable sources of genes for disease resistance and stress tolerance for cultivated crops. Furthermore, botanicals have been the source of many compounds with medicinal and/or industrial applications. Crop to weed introgressions also are seen as a way for genes such as those that confer herbicide resistance, pest resistance, or

stress tolerance, to move to a weed, changing its competitive relationship with the crop.

Conferring herbicide resistance through hybrid bridges

Herbicide resistance can occur in a population because of evolved mutation, directed mutagenesis, and genetic engineering. It can be introgressed from resistant to susceptible populations of the same species. Herbicide resistance also can be introgressed from resistant to susceptible populations of different wild or weedy species. Evolved ALS resistance was transfered from *Amaranthus palmeri* to *Amaranthus rudis* (Wetzel et al., 1999) and from *Lactuca serriola* (prickly lettuce) to *L. sativa* L. 'Bibb' (cultivated lettuce) (Mallory-Smith et al., 1993).

Herbicide resistance can be transferred via introgression from a crop to related weed species. Introgression conferring herbicide resistance between a crop and a weed may involve the transmission of a gene that is the result of mutagenesis or genetic engineering.

The most well documented case of herbicide resistance mediated by a transgene being transferred from a crop to a weed has been the canola - wild mustard system (Mikkelsen et al., 1996). No physiological cost was found when glufosinate resistance is transferred between *Brassica napus* and *B. rapa* (Snow et al., 1999), so the resistance gene may remain in the *B. rapa* population even if the selection pressure from the herbicide application ceases. Controlled crosses have been used to document the potential for herbicide resistance to be transferred from

crops to wild relatives. Herbicide resistance has been transferred (with resistance to beet necrotic yellow vein virus as well) from sugar beet (*Beta vulgaris* ssp. *vulgaris*) to wild beets (*Beta vulgaris* ssp. *maritima*) and has been found to be Mendelianly inherited (Dietz-Pfeilstetter and Kirchner, 1998).

Wheat x jointed goatgrass F_1s as hybrid bridges

Jointed goatgrass was introduced to North America, so there is little concern about the genetic integrity of North America jointed goatgrass population(s) being compromised due to introgression of wheat genes. Members of the *Aegilops* genus have been investigated for traits that could be useful in cultivated wheat (Gill and Raup, 1987). However, if jointed goatgrass were to be used as a germplasm source, most likely samples would be taken from the species' native range. The concern about introgression between imidazolinone-resistant wheat and jointed goatgrass is that the jointed goatgrass may become resistant to imidazoline herbicides.

In order for a wheat gene to be uniformly inherited in jointed goatgrass, it must be transferred from wheat to jointed goatgrass. The imidazolinone-resistance gene could potentially be transferred from wheat to jointed goatgrass by: gene recombination through translocations, through chromosome retention or substitution, and because the resistance gene is on the D genome, through homologous chromosome pairing and recombination (Wang et al., 2000a).

Herbicide-resistant wheat x jointed goatgrass hybrids were found in test plots of the imidazolinone-resistant wheat (Seefeldt et al., 1998).

According to Wang et al. (2000b), there are three deterrents to gene flow from wheat to jointed goatgrass: 1) the sexual incompatibility of the species, 2) the level of sterility of the F_1 and early backcross generations, and 3) the instability of the gene in the new host. The first obstacle, that of sexual incompatibility, is no longer thought to be true, as hybridization events have been well documented. The level of sterility in the F_1 and early backcross generations, the second barrier, is high, but not insurmountable. Spikes may have 30 florets, and hybrids with up to 76 tillers have been found in the field (Crémieux and Morrison, personal communication); therefore, the opportunity for progeny to be produced on F_1 hybrids exists, and is greater for every successive generation. The third obstacle to gene flow between wheat and jointed goatgrass, the instability of the wheat gene in the new host, has not been evaluated.

Genomic *in situ* hybridization (GISH) was used to identify the A/B chromosome segments in synthetic fertile backcross progenies, in an effort to estimate the frequency of wheat chromosome translocation and retention (Wang et al., 2000a). The ultimate goal of that undertaking was to provide information to assess the biological risk of releasing herbicide-resistant cultivars.

A procedure for GISH was developed that allowed A/B genome chromosomes to be visualized in BC_2S_2 individuals. Both wheat chromosome retentions and translocations were found (Wang et al., 2000a). The extra, 1 to 3,

chromosomes in cells with mitotic numbers greater than 28 (2n for jointed goatgrass) were found to be univalent A/B genome chromosomes. A translocation of an A/B genome segment to a jointed goatgrass chromosome also was visualized. Some selfed-progeny, of backcrosses carrying translocations from wheat, were homozygous for these translocations. The authors found this situation to be analogous to alien chromosome introgressions into wheat, which have long provided disease resistance to commercial cultivars (Fedak, 1999; Wang et al., 2000a). All BC_2S_2 individuals examined had 14 C genome chromosomes.

Analyses of chromosome numbers in various backcross generations have provided some information about the potential for herbicide transfer through chromosome retention. BC_1 plants have been produced both from reduced and unreduced hybrid gametes (Zemetra et al., 1991). The D genome chromosomes of wheat and jointed goatgrass have been observed to pair (Kosegi et al., 1998)

SELECTION PRESSURE INCREASES GENE FREQUENCY

Gene frequency in a population is determined by migration of alleles, rate of mutation, and the severity of the selection pressure. In the case of herbicide resistance, the strength of the selection pressure is a function of the efficacy of the herbicide and the frequency of the application. Herbicide resistant plants may evolve after only a few years of repeated application of an herbicide with the same site of action (Jasieniuk et al., 1996). An herbicide-resistant crop provides a large

source of resistance alleles for migration into a susceptible population, should the crop have weeds with sexual compatibility.

The resistance allele frequencies of two populations with the same initial frequencies may diverge when one population undergoes selection and the other does not. The population which undergoes selection pressure would be expected to have a higher frequency of resistance alleles than the population that does not undergo selection pressure. Deviations from expected gene frequencies can result from many circumstances including relative fitness of alleles, relative fitness of linked traits, and non-random mating, and genetic drift. Small populations are especially sensitive to the effects of drift. An allele is more likely to become fixed in a small population due to chance. This is because when there are overall fewer events, the chance that the actual outcomes will cover the range of all possible outcomes is less than when there are more overall events.

MICROSATELLITES AS A MEANS OF STUDYING D GENOME INHERITANCE

Molecular markers can be used to follow genes through generations and to make estimations of genetic diversity in crops. Because the development of some classes of molecular markers is a resource consuming enterprise, they exist for fewer weeds than crops. Molecular markers have, however, been used in weed science to look at diversity, to verify theories of introduction events, as well as to document crop to weed gene flow (Gressel, 2000).

Microsatellites or simple sequence repeats

A microsatellite (Litt and Luty, 1989) or simple sequence repeat (SSR) (Tautz et al., 1986) is a very short DNA sequence of 1 to 6 nucleotides tandemly repeated. SSRs are found in all eukaryotes, and have been reported in data base searches of at least 54 plants (Wang et al., 1994). When DNA replication is interrupted in the region of a SSR, it is less likely that the DNA polymerase will resume replication in exactly the same spot than in a non-repeating region. This phenomenon is referred to as slippage replication (Levinson and Gutman, 1987), and in combination with unequal crossing over and non-functional DNA mismatch repair genes (Strand et al., 1993) has contributed to the evolution of SSRs. If the DNA polymerase resumes replication somewhere other than where replication was interrupted, the number of times the short nucleotide sequence is repeated changes in the strand of DNA that is being replicated. Therefore, the "size" of the SSR changes in the replicated strand. In a relatively short amount of genetic time, SSRs come to have different numbers of repeats in different populations. These mutations are believed to be tolerated.

SSRs can be used as molecular markers

SSRs can be utilized as highly polymorphic, codominant markers. To develop SSRs as markers, genomic DNA of a particular species is digested with a restriction enzyme. The resulting fragments are inserted into vectors, and cloned in

bacteria. Clones containing SSRs are identified by probing with AT or GC polymers. The clones identified to contain SSRs are then sequenced. Primers are designed for the flanking ends of the SSR. Primers also can be made for SSR sequences found by searching sequence databases (Weber and May, 1989; Senior and Heun, 1993). To find the genomic location of a particular SSR, mapping populations from crosses between individuals that are polymorphic with respect to each other are used. Relative position of an SSR (or any other marker) locus can be determined by comparing the segregation of an SSR in the mapping population relative to other markers. The more frequently a new marker segregates with another marker, the more likely the 2 markers are to be close together. By making these two-way comparisons between all possible markers, including those whose loci have already been mapped, markers can be ordered.

To visualize SSR alleles in the progeny, DNA is amplified with specific SSR primers using the polymerase chain reaction (PCR). This process allows DNA to denature, primers to anneal to the template DNA and replicate the area flanked by the primers. Over several iterations, the region of DNA flanked by the primer is at a higher concentration than the rest of the DNA in the reaction. Because amplified SSR alleles will be of different sizes in the two polymorphic parental lines, they can be electrophoretically separated. These amplification products can be resolved in gels made of agarose (Senior and Heun, 1993), or non-denaturing acrylimide stained with ethidium bromide or silver staining (Love et al., 1990) or denaturing acrylimide radioactive sequencing gels (Akkaya et al., 1992).

When SSR primers have been generated, the DNA of the population(s) of interest can be amplified using PCR. If only one primer pair is used, generally only one SSR locus is amplified. The more closely related 2 lines are the more likely the SSRs at any particular locus of these two lines will be of the same size.

Gatersleben wheat microsatellites

An SSR map has been made for wheat (Röder et al., 1998b). The loci, designated *Xgwm* for Gatersleben Wheat Microsatellite, were integrated into an existing restriction fragment length polymorphism (RFLP) linkage map created by the International Triticeae Mapping Initiative (ITMI). Genomic libraries of the two varieties were screened for di, tri, and tetranucleotide repeats (Ma et al., 1996). The isolates were cloned and sequenced. The 230 primer sets used amplified 279 loci. Some of the wheat microsatellites were assigned to chromosomes through the use of nullisomic-tetrasomic and ditelosomic Chinese Spring lines (Plaschke et al., 1996). Many markers were placed on linkage maps, 65 markers were placed to a likelihood of odds ratio (LOD) score greater than 2.5, meaning that the likelihood that these markers are linked to previously mapped loci is sufficiently high to place them with confidence in the existing framework. The other 214 loci were mapped to the most likely interval, meaning that although their general location could be ascertained, their exact order is not known. Group 2 chromosomes have been physically mapped using Chinese Spring deletion stocks (Röder et al., 1998a).

Seventy-one of the 279 loci originally mapped amplified were assigned to the D genome (Röder et al., 1998). Of these 71 loci, 46 are non-orthologous, and 18 initially were mapped at a LOD greater than 2.5. Later, 65 microsatellite markers, isolated from *Ae. tauchii*, were added to the D genome map (Pestova et al., 2000a).

The wheat microsatellites have been utilized in several studies. They have been used in the authentication of inter-varietal chromosome substitution lines (Pestova et al., 2000b) and to evaluate the maintenance of heterogeneity of seed bank accessions (Börner et al., 2000). They have also been drawn on to map wheat genes (Korzun et al., 1997; Korzun et al., 1998; Huang et al., 2000; Salina et al., 2000).

Diversity studies have employed wheat microsatellites extensively, often in conjunction with other marker types. Wheat microsatellites have been used to look at diversity in wheat including, within European wheat varieties (Bohn et al., 1999; Stachel et al., 2000) and between historical and current Argentinean wheat germplasm collections (Manifesto et al., 2001). Diversity studies of particular regions of wheat chromosomes also have made use of wheat microsatellites (Shah et al., 2000). Wheat microsatellites have been used to look at diversity in yellow rust resistant *T. dicoccoides* Korn. accessions (Fahima et al., 1998) as well.

The versatility of wheat microsatellites has been attributed to their codominant inheritance, genome specificity, and their straightforward use (Plaschke et al., 1995; Korzun, et al., 1997; Röder et al., 1995). Wheat

microsatellites have been used in species other than *T. aestivum*, including the genus *Elymyus* (Sun et al., 1997), *T. dicoccoides* (Fahima et al., 1998). Microsatellite markers, designated gdm (Gatersleben D-genome Microsatellites), developed for use in *Ae. tauchii* have been used to amplify fragments in wheat (Salina et al., 2000)

Wheat microsatellite primer sets have been used to study D genomes in species other than wheat including *Ae. tauchii*. Lelley et al. (2000) estimated genetic relationships between 60 *Ae. tauchii*, the wheat D genome donor, genotypes from various geographic locations, and between the *Ae. tauchii* and 60 European wheat varieties, in an attempt to pinpoint the geographic location of hexaploid wheat origin. They believe that SSRs may have utility as a tool for studying polyploid evolution. Their system exploits the fact that although the D genomes in wheat and jointed goatgrass are similar, they are not the same. The D genomes are sufficiently conserved that most primers developed to amplify SSRs in wheat also amplify SSRs in *Ae. tauchii*.

The D genomes in wheat and *Ae. cylindrica* are sufficiently similar that the wheat-generated primers will amplify SSRs in *Ae. cylindrica*. They ought to be evolutionarily distinct enough that most of the amplification products between the two species should be able to be visualized as distinct bands in an agarose gel system.

RETENTION OF WHEAT ALLELES IN IMIDAZOLINONE-RESISTANT WHEAT X JOINTED GOATGRASS RECURRENT BACKCROSS GENERATIONS

INTRODUCTION

Wheat (*Triticum aestivum* L), resistant to either imazamox or glyphosate has been grown in field trials, and will probably be marketed in the Pacific Northwest (PNW) by 2005. Since there is no selective control for jointed goatgrass in wheat, these herbicide-resistant wheats (R-wheats) allow compounds that are normally herbicidal to wheat to be used to control jointed goatgrass (*Aegilops cylindrica* Host) (JGG) in winter and spring wheat. However, it is possible that these herbicide-resistant cultivars will have limited utility, because hybrids of jointed goatgrass and wheat are produced. These hybrids may serve as a hybrid bridge for the introgression of the herbicide-resistance gene from wheat into jointed goatgrass.

Viable wheat x jointed goatgrass F_1 hybrids are possible in part because wheat (2n = 6x = 42; AABBDD genomes) and jointed goatgrass (2n = 4x = 28; CCDD) have the D genome in common. F_1 hybrids with partial female fertility were found in jointed goatgrass infested wheat fields (Mallory-Smith et al., 1996). To assess the risk of gene flow from herbicide-resistant wheat to jointed goatgrass, more information about the genetics of the wheat x jointed goatgrass hybrids and successive backcross generations was sought. A variety of investigations were undertaken, including examinations of fertility (Snyder et al., 2000; Zemetra et al.,

1998; Wang et al., 2000b), retention of A and B genome chromosomes (Wang et al., 2000a), and retention of glutenin genes (Crémieux, 2000). Simultaneously, imazamox-resistant F_1 hybrids were found in test plots of imidazolinone-resistant wheat (Seefelt et al., 1998). Among the seven BC_1 progeny of these hybrids, six individuals were resistant to imazamox.

Herbicide-resistance alleles can enter a population through mutation, migration of alleles from a resistant population, or a combination of these two methods. Theoretically speaking, resistance to imazamox in jointed goatgrass populations may appear via mutation or via the R-wheat x jointed goatgrass hybrid bridge. Once a herbicide-resistance gene is in a population, resistance allele frequencies are influenced by the strength of the selection pressure, which is dependent on the efficacy of the herbicide and the frequency of its use. Intensifying the selection pressure acting on a population will, in theory, increase the resistance allele frequency in a population. Allele frequencies also can change due to allelic fixation, where through chance, rather than selection, some alleles are lost and others become fixed. Small populations are especially sensitive to this force, because when there are fewer events, it is less likely that all possible outcomes will be respresented.

A group of wheat SSRs primers, referred to as gwms, can be used to amplify D genome products in species other than wheat, including *Aegilops tauchii* (Lelley et al., 2000). Amplification products of the wheat and jointed goatgrass D genomes may be sufficiently variable to be distinguished from one another in F_1

wheat x jointed goatgrass hybrids. Thus, SSRs could be used as markers for the identification of wheat D genome alleles in recurrent backcrosses to jointed goatgrass.

In this study, the retention of a herbicide-resistance gene in jointed goatgrass backcrosses with or without selection pressure was compared. Specifically, the retention of the herbicide-resistance gene, with or without selection pressure, was compared to the retention of the wheat D genome specific SSR markers.

The objectives of this study were to: (1) screen D genome specific gwm markers for products that are polymorphic between wheat and jointed goatgrass, (2) determine the retention of the imidazolinone-resistance gene under selection pressure at the BC_2S_1 generation; (3) determine the retention of wheat alleles not undergoing selection pressure over two backcross generations, BC_1 and BC_2.

MATERIALS AND METHODS

Plant material

The R-wheat, which served as a female parent for the production of the F_1, was an advanced generation (FS_4) of an introgression of Fidel (R-wheat) into Madsen (soft white winter wheat). BC_1 seed was provided by the University of Idaho (UI). The seed was produced on F_1 hybrids resulting from controlled pollinations between an imidazolinone-resistant wheat (R-wheat) and a non-

imidazolinone-resistant jointed goatgrass. The F_1 hybrids (R-wheat x jointed goatgrass) served as female parents, and jointed goatgrass was used as the pollen donor, to produce BC_1 seed.

The BC_1 seed was separated into two sub-populations. Half of the seed was sent to Oregon State University (OSU) and the other half remained at UI. The seed that remained at UI is referred to as the unselected, or BC_1u, sub-population. The BC_1u were germinated, tissue removed for DNA extraction, and they were used as the female for crosses to jointed goatgrass to produce BC_2u seed. The BC_2u seed was germinated, DNA was extracted, and the plants were allowed to set seed. The majority of the BC_2u heads were allowed to self-pollinate, resulting in BC_2S_1u seed. A few backcrosses were made to jointed goatgrass, resulting in BC_3u seed. The BC_2S_1u and the BC_3u and were planted and sprayed with 26.9 g ai/ha imazamox at the 2 to 3 leaf stage.

The half of the BC_1 seed that was sent to OSU underwent selection for the herbicide-resistance gene at each generation, and is referred to as the selected, or BC_1s, sub-population. After the BC_1s were germinated and leaf tissue taken for DNA extraction, the plants were sprayed with imazamox at a rate of 26.9 g ai/ha. The survivors were backcrossed to jointed goatgrass to produce BC_2s seed. The BC_2s seed was germinated, DNA was extracted, and the plants were sprayed with imazamox (26.9 g ai/ha) before either self-pollinated to produce BC_2S_1s or backcrossed to jointed goatgrass to produce BC_3s seed. However, no seed was produced by the BC_2s.

Handling of the BC_1S and BC_2S generations

The seed was surface sterilized with a solution of 4 ml of sodium hypochlorite (10% w/v) and 2 drops of Tween 20 for every 12 ml of deionized water. To germinate, the seed was placed in sterile petri dishes on distilled water-saturated filter paper and kept in a dark cabinet, at room temperature. Seeds were germinated in November 1999 (BC_1S) and September 2000 (BC_2S). Jointed goatgrass, to be used as a pollen parent, was concurrently germinated in the same manner, but was not surface sterilized.

Approximately 7 days after germination, seedlings were transplanted into 5-cm pots filled with potting soil (Sunshine Mix #1, SunGro Horticulture, Box 189, Seva Beach, Alberta, Canada TOE 2BO). Seedlings were grown in the greenhouse with temperature settings of 22 C/18 C day/night, although actual temperatures were variable. Natural daylight was supplemented with 12 hours of artificial light from high intensity discharge 400 W sodium lamps. The plants were watered as necessary.

For the purpose of DNA extraction, cuttings (10 cm for the BC_1S generation and 4 cm for the BC_2S) were made from the youngest leaves. The change in length of harvested tissue, was due to the acquisition of improved maceration equipment, which allowed for smaller tissue samples to be harvested for the same DNA yield in the BC_2S generation. These samples were kept at -80° C until DNA was extracted. The seedlings were vernalized in a growth chamber for 12 weeks at 8 C with 10 h of light.

The plants were allowed to acclimate to the greenhouse after vernalization, prior to imazamox application. The herbicide was applied under 213.7 kPa of pressure, 187.3 l/ha flow rate, at an application rate equivalent to 26.9 g ai/ha. The BC_1S plants that survived the imazamox application were transplanted to 5 liter bags. These plants were assumed to carry the imidazolinone-resistance gene.

BC_1S seed was produced by backcrossing the BC_1S, as the female parent, to jointed goatgrass, via the approach cross method. A BC_1S head that was to be used for crossing was emasculated just before it completely emerged from the flag leaf. The head was completely exposed by pulling back the flag leaf. The middle floret was plucked from each flower with forceps, leaving the two outside florets. The flowers were trimmed with scissors to make the anthers more accessible. After the anthers had been removed with forceps, the head was covered with a glycine bag, and the bag attached to a stake. Two days after emasculation, a 5-ml vial was filled with water, and attached to the stake just below the head. A jointed goatgrass spike with exposed anthers was cut, and positioned in the vial so that it was directly over the BC_1 head. The heads were bagged together in 8 cm of 45-mm dialysis tubing (VWR Scientific). The dialysis tubing allows for gas exchange, as well as for increased visibility. Bags were made by wetting the portion of the dialysis tubing to be sealed, pressing that portion of the tubing together. Jointed goatgrass heads were replaced as their anthers whitened, for as long as the BC_1S stigmas appeared receptive.

The progeny, BC_2s, were handled in the same manner as the BC_1s generation and were germinated in September 2000. Although some heads were crossed to jointed goatgrass, in the same manner as the BC_2s, most heads were allowed to self-pollinate. To ensure self-pollination, the heads were covered, prior to anthesis, with dialysis tubing bags.

Retention of unselected genes

SSR marker screening

SSR loci were used as unselected markers. Primers flanking these loci have been designated "gwm" (Gatersleben wheat microsatellites) (Röder et. al. 1998b). Non-orthologous D-genome gwms were screened using 5 DNA samples: JGG, an advanced generation of Madsen x Fidel introgression (FS_4) carrying the imadazolinone resistance gene (R-wheat), the F_1 of the JGG and the R-wheat, as well as the wheat cultivar Opata 85 and the synthetic hexaploid M6 (W-79845). The amplification product(s) of each primer pair were assessed for variation in molecular size between JGG and FS_4 generation R-wheat that could be clearly discerned in a 3% agarose gel (5-10 base pairs). This ease of discernability corresponds to distinctness of JGG banding patterns versus F_1 banding patterns. Observed molecular weight estimates of Opata 85 and M6 were used as controls and compared to values published by Röder et al. (1998b). Two gwm markers

were chosen for each chromosome of the D genome, one from each arm of the chromosome when possible, for a total of 14 unselected markers.

In the BC_1 generation, the rate of retention of unselected markers was evaluated in the two BC_1 subpopulations, the UI BC_1u, and the OSU BC_1s, as well as in the BC_1 generation as a whole. For every primer pair, each plant was scored according to whether it had the amplification product of the size of that was expected for JGG (homozygote band pattern), or the amplification products of the sizes expected from both JGG and R-wheat (the hybrid or heterozygote band pattern).

DNA extraction

The BC_1u and BC_2u DNA were extracted by Jenny Hansen at UI. The method of DNA extraction used at (OSU) is a standard phenol/chloroform nuclear isolation method (Sambrook et al., 1989) that was optimized by Dr. Isabel Vales, Oregon State University.

For the BC_1s generation, 10 cm (approximately 0.1g) of leaf tissue was harvested, and for the BC_2 generation, 4 cm (30-50 ng) was used. After samples were weighed and cut into 2-3 mm pieces, they were stored in 1.5 ml eppendorf tubes at –80° C. The first step of the extraction process, the maceration of the leaf tissue, was different for the BC_1s and the BC_2s generations because in 2000 a tissue grinder (Retsch MM30) was acquired by the Department of Crop and Soil Science at OSU, allowing for better DNA extraction yield. Otherwise, the method was the

same for both generations. In the BC_1s generation, each unopened tube was submerged in liquid nitrogen for 30 to 40 seconds. The sample tissue was then crushed to powder with a mini-pestle. The samples from the BC_2s generation were pulverized by placing a metal bead into the eppendorf tubes with lysis buffer, and placed in the tissue grinder.

The remainder of the extraction process was the same for the BC_1s and BC_2s generations. First, 0.5 ml of lysis solution which is comprised of a 1:1 solution of nuclei isolation buffer (10mM Tris-HCl pH 9.5, 10 mM EDTA, 100 mM KCl, 0.5 sucrose, 4 mM spermidine, 1.0 mM spermine, 0.1% mercaptoethenol) (Liu and Whittier, 1994) and sarkosyl 10% were added to the leaf powder. After the leaf-lysis buffer suspension was vortexed to homogeneity, 0.5 ml of 1:1 phenol /chloroform was added and the tubes were inverted 20 times.

The samples were centrifuged for 4 minutes at 4000 rpm, and the aqueous layer was pipetted from each sample into a fresh tube. A 40 µl of sodium acetate (pH 5) was added, followed by the addition 1.1 ml cold 100% ethanol. The tubes were inverted repeatedly until the threads of DNA became visible. Following a second centrifugation (4 min, 4000 rpm), the ethanol was decanted. A volume of 1.5 ml of cold 70% ethanol was added. After a third centrifugation (2 min, 4000 rpm), the 70% ethanol was decanted, and the tubes were inverted to dry upside down for 0.5 h. The DNA pellets were dissolved in 50 to 100 ml of TE buffer containing 10 µg/µl RNase and left overnight at room temperature to encourage

RNase activity. DNA concentration was determined in a 1% agarose gel against dilutions (25, 50, 100, 200 ng) of a lambda DNA standard.

Reaction conditions for SSR amplification

The reaction mixture and conditions for amplifications used were similar to those described by Röder et al. (1998b). For a 25 µl volume, ddH_20, buffer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide, one unit of *Taq* polymerase, 250 nM of each primer, and 50-100 ng of DNA were combined on ice. The reaction tubes were placed in a thermocycler. Reactions also were prepared with the same proportions of components, but in a 10 µl volume, and with 20% sucrose in cresol red equal to the volume of buffer used. When cresol red was used, the amount of water was reduced to keep the overall volume at 10 µl.

Three programs, differing only in annealing temperatures, were used to amplify the DNA. The program began with 3 min at 94 C, followed by 45 cycles of 1 min at 94 C, then a 1 min annealing step at either 50, 55, or 60 C, followed by 2 min at 72 C. The final extension step of 10 minutes at 72 C was followed by storage at 4 C.

For visualization, amplification products were loaded on 3% agarose gels containing ethidium bromide, and run under approximately 55 to 105 watts of voltage for 1 to 3 h. UV light was used to illuminate the gels, which were documented with an instant photograph.

Marker scoring

BC_1 and BC_2 plants were evaluated for retention of loci corresponding to each of the 14 unselected markers using the instant photographs. For each primer pair, a photo of a gel with the five controls (JGG, R-wheat, F_1, Opata 85, M6), and all samples for that generation (from the sub-population to that eventually underwent selection pressure [Oregon] and the sub-subpopulations not undergoing selection pressure [Idaho]) were scored. Samples with only the amplification product(s) the same size as that of the jointed goatgrass standard were scored as not retaining the particular unselected marker from the R-wheat. A notation of 0 was used, since the plants lacked the wheat allele at that locus. Sample plants with the amplification products for both JGG and R-wheat (resembled the F_1) were recorded to have retained the unselected marker at that locus. A notation of 1 was used because the plant had a wheat allele at that locus. This procedure was repeated for all 14 markers.

Every plant in the BC_1 and BC_2 generations was given a wheat allele retention profile comprised of a yes (1) or no (0) for each of the 14 unselected markers (the amplification products of the SSRs). The BC_1s and the BC_2s were scored for retention of the imidazolinone-resistance gene as well.

Analysis

Chi-square tests were used to compare marker retention rates to expected retention rates. A significance level of 0.05 was used. The percentage of the alleles of each plant that were inherited from wheat was estimated as the number of markers scored 1 for each plant divided by twice the number of loci evaluated for that plant, to account for both alleles at each loci.

RESULTS AND DISCUSSION

Amplification of jointed goatgrass alleles with wheat SSR markers

The 42 wheat chromosomes are organized into three genomes, A, B, and D. Each genome is comprised of 7 pairs of chromosomes, which are labeled 1 through 7. For example, chromosome 1A is orthologous to chromosome 1B and chromosome 1D, so each numbered chromosome has common ancestry with a chromosome (designated by the same number) in each of the other 2 genomes designated by the different letters, A, B, or D. Each chromosome has a long (L) and short (S) arm.

The locations of 14 D genome loci amplified by the gwm primers used in this study will be given by chromosome number and arm. Specific locations, sequence data, and fragment size were previously published (Röder et al., 1998b).

The goal of the primer selection screening process was to find one gwm primer to use as an unselected marker for each arm of the 7 chromosome pairs of the D genome, for a total of 14 markers. Markers were sought to amplify products in both the R-wheat and jointed goatgrass, or to amplify a product in R-wheat, but not in jointed goatgrass (null alleles). Screening began with the non-orthologous gwms, those that specifically amplified D genome products. This strategy was employed because A and B chromosomes are known to be present in some advanced backcross generations (Wang et al., 2000 a). If primers amplifying products from more than one genome are used, the D genome allele from wheat could be lost and the jointed goatgrass allele could become fixed at that locus. This event would then be under counted because the A or B genome amplification products, indistinguishable from the D genome wheat amplification product in the agarose, would mask the absence of the wheat D genome allele.

The markers selected for use in this project are given in Table 1. Initially, the protocol for amplification given by Röder et al. (1998b) was used. Annealing temperatures for some markers were lowered or raised from those published. These modifications are noted in Table 1. The sizes of the amplification products, as estimated in 3% agarose gel, are given in Table 2.

Table 1. Modification from amplification reaction conditions of wheat SSRs.

Marker	Location	Annealing temperatures[a]		Other changes
		Published[b]	Used	
gwm232	1DL	55	55	
gwm106	1DS	60	50	
gwm349	2DL	55	55	
gwm261	2DS	55	60	
gwm3	3DL	55	50	
gwm161	3DS	60	60	
gwm194	4DL	50	50 or 47	2x primer
gwm624	4DL	60	50	
gwm212	5DL	60	60	
gwm190	5DS	60	60	
gwm325	6DS	60	60	
gwm469	6DS	60	60	
gwm37	7DL	60	50	
gwm44	7DS	60	60	

[a]Celcius scale

[b]Roder et al., 1998

Table 2. Size[a] of wheat microsatellite amplification products in wheat and jointed goatgrass.

		Values of standards[b]		Estimates	
Marker	Location	Size of Opata	Size of M6	Size of Fs_4	Size of JGG
gwm232	1DL	140	144	140	145
gwm106	1DS	-[c]	81	130	-
gwm349	2DL	243	-	230	-
gwm261	2DS	164	194	180	170
gwm3	3DL	84	-	90	105
gwm161	3DS	154	145	152	135
gwm194	4DL	136	131	220	210
gwm624	4DL	187	179	140	120
gwm212	5DL	102	117	100	90
gwm190	5DS	201	253	190	220
gwm325	6DS	133	138	135	115
gwm469	6DS	172	170	200	150
gwm37	7DL	189	-	190	200
gwm44	7DS	178	176	180	140

[a]Size is expressed in base pairs.
[b]Roder et al., 1998.
[c]Allele null at these loci.

Utility of gwms for discerning D genome alleles

Because gwms have been used to amplify products in species other that wheat, *Ae. tauchii* (Lelley et al., 2000), and SSRs developed from the *Ae. tauchii* sub *tauchii* and *stangulata*, amplify wheat products, (Stachel et al., 2000), it was anticipated that the gwms would amplify products in jointed goatgrass.

It was not determined that the jointed goatgrass amplification products were amplifications of the D genome. Theoretically the primers could have amplified

orthologous loci from the C genome of jointed goatgrass instead. Sixty-five percent of the 71 D genome primers that were available for this study amplified an othologous product in the A or B genomes as well as the D genome. The chance that one of the 14 markers used in this study amplified a C genome product, in addition to, or instead of, a D genome product in jointed goatgrass, is high if an assumption can be made. That assumption is that the C genome of jointed goatgrass is at least half (because there is only one C genome, and two A and B genomes) as genetically similar to the D genome of jointed goatgrass as the A or B genomes of wheat are genetically similar to the D genome of wheat. This should not affect the overall results, as the C genome is carried by the recurrent parent (jointed goatgrass) and should therefore be present in all generations.

The size estimates of the R-wheat and jointed goatgrass were made in 3% agarose gel, with a 100 base pair (bp) ladder. In an agarose gel system, sizes can be estimated to within 5 to10 bp. In this study, slightly more precise size estimates were assigned to the amplification products. This is justified because the wheat standards, Opata 85 and the synthetic M6, from the mapping population used to create the linkage maps for gwm markers were included. The R-wheat and jointed goatgrass product sizes can be estimated relative to these standards because the sizes of the standards are known.

Other researchers have used automated laser fluorescence (Plaschke et al., 1995), and PAGE electrophoresis with silver (Stachel et al., 2000), or EtBr (Röder et al., 1995) staining to visualize these markers. For this study, the convenience

and low cost of using an agarose gel, as well as the requirement of only needing to discern the homozygous allele band pattern from the heterozygous band pattern, made agarose a good choice. In diversity studies, where the maximum number of alleles possible needs to be distinguished at each loci, visualization systems with greater resolution than agarose may be more important.

BC_1 generation

Germination in the BC_1 generation

A total of 79 BC_1 seeds were produced and bulked (Table 3). Ten of the 39 BC_1s seeds that were planted germinated. Of the 20 BC_1u seeds planted in the BC2u generation, 13 germinated.

Table 3. Germination and fertility summary of BC_1, BC_2, BC_3, and BC_2S_1 generations.

Amount	BC_1s selected	BC_1u unselected	BC_2s selected	BC_2u unselected	BC_3u unselected	BC_2S_1u unselected
Seed produced	39	40	56	249	747	3943
Seed planted	39	40	56	137	628	1763
Seed germinated	10	13	14	104	509	1530
Plants resistant	5	n.a.	3	n.a.	20	127
Plants that set seed	5	12	0	102	n.a.	n.a.
Seed produced (total)	56	249	0	3875	n.a.	n.a.

Inheritance of the wheat SSRs in the BC_1 generation

Overall, the 14 wheat SSRs (gwm) used as unselected markers for wheat alleles were inherited in a Mendelian fashion in the BC_1 generation (Table 4; Table 5). Chi-square analysis of all BC_1, BC_1s and BC_1u combined, resulted in only 1 marker not fitting the expectation of 1:1 inheritance in the BC_1 generation at a 0.05 level of significance. The deviating marker was located on 3DL, and deviated in the direction of more heterozygotes than expected.

Table 4. Wheat allele count for BC_1 generation, by locus and by plant

		BC_1 plants, selected sub-population										BC_1 plants, unselected sub-population															
Marker	Location	2	4	7	9	18	19	27	30	36	38	1i	2i	3i	4i	5i	6i	7i	8i	9i	10i	11i	12i	13i	BC_1s	BC_1u	both
gwm232	1DL	1	1	1	0	1	1	1	1	1	0	1	0	0	1	0	0	0	1	1	1	0	1	1	8	7	15
gwm106	1DS	1	0	0	1	1	1	0	0	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0	6	9	15
gwm349	2DL	1	1	0	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	5	11	16
gwm261	2DS	0	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	1	1	1	1	0	5	7	12
gwm3	3DL	0	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	7	12	19
gwm161	3DS	0	0	1	1	0	1	1	1	1	0	0	1	0	1	0	0	0	1	0	1	1	1	1	6	7	13
gwm194	4DL	1	1	1	0	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	1	1	6	5	11
gwm624	4DL	0	1	1	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	4	3	7
gwm212	5DL	0	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	1	1	0	1	4	5	9
gwm190	5DS	1	0	1	1	0	1	1	0	1	0	1	1	0	0	1	1	1	1	1	0	1	1	1	6	10	16
gwm325	6DS	0	1	1	0	1	0	1	1	1	0	0	0	0	1	1	0	0	1	1	1	1	1	0	6	7	13
gwm469	6DS	0	1	1	0	0	0	1	0	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1	5	10	15
gwm37	7DL	1	0	1	0	0	1	1	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	0	7	8	15
gwm44	7DS	0	1	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	7	8	15
herbicide resistance		S (0)	S (0)	R (1)	R (1)	S (0)	S (0)	R (1)	S (0)	R (1)	R (1)	na	na	na	na	na	na	na	na	na	na	na	na	na			
	total wheat alleles	6	8	10	7	5	9	12	8	11	6	5	6	7	12	7	3	8	11	9	9	10	13	9			

Table 5. Chi-square values for the Mendelian inheritance of wheat alleles in the BC_1 generation, plants designated as heterozygous for a particular locus have a wheat allele at that locus.

Selected

		No. of plants			
Marker	Location	Heterozygous	Homozygous	$\chi 2$	Probability
gwm232	1DL	8	2	3.6	0.06
gwm106	1DS	6	4	0.4	0.53
gwm349	2DL	5	5	0	1
gwm261	2DS	5	5	0	1
gwm3	3DL	7	3	1.6	0 21
gwm161	3DS	6	4	0.4	0 53
gwm194	4DL	6	4	0 4	0.53
gwm624	4DL	3	7	1 6	0 21
gwm212	5DL	4	6	0.4	0 53
gwm190	5DS	6	4	0 4	0 53
gwm325	6DS	6	4	0.4	0 53
gwm469	6DS	4	6	0 4	0 53
gwm37	7DL	7	3	1 6	0 21
gwm44	7DS	7	3	1 6	0 21

Unselected

		No. of plants			
Marker	Location	Heterozygous	Homozygous	$\chi 2$	Probability
gwm232	1DL	7	6	0.08	0 78
gwm106	1DS	9	4	1.92	0.17
gwm349	2DL	11	2	6.23	0 01
gwm261	2DS	7	6	0 08	0 78
gwm3	3DL	12	1	9 31	0
gwm161	3DS	7	6	0.08	0 78
gwm194	4DL	4	9	1.92	0 17
gwm624	4DL	3	10	3 77	0 05
gwm212	5DL	5	8	0 69	0 41
gwm190	5DS	10	3	3 77	0 05
gwm325	6DS	7	6	0 08	0 78
gwm469	6DS	10	3	3 77	0 05
gwm37	7DL	8	5	0.69	0 41
gwm44	7DS	8	5	0 69	0 41

Table 5. (cont.) Chi-square values for the Mendelian inheritance of wheat alleles in the BC_1 generation. Plants designated as a heterozygous for a particular locus have a wheat allele at that locus.

Selected and Unselected					
		No. of plants			
Marker	Location	Heterozygous	Homozygous	$\chi 2$	Probability
gwm232	1DL	15	8	2 13	0 14
gwm106	1DS	15	8	2 13	0 14
gwm349	2DL	16	7	3 52	0.06
gwm261	2DS	12	11	0.04	0 84
gwm3	3DL	19	4	9 78	0
gwm161	3DS	13	10	0.39	0.53
gwm194	4DL	10	13	0 39	0 53
gwm624	4DL	6	17	5.26	0 02
gwm212	5DL	9	14	1.09	0 3
gwm190	5DS	16	7	3.52	0.06
gwm325	6DS	13	10	0 39	0 53
gwm469	6DS	14	9	1 09	0 3
gwm37	7DL	15	8	2.13	0.14
gwm44	7DS	15	8	2.13	0 14

No markers deviated in the BC_1s sub-population. Two markers deviated in the BC_1u sub-population, ($p < 0.05$). They were located at 3DL and 2DL. Both markers deviated overall (Table 5). Additionally there were 3 markers, located on 4DL, 5DS, and 6DS that were at the threshold for rejecting the model of 1:1 inheritance of wheat alleles in the BC_1 generation.

Due to the sample size used in this study, it is difficult to distinguish if the segregation distortions observed have a biological basis. On the other hand, multiple markers showing Mendelian segregation suggests that the inheritance of SSR loci generally was normal.

Fifty percent of BC_1 plants would be expected to have a wheat allele at any one locus. In general, as many or more plants than expected had a wheat allele at any particular locus (Table 6). A marker on 3DL that deviated in the combined BC_1 sub-populations, amplified a wheat allele in 83% of the BC_1 plants. All markers which deviated, or were just at the threshold of deviation, from the model of 1:1 inheritance in the BC_1u sub-population were present in more than 50% of the plants, except the one located at 4DL, which was present only in 23% of the BC_1u plants.

Table 6. Percentage of plants with particular wheat alleles.

		Selected		Unselected		Selected and Unselected	
Marker	Location	No. of plants	% of plants	No. of plants	% of plants	No. of plants	% of plants
gwm232	1DL	8	80	7	54	15	65
gwm106	1DS	6	60	9	69	15	65
gwm349	2DL	5	50	11	85	16	70
gwm261	2DS	5	50	7	54	12	52
gwm3	3DL	7	70	12	92	19	83
gwm161	3DS	6	60	7	54	13	57
gwm194	4DL	6	60	5	38	11	48
gwm624	4DL	4	40	3	23	7	30
gwm212	5DL	4	40	5	38	9	39
gwm190	5DS	6	60	10	77	16	70
gwm325	6DS	6	60	7	54	13	57
gwm469	6DS	5	50	10	77	15	65
gwm37	7DL	7	70	8	62	15	65
gwm44	7DS	7	70	8	62	15	65
mean			59		60		59

Alleles from the D genome of wheat also were assessed as a percentage of total D genome alleles. The expectation is that 25% of the total alleles in the BC_1 generation would be descended from wheat. In this study, 28% ± 8% of the BC_1 alleles originated from wheat. In the BC_1s sub-population, 27% ± 9 of alleles originated from wheat. In the BC_1u sub-population, 28% ± 7 of alleles originated from wheat. The percentages of alleles, overall and in both subpopulations, that originated from wheat was not different than the expectation of 25%.

A chi square test of homogeneity of samples was performed. At the ($p < 0.05$) level of significance there was not evidence to suggest that the plants were different with respect to percentage of D genome alleles that originated from wheat. Because the plants are homogenous, a chi square goodness of fit could be performed for the population as a whole. At the ($p < 0.05$) level of significance, there is no evidence to suggest that the population does not fit the expectation of 25% alleles of wheat origin.

Table 7. Percentage of the D genome that originated from wheat, by plant, in the BC_1 generation.

Selected		
	Wheat alleles	
Plant ID	No.	% genome
2	6	20
4	8	27
7	10	33
9	6	20
18	5	17
19	9	30
27	12	40
30	8	27
36	10	33
38	6	20
mean		27 ± 9
Unselected		
1i	5	17
2i	6	20
3i	6	20
4i	12	40
5i	7	23
6i	3	10
7i	8	27
8i	11	37
9i	9	30
10i	9	30
11i	10	33
12i	13	43
13i	9	30
12i	13	43
13i	9	30
mean		28 ± 7
Selected and Unselected		
mean		28 ± 8

Inheritance of herbicide resistance in the BC_1 generation

Five of the 10 BC_1s plants survived treatment of imazamox, and were assumed to have inherited the herbicide resistance gene (Table 4). This survival rate fits the 1:1 model of inheritance. As no markers in the BC_1s sub-population deviated from this model, the inheritance of the imidazolinone-resistance gene was not different from that of any of the 14 unselected markers in this generation, within the selected sub-population. The number of heterozygotes does not differ from the inheritance of 3 markers in the BC_1u sub-population.

Seed Set of the BC_1 generation

All five BC_1s plants set seed, but the number varied, 1 for plant 9; 6 for plants 36 and 38; 8 for plant 7; and 46 for plant 27. Twelve of 13 BC_1u plants set at least 6 seed (Appendix 1-A).

BC_2 generation

Germination of the BC_2 generation

Fourteen of the 74 BC_2s seeds (19%) that germinated resulted in plants (Table 3). This rate is lower than the rate in the BC_1s generation (26%). One hundred and four of the 193 BC_2u seeds planted germinated.

Inheritance of the wheat SSRs in the BC_2 generation

Since 1 BC_1u progeny line had only 3 progeny to collect DNA from, the BC_2u population was sub-sampled. Three BC_2u progeny of each BC_1u plant producing seed were sampled, for 36 total samples (Table 8). Sub-sampling in this instance balances the contribution of each BC_1u parent to the BC_2u generation. At the BC_2 generation, the expectation is of a 1 in 4 chance that a wheat allele will be present at any given locus. The inheritance of 3 markers deviated ($p<.05$) significantly from this model in the BC_2u sub-population (Table 9). The markers were located on 2DL, 3DL, and 5DS. That the markers located on 2DL and 3DL deviate from the expected ratios in the BC_2 generation is to be expected, as in the BC_1u both deviated from the expected ratios (Table 5). The inheritance of the other 11 gwm loci observed did not deviate significantly from the model of 1 heterozygote to 3 homozygotes (Table 9).

Table 8. Wheat allele count for BC_2 generation, by locus and by plant.

Marker	Location	BC$_2$ plants selected sub-population													
		1a	2a	3a	4a	4b	4c	4d	8c	20c	27-3d	7-17a	9-18a	36-2a	36-2b
gwm232	1DL	1	1	0	0	1	1	0	0	1	0	0	0	1	0
gwm106	1DS	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gwm349	2DL	1	0	1	1	1	0	1	1	1	1	0	0	0	0
gwm261	2DS	0	1	0	0	0	0	1	0	1	1	1	1	0	0
gwm3	3DL	1	1	1	0	0	0	1	1	1	1	0	0	1	1
gwm161	3DS	1	1	1	1	0	1	1	0	0	1	1	0	0	1
gwm194	4DL	1	1	1	0	0	0	0	0	1	1	1	0	0	0
gwm624	4DL	0	0	0	0	0	0	0	0	0	0	1	0	0	0
gwm212	5DL	0	1	0	0	0	1	0	1	1	0	1	0	0	0
gwm190	5DS	0	0	0	0	0	0	1	0	0	0	0	0	1	0
gwm325	6DS	0	0	0	1	1	0	1	0	1	1	1	0	0	0
gwm469	6DS	0	0	0	1	1	0	0	0	1	0	0	0	1	0
gwm37	7DL	0	0	1	1	1	1	1	1	1	0	0	0	1	1
gwm44	7DS	1	1	1	0	1	1	1	1	1	0	0	0	1	1
herbicide resistance		S(0)	S(0)	R(1)	S(0)	S(0)	S(0)	S(0)	S(0)	S(0)	R(1)	R(1)	S(0)	S(0)	S(0)
	total wheat alleles	6	7	6	5	6	5	8	5	10	6	6	1	6	4

Marker	BC$_2$ plants selected sub-population																	
	890-3	890-6	890-19	891-2	891-3	891-8	892-1	892-2	892-5	893-1	893-2	893-17	895-2	895-11	895-13	896-1	896-5	896-10
gwm232	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
gwm106	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	1
gwm349	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	1
gwm261	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1
gwm3	1	0	1	0	0	1	1	0	1	1	0	0	0	1	1	0	1	0
gwm161	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0
gwm194	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
gwm624	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
gwm212	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
gwm190	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1
gwm325	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
gwm469	0	0	0	0	0	0	0	0	0	1		1	0	0	0	1	1	0
gwm37	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0
gwm44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
herbicide resistance	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
total wheat alleles	4	3	3	1	2	2	4	4	5	7	6	3	1	2	2	2	7	4

Marker	897-1	897-6	897-18	898-4	898-8	898-10	899-5	899-7	899-18	900-3	900-14	900-15	901-5	901-8	901-19	902-1	902-3	902-4	BC$_2$s	BC$_2$u	both
gwm232	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	1	6	8	14
gwm106	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	11	11
gwm349	0	1	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	8	15	23
gwm261	0	0	0	1	0	1	1	0	0	0	0	0	1	1	1	0	0	0	6	12	18
gwm3	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	0	1	0	9	18	27
gwm161	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	1	0	1	9	13	22
gwm194	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	6	9	15
gwm624	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
gwm212	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	5	6	11
gwm190	1	0	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1	0	2	14	16
gwm325	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	7	13
gwm469	1	1	0	1	1	0	0	0	0	1	0	0	0	0	1	1	0	0	4	11	15
gwm37	0	0	0	1	1	1	0	0	0	1	1	1	1	1	0	0	0	0	9	12	21
gwm44	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	10	4	14
herbicide resistance	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na			
total wheat alleles	4	4	2	6	6	5	3	4	3	5	4	4	4	5	6	6	5	3			

BC_2s plants were scored for each of the 14 unselected markers (Table 8). Because the sample is skewed to the contribution of BC_1s plant number 27, which produced 10 of the 14 BC_2s seeds that germinated, chi-square tests for goodness of fit to the model of 1 wheat allele to 3 jointed goatgrass alleles, would be invalid. A marker would be expected to be present in 25% of all BC_2 plants.

Table 9. Chi-square values for the Mendelian inheritance of wheat alleles in the BC_2u generation, plants designated heterozygous for a particular locus carry a wheat allele at that locus.

		No.of plants			
Marker	Location	Heterozygous	Homozygous	$\chi 2$	Probability
gwm232	1DL	8	28	0.15	0.7
gwm106	1DS	11	25	0.59	0 44
gwm349	2DL	15	21	5.33	0.02
gwm261	2DS	13	23	2.37	0 12
gwm3	3DL	18	18	12	0
gwm161	3DS	13	23	2 37	0 12
gwm194	4DL	10	26	0 15	0 7
gwm624	4DL	8	28	0.15	0 7
gwm212	5DL	13	23	2.37	0 12
gwm190	5DS	16	20	7 26	0 01
gwm325	6DS	8	28	0.15	0 7
gwm469	6DS	11	25	0.59	0 44
gwm37	7DL	12	24	1.33	0.25
gwm44	7DS	8	28	0.15	0.7

Percentages of plants with each wheat allele were recorded (Table 10). The higher mean percentage of BC_2s plants with any given wheat allele (41%± 22) is due to the unbalanced contribution of BC_1s 27. Plant 27, the maternal parent of 10 of 14 BC_2s, was a heterozygote at 12 of 14 loci, and without it the mean percentage

of BC_2s plants with a wheat allele at any particular locus is 30%±17. None of the means were different than the expectation of 25%.

Table 10. Percentage plants with each wheat allele, by primer.

		Selected		Unselected		Selected and Unselected	
Marker	Location	No. of plants	% of plants	No. of plants	% of plants	No. of plants	% of plants
gwm232	1DL	6	43	8	22	14	28
gwm106	1DS	0	0	11	31	11	22
gwm349	2DL	8	57	15	42	23	46
gwm261	2DS	6	43	12	33	18	36
gwm3	3DL	9	64	18	50	27	54
gwm161	3DS	9	64	13	36	22	44
gwm194	4DL	6	43	9	25	15	30
gwm624	4DL	1	7	1	3	2	4
gwm212	5DL	5	36	6	17	11	22
gwm190	5DS	2	14	14	39	16	32
gwm325	6DS	6	43	7	19	13	26
gwm469	6DS	4	29	11	31	15	30
gwm37	7DL	9	64	12	33	21	42
gwm44	7DS	10	71	4	11	14	28
mean			41±22		28±12		32±12

Alleles from the D genome of wheat also were assessed as a percentage of total D genome alleles in the BC_2 generation (Table 11). The average percentage of loci with wheat alleles was 16% in all the BC_2 combined, and 21% in the selected sub-population, and 14% in the unselected sub-population. At this generation, if all alleles were inherited Mendelianly, the expectation would be that the genome of a BC_2 plant would be 12.5% wheat alleles.

A chi square test of homogeneity of samples for the percentage of D genome alleles originating from wheat was performed on the BC_2 generation plants. For the selected and unselected sub-populations combined, at the ($p < 0.05$) level of significance, there is not evidence to suggest that the plants are different with respect to percentage of D genome alleles that originated from wheat. Because the plants are homogenous, a chi square goodness of fit could be performed for the population as a whole. At the ($p < 0.05$) level of significance, evidence exists to suggest that the population does not fit the expectation of 12.5% alleles of wheat origin. By dropping one sample, plant 20c, there is no evidence to suggest that the population does not fit the expectation of 12.5% alleles originating from the wheat D genome. At the ($p < 0.01$) level of significance, there is no evidence to suggest that the population does not fit the expectation of 25% alleles of wheat origin, without dropping any samples.

Table 11. Percentage of the D genome that originated from wheat, by plant, in the BC_2 generation.

Selected			Unselected		
	Wheat Alleles			Wheat Alleles	
Plant ID	No.	% genome	Plant ID	No.	% genome
1a	6	21	890-6	3	11
2a	7	25	890-19	3	11
3a	6	21	891-2	1	4
4a	5	18	891-3	2	7
4b	6	21	891-8	2	7
4c	5	18	892-1	4	14
4d	8	29	892-2	4	14
8c	5	18	892-5	5	18
20c	10	36	893-1	7	25
27-3d	6	21	893-2	6	21
7-17a	6	21	893-17	3	11
9-18a	1	4	895-2	1	4
36-2a	6	21	895-11	2	7
36-2b	4	14	895-13	2	7
			896-1	2	7
mean		21	896-5	7	25
			896-10	4	14
			897-1	4	14
			897-6	4	14
			897-18	2	7
			898-4	6	21
			898-8	6	21
			898-10	5	18
			899-5	3	11
			899-7	4	14
			899-18	3	11
			900-3	5	18
			900-14	4	14
			900-15	4	14
			901-5	4	14
			901-8	5	18
			901-19	6	21
			902-1	6	21
			902-3	5	18
			902-4	3	11
			mean		14
Selected and Unselected			mean		16

Inheritance of herbicide resistance in the BC_2 generation

Three plants BC_2s, from two different mother plants, 7 and 27, survived herbicide application (Table 3). The expectation was that half of the plants would survive. Overall this is was not the result (3 of 14), but this may be due to the small sample size.

Seed set in the BC_2 generation

At the BC_2 generation, most heads were allowed to self-pollinate, producing selfed-seed, BC_2S_1. Heads were also backcrossed to jointed goatgrass. One BC_2s plant produced seed. Most BC_2u plants produced both BC_3 and BC_2S_1 seed. (Appendix 1-A).

BC_3 generation

The BC3s seed had an overall germination rate of over 90% (Table 3; Appendix A-2). The expectation for the overall inheritance of resistance at this generation is 1 R:7 S, if the sizes of the progeny groups had been uniform. It would be expected that the ratio of BC_3 progeny groups with 1R:1S inheritance to those groups with all susceptible individuals would be 1 (1R:1S):3 (all S). Six of 12 BC_1 parents produced BC_2 lines which produced resistant BC_3 progeny.

BC_2S_1 generation

It would be expected that the ratio of BC_2S_1 progeny groups with 3R:1S inheritance to those groups with all susceptible individuals would be 1 (3R:1S): 3 all S. Ten of 12 BC_1 parents produced BC_2 lines that produced BC_2S_1 individuals which survived the herbicide application (Table 12). Four BC_1 parents produced BC_2 lines that produced resistant BC_2S_1 individuals but not resistant BC_3 individuals, which although unexpected can be attributed to sample size.

Table 12. Number of BC_2 lines with resistant and susceptible BC_3 and BC_2S_1 individuals, per BC_1 parent.

	No.of BC_2 lines with			
	BC_3 progeny		BC_2S_1 progeny	
BC_1u parent ID	Some resistant	All susceptible	Some resistant	All susceptible
1i	0	19	7	12
2i	1	9	3	7
3i	0	9	5	4
4i	2	5	2	5
6i	2	4	4	2
7i	1	6	1	6
8i	0	8	0	8
9i	1	3	3	1
10i	1	5	2	4
11i	0	8	0	8
12i	0	11	1	10
13i	0	4	0	4

This thesis introduces wheat SSRs as a tool for tracking retention of specific regions of the wheat D genome in wheat x jointed goatgrass hybrids through successive backcross generations. Comparisons of herbicide resistance populations with and without selection pressure cannot be made due to the lack of seed production by the BC_2s plants.

Both the gwm markers and the herbicide resistance were inherited Mendelianly in the BC_1 generation. In the combined BC_1 sub-populations, 1 marker, located on 3DL, deviated from the expected inheritance ($p<0.05$). In general, deviation was in the direction of increased heterozygosity, but overall the deviation was not significant.

In the BC_2 generation a balanced sub-sample of BC2u individuals was used. Three markers, located at 2DL, 3DL, and 5DS deviated from an 1 wheat to 3 jointed goatgrass allele inheritance ($p<0.05$). All three markers were present in more plants than would have been expected, which could mean that selection is acting to maintain wheat genes in these regions of the chromosome.

CONCLUSIONS

The objectives of this study were to; (1) screen D genome specific gwm markers for products that are polymorphic between wheat and jointed goatgrass, (2) determine the retention of the imidazolinone resistance gene under selection

pressure at the BC_2S_1 generation; (3) determine the retention of wheat alleles not undergoing selection pressure over two backcross generations, BC_1 and BC_2.

The gwm markers amplify jointed goatgrass products. Wheat SSRs will be used in jointed goatgrass diversity studies and to create a map of jointed goatgrass. Polymorphisms can be observed in 3% agarose gels between the imidazolinone-resistant wheat alleles and jointed goatgrass loci amplified by the gwm markers. Wheat SSRs are a convenient method for following the inheritance of wheat alleles though recurrent backcrosses of jointed goatgrass to wheat x jointed goatgrass hybrids.

The retention of the imidazolinone-resistance gene under selection pressure at the BC_2S_1 generation was not determined in this study, due to the infertility of the BC_2s sub-population.

The retention rates of unselected wheat D genome alleles in the BC_1 and BC_2 generations did not differ significantly from the expected Mendelian inheritance for the majority of unselected makers. This study suggests that wheat D genome alleles are neither preferentially inherited nor lost when wheat x jointed goatgrass F_1 hybrids are recurrently backcrossed to jointed goatgrass. This implies that wheat D genome alleles are selectively neutral to jointed goatgrass alleles in backcross generations where jointed goatgrass is the recurrent parent.

FURTHER RESEARCH

These comments should be prefaced with the explanation that when this project was conceived, it was intended that herbicide resistance would be compared to just one unselected marker, isozymes of the *Ep-V1* endopepsidase (McMillin et al., 1986). This method proved to be inappropriate for single plants. Wheat SSRs were considered as an unselected marker alternative after the BC_1 plants had already been produced.

If this study were to be repeated, there are several points for improvement. The uniformity of the jointed goatgrass used as a pollen donor should be ascertained. The makers should be re-screened, and all available wheat SSRs evaluated for use. Other visualization systems ought to be considered. The subpopulations should be raised in the same environment, or at the very least with the protocols of growth conditions standardized. Finally, and most importantly, initial sample size should be increased to reduce limitations of inference.

UNIFORMITY OF JOINTED GOATGRASS

One priority would be to verify assumptions about the jointed goatgrass population used in this study. The jointed goatgrass used in this experiment came from a single collection in Idaho. Whenever jointed goatgrass plants were needed, seed was taken from this collection. For the purposes of this experiment, the F_1 hybrids created with this seed were considered to be genetically identical. This

supposition is made on the basis that jointed goatgrass, at least in the PNW, is genetically uniform. Evidence exists that this is a valid assumption. Glutenin (Morrison, personal communication) and RAPD analysis (Westra et al., 2000) have shown very little genetic variation. A sample of 10 jointed goatgrass plants from the population used in this study was tested for uniformity of amplification products with 4 randomly selected gwm markers. The 10 samples had completely uniform amplification products (data not shown).

Despite suggestions that the PNW jointed goatgrass population is genetically uniform, more assurance that the jointed goatgrass population used as a pollen parent in all generations is homogenous would be desirable. One way this could be achieved is to select the primers that will be used in a subsequent study before making the initial F_1 hybrids. Jointed goatgrass could be screened for amplification uniformity across all markers. Plants, uniform for the markers to be used in the new study, should be selfed. Enough jointed goatgrass seed from these uniform plants should be collected at this generation to provide the pollen parents for all the F_1s, as well as the BC_1, BC_2, and BC_3 generations.

To further ensure uniformity of pollen parents, each planting of jointed goatgrass to be used as pollen parents should be screened for uniformity of the markers to be used, or at the very least a subset of those markers. Non-uniform plants should not be used.

DNA should be extracted from jointed goatgrass used as pollen parents, and both the plant and the sample given an identification number. As part of the

crossing data, the specific jointed goatgrass plant used should be recorded. Different jointed goatgrass plants may be related to different success rates when backcrossing (personal observation). If true, this variability may be eliminated by the increased uniformity of the jointed goatgrass in the protocol for screening. Recording the pollen parent used would require a trivial amount of time, and the collection of DNA samples, and even marker evaluation for each potential pollen parent would not greatly increase the workload, as the backcrossed plants have to be evaluated anyway.

SELECTING SSRS

If this experiment were refined and undertaken again, the SSR markers should be re-screened, and possibly new ones selected. The screening process of this study could be improved by visualizing all markers for a chromosome arm on the same gel. Other changes that could be made to improve this study would be to have standards available that more closely resemble the sample material, and to evaluate additional markers. During the screening process in this study, primers amplified R-wheat, F_1, and jointed goatgrass samples, as well as the Opata 85 and M6 standards. In some cases, the amplification products of the F_1 could not be explained by the amplification products of the two parents, R-wheat and jointed goatgrass. In most cases, it was not necessary to use these primers, and others were chosen. It would, however, be better if the wheat, F_1, and jointed goatgrass used to

screen the primers, and as standards with later generations, could be the same as the F_1s and jointed goatgrass used to produce the BC_1.

Since the markers were selected for this study, more markers have been added for the D genome (Pestsova et al., 2000a) and other wheat SSRs exist in addition to the set used in this study (Stepenson et al., 1998). Furthermore, when the gwm primers were screened for this study there were factors biasing the selection and evaluation of the primers. One quarter of the primers were unknowingly screened at $1/10^{th}$ the concentration in the amplification reactions due to a difference in the concentration of primer stocks. Another 25% of the primers had not arrived from a collaborator. Although the primer concentrations were corrected when later stock solutions were diluted, and where necessary, primers from the set that arrived after the initial screening took place, have been used, the selection of primers may be biased by the initial conditions and availability of the primers. Primers that would provide clearer results may exist. Furthermore, not all of the primers available were screened. If a satisfactory primer were found for a particular chromosome arm, time was not spent to discover if other primers located on that arm gave less ambiguous results.

CONSIDERATION OF OTHER VISUALIZATION METHODS

Changing the visualization method of the amplification products should be considered if the study were to be repeated. This decision should be made before the primers are screened. To some extent the better resolution provided by

visualizing the amplification products in systems with greater resolving capacity than agarose (12% PAGE stained with either EtBr or silver, or automated laser fluorescence) might reduce or negate the need to re-screen all possible primers. The increased resolution, combined with wheat, F_1, and jointed goatgrass standards that accurately reflect the genetic variation of the recurrent backcross generations, may be enough to reduce ambiguity in scoring. On the other hand, if the relative simplicity of the agarose gel system were valued, re-screening the primers, to identify those whose wheat and jointed goatgrass amplification products have the most disparate sizes, would improve the quality of the results, and reduce the amount of time spent scoring each generation.

INCREASING SAMPLE SIZES

If sample sizes were increased, more questions could be addressed about the inheritance of wheat alleles in the experimental material. It is important, if the goal of repeating this study is to compare the rates of retention of the imidazolinone-resistance gene with and without selection pressure, it is valid to make comparisons between the selected and unselected subpopulations at each generation. These comparisons would be easier to make if the sample sizes at each generation were the same.

It is difficult to say how many BC_1s would be needed. One way to make this determination is to decide how many independent BC_2S_1/ BC_3 are desired in the sub-population under selection. The number of BC_1 expected to germinate

needs to be at least 4 times that number, to allow for the reduction in the selected population through the herbicide application. If 30 independent lines are needed for each BC_2S_1 subpopulation, 615 BC_1 would have to be produced in total, as 39% overall germination of BC_1 seed was observed in this study. In the BC_2u sub-population, individuals could be selected to keep samples balanced, and the time commitment reasonable. Likewise, herbicide retention should be screened in as many progeny groups of the BC_2u as the BC_2s. For purposes of comparing between generations, a sub-sample of the BC_2S_1u data, equal to the numbers in the final BC_2S_1s generation could be made.

Increasing initial sample sizes will decrease founder effects, and possibly allow inheritance of the imidazolinones-resistance gene to be compared between generations and sub-populations. Increased sample sizes might also allow other questions to be addressed such as (1) are certain markers associated with overall fertility; (2) are different markers associated with self-fertility than those associated with fertile backcrosses to jointed goatgrass; (3) are any markers associated with resistance to imazamox resistance; (4) does the overall percentage of the genome that is wheat affect the fertility of the plant; (5) are the retention ratios observed for certain markers in this study that did not fit the model of Mendelian inheritance actually a product of selection.

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Appendix 1. Fertililty of BC_1 and BC_2 generations, plant by plant.

	Plant ID	Cross*		Plant ID	Cross*	Selfed		Plant ID	Cross*	Selfed
BC_1s	7	8/192	BC_2u	890-1	11/14	134/616	BC_2u (cont.)	896-1	6/24	0/350
	9	1/176		890-2	1/16	67/754		896-4	5/18	0/206
	27	46/208		890-3	13/14	246/340		896-5	6/24	0/576
	36	6/240		890-4		9/126		896-6	1/12	0/108
	38	6/184		890-5	10/14	140/826		896-7	1/18	0/758
				890-6	14/18	139/492		896-9	4/16	0/16
BC_1u	1i	51/81		890-7	20/32	30/578		896-10	7/16	5/514
	2i	18/98		890-8	19/22	121/572		897-1	13/18	0/468
	3i	15/78		890-9		35/302		897-6	8/14	55/564
	4i	32/94		890-10	8/18	44/112		897-9		0/106
	5i	4/174		890-11	9/24	57/916		897-11	3/18	0/580
	6i	8/86		890-12	4/18	32/270		897-14	3/16	1/140
	7i	11/110		891-13		183/384		897-15		11/136
	8i	20/146		890-14	17/18	75/114		897-16	5/16	0/40
	9i	10/166		890-15	5/20	41/96		897-17	10/16	
	10i	25/144		890-16	8/34	9/118		897-18	11/24	0/1034
	11i	23/82		890-17	18/22	64/406		898-1	1/16	0/46
	12i	26/100		890-18	9/18	24/440		898-4	8/26	8/710
	13i	6/68		890-19	20/22	220/566		898-8	12/22	40/674
				890-20				898-10	5/14	0/924
BC_2s	7-17	0/24		891-1	7/20	33/290		899-5	13/40	1/280
	27-3b	0/64		891-2	5/14	65/480		899-7	5/22	7/440
	27-20c	0/64		891-3	6/18	47/482		899-8	6/20	1/268
				891-4	7/20	1/574		899-15	3/50	0/146
				891-5	6/16	3/318		899-17	4/22	0/498
				891-6	13/20	21/260		899-18	14/18	46/722
				891-7	6/16	14/652		900-3	3/22	26/682
				891-8	13/16	53/246		900-8	14/16	13/336
				891-9		1/20		900-10	4/42	1/466
				891-10		192/762		900-11	7/30	7/138
				892-1	21/24	252/752		900-13	1/18	10/742
				892-2	14/18	350/858		900-14	12/14	24/74
				892-3	20/26	18/408		900-15	8/22	57/212
				892-4	3/18	44/456		900-19	5/32	0/18
				892-5	16/18	268/670		901-1	7/16	0/478
				892-6	10/16	51/132		901-5	8/14	21/778
				892-7	1/18	24/504		901-7	1/12	1/532
				892-8	16/16			901-8	10/18	4/630
				892-10	16/18	12/22		901-9		3/62
				893-1	6/16	8/220		901-10	4/18	2/256
				893-2	7/18	13/328		901-11	5/20	0/518
				893-4		0/94		901-13	6/24	3/136
				893-8	3/18	1/500		901-15		42/102
				893-10	9/20	1/412		901-18	2/18	2/488
				893-13	6/78	0/134		901-19	9/20	56/430
				893-14	8/16	1/396		902-1	16/20	2/660
				893-17	11/88	55/540		902-2	12/20	
				895-1	2/18	1/218		902-3	1/16	0/44
				895-2	7/14	144/624		902-4	10/16	4/760
				895-5	7/12	24/706				
				895-11		39/174				
				895-12	9/12	46/488				
				895-13	4/16	21/102				

* all crosses made with jointed goatgrass as the pollen parent

Appendix A-2. Germination rates and resistance to imazamox treatment for BC_3u and BC_2S_1 progeny of BC_2u plants.

Parents		No. of BC_3u seeds					No. of BC_2S_1u seeds					
BC1u	BC2u	produced	planted	germinated	survived	% survival	produced	planted	germinated	survived	% survival	BC2u
1i	890-1	11	12	10	0	0	134	66	64	2	3	890-1
1i	890-2	1	0	0	0		67	35	33	1	3	890-2
1i	890-3	13	11	11	0	0	246	48	45	0	0	890-3
1i	890-4	0	0	0	0		9	0	0	0	0	890-4
1i	890-5	10	11	11	0	0	140	134	129	0	0	890-5
1i	890-6	14	14	14	0	0	139	45	45	0	0	890-6
1i	890-7	20	21	18	0	0	30	15	14	0	0	890-7
1i	890-8	19	19	18	0	0	121	61	61	0	0	890-8
1i	890-9	0	0	0	0		35	21	20	1	5	890-9
1i	890-10	8	8	8	0	0	44	63	60	3	5	890-10
1i	890-11	9	9	8	0	0	57					890-11
1i	890-12	4	2	2	0	0	32	2	2	0	0	890-12
1i	891-13	0	16	13	0	0	183	78	63	0	0	891-13
1i	890-14	17	16	14	0	0	75	53	51	2	4	890-14
1i	890-15	5	2	2	0	0	41	30	29	1	3	890-15
1i	890-16	8	8	5	0	0	9	9	7	0	0	890-16
1i	890-17	18	18	17	0	0	64	46	33	0	0	890-17
1i	890-18	9	9	7	0	0	24	18	15	5	33	890-18
1i	890-19	20	20	19	0	0	220	0	0	0	0	890-19
2i	891-1	7	0	0	0	0	33	25	24	4	17	891-1
2i	891-2	5	0	0	0	0	65	33	26	0	0	891-2
2i	891-3	6	5	3	0	0	47	33	30	0	0	891-3
2i	891-4	7	5	3	0	0	1	0	0	0	0	891-4
2i	891-5	6	2	1	0	0	3	9	9	0	0	891-5
2i	891-6	13	7	4	0	0	21	0	0	0	0	891-6
2i	891-7	6	5	2	1	50	14	12	12	0	0	891-7
2i	891-8	13	17	15	0	0	53	30	27	1	4	891-8
2i	891-9	0	1	0	0	0	1	0	0	0	0	891-9
2i	891-10	0	0	0	0	0	192	31	28	2	7	891-10

Appendix A-2. (cont.) Germination rates and resistance to imazamox treatment for BC_3u and BC_2S_1 progeny of BC_2u plants.

Parents		No. of BC_3u seeds					No. of BC_2S_1u seeds					
BC1u	BC2u	produced	planted	germinated	survived	% survival	produced	planted	germinated	survived	% survival	BC2u
3i	892-1	21	21	20	0	0	252	55	48	2	4	892-1
3i	892-2	14	14	11	0	0	350	34	34	0	0	892-2
3i	892-3	20	12	10	0	0	18	15	13	0	0	892-3
3i	892-4	3	1	1	0	0	44	42	40	0	0	892-4
3i	892-5	16	16	11	0	0	286	30	30	0	0	892-5
3i	892-6	10	11	9	0	0	51	39	37	2	5	892-6
3i	892-7	1	0	0	0	0	24	20	16	9	56	892-7
3i	892-8	16	16	15	0	0	0	30	30	6	20	892-8
3i	892-10	16	0	0	0	0	12	29	28	11	39	892-10
4i	893-1	6	5	2	0	0	8	9	8	0	0	893-1
4i	893-2	7	3	2	0	0	13	10	8	2	25	893-2
4i	893-8	3	1	0	0	0	1	2	2	0	0	893-8
4i	893-10	9	9	7	0	0	1	0	0	0	0	893-10
4i	893-13	6	3	3	2	67	0	39				893-13
4i	893-14	8	10	5	0	0	1	46				893-14
4i	893-17	11	11	10	1	10	55	21	23	20	87	893-17
6i	895-1	2	3	2	1	50	1	1	1	1	100	895-1
6i	895-2	7	9	8	2	25	144	37	35	28	80	895-2
6i	895-5	7	8	7	0	0	24	14	12	8	67	895-5
6i	895-11	0	0	0	0	0	39	18	16	0	0	895-11
6i	895-12	9	7	5	0	0	46	30	29	0	0	895-12
6i	895-13	4	3	2	0	0	21	29	28	3	11	895-13

Appendix A-2. (cont.) Germination rates and resistance to imazamox treatment for BC_3u and BC_2S_1 progeny of BC_2u plants.

Parents		No. of BC_3u seeds					No. of BC_2S_1u seeds					
BC1u	BC2u	produced	planted	germinated	survived	% survival	produced	planted	germinated	survived	% survival	BC2u
7i	896-1	6	3	2	0	0	0	3	3	0	0	896-1
7i	896-4	5	4	3	0	0	0	3	3	0	0	896-4
7i	896-5	6	4	1	0	0	0	3	2	0	0	896-5
7i	896-6	0	0	0	0	0	0	1	1	0	0	896-6
7i	896-7	0	1	0	0	0	0	1	1	0	0	896-7
7i	896-9	4	4	3	3	100	0	2	2	2	100	896-9
7i	896-10	7	11	8	0	0	5	0	0	0	0	896-10
8i	897-1	13	12	11	0	0	0	44	42	0	0	897-1
8i	897-6	8	2	2	0	0	55	27	21	0	0	897-6
8i	897-11	8	1	0	0	0	0	10	8	0	0	897-11
8i	897-14	3	1	1	0	0	1	2	2	0	0	897-14
8i	897-15		10	6	0	0	11	0	0	0	0	897-15
8i	897-16	5	2	1	0	0	0	3	2	0	0	897-16
8i	897-17	10	0	0	0	0	0	0	0	0	0	897-17
8i	897-18	11	11	8	0	0	0	0	0	0	0	897-18
9i	898-1	1	0	0	7	0	0	1	1	0	0	898-1
9i	898-4	8	10	10	0	0	8	7	6	1	17	898-4
9i	898-8	12	11	10	2	20	40	31	25	1	4	898-8
9i	898-10	5	2	1	0	0	0	3	2	0	0	898-10
10i	899-5	13	13	13	1	8	1	0	0	0	0	899-5
10i	899-7	5	7	7	0	0	7	9	8	4	50	899-7
10i	899-8	6	0	0	0	0	1	0	0	0	0	899-8
10i	899-15	3	1	0	0	0	0	2	2	0	0	899-15
10i	899-17	4	5	3	0	0	0	0	0	0	0	899-17
10i	899-18	14	8	0	0	0	46	3	3	2	67	899-18

Appendix A-2. (cont.) Germination rates and resistance to imazamox treatment for BC_3u and BC_2S_1 progeny of BC_2u plants.

Parents		No. of BC_3u seeds					No. of BC_2S_1u seeds					
BC1u	BC2u	produced	planted	germinated	survived	% survival	produced	planted	germinated	survived	% survival	BC2u
11i	900-3	3	2	0	0	0	26	19	15	0	0	900-3
11i	900-8	14	0	0		0	13	10	10	0	0	900-8
11i	900-10	4	1	0	0	0	1	1	1	0	0	900-10
11i	900-11	7	7	4	0	0	7	0	0	0	0	900-11
11i	900-13	1	2	2	0	0	10	8	8	0	0	900-13
11i	900-14	12	12	10	0	0	24	15	15	0	0	900-14
11i	900-15	8	8	8	0	0	57	30	9	0	0	900-15
11i	900-19	5	4	1	0	0	0	0	0	0	0	900-19
12i	901-1	7	8	7	0	0	0	0	0	0	0	901-1
12i	901-5	8	3	2	0	0	21	8	7	0	0	901-5
12i	901-7	1	0	0	0	0	0	0	0	0	0	901-7
12i	901-8	10	12	12	0	0	4	0	0	0	0	901-8
12i	901-9	0	2	2	0	0	3	0	0	0	0	901-9
12i	901-10	4	5	5	0	0	2	0	0	0	0	901-10
12i	901-11	5	5	4	0	0	0	3	3	0	0	901-11
12i	901-13	6	3	3	0	0	3	3	3	0	0	901-13
12i	901-15	0	0	0	0	0	42	20	20	0	0	901-15
12i	901-18	2	1	1	0	0	2	0	0	0	0	901-18
12i	901-19	9	9	6	0	0	56	38	34	3	9	901-19
13i	902-1	16	12	12	0	0	2	0	0	0	0	902-1
13i	902-2	12			0	0	0	0	0	0	0	902-2
13i	902-3	1	1	0	0	0	0	1	1	0	0	902-3
13i	902-4	10	7	5	0	0	4	5	5	0	0	902-4