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Title: Seed Protein and Chromosome Number Analyses of Experimental Wheat x Jointed Goatgrass (*Aegilops cylindrica* Host) Hybrid Derivatives.

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The occurrence of seed-producing wheat x jointed goatgrass hybrids in infested wheat fields suggests the possibility of gene flow between the two species. This study investigates 'Madsen' wheat x jointed goatgrass F_1 and reciprocal backcross derivatives produced in experimental field plantings. Electrophoresis of the high molecular weight (HMW) glutenin seed proteins, chromosome counts, and morphological studies were used to better understand the genetics of these hybrids, and to provide a baseline for evaluating hybrids collected in natural populations. The HMW glutenin profiles are a useful diagnostic tool because the banding patterns, in the 68-120 kDa molecular weight range, are species-unique (three bands for goatgrass, four bands for wheat) and can be used to trace parentage in the hybrid seed on the basis of band contribution. Experimental hybrids show considerable diversity in banding profiles (9 patterns of three to six bands). Diversity in number of different

glutenin profiles and number of subunits per seed decreases in more advanced generations (BC_2 and BC_1S_1). Chromosome counts confirm the direction of the crosses and vary as follows: 35 chromosomes for F_1; 36 to 57 for BC_1; 28 to 49 for BC_2; and 33 to 52 for BC_1S_1. A chromosome number of 28 suggests that jointed goatgrass (2n=4x=28) was the recurrent backcross pollen donor, while numbers closer to 42 and above point to wheat (2n=6x=42) as the pollen donor. Partial female fertility was found in all generations, as well as full self-fertility in BC_2 and BC_1S_1 plants. Analysis of the HMW glutenin profiles of the progeny seeds verifies that hybridization can go in either direction, with most banding patterns similar to either jointed goatgrass or wheat. The resulting potential for gene flow from wheat to jointed goatgrass calls for continued study of these hybrid derivatives.

Seed Protein and Chromosome Number Analyses of Experimental Wheat x Jointed Goatgrass (*Aegilops cylindrica* Host) Hybrid Derivatives

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Lisèle Crémieux, Author

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INTRODUCTION

Jointed goatgrass (*Aegilops cylindrica* Host) is one of the more weedy species of winter wheat (*Triticum aestivum* L.) fields. It is widely distributed in wheat-growing regions, infesting 7.5 million acres of winter wheat and fallow land in the western U.S. (Thompson, 1999).

Jointed goatgrass (2n=28; CCDD genomes) and bread wheat (2n=42; AABBDD genomes) are genetically related and share the D genome. The growth and development of jointed goatgrass resemble that of winter wheat (Donald and Ogg, 1991), and selective control of jointed goatgrass with current cultural, mechanical, and chemical methods is difficult (Fleming *et al.*, 1988). Herbicide-resistant wheat was reported as the most promising method to selectively remove jointed goatgrass from infested wheat field (Zemetra *et al.*, 1998). Wheat cultivars resistant to imidazolinone or glyphosate herbicides are currently undergoing field testing (Mallory-Smith *et al.*, 1996).

The close relationship between wheat and jointed goatgrass is reflected by the frequency of hybridization between the two species. The D genomes of jointed goatgrass and wheat are very similar and, if a hybrid is produced, they will form chromosome associations and the hybrid will be viable (Zemetra *et al.*, 1998). Indeed, hybrids between wheat and jointed goatgrass are frequently found in infested wheat

fields. They were however considered sterile and of no consequence. The recent discovery of partially female-fertile wheat x jointed goatgrass hybrids in infested wheat fields (Mallory-Smith *et al.*, 1996), along with the imminent release of herbicide-resistant wheat, raises the possibility of gene flow between wheat and jointed goatgrass following hybridization and recurrent backcrossing (Seefeldt *et al.*, 1998; Zemetra *et al.*, 1998). Advanced backcross generations of a wheat x jointed goatgrass hybrid to wheat or jointed goatgrass have been produced (Zemetra *et al.*, 1998), but little is known about the genetics of the hybrids.

The High Molecular Weight (HMW) glutenin seed protein profiles are a useful diagnostic tool because the banding patterns, in the 68-120 kDa molecular weight range are species-unique (3 bands for goatgrass and 4 bands for wheat), and remain distinctive in the combined banding pattern formed in a hybrid seed. HMW subunits are coded at the Glu-1 loci on group 1 homoeologous chromosomes. The A, B, and D genomes of wheat and the C and D genomes of jointed goatgrass have sets of storage proteins that are electrophoretically distinguishable from each other (Payne *et al.*, 1984), so the relative contribution of each parent to the hybrid seed electrophoretic banding pattern can be traced.

In this study, electrophoresis of the HMW glutenin seed proteins and chromosome counts were used to evaluate wheat x jointed goatgrass F_1 hybrids and reciprocal backcross derivatives produced experimentally. The objectives of the study were to: (1) describe the HMW glutenin patterns of three generations of hybrid seed and test for the presence of wheat glutenin genes in the progeny of self-fertile backcross plants, (2) document the chromosome number ranges of wheat and jointed

goatgrass backcross plants, and (3) determine if self-fertility can be restored in naturally-occurring jointed goatgrass backcrosses. This information will establish a baseline for evaluating hybrids collected in natural populations.

LITERATURE REVIEW

Introduction

Jointed goatgrass, *Aegilops cylindrica* Host, is one of the most troublesome annual grass weeds in U.S. winter wheat (*Triticum aestivum* L.) production. This weed was probably introduced into North America as a contaminant in winter wheat grains brought from Eurasia in the early 20th century. It has been spreading rapidly and is now widely distributed in all the wheat-growing regions, although more prevalent in the Pacific Northwest and the plains of the Midwest (Donald and Ogg, 1991).

Jointed goatgrass is found mostly in wheat fields, but also in other cereal grain fields, feedlots, fencerows, waste areas, and roadsides, where the soil has been broken or disturbed by animals or mechanical implements. It spreads by seed and has been introduced to some areas by custom combines or by planting contaminated wheat seed (Miller and Whitson, 1987). Current farming practices, such as the use of less competitive, semi-dwarf wheats, shorter crop rotations, increased fertilizer use, and reduced frequency and depth of tillage have increased the prevalence of jointed goatgrass (Thompson, 1999). This weed now infests 5 million acres of winter wheat plus 2.5 million acres of fallow land in the western U.S. and is spreading at the rate of 50,000 acres or more per year (Thompson, 1999).

Jointed goatgrass competes with winter wheat for nutrients, moisture, and sunlight, greatly reducing grain yields (Fleming *et al.*, 1988). Because it tillers profusely, as few as 54 jointed goatgrass plants per square meter in an infested wheat

field can reduce wheat yields by over 20%, and yield losses of 50% are common (Thompson, 1999). Grain that contains jointed goatgrass cannot be certified and is subject to price discount at the elevator (Swan, 1984). The seed 'joints' are difficult to separate from wheat and no method is 100% efficient. Jointed goatgrass costs farmers $45 million annually in direct yield losses and reduced grain value. Indirect costs, such as increased tillage and herbicide use, change to less profitable crops, reduced farmland value, and threat to U.S. wheat exports, exceed $90 million annually (Thompson, 1999).

Because jointed goatgrass is genetically related to bread wheat, and its growth cycle is synchronized with winter wheat (Donald and Ogg, 1991), selective control of jointed goatgrass in winter wheat with current cultural, mechanical, and chemical methods is difficult (Fleming *et al.*, 1988). Control is limited to cultural methods, such as spring cropping, double or triple fallow, crop rotations, and seed sanitation. The most promising method to remove jointed goatgrass from an infested field is to use herbicide-resistant wheat (Zemetra *et al.*, 1998). An imidazolinone herbicide-resistant wheat is currently undergoing field testing in the Pacific Northwest (Mallory-Smith *et al.*, 1996), and glyphosate-resistant wheat cultivars are under development. A major concern with this approach is the possibility for gene flow from wheat to jointed goatgrass following hybridization and recurrent backcrossing (Seefeldt *et al.*, 1998; Zemetra *et al.*, 1998). Indeed, jointed goatgrass and wheat can cross, and seed-containing hybrids were recently discovered in Idaho wheat fields (Mallory-Smith *et al.*, 1996). To determine the probability of resistance being transferred from wheat to

jointed goatgrass, information on the genetics of jointed goatgrass and the interspecific hybrids is needed.

Hybridization of wheat and jointed goatgrass

Jointed goatgrass and wheat are members of the same tribe

Jointed goatgrass and cultivated wheat are both members of the tribe Triticeae. They are very closely related, sharing phylogenetic relationships. Jointed goatgrass was placed in the genus *Triticum,* as *Triticum cylindricum* (Host) Ces., Pass. & Gib., by several systematic botanists (Gould and Shaw, 1983), but is still most commonly recognized as *Aegilops cylindrica* Host.

Morphology and growth habit

The growth and development of jointed goatgrass resemble that of winter wheat (Fleming *et al.*, 1988). In vegetative characters, jointed goatgrass seedlings are very similar to winter wheat except that the coleoptiles are a deeper purple in color and the seedlings more slender (Johnston and Parker, 1929). Leaves are shorter than wheat leaves, with short and hairy auricles at the base of the blade. Unlike wheat, the leaf blades of jointed goatgrass seedlings have fine evenly spaced hairs on the margins near the base of the leaf blade. It usually tillers more profusely than wheat, producing over 100 tillers per plant (Gealy, 1988).

When jointed goatgrass heads the plants can be easily distinguished from wheat (Johnston and Parker, 1929). The spikes are very slender and cylindrical, more than 10 times as long as wide. The seed head is 7 to 18 cm long and contains 5 to 12 spikelets, which fit into the contour of the rachis (Miller and Whitson, 1987). Each spikelet is about 1.3 cm long, and consists of a pair of outer glumes which contains from one to five flowers (Swan, 1984). The glumes have several veins and a keel on one side extending into a single awn (Miller and Whitson, 1987). The awns are short, the spikelets at the apex are the only ones to bear long awns (Johnston and Parker, 1929). The stems of the mature plants are much shorter and more slender than those of wheat. Jointed goatgrass usually grows 38 to 76 cm tall, or about one-half the height of wheat. The straw is weak, often allowing the spikes to extend away from the crown in all directions, but many spikes also are borne upright. The peduncles at the time of maturity become purplish then brown in color, a characteristic which helps locate *Aegilops* plants in wheat fields (Johnston and Parker, 1929).

Jointed goatgrass reaches maturity before winter wheat. The spikes then disarticulate at each node of the rachis allowing spikelets to fall to the ground. The apical spikelets mature first and ripening proceeds down the spike toward the base, the spikelets falling to the ground as soon as they mature (Johnston and Parker, 1929). Each spikelet generally has one or two, sometimes three viable seeds, which may or may not germinate at the same time (Miller and Whitson, 1987). The caryopsis of jointed goatgrass has a reddish-brown color (Priadcencu *et al.*, 1967), and unlike wheat, the lemma and palea adhere to it (Johnston and Parker, 1929).

A single jointed goatgrass plant can produce up to 100 spikes, 1500 spikelets or joints, and up to 3000 seeds. However, approximately 130 seeds per plant is typical when growing in a wheat crop with adequate moisture (Donald and Ogg, 1991).

Genetics

Bread wheat (*T. aestivum* L.) is an allohexaploid (2n=42), consisting of three different genomes: A, B, and D. Each genome is composed of seven pairs of chromosomes, giving a total of 21 pairs in bread wheat nuclei. Bread wheat has its origin by amphiploidy involving hybridization between the tetraploid species *T. dicoccum* Schübler (AABB) and the wild diploid species *Ae. tauschii* Cosson. (DD) (Johnson, 1972). *Triticum urartu* Gandilyan is the donor of the A genome, and *Ae. speltoides* Tausch is believed to be the most likely donor candidate of the B genome of the polyploid wheats (Daud and Gustafson, 1996).

Aegilops cylindrica is an allotetraploid (2n=28) possessing the CC and DD genomes. The C and D genomes originated from the diploid species *Ae. markgrafii* (Greuter) Hammer and *Ae. tauschii,* respectively (van Slageren, 1994).

Jointed goatgrass and wheat share a common ancestor, *Ae. tauschii* Cosson, the donor of the D genome (Kimber and Sears, 1987). The D genomes of jointed goatgrass and wheat are very similar and, if a hybrid is produced, they will form chromosome associations and the hybrid will be viable (Koszegi *et al.*, 1998; Zemetra *et al.*, 1998).

Jointed goatgrass and wheat hybridize

"The close relationship between jointed goatgrass and wheat is reflected in the ease with which they may be crossed, and the amount of natural crossing which occurs in the field" (Johnston and Parker, 1929).

Occurrence of wheat x jointed goatgrass hybrids

Jointed goatgrass x wheat hybrids were first described in 1917 (as *Aegilops sancti-andreae* Deg. Hybr. Nov.) by von Degen in Hungary. Since then, natural hybrids between jointed goatgrass and wheat have been examined by several authors in the native distributions of jointed goatgrass in Eastern Europe and Asia (Belea, 1968; van Slageren, 1994). In the US, natural crossing was first reported in Kansas (Mayfield, 1927; Johnston and Parker, 1929), where the amount of such crossing apparently depends considerably on the season, there being very many hybrid plants in some seasons and very few in others (Johnston and Parker, 1929). Hybrids are easily and consistently found in the wheat fields of the Pacific Northwest (PNW) (personal observations; Watanabe, 1999). They also are found in roadside locations and along the borders of bread wheat fields (Johnston and Heyne, 1960; van Slageren, 1994).

Controlled crosses are easily made. Crosses carried out show that *Ae. cylindrica* interbreeds at a frequency of 30.5% with wheat (Priadcencu *et al.*, 1967). Zemetra *et al.* (1991) tested the non-specificity of the production of jointed goatgrass x wheat hybrids by crossing jointed goatgrass to several different PNW cultivars, and

determined that the occurrence of these fertile hybrids was not related to the genetic makeup of a specific cultivar.

Wheat is primarily self-pollinated. However, under natural conditions 1 to 2% out-crossing does occur (Kimber and Sears, 1987; Poehlman and Sleper, 1995). Moreover, some cultivars (such as 'Madsen') have the potential for environmentally induced self- sterility, which increases the potential for wheat x jointed goatgrass hybrids to occur (Zemetra *et al.*, 1991). The rate of out-crossing of jointed goatgrass has not been reported. It is believed to be primarily self-pollinated, but observations in Eastern Oregon fields show that jointed goatgrass cross-pollinates (personal observations).

Morphology of the hybrids

Naturally occurring F_1 hybrid plants are vigorous plants (Watanabe, 1999; van Slageren, 1994). They are as tall or taller (on average 15 cm taller) than the surrounding wheat (Mayfield, 1927; Johnston and Parker, 1929; Seefeldt *et al.*, 1998), easily attaining 50-90 cm in height (van Slageren, 1994). Tiller numbers of 8 to 10 have been reported for F_1 hybrids (Seefeldt *et al.*, 1998). However, hybrids found in Oregon wheat fields have up to 76 tillers (personal observations).

Plants of the first generation are uniform and phenotypically intermediate (Johnston and Parker, 1929; Mayfield, 1927; Priadcencu *et al.*, 1967; Belea, 1968). The spikes are intermediate in size, shape, and awning between those of wheat and jointed goatgrass. They are compact and cylindrical in shape like jointed goatgrass

(Johnston and Parker, 1929), but the spikelets do not so closely flatten to the rachis (Belea, 1968). The toughness of the glume also is inherited from the jointed goatgrass parent (Belea, 1968). From the wheat parent comes the tough rachis trait, as well as the keeled glumes. Spike disarticulation is of the whole-spike type with the break point located just above the first node of the rachis. This trait is inherited from the jointed goatgrass parent. Although jointed goatgrass is typically described as having spikelet disarticulation with joints breaking off at each node (Johnston and Heyne, 1960), it also exhibits whole spike disarticulation (Laura Morrison, personal communication). The lateral glumes have one usually short awn extending from the keel and a blunt dorsal tooth (van Slageren, 1994). The terminal glumes have two long awns (3-5 cm), giving the hybrid spike a similar appearance to the jointed goatgrass parent (Belea, 1968). F_1 hybrids show the dark-brown color of the mature jointed goatgrass.

Hybrid sterility

Naturally occurring hybrids (Johnston and Heyne, 1960; Johnston and Parker, 1929; Mayfield, 1927), as well as products of controlled crosses (Priadcencu *et al.*, 1967; Johnston and Parker, 1929), have frequently been described as sterile.

Winter wheat x jointed goatgrass F_1 hybrids have 35 chromosomes (21 from wheat and 14 from jointed goatgrass) with the ABCDD genomic constitution. One cause of sterility in the hybrids is the lack of chromosome pairing during meiosis,

except for the D genomes, leading to unbalanced distribution of chromosomes in the gametes and non-viable gametes (Seefeldt *et al.*, 1998; Zemetra *et al.*, 1993).

A genetic sterility system (gametocidal gene), which operates in synthetic wheat x jointed goatgrass hybrids, has also been identified (Endo, 1988, 1996). Endo (1988) found that in jointed goatgrass x wheat hybrids backcrosses to wheat, a high degree of chromosome modification, deletions and translocations, occurred in some of the plants. This high degree of aberrant chromosome behavior appears to be due to a 'gametocidal' chromosome in jointed goatgrass. Backcross progeny that lacked a specific C chromosome suffered chromosome deletions and rearrangements. One hypothesis on the activity of the gametocidal gene is that its presence suppresses a gene on a different chromosome that when expressed causes rearrangement of the chromosomes. The action of this gametocidal chromosome from jointed goatgrass would help explain the high degree of sterility of the hybrid progeny. This sterility system does not operate in the first generation hybrids, but causes sterility in the first backcross generation if wheat is the recurrent parent.

Naturally occurring hybrids have recently been shown to produce viable seed with 2 to 3% fertility (Mallory-Smith *et al.*, 1996; Seefeldt *et al.*, 1998). In a two-year survey of hybrids in eastern Oregon, 45% of the hybrid plants collected had one or more fertile spikes, with an average of 1.3 seeds per spike (Morrison, Mallory-Smith and Cremieux, unpublished data). Fertility in the hybrid is reduced compared to wheat, but is not always zero.

Genetics of the hybrids

Zemetra *et al.* (1993) found viable seeds on supposedly sterile plants and determined by checking chromosome number and pairing that progeny were not the result of self-pollination, but rather cross-pollination, with wheat supplying the viable pollen. They concluded that viable progeny of the jointed goatgrass x wheat hybrids were not from self-fertile hybrid plants but from a male-sterile, partially female-fertile hybrid plant pollinated by wheat. Partially female-fertile hybrids overcome instability caused by the three unmatched genomes (ABC) by having a disproportionate number of univalents migrate to one pole in female gametogenesis (unreduced gametes). Hybrid derivatives studied by Zemetra *et al.* (1993) resulted from fertilization of both reduced and unreduced gametes. Seefeldt *et al.* (1998) studied seven first-generation backcross (BC_1) plants and found chromosome numbers ranging between 39 and 54. The plant with 54 chromosomes may have resulted from fertilization of an unreduced gamete from the hybrid, as this number exceeds the 42 chromosomes of bread wheat.

The BC_1 generation may result from backcrossing to wheat or jointed goatgrass (Seefeldt *et al.*, 1998). Backcrossing to wheat and subsequent selfing would tend to return the chromosome number to 42, whereas backcrosses to jointed goatgrass would tend to return the chromosome number to 28 (Seefeldt *et al.*, 1998).

BC_1 plants are usually sterile due to unequal chromosome segregation during meiosis and/or activity of a gametocidal gene in jointed goatgrass. Only 10% of the wheat BC_1 progeny obtained by Zemetra *et al.* (1993) were fertile or partially fertile. They were phenotypically similar to wheat and carried a greater percentage of wheat

chromosomes, allowing for greater chromosome pairing and more normal chromosome segregation and division. Snyder *et al.* (2000) reported up to 80% female fertility for wheat BC_1 plants. Enno obtained high seed fertility (90-100%) in experimental BC_2 lines derived from backcrosses of a jointed goatgrass x wheat hybrid to wheat.

Backcrossing to jointed goatgrass instead of wheat appears to be possible but occurs at a low frequency. Zemetra *et al.* (1998) produced BC_1 and BC_2 seeds at a frequency of 1.2 and 1.9 percent, respectively, and concluded that the probability of recovering BC_2 seeds with jointed goatgrass as the recurrent parent is 0.02% in experimental crosses, with a lower frequency expected in the field. Other experimental studies showed that advanced backcross generations to jointed goatgrass and their selfed backcross lines can reach relatively high levels of seed production: up to 73% fertility for BC_3 plants (Zemetra *et al.*, 1998), and up to 93% for BC_2S_2 lines (Wang *et al.*, 2000a).

Potential for gene flow between wheat and jointed goatgrass

The greatest perceived risk of releasing and growing genetically modified crops on a commercial scale is the possibility of sexual transfer of a crop's engineered genes to a weedy species through crop-weed hybridization and recurrent backcrossing, a process called introgression (Ellstrand and Hoffman, 1990). Hybridization is known to occur between many crops and their wild or weedy relatives, and the potential for gene flow from the cultivated crop to a weed population by introgressive hybridization

has been demonstrated in several crops (Raybould and Gray, 1994). An example is the transfer of herbicide tolerance from oilseed rape *(Brassica napus*, AACC, 2n=38) to its weed relative *Brassica campestris* (AA, 2n=20) (Jorgensen and Andersen, 1994).

Wheat resistant to the imidazolinone herbicide, imazamox, has been produced (Newhouse *et al.*, 1992). Since wheat can hybridize with jointed goatgrass and the hybrids are not completely sterile, the F_1 plants could serve as a bridge to transfer the herbicide-resistance gene from wheat to jointed goatgrass following recurrent backcrossing to jointed goatgrass. Seefeldt *et al.* (1998) demonstrated that the herbicide-resistance gene could be transferred to winter wheat x jointed goatgrass hybrids and the progeny of those hybrids. Two resistant hybrid plants were discovered in research plots where the imazamox herbicide-resistant wheat (FS-4 IR) was tested. Approximately 2% of the spikelets produced BC_1 seed and the majority of the seed produced by the hybrids possessed the resistance gene (Seefeldt *et al.*, 1998).

The current source of imidazolinone herbicide resistance in wheat is controlled by a single, semi-dominant gene (Newhouse *et al.*, 1992), which increases the risk of transfer by hybridization and backcrossing (Zemetra *et al.*, 1998). Since wheat and jointed goatgrass have both shared and nonshared genomes, the retention of the herbicide-resistance gene may also be related to the chromosome location of the gene (Zemetra *et al.*, 1998). The gene for resistance to imazamox in FS-4 IR wheat is thought to be located in the D genome, which is shared by wheat and jointed goatgrass, thus increasing the potential for transfer. If the resistance gene were located in the A or B genome, there would be a reduced probability of retention of the resistance gene in backcrosses to jointed goatgrass because as the number of backcross

generations increases, the chromosomes not found in the recurrent parent are eliminated (Zemetra *et al.*, 1998). However, it is possible that the herbicide-resistance gene could be retained by chromosome translocation from the A or B genome to the C or D genomes of jointed goatgrass. Chromosome addition or substitution could also lead to the retention of the herbicide-resistance gene. BC_2S_1 plants were shown to have chromosome numbers ranging from 28 to 40, indicating that extra chromosomes were present (Zemetra *et al.*, 1998).

This rapid transfer of herbicide-resistance from wheat to jointed goatgrass shows the need to adopt strategies that will reduce the movement of the resistance gene to the weed. A better understanding of the hybrids is needed.

High Molecular Weight glutenins as a diagnostic tool

The endosperm of cereals contains a great number of nonenzymatic storage proteins (Bietz, 1987). In wheat, the main storage proteins are the glutenins, composed of high- and low- molecular weight families, and the gliadins, composed of α/β, γ, and ω families (Kreis *et al.*, 1985). Glutenin forms about 45% of the total endosperm protein (Payne *et al.*, 1980a). Glutenin polymers comprise two main types of subunits, which were classified according to their electrophoretic mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne and Corfield, 1979). The subunits with the slowest mobility are referred to as the high-molecular-weight glutenin subunits (HMW-GS) and the group with faster mobility as the low-molecular-weight glutenin subunits (LMW-GS) (Werner *et al.*, 1992). The HMW glutenin

subunits appear as the heaviest fraction of the endosperm proteins, their molecular weight ranging between 120 kDa and 68 kDa (Galili and Feldman, 1983b).

Advantages of the technique

The HMW glutenin subunits are well-resolved in SDS-PAGE (Galili and Feldman, 1983c). The spectra of bands produced by this technique are diagnostic for the species (Johnson *et al.*, 1967; Ladizinsky and Hymowitz, 1979). Accessions of cultivated plants from different geographical areas and adapted to diverse ecological zones possess essentially the same profile (Johnson, 1972). Seed protein profiles are species-specific, and also serve as a very stable diagnostic trait. Composition of seed protein is only slightly affected by environmental conditions or seasonal fluctuations, and storage proteins are not likely to be changed in dry mature seed (Ladizinsky and Hymowitz, 1979). The rate of expression of the HMW glutenins is mainly affected by structural genes themselves, contrary to the gliadins, for which expression can be affected by nitrogen fertilizers (Galili and Feldman, 1985).

The additive nature of the seed protein profile makes it a useful tool in hybridization studies. The seed protein profile of synthetic allopolyploids represents an exact summation of the parental profiles (Jonhson *et al.*, 1967; Ladizinsky and Hymowitz, 1979). The homology among bands of different species based on similarity in molecular size and mobility can be used as a criterion of genetic affinity from which evolutionary relationships may be inferred. Seed protein electrophoresis is therefore a

useful tool in studies concerning the origin of polyploid plants and the evolution of cultivated plants (Ladizinsky and Hymowitz, 1979).

Genetic control of the HMW glutenins

The HMW glutenins have been studied extensively because of their association with bread-making quality and usefulness for varietal identification (Burnouf and Bouriquet, 1980; Moonen *et al.*, 1982), and their genetic control is known (Galili and Feldman, 1983c).

HMW subunits are coded at the Glu-1 loci on the long arm of group 1 homoeologous chromosomes (1A, 1B and 1D in wheat; 1C and 1D in jointed goatgrass) (Payne, 1987; Payne *et al.*, 1980b; Payne *et al.*, 1984; Lawrence and Shepherd, 1980; Galili and Feldman 1983a). In bread wheat, the HMW glutenin genes are located at similar positions on homoeologous chromosomes in the three genomes, which supports the concept that they are homoeoallelic (Payne *et al.*, 1982; Shewry *et al.*, 1989). Glutenin subunit genes on chromosomes 1A, 1B, and 1D show close linkage to the centromeres in crosses, the mean map distance being 9 cM (7.7, 9.3, 10.2 cM respectively) (Payne *et al.*, 1982b). However, the Glu-1 loci appear to be physically located more distally on the long arms of the group 1 chromosomes (Shewry *et al.*, 1989).

Each locus consists of two tightly linked genes encoding a faster migrating x-type subunit and a slower migrating y-type subunit (Payne *et al.*,1981b; Shewry *et al.*, 1984, Payne *et al.*, 1983, Werner *et al.*, 1992). So hexaploid wheat contains six

glutenin genes, although only three, four or five subunits are present in most cultivars, presumably because of gene inactivation (Anderson and Greene, 1989; Shewry *et al.*, 1992; Werner *et al.*, 1992). In any given wheat variety, the 1A chromosome expresses either one (x-type) or no HMW subunit, the 1B chromosome expresses either one (x-type) or two (one x- and one y-type) HMW subunits, and the 1D chromosome almost always expresses two HMW subunits (one x-type and one y-type) (Payne *et al.*, 1981b; Werner *et al.*, 1992).

The glutenin subunits which are controlled by the long arm of chromosome 1B tend to occupy the central part of the HMW glutenin fraction in the gel, while subunits controlled by chromosome 1D and 1A tend to occupy the upper and lower parts of this fraction (Galili and Feldman, 1983b).

Polymorphism of the HMW glutenins

Protein studies have shown that HMW glutenins are highly polymorphic in wild wheats (Ciaffi *et al.*, 1996) and also display significant variation in modern cultivars (Shewry *et al.*, 1989), because of the presence of different alleles at each of the three gene loci. Polymorphism in the number and electrophoretic properties of HMW subunits present in different genotypes of bread wheat has been studied by a number of workers (Lawrence and Shepherd, 1980; Payne *et al.*, 1980a,b, 1981a,b; Burnouf and Bouriquet, 1980; Moonen *et al.*, 1982; Galili and Feldman, 1983b, Branlard and Le Blanc, 1985). Galili and Feldman (1983b) detected a total of 23 distinct proteins that migrate in the HMW glutenin subunit region, many of these

proteins being products of allelic genes. Payne and Lawrence (1983) summarized the frequencies of alleles at the three glutenin loci of about 300 cultivars of bread wheat. Glu-D1 has six allelic forms (involving eight different polypeptides), with molecular weights varying between 108-106 kDa for the slowly migrating subunits and 84-78 kDa for the rapidly migrating ones (Galili and Feldman, 1983b). The 14 different polypeptides coded by 11 allelic forms at Glu-B1 had molecular weights varying between 92-102 kDa for the slowly migrating subunits and 88-90 kDa for the rapidly migrating ones (Galili and Feldman, 1983b). Payne and Lawrence (1983) found only three allelic forms (one of which is null or silent) at the Glu-A1 locus, while Galili and Feldman (1983b) found that Glu-A1 encoded for five different subunits, two of which were relatively faint. The molecular weights of the glutenin subunits coded at the Glu-A1 locus ranged between 105.5-114 kDa for the major subunits and between 100-103 kDa for the minor subunits (Galili and Feldman, 1983b).

The HMW glutenins exhibit less variation in jointed goatgrass. A study of 151 accessions of jointed goatgrass representative of the geographic distributions across Europe, Asia, and the USA showed a 3-band profile, although one or two of these bands may be absent. Variation from the 3-band profile is rare, having been found in only five accessions from Turkey (Laura Morrison, personal communication). Isozyme studies (Watanabe, 1997; Watanabe *et al.*, 1994) and random amplified polymorphic DNA (RAPD) analysis (Okuno *et al.*, 1998) also show low genetic diversity in jointed goatgrass. The low variation revealed by these three measures suggests an overall low genetic variation in the species.

This uniformity of the HMW glutenin profiles in jointed goatgrass and within each wheat cultivar is useful for hybrid studies because their unique profiles remain distinctive in the hybrids.

Conclusion

Jointed goatgrass is a serious annual weed in winter wheat and also a wild relative of bread wheat. The close relationship between wheat and jointed goatgrass is reflected in the frequency of hybridization of jointed goatgrass and wheat and by the fact that hybrids are not completely sterile (Mallory-Smith *et al.*, 1996).

A concern with the development of herbicide-resistant wheat is the potential for transfer of herbicide resistance from wheat to jointed goatgrass through introgressive hybridization. Advanced backcross generations of a wheat x jointed goatgrass hybrid to wheat or jointed goatgrass have been produced (Zemetra *et al.*, 1998). However, current knowledge on the genetics of the hybrids is limited.

The HMW glutenin technique has shown its usefulness in hybridization studies (Ladizinsky and Hymowitz, 1979). HMW subunits, coded at loci on homoeologous group 1 chromosomes of wheat and jointed goatgrass, can be used as markers to identify plants carrying wheat glutenin genes. The protein spectra indicate genome donors and are a valuable way to define hybrid genotypes.

MATERIAL AND METHODS

Plant material

Experimental backcross (BC) hybrid material was obtained from field and greenhouse studies conducted in 1995-1997 (Snyder *et al.*, 2000). Seeds include three successive generations:

- F_1 hybrids from controlled pollinations, using Madsen (soft white winter wheat) as the female parent, and jointed goatgrass as the male parent (Zemetra *et al.*, 1998).
- A first backcross (BC_1) generation harvested from 1996-1997 experiments, where wheat x jointed goatgrass F_1 hybrids were open-pollinating in 100% jointed goatgrass or 100% wheat plots.
- A selfed backcross generation (BC_1S_1) obtained from the 1997 and 1998 greenhouse experiments, with no bagging of the spikes.
- A second backcross generation (BC_2) harvested from the 1997-1998 experiment, where jointed goatgrass BC_1 plants and wheat BC_1 plants were open-pollinating in jointed goatgrass plots.

A total of 155 seeds were used in this study (Table 1).

For each seed, about 1/3 of the endosperm was cut and used for the High Molecular Weight (HMW) glutenin study, while the embryo part was germinated for morphological evaluation and chromosome counts.

Table 1: Origin and number of seeds studied.

Seed Category	Mother Plants	Number studied
Jointed goatgrass BC_1	F_1 hybrids grown in 100% jointed goatgrass plot	25
Wheat BC_1	F_1 hybrids grown in 100% wheat plot	25
Jointed goatgrass BC_2	Jointed goatgrass BC_1 plants grown in 100% jointed goatgrass plot	25
Wheat-Jointed goatgrass BC_2	Wheat BC_1 plants grown in 100% jointed goatgrass plot	25
Jointed goatgrass BC_1S_1	Jointed goatgrass BC_1 plants grown in the greenhouse	25
Wheat BC_1S_1	Wheat BC_1 plants grown in the greenhouse	25
F_1	Madsen x jointed goatgrass controlled cross	2
Jointed goatgrass	Jointed goatgrass parent	2
Madsen	Wheat parent	1
		155

Greenhouse studies

The embryo-half of each seed was placed in a germination box with two layers of germination paper and two layers of blotter paper soaked with distilled water. After germination, the seedlings were transplanted into 5-cm pots containing Sunshine Mix #1[1] and fertilized. The plants were vernalized for 8 weeks in a growth chamber set at 8 C, and 8 hours light. Germination and survival after vernalization percentages for each seed category are shown in Appendix, Table A2.

1. Sunshine Mix #1: SunGro Horticulture, Box 189, Seva Beach, Alberta, Canada T0E 2B0

On September 20, 1999, the plants were placed in the greenhouse. The temperature and photoperiod in the greenhouse were set at 22C/18C and 16h/8h day/night, respectively. The lighting was natural daylight supplemented with artificial light from high intensity discharge 400W sodium lamps. The pots were watered daily.

After 2 weeks of acclimatization to greenhouse conditions, root tips were collected from each seedling for chromosome counting. At that time, the seedlings were transplanted into 4.5x4x9 cm black 3-mil bags containing Sunshine Mix #1 and fertilized.

The morphological characteristics scored during growth included leaf width (broad, narrow, or intermediate) and plant posture (rosette, upright, or intermediate). The heading date (spike appearing 1 cm above the flag leaf) of the first tiller and the number of mature reproductive tillers at harvest were recorded. One spike from each plant was bagged just before the start of flowering, using standard glassine wheat crossing bags. Observations on anthers (fertile or sterile) and pollination (self- or cross-pollination) were made. A plant was classified as cross-pollinating if at least one open spikelet was observed during anthesis and as self-pollinating if no open spikelets were observed. At maturity, the spikes from each plant were harvested and threshed individually. The degree of disarticulation (disarticulation as a whole spike, disarticulation at the spikelet level, or no disarticulation), the number of spikes, and the number of seeds per spike and seeds per plant were recorded. One spike per plant was saved for morphological evaluation.

High Molecular Weight glutenin analysis

The endosperm part of the seeds was used in the HMW glutenin study. The endosperm proteins were fractionated in Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE).

Extraction of endosperm proteins

The sample buffer, consisting of 10% v/v glycerol, 3% w/v SDS, 5% v/v 2-mercaptoethanol, 0.1% w/v bromophenol blue, and 62.5 mM Tris-HCl, was added in quantity proportional to the seed weight (with 1 ml of sample buffer per 12 mg of seed). After 2 hours at room temperature, the samples were brought to 70°C for 30 minutes in a water bath, and then centrifuged at 12,000 rpm for 3 minutes.

Fractionation of the proteins

The polyacrylamide gels used for electrophoresis were 10-by-10-cm 12-well Owl's PAGE-ONE gels, composition Acrylamide/Bis-Acrylamide 4-20%, and 1.0 mm thick. Electrophoresis was carried out in a mini-vertical gel system (E-C Apparatus Corporation EC120) with an electrophoresis buffer (running buffer) containing glycine (148% w/v, 0.04M), Tris (30% w/v), and SDS (10% w/v), for a pH of 8.3.

A sample of 5µl of supernatant was loaded directly on each lane of the gel, the gel-loading tip being rinsed in running buffer between each sample. Ten samples were

loaded on each gel, the two remaining wells containing the molecular weight standard (Novex Mark12 Wide Range Protein Standard), or one of the wheat standards. The wheat cultivars used as standards for gel electrophoresis of the HMW glutenin proteins included Chinese Spring (CS), a genetic research standard, Madsen (Md), the female parent of the F_1 hybrid, and Stephens (St), a cultivar commonly grown in Oregon.

The gels were run for 1 h or until the bromophenol blue dye reached 5 mm from the bottom of the gel, at a constant 250 V.

Staining, destaining, and drying of SDS-PAGE gels

At the termination of the runs, the gels were removed from the electrophoresis system and placed overnight in a stain box containing about 50 ml of stain solution (2.5% v/v TCA, 6% v/v glacial acetic acid, and 250 mg coomassie blue dissolved in 170ml methanol). The gels were then destained overnight in 50 ml destaining solution (6% v/v Brij and 2% v/v TCA), and dried in cellophane on Promega plastic frames.

Estimation of the molecular weights

The molecular weight of the various endosperm protein subunits was estimated by comparison with protein markers of known molecular weight (Novex Mark12 Wide Range Protein Standard), which were fractionated in a parallel lane. The protein markers were myosin (200 kDa), ß-galactosidase (116.3 kDa), phosphorylase b (97.4

kDa), bovine serum albumin (66.3 kDa), glutamic dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

Chromosome counts

Root tips for mitotic chromosome number counts were collected 2 weeks after the end of vernalization. For each seedling, five root tips were placed in a petri dish on filter paper moistened with the colchicine pretreatment (40 ml dH2O, 0.02 g colchicine, 0.01 g 8-OH-quinoline, 10 drops DMSO). After 2 ½ to 3 hours in the dark, the roots were transferred to 2% aceto-orcein for staining, and refrigerated for at least 2 days.

The root tips were squashed for chromosome counting as follows: one root tip was placed in 45% acetic acid in a vial and boiled over an alcohol lamp for 5 seconds. It was then transferred onto a slide, the tip was cut off, and macerated with an arrow head needle to release the mitotic cells. A small drop of 45% acetic acid was then added, and a cover slip was placed on top. The excess acetic acid was removed. The slide was warmed over a spirit lamp and squashed with the thumb.

Chromosome counts were performed using a compound microscope. Five to 10 cells per plant were used to confirm the chromosome number.

RESULTS

Wheat x jointed goatgrass F_1 hybrids and reciprocal backcross derivatives (BC_1, BC_2, and BC_1S_1) were analyzed using the HMW glutenin technique, chromosome counts, and morphological evaluation. The seeds used were produced in a previous study (Snyder *et al.*, 2000), where F_1 hybrids were completely surrounded by either wheat or jointed goatgrass. The BC_1 were therefore classified as either wheat BC_1 or jointed goatgrass BC_1. However, the backcrosses were not in isolation plots and the pollen donor was not controlled. The present analysis revealed that the majority of the backcross seed produced on the F_1 hybrids in the jointed goatgrass plots had wheat instead of goatgrass as the paternal parent and that some seeds produced in the wheat plots had jointed goatgrass as the paternal parent. This study permitted determination of the pollen parent donor for most of the BC_1 plants (Appendix, Table A1). When such a determination was not possible, the plants are described as BC_1s with an unknown pollen donor. The same approach was applied to BC_2 and BC_1S_1 plants.

Most of the BC_1 plants had wheat as the paternal parent (Table 2). Jointed goatgrass might have been the paternal parent of four of the 39 BC_1 plants studied. Since most BC_1s were to wheat, and the BC_1 plants were surrounded by jointed goatgrass, it is assumed that most of the BC_2 plants resulted from a first backcross to wheat and a second backcross to jointed goatgrass. However, at least two BC_2 plants had jointed goatgrass as the recurrent pollen donor, and four BC_2 plants might have had wheat as the recurrent paternal parent, or have resulted from selfing of a wheat

BC_1. In the BC_1S_1 generation, two plants might have been produced by a jointed goatgrass BC_1, while 32 plants might have had wheat as pollen donor in the first backcross. These numbers are consistent with the results for the BC_1 generation.

Table 2: Pollen donor parent as determined by the results of the chromosome count, HMW glutenin and greenhouse studies.

Seed category	Pollen donor in the first backcross	Pollen donor in the second backcross	Number of plants	
BC_1	Jointed goatgrass	-	2	} 4
	Probably jointed goatgrass	-	2	
	Wheat	-	21	} 32
	Probably wheat	-	11	
	Unknown	-	3	
BC_2	Jointed goatgrass	Jointed goatgrass	2	
	Wheat	Wheat *	4	
	Probably wheat	Probably jointed goatgrass	35	
BC_1S_1	Jointed goatgrass	Self	2	
	Wheat	Self	28	
	Probably wheat	Self	4	} 32
	Unknown	Self	5	

* There is a possibility that these 4 plants selfed instead of cross-pollinating.

Greenhouse studies

Fifty seeds from each category (Table 1: BC_1, BC_2 and BC_1S_1), as well as the wheat and jointed goatgrass parents and two F_1 hybrid seeds were germinated, transferred to the greenhouse, and allowed to mature. The average germination was 84, 92, and 80% for BC_1, BC_2, and BC_1S_1, respectively, and 78 to 82% of the seedlings survived vernalization and produced reproductive tillers (Appendix, Table A2). The morphological characteristics of the hybrids were studied.

Vegetative characteristics

The growth habit (rosette, upright, or intermediate) and leaf width (broad, narrow, or intermediate) of the hybrids was scored. A wide range of phenotypic variation was found in the hybrids. Some were very similar to wheat, some were very similar to jointed goatgrass, but most plants had an intermediate phenotype (Appendix, Table A3). These results are similar to those reported by Snyder *et al.* (2000).

Reproductive characteristics

The reproductive characteristics recorded included heading date, number of reproductive tillers, pollination method (cross-pollination for plants with at least one open spikelet, or self-pollination for plants with no open spikelets), and anther fertility. The presence or absence of viable pollen in the anthers gives an indication of selfing potential. Spike appearance (wheat-, jointed goatgrass- or hybrid-like) and disarticulation mechanism (no disarticulation, disarticulation as a whole spike, disarticulation at the spikelet level) also were recorded. The type of dispersal unit can be used as an additional element to confirm the pollen parent donor: a tough rachis is a wheat characteristic, while disarticulation at the spike and spikelet level is characteristic of the weed species.

In the field, jointed goatgrass usually matures a few days earlier or at the same time as winter wheat (McGregor, 1987). In this greenhouse study, jointed goatgrass headed 45 days later than the wheat plant (Table 3) and it also matured after the wheat.

The light and temperature conditions in the greenhouse, particularly due to the heading dates occurring from November to January, may have altered the relative timing relationship from that observed in the field.

Table 3: Reproductive characteristics of wheat, jointed goatgrass, their F_1 hybrids and their backcross derivatives.

	Wheat	Jointed goatgrass	F_1	BC_1	BC_2	BC_1S_1
Number of plants	1	2	2	39	41	39
Tillers per plant	5	45; 65	22; 25	7-37	10-116	6-48
Days to heading[a]	123	168; 169	109; 105	116-192	106-182	106-187
Self-pollinating plants[b] (fertile anthers)	1	2	0	0	2	3
Cross-pollinating plants						
Fertile anthers	0	0	0	3	11	24
Sterile anthers	0	0	2	36	28	12
Plants by spike morphology						
Jointed goatgrass	0	2	0	3	15	2
Wheat	1	0	0	3	2	3
Hybrid	0	0	2	33	21	34
Plants by dispersal unit						
No disarticulation	1	0	0	7	3	11
Spike	0	0	2	31	28	26
Spikelet	0	2	0	1	10	2

a- Days to heading from germination, including 8 weeks of vernalization at 8C.

b- A plant was classified as self-pollinating if no open spikelets were observed during anthesis, and as cross-pollinating if at least one open spikelet was observed during anthesis.

The F_1 hybrids headed first, 8 to 14 days before the wheat parent. The F_1 hybrids have a tiller number intermediate between the wheat and the jointed goatgrass parent (Table 3). As expected, wheat self-pollinated, and had fertile anthers. Jointed goatgrass is usually classified as a primary self-pollinating species (van Slageren, 1994). The fact that in this study jointed goatgrass had some open spikelets might be due to the greenhouse conditions, but cross-pollination has also been observed in wheat fields (personal observations). The F_1 hybrids were completely male sterile and were cross-pollinating.

BC_1 plants had 7 to 37 reproductive tillers, and heading dates ranged from 116 to 192 days after germination (Table 3 and Appendix, Table A4). Most of the plants were not self-fertile. Their anthers were sterile-like in appearance and there was no evidence of pollen. Only one wheat BC_1 and two jointed goatgrass BC_1s had anthers that were visibly producing pollen. All BC_1 plants had open spikelets (Table 3).

Three plants with jointed goatgrass-looking spikes and three plants with wheat-looking spikes were recovered after only one backcross to jointed goatgrass and wheat, respectively. However, in the BC_1 generation, a wide variation in spike morphology was observed (Appendix, Table A4). For most plants, disarticulation was of the whole-spike type. Disarticulation at the spikelet level was observed in one jointed goatgrass BC_1 plant, while seven plants had a tough rachis.

BC_2 plants had up to 116 tillers. A high number of tillers is a jointed goatgrass characteristic. The two BC_2 plants with jointed goatgrass as the recurrent pollen donor had 46 and 79 tillers, while the four BC_2 plants with wheat as the recurrent pollen donor had 10, 11, 11, and 19 tillers. Except for those four wheat BC_2s, the plants in the

BC_2 group had spikes more similar to jointed goatgrass. Disarticulation at the spikelet level was found in 10 BC_2 plants, while three plants had a tough rachis.

Male fertility increased in BC_2 and BC_1S_1 plants: 13 out of 41 BC_1 plants and 27 out of 39 BC_1S_1 plants had anthers that were visibly producing pollen. However, most of the plants had open spikelets (Table 3). With an average of 32 tillers per plant, the BC_2 plants were more prolific than the BC_1 and BC_1S_1 plants (15 and 17 tillers per plant, respectively). Most of the original BC_1s were to wheat, and in the next generation, either selfed to produce BC_1S_1 plants or crossed with jointed goatgrass (most BC_2). The high expression of jointed goatgrass morphological traits (spike appearance, high number of tillers, disarticulation at the spike or spikelet level) in the BC_2 plants (Appendix, Table A4) shows how one cross to jointed goatgrass can affect plant morphology.

Seed production in the greenhouse

Fertility was determined by allowing the plants to set seed both in selfing bags and by open-pollination in the greenhouse (Appendix, Table A5). The two F_1 plants produced no seeds. In the BC_1 generation, 90% of the plants were sterile (Table 4). Only four plants showed partial fertility and produced a few seeds (1 to 11, with 1 or 2 seeds per spike). Two of the seed-producing BC_1 plants apparently had wheat as the paternal parent, and the two others jointed goatgrass. Three of the four seed-producing BC_1s had fertile-like anthers, producing seeds in the unbagged spikes but no seeds in the bagged spikes. Seeds produced in unbagged spikes may have resulted from self-

pollination. In a previous study where 1461 (1997) and 757 BC_1 (1998) plants were grown in the greenhouse, seed set was observed on 59 (4.1%) and 16 (2.1%) individuals, respectively (Snyder *et al.*, 2000), showing that a small proportion of the BC_1 plants can recover some partial self-fertility.

Table 4: Hybrid fertility and seed production.

	BC_1	BC_2	BC_1S_1
Sterile plants	**35 (90%)**	**27 (66%)**	**15 (38%)**
Fertile Plants	**4**	**14**	**24**
Female-fertile plants[a]	4 (10%)	8 (19 %)	10 (26%)
Self-fertile plants[b]	0	6 (15 %)	14 (36%)
Fertile spikes	**14**	**220**	**205**
Fertile spikes/plant[c]	3.5 (1-9)	15.7 (1-53)	8.5 (1-18)
% fertile spikes/plant[c]	18% (3-33%)	44% (3-100%)	64.4% (6-100%)
Seeds	**17**	**1453**	**1912**
Seeds/fertile plant[c]	4.25 (1-11)	103.8 (1-295)	79.7 (1-403)
Seeds/fertile spike[c]	1.2 (1-2)	6.6 (1-36)	9.4 (1-37)
Seeds/bagged spike[c]	0	11 (1-29)	9.8 (1-25)

a- Female-fertile plants: seed production in the unbagged spikes, no seed production in the bagged spike.
b- Self-fertile plants: seed production in both bagged and unbagged spikes.
c- Average (range)

The BC_2 and BC_1S_1 generations showed a marked increase in seed set. Partial-female fertility as well as partial and complete self-fertility were found in the advanced generations (Table 4). Seed production was low in partially-female fertile plants (in unbagged spikes), and high in self-fertile plants (in bagged and unbagged spikes).

The two self-fertile BC_2 plants that had jointed goatgrass as the recurrent pollen parent produced a total of 207 and 336 seeds in 71 and 46 spikes, respectively. Of the four wheat BC_2 plants, one was sterile, two were partially-female fertile and produced a total of 4 and 11 seeds (in unbagged spikes), and one was self-fertile and produced 295 seeds (in bagged and unbagged spikes). Nine additional BC_2 plants (probably wheat BC_1 – jointed goatgrass BC_2) set seed. They had a jointed goatgrass appearance, and produced up to 234 seeds per plant (Appendix, Table A5).

BC_1S_1 plants showed the highest percentage of partial and complete self-fertility (Table 4). Self-fertile plants produced up to 403 seeds per plant. Most of the BC_1S_1 had wheat as the pollen donor in the first backcross. However, one of the two plants presumably resulting from selfing on a jointed goatgrass BC_1 recovered some partial self-fertility, producing 1 to 2 seeds per spike, for a total of 25 seeds.

High Molecular Weight glutenin analysis

The variation of HMW glutenin subunits composition was determined in three generations of seeds by the use of one-dimensional SDS PAGE.

F_1 hybrids

As expected, the banding profile of the F_1 hybrid was the sum of the bands of the parents (Figure 1).

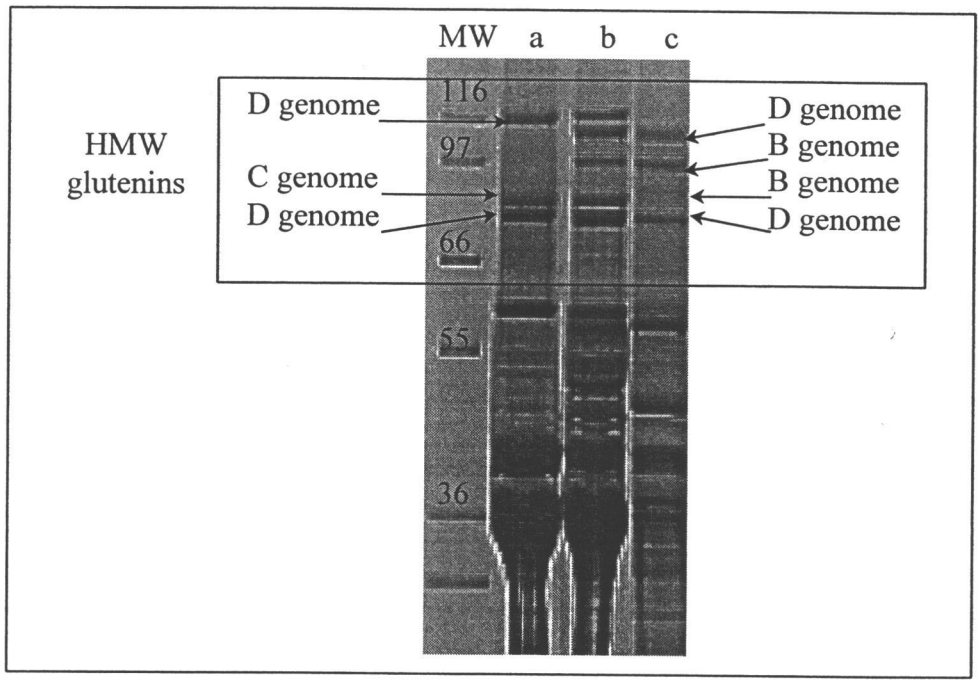


Figure 1: High Molecular Weight subunits composition of wheat, jointed goatgrass, and their F1 hybrid: (a) 3 band-pattern unique to jointed goatgrass, (b) F_1 hybrid, with both jointed goatgrass and wheat bands (c) 4-band pattern unique to wheat (Madsen). Reconstructed SDS-Page gel.

The lower wheat B-band was faint in 'Madsen', and not scored in the analysis of banding patterns of the backcross plants. Also, to simplify the analysis, and following Galili *et al.* (1988), the slower migrating wheat subunits that appeared as a closely migrating doublet were considered as a single band. The analysis was, therefore, based on the presence or absence of six HMW glutenin subunits (three wheat subunits and three jointed goatgrass subunits).

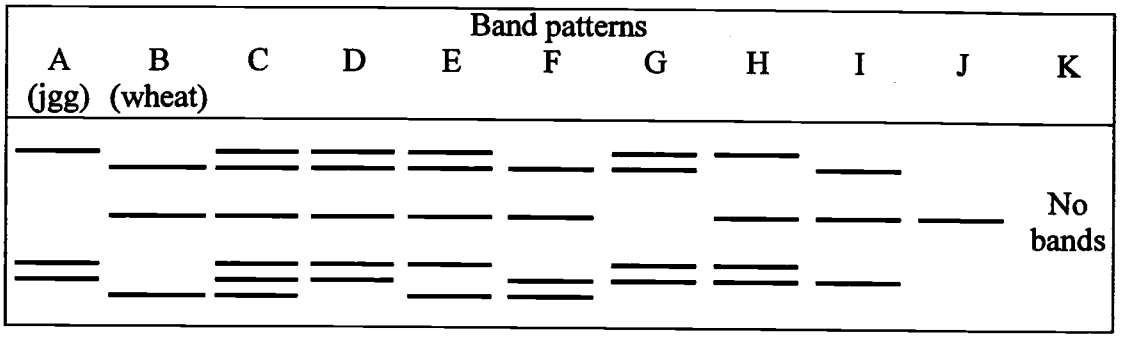
BC_1 generation

The HMW glutenin profiles of the experimental hybrids were very diverse, with banding patterns that combined different contributions from the patterns of wheat and jointed goatgrass (Table 5 and Appendix, Table A6). The majority (56%) of the BC_1 plants had a F_1-hybrid banding pattern, with all the wheat bands and all the jointed goatgrass bands. Three plants were missing distinctive wheat bands, which indicates that jointed goatgrass might have been the paternal parent. Indeed, if wheat had been the pollen donor, all the wheat glutenin subunits should be present in the BC_1 progeny. The absence of wheat bands in the HMW glutenin profile of a BC_1 seed suggests a jointed goatgrass backcross. The absence of jointed goatgrass subunits in 17 BC_1 seeds suggests a wheat backcross.

One BC_1 plant had a HMW glutenin pattern of only wheat bands, and no BC_1 plant was found that displayed only jointed goatgrass subunits.

In two lanes, only one band (the upper B genome band) appeared. The two seeds germinated and produced plants with 52 and 56 chromosomes, indicating that complete genomes of wheat and jointed goatgrass were present in the diploid and haploid state, respectively (cf. section on chromosome counts). The absence of bands might be due to protein degradation, manipulation error (excess of sample buffer, or electrophoretic run of insufficient amount of sample), and/or a gel artifact. This pattern was not considered a characteristic hybrid HMW glutenin profile.

Table 5: The HMW glutenin banding profiles observed and corresponding number of plants in each seed category.



Pattern	Description	Number of plants		
		BC_1	BC_2	BC_1S_1
C	**6 bands**: F_1 hybrid banding pattern	**28**	**26**	**3**
	5 bands: 1 lower D-genome band missing	**12**	**8**	-
D	• lower wheat D-genome band missing	1	6	-
E	• lower jointed goatgrass D-genome band missing	11	2	-
	4 bands	**7**	**8**	**9**
F	• 3 wheat bands + lower jointed goatgrass D-genome band	5	-	6
G	• 3 jointed goatgrass bands + upper wheat D-genome band	1	4	-
H	• 3 jointed goatgrass bands + upper wheat B-genome band	1	4	3
	3 bands:	**1**	**7**	**36**
B	• wheat banding pattern	1	4	31
A	• jointed goatgrass banding pattern	-	3	1
I	• wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level	-	-	4
J	**1 band**: upper B-genome band	**2**	-	-
K	**No bands**	-	**1**	**2**

BC_2 and BC_1S_1 generation

In the BC_2 generation, about half of the seeds still retained all of the wheat and jointed goatgrass bands and showed a 6-band HMW glutenin profile (Table 5). The four BC_2 plants resulting from two successive wheat backcrosses had a wheat banding pattern. Three BC_2 seeds had a HMW glutenin profile comprised of only jointed goatgrass bands, and might have had jointed goatgrass as recurrent pollen donor. Sixteen seeds had a 4- or 5-band profile: 14 plants had lost one or two wheat bands, and two plants had lost one jointed goatgrass subunit. The absence of wheat subunits in BC_2 lends support to jointed goatgrass as the pollen donor in the second backcross. Jointed goatgrass was indeed probably the pollen donor for most crosses, since there were no wheat plots for the production of the BC_2 generation.

Diversity decreased in the BC_1S_1 seed category: the number of banding patterns decreased from eight in the BC_1 generation to six in the BC_1S_1 generation ('no-band' not considered here, since the seeds did not germinate), and the number of glutenin subunits per line also decreased. Only three seeds retained all six bands (three from wheat and three from jointed goatgrass). While most of the BC_1 seeds had five or six HMW glutenin subunits, 45 plants out of the 50 BC_1S_1 studied had a 3- or 4-band profile. These might be due to the loss of unmatched chromosomes during meiosis and/or complex genetic interactions related to seed protein expression (gene silencing).

Most BC_1S_1 seeds had a wheat banding pattern, or a 4-band glutenin profile comprised of the three wheat bands plus one jointed goatgrass band. They might have

been produced by selfing of a wheat BC_1. Only one seed had a jointed goatgrass banding pattern and might have resulted from selfing of a jointed goatgrass BC_1. The 3-band pattern comprised of wheat bands with the lower D-genome band at jointed goatgrass D-genome band level is unique to the BC_1S_1 seed category. This banding pattern might be the result of chromosomal rearrangements during meiosis, chromosome aberrations and/or gene silencing.

The decrease in HMW glutenin subunits diversity observed in the BC_1S_1 seed, compared to the BC_1 generation, would also be expected in the BC_2 seed, to an even greater extent, if the same parent were the pollen donor in the two backcross. However, in this study, most BC_2 plants were produced by a wheat pollen parent in the first generation and a jointed goatgrass pollen parent in the second generation. This could partially explain why half of the BC_2 plants still had both sets of bands. BC_2 seeds that had only wheat or jointed goatgrass as the paternal parent had the respective 3-band recurrent pollen donor HMW glutenin profile.

Analysis of the seed progeny

BC_1S_1, BC_2S_1 and BC_1S_2 seeds were collected from BC_1, BC_2, and BC_1S_1 hybrid derivatives, respectively. Seed protein analysis was done on 1 to 10 seeds per plant, from both bagged and unbagged spikes. No difference in HMW glutenin banding profile was found between seeds from bagged spikes and seeds from unbagged spikes, suggesting that seeds in unbagged spikes might have been produced by selfing.

The progeny of plants with a wheat or jointed goatgrass banding pattern maintained the characteristic wheat or jointed goatgrass parental banding pattern, respectively (Appendix, Table A7). For all three parental generations (BC_1, BC_2, and BC_1S_1), the seed harvested from plants showing four or five HMW glutenin subunits either maintained the same banding pattern as the mother plant, or developed the 3-band pattern of wheat or jointed goatgrass, depending on which served as the pollen donor parent. The ratio between seeds with three HMW glutenin subunits and seeds with four subunits did not seem to follow any segregation pattern.

BC_1 and BC_2 plants with a 6-band HMW glutenin profile produced seeds that had lost one or two of the parental glutenin subunits. Depending on the origin of the pollen in the last backcross, either wheat or jointed goatgrass bands were lost. Thus, the hybrid HMW glutenin banding pattern returned to the distinctive 3-band pattern of the wheat or jointed goatgrass pollen parent. One exception is a BC_2S_1 seed that lost one wheat band and one jointed goatgrass band compared to its BC_2 mother plant. Seeds with less than three bands were visibly abnormal (shriveled seeds) and did not germinate.

Chromosome counts

The chromosome number of the plants was checked using the mitotic aceto-orcein squash technique for root tips. Winter wheat x jointed goatgrass F_1 hybrids are pentaploid with 35 chromosomes (21 from wheat and 14 from jointed goatgrass) and ABCDD as the genomic constitution (Table 6).

Table 6: Chromosome numbers and genomic constitution of wheat, jointed goatgrass, and the first hybrid generation.

	Number of chromosomes	Genomic composition
Wheat	42	AABBDD
Jointed goatgrass	28	CCDD
F_1 hybrid	35	ABCDD

BC_1 generation

A great variation in number of chromosomes was found in BC_1 plants, with chromosome counts ranging from 36 to 57 chromosomes (Table 7). Those results are in agreement with Seefeldt *et al.* (1998), and Zemetra *et al.* (1991), who report chromosome numbers varying between 39 and 54, and 38 and 56, respectively.

Table 7: Chromosome numbers observed in the BC_1 plants.

Chromosome number	36-38	42	43-44	45-46	47-48	50	52-53	54	55-56	57
Number of plants	2	2	4	7	4	1	4	7	5	1

As discussed previously, the BC_1 generation material used in this study may result from a backcross with wheat or jointed goatgrass. It is assumed that no selfing occurred in the F_1 hybrids. If F_1 were self-fertile, chromosome doubling resulting in F_2 plants with 70 chromosomes would be expected. (Zemetra *et al.*, 1991). However, wheat x jointed goatgrass hybrids are male-sterile (Farooq *et al.*, 1995, Zemetra *et al.*,

1998; personal observations), and chromosome counts showed no individuals with 70 chromosomes, which suggests that the hybrid progenies produced on the F_1 plants are indeed BC_1s, with wheat or jointed goatgrass as parent pollen donor.

The wheat BC_1s can be classified into two general groups according to their chromosome numbers: 17 plants have chromosome numbers in the 52 to 57 chromosome range and four plants in the 42 to 48 chromosome range. Ten additional plants probably resulting from a wheat backcross have chromosome numbers in the 42 to 48 chromosome range. Plants with 52-56 chromosomes may have resulted from the union of a wheat gamete (21 chromosomes) and an unreduced (35 chromosomes) or partially unreduced (31-34 chromosomes) hybrid gamete. Plants with 42-48 chromosomes may have resulted from the fusion of a wheat gamete and a reduced or partially reduced hybrid gamete with 21-27 chromosomes. The variable number of chromosomes donated by the hybrid (21-27) may be due to random chromosome segregation during meiosis.

The plant with 57 chromosomes is believed to have resulted from the union of an unreduced hybrid gamete with a hyperploid chromosome number of 36 and a 21-chromosome wheat gamete. An unreduced gamete with more than 35 chromosomes may be produced by the lack of chromatid separation of a given chromosome during the second division of meiosis.

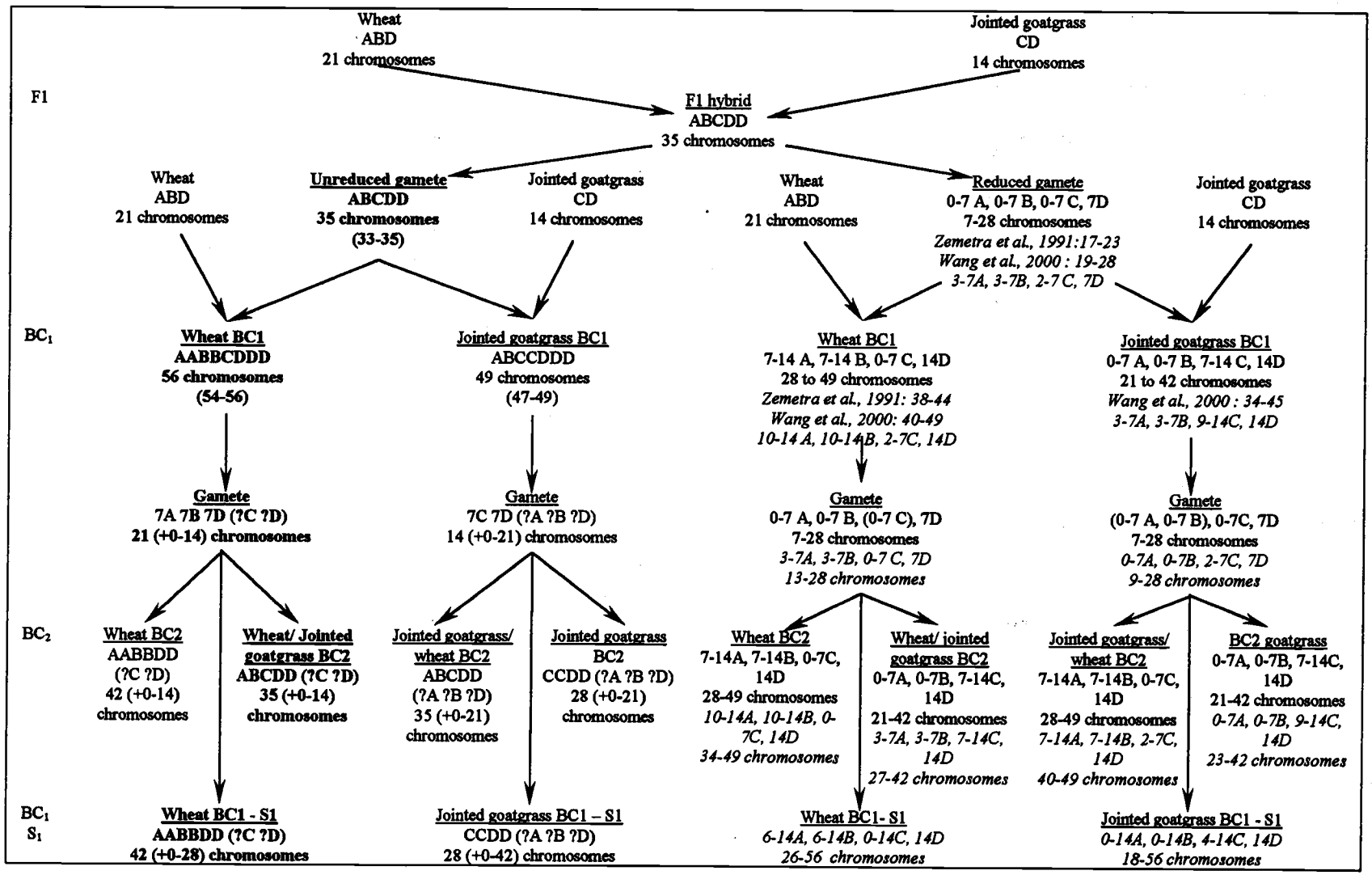
When a reduced F_1 hybrid gamete is fertilized by jointed goatgrass pollen (14 chromosomes), the resulting BC_1 plants are expected to have lower chromosome numbers. Indeed, the two presumed jointed goatgrass BC_1s in this study have 36 and 38 chromosomes. Their F_1 hybrid parents produced 22- and 24-chromosome gametes,

respectively. However, if meiotic restitution occurs in the F_1 hybrid, the BC_1s for which jointed goatgrass is the male parent can have up to 49 chromosomes (Figure 2). One of the BC_1 plants with 45-46 chromosomes had a jointed goatgrass appearance.

The plant with 50 chromosomes might have resulted from the union of an unreduced hybrid gamete carrying a hyperploid chromosome number of 36 with a 14-chromosome jointed goatgrass gamete.

Figure 2: Theoretical minimum and maximum number of chromosomes in the BC_1, BC_2 and BC_1S_1 following meiotic restitution or absence of meiotic restitution in the F_1 hybrid. The assumption was made that meiotic restitution is inhibited in advanced generation by increased bivalent pairing due to the addition of AB chromosomes from wheat or C chromosomes from jointed goatgrass.

Figure 2


Wheat
ABD
21 chromosomes
Jointed goatgrass
CD
14 chromosomes
F1
F1 hybrid
ABCDD
35 chromosomes
Wheat
ABD
21 chromosomes
Unreduced gamete
ABCDD
35 chromosomes
(33-35)
Jointed goatgrass
CD
14 chromosomes
Wheat
ABD
21 chromosomes
Reduced gamete
0-7 A, 0-7 B, 0-7 C, 7D
7-28 chromosomes
Zemetra et al., 1991:17-23
Wang et al., 2000 : 19-28
3-7A, 3-7B, 2-7 C, 7D
Jointed goatgrass
CD
14 chromosomes
BC1
Wheat BC1
AABBCDDD
56 chromosomes
(54-56)
Jointed goatgrass BC1
ABCCDDD
49 chromosomes
(47-49)
Wheat BC1
7-14 A, 7-14 B, 0-7 C, 14D
28 to 49 chromosomes
Zemetra et al., 1991: 38-44
Wang et al., 2000: 40-49
10-14 A, 10-14B, 2-7C, 14D
Jointed goatgrass BC1
0-7 A, 0-7 B, 7-14 C, 14D
21 to 42 chromosomes
Wang et al., 2000 : 34-45
3-7A, 3-7B, 9-14C, 14D
Gamete
7A 7B 7D (?C ?D)
21 (+0-14) chromosomes
Gamete
7C 7D (?A ?B ?D)
14 (+0-21) chromosomes
Gamete
0-7 A, 0-7 B, (0-7 C), 7D
7-28 chromosomes
3-7A, 3-7B, 0-7 C, 7D
13-28 chromosomes
Gamete
(0-7 A, 0-7 B), 0-7C, 7D
7-28 chromosomes
0-7A, 0-7B, 2-7C, 7D
9-28 chromosomes
BC2
Wheat BC2
AABBDD
(?C ?D)
42 (+0-14)
chromosomes
Wheat/ Jointed goatgrass BC2
ABCDD (?C ?D)
35 (+0-14)
chromosomes
Jointed goatgrass/ wheat BC2
ABCDD
(?A ?B ?D)
35 (+0-21)
chromosomes
Jointed goatgrass
BC2
CCDD (?A ?B ?D)
28 (+0-21)
chromosomes
Wheat BC2
7-14A, 7-14B, 0-7C, 14D
28-49 chromosomes
10-14A, 10-14B, 0-7C, 14D
34-49 chromosomes
Wheat/ jointed goatgrass BC2
0-7A, 0-7B, 7-14C, 14D
21-42 chromosomes
3-7A, 3-7B, 7-14C, 14D
27-42 chromosomes
Jointed goatgrass/ wheat BC2
7-14A, 7-14B, 0-7C, 14D
28-49 chromosomes
7-14A, 7-14B, 2-7C, 14D
40-49 chromosomes
BC2 goatgrass
0-7A, 0-7B, 7-14C, 14D
21-42 chromosomes
0-7A, 0-7B, 9-14C, 14D
23-42 chromosomes
BC1 S1
Wheat BC1 - S1
AABBDD (?C ?D)
42 (+0-28) chromosomes
Jointed goatgrass BC1 – S1
CCDD (?A ?B ?D)
28 (+0-42) chromosomes
Wheat BC1- S1
6-14A, 6-14B, 0-14C, 14D
26-56 chromosomes
Jointed goatgrass BC1 - S1
0-14A, 0-14B, 4-14C, 14D
18-56 chromosomes

BC_2 and BC_1S_1 generation

In BC_2 plants, chromosome numbers varied between 28 and 49 (Table 8). A chromosome number of 28 suggests that jointed goatgrass was the recurrent parent pollen donor, while numbers closer to 42 and above point to wheat as the recurrent pollen donor. For the two plants with 28 chromosomes, the pollen parent's identity is confirmed by the jointed goatgrass appearance of the plant (high number of tillers, narrow leaves, cylindrical spike that breaks at the joints), and the HMW glutenin profile. These two plants were self-fertile, producing 207 and 336 seeds respectively. These BC_2S_1 seeds also had a jointed goatgrass HMW glutenin banding profile. The BC_2 plants resulting from recurrent wheat backcrosses had 42, 44, and 49 chromosomes. These numbers are in agreement with a study by Koszgegi *et al.* (1998), which reports chromosome numbers between 42 and 48 for wheat BC_2s.

Table 8: Chromosome numbers observed in the BC_2 plants.

Chromosome number	28	30	33	34	35	36	37	38	39	40	41	42	44	49
Number of plants	2	1	1	3	4	9	7	5	1	1	2	1	1	1

Most BC_2 plants had a chromosome number between 34 and 38. These numbers would be expected in plants resulting from a first backcross between an unreduced F_1 hybrid gamete (35 chromosomes) and a wheat gamete (21 chromosomes), elimination of unpaired jointed goatgrass chromosomes during meiosis

(14 C and D chromosomes), and a second backcross to jointed goatgrass (14 chromosomes) (Figure 2). This scenario is consistent with the interpretation of the evidence for the BC_1 plants (mostly backcrosses of unreduced F_1 hybrid gametes to wheat) and the design of the experimental plan for production of the BC_2 generation (only jointed goatgrass present).

In the BC_1S_1 generation, the chromosome numbers ranged between 33 and 52 chromosomes (Table 9).

Table 9: Chromosome numbers observed in the BC_1S_1 plants.

Chromosome number	33	40	41	42	43	44	45	46	47	48	49	52
Number of plants	2	1	1	3	4	9	7	5	1	1	2	1

The plant with 33 chromosomes may have resulted from selfing of a jointed goatgrass BC_1. It had a jointed goatgrass-like morphology and HMW glutenin banding pattern. Most plants in the BC_1S_1 generation were the product of selfing in wheat BC_1s, and had chromosome numbers between 40 and 48. The elimination of unpaired jointed goatgrass chromosomes during meiosis in the BC_1 plants with a high number of chromosomes returned the chromosome number to 42 (and up to 48). Wheat BC_1-S_1 with fewer than 42 chromosomes may have resulted from a partially unreduced gamete or from a reduced gamete in the F_1 hybrid (missing one or two wheat chromosomes) (Figure 2). Since the chromosomes in hexaploid wheat are triplicate, partial or whole chromosome deletions are usually not lethal, since essential genes on

eliminated chromosomes are present on their homoeologues (Sears, 1954; Payne *et al.*, 1984). Chromosomes numbers of 49 and 52 are not yet explained.

DISCUSSION

The morphological, protein, and chromosome count evidence suggest that thirty-two BC_1 plants in this study had wheat as the paternal parent, while only two to four BC_1 plants had jointed goatgrass as the paternal parent. Zemetra *et al.* (1998) have found that in controlled crosses, the frequency of seed set on hybrids was similar when the F_1 hybrids were pollinated by wheat or jointed goatgrass (2.0 and 2.2% respectively). In the experimental plots which produced the BC_1 material for this study, although by design both wheat and jointed goatgrass had an equal chance to serve as the paternal parent of BC_1 plants, the pollination success ratio appears to be in favor of wheat. However, when the BC_1 hybrid seed was planted in jointed goatgrass plots for the production of the BC_2 generation, most of the progeny BC_2 plants were very similar to jointed goatgrass. Only four of these BC_2 plants appear to have wheat as the paternal parent in the second backcross. This difference in pollen donor success between the BC_1 and BC_2 generations can be explained in several ways: difference in climatic conditions between the two years, influencing either or both tillering, flowering time, and pollen movement; higher density of jointed goatgrass in the BC_2 experimental plots.

High densities of jointed goatgrass are found in eastern Oregon wheat fields (personal observations), and situations where more jointed goatgrass pollen than wheat pollen is available to the hybrids may occur in some wheat fields. This research also indicates that it is possible that wheat and jointed goatgrass may alternate as pollen donor, leading to complex field population dynamics. A study that mimics the winter

wheat/fallow rotation typical to Eastern Oregon has been started at the OSU Lewis Brown Research Farm to verify if observations in controlled crosses and experimental plots also apply in field conditions. This field has been planted with 'Madsen' and jointed goatgrass for monitoring the hybrid population development and dynamics over a five-year period.

Greenhouse studies

Growth habit and leaf and spike morphology of the BC_1 hybrids varied from wheat-like to jointed goatgrass-like. If the hybrids are recurrently backcrossed to wheat, their progenies become more and more like wheat, and if they are recurrently backcrossed to jointed goatgrass, their progenies gradually look more and more like jointed goatgrass. BC_2 and BC_1S_1 plants were found that looked like jointed goatgrass or wheat, indicating that one to two backcrosses are enough to return the plant appearance to one of the parents.

The pollen donor parent of the BC_1 plants cannot be identified by morphological traits such as coleoptile color, plant height, growth habit, leaf and spike morphology (Snyder *et al.*, 2000). In this study, wheat BC_1 and BC_1S_1 plants were found that were more similar to jointed goatgrass than wheat. Although morphology cannot be used to determine the pollen donor, a morphological characterization of the hybrids can supplement results from other techniques.

Stable, self-fertile hybrids can develop in one to two backcross generations. In a study by Zemetra *et al.* (1998), self-fertile plants were produced after two

backcrosses of wheat x jointed goatgrass hybrids to jointed goatgrass. In this study, as well as in the previous study by Snyder *et al.* (2000), both wheat BC_1 and jointed goatgrass BC_1 plants grown in the greenhouse showed some partial self-fertility. Seed set in both unbagged and bagged spikes increased in the BC_2 and BC_1S_1 plants: a total of 1459 seeds were produced on 14 plants in the BC_2 generation, and 1912 seeds were harvested from 24 BC_1S_1 plants.

High Molecular Weight glutenin analysis

Nine HMW glutenin banding profiles of 3 to 6 bands were identified. All banding patterns were found in varying combinations of at least 2 seed categories (Table 5), and three glutenin profiles were found in all three seed categories (BC_1, BC_2, and BC_1S_1). The jointed goatgrass banding pattern was not found in the BC_1 generation, but this could be due to the low number of jointed goatgrass BC_1 plants studied. A 3-band HMW glutenin pattern was unique to the BC_1S_1 generation. Since most patterns were found in more than one seed category, the HMW technique alone cannot be used to determine the hybrid generation.

The HMW glutenin technique can provide some evidence for determining the paternal parent in the first backcross generation. Indeed, all of the glutenin subunits of the pollen donor should be present in the seed resulting from the backcross. Missing wheat bands suggest that jointed goatgrass was the pollen donor parent, and vice versa.

The HMW glutenin technique does not provide a rapid screening for hybrids because of false negatives. HMW glutenin patterns consisting of only wheat bands or only jointed goatgrass bands were found in hybrids, as early as in the first backcross generation, and most BC_1S_1 seeds had wheat or jointed goatgrass banding patterns. The HMW glutenins are coded on the long arm of the group 1 homoeologous chromosomes, i.e. chromosomes 1A, 1B, 1D, and 1C. Therefore, this seed protein trait is limited to characterizing only this component of the hybrid genotype.

Because differences in gel concentrations will influence molecular weight values and due to the close association of the HMW glutenin subunits, consistent molecular weight values are difficult to obtain with SDS-PAGE (Ng and Bushuk, 1989; Shewry *et al.*, 1984). The Payne numerical nomenclature system (Payne and Lawrence, 1983) is more widely used among breeders and cereal chemists than the molecular weights. While this system is useful for labeling subunits from the wheat parent, it does not have the capability for dealing with the hybrid banding patterns. For this technique to become more useful as a screening tool, band value assignment should be developed using either the Ng and Bushuk system for corrected molecular weights or an expanded version of the Payne nomenclature.

Chromosome counts

Chromosome counts in the BC_1 generation ranged from 36 to 57 chromosomes. A plant with 36 chromosomes is a jointed goatgrass backcross, and plants with 51-56 chromosomes are wheat backcrosses. BC_1 plants with 43 to 50

chromosomes could result from pollination by wheat or by jointed goatgrass. The paternity of BC_1 plants cannot always be determined by counting the total number of chromosomes (Wang *et al.*, 2000b). HMW glutenin banding patterns and morphology of the plant provide additional clues. However, for BC_1 plants with 43 to 50 chromosomes and displaying a 6-band HMW glutenin pattern, the pollen donor parent cannot be determined with certainty. Three out of the 39 BC_1 plants studied fell into this category.

Almost half (48%) of the BC_1 plants studied were the product of meiotic restitution in the F_1 hybrid. This frequency of meiotic restitution is much higher than those reported in previous studies with controlled crosses (Wang *et al.*, 2000b, Farooq *et al.*, 1995). These previously reported percentages of meiotic restitution might not include partial meiotic restitution while the percentage in this study includes both partial and complete meiotic restitution. If meiotic restitution happens after homologous chromosomes pair in metaphase of the first division of meiosis, there is a possibility for recombination between the respective D-genome chromosomes of wheat and jointed goatgrass.

The D genome of jointed goatgrass and wheat was donated by the same diploid parent, *Aegilops tauschii* Cosson (Kimber and Zhao, 1984). The D genomes of both jointed goatgrass and wheat have remained very similar to the D genome of *Ae. tauschii* (Linc *et al.*, 1999; Gill *et al.*, 1991). Koszgegi *et al.* (1998) studied chromosome pairing in wheat x jointed goatgrass F_1 hybrids and discovered that the D-genome chromosomes from wheat and jointed goatgrass form chromosome associations (maximum 6 bivalent rings and 1 bivalent rod) at meiotic metaphase I.

They concluded that the D-genome chromosomes in backcross lines should be highly recombined and derived from both the D genomes of wheat and jointed goatgrass. Farooq *et al.* (1995) reported on the possibility of crossing over between D-genome chromosomes of wheat and jointed goatgrass in wheat BC_1 lines with a 49-chromosome number.

Intergenomic translocations also need to be taken into account when evaluating risk of transfer of herbicide resistance between wheat and jointed goatgrass. Translocations between the A and B genome have taken place during the evolution of hexaploid wheat (Nelson *et al.*, 1995). Translocation of genes (e.g. herbicide-resistance gene) of the A or B genomes to a chromosome of the D genome in hybridization events between jointed goatgrass and wheat is theoretically possible.

As well as describing HMW glutenin banding patterns and chromosome numbers for three backcross generations, this study has shown that for a wheat x jointed goatgrass F_1 hybrid, backcross hybridization can go in either direction. Evidence to support this conclusion includes: (1) a BC_2 plant with a jointed goatgrass banding pattern, 28 chromosomes, and jointed goatgrass plant morphology, and (2) a BC_2 plant with a wheat banding pattern, 42 chromosomes, and wheat plant morphology.

Additional information on the genetic composition of these hybrids is needed to determine if intragenomic (within the D genome) and intergenomic translocations can occur during the introgression process, i.e. if wheat genetic material is present in plants resulting from recurrent jointed goatgrass backcross, and vice versa. Using the Genomic In Situ Hybridization (GISH) technique, Wang *et al.* (2000a) observed

retention of A/B genome chromosomes in jointed goatgrass backcross lines (BC_2S_1), and translocations between chromosomes of the wheat A/B genome and the jointed goatgrass C genome. To determine if translocation can occur between the D genomes of wheat and jointed goatgrass, molecular markers such as microsatellites must be used.

The results of the present study are currently being used in studies of population structure in natural hybrids collected in Oregon wheat fields, and the experimental hybrid lines are being maintained for future genetic study involving GISH and microsatellites.

SUMMARY AND CONCLUSIONS

Jointed goatgrass, one of the more weedy species of winter wheat fields, is a wild tetraploid wheat. Jointed goatgrass (CD genomes) and bread wheat (ABD genomes) are genetically related by the D genome. Hybrids between wheat and jointed goatgrass are frequently found in infested wheat fields. The discovery of seed-containing hybrids in infested wheat fields (Mallory-Smith *et al.*, 1996) raises the possibility of gene flow between wheat and jointed goatgrass. The imminent release of herbicide-resistant wheat cultivars increases the necessity to better study the hybrids between wheat and jointed goatgrass and to determine the gene flow potential.

Electrophoresis (SDS-PAGE technique) of the High Molecular Weight (HMW) glutenin seed proteins, chromosome counts, and morphological evaluation were used to analyze wheat x jointed goatgrass hybrids and reciprocal backcross derivatives (BC_1, BC_2, and BC_1S_1) produced in field plots.

The main findings are as follows:

1. Most seeds produced on the F_1 hybrids in the jointed goatgrass plots had wheat instead of jointed goatgrass as the pollen parent. These results emphasize the importance of isolation of the research material because of pollen transport via wind. Thirty two BC_1 plants in this study might have had wheat as the paternal parent, while only two to four BC_1 plants had jointed goatgrass as the pollen parent. However, when the BC_1 hybrids were placed in jointed goatgrass plots for the production of the BC_2 generation, the progeny plants obtained were very similar to jointed goatgrass.

2. BC_2 and BC_1S_1 plants were found that looked like jointed goatgrass or wheat.
3. Stable, self-fertile hybrids were produced in one to two backcross generations. Partial female fertility was observed in all generations. Partial and complete self-fertility was observed in BC_2 and BC_1S_1 plants.
4. Gel electrophoresis of the HMW glutenin seed proteins revealed 9 patterns containing 3 to 6 bands. Diversity in number of different patterns and number of subunits/seeds decreased with advanced generations. Jointed goatgrass-like and wheat-like glutenin patterns were found in BC_2 and BC_1S_1 seeds.
5. The HMW glutenin technique alone cannot be used to determine the hybrid generation and, in most cases, the pollen donor parent.
6. Chromosome numbers ranged from 36 to 57 in the BC_1 plants, 28 to 49 in the BC_2 plants, and 33 to 52 in the BC_1S_1 plants.
7. Backcross hybridization can go be to either parent species. Evidence to support this conclusion includes: (1) a BC_2 plant with a jointed goatgrass banding pattern, 28 chromosomes, and a jointed goatgrass-like plant morphology, and (2) a BC_2 plant with a wheat banding pattern, 42 chromosomes, and a wheat-like plant morphology.

The combined HMW glutenins, chromosome counts, and morphological studies contribute to establishing a baseline for evaluating hybrids collected in natural populations. They document the rapid establishment of stable wheat x jointed goatgrass hybrids and offer a relatively inexpensive set of tools for selecting hybrid material for advanced genetic studies.

Indeed, additional information on the genetic composition of the hybrids is needed to determine if, during the introgression process, recombination can occur between the D genome of wheat and the D genome of jointed goatgrass and/or the A and B genomes of wheat and the C and D genomes of jointed goatgrass. Fluorescence in Situ Hybridization (FISH) on mitotic root tip cells and the use of molecular markers unique to wheat or to jointed goatgrass to screen the D genome chromosomes would be a way to address these questions.

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APPENDIX

Table A1-1. Reassessment of the pollen donor parent: BC_1 seeds harvested from F_1 hybrids in jointed goatgrass plots.

BC_1 plant number	Chromo-some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	Dispersal unit	Number of tillers	Glutenin profile of the BC_1 progeny	Pollen donor
1	ND	6	NM	wheat	spike	10	no seed	unknown
2	46~	6	NM	hybrid	none	8	no seed	unknown
3	NG	5	wheat?	---	---	---	---	---
4	54	4	jgg	hybrid (wheat)	spike	7	wheat	wheat
5	54	4	jgg	hybrid	spike	9	no seed	wheat
6	36	4	wheat	hybrid (jgg)	spike	13	no seed	jgg
7	43	4	jgg	hybrid (jgg)	spike	12	no seed	wheat
8	43	5	jgg?	hybrid	none	16	no seed	wheat?
9	52-54	6	NM	hybrid (wheat)	spike	12	no seed	wheat
10	NG	6	NM	---	---	---	---	---
11	45	5	jgg?	hybrid	spike	12	no seed	wheat?
12	46-47	4	jgg	hybrid	spike	30	N/D	wheat
13	47	5	jgg?	hybrid (wheat)	spike	11	no seed	wheat?
14	NG	4	wheat	---	---	---	---	---
15	45-46	6	NM	jgg	spike	15	no seed	jgg?
16	42	wheat	jgg	(hybrid) wheat	spike	20	no seed	wheat
17	46	6	NM	hybrid (wheat)	spike	22	no seed	wheat?
18	54	6	NM	hybrid (wheat)	spike	14	no seed	wheat
19	38	6	NM	jgg	spikelets	37	N/D	jgg
20	42	6	NM	wheat	none	37	no seed	wheat?
21	56	6	NM	hybrid (wheat)	spike	11	no seed	wheat
22	NG	6	NM	---	---	---	---	---
23	NG	6	NM	---	---	---	---	---
24	57	6	NM	hybrid (wheat)	spike	11	no seed	wheat
25	ND	5	jgg?	hybrid	spike	14	no seed	wheat?

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.

Table A1-2. Reassessment of the pollen donor parent: BC_1 seeds harvested from F_1 hybrids in wheat plots.

BC_1 plant number	Chromo-Some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	Dispersal unit	Number of tillers	Glutenin profile of the BC_1 progeny	Pollen donor
26	48	5	jgg?	wheat	spike	28	no seed	**wheat?**
27	48	6	NM	hybrid	no	24	no seed	**wheat?**
28	50-51	6	NM	jgg	spike	33	1 or 2 wheat bands missing	**jgg?**
29	55	6	NM	hybrid (jgg)	spike	17	no seed	**wheat**
30	NG	5	jgg?	---	---	---	---	---
31	52	1	both	hybrid (wheat)	spike	11	no seed	**wheat**
32	45	5	jgg?	hybrid (jgg)	hard	17	no seed	**wheat?**
33	NG	6	NM	---	---	---	---	---
34	48	6	NM	hybrid (wheat)	spike	10	no seed	**wheat?**
35	56	6	NM	hybrid (jgg)	no	13	no seed	**wheat**
36	NG	6	NM	---	---	---	---	---
37	53	6	NM	hybrid (wheat)	spike	21	no seed	**wheat**
38	52-53	6	NM	hybrid	spike	11	no seed	**wheat**
39	54	5	jgg?	hybrid (wheat)	spike	9	no seed	**wheat**
40	54	5	jgg?	hybrid (jgg)	spike	10	no seed	**wheat**
41	54	6	NM	hybrid (wheat)	spike	14	no seed	**wheat**
42	NG	6	NM	---	---	---	---	---
43	NG	6	NM	---	---	---	---	---
44	46	4	jgg	hybrid (wheat)	no	8	no seed	**wheat**
45	44	6	NM	hybrid (jgg)	spike	11	no seed	**unknown**
46	54	6	NM	hybrid (wheat)	spike	5	no seed	**wheat**
47	44	5	jgg?	hybrid (wheat)	spike	16	no seed	**wheat?**
48	56	1	both	hybrid (wheat)	spike	9	no seed	**wheat**
49	56	6	NM	hybrid (wheat)	spike	10	no seed	**wheat**
50	NG	4	jgg	---	---	---	---	---

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.

Table A1-3. Reassessment of the pollen donor parents: BC_2 seeds harvested from BC_1 hybrids produced in jointed goatgrass plots and grown in jointed goatgrass plots.

BC_2 plant number	Chromo-some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	dispersal unit	Number of tillers	Glutenin profile of the BC_2 progeny	Pollen donor	
								BC1	BC2
51	37	4	wheat	hybrid (jgg)	spike	21	no seed	a	jgg
52	34	6	NM	hybrid (wheat)	spike	24	no seed	a	b
53	37	6	NM	hybrid (jgg)	spike	27	no seed	a	b
54	NG	6	NM	---	---	---	---	---	---
55	38	6	NM	hybrid (jgg)	spike	23	no seed	a	b
56	ND	6	NM	hybrid (jgg)	spike	15	no seed	a	b
57	NG	6	NM	---	---	---	---	---	---
58	NG	jgg	wheat	---	---	---	---	---	---
59	NG	6	NM	---	---	---	---	---	---
60	36	6	NM	hybrid (jgg)	spike	26	no seed	a	b
61	35	6	NM	hybrid (jgg)	spike	23	no seed	a	b
62	NG	6	NM	---	---	---	---	---	---
63	37	4	wheat	hybrid (jgg)	spike	28	no seed	a	jgg
64	38	6	NM	jgg	spike	28	no seed	a	
65	37	4	wheat	jgg	spikelets	51	no seed	a	jgg
66	36	4	wheat	jgg	spike	30	jgg (7), jgg + 1 wheat band (3)	a	jgg
67	39	6	NM	hybrid (jgg)	spike	16	no seed	a	b
68	33	4	wheat	jgg purple	spikelets	25	jgg + 1 wheat band	a	jgg
69	41	6	NM	hybrid (jgg)	spike	31	jgg + 1 wheat band	a	b
70	38	6	NM	hybrid (jgg)	spike	25	no seed	a	b
71	NG	6	NM	---	---	---	---	---	---
72	38~	6	NM	jgg dark	spike	25	no seed	a	b
73	28	jgg	wheat	jgg	spikelets	79	jgg	jgg	jgg
74	34	6	NM	jgg	spike	29	no seed	a	b
75	35	6	NM	jgg	spikelets	51	mixed pattern	a	b

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.
a: evidence inconclusive; probable wheat.
b: evidence inconclusive; probable jointed goatgrass.

Table A1-4. Reassessment of the pollen donor parents: BC_2 seeds harvested from BC_1 hybrids produced in wheat plots and grown in jointed goatgrass plots.

BC_2 plant number	Chromo-some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	dispersal unit	Number of tillers	Glutenin profile of the BC_2 progeny	Pollen donor	
								BC1	BC2
76	49	wheat	jgg	hybrid (wheat)	spike	10	wheat	**wheat**	**wheat**
77	36	5	wheat?	hybrid (jgg)	spike	22	no seed	**a**	**jgg?**
78	41	6	NM	hybrid (jgg)	spike	15	no seed	**a**	**b**
79	36	6	NM	hybrid (jgg)	spike	27	no seed	**a**	**b**
80	37	6	NM	hybrid (jgg)	spike	29	no seed	**a**	**b**
81	30	6	NM	hybrid (jgg)	spike	24	no seed	**a**	**b**
82	37	4	wheat	jgg dark	spikelets	116	jgg (4), jgg + 1 wheat band (5)	**a**	**jgg**
83	40	5	wheat?	hybrid (jgg)	spike	27	no seed	**a**	**jgg?**
84	38	4	wheat	jgg	spikelets	45	jgg	**a**	**jgg**
85	NG	6	NM	---	---	---	---	—	—
86	36	5	wheat?	hybrid (jgg)	spikelets	58	no seed	**a**	**jgg?**
87	36	5	jgg?	jgg	spikelets	35	N/D	**a**	**b**
88	NG	0	all	---	---	---	---	—	—
89	NG	5	jgg?	---	---	---	---	—	—
90	35	6	NM	jgg	spike	27	no seed	**a**	**b**
91	35	5	wheat?	hybrid (jgg)	spike	14	no seed	**a**	**jgg?**
92	34	4	wheat	jgg	spike	31	jgg(1), jgg + 1 wheat band (4)	**a**	**jgg**
93	36	6	NM	hybrid (jgg)	spike	25	no seed	**a**	**b**
94	36	6	NM	hybrid (jgg)	spike	44	no seed	**a**	**b**
95	28	jgg	wheat	jgg	spikelets	46	jgg	**jgg**	**jgg**
96	37	5	wheat?	hybrid (jgg)	spike	39	jgg	**a**	**jgg?**
97	36	5	wheat?	jgg	spikelets	61	no seed	**a**	**jgg?**
98	ND	wheat	jgg	hybrid (wheat)	no	11	wheat	**wheat**	**wheat**
99	41-42	wheat	jgg	wheat	no	19	wheat	**wheat**	**wheat**
100	44	wheat	jgg	wheat	no	11	no seed	**wheat**	**wheat**

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.
a: evidence inconclusive; probable wheat.
b: evidence inconclusive; probable jointed goatgrass.

Table A1-5. Reassessment of the pollen donor parent: BC_1S_1 seeds harvested from BC_1 hybrids produced in jointed goatgrass plots and grown in the greenhouse.

BC_1S_1 plant number	Chromo-some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	Dispersal unit	Number of tillers	Glutenin profile of the BC_1S_1 progeny	Pollen donor for BC_1
101	43	wheat	jgg	wheat	no	12	wheat	**wheat**
102	40	3	1w, 2jgg	hybrid (wheat)	spike	15	no seed	**wheat?**
103	40	wheat	jgg	hybrid (wheat)	no	17	wheat	**wheat**
104	NG	wheat	jgg	---	---	---	---	**---**
105	44	wheat	jgg	hybrid (wheat)	no	14	wheat	**wheat**
106	NG	no band	all	---	---	---	---	**---**
107	NG	wheat	jgg	---	---	---	---	**---**
108	41	wheat	jgg	hybrid (wheat)	no	16	no seed	**wheat**
109	44	wheat	jgg	hybrid (jgg)	spike	48	no seed	**wheat**
110	45	wheat	jgg	hybrid (jgg)	spike	17	wheat	**wheat**
111	49~	4	wheat	hybrid (wheat)	spike	40	no seed	**unknown**
112	52~	4	wheat	(hybrid) jgg	spike	38	no seed	**unknown**
113	NG	wheat	jgg	---	---	---	---	**---**
114	ND	4	wheat	jgg purple	spikelets	11	no seed	**jgg**
115	33	jgg	wheat	jgg	spikelets	52	jgg	**jgg**
116	NG	4	jgg	---	---	---	---	**---**
117	46	3	1w, 2jgg	hybrid (wheat)	spike	17	3 bands: 2 wheat, 1 jgg	**wheat?**
118	NG	4	jgg	---	---	---	---	**---**
119	49	4	jgg	(hybrid) wheat	spike	7	wheat	**wheat**
120	49	6	NM	(hybrid) jgg	spike	27	no seed	**unknown**
121	42	wheat	jgg	wheat	no	16	no seed	**wheat**
122	46	wheat	jgg	hybrid (wheat)	spike	1	no seed	**wheat**
123	43	wheat	jgg	hybrid (jgg)	spike	20	N/D	**wheat**
124	44	wheat	jgg	hybrid (wheat)	spike	14	wheat	**wheat**
125	42	wheat	jgg	hybrid	no	11	wheat	**wheat**

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.

Table A1-6. Reassessment of the pollen donor parent: BC_1S_1 seeds harvested from BC_1 hybrids produced in wheat plots and grown in the greenhouse.

BC_1S_1 plant number	Chromo-some number	Number of glutenin subunits	Missing glutenin subunits	Spike morphology	Dispersal unit	Number of tillers	Glutenin profile of the BC_1S_1 progeny	Pollen donor for BC_1
126	46	wheat	jgg	hybrid (wheat)	spike	15	N/D	**wheat**
127	47	wheat	jgg	hybrid (wheat)	spike	10	no seed	**wheat**
128	NG	4	jgg	---	---	---	---	---
129	46	wheat	jgg	hybrid (wheat)	spike	12	N/D	**wheat**
130	42	wheat	jgg	hybrid (wheat)	no	26	wheat	**wheat**
131	48	6	NM	hybrid (jgg)	spike	27	no seed	**unknown**
132	48	wheat	jgg	hybrid	no	17	no seed	**wheat**
133	42	wheat	jgg	wheat	no	10	wheat	**wheat**
134	48	4	jgg	hybrid	spike	10	wheat(2), wheat + 1 jgg band (3)	**wheat**
135	48	4	jgg	hybrid (wheat)	spike	17	N/D	**wheat?**
136	NG	3	1w, 2jgg	---	---	---	---	---
137	NG	wheat	jgg	---	---	---	---	---
138	47	wheat	jgg	hybrid	spike	15	wheat (4), 3 bands: 2 wheat, 1 jgg (5)	**wheat**
139	46	wheat	jgg	hybrid	spike	11	wheat	**wheat**
140	44	wheat	jgg	hybrid (wheat)	spike	6	wheat	**wheat**
141	44	3	1w, 2jgg	hybrid	no	9	wheat (1), 3 bands: 2 wheat, 1 jgg (1)	**wheat?**
142	45	wheat	jgg	hybrid	spike	13	wheat	**wheat**
143	46	wheat	jgg	hybrid (jgg)	spike	21	wheat	**wheat**
144	NG	No band	6	---	---	---	---	---
145	49-50	6	NM	(hybrid) jgg	spike	18	no seed	**unknown**
146	40	wheat	jgg	hybrid (wheat)	no	13	wheat	**wheat**
147	NG	wheat	jgg	---	---	---	---	---
148	46	wheat	jgg	hybrid (wheat)	spike	13	no seed	**wheat**
149	47	wheat	jgg	hybrid (wheat)	spike	14	no seed	**wheat**
150	47	wheat	jgg	hybrid (wheat)	spike	9	wheat	**wheat**

ND: Not Determinated.
NG: Not Germinated.
NM: None Missing.
?: best probability.

Table A2: Percentages of germination and survival after vernalization.

	% germination	% survival after vernalization
BC_1	84	78
BC_2	92	82
BC_1S_1	80	78
F_1	100	100
Jointed goatgrass	100	100
Madsen	100	100

Table A3. Vegetative characteristics of the BC_1, BC_2, and BC_1S_1 plants.

BC_1			BC_2			BC_1S_1		
Plant number	Leaf width[a]	Growth habit[b]	Plant number	Leaf width[a]	Growth habit[b]	Plant number	Leaf width[a]	Growth habit[b]
1	I	I	51	N	I	101	B	I
2	I	U	52	I	I	102	I	U
4	I	U	53	N	R	103	I	I
5	I	U	55	N	R	105	I	I
6	I	I	56	N	I	108	I	I
7	N	I	60	B	I	109	I	I
8	I	I	61	N	R	110	I	R
9	I	I	63	N	R	111	I	I
11	I	I	64	I	I	112	I	I
12	N	I	65	N	I	114	N	I
13	I	I	66	N	I	115	I	U
15	N	I	67	I	I	117	I	I
16	I	R	68	I	I	119	I	I
17	I	R	69	I	I	120	I	R
18	I	I	70	I	I	121	I	I
19	N	I	72	I	R	122	N	U
20	I	I	73	N	U	123	I	I
21	I	I	74	N	I	124	I	I
24	I	U	75	N	I	125	I	I
25	I	I	76	I	I	126	I	I
26	I	R	77	I	R	127	I	I
27	N	U	78	I	I	129	I	R
28	N	U	79	I	R	130	I	R
29	N	U	80	I	R	131	I	R
31	I	U	81	I	R	132	I	U
32	I	I	82	N	U	133	I	U
34	I	U	83	N	R	134	I	R
35	I	I	84	N	I	135	I	I
37	I	I	86	N	U	138	I	I
38	I	I	87	I	R	139	I	U
39	I	I	90	N	R	140	I	U
40	I	I	91	I	R	141	I	I
41	I	I	92	N	I	142	I	I
44	I	U	93	I	R	143	N	R
45	I	I	94	I	R	145	I	R
46	I	I	95	N	U	146	I	R
47	B	I	96	N	I	148	I	R
48	B	I	97	N	I	149	I	I
49	I	I	98	B	I	150	I	I
			99	B	I			
			100	B	U			

a- B= broad; N= narrow, I= Intermediate

b- U= upright, R= rosette, I= Intermediate

Table A4-1. Reproductive characteristics of wheat, jointed goatgrass, and the F_1 hybrids.

	Reproductive tillers	Days to heading[a]	Pollination[b]	Anther fertility[c]	Dispersal unit
Jointed goatgrass	45	169	CP	F	Spikelets
Jointed goatgrass	65	168	CP	F	Spikelets
Wheat	5	123	SP	F	None
F_1 hybrid	25	115	CP	S	Spike
F_1 hybrid	22	109	CP	S	Spike

a- Days to heading from germination, including 8 weeks of vernalization at 8C
b- CP: Cross-Pollination (open spikelets); SP: Self-pollination (closed spikelets).
c- F: Fertile anthers; S: Sterile anthers.

Table A4-2. Reproductive characteristics of the BC_1 plants.

Plant Number	Reproductive tillers	Days to heading[a]	Pollination method[b]	Anther fertility[c]	Spike morphology	Dispersal unit
1	10	138	CP	S	Wheat	Spike
2	8	130	CP	S	Hybrid	No
4	7	147	CP	F	Hybrid (wheat)	Spike
5	9	142	CP	S	Hybrid	Spike
6	13	151	CP	S	Hybrid (jgg)	Spike
7	12	146	CP	S	Hybrid (jgg)	Spike
8	16	139	CP	S	Hybrid	No
9	12	138	CP	S	Hybrid (wheat)	Spike
11	12	133	CP	S	Hybrid	Spike
12	30	160	CP	S	Hybrid	Spike
13	11	141	CP	S	Hybrid (wheat)	Spike
15	15	180	CP	S	Jgg	Spike
16	20	145	CP	S	(Hybrid) wheat	Spike
17	22	122	CP	S	Hybrid (wheat)	Spike
18	14	128	CP	S	Hybrid (wheat)	Spike
19	37	150	CP	F	Jgg	Spikelets
20	37	146	CP	S	Wheat	No
21	11	119	CP	S	Hybrid (wheat)	Spike
24	11	133	CP	S	Hybrid (wheat)	Spike
25	14	169	CP	S	Hybrid	Spike
26	28	133	CP	S	Wheat	Spike
27	24	138	CP	S	Hybrid	No
28	33	128	CP	F	Jgg	Spike
29	17	132	CP	S	Hybrid (jgg)	Spike
31	11	128	CP	S	Hybrid (wheat)	Spike
32	17	122	CP	S	Hybrid (jgg)	No
34	10	132	CP	S	Hybrid (wheat)	Spike
35	13	192	CP	S	Hybrid (jgg)	No
37	21	131	CP	S	Hybrid (wheat)	Spike
38	11	130	CP	S	Hybrid	Spike
39	9	132	CP	S	Hybrid (wheat)	Spike
40	10	140	CP	S	Hybrid (jgg)	Spike
41	14	133	CP	S	Hybrid (wheat)	Spike
44	8	126	CP	S	Hybrid (wheat)	No
45	11	116	CP	S	Hybrid (jgg)	Spike
46	5	116	CP	S	Hybrid (wheat)	Spike
47	16	122	CP	S	Hybrid (wheat)	Spike
48	9	121	CP	S	Hybrid (wheat)	Spike
49	10	137	CP	S	Hybrid (wheat)	Spike

a- Days to heading from germination, including 8 weeks of vernalization at 8C
b- CP: Cross-Pollination (open spikelets); SP: Self-pollination (closed spikelets).
c- F: Fertile anthers; S: Sterile anthers.

Table A4-3. Reproductive characteristics of the BC_2 plants.

Plant Number	Reproductive tillers	Days to heading[a]	Pollination method[b]	Anthers fertility[c]	Spike morphology	Dispersal unit
51	21	128	CP	S	Hybrid (jgg)	Spike
52	24	109	CP	S	Hybrid (wheat)	Spike
53	27	122	CP	S	Hybrid (jgg)	Spike
55	23	110	CP	S	Hybrid (jgg)	Spike
56	15	128	CP	S	Hybrid (jgg)	Spike
60	26	110	CP	S	Hybrid (jgg)	Spike
61	23	111	CP	S	Hybrid (jgg)	Spike
63	28	131	CP	F	Hybrid (jgg)	Spike
64	28	134	CP	S	Jgg	Spike
65	51	164	CP	S	Jgg	Spikelets
66	30	141	CP	F	Jgg	Spike
67	16	114	CP	S	Hybrid (jgg)	Spike
68	25	130	SP	F	Jgg purple	Spikelets
69	31	122	CP	S	Hybrid (jgg)	Spike
70	25	111	CP	S	Hybrid (jgg)	Spike
72	25	115	CP	S	Jgg dark	Spike
73	79	182	CP	F	Jgg	Spikelets
74	29	128	CP	S	Jgg	Spike
75	51	128	CP	F	Jgg	Spikelets
76	10	128	CP	F	Hybrid (wheat)	Spike
77	22	128	CP	S	Hybrid (jgg)	Spike
78	15	159	CP	S	Hybrid (jgg)	Spike
79	27	126	CP	S	Hybrid (jgg)	Spike
80	29	117	CP	S	Hybrid (jgg)	Spike
81	24	111	CP	S	Hybrid (jgg)	Spike
82	116	134	CP	F	Jgg dark	Spikelets
83	27	157	CP	S	Hybrid (jgg)	Spike
84	54	128	CP	F	Jgg	Spikelets
86	58	140	CP	S	Hybrid (jgg)	Spikelets
87	35	151	SP	F	Jgg	Spikelets
90	27	145	CP	S	Jgg	Spike
91	14	134	CP	S	Hybrid (jgg)	Spike
92	31	110	CP	F	Jgg	Spike
93	25	122	CP	S	Hybrid (jgg)	Spike
94	44	114	CP	S	Hybrid (jgg)	Spike
95	46	146	CP	F	Jgg	Spikelets
96	39	122	CP	S	Hybrid (jgg)	Spike
97	61	128	CP	F	Jgg	Spikelets
98	11	106	CP	S	Hybrid (wheat)	No
99	19	110	CP	S	Wheat	No
100	11	124	CP	?	Wheat	No

a- Days to heading from germination, including 8 weeks of vernalization at 8C
b- CP: Cross-Pollination (open spikelets); SP: Self-pollination (closed spikelets).
c- F: Fertile anthers; S: Sterile anthers.

Table A4-4. Reproductive characteristics of the BC_1S_1 plants.

Plant Number	Reproductive tillers	Days to heading[a]	Pollination method[b]	Anthers fertility[c]	Spike morphology	Dispersal unit
101	12	110	CP	F	Wheat	No
102	15	122	CP	F	Hybrid (wheat)	Spike
103	17	128	SP	F	Hybrid (wheat)	No
105	14	116	CP	F	Hybrid (wheat)	No
108	16	115	CP	S	Hybrid (wheat)	No
109	48	122	CP	S	Hybrid (jgg)	Spike
110	17	106	CP	F	Hybrid (jgg)	Spike
111	40	110	CP	F	Hybrid (wheat)	Spike
112	38	151	CP	S	(Hybrid) jgg	Spike
114	11	161	CP	S	Jgg purple	Spikelets
115	52	140	CP	F	Jgg	Spikelets
117	17	111	CP	S	Hybrid (wheat)	Spike
119	7	132	CP	F	(Hybrid) wheat	Spike
120	27	128	CP	S	(Hybrid) jgg	Spike
121	16	112	CP	S	Wheat	No
122	1		CP	S	Hybrid (wheat)	Spike
123	20	111	CP	F	Hybrid (jgg)	Spike
124	14	106	CP	F	Hybrid (wheat)	Spike
125	11	122	CP	F	Hybrid	No
126	15	119	CP	F	Hybrid (wheat)	Spike
127	10	122	CP	S	Hybrid (wheat)	Spike
129	12	128	CP	S	Hybrid (wheat)	Spike
130	26	122	CP	F	Hybrid (wheat)	No
131	27	128	CP	F	Hybrid (jgg)	Spike
132	17	119	CP	F	Hybrid	No
133	10	109	CP	F	Wheat	No
134	10	122	CP	F	Hybrid	Spike
135	17	138	CP	F	Hybrid (wheat)	Spike
138	15	131	CP	F	Hybrid	Spike
139	11	128	SP?(CP)	F	Hybrid	Spike
140	6	187	SP?	F	Hybrid (wheat)	Spike
141	9	122	CP	F	Hybrid	No
142	13	138	SP	F	Hybrid	Spike
143	21	126	CP	F	Hybrid (jgg)	Spike
145	18	128	CP	S	(Hybrid) jgg	Spike
146	13	163	CP	F	Hybrid (wheat)	No
148	13	117	CP	F	Hybrid (wheat)	Spike
149	14	128	CP	S	Hybrid (wheat)	Spike
150	9	122	CP	F	Hybrid (wheat)	Spike

a- Days to heading from germination, including 8 weeks of vernalization at 8C
b- CP: Cross-Pollination (open spikelets); SP: Self-pollination (closed spikelets).
c- F: Fertile anthers; S: Sterile anthers.

Table A5. Seed production on the BC_1, BC_2, and BC_1S_1 plants.

Plant Number	Probable paternal parent(s)	Spikes threshed	Sterile spikes	Fertile spikes	Total seeds	Average seeds/spike	Seeds in bagged spikes
BC_1							
4	wheat	6	4	2	3	1.5	0
12	wheat	29	27	2	2	1	0
19	jgg	31	30	1	1	1	0
28	jgg?	32	23	9	11	1.2	0
BC_2							
66	wheat-jgg?	29	1	28	198	7.1	9
68	wheat-jgg?	24	0	24	234	9.8	11
69	wheat-jgg?	30	29	1	4	4.0	0
73	jgg-jgg	71	18	53	207	3.9	
75	wheat-jgg?	50	45	5	5	1.0	0
76	wheat-wheat	9	7	2	4	2.0	1
82	wheat-jgg?	100	73	27	52	1.9	5
84	wheat-jgg?	53	34	19	80	4.2	0
87	wheat-jgg?	34	33	1	1	1.0	0
92	wheat-jgg?	31	17	13	27	2.1	0
95	jgg	46	15	31	336	10.8	13
96	wheat-jgg?	38	37	1	4	4.0	0
98	wheat-wheat	10	0	10	295	29.5	29
99	wheat-wheat	18	12	6	12	2	0
BC_1S_1							
101	wheat	11	0	11	264	24.0	15
103	wheat	16	1	15	135	9.0	17
105	wheat	13	7	6	7	1.2	0
110	wheat	15	1	14	103	7.4	11
115	jgg	47	30	17	25	1.5	1
117	wheat?	16	13	3	6	2.0	0
119	wheat?	6	2	4	9	2.3	5
123	wheat	18	17	1	1	1.0	0
124	wheat	13	1	12	210	17.5	22
125	wheat	10	5	5	9	1.8	4
126	wheat	14	11	3	3	1.0	0
129	wheat	11	10	1	2	2.0	0
130	wheat	25	0	25	403	16.1	6
133	wheat	8	1	7	73	10.4	0
134	wheat?	9	0	9	156	17.3	0
135	wheat?	16	14	2	2	1.0	0
138	wheat	14	0	14	103	7.4	9
139	wheat	10	1	9	187	20.8	25
140	wheat	5	0	5	9	1.8	2
141	wheat?	8	5	3	6	2.0	0
142	wheat	12	0	12	127	10.6	12
143	wheat	20	2	18	56	3.1	5
146	wheat	12	8	4	6	1.5	0
150	wheat	8	3	5	10	2.0	3

?: best probability for the pollen donor parent(s)

Table A6. HMW glutenin banding profiles of the BC_1, BC_2, and BC_1S_1 seeds.

Jgg	Wheat	F_1								No bands
BC1 plant number										
	16	1	28	3	8	4	6	14	31	
		2	29		11	5			48	
		9	33		13	7				
		10	34		25	12				
		15	35		26	44				
		17	36		30					
		18	37		32					
		19	38		39					
		20	41		40					
		21	42		47					
		22	43		50					
		23	45							
		24	46							
		27	49							
BC2 plant number										
58	76	52	70	83	89		65	51		88
73	98	53	71	86	87		68	63		
95	99	54	72	91			82	66		
	100	55	74	96			84	92		
		56	75	97						
		57	78	77						
		59	79							
		60	80							
		61	81							
		62	85							
		64	90							
		67	93							
		69	94							
BC1S1 plant number										
115	101	129	120			116		111	102	106
	103	130	131			118		112	117	144
	104	132	145			119		114	136	
	105	133				128			141	
	107	137				134				
	108	138				135				
	109	139								
	110	140								
	113	142								
	121	143								
	122	146								
	123	147								
	124	148								
	125	149								
	126	150								
	127									

Table A7. HMW glutenin banding profiles of the seed-producing hybrids and of their respective seed progeny.

HMW glutenin profile of the mother plant	**HMW glutenin profile of the seed progeny seed (and number of seeds displaying that pattern)**
Wheat BC1	Wheat BC1 - S1
4 bands: 3 wheat bands + lower jointed goatgrass D-genome band	Wheat (1)
Jointed goatgrass (?)BC1	Jointed goatgrass BC1 - S1
6 bands	5 bands: lower wheat D-genome band missing (1) 4 bands: jointed goatgrass + upper wheat B-genome band (1) no bands (1)
Wheat BC2	Wheat BC2 – S1
Wheat	Wheat (1)
Wheat	Wheat (10)
Wheat	Wheat (2) 2 bands: wheat D-genome bands (1)
Jointed goatgrass BC2	Jointed goatgrass BC2 – S1
Jointed goatgrass	Jointed goatgrass (5)
Jointed goatgrass	Jointed goatgrass (10)
Wheat/jointed goatgrass BC2	BC2S1
6 bands	4 bands: 3 jointed goatgrass bands + upper wheat D-genome band (1)
6 bands	5 bands: lower jointed goatgrass D-genome band missing (1) 4 bands: lower jointed goatgrass D-genome band and upper wheat B-genome band missing (1)
5 bands: lower wheat D-genome band missing	Jointed goatgrass (1)
4 bands: 3 jointed goatgrass bands + upper wheat D-genome band.	3 bands: upper jointed goatgrass and wheat D-genome bands + jointed goatgrass C-genome band (1) 4 bands: 3 jointed goatgrass bands + upper wheat D-genome band (9)
4 bands: 3 jointed goatgrass bands + upper wheat D-genome band.	Jointed goatgrass (5)
4 bands: 3 jointed goatgrass bands + upper wheat B-genome band.	Jointed goatgrass (4) 4 bands: 3 jointed goatgrass bands + upper wheat D-genome band (5) no bands (1)
4 bands: 3 jointed goatgrass bands + upper wheat B-genome band.	Jointed goatgrass (7) 4 bands: 3 jointed goatgrass bands + upper wheat B-genome band (3)
4 bands: 3 jointed goatgrass bands + upper wheat B-genome band.	Jointed goatgrass (1) 4 bands: 3 jointed goatgrass bands + upper wheat B-genome band (4)

Table A7. Continued

HMW glutenin profile of the mother plant	**HMW glutenin profile of the seed progeny seed (and number of seeds displaying that pattern)**
<u>Jointed goatgrass BC1 – S1</u> Jointed goatgrass	<u>Jointed goatgrass BC – S2</u> Jointed goatgrass (4) 2 bands: jointed goatgrass upper D-genome and C-genome bands
<u>Wheat BC1 - S1</u> 4 bands: wheat + lower jointed goatgrass D-genome band	<u>Wheat BC1 – S2</u> Wheat (3)
4 bands: wheat + lower jointed goatgrass D-genome band	Wheat (2) Wheat + lower jointed goatgrass D-genome band (3)
Wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level	Wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level (2)
Wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level	Wheat (1) Wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level (1)
Wheat	Wheat (4) Wheat banding pattern with lower D-genome band at jointed goatgrass D-genome band level (5) No bands (1)
Wheat	Wheat (9) 2 bands: wheat D-genome bands (1)
Wheat	Wheat (1) 1 band: upper wheat D-genome band (1)
Wheat Wheat	Wheat (9) No band (1) Wheat (9) No band (1)
Wheat	Wheat (10)
Wheat	Wheat (10)
Wheat	Wheat (10)
Wheat	Wheat (10)
Wheat	Wheat (3)
Wheat	Wheat (5)
Wheat	Wheat (10)
Wheat	Wheat (2)
Wheat	Wheat (2)
Wheat	Wheat (3)

Table A8. Chromosome numbers observed for the BC_1, BC_2, and BC_1S_1 plants

BC_1 generation

Plant Number	Chromosome number
Jointed goatgrass BC_1	
6	36
19	38
Probable jgg BC_1	
15	45-46
28	50-51
Wheat BC_1	
4	54
5	54
7	43
9	52-54
12	46-47
16	42
18	54
21	56
24	57
29	55
31	52
35	56
37	53
38	53
39	52-53
40	54
41	54
44	46
46	54
48	56
49	56
Probable wheat BC_1	
8	43
11	45
13	47
17	46
20	42
26	48
27	48
32	45
34	48
47	44
Unknown pollen donor	
2	46
45	44

BC_2 generation

Plant Number	Chromosome number
Jointed goatgrass BC_2	
73	28
95	28
Wheat BC_2	
76	49
99	41-42
100	44
Wheat/Jointed goatgrass	
51	37
52	34
53	37
55	38
60	36
61	35
63	37
64	38
65	37
66	36
67	39
68	33
69	41
70	38
72	38~
74	34
75	35
77	36
78	41
79	36
80	37
81	30
82	37
83	40
84	38
86	36
87	36
90	35
91	35
92	34
93	36
94	36
96	37
97	36

BC_1S_1 generation

Plant Number	Chromosome number
Jointed goatgrass BC_1S_1	
115	33
Wheat BC_1S_1	
101	43
103	40
105	44
108	41
109	44
110	45
119	49
121	42
122	46
123	43
124	44
125	42
126	46
127	47
129	46
130	42
132	48
133	42
134	48
138	47
139	46
140	44
142	45
143	46
146	40
148	46
149	47
150	47
Probable wheat BC_1S_1	
102	40
117	46
135	48
141	44
Unknown pollen donor	
111	49
112	52
120	49
131	48
145	49-50