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ORIGINAL PAPER

Introgression of an imidazolinone-resistance gene from winter wheat (*Triticum aestivum* L.) into jointed goatgrass (*Aegilops cylindrica* Host)

Alejandro Perez-Jones · Carol A. Mallory-Smith · Jennifer L. Hansen · Robert S. Zemetra

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Abstract Imidazolinone-resistant winter wheat (*Triticum aestivum* L.) is being commercialized in the USA. This technology allows wheat growers to selectively control jointed goatgrass (*Aegilops cylindrica* Host), a weed that is especially problematic because of its close genetic relationship with wheat. However, the potential movement of the imidazolinone-resistance gene from winter wheat to jointed goatgrass is a concern. Winter wheat and jointed goatgrass have the D genome in common and can hybridize and backcross under natural field conditions. Since the imidazolinone-resistance gene (*Imi1*) is located on the D genome, it is possible for resistance to be transferred to jointed goatgrass via hybridization and backcrossing. To study the potential for gene movement, BC_2S_2 plants were produced artificially using imidazolinone-resistant winter wheat (cv. FS-4) as the female parent and a native jointed goatgrass collection as the male recurrent parent. FS-4, the jointed goatgrass collection, and 18 randomly selected BC_2S_2 populations were treated with imazamox. The percentage of survival was 100% for the FS-4, 0% for the jointed goatgrass collection and 6 BC_2S_2 populations, 40% or less for 2 BC_2S_2 populations, and 50% or greater for the remaining 10 BC_2S_2 populations. Chromosome counts in BC_2S_3 plants showed a restoration of the chromosome number of jointed goatgrass, with four out of four plants examined having 28 chromosomes. Sequencing of *AHASL1D* in BC_2S_3 plants derived from BC_2S_2-6 revealed the sexual transmission of *Imi1* from FS-4 to jointed goatgrass. *Imi1* conferred resistance to the imidazolinone herbicide imazamox, as shown by the in vitro assay for acetohydroxyacid synthase (AHAS) activity.

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A. Perez-Jones (✉) · C. A. Mallory-Smith
Department of Crop and Soil Science,
Oregon State University,
Corvallis, OR 97331-3002, USA
e-mail: perezjoa@oregonstate.edu

J. L. Hansen · R. S. Zemetra
Department of Plant, Soil, and Entomological Sciences,
University of Idaho, Moscow, ID 83844-2339, USA

Introduction

Jointed goatgrass (*Aegilops cylindrica* Host) is a winter annual grass weed that infests over 3 million ha of winter wheat (*Triticum aestivum* L.) in the Pacific Northwest and Great Plains regions of the USA (Dewey 1996). Jointed goatgrass reduces winter wheat yields by interference and lowers harvested grain quality. Average yield loss with moderate to dense jointed goatgrass infestations has been estimated to be 25% (Anderson 1993; Donald and Ogg 1991) and it also has been estimated that the economic cost of jointed goatgrass to winter wheat producers in the western United States is $145 million annually (Ogg 1993).

Jointed goatgrass and winter wheat are closely related. Therefore, the development of selective herbicides to control jointed goatgrass in winter wheat has been problematic. Jointed goatgrass is an allotetraploid ($2n = 4x = 28$; CCDD) of the Triticeae tribe (Poaceae family) with 2 genomes and 28 chromosomes that originated from two species. The C

genome was contributed by *Ae. markgrafii* (Greuter) Hammer ($2n = 2x = 14$; CC) and the D genome was contributed by *Ae. tauschii* Coss. ($2n = 2x = 14$; DD) (Johnson 1967; Linc et al. 1999). Winter wheat also belongs to the Triticeae tribe and the Poaceae family, and is an allohexaploid ($2n = 6x = 42$; AABBDD) with three genomes and 42 chromosomes. As in jointed goatgrass, each genome contains seven chromosomes and originated from a different ancestor. Thus, *T. aestivum* arose from a combination of AB genomes from *T. turgidum* and the D genome from *Ae. tauschii* (Feldman and Sears 1981; Kimber and Sears 1987).

Acetohydroxyacid synthase (AHAS; EC 4.1.3.18), also known as acetolactate synthase (ALS), is the first enzyme involved in the synthesis of the branched chain amino acids (i.e., valine, leucine, and isoleucine) (Umbarger 1978). AHAS catalyzes two parallel physiological reactions: the condensation of two molecules of pyruvate to form acetolactate and carbon dioxide, and the condensation of one molecule of pyruvate with one molecule of 2-ketobutyrate to form acetohydroxybutyrate and carbon dioxide. Acetolactate is a precursor for valine and leucine synthesis, and acetohydroxybutyrate is a precursor for isoleucine synthesis (Kishore and Shaw 1988; Stidham and Singh 1991). AHAS is a nuclear encoded chloroplastic enzyme in plants (Shaner and Singh 1997), and is the site of action of five structurally different herbicide families, including the imidazolinones (e.g., imazethapyr) (Shaner et al. 1984), the sulfonylureas (e.g., chlorsulfuron) (Ray 1984), the triazolopyrimidines (e.g., flumetsulam) (Kleschick et al. 1990), the pyrimidinylthiobenzoates (e.g., pyrithiobac-sodium) (Takahashi et al. 1991), and the sulfonylaminocarbonyltriazolinones (e.g., flucarbazone-sodium) (Santel et al. 1999). The inhibition of AHAS blocks the production of branched chain amino acids and causes death in plants; however, other physiological consequences, such as inhibition of growth and cell division, disruption of protein synthesis and photosynthate transport, and buildup of 2-ketobutyrate, have been described as secondary effects of AHAS inhibition and are also implicated in the mechanism of plant death (Shaner 1991; Schloss 1994).

In higher plants, a single amino acid substitution in the AHAS enzyme can confer high levels of resistance to AHAS inhibitors (Saari et al. 1994). Several point mutations conferring different levels and patterns of resistance to AHAS inhibitors have been detected (Tranel and Wright 2002). The mutations usually occur in five highly conserved amino acids of the catalytic subunit of AHAS, including alanine at position 122 (Ala_{122}) (Wright et al. 1998), proline at position 197 (Pro_{197}) (Guttieri et al. 1992), alanine at position 205 (Ala_{205}) (White et al. 2003), tryptophan at position 574 (Trp_{574}) (Bernasconi et al. 1995), and serine at position 653 (Ser_{653}) (Sathasivan et al. 1991). Ala_{122}, Pro_{197}, and Ala_{205} are located near the amino terminal end in domains C, A, and D, respectively, whereas Trp_{574} and Ser_{653} are located near the carboxyl terminal end in domains B and E, respectively (Tranel and Wright 2002).

Imidazolinone-resistant winter wheat was developed by mutagenizing a population of winter wheat seed (cv. Fidel) with sodium azide (Newhouse et al. 1992). The M_2 seeds were screened by using a seed treatment of imazethapyr [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 *H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid] followed by a pre-emergence application of the same herbicide. Four resistant plants were selected and named Fidel selection (FS) 1 to 4. Winter wheat has three homoeologous AHAS genes that are located on the long arms of chromosomes 6D, 6B, and 6A (Anderson et al. 2004). The mutation of the AHAS gene on 6DL (*AHASL1D*) was named *Imi1* and was found to confer an amino acid substitution of Ser_{653} to asparagine (Asn) in domain E, which confers high resistance only to imidazolinone herbicides (Pozniak et al. 2004). FS-4 has the *Imi1* mutation and has been used as a trait donor for breeding imidazolinone-resistant winter wheat cultivars (Tan et al. 2005), including ORCF-101 (Dr. J. Peterson, Oregon State University, personal communication) and Idaho 587 (Souza et al. 2006).

Imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 *H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid] is an imidazolinone herbicide that controls a large number of broadleaf and grass weeds (Blackshaw 1998), and can be used at field rates of 0.036 to 0.072 kg ai ha^{-1} to selectively control jointed goatgrass in imidazolinone-resistant winter wheat with negligible crop injury (Ball et al. 1999; Pester et al. 2001; Geier et al. 2004). However, the potential for the sexual transfer of the imidazolinone-resistance gene is an ecological risk because jointed goatgrass and winter wheat are related genetically, and can hybridize and backcross under natural field conditions (Mallory-Smith et al. 1996; Zemetra et al. 1998; Guadagnuolo et al. 2001; Stone and Peeper 2004). Because jointed goatgrass and winter wheat have a common ancestor (*Ae. tauschii*), D genome chromosomes in F_1 hybrids between these two species form bivalents during meiosis (Kimber and Zhao 1983; Wang et al. 2001) and normal segregation of loci on these chromosomes has been documented (Kroiss et al. 2004). Hybrids between winter wheat and

jointed goatgrass are partially female fertile and can backcross under natural field conditions and set seed (Snyder et al. 2000; Morrison et al. 2002a; Morrison et al. 2002b). Because imidazolinone-resistance in winter wheat is controlled by a single semidominant gene (Newhouse et al. 1992) located on the D genome (Anderson et al. 2004), it is possible for resistance to be transferred to jointed goatgrass via hybridization and backcrossing. The transfer of the herbicide-resistance gene located on the A or B genome also could occur through translocation or transmission of an extra chromosome (Wang et al. 2000).

The production of imidazolinone-resistant hybrids between imidazolinone-resistant winter wheat (cv. FS-4) and jointed goatgrass by natural hybridization has been reported (Seefeldt et al. 1998; Hanson et al. 2005). However, it is still unclear if the imidazolinone-resistant gene can be introgressed into a jointed goatgrass population and become stabilized, and if so what level of resistance can be obtained after the introgression of the imidazolinone-resistant gene. Therefore, the objectives of this research were to: (1) select a BC_2S_2 population, derived from artificial crosses between the imidazolinone-resistant winter wheat cultivar FS-4 and a native collection of jointed goatgrass, resistant to the herbicide imazamox; (2) to confirm the presence of the imidazolinone-resistant gene derived from FS-4; and (3) to determine the level of herbicide resistance in the selected backcross population.

Materials and methods

Plant material

Imidazolinone-resistant winter wheat by jointed goatgrass backcross progeny were developed by controlled crosses in a greenhouse as follows: imidazolinone-resistant winter wheat (cv. FS-4) was used as the female parent and crossed manually with a native jointed goatgrass collection from Idaho; the F_1 hybrids were backcrossed twice using the same jointed goatgrass collection as the male recurrent parent to restore self-fertility. The second backcross generation (BC_2) originated from ten BC_1 plants. The BC_1 and BC_2 generations underwent selection for the imidazolinone-resistance gene using imazamox at 0.044 kg ai ha^{-1} as the selection agent reducing the number of donor BC_1 plants to seven. Spikes from the surviving BC_2 plants were bagged and allowed to self-pollinate twice, to produce the second backcross-second self (BC_2S_2) generation. Eighteen BC_2S_2 populations derived from seven BC_1 plants were evaluated for imazamox resistance. Progeny of one BC_2S_2 population designated BC_2S_2-6 were selfed to produce a BC_2S_3 generation to produce seed for additional testing.

Test for imazamox resistance

The test for imazamox resistance was conducted in the greenhouse. The imidazolinone-resistant winter wheat cultivar FS-4, the native jointed goatgrass collection, and the 18 BC_2S_2 populations were grown in flats ($52 \times 26 \times 7$ cm^3) containing potting mix (Sunshine Mix #1, Sun Gro Horticulture Inc., Bellevue, WA) at 24/20°C day/night temperature. Supplemental lighting was provided for a 16 h photoperiod. The plants were sprayed at the three leaf stage with imazamox at 0.044 kg ai ha^{-1} using an overhead compressed-air sprayer calibrated to deliver 187 L ha^{-1}. The nonionic surfactant R-11® (Wilbur-Ellis Company, Fresno, CA) at 0.25% v/v was added to the herbicide solution. There were a total of 6 flats, and each flat contained 10 rows with 12 plants per row. For each line, there were three replications that were randomly distributed among the flats. Thus, for each of the BC_2S_2 populations, the winter wheat cultivar FS-4, and the jointed goatgrass collection, a total of 36 plants were treated. Three weeks after treatment, the plants were evaluated and scored as dead or alive to determine the percentage of plant survival.

Whole-plant bioassay

Seeds of the imidazolinone-resistant winter wheat cultivar FS-4, susceptible jointed goatgrass, and the BC_2S_3 generation from the putative imidazolinone-resistant BC_2S_2-6 population were planted in 267-mL plastic pots in the greenhouse as described previously. Plants at the three-leaf stage were sprayed with imazamox as described previously at 0.0001, 0.0004, 0.001, 0.004, 0.014, 0.044, 0.139, and 0.438 kg ai ha^{-1}. Three weeks after treatment, individual plants were scored as dead or alive to determine the percentage of plant survival. The experiment was conducted twice with 6 replications for each herbicide treatment.

Chromosome analysis

Somatic chromosome counts of four BC_2S_3 individuals from the putative imidazolinone-resistant BC_2S_2-6 population were conducted using root-tip cells. Seeds were germinated in Petri dishes, and root-tips were

collected from primary roots and placed in 1°C water for 24 h, before fixing in Farmer's solution (3:1 ethanol-acetic acid). After a minimum of 3 d, the roots were transferred to 2% acetocarmine for staining (Tsuchiya 1971). Chromosome counts were performed using a light microscope (Zeiss Axioskop 2; Carl Zeiss Inc., Thornwood, NY).

Cloning and sequencing of *AHASL1D*

Total DNA from FS-4, the jointed goatgrass collection, and the putative imidazolinone-resistant BC_2S_2-6 derived BC_2S_3 generation was extracted from leaf tissue using a DNA isolation kit (DNeasy® Plant Mini Kit, Qiagen Inc., Valencia, CA). Polymerase chain reaction (PCR) with two sets of primers were used to amplify domain E of the AHAS gene on chromosome 6DL (*AHASL1D*). In a first reaction, primers CM-F (5′-CCGCCGCAATATGCTATCCAG-3′; GeneBank accession number CS176837) and CM-R (5′-GTAGGACAAGAAACTTGCATG-3′; GeneBank accession number CS176838) were used to amplify a 852 bp fragment of the three AHAS genes located on chromosomes 6D, 6B, and 6A. In a second reaction, in which the PCR products from the first reaction were used as templates, the genome specific primers 1AD-F (5′-GGGAGGCGATCATTGCCACT-3′; GeneBank accession number CS176841) and 1D-R (5′-GCACATCCCTACAAAAGAGAAGAT-3′; GeneBank accession number CS176843) were used to amplify a 775 bp fragment of *AHASL1D* that includes the *Imi1* mutation (Ser_{653} to Asn) (Zhao et al. 2005). PCR reactions were performed in 25 μL reactions containing 1X PCR buffer, 0.2 µM of each primer, 0.2 mM of each deoxynucleotide, 0.75 unit of *Taq* DNA polymerase (Fermentas Inc., Hanover, MD), and 80 to 100 ng of template DNA in a Primus96 plus thermocycler (MWG Biotech Inc., High Point, NC). The cycling program consisted of one denaturation step of 3 min at 94°C, 30 cycles of 30 sec at 94°C, 1 min at 55°C (primers CM-F/CM-R) or 65°C (primers 1AD-F/1D-R), and 45 sec at 72°C, followed by a final extension step of 10 min at 72°C. The amplified fragments were cloned using a TOPO TA Cloning® Kit (Invitrogen Corporation, Carlsbad, CA), purified using a QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced using an automatic ABI PRISM® 3771 DNA sequencer (Perkin-Elmer Applied Biosystem, Foster City, CA) with fluorescence dye-labeled dideoxynucleotides. DNA extraction and amplification of *AHASL1D* was performed in four plants of the winter wheat cultivar FS-4, the jointed goatgrass collection, and the BC_2S_3 generation derived from BC_2S_2-6. To exclude PCR errors, six clones per PCR product were sequenced and aligned.

In vitro assay for AHAS activity

AHAS activity of two imidazolinone-resistant (cv. ORCF-101 and Idaho 587) and one imidazolinone-susceptible (cv. Madsen) winter wheat cultivars, the jointed goatgrass collection, and the putative imidazolinone-resistant BC_2S_2-6 derived BC_2S_3 generation was assayed colorimetrically by converting acetolactate produced by AHAS to acetoin, according to Singh et al. (1988) with some modifications. For each of the winter wheat cultivars, the jointed goatgrass collection, and the BC_2S_3 generation derived from the putative imidazolinone-resistant BC_2S_2-6 population, 36 plants were grown in 267 mL plastic pots (three plants per pot) in the greenhouse as described previously. Two weeks after planting, 4 g of leaf tissue were harvested for crude enzyme extraction. The leaf tissue was ground in a mortar and pestle with liquid N_2, and homogenized with cold 2 × reaction buffer [50 mM K_2HPO_4, pH 7.5; 200 mM sodium pyruvate; 1.25 mM $MgCl_2$; 1.25 mM thiamine pyrophosphate; 2.5 µM flavin adenine dinucleotide]. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 18,000×*g* at 4°C for 15 min. The crude enzyme extract was used immediately for the enzyme assay. AHAS enzyme activity was assayed in 96-well microtiter plates by mixing 50 μL of crude enzyme extraction and 50 μL of imazamox solution at 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{0}, 10^{1}, 10^{2}, 10^{3}, and 10^{4} µM and incubated for 1.5 h at 37°C. The reaction was stopped by the addition of 25 μL 1.8 N H_2SO_4 and heated at 60°C for 20 min to facilitate decarboxylation of acetolactate to acetoin. Acetoin content was measured by the method of Westerfeld (1945) by adding 175 μL of 0.25% creatine and 2.5% α-naphtol solution in 2.5 N NaOH to each well. Color was allowed to be developed at 60°C for 20 min and absorbance was determined at 535 nm in a VERSAmax™ microtiter plate reader (Molecular Devices, Sunnyvale, CA). Background absorbance was determined by adding 25 μL 1.8 N H_2SO_4 before incubation. The experiment was conducted twice with eight replications for each herbicide concentration.

Statistical analysis

Dose-response curves for the in vitro AHAS assay were obtained by a non-linear regression using the log-logistic equation (Streibig 1988; Streibig et al. 1993):

$$y = C + \frac{D - C}{1 + \left(x/I_{50}\right)^{b}}$$

where y represents AHAS activity (percentage of control) at herbicide concentration x, C is the mean response at very high herbicide concentration (lower limit), D is the mean response when the herbicide concentration is zero (upper limit), b is the slope of the line at I_{50}, and I_{50} is the herbicide concentration required for 50% enzyme inhibition. The regression parameters were obtained using PROC NLIN in SAS v8.02 (SAS Institute Inc, Cary, NC) and compared to test significant differences using a sum of square reduction test (Seefeldt et al. 1995). The level of resistance was determined by calculating the ratio of the I_{50} of the resistant genotype to the I_{50} of the susceptible genotype. Analysis of variance for the in vitro AHAS assay showed no significant interaction between experiments and herbicide concentrations; therefore, data from both experiments were combined. Thus, data are presented as means of two experiments with eight replications for each herbicide concentration.

Results

Test for imazamox resistance

The percentage of plant survival after imazamox treatment was 100% for the imidazolinone-resistant winter wheat cultivar FS-4, 0% for the jointed goatgrass collection and six BC_2S_2 populations, 40% or less for two BC_2S_2 populations, and 50% or greater for the remaining ten BC_2S_2 populations (Fig. 1). The putative imidazolinone-resistant BC_2S_2 population with the highest level of survival was selected and designated BC_2S_2-6. The surviving progeny of BC_2S_2-6 were allowed to produce selfed seed and the BC_2S_3 generation derived from the BC_2S_2-6 population was used to conduct a whole-plant bioassay and an in vitro assay for AHAS activity to confirm imidazolinone-resistance.

Whole-plant bioassay

The percentage of plant survival in the imidazolinone-resistant winter wheat cultivar FS-4 after treatment with imazamox was 100%, even at the highest imazamox rate of 0.438 kg ai ha^{-1} which is equivalent to tenfold, the recommended field rate of 0.044 kg ai ha^{-1} (Fig. 2). None of the jointed goatgrass plants survived at the imazamox rate of 0.044 kg ai ha^{-1} or greater. On the other hand, the percentage of plant survival of the BC_2S_3 generation from BC_2S_2-6 was 85% at the recommended field rate, and only decreased to 73% when the herbicide rate was increased tenfold (Fig. 2), confirming resistance of the selected population to imazamox.

Chromosome analysis

Somatic chromosome counts were conducted using root-tip cells from four BC_2S_3 generation plants from the putative imidazolinone-resistant BC_2S_2-6 population. Four out of four plants had 28 chromosomes (Fig. 3), indicating that the normal number of chromosomes for jointed goatgrass ($2n = 4x = 28$) had been restored.

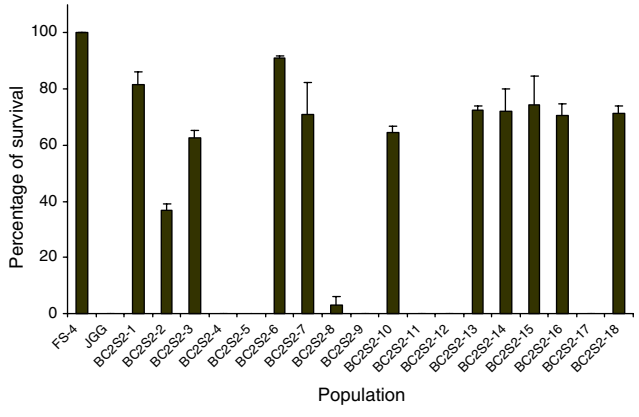

Fig. 1 Percentage plant survival of imidazolinone-resistant winter wheat cultivar FS-4, a jointed goatgrass collection, and 18 BC_2S_2 populations 3 weeks after imazamox treatment at 0.044 kg ai ha^{-1}. *Vertical bars* represent standard errors of the mean

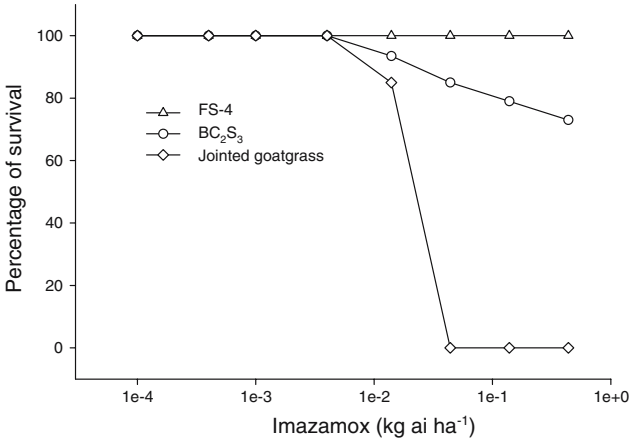

Fig. 2 Percentage plant survival of imidazolinone-resistant winter wheat cultivar FS-4, a jointed goatgrass collection, and the BC_2S_3 generation derived from BC_2S_2-6 as influenced by imazamox rate 3 weeks after treatment

Fig. 3 Somatic mitotic metaphase of one BC_2S_3 plant derived from BC_2S_2-6 after acetocarmine staining showing $2n = 4x = 28$ chromosomes

Cloning and sequencing of *AHASL1D*

PCR with two sets of primers were used to amplify a fragment containing domain E of *AHASL1D* from FS-4, the jointed goatgrass collection, and the imidazolinone-resistant BC_2S_2-6 population. The imidazolinone-resistant winter wheat cultivar FS-4 carries *Imi1*, a single point mutation from guanine to adenine that results in a serine to asparagine substitution in domain E, which confers resistance to imidazolinone herbicides. Both the susceptible and the resistant alleles were found in the BC_2S_3 generation of BC_2S_2-6 (Fig. 4) confirming the sexual transfer of the imidazolinone-resistance gene *Imi1* from winter wheat to jointed goatgrass. The presence of both the susceptible and resistant alleles in the BC_2S_3 generation indicated that the BC_2S_2-6 population was heterogeneous for the resistance allele resulting in progeny that would vary in their level of herbicide resistance.

In vitro assay for AHAS activity

The concentration of imazamox required to inhibit the in vitro activity of AHAS by 50% when compared to the untreated control (I_{50}) was determined in the BC_2S_3 generation derived from BC_2S_2-6, the jointed goatgrass collection, and in the imidazolinone-resistant winter wheat cultivars ORCF-101 and Idaho 587, and the imidazolinone-susceptible winter wheat cultivar Madsen (Fig. 5). The I_{50}'s for the imidazolinone-resistant BC_2S_3 generation and the jointed goatgrass collections were 4.09 and 1.40 μM, respectively (Table 1). Thus, the I_{50} for the BC_2S_3 generation derived from the imidazolinone-resistant BC_2S_2-6 population was approximately three times greater than in the susceptible jointed goatgrass. In winter wheat, the I_{50}'s for the imidazolinone-resistant cultivars ORCF-101 and Idaho 587, and the susceptible cultivar Madsen were 6.20, 8.72, and 1.70 μM, respectively (Table 1). Thus, the I_{50}'s for the imidazolinone-resistant winter wheat cultivars ORCF-101 and Idaho 587 were approximately four to five times greater than in the susceptible winter wheat cultivar Madsen.

Discussion

Twelve of the 18 BC_2S_2 populations tested had some level of resistance to the herbicide imazamox. This high percentage (61%) of resistant populations might be expected because the BC_1 and BC_2 generations underwent selection for the imidazolinone-resistance gene using the herbicide imazamox. One of the putative resistant BC_2S_2 populations was selected (BC_2S_2-6) and

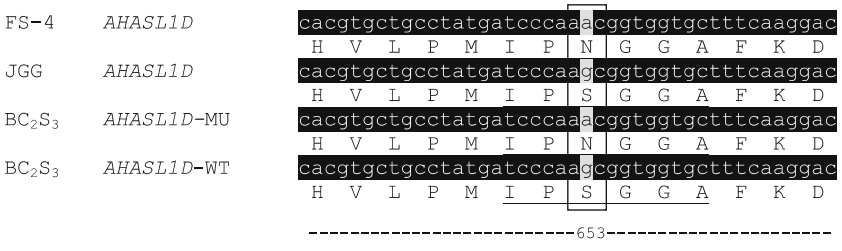

Fig. 4 Partial nucleotide and deduced amino acid sequence alignment of the AHAS gene on chromosome 6DL (*AHASL1D*) in imidazolinone-resistant winter wheat cultivar FS-4, a jointed goatgrass collection, and the BC_2S_3 generation derived from BC_2S_2-6. The *underlined* sequences indicate domain E. The *boxed* codon indicates mutation *Imi1*, a serine (*S*) to asparagine (*N*) substitution at amino acid 653 (amino acid number based on *Arabidiopsis thaliana* sequence). *MU* mutant allele, *WT* wild type allele

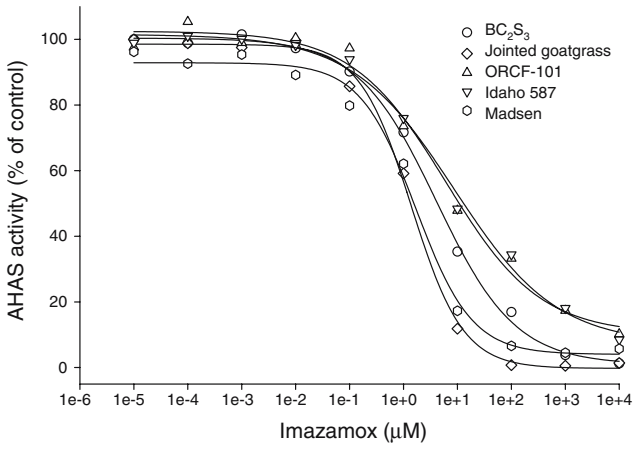

Fig. 5 In vitro AHAS activity of the BC_2S_3 generation derived from BC_2S_2-6, the goatgrass collection, and the winter wheat cultivars ORCF-101, Idaho 587, and Madsen, as influenced by imazamox concentration

seed was increased to produce a BC_2S_3 generation for additional testing. The whole-plant bioassay on the BC_2S_3 generation confirmed that the BC_2S_2-6 population was resistant to imazamox, with 73% plant survival when treated with tenfold, the recommended herbicide rate. The lower level of survival of the BC_2S_3 generation derived from BC_2S_2-6 compared to the imazamox-resistant wheat can be explained by the results of the sequence analysis that showed that BC_2S_2-6 was heterogeneous for the resistance allele and the population derived from this line was still segregating for herbicide resistance and some susceptible plants would have been expected.

Chromosome counts of BC_2S_3 plants derived from BC_2S_2-6 showed the elimination of alien A- and B-genome chromosomes and a continued restoration of the chromosome number of jointed goatgrass ($2n = 4x = 28$), with four out of four plants showing 28 chromosomes. This outcome was expected since the winter wheat by jointed goatgrass F_1 hybrids were backcrossed twice using jointed goatgrass as the male recurrent parent to restore self-fertility.

Cloning and sequencing of *AHASL1D* in plants of the BC_2S_3 generation of BC_2S_2-6 revealed the sexual transfer of the imidazolinone-resistance gene *Imi1* from FS-4 to jointed goatgrass. It has been shown previously that normal genetic recombination between homologous D genome chromosomes of winter wheat and jointed goatgrass in backcross progenies can occur (Kroiss et al. 2004). *Imi1* is located in chromosome 6DL (Anderson et al. 2004; Pozniak et al. 2004); therefore, the introgression of *Imi1* from winter wheat into the D genome of jointed goatgrass after hybridization and backcrossing was predicted.

The in vitro assay for AHAS enzyme activity confirmed that winter cultivars ORCF-101 and Idaho 587, and the selected BC_2S_2-6 population possess a modified AHAS enzyme with decreased sensitivity to imazamox. The level of resistance observed at the enzyme level in the BC_2S_3 plants originating from BC_2S_2-6 is lower when compared to the level of resistance observed in the imidazolinone-resistant winter wheat cultivars. Imidazolinone-resistance conferred by *Imi1* is inherited as a single, incompletely dominant nuclear gene (Newhouse et al. 1992; Pozniak and Hucl 2004; Pozniak et al. 2004). Resistance to AHAS inhibitors in other species has been also described as a partial dominant trait inherited as a single nuclear gene (Mallory-Smith et al. 1990; Boutsalis et al. 1999; Kolkman et al. 2004). The selected BC_2S_2-6 population contained both the susceptible and the resistant alleles, which can explain the lower level of resistance to imazamox observed at the enzyme level.

Since the introduction of genetically modified crops, gene flow between cultivated crops and their wild relatives has been an environmental concern (Raybould and Gray 1993). Gene flow from cultivated crops to their wild relatives has been documented in several crop species, including sunflower (*Helianthus annuus* L.) (Linder et al. 1998), rice (*Oryza sativa* L.) (Chen et al. 2004), and canola (*Brassica napus* L.) (Warwick et al. 2003). The introgression of genes from cultivated crops to their wild relatives after hybridization can increase the ability of the wild relatives to adapt to certain agricultural environments, and also their ability to compete with domesticated crops or other wild species (Ellstrand et al. 1999). However, gene introgression from a cultivated crop to its wild relative is not

Table 1 Nonlinear regression parameter estimates and standard errors for AHAS activity response curves of Fig. 5. The model fitted corresponded to: AHAS activity (percentage of control) = $C + [(D - C) / (1 + (x/I_{50})^b)]$

Genotype	D (±SE)	C (±SE)	b (±SE)	I_{50} (±SE)	R^2
BC_2S_3	100.40 (2.64)	0.93 (4.24)	0.59 (0.10)	4.09 (1.33)	0.99
Jointed goatgrass	98.54 (2.31)	0.26 (2.87)	0.90 (0.16)	1.40 (0.30)	0.99
ORCF-101	102.50 (1.46)	10.26 (2.78)	0.50 (0.05)	6.20 (1.40)	0.99
Idaho 587	101.50 (1.60)	7.15 (3.48)	0.46 (0.05)	8.72 (2.36)	0.99
Madsen	92.88 (2.14)	4.02 (2.75)	0.84 (0.14)	1.70 (0.40)	0.99

a simple process and requires the hybridization between the two species, followed by repeated backcrosses to the wild relative and the stabilization of the gene in the new host genome (Stewart et al. 2003).

The imidazolinone-resistant winter wheat cultivar FS-4 carries *Imi1* and was used as a trait donor in the development of the winter wheat cultivars ORCF-101 and Idaho 587 that are being commercialized in the Pacific Northwest of the USA. Although the development of imidazolinone-resistant winter wheat allows selective control of jointed goatgrass, the introgression of the herbicide-resistance gene from winter wheat to jointed goatgrass via a hybrid bridge would eliminate this benefit. Since *Imi1* is located in the long arm of chromosome 6D, it can be transferred from winter wheat to jointed goatgrass after hybridization and backcrossing as demonstrated in this study. Nevertheless, it is still unclear how long it will take for this to happen in the field under natural field conditions. In fact, the evolution of a herbicide-resistant jointed goatgrass population due to strong selection pressure by continuous applications of the same herbicide (e.g., imazamox) might be of more concern. The repeated use of the same herbicide, or different herbicides with the same mechanism of action, may lead to the selection of herbicide-resistant populations in the target weed species (Maxwell and Mortimer 1994; Jasieniuk et al. 1996). Moreover, AHAS inhibitors, such as imazamox, have an increased risk of selecting resistant populations when compared to herbicides with other mechanisms of action because there are several point mutations within the gene encoding the target enzyme AHAS that can confer herbicide resistance (Saari et al. 1994; Preston and Mallory-Smith 2001). A simulation of continuous, no-till, imidazolinone-resistant winter wheat production resulted in rapid development of imazamox-resistant jointed goatgrass without hybridization, and in extremely rapid resistance development with hybridization (Hanson et al. 2002). Imidazolinone-resistant winter wheat is and will be an important tool to control and manage jointed goatgrass. However, alternating this technology with conventional nonresistant winter wheat or other crops, in which AHAS-inhibiting herbicides are not used, will decrease the selection pressure by the herbicide. In addition, it will reduce the likelihood of a hybridization and backcrossing event. Thus, the risk of development of imazamox resistance in jointed goatgrass populations will be reduced.

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