

Genetic Analysis of Imidazolinone Resistance in Mutation-Derived Lines of Common Wheat

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ABSTRACT

The imidazolinone herbicides possess high biological potency at low application rates, and thus are an attractive alternative for weed control. The induction of genes conferring resistance by mutagenesis could facilitate the use of imidazolinones as an alternative weed control system in spring wheat (*Triticum aestivum* L.). Six M_{36} spring wheat lines resistant to imidazolinone herbicides were identified following seed mutagenesis and were selected for genetic study. The lines were designated as 1A, 9A, 10A, 11A, 15A, and 16A. BW755 carries a previously characterized partially dominant resistance gene (*FS-4*). On the basis of analysis of F_1 , F_2 , backcross (BC_1), F_1 and $F_{2.3}$ populations, resistance in lines 1A, 9A, 10A, 11A, and 16A is a partially dominant trait inherited as a single nuclear gene. Resistance in Tea-IIIMI 15A is dominant and is inherited as two independent nuclear-coded genes. Allelism studies indicated that resistance genes in 1A, 9A, 10A, 16A, and one of the resistance genes in 15A are allelic to *FS-4*. All crosses between resistant lines and 11A produced segregating F_2 and $F_{2.3}$ populations suggesting the presence of a unique resistance gene in 11A. The resistance genes were named on the basis of the recommended rules for gene symbolization in wheat. The *FS-4* allele was redesignated as *Imi1*. The resistance gene in 11A and the second resistance gene in 15A were designated as *Imi2* and *Imi3*, respectively. Results from these studies indicate that higher levels of imidazolinone resistance in wheat could be achieved by stacking two or more genes into a single genotype.

ACETOHYDROXYACID SYNTHASE (AHAS; EC 4.1.3.18), also known as acetolactate synthase (ALS), is the first enzyme that catalyzes the biochemical synthesis of the branched chain amino acids valine, leucine, and isoleucine (Singh, 1999). This enzyme is the site of action of five structurally diverse herbicide families, including the imidazolinones (Shaner et al., 1984), the sulfonylureas (Ray, 1984), the triazolopyrimidines (Subramanian and Gerwick 1989), the pyrimidylxybenzoates (Subramanian et al., 1990), and the sulfonylaminocarbonyl-triazolinones (Santel et al., 1999). The imidazolinones are environmentally attractive because they possess high biological potency, making them very effective at low application rates (Newhouse et al., 1991). Since branched chain amino acid biosynthesis does not occur in animals, the imidazolinones are relatively nontoxic to animals. Although these herbicides control a wide spectrum of weeds, wheat is sensitive to most imidazolinone herbicides (Newhouse et al., 1992; Southan and Copeland, 1996). A mutation conferring high levels of resistance to all of the imidazolinones is desirable and

would enhance the weed control options available to wheat producers world-wide (Newhouse et al., 1992).

Plants resistant to the imidazolinones, sulfonylureas, triazolopyrimidines, and pyrimidylxybenzoates have been successfully produced by seed, microspore, pollen, and callus mutagenesis, and somatic cell selection in maize (*Zea mays* L.) (Newhouse et al., 1991), *Arabidopsis thaliana* (L.) Heynh. (Haughn and Somerville, 1986; Sathasivan et al., 1991; Mourad et al., 1993), sugar beet (*Beta vulgaris* L.) (Hart et al., 1992; Wright and Penner, 1998), canola (*Brassica napus* L.) (Swanson et al., 1989), cotton (*Gossypium hirsutum* L.) (Subramanian et al., 1990; Rajasekaran et al., 1996), soybean [*Glycine max* (L.) Merr.] (Sebastian et al., 1989), tobacco (*Nicotiana tabacum* L.) (Chaleff and Ray, 1984; Creason and Chaleff, 1988), and common wheat (Newhouse et al., 1992). In nearly all cases, a single, partially dominant nuclear gene conferred resistance. Four imidazolinone resistant plants were previously isolated following seed mutagenesis of *T. aestivum* cv. Fidel (Newhouse et al., 1992). Inheritance studies confirmed that a single, partially dominant gene conferred resistance. On the basis of allelism studies, the authors concluded that the resistance genes in the four identified lines were alleles located at the same locus. The allele was denoted as *FS-4* (Newhouse et al., 1992). In the current research, seeds of *T. aestivum* cv. CDC Teal (Hughes and Hucl, 1993) were mutagenized with ethyl methane sulfonate (EMS) and M_2 plants resistant to imazamox, an imidazolinone herbicide, were identified and selected. Six homozygous resistant lines tracing back to individual M_3 plants were selected for genetic analysis of the imidazolinone resistance trait. The objectives of this research study were to determine the inheritance of imidazolinone resistance in the six selected lines, and to determine the allelic relationships among the lines as well as the previously characterized resistance gene (*FS-4*) in BW755.

MATERIALS AND METHODS

Seed Mutagenesis and Selection of Resistant Lines

Approximately 40 000 seeds of *T. aestivum* cv. CDC Teal (Hughes and Hucl, 1993) were mutagenized by means of the modified procedures described by Washington and Sears (1970). Seeds were presoaked in distilled water for 4 h, followed by treatment with 0.3% (v/v) EMS for 6 h. Seeds were rinsed continually with tap water for 4 h and allowed to dry for approximately 4 h before being planted in the field. The M_1 plants were selfed and the seed was harvested in bulk. Approximately 2×10^6 M_2 plants were grown in the field the following year and were sprayed at Haun stage 2.0 (Haun, 1973) with imazamox [(*RS*)-2-(4-isopropyl-4-methyl-5-oxo-

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Abbreviations: AHAS, acetohydroxyacid synthase; a.i., active ingredient; R, resistant; I, intermediate; S, susceptible.

2-imidazolin-2-yl)-5-methoxymethylnicotinic acid] at a rate of 40 g a.i. ha⁻¹ in a spray volume of 100 L ha⁻¹. Sunit adjuvant (1.25% v/v) (BASF, Toronto, Ontario, Canada) was added to the spray solution. This rate was chosen to select only those mutants with moderate to high levels of resistance. M₂ plants resistant to imazamox were identified, transplanted to pots in a walk-in growth chamber, and harvested individually. Each seed from the M₂ mother plant was grown as an M₃ plant in a walk-in growth chamber without imazamox challenge, selfed, and harvested individually. The resulting M_{3,4} lines were grown in a walk-in growth chamber and screened with imazamox. Since plants are generally more sensitive to herbicide under controlled environment conditions, the M_{3,4} lines were screened by application of half the rate of imazamox used in the field (20 g a.i. ha⁻¹). Herbicide treatments were applied to plants at Haun stage 2.0 (Haun, 1973) with a traveling cable sprayer calibrated to spray 100 L ha⁻¹ using an 8001 EVS nozzle at a pressure of 275 kPa. All lines survived imazamox treatment and were selfed and harvested. M_{3,5} lines were screened with 40 g a.i. ha⁻¹ in the field to confirm the resistant phenotype, selfed and harvested. M_{3,5} lines were homozygous for the trait, as progeny testing detected no segregation for resistance to imazamox. Six M_{3,6} lines with moderate to high levels of resistance to imazamox were selected for genetic study. The lines were designated as the TealIMI lines 1A, 9A, 10A, 11A, 15A, and 16A.

Inheritance and Allelism Studies

To determine the genetic control of resistance to the imidazolinones, reciprocal crosses between the six homozygous resistant M_{3,6} lines and CDC Teal (susceptible to imidazolinones) were made. Randomly selected F₁ plants from each of the crosses were backcrossed to CDC Teal to form backcross (BC₁)F₁ populations. To investigate allelism, all possible intercrosses between the six mutants and BW755 (Grandin*3/Fidel-*FS-4*) were made. BW755 is a spring wheat line that is homozygous for the *FS-4* allele. Parental lines were grown in a walk-in growth chamber with a 16-h photoperiod and a 24°C day and 16°C night temperature regime. Spikes that were 75% emerged from the boot were emasculated, covered with glassine bags, and then pollinated 2 to 3 d after the emasculation date. Randomly selected F₂ plants from all segregating crosses were selfed to produce F_{2,3} families. Parental, F₁, BC₁F₁, F₂ plants, and F_{2,3} families were tested for reaction to imazamox.

All experiments were conducted in a walk-in growth chamber with a 16-h photoperiod and a 23°C day and 16°C night temperature regime. A completely random design was used for all experiments. The F₁ and F₂ populations were screened in the same experiment along with appropriate resistant lines and CDC Teal as controls. The BC₁F₁ and F_{2,3} populations were screened in two separate experiments along with appropriate parental lines as controls. To ensure uniform emergence of seedlings, seeds were pregerminated at 15°C in 9-cm Petri dishes containing approximately 2 mL of dH₂O. Once all seeds had germinated, the seedlings were planted into 8- by 16-celled flats containing Redi-earth (W.R. Grace and Company, Ajax, Ontario, Canada). Herbicide treatments were applied to plants at Haun stage 2.0 (Haun 1973) with a traveling cable sprayer calibrated to spray 100 L ha⁻¹. Imazamox was applied to plants at a rate of 20 g a.i. ha⁻¹ with an 8001 EVS nozzle at a pressure of 275 kPa. Merge surfactant (0.5% v/v) was added to the herbicide solution before application. For Mendelian analysis of the segregating populations, plants were scored into resistant (R), intermediate (I), susceptible (S) categories 15 d after herbicide application and tested for goodness

of fit to various one gene, two gene, and three gene models by Chi-square analysis. Resistant plants were phenotypically unaffected following herbicide treatment, whereas I plants were characterized by delayed growth, darkening (dark-green pigmentation) of the first two leaves, and the emergence of coleoptilar tillers. Susceptible plants were characterized by failure to develop new leaves, extensive leaf chlorosis, and eventually, plant death. Although minor variation in phenotypic expression was observed from plant to plant, leaf number and color served as excellent indicators of R and I reactions. Yates correction for continuity was used to adjust the Chi-square value when only a single degree of freedom was used in the analysis (Steel and Torrie, 1980, p. 633).

RESULTS AND DISCUSSION

Inheritance of Imidazolinone Resistance

At an imazamox application rate of 20 g a.i. ha⁻¹, plants from parental, F₁, F₂, BC₁F₁, and F₃ populations could easily be scored into one of three discrete phenotypic classes (R, I, or S) 15 d after herbicide application (Fig. 1). The resistant lines were used as controls in all experiments and consistently produced a resistant phenotype when sprayed with 20 g a.i. ha⁻¹ of imazamox. In all experiments, CDC Teal was killed 15 d after application of imazamox.

No phenotypic differences were observed between F₁ plants derived from reciprocal crosses, indicating that resistance to imazamox is not cytoplasmically inherited. As such, F₁ data from reciprocal populations were pooled (Table 1). All F₁ plants evaluated survived application of imazamox (Table 1). With the exception of cross CDC Teal/15A, the F₁ plants resulting from each of the resistant lines crossed with CDC Teal displayed an I reaction (Table 1). Since the F₁ plants were phenotypically intermediate between the two parents, it was concluded that resistance to imazamox in these lines was a partially dominant trait with higher levels of resistance in the homozygous condition. Previous genetic analysis of resistance to imidazolinones and sulfonylureas in common wheat (Newhouse et al., 1992), *A. thaliana* (Haughn and Somerville, 1986), maize (Newhouse et al., 1991), canola (Swanson et al., 1989), and soybean (Sebastian et al., 1989) also indicated that resistance was partially dominant.

Test of cytoplasmic inheritance was conducted in the F₂ generation by testing homogeneity of deviations from segregation ratios between the two reciprocal F₂ populations. Chi-square analysis revealed no significant deviations between reciprocal populations (Table 1), confirming the absence of cytoplasmic inheritance. Since cytoplasmic inheritance was absent, data from the two reciprocal populations were combined and a total Chi-square on pooled F₂ data was calculated (Table 1).

The expected genotypic segregation ratio in an F₂ population segregating for a single resistance gene would be 1(RR): 2(Rr): 1(rr). With the exception of Teal/15A, all F₂ populations resulting from susceptible × resistant crosses gave a good fit to a 1:2:1 R:I:S ratio, indicating segregation of a single gene for resistance to imazamox (Table 1). Since the F₂ data from these crosses fit a 1:2:1 R:I:S ratio, the intermediate phenotype is

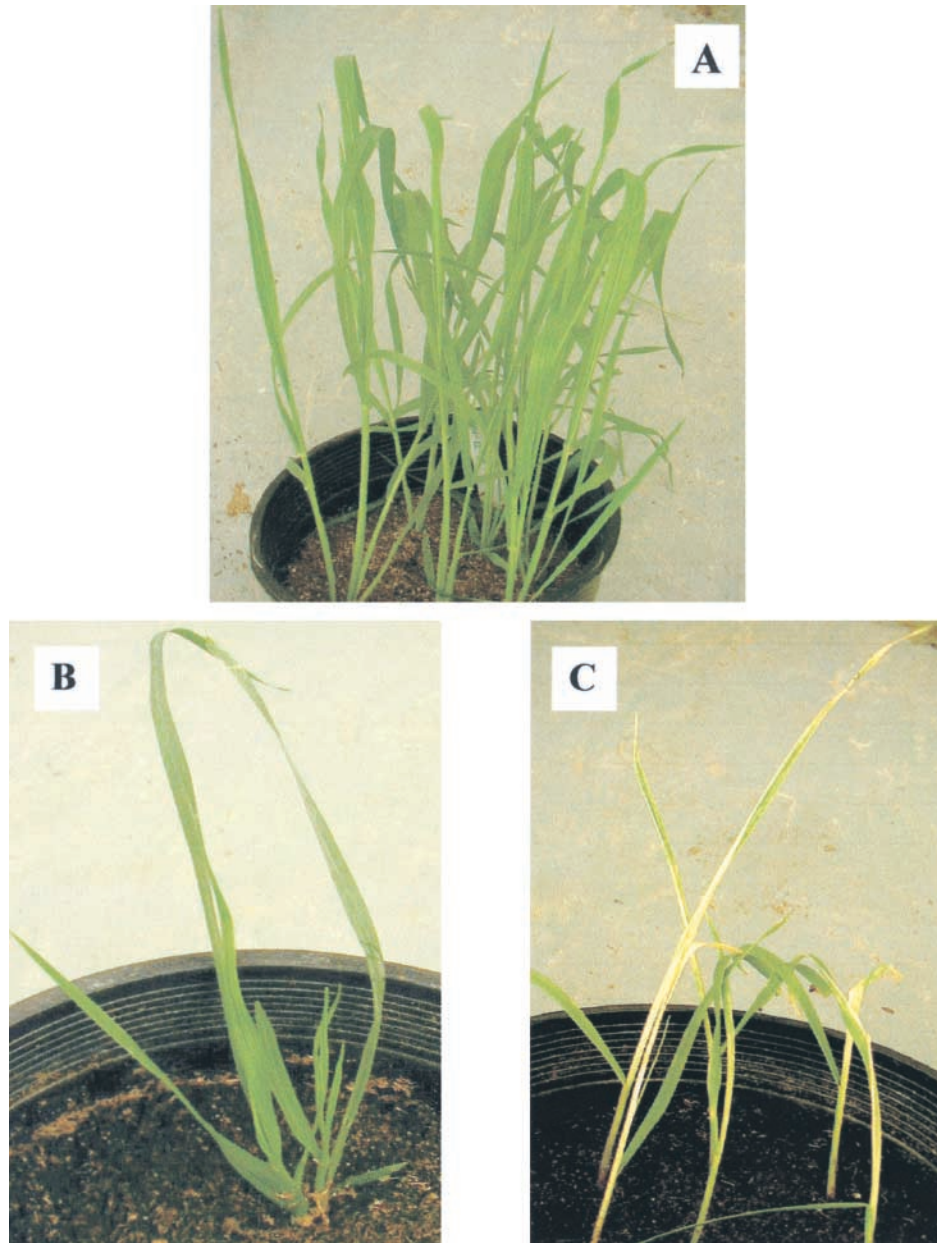


Fig. 1. Three distinct phenotypes observed in segregating populations 15 d after application of 20 g a.i. ha⁻¹ of imidazox. Phenotypes were characterized as A = resistant (R) reaction (no damage), B = intermediate (I) reaction, C = susceptible reaction.

indicative of a heterozygous genotype. These results suggest that resistance mechanisms are additive, and homozygous individuals have a higher level of resistance than heterozygous individuals. Newhouse et al. (1992) also concluded that lines heterozygous for *FS-4* had lower levels of resistance than lines homozygous for *FS-4*.

To confirm the results from the F₂ population, F₁ plants were backcrossed to CDC Teal, and the resulting progeny were evaluated for reaction to imidazox. If a single gene was conferring resistance, two genotypes, produced in equal frequencies, would be expected in the BC₁F₁ populations, namely *Rr* and *rr*. When F₁ plants from crosses Teal/1A, Teal/9A, Teal/10A, Teal/11A, and Teal/16A were backcrossed to the susceptible parent, resulting BC₁F₁ populations gave a good fit to a

1(*Rr*): 1(*rr*) I: S ratio, confirming the single locus hypothesis (Table 1).

If two unlinked genes for resistance were segregating, the expected genotypic segregation ratios would be 9(*R₁-R₂-*): 2(*R₁r₁R₂r₂*): 2(*r₁r₁R₂r₂*): 1(*R₁R₁r₂r₂*): 1(*r₁r₁R₂R₂*): 1(*r₁r₁r₂r₂*). On the basis of the results from the crosses segregating for a single resistance gene, genotypes heterozygous at a single resistance locus produced an I phenotype. Genotypes *R₁r₁r₂r₂* and *r₁r₁R₂r₂* are heterozygous at a single locus and were expected to produce an I phenotype. The F₂ population data from the cross Teal/15A did give a good fit to an 11 (9 *R₁-R₂-*; 1 *R₁R₁r₂r₂*; 1 *r₁r₁R₂R₂*): 4(2 *R₁r₁r₂r₂*; 2 *r₁r₁R₂r₂*): 1(*r₁r₁r₂r₂*) R:I:S ratio with a Chi-square *P* value of 0.10, indicating independent segregation of two resistance genes (Table 1). The ex-

Table 1. Reaction [resistant (R), intermediate (I), susceptible (S)] to imazamox in F₁, F₂ and BC₁F₁ populations resulting from crosses between CDC Teal and resistant lines and Chi-square tests of single locus and two locus models (CDC Teal/TealIMI 15A) for control of resistance.

Cross	F ₁ Populations†			F ₂ Populations					BC ₁ F ₁ Populations‡					
	Number of plants scored			Number of plants scored			Ratio tested	χ ² P value‡	HRC§ χ ² P value	Number of plants scored			Ratio tested#	χ ² P value‡
	R	I	S	R	I	S				R	I	S		
Teal/1A	0	10	0	198	437	201	1:2:1	0.42	0.52	0	33	27	0:1:1	0.52
Teal/9A	0	10	0	167	325	141	1:2:1	0.27	0.49	0	48	39	0:1:1	0.39
Teal/10A	0	11	0	237	461	231	1:2:1	0.94	0.10	0	27	20	0:1:1	0.38
Teal/11A	0	15	0	163	342	189	1:2:1	0.35	0.28	0	33	27	0:1:1	0.71
Teal/15A	20	0	0	638	260	72	11:4:1	0.10	0.24	17	45	16	1:2:1	0.39
Teal/16A	0	14	0	202	385	168	1:2:1	0.20	0.57	0	26	23	0:1:1	0.78

† The F₁ data represents the pooled data from reciprocal crosses.

‡ Chi-square P value represents the probability that deviations from the tested ratio are due to chance alone. Chi-square P values greater than 0.05 indicate that observed values were not significantly different from expected values.

§ Homogeneity of reciprocal crosses (HRC). Chi-square P-values greater than 0.05 indicate that reciprocal F₂ populations were homogeneous, and data from the two reciprocal populations were pooled.

¶ CDC Teal was used as the recurrent parent.

Ratios tested were based on the results of the F₂ generation.

pected genotypes in the BC₁F₁ population would be $R_1r_1R_2r_2$, $R_1r_1R_2R_2$, $r_1r_1R_2r_2$, and $r_1r_1R_2R_2$, each produced in equal frequency. The BC₁F₁ population resulting from cross CDC Teal*2/15A gave good fit to a 1 ($R_1r_1R_2r_2$):2 ($R_1r_1R_2R_2$; $r_1r_1R_2r_2$):1 ($r_1r_1R_2R_2$) R:I:S ratio with a Chi-square P value of 0.39, confirming the results observed in the F₂ populations that resistance in TealIMI 15A is conferred by two, independently segregating resistance genes (Table 1).

Twenty F₁ plants resulting from the cross Teal/15A were rated as resistant (Table 1). Evaluation of F₂ populations from this cross indicated that two independently segregating loci were involved in conferring resistance in this line (Table 1). The F₁ plants are heterozygous at each of the resistant loci, and if each of the resistant alleles alone would confer partial dominance, additively, two alleles would produce a resistant reaction.

To confirm the results of the F₂ and BC₁F₁ populations, progeny tests of individual F₂ plants (F_{2,3} families) were evaluated for reaction to imazamox. F₂ plants with the genotype RR are homozygous and would produce F₃ progeny all resistant to imazamox, whereas F₂ plants with genotype rr would produce F₃ progeny that were all susceptible. The F₂ plants with genotype Rr would produce F₃ progeny that were segregating for resistance. Therefore, for segregation of a single gene, the expected segregation ratio for F_{2,3} families is 1 homozygous resistant: 2 segregating for resistance: 1 homozygous susceptible families. If two resistance genes conferred resis-

tance to imazamox, F₂ plants homozygous for one or both resistance genes (1 $R_1R_1R_2R_2$; 2 $R_1R_1R_2r_2$; 1 $R_1R_1r_2r_2$; 2 $R_1r_1R_2R_2$; 1 $r_1r_1R_2R_2$) would produce F₃ progeny that were all resistant to imazamox. Plants heterozygous at both loci (4 $R_1r_1R_2r_2$) or heterozygous at one locus, and homozygous susceptible at the other (2 $R_1r_1r_2r_2$; 2 $r_1r_1R_2r_2$) would produce segregating F₃ progeny, whereas F₂ plants with genotype $r_1r_1r_2r_2$ would produce F₃ progeny that were all susceptible to imazamox. Therefore, the expected F_{2,3} family segregation ratio is 7 resistant: 8 segregating: 1 homozygous susceptible.

Since it is speculated from F₂ data that resistance in lines 1A, 9A, 10A, 11A, and 16A is controlled by a single major gene, F_{2,3} families should segregate and fit a 1:2:1 homozygous resistance: segregating: homozygous susceptible family ratio. Evaluation of F_{2,3} families indicated that crosses Teal/1A, Teal/9A, Teal/10A, Teal/11A, and Teal/16A all fit a 1:2:1 resistant: segregating: susceptible F_{2,3} family ratio with Chi-square P values of 0.64, 0.66, 0.52, 0.40, and 0.94, respectively (Table 2). These results confirm the results of the F₂ and BC₁F₁ data that resistance in lines 1A, 10A, 9A, 11A, and 16A is controlled by a single major gene. The F₂ data resulting from the cross Teal/15A gave a good fit to a 11:4:1 R:I:S ratio (Table 1). If this is the case, F_{2,3} families should segregate and fit a 7:8:1 resistant: segregating: susceptible ratio. F_{2,3} families from the cross Teal/15A fit the expected 7:8:1 ratio (Table 2), confirming the results of the F₂ and BC₁F₁ populations that resistance

Table 2. Evaluation of resistance to imazamox in F_{2,3} families resulting from crosses between resistant lines and CDC Teal and Chi-square tests of single-locus and two-locus models (Teal/15A) for control of resistance.

Cross	Number of F _{2,3} families scored			Total families scored	Ratio tested (R:Seg:S)†	χ ² P value‡
	Resistant (R)	Segregating (Seg)	Susceptible (S)			
Teal/1A	12	28	10	50	1:2:1	0.64
Teal/9A	15	23	12	50	1:2:1	0.66
Teal/10A	9	27	14	50	1:2:1	0.52
Teal/11A	14	26	8	48	1:2:1	0.40
Teal/15A	36	55	9	100	7:8:1	0.21
Teal/16A	12	25	11	48	1:2:1	0.94

† Family segregation ratios tested were based on the results of the F₂ and BC₁F₁ populations.

‡ Chi-square P value (2 df) representing the probability that deviations from the tested ratio are due to chance alone. Chi-square P-values greater than 0.05 indicate that observed values were not significantly different from expected values.

Table 3. Single plant evaluation of imazamox resistance in F₂ population resulting from intercrosses between all resistant lines with the exception of TealIMI 11A.

Cross	Number of F ₂ plants scored			Total
	Resistant (R)	Intermediate (I)	Susceptible (S)	
1A/9A	506	0	0	506
1A/10A	566	1	0	567
1A/15A	501	0	0	501
1A/16A	814	0	0	814
1A/BW755	509	0	0	509
9A/10A	602	1	0	603
9A/15A	424	0	0	424
9A/16A	487	1	0	488
9A/BW755	499	0	0	499
10A/15A	547	0	0	547
10A/16A	569	0	0	569
10A/BW755	686	0	0	686
15A/16A	498	0	0	498
15A/BW755	610	0	0	610
16A/BW755	508	1	0	509

in 15A is conferred by two, independent loci. We believe that this is the first reported instance where two independently segregating imidazolinone resistance genes have been identified in a single line following seed mutagenesis.

The results of the genetic study indicate that resistance in TealIMI lines 1A, 9A, 10A, 11A, and 16A is inherited as a partially dominant trait conferred by a single nuclear coded gene. This pattern of inheritance is consistent with other findings that have reported the genetic control of resistance to AHAS inhibiting herbicides. In maize, soybean, *A. thaliana*, and tobacco, resistance to AHAS inhibitors is partially dominant and inherited as a single nuclear gene (Chaleff and Ray, 1984; Newhouse et al., 1991; Sathasivan et al., 1991). Resistance in all cases was due to the presence of a herbicide resistant form of AHAS. Following seed mutagenesis, Sebastian and Chaleff (1987) isolated four soybean lines with increased tolerance to chlorsulfuron {2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide}, a sulfonylurea. However, contradictory to this research, genetic analysis of resistance in all four lines indicated that resistance was inherited as a single recessive gene. However, resistance in these lines was not due to an altered form of AHAS.

Resistance in TealIMI 15A was dominant and was

found to be conferred by two, unlinked resistance genes. The F₁ plants resulting from the cross Teal/15A were phenotypically identical to lines homozygous for a single resistance gene at 20 g a.i. ha⁻¹ of imazamox, suggesting that resistance is additive. With resistance under additive gene action, lines homozygous for a single resistance gene can be selected in a single generation. In this study, plants heterozygous at a single resistance locus could be easily identified in segregating populations. Since resistance is additive, selection of lines containing more than one resistance gene should be possible by applying a higher application rate of imazamox.

Allelism Studies

To determine the allelic relationships of the resistance genes, all possible intercrosses between resistant lines were evaluated. If resistance genes in two separate lines are alleles at the same locus, no intermediate or susceptible progeny would be observed in an F₂ population resulting from crossing the two resistant lines. No susceptible plants were observed in the F₂ populations resulting from the intercrosses between lines BW755, 1A, 9A, 10A, 15A, and 16A (Table 3). A single intermediate plant was observed in F₂ populations 1A/10A, 9A/10A, 9A/16A and 16A/BW755 (Table 3) and it is likely that these plants were misclassified as I and should be included in the R category. Since no segregation was observed in these populations, it was concluded that the resistance genes in these lines are either alleles at the *FS-4* locus, or are very tightly linked. Since cross 15A/BW755 did not produce a segregating F₂ population (Table 3) one of the resistance genes present in TealIMI 15A is allelic to *FS-4*. Since these populations were not segregating in the F₂ generation, F_{2,3} families from these crosses were not evaluated.

All intercrosses involving 11A segregated in the F₂ generation, indicating the presence of a unique resistance gene in 11A (Table 4). If two independently segregating resistance genes were present as the result of crossing two lines, each carrying a single resistance gene, an 11(9 *R₁-R₂*; 1 *R₁R₁R₂r₂*; 1 *r₁r₁R₂R₂*): 4(2 *R₁r₁r₂r₂*; 2 *r₁r₁R₂r₂*): 1(*r₁r₁r₂r₂*) R:I:S ratio would be expected in the F₂ population. None of the F₂ populations from crosses

Table 4. Reaction to imazamox in F₂ populations resulting from crosses between resistant lines and TealIMI 11A.

Cross	Number of F ₂ plants scored			Total	Ratio tested	χ ² <i>P</i> value†
	Resistant (R)	Intermediate (I)	Susceptible (S)			
11A/1A	567 (605)‡	246 (220)	67 (55)	880	11:4:1 R:I:S	0.00
	813 (825)		67 (55)		15:1 R+I:S§	0.28
11A/9A	313 (338)	133 (123)	46 (31)	492	11:4:1 R:I:S	0.00
	446 (461)		46 (31)		15:1 R+I:S§	0.00
11A/10A	344 (415)	213 (151)	46 (38)	603	11:4:1 R:I:S	0.00
	557 (565)		46 (38)		15:1 R+I:S§	0.19
11A/15A	430 (458)	70 (48)	14 (8)	614	57:6:1 R:I:S	0.00
	500 (506)		14 (8)		63:1 R+I:S§	0.19
11A/16A	408 (439)	182 (160)	48 (40)	638	11:4:1 R:I:S	0.03
	590 (598)		48 (40)		15:1 R+I:S§	0.21
11A/BW755	473 (505)	215 (184)	47 (46)	735	11:4:1 R:I:S	0.00
	688 (689)		47 (46)		15:1 R+I:S§	0.93

† Chi-square *P* value representing the probability that deviations from the tested ratio are due to chance alone. Chi-square *P* values greater than 0.05 indicate that observed values were not significantly different from expected values.

‡ Values in brackets present the number of F₂ plants expected in each phenotypic class based on the segregation ratio tested.

§ Intermediate plants were combined with resistant plants before chi-square testing. See text for details.

11A/BW755, 11A/1A, 11A/9A, 11A/10A, and 11A/16A fit the expected 11:4:1 R:I:S ratio, because of an excess of I and S segregants (Table 4). Various other two-gene hypotheses were tested, but in all cases, the observed values deviated significantly from the expected values. Since an excess of I and S segregants were observed, linkage could be discounted, since linkage would result in an excess of parental phenotypes (i.e., resistance in this case) in segregating populations. However, if I phenotypes are combined with those that produce a resistant reaction, a 15:1 R+I:S ratio would be expected if the two genes segregated independently. The F₂ populations from crosses 11A/BW755, 11A/1A, 11A/10A, and 11A/16A did fit the expected 15:1 R+I:S ratio suggesting independent segregation of two major resistance genes in these populations (Table 4). Cross 11A/9A, however, did not fit the expected 15:1 R+I:S segregation ratio because of an excess of susceptible segregants (Table 4). Various other two-gene hypotheses were tested, but all observed values deviated significantly from the expected values. Since F₂ data is based on segregation data collected from single plants, an error in rating could distort segregation ratios. Single plant evaluation is only useful for providing preliminary estimates of the number of genes controlling resistance and results should be confirmed from family data. Since segregation data of F_{2,3} families are based on a population of plants, a rating error would be less likely to distort segregation ratios. F_{2,3} family ratios from the crosses 11A/BW755, 11A/1A, 11A/9A, 11A/10A, and 11A/16A all gave a good fit to a 7:8:1 resistant: segregating: susceptible ratio, confirming the presence of two segregating genes (Table 5). These results confirm that the resistance gene in 11A is independently inherited from those in lines 1A, 9A, 10A, 15A, 16A, and BW755.

Cross 11A/15A produced a segregating F₂ population as indicated by the presence of I and S phenotypes (Table 4). Since 15A is carrying two resistance alleles, one allelic to *FS-4*, a segregating F₂ population in cross 11A/15A would indicate the presence of three segregating genes. Given the assumption that genotypes heterozygous at a single resistance gene locus would display an I phenotype, the expected segregation ratio for three genes would be 57:6:1 R:I:S. Similarly, if plants with an intermediate phenotype are included as resistant, the expected segregation ratio would be 63:1 R+I:S. The

F₂ plants did not fit the expected 57:6:1 R:I:S ratio, but did fit the expected 63:1 R+I:S ratio, indicating the independent segregation of three loci (Table 4). These results suggest that the second gene in 15A is not allelic to the resistance allele in 11A. F_{2,3} families were not screened as approximately 190 families would have to be screened to ensure a 95% probability of observing at least one susceptible family (Hanson, 1959).

Recommended rules for gene symbolization in wheat suggest that two or more genes having phenotypically similar effects should be designated by a common basic symbol that describes the phenotypic purpose of the gene(s) (McIntosh et al., 1998). Thus, the *FS-4* resistance gene characterized by Newhouse et al. (1992) was redesignated as *Imi1*. *Imi* stands for imidazolinone resistance and indicates that resistance is a dominant trait and that this is the first allele identified. Segregating F₂ and F_{2,3} population data suggest that 15A and 11A carry two new, independently segregating resistance genes (Tables 4 and 5). The designations for these genes are *Imi2* for the 11A resistance gene and *Imi3* for the second resistance gene in 15A.

Seed mutagenesis has proven to be a viable alternative to cell culture or transformation to develop imidazolinone resistant wheat. Following seed mutagenesis of CDC Teal, six lines were selected with moderate to high levels of resistance to the imidazolinones. The results of this study indicate that three unlinked resistance genes are present in the TealIMI lines namely *Imi1* (1A, 9A, 10A, 15A, and 16A), *Imi2* (11A), and *Imi3* (15A). Resistance to AHAS-inhibiting herbicides in maize (Bernasconi et al., 1995), sugar beet (Wright et al., 1998), cotton (Rajasekaran et al., 1996), canola (Wiersma et al., 1989; Hattori et al., 1995), *A. thaliana* (Haughn et al., 1988; Sathasivan et al., 1991; Chang and Duggleby, 1998; Lee et al., 1999), and tobacco (Lee et al., 1988; Hartnett et al., 1990) is the result of a single point mutation to the gene(s) encoding the catalytic subunit of the AHAS enzyme, resulting in the production of AHAS with reduced sensitivity to the herbicide inhibition. It is also likely that each of the three resistance genes identified are structural genes coding for herbicide-insensitive forms of AHAS, one for each of the three genomes in common wheat. Since common wheat is a hexaploid, multiple AHAS loci would be expected. Other polyploid species have been found to

Table 5. Evaluation of imazamox resistance in F_{2,3} families resulting from segregating inter-crosses between resistant lines and TealIMI 11A.

Cross	Number of F _{2,3} families scored			Total families scored	Ratio tested (R:Seg:S)†	χ ² P value‡
	Resistant (R)	Segregating (Seg)	Susceptible (S)			
BW755/11A	32	42	7	81	7:8:1	0.57
11A/1A	33	58	9	100	7:8:1	0.07
11A/9A	36	49	5	90	7:8:1	0.77
11A/10A	34	59	7	100	7:8:1	0.14
11A/16A	45	47	7	99	7:8:1	0.86
11A/15A§	–	–	–	–	–	–

† Family segregation ratios tested were based on the results of the F₂ populations examined.

‡ Chi-square P value (2 df) representing the probability that deviations from the tested ratio are due to chance alone. Chi-square P values greater than 0.05 indicate that observed values were not significantly different from expected values.

§ Family ratios were not tested because of the large number of single plants that would have to be evaluated in each family to ensure adequate power of test. See text for details.

have more than one copy of AHAS. In tobacco, an allotetraploid, two AHAS genes have been identified and characterized (Mazur et al., 1987). Maize possesses two constitutively expressed AHAS genes (Fang et al., 1992). In allotetraploid canola and cotton, an AHAS multigene family consisting of five and six members, respectively, is present (Rutledge et al., 1991; Grula et al., 1995).

Since three independently segregating resistance genes were identified in the TealIMI populations, a simple backcrossing program to introduce one or more of the resistance genes into other spring wheat genetic backgrounds is feasible. Higher levels of resistance are desirable to ensure minimal plant injury at rates that are required for adequate weed control (Newhouse et al., 1991). Higher levels of resistance to AHAS inhibiting herbicides have been observed in polyploid species when multiple resistance alleles are present. Swanson et al. (1989) combined two semidominant imidazolinone resistance genes from two canola lines, representing two unlinked genes, to produce an F₁ hybrid that was superior in imidazolinone resistance than either of the parents alone. The authors concluded that resistance mechanisms were additive because a higher level of resistance was observed in lines carrying more than one resistance gene. Since resistance is additive, selection of common wheat lines homozygous for one or more genes should be possible with high application rates of imazamox.

CONCLUSIONS

With the exception of TealIMI 15A, resistance in all lines was partially dominant and inherited as a single nuclear gene. TealIMI 15A carries two resistance genes. In this study, three independently segregating resistance loci were identified. On the basis of recommended rules for gene symbolization in wheat, the genes were designated *Imi1*, *Imi2*, and *Imi3*. Multiple resistance genes conferring resistance to the sulfonylureas and the imidazolinones have been identified in other polyploid species. Higher levels of resistance to imidazolinones in spring wheat should be possible by combining *Imi1*, *Imi2*, and *Imi3* into a single genotype. It has been previously shown (Newhouse et al. 1992) that *Imi1* codes for a herbicide resistant form of AHAS. Studies must be conducted to determine if *Imi2* and *Imi3* also code for an altered, imidazolinone resistant AHAS enzyme. The resistant lines should also be evaluated in the field to determine the level of whole plant resistance to the imidazolinones.

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REFERENCES

- Bernasconi, P., A.R. Woodworth, B.A. Rosen, M.V. Subramanian, and D.L. Siehl. 1995. A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *J. Biol. Chem.* 270:17381–17385.
- Chaleff, R.S., and T.B. Ray. 1984. Herbicide resistant mutants from tobacco culture. *Science* 223:1148–1151.
- Chang, A.K., and R.G. Duggleby. 1998. Herbicide-resistant forms of *Arabidopsis thaliana* acetohydroxyacid synthase: Characterization of the catalytic properties and sensitivity to inhibitors of four defined mutants. *Biochem. J.* 333:765–777.
- Creason, G.L., and R.S. Chaleff. 1988. A second mutation enhances resistance of a tobacco mutant to sulfonylurea herbicides. *Theor. Appl. Genet.* 76:177–182.
- Fang, L.Y., P.R. Gross, C.H. Chen, and M. Lillis. 1992. Sequence of two acetohydroxyacid synthase genes from *Zea mays*. *Plant Mol. Biol.* 18:1185–1187.
- Grula, J.W., R.L. Hudspeth, S.L. Hobbs, and D.M. Anderson. 1995. Organization, inheritance and expression of acetohydroxyacid synthase genes in the cotton allotetraploid *Gossypium hirsutum*. *Plant Mol. Biol.* 28:837–846.
- Hanson, W.D. 1959. Minimum family sizes for planning of genetic experiments. *Agron. J.* 51:711–716.
- Hart, S.E., J.W. Saunders, and D. Penner. 1992. Chlorsulfuron resistant sugar beet: Cross-resistance and physiological basis of resistance. *Weed Sci.* 40:378–383.
- Hartnett, M.E., C.F. Chui, C.J. Mauvais, R.E. McDevitt, S. Knowlton, J.K. Smith, S.C. Falco, and B.J. Mazur. 1990. Herbicide resistant plants carrying mutated acetolactate synthase genes. *Am. Chem. Soc.* 421:459–473.
- Hattori, J., D. Brown, G. Mourad, H. Labbe, T. Ouellet, G. Sunohara, R. Rutledge, J. King, and B. Miki. 1995. An acetohydroxyacid synthase mutant reveals a single site involved in multiple herbicide resistance. *Mol. Gen. Genet.* 246:419–425.
- Haughn, G.W., and C. Somerville. 1986. Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. *Mol. Gen. Genet.* 204:430–434.
- Haughn, G.W., J. Smith, B. Mazur, and C. Somerville. 1988. Transformation with a mutant *Arabidopsis* acetolactate synthase gene renders tobacco resistant to sulfonylureas. *Mol. Gen. Genet.* 211:266–271.
- Haun, J.R. 1973. Visual quantification of wheat development. *Agron. J.* 65:116–119.
- Hughes, G.R., and P.J. Hucl. 1993. CDC Teal hard red spring wheat. *Can. J. Plant Sci.* 73:193–197.
- Lee, K.Y., J. Townsend, J. Tepperman, M. Black, C.F. Chui, B. Mazur, P. Dunsuir, and J. Bedbrook. 1988. The molecular basis of sulfonylurea resistance in tobacco. *EMBO J.* 7:1241–1248.
- Lee, Y.T., A.K. Chang, and R.G. Duggleby. 1999. Effect of mutagenesis at serine-653 of *Arabidopsis thaliana* acetohydroxyacid synthase on the sensitivity to imidazolinone and sulfonylurea herbicides. *FEBS Lett.* 452:341–345.
- Mazur, B.J., C.F. Chui, and J.K. Smith. 1987. Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. *Plant Physiol.* 85:1110–1117.
- McIntosh, R.A., G.E. Hart, K.M. Devos, M.D. Gale, and W.J. Rogers. 1998. Catalogue of gene symbols. Volume 5. *In* A.E. Slinkard (ed.) Proceedings of the 9th International Wheat Genetics Symposium. Saskatoon, SK. 2–7 Aug. 1998. University Extension Press, University of Saskatchewan, Saskatoon.
- Mourad, G., B. Pandey, and J. King. 1993. Isolation and genetic analysis of a triazolopyrimidine-resistant mutant of *Arabidopsis*. *J. Hered.* 84:91–96.
- Newhouse, K., B.K. Singh, D.L. Shaner, and M. Stidham. 1991. Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinones. *Theor. Appl. Genet.* 83:65–70.
- Newhouse, K., W. Smith, M. Starrett, T. Schaefer, and B.K. Singh. 1992. Tolerance to imidazolinone herbicides in wheat. *Plant Physiol.* 100:882–886.
- Rajasekaran, K., J.W. Grula, and D.M. Anderson. 1996. Selection and characterization of mutant cotton (*Gossypium hirsutum* L.) cell lines resistant to sulfonylurea and imidazolinone herbicides. *Plant Sci.* 199:115–124.
- Ray, T.B. 1984. Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiol.* 75:827–831.
- Rutledge, R.G., T. Ouellet, J. Hattori, and B.L. Miki. 1991. Molecular characterization and genetic origin of the *Brassica napus* acetohydroxyacid synthase multigene family. *Mol. Gen. Genet.* 229:31–40.

- Santel, H.J., B.A. Bowden, V.M. Sorenson, K.H. Mueller, and J. Reynolds. 1999. Flucarbazone-sodium—a new herbicide for grass control in wheat. *Proc. West. Soc. Weed Sci.* 52:124–125.
- Sathasivan, K., G.W. Haughn, and N. Murai. 1991. Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var Columbia. *Plant Physiol.* 97:1044–1050.
- Sebastian, S.A., and R.S. Chaleff. 1987. Soybean mutants with increased tolerance for sulfonylurea herbicides. *Crop Sci.* 27:948–952.
- Sebastian, S.A., G.M. Fader, J.F. Ulrich, D.R. Forney, and R.S. Chaleff. 1989. Semi-dominant soybean mutation for resistance to sulfonylurea herbicides. *Crop Sci.* 29:1403–1408.
- Shaner, D.L., P.C. Anderson, and M.A. Stidham. 1984. Imidazolinones: Potent inhibitors of acetohydroxyacid synthase. *Plant Physiol.* 76:545–546.
- Singh, B.K. 1999. Biosynthesis of valine, leucine and isoleucine. p. 227–247. *In* B.K. Singh (ed.) *Plant amino acids*. Marcel Dekker Inc., New York.
- Southan, M.D., and L. Copeland. 1996. Physical and kinetic properties of acetohydroxyacid synthase from wheat leaves. *Physiol. Plant.* 98:824–832.
- Steel, R.G.D., and J.H. Torrie. 1980. *Principles and procedures of statistics*. McGraw-Hill, New York.
- Subramanian, M.V., and B.C. Gerwick. 1989. Inhibition of acetolactate synthase by triazolopyrimidines. p. 277–288. *In* J.R. Whitaker et al. (ed.) *Biocatalysis in agricultural biotechnology*. ACS Symposium Series. American Chemical Society, Washington, DC.
- Subramanian, M.V., H.Y. Hung, J.M. Dias, V.W. Miner, J.H. Butler, and J.J. Jachetta. 1990. Properties of mutant acetolactate synthases resistant to triazolopyrimidine sulfonanilide. *Plant Physiol.* 94:239–244.
- Swanson, E.B., M.J. Hergesell, M. Arnoldo, D.W. Sippell, and RSC Wong. 1989. Microspore mutagenesis and selection: Canola plants with field tolerance to imidazolinones. *Theor. Appl. Genet.* 78:525–530.
- Washington, W.J., and E.R. Sears. 1970. Ethyl methanesulfonate-induced chlorophyll mutations in *Triticum aestivum*. *Can. J. Cytol.* 12:851–859.
- Wiersma, P.A., M.G. Schmiemann, J.A. Condie, W.L. Crosby, and M.M. Moloney. 1989. Isolation, expression and phylogenetic inheritance of an acetolactate synthase gene for *Brassica napus*. *Mol. Gen. Genet.* 219:413–420.
- Wright, T.R., and D. Penner. 1998. Cell selection and inheritance of imidazolinone resistance in sugar beet (*Beta vulgaris*). *Theor. Appl. Genet.* 96:612–620.
- Wright, T.R., N.F. Bascomb, S.F. Sturmer, and D. Penner. 1998. Biochemical mechanism and molecular basis for ALS-inhibiting herbicide resistance in sugar beet (*Beta vulgaris*) somatic cell selections. *Weed Sci.* 46:13–23.