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Imidazolinone-tolerant crops: history, current status and future†

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Abstract: Imidazolinone herbicides, which include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin, control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS). AHAS is a critical enzyme for the biosynthesis of branched-chain amino acids in plants. Several variant AHAS genes conferring imidazolinone tolerance were discovered in plants through mutagenesis and selection, and were used to create imidazolinone-tolerant maize (*Zea mays* L), wheat (*Triticum aestivum* L), rice (*Oryza sativa* L), oilseed rape (*Brassica napus* L) and sunflower (*Helianthus annuus* L). These crops were developed using conventional breeding methods and commercialized as Clearfield* crops from 1992 to the present. Imidazolinone herbicides control a broad spectrum of grass and broadleaf weeds in imidazolinone-tolerant crops, including weeds that are closely related to the crop itself and some key parasitic weeds. Imidazolinone-tolerant crops may also prevent rotational crop injury and injury caused by interaction between AHAS-inhibiting herbicides and insecticides. A single target-site mutation in the AHAS gene may confer tolerance to AHAS-inhibiting herbicides, so that it is technically possible to develop the imidazolinone-tolerance trait in many crops. Activities are currently directed toward the continued improvement of imidazolinone tolerance and development of new Clearfield* crops. Management of herbicide-resistant weeds and gene flow from crops to weeds are issues that must be considered with the development of any herbicide-resistant crop. Thus extensive stewardship programs have been developed to address these issues for Clearfield* crops.

Keywords: imidazolinone tolerance; weed control; acetohydroxyacid synthase (AHAS); herbicide; mutant; Clearfield*

1 INTRODUCTION

It is increasingly difficult to discover a new herbicide and even more difficult to find one with a novel mode of action.[1] Today, approximately 500 000 compounds must be screened to discover a potential herbicide compared with one per 500 compounds screened in the 1940s.[1] Given the difficulty of discovering new herbicides, expanding the utility of existing herbicides that have a broad weed-control spectrum and good environmental profile through genetically enhanced resistance is a useful strategy for advancing the development of selective herbicides. Crop resistance to herbicides is typically conferred by one of three mechanisms: resistance at the site of action, metabolic detoxification and prevention of the herbicide from reaching the site of action.[2] Developing one or more of these three mechanisms through genetic modification may provide herbicide resistance in a crop.

Imidazolinone herbicides control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS), which is a critical enzyme for the biosynthesis of branched-chain amino acids in plants. These herbicides control a wide spectrum of grass and broadleaf weeds, are effective at low application rates, have low mammalian toxicity, and possess a favorable environmental profile. Thus imidazolinone herbicides have many ideal characteristics for utilization in a herbicide-resistant crop. Furthermore, imidazolinone-tolerant plants with altered AHAS genes and enzymes have been discovered in many crops. This makes it possible to develop imidazolinone-tolerant crops based on the resistance mechanism at the site of action for these crops. Since the commercial launch of imidazolinone-tolerant maize in 1992, four other imidazolinone-tolerant crops have been developed and commercialized using conventional breeding methods. Meanwhile, four imidazolinone active ingredients have been registered on different imidazolinone-tolerant crops, either individually or in

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combinations. Extensive research and development on this multi-trait and multi-herbicide technology have been carried out through cooperation between public and private sectors. Significant amounts of data and information have been generated and published since the last review of imidazolinone-tolerant crops.[3] This review summarizes the history, current status and future development of imidazolinone-tolerant crops. The review discusses the interaction between imidazolinones and the AHAS enzyme, and the development, characterization and utility of the five commercialized imidazolinone-tolerant crops. It also presents strategies to minimize outcrossing of the tolerant trait to closely related weed species. Potential for the development of other imidazolinone-tolerant crops is also highlighted.

2 IMIDAZOLINONE HERBICIDES AND THE AHAS ENZYME

2.1 Imidazolinone herbicides

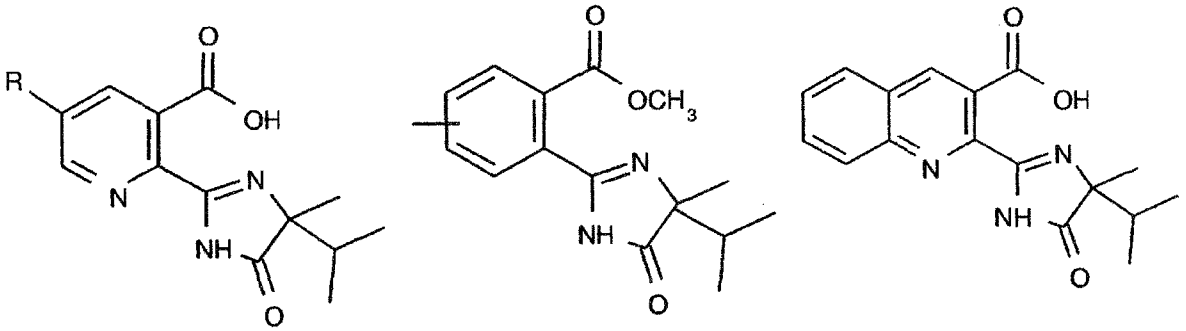
Imidazolinones are among the five chemical families of AHAS-inhibiting herbicides. The other four families are sulfonylureas, triazolopyrimidines, pyrimidinyl-thiobenzoates and sulfonylamino-carbonyltriazolinones.[4] Imidazolinones include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin (Fig 1). As the names indicate, all imidazolinones have an imidazole moiety in their molecular structure.[5] They are further divided into three groups based on the second cyclic structure of their molecules excluding the imidazole ring. Imazaquin has a quinoline moiety, imazamethabenz has a benzene ring and the other imidazolinones have a pyridine ring. The imidazolinones with the pyridine ring are distinguished by four analogs that differ only at position five of the pyridine ring. Imazapyr, imazapic, imazethapyr and imazamox have respectively hydrogen (H), methyl (CH_3), ethyl (CH_3-CH_2) and methoxymethyl (CH_3-O-CH_2) functional groups at position five of the pyridine ring (Fig 1). Only this group of imidazolinone compounds is used with imidazolinone-tolerant crops.

Because all six imidazolinone compounds have the imidazole ring, there must be a strong link between this and AHAS inhibition. The inhibition difference of AHAS activity among the three groups of imidazolinones carrying quinoline, benzene and pyridine rings suggests that the second cyclic structure—other than the imidazole ring—of the imidazolinones may also participate in AHAS inhibition.[6] In contrast, only a small difference in AHAS inhibition has been observed among the four imidazolinone analogs that have the pyridine ring (Tan S, unpublished data). The different functional groups at position five of the pyridine ring play a smaller role in herbicide inhibition of AHAS than the other two molecular structures mentioned earlier. The functional groups, however, are related to certain characteristics of the imidazolinone herbicides such as metabolism in plants.[7]

2.2 Interaction between imidazolinone herbicides and the AHAS enzyme

Analysis of the crystal structure of yeast AHAS and other studies suggest that the AHAS enzyme of eukaryotes may be composed of a catalytic sub-unit and a regulatory sub-unit.[8–10] The catalytic sub-unit is most likely a homodimer formed by the folding of two large sub-units (LSU); each LSU is a monomer of an AHAS polypeptide and has three domains of similar size, α, β and γ.[9,11–13] The regulatory sub-unit is believed to be an AHAS small sub-unit (SSU) in eukaryotes.[8,10] A putative regulatory AHAS SSU has been cloned and expressed in several plant species.[8,14–16]

Structural modeling of plant AHAS, crystal structure analysis of yeast AHAS, and the available data on known AHAS mutations suggest that the binding site of AHAS-inhibiting herbicides is near the active site located at the interface of the two LSU monomers in the catalytic sub-unit of AHAS.[9,12] Herbicide-tolerant mutations are spread through all three domains, but the protein folds in a way that places all the amino-acid substitutions of interest at the interface of the two monomers where the herbicide-binding site is proposed.[13] Substitution of some amino acids in the proposed herbicide-binding pocket resulted in an increased resistance of AHAS to imidazolinone herbicides, while substitution of other



Pyridine imidazolinone

Benzene imidazolinone (imazamethabenz methyl ester)

Quinoline imidazolinone (imazaquin)

Figure 1. Imidazolinone herbicides; imazapyr: R = H, imazapic: R = CH_3, imazethapyr: R = CH_3-CH_2, and imazamox: R = CH_3-O-CH_2.

amino acids in the pocket resulted in increased sensitivity of AHAS to imidazolinones.[12,13] Both observations indicate that the amino acids of the proposed herbicide-binding pocket do interact with the herbicides. It is generally believed that different classes of herbicides bind to distinct but overlapping sites within the pocket.[9,12,17–19] Based on molecular modeling of AHAS–imidazolinone interaction, the binding pocket has been proposed to be at the entry site for the substrate of the AHAS enzyme.[12] Results from the herbicide docking model, in combination with crystal structure analysis of the AHAS, suggest that both sulfonylureas and imidazolinones may bind in the substrate access channel and impede the binding of the substrate to AHAS.[9,13,20]

2.3 AHAS gene mutation and imidazolinone-tolerance trait

The primary structure of AHAS LSU is composed of about 670 amino acids, varying from species to species.[9,12,21,22] Most of the imidazolinone-tolerant mutations come from amino-acid substitutions in domains α and γ, and many researchers refer to the two regions as domains A and B or regions A and B.[23–26]

Several authors have reviewed known mutations of the AHAS genes that confer resistance to AHAS-inhibiting herbicides in plants.[1,19,24–26] The most commonly occurring mutations that confer resistance to AHAS inhibitors are at positions of Ala122, Pro197, Ala205, Trp574, and Ser653 of the AHAS LSU (Fig 2). The mutation at Ser653 confers tolerance to imidazolinones but not cross-tolerance to other AHAS inhibitors, a preferable characteristic for the

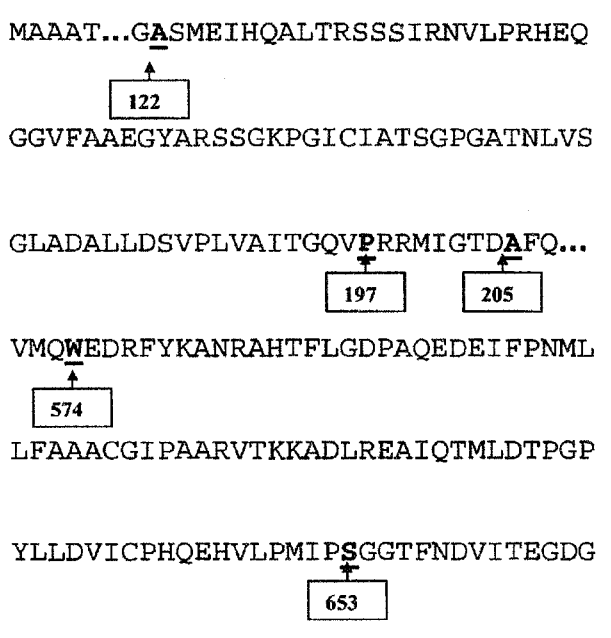


Figure 2. Partial amino-acid sequence of *Arabidopsis thaliana* AHAS protein showing five amino-acids which are commonly substituted from mutations in plants. The substitution confers resistance to AHAS-inhibiting herbicides.

development of imidazolinone-tolerant crops.[24,26–29] The mutation at Trp574 is generally cross-tolerant to the different families of AHAS-inhibiting herbicides and has been used for the development of imidazolinone-tolerant crops.[22,24,26] Mutations at Ala122 and Ala205 exhibit acceptable tolerance to imidazolinones and are also a good choice for the development of imidazolinone-tolerant crops.[24–26,30] In contrast, the mutation at Pro197 generally has no or low tolerance to imidazolinones but good tolerance to sulfonylureas.[24,26,31–33] The majority of commercialized imidazolinone-tolerant crops are currently developed from either one or a combination of Ala205, Trp574, and Ser653 mutations.[22,28,34,35] All commercialized imidazolinone-tolerant crops have been developed through selection or mutagenesis, utilizing conventional plant-breeding techniques, and are therefore non-transgenic.[3,36–39] The imidazolinone tolerance is based on a target-site mutation that reduces the sensitivity of AHAS enzyme to imidazolinone herbicides. Other mechanisms for herbicide tolerance (metabolism and reduced uptake and translocation to the active site) have not been exploited in the development of imidazolinone-tolerant crops, but these inherent attributes of the crops may contribute to their overall tolerance.

3 COMMERCIALIZED IMIDAZOLINONE-TOLERANT CROPS

3.1 Imidazolinone-tolerant maize

Development of imidazolinone-tolerant maize began in 1982.[3] Tissue culture selection of cell callus of the maize hybrid A188 × B73 with imazaquin resulted in several imidazolinone-tolerant lines: XA17, XI12, QJ22, XS40, ZA54, UV18, AC17 and QT15.[3,40–42] XA17 and XI12 were subsequently introduced into commercial maize varieties and first marketed in 1992 as IMI corn, and currently as Clearfield* corn. Several imidazolinone-tolerant maize lines including mutant 1 and mutant 2 were also successfully obtained by using the chemical mutagen ethyl methanesulfonate (EMS) to mutagenize pollen from the inbred maize line UE95. Imidazolinone-tolerant maize from this source was subsequently commercialized.[3,22,30]

Maize is a diploid species with a chromosome number of $2n = 2x = 20$.[43] Two AHAS genes or loci, *als1* and *als2*, have been reported with 95% homology.[44] XA17 and XI12 are allelic mutations of *als2*, while QJ22 and XS40 are believed to be mutations of *als1* (Table 1).[3,28] DNA sequencing data reveal that the XI12 mutation has a single nucleotide substitution at codon 653 in reference to *A thaliana* (L) Heynh (Table 1) (all codon or amino-acid position numbers mentioned in this review are in reference to *A thaliana*).[28] As a result, serine was substituted by asparagine at position 653 of the AHAS protein. Although QJ22 and XI12 are located at different loci, QJ22 has the same mutation as XI12, ie Ser653-to-Asn653.[28] Similarly, mutant

Table 1. Mutations of the AHAS gene conferring imidazolinone tolerance in maize (*Zea mays*)[a]

	Maize					
Mutant	XI12	QJ22	Mutant 2	XA17	ICI 8532 IT	Mutant 1
Codon in reference to *Arabidopsis thaliana*	653	653	653	574	155	122
Other names	IT	None	IT	IR	IT	None
Amino-acid change	Serine to asparagine	Serine to asparagine	Serine to asparagine	Tryptophan to leucine	Alanine to threonine	Alanine to threonine
Selection method	Cell culture	Cell culture	Pollen mutagenesis	Cell culture	Pollen mutagenesis	Pollen mutagenesis
Imidazolinone resistance	R	R	R	R	R	R
Sulfonylurea resistance	S	S	S	R	S	S
Triazolopyrimidine resistance	No published data	No published data	S	R	S	No published data
Pyrimidinylthiobenzoate resistance	No published data	No published data	No published data	R	R	No published data

[a] R = resistant and S = susceptible.

2 selected from pollen mutagenesis has the same mutation as XI12 and QJ22.[28,30] In contrast, the XA17 mutation has a single nucleotide substitution at codon 574 (Table 1).[22] As a result, tryptophan was replaced with leucine at position 574 of the AHAS protein. RSC or IMR maize obtained through tissue culture selection and tolerant to AHAS inhibitors is believed to have the same mutation as XA17 based on the sensitivity of its AHAS enzyme to imazethapyr, nicosulfuron, and primisulfuron.[45,46] Mutant 1 has a single nucleotide substitution at codon 122, and consequently alanine was replaced with threonine in AHAS primary structure (Table 1).[30] One atypical mutation was reported in maize variety ICI 8532 IT which has a mutation at codon 155. The mutation resulted in a substitution of alanine by threonine in AHAS enzyme (Table 1).[22]

Mutation Ser653-to-Asn653, occurring in XI12, QJ22, and mutant 2, confers only tolerance to imidazolinones (Table 1).[30,36,41,42,46] In contrast, mutation Trp574-to-Leu574 of XA17 confers tolerance not only to imidazolinones but also to all other families of AHAS inhibitors including sulfonylureas, triazolopyrimidines, and pyrimidinylthio-benzoates.[22,36,42,45–48] Mutation Ala155-to-Thr155 confers tolerance to imidazolinones and pyrimidinylthiobenzoates but not to sulfonylureas or triazolopyrimidines.[22,45,47] Mutation Ala122-to-Thr122 confers only tolerance to imidazolinones.[30]

3.2 Imidazolinone-tolerant oilseed rape

Microspores of the oilseed rape variety Topas were isolated, mutagenized using ethyl nitrosourea, and developed into embryos and eventually haploid plantlets which were then doubled with colchicine.[49] Five double-haploids survived soil treatment with imazethapyr, and two of the lines showed superior tolerance to imazethapyr. The two most tolerant mutants were P1 and P2, also referred to as PM1 and PM2.[3,49] All imidazolinone-tolerant oilseed rape varieties were developed on the basis of PM1 and PM2 mutants and were first marketed as Smart canola in 1995. They are currently marketed as Clearfield* canola.

Oilseed rape (*B napus*) is an allotetraploid (2n = 38) with two genomes, A and C; genome A has 10 chromosomes, whereas genome C has nine.[43] *Brassica napus* is believed to originate from an interspecific cross between *Brassica campestris* L, the A genome donor, and *Brassica oleracea* L, the C genome donor.[43,50] Five AHAS loci have been reported in oilseed rape.[50] AHAS2, AHAS3 and AHAS4 originate from the A genome, whereas AHAS1 and AHAS5 originate from the C genome.[50] AHAS1 and AHAS3 are the only genes that are constitutively expressed and encode the primary AHAS activities essential to growth and development in *B napus*. On the basis of the fact that imidazolinone tolerances of PM1 and PM2 are unlinked and additive, Rutledge *et al*[50] predicted that the alleles of PM1 and PM2 mutants correspond to

AHAS1 and AHAS3. It was easy to introgress one of the resistant genes from *B napus* to *B juncea* (L) Coss, which has genomes A and B, by inter-specific crossing; however, it was difficult to introgress the other resistant gene in *B napus* to *B juncea*.[51] The two species share the genome A where AHAS3 originates.[43] The difficulty of introgressing one but not the other mutation from *B napus* to *B juncea* also suggests that the two AHAS genes conferring imidazolinone tolerance in *B napus* are derived from AHAS1 and AHAS3.[51]

The imidazolinone-tolerance traits in oilseed rape were found to be the result of single amino-acid modifications of the AHAS enzymes.[52] PM1 has a single nucleotide substitution at codon 653, resulting in an asparagine substituted for serine in the AHAS1 primary structure (Table 2). In comparison, PM2 has a single nucleotide substitution at codon 574, and consequently tryptophan is replaced by leucine in the AHAS3 protein (Table 2).[53] PM1 and PM2 mutations in *B napus* are similar to XI12 and XA17 mutations in maize (Tables 1 and 2). PM1 is tolerant to imidazolinones only, but PM2 is cross-tolerant to both imidazolinones and sulfonylureas.[3] Although both PM1 and PM2 confer tolerance to imidazolinones, the tolerance level contributed by PM2 is much higher than that from PM1.[3,49] The highest level of tolerance to imidazolinone herbicides is obtained when PM1 and PM2 mutations are stacked and homozygous.

3.3 Imidazolinone-tolerant rice

Seeds of the rice variety AS3510 were mutagenized with EMS. M_2 plants were sprayed with imazethapyr. A single surviving plant was identified, and the progeny of this rice plant showed tolerance to several AHAS-inhibiting herbicides.[38] This mutant line was referred to as 93AS3510, and subsequently two imidazolinone-tolerant rice varieties, CL121 and CL141, were developed with this tolerance trait and were first marketed in the USA in 2001.[54,55] Seeds of the rice cultivar Cypress were also mutagenized with EMS.[35] M_2 plants were foliar treated with imazapyr or imazapic. Twelve plants survived the treatment and were confirmed to have tolerance. The seven most tolerant lines, PWC16, PWC23, CMC29, CMC31, WDC33, WDC37 and WDC38, were selected for further characterization.[35] Subsequently, two imidazolinone-tolerant rice varieties, CL161 and XL8, were developed from the mutations of this source and first marketed in 2003.[55]

Rice is a diploid species with a chromosome number of $2n = 2x = 24$.[43] It is expected that a single AHAS locus exists in rice. DNA sequencing reveals that both 93AS3510 and PWC16 mutants have single codon changes in their AHAS genes that are responsible for herbicide tolerance (Table 2).[56] The position of the mutation for 93AS3510 is codon 654 where glycine was substituted by glutamic acid in the encoded AHAS protein.[56] The position of the target site mutation for the PWC16 is at codon

Table 2. Mutations of the AHAS gene conferring imidazolinone tolerance in rice (*Oryza sativa*), wheat (*Triticum aestivum*), oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*)

	Rice		Wheat		Oilseed rape		Sunflower
Mutant	93AS3510	Several	Several	TeaIMI 11A	PM1	PM2	Two
Codon in reference to *A thaliana*	654	653	653	653	653	574	205
Amino-acid change	Glycine to glutamic acid	Serine to asparagine	Serine to asparagine	Serine to asparagine	Serine to asparagine	Tryptophan to leucine	Alanine to valine
Locus	No data	No data	ALS1	ALS2	AHAS1	AHAS3	No data
Genome	A	A	D	B	C	A	–
Selection method	Seed mutagenesis	Seed mutagenesis	Seed mutagenesis	Seed mutagenesis	Microspore mutagenesis	Microspore mutagenesis	Natural selection

653 where serine is substituted by asparagine in the encoded AHAS protein (Table 2).[56] The mutations in PWC23, CMC29, WDC33 and WDC38 are the same as that of PWC16. They all have the same mutation as the XI12 maize mutant and the PM1 oilseed rape mutant (Tables 1 and 2). Because of this, similar characteristics of the Ser653-to-Asn653 mutation among these three crops are expected.

3.4 Imidazolinone-tolerant wheat

Seeds of the French winter wheat cv Fidel were mutagenized with sodium azide.[57] The M_2 seeds were screened by using a seed treatment with imazethapyr followed by a pre-emergence application of imazethapyr. Four tolerant plants were selected and named FS1 (Fidel selection 1), FS2, FS3 and FS4, respectively.[57] Subsequently, these four imazethapyr-tolerant Fidel selections have been used as trait donors for breeding imidazolinone-tolerant wheat varieties which were first marketed in 2001. More wheat mutants were discovered also through seed mutagenesis.[58] Seeds of spring wheat, cv Teal, were treated with EMS, and M_2 plants were sprayed with imazamox. Six lines with moderate to high levels of imazamox tolerance were selected for further genetic study. The lines were designated as TealIMI lines 1A, 9A, 10A, 11A, 15A and 16A. Two distinctive mutations, different from FS4, were discovered from lines 11A and 15A. Line 15A had the FS4 mutation and another novel mutation. TealIMI 11A possessed a non-allelic mutation to FS4.[58]

Wheat is a hexaploid with a chromosome number of $2n = 6x = 42$ and has three genomes: A, B and D.[43] Three AHAS genes have been confirmed in wheat recently, and the three homologous loci are located on the long arm of chromosomes 6D, 6B and 6A (Table 2).[58–60] The mutation of the AHAS gene on 6DL has been named Imi1, and the two mutations discovered from Teal have been named Imi2 and Imi3.[58] On the basis of studies of inheritance of imidazolinone tolerance and allelism of the traits, Imi1 of FS4 or TealIMI 15A, Imi2 of TealIMI 11A, and Imi3 of TealIMI 15A are all semi-dominant and are unlinked. Higher levels of imidazolinone tolerance in wheat can be achieved by stacking two or more tolerant genes into a single genotype.[57,58] Imi2 and Imi3 are believed to be on genomes B and A, respectively.[60] DNA sequencing data show that both Imi1 and Imi2 have an amino-acid substitution of Ser653-to-Asn653 in the AHAS enzyme that is analogous to the XI12 mutation in maize, the PM1 mutation in oilseed rape, and the PWC16 mutation in rice (Tables 1 and 2).[60] Winter wheat with a single homozygous FS4 gene has an acceptable imidazolinone tolerance. For spring wheat, two homozygous imidazolinone-tolerant AHAS genes are required and stacked to achieve acceptable tolerance to imidazolinone herbicides.

3.5 Imidazolinone-tolerant sunflower (*Helianthus annuus* L)

An imazethapyr-tolerant wild sunflower population was discovered in a soybean field near Rossville, Kansas, USA.[37] Seeds of the tolerant wild sunflower population were collected and grown as imidazolinone-tolerant gene donors to introduce the tolerance trait into cultivated sunflowers.[61–63] IMISUN-1 is the BC_2F_2 seed derived from imidazolinone-tolerant BC_2F_1 plants from the cross HA89*3/*H annuus*, and IMISUN-2 is the BC_2F_2 seed derived from imidazolinone-tolerant BC_2F_1 plants from the cross RHA409//RHA376*2/*H annuus*.[62] Further pedigree breeding led to the development of maintainer HA425 (BC_2F_6) from IMISUN-1, and restorers RHA426 and RHA427 from IMISUN-2.[63] The seeds of IMISUN-1 and IMISUN-2 were made available for sunflower breeders to develop imidazolinone-tolerant sunflowers.[62,63] Several commercial seed companies have introduced the imidazolinone-tolerance trait into their own sunflower lines, and imidazolinone-tolerant sunflower varieties were first commercialized as Clearfield* sunflower in the USA, Argentina and Turkey in 2003.

Common sunflower is a diploid with $2n = 2x = 34$.[43] Although the copy number of AHAS genes in *H annuus* is still unknown, White *et al*,[25] on the basis of their sequencing the AHAS gene of common sunflower, suggest that at least two copies of the AHAS genes exist in common sunflower. Bruniard[34] suggests that there are three putative AHAS genes in common sunflower. DNA sequencing reveals a mutation in the AHAS gene conferring imidazolinone tolerance (Table 2). DNA sequence of HA425 is different from susceptible common sunflower at codon 205; valine is substituted for alanine.[34] Similarly, White *et al*[25] sequenced DNA of an imazethapyr-tolerant biotype from a wild sunflower population near Howard, South Dakota, USA and also found the Ala205-to-Val205 mutation. Two other herbicide-tolerant AHAS mutants have also been reported in sunflowers. They are tolerant to tribenuron and have some tolerance to imazamox.[64]

The sunflower mutant from Kansas is highly tolerant to imazamox, slightly tolerant to thifensulfuron and chlorimuron, but not tolerant to cloransulam-methyl or pyrithiobac.[34,65] The mutant discovered in South Dakota also shows a high tolerance to imazethapyr and slight tolerance to chlorimuron.[66] Inheritance of imidazolinone tolerance is not as clear as expected from the DNA sequencing result. Miller and Al-Khatib[61] studied the inheritance pattern of the trait and concluded that the tolerance appears to be controlled additively by at least two genes. In studying the tolerance of the mutant from Kansas, Bruniard[34] observed that the progeny of an intermediate-tolerant type did not segregate, suggesting that at least two genes are involved in the total tolerance. He proposed a model for tolerance with one semi-dominant gene and a second modifier gene. He also pointed out

that some breeders observed distorted ratios from the proposed model if the population was derived from different genetic backgrounds, especially the population involved with line 87CAEB.

4 BENEFITS AND ADVANTAGES OF IMIDAZOLINONE-TOLERANT CROPS

The Clearfield* production system, by combining imidazolinone-tolerant crops with imidazolinone herbicides, is able to control certain weeds that no other herbicide can control in some crops. Red rice (*Oryza sativa* L) is a very difficult weed to control in cultivated rice because of its taxonomic and physiological similarities to commercial rice, and red rice is considered to be one of the most troublesome weeds of cultivated rice in many rice production areas of the world.[54,55,67,68] With the absence of a herbicide for red rice control in commercial rice, controlling red rice with traditional rice herbicides has mostly been unsuccessful.[54,67] Imidazolinone herbicides are very effective for controlling red rice in imidazolinone-tolerant rice.[54,67,69–74] Jointed goatgrass (*Aegilops cylindrica* Host) is a problematic weed in winter wheat in the USA. Before imidazolinone-tolerant wheat was developed, there were no registered herbicides that would selectively control this weed without injuring the wheat.[59] Imidazolinone herbicides have demonstrated effective control of jointed goatgrass but have no selectivity to conventional wheat.[75,76] With imidazolinone-tolerant wheat, farmers can use imidazolinone herbicides to solve the problem of jointed goatgrass in wheat.

Besides weeds that other herbicides cannot control, the Clearfield* production system is also able to control a broad spectrum of weeds in several crops in which imidazolinone-tolerant varieties are available. Field tests in the USA have demonstrated the efficacy of imidazolinones on the complex of weeds that infest maize, including some difficult-to-control weeds such as shattercane [*Sorghum bicolor* (L) Moench] and johnsongrass [*Sorghum halepense* (L) Pers].[3,36,77,78] Imidazolinone herbicides also control the most troublesome weeds in oilseed rape such as wild mustards [*Brassica kaber* (DC) LC Wheeler] and stinkweed [*Pluchea camphorata* (L) DC], many weeds that infest rice, including barnyardgrass [*Echinochloa crus-galli* (L) Beauv], and many difficult-to-control weeds in wheat such as cheat (*Bromus secalinus* L) and Italian ryegrass (*Lolium multiflorum* Lam).[36,49,70–73,76,79] Imazethapyr and imazamox control several important broadleaf weeds such as *Xanthium pensylvanicum* Wallr and *Brassica* spp in sunflower, and give farmers more flexibility to grow sunflowers in areas where broadleaf weeds are problems or where soil-applied herbicides are not compatible with conservation tillage practices.[61]

The Clearfield* production system is a very effective tool to control parasitic weeds. Witchweed (*Striga* spp) is a severe problem in Africa.[1,80] Imazapyr drenched at 30 g AE ha^{-1} achieved 100% suppression of witchweed capsule formation. The use of the Clearfield* production system in Kenya increased the maize harvest index by 17% in *Striga*-infested soils.[81] A tripling of the maize yield by imazapyr seed dressing at 30 g AE ha^{-1} to control *Striga* was also reported.[1] Similarly, the combination of imidazolinone herbicides and Clearfield* sunflowers is also a very effective tool for the control of the parasitic weed broomrape (*Orobanche* spp) in sunflower.[82]

Since maize and rice are often rotated with soybeans, and imidazolinones are common herbicide choices for soybeans, using imidazolinone-tolerant maize and rice in rotation with soybeans eliminates any risk of maize or rice injury resulting from carryover of residual imidazolinone herbicides from the previous year in soybeans.[3,54] IR maize with the XA17 gene can prevent maize injury caused by carryover of residual sulfonylurea herbicides which are commonly registered on wheat and other crops.[83] Similarly, Clearfield* canola can grow in rotations where the rotational crop uses imidazolinones and sulfonylureas and the residues might damage a following oilseed rape crop.[49]

Conventional maize can metabolize some sulfonylureas, rendering them non-toxic to maize. This is the basis for selectivity of these herbicides. Organophosphate insecticides were found to interfere with sulfonylurea metabolism.[84] As a result, the sulfonylurea herbicide can reach and inhibit the AHAS enzyme and cause conventional maize injury when applied to an organophosphate insecticide-treated plant. IR or IMR maize is cross-tolerant to all AHAS-inhibiting herbicides and can prevent maize injury caused by the interaction between AHAS-inhibiting herbicides and organophosphate insecticides.[46,85] Some growers chose IR and IMR maize hybrids specifically for this characteristic.

Besides the benefits of weed control, Clearfield* crops currently have an advantage in the process of commercialization with fewer regulatory hurdles compared with transgenic herbicide-tolerant crops. Because Clearfield* crops were all developed using traditional breeding methods, there is no additional regulatory restriction on their commercialization over any other conventionally developed crop except approval from Canadian regulatory agencies which review all plants with novel traits, transgenic or non-transgenic.[52] As a result, Clearfield* crops are more readily accessible to farmers than transgenic herbicide-tolerant crops. A good example is the development of herbicide-tolerant rice and wheat. Farmers have been commercially growing Clearfield* rice and wheat since 2001. In contrast, glyphosate-tolerant rice and wheat and glufosinate-tolerant rice still have not been commercialized even though they have been also developed.[55,86]

With its distinctive advantages over other weed management programs and its easy access to farmers, the Clearfield* production system has been popularly adopted by farmers who grow the crops in which

Table 3. Clearfield* production system combines imidazolinone-tolerant crops with imidazolinone herbicides for different geographic regions

Commercialized imidazolinone-tolerant crops	Registered imidazolinone in region			
	North America	South America	Europe (including Turkey)	Australia
Maize	Imazapyr Imazethapyr	Imazapyr Imazethapyr Imazapic	Imazamox	
Oilseed rape	Imazamox Imazethapyr			Imazapyr Imazapic
Rice	Imazethapyr	Imazethapyr Imazapic		
Wheat	Imazamox			Imazapyr Imazapic
Sunflower	Imazamox	Imazapyr	Imazamox Imazapyr	

Clearfield* varieties are available. For instance, the estimated amount of Clearfield* maize seeds was enough to plant approximately 4.9 million hectares in the USA in 2002, about 15% of the total maize planting hectares in the USA.[87] Another example of the popular adoption is the Clearfield* production system in canola. About 20% of the 4–4.9 million hectares of canola in Canada in 2000 and 2001 were Clearfield* canola.[88,89] Besides sharing a significant portion of the market in maize and canola, the Clearfield* production system at present is the only commercialized herbicide-tolerant technology in rice, wheat and sunflower. Clearly, the Clearfield* production system has made a significant impact to each of its commercialized crops. By 2004, five imidazolinone-tolerant crops in combination with four imidazolinone herbicides have been commercialized as the Clearfield* production system in different regions of the world (Table 3).

5 STEWARDSHIP FOR IMIDAZOLINONE-TOLERANT CROPS

Genes can be exchanged between plants of the same or sometimes different species through cross-pollination. As a result, outcrossing of certain crop traits such as herbicide resistance to closely related weed plants or gene flow from crops to weed species is a major concern.[55,90–94] Concerns about gene flow from cultivated rice to red rice and from cultivated sunflower to wild sunflower are particularly relevant to imidazolinone-tolerant crops. If weeds gain the herbicide-tolerance trait from the crops, the herbicide will fail to control the weeds effectively and may result in herbicide-resistant populations. Therefore, herbicide-resistant crop systems must be integrated with proper stewardship to minimize outcrossing and survival of weed-crop hybrids. This will ensure long-term success of the system. As a key component of the Clearfield* production system, stewardship programs have been developed and implemented for several imidazolinone-tolerant crops. These are aimed at preventing weed resistance resulting from trait outcrossing as well as from selecting spontaneous mutations in the field.

Stewardship for the Clearfield* production system may include both required and recommended practices. Seed producers of imidazolinone-tolerant crops are required to follow strict guidelines which ensure that the fields used for seed production are free of key weeds such as red rice and jointed goatgrass that may cross-pollinate with imidazolinone-tolerant crops. This measure minimizes the possible flow of resistant traits to closely related weed species and prevents incidental transfer of weed seeds through crop seeds. Growers may be required to purchase registered or certified seeds each year, and saving seeds for a second crop is prohibited. Again this step ensures that the imidazolinone-tolerant crop is relatively free of weed seeds and limits contamination from volunteer conventional crop varieties. Growers may also be required to sign a stewardship agreement and to complete stewardship training before using the Clearfield* technology. Imidazolinone herbicides should be applied following the label and at the label rate to achieve optimum weed control.

Rotating crops is strongly encouraged in the Clearfield* production system. Imidazolinone-tolerant crops should be grown a maximum of two out of every four years in the same field. This will reduce the risk of developing herbicide-resistant weeds. Rotating herbicides with different modes of action in the same field is also recommended. When weeds with the potential for gene flow from the crop, such as red rice and jointed goatgrass, are present in imidazolinone-tolerant rice, sunflower or wheat, growers should use the imidazolinone herbicide registered for use on that crop. This will reduce the opportunity for movement of the imidazolinone-tolerance trait to those weeds. Growers should also employ other weed-management practices that can control resistant weeds. Controlling key weeds in areas adjacent to imidazolinone-tolerant crops will further decrease the possibility for outcrossing. Controlling weeds and imidazolinone-tolerant volunteer plants effectively following use of imidazolinone-tolerant crops completes the stewardship recommendations.

6 OTHER CROPS WITH A POTENTIAL FOR THE DEVELOPMENT OF IMIDAZOLINONE-TOLERANCE TRAITS

Imidazolinone-tolerant mutations have been discovered in several crops not mentioned earlier, including sugarbeet (*Beta vulgaris* L), cotton (*Gossypium hirsutum* L), soybean [*Glycine max* (L) Merr], lettuce (*Lactuca sativa* L), tomato (*Lycopersicon esculentum* Mill) and tobacco (*Nicotiana tabacum* L). Three mutants from sugarbeet have been reported to have resistance to AHAS-inhibiting herbicides.[23,95] Mutant Sir-13 has

an amino-acid substitution of Ala122-to-Thr122 and is tolerant to imidazolinones but not to sulfonylureas and triazolopyrimidines. In contrast, Mutant Sur has an amino-acid substitution of Pro197-to-Ser197 in the AHAS and is tolerant to sulfonylureas and triazolopyrimidines, but not to imidazolinones. Mutant 93R30B of sugarbeet has both Sir-13 and Sur, and is cross-tolerant to all three families of the tested AHAS-inhibiting herbicides. A homozygous Sir-13 is more tolerant to herbicides than a heterozygote, indicating that the trait is semi-dominant. This mutant has the same mutation as mutant 1 of maize.

Two mutants from cotton have been reported to have a resistance to AHAS-inhibiting herbicides.[96] One of them, DO-2, exhibits a high level of resistance to imazethapyr and pyrimidinylthiobenzoates but a relatively low tolerance to sulfonylurea and triazolopyrimidine. This is very similar to the cross-tolerant pattern of ICI 8532 IT maize. The other mutant, named PS-3, has a high resistance to triazolopyrimidines and one of the tested sulfonylureas but a very low tolerance to imidazolinones, pyrimidinylthiobenzoates and other tested sulfonylureas. Besides mutant cotton lines, a transgenic imazaquin-resistant cotton has also been reported but has not been commercialized.[97]

Soybeans metabolize certain imidazolinones quickly, and as a result, these imidazolinone herbicides are safe to use on soybeans without needing a target-based (AHAS) resistance.[98] Several mutant lines that are tolerant to AHAS-inhibiting herbicides have been discovered in soybean.[99,100] These soybean mutant lines are all tolerant to sulfonylurea herbicides. Among the mutant lines, W20 and W4-4 have a significant tolerance to one of the three imidazolinones tested.

Lettuce that is tolerant to AHAS-inhibiting herbicides has been created by introgressing a mutated AHAS gene from a tolerant prickly lettuce (*Lactuca serriola* L) discovered in Idaho, USA.[31] The altered AHAS has an amino-acid substitution of Pro197-to-His197.[101,102] This mutation also resulted in reduced feedback inhibition of AHAS by leucine, isoleucine and valine.[101] For vegetable crops other than lettuce, several tomato lines which were selected *in vitro* are reported to have some imazethapyr tolerance.[103]

A mutant that is tolerant to AHAS-inhibiting herbicides has been reported in tobacco and named KS-43.[96] It is cross-tolerant to all tested AHAS-inhibiting herbicides including imidazolinones, sulfonylureas, triazolopyrimidines and pyrimidinylthiobenzoates. In addition, other tobacco mutants such as S4 and SU-27D5 which confer resistance to AHAS inhibitors have been discovered from tobacco cell cultures.[21,104]

7 CONCLUSIONS

The mechanism responsible for all commercially-important imidazolinone-tolerant crops involves target-based tolerance—ie variants of the AHAS gene. Imidazolinone-tolerant maize was developed from selections of cell culture and pollen mutagenesis. Similarly, imidazolinone-tolerant oilseed rape was derived from the mutagenesis of microspores. By comparison, imidazolinone-tolerant wheat and rice were developed from chemical mutagenesis of seeds. Different from other crops, imidazolinone-tolerant sunflower was obtained by selecting naturally occurring tolerant mutants in wild sunflower and transferring the trait to cultivated types. Because the imidazolinone tolerance was achieved without inserting foreign DNA, all commercialized imidazolinone-tolerant crops are non-transgenic, and may be marketed as non-GMO Clearfield* crops.

Commercialized imidazolinone-tolerant maize, rice, oilseed rape and wheat have all utilized a Ser653-to-Asn653 substitution in the AHAS enzyme. Imidazolinone-tolerant maize and oilseed rape also have used Trp574-to-Leu574 substitutions in the AHAS enzyme. Trp574-to-Leu574 mutation is the only documented imidazolinone-tolerance mutation that also confers a high tolerance to all other families of AHAS inhibitors. Besides position 653 and 574 mutations, other unique AHAS gene mutations conferring imidazolinone tolerance have been reported in maize and rice. In contrast, imidazolinone-tolerant sunflower has an altered AHAS gene that encodes the AHAS protein with an Ala205-to-Val205 substitution of amino acids. All commercialized imidazolinone-tolerance traits are semi-dominant, and the tolerance level depends on mutation type and zygosity of the traits and also the chemical type and rate of the herbicides.

The combination of non-transgenic imidazolinone-tolerance traits and imidazolinone herbicides is the basis of the Clearfield* production system. Imazamox, imazethapyr, imazapyr and imazapic have been registered for imidazolinone-tolerant crops. They may be marketed as a single active ingredient, as a mixture of two imidazolinones, or as a combination with other herbicides depending on crops and growing regions. The Clearfield* production system offers control of many weeds missed by other herbicides and adds an effective weed-control tool to maize, oilseed rape, rice, wheat and sunflower. The system is particularly effective for parasitic weed control. The Clearfield* production system can prevent crop injury caused by herbicide carryover and herbicide–insecticide interaction. Stewardship programs for imidazolinone-tolerant crops have been developed and implemented to reduce gene flow and weed resistance and to preserve these effective weed-management tools. AHAS gene mutations in other crops provide the possibility to develop more imidazolinone-tolerant crops.

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