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Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops

Review Article

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Summary. Acetohydroxyacid synthase (AHAS) inhibitors interfere with branched-chain amino acid biosynthesis by inhibiting AHAS. Glyphosate affects aromatic amino acid biosynthesis by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glufosinate inhibits glutamine synthetase and blocks biosynthesis of glutamine. AHAS gene variants that confer tolerance to AHAS inhibitors have been discovered in plants through selection or mutagenesis. Imidazolinone-tolerant crops have been commercialized based on these AHAS gene variants. A modified maize EPSPS gene and CP4-EPSPS gene from *Agrobacterium* sp. have been used to transform plants for target-based tolerance to glyphosate. A *gox* gene isolated from *Ochrobactrum anthropi* has also been employed to encode glyphosate oxidoreductase to detoxify glyphosate in plants. Glyphosate-tolerant crops with EPSPS transgene alone or both EPSPS and *gox* transgenes have been commercialized. Similarly, *bar* and *pat* genes isolated from *Streptomyces hygroscopicus* and *S. viridochromogenes*, respectively, have been inserted into plants to encode phosphinothricin *N*-acetyltransferase to detoxify glufosinate. Glufosinate-tolerant crops have been commercialized using one of these two transgenes.

Keywords: Acetohydroxyacid synthase – Acetolactate synthase – 5-Enolpyruvylshikimate-3-phosphate synthase – Imidazolinone – Glyphosate – Glufosinate – Herbicide tolerance

Abbreviations: AHAS, acetohydroxyacid synthase; AMPA, aminomethylphosphonic acid; EPSP, 5-enolpyruvylshikimate-3-phosphate; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; GOX, glyphosate oxidoreductase; GS, glutamine synthetase; PAT, phosphinothricin *N*-acetyltransferase

Herbicidal inhibitors of amino acid biosynthesis

Most herbicides control weeds by targeting and inhibiting a protein or enzyme in plants (OECD, 1999; Cole et al., 2000). Amino acid biosynthesis-inhibiting herbicides inhibit enzymes of the plant and consequently block the biosynthesis of the building blocks of proteins, amino acids. Because animals do not synthesize all needed amino acids but obtain some amino acids from plants or bacteria, amino acid biosynthesis-inhibiting herbicides tend to have less impact on animals than herbicides with other modes of action (Reade and Cobb, 2002). Due to this effect, amino acid biosynthesis pathways are very desirable targets for new herbicide discovery. In fact, a significant portion of newly commercialized herbicides belong to the acetohydroxyacid synthase (AHAS) inhibitors (DeFelice, 1998; Cole et al., 2000). The same trend is also evident with herbicide-tolerant crops. Crops that are tolerant to amino acid biosynthesis inhibitors have dominated the herbicide-tolerant crop market (Duke, 2005).

There are three major enzymes that can be inhibited by commercial amino acid biosynthesis-inhibiting herbicides (Fig. 1) (LaRossa and Falco, 1984; Bender, 1985; Mousdale and Coggins, 1991; Vaughn and Duke, 1991; Singh and Shaner, 1995). They are AHAS (EC 4.1.3.18), also called acetolactate synthase (ALS), in the branched-chain amino acid biosynthesis pathway, EPSP synthase (EC 2.5.1.19) in the shikimate pathway, and GS (EC 6.3.1.2) in ammonium assimilation (LaRossa and Falco, 1984; Kishore and Shah, 1988; Duke, 1990, Mousdale and Coggins, 1991; Sherman et al., 1996; Reade and Cobb, 2002).

Herbicidal inhibitors of branched-chain amino acid biosynthesis

AHAS catalyzes two reactions in the branched-chain amino acid biosynthesis pathway: the condensation of two pyruvate molecules to acetolactate and the synthesis of

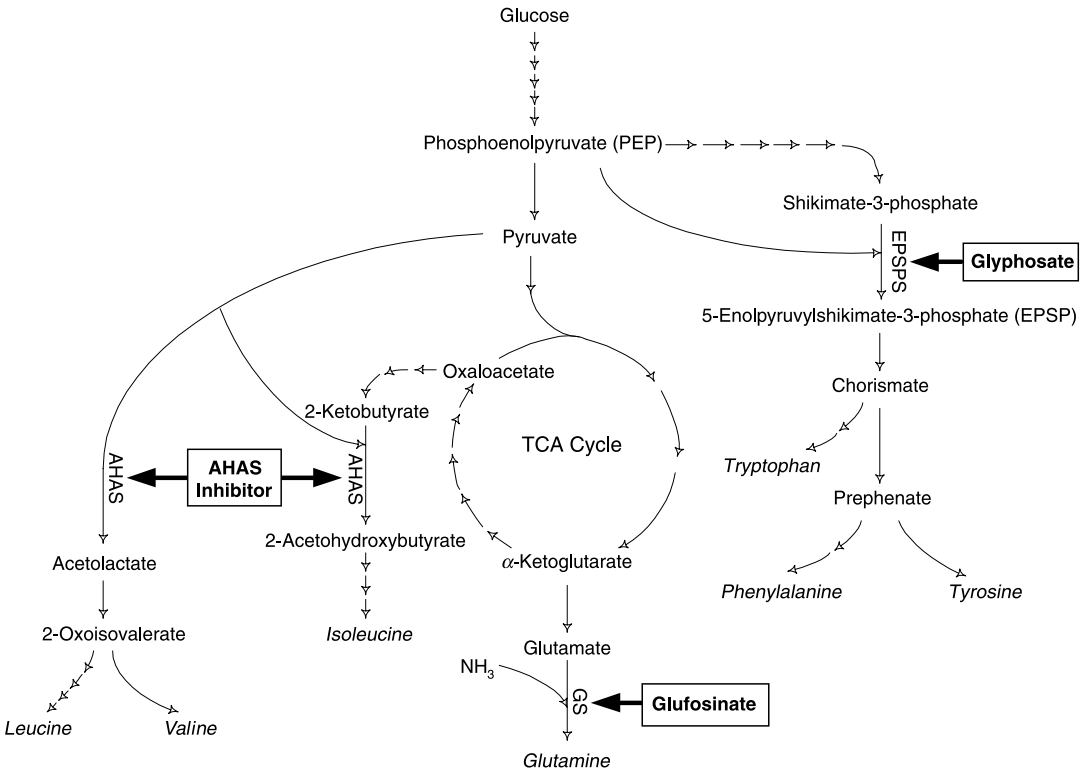

Fig. 1. Biosynthesis pathways of branched-chain amino acids, aromatic amino acids, and glutamine in plants showing three enzymes that are inhibited by amino acid biosynthesis inhibitors. Acetohydroxyacid synthase (*AHAS*) inhibitors, glyphosate, and glufosinate inhibit AHAS, 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*), and glutamine synthetase (*GS*), respectively

2-acetohydroxybutyrate from pyruvate and 2-ketobutyrate (Fig. 1) (Bender, 1985; Singh and Shaner, 1995). Acetolactate leads to the final synthesis of leucine and valine, while 2-acetohydroxybutyrate is a precursor of isoleucine. On the other hand, the product amino acids function as feedback regulators of the activity of AHAS (Stidham and Singh, 1991; Singh and Shaner, 1995; Shaner et al., 1996). AHAS is a nuclear-encoded protein which is transported into plastids where the biosynthesis of the branched-chain amino acids primarily occurs (Vaughn and Duke, 1991; Singh and Shaner, 1995; Saari and Mauvais, 1996; Reade and Cobb, 2002).

There are five chemical families of AHAS-inhibiting herbicides that have been commercialized (Fig. 2) (Shaner and Singh, 1997; Vencill, 2002; Mallory-Smith and Retzinger, 2003). They are imidazolinones, sulfonylureas, triazolopyrimidines, pyrimidinylthio(or oxy)-benzoates, and sulfonylamino-carbonyltriazolinones. About 30 commercial herbicides are from the family of sulfonylureas, but only 2–6 are from each of the rest chemical families (Mallory-Smith and Retzinger, 2003; Herbicide Resistance Action Committee, 2005).

The AHAS inhibitors have been postulated to bind to the AHAS enzyme at the entry site for the AHAS substrate or the substrate access channel (Ott et al., 1996; Pang et al., 2003). It is generally believed that different families of AHAS inhibitors bind to AHAS at different but overlapping sites (Singh and Shaner, 1995; Ott et al., 1996). The inhibition of AHAS by imidazolinones is uncompetitive with respect to pyruvate (Stidham and Singh, 1991; Shaner and Singh, 1997). Herbicide binding reduces or blocks AHAS catalytic activity and results in the deficiency of branched-chain amino acids. Consequently, plants die from starvation for leucine, valine, and isoleucine (Shaner and Singh, 1992).

Glyphosate inhibition of aromatic amino acid biosynthesis

EPSP synthase catalyzes EPSP synthesis from phosphoenolpyruvate (PEP) and shikimate-3-phosphate in the shikimate pathway. EPSP leads to the final synthesis of tryptophan, phenylalanine, and tyrosine (Fig. 1) (Bender, 1985; Cole, 1985; Dill, 2005). EPSP synthase is nuclear-encoded, and the biosynthesis of aromatic amino acids primarily occurs in plastids (Vaughn and Duke, 1991; Reade and Cobb, 2002).

Glyphosate interferes with aromatic amino acid biosynthesis by inhibiting EPSP synthase (Figs. 1 and 2) (Cole,

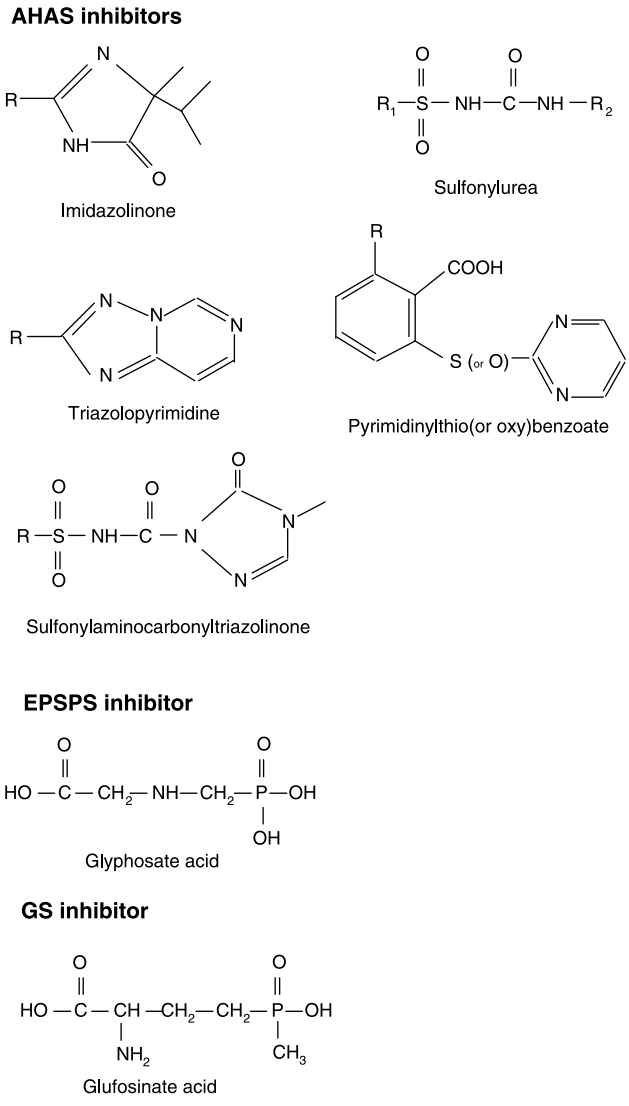

Fig. 2. Basic molecular structures of herbicides that inhibit amino acid biosynthesis. AHAS inhibitors belong to five distinctive families of chemicals. R, R_1, and R_2 are additional moieties besides the basic structures

1985; Kishore and Shah, 1988; OECD, 1999). It is believed that there is a close overlap of the binding sites of PEP and glyphosate on the EPSP synthase (Franz et al., 1997; Dill, 2005). Glyphosate is competitive with respect to PEP in binding to EPSP synthase but uncompetitive with respect to shikimate-3-phosphate (Mousdale and Coggins, 1991; Vaughn and Duke, 1991; Franz et al., 1997; Dill, 2005). However, in a review Reade and Cobb (2002) pointed out that glyphosate does not bind to the active site of EPSP synthase but binds to a possible allosteric site, resulting in structural change at the active site and preventing PEP binding. Glyphosate binding reduces or blocks EPSP synthase catalytic activity and causes a deficiency of aromatic amino acid supply; consequently, plants die from starvation for tryptophan, phenylalanine, tyrosine, and secondary products from the shikimate pathway, and drainage of vital carbon into a useless pool of shikimate (Shaner and Singh, 1992).

Glufosinate inhibition of glutamine biosynthesis

GS is a nuclear-encoded protein that catalyzes conversion of L-glutamate to L-glutamine by assimilating ammonia in the cytoplasm and plastids but predominately in the chloroplast of green tissues (Vaughn and Duke, 1991; Reade and Cobb, 2002) (Fig. 1). This reaction is a major mechanism for ammonia assimilation in plants (Bender, 1985; Mousdale and Coggins, 1991). Glufosinate is racemic, and its L-isomer is a GS inhibitor (Fig. 2). Molecular structures of L-glufosinate and L-glutamate are very similar, and the inhibition is competitive with respect to substrate L-glutamate (Hess, 2000; Reade and Cobb, 2002). In contrast, D-glufosinate is not a GS inhibitor and has no herbicidal activity (OECD, 2002; Ruhland et al., 2004). Glufosinate inhibition of GS can result in a glutamine deficiency and accumulation of ammonia in the plant (Fig. 1). However, ammonia accumulation is not directly responsible for the toxic effects of glufosinate. Glufosinate indirectly inhibits light reaction in photosynthesis, and inhibition of electron flow in photosynthesis under light causes membrane disruption (Hess, 2000).

Besides the aforementioned three enzymes of commercial herbicide targets, several commercially failed herbicidal-target sites in amino acid biosynthesis pathways have been summarized by Gressel (2002a), such as imidazoleglycerol phosphate dehydratase in histidine biosynthesis, keto acid reductoisomerase in the branched-chain amino acid biosyntheis, and shikimate dehydrogenase in the aromatic amino acid biosynthesis. Conversely, a few possible new herbicidal targets in amino acid biosynthesis pathways have been projected by Wakabayashi and Boger (2002). Examples are anthranilate synthase in the shikimate pathway and asparagine synthetase in ammonia assimilation. They concluded that amino acid biosynthesis will be one of the major target domains upon which the most promising new herbicides will act.

Characterization of crops tolerant to amino acid biosynthesis-inhibiting herbicides

There are numerous possible strategies to obtain crop tolerance to herbicides. They include altering, amplifying,

and overexpressing the target gene; bypassing the target enzyme with an alternative pathway; detoxifying herbicide (also including the use of safeners and antibodies); preventing herbicide from reaching the target by restricting uptake and translocation; sequestering herbicide; and increasing substrate flux (Bright, 1992; Dekker and Duke, 1995; Sherman et al., 1996; Gressel, 2002a; Kirkwood, 2002; Matringe et al., 2005). However, only two strategies have been successfully employed in commercial herbicide-tolerant crops: alteration of the target gene and detoxification of herbicide through metabolism (Duke et al., 2002; Kirkwood, 2002; Duke, 2005). Alteration of the target is a defensive approach by making the target enzyme less sensitive or insensitive to the herbicide, whereas detoxification of herbicide is an offensive approach by employing a metabolism enzyme to attack and degrade the herbicide before the herbicide reaches the target.

Genes exist to make crops tolerant to most herbicide classes (Duke, 2005). However, because of their low toxicity to mammals and high activity in controlling weeds, amino acid biosynthesis-inhibiting herbicides are ideal choices for developing herbicide-tolerant crops (Vaughn and Duke, 1991). Among the currently commercial herbicide-tolerant crops, three major ones are based on tolerance to herbicidal inhibitors of amino acid biosynthesis (Duke, 2005). They are imidazolinone-, glyphosate-, and glufosinate-tolerant crops with trade names of CLEARFIELD*, Roundup Ready®, and LibertyLink®, respectively.

These three types of herbicide-tolerant crops not only dominate the herbicide-tolerant crop market but also account for a significant portion of the entire crop market where herbicide-tolerant traits have been commercialized. For instance, about 80–86% canola in Canada in 1999, 2000, and 2001 were planted to herbicide-tolerant varieties. Except for the less than 1% bromoxynil-tolerant canola, the herbicide-tolerant canola hectares were all imidazolinone-, glyphosate-, or glufosinate-tolerant canola in 2000 and 2001 (Kirkwood, 2002; Simard et al., 2002; Beckie et al., 2004).

Besides these three types of herbicide-tolerant crops, sulfonylurea-tolerant crops are also based on tolerance to herbicidal inhibitors of amino acid biosynthesis (Saari and Mauvais, 1996; Duke, 2005). However, because there are about 30 commercial sulfonylurea herbicides, and many crops are naturally tolerant to one or more of the sulfonylurea herbicides, there is no strong incentive for developing sulfonylurea-tolerant crops (Dekker and Duke, 1995; Saari and Mauvais, 1996). Nevertheless, some sulfonylurea-tolerant crops have been developed through transformation, mutagenesis, or selection, and sulfonylurea-tolerant soybeans developed from a mutated AHAS gene have certain commercial impact (Sebastian et al., 1989; Saari and Mauvais, 1996; Fabie and Miller, 2002; Duke, 2005; FDA, 2005).

Imidazolinone-tolerant crops

Imidazolinone-tolerant crops have been developed through selecting naturally occurring AHAS gene variants or mutations from chemical mutangenesis (Newhouse et al., 1991; Shaner et al., 1996; Tan et al., 2005). The altered AHAS enzyme becomes less sensitive to imidazolinone herbicides; consequently, the syntheses of acetolactate and 2-acetohydroxybutyrate are less affected by imidazolinones in the tolerant plants (Fig. 1) (Newhouse et al., 1991; Shaner et al., 1996). Since the trait is obtained through traditional breeding methods of mutagenesis and selection, and no foreign gene has been inserted into the plant, imidazolinone-tolerant crops are considered non-transgenic and are accepted in all major markets.

The AHAS enzyme consists of large catalytic subunits and small regulatory subunits (Tan et al., 2005). Each AHAS large subunit is a monomer of an AHAS polypeptide with about 670 amino acids. There are five commonly occurring mutations in the AHAS large subunit gene that confer tolerance to AHAS-inhibiting herbicides. The codon numbers of these mutations are 122, 197, 205, 574, and 653 in reference to *Arabidopsis thaliana* (L) Heynh (Gressel, 2002a; Tranel and Wright, 2002; Christoffers et al., 2004; Tan et al., 2005). Although the amino acids expressed from those codons spread on the entire primary structure of the AHAS, they are folded in the adjacent area in the quaternary structure of the AHAS enzyme (Ott et al., 1996). This area has been proposed as the binding site of the AHAS inhibitors. Interestingly, the codon 574 mutation encodes AHAS that is cross resistant to all families of AHAS inhibitors (Tan et al., 2005; Tranel et al., 2005). The mutation at codon 197 results in AHAS that is more tolerant to sulfonylureas than imidazolinones. AHAS expressed from mutants with mutations at codons 122, 205, and 653 is resistant to imidazolinones.

Five imidazolinone-tolerant crops have been commercialized since 1992. Imidazolinone-tolerant maize, wheat, rice, and canola all have an S653N AHAS gene mutation, whereas imidazolinone-tolerant maize and canola also have a W574L AHAS gene mutation (Table 1) (Tan et al., 2005). In contrast, imidazolinone-tolerant sunflower

Table 1. Major mutants of AHAS genes that were obtained through mutagenesis or selection and have been used in developing commercial imidazolinone-tolerant crops

Mutation in reference to *A. thaliana*	A205V	W574L	S653N	G654E
Maize		XA17	XI12	
Wheat			FS4, Teal 11A	
Rice			PWC16	93AS3510
Canola		PM2	PM1	
Sunflower	IMISUN-1 or IMISUN-2			

has an A205V mutation of AHAS gene. All mutated and imidazolinone-tolerant AHAS genes are semidominant; as a result, crop tolerance increases with gene dosage. Besides these five commercial imidazolinone-tolerant crops, AHAS gene variants conferring imidazolinone tolerance have also been discovered in several other crops (Tan et al., 2005).

Four imidazolinone herbicide active ingredients have been registered on imidazolinone-tolerant crops in different regions of the world. They include imazamox, imazethapyr, imazapyr, and imazapic, and they can be applied as a single active ingredient, as a combination of two, or in combination with other herbicides for a season-long weed control. The combination of multiple traits and multiple herbicides of the imidazolinone-tolerant crop technology makes it easy to meet a wide range of weed control needs around the world.

Glyphosate-tolerant crops

A similar approach as development of the imidazolinone-tolerant trait was pursued in the development of glyphosate-tolerant traits. However, no plant mutants achieved a commercial level of tolerance to glyphosate from traditional mutagenesis (Kishore and Shah, 1988; OECD, 1999; Dill, 2005). As a result, transgenes have been used to obtain a commercial level of tolerance (OECD, 1999).

Both target alteration and herbicide detoxification strategies have been employed in the development of glyphosate-tolerant crops (Kishore and Shah, 1988; Barry et al., 1992; Padgette et al., 1996; Franz et al., 1997; OECD, 1999; Dill, 2005). EPSPS gene from *Agrobacterium* sp. strain CP4 called CP4-EPSPS gene was inserted into the plant to encode an alternative EPSP synthase that is less sensitive to glyphosate than the endogenous EPSP synthase. The glyphosate-tolerant CP4-EPSPS protein consists of a single polypeptide with 455 amino acids, and its amino acid sequence is typically 48.5–59.3% similar and 23.3–41.1% identical to native EPSP synthase of plants and bacteria (Padgette et al., 1996; Dill, 2005).

Besides the CP4-EPSPS gene, a maize EPSPS transgene obtained through site-directed mutagenesis and with double mutations T102I and P106S was also used by stable insertion as EPSPS transgene in a maize plant to achieve commercial tolerance to glyphosate (Lebrun et al., 2003; Dill, 2005, Pline-Srnic, 2005). This transgenic event is known as GA21. As a result, the transformed plant with the inserted CP4-EPSPS or the modified maize EPSPS transgene has an alternative EPSP synthase that is less or insensitive to glyphosate compared to endogenous EPSP synthase. Consequently, the transformed plant has a normal synthesis of EPSP even though glyphosate inhibits the endogenous EPSP synthase and kills the non-transformed plant (Fig. 1).

Despite some cut (rootless) plants and plant cell cultures have a fairly extensive degradation of glyphosate to aminomethylphosphonic acid (AMPA), most plants have little or no endogenous ability to metabolize glyphosate (Coupland, 1985; Vaughn and Duke, 1991; Komoba et al., 1992; Franz et al., 1997; Dill, 2005). For the detoxification strategy, the *gox* gene isolated from *Ochrobactrum anthropi* (formerly *Achromobacter* sp.) strain LBAA was inserted into the plant. The *gox* gene encodes glyphosate oxidoreductase (GOX), and the enzyme can degrade glyphosate to glyoxlate and AMPA: $HOOC–CH_2–NH–CH_2–PO_3H_2 \rightarrow HOOC–CHO + NH_2–CH_2–PO_3H_2$ (Barry et al., 1992; Komoba et al., 1992; Padgette et al., 1996; OECD, 1999; Dill, 2005). As a result, GOX-catalyzed metabolism of glyphosate reduces the amount of glyphosate that can reach the target enzyme EPSP synthase, and thus reduces the possibility of glyphosate injury to the plant. Further modification of the coding sequence and ultimately a complete resynthesis of the coding region was reported to increase *gox* expression (Barry et al., 1992; OECD, 1999).

GOX has to be employed in combination with a glyphosate-insensitive EPSPS because detoxification mechanism alone is not sufficient to be resistant to commercial rate of glyphosate (Dill, 2005). A recent study shows that AMPA can cause injury to both glyphosate-tolerant and conventional soybeans (Reddy et al., 2004). The reported herbicidal activity of AMPA diminishes the effectiveness of using GOX metabolism of glyphosate as a strategy for enhancing glyphosate tolerance.

Table 2. Major transgenic events of important glyphosate-tolerant crops and turf grass that have been reviewed or deregulated by the regulatory agencies in the USA or Canada for planting, food, or feed use. The underlined events have been commercialized

Enzyme encoded by transgene	EPSPS		GOX	Altered GOX
Source of the transgene	*Agrobacterium* sp. strain CP4	Modified maize EPSPS gene	*Ochrobactrum anthropi* strain LBAA	*Ochrobactrum anthropi* strain LBAA
Maize	MON 802, MON 805, MON 830, MON 831, MON 832, NK603	GA21	MON 802, MON 805, MON 830, MON 831, MON 832	
Soybean	40-3-2			
Cotton	1445, 1698			
Canola	GT200 or RT200, GT73 or RT73		GT200 or RT200	GT73 or RT73
Wheat	MON 71800			
Sugarbeet	H7-1, GTSB77			GTSB77
Creeping bentgrass	ASR368			

Glyphosate-tolerant maize, soybean, cotton, and canola have been commercialized (Biotechnology Industry Organization, 2004; Duke, 2005). Most of the commercial glyphosate-tolerant crops depend on the target-based tolerance (Table 2) (Australian Office of the Gene Technology Regulator, 2005). Although glyphosate-tolerant canola, maize, wheat, and sugarbeet have been developed with both the alternative EPSPS transgene and *gox* gene, only glyphosate-tolerant canola with both genes has been commercialized (Dill, 2005; Pline-Srnic, 2005). Table 2 lists major glyphosate-tolerant events in economically important crops and turfgrass that have been reviewed or deregulated by the regulatory agencies in the USA or Canada for planting, food, or feed use (AgBios, 2005; Canadian Food Inspection Agency, 2005; FDA, 2005; Health Canada, 2005; USDA, 2005a). Apparently, only a few events have been commercialized (Table 2) (Biotechnology Industry Organization, 2004). Besides the reviewed or deregulated events, the US Department of Agriculture (2005b) also discloses and updates a list of all regulated and field-tested glyphosate- and glufosinate-tolerant events in the USA.

Glufosinate-tolerant crops

Similar to the development of imidazolinone-tolerant crops, efforts were also made to discover GS gene mutants that are naturally tolerant to glufosinate. These were unsuccessful (Vasil, 1996). As a result, transgenic techniques had to be used to develop glufosinate-tolerant crops. Different from glyphosate-tolerant crops, only the detoxification strategy has been employed in the development of glufosinate-tolerant crops (Vasil, 1996; OECD, 2002).

Endogenous metabolism of glufosinate in plants is limited and can be too slow to degrade the herbicide before the herbicide causes injury or kills the plants (Fig. 3) (Komoba and Sandermann, 1992; Muller et al., 2001;

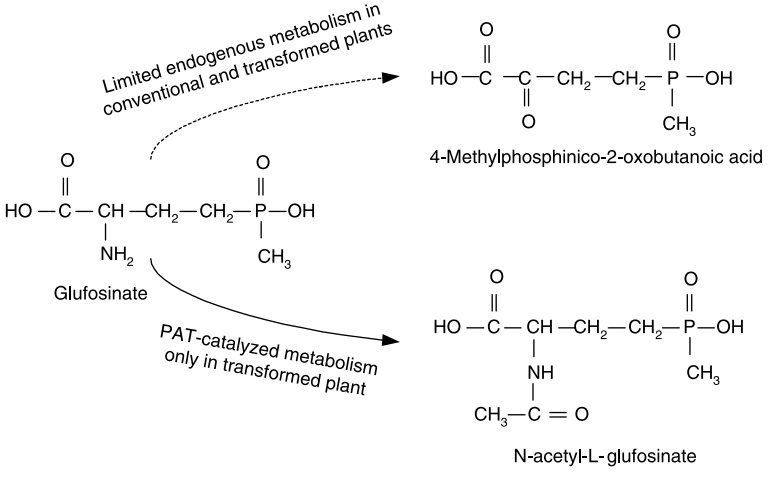

Fig. 3. Transformed glufosinate-tolerant plants have the PAT enzyme encoded by the transgene *bar* or *pat*. The PAT can detoxify glufosinate rapidly. Reduction of glufosinate by the PAT-catalyzed metabolism in the transformed plant eliminates or decreases the chance for glufosinate to reach and inhibit GS. This is the basis for glufosinate tolerance of the transformed plant

Table 3. Major transgenic events of important glufosinate-tolerant crops that have been reviewed or deregulated by the regulatory agencies in the USA or Canada for planting, food, or feed use. The underlined events have been commercialized

Transgene	*bar*	*pat*
Source of transgene	*Streptomyces hygroscopicus*	*Streptomyces viridochromogenes*
Maize	CBH-351, DLL25	1507, T14, T25
Cotton	LLCotton25	
Canola		HCN92 or Topas19/2, T45 or HCN28
Rice	LLRICE06, LLRICE62	
Soybean	W62, W98	A2704-12, A2704-21, A5547-35, A5547-127, GU262
Sugarbeet		T120-7

OECD, 2002). Therefore, foreign genes have to be inserted to encode an enzyme that can detoxify L-glufosinate fast enough to prevent the herbicide from reaching the target enzyme. The expressed foreign enzyme is called phosphinothricin *N*-acetyltransferase (PAT). PAT can rapidly convert L-glufosinate to non-phytotoxic metabolite *N*-acetyl-L-glufosinate (NAG) through acetylation of glufosinate, consequently reducing or eliminating the possibility for glufosinate to inhibit GS (Fig. 3) (D'Halluin et al., 1992; Droge et al., 1992; Rasche, 1995; Vasil, 1996; Muller et al., 2001; OECD, 2002; Ruhland et al., 2004). NAG has not been found in nontransgenic plants (OECD, 2002). The herbicidally inactive D-glufosinate appears to be stable in plants due to the L-specific acetylation activity of the PAT enzyme (Droge et al., 1992).

Two sources of genes have been used to transform glufosinate-tolerant crops (Table 3). One gene, called *bar*, is from *Streptomyces hygroscopicus* and encodes the PAT enzyme. The other gene, named *pat*, is from *S. viridochromogenes* and also expresses the PAT enzyme (Rasche, 1995; Vasil, 1996; Wehrmann et al., 1996). The two genes are highly homologous even though they are from different species (Vasil, 1996). The PAT enzymes encoded by *bar* and *pat* genes are structurally and functionally equivalent and have a comparable performance in transgenic plants, and the most important intrinsic characteristics of the two PAT enzymes are similar (Wehrmann et al., 1996). If a PAT enzyme is used as a selectable marker in transgenic plants, lower levels of PAT activity are required compared to the level required in glufosinate-tolerant crops (OECD, 2002).

Glufosinate-tolerant maize, cotton, and canola have been commercialized (Biotechnology Industry Organization, 2004; Duke, 2005). However, almost every crop has now been transformed with the *bar* or *pat* gene because these genes are excellent selectable markers for plant transformation (Dekker and Duke, 1995; Duke et al., 2002). Among the glufosinate-tolerant events that have been reviewed or deregulated by the regulatory agencies in the USA or Canada, some glufosinate-tolerant crops employ either *bar* or *pat* gene but others use exclusively only one of them (Table 3) (Biotechnology Industry Organization, 2004; AgBios, 2005; Canadian Food Inspection Agency, 2005; FDA, 2005; Health Canada, 2005; USDA, 2005a).

Comparison and applications of amino acid biosynthesis inhibitors and their tolerance traits

Because imidazolinone, glyphosate, and glufosinate belong to different families of chemicals, there are some distinctive properties among the three types of herbicides (Table 4) (Vencill, 2002). Differences in action sites and metabolism in plants of these three families of herbicides also result in some unique characteristics of their tolerant crops (Table 4). For instance, imidazolinones have both foliar and soil activities on weeds, but glyphosate and glufosinate can control weeds only by foliar application. Glufosinate kills weeds faster than either glyphosate or imidazolinones. Imidazolinone-tolerant crops are dependent on target-based tolerance. In contrast, glufosinate-tolerant crops are solely based on the detoxification of the herbicide. Glyphosate-tolerant crops employ both target- and metabolism-based tolerances.

Amino acid biosynthesis-inhibiting herbicides had already played a very important role in weed management before the development of herbicide-tolerant crops. The combination of these herbicides and their tolerance traits allows farmers to expand the utility of the herbicides and to manage weeds more effectively (Rasche, 1995; Dill, 2005; Tan et al., 2005). The mutated AHAS, CP4-EPSPS, *gox*, *bar*, and *pat* genes are not only used for developing herbicide-tolerant crops but also widely used as molecular markers for plant transformation and study of gene flow (D'Halluin et al., 1992; Chandler, 1995; Dekker and

Table 4. Comparison of three types of amino acid biosynthesis inhibitors and their tolerant crops

Inhibitor	Imidazolinones	Glyphosate	Glufosinate
Herbicide			
Target enzyme	AHAS	EPSPS	GS
Enzyme inhibition with respect to substrate	Uncompetitive	Uncompetitive, competitive	Competitive
Use rate (g ae/ha)	20–1,700	160–4,200	320–1,560
Application method	Foliar, soil	Foliar	Foliar
Application timing to weeds	Pre-emergence, post-emergence	Post-emergence	Post-emergence
Weed control activity	Broad spectrum	Non-selective	Non-selective
Acute rat oral toxicity (LD_{50} mg/kg)	>5000	5600	1910
Tolerant crop			
Mechanism of tolerance	Tolerant AHAS	Tolerant EPSPS, detoxification of glyphosate	Detoxification of glufosinate
Method of development	Mutagenesis or selection	Genetically engineered	Genetically engineered
Foreign gene inserted	No	Yes	Yes
Foreign gene source	None	*Agrobacterium* sp. strain CP4, *Ochrobactrum anthropi* strain LBAA	*Streptomyces hygroscopicus*, *S. viridochromogenes*
Modified or inserted target-site gene	Variant AHAS gene	CP4-EPSPS or modified maize EPSPS gene	None
Inserted metabolism gene	None	*gox* gene	*bar* or *pat* gene
Organism classification	Non-transgenic	Transgenic	Transgenic

Duke, 1995; Gealy et al., 2003; Berry et al., 2004; Ray et al., 2004; Watrud et al., 2004; Goodwin et al., 2005). These genes in combination with amino acid biosynthesis inhibitors allow molecular biologists to detect and monitor their traits of interest more accurately. Knowledge obtained from gene flow studies by using herbicide-tolerant traits as markers will lay a foundation for understanding ecological impacts of future economically important and transgenic traits. Discovery and development of amino acid biosynthesis inhibitors have contributed to the advancement of plant biochemistry significantly. Similarly the development of crops that are tolerant to amino acid biosynthesis inhibitors have made significant contributions to the advancement of plant molecular biology (Gressel, 2002b).

Conclusions

Herbicides have a close relationship with proteins and amino acids. Glyphosate, glufosinate, and the AHAS-inhibiting herbicides not only interact with proteins by inhibiting enzymes as other herbicides do but also block the biosynthesis of enzyme building blocks, amino acids, making the relationship between this group of herbicides and proteins even closer. Interestingly, this close interaction has been further demonstrated by the development of crops that are tolerant to amino acid biosynthesis-inhibiting herbicides. In tolerant plants, substituting amino acids in the AHAS and EPSPS enzymes makes the enzymes tolerant to the AHAS inhibitors and glyphosate. Additionally, inserting new enzymes, GOX and PAT, in the transformed plants reverses the interaction between herbicides and enzymes. Instead of herbicides attacking and inhibiting enzymes, the new GOX and PAT enzymes in transformed plants can attack herbicides glyphosate and glufosinate and degrade them into non- or less-herbicidal metabolites, making the plants tolerant to the herbicides. The close interaction of amino acid-inhibiting herbicides with proteins and amino acids have provided not only more effective weed control methods for farmers but also better research tools for biochemists and molecular biologists.

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