Herbicide Tolerant Crops and Weed Management

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Abstract: ***The development of weed resistances to manyselective herbicides and the prohibitive expense anddifficulty associated with the development of newherbicides, a need has arisen to seek alternatives toaddress these challenges. Along with the efforts todiscover new herbicide target sites in plants, biotechnologyis making major contributions in broadening cropselectivity to the already existing and effectiveherbicides. Further efforts to create more herbicidetolerant crops are needed to ensure more economicalcrop production and safeguard environmental quality byreducing the demand for and the number of selectiveweed killing chemicals required for economical chemical crop protection.***

Keywords: *Herbicide, weed, disease, resistance*

1. Introduction

Farmer must control weeds that compete with their crops for water, nutrients and sunlight. A number of options for minimizing the impact of weeds on crop productivity are available to growers: one option is the application of herbicides. Herbicide treatment in crop plantings has allowed economically viable weed control [1] and increased productivity. The most preferred herbicides today are those that combine weed killing potency with low- or noenvironmentalpersistence. However, the very effectivebroad spectrum herbicides available also lack selectivity, thus limiting their use in some cropping operations. Onthe other hand, the continuous use of the few availableselective herbicides is speeding up the development ofherbicide resistance in weeds; hence making it difficult toachieve effective control in some crops [15].

Virtually all crops have some degree of innate tolerance to some herbicides but not others. In the 1940's, this selective tolerance started to be used to control weeds in corn fields. Today growers all over the world minimize the negative impact of weeds by using herbicides and herbicide tolerant crops, including all of the major commodity crops, as well as small acreage horticultural crops, such as vegetables.An ancillary benefit of herbicides is the impact their use has on soil erosion. The use of herbicides and herbicide tolerant crops enables growers to use minimal tillage or no-till techniques, which significantly decreases the impact of agriculture on soil erosion.

Since the 1990s herbicide tolerant crops, developed with modern biotechnology, have significantly increased growers' profits by decreasing their input costs, increasing yields, or both, in some cases as well as save soil from erosion. As a result of these benefits, the rate of adoption of biotech herbicide tolerant crops has been quite rapid. For example in Argentina glyphosate-tolerant soybeans were planted on over 98% of soybean acres within 5 years of introduction.

Two approaches can be pursued to achieve this goal.The first is the design of specific chemicals with broadselectivity for crops. This approach, however, isexpensive and the products thereof may be uneconomical for use by growers, not to mention that it is also away to increase the already growing chemical load to theenvironment. According to Gressel (2002), it has become increasingly difficult to discover new herbicidesand even harder to come up with one that has a novelmode of action. In the 1940s, only about 500 compounds needed to be screened to select a potential herbicide [17].

By 1989, it was estimated that thediscovery of one selective herbicide involved thescreening of more than 30 000 compounds and afteridentification these compounds had to be further modified to improve their toxicity to target weeds andtheir rapid metabolism in crops (Parry, 1989). In addition"chemical handles" have to be designed to aid the rapiddelivery of new chemicals into the target weed plant systems (Owen and DeBoer, 1995). Today the discovery of a potential herbicide requires the screeningof nearly 500 000 compounds (Tan et al., 2005).The second and more popular approach to cropherbicide selectivity is the development of crop cultivarswith tolerance to the already existing effective broad spectrum herbicides so as to expand the crop options in which they can be used. Two methods can be used todevelop crops with resistance to herbicides.Conventional plant breeding utilizing lines that are knownto be tolerant to specific herbicides is one approach thatcould confer resistance to susceptible crops from closelyrelated species. However, this approach has limitationsin that naturally herbicide resistant plants are found moreamong weed species than in crops. Also, conventionalplant breeding takes a long time to produce a singleuseful line. A faster approach is the use of biotechnologytechniques such as *in vitro* cell culture, mutagenesis [9] and selection in physiologically inhibitory concentrations of herbicides (also referred to as brute force selection) orgenetic transformation of already existing crop cultivarswith genes than confer resistance to herbicides.The purpose of this communication is to summarizethe results of studies towards the development of cropherbicide selectivity using biotechnology techniques andhighlight some of the crop products that have been developed using these techniques.

2. Resistance Definitions and Development

Herbicide resistance is the inherited ability of a plantto survives and reproduce following selection with adose of herbicide normally lethal to the wild type ofthe plant. The development of herbicide resistance inweeds is an evolutionary process. Weed populations are extremely diverse genetically. In some cases, the geneticvariation within weed populations includes the inherent abilities to resist some herbicides. However, the frequencyof such variation in a normal weed population is very low. However, if an herbicide is applied repeatedly onthose populations (or herbicides from the same herbicide group are applied), the entire picture can change.As the majority of the susceptible biotypes are controlled after repeated applications, the few resistant biotypesare provided with a unique opportunity to proliferate. Therefore, the use of an herbicide or herbicides from the same herbicide group continuously for many years can drastically decrease the number of susceptiblebiotypes within the natural weed population and dramaticallyincrease the number of resistant biotypes. Inresponse to widespread use of a particular family of herbicides, weed populations can change in genetic compositionsuch that the frequency of resistance gene(s) and resistant individuals increases. Thus, weed populationsbecome adapted to the intense selection imposed by herbicides [2].

3. What are some of the advantages of GM foods?

The world population has topped 6 billion people and is predicted to double in the next 50 years. Ensuring an adequate food supply for this booming population is going to be a major challenge in the years to come. GM foods promise to meet this need in a number of ways:

• **Pest resistance** Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries. Farmers typically use many tons of chemical pesticides annually. Consumers do not wish to eat food that has been treated with pesticides because of potential health hazards, and run-off of agricultural wastes from excessive use of pesticides and fertilizers can poison the water supply and cause harm to the environment. Growing GM foods such as B.t. corn can help eliminate the application of chemical pesticides and reduce the cost of bringing a crop to market.

• **Herbicide tolerance**For some crops, it is not cost-effective to remove weeds by physical means such as tilling, so farmers will often spray large quantities of different herbicides (weed-killer) to destroy weeds, a time-consuming and expensive process, that requires care so that the herbicide doesn't harm the crop plant or the environment. Crop plants genetically-engineered to be resistant to one very powerful herbicide could help prevent environmental damage by reducing the amount of herbicides needed. For example, Monsanto has created a strain of soybeans genetically modified to be not affected by their herbicide product Roundup ®.

A farmer grows these soybeans which then only require one application of weed-killer instead of multiple applications, reducing production cost and limiting the dangers of agricultural waste run-off.

• **Disease resistance** There are many viruses, fungi and bacteria that cause plant diseases. Plant biologists are working to create plants with genetically-engineered resistance to these diseases.

• **Cold tolerance** Unexpected frost can destroy sensitive seedlings. An antifreeze gene from cold water fish has been introduced into plants such as tobacco and potato. With this antifreeze gene, these plants are able to tolerate cold temperatures

• **Drought tolerance/salinity tolerance**As the world population grows and more land is utilized for housing instead of food production, farmers will need to grow crops in locations previously unsuited for plant cultivation.

• **Pharmaceuticals** Medicines and vaccines often are costly to produce and sometimes require special storage conditions not readily available in third world countries. Researchers are working to develop edible vaccines in tomatoes and potatoes. These vaccines will be much easier to ship, store and administer than traditional injectable vaccines.

4. Cell culture and Selection

Plant tissue culture represents the simplest of the biotechnologies available to plant scientists today. The realization that certain *in vitro* conditions could induce heritable changes, called soma clonal variations, in the genomes of plant cells opened an avenue for theselection of various desirable traits from *in vitro* cultures, including herbicide resistance (Maliga, 1984). Using cellculture procedures, BASF Inc. produced a corn hybrid (DK404SR) that is resistant to the sulfonylurea herbicide, Sethoxidim. Cell culture [3] under lethal concentrations of certainherbicides also results in gene amplification in survivingcells that leads to resistance through the overproduction of enzymes targeted by herbicides. A petunia cell linewith resistance to glyphosate was selected in thismanner and plants regenerated from it survived lethallevels of glyphosate (Steinrucken et al., 1986). This resistance was found to be due to amplification of thegene encoding 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that caused its overproduction in the cells (Steinrucken et al., 1986). Other *in vitro*cell selection studies have developed resistance to paraquat in tomato cells (Thomas andPratt, 1982), resistance to glyphosate in carrot and groundnut cells [5] and resistance to a Protoporphyrinogen oxidase [7] (PPO)inhibitor in soybean cells [6] however, no viable plant regeneration was reported in thesestudies.

5. Mutagenesis

Chemical or physical mutagenesis of seed, microspores or pollen followed by selection under herbicide selective pressure has also been utilized to develop crop resistance to herbicides. The most common mutagen employed is ethyl methanesulfonate (EMS), which is efficient in producing chloroplast mutants [4]. In this method, seeds or pollen are treated with EMS then grown either *in vitro* or *in vivo* in the presence of an herbicide. Surviving plants are selected and grown to maturity to provide seed that is used for further screening with herbicides. Utilizing this method, [8] developed 21 Brazilian rice lines that were resistant to glyphosate. [10] Produced atrazine resistant *Solanummelongena*plants by mutagenizing seeds followed by germination and *invitro* regeneration of plants from the resultant seedling cotyledons. This change prevents the binding of the herbicide to the enzyme active site, thus maintaining normal enzyme function. Recently, [11] reported the production of atrazine-resistant pepper (*Capsicum annuum*) plants regenerated from three-week old seedling cotyledons obtained from EMStreated seeds. They also observed maternal inheritance of the atrazine resistance trait.

6. Genetic Transformation

A number ofGM crops expressing various traits have beencommercialized and several are at various stages ofdevelopment (TRANSGEN 2005). Herbicide tolerance is the most common trait in commercial transgenic crops, being part of 82% of all transgenic crops in the year 2003[12]. Several techniques are now available for the transfer ofgenes (genetic engineering) into crop plants, including*Agrobacterium*-mediated gene transfer, micro-projectile (or particle) bombardment, polyethylene glycol-mediatedDNA transfer and cell (protoplast) electroporation. Themost commonly employed techniques in developingherbicide resistant crops are the *Agrobacterium* and theparticle bombardment methods, respectively (Tsaftaris, 1996). Herbicide tolerance via genetic transformationcan is conferred by one or a combination of these four mechanisms [18]:

Introduction of a gene(s) coding for an herbicidedetoxifying enzyme(s);
Introduction of gene(s) coding for a herbicideinsensitive form of a normal functioning enzyme orover expression of the genes coding for a herbicidetarget enzyme such that the normal metabolicfunctioning is still achieved in the plant even thoughsome of the enzyme is inhibited;

Modification of the herbicide target enzyme in such away that the herbicide molecule does not bind to itand;

The more recently described engineering of activeherbicide efflux from plant cells.these mechanisms have variously been explored inthe production of crops that are resistant/ tolerant to various herbicide classes as discussed bellow.

7. Types of Resistance

In some cases, resistant weeds can also survive the applicationof herbicides other than the herbicide towhich they have developed resistance (i.e., the selectingherbicide). In such cases, resistant weeds are consideredto have cross- or multiple resistance. Cross-resistance occurs when one resistance mechanism (e.g., enhancedherbicide metabolism) allows the plant to withstandherbicides from different chemical classes. However,when a plant has multiple resistance it possesses two ormore distinct resistance mechanisms (e.g., two or morealtered sites of action), which allow the plant to resistherbicides from different chemical classes (Hall et al.,1994). For example, a population of smooth pigweed(*Amaranthushybridus*) from Illinois has resistance toatrazine, a photosynthesis-inhibiting herbicide; multiple resistance to primisulfuron (Beacon), a sulfonylurea herbicide; and cross-resistance to imazamox (Raptor), animidazolinone herbicide [16].On the contrary, in some weeds, resistance to oneherbicide results in increased susceptibility to anotherherbicide or other abiotic factors, such as standardcultivation practices, and/or biotic factors, such as effectsof insect pests or infection by viruses and fungi.This phenomenon is known as negative cross-resistance (Gressel and Segel, 1990) and could be exploited insome resistant weed species. For example, Salhoff and Marton [19] reported that triazine resistant *Kochia*biotypes from Idaho were more sensitive to 2, 4-D thansusceptible biotypes.

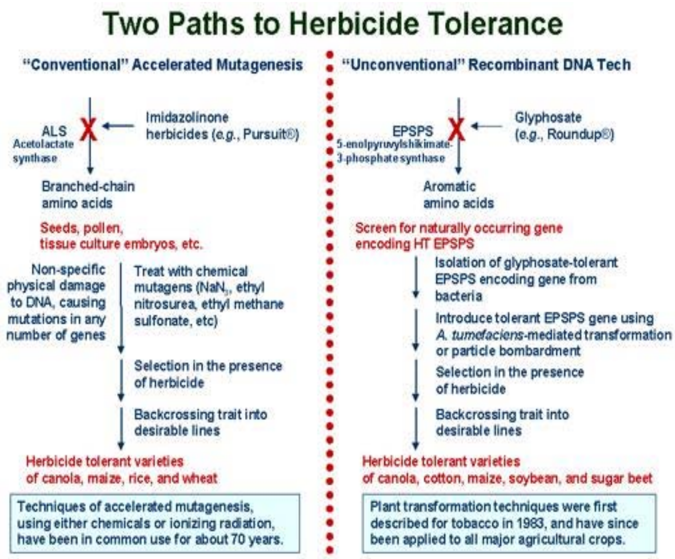

Figure 1: Two paths to Herbicide Tolerance

8. Current Herbicide Technologies

Besides glyphosate, mostcurrent herbicides used for weed management in corn, soybean and cotton are selective and typically used in mixtures to controla broad spectrum of weed species.

Glyphosate

Glyphosate is a non selective, broad-spectrumfoliar herbicide with no soil residual activity that has been usedfor >30 years to manage annual, perennial, and biennial herbaceousgrass, sedge, and broadleaf weeds as well as unwanted woodybrush and trees. Glyphosate is labeled to control over 300 weedspecies. Many glyphosate formulations and salts are commerciallyavailable; the most common salts are the monopotassium and isopropylamine. The type and amount of adjuvant included inthe various formulations differ greatly and strongly influence weedcontrol. Glyphosate strongly competes with the substrate phosphoenolpyruvate(PEP) at the EPSPS enzyme-binding site in thechloroplast, resulting in the inhibition of the shikimate pathway.

Products of the shikimate pathway include the essential aromaticamino acids tryptophan, tyrosine, and phenylalanine and other important plant metabolic products Favourable physicochemical characteristics, low cost, tight soilsorption, application flexibility, low mammalian toxicity, and availability of GR crops have helped make glyphosate the mostwidely used herbicide in the world.32 A key advantage forglyphosate has been the consistent control of weeds almostwithout regard to size.

Glufosinate

Glufosinate is a non selective, broad-spectrumfoliarherbicide with no soil residual soil activity that inhibits glutaminesynthetase [GS; EC 6.3.1.2], an enzyme that catalyzes the conversionof glutamate plus ammonium to glutamine as part of nitrogenmetabolism.31 Glufosinate is faster acting and controls key broadleafweeds such as morningglories (Ipomoea spp.), hemp sesbania(Sesbaniaherbacea (P. Mill.) McVaugh), Pennsylvania smartweed (Polygonumpensylvanicum L.), and yellow nutsedge (Cyperusesculentus L.) better than glyphosate. However, glufosinate isused at higher rates and has historically been more expensivethan glyphosate. Cost and more restrictive application timingrelative to weed size are probably its greatest disadvantagescompared to glyphosat.

Synthetic Auxins

Synthetic auxin herbicides act as auxinagonists by mimicking the plant growth hormone indole-3-aceticacid (IAA), disrupting growth and development processes, andeventually causing plant death, particularly in broadleaf species.31Growers have used auxin herbicides widely for over 60 years asselective herbicides in monocotyledonous crops. Auxinscontrola broad spectrum of broadleaf weeds, including key weeds thathave evolved resistance to glyphosate. Some synthetic auxinssuch as dicamba have fair soil residual activity with a half-life from7 to 21 days. Relatively few weeds have evolved resistance toauxin herbicides, which is noteworthy considering their longtermand widespread use. For example, only six weed specieshave evolved resistance to dicamba after 50 years of widespreaduse in cereal and noncrop environments.

HPPD Inhibitors

The enzyme 4-hydroxyphenyl pyruvate dioxygenase [HPPD; EC 1.13.11.27] converts 4-hydroxyphenyl pyruvateto homogentisate, a key step in plastoquinone biosynthesis. This is themost recently discovered herbicideMOA, and active analogue testingcontinues to generate new products.37 Inhibition of HPPD causesbleaching symptoms on new growth by indirectly inhibiting carotenoidsynthesis due to the requirement of plastoquinone as cofactor of phytoenedesaturase [PDS; EC 1.14.99].38 Visible injury depends oncarotenoid turnover and thus is slower to appear on older tissues thanyoung leaves.31 HPPD-inhibiting herbicides control a number ofimportant weed species and may have soil residual activity, and noweeds have been formally reported to be resistant to this MOA yet.Corn is naturally tolerant to key HPPD herbicides, but soybeans andcotton are generally sensitive.

ALS Inhibitors

Herbicides that inhibit acetolactate synthase(ALS; EC 2.2.1.6), also known as acetohydroxyacid synthase(AHAS), were discovered in the mid-1970s and are still widelyused.39,40 The ALS enzyme is a keystep in the biosynthesis of theessential branched-chain amino acids valine, leucine, and isoleucine.ALS is a nuclear encoded enzyme that moves to the chloroplastvia a transit peptide. More than 50 different ALS-inhibitingherbicides from five different chemical classes (sulfonylureas,imidazolinones, triazolopyrimidines, pyrimidinylthiobenzoates,and sulfonylamino-carbonyl-triazolinones) are commerciallyavailable. The characteristics of ALS herbicides vary in their soilresidual properties, crop response, and types of weeds that areeffectively controlled.

PPO Inhibitors

Protoporphyrinogen oxidase (PPO; EC 1.3.3.4)is an essential enzyme that catalyzes the last common step in thebiosynthesis of heme and ultimately chlorophyll by the oxidation of protoporphyrinogen IX to protoporphyrin IX. PPO-inhibitingherbicides cause the accumulation of protoporphyrinogen IX,which is photoactive, and exposure to light causes the formationof singlet oxygen and other oxidative chemicals that cause rapidburning and desiccation of leaf tissue. The soil residual and fastaction characteristics of PPO herbicides complement the lack ofsoil residual and the slow activity of glyphosate.

ACCase Inhibitors

Acetyl coenzyme A carboxylase [ACCase;EC 6.4.1.2] is the first step of fatty acid synthesis and catalyzes theadenosine triphosphate (ATP)-dependent carboxylation of malonyl-CoA to form acetyl-CoA in the cytoplasm, chloroplasts,mitochondria, and peroxisomes of cells.43 ACCase-inhibitingherbicides generally inhibit the ACCase activity of monocotspecies and not dicots. The three chemical classes of ACCaseinhibitors are cyclohexanediones (DIMs) (e.g., sethoxydim), aryloxyphenoxypropionates (FOPs) (e.g., quizalofop), and phenylpyrazolines(DENs) (e.g., pinoxaden). The ability to useACCase herbicides selectively in corn would be useful, but thetendency of weeds to evolve resistance to this herbicide classwould limit its utility to being part of a weed managementsystem.

9. Summary and Conclusion

Following the development of weed resistances to manyselective herbicides and the prohibitive expense anddifficulty associated with the development of newherbicides, a need has arisen to seek alternatives toaddress these challenges. Along with the efforts todiscover new herbicide target sites in plants, biotechnologyis making major contributions in broadening cropselectivity to the already existing and effectiveherbicides. Further efforts to create more herbicidetolerant crops are needed to ensure more economicalcrop production and safeguard environmental quality byreducing the demand for and the number of selectiveweed killing chemicals required for economical chemicalcrop protection.

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