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# Development of herbicide resistant crops through induced mutations

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## Abstract

Herbicide resistance is an innate characteristic of crop plants. It enables them to survive and propagate even in the presence of lethal doses of herbicides in the surroundings. Genetic tolerance in crops towards herbicides may have several benefits. It may increase safety margins between weed and crop sensitivity and also expands applicability of a particular herbicide. Besides, it can also lower the operating cost for weed control as compared to manual weeding and crop rotation which is normally prohibited by herbicide persistence. Herbicide resistant crops are developed through transformation of a plant with either native or mutant resistant genes, seed mutagenesis, plant cell or tissue culture and through other traditional plant breeding techniques. Seed mutagenesis is a non-transgenic approach, which is found to be most economical and perfect approach. Moreover, all commercial herbicide tolerant crops were derived from single nucleotide substitution of genes and trait can be incorporated into elite varieties because of incomplete dominance and non-pleiotropic effect of the alleles of all commercial herbicide tolerant mutations.



## Introduction

The increase in crop productivity has been resulted from cultivars which were developed through the genetic improvement for high seed yield and disease resistance. Some non-genetic crop management practices have also been developed in past for diseases and insect control through the use of chemical protectants [1]. But besides all these achievements, there is still a yield gap which exists due to inadequate weed control. Poor weed control due to the sensitivity of crops to herbicides is a major hurdle for their production all over the world. Overall, weeds produced the highest potential loss (34%) of agriculture production [2] and eat the food of about 1 billion inhabitants [3]. In conventional agriculture, only selective herbicides, which do not have any harmful effect, on crop plants are being used. But these herbicides are not much successful against weed control. Due to ineffectiveness of these herbicides, farmers use spray combinations of herbicides at different stages of crop. Therefore, by growing herbicide tolerant crops, higher doses of herbicides or even non-selective herbicides may become helpful to control the weeds in a single quick application. The first herbicide tolerant plant developed in the laboratory was tobacco mutant plant, obtained through selection of cell lines in tissue culture [4]. This tolerance was induced in regenerated plants by making some alteration in acetolactate synthase (ALS) enzyme that was much less sensitive to inhibition by sulfonylurea herbicides as compared to normal enzyme [5]. The second approach for the development of herbicide resistant plants is the transformation of resistance genes from a reliable source to the host plant [6]. Another effective method applied for the development of such plants is seed mutagenesis. This approach is most reliable as it is followed by extensive screening then selection of truly resistant plants from large populations [7]. Screening at whole plant level has also been in use to identify induced variants of tomato [8] and natural variants of soybean [9] conferring tolerance to herbicides. This article provides an overview of the concept, development and examples of the herbicide resistant crops.

## Methods

### Search Strategy and Selection Criteria

A systematic search was carried out from Google Scholar and Google Web Browser by providing key

terms “herbicide resistant crops”, mode of action of herbicides, techniques to develop herbicide resistant crops, seed mutagenesis and herbicide resistant crops, mechanisms of herbicide resistance, herbicide resistant mutations, characterization of herbicide resistant mutants, inheritance of herbicide resistance etc. The literature found was further screened for inclusion according to their contents. In this study, 51 peer reviewed research articles were selected.

## Discussion

### Different Techniques to Develop Herbicide Resistant Crops

In traditional plant breeding, plant breeders select and breed herbicide resistant crop varieties with agronomically desirable traits. Three main techniques are being used commonly. The first one is to find herbicide resistant cultivars and cross them with indigenous agronomically desirable cultivars. Second technique to find herbicide resistance is by selecting a cultivar possessing both agronomically desirable traits as well as herbicide resistance. Faulkner used the third technique of deliberately causing mutations in existing cultivars and then selecting the mutations that confers herbicide resistance or tolerance [10]. Hybridization has been used to transfer herbicide tolerance trait from an atrazine tolerant weed to produce atrazine tolerant crops [11]. Atrazine tolerance into foxtail millet was introduced from the weed green bristle grass [12] and sulfonylurea tolerance into domestic lettuce was introduced from prickly lettuce [13]. Gaur *et al.*, [14] performed screening of 300 diverse chickpea genotypes against two different herbicides (imazethapyr and metribuzin) and found large genetic variation for tolerance to these herbicides. Several genotypes tolerant to imazethapyr (ICC 7867, ICC 13441, ICC 13357, ICC 13187, ICC 3239, and ICC 1710) and metribuzin (ICC 1164, ICC 8195, ICC 9586, ICC 283, and ICC 1205) were identified. Sebastian [15] selected a line tolerant to sulfonylureas (chlorsulfuron) by chemically mutating the soybean seeds. Tonnemaker [16] also used the similar technique to develop sulfonylurea tolerant canola.

Modern techniques including tissue or plant cell culture and transformation were used in past decades for the development of herbicide tolerant crops [17,18]. Sugar beet tolerant to chlorsulfuron has been selected through cell culture [19]. *Nicotiana tabacum* plants

transformed with mutant plant acetolactate synthase (ALS) genes conferred high levels of herbicide resistance [20]. The tobacco *SURB-Hra* gene was used to transform sugar beet, melon, oilseed rape, tomato, alfalfa and lettuce. Few transformants of tomato from all the heterologous species to sulfonylurea herbicide resistance subjected to transformation, showed efficient expression of the tobacco ALS gene. Mutant gene was found to be responsible for 20-60% of ALS activity [21].

### **Seed Mutagenesis: A perfect non-transgenic approach**

In last few decades, considerable efforts were put in for the development of imidazolinones/sulfonylureas resistant crops by seed mutagenesis. This tolerance/resistance against these herbicides has been developed in many crops including Soybean (*Glycine max* L. Merr.), Sunflower (*Helianthus annuus* L.), Wheat (*Triticum aestivum* L.), Corn (*Zea mays* L.), Lentil (*Lens culinaris* Medik.) and Canola [22,23]. These modified crops provided numerous benefits to producers including increased and economical weed control and ultimately increased economic return. All these crops resistant/tolerant to the imidazolinone and sulfonylurea herbicides are non-transgenic because tolerance to herbicides was incorporated in them through physical/chemical mutagenesis following selection, a conventional breeding method. Seed mutagenesis followed by selection has been utilized widely under herbicide selective pressure to develop resistance in crops to herbicides [24].

Many mutagens including physical (gamma irradiation) and chemical (ethyl methanesulfonate, N-nitroso-N-methylurea, ethylnitrosourea and sodium azide) have been used for seed as well as pollen mutagenesis in different crops [25]. The mutations derived from these mutagens were screened for the selection of herbicide tolerant mutants. Chemical mutagenesis was found to be an important source of creating genetic variability as most of the herbicide tolerant mutants were developed through this type of mutagenesis. Mutagenesis is also known for alteration in genes that produces heritable changes in plants [26]. Among the chemical mutagens, ethyl methanesulfonate (EMS) was the most effective one [23]. These chemical mutagens were used to increase the probability of selecting resistant mutants in soybean [7,15] and tobacco [4]. Kueh [27] used sodium azide as a chemical

mutagen to develop barley mutants resistant to trans-4-hydroxy-L-proline. Haughn [28] used EMS for the development of sulfonylurea resistant mutants of *Arabidopsis thaliana*. EMS causes point mutations or small nucleotide changes within the genome as compared to other chemical and physical mutagens which causes mutations like deletion of the large section of the genome which causes major changes and also disrupts the characteristics of the variety [29]. EMS is easily available and easy to use and also don't affect the general characteristics of the treated plant material.

Gamma irradiation was also used in seed mutagenesis for the selection of herbicide tolerant mutants. But this method of seed mutagenesis was found less effective as compared to chemical mutagenesis because of non-development of any commercial trait. Malkawi [30] treated two lentil cultivars with 90, 100 and 110 gray doses of gamma irradiations to develop plants tolerant/resistant to chlorsulfuron herbicide. Kumar and Singh [31] used physical mutagenesis such as gamma irradiation for the development of male sterile barley mutants. Spontaneous mutations were also screened in different crops for the discovery of herbicide tolerant mutants through direct herbicide selection.

### **Herbicides Targets in Amino Acid Biosynthetic Pathways**

Herbicides have different inhibiting pathway with different primary targets. For example, glyphosate inhibits aromatic amino acid biosynthesis pathway with 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase as its primary target. The mode of action of glyphosate is to inhibit the biosynthesis of aromatic amino acids (Tryptophan, Tyrosine and Phenylalanine). Glyphosate targets EPSP synthase through identification of shikimic acid as an intermediate. Glutamine synthase is the primary target of phosphinothricin that inhibits glutamine biosynthesis pathway [32].

Chlorsulfuron (sulfonylurea) and imazapyr (imidazolinone) herbicides inhibits branched chain amino acid biosynthesis pathway with acetolactate synthase as their primary target. The mode of action of Imidazolinone/sulfonylurea herbicides is the inhibition of biosynthesis of three essential amino acids (Valine, Isoleucine, and Leucine) in amino acid biosynthetic pathway [33]. Acetolactate synthase (ALS) or Acetohydroxy acid synthase (AHAS) is the site of action of these herbicides [34]. Genetic and biochemical

studies had confirmed the mode of action of sulfonylureas that they act by inhibition of ALS. Sulfonylurea resistant mutants of *Arabidopsis thaliana*, *Nicotiana tabacum*, *Saccharomyces cerevisiae* and *Saccharomyces typhimurium* were isolated and most of them showed acetolactate synthase activity insensitive to the herbicides and the activity of resistant enzyme cosegregated with cellular resistance in genetic crosses [5,28,35].

### Mechanism of Herbicide Resistance

Kandasamy [36] reviewed three mechanisms of herbicide resistance:

- i) Exclusionary Resistance Mechanism - in which plants exclude the herbicide molecules where they produce the toxic response.
- ii) Site of Action Resistance - in which specific site in the plant become resistant against the herbicide.
- iii) Site of Action Over Production - in which plants confer resistance by over production of the target site. Over production of the target site dilutes the herbicides that reaches the target site and the over produced enzyme thus remains active to complete normal function and growth.

Exclusionary resistance mechanism involves morphological and physiological adaptations of the individual plant species. The movements of herbicides into and through the plants influenced by the structural characteristics of plant organs like root, leaf and vascular system. In addition, herbicides uptake, translocation, compartmentation and metabolic detoxification also play role in exclusionary resistance mechanism. Oxidation, reduction, hydrolysis and conjugation of herbicides to amino acids, glucose or glutathione are the biochemical reactions that detoxify herbicides. Altered site of action resistance occurs due to differential sensitivity of molecular target sites i.e. the sites of herbicide inhibition and activity. Most of the knowledge about physiological, biochemical and molecular nature of herbicide activity and inhibition sites came from understanding of herbicide resistant weeds. Imidazolinone, sulfonylureas and triazopyrimidine inhibits branched chain amino acids as their primary molecular target site of action is the enzyme acetolactate synthase (ALS). Over production of the molecular site of action of the herbicide in plant can confer resistance due to gene amplification (multiple copies of the gene

encoding the target site enzyme) and by increased target site enzyme expression.

### Herbicide Resistance: Molecular and Biochemical Basis

Sathasivan [37] presented the first report on the molecular basis of imidazolinone resistance in plants. To understand the molecular basis of herbicide resistance, they isolated the acetolactate synthase gene from GH90 (an imazapyr resistant mutant of *Arabidopsis thaliana*). A single point mutation (from G to A at nucleotide 1958) of the ALS coding sequence was detected through DNA sequence analysis. This single point mutation resulted in Ser to Asn substitution. Newhouse [38] studied the biochemical basis of imidazolinone tolerant corn lines XI12 and QJ22 by analyzing the activity of AHAS enzyme. XI12 approximately showed 80% amount of resistant AHAS activity of the total extractable AHAS activity whereas line QJ22 showed only 20% of the total extractable AHAS activity. Similarly, Newhouse [39] studied biochemical basis of imidazolinone resistant wheat lines (FS1, FS2, FS3 and FS4) in order to check the resistant AHAS activity. Initial analysis showed relatively low level of resistant activity. To clarify the biochemical basis, the line FS4 and the wild type was sprayed with herbicide and AHAS extraction was performed on harvested leaf material three days after spraying. This spray treatment inhibited AHAS (endogenous sensitivity) and provided a clear picture of resistance in selected FS4 line.

### Herbicide Resistant Mutations

Tan [23] described herbicide resistant crops bred through mutagenesis induced amino acid substitutions. Few examples are presented here.

- ☞ Tolerance to imidazolinone/sulfonylurea herbicides is bred from mutagenesis induced amino acid substitutions that occur at various positions i.e., Pro197, Ala205, Ala122, Ser653 and Trp574.
- ☞ Imidazolinone tolerant rice, maize, oilseed rape and wheat have an altered AHAS with the S653N amino acid substitution.
- ☞ Additionally, AHAS with the W574L amino acid substitution was responsible for imidazolinone tolerant maize and oilseed rape.
- ☞ Similarly, imidazolinone tolerant sunflower has A205V while sulfonylurea tolerant soybean has a P197S AHAS gene mutation.



- ✧ Sathasivan [37] detected single point mutation from G to A at nucleotide 1958 in GH90 (imazapyr resistant mutant of *Arabidopsis thaliana*) through DNA sequence analysis.
- ✧ Sulfonylurea resistant mutant of *Arabidopsis thaliana* GH50 was developed with P197S ALS gene mutation.
- ✧ Similarly, sulfonylurea resistant mutant of *Nicotiana tabacum* Sura-C3 was developed with Proline to Glutamine substitution at 196 amino acid position.
- ✧ Imidazolinone and sulfonylurea resistant mutant (SuRB-S4HRA) of *Nicotiana tabacum* has ALS with the Pro196Ala and Trp573Leu amino acid substitutions.
- ✧ Likewise, imidazolinone tolerant lentil (RH44) has been developed from the amino acid substitution in which serine was substituted for an asparagine in the AHAS gene [40].

#### Inheritance of Herbicide Resistance

Herbicide tolerant traits can be incorporated into elite varieties because of incomplete dominance and non-pleiotropic effect of the alleles of all commercial herbicide tolerant mutations. This can be done through crossing of the elite variety with an herbicide resistant trait donor except for the triazine tolerant mutation trait which is linked with several agronomic traits and also inherited maternally [23].

Saari *et al.*, [41] reported that in most crops herbicide resistance to ALS is mostly conferred by dominant or partially dominant genes. Newhouse [38] found semi-dominant resistance in maize against imidazolinones. Genetic analysis of imidazolinone resistant corn was performed by crossing XA17 (imidazolinone resistant mutant) with B73 (imidazolinone susceptible inbred). Genetic stability of the said trait was found in the progeny. The progenies were further analyzed via backcrossing to B73 and by selfing. Backcrossed progeny exhibited 1/1 ratio as expected for single point mutation in the genome. Selfed progeny showed 3:1 ratio of resistant to susceptible plants. The further genetic analysis showed that the resistance was inherited as semi-dominant, single allele. Newhouse [39] observed semi dominant and dominant resistance in five lines of wheat. Chlorsulfuron resistance was found consistent with a semi dominant mode of inheritance in brassica [42].

#### Herbicide Resistant Crops: Few Examples

- ✧ Isolation of four mutant soybean lines (1-126A, 1-166A, 1-183A and 1-184A) with increased chlorsulfuron tolerance following seed mutagenesis with ethylnitrosourea (ENU) has been reported in literature [7]. In this study, mutant 1-184A displayed more tolerance for pre-plant incorporation and post emergence levels of chlorsulfuron herbicide as compared to wild type “Williams”. ALS assay showed that mutants do not contain an altered form of acetolactate synthase.
- ✧ Pozniak and Hucl [43] studied genetics of herbicide resistant mutants with target site acetolactate synthase resistance in common wheat.
- ✧ Imidazolinone resistant maize lines have been developed through pollen mutagenesis [44,45].
- ✧ Sulfonylurea and triazopyrimidine herbicides resistant canola has also been developed [46].
- ✧ A four step procedure for the development of imidazolinone resistant wheat including seed mutagenesis with sodium azide, seed soaking in herbicide, pre-emergence herbicide application and screening and selection of herbicide resistant mutants have also been proposed previously [39]. This procedure resulted in four lines (FS1, FS2, FS3 and FS4) out of 120000 M<sub>2</sub> plants that were confirmed to be imidazolinone resistant.
- ✧ Imidazolinone canola was developed through microspore mutagenesis as a collaborative research program between Allelix Crop Technologies and American Cyanamid [47].
- ✧ Malkawi [30] studied ALS activity and sensitivity of gamma irradiated lentil cultivars (Jordan 1 and Jordan 2) against chlorsulfuron herbicide. In this study, both cultivars were subjected to 90, 100 and 110 Gray doses of gamma irradiation. The tolerance resulted due to an alteration of the ALS gene which leads to overproduction of altered ALS enzyme.
- ✧ Imidazolinone tolerant RH44 is one of the latest lentil mutant derived by chemical induced mutagenesis. In the development of this mutant, seeds from many lentil cultivars were treated as a batch with ethyl methanesulfonate (EMS) and then planted. Whole plant selection procedures for herbicide tolerance were used and one tolerant mutant was selected and designated as line RH44 [48].

Attempt to develop Glyphosate (Roundup Ultra) tolerant wheat genotype was made earlier under a project entitled "Mutation Breeding for Tolerance to Round Up, *Rhizoctonia* Root Rot and Drought" (Project # 3019-5850). The first report of this project was presented in 2008 and patent was filed in 2007 by Washington State University. In this project three approaches were used to develop glyphosate tolerant wheat germplasm including large scale screening of EMS mutagenized populations, re-mutagenesis and screening for enhanced glyphosate resistance and gene pyramiding of independently isolated mutations for increased resistance. Large scale screening of wheat cultivars Hollis, Louise, Macon and Tara 2002 was performed in field and greenhouse (Plant Growth Center). Screening was performed against 1X, 2X and 4X doses of glyphosate. A population totaling over 7 million plants was screened under field and greenhouse conditions. Retest experiments were also performed after seed increase. A single line Louise FRI-62 showed 99% survival at 2X dose of glyphosate. This line was re-mutagenized using EMS for tolerance to 4X dose of glyphosate through two gene enhanced resistance. Further, gene pyramiding approach was adopted to increase the resistance level by combining independently isolated mutations. For this purpose 147 crosses were made between some of the individual tolerant plants and also this approach was found to be most effective for increasing tolerance to glyphosate [49].

### Herbicide Resistance: Management Strategies

Natural selection and evolution are the processes responsible for the survival of different plant species around the world. Among plants, weeds can survive a variety of environmental conditions due to availability of tremendous genetic variation that allows them to survive. Through selection, where herbicide is the selection pressure, resistant plants survive and reproduce while susceptible plants are killed [50]. Weeds possess the traits that enhance the occurrence of resistance. If an herbicide with one mode of action is continually used then resistant plants become dominant in the population. High rate of seed production per year can also increase the probability of resistance to occur. The presence of tremendous genetic variation coupled with high rate of seed production evolves rapid

resistance in a population. The frequency of weeds resistant to herbicides is an important factor in determining the rate of selection among weed species for resistance. For example, weeds resistant to triazine herbicide were evolved after 10 years of its continual use whereas resistance to sulfonylurea herbicides took only 4-5 years to evolve. Resistance to sulfonylurea herbicides, the proportion of resistant plants estimated was found to be 1 in 1 million individuals i.e. if there is weed population density of 10 plants per m<sup>2</sup>, and then there is chance of one resistant individual in every 10 hectares of weed population [51].

Multiple control strategies are needed to manage the herbicide resistant weeds so that they do not survive long enough to produce resistant seeds. Management actions can reduce the selection pressure for resistance that ultimately reduces the rate of resistance evolution. The prevention and management actions of delayed herbicide resistance in weeds may include:

- Herbicide rotation- Rotation of herbicides with different mode of actions reduces the chance of occurring resistant weed individuals in a population.
- Crop rotation- Rotation of crops having different season of growth, different registered herbicides and alternate methods of weed control is effective management practice for delayed herbicide resistance in weeds.
- Monitoring after herbicide application- Checking of weedy patches after herbicide spray to find out any single resistant plant before seed set.
- Non chemical control techniques- Hand weeding, mulching with synthetic and organic materials and solarizing the soil reduces the chances that a plant will produce seed.
- Clean equipment
- Short residual herbicides
- Certified seed
- Fallow tillage
- Close cultivation

### Conclusion

The concept of herbicide resistant crops was developed during 1979 and considerable achievements have been made in this field. These crops can be developed through traditional plant breeding techniques i.e. through crossing of resistant cultivar with agronomically desirable cultivar, selection within agronomically

desirable cultivars and through seed mutagenesis. Modern plant breeding techniques including plant transformation with the resistant genes either native or mutant and plant cell or tissue culture techniques are also helpful for the generation of these crops. Seed mutagenesis was found to be easy and economical approach but it demands considerable efforts and time to produce these crops. Both physical and chemical mutagens were used for seed or pollen mutagenesis but chemical mutagenesis and especially EMS were most effective. All commercial herbicide tolerant crops were derived from single nucleotide substitution of genes. Herbicide tolerant traits can be incorporated into elite varieties because of incomplete dominance and non-pleiotropic effect of the alleles of all commercial herbicide tolerant mutations. Herbicides selection pressure led to the evolution of herbicide resistant weeds. Although the frequency of evolution of resistant weeds is less but the prevention and management strategies to avoid or delay the resistance are necessary.

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