Review Paper:

Breeding for herbicide tolerance in crops: a review

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Abstract

Weeds compete with crop plants for water, nutrients, sunlight, and space and also harbor insect and disease pests. With continuously increasing labor cost, manual weeding has become an expensive field operation for any crop and farmers are increasingly opting for cultivars tolerant to herbicides. Herbicide-tolerant cultivars offer opportunity of controlling weeds through need-based applications of herbicides. Herbicide tolerant cultivars have been developed in many crops by exploiting already available genetic variability in the germplasm or by creating mutations or by transgenic. A large genetic variation for tolerance to herbicide exists among various crops such as maize, wheat, rice, sunflower, soybean, chickpea, alfalfa etc. Herbicide tolerance in germplasm or in mutant lines may be due to altered binding site of target enzyme for herbicide, improved herbicide metabolism, sequestration of herbicide molecule and overexpression of target protein.

Various mutations have been reported which may include SNP or insertion/deletion mutation conferring the herbicide tolerance e.g. a point mutation in the AHAS1 gene at C675 to T675 resulting in an amino acid substitution from Ala205 to Val205 confers resistance to IMI in chickpea. Herbicide resistant crop via transgenic has also been developed by taking resistant genes from various sources such as bacteria or other plant and incorporating them into crop to make them herbicide resistant. Transgenic herbicide tolerant crops occupy 47% of total area under transgenic in world. The disadvantage accompanied with herbicide tolerant crops is super weeds. Super weeds are the plants which are crop related weeds species on which the resistance genes got transferred by natural outcrossing.

Keywords: Weeds, herbicides, herbicide tolerance, transgenic, breeding methods.

Introduction

Weeds are one of the most limiting factors in crop cultivation. Weeds compete with crop plants for water, nutrients, sunlight and space and also carry insect-pests and diseases. If not controlled in the beginning, weeds can cause significant reduction in crop yield and also deteriorate the quality. Gharde et al[24] have reported that in India alone, the losses due to weeds are estimated to be 11 billion USD per year, which varied from 13.8% in transplanted rice to 76% in soybean. Among all biotic stresses, weeds cause the highest potential loss (34%), with insect (18%) and pathogens (16%) being less important[46]. Thus, effective weed control in any crop production system is a pre-requisite if high yields and good quality are to be achieved.

Initially, hand weeding dominated most weeding practices, but it was gradually replaced (in rural India wage rate for men increased up to 400% from INR 67 in 2004 to INR 276 in 2018, www.indiastat.com accessed on 22 August 2018) with mechanical control. Mechanical weed control practices are now viewed to be unsatisfactory due to the high-energy requirements and other associated costs, plus the perceived facilitation of soil erosion and compaction.[53] Therefore, it has now been largely replaced by chemical weed control that can eliminate weeds from crop plantings with minimal soil disturbance. Herbicide usage in world has been increased 15-fold after the introduction of transgenic herbicide tolerance cultivars which offer opportunity of controlling weeds through need-based applications of herbicides.[4]

Herbicides have severe morphological and physiological effects on plants such as necrosis, delayed flowering, deformed flower, stunted growth, cupping of Leaves, burning symptoms etc.[50] An ideal herbicide should have high weed killing potency with low environmental persistence. Most of the broad-spectrum herbicides lack selectivity, thus limiting their use in some cropping operations but are very useful for complete weed control. Use of broad-spectrum herbicide is limited because of its sensitivity to crop. Continuous use of the selective herbicides has led to the development of herbicide resistance in weeds.[11,56] Thus, a genotype with herbicide tolerance is required in current perspective. Breeding for herbicide tolerance is being necessitated by increase in cost of hand weeding, shortage of agricultural labours, decrease in cost of herbicide, increase in the effectiveness of new herbicide molecule and need of mechanization in intensive agriculture.

Breeding for any crop trait is depending upon the availability of variation for that trait, if not available, creation of variability and their subsequent utilization in crop improvement. Crop genotype tolerant to herbicide endows

us with an opportunity to have mechanized agriculture. Therefore, in this review we have elaborated the recent methodologies applied for herbicide tolerance crop breeding, examples of varieties released and future course of action.

Mechanism of Action of Herbicides: Herbicides are often classified according to their site of action, movement inside plants, target enzyme, physiological process affected etc. and therefore, herbicides within the same site of action class will produce similar symptoms on susceptible plants.[60] Knowledge of mode of action of herbicide is required for understanding crop management and its efficient utilization. Classification by mechanism of action indicates the first enzyme, protein, or biochemical step affected in the plant following application.[17] The most commonly used herbicides and their mode of action are given in table 1.

Table 1
Commonly used herbicides with their mode of action (modified from Sherwani et al[60])

Mode of Action	Example
Amino Acid Synthesis Inhibitors	Imazethapyr, Glyphosate
Lipid synthesis Inhibitors	Cyclohexanediones
Seedling Growth Inhibitors	Pendimethalin, Alachlor
Photosynthesis Inhibitors	Atrazine, Metribuzin
Cell membrane Disruptors	Paraquat
Growth Regulators	2,4-D, 2,4,5-T, Dicamba
Pigment synthesis Inhibitor	Bromoxynil

The main mechanisms of action herbicides according to Weed Science Society of America (http://wssa.net/wp-content/uploads/WSSA-Mechanism-of-Action.pdf) are:

1. ACCase inhibitors: Inhibition of Acetyl coenzyme A carboxylase (ACCase) affects lipid metabolism affecting cell membrane production in the meristems of the grass plant.

2. ALS inhibitors: Inhibition of Acetolactate synthase (ALS) enzyme (also known as acetohydroxyacid synthase or AHAS) causes reduction in the synthesis of branched-chain amino acids (valine, leucine and isoleucine) affecting meristematic tissue.

3. EPSPS inhibitors: Inhibition of enolpyruvylshikimate 3-phosphate synthase enzyme (EPSPS) creates problem in synthesis of aromatic amino acids (tryptophan, phenylalanine and tyrosine).

4. Synthetic auxins: It mimics plant hormone and thus affects various process cell growth, cell division, cell differentiation, morphogenesis etc.

5. Photosystem-II inhibitors: It creates problem in electron flow from water to $NADPH^{2+}$ in photosynthesis. As a result, oxidation reactions occur in excess resulting in plant death.

6. Photosystem-I inhibitors: It removes electron from the normal pathway through FeS to Fdx to NADP leading to direct discharge of electrons on oxygen. Production of this extra amount of reactive oxygen species is not tolerated by the cell leading to plant death.

Other mechanism of herbicide mode of action includes mitosis inhibitors (Benzamide, Dinitroaniline etc.), glutamine synthetase inhibitors (Gluphosinate), carotenoid inhibitors (amides, anilidex etc.), protoporphyrinogen oxidases inhibitors (diphenylethers, oxadiazoles) etc.

Breeding for herbicide tolerance: The discovery of herbicide-resistant weeds in the early 1970s in mustard field, created an interest in mimicking this unintentional development for use in herbicide resistance crop breeding (http://www.fao.org/docrep/006/y5031e/y5031e0i.htm.

Significant progress in molecular genetics has led us to identify[33], locate[2] and transfer the herbicide tolerance gene.[68] To match the biology of the crop to the chemistry of herbicide, we are now in continuous process of studying the physiology of herbicide action.[16,21] Herbicide Resistant Crops (HRCs) have been grown commercially since 1984, when the first triazine-resistant oilseed rape cultivar (OAC Triton) was introduced in the Canadian market. This cultivar was developed by methods of traditional breeding. Triazine resistance from *Brassica rapa* L. had been backcrossed into a commercial variety of oilseed rape.[26] Nowadays major HRCs have been produced by genetic engineering, the technology which has unintentionally placed these crops in a fierce debate between those in favour and those against the introduction and commercial use of genetically modified (GM) crops and made HRCs popular.

Source of Herbicide tolerance: Herbicide tolerance is a relative phenomenon i.e. even a herbicide resistant plant may become susceptible at higher dose of herbicide. Therefore, we generally go for evaluating the genotypes at three times recommended dose of herbicide.[52] The following are the sources for variability for herbicide tolerance:

1. Germplasm collections: Conventionally the herbicide tolerance can be found in the crop species itself or its wild and weedy relatives. Several authors have reported the presence of large amount of genetic variability for several herbicide in the germplasm of different crop plants such as in chickpea[13,23,52,67], rice[39], groundnut[40] etc.

2. Artificial random mutagenesis: Herbicides generally target a specific enzyme[17] and even a small artificial mutation created by random mutagenesis at specific site so that enzyme can be a source of tolerance to herbicide e.g. EMS mutagenesis in arabidopsis for sulfonylurea and imidazolinone[35], in soybean for sulfonylurea[58], in sunflower for imidazolinone[54] etc. Similarly, mutation by sodium azide has led to development of tolerance to imidazolinone in wheat.[45]

3. Site specific mutagenesis: With advancement of molecular biology and nucleotide sequence data, it has now become easy to target specific domain[70] of an enzyme and create site specific mutation using genome editing tools e.g. in maize, modified Acetolactate synthase (ALS) enzyme having tolerance to herbicide chlorsulfuron have been developed using CRISPR-Cas9 technology.[66]

4. Transgenic: Several bacterial genes are known to confer tolerance to herbicide. Transfer of these genes is possible using modern molecular biological tools. For example, gene for tolerance to herbicides "*aroA*" has been transferred from *Agrobacterium sp*. strain CP4 to several crops for tolerance to Glyphosate.[47]

Molecular mechanism of herbicide tolerance: To effectively utilize the genotype for tolerance to herbicide, we must understand the molecular basis of it. It has been a matter of curiosity and need of the hour to understand the genetic, physiological and biochemical basis of tolerance to herbicide.[1] We can only utilize (with best of method), modify (with gene editing tools), engineer (novel mechanism in a species) for tolerance to herbicide, if we fully understand the mechanism behind it.[22] Based on the several studies, following mechanisms of resistance to herbicide are proposed:

1. Altered target site: Enzymes generally have domains for binding with several biomolecules. These domains are specific to different substrate or other interacting enzymes. Herbicides generally target specific domain of target protein, where it acts to disrupt a particular plant process or function (mode of action). If this target site is somewhat altered (change in amino acids) without affecting its function, the herbicide no longer binds to the site of action and is unable to exert its phytotoxic effect.[16]

2. Enhanced metabolism: Plants have many mechanisms to degrade or solubilize the toxic compounds, this mechanism can be used by plants to degrade herbicides. Therefore, a plant having enhanced metabolism of herbicide detoxifying enzymes can easily overcome the toxic effects.[29]

3. Compartmentalization or sequestration: Plants have several inherent mechanisms to exclude the exo- or endogenous toxins such as by encompassing the toxins in vacuole, releasing them with exudates, restricting their movement within plants etc. and thus preventing their harmful effects.[12,27] In this case, a herbicide may be inactivated either through binding (sequestration) or removed from metabolically active regions of the cell to inactive regions (compartmentalization) such as cell wall, vacuole etc.

4. Over-expression of the target protein: Herbicide targets a specific protein and this target enzyme is produced in large quantities by the plant, then the effect of the herbicide becomes insignificant.[44,51]

In recent years, there are several examples of approaches used in breeding of herbicide tolerance. In narrow-leaf lupin, two highly tolerant Metribuzin mutants, 'Tanjil-AZ-55' and 'Tanjil-AZ-33', were identified by Si et al[61] and in lentil, a variety 'RH44' with tolerance to Imidazolinone was developed by mutagenesis by Slinkard et al.[63] Kolkman et al[38] had identified, cloned, and sequenced three Aceto-hydroxy acid synthase (*AHAS*) genes (*AHAS1*, *AHAS2*, and *AHAS3*) from herbicide-resistant (mutant) and susceptible (wild type) sunflower. They found that it as 48 SNPs in *AHAS1*, single 6bp deletion in *AHAS2* gene and single SNP in *AHAS3* gene, which is conferring tolerance to herbicide sulfonylurea and imidazolinone. *AHAS1* from imazethapyr-resistant inbred harboured a C-to-T mutation in codon 205 (*ala* to *val*), (*Arabidopsis thaliana* codon nomenclature), conferring resistance to IMI herbicides, whereas *AHAS1* from chlorsulfuron-resistant inbred harboured a C-to-T mutation in codon 197(*pro* to *leu*), conferring resistance to SU herbicides.

Mutation of proline at 197th position in *AHAS* gene is one of the most common mutations found in plants resistant to *AHAS*-inhibiting herbicides. Substitution of Pro197 with at least eight different amino acids has produced SU resistance in *Lactuca*, *Kochia*, *Brassica*, *Sisymbrium*, *Amaranthus* and *Arabidopsis* species.[35,71] The crystal structure of yeast AHAS in complex with chlorimuron-ethyl, an SU herbicide, revealed that both Pro197 and Ala205 make hydrophobic contact with the inhibitor which binds in the substrate access channel and blocks entry of substrate into the active site of the enzyme.[48]

Thompson et al[68] and Taran et al[67] have reported two homologous AHAS genes namely *AHAS1* and *AHAS2* sharing 80 % amino acid sequence similarity in the chickpea genome. They have reported that a point mutation in the *AHAS1* gene at C675 to T675 results in an amino acid substitution from Ala205 to Val205 which confers the resistance to IMI in chickpea. These genes were inherited in semi-dominant fashion. Similarly, Li et al reported that an Ala122 mutation on chromosome 6D in wheat (*Triticum aestivum* L.) is responsible for the IMI resistance. Ghio et al[25] recently reported that a change from Pro197 to Ser197 confers the resistance to SU herbicides in soybean.

Conventional approaches to develop herbicide tolerant crops: Breeding with conventional approaches is cheaper, less regulated and highly adapted. Apart from this transgenic and gene editing, it can be used as non-conventional breeding approaches which are highly regulated, difficult to release varieties and having less consumer acceptance[8]. There are various ways by which Herbicide Tolerant / Resistant Crops can be developed.[36] All these methods have been successfully applied for development of herbicide tolerant crops. These are:

- Screening for natural variation in various germplasm lines of diverse source.

- Using available genes in gene pool or genes present in wild relatives.
- Use of random mutagenesis for creating variation for tolerance to herbicide.

Germplasm Screening: Evaluation of germplasm is done by screening them under sprayed condition. It can be done by growing lines and spraying various doses of herbicide application and evaluating them on various parameters such as average plant stand, yield difference between control and sprayed, leaf damage score on 0-5 scale, floral development, days to flowering and days to maturity, harvest index, photosynthetic ability, Normalized Difference Vegetation score (NDVI) etc.[13,23,52,67] The best line selected can be further screened intensively at multi-location in multi-year and later can be used in development of herbicide tolerant cultivar by various breeding methods (Figure 1).

Wild relatives and wild species: Idea of using wild relatives and species in breeding for herbicide tolerance has come from an unwanted phenomenon of superweeds.[3] Application of more herbicide in fields has resulted into the selection among the related weed species (crop wild relatives acting as weeds) and subsequently failure of that herbicide against that weeds. Therefore, wild relatives of cultivated crop species can be screened via same procedures as done for germplasm materials. Gene-flow is very common among crop wild relatives to crops and among wild relatives.[64]

Once the resistance gene is present in crop volunteers or related weed species, then it is expected that the same weed control practices (consistent sprayings with herbicides having the same mode of action) which cause herbicide resistance to occur in naturally tolerant/resistant weed biotypes, will lead to a rapid build-up of HR-weeds and volunteers.[15]

Mutant Screening: Mutation is a great source of herbicide tolerance in the crops where enough variability for herbicide tolerance is not available in crop germplasm[5,6]. Mutation can be generated by physical and chemical mutagenesis which can be effectively utilized in developing herbicide tolerant lines[49,54]. Screening for herbicide tolerance in mutants is same as that of germplasm screening. Lines identified can be directly released as herbicide tolerant cultivars[43] or can be incorporated in breeding programs.[57]

Non-conventional approaches for herbicide tolerance: Herbicide crops can also be produced by novel breeding approaches such as somaclonal variation,[7] site directed mutagenesis[9], gene targeting[19,37], gene editing[59], transgenic[20] etc. These approaches are nowadays used commonly to develop herbicide tolerant crops.[41] These approaches can be characterized under following:

1. Somaclonal variation: Genetic changes occurred during the tissue culture operation have been effectively utilized in development of herbicide tolerance in several cases such as in *Brassica napus* L.(cv Topas) for tolerance to chlorsulfuron (CS), for difenzoquat in wheat[7], for glyphosate in tobacco[62], for atrazine in soybean[73] etc.

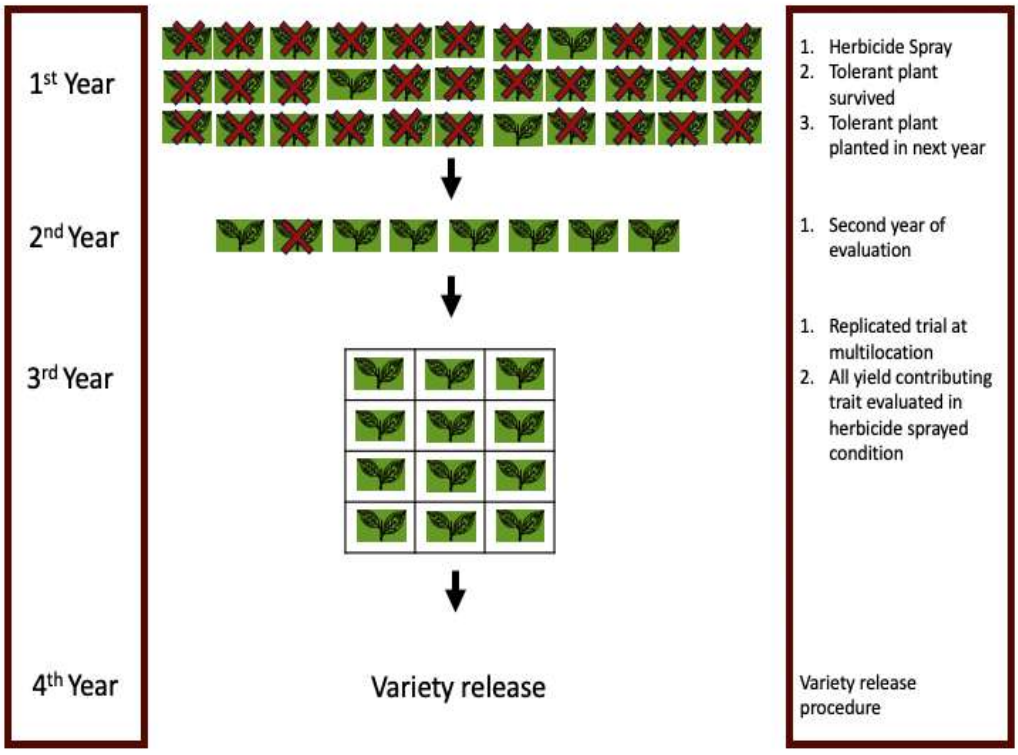

Figure 1: Germplasm screening for herbicide tolerance (a schematic representation)

2. Site-directed mutagenesis: Gene targeted by herbicide can be mutated at herbicide binding site in a way that it does not affect other function of that gene.[18,69] Site directed mutagenesis approach by oligo-directed mutagenesis[19,55] and engineered nucleases[41] can be used for generating herbicide tolerant cultivars.

3. Gene editing: Homologous recombination and replacement of herbicide target domain of target enzyme with mutated version not suited for herbicide binding can be a good approach for herbicide tolerance.[10,41,55,65]

4. Transgenic: Herbicide tolerance is the most common trait in commercial transgenic crops as 47% (80.7 Mha) of total area under transgenic crop in the world is under transgenic herbicide tolerance (189.8 Mha) (ISAAA website). Transgenesis for herbicide tolerance involves the identification of an herbicide resistance gene from a plant or microorganism, its isolation and manipulation for efficient plant expression (if it is of microbial origin) and its subsequent delivery, stable integration and expression in the cells of the target crop plant.[34] For the most part, genes coding for useful herbicide resistance in crops are isolated from herbicide degrading soil microorganisms.

The most commonly employed techniques in developing herbicide resistant crops via transgenic are the *Agrobacterium* and the particle bombardment methods respectively[72]. Herbicide tolerance via genetic transformation can be conferred by one or a combination of these four mechanisms:

1. Introduction of a gene(s) coding for an herbicide detoxifying enzyme(s).[14]

2. Introduction of gene(s) coding for herbicide insensitive form of a normal functioning enzyme or over expression of the genes coding for a herbicide target enzyme such that the normal metabolic functioning is still achieved in the plant even though some of the enzyme is inhibited.[30]

3. Modification of the herbicide target enzyme in such a way that the herbicide molecule does not bind to it.[69]

4. Engineering for active herbicide efflux from plant cells.[69]

Table 2
Transgenic varieties/hybrids approved for cultivation in USA (ISAAA website)

Crop	Herbicide	Year	Commercial name	Company
Cotton	Glyphosate	1996	Roundup Ready	Monsanto
	Bromoxynil	1995	BXN	Calgene
	Sulphonylurea	1996		Dupont
	Isoxaflutole		Liberty Link® GT27™	BASF
Maize	Glufosinate	1996		Dekalb
	Glufosinate	1996	Liberty Link	Agro-Evo
	Sethoxydin		SR	
	Imidazolinone		IMI	
	Sulfonylurea, Imidazolinone, Glyphosate	2009	Optimum™ GAT	Corteva Agriscience
	Glyphosate, Glucosinolate	2013	Agrisure	Syngenta
Soybean	Glyphosate	1995	Roundup Ready	Monsanto
	Glufosinate		Liberty link	BASF
	sulphonylurea		STS	
	Imidazolinone	2014	Cultivance	BASF
Canola	Oxynil		Navigator	Bayer
			Optimum	
	Glyphosate		Roundup Ready	
	Glyphosate, Glucosinolate	2016	InVigor	Bayer
Carnation	Sulfonylurea		Moondust, Moonshadow Moonshade Moonlite Moonaqua Moonvista	Florigene
Linseed	Sulfonylurea		CDC Triffid Flax	University of Saskatchewan
Alfalfa	Glyphosate	2005	Roundup Ready	Monsanto

Table 3
Non-transgenic varieties tolerant to herbicide released for cultivation

Crop	Method	Name of variety	Developer/Organisation
Rice		Clearfield	BASF
Wheat		Clearfield	BASF
Sunflower		Clearfield	BASF
Narrow-leaf lupin	Advanced breeding lines	Coromup	Si et al[61]
Soybean	Advanced breeding lines	Tracy-M	Hartwig[28]
Oilseed Rape	Traditional Breeding	OAC-Triton	Hall et al[26]
Wheat	Modified Bulk & Pedigree	ND901CL	Mergoum et al[42]

Commercially successful varieties: Transgenic varieties occupy 40% of total global area under transgenic in USA [International Service for the Acquisition of Agri-biotech Applications (ISAAA), 2019]. Transgenic varieties for herbicide tolerance approved for cultivation are given in table 2 (ISAAA website). List of Non-transgenic varieties is given in table 3.

Disadvantages of herbicide tolerant crops
1. Mammalian toxicity due to increased usage of herbicide.
2. Ecotoxicity (side effects on soil microorganisms and agricultural flora or fauna).
3. Raising herbicide resistant weeds and volunteers' crop.
4. Yield performance is affected.
5. Single selection pressure and weed resistance.
6. Shifts in weed species (minor weeds may become major).
7. Gene escape (transfer of transgenic trait into related wild weedy species by pollination).
8. Gene flow and contamination to organic crops.
9. Drift and non-target movement of resistance gene.

Advantages of transgenic herbicide tolerant crops
1. Facilitate low or no tillage.
2. Broader spectrum of weeds controlled.
3. Reduced crop injury.
4. Reduced herbicide carry-over.
5. Use of herbicides that are more environmentally friendly.
6. New mode of action for resistance management.
7. Crop management flexibility and simplicity.
8. Superweeds - A wild plant that has been accidentally pollinated by a genetically modified plant and now contains that plant's abilities to resist herbicides and insects. Certain invasive grasses and weeds have proven themselves to be resistant to glyphosate, meaning that the herbicide is no longer effective against these plants. Examples of weeds resistant to Glyphosate are Common Ragweed, Italian Ryegrass etc.

Conclusion
Herbicide tolerant crops are now required to popularize the mechanized agriculture. Based on the wide amount of genetic variability available in various crops and with advancement of molecular biology tools, we can now be sure to develop herbicide tolerant cultivars.

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