

Genome Editing and Transgenic for Development of herbicide-resistant crops

**Ipsita Sahu¹, Yogendra Singh²,
Manoranjan Biswal³,
Shivani Jha¹ and V.K.Yadav⁴**

¹. M.Sc Research Scholar,
Biotechnology Centre, JNKVV,
Jabalpur (M.P)

². Assistant Professor (Senior
Scale)-Biotechnology, Department
of Genetics and Plant Breeding,
JNKVV, Jabalpur (M.P)

³. Scientist , College of Agriculture,
Iroisemba , CAU, Imphal
(Manipur)

⁴. Professor , Department of Plant
Pathology, JNKVV, Jabalpur (M.P)



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*Corresponding Author

Ipsita Sahu*

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INTRODUCTION

The introduction of herbicides has provided a revolutionary tool for managing the difficult task of weed control contributing significantly towards global food security and sustainability. However, despite the significant contribution herbicides have made towards production by providing a consistent, efficient, rapid and economic approach to weed management, the development of herbicide resistance in many weed species is generating global concerns. Herbicide-resistant weeds are a major threat to sustainable agriculture, as they render herbicides less effective, increase costs, and decrease crop yields. Repeated use of the same herbicide modes of action (MOAs), simplified crop rotations and intensive tillage have accelerated the evolution of resistant biotypes across numerous weed species. Tackling resistance requires both non-chemical integrated weed management (IWM) and novel biotechnology-based tools that reduce reliance on single chemical MOAs while offering targeted control of problematic species

Biotechnological Approaches for Improved Weed Management

- Development of herbicide-resistant crops through transgenic approaches
- Improvement of biocontrol agents
- Development of transgenic allelopathy in crops.
- Development of herbicide-resistant crops through genome editing approaches

A. Development of Herbicide-resistance crops using Transgenic Methods

Crops resistant to broad-spectrum herbicides, especially glyphosate, have been developed through the extensive use of transgenic technology. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an important enzyme in the shikimate pathway necessary for the production of aromatic amino acids in plants, is inhibited by glyphosate.

Over expression of EPSPS gene

Early research showed that glyphosate tolerance might be conferred by overexpression of the native EPSPS gene. First identified in petunia, an overexpressed EPSPS gene was subsequently transferred into agricultural plants (Steinrücken et al., 1986), resulting in increased tolerance. It has been found that transgenic crops, like tobacco (Daniell et al. 1998). and carrot (Hauptmann et al., 1988), can withstand glyphosate at concentrations two to four times higher than those that kill wild-type plants.

Use of mutant EPSPS: -

Salmonella typhimurium was shown to have a glyphosate-resistant mutant EPSPS gene. This mutation prevented glyphosate binding by changing an amino acid from proline to serine through a single base substitution. (Stalker et al., 1985). This mutant EPSPS gene was tagged with chloroplast specific transit EPSPS enzyme that freely enter chloroplast & confer glyphosate resistance. e.g. tobacco (Garg et al., 2014), maize (Heck et al., 2005).

Detoxification of glyphosate

Some soil microbes include enzymes called glyphosate oxidoreductase that can break down glyphosate into non-toxic substances

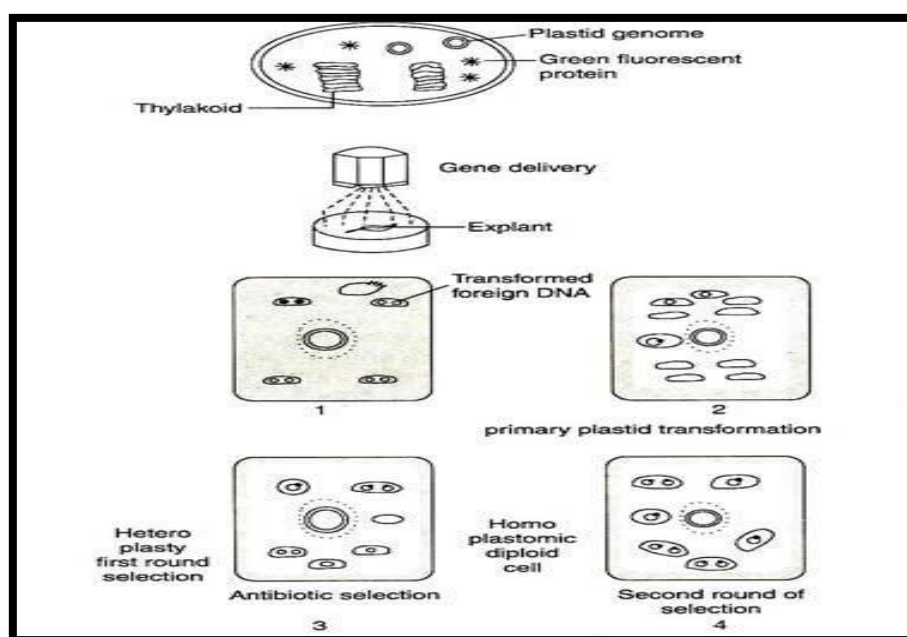
like glyoxylate. After being extracted from microbial sources, such as *Ochrobactrum anthropi*, the genes encoding these detoxifying enzymes were inserted into agricultural plants. Through active herbicide detoxification, transgenic oilseed rape (Miki and McHugh, 2004). that expressed these genes showed improved glyphosate tolerance.

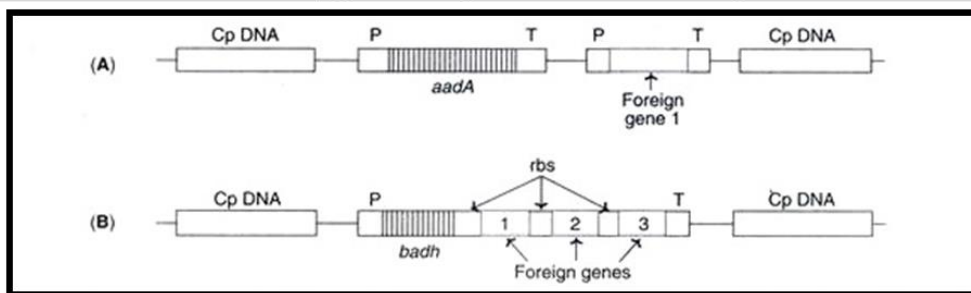
Use of combined strategy

High resistance is achieved by combining multiple strategies, including EPSPS mutant, detoxification and over expression genes were employed in the same crop plant thus provides robust tolerance and reduces the likelihood of resistance breakdown.

B. Chloroplast Engineering:

Genetic engineering of chloroplasts (plastids) is a significant development in plant biotechnology. High transgenic expression, accurate gene insertion by homologous recombination, and a lower chance of transgene escape are only a few benefits of plasmid transformation over nuclear transformation. Chloroplasts are maternally inherited; hence there is no danger of gene transfer through pollen to related weeds. This is because pollen does not contain transgene. Multi-gene transfer can be conveniently carried out in chloroplasts which is rather difficult with nuclear genome





Source: Agrawal, S., Karcher, D., Ruf, S., Erban, A., Hertle, A. P., Kopka, J., et al. (2022). Riboswitch-mediated inducible expression of an astaxanthin biosynthetic operon in plastids. *Plant Physiol.* 188, 637–652. doi: 10.1093/plphys/kiab428

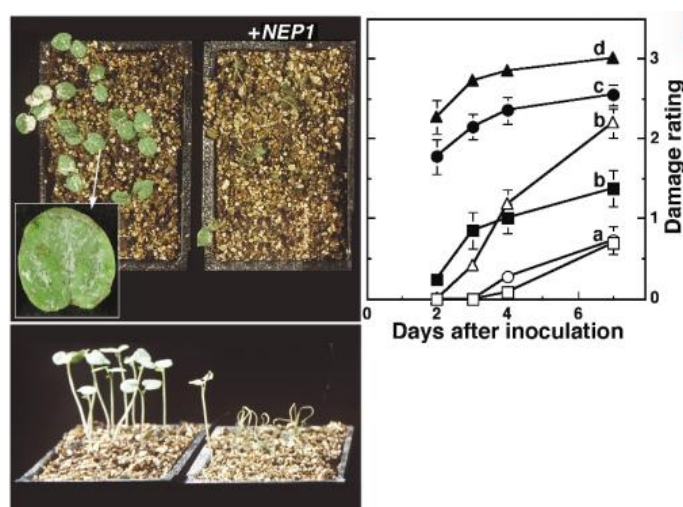
C. Development of Allelopathy in Transgenic Crops

The ability of some plants to release biochemical substances (allelochemicals) that prevent nearby plants, particularly weeds, from growing and developing is known as allelopathy. Enhancing allelopathic features in crops via genetic engineering provides an environmentally safe and natural way to reduce weeds. The identification and cloning of the genes in charge of allelochemical production is necessary for the creation of allelopathic transgenic crops. For many allelochemicals, however, our inadequate understanding of their biosynthesis processes continues to be a significant limitation. Making expressed sequence tag (EST) libraries from tissues with high allelochemical production and comparing mRNA expression profiles across

tissues expressing and not expressing allelopathic characteristics are two methods used in gene discovery. Candidate genes can be inserted into crop plants after they have been discovered to improve their capacity to suppress weeds.

D. Improvement of Biocontrol Agents

Many fungi of native origin are pathogenic to specific species of weeds. Such fungi are used to kill the weeds selectively and eliminate them from the area. These weed-killing fungi are called mycoherbicides eg. *Alternaria alternata* is used to control the weed Water hyacinth A weak mycoherbicidal strain of *Colletotrichum coccodes*, virulence was increased nine-fold by transferring NEP1 encoding a phytotoxic protein, and was more rapidly effected *Abutilon theophrasti* weed (Amsellem et al., 2002 }



Source: Enhanced weed suppression by *Colletotrichum coccodes* transformed with the NEP1 (Necrosis- and Ethylene-inducing Protein 1) gene, showing increased pathogenicity against *Abutilon theophrasti* compared to the wild-type strain. (Adapted from Amsellem et al., 2002).

E. Genome Editing for Weed Management and Herbicide Tolerance

The development of herbicide-tolerant crops and the possible targeting of weed genomes have made genome editing technologies, especially CRISPR/Cas systems, accurate and effective tools. In contrast to transgenic methods, genome editing allows for focused changes without introducing foreign DNA, which enhances public perception and regulatory acceptability.

CRISPR-based editing has been utilised to produce new tolerance traits, change metabolic pathways, and tweak herbicide target genes. Genome editing provides potential for cutting-edge weed control techniques, such as direct genetic suppression of weeds and quick generation of competitive, climate-resilient crop varieties, when combined with advancements in genomics, transcriptomics, and low-cost sequencing.

Plant	Genome Editing System	Target Gene	Target Herbicide	Reference
Rice	CRISPR-Cas9	ALS	Bispyribac-sodium	Sun et al., 2016
	CRISPR-Cas9	ALS	Imazethapyr	Wang et al., 2020
	TALEN	ALS	Bispyribac-sodium	Li et al., 2016
	Target-AID	OsALS	Imazamox	Shimatani et al., 2018
	ABE	OsTubA2	Dinitroaniline	Liu et al., 2020
	CBE	OsACCase	Gallant	Liu et al., 2020
	BEMGE	OsALS1	Bispyribac-sodium	Kuang et al., 2020
	STEMEs	ACCase	Haloxifop	Li et al., 2020
	CRISPR-Cas9	EPSPS	Glyphosate	Li et al., 2016
	ABE	ACCase	Haloxifop	Li, et al. 2018
	Prime editing	OsALS	Bispyribac sodium	Butt et al., 2020
	Prime editing	ALS	Imidazolinone	Hua et al., 2020
	Prime editing	OsACCase1	Haloxifop	Xu et al., 2020
Watermelon	CBE	CIALS	Tribenuron	Tian, 2018
Wheat	n/dCas9-PBE	TaALS, ACCase	Sulfonylurea, Imidazolinone and Aryloxyphenoxy propionate	Zhang et al., 2021
	n/dCas9-PBE	TaALS	Nicosulfuron	Zong et al., 2018
Maize	CBE	ZmALS	Sulfonylurea	Li et al., 2020
Maize	Cas9-gRNA	ALS	Chlorsulfuron	Svitashev et al., 2015
Arabidopsis	CBE	ALS	Tribenuron-methyl	Chen et al., 2017
Oilseed rape	CBE	BnALS1	Tribenuron-methyl	Wu et al., 2020
Tomato and Potato	CBE	ALS	Chlorsulfuron	Veillet et al., 2019
Soybean	CRISPR-Cas9	ALS1	Chlorsulfuron	Li et al., 2015
Flax	ssODN and CRISPR/Cas9	EPSPS	Glyphosate	Saueret al., 2016
Chile peeper	Intragenic method	EPSPS	Glyphosate	Ortega et al., 2018
Cassava	Cas9-gRNA	EPSPS	Glyphosate	Hummel et al., 2018
Potato	GVR	ALS1	Imidazolinone	Butler et al., 2016

SUMMARY AND CONCLUSION

Food security and agricultural sustainability are seriously threatened by the ongoing evolution of herbicide-resistant weed biotypes. Herbicides are still an essential part of contemporary agriculture, but their long-term efficacy has been greatly diminished by their indiscriminate and frequent usage. To accomplish long-lasting and ecologically safe weed control, integrated weed management techniques must be strengthened with cutting-edge biotechnological Intervention. Herbicide-resistant crops have been developed thanks in large part to transgenic techniques, especially the introduction of mutant genes, overexpression of EPSPS, and herbicide detoxifying mechanisms. By guaranteeing high-level transgenic expression and reducing the

possibility of gene flow, chloroplast engineering enhances these tactics even more. Furthermore, the improvement of allelopathy the genetic improvement of biocontrol agents offers promising non-chemical alternatives for weed suppression. in weed control research, genome editing technologies like CRISPR/Cas systems constitute a paradigm shift. These methods make it possible to precisely and specifically alter plant genomes, which speeds up the creation of crops that are resistant to herbicides and creates new opportunities for direct genetic control of weeds. Genome editing has the ability to completely transform weed management techniques when combined with omics technologies and systems biology methodologies

In conclusion, sustainable agriculture requires the combination of cutting-edge biotechnology tools with traditional weed control techniques. For these technologies to be successfully implemented in field settings, more research, biosafety evaluation, and governmental backing are needed.

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