Explore Effective Prevention and Control Methods of *Amaranthus Retroflexus L.* by Revealing the Weed Resistance Mechanism

Xieyu Zhang*, Yingying Zhang
Shijiazhuang Foreign Language School, Hebei, China
**Corresponding Author*

Abstract: Due to single and the frequent application of herbicides, the resistance of *Amaranthus retroflexis* is constantly evolving. This paper reviews the research progress and research methods of the resistance mechanism of *Amaranthus retroflexis L.* in recent years. The mechanism of action of Non-target-site resistance (NTSR) is usually more complex than that of target resistance (TSR), and to herbicides with dissimilar modes of action it can produce cross-resistance. In addition, the effects of *Amaranthus retroflexis* on the mutation of different herbicide targets and the alteration and selection of NTSR by P450 enzyme inhibitors are also discussed. Understanding the prevalence of resistance mechanisms in *Amaranthus retroflexis* and the coexistence of TSR and NTSR will be critical for designing sustainable weed management strategies to prevent further evolution and selection of *Amaranthus retroflexis* resistance to herbicides.

Keywords: *Amaranthus retroflexus L.*; Target Resistance (TSR); Non-Target-Site Resistance (NTSR); Weed Resistance; P450 Enzyme Inhibitors

1. Introduction

Weeds have been a main biological cause of the loss of crop yield since the beginning of agriculture. Weeds cause an average loss of 34% of crop yields worldwide, i.e. [1]. In the United States alone, the annual cost of crop yield loss due to weeds exceeds $26 billion [2]. Therefore, weeds are a significant threat to food security.

Amaranthus retroflexus L. belongs to the genus Amaranthus in the the Amaranthaceae family. It is a self-pollinating, annual dicotyledonous plant. Native to the U.S., it first appeared in China in 1905 as an invasive weed [3].The emerging characteristics of panicles composed mostly of spikes, the strong fertility of the panicle, and the ability of bearing 1-30,000 seeds per plant, the wide and adaptable habitat, and its features of spreading everywhere with human activities as a companion plant, has all led it to become widely distributed in the northeast, north, northwest and other regions of our country. It mainly grows in fields growing soybean and corn, and has also been reported in wheat, sweet potato and other fields. Owing to its adaptability advantage and strong reproductive ability, *Amaranthus retroflexus* has been recognized as a malignant weed which competes with crops for nutrients and water, often resulting in a significant decrease in yield and a bad impact on farming production.

The commercialization of ALS and other inhibitor herbicides brought about new methods of chemical control for *Amaranthus retroflexus* and is favored by farmers due to its advantages of broad spectrum of weed killing, low application quantity and high crop safety [4]. However, the long-term, single-usage of the herbicides has caused the appearance of the selectivity of weed resistance, and the dose recommended in the field has been ineffective for the control of the weed. Resistant weeds reduces the control effect of corresponding herbicides, making it difficult to select herbicides, and increases the need for mutiple types and dosages of herbicide combinations, which increase the cost of weed control and cause crop yield loss, as well as aggravate the risk of pesticide damage and pollution on the environment. At present, weed resistance is one of the important issues that threaten our country's food security.

Up to now, *Amaranthus retroflexus* has evolved to possess a variety of herbicide resistance, including photosystem II (PS II), acetyllactate synthase (ALS), and protoporphyrinogen oxidase (PPO) inhibitors [1].

Weed resistance is mainly achieved through two mechanisms: target resistance (TSR) and non-target-site resistance (NTSR). Target

resistances such as acetyl lactate synthase (ALS), also known as acetylhydroxy acid synthase (AHAS), are present in chloroplasts to catalyze the biosynthesis of branched-chain amino acids (leucine, isoleucine, and valine) in plants. ALS inhibitors mainly achieve herbicidal effects by inhibiting the activity of ALS enzymes, preventing substrates from binding to active sites and cutting off the synthesis of branched-chain amino acids [2].

In the process of weed resistance research, the study of non-target resistance is relatively rare but more complex, so it is of great significance to further explore effective weed prevention and control measures to continuously clarify the molecular basis of the resistance mechanism of *Amaranthus retroflexus*.

2. Theory of Weed Resistance Mechanism

The efficacy of herbicides usually depends on the amount of the herbicide that enters the plant cell and how long its active form interacts with its site of action, also known as the target site [5]. To fully understand the mechanisms of herbicide resistance, the mechanism of action (MOA) of herbicides must be known.

The mechanism of weed resistance mainly includes two points: first, target resistance (TSR), most herbicides act on a specific target enzyme in plants, and mutations in the functional site of the target gene will lead to the inactivation of the target enzyme, which makes the weeds become resistant. To make it more specific, single nucleotide mutations in genes encoding proteins bound to herbicides can lead to changes in single amino acids that disrupt the ability of herbicides to bind to proteins without disrupting the function of enzymes. In general, most target proteins have few amino acids in or near the herbicide-binding sites, as amino acid substitution causes TSR. Most target site mutations occur at or near the herbicide site, but some occur elsewhere in the protein structure. Target site mutations are identified by amino acids and their location in proteins, numbered starting with the protein's start codon. In some cases, mutations can produce very high levels of resistance, while in others, mutations can produce low levels (but significant) resistance. For example, the development of herbicide resistance to ALS inhibition by weeds is mainly due to amino acid substitution of ALS genes, resulting in a decrease in the affinity between herbicides and ALS in weeds, and can no longer interfere with the normal synthesis of amino acids [6]. In addition, weeds develop resistance to photosystem II. (PSII.) inhibitors, which change the spatial configuration of membrane proteins by binding to D-1 protein on the PSII. complex, hinder the electron transport process, inhibit the production of ATP and NADPH, block the carbon reduction cycle, and affect the normal photosynthesis of weeds. However, due to mutations in psbA, the encoding gene that controls D-1 protein on chloroplasts, the binding ability of herbicides to D-1 proteins is reduced, resulting in weed resistance [2]. PPO is the last kind of common enzyme in the tetrapyrrole biosynthesis pathway to produce heme and chlorophyll, and is an essential herbicide target. Two subunits of PPO, plastid PPO1 and mitochondrial PPO2, were targeted by PPO inhibitory herbicides and were encoded by two nuclear genes, PPX1 and PPX2, respectively, and mutations in PPX1 and PPX2 genes led to weed resistance to PPO herbicide inhibitors.

The second is non-target resistance (NTSR), which refers to weeds enhancing their metabolic pathways to herbicides, rapidly metabolizing herbicide toxicity into non-toxic products or reducing their toxicity, thereby causing herbicides to lose their efficacy. Substances involved in the metabolic degradation of herbicides include cytochrome monooxygenase (P450) and antioxidant enzyme system [6]. Cytochromes are involved in the weed detoxification process and improve weed herbicide resistance through overexpression. Herbicide metabolism is often described as a three-stage process, although some herbicides do not require a first step. The first stage involves minor modifications of the herbicide molecules, making them easy to further modifications. The second phase consists of combining a modified herbicide with another compound (sugar, glutathione, etc.) to facilitate the final step. The third stage uses transport enzymes to transfer herbicides to cell vacuoles (often described as the cells' trash cans) or the intracellular space outside the cell. Moving herbicides to these areas separates the herbicide from the target site. Some herbicides are further degraded in the vacuoles. Metabolism-based resistance is increasing, and it poses a unique threat compared to other resistance mechanisms.

The multiple mechanisms that cause NTSR are complicated and involve diverse gene types, many of which exist in plants as gene families.

This can lead to difficulty in identifying specific genes associated with specific cases of resistance. The expression of gene family members can be difficult to distinguish with traditional sequencing techniques and may be specifically regulated by expression at different stages of development or after herbicide treatment. Sequence variation between gene family members occurs normally and plays an evolutionary role in functional diversification, so mutations that alter the specificity of herbicide detoxification substrates may also occur. By far the most critical gene families for NTSR are p450 and GSTs. Elucidating the complicated mechanisms of NTSRs and understanding their relationship to the overall stress response pathway in plants is the focus of future research. Table 1 is the comparison between TSR and NTSR.

Table 1. Mechanism Comparison of TSR and NTSR

Aspect of Comparison	Target-Site Resistance (TSR)	Non-Target-Site Resistance (NTSR)
Core Mechanism	Direct Alteration of the Herbicide's Target.	Reduced Herbicide Availability at the Target Site.
Primary Action	Modifies the target protein itself.	Modifies herbicide movement or fate within the plant.
Key Molecular Events	• Single Nucleotide Polymorphisms (SNPs) causing amino acid substitutions in the target enzyme.	• Enhanced Metabolic Detoxification: Over-expression of enzymes like Cytochrome P450s, Glutathione S-Transferases (GSTs), and Glycosyltransferases to degrade or sequester the herbicide.
Genetic Basis	Typically involves point mutations in a single gene. The genetic change is specific and heritable.	Involves multiple genes from large gene families (e.g., P450s, GSTs). The genetic basis is more complex and less predictable.
Impact on Herbicide Binding	Directly prevents or reduces the herbicide from binding to its target site.	Indirectly reduces the concentration of active herbicide that can interact with the target.
Resistance Level	Often confers high-level, qualitative resistance (a single mutation can confer strong resistance).	Often confers lower-level, quantitative resistance (the combined effect of multiple small changes).
Cross-Resistance Potential	Generally confers resistance to herbicides with the same mode of action.	Can confer cross-resistance to herbicides with different modes of action, including those not yet used.
Example	A mutation in the ALS gene (e.g., Pro197Ala) prevents ALS-inhibiting herbicides from binding.	Over-expression of P450 enzymes allows a weed to rapidly break down multiple herbicides before they can act.

3. Weed Resistance Research Methods

Among the tested *Amaranthus* seeds, the suspected resistant populations were collected from farmland that had used a single herbicide for many years, while the sensitive groups were collected from plots without a history of herbicide use. Firstly, the resistant and sensitive populations were confirmed by whole plant dose-response test.

In vitro target enzyme activity was determined by statistical analysis to determine the RI value (i.e., resistance multiple), and the molecular basis of resistance was determined by extracting individual RNA from two populations of *Amaranthus retroflexus* , which was amplified and compared with published primers.

Table 2 is the steps of for testing weed resistance.

Table 2. Steps for Testing Weed Resistance

Step	Stage	Key Activities	Purpose & Notes
1	Sample Collection	• Collect ripe seeds from suspected resistant and nearby susceptible weed populations.	To obtain representative plant material for testing.
2	Seed Preparation	• Clean seeds (remove debris).	To ensure uniform and high germination for reliable bioassays.

3	Initial Screening	• Grow seedlings to 2–4-leaf stage (species-dependent).	To rapidly identify putative resistant populations.
4	Detailed Dose-Response	• Treat plants with a range of herbicide doses (e.g., 0.25× to 8× the label rate).	To quantify the level (fold-resistance) of resistance.
5	Molecular Analysis	• Extract DNA from plant tissue.	To determine the genetic basis (target-site vs. non-target-site) of resistance.
6	Data Integration & Reporting	• Compare phenotypic and genotypic data.	To guide growers and advisors in resistance management.
7	Monitoring & Follow-up	• Establish periodic sampling of key fields.	

The presence of non-target resistance can be assessed by HPLC-MS using malathion pre-assay and herbicide metabolism assay. In order to determine whether the resistant population of Anorci anti-branch showed non-target resistance to PPO inhibitor herbicides, especially metabolic resistance, the P450 enzyme inhibitor malathion could be used for pretreatment, applied 1 h before the herbicide application, photographed and recorded after 14 days, and the plant height and fresh weight data were statistically analyzed [6,7].

4. Further Discussion

In the 80s and 90s of the 20th century, the first herbicide-resistant weeds discovered were investigated. In most cases, resistance is granted through a target mechanism determined by the dominant allele of a single nuclear gene site.

In the molecular basis of ALS inhibitor herbicide resistance, the resistance multiple and I50 value of the resistant population were higher than those of the sensitive population. Huang et al. showed that the resistance to imidazolinone in the resistant group was 19.16 times that of the sensitive group. The results of in vitro ALS activity assay showed that the I50 value of imidazolinone in the resistant population was 21.33 times higher than that of the sensitive population. However, qRT-PCR analysis showed that there was no distinctions in ALS gene expression between resistant and sensitive populations. Sequence analysis showed that Asp-376-Glu was replaced in ALS in resistant populations. The ALS-R and ALS-S genes were fused with the CaMV 35S promoter, respectively, and introduced into Arabidopsis. Compared with Arabidopsis thaliana with transgenic ALS-S, the expression resistance of transgenic ALS-R was 13.79 times, which verified the resistance confered by Asp-376-Glu mutation [7]. The RI values were obtained in the resistance test of A. auran R1/R2 to nicosulfuron, which were 14.50 and 44.24, respectively. ALS sequence analysis showed mutations in Ser-653-Asn in R1 and Trp-574-Leu amino acid in R2. These two target mutations seem to play a major role in the resistance of Amaranth to nicosulfuron, and the Ser-653-Asn mutation is the first to be reported in Amaranth resistance [8]. Chen et al. also found these two amino acid mutations in the basis of resistance to imidazolinone in the A. reincarnan-resistant population, and also reported the third amino acid mutation in this resistant population, Ala-205-Val [9].

Target and non-target resistance may coexist in weed resistance. Huang et al. showed that the original porphyrinogen oxidase (PPO) was replaced by Arg-128-Gly in the study of the resistance molecule of A. rebel against fluorosulfonamide ether through gene sequence analysis. The response of transgenic PPO2 Arabidopsis thaliana to broad bean was verified, and the Arg-128-Gly mutation did improve the resistance of broad bean. This is the first reported target of resistance to PPO inhibitor herbicides in A. reverse. However, it also questioned whether Amaranth reverse has non-target resistance to PPO inhibitors and herbicides [6]. Cao et al. conducted a comparative analysis of resistant plants pretreated with metabolic enzyme inhibitors by HPLC-MS, showing that P450-mediated non-target resistance is helpful to resistance to flusulfame ether. GR50 values were similar in sensitive populations with or without malathion pretreatment. However, the GR50 value of the resistant population treated with malathion plus flusulfame ether decreased from 293.3 g a.i. ha− 1 to 158.3 g a.i. ha− 1. The GR50 value decreased by 46%, indicating that malathion may reverse the level of resistance of weed populations to herbicides [10].

Essentially, NTSR appears to be a more general adaptive response to herbicides than TSR. The NTSR mechanism is a subset of plant responses to abiotic stresses. NTSR can be constitutive,

stress-induced, or partially both. Constitutive NTSR has been shown to be associated with higher secondary metabolism in plants. The current hypothesis of NTSR induction is that herbicide application is a stress that triggers response pathways in all weed individuals, regardless of their sensitivity to herbicides. Genetic variation in the amplitude of response between individuals leads to variation in susceptibility, which is the basis for inducing the evolution of NTSR. NTSR mechanisms are expected to be diverse and vary between and within species. This is consistent with the evidence of complex polygenic control of NTSRs in weeds, although single-gene NTSRs can be present.

Li et al. also confirmed this in the study of the resistance mechanism of benzophenone in the multi-resistant *Amaranthus retroflexus* population, and the P450 inhibitor malathion significantly reduced the resistance rate of the weed population before phenelzine treatment. Malathion did not lead to a significant increase in the sensitivity of the sensitive population to benzodone, while the GR50 value of p-phenylacetone decreased by 50% in the resistant population after malathion treatment [11].

Multiple resistance occurred in the resistant population of *Amaranthus retroflexus*. Cao et al. also amplified and sequenced the PPX1, PPX2, and ALS gene regions covering potential mutation sites in resistant and sensitive populations. Finally, it was found that the target site of PPX2 mutation Arg-128-Gly mutation and ALS gene Ala-205-Val substitution and metabolic enhancement coexisted in the multidrug-resistant *Amaranthus* resistance population. This finding implies that the participation of metabolic enhancement can complicate weed resistance mechanisms, and even lead to resistance to agents without a history of use [10]. The independent evolution of resistance from multiple populations on short time scales requires very large population sizes, so that almost all beneficial mutations have non-negligible changes and are present at the time of selection. In weeds, large effective population sizes appear often due to their high capacity in reproduction and the emergence of soil seed banks.

5. Results and Suggestions

Mutations at target sites lead to the levels of resistance to herbicides being high, while increased metabolism involves other mechanisms of action, even herbicides with no history of use into developing resistance. The coexistence of non-target and target resistance complicates weed control, requiring other strategies to control weeds. Therefore, it is necessary to monitor the occurrence of *Amaranthus retroflexus* resistance in production, consider the rotation of different herbicides, and strictly control the amount of usage of herbicides. Accelerating the revelation of the mechanism of non-target resistance of *Amaranthus retroflexus* and developing compounds which can reverse non-target resistance will help improve the evolution of *Amaranthus retroflexus* resistance.

Global resistance to weed herbicides in cultivated land is rapidly increasing and threatens global food security. Despite resistance management strategies in the 90s of the 20th century, all major modes of herbicide action are resistant. While earlier studied cases of resistance were highly herbicide-specific, primarily under single-gene control, today's most high-profile cases often include resistance to multiple modes of action, under polygenic control, and from pre-existing stress response pathways. While "omics" approaches should be able to unravel the genetic basis of complex resistance, the emergence, selection, and spread of herbicide resistance in weed populations can only be fully elucidated by focusing on evolutionary dynamics and implementing comprehensive modeling efforts.

In view of the current situation of herbicide resistance of *Amaranthus retroflexus L*., in addition to strengthening the application and management of herbicides, comprehensive management measures should be actively taken in field production, improving tillage and cultivation methods, and physical grass control and ecological weeding methods should be tried to effectively delay the development of *Amaranthus* resistance. In the follow-up weed prevention and control management, strengthen the regular monitoring of the resistance level of the Amaranthus population, continue to understand the evolution trend of weed resistance, and formulate corresponding weed prevention and control strategies.

References

[1] Li J., Li M., Gao X.X., Fang F., Dong L.H. Advances in research on weed resistance

and its mechanism. Shandong Agricultural Sciences, 2016, 48(12):165-170

[2] Qiu F.X., Du G.P., Liu K.L., Mao A.X., Luo Kun. Advances in weed herbicide resistance and management strategies. Weed Science, 2015, 33(02):1-6

[3] Ministry of Ecology and Environment of the People's Republic of China. 2014. Announcement on the publication of the list of invasive alien species in China (the third batch) [2019-09-09]. http://sts.mee.gov.cn/swaq/lygz/201708/t20170828_420477.

[4] Ren S.Y. Main types of chemical herbicides in maize field and their application technology. Rural Science and Technology, 2022, (7):74-76

[5] Wang R.M, Zhang M.Z., Cao S.H., et al. Resistance level and mechanism of crabgrass to nicosulfuron. Pesticides, 2023, 62(6):464-468.

[6] Huang Z.F., Cui H.L., Wang C.Y., et al. Investigation of resistance mechanism to fomesafen in Amaranthus retroflexus L. Pesticide Biochemistry and Physiology, 2020, 165:104560.

[7] Huang Z.F., Chen J.Y., Zhang C.X., et al. Target-site basis for resistance to imazethapyr in redroot amaranth (*Amaranthus retroflexus* L.). Pesticide Biochemistry and Physiology, 2016, 128: 10-15.

[8] Huang Z.F., Huang H.J., Chen J.Y., et al. Nicosulfuron-resistant *Amaranthus retroflexus* L. in Northeast China. Crop Protection, 2019, 122:79-83.

[9] Chen J.Y., Huang Z.F., Zhang C.X., et al. Molecular basis of resistance to imazethapyr in redroot pigweed (*Amaranthus retroflexus* L.) populations from China. Pesticide Biochemistry and Physiology, 2015, 124:43-47.

[10] Cao Y., Huang H.J., Wei S.H., et al. Target gene mutation and enhanced metabolism confer fomesafen resistance in an *Amaranthus retroflexus* L. population from China. Pesticide Biochemistry and Physiology, 2022, 188:105256.

[11] Li W.Y., Cao Y., Liu Z., et al. Investigation of resistance mechanisms to bentazone in multiple resistant *Amaranthus retroflexus* populations. Pesticide Biochemistry and Physiology, 2022, 186:105164.

