Minireview

Insight into the mode of action of 2,4-Dichlorophenoxyacetic acid (2,4-D) as an herbicide

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Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) was the first synthetic herbicide to be commercially developed and has commonly been used as a broadleaf herbicide for over 60 years. It is a selective herbicide that kills dicots without affecting monocots and mimics natural auxin at molecular level. Physiological responses of dicots sensitive to auxinic herbicides include abnormal growth, senescence and plant death. The identification of auxin receptors, auxin transport carriers, transcription factors response to auxin, and cross-talk among phytohormones have shed light on the molecular action mode of 2,4-D as an herbicide. Here, I highlight the molecular action mode of 2,4-D according to the latest findings, emphasizing the physiological process, perception and signal transduction under herbicide treatment.

Keywords: 2,4-D (2,4-Dichlorophenoxyacetic acid); auxin; abscisic acid; ethylene; herbicide; metabolism

INTRODUCTION

It is well known that undesired plants compete with crops for water, sunshine, carbon dioxide, space and nutrients. Herbicides are agrochemicals used to control the growth of undesired weeds, and aim to significantly increase crop productivity. Most herbicides are small molecules that normally do not cause intrinsic toxicities but inhibit specific molecular target sites within critical plant biochemical and/or physiological pathways, which often cause catastrophic and lethal consequences. Herbicides are generally classified as either nonselective or selective. A nonselective herbicide is used to kill or damage all growth and is generally reserved for agricultural use or for clearing large or heavily overgrown areas; “Roundup” (developed by Monsanto Company) is one of many such herbicides. On the other hand, a selective herbicide is used to control certain types of weeds, and usually works through some type of hormone disruption. Synthetic auxin is a selective herbicide and serves as one of the most important herbicides to control weed growth in agriculture worldwide. Natural auxins are important phytohormones consisting of indole-3-acetic-acid (IAA) and its related endogenous molecules including 4-chloroindole-3-acetic acid, phenylacetic acid, and indole-3-butyric acid, all of which have similar responses in plants. Academic and industrial laboratories synthesized several synthetic auxins including 1-NAA, 2,4-D, and MCPA as early as the 1940s. Synthetic auxin herbicides opened a new era of weed control in modern crop production due to their selective action, and preferential control of dicot weeds in cereal crops (Grossmann 2003). Auxinic herbicides have been widely used to control dicot weeds in domestic lawns, commercial golf courses, and crops. However, the underlying molecular mechanism of how auxinic herbicides selectively kill dicots and spare monocots is not understood yet (Grossmann 2000; Kelley and Riechers 2007; McSteen 2010). The mechanisms of auxin biosynthesis, transport, and signal transduction are conserved in monocots and dicots make this question more complex (McSteen 2010). Early research has proposed that the selectivity of auxinic herbicide is because of either limited translocation or rapid degradation of exogenous auxin, altered vascular anatomy, or altered perception of auxin in monocots (Monaco et al. 2002; Kelley and Riechers 2007). Auxin transport is influenced by plant vascular systems (Mattsson et al. 1999; Scarpella et al. 2006). The difference in vascular tissue structure between dicots and monocots may contribute to the selectivity of auxinic herbicides. In monocot stems, the vascular tissues (the phloem and xylem) are scattered in bundles, and lack a vascular cambium; in dicot stems, the vascular tissues are formed in rings and possess a cambium.

The auxinic herbicide family contains four major chemical groups (Figure 1), including quinolinecarboxylic acids (quinmerac and quinclorac), pyridinecarboxylic acids (fluroxypr, triclopyr, clopyralid and picloram), a benzoic acid (dicamba), and phenoxyalkanoic acids (2,4-D, 2,4-DP, 2,4-DB, 2,4,5-T, MCPA, MCPB, and mecoprop). 2,4-D was one of the first synthetic auxin herbicides to be widely and commonly used

to control annual and perennial weeds (Peterson 1967). It was developed during World War II as one of many so-called phenoxy herbicides by aiming to increase crop yields for a nation at war (Quastel 1950). It was commercially released in 1946 becoming the first successful selective herbicide and allowed for greatly enhanced weed control in wheat, maize, rice, and other similar cereal crops because it specifically targets dicots. This herbicide family is said to have "initiated an agricultural revolution and laid the corner stone of present-day weed science" when it was first marketed in the 1940s. The low cost of 2,4-D has led to continued usage today and it remains one of the most commonly used herbicides in the world. There are over 600 2,4-D products currently on the market. It has also proven to be a useful chemical probe of auxin action because of the potent and stable xenobiotic compound that is not subject to the many endogenous homeostatic and metabolic mechanisms that can affect IAA (Ljung et al. 2002). Further herbicide research has led to the discovery and development of more commercial auxinic herbicides and considerably more experimental compounds that act via the auxin mode of action (Walsh et al. 2006).

Metabolic and physiological processes of 2,4-D

2,4-D is a synthetic small molecule that plants cannot degrade *in vivo*; however, members of a class of bacterial enzymes aryloxyalkanoate dioxygenases (AADs) can efficiently cleave 2,4-D into nonherbicidal dichlorophenol and glyoxylate (Wright et al. 2010). *Sphingobium herbicidovorans* is a wide-spread soil bacterium that can degrade a number of chemicals in the environment including aromatic and chloroaromatic compunds, phenols, herbicides, and polycyclic hydrocarbons (Lal et al. 2006). AAD-1 and AAD-12 proteins are derived from *Sphingobium herbicidovorans*. AAD-1 protein can degrade 2,4-D and aryloxyphenoxypropionate herbicides. AAD-12 can cleave pyridyloxyacetate auxins such as triclopyr and fluroxypyr2, which are structurally diverse members of the synthetic auxin herbicide family related to 2,4-D, and provides extended spectra of weed control. Crops like soybean and maize expressed this type of gene resistance to 2,4-D in field. Transgenic *Arabidopsis* plants expressing either *AAD-1* or *AAD-12* exhibited up to a 64-fold resistance to untransformed plants, and this application rate is about 5- to 10-fold higher than typical field use rates of 2,4-D (Wright et al. 2010).

The symptoms induced in plants by auxinic herbicides are similar to those induced by high exogenous doses of the natural auxin, IAA. At low doses, it promotes plant growth while at high doses it drives plant overgrowth, including cupping and stunting of leaves, brittleness, stunting and twisting of stems, and general abnormal growth (Grossmann 2009). According to the U.S Environmental Protection Agency (EPA), 2,4-D mainly kills plants in three ways: altering the plasticity of the cell walls, influencing the amount of protein production, and increasing ethylene production. When applied to dicotyledonous plants at effective doses, 2,4-D is absorbed through roots, stems and leaves, and is translocated to the meristems of the plant (Munro. et

al. 1992). Roots become thickened and stunted, phloem and xylem tissue in the stem disintegrates or blocks, and leaf growth ceases. Uncontrolled, unsustainable growth ensues, causing stem curl-over, leaf withering, and eventual plant death. Even plant species resistant to 2,4-D may become injured if it is applied during rapid cell division (tillering or flowering) or during rapid growth conditions. In conclusion, the application of auxinic herbicides interfere with plant physiological processes in three stages: First, stimulation of abnormal growth and the initiation of gene expression with characteristics such as stem curling, tissue swelling and the up-regulation of *NCED* genes which encode key regulatory enzymes in ABA biosynthesis and *ACS* genes which encode the rate-limiting enzyme of ethylene biosynthesis; second, inhibition of abnormal growth and physiological responses, such as stomatal closure and ROS production; and ending with senescence and cell death, including disruption of chloroplasts, and tissue necrosis (Grossmann 2009).

Early stage of 2,4-D at the molecular level: perception by receptor

Auxin was one of the first plant hormones to be discovered more than seventy years ago. It plays a crucial role in plant growth and development from embryogenesis, through all stages of development, including formation of reproductive structures and growth of the gametophyte to the regulation of plant senescence. The regulation of auxin signaling, from perception to nuclear events, and auxin transport are central to a fundamental understanding of plant growth and development. After several years of extensive study, there have been remarkable advances in understanding the molecular mechanisms of auxin. Recent research highlights include the identification of auxin receptors, modeling of auxin-dependent regulation of root and shoot meristem function, the identification of protein kinases and endocytic pathways that regulate auxin transport, and characterization of novel auxin biosynthetic pathways (Sieburth and Lee 2010). 2,4-D mimics natural auxin at the molecular level, and progress in identification of auxin signal components have helped unravel the molecular mechanisms involved in how 2,4-D acts as an herbicide.

In contrast to the main role of natural auxin, which is to promote plant growth, auxinic herbicides, like 2,4-D, kill plants. 2,4-D is structurally and functionally analogous to the natural auxin indole-3-acetic acid (IAA). That is, 2,4-D is not only structurally similar to IAA (Figure 2), but is also biologically active as an auxin in plants. Although 2,4-D looks and acts like an auxin, plants cannot metabolize this phenoxy herbicide as they can with IAA. This turns out to be the key reason 2,4-D is able to kill sensitive plants. Although 2,4-D has been used in agriculture for more than half century, the molecular mode of 2,4-D action is far from completely characterized. However, because of this structural similarity to auxin, 2,4-D, it was thought to act like auxin at the molecular level. Identification of important elements in the auxin signaling pathway provided basic clues about the molecular mode action of 2,4-D. Cell-to-cell auxin transport is largely controlled by the activity of the plasma membrane (PM)-resident auxin transporters that include the amino acid permease-like AUXIN

RESISTANTS/LIKE AUX (AUX/LAX) proteins, which mediate auxin influx (Bennett et al. 1996; Yang et al. 2006; Swarup et al. 2008); the PIN-FORMED (PIN) efflux carriers, which mediate auxin efflux, and the MULTIDRUG RESISTANCE/P-GLYCOPROTEIN (PGP) class of ATP-binding cassette auxin transporters (Luschnig et al. 1998; Petrasek et al. 2006). The natural auxin IAA enters the cell through auxin-influx carriers and rapidly controls auxin-responsive gene expression by regulating the degradation of Aux/IAA repressor proteins. Aux/IAA proteins are negative regulators of auxin-responsive genes (Mockaitis and Estelle, 2008). At low concentrations of auxin, Aux/IAA binds to ARF, thus repressing the expression of auxin inducible gene; at high auxin concentration, auxin serves as "molecular glue" that brings Aux/IAA protein to F-box protein TIR1 and mediates the degradation of Aux/IAA proteins. Thereby ARF is alleviated from Aux/IAA allowing the homo-dimerization of ARFs, and binding to AuxREs, and the subsequent activation of auxin response genes (Tan et al. 2007) (Figure 3). *Arabidopsis* encodes five TIR1-like F-box proteins (AFB1 to AFB5), with AFB1, AFB2, and AFB3 showing the closet homology to TIR1 (Dharmasiri et al. 2005b; Parry et al. 2009). Like TIR1, the AFB1, AFB2, and AFB3 proteins act as auxin receptors and bind to IAA at different affinity. However, an extensive phylogenetic analysis revealed that the AFB4/AFB5 clade has diverged significantly from the other members of the TIR1/AFB family, which contain an amino-terminal extension of unknown function (Dharmasiri et al. 2005b; Parry et al. 2009; Greenham et al. 2011). The identification of receptors for auxin perception may shed new light on herbicide selectivity. Several experiments show different AFBs bind to different types of auxinic herbicides. The main reason for the selectivity of different auxinic herbicides by AFBs may be based on the structure or size of their auxinic binding pockets controlled by the aromatic ring size in auxinic herbicides (Calderon-Villalobos et al. 2010). For example, AFB5 plays a role in synthetic auxin selectivity. The AFB5 mutant was found to be resistant to the auxinic herbicide picloram, suggesting SCF^{AFB5} may be the main receptor for picloram (Walsh et al. 2006). TIR1 is required for 2,4-D perception. TIR1 contains a binding pocket that can bind 2,4-D (Calderon-Villalobos et al. 2010). Auxin insensitive mutants showed different components of the auxin-mediated signaling pathway in *Arabidopsis,* that act in auxin transport (*axr4-2*) and as an auxin receptor (*tir1-1*), were identified to be resistant to 2,4-D. In detail, auxin insensitive mutant *axr4* is defective in auxin responses due to the mislocalization of AUX1, an auxin influx carrier, and is thus considered to be a gene involved in auxin transport (Dharmasiri et al., 2006). Previous reports also show membrane-localized AUX1 is required for the uptake of 2,4-D by the cell, implying 2,4-D enters cells through AUX1 (Marchant et al. 1999). As mentioned above, the TIR1 homologs AFB1, AFB2, and AFB3 are closely related to TIR1, with amino acid identity as high as 67-72% to each other. 2,4-D binds to the TIR1/AFB1-3 nuclear auxin receptors resulting in ubiquitin-dependent degradation of Aux/IAA repressors during the auxin signaling pathway (Dharmasiri et al. 2005a, 2005b). However, *tir1-1* is clearly resistant to 2,4-D, and plants deficient in AFB1, AFB2 and AFB3 proteins are more sensitive to 2,4-D than *tir1-1* indicating

TIR1 is the main receptor for 2,4-D (Parry et al. 2009). In a slightly weaker form than IAA, 2,4-D also acts as a "molecule glue" to mediate the interaction between Aux/IAA proteins and TIR1 F-box protein, and promote the degradation of Aux/IAAs to activate the ARF family, and thus, activate the regulation of auxin responsive genes (Figure 3). The dichlorophenyl ring and the two chlorines of 2,4-D goes into the pocket of TIR1 mimicking the double-rings of IAA. The whole structure of 2,4-D is accommodated by the overall shape and generally hydrophobic properties mimicking the whole structure of IAA and fits into the TIR1 cavity (Figure 2). The pocket for binding auxin in TIR1 is defined by two highly selective polar residues (Arg 403 and Ser 438) and together with a less selective hydrophobic environment forms a fixed cavity. The different AFB pockets fit different auxinic herbicides and partly explains how the auxin receptor can potentially bind a variety of auxinic herbicide (Tan et al. 2007). *afb5* mutant is sensitive to 2,4-D suggesting *afb5* is not a 2,4-D receptor (Walsh et al. 2006). Moreover, 2,4-D is also recognized by AUXIN BINDING PROTEIN1 (ABP1), which acts as a plasma membrane auxin receptor, suggesting there are multiple pathways to sensor 2,4-D (Sauer and Kleine-Vehn 2011; Simon and Petrasek 2011). However, unlike IAA, 2,4-D is not a good substrate for the auxin-binding protein ABP1 (Lobler and Klambt, 1985) and is poorly transported by auxin efflux carriers (Delbarre et al. 1996). Many heterogeneous synthetic substances have auxin activity, complicating studies of structure-activity and the search for a common mode of action (Ferro et al. 2010).

2, 4-D works as an herbicide through interplay with other hormones

Natural auxins are usually inactivated very quickly by conjugation and degradation (Ljung et al. 2002), while 2,4-D is retained for long periods of time, and therefore works as an herbicide. Auxin and auxinic herbicides induce growth by cell elongation as opposed to cell division. Hormone interplay is important in the regulation of plant growth and development (Aviles-Arnaut and Delano-Frier 2012; Xu et al. 2013). There are several possible mechanisms involved in 2,4-D controlled plant death mediated by multiple hormones such as ethylene and abscisic acid.

First, one of the most well-known effects of excess auxinic herbicides on dicots is the overproduction of the plant hormone ethylene (Grossmann 2003, 2009)(Figure 3). Unlike natural auxins, which are rapidly degraded by plants 2,4-D lasts for a long time resulting in the overproduction of ethylene which may result in a number of herbicide related responses, including epinasty and senescence. Ethylene has the simplest structure among phytohormones but plays an important role in a wide range of physiological reactions involved in plant developmental processes and environmental stresses (Bleecker and Kende 2000). The synthesis of ethylene is induced by various environmental stimuli and is involved in plant response to drought, wound, defense against pathogens and auxinic herbicides (Wang et al. 2002). The ethylene biosynthesis in plants starts from the production of *S*-adenosyl-methionine (SAM) through methionine and ATP combination which is catalyzed by

the enzyme SAM synthase. A further reaction, which also known as the rate-limiting step of ethylene biosynthesis is the conversion of *S*-adenosyl-methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS). The final step for ethylene production is catalyzed by ACC oxidase (ACO) producing ethylene, hydrogen cyanide and carbon dioxide from ACC. The hydrogen cyanide is converted into a harmless compound by another enzyme. Both ACS and ACO are encoded by multigene families in plants, and their expression patterns are regulated by several environmental stimuli including 2,4-D (Lin et al. 2009). In the potato plant, various auxins with different concentrations induce an increase of ethylene production. 2,4-D at 10 μM, NAA at 50 μM, and IAA at 100 μM were found to be the most effective doses in stimulating ethylene production, respectively (Arteca 1982). In *Arabidopsis*, the inflorescence tip and root produced the highest amount of ethylene in response to IAA (Abeles et al. 1992). Tests on IAA, 2,4-D, or NAA induced ethylene production in inflorescence revealed that ethylene production increased with IAA concentration from 1 μM to 100 μM and reached a plateau at 500 μM, while 2,4-D and NAA elicited much greater ethylene production than IAA at all concentrations tested in inflorescence stalks (Arteca and Arteca 2008). Further research showed auxin-insensitive mutants *axr1-12* and *axr1-3* (*axr1* works with RUB1-conjugation enzymes to regulate the activity of $SCF^{TIR1/AFB}$) produced less IAA-induced ethylene than the control, while *axr2-1* (gain-of-function mutant of *IAA7*) produced barely detectable levels of ethylene in *Arabidopsis* (Arteca and Arteca 2008). These results further showed that the induction of ethylene production in auxinic herbicides like 2,4-D depends on the auxin signaling pathway. Although the exact role of ethylene in the molecular mode of action of 2,4-D is still not fully understood, it is clearly a secondary response in plants exposed to 2,4-D.

Another effect of excess ethylene production in response to 2,4-D is the stimulation of abscisic acid (ABA) production (Figure 3). The rate-limiting factor of ABA biosynthesis is the conversion of 9-*cis*-neoxanthin to *cis*-xanthoxin by 9-*cis*-epoxycarotenoid dioxigenases (NCED), and the plastid enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED) catalysis is encoded by a family of *NCED* genes (Nambara and Marion-Poll, 2005). Several experiments show 2,4-D induces the expression of *NCED* genes, consequently increasing the production of ABA (Hansen and Grossmann 2000). 2,4-D may induce ethylene to induce the production of ABA and ABA directly mediates plant death via stomatal closure.

Reactive Oxygen Species and NO are small molecules mediated in 2,4-D toxicity

ROS is considered the main toxic effect caused by the application of 2,4-D. It is involved in 2,4-D-induced epinasty by promoting cell expansion and vascular tissue proliferation and signals molecules to induce cellular response against stress conditions (Mittler et al. 2004; Pazmino et al. 2011). The increase of ROS production induced by 2,4-D is a direct consequence of the activation of specific enzymes such as xanthine

oxido-reductase (XOD) involved in ureide metabolism, acyl-CoA oxidase (ACX) involved in fatty acid β-oxidation and jasmonic acid biosynthesis, and lipoxygenase (LOX) (Pazmino et al. 2011). NADPH oxidases from the plasma membrane have been considered as another main resource of ROS induced by 2,4-D. Another important small molecule, nitric oxide (NO), appears to be key in 2,4-D response. Under 2,4-D treatment, NO production is reduced and S-nitrosylation of peroxisomal proteins increases in pea plants (Ortega-Galisteo et al. 2012). The precise molecular mechanism of reduced NO production in 2,4-D application is still poorly understood.

Mutants resistant to 2,4-D

To date, several genes with diverse functions have been recognized as resistant to 2,4-D (Table 1). Transient activation of *PDR5* and *TPO1* in yeast shows resistance to 2,4-D (Teixeira and Sa-Correia 2002). PDR5 encodes an ABC plasma membrane multidrug transporter and TPO1 encodes a plasma membrane drug/H^+ antiporter belonging to the MFS known to confer resistance to a number of chemically and structurally unrelated compounds. *AtPDR9*, a *PDR5* homolog in *Arabidopsis* encoding a MDR transporter belonging to the ABC superfamily, was shown to be involved in auxinic herbicide resistance. Gain-of-function mutant of *AtPDR9* exhibits increased tolerance to 2,4-D and loss-of-function mutations in *PDR9* confer 2,4-D hypersensitivity, without any effect on IAA and indole-butyric acid (IBA). This suggests that PDR9 transporter 2,4-D effluxes out of plant cells specifically without influencing endogenous auxin transport (Ito and Gray 2006). In conclusion, plant cells may uptake 2,4-D by AUX1 and export it by PDR9 other than PIN genes. *TPO1* homologs in *Arabidopsis, AtTPO1* (At5gl3750) expressed in yeast, confers resistance to herbicide 2,4-D (Cabrito et al. 2009). Further microarray analysis of yeast exposed to 2,4-D revealed 11 genes encoding multidrug transporters of the ATP-binding cassette (ABC) superfamily or the major facilitator superfamily (MFS) are up-regulated upon exposure to 2,4-D (Teixeira et al. 2006). Further characterization of how plant ortholog proteins function in 2,4-D will better unravel the mystery of how 2,4-D works. These results show other transporters in plasma membrane may account for the import or export of 2,4-D except auxin influx carrier and auxin efflux carriers.

Yutaka Oono's group focuses on isolating novel anti-auxin resistant mutants (named *aar*) in *Arabidopsis* by screening mutants for root growth resistance to a putative anti-auxin, *p*-chlorophenoxyisobutyric acid (PCIB), which inhibits auxin action by interfering with the upstream auxin-signaling events. Several genes specifically affected by 2,4-D were identified using this method. aarl is a small acidic protein (SMAP1) functioning as a regulatory component that mediates responses to 2,4-D. It may work upstream of auxin signaling events since SMAP1 may be an accessory protein that stabilizes the auxin signaling complex, and the multi-gene deletion

alleles of *aar1* and the RNAi plants display 2,4-D resistant phenotypes. *aar1* is a unique auxin-signaling mutant that exhibits 2,4-D specific resistance without any changes in 2,4-D transport or metabolism, implying that plants have a partially distinct downstream response pathway for 2,4-D (Rahman et al. 2006). Further studies characterize SMAP1 protein as physically interacting with the RUB modification components, CONSTITUTIVE PHOTOMORPHOGENIC9 SIGNALOSOME (CSN) and AXR1 to regulate growth and development of *Arabidopsis* under limiting AXR1 or CSN function (Nakasone et al. 2012). *aar2-1* is a T-DNA insertion of the *AtCUL1* gene (*AXR6*), which encodes a scaffold subunit of the SCF ubiquitin ligase complex (Hellmann et al. 2003). Several *aar* mutants with a single nucleotide replacement were mapped to the *tir1* locus. Fine-mapping of *aar3* mutant shows the *AAR3* gene encodes a DCN1-like protein and regulates the response to 2,4-D in *Arabidopsis* roots (Biswas et al. 2007). Auxin resistance in root growth is generally associated with auxin transport or auxin signaling (Hobbie and Estelle, 1994). Generally, it is assumed that the gravity response in the root is tightly regulated by the polar auxin-transport system and that any perturbation of this system results in root (Luschnig et al. 1998; Muller et al. 1998; Marchant et al. 1999). Several studies show *aar* mutants are defective in auxin-related signaling rather than auxin transport. First, the normal gravity response observed in *aar* roots confirms that the mutations do not affect the auxin-transport system (Biswas et al. 2007). Second, PCIB does not require known auxin influx or efflux carrier proteins to enter or exit from the cell as was shown by analyzing *aux1* and *tir1-1* mutants, which are sensitive to PCIB (Oono et al. 2003). Finally, sequence motifs of the protein encoded by the *AAR3* gene and the identification of TIR1 and AtCUL1 alleles in PCIB screening suggest that PCIB targets the signaling component rather than auxin-transport system (Biswas et al. 2007).

In rice, overexpression of two *Aux/IAAs* (*OsIAA1* and *OsIAA4*) exhibit significant resistance to 2,4-D (Song et al. 2009b; Song and Xu 2013). Rice is a model monocot plant, which is resistant to auxinic herbicides. However, at some stages such as rapid cell division like tillering or during rapid growth conditions it shows sensitivity to 2,4-D, thus, elucidation of the mechanisms involved in monocot plant responses to 2,4-D will improve the usage of 2,4-D in rice production.

Other functions of 2,4-D

Auxins are best known for their two main uses: they form the active ingredient in rooting hormone mixtures, and they are used for the selective control of broadleaf weeds. The main function of 2,4-D at low concentrations mimics auxin to promote cell division and elongation, and as an herbicide at high concentrations to control broad-leaf growth. In tissue culture 2,4-D can replace IAA as an hormone supplement for normal cell development in plant-cell culture mediums. Besides these main functions, there is other usage of 2,4-D. Rice is

one of the most important food crops in the world, but suffers heavily from insect pests. It is known that an herbivore attack stimulates a variety of plant hormones including JA, SA and ET, which subsequently regulate defensive responses (Zhou et al. 2009). 2,4-D can be used as a potent rice defense elicitor. Low doses of 2,4-D induced a strong defensive reaction upstream of the jasmonic acid and ethylene pathways, and significantly increased trypsin proteinase inhibitor activity and volatile production. In a field experiment, 2,4-D sprayed on rice attracted the brown planthopper *Nilaparvata lugens* and its main egg parasitoid *Anagrus nilaparvatae* (Xin et al. 2012). To study the auxin signaling transduction, 2,4-D has been successfully used as an exogenous source of auxin in experiments and mutant screens, thus demonstrating the utility of 2,4-D as a chemical surrogate for IAA to dissect auxin response.

PERSPECTIVE AND CONCLUSION

2,4-D was the first herbicide to be used worldwide and it continues to be one of the most important herbicides. Despite its long history of usage in agriculture, the precise molecular mechanism of how it works as an herbicide is still poorly understood. Although much progress has been achieved in the past decade on the signal transduction of auxin, which partly explains the molecular mechanism of 2,4-D as an herbicide, the detailed mechanisms of how 2,4-D kills broadleaf plants without affecting monocot plants is still far from completely understood. Further study the auxin signaling transduction pathway and especially focus on genes like SMAP that specifically respond to 2,4-D will help elucidate the molecular mode action of 2,4-D. Unraveling the molecular mechanism of 2,4-D will not only contribute to research on how hormones work at a molecular level, such as the cross-talk between hormones, but will also provide basic information for better use of this herbicide in agriculture.

REFERENCES

Abeles FB, Morgan PW, Salveit MJ (1992) *Ethylene in plant biology.* 2nd ed. Academic Press, New York

Arteca RN (1982) Influence of IAA, NAA and 2,4-D on ethylene production by potato discs (*Solanum Tuberosum* L. cv. Red Pontiac). **Am Potato J** 59: 267-274

Arteca RN, Arteca JM (2008) Effects of brassinosteroid, auxin, and cytokinin on ethylene production in *Arabidopsis thaliana* plants. **J Exp Bot** 59: 3019-3026

Aviles-Arnaut H, Delano-Frier JP (2012) Characterization of the tomato prosystemin promoter: organ-specific expression, hormone specificity and methyl jasmonate responsiveness by deletion analysis in transgenic tobacco plants. **J Integr Plant Biol** 54: 15-32

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA (1996) *Arabidopsis* AUX1 gene: A permease-like regulator of root gravitropism. **Science** 273: 948-950

Biswas KK, Ooura C, Higuchi K, Miyazaki Y, Van Nguyen V, Rahman A, Uchimiya H, Kiyosue T, Koshiba T, Tanaka A, Narumi I, Oono Y (2007) Genetic characterization of mutants resistant to the antiauxin p-chlorophenoxyisobutyric acid reveals that AAR3, a gene encoding a DCN1-like protein, regulates responses to the synthetic auxin 2,4-dichlorophenoxyacetic acid in *Arabidopsis* roots. **Plant Physiol** 145: 773-785

Bleecker AB, Kende H (2000) Ethylene: A gaseous signal molecule in plants. **Annu Rev Cell Dev Biol** 16: 1-18

Cabrito TR, Teixeira MC, Duarte AA, Duque P, Sa-Correia I (2009) Heterologous expression of a Tpo1 homolog from *Arabidopsis thaliana* confers resistance to the herbicide 2,4-D and other chemical stresses in yeast. **Appl Microbiol Biotechnol** 84: 927-936

Calderon-Villalobos LI, Tan X, Zheng N, Estelle M (2010) Auxin perception--structural insights. **Cold Spring Harb Perspect Biol** 2: a005546

Delbarre A, Muller P, Imhoff V, Guern J (1996) Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. **Planta** 198: 532-541

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. **Nature** 435: 441-445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jurgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. **Dev Cell** 9: 109-119

Dharmasiri S, Swarup R, Mockaitis K, Dharmasiri N, Singh SK, Kowalchyk M, Marchant A, Mills S, Sandberg G, Bennett MJ, Estelle M (2006) AXR4 is required for localization of the auxin influx facilitator AUX1. **Science** 312: 1218-1220

Ferro N, Bredow T, Jacobsen HJ, Reinard T (2010) Route to novel auxin: Auxin chemical space toward biological correlation carriers. **Chem Rev** 110: 4690-4708

Greenham K, Santner A, Castillejo C, Mooney S, Sairanen I, Ljung K, Estelle M (2011) The AFB4 auxin receptor is a negative regulator of auxin signaling in seedlings. **Curr Biol** 21: 520-525

Grossmann K (2000) Mode of action of auxin herbicides: A new ending to a long, drawn out story. **Trends Plant Sci** 5: 506-508

Grossmann K (2003) Mediation of herbicide effects by hormone interactions. **J Plant Growth Regul** 22: 109-122

Grossmann K (2009) Auxin herbicides: Current status of mechanism and mode of action. **Pest Manag Sci** 66: 113-120

Hansen H, Grossmann K (2000) Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. **Plant Physiol** 124: 1437-1448

Hellmann H, Hobbie L, Chapman A, Dharmasiri S, Dharmasiri N, del Pozo C, Reinhardt D, Estelle M (2003) *Arabidopsis* AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. **EMBO J** 22: 3314-3325

Hobbie L, Estelle M (1994) Genetic approaches to auxin action. **Plant Cell Environ** 17: 525-540

Ito H, Gray WM (2006) A gain-of-function mutation in the *Arabidopsis* pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. **Plant Physiol** 142: 63-74

Kelley KB, Riechers DE (2007) Recent developments in auxin biology and new opportunities for auxinic herbicide research. **Pestic Biochem Physiol** 89: 1-11

Lal R, Dogra C, Malhotra S, Sharma P, Pal R (2006) Diversity, distribution and divergence of lin genes in hexachlorocyclohexane-degrading sphingomonads. **Trends Biotechnol** 24: 121-130

Lin Z, Zhong S, Grierson D (2009) Recent advances in ethylene research. **J Exp Bot** 60: 3311-3336

Ljung K, Hull AK, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. **Plant Mol Biol** 49: 249-272

Lobler M, Klambt D (1985) Auxin-binding protein from coleoptile membranes of corn (*Zea mays* L.). II. Localization of a putative auxin receptor. **J Biol Chem** 260: 9854-9859

Luschnig C, Gaxiola RA, Grisafi P, Fink GR (1998) EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. **Genes Dev** 12: 2175-2187

Marchant A, Kargul J, May ST, Muller P, Delbarre A, Perrot-Rechenmann C, Bennett MJ (1999) AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. **EMBO J** 18: 2066-2073

Mattsson J, Sung ZR, Berleth T (1999) Responses of plant vascular systems to auxin transport inhibition. **Development** 126: 2979-2991

McSteen P (2010) Auxin and monocot development. **Cold Spring Harb Perspect Biol** 2: a001479

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. **Trends Plant Sci** 9: 490-498

Mockaitis K, Estelle M (2008) Auxin receptors and plant development: A new signaling paradigm. **Annu Rev Cell Dev Biol** 24: 55-80

Monaco TJ, Weller SC, Ashton FM (2002) *Weed Science: Principles and Practices.* Wiley-Blackwell, New York

Muller A, Guan C, Galweiler L, Tanzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K (1998) AtPIN2 defines a locus of *Arabidopsis* for root gravitropism control. **EMBO J** 17: 6903-6911

Munro. IC, Carlo. GL, Orr. JC, Sund. KG, Wilson. RM, Kennepohl. E, Lynch. BS, Jablinske M (1992) A comprehensive, integrated

review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. **J Am Coll Toxicol 11**: 560-664

Nakasone A, Fujiwara M, Fukao Y, Biswas KK, Rahman A, Kawai-Yamada M, Narumi I, Uchimiya H, Oono Y (2012) SMALL ACIDIC PROTEIN1 acts with RUB modification components, the COP9 signalosome, and AXR1 to regulate growth and development of *Arabidopsis*. **Plant Physiol** 160: 93-105

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. **Annu Rev Plant Biol** 56: 165-185

Oono Y, Ooura C, Rahman A, Aspuria ET, Hayashi K, Tanaka A, Uchimiya H (2003) p-Chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root. **Plant Physiol** 133: 1135-1147

Ortega-Galisteo AP, Rodriguez-Serrano M, Pazmino DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: Changes under abiotic stress. **J Exp Bot** 63: 2089-2103

Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, Itoh H, Lechner E, Gray WM, Bennett M, Estelle M (2009) Complex regulation of the TIR1/AFB family of auxin receptors. **Proc Natl Acad Sci USA** 106: 22540-22545

Pazmino DM, Rodriguez-Serrano M, Romero-Puertas MC, Archilla-Ruiz A, Del Rio LA, Sandalio LM (2011) Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in pea plants: Role of reactive oxygen species. **Plant Cell Environ** 34: 1874-1889

Peterson GE (1967) The Discovery and Development of 2,4-D. **Agricul His** 41: 243-254

Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertova D, Wisniewska J, Tadele Z, Kubes M, Covanova M, Dhonukshe P, Skupa P, Benkova E, Perry L, Krecek P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zazimalova E, Friml J (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. **Science** 312: 914-918

Quastel JH (1950) 2,4-dichlorophenoxyacetic acid (2,4-D) as a selective herbicide in agricultural control chemicals, Washington, DC. **Am Chem Soc** 45: 244-249

Rahman A, Nakasone A, Chhun T, Ooura C, Biswas KK, Uchimiya H, Tsurumi S, Baskin TI, Tanaka A, Oono Y (2006) A small acidic protein 1 (SMAP1) mediates responses of the *Arabidopsis* root to the synthetic auxin 2,4-dichlorophenoxyacetic acid. **Plant J** 47: 788-801

Sauer M, Kleine-Vehn J (2011) AUXIN BINDING PROTEIN1: The outsider. **Plant Cell** 23: 2033-2043

Scarpella E, Marcos D, Friml J, Berleth T (2006) Control of leaf vascular patterning by polar auxin transport. **Genes Dev** 20: 1015-1027

Sieburth LE, Lee DK (2010) BYPASS1: how a tiny mutant tells a big story about root-to-shoot signaling. **J Integr Plant Biol** 52: 77-85

Simon S, Petrasek J (2011) Why plants need more than one type of auxin. **Plant Sci** 180: 454-460

Song Y, Xu ZF (2013) Ectopic Overexpression of an AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) gene OsIAA4 in rice induces morphological changes and reduces responsiveness to auxin. **Int J Mol Sci** 14: 13645-13656

Song Y, You J, Xiong L (2009b) Characterization of OsIAA1 gene, a member of rice Aux/IAA family involved in auxin and brassinosteroid hormone responses and plant morphogenesis. **Plant Mol Biol** 70: 297-309

Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, Levesque MP, Carrier D, James N, Calvo V, Ljung K, Kramer E, Roberts R, Graham N, Marillonnet S, Patel K, Jones JD, Taylor CG, Schachtman DP, May S, Sandberg G, Benfey P, Friml J, Kerr I, Beeckman T, Laplaze L, Bennett MJ (2008) The auxin influx carrier LAX3 promotes lateral root emergence. **Nat Cell Biol** 10: 946-954

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. **Nature** 446: 640-645

Teixeira MC, Fernandes AR, Mira NP, Becker JD, Sa-Correia I (2006) Early transcriptional response of Saccharomyces cerevisiae to stress imposed by the herbicide 2,4-dichlorophenoxyacetic acid. **FEMS Yeast Res** 6: 230-248

Teixeira MC, Sa-Correia I (2002) Saccharomyces cerevisiae resistance to chlorinated phenoxyacetic acid herbicides involves Pdr1p-mediated transcriptional activation of *TPO1* and *PDR5* genes. **Biochem Biophys Res Commun** 292: 530-537

Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, Matsumura W, Davies JP (2006) Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2,4-dichlorophenoxyacetic acid or indole-3-acetic acid in *Arabidopsis*. **Plant Physiol** 142: 542-552

Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. **Plant Cell** 14 Suppl: S131-151

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. **Proc Natl Acad Sci USA** 107: 20240-20245

Xin Z, Yu Z, Erb M, Turlings TC, Wang B, Qi J, Liu S, Lou Y (2012) The broad-leaf herbicide 2,4-dichlorophenoxyacetic acid turns rice into a living trap for a major insect pest and a parasitic wasp. **New Phytol** 194: 498-510

Xu Z, Zhang C, Zhang X, Liu C, Wu Z, Yang Z, Zhou K, Yang X, Li F (2013) Transcriptome profiling reveals auxin and cytokinin regulating somatic embryogenesis in different sister lines of cotton cultivar CCRI24. **J Integr Plant Biol** 55: 631-642

Yang Y, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E (2006) High-affinity auxin transport by the AUX1 influx carrier protein. **Curr Biol** 16: 1123-1127

Zhou G, Qi J, Ren N, Cheng J, Erb M, Mao B, Lou Y (2009) Silencing OsHI-LOX makes rice more susceptible to chewing herbivores, but enhances resistance to a phloem feeder. **Plant J** 60: 638-648

Table 1 Mutants resistant to 2,4-Dichlorophenoxyacetic acid

Gene name	description	species	Experiment condition	reference
PDR5	ABC plasma membrane multidrug transporter	yeast	transient activation in yeast	Teixeira and Sa-Correia 2002
TPO1	plasma membrane drug/H+ antiporter	yeast	transient activation in yeast	Teixeira and Sa-Correia 2002
AtPDR9	a MDR transporter belonging to the ABC superfamily	*Arabidopsis*	Gain-of-function and loss-of-function in Arabidopsis	Ito and Gray 2006
AtTPO1	plasma membrane drug/H+ antiporter in *Arabidopsis*	*Arabidopsis*	Overexpression in yeast	Cabrito et al. 2009
aar1	small acidic protein	*Arabidopsis*	multi-gene deletions and RNAi	Rahman et al. 2006
aar2	AtCUL1	*Arabidopsis*	T-DNA insertion and semidominant mutant	Hellmann et al. 2003
aar3	DCN1-like protein	*Arabidopsis*	Recessive mutant	Biswas et al. 2007
OsIAA1	Aux/IAA protein	rice	Overexpression in rice	Song et al. 2009b
OsIAA4	Aux/IAA protein	rice	Overexpression in rice	Song et al. 2013

Figure 1. Structures of auxinic herbicides belonging to different chemical families

Figure 2. Structural similarities between natural auxin, IAA; and an auxinic herbicide, 2, 4-D

Figure 3. A proposed model of the molecular mechanism on 2,4-D works as herbicide

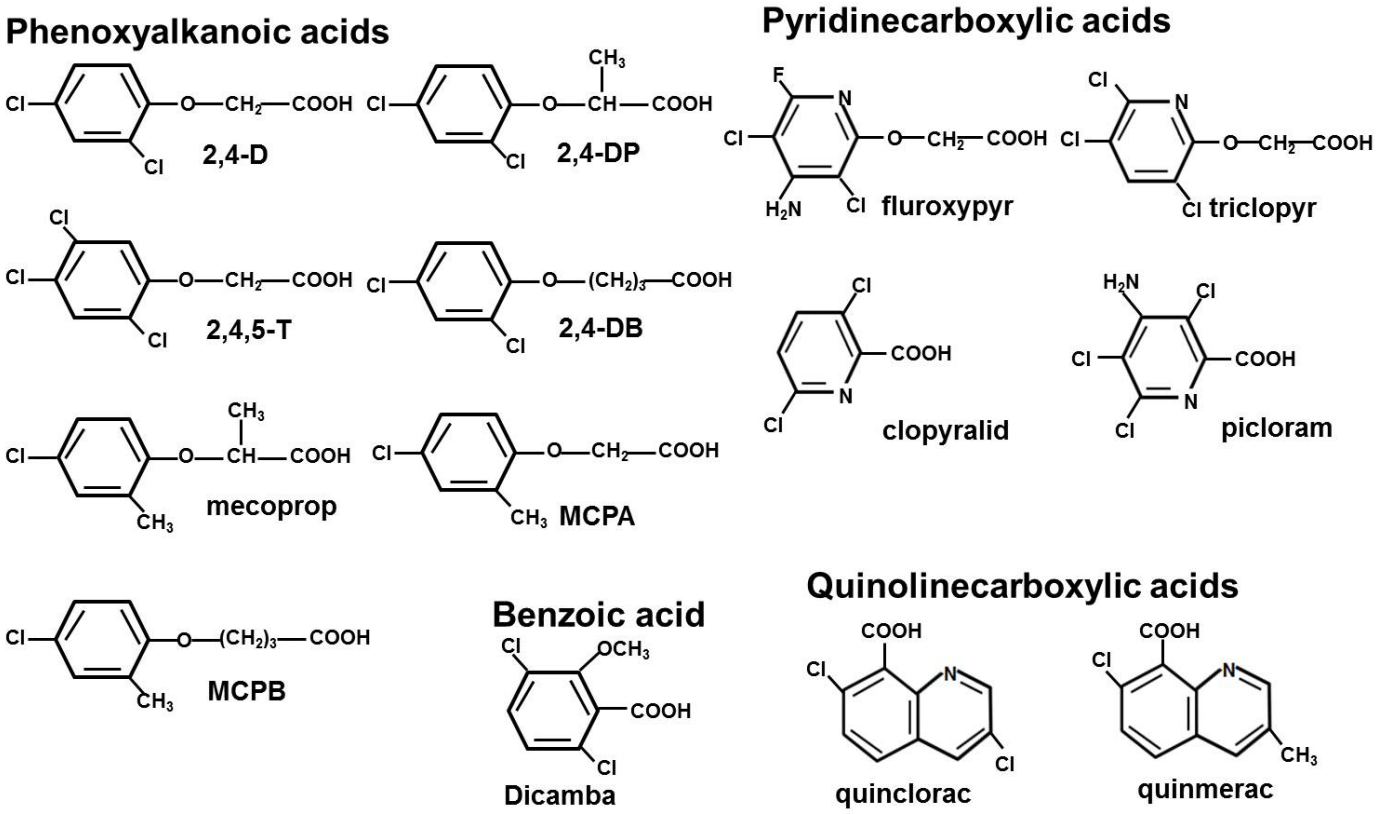

Figure 1

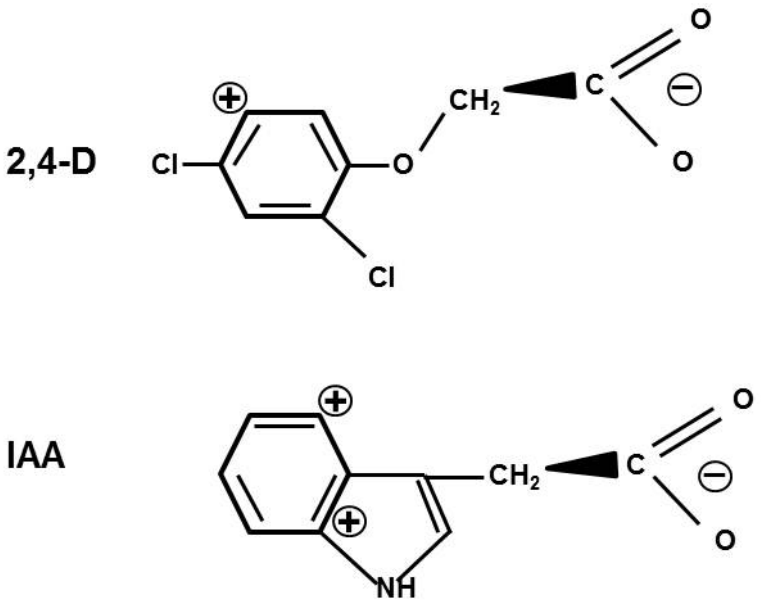

Figure 2

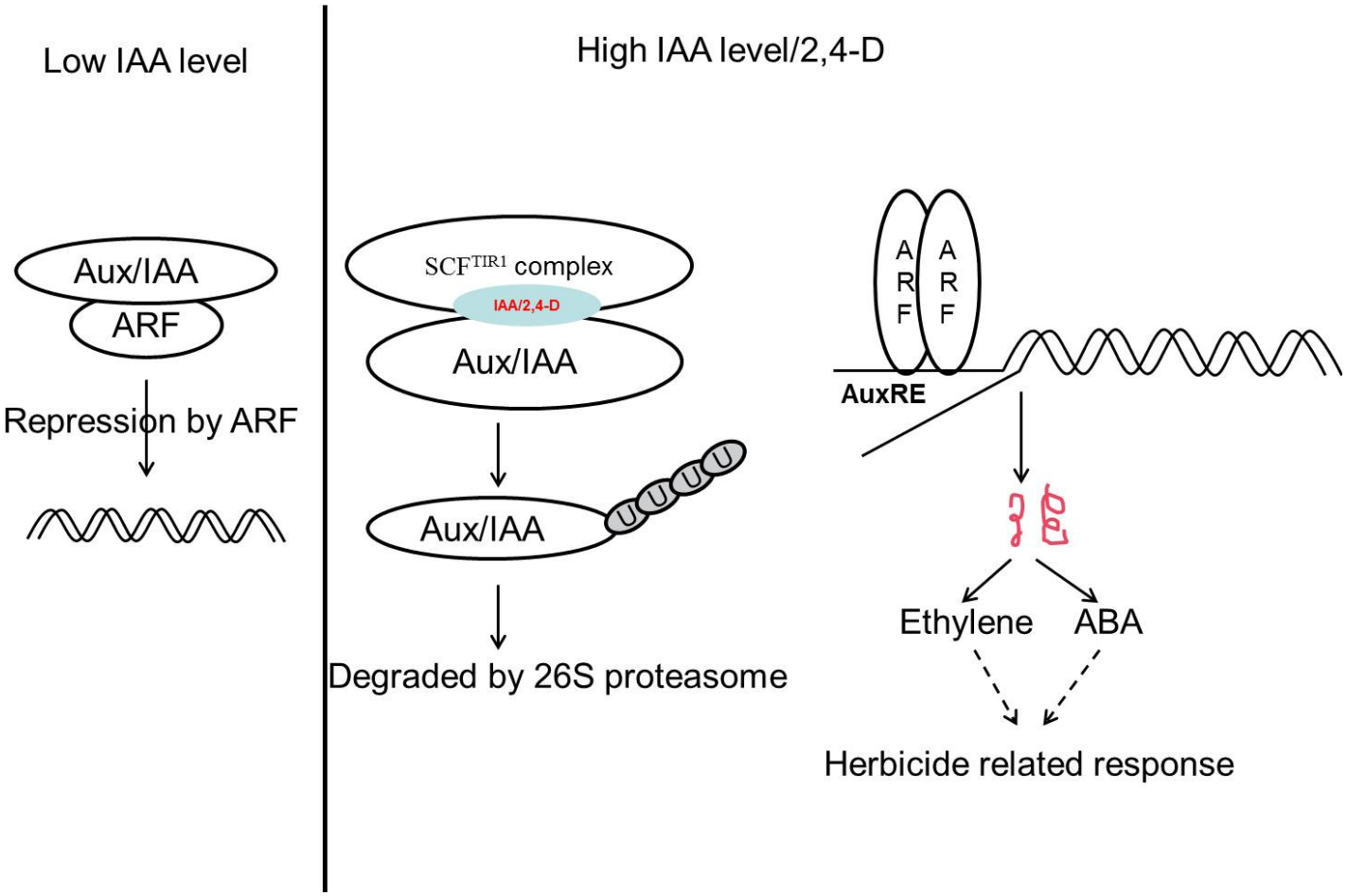

Figure 3