Supporting Information

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SI Materials and Methods

Characterization of the Resistance of the Parental Lines to Dicamba, 2,4-D, and Fluroxypyr. The resistant parental lines were subjected to a whole-plant dose–response study in the growth chamber at Monsanto (St. Louis, MO). Kochia seeds were planted in Redi-Earth (Hummert International) in 3.5-inch plastic pots and thinned to one per pot. When plants reached 10–15 cm in height, they were sprayed with dicamba (Clarity; BASF Corporation), 2,4-D (2,4-D Amine; Alligare, LLC), or fluroxypyr (Starane Ultra; Dow AgroSciences) at eight different rates using a cabinet spray chamber calibrated to deliver 140 L ha^{-1} with a TTi nozzle (TTI110015). Visual ratings and photos were taken at both 14 and 21 DAT. At 21 DAT, aerial tissues were harvested just at the soil surface, and fresh weights were measured at 21 DAT. Dose–response curves were fitted using the four-parameter log-logistic model (54, 55) through the drc package in R (version 3.4.2):

$$Y = c + \frac{d - c}{(1 + \exp\{b(\log(x) - \log(e))\})},$$

where Y is the visual control or fresh weight, the upper limit d = 100, the lower limit c = 0, b is the slope, and e is the ED_{50} for each herbicide. R/S ratio was calculated by dividing the ED_{50} values of the resistant line by the ED_{50} values of the sensitive line. Model fit, parameters estimates, SEs, and significances were all tested through the drc package in R.

For GUS staining of mature leaf, flowers, and siliques, tissues were harvested from a mature plant, whereas for young tissues, seedlings were harvested at 1, 2, 3, 4, 7, 10, and 15 days after pollination (DAP). For dicamba treatments, 19 DAP seedlings on plates were either left untreated or sprayed with the equivalent of a 0.5-lb $acre^{-1}$ rate of dicamba. Water was used for the untreated control. After 24 h, untreated and dicamba-treated plants were harvested and stained (56).

Transcriptome Generation and Analysis. To enable transcriptome sequencing, leaf tissues were harvested separately from greenhouse-grown S and R kochia biotypes and frozen on liquid nitrogen. Total RNA was isolated from three biological replicates for each tissue using TRIzol (Invitrogen) according to the manufacturer's protocol. RNA quantity was determined with a Nanodrop 8000 (Thermo-Fisher) spectrophotometer, and integrity was assessed by the BioAnalyzer (Agilent) assay with RNA integrity number greater than six. Two milligrams of total RNA was used for sequencing library preparation with the Illumina TruSeq RNA Sample Prep Kit V2 following the manufacturer's protocol. qPCR (SYBR Green PCR Master Mix; Applied Biosystems) was utilized to quantify sequencing libraries. Sequencing was performed with HiSeq2000 sequencing using 50-bp reads as described previously (57). Paired end reads were used to build kochia transcriptome assemblies for R (82,123,945 pairs) and S (68,928,684 pairs) biotypes. Reads were first quality trimmed using the FASTX toolkit (version 0.0.13; minimum 50% of bases with quality score ≥30) and then assembled using Trinity (version r2013_08_14; default settings, minimum transcript length = 300).

Fitness Cost Determination for the *KsIAA16R* Allele Through Greenhouse Studies. Three segregating F2 lines were generated independently from the above-mentioned parental lines to control the genetic background for unbiased measurement of the fitness cost. (*i*) Resistant and sensitive parents were hand pollinated. (*ii*) The genotypes of the resulting F1 progenies (heterozygous, RS) were confirmed through the TaqMan assay. (*iii*) Three F1 plants were then selfed to produce three independent F2 lines. A total of 1,440 F2 seeds (480 from each of the three F2 lines) were surface sterilized with 1% sodium hypochlorite and then placed onto 2 mL of agarose in a 48-well plate. Seeds were then allowed to germinate in a growth chamber (22 °C constant temperature, 16-/8-h day/night photoperiod) for 14 d. Germinated seeds were recorded daily, and both germinated seedlings and nongerminated seeds were genotyped through the TaqMan assay at the end of the germination test. Of the total of 1,440 F2 seeds that were included for the germination test, 92–97% were successfully genotyped. Uniform-sized seedlings with known genotypes were then transplanted into 20-L plastic pots filled with a commercial potting media fertilized with 3.6 g L^{-1} of osmocote 14-14-14. Each pot contained eight plants of RR, RS, or SS (monoculture) or a 4:4 ratio of RR/SS or RS/SS to introduce competition (equivalent to 168 plants $meter^{-2}$) under standard greenhouse conditions. Five pots of each culture condition were grown for each of three independent F2 lines, and the experiment was repeated in time. The pots were randomized weekly, and the plants were challenged with drought stress through minimum watering. Plant height was measured weekly until harvest, and RGR was calculated using the formula: RGR = (plant height at harvest − plant height at transplanting)/ plant height at transplanting. The flowering time was recorded every 2 d. Plants above the soil surface were harvested in batches based on maturity and then air dried in paper bags in the greenhouse for 6 wk before weighing for biomass and seed cleaning. Reproductive parts of the harvested plants were separated and cleaned using a coarse-mesh screen (2 mm). Seeds were then further cleaned to remove small chaff and other debris by using an air-propelled column blower. The total seed production per plant was weighed, and 100 seeds from each plant were counted and weighed to calculate the 1,000-seed mass. All data were subjected to ANOVA in R (version 3.4.2). Since there is no difference among the F2 lines, all three lines were pooled. Means of different fitness traits of different genotypes were separated using Fisher's protected least significant difference test at $P < 0.05$.

Fig. S1. Auxin transport and signaling pathways. AUX1/LAX family proteins function as importers of auxins, and PIN and ABCB family proteins function as exporters of auxins. Auxin perception takes place in the nucleus, where auxin binds to and facilitates the interaction between TIR/AFB and AUX/IAA proteins in their respective complexes; this leads to the ubiquitination (U) and degradation of the AUX/IAA proteins, thereby relieving transcriptional repression and allowing transcription of auxin-responsive genes.

Fig. S2. The kochia R biotype is cross-resistant to 2,4-D and fluroxypyr. Greenhouse-grown 10- to 15-cm-tall kochia plants were sprayed with dicamba at 0, 140, 280, 560, 2,240, 4,480, 6,720, 8,960, or 17,920 g acid equivalents (a.e.) ha^{-1}; 2,4-D at 0, 70, 140, 280, 560, 1,120, 2,240, 4,480, 6,720, or 8,960 g a.e. ha^{-1}; or fluroxypyr at 0, 17.5, 35, 70, 140, 280, 420, 560, 1,120, or 2,240 g a.e. ha^{-1} in a track sprayer. Visible injury at 14 DAT for R (*Upper*) and S (*Lower*) biotypes is shown in *A* for dicamba, *C* for 2,4-D, and *E* for fluroxypyr (*E*). Fresh weights were determined at 21 DAT and used to calculate dose–response curves using R software. The curves for R kochia (black circles) and S kochia (white circles) are shown for dicamba in *B*, 2,4-D in *D*, and fluroxypyr in *F*. UTC, untreated control.

Fig. S3. A molecular marker accurately detects the degron domain mutation in *KsIAA16*. *A* shows the detection of different genotypes using the TaqMan marker developed to distinguish the *KsIAA16R* and S alleles as described in *Materials and Methods*. *B* shows the results of a spray test of F1 individuals scored as "self-pollinated" (SS) or "heterozygous," thus resulting from the cross-pollination (RS). *Upper* shows the unsprayed check, and *Lower* shows 21 DAT with 1,120 g acid equivalents ha^{-1} (2× field use rate) of dicamba. Note that all plants designated as RS survived the spray and that all those designated as SS were killed, indicating that the marker is effective in identifying the R allele and that the trait is dominant.

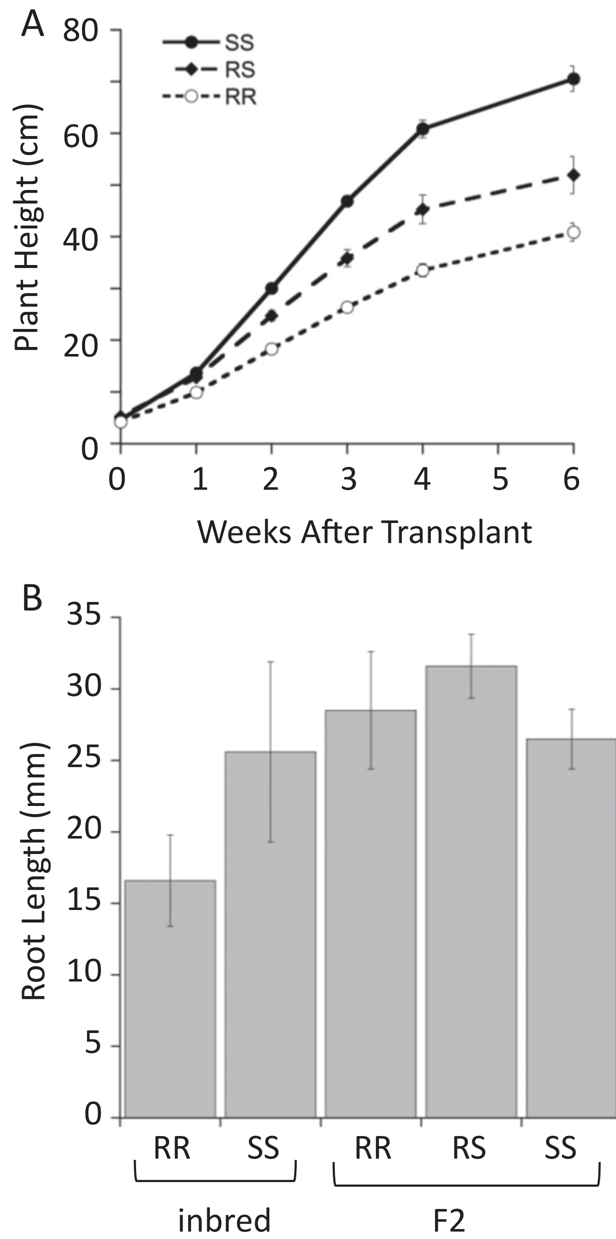

Fig. S4. Fitness data for *KsIAA16R*. *A* shows a graph of the plant height of F2 kochia plants growing in soil in absence of competition at a density of eight plants per 5-gallon pot; $n = 48$ for RR, $n = 32$ for RS, and $n = 80$ for SS. *B* shows the root length of 7-d-old seedlings by genotype of either the inbred parental lines or a segregating F2 population; $n = 17$ for RR parental, $n = 23$ for SS parental, $n = 23$ for RR F2, $n = 38$ for RS F2, and $n = 28$ for SS F2. Each data point represents the mean, and error bars represent SEs.

Table S1. GenBank accession numbers for sequences identified from *Kochia scoparia* and those used as query sequences

Gene name	GenBank accession no.
KsTIR1	MF376151
KsAFB2	MF376153
KsAFB5	MF376154
KsAFB6	MF376152
KsIAA16S	MF376149
KsIAA16R	MF376150
KsIAA16 genomic	MF465806
KsIAA1	MF376158
KsIAA3	MF376159
KsIAA6	MF376162
KsIAA7	MF376155
KsIAA8	MF376157
KsIAA9	MF376156
KsIAA13	MF376160
KsIAA18	MF376161
KsIAA31	MF376163
AtIAA7	AAC49048
AtAUX1	CAA67308
AtABP1	NP_192207
AtTIR1	Q570C0

Table S2. *Arabidopsis* AUX/IAA degron domain mutants

	Amino acid at degron position				
Mutant (protein)	1	2	3	4	5
Consensus	G	W	P	P	V/I
crane1 (IAA18)	**R**	W	P	P	V
crane2 (IAA18)	**E**	W	P	P	V
axr5-1 (IAA1)	G	W	P	**S**	V
shy2-1 (IAA3)	G	W	**S**	P	V
shy2-3 (IAA3)	**E**	W	P	P	V
shy2-6 (IAA3)	G	W	P	**L**	V
axr2-1 (IAA7)	G	W	**S**	P	V
bdl (IAA12)	G	W	**S**	P	I
slr1-1 (IAA14)	G	W	P	**S**	V
slr1-2 (IAA14)	G	W	**S**	P	V
slr1-3 (IAA14)	G	W	**A**	P	V
axr3-1 (IAA17)	G	W	P	**L**	V
axr3-3 (IAA17)	G	W	P	P	**G**
axr3-101 (IAA17)	**E**	W	P	P	V
msg2-1 (IAA19)	G	W	P	**S**	V
msg2-2 (IAA19)	**R**	W	P	P	V
msg2-3 (IAA19)	G	W	P	**L**	V
msg2-4 (IAA19)	G	W	**L**	P	V
iaa28-1 (IAA28)	G	W	**L**	P	V
iaa16-1 (IAA16)	G	W	**L**	P	V

Amino acids differing from the consensus are indicated in bold.