Supplementary material

Table S1. Data of RNA extraction of *L. rigidum* sensitive biotype.

Sample	Dil to 20 ng/ul in 20ul **1ug**						
	Dil	**Qubit ng/ul**		**Average**	**origin c.**	**RNA**	**H20**
L. rigidum	20	18,50	18,50	18,50	370,00	1,08	18,92

Sample	KAPA			Tapestation			QPCR		Qubit	QPCR dil to 8nM	
	index		**Qubit ng/ul**	TS ng/ul	Av Mw	conc. nM	conc. nM	ng/ul	nM	ul templát	EB
L. rigidum	21	GTTTCGGA	7,64	8,82	260	52,1	58,64	9,42	44,5221445	2,73	17,27

Figure S1. Gel picture of RNA sample of *L. rigidum* sensitive biotype.
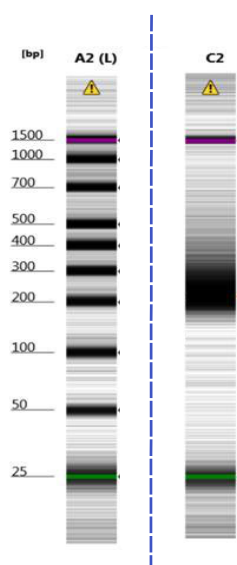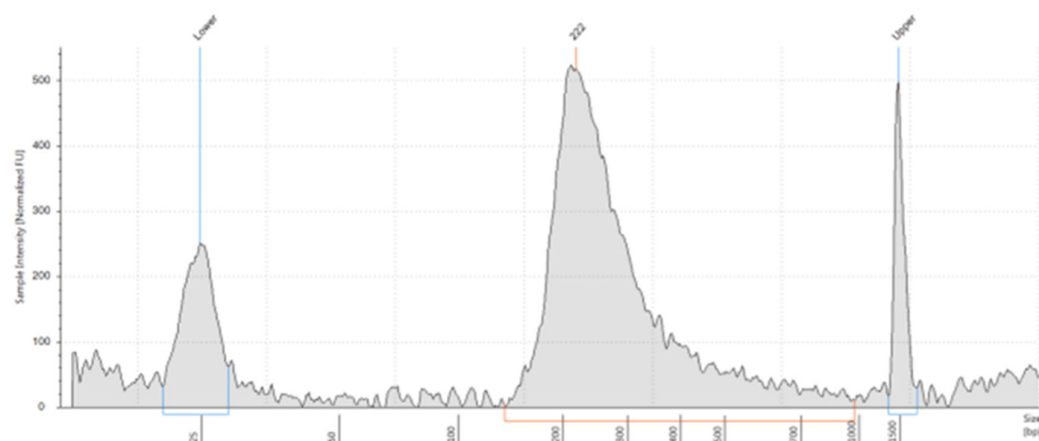
A2(L): RNA ladder; C2: sample

Figure S2. Data of library preparation of *L. rigidum* sensitive biotype.
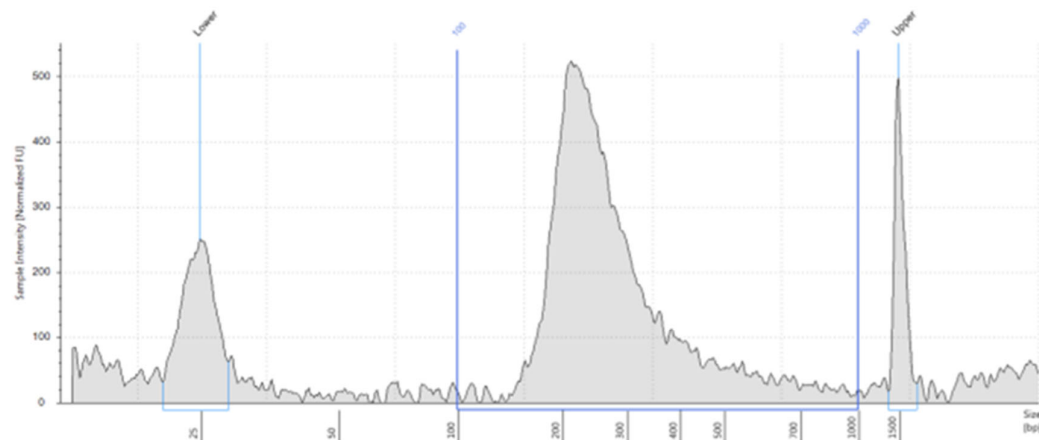
C2: Plant pool

Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
C2	2050	Plant pool	⚠	Caution! Expired ScreenTape device

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Peak Comment	Observations
25	424	-	26100	-		Lower Marker
222	2050	-	14200	100.00		
1500	250	250	256	-		Upper Marker

Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
100	1000	299	2070	12500	79.36		■

Figure S3. Statistic data and R script of drc analysis.

```
Model fitted: Log-logistic (ED50 as parameter) (4 parms)

Parameter estimates:

                       Estimate Std. Error  t-value   p-value
hill:(Intercept)        6.96824    1.79325   3.8858 0.0004822 ***
min_value:(Intercept) 29.12218    1.34760  21.6105 < 2.2e-16 ***
max_value:(Intercept) 99.42234    0.74323 133.7699 < 2.2e-16 ***
ED_50:(Intercept)       3.56898    0.11110  32.1247 < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error:

 3.132974 (32 degrees of freedom)
```

```
Model fitted: Log-logistic (ED50 as parameter) (4 parms)

Parameter estimates:

                       Estimate Std. Error t-value   p-value
hill:(Intercept)       3.479764   0.185353  18.774 2.531e-11 ***
min_value:(Intercept) 0.601996   0.674921   0.892    0.3875
max_value:(Intercept) 91.959942  1.279306  71.883 < 2.2e-16 ***
ED_50:(Intercept)      0.793142   0.014352  55.263 < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error:

 1.754994 (14 degrees of freedom)
```

```r
library(drc)
#data average
library(readr)
average_data <- read_delim("average_data.csv",";",
          escape_double = FALSE,
          locale = locale(decimal_mark = ",",
                          grouping_mark = ""), trim_ws = TRUE)
#Reshaping wide format to long format with gather() function
library(tidyr)
long.data.average <- gather(average_data, Reps, Value, Rep1:Rep3)
head(long.data.average)
# Fitting log-logistic model
curved_fit <- drm(
  formula = Value ~ conc,
  data = long.data.average,
  fct = LL.4(names = c("hill", "min_value", "max_value", "ED_50")))
summary(curved_fit)
# plot the data with plot()
plot(curved_fit, broken = TRUE, type = "all",
     xlab = "Lolium", xlim = c(0.25, 8),
     ylab = "Biomass (C%)")
#preparation for ggplot2
## new dose levels as support for the line
newdata <- expand.grid(conc=exp(seq(log(0.25), log(8), length=100)))
pm <- predict(curved_fit, newdata=newdata, interval="confidence")
View(newdata)
View(pm)
# new data with predictions
```

```
newdata$p <- pm[,1]
newdata$pmin <- pm[,2]
newdata$pmax <- pm[,3]
View(newdata)
#plot with ggplot2
library(ggplot2)
# need to shift conc == 0 a bit up, otherwise there are problems with coord_trans
long.data.average$conc0 <- long.data.average$conc
long.data.average$conc0[long.data.average$conc0 == 0] <- 0.25
# plotting the curve
dose_curve <- ggplot(long.data.average, aes(x = conc0, y = Value)) +
  geom_point() +
  geom_ribbon(data=newdata, aes(x=conc, y=p, ymin=pmin, ymax=pmax), alpha=0.2) +
  geom_line(data=newdata, aes(x=conc, y=p)) +
  coord_trans(x="log") +
  xlab("Lolium Dose (x)") + ylab("Biomass (C%)")+
  theme_bw()+
  theme(text = element_text(size=16), #edit the size of the text
        axis.text = element_text(size = 14)) +
  scale_x_discrete(limits = c(0.25, 1, 2, 4, 8)) #change legend for x-axis
show(dose_curve)
```

Table S2. Primer sequences that were used for amplification of three part of *ahas* gene in *L. rigidum*
Numbering of location was based on *in silico* identified mRNA sequence of *ahas* gene (MK492446).

Primer name	Length bp	Ann. °C	Primer loc. bp	Sequence (5′–3′)	Covered amino acid sub. point
Lolriahas1F	418	58	207-624	CAAGGGCGCCGACATCCT	122, 197, 205
Lolriahas1R				CTTCTTCCTCGCCTCCTCCG	
Lolriahas2F	259	58	917-1175	GTCTTGGCAACTTCCCCAG	376, 377
Lolriahas2R				GATGTCAAGCTCGCTTTGCA	
Lolriahas3F	399	56	1531-1929	GGTAGCTTCCTCATGAACATTC	574, 653, 654
Lolriahas3R				GATGGCAGGATTGCGTATTAA	

Figure S4. The cds of *ahas* gene in *L. rigidum* indicating regions amplified by specific primers. Coloured lines show positions of the three primer pairs. Numbers between round brackets are start and end positions of primers numbered on cds sequence (MK492446). Vertical grey lines show amino acid positions effected to herbicide resistance-conferring amino acid substitutions. The number after the amino acid refers to the amino acid position in the *ahas* gene of *Arabidopsis thaliana*.
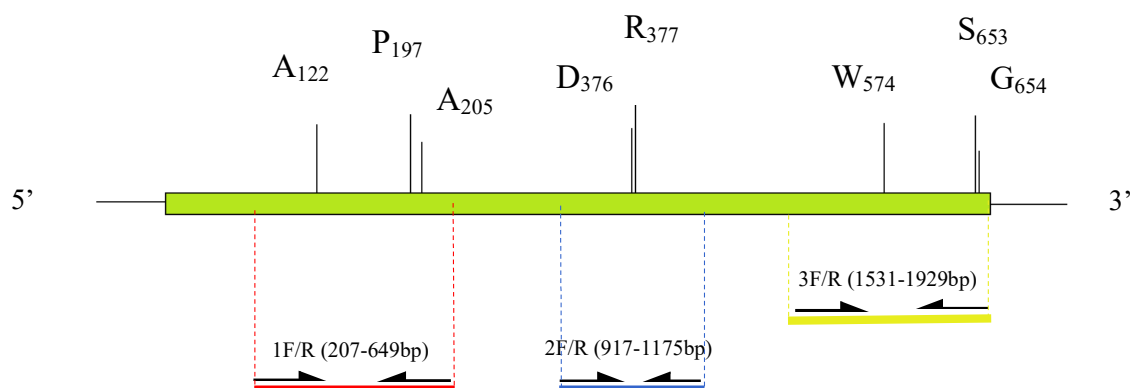
Figure S5. PCR products and bioanalyzer analysis of three part of *ahas* gene in *L. rigidum* (Lolriahas1-3).

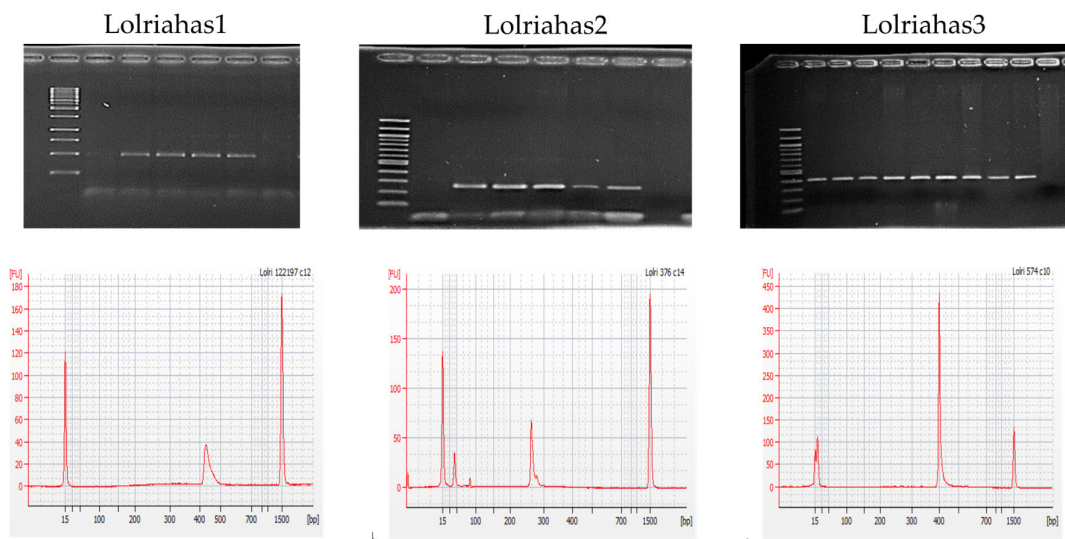| Lolriahas1	Lolriahas2	Lolriahas3

Figure S6. Amino acid substitution at Pro197 position in *L. rigidum*. Reference sequence (R) is *A. thaliana* AHAS enzyme. Yellow boxes represent the known mutation points Pro197 and Ala205. Below wild type and resistant AHAS sequences of *L. rigidum* are visualized. Coloured boxes represent the detected mutations at Pro197 position.

A. thaliana sens. biotype 171 GATNLVSGLADALLDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVED 230

L. rigidum sens. biotype   GATNLVSALADALLDSIPMVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVLDVED

Mutation Pro197Thr   GATNLVSALADALLDSIPMVAITGQVTRRMIGTDAFQETPIVEVTRSITKHNYLVLDVED

Mutation Pro197Ala   GATNLVSALADALLDSIPMVAITGQVARRMIGTDAFQETPIVEVTRSITKHNYLVLDVED

Mutation Pro197Ser   GATNLVSALADALLDSIPMVAITGQVSRRMIGTDAFQETPIVEVTRSITKHNYLVLDVED

Mutation Pro197Gln   GATNLVSALADALLDSIPMVAITGQVQRRMIGTDAFQETPIVEVTRSITKHNYLVLDVED

Figure S7. Electropherogram of a single nucleotide substitution of *ahas* gene in *L. rigidum*. Nucleotide change of CCG to ACG was detected at the 197 amino acid position that resulted proline/threonine substitution numbered 273-275.
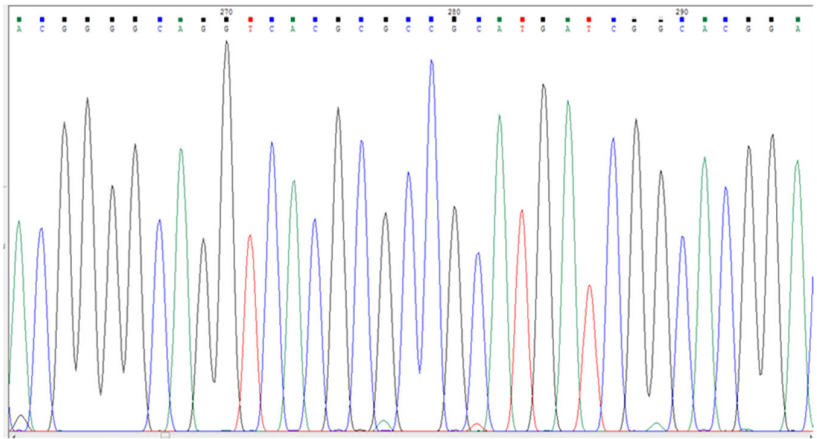

Figure S8. Amino acid substitution at Trp574 position in *L. rigidum*. Reference sequence (R) is *A. thaliana* AHAS enzyme. Yellow boxes represent the known mutation point. Wild type and resistant AHAS sequences of *L. rigidum* are visualized. Green box represents the detected Trp574Leu mutation

A. thaliana sens. biotype	566	QHLGMVMQ**W**EDRFYKANRAHTFLGDP 591
L. rigidum sens. biotype		QHLGMVVQ**W**EDRFYKANRAHTYLGNP
Mutation Thr574Leu		QHLGMVVQ**L**EDRFYKANRAHTYLGNP

Figure S9. Nucleotide mutation at Asp376Asp GAT/GAC position of AHAS-resistant biotype in *L. rigidum*.

A. thaliana sens. biotype	1098	GGGGTAAGGTTTGAT**GAT**CGTGTCACGGGTAA 1131
L. rigidum sens. biotype		GGCGTGCGGTTTGAT**GAT**CGCGTGACTGGGAA
Mutation Asp376		GGCGTGAGGTTTGAT**GAC**CGCGTGACTGGGAA

Table S3. Amino acid substitutions of total 50 AHAS-resistant biotype in *L. rigidum*.

Amino acid substitution in *L rigidum* population (total 50 biotype)	Biotypes
Pro197Thr	16
Pro197Ser	14
Pro197Gln	11
Pro197Ala	3
Trp574Leu	6