

Supplementary data

Both Allene Oxide Synthases genes are Involved in the Biosynthesis of Herbivore-Induced Jasmonic Acid and Herbivore Resistance in rice

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Table S1. Volatile compounds emitted from non-manipulated and SSB-infested plants (24 h) of *as-aos1*, *as-aos2* and wild-type plants.

Table S2. Primers and probes used in this study.

Figure S1. The nucleotide and deduced amino acid sequence of *OsAOS1* and *OsAOS2*.

Figure S2. Alignment of the nucleotide and amino acid sequence of *OsAOS1* and *OsAOS2*.

Figure S3. Expression levels of *OsAOS1* and *OsAOS2* in rice plants that were treated with SA.

Figure S4. The transformation vector used to generate *as-aos1* and *as-aos2* lines.

Figure S5. DNA gel-blot analysis of *as-aos1* and *as-aos2* lines.

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Figure S8. Growth phenotypes of *as-aos1*, *as-aos2* and WT plants at one-week-old seedling stage, tillering stage and heading stage.

Figure S9. The setup used for herbivore bioassays.

Table S1. Volatile compounds emitted from non-manipulated and SSB-infested plants (24 h) of *as-aos1*, *as-aos2* and wild-type lines. Data represent mean amount (% of internal standard peak area, $\pm$ SE) of five replications. Letters in the same row indicate significant differences among treatments ($P < 0.05$, Tukey's HSD post-hoc test).

No.	Chemical	WT	as1-5	as2-20	WT+SSB	as1-5+SSB	as2-20+SSB
1	2-heptanone	3.52 $\pm$ 0.82b	1.07 $\pm$ 0.05b	2.64 $\pm$ 1.85b	41.39 $\pm$ 7.63a	36.59 $\pm$ 12.05a	19.8 $\pm$ 7.37ab
2	2-heptanol	2.68 $\pm$ 0.14c	2.83 $\pm$ 0.94c	1.81 $\pm$ 0.96c	34.06 $\pm$ 4.65a	15.75 $\pm$ 4.01b	12.76 $\pm$ 4.58b
3	α -thujene	0.63 $\pm$ 0.25bc	0.40 $\pm$ 0.19c	0.55 $\pm$ 0.36c	3.06 $\pm$ 0.45a	0.81 $\pm$ 0.33bc	0.86 $\pm$ 0.4bc
4	α -pinene	0.71 $\pm$ 0.33a	0.18 $\pm$ 0.1a	0.66 $\pm$ 0.49a	0.45 $\pm$ 0.23a	0.79 $\pm$ 0.34a	0.58 $\pm$ 0.24a
5	myrcene	5.53 $\pm$ 1.42a	4.03 $\pm$ 0.68a	3.12 $\pm$ 1.17a	4.03 $\pm$ 0.42a	5.23 $\pm$ 0.58a	4.44 $\pm$ 0.88a
6	(+)-limonene	8.74 $\pm$ 1.1c	9.57 $\pm$ 6.17bc	6.09 $\pm$ 3.41c	21.39 $\pm$ 3.83a	22.74 $\pm$ 3.52a	10.89 $\pm$ 0.58bc
7	(E)-linalool oxide	0.95 $\pm$ 0.19b	1.78 $\pm$ 1.56ab	0.86 $\pm$ 0.12b	7.29 $\pm$ 1.75a	4.76 $\pm$ 1.76ab	3.63 $\pm$ 1.25ab
8	linalool	12.93 $\pm$ 6.8bc	4.28 $\pm$ 0.12c	7.47 $\pm$ 1.15bc	87.45 $\pm$ 14.05a	61.99 $\pm$ 23.6ab	28.72 $\pm$ 6.28bc
9	methyl salicylate	1.68 $\pm$ 0.79a	0.89 $\pm$ 0.14a	1.11 $\pm$ 0.19a	2 $\pm$ 1.03a	2.39 $\pm$ 0.8a	2.49 $\pm$ 1.24a
10	unknown 1	3.08 $\pm$ 1.08a	1.56 $\pm$ 0.12a	5.03 $\pm$ 2.07a	1.21 $\pm$ 0.33a	0.95 $\pm$ 0.24a	0.45 $\pm$ 0.05a
11	unknown 2	0.77 $\pm$ 0.54a	0.69 $\pm$ 0.01a	1.35 $\pm$ 0.49a	2.22 $\pm$ 0.56a	4.16 $\pm$ 2.36a	2.89 $\pm$ 1.47a
12	α -copaene	4.62 $\pm$ 1.65b	3.79 $\pm$ 1.56b	2.94 $\pm$ 1.48b	11.11 $\pm$ 2.44a	4.66 $\pm$ 1.19b	4.87 $\pm$ 1.04b
13	n-tetradecane	0.35 $\pm$ 0.08b	0.21 $\pm$ 0.13b	0.37 $\pm$ 0.04b	33.40a	1.45 $\pm$ 0.49b	1.19 $\pm$ 0.59b
14	sesquithujene	2.05 $\pm$ 1.18a	1.5 $\pm$ 0.35a	3.58 $\pm$ 1.58a	3 $\pm$ 0.8a	2.79 $\pm$ 0.57a	11.81 $\pm$ 6.66a
15	(-)- α -cedrene	1.66 $\pm$ 0.56a	0.94 $\pm$ 0.21a	2.86 $\pm$ 1.07a	1.21 $\pm$ 0.25a	1.3 $\pm$ 0.23a	9.46 $\pm$ 5.42a
16	(E)- β -caryophyllene	4.86 $\pm$ 0.87b	1.95 $\pm$ 0.16b	1.92 $\pm$ 0.52b	16.2 $\pm$ 1.16a	5.72 $\pm$ 1.48b	5.9 $\pm$ 1.23b
17	(E)- α -bergamotene	2.09 $\pm$ 1.46ab	2.15 $\pm$ 1.22ab	0.97 $\pm$ 0.2b	9.68 $\pm$ 3.23ab	10.34 $\pm$ 2.46a	5.06 $\pm$ 0.99ab
18	sesquisabinene	2.14 $\pm$ 1.47a	1.9 $\pm$ 1.09a	2.99 $\pm$ 1.05a	8.66 $\pm$ 2.77a	8.62 $\pm$ 2.02a	5.84 $\pm$ 1.83a
19	(E)- β -farnesene	1.98 $\pm$ 0.39a	1.68 $\pm$ 0.16a	3.17 $\pm$ 1.27a	3.98 $\pm$ 1.29a	3.57 $\pm$ 0.94a	4.02 $\pm$ 1.85a
20	ar-curcumene	3.53 $\pm$ 2.47a	2.62 $\pm$ 0.64a	3.04 $\pm$ 1.1a	6.88 $\pm$ 1.75a	7.41 $\pm$ 1.27a	12.22 $\pm$ 5.32a
21	Zingiberene	6.81 $\pm$ 2.47a	3.77 $\pm$ 1.32a	12.81 $\pm$ 7.69a	22.54 $\pm$ 7.63a	19.73 $\pm$ 5.38a	12.1 $\pm$ 3.42a
22	β -bisabolene	5.06 $\pm$ 3.49a	2.86 $\pm$ 0.37a	5.82 $\pm$ 2.61a	13.08 $\pm$ 4.08a	12.27 $\pm$ 2.94a	20.4 $\pm$ 9.6a
23	β -sesquiphellandren	5.99 $\pm$ 3.49a	3.75 $\pm$ 0.75a	2.79 $\pm$ 0.24a	18.64 $\pm$ 5.98a	18.82 $\pm$ 4.38a	11.64 $\pm$ 2.98a
24	(E)- γ -bisabolene	2.94 $\pm$ 1.29a	2.52 $\pm$ 1.87a	2.25 $\pm$ 1.05b	10.4 $\pm$ 3.51a	7.9 $\pm$ 2.46a	2.54 $\pm$ 1.99a
Total		85.3 $\pm$ 34.33	56.92 $\pm$ 19.91	76.2 $\pm$ 32.16	363.33 $\pm$ 69.82	260.74 $\pm$ 75.4	194.56 $\pm$ 67.26

Table S2. Primers and probes used in this study

Gene name	TIGR ID	Description	Forward primer (5'---3')	Reverse primer (5'---3')	Probe (5'---3')
<i>OsAOS1</i>	Os03g55800.1	RT-PCR	GCTAGTAGCTAGCTCGGGGA	CAGTGCAACTCCGTATCCGT	
<i>OsAOS2</i>	Os03g12500.1	RT-PCR	TTTGCCATCGTGGACACACT	GCTATGTACGTGGGGGAAGG	
<i>OsAOS1</i>	Os03g55800.1	QRT-PCR	TGATCACCAAGTGGGTGCTG	CGAGTGGAGGAGCGTGTCC	CTCAGCCCCTGCTCAGCCTC
<i>OsAOS2</i>	Os03g12500.1	QRT-PCR	TGCCCATGATCATCGAGGAT	TGTAGTCGGAGCTGATGAGGAA	CTCCTCCACACGCTGCCGCTG
<i>OsACT</i>	Os03g50885	QRT-PCR	TGGACAGGTTATCACCATTGGT	CCGCAGCTTCCATTCCTATG	CGTTTCCGCTGCCCTGAGGTCC

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(b)

[illegible]

Figure S1. The nucleotide and deduced amino acid sequence of OsAOS1 (a) and OsAOS2 (b). Gray shading indicted P450 family, red frame indicted phosphorylation site.

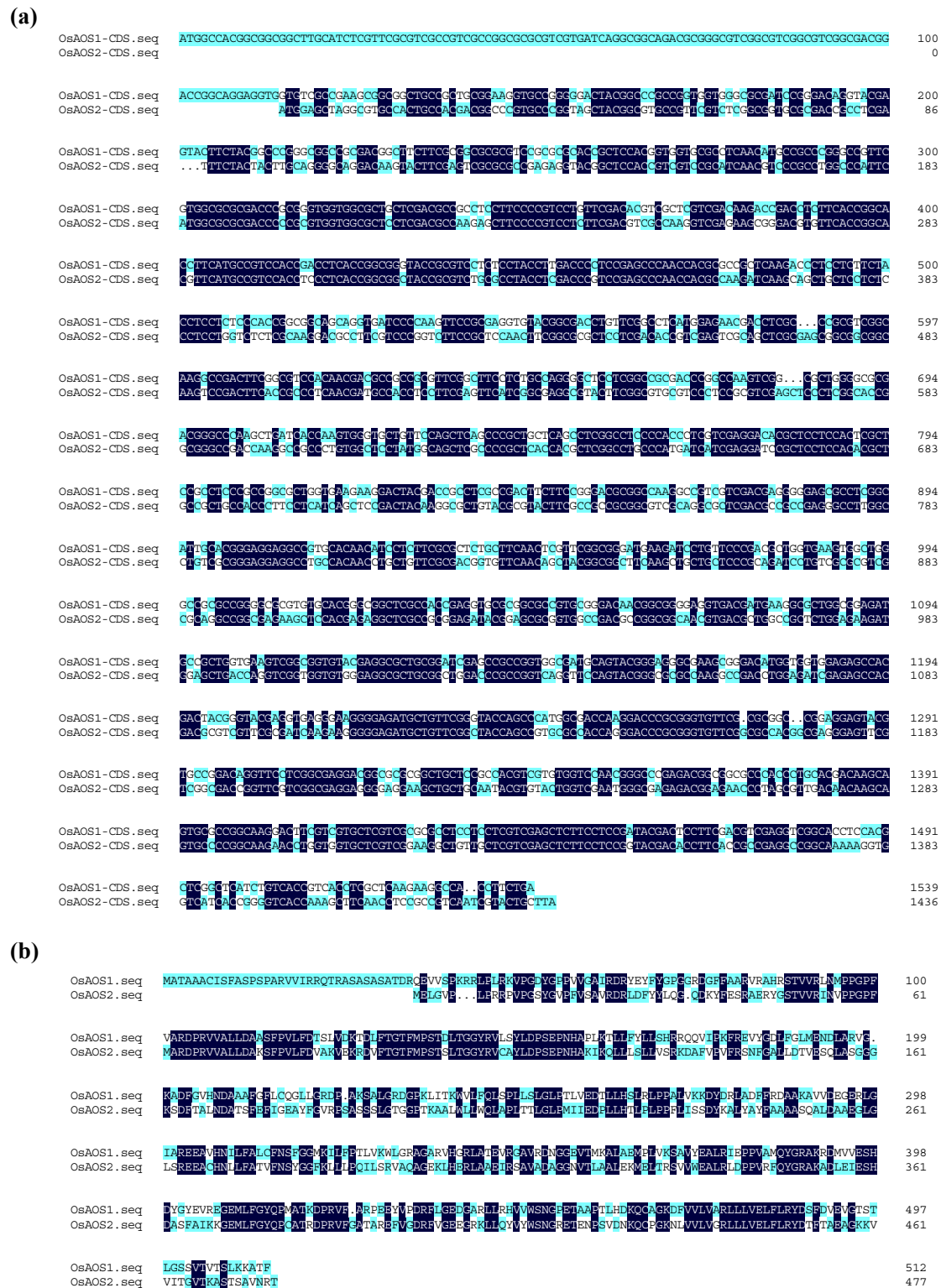


Figure S2. Alignment of the nucleotide (a) and amino acid (b) sequence of OsAOS1 and OsAOS2. The blue shading indicates the bases that are identical in the two sequences.

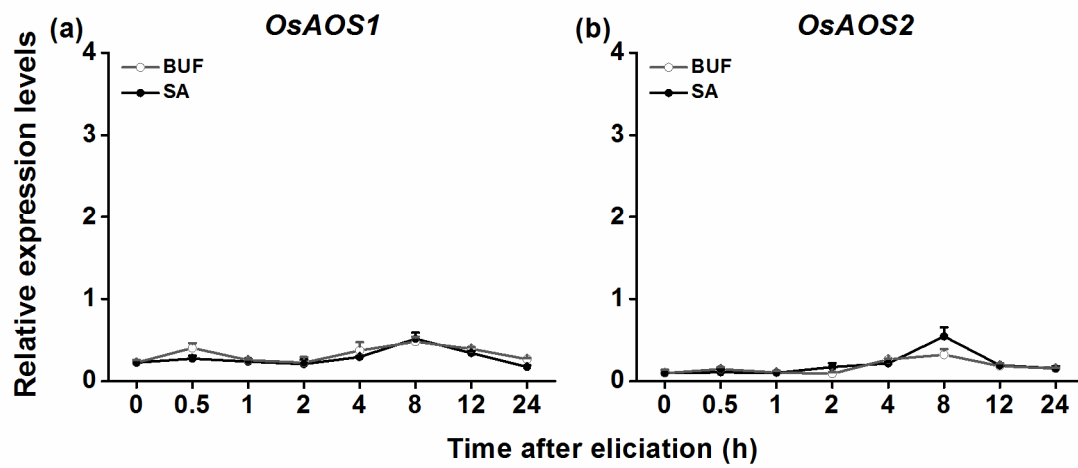


Figure S3. Expression levels of *OsAOS1* and *OsAOS2* in rice plants that were treated with SA. Mean expression levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* (a) and *OsAOS2* (b) in rice leaf sheaths that were treated by buffer (BUF) or salicylic acid (SA).

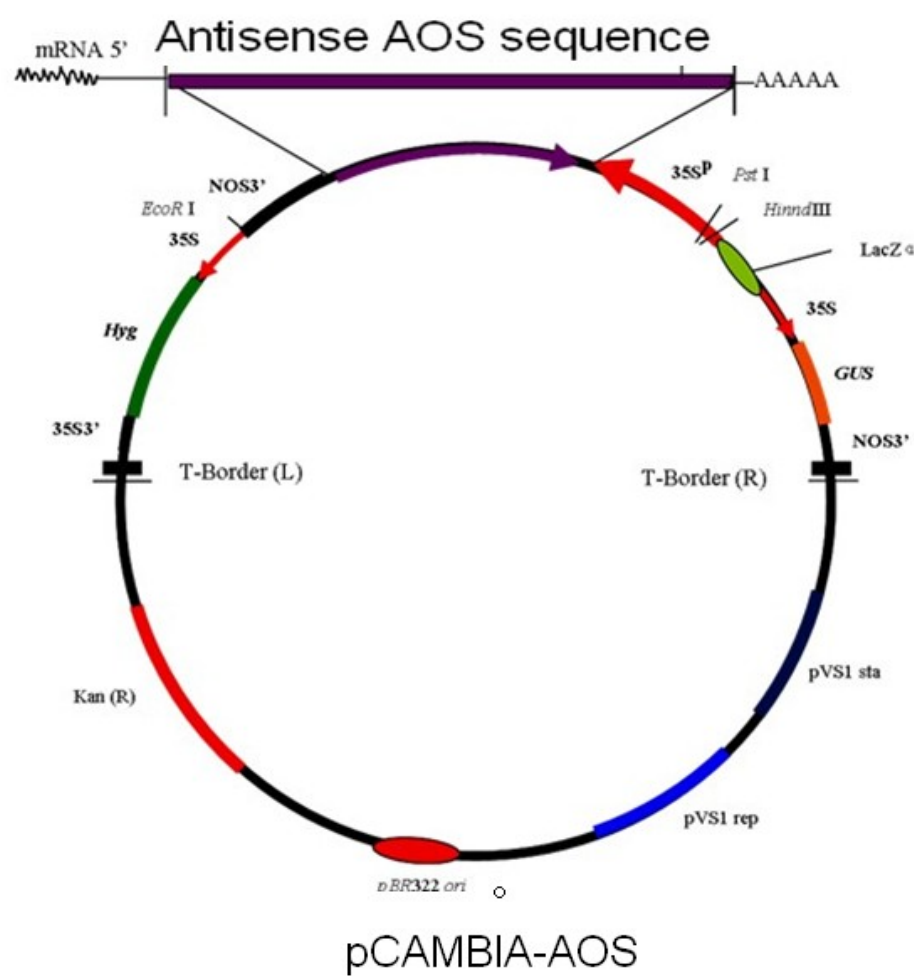


Figure S4. The transformation vector used to generate the *as-aos1* and *as-aos2* lines.

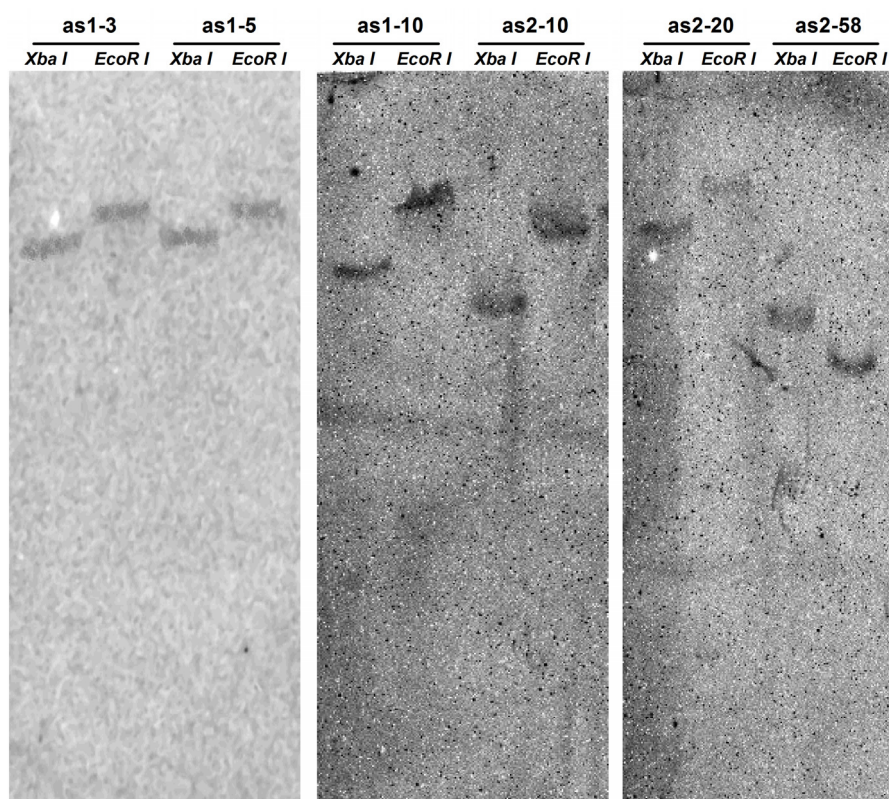


Figure S5. DNA gel-blot analysis of *as-aos1* and *as-aos2* lines plants. Genomic DNA was digested with *Xba*I or *Eco*RI. The blot was hybridized with a probe specific for reporter gene *gus*. Hybridization was created using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). All *as-aos* lines have a single insertion of the transgene.

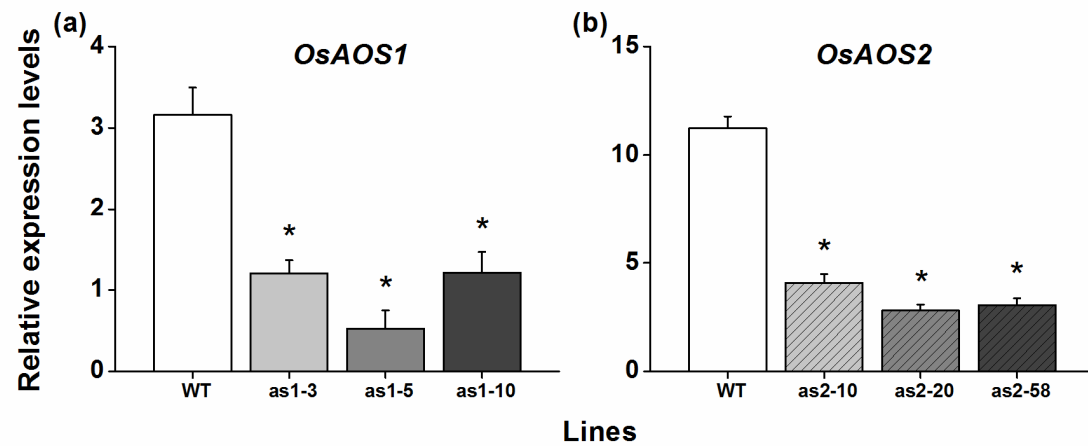


Figure S6. Expression levels of *OsAOS1* and *OsAOS2* in *as-aos1*, *as-aos2* and wild-type plants that were infested by SSB. (a) Mean transcript levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* in *as-aos1* lines and WT plants that were individually infested by SSB for 1 h. (b) Mean transcript levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS2* in *as-aos2* lines and WT plants that were individually infested by SSB for 1 h. Asterisks indicate significant differences between treatments and controls ($*P < 0.05$, Tukey's HSD post-hoc test).

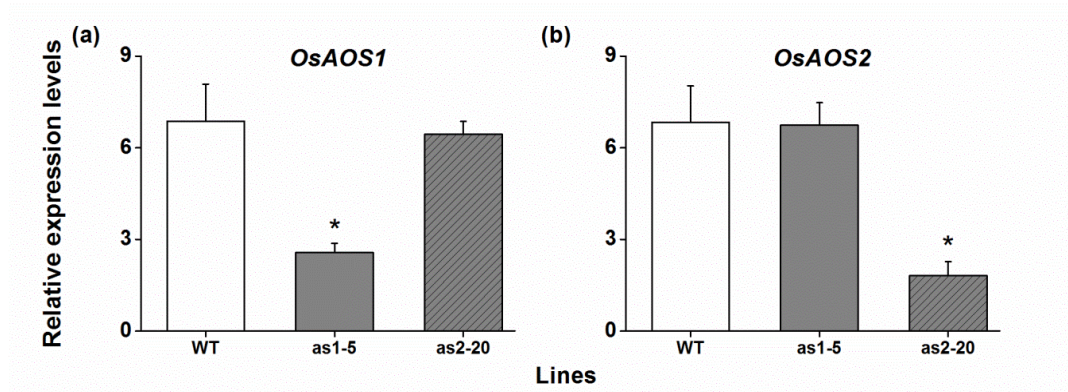


Figure S7. Expression levels of *OsAOS1* and *OsAOS2* in *as-aos1*, *as-aos2* and WT plants that were infested by SSB. Mean transcript levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* (a) or *OsAOS2* (b) in *as-aos1* (as1-5), *as-aos2* (as2-20) and WT plants that were individually infested by SSB for 3 h. Asterisks indicate significant differences in *as-aos* lines compared with WT plants ($*P < 0.05$, Tukey's HSD post-hoc test).



Figure S8. Growth phenotypes of *as-aos1*, *as-aos2* and WT plants at one-week-old seedling stage, tillering stage and heading stage.



Figure S9. The setup used for herbivore bioassays.