

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*.

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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	TTTGCATCGTGGACACACT	GCTATGTACGTGGGGGAAGG	
<i>OsAOS1</i>	Os03g55800.1	QRT-PCR	TGATCACCAAGTGGGTGCTG	CGAGTGGAGGAGCGTGTCC	CTCAGCCGCTGCTCAGCCTC
<i>OsAOS2</i>	Os03g12500.1	QRT-PCR	TGCCCATGATCATCGAGGAT	TGTAGTCGGAGCTGATGAGGAA	CTCCTCCACACGCTGCCGCTG
<i>OsACT</i>	Os03g50885	QRT-PCR	TGGACAGGTTATCACCATTGGT	CCGCAGCTCCATTCTATG	CGTTTCCGCTGCCCTGAGGTCC



(a)

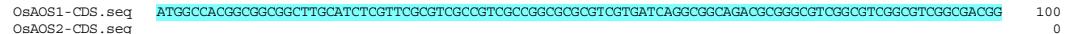
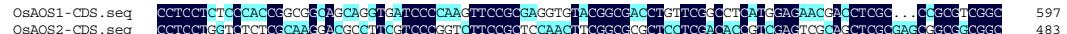
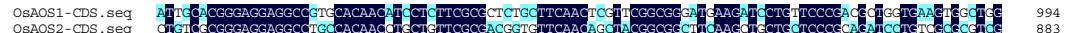
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GGGGATGCCACCGGGCGGCCTGGCATCTGGCTCGCTGCCTGCCGCGGCCGGTGTGATCAGGGCCAGACGCCGGCTCGCGCACGGCAGGAAGGGGGTTGG
241 M A T T A A C I S F A [S P S P A R V V I F R R Q T] R A S A S A S A T D R Q E V V
TCGGCAGGGCCTCGGGCCTGGGGACTACGGCCGGCGCGTGTGGCGCGGGATCGGGGAGGGCTACGGGGAGGTAACGGTAGCTACGGGGGGGCCGGGCGAACGGCAGGGGGTTGG
361 [S P K R R L P L R K V P G D Y G P P V V G A I R D R Y E Y F Y G P G G R D G G F F
GGGGCGCCGCTCGGGGCCACGGCTCCAGGGGGTGCTCAACATGCCGCCGGCGGCGTCTGGCCGGCGGCGACGGCGGTGTGSGCGCTGCTCGAOGCCGCTCTTCCCCTGG
481 A A R V R A H R S [S P V V R L N M P P G P F V A R D P R V V A L L D A A S F P V L
TTCGACACGTGGCTCGACAAGACGGACCTCTCCACCGGACCTTCTGGCCGCCACGGCTACCGGGGGTACCGGCGGTCTCGGCGCTCTCCACCTTGACCCCTCCGAGCCCCAACACCGC
601 F D T [S L V D K T D L F T G T F M P S T D L T G G Y R V L [S P E P N H A
CGGCTCAAGACCCCTCCCTCTTCTACCTTCTCCACCGGCCGGCAGCGGTATCCAAAGTTCCTGGCGGGGTGTGACGGSGACCTGTGCGCCCTCAAGAGAACGACCTCGGCCGCC
721 P L K T L L F Y L L S H R R Q V T P F R E V Y G D L F G L M E N D L A R V G
AAGGGCGACTCGGGGTGACAGGCGGCCGGTCTGGCCCTCCACCGCCAGGGGCTCCGGCGGCCAGGGCAACGGCCGGGGGTGAGCTGG
841 K A D F V H N D F G L C Q G L L G R D P A K [S A L G R D G P K L I T K
TGGGGCTGCTCAGCTCAGGCCCTGGCTACGGCCCTCCACCTCGTGTGGAGGGACGGCTCCACTCTGGCCCTCCGCGGCTGGTAAGAAGGGACTACGGACCGCC
W V L F Q L S P L L S L G L P T L V E D T L L H S [S P E R L L P P A L V K E D Y D R L
961 GCGGACTCTCTCGGGGAGCGGCCAACGGCCGCTGGTACGAGGAGGGGGCGCCCTGGCATGGCAGACACATCCTCTCGGGGTCTGCTCAACTCGTCTCGGG
A D F R D A A K V V D E G E R L G I A H E V N I L L F A L C F N [S P G C
1081 ATGAAGAGTCCTGTTCCGAGCTGGTGAAGTGGCTGGCGCAGGGGCGGGCGGTGTGACGGGGCTCGGACCCGGAGGTGCGGGGCCAGACGGGGGAGGTGACGATG
1201 M K I L F P T L V K L L G R A G A R V G E R L A T E T V R G A V R D N G G C E V T I M
AAGGGCTGGCGGAGATGCCGTGGTAGTGGCTGGTAGCTGGCAGGTGGCATGGAGGGCTGGATGCGATGAGCTAGGGAGGGAGCATGGTGAGGAGGGAGC
1321 K A L H A N N P L V E S A L R I P E V A N Q V R D M V V V E S H D
TACGGGTACGGAGGTGAGGGAGAGATGCTGGGTTACGGCCATGGGACCAAGGGCCGGGGTTCTGGCCGGGGAGTAGCTGGTAGGCTCTGGGGAGAC
1441 Y G V Y R E G G L F G Y Q P H A T [D P T L V F R V F A R E E [S V F D R F L G E D
GCGCCGCGGCCTCGTCGCGGCCAGCTGTGTGGTCACCGGCCCGGGCCCTGGCATGGACAAGCAGTCGCGCCGAGGACTTCCTCGCCGCTCTCTCC
1561 C A R L L R H V W S N G P E [S A A P T L Q D H D K Q C A G K D F V V L V A R L L L
GTCGAAGCTCTCCCGATACGAGCCCTCGACGGTGGCAACCTCTCGTCACTGTGACCTCGTCAAGTGTGACCTCTCGTCAAGAGGGCACCTCTCGACCGGG
V E L F L R Y D [S F D V E V G T S T L G S [S P V T V T [S L K K A T F *
1681 TCCGGCGCCCGTCACTTATTATTCCTCCTCTCTCTCTCAATAATAATTAGTAGTATAAGTAATTTTGACCTGAGTGTGAGTCAAGCTTGAGGATACGGATTGA
1801 CTGGTAGTGAATTTCTCTGGTAGATAATACTAGTAGAAGATAATTTTTTACCAAATTACTTAATAATATAATATTTTCAAAATTAACTTTAACTAAA
1801

(b)

1 AGAGACTGTCGFGGGTGAATTTACAGTCCTCCCTCTCATTTCCTGTGCCACCTCATCACATTTCGACATGGAGCRACTACATACCGGGCTCAAGCTAATTTTTCAATTGTAGCTG
121 TCTTACTAGTCAGCTGTGGCTCAAATTTCCTTCTCAGAAGAACGCCGAGAACGGCGAGAACGGCGAGAACGGGAGAACGGGCTTACCCGGGAGCGGGTT
241 GCGGAGTAGTGGCTGTCAGGGTAAAGTTCTGGAGGTACATCGTAAACGGTAGATCAAAGAACACAACACTCGAGGAAAGCTAGTACGAGTAGTAGTCAACAAACAGCTAG
361 CGATAGCTAGCTAGAAAGAGGTAGCTAGGGGTGGACTGGAGCTAGGGGGCTTGAGGTGACGGGGGCTGCGGCGTTCTCGGGGGGGGGAGACGGCTCG
481 M E L G V P L P R R P V F G [S Y G V P F V S A V R D R L
ATTCTACTACCTSCAGGGGAGGACAAGTACTCGGAGGGCCGGAGGGTCCGGCGCCGGAGAGGTACGGCTCCGGCCATCGCAGCTGGGGCGGCCATTCTGGGGGGGGGGGGGGGG
D F Y Y L O G O D K [Y F E S R A E R [Y G S T V P R I N V P F G P F M A R D P R V
601 TGCGGCTCTCGAGGCCAGCTCTCCCGTCTCTCGACGTCGCCAGGGGGAGGG
721 V A L L D A K S F P V L F D R V A K V E K R D V F T G T L M P S T [S I L T G G V R V
GGCGCTACCTCGACCCGCTCGAGGCCAACACGGCAGAGATCAGCGAGCTGCTCTCCTCTCTGCTCTCTGCAAGGAGCGCCCTCTGGCTCCCTACCCGGGGGCTACCGGGGT
841 C A V L F C T Q P V N H A K P S E R U S L L V [S R K D A F P T L V F R S N F G A L
TCGACACCGTGAGSTCGCACGCTCGAGGG
961 L D T V E S Q L A S G G G P T R T K A L N D A T [R Q C A G T L C A C C T C T C T C C T C G G C G C G C T C C G G C T C C G G C T C C
S S L G T G G P T R W Q L A P L T T L G L P H I X I E D P L L H T L
CGCTGCCACCCCTCTCATCGCTCGACTAACGGCTGTACGGGTACTCTCGCGCGGCCGGGGCGTGAGGGCGCTCGAGGAGGGGGGGGGGGGGGGGGGGGGGGGGGG
1081 P L P P F L I S D Y K A L [Y A V A A A S Q L D A A E R G L G L [S P R E E E A C
ACACCTCTGTGTGGGAGGGGTGTTCAACAGCTAGGGCTCGGTGCGCCAGATCTGTGAGCTGGCGAGGGGGAGGGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
1201 H N L L F A T V F N S G Y G F K L L L F Q L L R V A Q R G E K L H E R L A E
1321 TACGGAGCGGGTGGCCGAGCGGGGCGGCAACGCTGAGCTGGCCGTCTCGAGGGAGGAGCTGGAGCTGGCCGTCTGGGGGGGGGGGGGGGGGGGGGGGGGG
1441 I R S A V A D A G G N V T A L A K E M H E L [T R S V V W C E A L R L D F P V R F Q
ACGGGGCGGCCAAAGCGGACCTGGAGAGTAGCAGAGGGGAGGAGCTGGCTGTGGAGTCAAGAAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
1561 Y G R A K A D L E I E S H D A S F A I K R G E M I L F Q Y G P C A T R D F P V R F Q
CCACGGGGAGGG
1681 A T A R E F T C V G G F G E E G R V G L L Q Y V W S N G R E [E N P S V D N K V
GCCCCGGCAAGAACGGCTGGTGGGGCTGGGGAGGG
1801 C G F K N L V V L V G R L L L V E L F L R Y D F T E A R G K V V I T G V T K
CTCAACCTCCGGCGTCAATCTGACTGTCTAGCCGGGGCATACTTCAGGGCTGGGTGCGTCACTCACCACTCAACGCTGGCTCCTACOGCATATGCAAA
1801 A S T [S A V N R T A *
ATTCTTGATGGCTAACACTACTAATAAGCTGTATGTCTGTATTTCGTGATTCATATTGTGATTGTTGTGATTCGATTGATTTGTTGTTGATTGTTGTTGTTGTTGTTG
2041 TGACAGTGTCTTAAACACCCTCAATAATACTGAGTCAATCTGGCTGAGCTGCTGG
2161 CGTCTCTCCCAACGGCTAGCATGACTCTATACTCTCTCTGAGTAAACAAAGTAACTCTTACCTCTGTGTTGCTCTGAGCTGGGGGGGGGGGGGGGGGGGGGGGG
2281 GACAGCGAGTTAAATCTTGTCTTAATAAAAAAAATATAACCTTTTATTGGTAGCTATTCTGTTGCTCTGAGCTGTTGAGCTATTCTGCTATTGGGGGGGGGGGGGGGG
2401 TATATTAATTAAATTAATAAGCAGTAACTTAAAGCTCAAGCTCAACGGGATCATATAAA

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

(a)

OsAOS1-CDS.seq		100
OsAOS2-CDS.seq	0	
OsAOS1-CDS.seq		200
OsAOS2-CDS.seq	86	
OsAOS1-CDS.seq		300
OsAOS2-CDS.seq	183	
OsAOS1-CDS.seq		400
OsAOS2-CDS.seq	283	
OsAOS1-CDS.seq		500
OsAOS2-CDS.seq	383	
OsAOS1-CDS.seq		597
OsAOS2-CDS.seq	483	
OsAOS1-CDS.seq		694
OsAOS2-CDS.seq	583	
OsAOS1-CDS.seq		794
OsAOS2-CDS.seq	683	
OsAOS1-CDS.seq		894
OsAOS2-CDS.seq	783	
OsAOS1-CDS.seq		994
OsAOS2-CDS.seq	883	
OsAOS1-CDS.seq		1094
OsAOS2-CDS.seq	983	
OsAOS1-CDS.seq		1194
OsAOS2-CDS.seq	1083	
OsAOS1-CDS.seq		1291
OsAOS2-CDS.seq	1183	
OsAOS1-CDS.seq		1391
OsAOS2-CDS.seq	1283	
OsAOS1-CDS.seq		1491
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OsAOS1-CDS.seq		1539
OsAOS2-CDS.seq	1436	

(b)

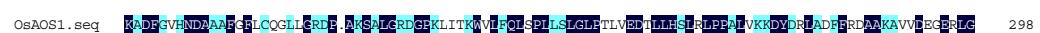
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OsAOS2.seq	61	
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OsAOS2.seq	161	
OsAOS1.seq		298
OsAOS2.seq	261	
OsAOS1.seq		398
OsAOS2.seq	361	
OsAOS1.seq		497
OsAOS2.seq	461	
OsAOS1.seq		512
OsAOS2.seq	477	

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.

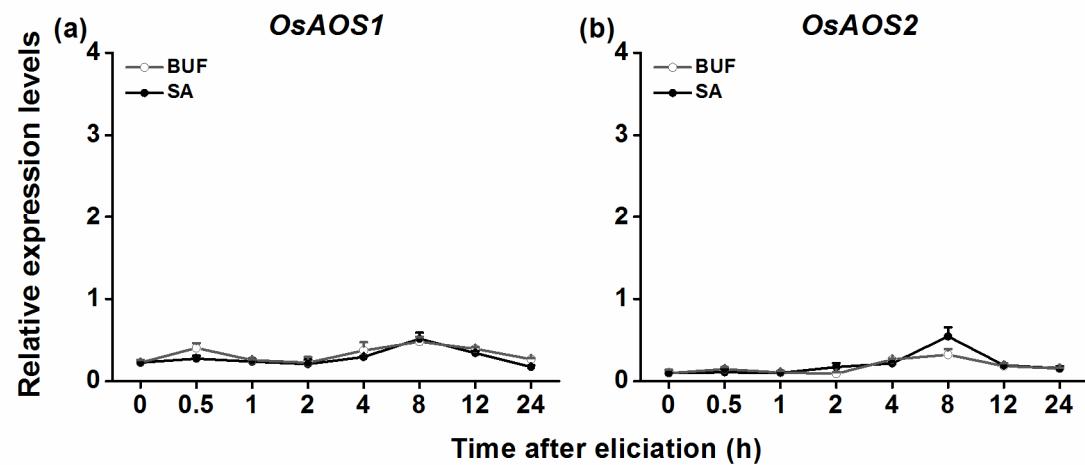


Figure S3. Expression levels of *OsAOS1* and *OsAOS2* in rice plants that were treated with SA. Mean expression levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* (a) and *OsAOS2* (b) in rice leaf sheaths that were treated by buffer (BUF) or salicylic acid (SA).

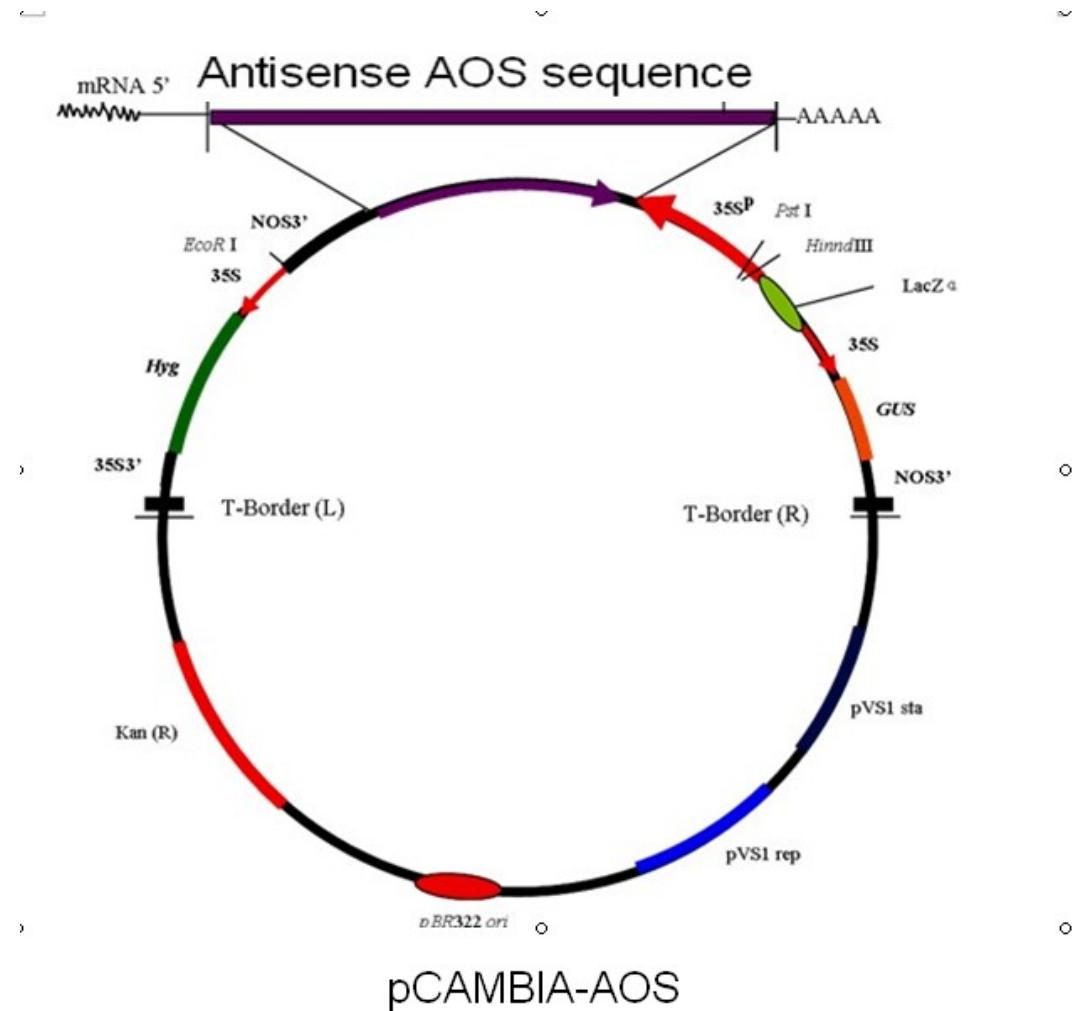


Figure S4. The transformation vector used to generate the *as-aos1* and *as-aos2* lines.

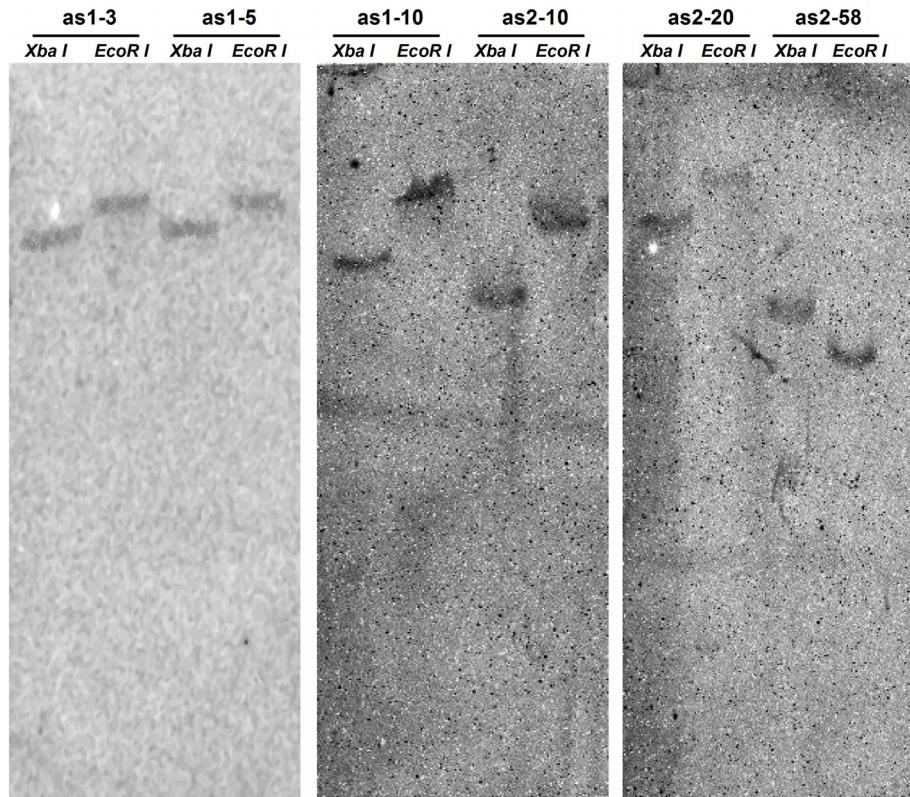


Figure S5. DNA gel blot analysis of as-*aos1* and as-*aos2* lines plants. Genomic DNA was digested with *Xba*I or *Eco*RI. The blot was hybridized with a probe specific for reporter gene *gus*. Hybridization was created using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). All as-*aos*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

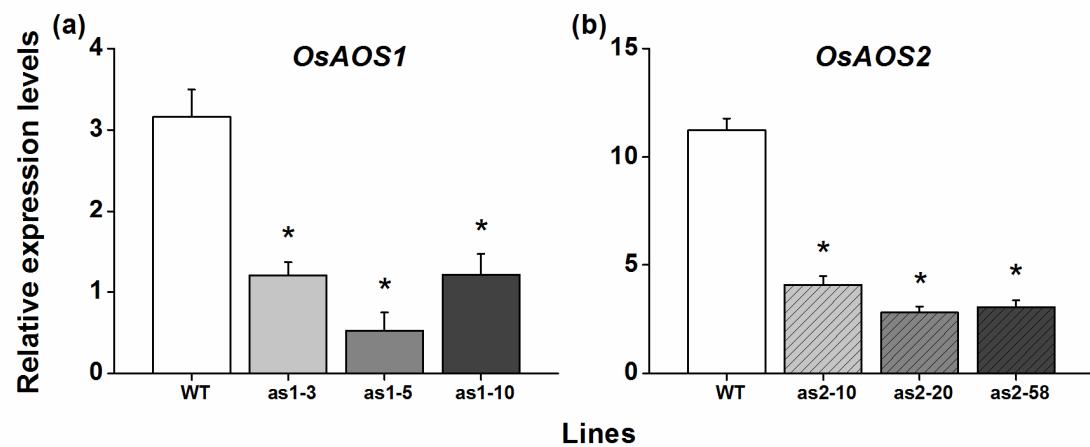


Figure S6. Expression levels of *OsAOS1* and *OsAOS2* in *as-aos1*, *as-aos2* and wild-type plants that were infested by SSB. (a) Mean transcript levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* in *as-aos1* lines and WT plants that were individually infested by SSB for 1 h. (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).

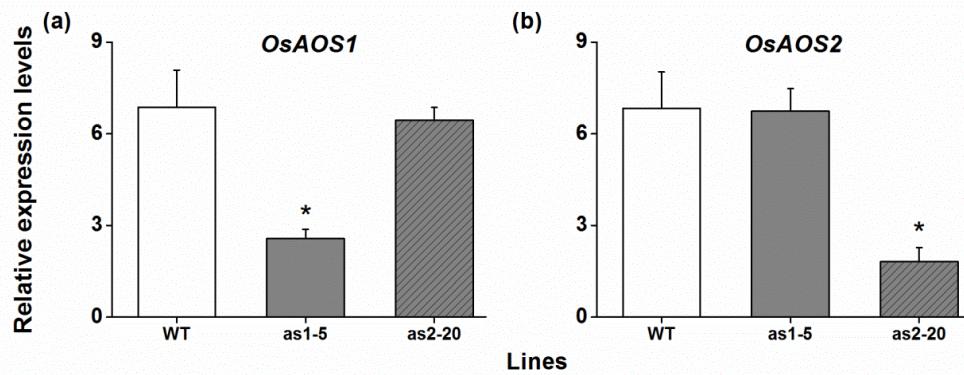


Figure S7. Expression levels of *OsAOS1* and *OsAOS2* in *as-aos1*, *as-aos2* and WT plants that were infested by SSB. Mean transcript levels (relative to expression levels of *OsACT*, +SE, $n = 5$) of *OsAOS1* (a) or *OsAOS2* (b) in *as-aos1* (as1-5), *as-aos2* (as2-20) and WT plants that were individually 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.