A The state of the

N-(3-oxo-hexanoyl)-L-homoserine lactone (oxo-C6-HSL)

N-3-oxo-octanoyl-*L*-homoserine lactone (oxo-C8-HSL)

N-3-oxo-dodecanoyl-L-homoserine lactone (oxo-C12-HSL)

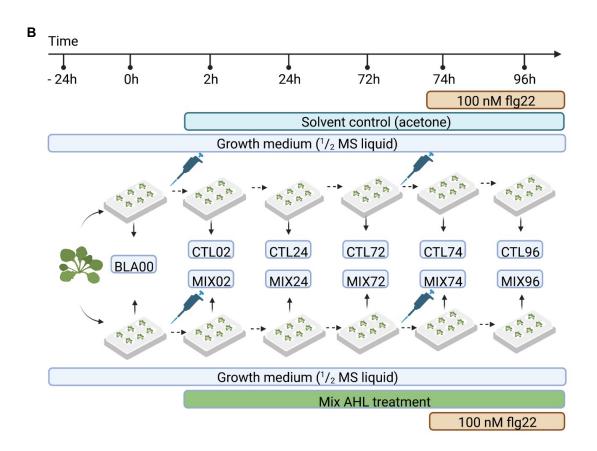
N-3-oxo-tetradecanoyl-L-homoserine lactone (oxo-C14-HSL)

Mix AHL

Each AHL at a final concentration of 6 µM was present in the working solution.

Solvent control

The same volume of acetone, as used for dilution of AHL molecules, was added into half-strength MS.

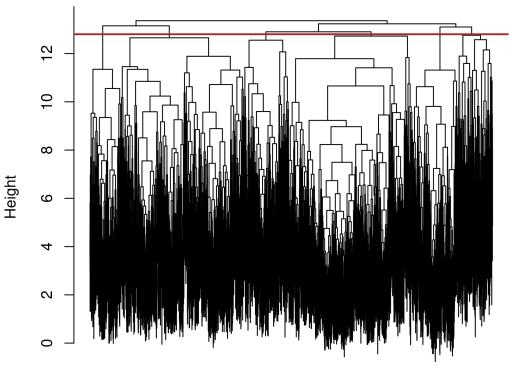


Supplementary Figure S1. Experimental setting and time schedule of the transcriptome analysis.

Mixed AHLs contain four AHL molecules with different acyl chain lengths, each at a final concentration of 6 μ M in the working solution (A). The same volume of acetone was added to the solvent control. Two-week old sterile *Arabidopsis thaliana* Col-0 plants were transferred into six-well plates and grown for additional 24h. The blank control for the transcriptome analysis was collected before adding mixed AHL (AHL mix) or the solvent (control). The samples were collected 2h, 24h, 72h, 74h, and 96h thereafter (B).

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Cluster Dendrogram



gene_dist
hclust (*, "complete")

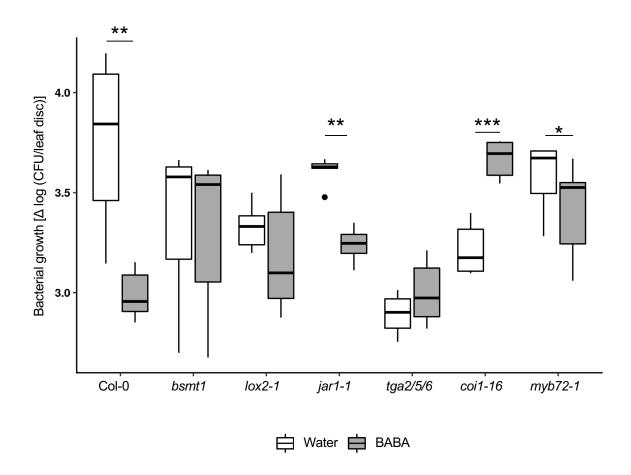
Supplementary Figure S2. Cluster dendrogram of gene expression patterns across the experimental time course.

Gene expression over time was analyzed using R package DESeq2 (Love et al., 2014) with the default setting in the comparison between gene expression full model (time + treat + treat: time) and gene expression reduced model (time + treat). Patterns of differentially expressed genes were identified by threshold (*p* adjusted < 0.05) in comparisons between control (acetone) and AHL mix-treated samples across the experimental time course. The method (termed Complete) was used to calculate the gene distance in the genes which have differential expression patterns. Six groups of gene expression patterns were identified. The brown line in the plot represented the gene distance of 12.5.

	AHL mix			Post	flg22	
	13.64	0.00	36.36	36.36		cellular response to amino acid stimulus (22)
	8.00	0.00	34.00	30.00		cellular response to organonitrogen compound (50)
	20.00	0.00	80.00	60.00		response to indolebutyric acid (5)
	9.09	0.00	36.36 35.94	33.33 25.07	24.20	response to amino acid (33)
	0.92	0.43	41.23	27.08		response to organonitrogen compound (690) response to chitin (325)
	0.00	0.00	24.24	18.18	24.24	response to glucose (66)
	0.00	0.00	22.54	16.90		response to hexose (71)
	0.00	0.00	22.67	16.00	25.33	response to monosaccharide (75)
	1.39	1.39	30.56	23.61	23.61	response to sucrose (72)
	1.35	1.35	31.08	22.97	22.97	response to disaccharide (74)
	0.64 2.11	0.00	21.02	14.65 35.31		response to carbohydrate (157)
	1.29	0.76	29.25	21.12	22.57	response to salicylic acid (473) response to alcohol (1316)
	1.44	0.52	27.97	20.85		response to account (1919) response to oxygen-containing compound (3257)
	2.13	2.13	36.17	14.89	12.77	response to unfolded protein (47)
	0.00	4.00	8.00	4.00		response to misfolded protein (25)
ਲ	1.33	0.48	26.28	18.93	20.50	response to lipid (2097)
. <u>ö</u>	1.45	0.59	26.20	19.12		response to organic substance (3718)
Ĕ	2.10	0.35	36.09	28.99		response to organic cyclic compound (859)
þe	0.48	0.48	19.23	11.30	17.31	response to auxin (416)
O	2.65 1.44	0.00	22.35	19.32 17.50	19.45	response to ethylene (264) response to hormone (2709)
유	1.35	0.84	28.61	20.59	22.62	response to abscisic acid (1185)
Biological process response to chemical	1.97	0.15	25.19	20.03	18.97	response to jasmonic acid (659)
	1.96	0.15	25.04	19.91	18.85	response to fatty acid (663)
ď	1.15	0.72	25.25	16.21		cellular response to hormone stimulus (1394)
lse	2.44	0.00	16.26	15.45	23.58	cellular response to ethylene stimulus (123)
<u>_</u>	1.03	0.69	25.15	15.79		hormone-mediated signaling pathway (1165)
SS	1.91	0.00	16.19	13.33	20.95	ethylene-activated signaling pathway (105)
8	0.30	0.59	26.71 25.84	15.43		cellular response to abscisic acid stimulus (337)
2	0.38	0.78	25.58	14.61 13.95		abscisic acid-activated signaling pathway (267) regulation of abscisic acid-activated signaling pathway (129)
<u>a</u>	0.00	0.00	9.09	9.09		induced systemic resistance, jasmonic acid mediated signaling pathway (11)
g	1.05	0.00	20.94	16.75	22.51	jasmonic acid mediated signaling pathway (191)
gi	1.00	0.00	21.11	17.09	22.11	cellular response to jasmonic acid stimulus (199)
<u>_</u>	6.82	2.27	34.09	27.27	29.55	cellular response to toxic substance (44)
. <u>e</u>	6.98	2.33	34.88	27.91	30.23	cellular detoxification (43)
ш	7.35	0.00	32.35	29.41	33.82	cellular response to nitrogen compound (68)
	1.39 6.67	0.69 1.67	26.57 35.00	18.50 25.00	20.71	cellular response to chemical stimulus (2303) cellular response to acid chemical (60)
	0.90	0.00	34.23	22.52	16.22	salicylic acid mediated signaling pathway (111)
	0.00	0.00	21.05	10.53	36.84	systemic acquired resistance, salicylic acid mediated signaling pathway (19)
	3.03	0.00	36.36	9.09	15.15	endoplasmic reticulum unfolded protein response (33)
	2.13	2.13	36.17	14.89	12.77	cellular response to unfolded protein (47)
	0.00	0.78	25.58	13.95	20.16	regulation of response to alcohol (129)
	0.30	0.59	26.71	15.43	21.36	cellular response to alcohol (337)
	0.00	0.78	25.58	13.95	20.16	regulation of cellular response to alcohol (129)
	10.00	0.00	20.00	10.00	50.00	cellular response to disaccharide stimulus (10)
	1.21	0.60	27.36	18.76	21.85	cellular response to sucrose stimulus (9) cellular response to oxygen-containing compound (1327)
	0.98	0.00	20.69	16.75	21.68	cellular response to dayyerr-containing compound (1327)
	1.38	0.66	25.74	17.59	20.51	cellular response to organic substance (1814)
	0.59	0.29	23.43	15.08	20.79	cellular response to lipid (683)
	1.32	0.33	31.91	21.71	19.08	cellular response to organic cyclic compound (304)
	1.40	0.00	35.66	25.87		cellular response to salicylic acid stimulus (143)
	2h	24h	72h	74h	96h	

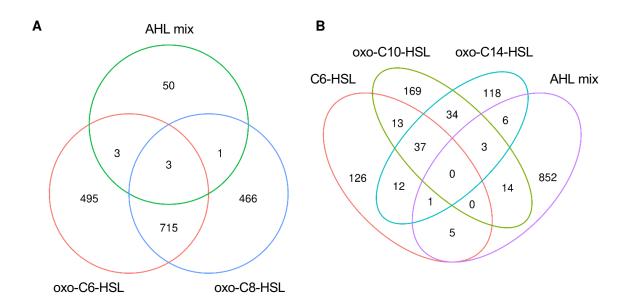
Supplementary Figure S3. Changes in the GO term "biological process response to chemical" during the experimental time course.

Analysis of the enriched GO terms among differentially expressed genes across the experimental time course, was performed using the R package ViSEAGO with default setting (Brionne et al., 2019). The GO term "biological process response to the chemical" was shown with the percentage of participating genes for different processes. The percentage was highlighted in green color in the plot. Total gene number of each process was indicted at the end of the process name (in brackets). Compared to others, response to jasmonic acid was very prominent among the enriched GO terms.



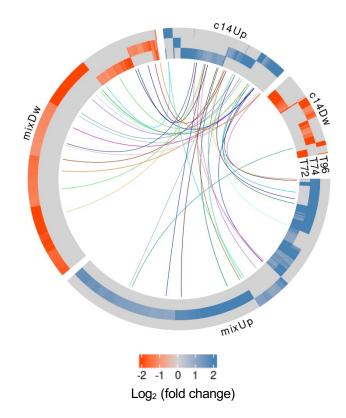
Supplementary Figure S4. BABA induces enhanced resistance in plants grown in a jar hydroponic system.

Three-week old Col-0 plant and several mutants deficient in jasmonate homeostasis were transferred to a sterile hydroponic system, based on glass jars, perlite, and ¼ MS medium. Plants were grown in a growth chamber for another 3-4 weeks. During the growth process, fresh ¼ MS was added weekly into the jars according to plants' requirement. Roots were pretreated with a final concentration of 250 μM β-aminobutyric acid (BABA) or water (control) for 72h and subsequently leaves were inoculated with the plant pathogens *Pseudomonas syringae* pv. *tomato* (*Pst*), $OD_{600nm} = 0.01$ (10^7 CFU/mL) in 10 mM MgCl₂. Bacterial proliferation was used to assess plant resistance and is expressed as difference in the number of Colony Forming Unit (CFU) in leaf discs between 96h and 2h time points (Δlog (CFU/leaf disc)). Boxes represent the interquartile range between the first and the third quartile and the middle line marks the median, whiskers indicate 1.5× interquartile range, points indicate outliers. Statistical analysis was performed by Student's *t*-test, * p < 0.05, *** p < 0.001, n ≥ 3. The results demonstrated that BABA-priming may be established in jar-grown plants and is independent of JAR1 and the MYB72 transcription factor however, requires LOX2, COI1, TGA2/5/6 transcription factors as well as BSMT1.



Supplementary Figure S5. Gene expression in response to AHL mix differs from that of response to single AHL molecules.

Information on differentially expressed genes (DEGs) after exposure to single AHL molecules including 6 μ M C6-HSL, 1 μ M oxo-C6-HSL, 10 μ M oxo-C8-HSL, 6 μ M oxo-C10-HSL and 6 μ M oxo-C14-HSL were extracted from previous studies (Zhao et al.2016; Liu et al.2022; Schenk et al. 2014). DEGs induced by AHL mix, compared to solvent control, were obtained in this study. The comparison of genes differentially regulated 24h post AHL treatment is presented in (A), and the comparison at 72h post AHL treatment is presented in (B). Many genes responded specifically to AHL mix, indicating that the response of plants exposed to AHL mix differs from that exposed to single AHL molecules.



Supplementary Figure S6. Responses to the flg22 challenge differs between plants primed with AHL mix and oxo-C14-HSL.

Information on gene expression upon response to 100 nM flg22 in oxo-C14-HSL (C14)-primed plants was extracted from Schenk *et al.* (2014). Information on gene expression of AHL mix-primed plants response to flg22 was obtained in this study. Data were collected for the 72h (T72) time point: 72h post AHL-priming and prior the flg22 challenge; 74h (T74): 2h post flg22 challenge); and 96h (T96): 24h post the flg22 challenge. The up-regulated genes were indicated in green and down-regulated were marked in red. The gaps in the gene list were marked with light gray. Differentially expressed genes (DEGs) were identified by threshold (p adjusted < 0.05) and the absolute \log_2 value of fold change > 1. DEGs common in oxo-C14-HSL- and AHL mix-primed plants are indicated by lines inside the plot.

Supplementary Tables

Supplementary Table S1. Jasmonic acid (JA)-related gene expression in a single AHL treatment.

GeneID	Log ₂ (FC)	AHLs	Time	Category	Gene_description	Gene_name
AT2G06050	-1.10	oxo-C6-HSL	24h	Biosynthesis	oxophytodienoate-reductase 3	OPR3
AT3G25760	-1.17	oxo-C6-HSL	24h	Biosynthesis	allene oxide cyclase 1	AOC1
AT3G45140	-2.16	oxo-C6-HSL	24h	Biosynthesis	lipoxygenase 2	LOX2
AT1G20510	-1.08	oxo-C8-HSL	24h	Biosynthesis	OPC-8 coenzyme A ligase1 (OPCL1)	OPCL1
AT2G06050	-1.38	oxo-C8-HSL	24h	Biosynthesis	oxophytodienoate-reductase 3	OPR3
AT3G25760	-1.14	oxo-C8-HSL	24h	Biosynthesis	allene oxide cyclase 1	AOC1
AT3G45140	-2.28	oxo-C8-HSL	24h	Biosynthesis	lipoxygenase 2	LOX2
AT3G53510	-1.05	oxo-C8-HSL	24h	Metabolism	ABC-2 type transporter family protein	JAT4

Note: data on Arabidopsis treated with *N*-(3-oxo-hexanoyl)-L-homoserine lactone (oxo-C6-HSL) and *N*-3-oxo-octanoyl-L-homoserine lactone (oxo-C8-HSL) at 24h, were extracted from previous studies (Zhao et al., 2016; Liu et al., 2022).

Supplementary Tables

Supplementary Table S2. List of oligonucleotides used in this study.

Accession number / Gene	Name	Sequence (5' -> 3')	Reference		
AT3G25760	RT-qAtAOC1-F	TCAGAACTTGGGAAATACCGAA	(Huang et al. 2021)		
AOC1	RT-qAtAOC1-R	TAAGAATTTTTGGGCTGTGTCG	(Huang et al., 2021)		
AT5G42650	RT-qAtAOS-F	AACACCAGCTCCAGCTCTATTCTT	(11, 22, 23, 4, 21, 0004)		
AOS	RT-qAtAOS-R	TTGACTCTGTACACCGTGGAGTT	(Huang et al., 2021)		
AT3G45140	RT-qAtLOX2-F	ATGAGCCTGTTATCAATGCTGC	(11 + 1 0040)		
LOX2	RT-qAtLOX2-R	AACACCAGCTCCAGCTCTATTCTT	(Hu et al., 2013)		
AT1G17420	RT-qAtLOX3-F	TATGGATTTGCGGCAGAGATCGGA	(0.1		
LOX3	RT-qAtLOX3-R	AGGCTCAGAACTCGGAACCAACAA	(Schenk et al., 2014)		
AT1G72520	RT-qAtLOX4-F	GGGATCAACCCGGTCAACATAGAAC	(Schenk et al., 2014) (Beynon et al., 2009)		
LOX4	RT-qAtLOX4-R	GTCCACCATAAACAAACGGTTCGTC			
AT1G76690	RT-qAtOPR2-F	CCAGAAGCATTAGGGCTG			
OPR2	RT-qAtOPR2-R	GGCTTCCCTCATTGGCAT			
AT2G06050	RT-qAtOPR3-F	ATTATGGCATGTTGGACGTG	(Huang et al., 2021)		
OPR3	RT-qAtOPR3-R	AACAAAACTCGCCACCTGTT			
AT3G48520	RT-CYP94B3-F	TGGCTTACACGAAGGCTTGTC	(Heitz et al., 2012)		
CYP94B3	RT-CYP94B3-R	AGTCCCACGAAACTGGAGGAT			
AT2G27690	RT-CYP94C1-F	GGCCCGGATTACGAAGAGTTT	(Heitz et al., 2012)		
CYP94C1	RT-CYP94C1-R	GGCCGGAACTTACCTTCGTT			
AT1G51760	RT-qAtIAR3-F	TCGGATTAAGCAAGTGAAGGA	(Smirnova et al., 2017)		
IAR3	RT-qAtIAR3-R	TTGCTTCTTGTACCCCTGCT			
AT1G44350	RT-qAtIAR3-F	GTGTCCCATATCCATCCAACGG			
ILL6	RT-qAtIAR3-R	AGACTAATGACCGCGGAAGAAG	(Smirnova et al., 2017		
AT5G05600	RT-qAtJAO2_2-F	CCTCCTTTATACCCTCCCATGAC	(Smirnova et al., 2017)		
JAO2-2	RT-qAtJAO2_2-R	TTTGCCTTGTGGACCTTGAGTTC			
AT3G55970	RT-qAtJAO3_1-F	AGCTCCTCATGCTTTCATCGTT	(0		
JAO3-1	RT-qAtJAO3_1-R	CGATCACTCTGTGTTCTACGCT	(Smirnova et al., 2017)		
AT2G38240	RT-qAtJAO4 1-F	GGAATTTACAAAAGCGTGGAACA	(One-in-out of all 0047)		
JAO4-1	RT-qAtJAO4 1-R	CGGGATATCACTTCTCGGGT	(Smirnova et al., 2017		
AT2G46370	RT-qAtJAR1-F	AACGCTACTGACCCTGAAGAAGC	(Hu et al., 2013)		
JAR1	RT-qAtJAR1-R	GGTGAAGTGTCACCATCAACCA			
AT1G19640	RT-qAtJMT-F	ATGTCCATGGCCAAAGAGGG	(Li et al., 2017)		
JMT	RT-qAtJMT-R	CGGAGCTCGCAGCATAGTAA			
	RT-qAtJAT1-F	TGGTTCTTCACTTGACGGAGAT	(Li et al., 2017)		
AT3G55090 JAT1	RT-qAtJAT1-R	AAGAGTCTTGGTCTTGGAGA			
	RT-qAtMYC2-F	GGTTGGGACGCAATGATTAGAGT			
AT1G32640 MYC2	RT-aAtMYC2-R	CCATCTTCACCGTCGCTTGTTG	(Schenk et al., 2014)		
	RT-gAtORA47-F	TCCACCGTCGATCTCCGTAGAAAA	+		
AT1G74930	RT-qAtORA47-R	GCGAATCTAGCAGCAGCTTCCTGA	(Hickman et al., 2017)		
ORA47	RT-qAtWRKY22-F	ATCTCCGACGACCACTATTG	(Schikora et al., 2011)		
AT4G01250	RT-gAtWRKY22-R	TCATCGCTAACCACCGTATC			
WRKY22	RT-qAtWRKY29-F	TCCGGTACGTTTTCACCTTC	(Schikora et al., 2011)		
AT4G23550	RT-qAtWRKY29-R	AGAGACCGAGCTTGTGAGGA			
WRKY29	RT-qAtUBQ4-F	GCTTGGAGTCCTGCTTGGACG			
AT5G25760	RT-qAtUBQ4-R	CGCAGTTAAGAGGACTGTCCGGC	(Schikora et al., 2011)		
UBQ4	INT-YALUBQ4-K	TOGONG LIANGAGGACTG TOGGGC			