

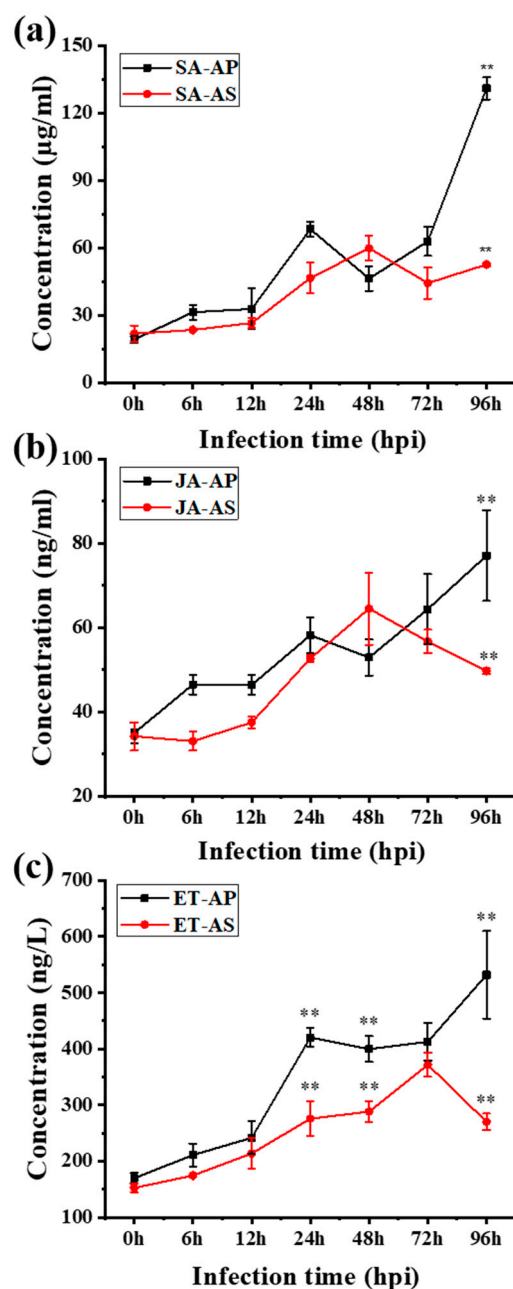
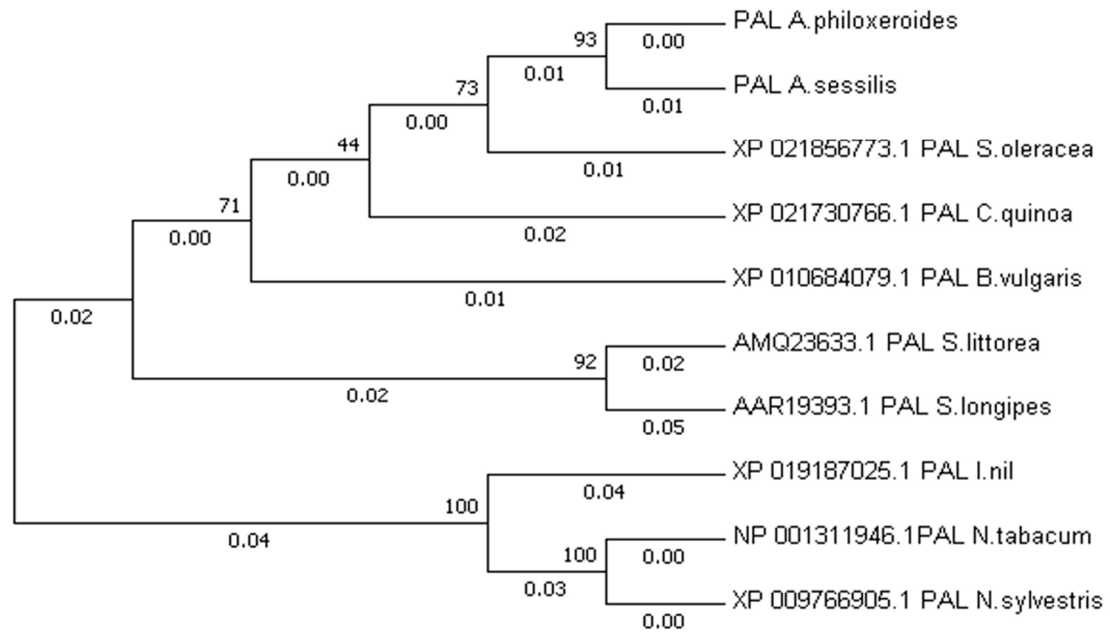
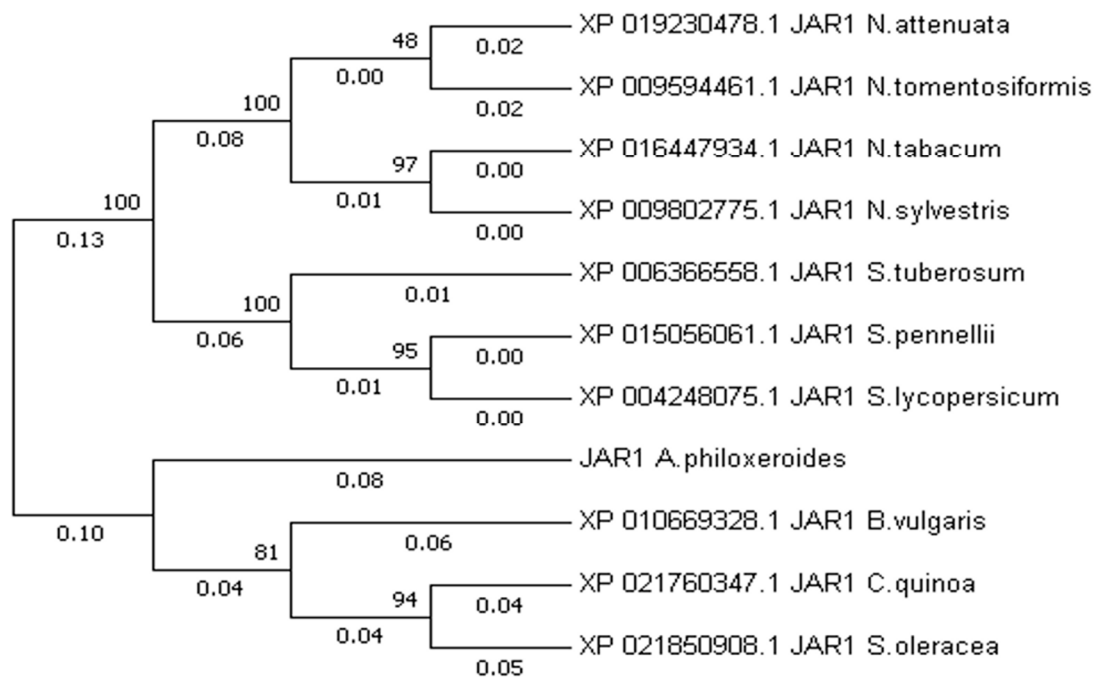


Figure S1. Endogenous hormone Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) contents in both *Alternanthera philoxeroides* and *A. sessilis* after *Rhizoctonia solani* inoculations. (a) SA, (b) JA and (c) ET. Four-week-old plants were inoculated with *R. solani* and un-inoculated leaves were used as a control (0h). Samples were harvested at the indicated time points for quantification using ELISA. Values are means $\pm$ SE ($n = 4$). The different letters indicate significant difference in hormone levels compared with control un-inoculated plant leaves assessed by Duncan's test at $P < 0.01$. Abbreviations: AP - *A. philoxeroides*, AS - *A. sessilis*.

(a) *PAL* (SA)



(b) *JAR1* (JA)



(c) *EIN3* (ET)

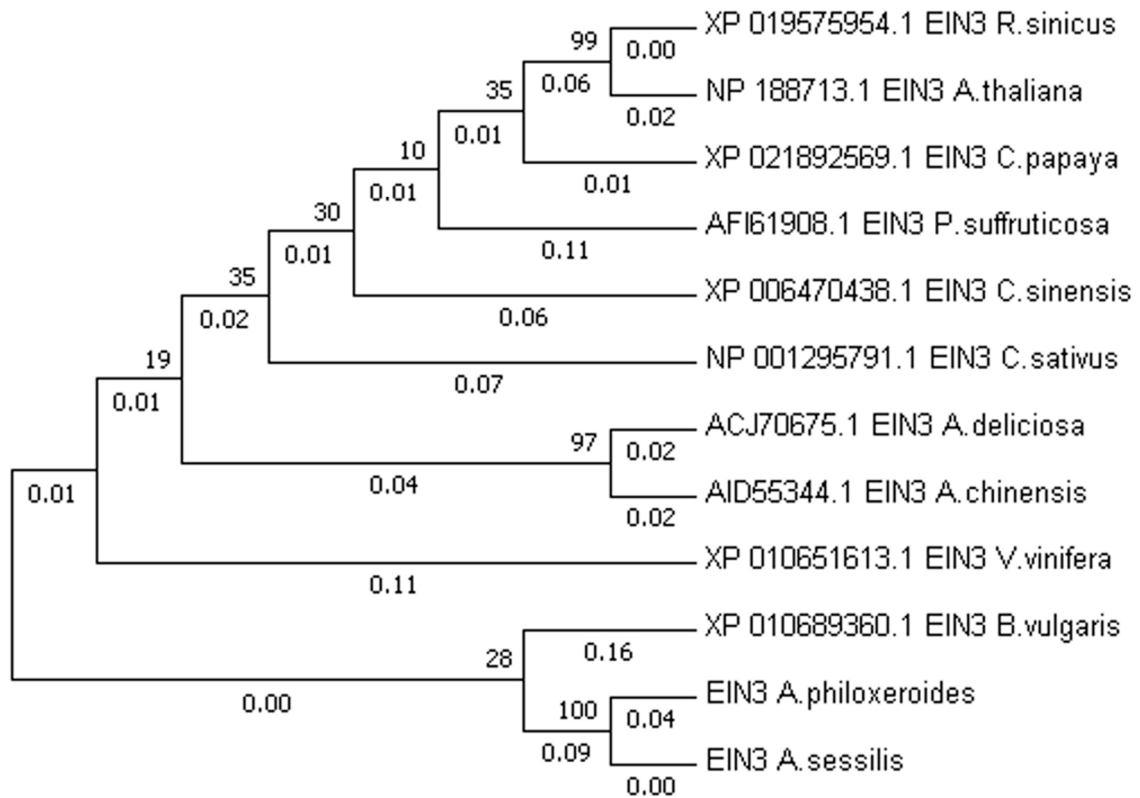
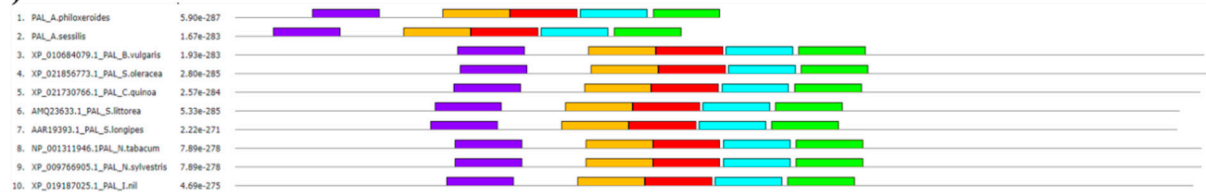


Figure S2. Comparative phylogenetic analysis of the selected hormone genes in both the invasive and native species, as well as other closely related species. A maximum likelihood analysis was performed for each gene: (a) *PAL* (Salicylic Acid), (b) *JAR1* (Jasmonic Acid), (c) *EIN3* (Ethylene). A maximum likelihood phylogenetic tree was constructed with 1000 bootstrap replicates. Bootstraps are shown on the nodes of the trees. Genetic distances are shown below each branch.

(a) *PAL* (SA)

1)

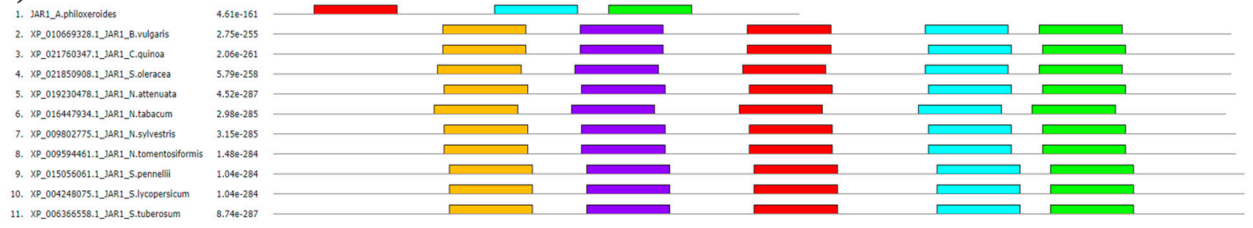


2)



(b) *JAR1* (JA)

1)

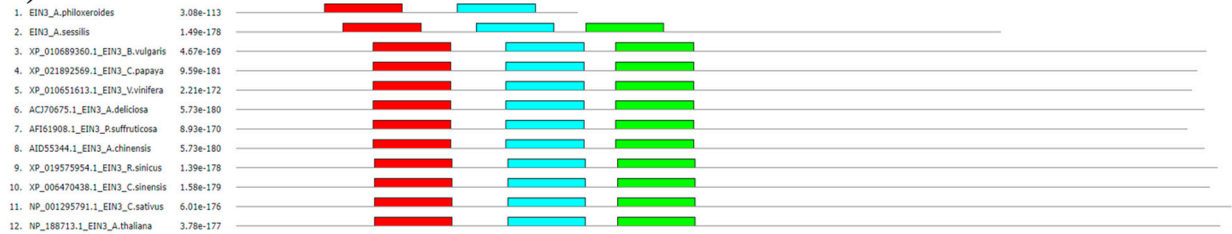


2)



(c) *EIN3* (ET)

1)



2)



Figure S3. Identification of conserved motifs in selected genes from both invasive and native species. (a) *PAL* (Salicylic Acid), (b) *JAR1* (Jasmonic Acid), (c) *EIN3* (Ethylene). For each gene the following are shown: 1) a conserved motif revealing five motifs from the full-length protein sequences of all analyzed species with greater e-value, and 2) conserved amino acid sequences and the conservation of each motif are represented as a logo in each gene with their bit score as height of amino acid.

(a1) PAL (SA) - *A. philoxeroides*



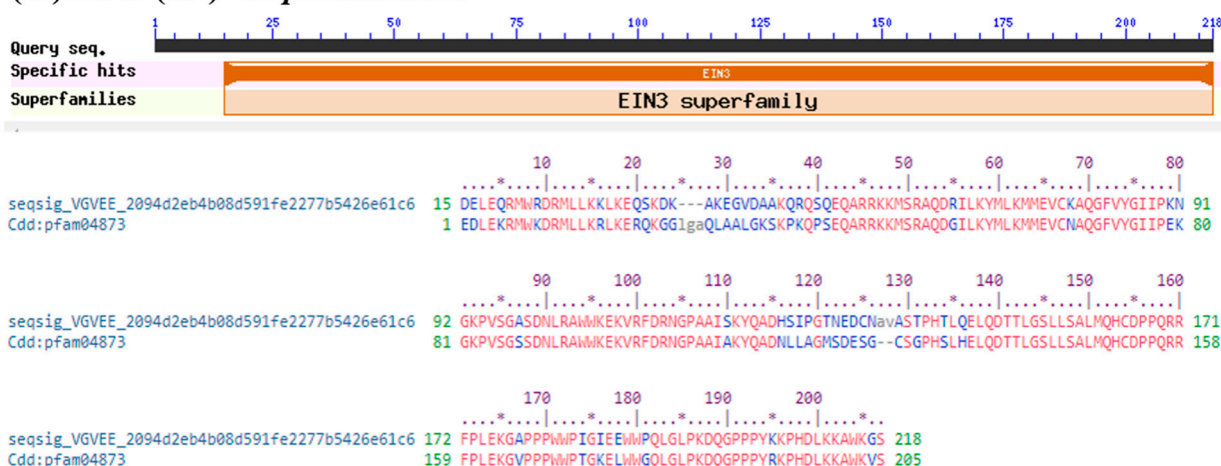
(a2) PAL (SA) - *A. sessilis*



(b) *JAR1* (JA) - *A. philoxeroides*



(c1) *EIN3* (ET) - *A. philoxeroides*



(c2) *EIN3* (ET) - *A. sessilis*

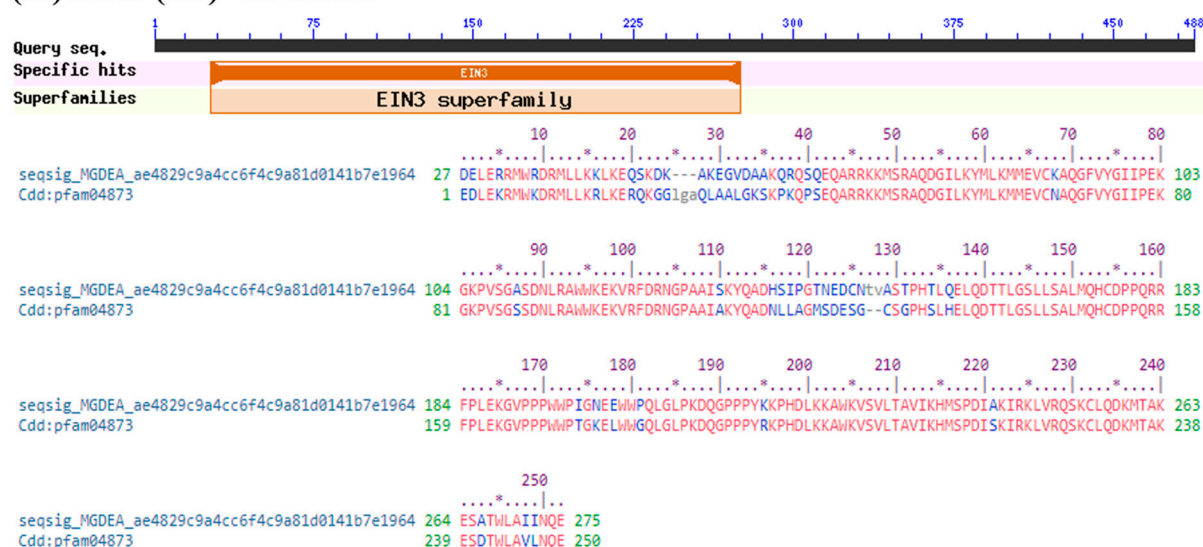
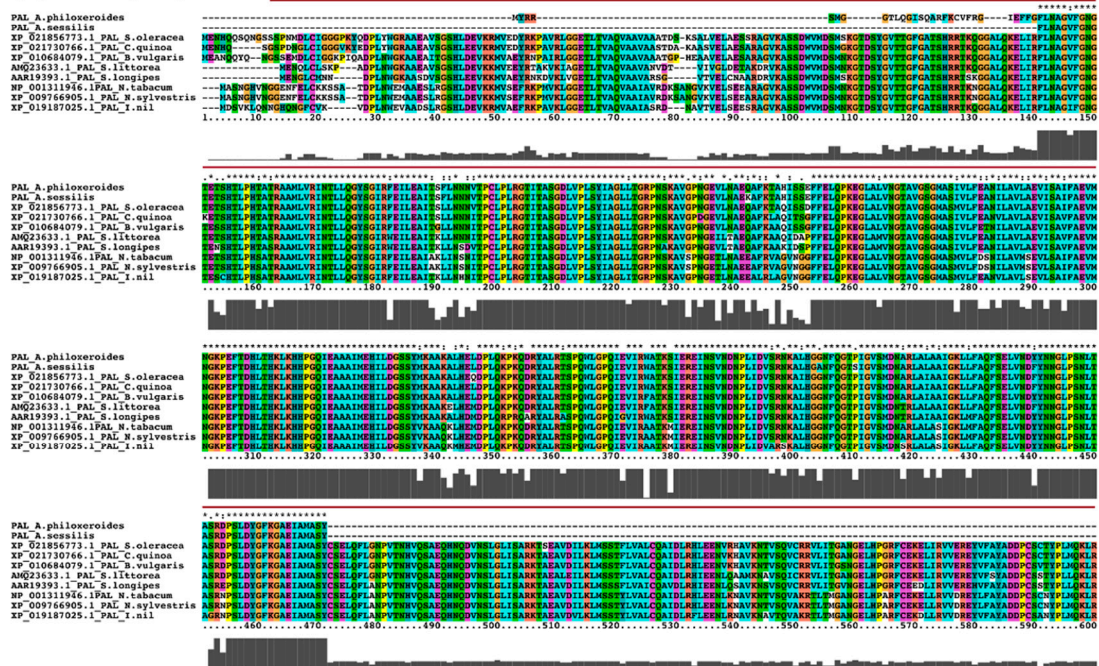
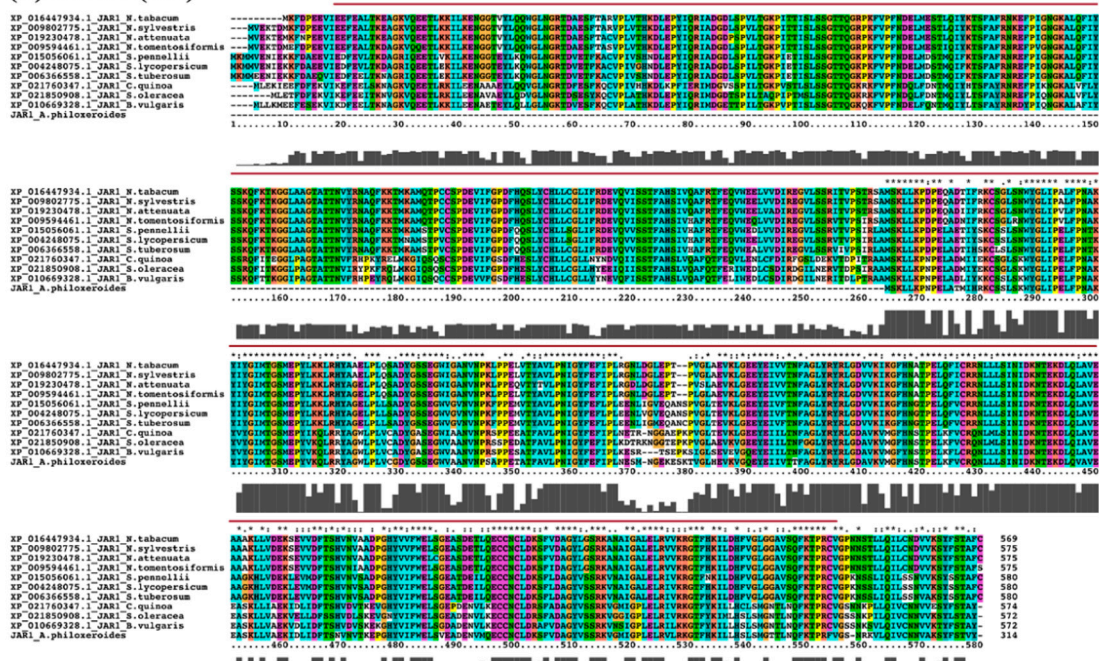


Figure S4. Identification and alignment of the conserved domain in each of the selected genes from both invasive and native species. We identified conserved domains for each gene: (a) *PAL* (Salicylic Acid), (b) *JAR1* (Jasmonic Acid), (c) *EIN3* (Ethylene). The upper figure represents the conserved domain, while the lower figure shows the respective domain peptide sequence aligned with related species using the NCBI domain database. We could not predict the conserved domain for *JAR1* due to the short number of amino acids from open reading frame.

(a) *PAL* (SA)



(b) *JAR1* (JA)



(c) *EIN3* (ET)

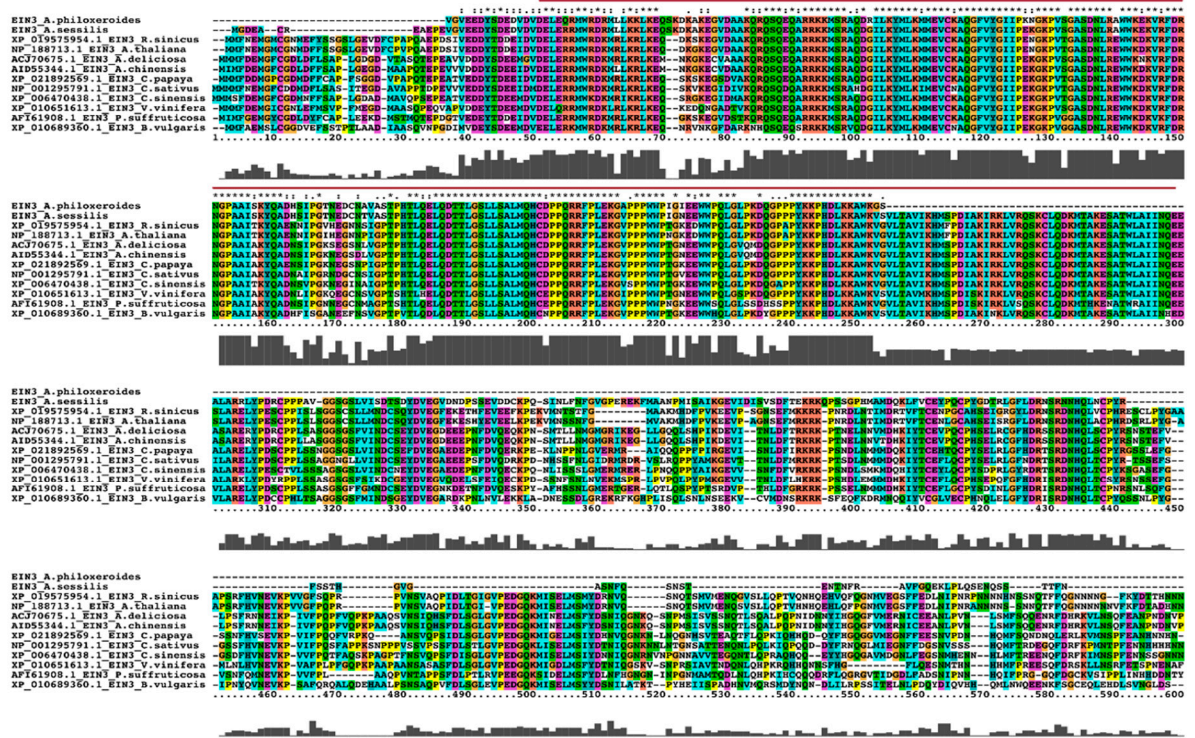


Figure S5. Multiple sequence alignments of predicted amino acid sequences of both *Alternanthera philoxeroides* and *A. sessilis* along with other closely related plants. (a) *PAL* (Salicylic Acid), (b) *JAR1* (Jasmonic Acid), (c) *EIN3* (Ethylene). The superfamily domain of each gene is highlighted with a red line. Star and colon symbols represent the conservation of amino acids and the dots and white space represent low conservation or mismatches respectively. Hyphens in the aligned sequences represent gaps in the sequence.

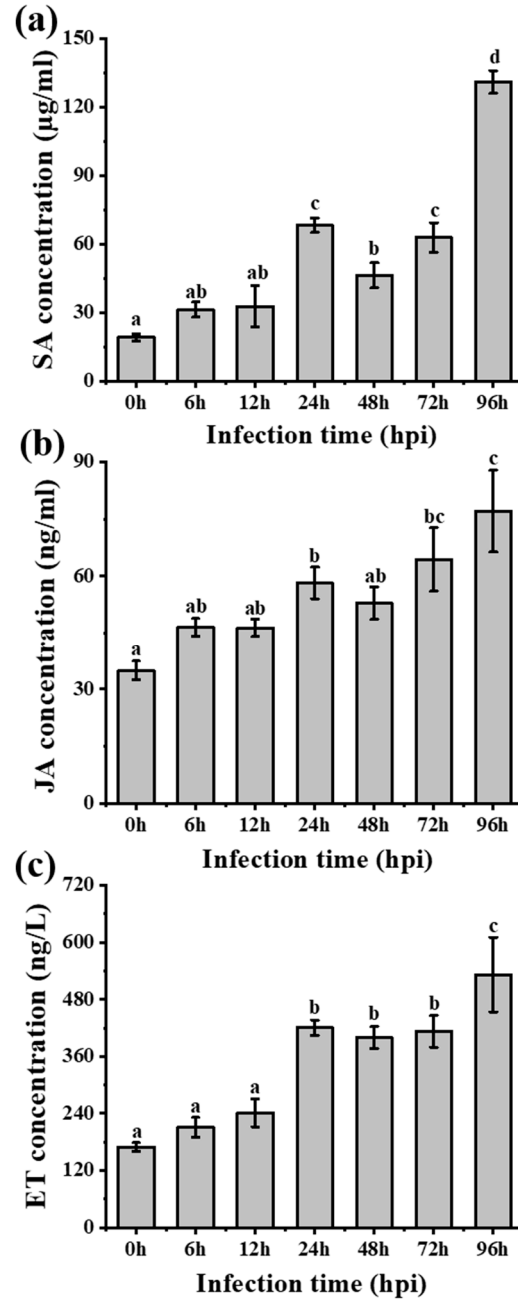


Figure S6. Endogenous hormone Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) contents in *Alternanthera philoxiroides* after infected with *Rhizoctonia solani*. (a) SA, (b) JA and (c) ET. Four-week-old plants were used for inoculations. Un-inoculated leaves were used as a control (0h). Samples were collected at different time intervals for quantification using ELISA. Values are means $\pm$ SE (n = 4). Data bars with different letters indicate they are significantly different compared with the un-inoculated leaves (using Duncan's test at $P < 0.05$).

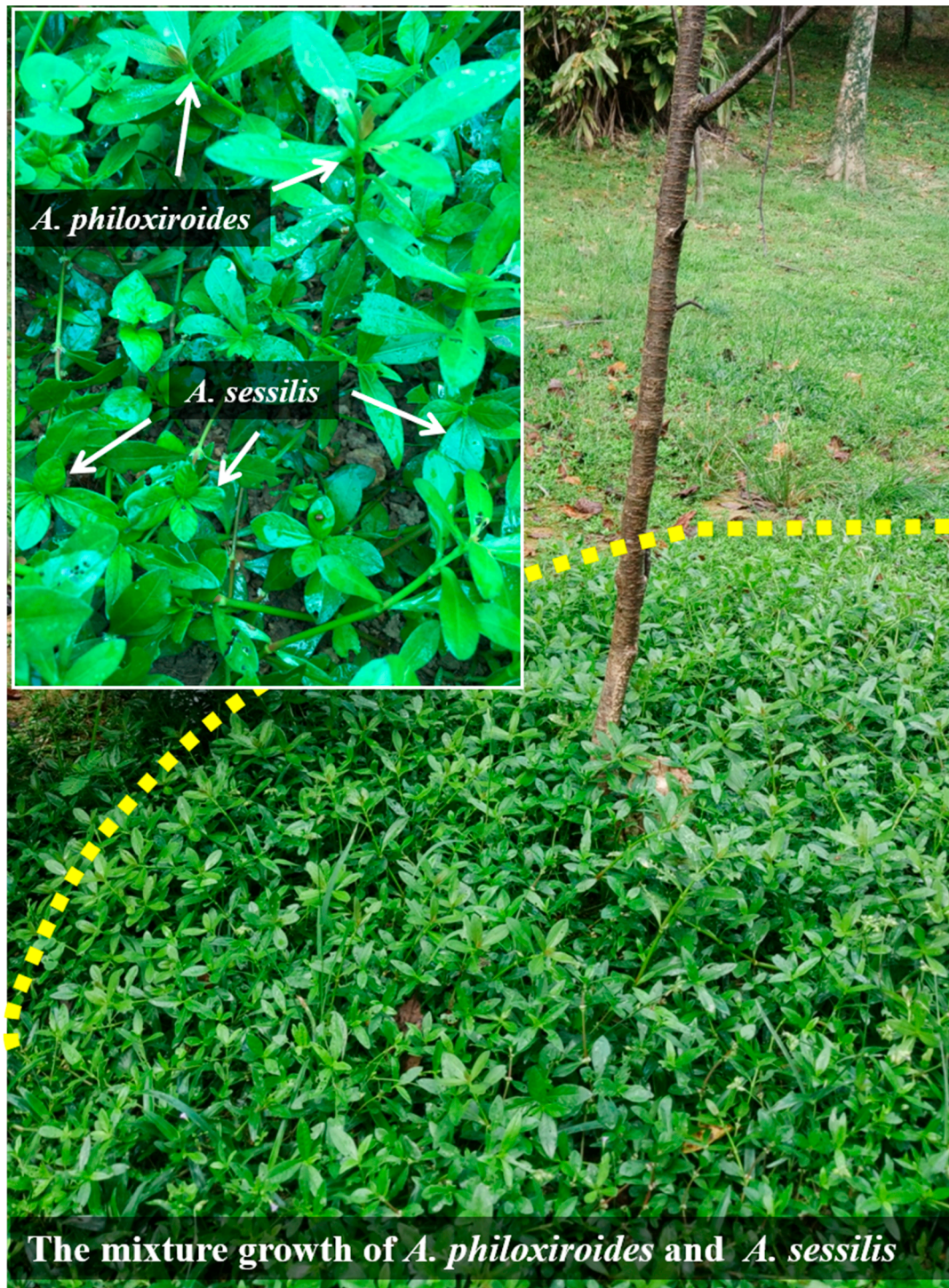


Figure S7. Sampling site of invasive *Alternanthera philoxeroides* and native *A. sessilis*. Samples were collected from the Fuzhou National Forest Park, Fuzhou, Fujian, China.

Table S1. Fold-change ratio of defense hormones and their responsive genes under different treatment conditions in invasive *Alternanthera philoxeroides* compared to native *A. sessilis*. Fold-change ratios are based on the relative amounts of signal molecules produced in *A. philoxeroides* and *A. sessilis* at the different time intervals. Fold-change ratios are calculated based on the comparative $2^{-\Delta\Delta CT}$ method normalized with the *Actin* gene at indicated time intervals after infection. Values highlighted in green show positive (higher) expression and red indicate negative (reduced) expression, compared with the control. Abbreviations: JA – Jasmonic acid, SA – Salicylic acid, ET – Ethylene, AP - *A. philoxeroides*, AS - *A. sessilis*. .

Treatment		Time (hpi)	JA-LOX		JA-JAR1		JA-PR6		SA-PAL		SA-PR3		ET-EIN3	
			AP	AS	AP	AS	AP	AS	AP	AS	AP	AS	AP	AS
<i>Rhizoctonia solani</i>		6	2.71	2.90	1.28	1.12	1.09	1.64	0.53	1.13	0.06	0.13	0.92	0.68
		12	0.43	1.09	0.60	0.92	0.69	0.83	1.30	2.21	0.04	0.16	1.25	0.79
		24	1.94	0.45	0.64	1.20	0.87	2.00	3.24	1.74	3.30	11.36	1.02	0.60
		48	0.79	1.89	0.47	1.59	0.60	2.32	8.81	3.02	10.24	10.62	1.67	0.98
		72	1.04	0.61	0.55	1.16	0.71	1.92	6.86	6.59	7.56	13.99	1.20	0.61
		96	1.75	0.32	0.62	0.66	0.97	0.93	4.96	7.58	7.72	4.84	2.66	0.83
MeJA pretreated before <i>R. solani</i> inoculations		6	0.27	4.15	0.32	1.90	0.27	0.80	0.21	5.11	4.01	1.91	0.49	9.88
		12	0.32	4.78	0.24	1.09	0.56	1.20	0.89	6.76	2.45	2.08	0.37	4.19
		24	0.40	1.88	0.64	1.97	0.57	1.32	0.37	3.21	6.95	10.54	0.66	1.93
		48	0.05	2.04	0.23	0.91	0.90	1.82	1.97	10.65	75.56	2.04	0.83	3.35
		72	0.10	1.44	0.07	0.67	0.39	1.04	3.23	8.73	73.16	1.41	0.62	2.46
		96	0.08	1.47	0.17	0.61	0.66	0.92	1.03	6.88	56.92	4.25	0.47	2.52
SA pretreated before <i>R. solani</i> inoculations		12	0.06	0.11	1.29	0.54	0.76	1.47	1.41	1.04	38.89	2.57	2.11	0.90
		24	1.70	0.80	1.18	0.73	2.13	1.36	1.62	1.70	56.04	1.20	1.53	1.46
		48	0.63	0.02	1.13	0.71	2.53	0.49	1.25	3.44	98.83	1.88	1.44	1.62
ET pretreated before <i>R. solani</i> inoculations		12	0.39	0.22	1.93	2.44	2.22	1.63	0.63	1.61	7.46	5.92	1.86	2.48
		24	2.01	1.39	1.78	1.91	1.41	2.88	0.87	1.50	1.33	2.31	1.01	2.71
		48	3.01	1.37	1.63	1.50	1.37	1.48	1.80	0.61	13.32	0.25	1.24	2.26
Systemic leaves		6	1.46	2.10	1.04	1.23	1.10	1.32	0.81	1.28	0.14	0.17	0.68	0.58
		12	0.78	1.11	0.60	0.68	0.80	0.49	0.83	1.46	0.02	0.04	0.76	0.37
		24	1.68	6.07	0.60	1.60	1.05	3.13	0.77	2.87	0.03	0.39	0.54	1.15
		48	1.56	3.37	0.86	1.49	1.33	1.65	1.33	1.28	0.18	0.24	0.58	1.06
		72	2.03	3.01	0.95	1.64	1.08	1.44	1.45	1.69	0.38	0.32	0.54	1.02
		96	1.81	3.80	0.74	1.85	1.03	1.86	1.31	1.46	0.05	0.21	0.47	0.70

Table S2. Primers used for quantitative RT-qPCR. *A species-specific primer was used for the *LOX* gene due to difficulty in finding common sequences between species. For other genes, a single common primer to both species was used for expression analysis.

Gene	*Source	Primers (5' to 3')	Product size (bp)	Reference
<i>PAL</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-TCTATGGATAATGCAAGGTTGG-3' R: 5'-GGTTTGAGGGAAGACCATTG-3'	106	This study
<i>PR3</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-ATCTCATGGAAC TACAAC TACGG-3' R: 5'-AGAAGCCTGTCTTGAAGGATATAA-3'	112	This study
<i>LOX</i>	<i>A. philoxeroides</i>	F: 5'-GGTCGCTGAAACAATCAATAAC-3' R: 5'-TCTTGATAACAGCTGGGACATC-3'	86	This study
<i>LOX</i>	<i>A. sessilis</i>	F: 5'-GGTCGCTGAAACGATCAATAAC-3' R: 5'-TCTTGATAACAGCTGGGACATC-3'	86	This study
<i>JAR1</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-CTACTCGAGCTGCTATGTCAAA-3' R: 5'-AGCCATACCATTGCTTAAACTG-3'	96	This study
<i>PR6</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-TGAAGCAAAGGTATGGGTGAA-3' R: 5'-CACCAAGGTCTGAAGGAGTAATAG-3'	101	This study
<i>EIN3</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-TGCTGAAGATGATGGAGGTTT-3' R: 5'-CACCAGGCTCGAAGATTGT-3'	106	This study
β -Tubulin	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-CTACCTCTTTAGTGCTCATCTTACC-3' R: 5'-CAAATGTGGGATGCCAAGAAC-3'	106	This study
<i>Elongation factor 1-alpha</i>	<i>A. philoxeroides</i> & <i>A. sessilis</i>	F: 5'-TTGCTTCTGACTCCAAGAATGA-3' R: 5'-GCATAACCATTTC AATCTGACC-3'	100	This study
<i>Actin</i>	<i>A. philoxeroides</i> , <i>A. sessilis</i> & <i>Beta vulgaris</i>	F: 5'-TCAATGTGCCTGCTATGTATGT-3' R: 5'-GTGACTAACACCATCACCAGAG-3'	104	This study & Li & Smigocki, 2016

Table S3. List of putative defense hormones and responsive genes isolated in both *Alternanthera philoxeroides* and *A. sessilis*.

Plant	Hormone	Gene	Function	Size (bp)	GenBank Accession
<i>A. philoxeroides</i>	Salicylic Acid	<i>PAL</i>	SA biosynthesis	440	MK790145
<i>A. sessilis</i>				436	MK790146
<i>A. philoxeroides</i>	Salicylic Acid	<i>PR3</i>	Acidic chitinase	304	MK790153
<i>A. sessilis</i>				303	MK790154
<i>A. philoxeroides</i>	Jasmonic Acid	<i>LOX</i>	JA biosynthesis	578	MK790155
<i>A. sessilis</i>				613	MK790156
<i>A. philoxeroides</i>	Jasmonic Acid	<i>JAR1</i>	Catalyzes the synthesis of several JA-amido conjugates	305	MK790147
<i>A. sessilis</i>				307	MK790148
<i>A. philoxeroides</i>	Jasmonic Acid	<i>PR6</i>	Proteinase inhibitor	172	MK790149
<i>A. sessilis</i>				172	MK790150
<i>A. philoxeroides</i>	Ethylene	<i>EIN3</i>	A transcription factor and positive regulator of ET-responsive gene expression	525	MK790151
<i>A. sessilis</i>				527	MK790152

Table S4. Sequence information of six defense hormones and responsive genes isolated from *Alternanthera philoxeroides* and *A. sessilis*. See Supplementary Table S2 for additional information regarding each isolated gene. Selected ORF (Open Reading Frame) indicates six-reading frames of each isolated gene sequence. Abbreviations: AP - *A. philoxeroides*, AS - *A. sessilis*, CDS - Coding DNA Sequence.

Predictions	Salicylic Acid				Jasmonic Acid				Ethylene			
	<i>PAL</i>		<i>PR3</i>		<i>LOX</i>		<i>JAR1</i>		<i>PR6</i>		<i>EIN3</i>	
	AP	AS	AP	AS	AP	AS	AP	AS	AP	AS	AP	AS
Nucleotide (bp)	451	448	305	306	307	622	310	311	175	174	535	537
Selected ORF	+2	+3	+1	+3	+1	+2	-3	+1	-2	-1	+3	+2
CDS length (bp)	418	414	165	303	282	270	132	123	147	147	447	480
Peptide length (aa)	139	138	55	101	94	90	44	41	49	49	149	160
Similarity (Gene family)	Phenylalanine ammonia-lyase		EP3-like protein		Lipoxygenase 6 gene family		JAR1-like proteins		No significant similarity		Ethylene insensitive 3-like protein	

Table S5. Functional analysis of three hormone genes in both *Alternanthera philoxeroides* and *A. sessilis*. Assembled contigs and annotated gene sequences were used for predicting protein isoelectric point (http://bioinformatics.org/sms2/protein_iep.html) and protein molecular weight (http://bioinformatics.org/sms2/protein_mw.html). We could not predict the gene from the assembled contigs of *JAR1* from *A. sessilis*. Abbreviations: AP - *A. philoxeroides*, AS - *A. sessilis*.

Predictions	PAL (SA)		JAR1 (JA)		EIN3 (ET)	
	AP	AS	AP	AS	AP	AS
Contig length (bp)	1411	1286	1284	1206	675	1698
Number of Exons	2	1	1	-	1	1
mRNA length (bp)	1080	993	945	-	654	1464
No. of amino acids	360	331	314	-	218	488
Isoelectric Point (pH)	7.37	6.68	8.04	-	9.34	6.34
Protein molecular weight (kDa)	39.01	35.67	35.29	-	24.98	54.95
No. of motifs	5	5	3	-	2	3
Representing domain	PLN02457 superfamily (PAL) (26 to 360 aa)	PLN02457 superfamily (PAL) (1 to 331 aa)	GH3 superfamily domain (1 to 293 aa)	-	EIN3 superfamily (15 to 218 aa)	EIN3 superfamily (27 to 275 aa)

Table S6. Screening and isolation of defense hormones and responsive gene sequences from both invasive *Alternanthera philoxeroides* and native *A. sessilis*. The highlighted green color in the table are the sequences of genes that were successfully isolated (see Supplementary Table S3 above).

S. No	Gene name and function	Primer Name	Primers (5' to 3')	Primer Length (bp)	Tm (°C)	GC (%)	Product size (bp)
Salicylic Acid regulated signaling pathway genes							
1	<i>NDR1</i> NDR1 (Non race-specific Disease Resistance 1); a plasma membrane protein, for CC-NBS-LRR subset of R genes.	AmNDR1-F AmNDR1-R	F: 5'-TGCTAGAGGGGTAAAATGGGA-3' R: 5'- ACCCAAAACACCCAACAACATAA-3'	21 23	58.15 59.22	47.62 39.13	250
2	<i>EDS1</i> PAD4 (PhytoAlexin Deficient 4); lipase-like. Act upstream of SA in basal resistance to biotrophic pathogens & ETI initiated by TIR-NBS-LRR subset of R genes.	AmEDS1-F AmEDS1-R	F: 5'-GTGGAATGAGGTAGCCCAGA-3' R: 5'-CGATCCAGTGTCTCGTTCT-3'	20 20	58.80 59.19	55 55	577
3	<i>NPR1</i> NPR1 (Nonexpresser of PR genes 1); transcription cofactor. Positive regulator of SA and negative regulator of JA.	AmNPR1-F AmNPR1-R	F: 5'-ACACAGAGCTTTGGAATCCG-3' R: 5'-ATGCACAACCGATCCTTTGG-3'	20 20	58.19 58.82	50 50	375
4	<i>PR1</i> Pathogenesis related protein 1	AmPR1-F AmPR1-R	F: 5'-TCATCCTTGCATCAACCCCT-3' R: 5'-AGCTGTCTGCCAAGTAAA-3'	20 20	59.00 58.95	50 50	328
5	<i>PAL</i> (see Table S2) Phenylalanine Ammonia Lyase	AmPAL-F AmPAL-R	F: 5'-TCACCCAGGACAGATTGAGG-3' R: 5'-GCTCGGAACAGTAAGAAGCC-3'	20 20	58.72 58.64	55 55	476
6	<i>PR2</i> PR-2 (endo-1,3-beta-glucanase)	AmPR2-F AmPR2-R	F: 5'-CGGCCATGCAAAATGTCCAA-3' R: 5'-GCCGCATACACAGTGTCTAC-3'	20 20	60.04 58.72	50 55	331
7	<i>PR3</i> (see Table S2) PR3-Acidic chitinase	AmPR3-F AmPR3-R	F: 5'-TCCATGCAACCCAAGTAAGC-3' R: 5'-GGTTATCCCTGGCGAAACG-3'	20 20	58.46 59.27	50 55	332
8	<i>PR5</i> PR-5 (thaumatin-like)	AmPR5-F AmPR5-R	F: 5'-CCATGGTTTTCAACGCTCCA-3' R: 5'-GTTAAACCCGTCGACAAGGC-3'	20 20	59.04 59.49	50 55	212
Jasmonic Acid regulated signaling pathway genes							
1	<i>JAR1</i> (see Table S2) (Jasmonate Resistant 1); jasmonate-amino synthetase; a member of the GH3 family. JAR1 enzyme that produces the JA-Ile conjugate.	AmJAR1-F AmJAR1-R	F: 5'-TGCAGTCTCTGATGAAGTGGT-3' R: 5'-GCATTGGGGAAGCTCAGG-3'	20 20	58.94 59.55	50 55	335
2	<i>COI1</i> (COronatine Insensitive 1); ubiquitin-protein ligase, required for jasmonate-regulated defense.	AmCOI1-F AmCOI1-R	F: 5'-AGGTAAAGCGGCTCAGGAT-3' R: 5'-GCCCCACATAACTGAGACCT-3'	20 20	59.09 59.18	50 55	352
3	<i>MYC2</i> JIN1 (Jasmonate INsensitive 1); MYC2; TF & a positive regulator of JA-responsive genes such as <i>VSP2</i> and <i>LOX2</i> , whereas it acts as a negative regulator of JA/ET-responsive genes such as <i>PDF1.2</i> .	AmMYC2-F AmMYC2-R	F: 5'-CGAGTTCGCCTTGTAATCGG-3' R: 5'-ATATCTCCCCTGATTCCGGC-3'	20 20	59.08 58.72	55 55	513
4	<i>LOX</i> (see Table S2)	AmLOX-F	F: 5'-TTCGCGTGATGAAGCITTT-3'	20	58.77	45	470

	JA-mediated defense pathway marker gene.	AmLOX-R	R: 5'-AATGGCCTCTTCGACACTCA-3'	20	59.02	50	
5	AOS Allene Oxide Synthase 1, chloroplastic.	AmAOS-F AmAOS-R	F: 5'-GTTCTCTCCTACCTCGACCC-3' R: 5'-TTCGTACGTGAGATCCCAA-3'	20 20	58.61 59.04	60 55	471
6	PR6 (see Table S2) PR6 proteinase inhibitor	AmPR6-F AmPR6-R	F: 5'-CTGGAGTTTGCAAGGGTTGT-3' R: 5'-AGCACCAAGGTCTGAAGGAG-3'	20 20	58.60 59.31	50 55	204
7	PR9 Peroxidase	AmPR9-F AmPR9-R	F: 5'-TTGTCAGGGGTGTGATGGA-3' R: 5'-AATTGCTTGAGAAACCGCA-3'	20 20	58.86 58.67	50 45	302
Ethylene regulated signaling pathway genes							
1	ACC synthase Aminocyclopropane-1-carboxylic acid synthase is an enzyme that catalyses the synthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC), a precursor for ethylene, from S-Adenosyl methionine.	AmACC-F AmACC-R	F: 5'-AGAACAGGGGTGGAGCTTTT-3' R: 5'-CCAAACACTGTGGCTCCATA-3'	20 20	59.15 57.80	50 50	254
2	EIN3 (see Table S2) EIN3 (Ethylene INsensitive 3); transcription factor. A positive regulator of ET-responsive gene expression	AmEIN3-F AmEIN3-R	F: 5'-GCGAGTGGTGGAAAGACAAG-3' R: 5'-GGACACTCACTTCCATGCC-3'	20 20	59.13 58.83	55 55	351
3	ERF1 AP2/ERF and B3 domain-containing transcription factor. A TF & positive regulator of JA and ET signaling.	AmERF1-F AmERF1-R	F: 5'-AAATGGTCATTGGGGTGCAC-3' R: 5'-GTCAGTGGCATAGTGGGCTT-3'	20 20	59.02 60.04	50 55	205
4	ERF2 Ethylene-responsive transcription factor ERF024. A TF & positive regulator of JA and ET signaling.	AmERF2-F AmERF2-R	F: 5'-GGGAAGTGGGTCTCGGAAAT-3' R: 5'-AGCTGCCTGAATGTCCCTT-3'	20 19	59.38 58.92	55 52.63	204
5	PDF1.2-A Defensin-like protein AX1. ET/JA-mediated defense pathway marker gene.	AmPDF1.2A-F AmPDF1.2A-R	F: 5'-TGAGATGAACATGGTGGTTGAG-3' R: 5'-ACGGGCACACAGATTCCAAT-3'	22 20	58.32 59.96	45.45 50.00	150
6	PDF1.2-B Defensin Ec-AMP-D2-like. ET/JA-mediated defense pathway marker gene.	AmPDF1.2B-F AmPDF1.2B-R	F: 5'-CAACTGCAGAGGTAGGACCA-3' R: 5'-TGTTTAGTGCAGAAGCATCGAC-3'	20 22	59.02 59.26	55.00 45.45	172