**Supplementary file 1A. Genes induced by FAC in all six *Nicotiana* species.** Green and red colors indicate e3 ubiquitin-protein ligase and transcription factors, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene ID** | **Annotation** | **Note** | **Best Blast Hit** |
| NIATv7\_g05178 | e3 ubiquitin-protein ligase pub23-like | e3 ubiquitin-protein ligase | XP\_004228871 |
| NIATv7\_g34426 | e3 ubiquitin-protein ligase pub23-like | e3 ubiquitin-protein ligase | XP\_004241334 |
| NIATv7\_g16579 | ap2 erf domain-containing transcription factor | Transcription Factor | XP\_004237817 |
| NIATv7\_g30738 | ap2 erf domain-containing transcription factor | Transcription Factor | XP\_004237144 |
| NIATv7\_g12925 | heat stress transcription factor a-4a-like | Transcription Factor | XP\_004243245 |
| NIATv7\_g36386 | transcription factor tt2-like | Transcription Factor | XP\_004236420 |
| NIATv7\_g09195 | PLATZ transcription factor family | Transcription Factor | XP\_009790819 |
| NIATv7\_g12331 | probable wrky transcription factor 28-like | Transcription Factor | XP\_004233015 |
| NIATv7\_g35285 | probable wrky transcription factor 41-like | Transcription Factor | XP\_004234275 |
| NIATv7\_g27487 | probable WRKY transcription factor 48 | Transcription Factor | XP\_009770697 |
| NIATv7\_g35699 | endochitinase pr4 |  | XP\_004237833 |
| NIATv7\_g00113 | auxin-regulated protein |  | NP\_001234635 |
| NIATv7\_g01329 | PREDICTED: uncharacterized protein LOC101261228 |  | XP\_004238950 |
| NIATv7\_g01695 | ring-h2 finger protein atl3-like |  | XP\_004235522 |
| NIATv7\_g04111 | vq motif-containing protein |  | XP\_004237898 |
| NIATv7\_g05562 | u-box domain-containing protein 21-like |  | XP\_004250456 |
| NIATv7\_g08158 | f-box protein skip27-like |  | XP\_004233817 |
| NIATv7\_g13317 | uncharacterized loc101205876 |  | XP\_004232247 |
| NIATv7\_g14560 | sugar transport protein 13-like |  | NP\_001234785 |
| NIATv7\_g14832 | isoflavone 2 -hydroxylase-like |  | XP\_004238116 |
| NIATv7\_g15963 | phospholipase a1- chloroplastic-like |  | XP\_004244860 |
| NIATv7\_g17751 | predicted protein |  | XP\_002322915 |
| NIATv7\_g17823 | probable indole-3-acetic acid-amido synthetase -like |  | XP\_004230721 |
| NIATv7\_g23001 | PREDICTED: uncharacterized protein LOC100854721 isoform 1 |  | XP\_003633026 |
| NIATv7\_g23271 | basic 7s globulin-like |  | NP\_001234249 |
| NIATv7\_g23435 | uncharacterized loc101216872 |  | XP\_002536881 |
| NIATv7\_g29183 | nac domain ipr003441 |  | XP\_002328928 |
| NIATv7\_g30658 | thioredoxin reductase 2-like |  | XP\_004232594 |
| NIATv7\_g31295 | mitochondrial chaperone bcs1-like |  | XP\_004234999 |
| NIATv7\_g40482 | 4-coumarate-- ligase 1-like |  | XP\_004235870 |
| NIATv7\_g18591 | LURP-one-related 14-like isoform X2 |  | XP\_009763498 |
| NIATv7\_g26461 | F-box PP2-B15-like |  | XP\_009795812 |
| NIATv7\_g28238 | exocyst complex component EXO70A1-like |  | XP\_009798500 |
| NIATv7\_g58651 | PREDICTED: uncharacterized protein LOC104242562 |  | XP\_009795939 |
| NIATv7\_g59916 | RING-H2 finger ATL3-like |  | XP\_009788965 |

**Supplementary file 1B. Genes which showed lower expression in samples induced by *M. sexta* OS than by FAC in both *N. attenuata* and *N. pauciflora.*** Numbers in each column indicate the TMM-normalized FPKM values.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | ***N. attenuata*** | | | | | | | | | | | | ***N. pauciflora*** | | | | | | | | | | | | **Annotation** |
| **Treatments** | Water | | | FAC | | | OS*Ms* | | | OS*Sl* | | | Water | | | FAC | | | OS*Ms* | | | O*SI* | | |
| **Replicates** | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| NIATv7\_g02972 | 1 | 1 | 2 | 24 | 42 | 31 | 17 | 21 | 8 | 22 | 33 | 11 | 1 | 1 | 1 | 5 | 5 | 4 | 2 | 2 | 2 | 3 | 3 | 3 | g-type lectin s-receptor-like serine threonine-protein kinase |
| NIATv7\_g09323 | 4 | 8 | 6 | 28 | 49 | 29 | 19 | 24 | 20 | 24 | 31 | 19 | 2 | 3 | 2 | 11 | 8 | 13 | 4 | 6 | 6 | 3 | 3 | 4 | ammonium transporter 1 member 1-like |
| NIATv7\_g12469 | 10 | 11 | 13 | 24 | 28 | 22 | 12 | 16 | 9 | 19 | 21 | 10 | 10 | 10 | 10 | 19 | 18 | 13 | 10 | 8 | 11 | 16 | 15 | 13 | dis3-like exonuclease 2-like |
| NIATv7\_g13385 | 13 | 22 | 12 | 38 | 43 | 53 | 26 | 21 | 28 | 30 | 44 | 32 | 10 | 10 | 10 | 31 | 24 | 20 | 13 | 16 | 15 | 22 | 17 | 15 | chalcone-flavanone isomerase-like protein |
| NIATv7\_g15963 | 1 | 1 | 1 | 8 | 15 | 9 | 5 | 5 | 3 | 7 | 9 | 3 | 7 | 5 | 8 | 41 | 35 | 31 | 18 | 21 | 21 | 10 | 11 | 9 | phospholipase a1- chloroplastic-like |
| **NIATv7\_g23173** | **8** | **7** | **10** | **20** | **37** | **21** | **12** | **13** | **10** | **16** | **21** | **11** | **6** | **7** | **6** | **19** | **17** | **14** | **10** | **11** | **8** | **12** | **11** | **8** | ***NtJAR*1.1** |
| NIATv7\_g42489 | 2 | 2 | 2 | 37 | 45 | 39 | 24 | 22 | 27 | 33 | 38 | 26 | 25 | 16 | 22 | 88 | 83 | 82 | 40 | 64 | 51 | 28 | 34 | 26 | u-box domain-containing protein 21-like |

**Supplementary file 1C. Summary of RNA-seq reads from six closely related *Nicotiana* species.**

**Supplementary file 1D. Primers used for qPCR and VIGS in this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene ID** | **Forward Primer** | **Reverse Primer** | **Experiment** |
| *NaLRRK1*(NIATv7\_g00304) | CTAGATTTTCTTGATACCCTTTCTT | CCATCTTCGCGGTCATTACTTT | Open reading frame clone |
| TGTTGCTGGTTCTGTCGGTT | CCGGCCTTCTGGTGAGATAC | qPCR for silencing efficiency |
| GCGGCGGTCGACATATTCCTCCAGAATATGCA | GCGGCGGGATCCTAACACAAGCTAGAGCCAC | VIGS construct |
| CACCATGAACTTAAGGTATGCTC | CCTTGCATTGAGAAGCATGCGAA | Subcellular localization |
| *Plasma membrane intrinsic protein 2a* | CACCATGGCAAAGGATGTGGAAGC | GACGTTGGCAGCACTTCTGAA | Subcellular localization |
| *Elongation Factor 1α (D63396)* | CCACACTTCCCACATTGCTGT | CGCATGTCCCTCACAGCAAAAC | qPCR |
| *NaGLA* | AGTAGCAGATGATGTTAGTACATGTA | ACATGTGAATATGCCCATGGCATACT | qPCR |
| *NaLOX3* | GGCAGTGAAATTCAAAGTAAGAG | CCCAAAATTTGAATCCACAACA | qPCR |
| *NaAOS* | GACGGCAAGAGTTTTCCCAC | TAACCGCCGGTGAGTTCAGT | qPCR |
| *NaAOC* | CTATATACCGGAGACCTAAAGAAGA | AGTATCCTCGTAAGTCAAGTACGAT | qPCR |
| *NaCYP94B3-like1/2* | TGAAACGCGTTCACTGTTGT | GAGTGCCTTATCGCCATTGT | qPCR |
| *NaCYP94C1* | ACACTGTTGCTTCTGCGTTG | GTACCATCGGGGAGCGTAT | qPCR |
| *NaTPI* | TCAGGAGATAGTAAATATGGCTGTTCA | ATCTGCATGTTCCACATTGCTTA | qPCR |
| *NaTD* | ATTTTGCCTGTAACCCCAAA | GCCCCCTGATGGATTATTCT | qPCR |
| *NaMyb8* | AACCTCAAGAAACTCAGGACATACAA | GATGAATGTGTGACCAAATTTTCC | qPCR |
| *NIATv7\_g00304* | AAATTCAACAAGGCCAAATCAG | TAAACTTCCATCAGTCTTTCCC | qPCR |
| *NIATv7\_g10386* | TGTTGCTGGTTCTGTCGGTT | CCGGCCTTCTGGTGAGATAC | qPCR |
| *NIATv7\_g21011* | TCAAATCATTGTTCTTGGCCTGG | GTTGTTTGAAGTCAAGTTAAGGACT | qPCR |
| *NIATv7\_g26672* | TTTGGAGCTTCTTACAGGCAAA | CAGGTGGTGTGTTTATGTTGTC | qPCR |
| *NIATv7\_g39903* | CCCTTCTTCCTCAACCACT | CATTAGGGAGGCTGCTGT | qPCR |
| *NIATv7\_g42200* | CGGTGGATCTACTATTTTTCCT | TGAGAAGTGGGGCTATGTGC | qPCR |
| *NIATv7\_g28967* | CGAGTCTTTTGGATTCGGTAA | ATGCTGTGACGCCATCATAC | qPCR |
| *NIATv7\_g26266* | CAAAGTTGAGCAGAAACAGAACG | GCTTCCATGTTGGATTTGGAAGT | qPCR |
| *NIATv7\_g08724* | AATTCCAAAATCAAGAGCAACCC | TTTCTGTTCGACTACTCTGCTCT | qPCR |
| *NIATv7\_g15549* | GGTTCTTTGAATTGGGGTTG | GGGTAATGCAGTTTTTCCAAG | qPCR |
| *NIATv7\_g17204* | GGAAGCGATTTGTCCTACTCTTC | AACAGCATTTCCACCAGAGAAAA | qPCR |
| *NIATv7\_g05178* | GAGCGAATCGAAAACCAGTC | GGGTCTAATCCCGATAAAAGG | qPCR |