**Supplemental Table S2.** ‘Draper’ genome gene IDs and genbank numbers

|  |  |  |
| --- | --- | --- |
| **Gene** | **‘Draper’ gene ID** | **Genbank** |
| *CHS* | augustus\_masked-VaccDscaff9-processed-gene-64.0 | JN654702.1 |
| *F3’5’H* | maker-VaccDscaff8-augustus-gene-360.23-mRNA-1 | MH321464.1 |
| *DFR* | augustus\_masked-VaccDscaff13-processed-gene-166.8 | KF960989 |
| *ANS* | maker-VaccDscaff43-augustus-gene-236.29 | JN654701.1 |
| *UFGT* | maker-VaccDscaff6-augustus-gene-420.36 | MH321467.1 |
| *ANR* | maker-VaccDscaff19-augustus-gene-256.19 | MH321471.1 |
| *LAR* | maker-VaccDscaff9-augustus-gene-315.13  | MH321470.1 |
| *MYBA1* | maker-VaccDscaff1486-snap-gene-0.3 | MH105054 |
| *MYBA2* | maker-VaccDscaff13-augustus-gene-105.26-mRNA-1 |  |
| *MYBPA1.1* | maker-VaccDscaff39-snap-gene-168.25 | JQ085966 |
| *MYBPA2.1* | maker-VaccDscaff32-augustus-gene-55.27 |  |
| *MYBPA2.2* | maker-VaccDscaff34-augustus-gene-10.31 |  |
| *MYBPA2.3* | maker-VaccDscaff33-snap-gene-307.38 |  |
| *MYBC2.1* | maker-VaccDscaff28-augustus-gene-197.19 |  |
| *MYBC2.2* | maker-VaccDscaff4-augustus-gene-330.20 |  |
| *MYBR3.1* | maker-VaccDscaff4-snap-gene-174.23 |  |
| *MYBR3.2* | maker-VaccDscaff1069-augustus-gene-0.8 |  |
| *WRKY2* | maker-VaccDscaff26-augustus-gene-44.51 |  |
| *WRKY3* | maker-VaccDscaff11-augustus-gene-207.26 |  |
| *WRKY5* | maker-VaccDscaff4-augustus-gene-398.15 |  |
| *WRKY6* | maker-VaccDscaff19-augustus-gene-368.29 |  |
| *NAC1* | maker-VaccDscaff30-augustus-gene-305.45 |  |
| *bHLH2* | maker-VaccDscaff19-augustus-gene-381.30 |  |
| *MATE8* | maker-VaccDscaff4-snap-gene-174.23 | KF875439.1 |
| *PAL* | maker-VaccDscaff10-augustus-gene-222.29 |  |
| *C4H* | augustus\_masked-VaccDscaff33-processed-gene-307.8 |  |
| *CHI* | maker-vaccdscaff26-snap-gene-196.29 | MH321461.1 |
| *F3H* | maker-VaccDscaff32-augustus-gene-323.35 |  |
| *DFR* | augustus\_masked-VaccDscaff13-processed-gene-166.8 | KF960989 |
| *LDOX* | maker-VaccDscaff6-augustus-gene-163.26 |  |
| *GST* | maker-VaccDscaff30-augustus-gene-282.25 |  |

|  |  |  |
| --- | --- | --- |
| Gene | Forward primer (5'-3') | Reverse primer (5'-3') |
| *VcMYBR3.1* | CACCAAAATGGCTGACTCAGAACAC (TOPO) | TCATTCGCTCGTTGAGTATCTGGAG |
| *VcMYBC2.1* | CACCAAAATGAGGAAGCCATGTTGTG (TOPO) | TCATGTAAAGAGAGGAAGGGTG |
| *VcMYBR3.1pro* | ATTGCTCGAGTGTATGGATAGGTACATGAGTAGGA (XhoI) | TTACGAATTCTTGGAGAGAGGGGGAGAG (EcoRI) |
| *VcMYBC2.1pro* | GGATCTGCAGGTGGAGTCTGTGCACTCCT (Pst I) | GTACCATGGTCTACTCACACAGAGTACATAAACCTC (NcoI) |
| *VcbHLH2pro* | attgGCGGCCGCTCTCACTATGTTTTGTTTGTGTAGGCTAGA (NotI) | gtaCCATGGCTCCGCCGCTGGCCAC (NcoI) |

**Supplemental Table S3.** Primers used for isolating and cloning sequences into vectors for functional analysis

|  |  |  |
| --- | --- | --- |
| Gene | Forward primer (5'-3') | Reverse primer (5'-3') |
| *VcANS* | TTCCACACTGTTTACCCTGAGG | CTTCTTCCAATCCCAAGCAGAC |
| *VcANR* | CCGACAATCACAAGAAGACC | GGTCACAACCTGTTATTGGG |
| *VcUFGT* | GGAAGATTGGTGTGAGAGTGG | TGTTCCCTCAATTCCTTCCCC |
| *VcbHLH2* | TAGAGAGTGATGCGTTGG | CATTAGTCAACGACGACTGC  |
| *VcMYBA1* | CAACCCATCCCAAACACAACC | CCACGTCATCATACCTCTGC |
| *VcMYBPA1.1* | TCCTAACGACCAACCACCA | GGGCTTTGGGTTATGGACTT |
| *VcMYBPA2.3* | TCCACAGCCAAACATGAAAA  | CCAAAGCAAAATCCCTTGAG  |
| *VcMYBC2.1* | AGGAGCATGGTCCAAACAAG | TTCCCACAACGAAGTAGTCC |
| *VcMYBR3.1* | GAGCGAATTCAGGCATCTGT  | CCCAAAAACCTTGAACACGA  |

**Supplemental Table S4.** Blueberry gene-specific primers used for RT-qPCR analysis

**Supplemental Table S5. Differentially expressed TFs identified in DEseq2 analysis.**

Mapman annotaton is shown for each gene ID, with gene IDs representing the same gene grouped. The amino acid sequence of the gene IDs were BLASTP queried against the Araport11 protein sequences dataset using TAIR BLAST 2.9.0+ and the Arabidopsis accession number and annotation of the best hit is displayed. The log2 fold change in expression between blueberry and bilberry stage 7 flesh is shown. Mean counts represents the mean across all samples. Genes identified previously and used in further analysis are provided with gene names. The predicted function of the Arabidopsis gene is shown.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **MSU Gene ID** | **Mapman Bin Annotation** | **Arabidopsis AtG**  | **Annotation** | **Log2Fold**  | **Mean Counts** | **Gene Name** | **Predicted function in Arabidopsis, if known** |
| vaccdscaff28-197.19 | MYB | AT4G38620.1  | MYB4 | 1.0 | 983 | MYBC2.1 | Repressor of phenylpropanoids 1 |
| vaccdscaff37-143.27 vaccdscaff38-163.31 vaccdscaff39-168.25vaccdscaff6-261.32 | MYB | AT3G13540.1 | MYB5 | 8.0, 8.3, 7.8, 7.1 | 669, 2108, 3314, 913 | MYBPA1.1 | Controls outer seed coat differentiation, mucilage synthesis and trichome formation 2,3 |
| vaccdscaff9-307.24 vaccdscaff35-237.26 vaccdscaff4-330.20 vaccdscaff36-113.22 | MYB | AT4G09460.1 | MYB6 | 5.8, 5.5, 9.1, 5.0 | 192, 216, 216, 147 | MYBC2.2 | Repressor of flavonoids 4 |
| vaccdscaff58-1.25 | MYB | AT1G16490.1  | MYB58 | 2.2 | 85 |  | Activator of lignin biosynthesis 5 |
| vaccdscaff16-61.39 vaccdscaff7-363.26 vaccdscaff18-336.27 | MYB | AT3G47600.1  | MYB94 | 2.0, 3.5, 1.6 | 725, 274, 585 | MYB4 | Activator of cuticular wax biosynthesis 6 |
| vaccdscaff13-105.27 | MYB | AT1G66370.1  | MYB113 | 8.1 | 1543 | MYBA2 | Activator of anthocyanin biosynthesis 7 |
| vaccdscaff13-106.35 vaccdscaff1486-0.3 | MYB | AT1G66370.1  | MYB113 | 3.8, 4.3 | 1818, 4963 | MYBA1 | Activator of anthocyanin biosynthesis 7 |
| vaccdscaff1069-0.8 | MYB | AT1G01380.1 | ETC1 | 2.5 | 122 | MYBR3.2 | Repressor of trichome formation 8 |
| vaccdscaff190-0.40 | MED2/29/32 | AT1G11760.1 | MEDIATOR 2 | 3.6 | 60 |  | Regulates phenylpropanoid biosynthesis 9 |
| vaccdscaff11-136.2 | DOF | AT2G34140.1 | CDF4  | 4.0 | 32 |  | Cell differentiation 10  |
| vaccdscaff31-277.12 vaccdscaff18-68.36 | HD-ZIP I/II | AT4G40060.1 | HB16 | 4.5, 2.1 | 78, 57 |  |  |
| vaccdscaff43-73.22 | REM | AT1G49480.3 | RTV1 | 5.8 | 78 |  | Floral regulator 11 |
| vaccdscaff49-0.0 vaccdscaff49-3.6 | RAV/NGATHA | AT2G46870.1 | NGATHA1 | 2.9, 3.0 | 26, 33 |  | Induces ABA biosynthesis during drought stress 12 |
| vaccdscaff44-18.25 | NLP | AT4G24020.1 | NLP7 | 5.5 | 249 |  | Modulates nitrate sensing and metabolism 13 |
| vaccdscaff27-275.4 vaccdscaff17-311.1 | GRAS | AT1G07530.1 | SCL14 | 1.9, 3.2 | 198, 217 |  | Activates stress-responsive genes 14 |
| vaccdscaff32-313.21 | MADS/AGL | AT2G03710.2 | SEP4 | 1.9 | 507 | MADS1 | Involved in the development of sepals, petals, stamens and carpels 15 |
| vaccdscaff16-355.5 | C2H2 | AT1G27730.1 | STZ  | 10.1 | 94 |  | Transcriptional repressor that is induced by several stress responses to inhibit plant growth 16 |
| vaccdscaff13-50.35 vaccdscaff30-305.45 | NAC | AT2G33480.1 | NAC041 | 4.6, 5.2 | 31, 45 | NAC1 | Involved in regulating mannan biosynthesis 17 |
| vaccdscaff24-191.23 vaccdscaff15-200.28 vaccdscaff19-273.26 vaccdscaff11-207.26 | WRKY | AT1G62300.1 | WRKY6 | 2.0, 1.7, 1.9, 2.7 | 434, 267, 294, 359 | WRKY3 | Involved in senescence, pathogen defense, low phosphate stress and ABA signalling 18-20 |
| vaccdscaff22-298.44 vaccdscaff24-64.33 vaccdscaff19-368.29 vaccdscaff43-79.16 | WRKY | AT2G38470.1 | WRKY33 | 3.7, 1.3, 3.0, 2.4 | 150, 387, 798, 833 | WRKY6 | Involved in fungal resistance 21 |
| vaccdscaff36-33.17 vaccdscaff9-377.26 vaccdscaff35-292.32vaccdscaff4- 398.15 | WRKY | AT1G80840.1 | WRKY40 | 2.7, 3.2, 2.7, 4.5 | 1277, 1064, 1137, 2178 | WRKY5 | Pathogen and ABA induced transcriptional repressor which regulate plant defense to microbial pathogens and represses ABA responses 22-24 |
| vaccdscaff21-44.36 | WRKY | AT4G11070.1 | WRKY41 | 7.9 | 109 | WRKY2 | Transcriptional repressor of anthocyanins and activator of *AtABI3*, which regulates seed maturation 25,26 |
| vaccdscaff22-182.19 | JUMONJI | AT1G30810.3 | JMJ15 | 1.2 | 267 |  | A histone demethylase that regulates flowering time and high salt and temperature tolerance 27-29 |
| vaccdscaff27-261.27 vaccdscaff17-294.20 vaccdscaff34-85.27 | TAZ | AT5G63160.1  | BT1 | 1.3, 1.8, 1.2 | 278, 814, 968 |  | Transcriptional repressor of nitrate uptake genes and regulates plant development 30,31 |
| vaccdscaff11-350.30 vaccdscaff15-339.25 vaccdscaff24-51.34 | bHLH | AT2G43060.1  | IBH1 | 4.2, 1.7, 2.3 | 294, 611, 1569 |  | Transcriptional repressor of cell elongation 32 |
| vaccdscaff21-15.39 vaccdscaff33-304.30 vaccdscaff26-12.21 vaccdscaff29-11.26 | TIFY | AT1G19180.3  | JAZ1 | 2.3, 2.9, 2.2, 2.5 | 1620, 1700, 1426, 5017 |  | Repressors anthocyanin biosynthesis in the absence of jasmonate 33 |
| vaccdscaff16-197.18 | LUG | AT4G32551.1  | RON2 | 4.3 | 182 |  | Regulates flower and leaf development 34,35 |

**Supplementary Table S6.** Composition of tissue culture media used for blueberry transformation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Micro-propagation | Co-cultivation | Selection | Shoot Proliferation | Rooting |
| WPM macronutrients36 | ✓ | ✓ | - | - | ✓ |
| Modified WPM macro /micro/Fe/vitamins37  | - | - | ✓ | ✓ | - |
| MS micronutrients38 | ✓ | ✓ | - | - | ✓ |
| Cupric sulfate | 5 µM | 5 µM | 5 µM | 5 µM | ✓ |
| MS iron38 | ✓ | ✓ | - | - | ✓ |
| B5 vitamins39 | ✓ | ✓ | - | - | ✓ |
| Zeatin  | 4 mg L-1 | - | - | 4 mg L-1 | 0.3 mg L-1 |
| 1-naphthaleneacetic acid (NAA) | - | 0.5 mg L-1 | 0.5 mg L-1 | - | - |
| Thidiazuron (TDZ) | - | 1 mg L-1 | 1 mg L-1 | - | - |
| Coconut milk | - | - | - | - | 2% |
| Acetosyringone | - | 200 µM | - | - | - |
| Kanamycin sulfate | - | - | 30 mg L-1 | 30 mg L-1 | - |
| Cefotaxime | - | - | 250 mg L-1 | 250 mg L-1 | 250 mg L-1 |
| Sucrose | 20 g L-1 | 30 g L-1 | 30 g L-1 | 30 g L-1 | 20 g L-1 |
| Bacterial agar | 7.5 L-1 | 7.5 L-1 | 7.5 L-1 | 7.5 L-1 | - |
| Gelrite | - | - | - | - | 4 g L-1 |

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