**Supplementary Table 1: Potato germplasm used in this study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genotype abbreviation | Description | Ploidy | Flower color | Reference |
| **DM** | Doubled monoploid DM 1-3 516 R44 | diploid | white | (Potato Genome Sequencing Consortium 2011) |
|  |  |  |  |  |
| **RH** | RH89-039-16 | diploid | purple | (Potato Genome Sequencing Consortium 2011) |
|  |  |  |  |  |
| **DRH** | F1 hybrid population between DM and RH |  |  | (Felcher *et al.* 2012) |
|  |  |  |  |  |
| **DRH F1 171** | Specific F1 hybrid between DM and RH used for transformation | diploid | white | (Peterson *et al.* 2016) |
|  |  |  |  |  |
| **DRHp 28-5 (DRHp)** | Advanced inbred line of DRH F1 28 after self-pollination for 5 generations | diploid | purple | (Nadakuduti *et al.* 2019) |
|  |  |  |  |  |
| **F1 monoploids (DRHw)** | Monoploids derived by anther culture of randomly selected DRH F1 plants; only one monoploid was selected from any given F1 | monoploid | whitea | First described in this paper |
|  |  |  |  |  |
| **M1, M8, M9, M10, M11b** | Monoploids derived by anther culture of Adapted Phureja PIs 225669, 243461 and 225709 | monoploid | purple | (Hardigan *et al.* 2016) |
|  |  |  |  |  |
| **M2, M3, M5, M7b** | Monoploids derived by anther culture of Adapted Phureja PIs 225669 and 225709 | monoploid | white | (Hardigan *et al.* 2016) |

aWe would have expected both white and purple flowered monoploids as the F1 plants segregated for flower color; however, only white-flowered monoploids were recovered among 13 regenerated plants; bFor full description of the pedigree of these monoploids, see Supplementary Figure 1 in Hardigan et al. (2016)

Supplementary Table 2: Differentially expressed genes identified in the bulked segregant analysis. The gene annotation, location, and PGSC\_ID are listed; as are each FDR corrected p-value and fold change, where a positive fold change indicated upregulation in the purple bulks. Genes relevant to the flavonoid biosynthetic pathway are shaded.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Annotation** | **Fold Change** | **FDR p-value** | **Chr** | **Chr region start** | **Chr region end** | **RPKM white 1** | **RPKM white 2** | **RPKM purple 1** | **RPKM purple 2** |
| AN2 | 532.18 | 7.34E-41 | chr10 | 51469784 | 51470105 | 0.19 | 0.18 | 121.62 | 79.31 |
| Major latex\_1 | 115.58 | 3.62E-09 | chr01 | 67618877 | 67620455 | 1.49 | 0.4 | 1.15 | 217.51 |
| Glutathione S-transferase\_3 | 68.02 | 6.42E-69 | chr02 | 36961220 | 36962529 | 3.39 | 2.14 | 213.54 | 162.78 |
| Caffeoyl-CoA O-methyltransferase | 37.08 | 1.64E-37 | chr09 | 54418734 | 54424093 | 6.6 | 7.42 | 348.85 | 170.9 |
| Anthocyanin 5-O-glucosyltransferase | 36.62 | 1.14E-53 | chr12 | 59388654 | 59391021 | 3.1 | 3.3 | 126.73 | 107.61 |
| Anthocyanin synthase | 28.36 | 1.39E-12 | chr08 | 53728301 | 53732590 | 1.12 | 1.03 | 29.11 | 31.83 |
| Anthocyanin permease\_2 | 27.79 | 2.49E-48 | chr03 | 43777495 | 43780468 | 4.55 | 4.83 | 150.37 | 110.24 |
| Anthocyanin acyltransferase\_26 | 18.19 | 1.10E-28 | chr12 | 56016125 | 56018266 | 4.47 | 4.99 | 106.3 | 65.85 |
| Flavonoid 3',5'-hydroxylase | 17.19 | 8.71E-09 | chr11 | 39417578 | 39420629 | 1.24 | 1.67 | 29.85 | 20.23 |
| Acyl:coa ligase acetate-coa synthetase\_1 | 13.81 | 7.28E-06 | chr02 | 36961117 | 36964883 | 1.06 | 1.44 | 21.08 | 13.38 |
| Anthocyanin 1 | 10.29 | 0.03 | chr09 | 47422300 | 47429221 | 1.12 | 0.84 | 12 | 8.11 |
| Homeobox protein\_8 | 5.31 | 1.16E-03 | chr03 | 60116827 | 60123799 | 3.34 | 3.79 | 21.33 | 16.58 |
| D-alanine-D-alanine ligase | 4.24 | 7.15E-03 | chr10 | 53674619 | 53681752 | 3.8 | 4.76 | 19.33 | 16.97 |
| Conserved gene of unknown function\_3010 | 3.95 | 8.65E-10 | chr06 | 43585357 | 43588353 | 13.94 | 16.86 | 66.67 | 54.86 |
| Phenylalanine ammonia-lyase\_3 | 3.91 | 6.66E-05 | chr05 | 51694755 | 51698709 | 7.99 | 8.37 | 36.26 | 27.68 |
| Aminotransferase family protein\_4 | 3.22 | 2.10E-03 | chr10 | 2074803 | 2078104 | 19.8 | 16.77 | 33.32 | 84.52 |
| ASR4 | 2.66 | 7.10E-29 | chr04 | 60205626 | 60207072 | 306.51 | 310.8 | 753.53 | 885.52 |
| Proton P-ATPase | 2.55 | 6.97E-04 | chr07 | 270950 | 275949 | 20.22 | 15.16 | 40.55 | 49.7 |
| Cytochrome P450 hydroxylase\_15 | 2.44 | 2.52E-12 | chr10 | 54538508 | 54540606 | 59.17 | 68.11 | 159.2 | 151.14 |
| GPI-anchored protein\_2 | 2.44 | 1.52E-03 | chr03 | 4985642 | 4986819 | 18.86 | 18.09 | 45.01 | 45.04 |
| Pectate lyase P59\_3 | 2.41 | 0.03 | chr03 | 31505868 | 31507745 | 14.98 | 11.85 | 29.4 | 35.33 |
| SAUR family protein\_35 | 2.41 | 4.95E-03 | chr10 | 55103086 | 55103856 | 18 | 17.49 | 52.97 | 32.59 |
| Pectate lyase P59\_1 | 2.36 | 0.03 | chr02 | 26017219 | 26019334 | 17.39 | 11.43 | 30 | 38.08 |
| P-coumaroyl quinate/shikimate 3'-hydroxylase\_3 | 2.35 | 1.03E-03 | chr10 | 59468653 | 59471590 | 20.79 | 24.2 | 59.02 | 46.66 |
| Pectate lyase\_7 | 2.34 | 9.51E-03 | chr05 | 5914722 | 5917535 | 19.18 | 13.55 | 34.24 | 42.25 |
| L-ascorbate oxidase homolog | 2.33 | 6.66E-05 | chr01 | 28857039 | 28858942 | 36.02 | 24.13 | 63.52 | 76.64 |
| Ntp201 | 2.31 | 3.63E-04 | chr01 | 1062928 | 1065071 | 30.05 | 20.6 | 52.96 | 64.01 |
| Chalcone synthase 2 | 2.31 | 1.19E-07 | chr05 | 48886959 | 48888729 | 703.22 | 1,042.37 | 1,918.86 | 2,105.95 |
| Pectinesterase\_5 | 2.3 | 6.79E-05 | chr01 | 57206298 | 57208265 | 40.76 | 25.46 | 67.52 | 84.54 |
| Monosaccharide transporter 1 | 2.29 | 4.67E-03 | chr01 | 3931847 | 3936000 | 23.83 | 16.23 | 44.2 | 47.7 |
| Cullin 1A | 2.27 | 1.37E-03 | chr06 | 1962708 | 1966421 | 28.67 | 17.78 | 53.34 | 52.12 |
| PGPS/D3\_2 | 2.22 | 4.66E-03 | chr03 | 46614170 | 46615243 | 26.63 | 17.48 | 42.76 | 55.35 |
| Gene of unknown function\_6940 | 2.22 | 0.02 | chr11 | 38321397 | 38324170 | 17.71 | 19.15 | 42.3 | 39.62 |
| Pectinesterase\_37 | 2.22 | 0.01 | chr06 | 59454833 | 59456909 | 23.09 | 15.18 | 38.77 | 46.21 |
| Conserved gene of unknown function\_2983 | 2.17 | 2.92E-05 | chr06 | 40610372 | 40612477 | 52.84 | 34.67 | 86.12 | 103.57 |
| Phenylalanine ammonia-lyase\_9 | 2.16 | 1.82E-06 | chr10 | 51926200 | 51930606 | 45.72 | 48.49 | 103.76 | 99.27 |
| Anthocyanidin 3-O-glucosyltransferase | 2.15 | 1.96E-08 | chr09 | 31520019 | 31521721 | 93.09 | 134 | 237.92 | 250.31 |
| KUP1\_2 | 2.12 | 0.02 | chr09 | 52350668 | 52354458 | 24.27 | 17.76 | 39.17 | 50.14 |
| Gene of unknown function\_2168 | 2.11 | 8.16E-07 | chr04 | 11393644 | 11394425 | 79.45 | 74.07 | 184.4 | 140.09 |
| Pectinesterase\_7 | 2.1 | 1.10E-03 | chr01 | 63309078 | 63311172 | 40.51 | 27.63 | 62.02 | 81.23 |
| Pectate lyase P56 | 2.01 | 9.27E-03 | chr03 | 31546129 | 31547779 | 32.03 | 22.6 | 48.71 | 61.16 |
| Fasciclin-like arabinogalactan protein 3 | 2 | 1.24E-03 | chr08 | 2394607 | 2395637 | 43.67 | 29.55 | 69.64 | 77.14 |
| RUBP carboxylase/oxygenase activase, chloroplastic | -2.01 | 2.58E-09 | chr10 | 50945735 | 50948506 | 400.12 | 476.9 | 203.43 | 232.82 |
| Photosystem I reaction center subunit X psaK | -2.02 | 5.50E-03 | chr08 | 1301974 | 1310102 | 58.96 | 66.02 | 29.92 | 32.05 |
| S-adenosylmethionine-dependent methyltransferase\_5 | -2.03 | 1.11E-03 | chr04 | 12347557 | 12349360 | 84.87 | 70.48 | 41.04 | 35.67 |
| Cytochrome P450\_203 | -2.03 | 8.42E-09 | chr10 | 57175786 | 57177298 | 190.1 | 215.51 | 100.29 | 99.67 |
| 21kD protein\_1 | -2.06 | 1.81E-03 | chr03 | 40102015 | 40102864 | 70.9 | 65.76 | 30.32 | 36.14 |
| Conserved gene of unknown function\_4854 | -2.06 | 4.04E-03 | chr10 | 51111644 | 51112600 | 59.21 | 65.41 | 33.08 | 27.39 |
| Conserved gene of unknown function\_3754 | -2.07 | 0.04 | chr08 | 5526693 | 5528435 | 43.57 | 42.87 | 17.87 | 23.98 |
| PSI-H\_1 | -2.07 | 3.63E-04 | chr06 | 49305821 | 49307605 | 74.15 | 88.44 | 38.89 | 39.51 |
| Beta-ketoacyl-coa synthase family protein\_4 | -2.12 | 7.71E-03 | chr08 | 40944428 | 40946611 | 55.69 | 50.14 | 21.59 | 28.35 |
| Beta-galactosidase\_7 | -2.12 | 5.59E-07 | chr03 | 60932522 | 60938528 | 298.13 | 251.46 | 150.66 | 108.48 |
| Desacetoxyvindoline 4-hydroxylase\_1 | -2.14 | 2.72E-05 | chr04 | 2625517 | 2628817 | 98.79 | 93.55 | 38.86 | 51 |
| 16kDa membrane protein | -2.18 | 1.36E-08 | chr06 | 54752175 | 54753353 | 149.48 | 174.53 | 73.37 | 75.44 |
| Fructose-1,6-bisphosphatase\_2 | -2.24 | 5.25E-06 | chr09 | 739209 | 741850 | 90.71 | 104.83 | 40.48 | 46.63 |
| P-coumaroyl quinate/shikimate 3'-hydroxylase\_5 | -2.28 | 2.95E-06 | chr10 | 59481981 | 59484716 | 94.67 | 103.89 | 49.64 | 37.52 |
| Gene of unknown function\_2136 | -2.28 | 1.68E-04 | chr04 | 7644725 | 7646446 | 78.39 | 63.59 | 29.87 | 32.4 |
| Phosphoribulokinase | -2.34 | 1.37E-08 | chr08 | 48800791 | 48805673 | 142.61 | 179.31 | 63.55 | 73.74 |
| UDP-glucose:glucosyltransferase\_17 | -2.35 | 0.04 | chr05 | 7189487 | 7192188 | 39.42 | 29.56 | 14.25 | 15.07 |
| Fructose-bisphosphate aldolase\_2 | -2.39 | 0.02 | chr02 | 20680813 | 20683256 | 33.72 | 37.69 | 11.4 | 18.5 |
| Peroxidase 12 | -2.46 | 0.02 | chr04 | 61089723 | 61091577 | 30.79 | 39.89 | 11.15 | 17.53 |
| Nb cell deth marker | -2.85 | 0.02 | chr03 | 49862776 | 49863532 | 23.81 | 27 | 9.58 | 8.23 |
| Zinc finger protein\_82 | -3.08 | 5.70E-04 | chr04 | 46475605 | 46480004 | 37.99 | 40.45 | 4.37 | 21.12 |
| CYP72A54\_6 | -3.08 | 0.04 | chr10 | 43658746 | 43666340 | 22.28 | 22.18 | 7.13 | 7.29 |
| Flavonoid glucoyltransferase UGT73E2\_1 | -3.67 | 3.56E-06 | chr10 | 52432622 | 52434505 | 48 | 41.18 | 14.67 | 9.61 |
| Conserved gene of unknown function\_4918 | -3.76 | 4.41E-19 | chr10 | 57306083 | 57307830 | 139.31 | 125.32 | 31.33 | 39.14 |
| Conserved gene of unknown function\_3926 | -3.83 | 4.11E-16 | chr08 | 41634497 | 41635721 | 112.52 | 114.09 | 21.27 | 37.91 |
| Conserved gene of unknown function\_1306 | -3.93 | 5.02E-04 | chr03 | 850686 | 851294 | 24.93 | 27.03 | 5.75 | 7.48 |
| Caryophyllene/alpha-humulene synthase\_1 | -4.07 | 1.36E-08 | chr06 | 41556836 | 41557802 | 52.4 | 83.75 | 14.93 | 18.49 |
| Patatin group O | -4.34 | 3.88E-04 | chr08 | 1649641 | 1654633 | 19.69 | 43.15 | 10.29 | 4.2 |
| Tropinone reductase I | -4.36 | 0.02 | chr10 | 56414553 | 56425560 | 17.8 | 13.92 | 4.2 | 3.08 |
| Pectin methlyesterase inhibitor protein 1 | -4.87 | 1.13E-15 | chr03 | 39768603 | 39769328 | 77.4 | 88.87 | 16.28 | 17.88 |
| Senescence-specific cysteine protease | -4.99 | 2.15E-25 | chr02 | 32269516 | 32271942 | 232.19 | 183.21 | 28.8 | 54.37 |
| Cytokinin oxidase/dehydrogenase\_1 | -5.05 | 6.88E-03 | chr01 | 64568551 | 64571106 | 18.22 | 13.85 | 0.94 | 5.41 |
| Metallocarboxypeptidase inhibitor\_1 | -11.54 | 3.57E-06 | chr07 | 3198623 | 3200152 | 14.8 | 37.92 | 0.85 | 3.72 |
| Cysteine protease inhibitor 1\_3 | -11.56 | 5.66E-03 | chr03 | 49685390 | 49686264 | 5.88 | 17.04 | 0.49 | 1.5 |
| Polyphenoloxidase | -24.73 | 4.31E-07 | chr02 | 34338338 | 34340696 | 16.96 | 23.5 | 0.6 | 1.03 |
| Calcium ion binding protein\_12 | #NAME? | 6.66E-05 | chr04 | 3712421 | 3714476 | 16.79 | 7.58 | 0 | 0 |

**Supplementary Table 3: Phenylalanine ammonia lyase (PAL) and Chalcone synthase (CHS) genes annotated in the potato genome DM 1-3 516 R44 v3.04, their length, position in the pseudomolecules and the tissues in which expression is greatest in DM and RH as reported in the University of Toronto BAR ePlant website**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| PAL genes | Size (kbp) | PGSC position | expression in DMb | expression in RHb |
| PGSC0003DMG400006637 | 1.03 | chr02:4449620..4450650 | Fruit, tubers | tuber sprouts, roots |
| PGSC0003DMG400031457 | 1.513 | chr03:17248040..17249560 | stolons | none |
| PGSC0003DMG400005492 | 4.664 | chr05:36342700..36347400 | carpels | none |
| PGSC0003DMG400023458a | 3.954 | chr05:51694756..51698709 | none | petioles, stems |
| PGSC0003DMG402021549 | 3.692 | chr09:5502194..5505885 | petals, carpels, stamen stolons, callus | tuber sprouts, roots |
| PGSC0003DMG402021549 | 2.807 | chr09:5510117..5512923 |  |  |
| PGSC0003DMG402021564 | 2.878 | chr09:5530471..5533348 | stolons, tubers | none |
| PGSC0003DMG402021564 | 0.604 | chr09:5539567..5540170 |  |  |
| PGSC0003DMG400019386 | 3.68 | chr10:6301474..6305153 | callus | none |
| PGSC0003DMG400031365a | 404.6 | chr10:51926201..51930606 | stamen, flowers | stamen, flowers, stem, petiole, tuber sprouts |
|  |  |  |  |  |
| CHS genes |  |  |  |  |
| PGSC0003DMG400001635 | 1.823 | chr01:87143395..87145217 | roots | tubers |
| PGSC0003DMG400027146 | 3.192 | chr05:48437160..48440351 | none | roots |
| PGSC0003DMG400019110a | 1.77 | chr05:48886960..48888729 | flowers, petals, carpels | flowers |
| PGSC0003DMG400029620 | 2.084 | chr09:58356999..58359082 | flowers, stamen | flowers, stamen |
| PGSC0003DMG400029621 | 2.51 | chr09:58361561..58364070 | stamen, fruit | flowers, stamen |
| PGSC0003DMG400008635 | 1.021 | chr12:58736647..58737667 | roots | petioles, stems |
| PGSC0003DMG400008633 | 1.192 | chr12:58741442..58742633 | none | petioles, stems |
| PGSC0003DMG400008634 | 1.557 | chr12:58746401..58747957 | fruit | flowers |
| PGSC0003DMG400008632 | 2.593 | chr12:58758677..58761269 | in vitro shoots, petiole | petioles, stems |
| PGSC0003DMG400016867 | 2.525 | chr12:58934717..58937241 | in vitro shoots, petiole | none |
| PGSC0003DMG400016873 | 1.921 | chr12:58954773..58956693 | sepals | none |

asignificantly more highly expressed in purple compared to white flowered bulk samples (Supplementary Table 1).b<http://bar.utoronto.ca/eplant_potato/>

**Supplementary Table 4:** **Summary of current and past designations of relevant *D/P/R* (tuber) and *F/D/P* (floral) loci involved in anthocyanin synthesis in potato**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| PGSC DM1-3 v4.03 Locusa | PGSC DM1-3 v4.03 Transcipt ID | PGSC DM1-3 v4.03 Locationb | PGSC annotation | Tissue expressing anthocyanins | Original gene symbol | Current gene symbol | Citation |
| PGSC0003DMG400003605 | PGSC0003DMT400009287 | chr02:40,293,862-40,297,510 | Dihydroflavonol 4-reductase | Tuber skin, flowers | *R* for tuber skin*, D* for flowers | *DFR* | (Van Eck *et al.* 1993; De Jong *et al.* 2004) |
| PGSC0003DMG400013965 | PGSC0003DMT400036281 | chr10:51,749,197-51,750,359 | Transcription factor R2R3-MYB | Tuber skin | *D* [*StAN2;* Jung et al. (2009)] | *StAN1* | (André *et al.* 2009; Payyavula *et al.* 2013; D'amelia *et al.* 2014) |
| PGSC0003DMG400013966 | PGSC0003DMT400036286PGSC0003DMT400036284 | chr10:51,725,169-51,726,374 | MYB transcription Factor | Tuber skin and flesh (Lui et al. 2016)  May be a pseudogene (Jung et al. 2009) | *StAN3* [Jung et al. (2009)] | *StAN2; StMYBA1c* | (André *et al.* 2009; Liu *et al.* 2016) |
| PGSC0003DMG400019217 | PGSC0003DMT400049460 | chr10:51,469,785- 51,470,105 | AN2 | Flower petals | *F* | *StFlAN2* | This article |
| PGSC0003DMG400000425 | PGSC0003DMT400001124  PGSC0003DMT400001125 | chr11:39417579-39420629 | Flavonoid 3',5'-hydroxylase | Tuber skin | *P* | *F3’5’h* | (Jung *et al.* 2005) |

a Locus identification was determined by BLAST searching the primer sequences provided by the authors listed in the citations against DM 1-3 v4.03

b The location is for the area of locus annotation and may encompass the complete coding sequence.

c This gene is found as both *StAN2* and *StMYBA1* in current publications.

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