Supplementaty Materials

A

```
                          GA
DM-GGH1   978  CAGTGGCGCCAGAAGTGCTCGATAGTCTCATATACAATTACAATCCCACTTATGGTGGGA
DM-GGH2  1018  CGGCTACCACCAAAGTCCTTGACAACCTCATCTACAATTACAGTCCTTCTTATGCTGGGA
DM-GGH3   893  CAGAAGCTCAAAAAGTACTCGACAATCTCATCTACAATTACAATCCCACTTATAGTGGAA

                         Fwd
                AGGCAGGGAAGGGTTATG
DM-GGH1  1038  AGGCAGGGAAGGGTTATGACGAGGTTTATCTCTTCACTCCGCATTCTTCCTCGAGCTCTA
DM-GGH2  1078  AGGTTCGGGGGAGTTTTGAGGAGGTCTACCTCTTCACTCCGCGTCCCACCTTGAGCTCTC
DM-GGH3   953  AGACTGGGAAAGGTTACGATGAGGTTTACGTTTTCAA.....CAGTCATGCTGTGAACTA

                                                       Rev
                                            CAACTGCACAATCTTATTGATGC
DM-GGH1  1098  TGTAGAGAAAAAAACACCAGTTTAATTGAGCAATAACCAACTGCACAATCTTATTGATCC
DM-GGH2  1138  TGTAGATGCAAGTGAAC.....TGCAAAAGCATTGAC..AATTGCATGATCAAAGA..TTAT
DM-GGH3  1008  A
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B

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                                              Fwd
                                   CTGGTATGGTTGTGACCTTTG
DM         766  CAGTTGGTCGTGTTGAGACTGGTGTGATCAAGCCTGGTATGGTTGTGACCTTTGGCCCTA
Kuras      814  CTGTTGGTCGTGTTGAGACTGGTGTGATCAAGCCTGGTATGGTTGTGACCTTTGGCCCTA
Desiree    838  CAGTTGGTCGTGTAGAGACTGGTGTGATCAAGCCTGGTATGGTTGTGACCTTTGGCCCTA
CIP800928  269  CTGTTGGTCGTGTTGAGACTGGTGTGATCAAGCCTGGTATGGTTGTGACCTTTGGCCCTA

DM         826  CTGGTTTGACAACTGAAGTCAAGTCTGTAGAGATGCACCACGAAGCTCTCCTGGAGGCA
Kuras      874  CTGGTTTGACAACTGAAGTCCAGTCTGTTGAGATGCACCACGAGGCTCTTCTGGAGGCA
Desiree    898  CTGGTTTGACAACTGAAGTCAAGTCTGTTGAGATGCACCACGAAGCTCTTCTGGAGGCA
CIP800928  329  CTGGTTTGACAACTGAAGTCAAGTCTGTTGAGATGCACCACGAAGCTCTTCTGGAGGCA

                            Rev
                 GGTGACAATGTTGGGTTCAA
DM         885  CTCCCTGGTGACAATGTTGGGTTCAATGTTAAGAATGTTGCTGTTAAGGATCTTAAGCG
Kuras      933  CTCCCTGGTGACAATGTTGGGTTCAATGTTAAGAATGTTGCTGTTAAGGATCTGAAGCG
Desiree    957  CTCCCTGGTGACAATGTTGGGTTCAATGTTAAGAATGTTGCTGTTAAGGATCTGAAGCG
CIP800928  388  CTCCCTGGTGACAATGTTGGGTTCAATGTTAAGAATGTTGCTGTTAAGGATCTGAAGCG
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Figure S1. Partial alignments of GGH (A) and EF1-α (B) sequences and primers used in qPCR reactions. GGH1, GGH2 and GGH3 transcripts sequences from the DM genotype were aligned in order to design primers specific of GGH1. EF1-α sequences from four potato genotypes were aligned to design primers within highly conserved regions.
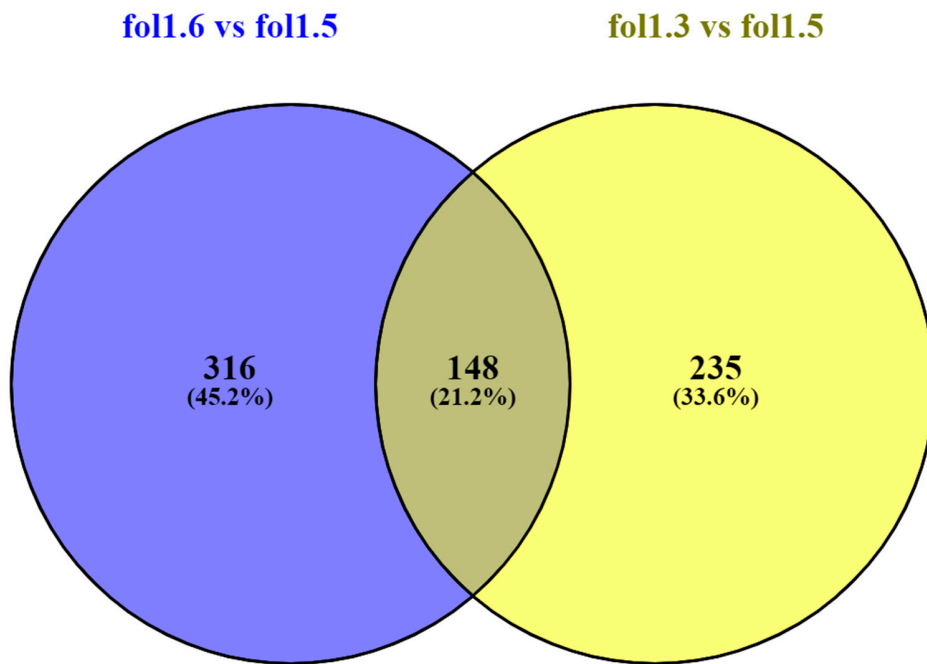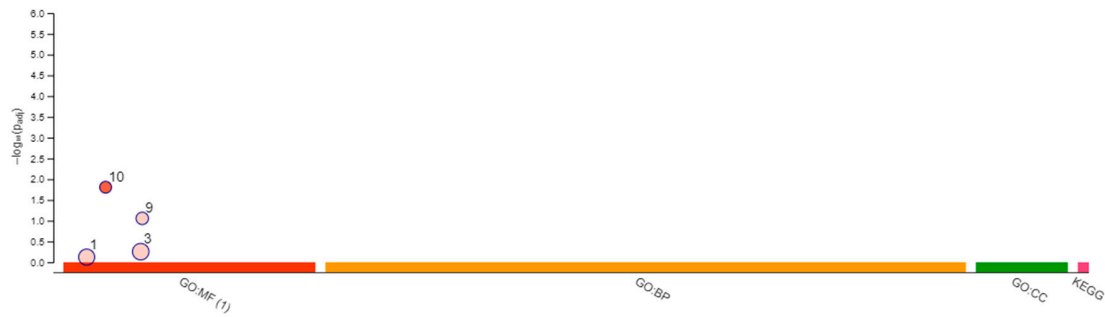
Figure S2. Venn diagram showing numbers of differentially expressed genes that are common or exclusive in comparisons fol 1.6 versus fol 1.5 and fol 1.3 versus fol 1,5. [1]

> fol1.6vsfol1.5
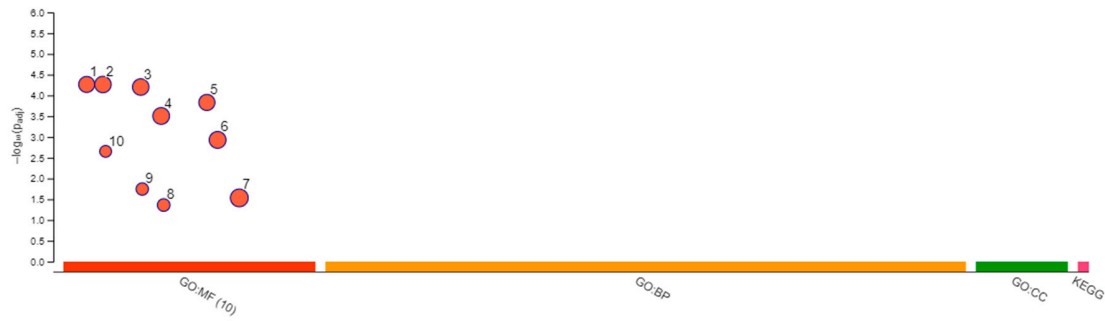
> fol1.3vsfol1.5

ID	Source	Term ID	Term Name	p_{adj} (fol1.6vsfol1.5)	p_{adj} (fol1.3vsfol1.5)
1	GO:MF	GO:0004497	monooxygenase activity	5.426×10^{-5}	7.515×10^{-4}
2	GO:MF	GO:0005506	iron ion binding	5.451×10^{-5}	1.000
3	GO:MF	GO:0016705	oxidoreductase activity, acting on paired donors, ...	6.269×10^{-5}	8.558×10^{-4}
4	GO:MF	GO:0020037	heme binding	3.122×10^{-4}	1.000
5	GO:MF	GO:0043531	ADP binding	1.473×10^{-4}	1.000
6	GO:MF	GO:0046906	tetrapyrrole binding	1.176×10^{-3}	1.000
7	GO:MF	GO:0048037	cofactor binding	2.929×10^{-2}	1.000
8	GO:MF	GO:0030247	polysaccharide binding	4.353×10^{-2}	1.000
9	GO:MF	GO:0016782	transferase activity, transferring sulfur-containing ...	1.790×10^{-2}	8.779×10^{-2}
10	GO:MF	GO:0008146	sulfotransferase activity	2.219×10^{-3}	1.560×10^{-2}

| version	e98_eg45_p14_bca6d38
date	10/30/2019, 9:50:51 AM
organism	stuberosum

g:Profiler

Figure S3. Functional enrichment analysis in comparisons between fol 1.6 and fol 1.5, and fol 1.3 and fol 1.5. Analyses were done using g:GOSt in g:Profiler [2]. A g:SCS threshold of 0.05 was used. The top panel is a Manhattan plot of enriched terms in the comparison between fol 1.6 and fol 1.5. The middle panel is a Manhattan plot of enriched terms in the comparison between fol 1.3 and fol 1.5. The bottom table provides detailed information such as data source, id and name of the term with corresponding p-value. The light circles in Manhattan plots represent insignificant terms.
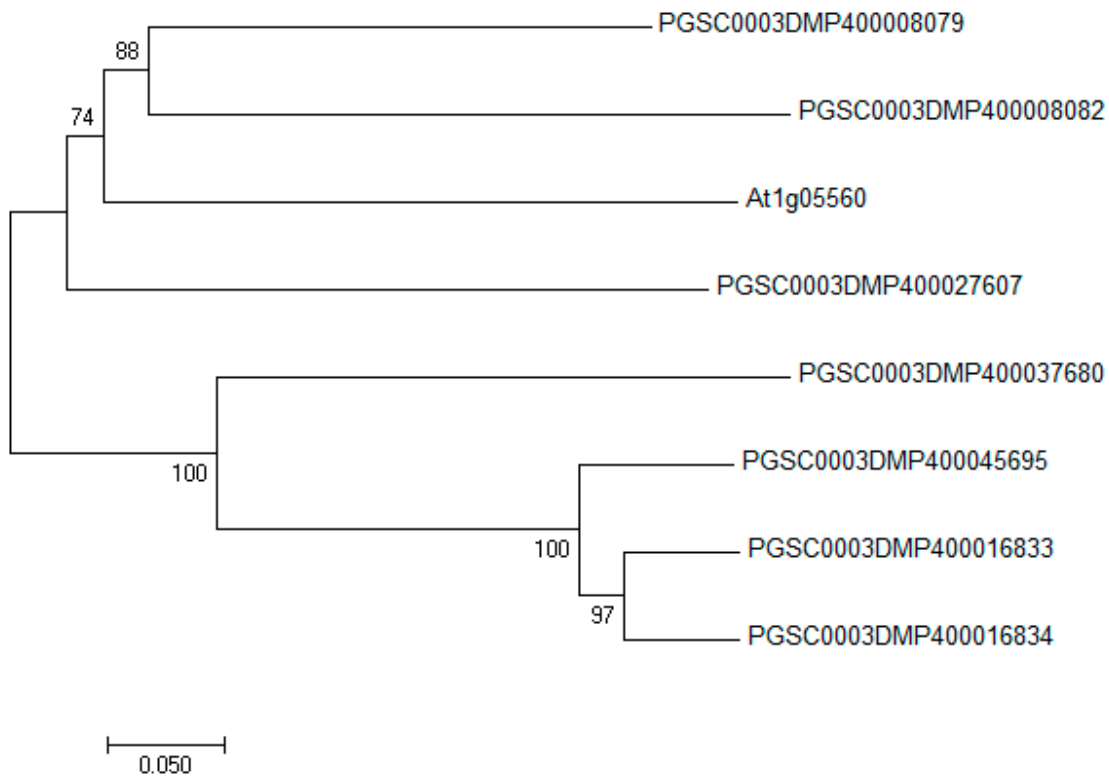
Figure S4. Phylogenetic tree of UDP-glucose glucosyltransferases from potato and Arabidopsis. Homologs of the At1g05560-encoded protein were searched in the potato genome by using tBLASTn search in Spud DB (http://potato.plantbiology.msu.edu/). The seven top matches were used for phylogenetic analysis in MEGA7.

The evolutionary history was inferred using the Neighbor-Joining method [3]. The optimal tree with the sum of branch length = 1.77531023 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [4]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method [5] and are in the units of the number of amino acid differences per site. The analysis involved 8 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 413 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [6].
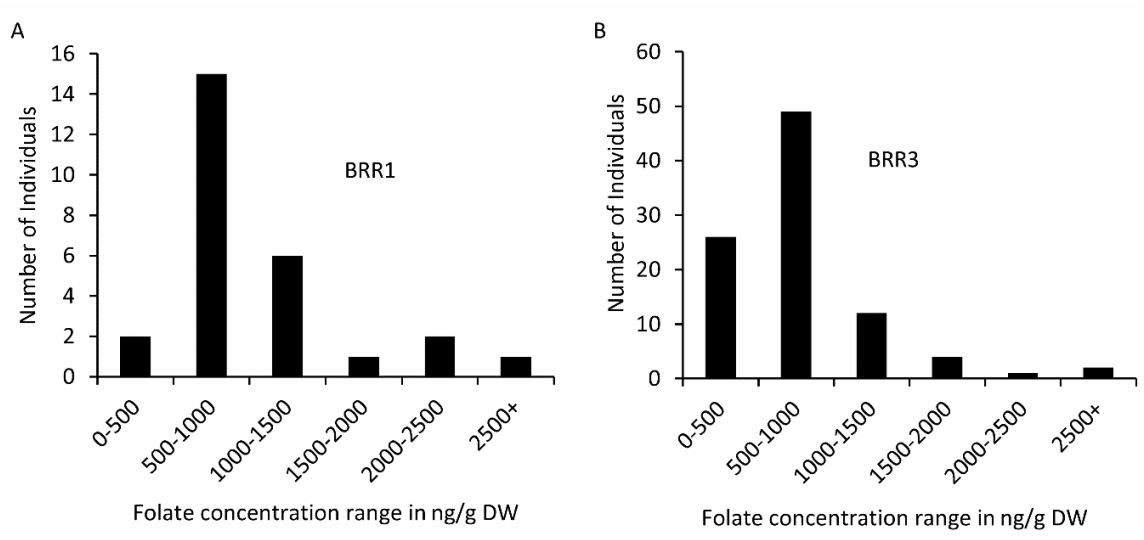
Figure S5. Histogram of number of individuals within folate concentration brackets. A, BRR1 population; B, BRR3 population.

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AtGGH1    1  -MIDNNCLYKEELNRNSYSGLAKEASESILLPSESGFD-------GSRSPVCSSPDPNLN
AtGGH2    1  --MWSYVWLPLVALSLFKDSIIMAKAATILLPSQTGFD-------ISRSPVCSAPDPNLN
AtGGH3    1  -----MWRFCFFLSLLFFDVSAVKSAESIFLPSQIGVEDSRVFESLSLSPVCSAADPNLN
StGGH1    1  MGNYLLILFLLLVCGIITSQSQLFVSNQILPPS------------------CPAPDPTLN
StGGH2    1  --------------MATHVKPQIIESQLNFPS-------------------CPATDPALN
StGGH3    1  --MSNYFLISFLTISLFLVVTAIEPAELFVPAG-----------------CPLPDHNLN

                                                               *
AtGGH1   53  YRPVIGILSHPGDG-------ASGRLTNDTSSTYIAASYVKFAEAGGARVIPLIYNEPEE
AtGGH2   52  YRPVIGILSHPGDG-------ASGRLSNATDASSIAASYVKLAESGGARVIPLIFNEPEE
AtGGH3   56  YKPVIGILTHPGEGRWDARLHSLKNYAYATNISYIAASYVKLAETGGARVIPLIYNEPEE
StGGH1   43  YRPIIGIVSHPGDG-------ATGRLSNATNVSYIAASYVKFAEMAGARVIPLIYTEPPE
StGGH2   28  YRPVIGIVSHPGDG-------ASGRLNNASDVSYIAASYVKFAEMAGARVIPIIYTEPPE
StGGH3   41  YRPVIGIISHPGHG-------DSGRINNSTGVSYIAASYVKLVESGGARVIPLLFDDSPQ

                                                    *       *
AtGGH1  106  VLFQKLELVNGVIFTGGWAKKYDYFEIVKKIFTKALERNDAGEHFPVYGICLGFELMSII
AtGGH2  105  ILFQKLELVNGVILTGGWAKEGLYFEIVKKIFNKVLERNDAGEHFPIYAICLGFELLTMI
AtGGH3  116  ILFQKLELVNGVIFTGGWAKTGLYYDVVEKIFNKVMEKNDAGEHFPVYAMCLGFEILSMI
StGGH1   96  VLNQKLNLVNGIIFTGGWAKDGLYFDVIKGIFQKVLEKNDAGEHFPLLAICLGYELLTMI
StGGH2   81  IINQKLNLVNGIIFTGGWSKKGLYFEVVKGIFEKVLEKNDAGEHFPLLAICLGFELLTMI
StGGH3   94  LLNQKLNLVNGVIFPGGWAKKGHYFETIKAIFRKVLEKNDAGEHFPLLAINHGFELLMMI

                                                             *
AtGGH1  166  ISQNRDILERFDAEDNASSLQFVDNVNNDGTLFQRFPPELLKKLSTDCLVMQKHHKYGITP
AtGGH2  165  ISQNRDIFEKMDARNSASSLQFVENVNIQGTIFQRFPPELLKKLGTDCLVMQNHRFGISP
AtGGH3  176  ISQNRDILERFNSVNYASSLQFFKNVNIEATVFQRFPPELLKKLSADCLVMQNHYFGISP
StGGH1  156  ITNDNNILEEFSAASQASTVQFVENVNIDGTVFGRFPPVLLKKMSIDCLVMQNHHFGISP
StGGH2  141  ISKDNNILEEFSASHQASTVQFVENIKFDGTVFGRFPPVLLKKMSTHCLVMQNHHFGISP
StGGH3  154  VSKDNNILEKFSVSNQATKLHFVETVNIEDTVFGRFPPTLIKKLSKECLVLQSHKYGLSP

                                               *  *  * **
AtGGH1  226  ANFQANPALSSFFEILTTCIDENSKTYVSTVKAKRYPITGFQWHPEKNAFEWGSSAIPHS
AtGGH2  225  QSFEGNIALSNFFKIVTTCVDDNGKVYVSTVQSTKYPVTGFQWHPEKNAFEWGSSKIPHS
AtGGH3  236  DNFQGNPYLSSFFNIVTTSADKDSKTFVSTIRSKRYPVTAFQWHPEKNAFEWGSSEIPHS
StGGH1  216  ERFQANKDLSSFFRVLTTSTDENNKVYVSTIQATRYPIAAFQWHPEKNAFEWGSSRIPHS
StGGH2  201  ERLQANNDLCGFFRVLTTSVDKKNKVYVSSVQAQHYPVTALQWHPEKNVFEWGSSQIPHT
StGGH3  214  DKFQANDDLSSFFIMLTTSTDTRNKVYVSTVKAENYPITALQWHPEKSAFEWGLSAIPHS

AtGGH1  286  EDAIQVTQHAASYLVSEARKSLN-RPESQKVLSNLIYNYKPTYCGYAGRGYDEVYIFTQP
AtGGH2  285  EDAIQVTQHAANHLVSEARKSLN-RPESKKVLSNLIYNYKPTYCGYAGIGYDEVYIFTQQ
AtGGH3  296  EDAIQVTQHAANYLVSEARKSMN-RPSSEKVLSNLIYNYKPTYSGYKGSGDDEVYIFT--
StGGH1  276  EDAIQVTTHVANYFISEARKSSN-KPVAREVLDSLIYNYNPTYGGKAGKGYDEVYLFTPH
StGGH2  261  EDAIQVTQHVANYFVSEARKSSNNKPATSKVLDNLIYNYSPSYAGKVRGSFEEVYLFTPR
StGGH3  274  EDAVQVTQLVANYFVSEARKSSN-KPEAQKVLDNLIYNYNPTYSGKTGKGYDEVYVFNSH

AtGGH1  345  RSRF--
AtGGH2  344  RSLL--
AtGGH3       ------
StGGH1  335  SSSSSM
StGGH2  321  PTLSSL
StGGH3  333  AVN---
```

Figure S6. Alignment of potato and Arabidopsis GGH proteins. Asterisks indicate conserved residues that are catalytically essential in human GGH or other conserved active site residues that may participate in substrate binding [7]. Alignment was done by CLUSTALW (https://www.genome.jp/tools-bin/clustalw). Shading was done by BOXSHADE (https://embnet.vital-it.ch/software/BOX_form.html). AtGGH1, At1g78660; AtGGH2, At1g78680; AtGGH3, At1g78670; StGGH1, PGSC0003DMG400007066; StGGH2, PGSC0003DMG400021256; StGGH3, PGSC0003DMG400035974.

Table S1. Folate metabolism-related genes in *Arabidopsis*, tomato, and potato. Orthologs of Arabidopsis genes in tomato and potato were retrieved from EnsemblPlants.

Gene name	Arabidopsis	Tomato	Potato	Chr.[1]	Start Pos.[2]	End Pos.
GTP cyclohydrolase I (GCHI)	At3g07270	Solyc06g083230	PGSC0003DMG400020105	6	58,218,330	58,221,998
Dihydroneopterin (DHN) triphosphate diphosphatase	At1g68760	Solyc03g043855.1	PGSC0003DMG400030259	3	6,301,879	6,303,243
Dihydroneopterin aldolase (DHNA)	At3g11750 At5g62980 At3g21730	Solyc10g079830.2	PGSC0003DMG400029847	10	58,006,909	58,009,753
Aminodeoxychorismate synthase (ADCS)	At2g28880	Solyc04g049360.3	PGSC0003DMG400009777	4	32,090,468	32,092,714
Aminodeoxychorismate lyase (ADCL)	At5g57850	Solyc11g071280.1.1	PGSC0003DMG400018587	11	42,627,656	42,632,239
6-Hydroxymethyldihydropterin pyrophosphokinase (HMDHP-PPK) / dihydropteroate synthase (DHPS)	At4g30000 (mito.) At1g69190 (cyto., only in Arabidopsis)	Solyc05g012090.3	PGSC0003DMG400028362	5	692,312	694,941
Dihydrofolate synthase (DHFS)	At5g41480	Solyc06g051900.3	PGSC0003DMG400002352	6	38,180,979	38,187,447
Dihydrofolate reductase (DHFR)	At2g16370 At4g34570 At2g21550	Solyc01g109830.3	PGSC0003DMG400000736	1	86,138,599	86,145,134
Folylpolyglutamate synthase (FPGS)	At5g05980 (FPGS1) At3g10160 (FPGS2) At3g55630 (FPGS3)	Solyc05G052920.3 Solyc04G016550.3	PGSC0003DMG400027193	5	48,208,214	48,215,644
UDP-glucose–*p*-aminobenzoate glucosyltransferase [3]	At1g05560	Solyc12g098590.1.1	PGSC0003DMG400004573 PGSC0003DMG400004574	12 12	59,388,655 59,391,505	59,391,021 59,393,303
γ-Glutamyl hydrolase (GGH)	At1g78660 (GGH1) At1g78680 (GGH2) At1g78670 (GGH3)	Solyc07g062270.3 Solyc07g062280.3 Solyc07g062280.3	PGSC0003DMG400007066 PGSC0003DMG400021256 PGSC0003DMG400035974	7 10 7	52,454,391 2,480,397 52,459,355	52,459,018 2,487,536 52,462,734
5-Formyltetrahydrofolate cycloligase (5-FCL)	At5g13050	Solyc03g113590.3	PGSC0003DMG400024570	3	53,569,914	53,573,217

[1] Chromosome number in potato; [2] Start and end position in the reference potato genome; [3] UDP-glucose-*p*-aminobenzoate glucosyltransferase belongs to a large gene family. Therefore, the closest orthologs to the characterized Arabidopsis gene (At1g05560) shown to be involved in *p*-aminobenzoate glucosylation [8] were identified in potato by BLAST and phylogenetic analyses (Figure S3).

References

1. Oliveros, J.C. An interactive tool for comparing lists with venn diagrams. *http://bioinfogp.cnb.csic.es/tools/venny/index.html* **2007**.
2. Raudvere, U.; Kolberg, L.; Kuzmin, I.; Arak, T.; Adler, P.; Peterson, H.; Vilo, J. G:Profiler: A web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* **2019**, *47*, W191-W198.
3. Saitou, N.; Nei, M. The neighbor-joining method - a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406-425.
4. Felsenstein, J. Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution* **1985**, *39*, 783-791.
5. Nei, M.; Kumar, S. *Molecular evolution and phylogenetics*. Oxford University Press: New York, 2000.
6. Kumar, S.; Stecher, G.; Tamura, K. Mega7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870-1874.
7. Orsomando, G.; de la Garza, R.D.; Green, B.J.; Peng, M.; Rea, P.A.; Ryan, T.J.; Gregory, J.F., 3rd; Hanson, A.D. Plant gamma-glutamyl hydrolases and folate polyglutamates: Characterization, compartmentation, and co-occurrence in vacuoles. *J. Biol. Chem.* **2005**, *280*, 28877-28884.
8. Eudes, A.; Bozzo, G.G.; Waller, J.C.; Naponelli, V.; Lim, E.K.; Bowles, D.J.; Gregory, J.F., 3rd; Hanson, A.D. Metabolism of the folate precursor p-aminobenzoate in plants: Glucose ester formation and vacuolar storage. *J. Biol. Chem.* **2008**, *283*, 15451-15459.