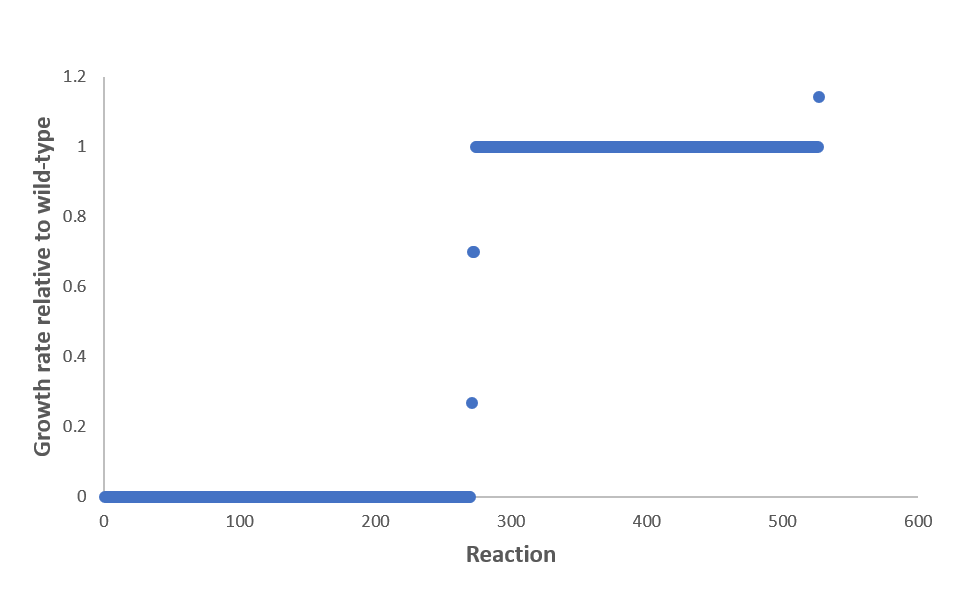
***In silico* prediction of candidate gene targets for the management of African cassava whitefly (*Bemisia tabaci,* SSA1-SG1-UG), the key vector of Cassava brown streak disease causative viruses**

Tadeo Kaweesi1,2, 5\*, John Colvin1, Lahcen Campbell3, Paul Visendi4, Gareth Maslen3, Titus Alicai5 and Susan E Seal1



**Figure S1: Robustness of the metabolic network “SSA1-SG1\_*Portiera*” iKT420 showing the effect of single reaction deletion on the growth rate of *B. tabaci* SSA1-SG1 and *Portiera* mutant relative to the wild-type, simulated using flux balance analysis. A total of 270 reaction deletions affected growth, therefore, were deemed indispensable/essential for the growth and survival of cassava *B. tabaci* SSA1-SG1**

**Table S1: Comparison of genomic features of *Portiera* genome from MEAM1, MED and SSA1-SG1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Description** | **MEAM1** | **MED** | **SSA1-SG1** |
| Size (bp) | 352068 | 357461 | 347165 |
| GC content (%) | 26.2 | 26.1 | 26.2 |
| Number of coding sequences | 282 | 290 | 291 |
| Number of subsystems | 66 | 71 | 65 |
| Number of RNAs | 36 | 36 | 36 |
| Number of unique hypothetical proteins | 23\* | 27\*\* | 31\*\*\* |

\*present in MEAM1 but missing in SSA1-SG1 \*\*present in MED but missing in SSA1-SG1 \*\*\*present in SSA1-SG1 but missing in MEAM1

**Table S2: Missing protein/function in *Portiera* genome from MEAM1, MED and SSA1-SG1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Unique protein/function** | **MEAM1** | **MED** | **SSA1-SG1** |
| 4-hydroxy-tetrahydrodipicolinate reductase (EC 1.17.1.8) | + | + | - |
| Inner membrane protein translocase component (YidC) | - | + | - |
| tRNA uridine 5-carboxymethylaminomethyl enzyme (GidA) | - | + | - |
| Cytochrome O ubiquinol oxidase subunit 1 | + | + | - |
| GTPase and tRNA-U34 5-formylation enzyme (TrmE) | - | + | - |
| SSU ribosomal protein S6p | - | + | - |

C:\Users\tk1853u\Downloads\Kawxxx\Predicted EAA rates.tif

**Figure S2:** *In* *silico* prediction of production rates of essential amino acid of the two-compartment metabolic model of *B. tabaci* SSA1-SG1 and *Portiera.*

**Table S4: Comparison of genome-scale metabolic models of *B. tabaci* SSA1-SG1 and MEAM1**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Description** | ***B. tabaci* SSA1-SG1** | | |  | | ***B. tabaci* MEAM1** | | |
|  | ***i*KT90** | ***i*KT330** | ***i*KT420\*\*** | |  | ***i*NA94** | ***i*NA332** | ***i*NA774\*\*\*** |
| Number of metabolic genes | 90 | 330 | 420 | |  | 94 | 332 | 774 |
| Number of intracellular reactions | 76 | 233 | 310 | |  | 76 | 236 | 774 |
| Number of metabolites | 150 | 251 | 402 | |  | 148 | 253 | 550 |
| Flux balance optimal solution (h-1) | 6.70 | 25.38 | 0.39 | |  | 12.02 | 26.55 | 0.20 |
| Total protein (mmol/gDw) |  |  | 6.20 | |  |  |  | 6.20 |
| Total cost for protein synthesis |  |  | 26.71 | |  |  |  | 26.71 |

\*\* Two compartment model for *B. tabaci* SSA1-SG1 & Portiera \*\*\* Three-compartment (Ankrah *et al*., 2017)

**Table S5:List of essential reactions in a two-compartment metabolic model of SSA1-SG1**

|  |  |
| --- | --- |
| **Reaction Flux (mmol gDW-1 h-1)** | **Reaction Flux (mmol gDW-1 h-1)** |
| |  |  | | --- | --- | | SSA1\_Por\_OCBT | 0.111152 | | SSA1\_Por\_METS | 0.046402 | | SSA1\_Por\_PRPPS | 0.116569 | | SSA1\_Por\_ATPPRT | 0.049133 | | SSA1\_Por\_PRATPP | 0.049133 | | SSA1\_Por\_PRAMPC | 0.049133 | | SSA1\_Por\_PRMICI | 0.049133 | | SSA1\_Por\_IG3PS | 0.049133 | | SSA1\_Por\_IGPDH | 0.049133 | | SSA1\_Por\_HSTPT | 0.049133 | | SSA1\_Por\_ASPK | 0.260487 | | SSA1\_Por\_ASAD | 0.260487 | | SSA1\_Por\_DHDPS | 0.159891 | | SSA1\_Por\_THDPS | 0.159891 | | SSA1\_Por\_SDPTA | 0.159891 | | SSA1\_Por\_SDPDS | 0.159891 | | SSA1\_Por\_HSD | 0.100596 | | SSA1\_Por\_HSK | 0.100596 | | SSA1\_Por\_THRS | 0.100596 | | SSA1\_Por\_DDPA | 0.277102 | | SSA1\_Por\_DHQS | 0.277102 | | SSA1\_Por\_DHQTi | 0.277102 | | SSA1\_Por\_SHK3Dr | 0.277102 | | SSA1\_Por\_SHKK | 0.277102 | | SSA1\_Por\_PSCVT | 0.277102 | | SSA1\_Por\_CHORS | 0.277102 | | SSA1\_Por\_ANS | 0.067436 | | SSA1\_Por\_ANPRT | 0.067436 | | SSA1\_Por\_PRAIi | 0.067436 | | SSA1\_Por\_IGPS | 0.067436 | | SSA1\_Por\_PPNDH | 0.209666 | | SSA1\_Por\_ACHBS | 0.113294 | | SSA1\_Por\_KARA2 | 0.113294 | | SSA1\_Por\_DHAD2 | 0.113294 | | SSA1\_Por\_ACLS | 0.310706 | | SSA1\_Por\_KARA1 | 0.310706 | | SSA1\_Por\_DHAD1 | 0.310706 | | SSA1\_Por\_IPPS | 0.16531 | | SSA1\_Por\_IPPMIb | 0.16531 | | SSA1\_Por\_IPPMIa | 0.16531 | | SSA1\_Por\_IPMD | 0.16531 | | |  |  | | --- | --- | | SSA1\_Por\_OMCDC | 0.16531 | | SSA1\_Por\_PDH | 0.165312 | | SSA1\_Por\_AKGDH | 0.209024 | | SSA1\_Por\_ATPS4rpp | 3.89506 | | SSA1\_Por\_CYTBO3\_4pp | 0.493353 | | SSA1\_Por\_NADH16pp | 0.493353 | | SSA1\_Por\_PSY1 | 4.93E-06 | | SSA1\_Por\_PSY2 | 4.93E-06 | | SSA1\_Por\_PDS1 | 4.93E-06 | | SSA1\_Por\_PDS2 | 4.93E-06 | | SSA1\_Por\_ZCAROTDH1 | 4.93E-06 | | SSA1\_Por\_ZCAROTDH2 | 2.47E-06 | | SSA1\_Por\_LCYBZC | 2.47E-06 | | SSA1\_Por\_HEMEOS | 2.47E-06 | | SSA1\_Por\_LIPOS | 2.47E-06 | | SSA1\_Por\_MTHFR2 | 0.046402 | | SSA1\_Por\_DPR | 2.91E-06 | | SSA1\_Por\_CLPNS160pp | 0.000318 | | SSA1\_Por\_DM\_LIPOPB | 2.47E-06 | | SSA1\_Por\_DM\_GLYC | 0.000318 | | SSA1\_Por\_O2tex | 0.246684 | | SSA1\_Por\_H2Otex | -2.83424 | | SSA1\_Por\_CO2tcy | 1.77185 | | SSA1\_Por\_UP\_2DHP | 2.91E-06 | | SSA1\_Por\_UP\_PYR | 0.992473 | | SSA1\_Por\_UP\_2OBUT | 0.113294 | | SSA1\_Por\_UP\_E4P | 0.277102 | | SSA1\_Por\_UP\_PEP | 0.554204 | | SSA1\_Por\_UP\_R5P | 0.116569 | | SSA1\_Por\_UP\_PG160 | 0.000636 | | SSA1\_Por\_UP\_GGDP | 9.87E-06 | | SSA1\_Por\_UP\_FRDP | 2.47E-06 | | SSA1\_Por\_UP\_OCTAPB | 2.47E-06 | | SSA1\_Por\_UP\_PYDX5P | 2.47E-06 | | SSA1\_Por\_UP\_THMPP | 2.47E-06 | | SSA1\_Por\_UP\_FMNH2 | 2.47E-06 | | SSA1\_Por\_UP\_THDP | 0.159891 | | SSA1\_Por\_UP\_MLTHF | 0.046402 | | SSA1\_Por\_UP\_COA | 0.049135 | | SSA1\_Por\_UP\_PHEME | 2.47E-06 | | SSA1\_Por\_UP\_NADP | 2.61E-05 | |

**Table S2: Continued**

|  |  |
| --- | --- |
| **Reaction Flux (mmol gDW-1 h-1)** | **Reaction Flux (mmol gDW-1 h-1)** |
| |  |  | | --- | --- | | SSA1\_Por\_UP\_NADH | 0.000109 | | SSA1\_Por\_UP\_DATP | 0.001809 | | SSA1\_Por\_UP\_DCTP | 0.000643 | | SSA1\_Por\_UP\_DGTP | 0.000643 | | SSA1\_Por\_UP\_DTTP | 0.001809 | | SSA1\_Por\_UP\_ADP | 0.255983 | | SSA1\_Por\_UP\_CTP | 0.006216 | | SSA1\_Por\_UP\_GTP | 0.010014 | | SSA1\_Por\_UP\_UTP | 0.006709 | | SSA1\_Por\_UP\_AMET | 4.93E-06 | | SSA1\_Por\_UP\_HCYS-L | 0.046402 | | SSA1\_Por\_UP\_ORN | 0.111152 | | SSA1\_Por\_UP\_ASN-L | 0.013436 | | SSA1\_Por\_UP\_ASP-L | 0.356567 | | SSA1\_Por\_UP\_CYS-L | 0.017419 | | SSA1\_Por\_UP\_GLN-L | 0.235647 | | SSA1\_Por\_UP\_GLY | 0.015866 | | SSA1\_Por\_UP\_PRO-L | 0.007604 | | SSA1\_Por\_UP\_SER-L | 0.083706 | | SSA1\_Por\_UP\_TYR-L | 0.006368 | | SSA1\_Por\_UP\_ARG-L | 0.010652 | | SSA1\_Por\_UP\_HIS-L | 0.004045 | | SSA1\_Por\_UP\_ILE-L | 0.021221 | | SSA1\_Por\_UP\_LEU-L | 0.02112 | | SSA1\_Por\_UP\_LYS-L | 0.021646 | | SSA1\_Por\_UP\_PHE-L | 0.007694 | | SSA1\_Por\_UP\_VAL-L | 0.01781 | | SSA1\_Por\_EF\_SUCC | 0.159891 | | SSA1\_Por\_EF\_G3P | 0.067436 | | SSA1\_Por\_EF\_PPI | 0.283837 | | SSA1\_Por\_EF\_PANT-R | 2.91E-06 | | SSA1\_Por\_EF\_PHPYR | 0.209666 | | SSA1\_Por\_EF\_3MOB | 0.145396 | | SSA1\_Por\_EF\_3MOP | 0.113294 | | SSA1\_Por\_EF\_4MOP | 0.16531 | | SSA1\_Por\_EF\_SUCCOA | 0.049133 | | SSA1\_Por\_EF\_DAD-5 | 4.93E-06 | | SSA1\_Por\_EF\_AMP | 0.198622 | | SSA1\_Por\_EF\_THF | 0.046402 | | SSA1\_Por\_EF\_AICAR | 0.049133 | | SSA1\_Por\_EF\_23DHDP | 0.159891 | | |  |  |  | | --- | --- | --- | | SSA1\_Por\_EF\_26DAP-LL | | 0.159891 | | SSA1\_Por\_EF\_ARGSUC | | 0.082103 | | SSA1\_Por\_EF\_HISP | | 0.049133 | | SSA1\_Por\_EF\_TRP-L | | 0.065903 | | SSA1\_Por\_EF\_THR-L | | 0.088147 | | SSA1\_Por\_EF\_MET-L | | 0.039207 | | SSA1\_Bt\_ASPTA | | 0.817173 | | SSA1\_Bt\_ASNS1 | | 0.135612 | | SSA1\_Bt\_DHDPRy | | 0.159891 | | SSA1\_Bt\_DAPE | 0.159891 | | | SSA1\_Bt\_DAPDC | 0.159891 | | | SSA1\_Bt\_GLNS | 0.842491 | | | SSA1\_Bt\_CBPS | 0.015376 | | | SSA1\_Bt\_P5CR | 998.112 | | | SSA1\_Bt\_P5CDr | 0.217739 | | | SSA1\_Bt\_GSC | 0.111152 | | | SSA1\_Bt\_ORNTAr | 0.111152 | | | SSA1\_Bt\_ARGSL | 0.111152 | | | SSA1\_Bt\_METAT | 4.93E-06 | | | SSA1\_Bt\_CYSTS | 0.113294 | | | SSA1\_Bt\_CYSTGL | 0.113294 | | | SSA1\_Bt\_HISTP | 0.049133 | | | SSA1\_Bt\_HISTD | 0.049133 | | | SSA1\_Bt\_ILETA | 0.113294 | | | SSA1\_Bt\_LEUTAi | 0.16531 | | | SSA1\_Bt\_PHETA1 | 0.209666 | | | SSA1\_Bt\_PHETHPTOX | 0.104398 | | | SSA1\_Bt\_VALTA | 0.145393 | | | SSA1\_Bt\_GTPCI | 0.104398 | | | SSA1\_Bt\_PTHPS | 0.104398 | | | SSA1\_Bt\_SPR | 0.104398 | | | SSA1\_Bt\_GHMT2r | 0.238214 | | | SSA1\_Bt\_MTHFD | 0.195203 | | | SSA1\_Bt\_MTHFC | 0.195203 | | | SSA1\_Bt\_RBFK | 2.47E-06 | | | SSA1\_Bt\_ANNA | 0.000135 | | | SSA1\_Bt\_DNGAL | 0.000135 | | | SSA1\_Bt\_NADK | 2.61E-05 | | | SSA1\_Bt\_MOHMT | 2.91E-06 | | | SSA1\_Bt\_PANTS | 2.91E-06 | | | SSA1\_Bt\_PNTK | 2.91E-06 | | |

**Table S2: Continued**

|  |  |
| --- | --- |
| **Reaction Flux (mmol gDW-1 h-1)** | **Reaction Flux (mmol gDW-1 h-1)** |
| |  |  | | --- | --- | | SSA1\_Bt\_PPNCL2 | 2.91E-06 | | SSA1\_Bt\_PPCDC | 2.91E-06 | | SSA1\_Bt\_PTPATi | 2.91E-06 | | SSA1\_Bt\_DPCOAK | 2.91E-06 | | SSA1\_Bt\_ALASm | 1.97E-05 | | SSA1\_Bt\_PPBNGS | 9.87E-06 | | SSA1\_Bt\_HMBS | 2.47E-06 | | SSA1\_Bt\_UPP3S | 2.47E-06 | | SSA1\_Bt\_UPPDC1 | 2.47E-06 | | SSA1\_Bt\_CPPPGO | 2.47E-06 | | SSA1\_Bt\_PPPGO | 2.47E-06 | | SSA1\_Bt\_FCLT | 2.47E-06 | | SSA1\_Bt\_GLUK | 1.88671 | | SSA1\_Bt\_GAPD | 3.92792 | | SSA1\_Bt\_PGK | 3.92792 | | SSA1\_Bt\_PGM | 3.92792 | | SSA1\_Bt\_ENO | 3.92792 | | SSA1\_Bt\_PYK | 3.37372 | | SSA1\_Bt\_PDH | 0.666063 | | SSA1\_Bt\_PCr | 0.978394 | | SSA1\_Bt\_CS | 0.665922 | | SSA1\_Bt\_ACONTa | 0.665922 | | SSA1\_Bt\_ACONTb | 0.665922 | | SSA1\_Bt\_SUCOAS | 0.049113 | | SSA1\_Bt\_SUCDi | 0.209004 | | SSA1\_Bt\_FUM | 0.504702 | | SSA1\_Bt\_MDH | 0.504702 | | SSA1\_Bt\_PGL | 0.531102 | | SSA1\_Bt\_RPI | 0.440854 | | SSA1\_Bt\_CYOO6m | 1.66454 | | SSA1\_Bt\_CYOR(q9)m | 3.32908 | | SSA1\_Bt\_TRDR | 0.004903 | | SSA1\_Bt\_PPA | 0.565679 | | SSA1\_Bt\_ACACT1r | 4.69E-05 | | SSA1\_Bt\_HMGCOAS | 4.69E-05 | | SSA1\_Bt\_HMGCOARx | 4.69E-05 | | SSA1\_Bt\_MEVK1x | 4.69E-05 | | SSA1\_Bt\_PMEVKx | 4.69E-05 | | SSA1\_Bt\_DPMVDx | 4.69E-05 | | SSA1\_Bt\_IPDDI | 1.23E-05 | | SSA1\_Bt\_DMATT | 1.23E-05 | | |  |  | | --- | --- | | SSA1\_Bt\_GRTT | 1.23E-05 | | SSA1\_Bt\_FRTT | 9.87E-06 | | SSA1\_Bt\_HCO3E | 1.10492 | | SSA1\_Bt\_CAT | 1.23E-06 | | SSA1\_Bt\_ASPCT | 0.015376 | | SSA1\_Bt\_DHORTS | 0.015376 | | SSA1\_Bt\_DHORD2 | 0.015376 | | SSA1\_Bt\_ORPRT | 0.015376 | | SSA1\_Bt\_ORPDC | 0.015376 | | SSA1\_Bt\_UMPK | 0.015376 | | SSA1\_Bt\_ATUD | 0.013567 | | SSA1\_Bt\_ATCM | 2.91E-06 | | SSA1\_Bt\_ATCD | -0.00064 | | SSA1\_Bt\_ATDCD | 0.000643 | | SSA1\_Bt\_ATDGD | 0.000643 | | SSA1\_Bt\_ATGD | 0.173724 | | SSA1\_Bt\_NDPK | 0.001809 | | SSA1\_Bt\_ATDTD | 0.001809 | | SSA1\_Bt\_PRDPAR | 0.125234 | | SSA1\_Bt\_PPRGL | 0.125234 | | SSA1\_Bt\_FPGFT | 0.125234 | | SSA1\_Bt\_PRFGS | 0.125234 | | SSA1\_Bt\_PRFGCL | 0.125234 | | SSA1\_Bt\_PRAIC | 0.125234 | | SSA1\_Bt\_PRAIS | 0.125234 | | SSA1\_Bt\_AIAL | 0.125234 | | SSA1\_Bt\_FPAIF | 0.174367 | | SSA1\_Bt\_ICH | 0.174367 | | SSA1\_Bt\_IMPD | 0.115055 | | SSA1\_Bt\_GMPS(glu) | 0.115055 | | SSA1\_Bt\_AGPT | 0.115055 | | SSA1\_Bt\_DGOTO | 0.000643 | | SSA1\_Bt\_ADSS | 0.059312 | | SSA1\_Bt\_AAL(fum) | 0.059312 | | SSA1\_Bt\_ATAM | 0.537788 | | SSA1\_Bt\_DAOTO | 0.001809 | | SSA1\_Bt\_DCDT | 0.000643 | | SSA1\_Bt\_TMDS | 0.001809 | | SSA1\_Bt\_DTMPK | 0.001809 | | SSA1\_Bt\_DM\_ALA-B | 2.91E-06 | | SSA1\_Bt\_DM\_PG160 | 0.000636 | |

**Table S2: Continued**

|  |  |
| --- | --- |
| **Reaction Flux (mmol gDW-1 h-1)** |  |
| |  |  | | --- | --- | | SSA1\_Bt\_DM\_OCTAPB | 2.47E-06 | | SSA1\_Bt\_DM\_DHBPT | 0.104398 | | SSA1\_Bt\_O2tex | 2.01563 | | SSA1\_Bt\_Pitex | -0.34944 | | SSA1\_Bt\_NH4tex | -3.69554 | | SSA1\_Bt\_UP\_hcys-L | 0.159695 | | SSA1\_Bt\_UP\_thmpp | 2.47E-06 | | SSA1\_Bt\_UP\_ribflv | 2.47E-06 | | SSA1\_Bt\_UP\_pdx5p | 2.47E-06 | | SSA1\_Bt\_UP\_nicrnt | 0.000135 | | SSA1\_Bt\_EF\_dad-5 | 4.93E-06 | | SSA1\_Bt\_EF\_pppi | 0.104398 | | Bt\_GROW | 0.312566 | | EX\_pppi(e) | 0.104398 | | EX\_dad-5(e) | 4.93E-06 | | EX\_o2(e) | -2.01563 | | EX\_h(e) | 3.43033 | | EX\_pi(e) | -0.34944 | | EX\_nh4(e) | -3.69554 | | EX\_hcys-L(e) | -0.1597 | | EX\_thmpp(e) | -2.47E-06 | | EX\_ribflv(e) | -2.47E-06 | | EX\_pdx5p(e) | -2.47E-06 | | EX\_nicrnt(e) | -0.00013 | |  |

**Table S3:** **Primers used in the validation of gene expression of selected genes**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target gene** | **Primer sequence** | **Amplicon length (bp)** | **Tm (0C)** |
| ENSSSA1UGT022145 (AQP1) | F - TTGTTTCGCAAGTTTGCCGT | 90 | 59.83 |
| R - GACTGATTGACGCCCTGGAT |  | 59.82 |
| ENSSSA1UGT002057 (SUC1) | F - AACACTGCGAATAGCGCATC | 86 | 59.35 |
| R - CGCCACTCTAGATGTTCGCA |  | 60.18 |
| ENSSSA1UGT002066 (SUC2) | F - CGGTAAGGTCTGAAACTGCGAT | 110 | 60.86 |
| R - GTTTTGCTAGATGTGCAAGGCA |  | 58.76 |
| Diaminopimelate decarboxylase (*LysA*) | F - ACTACAATTCTCGCCCTCGC | 86 | 60.18 |
| R - GTCATCAAAGGTCTCCCGCC |  | 60.74 |
| Arginosuccinate lyase (*ArgH*) | F - AAGCTCTGGTGTAAGGCACA | 98 | 59.24 |
|  | R - AGGATGTCTTGGGTCGCTTC |  | 59.75 |
| Branched-chain-amino-acid aminotransferase (*BCAT*) | F - CGTCCAGAGTCAGTGGCAA | 93 | 59.63 |
| R - GTTCATGGCTCCGGCTTCAG |  | 61.37 |
| Aspartate aminotransferase (*AAT*) | F - GGTCCTACCAGTTGTGCGAA | 95 | 59.97 |
| R - AGAATGGAGCCGAACCCAAG |  | 60.04 |
| 60S ribosomal protein L13a (*RPL13A*) | F - CATTCCACTACAGAGCTCCA | 101 | 60.00 |
| R - TTTCAGGTTTCGGATGGCTT |  | 60.00 |
| β-Tubulin (β-Tub) | F - TGTCAGGAGTAACGACGTGTTTG | 150 | 60.00 |
|  | R - TTCGGGAACGGTAAGTGCTC |  | 60.00 |
| 4-hydroxy-tetrahydrodipicolinate reductase (*dapB*) | F - TGGTAAAAGACTACCAGGCGAA | 118 | 59.37 |
| R - AGCTTGGTGTTTACAGCTGAGAG |  | 60.81 |



**Figure S2: Robustness analysis measuring sensitivity of the metabolic object function (growth rate of B. tabaci SSA1-SG1) to the quantitative flux levels through terminal reactions for (A) arginine biosynthesis, (B) lysine biosynthesis, (C) histidine biosynthesis, (D) leucine biosynthesis, (E) isoleucine synthesis and (F) valine biosynthesis.**



**Figure S3: Robustness analysis measuring sensitivity of the metabolic object function (growth rate of B. tabaci SSA1-SG1) to the quantitative flux levels through terminal reactions for (A) phenylalanine biosynthesis (B) methionine biosynthesis (C) threonine biosynthesis and (D) tryptophan biosynthesis**