Simultaneous manipulation of source and sink metabolism for improved crop yield

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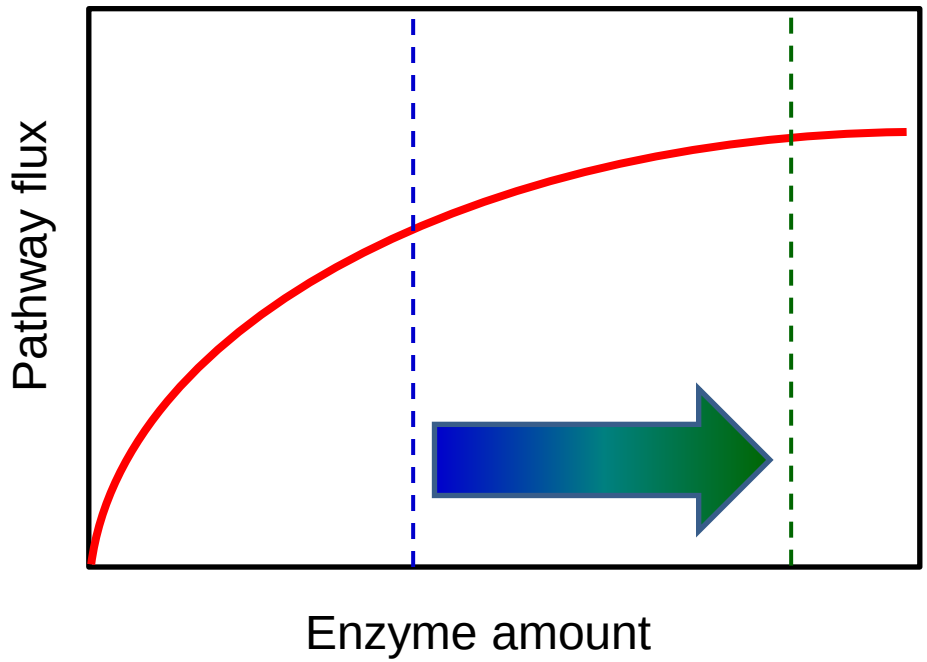
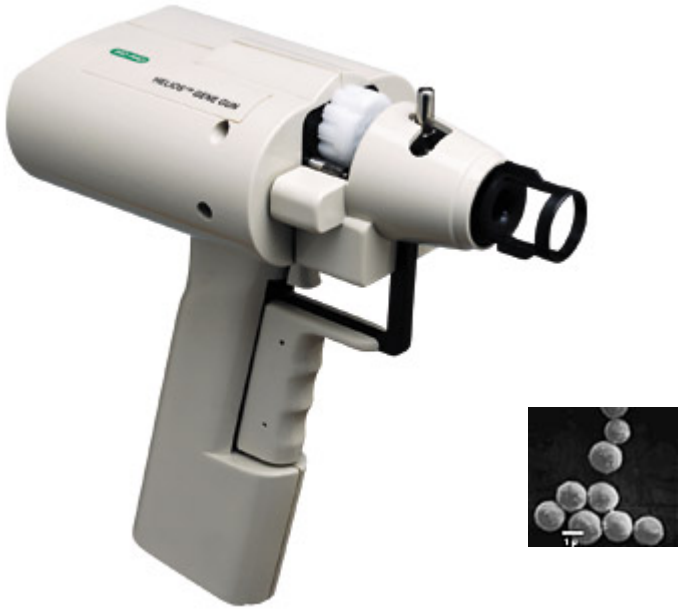
ERA-CAPS

ERA-NET for Coordinating Action in Plant Sciences


Pathway flux
Enzyme amount

Combinatorial co-transformation



- Each transgene construct (promoter-ORF-terminator) in a separate plasmid
- Use biolistic transformation to introduce all plasmids simultaneously

- Integration of transgene constructs in tandem at the same genomic locus

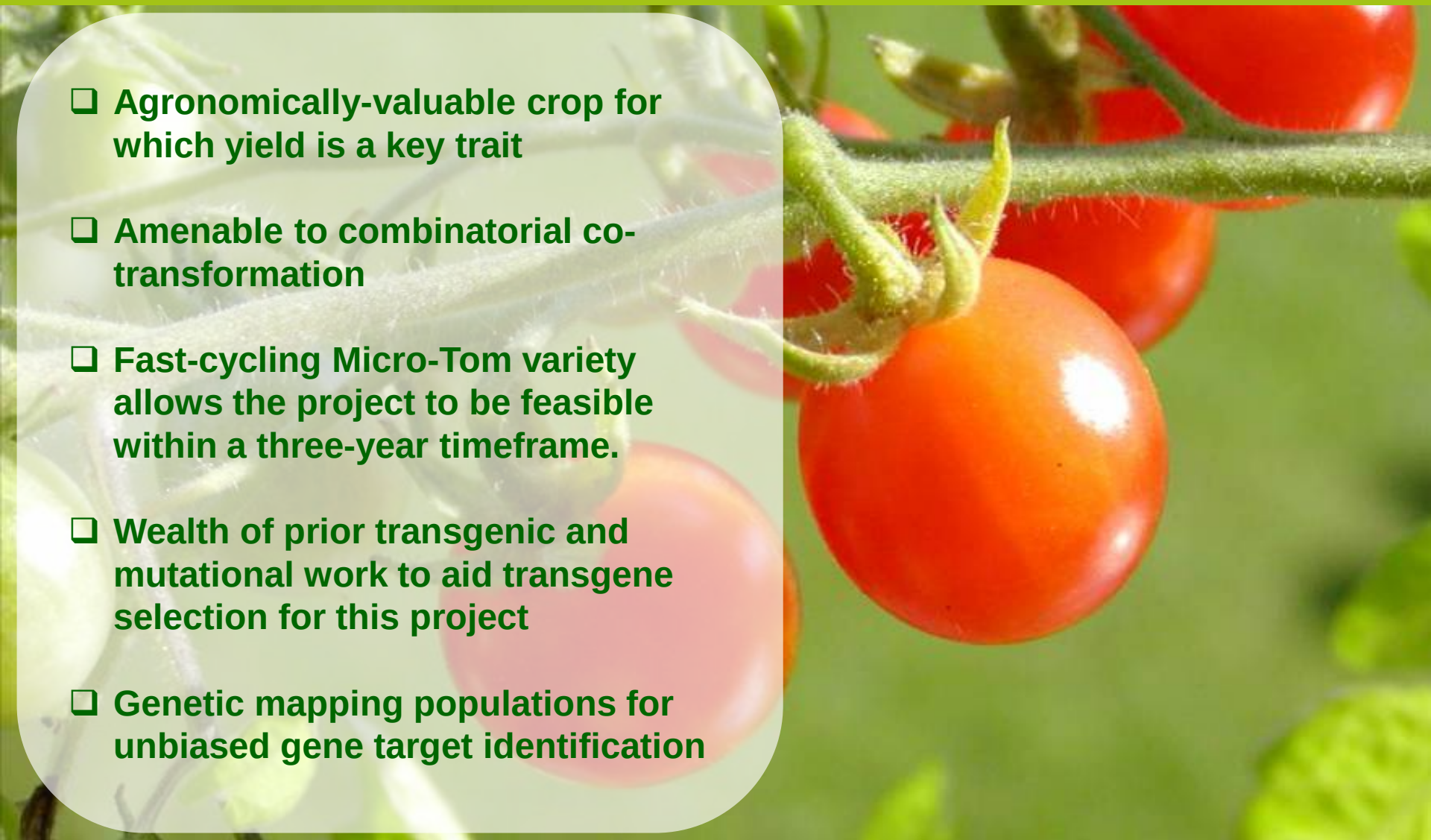
- Perform many independent transformations to create a combinatorial library of transgenics, each with a different combination of transgenes, with varying copy number and genomic arrangement

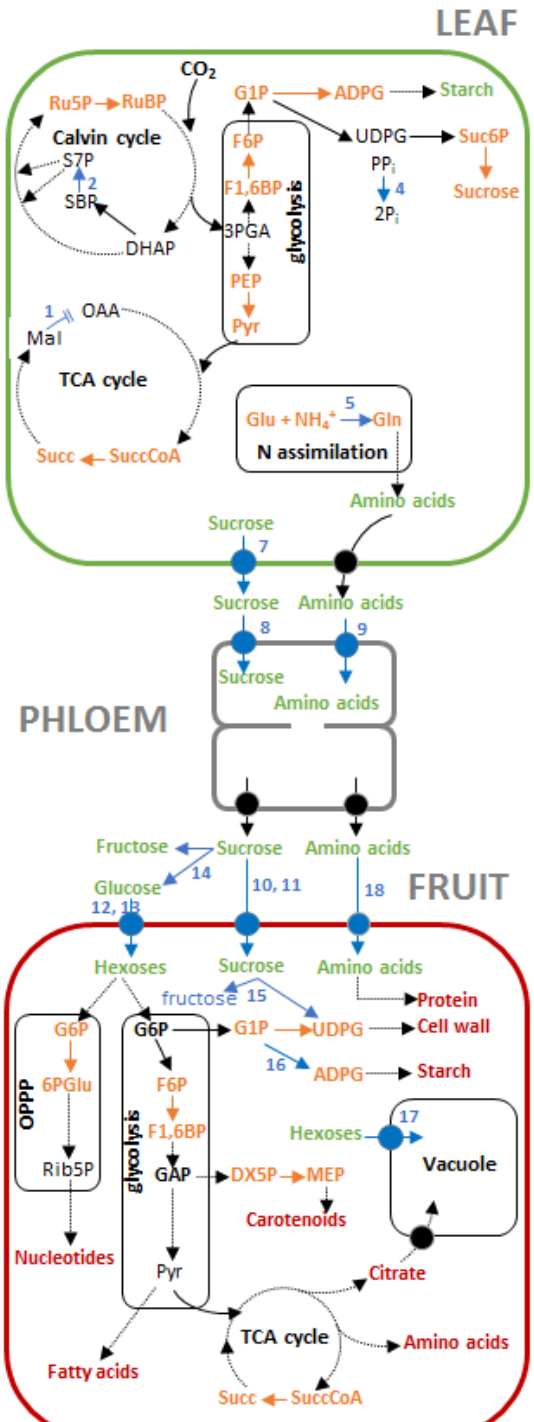
- No theoretical limit to number of transgenes that can be simultaneously introduced. Ralph Bock has previously introduced 15 transgenes into tobacco

Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P, Capell T (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. PNAS 105:18232–18237

Increasing tomato fruit yield

- Agronomically-valuable crop for which yield is a key trait
- Amenable to combinatorial co-transformation
- Fast-cycling Micro-Tom variety allows the project to be feasible within a three-year timeframe.
- Wealth of prior transgenic and mutational work to aid transgene selection for this project
- Genetic mapping populations for unbiased gene target identification



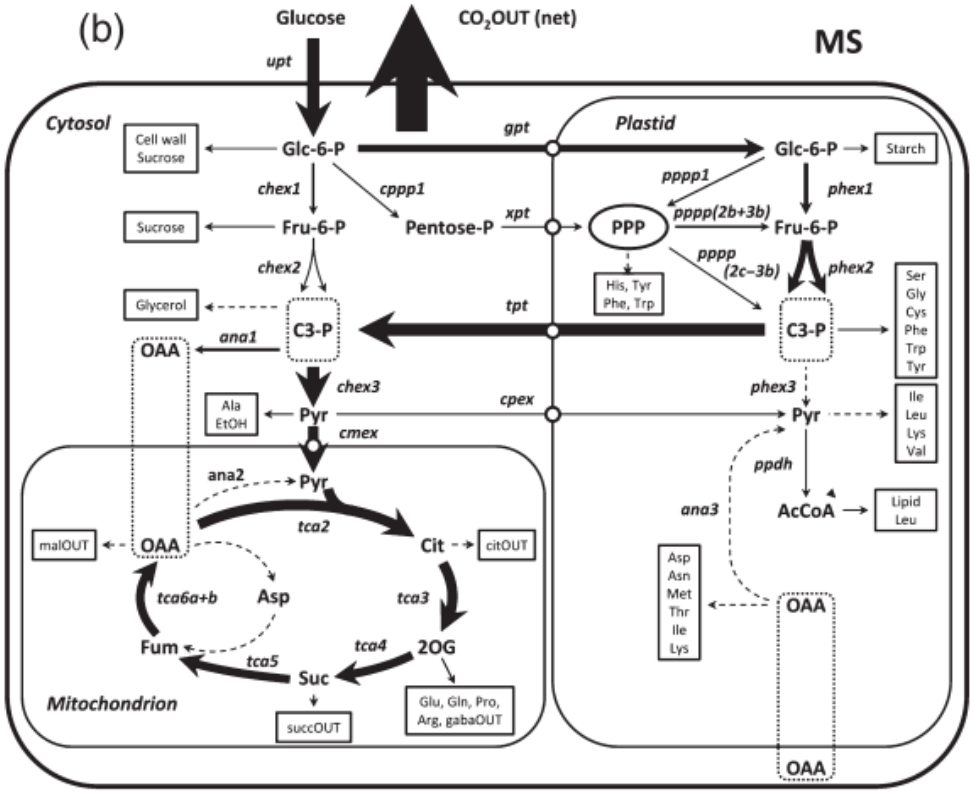


TARGET TRANSGENES

- 18 transgene targets selected based on their previously demonstrated effect in transgenic tomato or other plants
- Includes enzymes in leaf (Calvin-Benson cycle, TCA cycle, sucrose synthesis; N assimilation) and fruit (sucrose catabolism, starch synthesis)
- And transporters for phloem loading and unloading of sucrose and amino acids
- And genes to alter tomato plant development, e.g. knockdown of SPA encoding a plastid chaperone increases harvest index

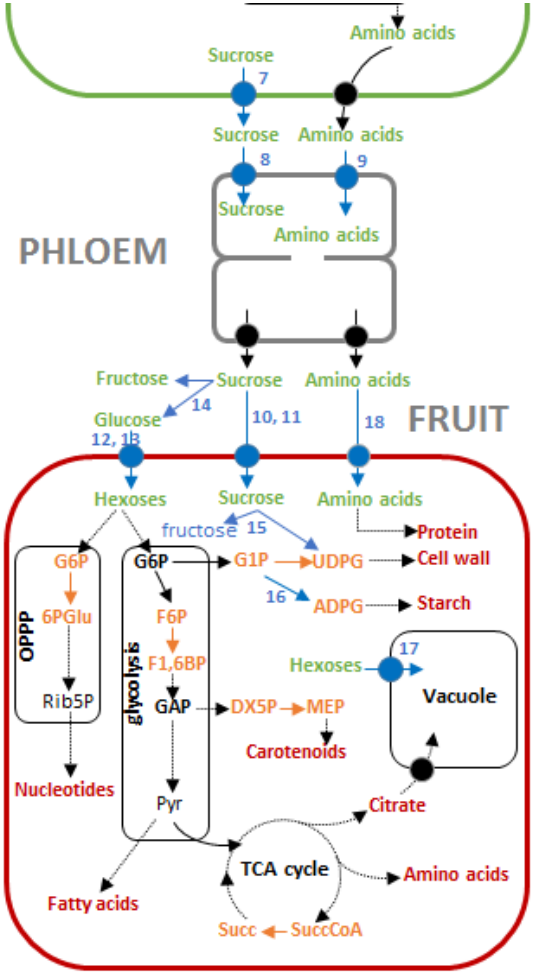
Identifying additional bottlenecks

Enzyme capacity bottlenecks



- Flux balance modelling and isotope labelling to predict fluxes in WT and engineered tomato leaves and fruit
- Fluxes compared to maximal catalytic activities of relevant enzymes

Identifying targets for increased N flow

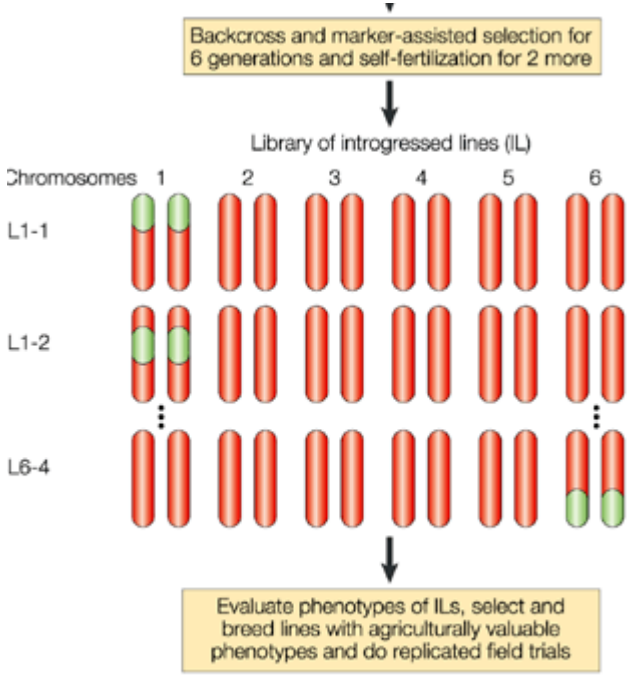


- In contrast to sucrose transport, relatively little is known about the source-to-sink flow of nitrogen in plants

- Maintaining an unloading gradient is crucial
- Given that tomatoes have extensive vacuolar amino acid stores, tonoplast transporters are likely to be key
- Based on our previous work we have identified 3 members of the CAT family and 3 of the SLC 32/36/38 sub-family as good targets
- Their localisation and function will be explored by transient and stable over-expression

Genetic approach for unbiased identification of additional transgene targets

Solanum pennellii x Solanum lycopersicum



- **From a series of field trials we have identified 3 harvest-index QTLs and 3 fruit nitrogen content QTLs**
- **Sub-introgression lines and backcross introgression lines will be used to identify candidate genes**

Outcomes

- Transgenic tomato plants with a step-change in yield
- New understanding of bottlenecks for source-to-sink carbon flow
- New insight into the role of tonoplast transporters in source-to-sink nitrogen flow
- Transfer of the best combination of transgenes to commercially-relevant varieties by crossing
- Super-transformed lines in which the best-performing transgenic lines are combined with additional transgene targets discovered in this project

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Gene	Promoter	Rationale
1. SlmMDH	RbcS	Knockdown of tomato mitMDH affects stomatal aperture leading to increased gas exchange and increased photosynthesis[9]
2. AtSBPase	RbcS	Overexpression of the Calvin-Benson cycle enzyme SBPase increases photosynthesis and growth in tobacco[11]
3. SlSPA	35S	Knockdown of this plastidial chaperone alters leaf carbon partitioning to favour sucrose export[1]
4. EcPPase	cyFBPase	Over-expression of PPase in the cytosol of leaf mesophyll cells alters carbon partitioning to favour sucrose export[7]
5. NtGS2	RbcS	Leaf-specific overexpression of chloroplast GS leads to increased N assimilation and growth in tobacco[8]
6. FpGLDH	35S	Constitutive over-expression of the glycine decarboxylase H protein leads to increased photosynthetic assimilation rate in tobacco[13]
7. AtSWEET11	RbcS	Leaf-specific over-expression of the SWEET sucrose effluxer[3]
8. AtSUC2	CoYMV	Companion cell-specific over-expression of a sucrose transporter to increase phloem loading capacity[4, 12]
9. AtAAP1	CoYMV	Companion cell-specific over-expression of a broad-specificity amino acid transporter[16]
10. AtSUC2 11. AtSUC9	Patatin B33	Increased capacity for uptake of sucrose into fruit parenchyma cells by overexpression of high and low affinity sucrose transporters[12]
12. AtSTP6 13. AtSTP3	Patatin B33	Increased uptake capacity of hexoses into fruit parenchyma cells by overexpression of high and low affinity transporters[2]
14. SpLIN5	Patatin B33	Fruit-specific over-expression of an allele of LIN5 encoding a kinetically superior apoplastic invertase that is linked to increased fruit sugar content[6]
15. AtSUS1	Patatin B33	Over-expression of sucrose synthase increases sink strength and yield in cotton[15]
16. ShAgpL1	native	Introgression of the ShAgpL1 gene into tomato leads to an extended period of fruit transient starch accumulation and increased sink strength[10]
17. AtTMT1	Patatin B33	Fruit-specific overexpression of a tonoplast hexose transporter to maintain unloading gradient by transfer of sugars to vacuole[14]
18. AtAAP6	Patatin B33	Increased uptake of amino acids into fruit parenchyma cells[16]

The population was constructed from a cross between the cultivated tomato *S. lycopersicum* (CV. M82) and the wild species *S. pennellii* (LA0716). To reduce the wild parent genomic representation all the lines were backcrossed to the cultivated parent for two to three generations. The BILs were then selfed for eight to nine generations, resulting in around 550 lines. The population was genotyped with the 10K SOLCAP SNP Chip analysis (Sim et al., 2012). Polymorphic markers between the two parents were used for the mapping analysis.