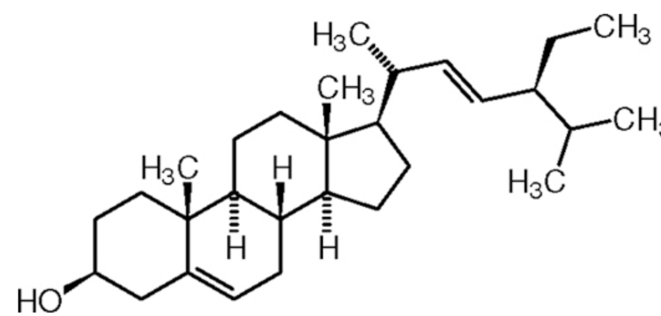
Enginyeria metabòlica d’esterols en tomàquet: Una eina per millorar la qualitat del fruit i la tolerància a condicions ambientals adverses?

Teresa Altabella



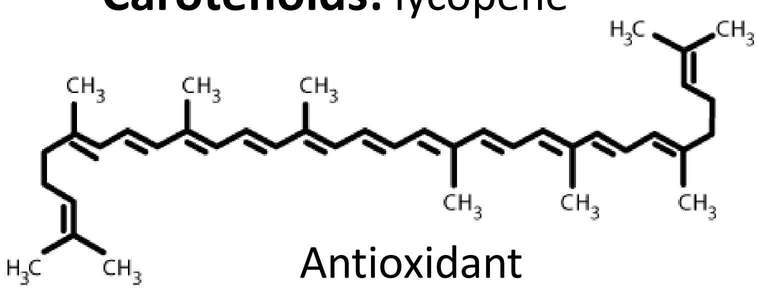
Tomato is an important component of the human diet

TOMATO

Solanum lycopersicum

(Solanaceae)

Carotenoids: lycopene

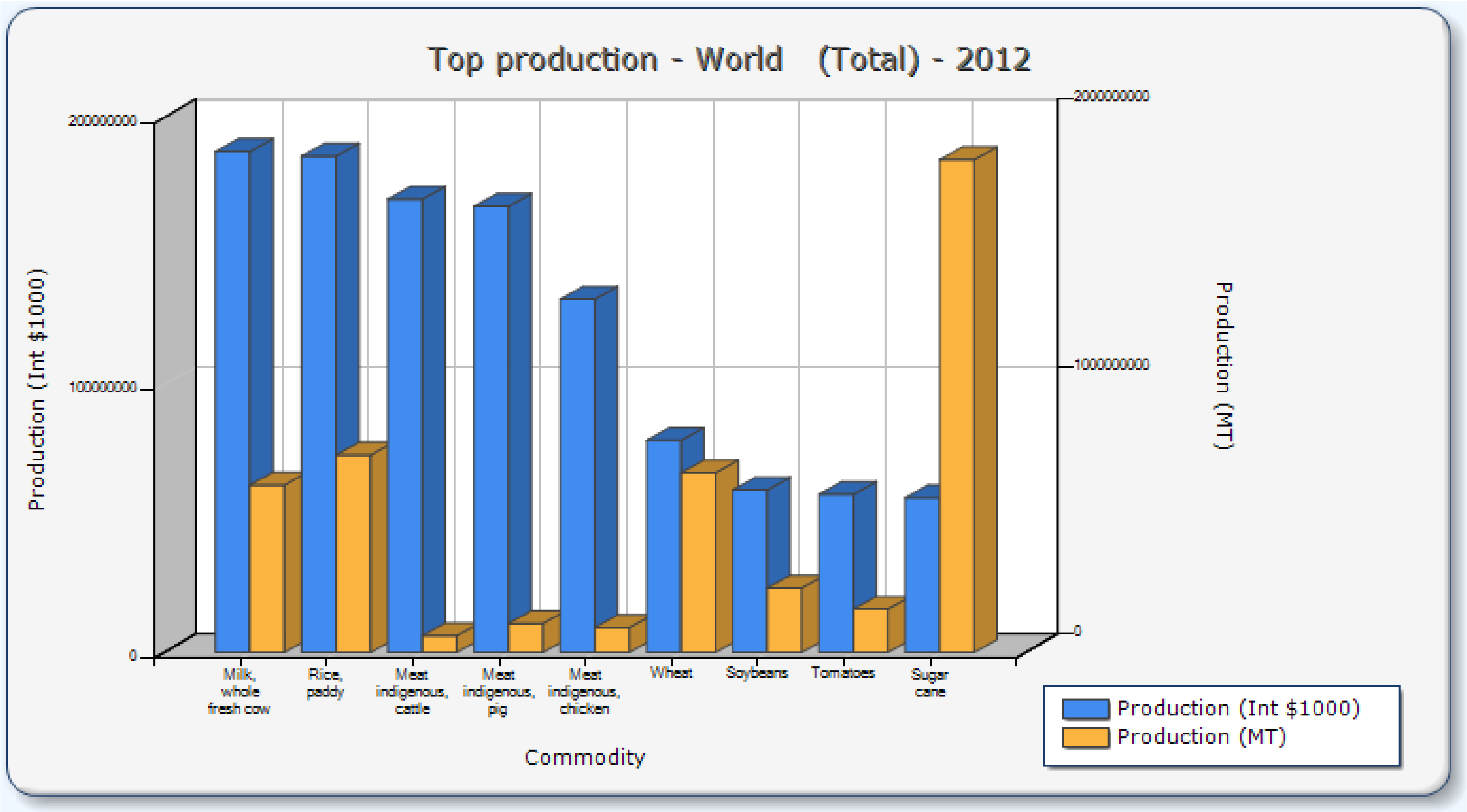


Antioxidant

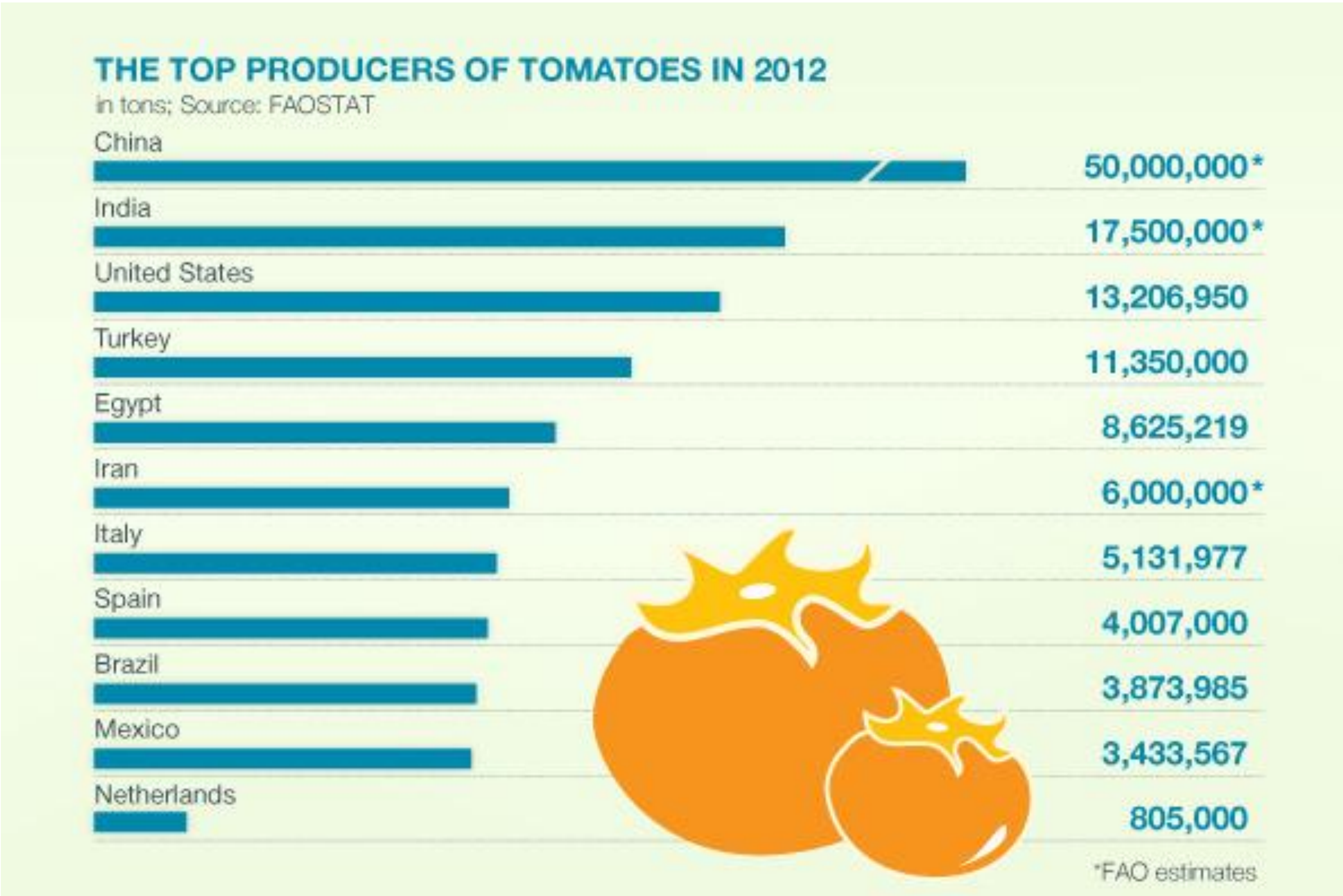
Nutrients in Tomatoes 1.00 cup raw (180.00 grams)

Nutrient	%Daily Value
vitamin C	38.1%
vitamin A	29.9%
vitamin K	17.7%
potassium	12.1%
molybdenum	12%
manganese	10.5%
fiber	8.6%
vitamin B6	7%
folate	6.7%
copper	5.5%
vitamin B3	5.3%
magnesium	4.9%
vitamin E	4.8%
vitamin B1	4.6%
phosphorus	4.3%
protein	3.1%
tryptophan	3.1%
choline	2.8%
iron	2.7%
Calories (32)	1%

Tomato is important as food and in the global economy



Tomato is important as food and in the global economy



Tomato as a model system for studying fleshy fruit ripening

- ✓ commercial importance
- ✓ relatively short development period
- ✓ easy propagation
- ✓ diploid
- ✓ genome: 950 Mb, 12 chromosomes

Sequenced genome (2012)
Sol Genomics Network
http://solgenomics.net/

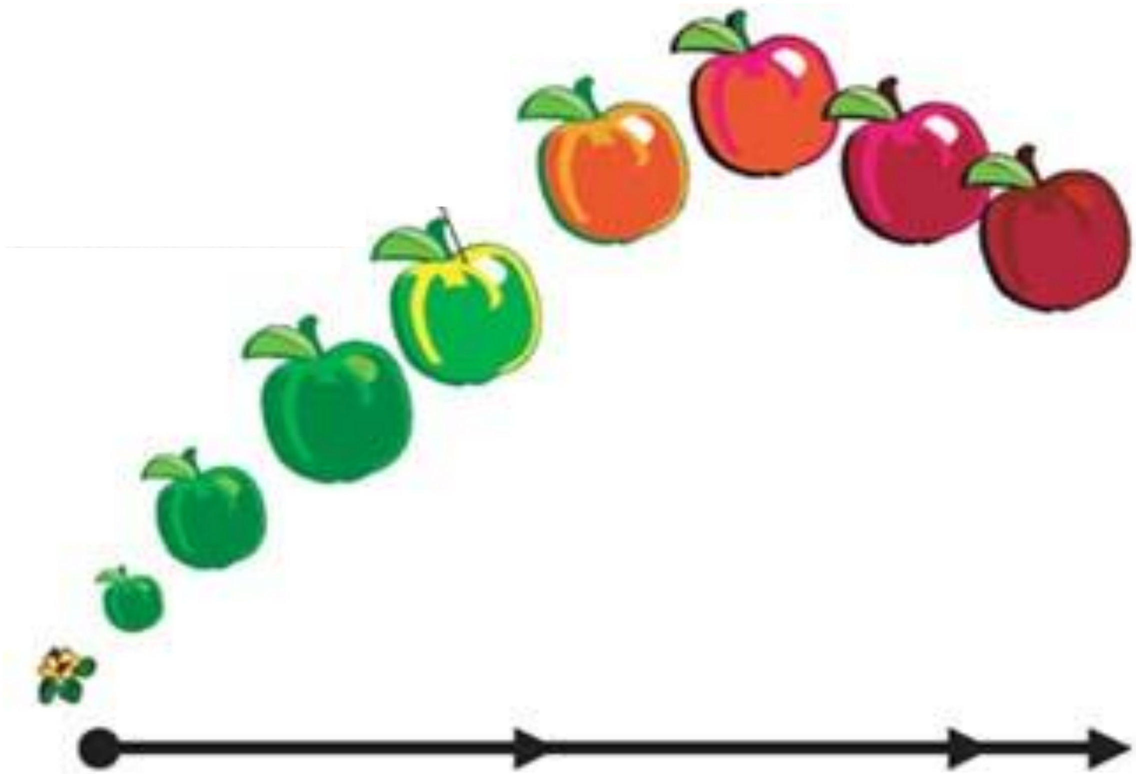
***S. lycopersicum* cv. Micro-Tom**

cv. Florida Basket X cv. Ohio 4013-3

dwarf phenotype: mutations in the recessive genes *dwarf* (*d*) and *miniature* (*mnt*)

- ✓ small size (1,357 plants / m^2)
- ✓ rapid life cycle (70-90 days from seeds to ripe fruit)
- ✓ easy transformation

Tomato fruit ripening



BIOCHEMICAL AND METABOLIC CHANGES
ORGANOLEPTIC QUALITY

Development

Cell division and expansion

Ripening

- Pigmentation
- Texture
- Flavor
- Aroma

Optimal characteristics for consumption

Over-Ripening

- Rapid softening
- Increased susceptibility to microbial infection

Harvest, transport, storage and marketing

Tomato fruit ripening

High susceptibility of the plant to different plagues

PESTICIDES

- Increase the production costs
- A risk factor for consumers and the environment

Development of pest resistant varieties is a major challenge in sustainable agriculture

BIOCHEMICAL AND METABOLIC CHANGES

ORGANOLEPTIC QUALITY

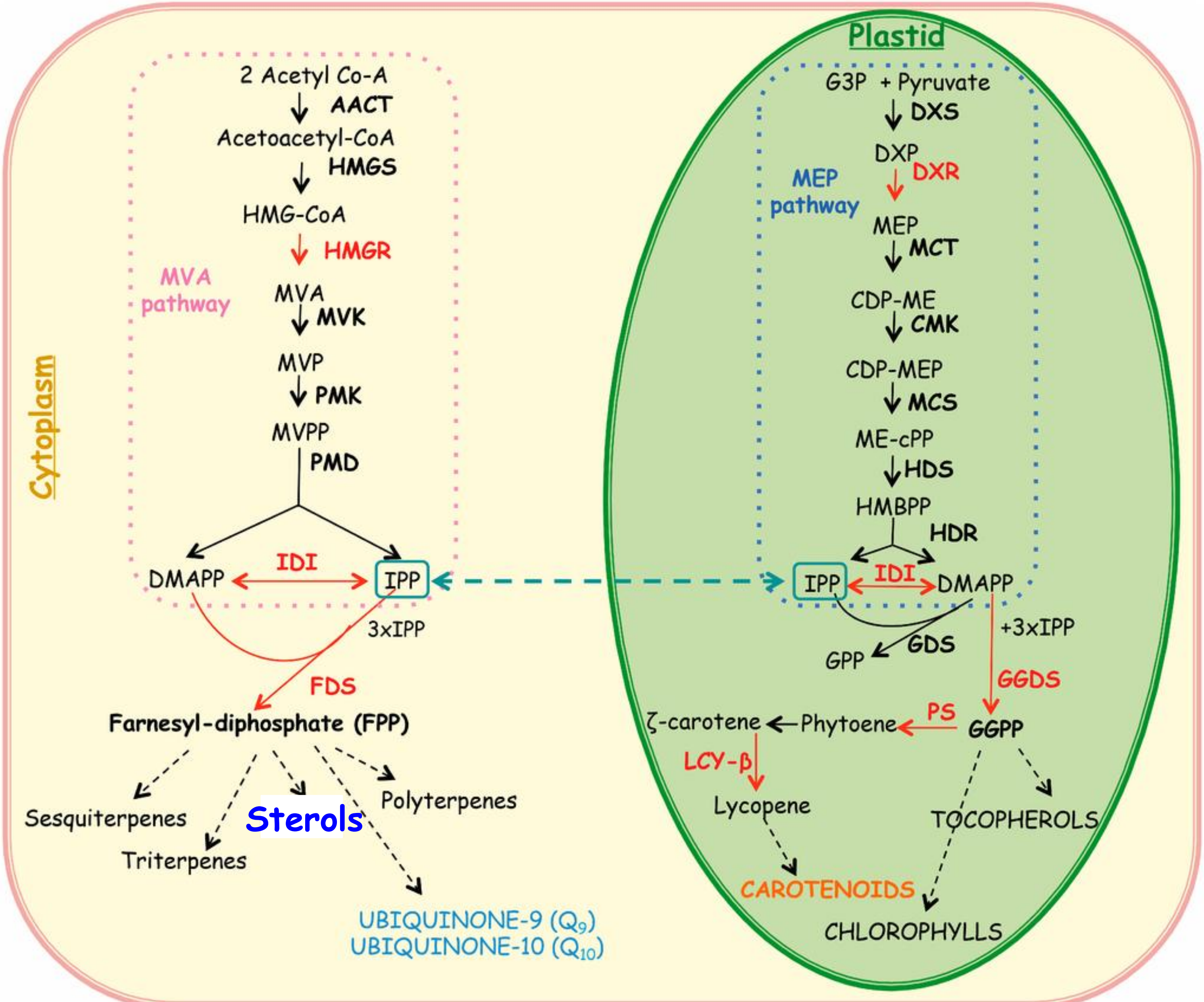
Over-Ripening

- Rapid softening
- Increased susceptibility to microbial infection

s

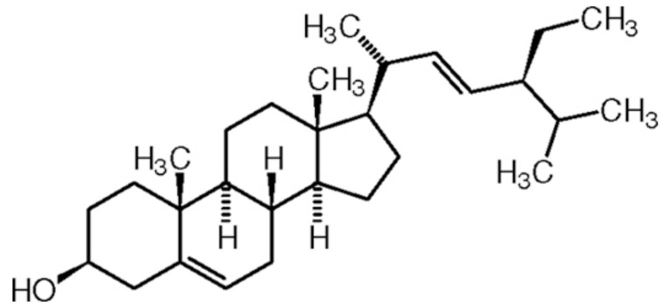
Harvest, transport, storage and marketing

Isoprenoid biosynthesis in plants

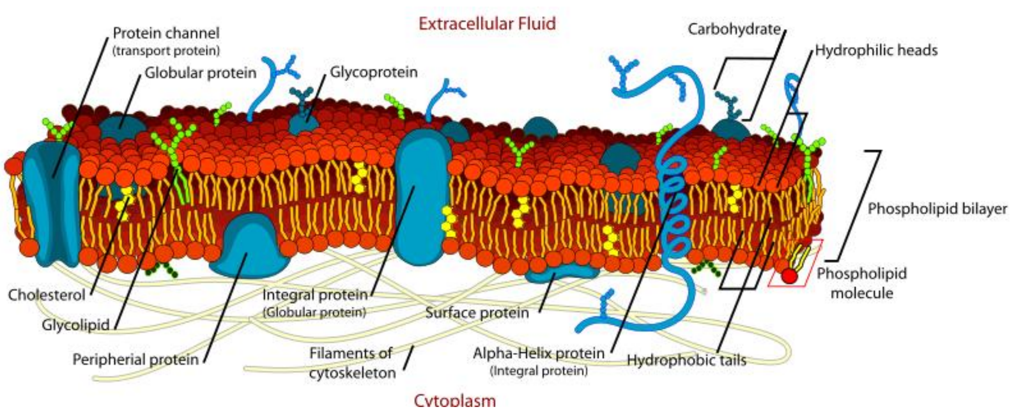


Rizzello et al. 2014

Sterols are essential components of cell membranes

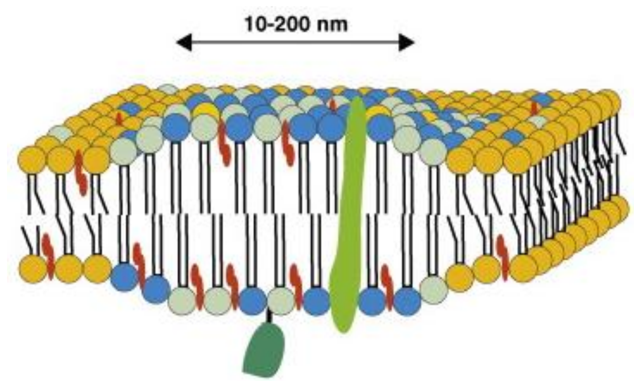


Cell membranes



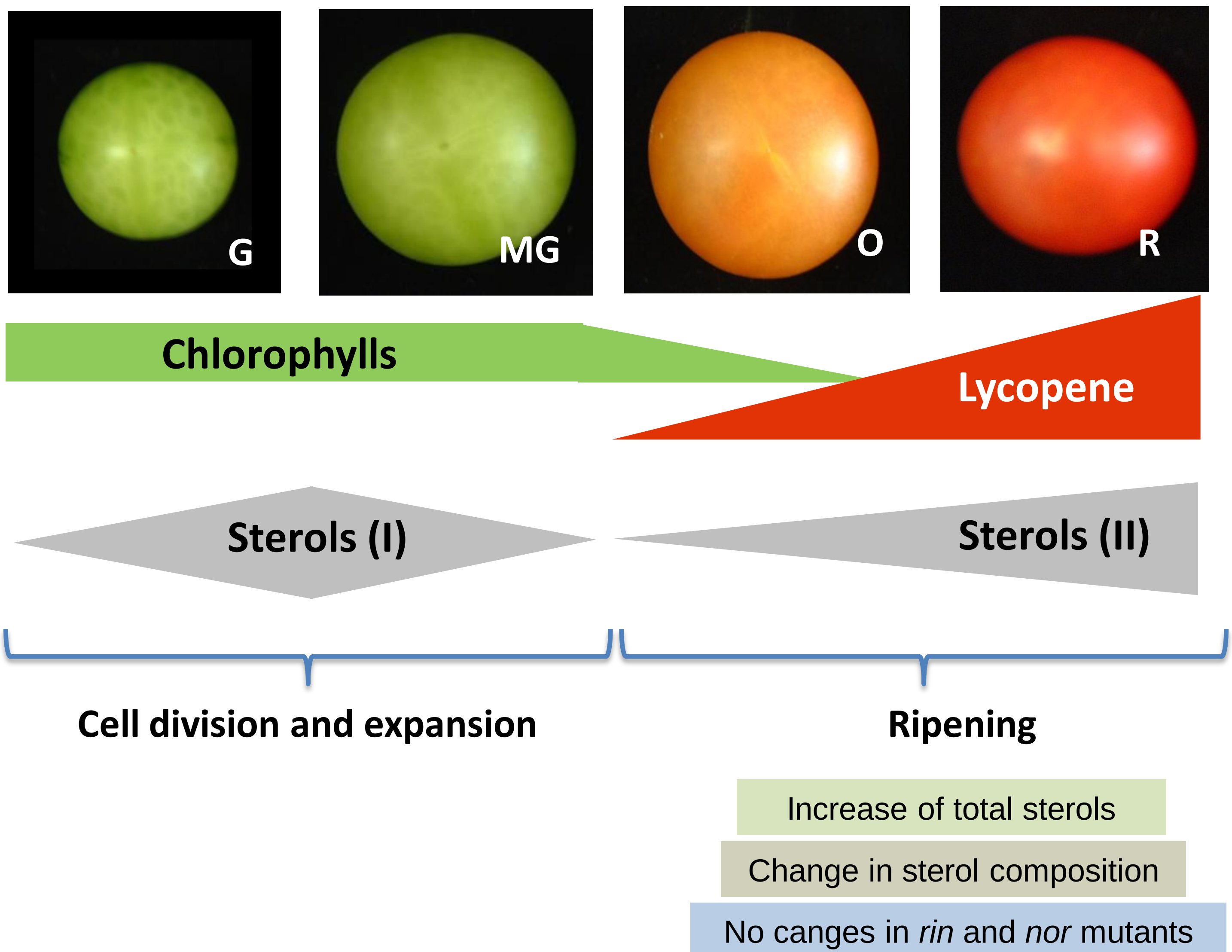
Modulate membrane fluidity and permeability

Lipid rafts

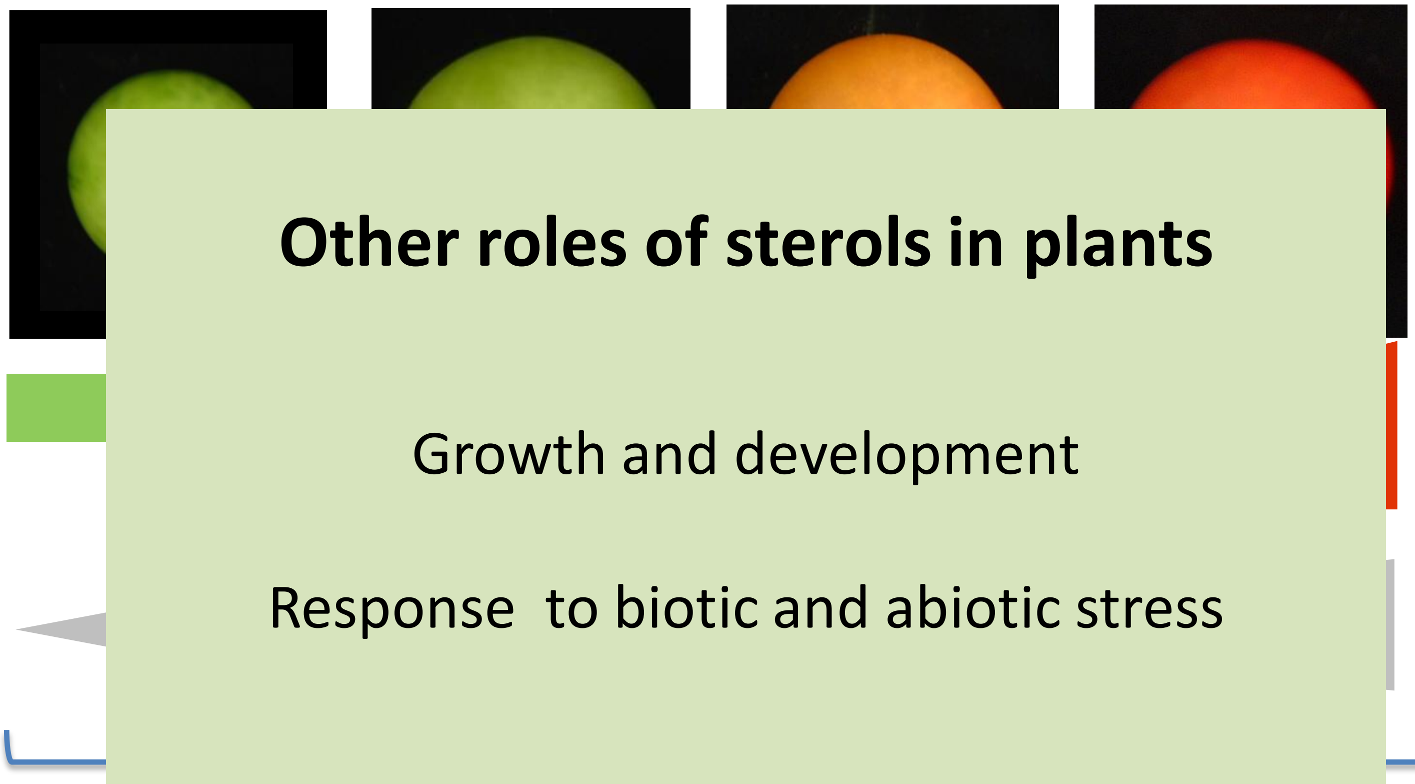


Signaling
Cell polarity
Trafficking
Secretion

Changes in sterol composition associated to tomato fruit development and ripenig



Changes in sterol composition associated to tomato fruit development and ripenig



Other roles of sterols in plants

Growth and development

Response to biotic and abiotic stress

Cell division and expansion

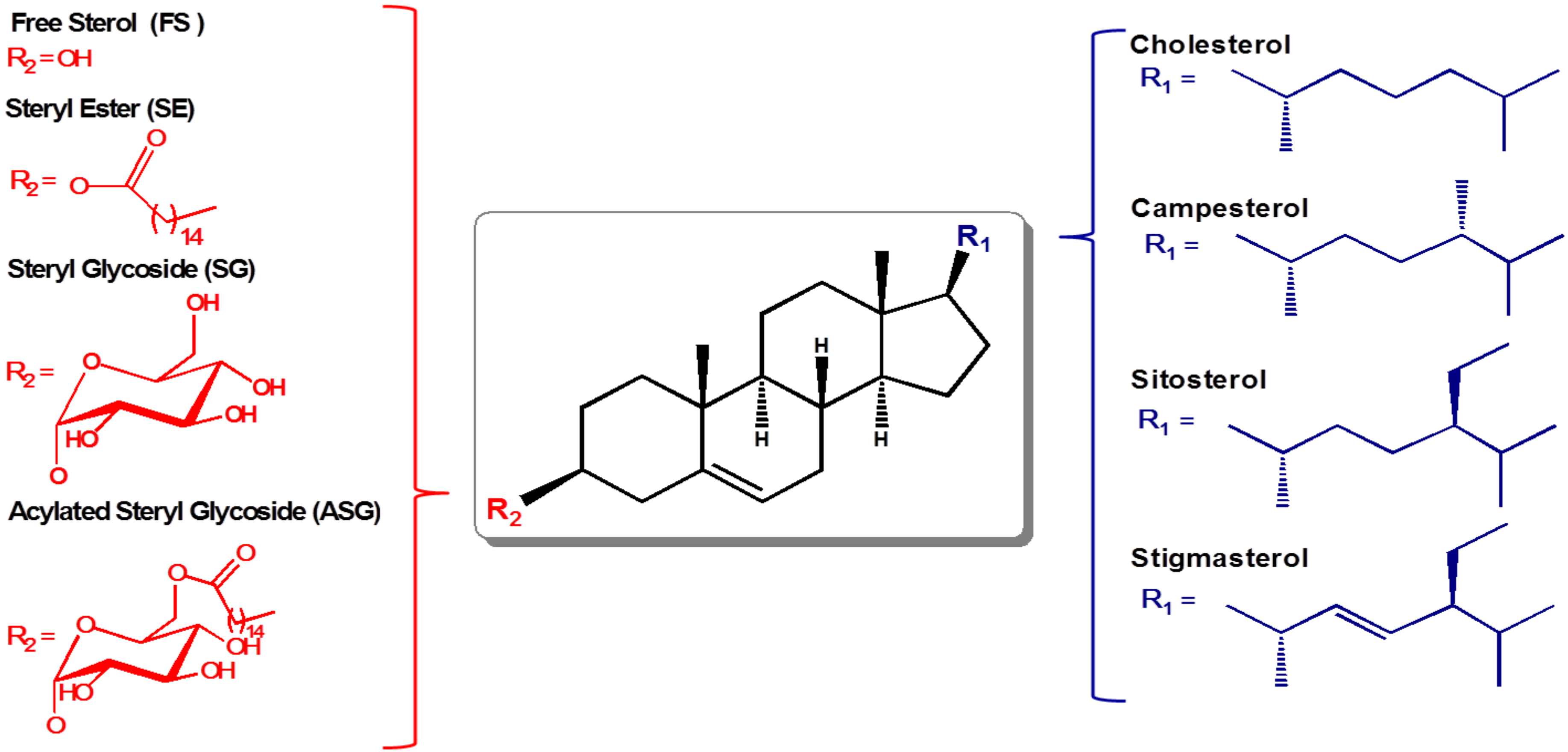
Ripening

Increase of total sterols

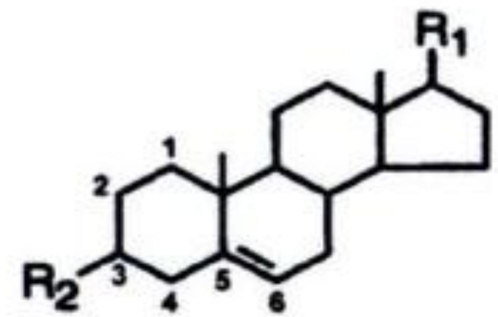
Change in sterol composition

No canges in *rin* and *nor* mutants

Plants contain a high diversity of free and conjugated sterols



Tomato (like other *Solanum* species) have an unusual sterol composition



Arabidopsis

Tomato

	Arabidopsis	Tomato
Free sterols	74 %	10 %
Steryl esters	15 %	8 %
Steryl glucosides	8 %	26 %
Acylated steryl glucosides	3 %	56 %

> 80%

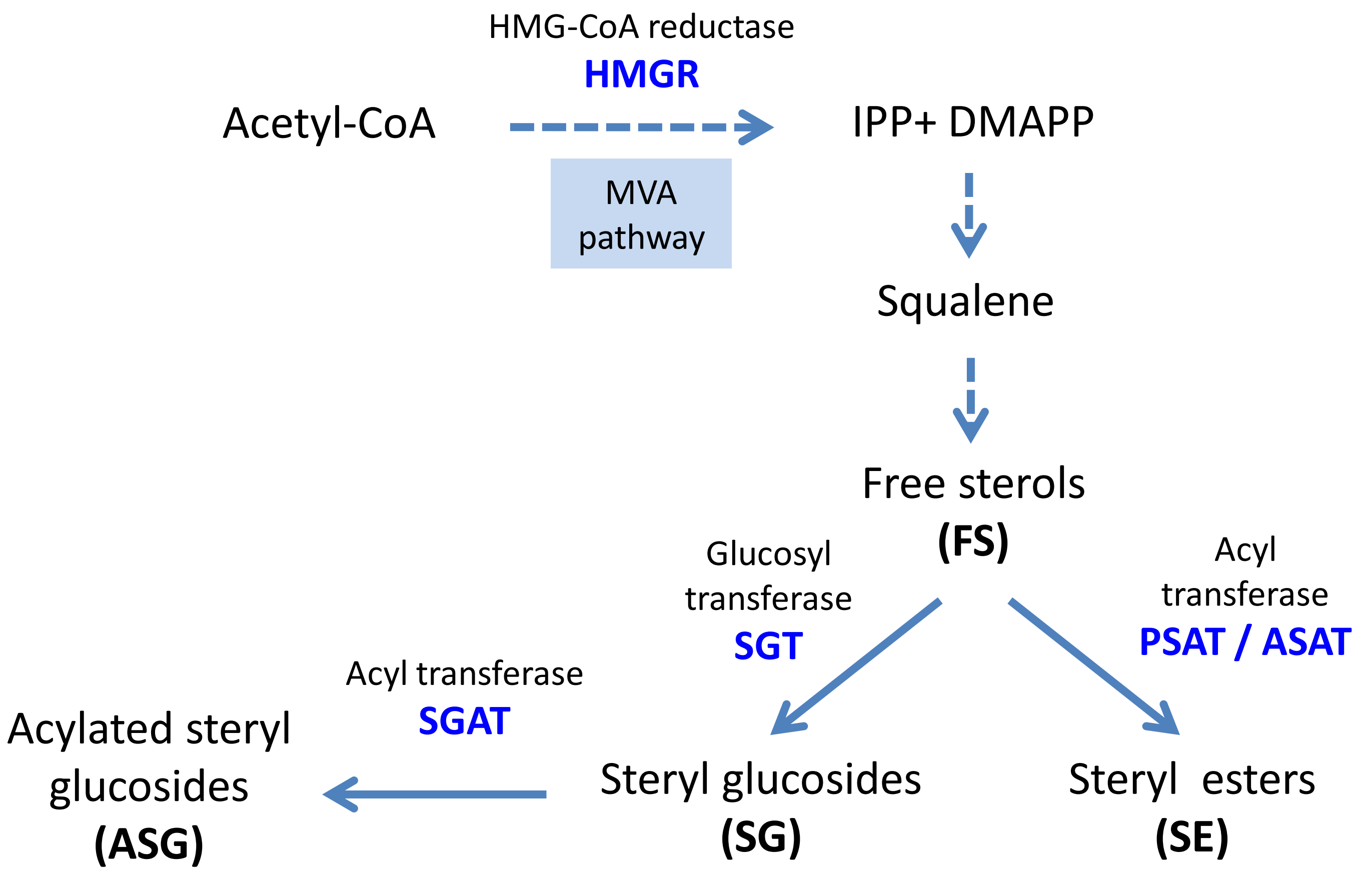
Tomato (like other *Solanum* species) have an unusual steryl lipid composition

The biological and evolutionary significance of this peculiar sterol composition is currently unknown

	Arabidopsis	Tomato
Free sterols	**74 %**	**10 %**
Steryl esters	**15 %**	**8 %**
Steryl glucosides	**8 %**	**26 %**
Acylated steryl glucosides	**3 %**	**56 %**

> 80%

Schematic representation of sterol metabolism in tomato



Objectives

1. Identification and characterization of the genes and enzymes involved in the biosynthesis of conjugated sterols in tomato.

2. Generation of genetically modified tomato lines with altered profiles of free and conjugated sterols (plant and/or fruit).

3. Evaluation of the effects of the perturbations in sterols metabolism on plant development, fruit development and ripening, and resistance to biotic and abiotic stresses.

4. Identification and characterization of mutant and allelic variants of genes involved in sterol metabolism using TILLING and EcoTILLING.

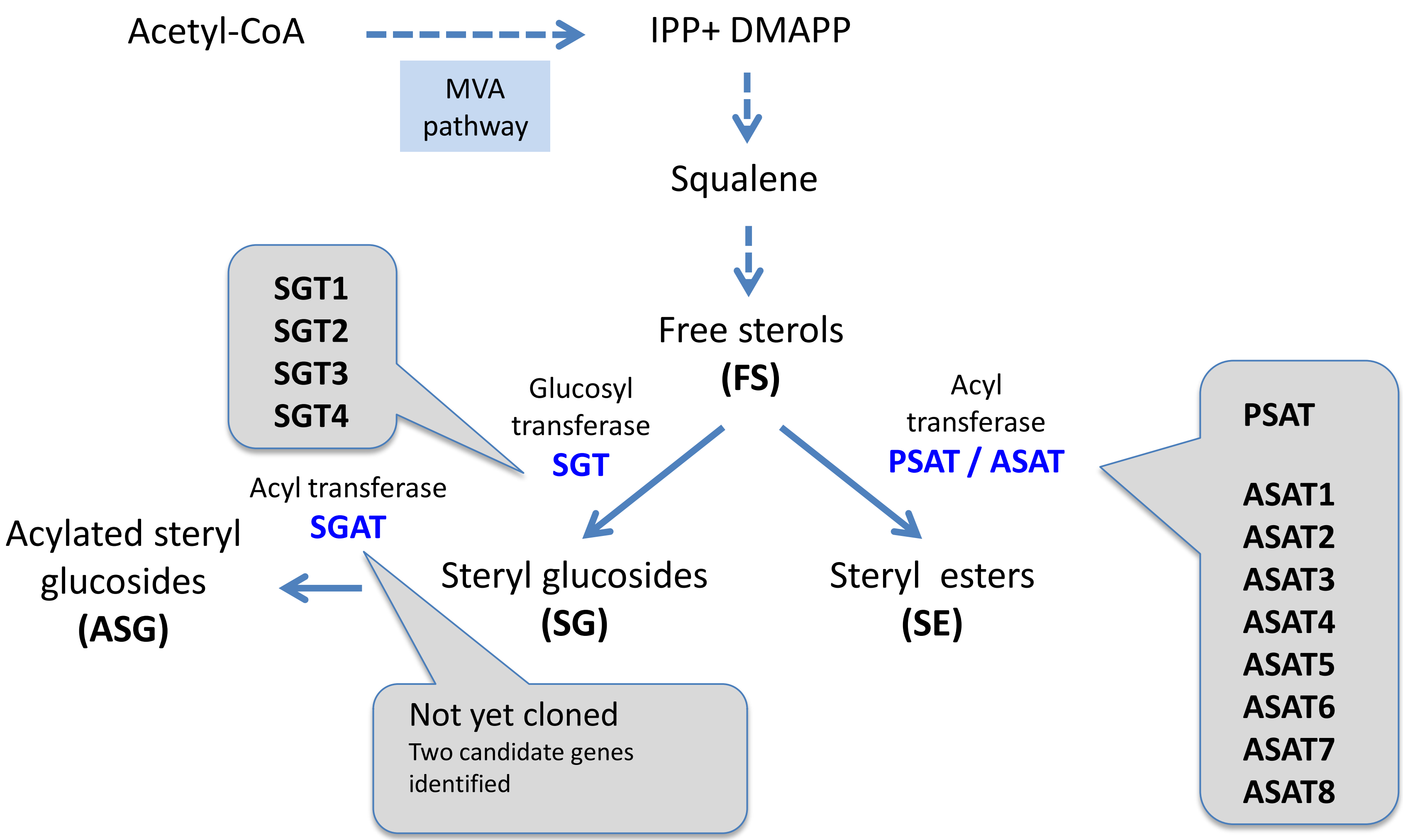
Objective 1: Identification and characterization of the genes and enzymes involved in the biosynthesis of conjugated sterols in tomato

1.1. Cloning and Functional validation of candidates enzymes (PSAT, ASAT, SGT)

1.2. Study of the expression pattern of the corresponding genes

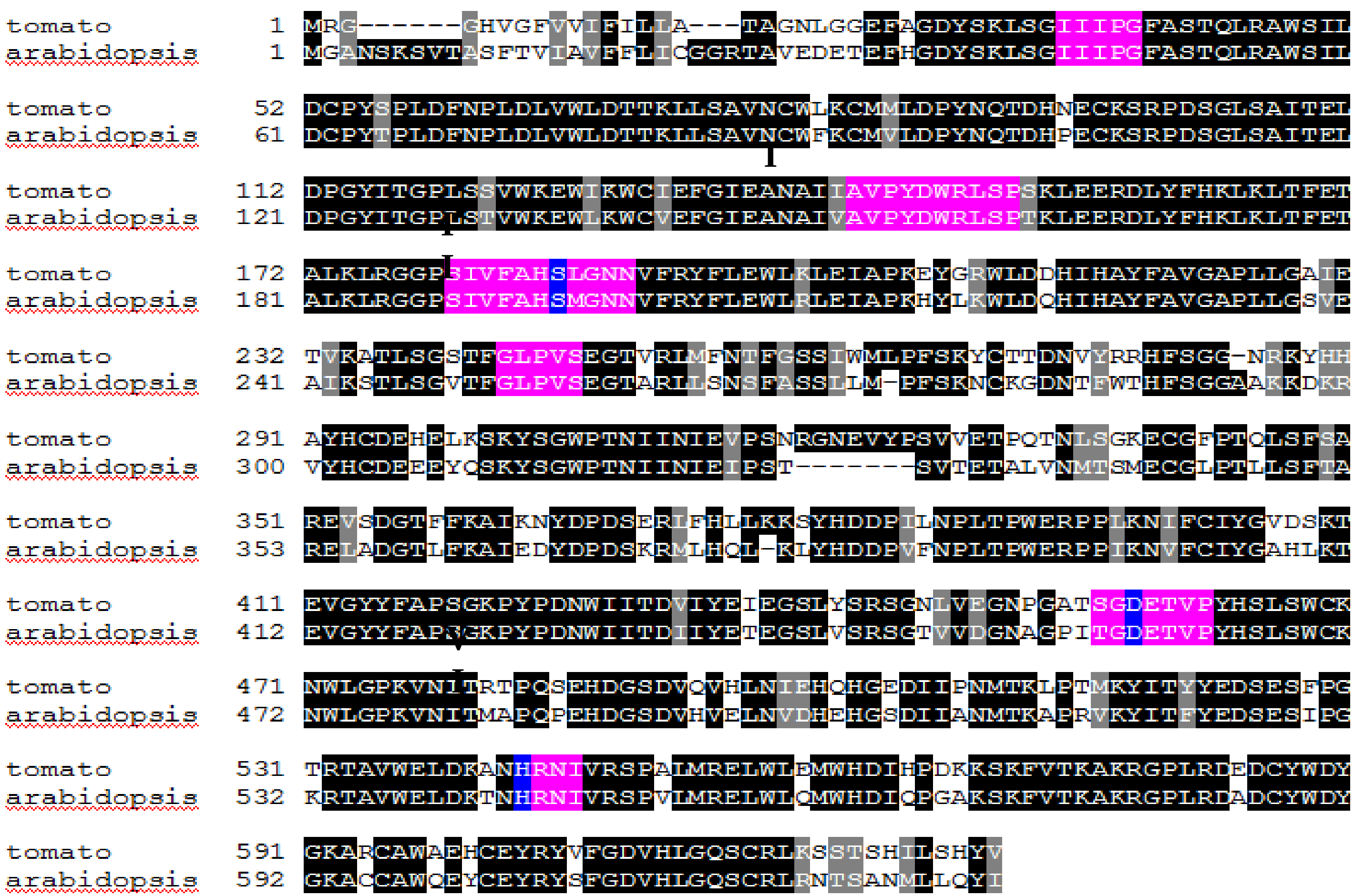
1.3. Study of the subcellular localization of the enzymes

Enzymes involved in sterol metabolism in tomato are encoded by gene families



Cloning and characterization of *PSAT* from *S. lycopersicum* (cv. Micro-Tom)

The *PSAT* cDNA cloned from mature green fruit RNA of *S. lycopersicum* cv. Micro-Tom (*SlPSAT)*, encodes a 630 aa protein that is 77% identical to the Arabidopsis PSAT1



PINK

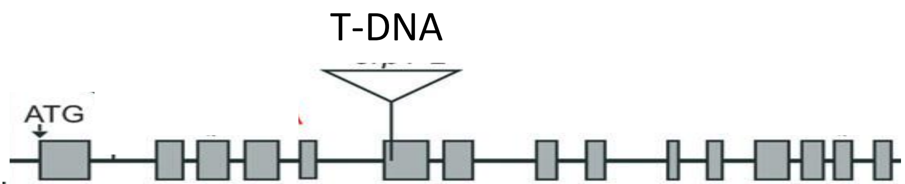
characteristic conserved domains in mammalian LCAT-type enzymes

BLUE

catalytic triad
S-D-H

Functional validation of the candidate SlPSAT enzyme

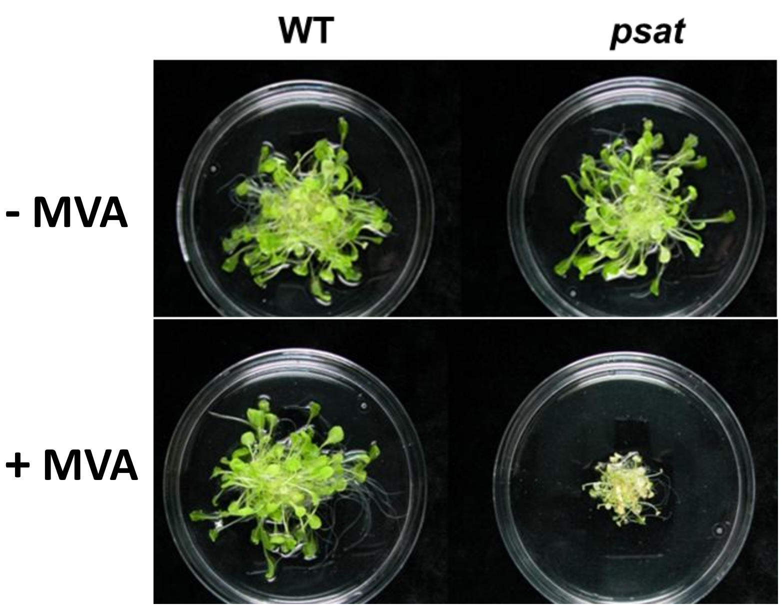
Complementation of the Arabidopsis *psat* knock-out mutant

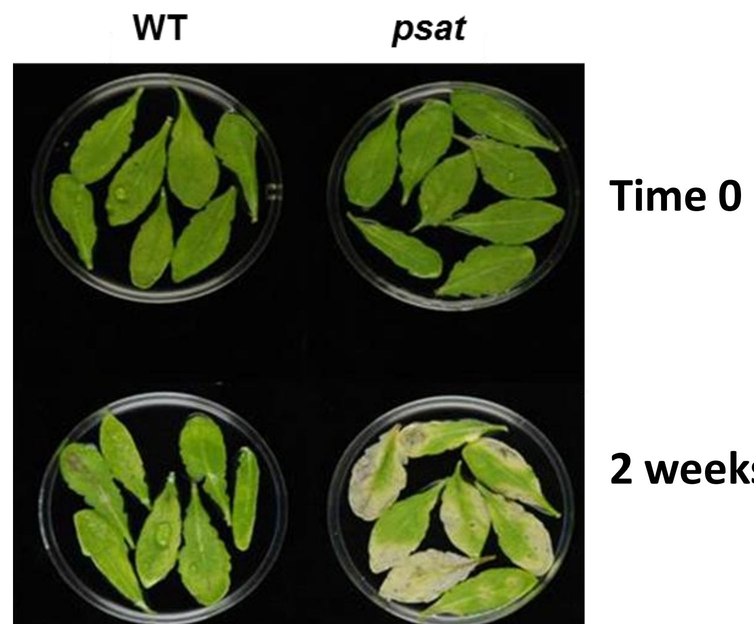


psat

PHENOTYPE ALTERATIONS

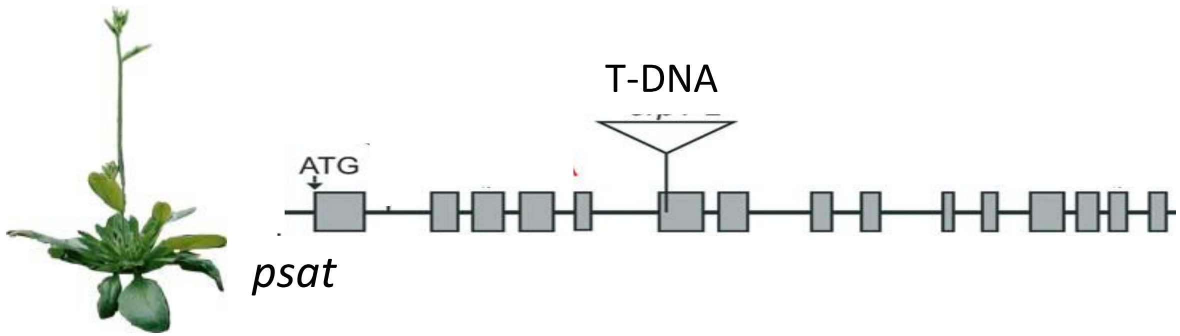
- Toxicity of MVA treatment
- Premature senescence





Functional validation of the candidate SlPSAT enzyme

Complementation of the Arabidopsis *psat* knock-out mutant



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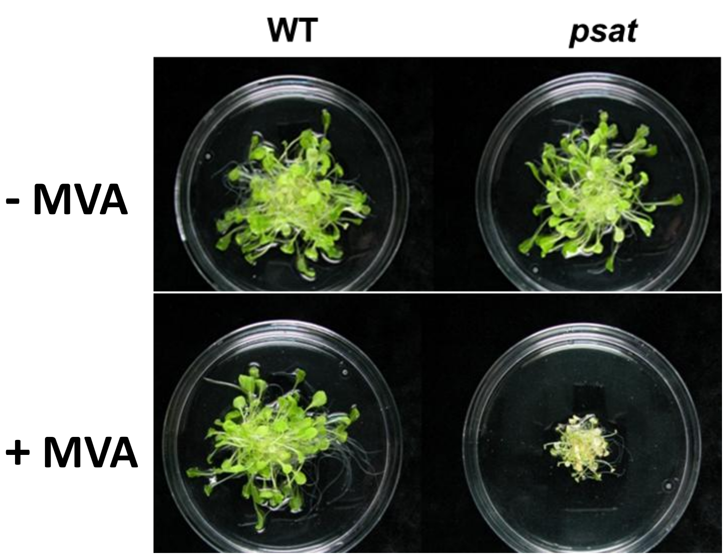
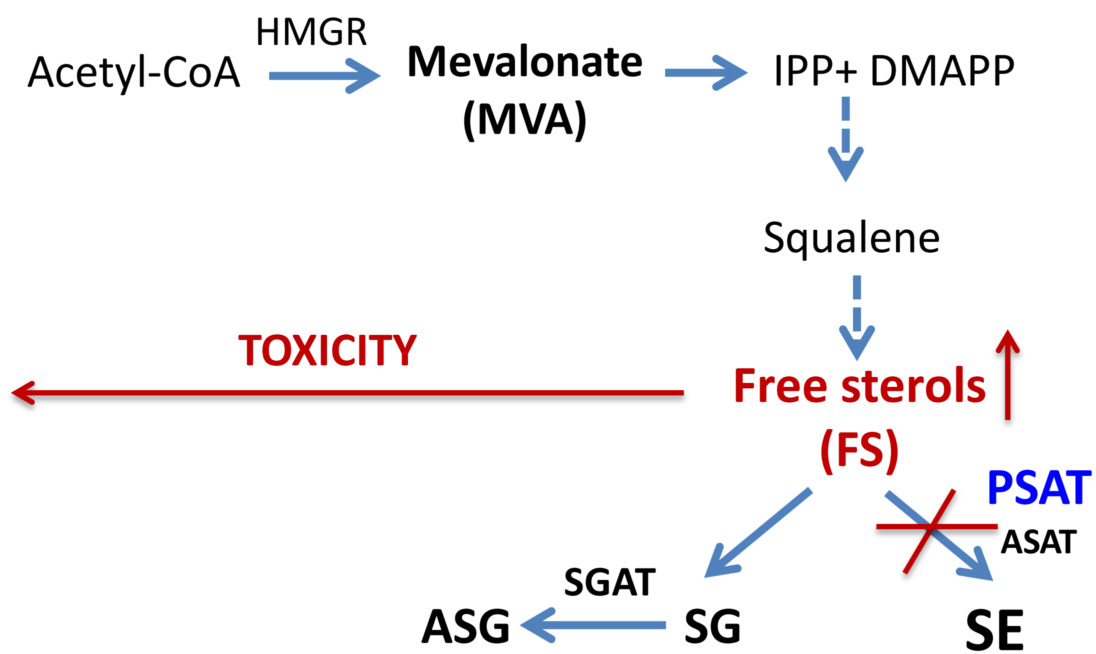
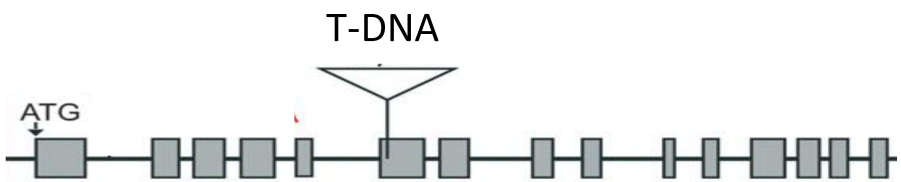




Functional validation of the candidate SlPSAT enzyme

Complementation of the Arabidopsis *psat* knock-out mutant



psat

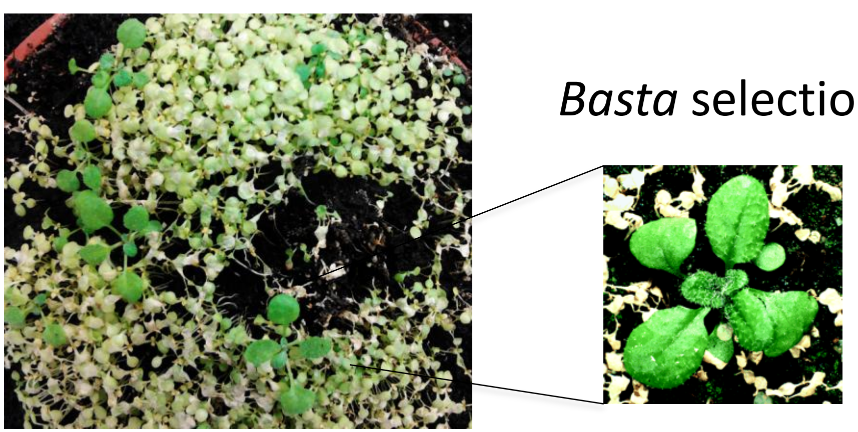
Floral dipping

Agrobacterium tumefaciens

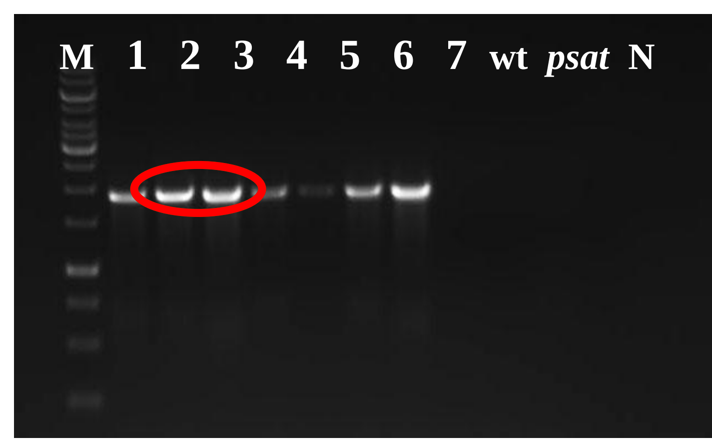
PHENOTYPE ALTERATIONS

- Toxicity of MVA treatment
- Premature senescence

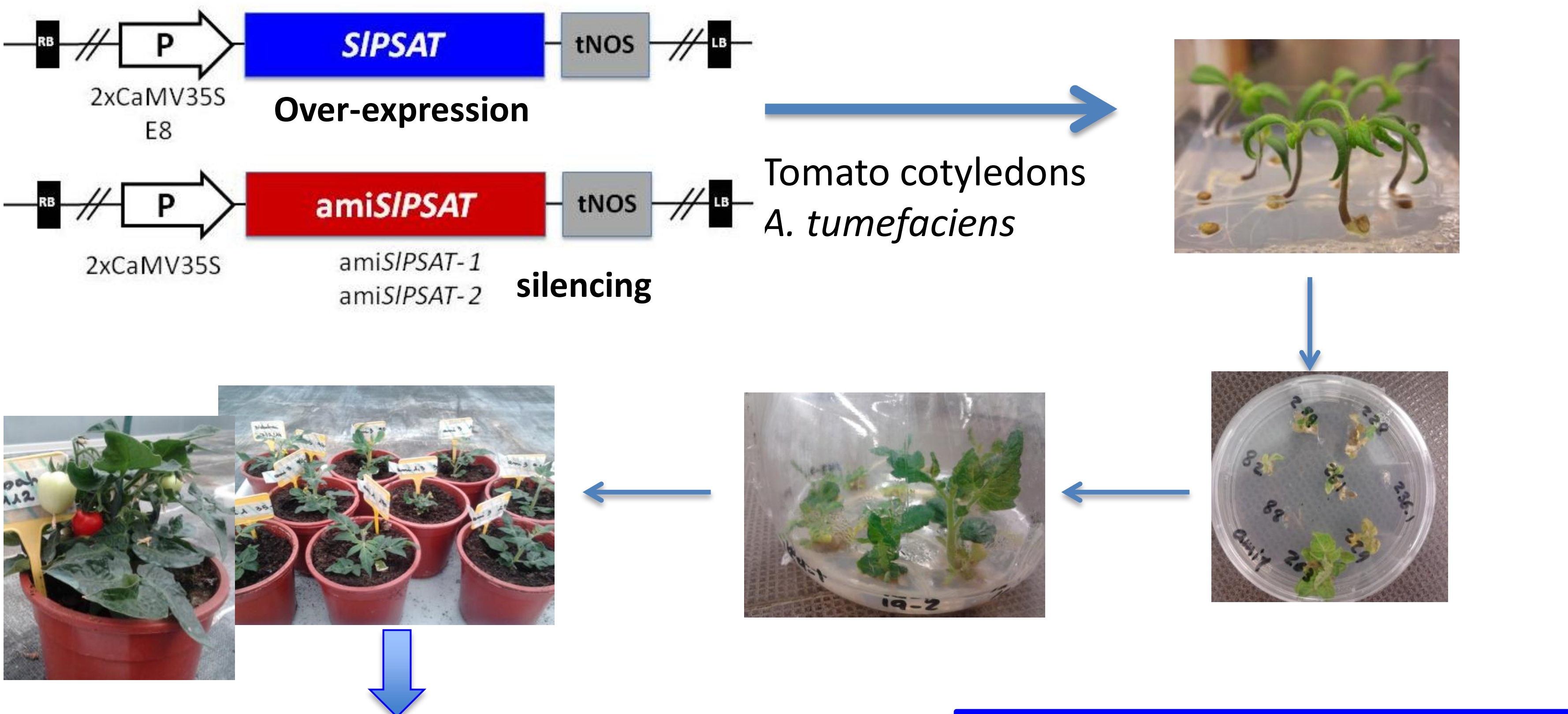
p35S::*SlPSAT*



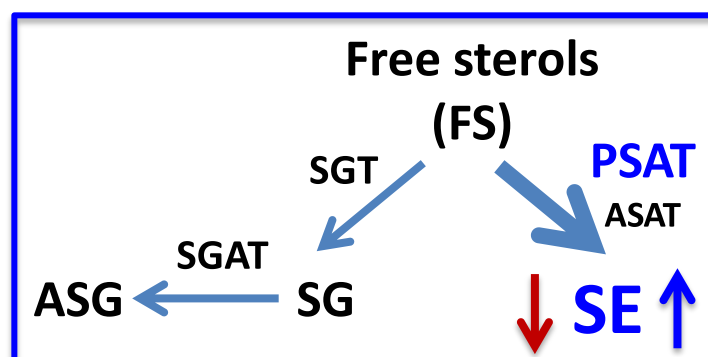
Basta selection



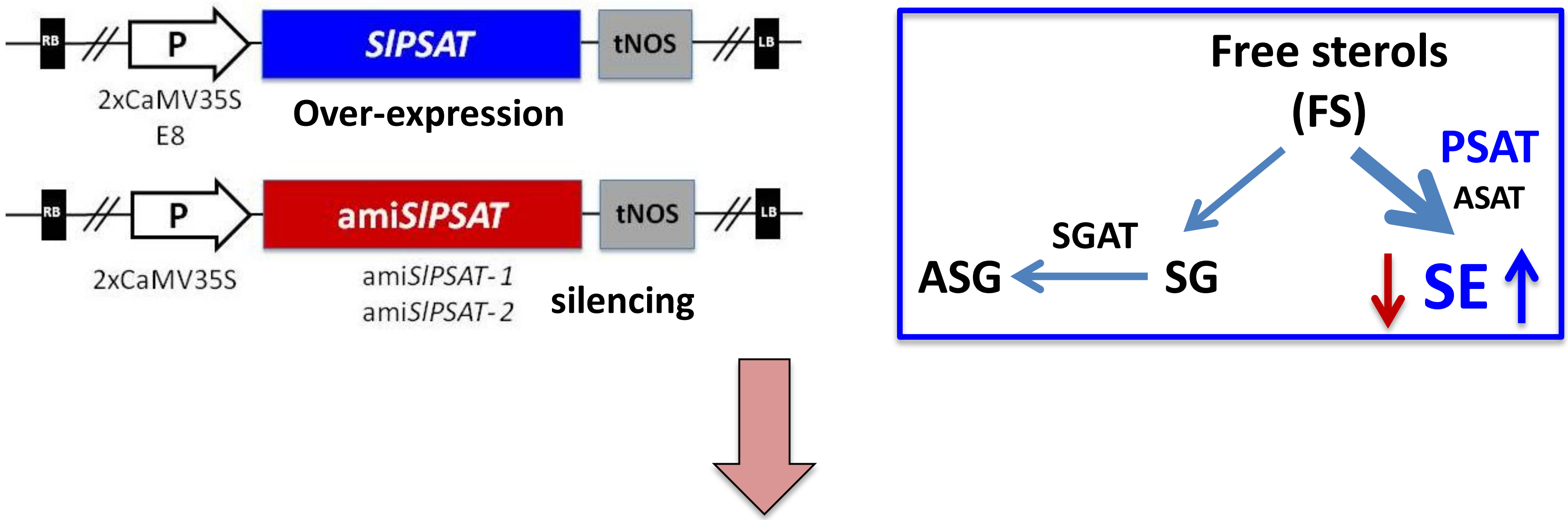
Generation of genetically modified tomato lines with altered profiles of free and conjugated sterols (plant and/or fruit)



- Presence of the transgen
- Copy number (1 copy plants)
- *SlPSAT* expression level
- Homozygous selection
- Sterol levels



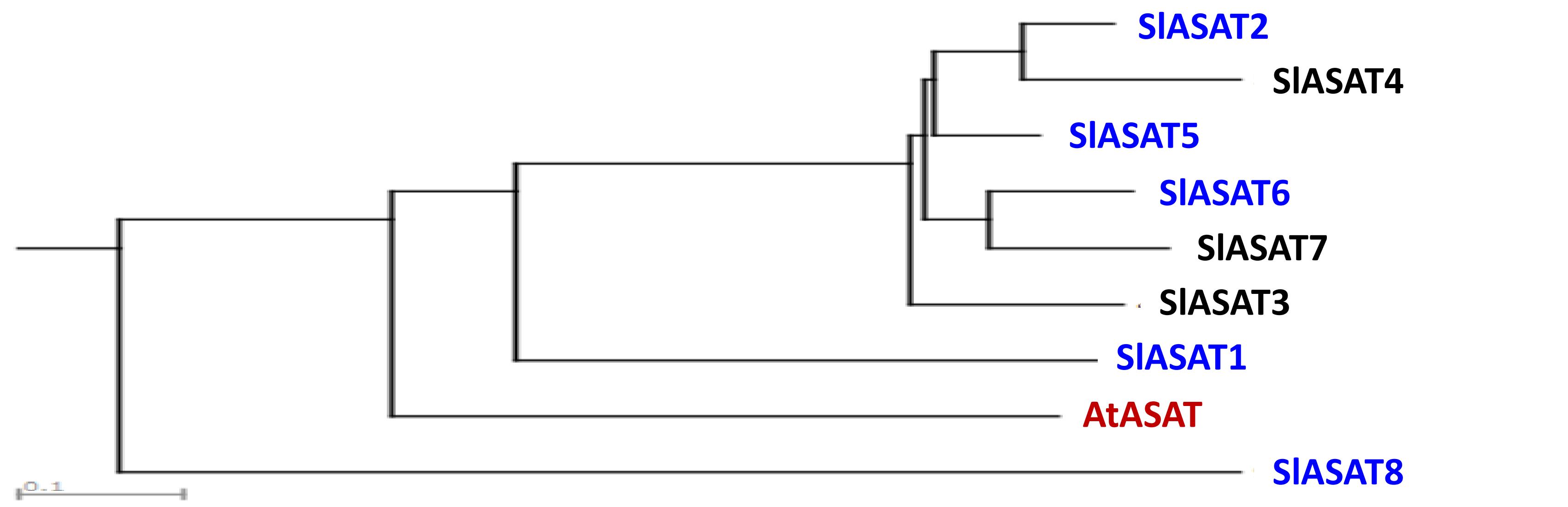
Evaluation of the effects of the perturbations in sterols metabolism on plant development, fruit development and ripening, and resistance to biotic and abiotic stresses.



- ✓ **Tolerance to pathogens**: *Botritis cinerea*, *Pseudomonas syringe*, *Phytophtora infestans*
- ✓ **Abiotic stress tolerance** : cold, drought
- ✓ **Plant productivity and fruit quality:** number and size of fruits, softening, sugars, acidity

Cloning and characterization of *ASAT* from *S. lycopersicum* (cv. Micro-Tom)

	AtASAT	SlASAT1	SlASAT2	SlASAT3	SlASAT4	SlASAT5	SlASAT6	SlASAT7	SlASAT8
AtASAT		49	43	42	45	45	43	44	33
SlASAT1	67		47	43	49	49	47	47	33
SlASAT2	76	66		82	75	82	75	72	34
SlASAT3	73	66	84		73	78	71.	69	32
SlASAT4	74	77	88	85		80	72	72	32
SlASAT5	70	68	87	87	85		78	78	34
SlASAT6	68	84	82	81	80	83		79	31
SlASAT7	73	70	81	81	79	82	83		33
SlASAT8	69	75	91	92	91	85	75	86	



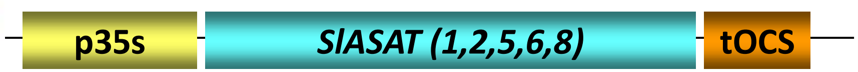
Functional validation of the candidate SlASAT enzymes

Complementation of the Arabidopsis *asat* knock-out mutant

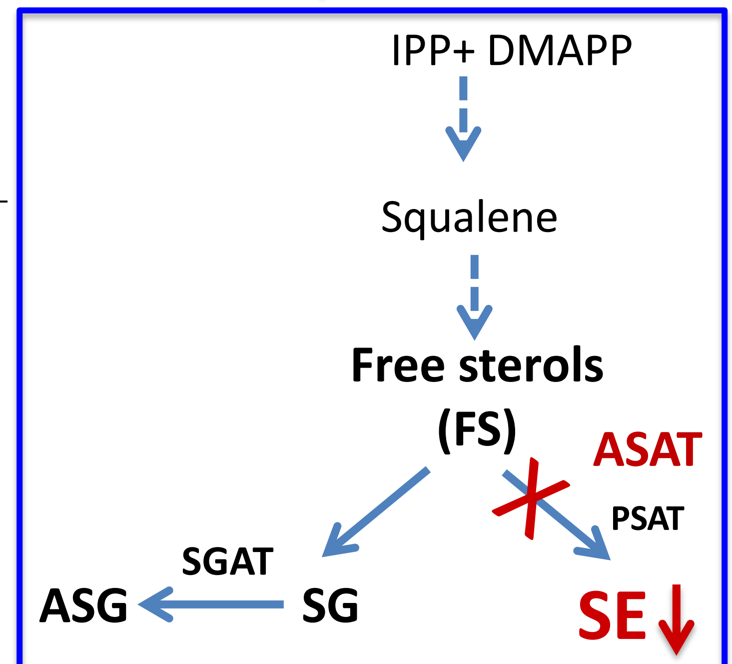
asat

Floral dipping
Agrobacterium tumefaciens

p35S::*SlASATs*



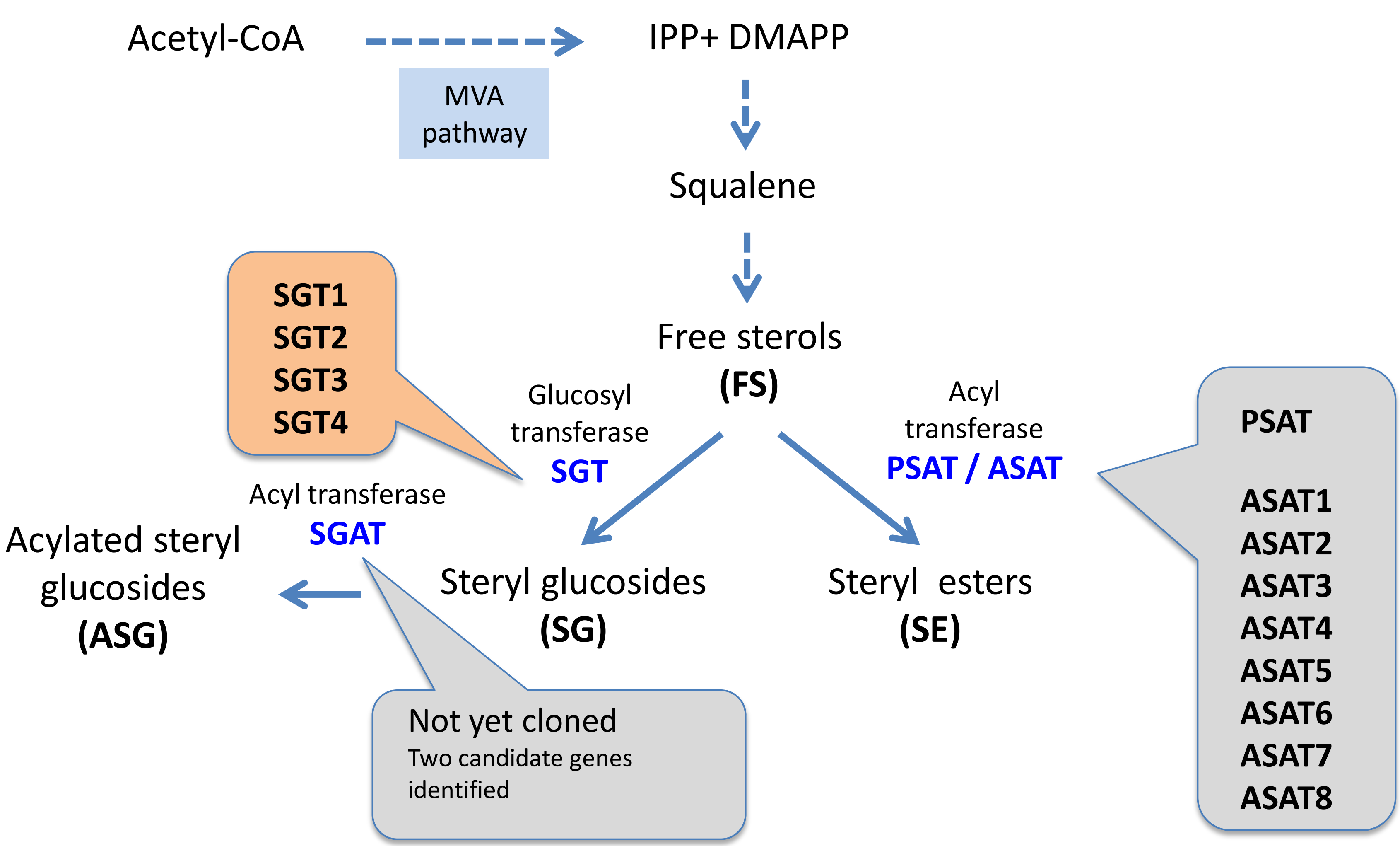
NO Visible phenotype alterations
Altered Sterol levels (Reduced SE)



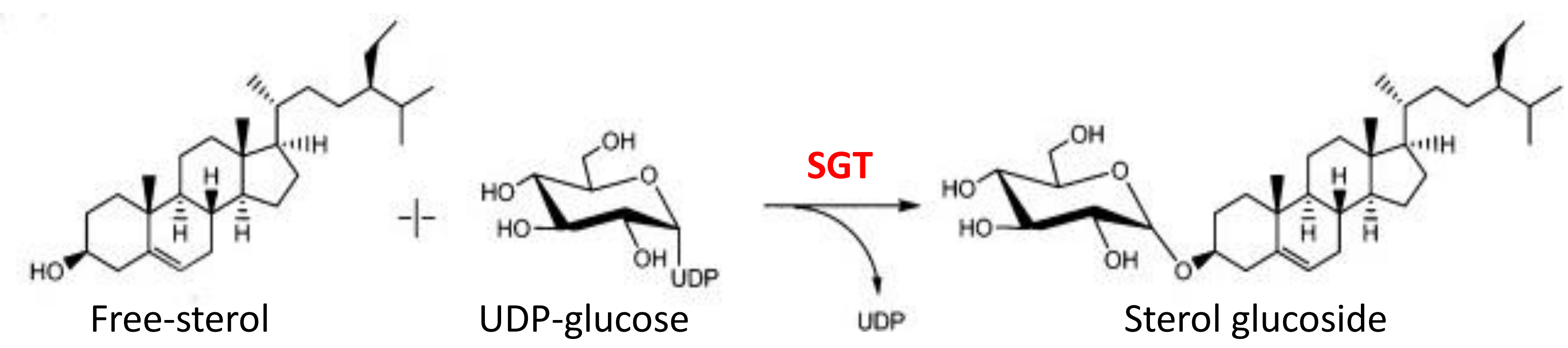
Basta selection

Lines with higher
SlASATs expression

Enzymes involved in sterol metabolism in tomato are encoded by gene families



Identification of 4 candidate genes encoding SGTs in tomato

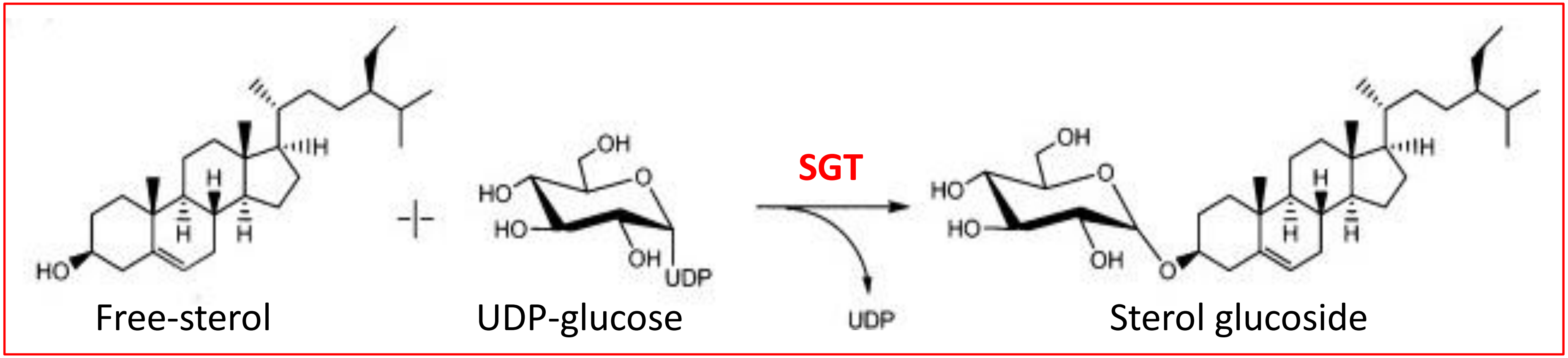


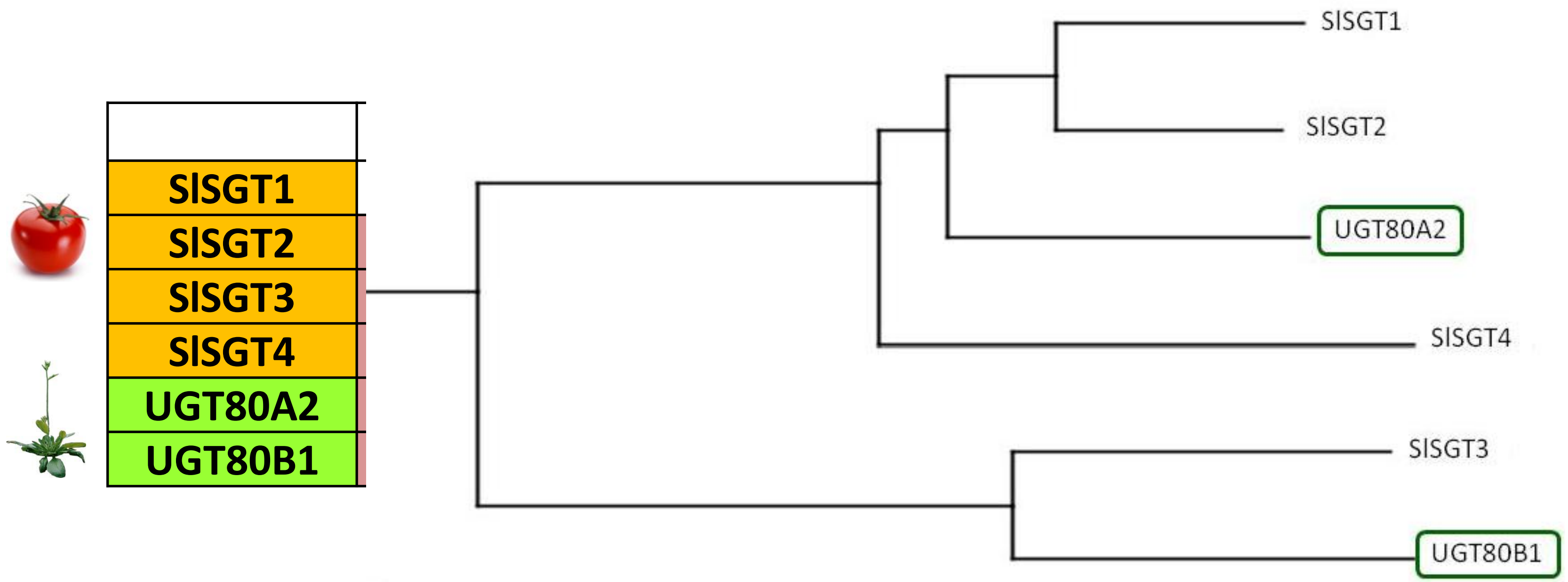
% identity

	SlSGT1	**SlSGT2**	**SlSGT3**	**SlSGT4**	**UGT80A2**	**UGT80B1**
SlSGT1		80	59	65	70	58
SlSGT2	85		59	74	74	59
SlSGT3	73	75		50	56	69
SlSGT4	77	85	64		67	51
UGT80A2	79	82	73	76		57
UGT80B1	73	74	79	68	74	

% similitude

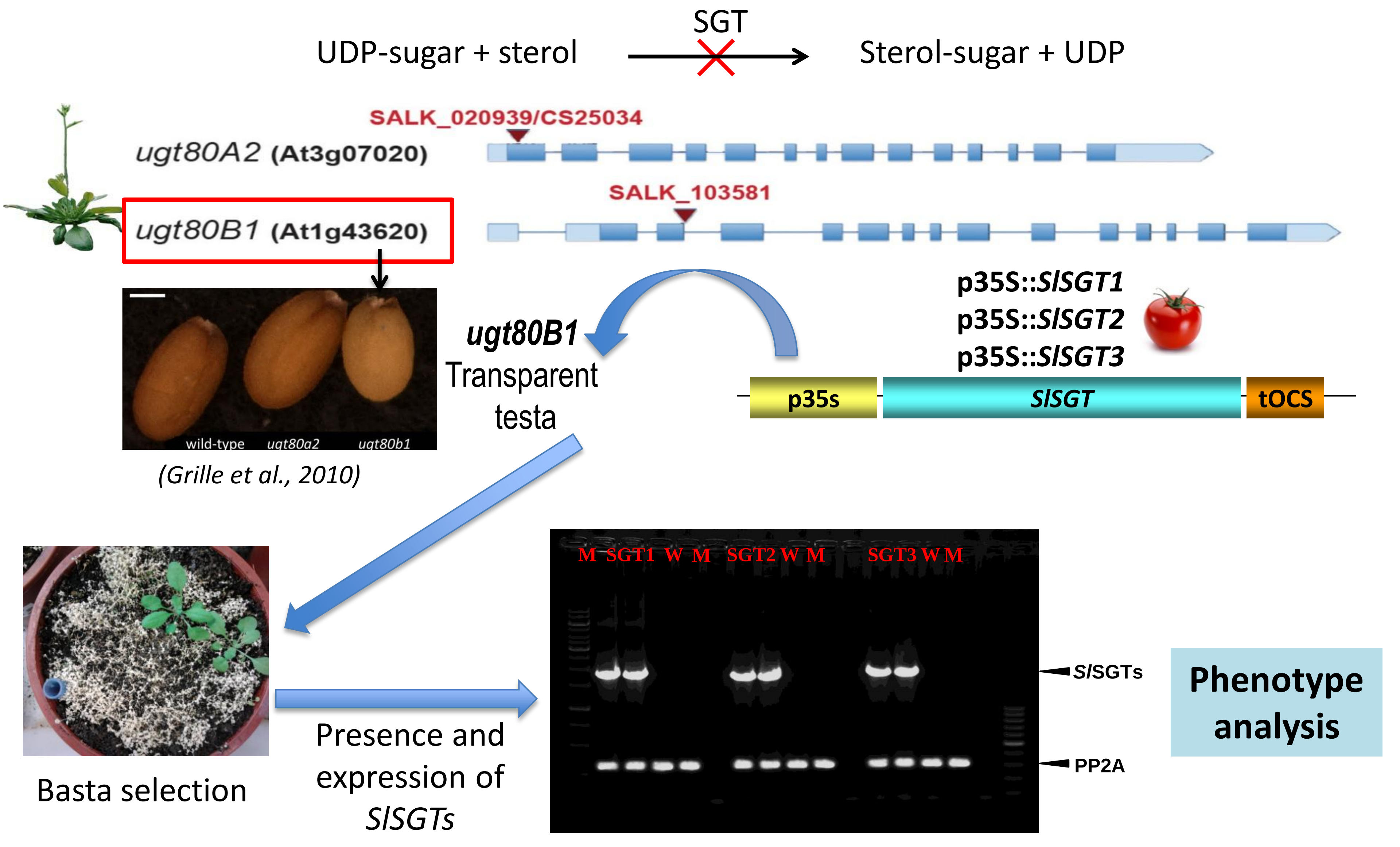
Identification of 4 candidate genes encoding SGTs in tomato





Functional validation of the candidate SlSGT enzymes

Complementation of the Arabidopsis *ugt80B1* knock-out mutant



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