Towards the identification of genes controlling major traits in tomato through sequencing of Micro-Tom EMS mutants

INRA
SCIENCE & IMPACT

université de BORDEAUX

L. Fernandez, EPGV 2014, Evry

EARLY FLESHY FRUIT DEVELOPMENT

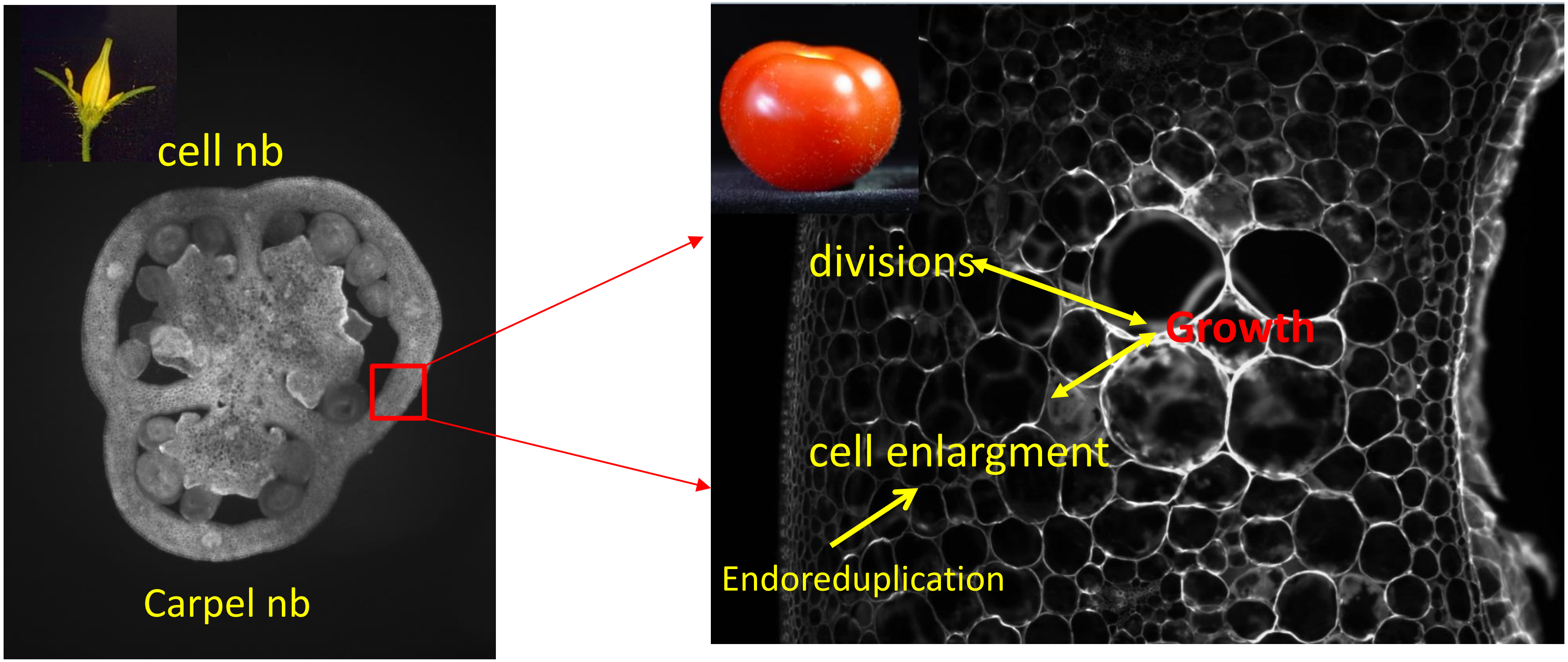
Water accumulation

Acid accumulation

Sugar accumulation

Fleshy trait acquisition

FRUIT SIZE A COMPLEX TRAIT

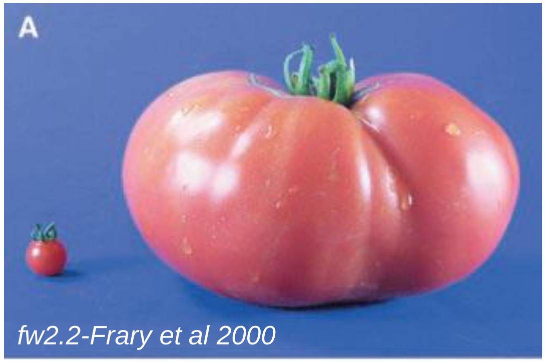


Asymetric divisions, cell growth, polarity, specification…

EXTREME PHENOTYPE ALLOWS THE IDENTIFICATION OF KEY GENES AND FUNCTIONS

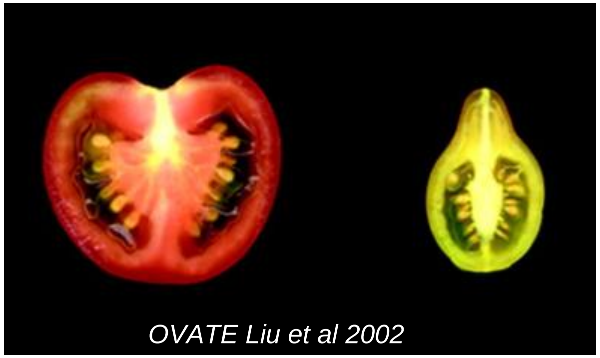
Wild species

Domesticated species



fw2.2-Frary et al 2000

Spontaneus mutants



OVATE Liu et al 2002

MicroTom

EMS mutant collection

Artificial mutants

8000 mutant families
3500 phenotyped families

Why MicroTom ?

- -recessive mutations (*dwarf,sp*)
- -385 plants = 4 m²
- - short life-cycle
- -genome sequenced

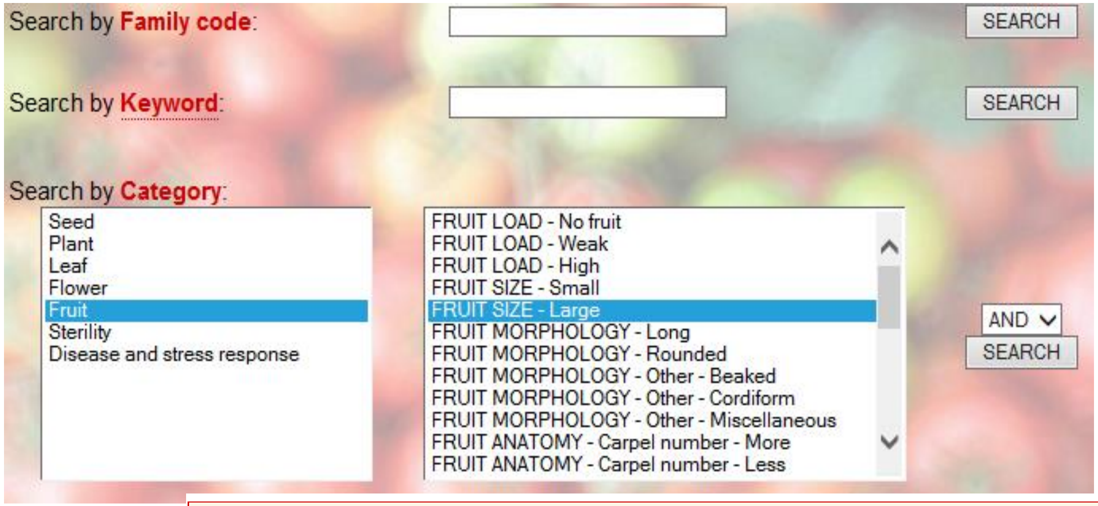
well-adapted as a model cultivar for functional genomics in tomato

EMS MUTANT COLLECTION AS A SOURCE OF PHENOTYPIC VARIATIONS

MicroTom Mutants DB

http://www.bordeaux.inra.fr/umr619/live/MMDB/

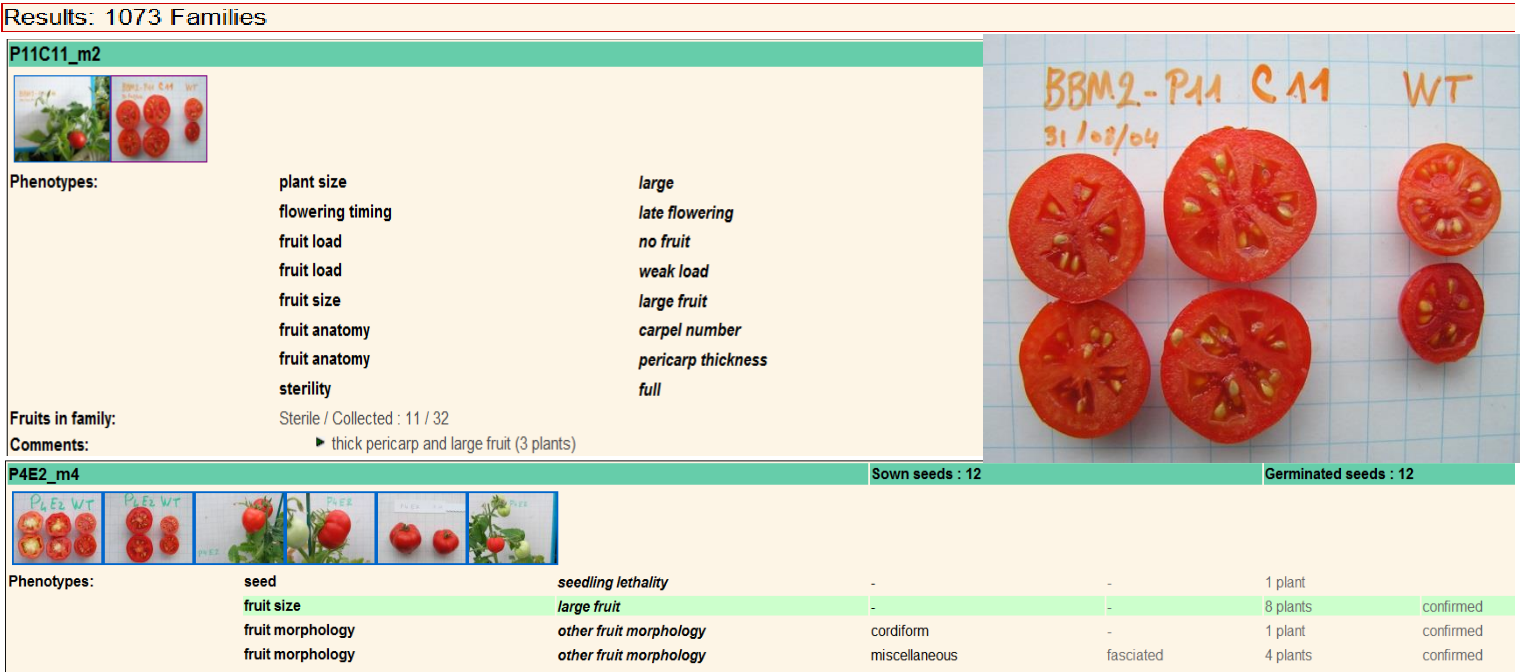
3500 phenotyped M2 families (> 30 000 annotations MMDB database) Just et al. 2013



Search by **Family code**:

Search by **Keyword**:

Search by **Category**: Fruit — FRUIT SIZE - Large



Results: 1073 Families

P11C11_m2

Phenotypes:	plant size	*large*
	flowering timing	*late flowering*
	fruit load	*no fruit*
	fruit load	*weak load*
	fruit size	*large fruit*
	fruit anatomy	*carpel number*
	fruit anatomy	*pericarp thickness*
	sterility	*full*

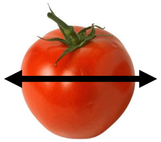
Fruits in family: Sterile / Collected : 11 / 32

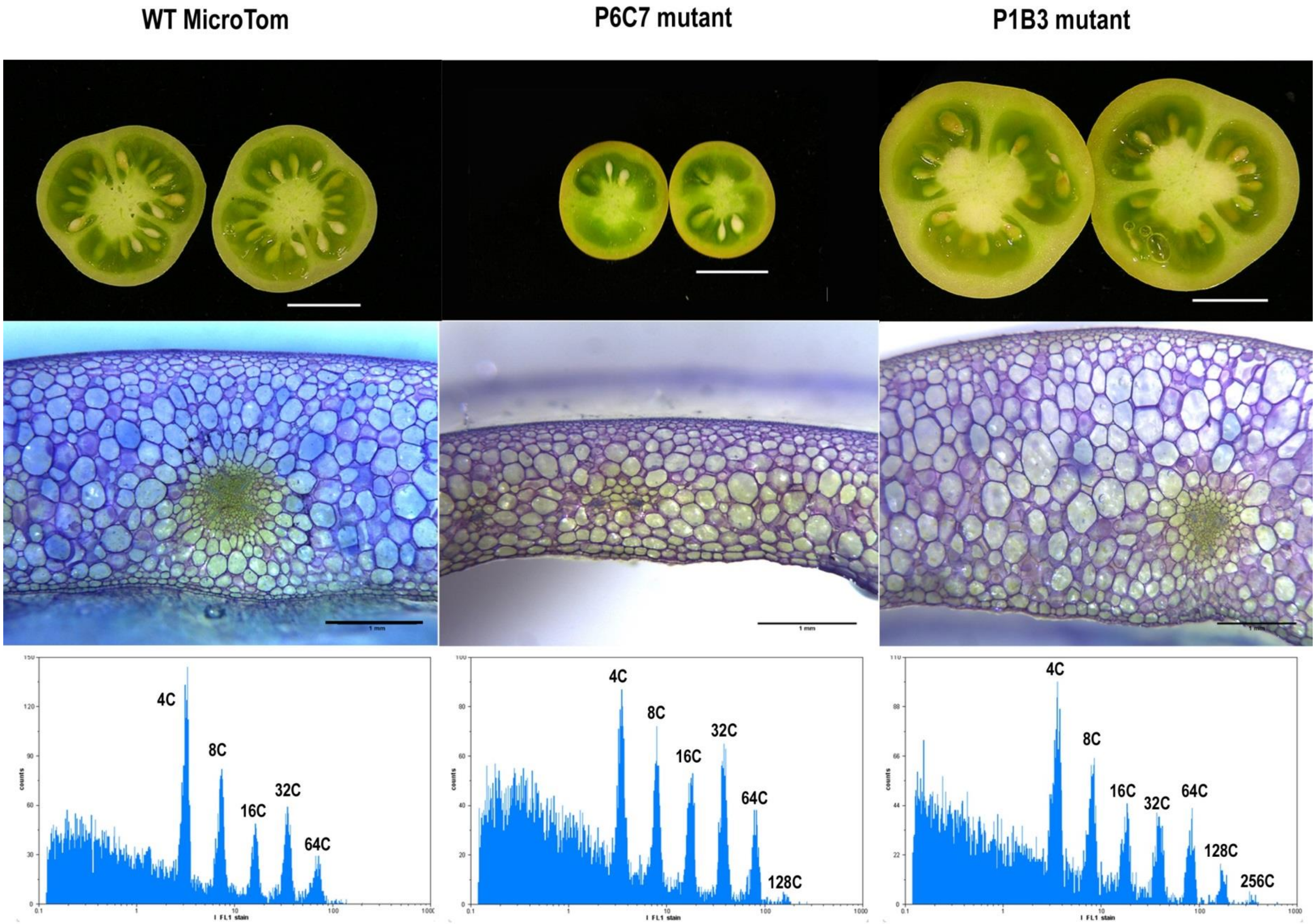
Comments:
- thick pericarp and large fruit (3 plants)

P4E2_m4 **Sown seeds : 12** **Germinated seeds : 12**

Phenotypes:	seed	*seedling lethality*	-	-	1 plant	
	fruit size	*large fruit*	-	-	8 plants	confirmed
	fruit morphology	*other fruit morphology*	cordiform	-	1 plant	confirmed
	fruit morphology	*other fruit morphology*	miscellaneous	fasciated	4 plants	confirmed

FRUIT GROWTH MUTANT PHENOTYPING





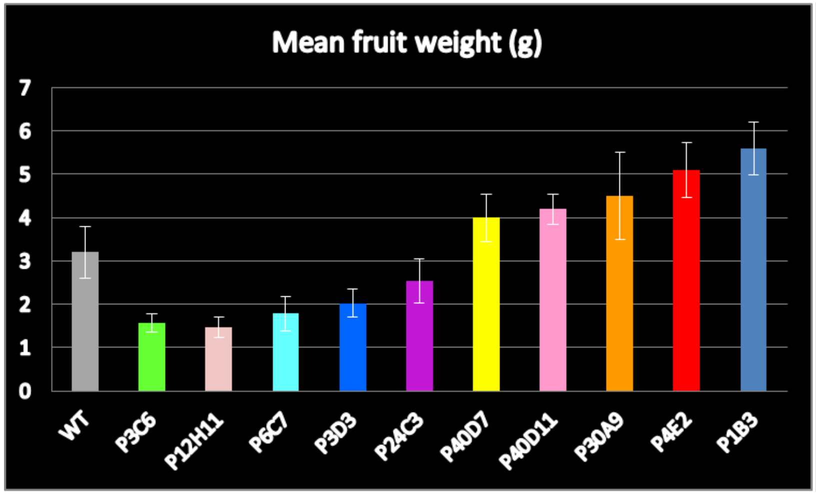
WT MicroTom

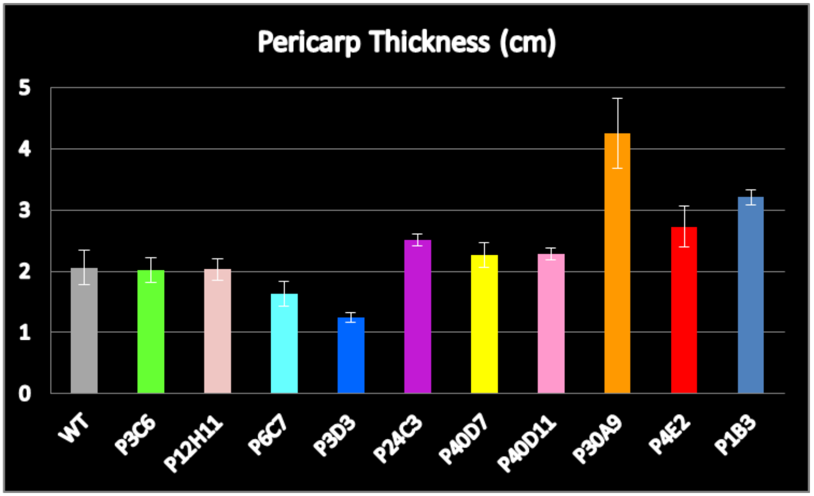
P6C7 mutant

P1B3 mutant

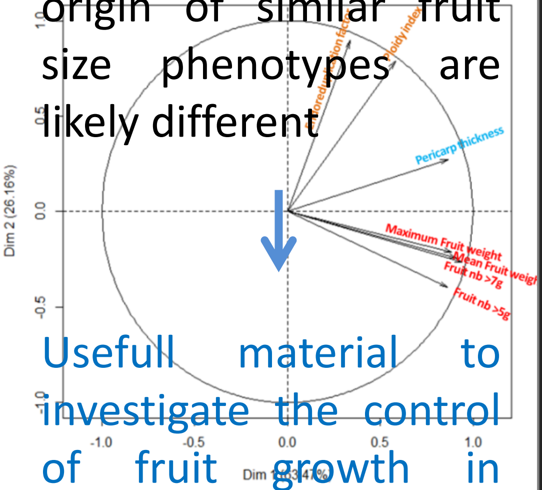
40% of confirmed phenotypes

CONTRASTED PATTERNS OF MUTANT FRUIT GROWTH ALTERATIONS

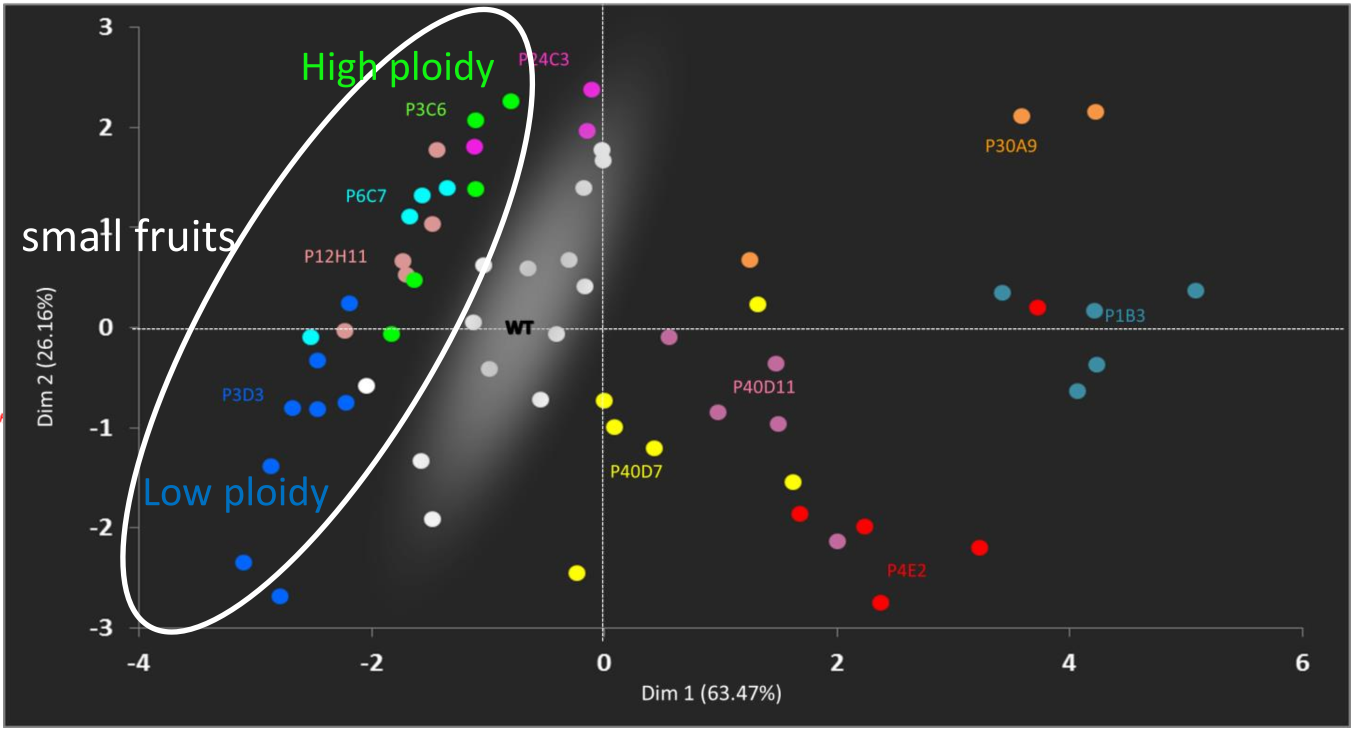




Mecanisms at the origin of similar fruit size phenotypes are likely different



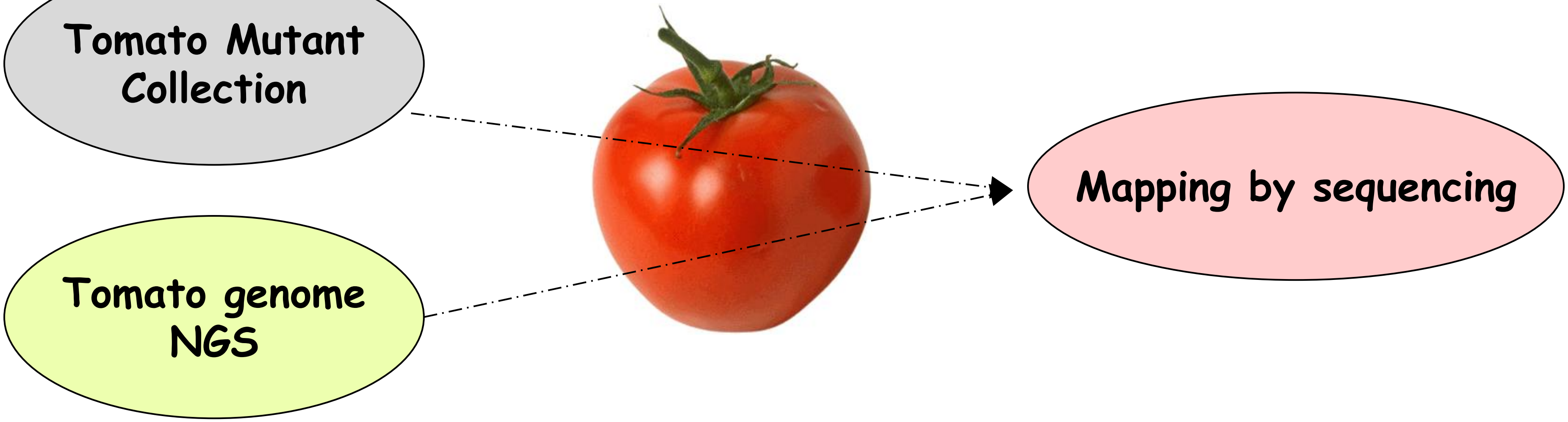
Usefull material to investigate the control of fruit growth in tomato



TOWARDS THE IDENTIFICATION OF UNDERLYING CAUSAL LOCUS

	P3C6	P12H11	P6C7	P3D3	P24C3	P40D7	P40D11	P30A9	P4E2	P1B3
Fruit size	small	small	small	small	small	large	large	large	large	large
Pericarp thickness	WT	WT	thin	thin	thick	WT	WT	thick	thick	thick
Pericarp ploidy	WT	WT	WT	low	high	WT	WT	high	low	high

Forward: from Phenotype to Gene



Tomato Mutant Collection

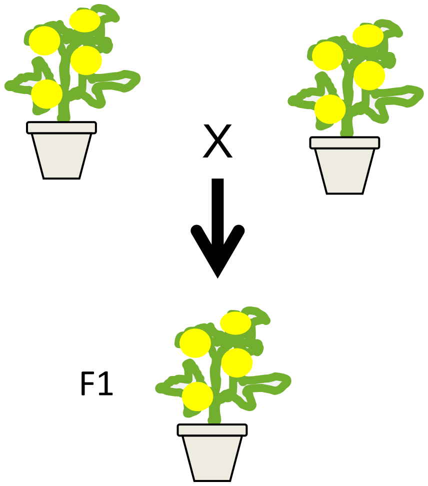
Tomato genome NGS

Mapping by sequencing

Classical mapping + NGS sequencing

Ariizumi et al 2014

1. allelism test → *Same causative gene*



pale yellow petal
EMS mutants

Mutant a

Mutant b

Classical mapping + NGS sequencing

Ariizumi et al 2014

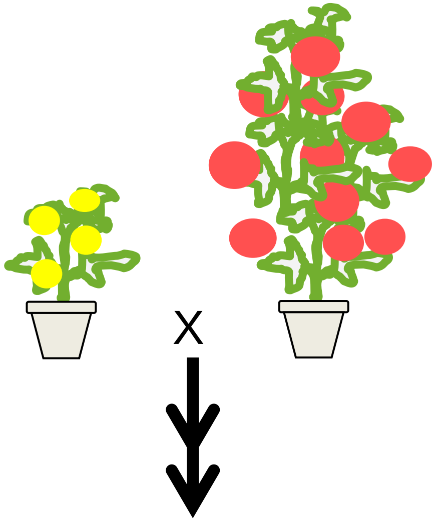
pale yellow petal
EMS mutants

1. allelism test → *Same causative gene*

2. Intraspecific F2 mapping population → *monogenic recessive mutation*
→ chr1 : ~3.4 cM interval - 222ORFs

Mutant a

Mutant b



F2

Classical mapping + NGS sequencing

Ariizumi et al 2014

pale yellow petal
EMS mutants

Mutant a

Mutant b

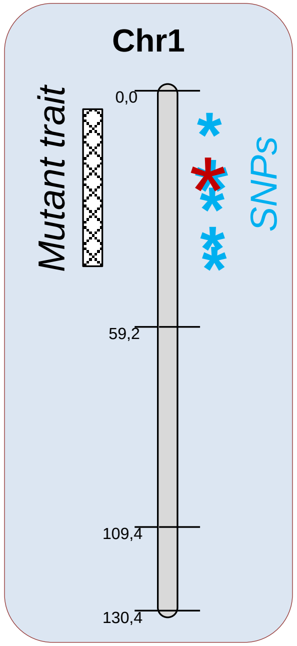
1. allelism test → *Same causative gene*

2. Intraspecific F2 mapping population → *monogenic recessive mutation*

→ chr1 : ~3.4 cM interval - 222ORFs

3. Direct sequencing of mutants (25X) → PYP1 (acyltransferase-related family)

mutant a

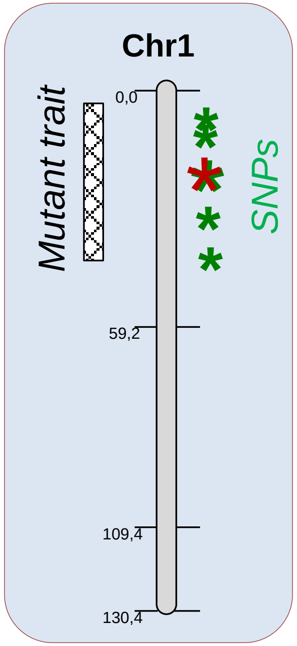


SNPs in common gene

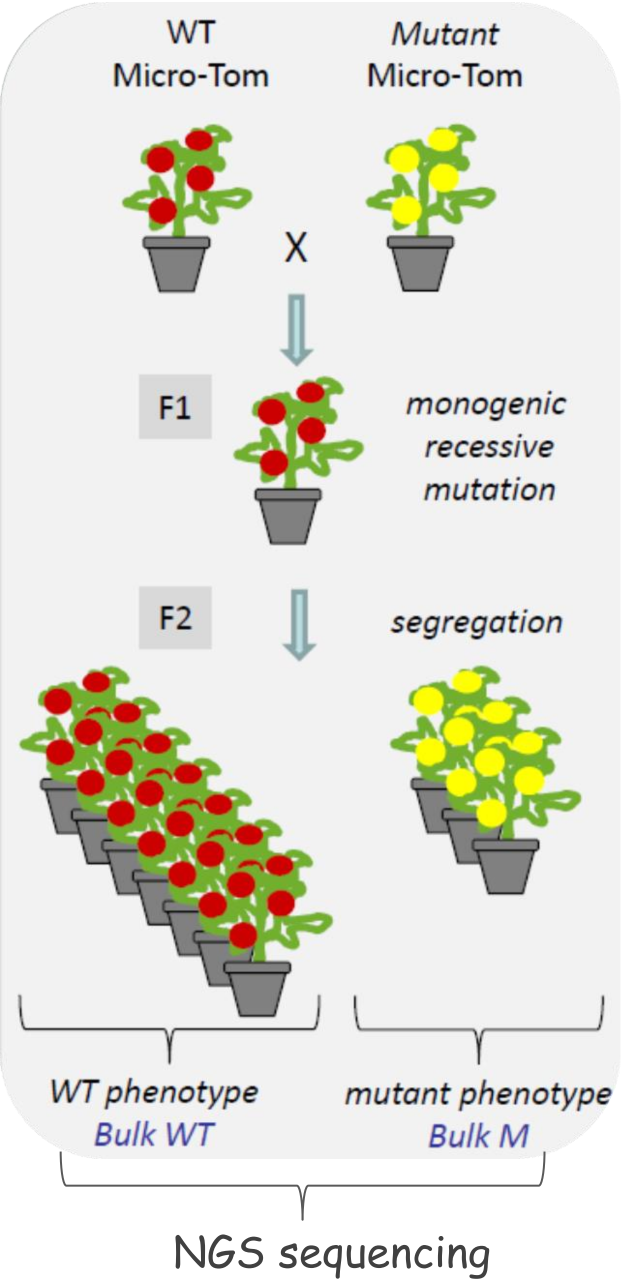
↓

causal mutation alleles a and b

mutant b



NGS MAPPING



WT
Micro-Tom

Mutant
Micro-Tom

X

F1

monogenic
recessive
mutation

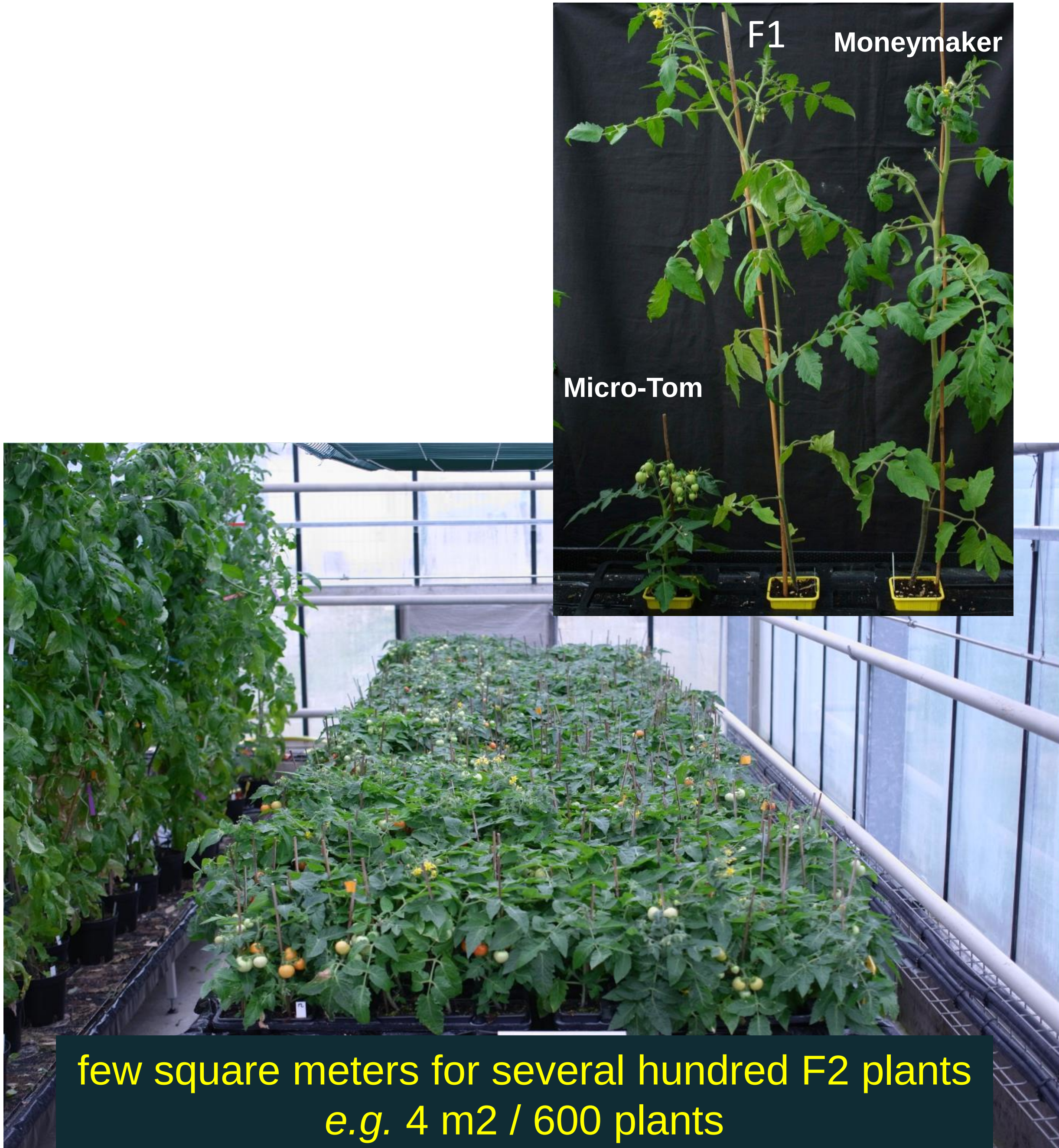
F2

segregation

WT phenotype
Bulk WT

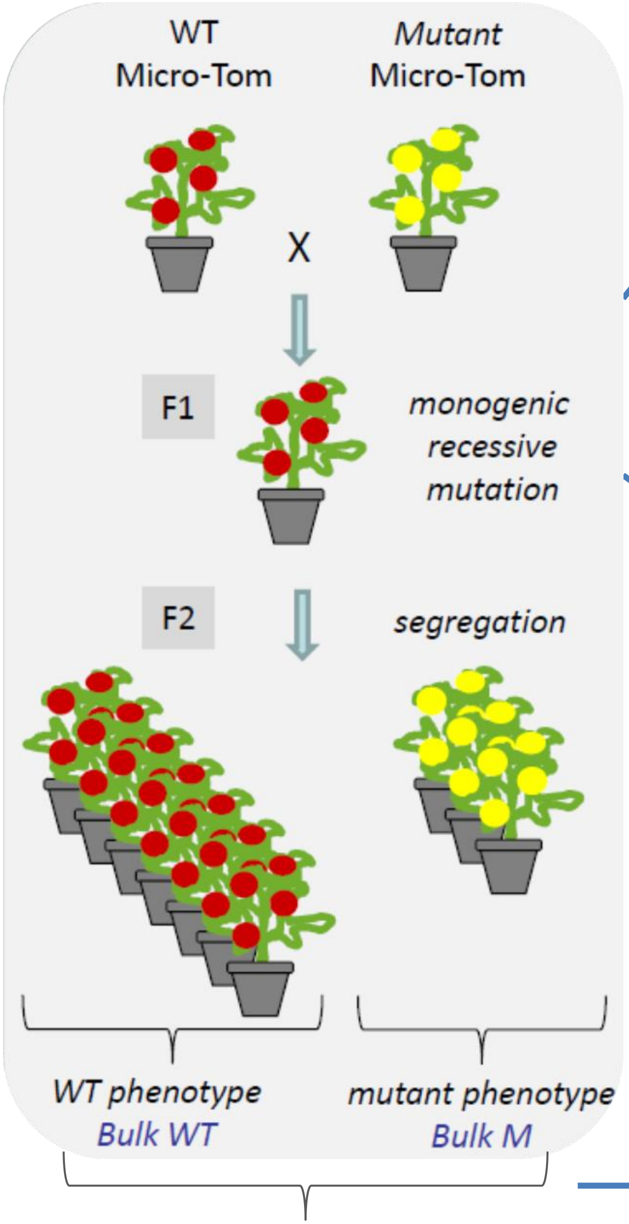
mutant phenotype
Bulk M

NGS sequencing



few square meters for several hundred F2 plants
e.g. 4 m2 / 600 plants

NGS MAPPING



WT
Micro-Tom

Mutant
Micro-Tom

X

F1

monogenic
recessive
mutation

F2

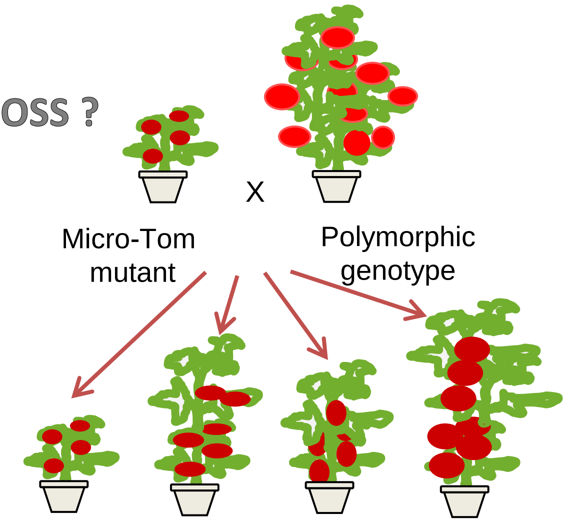
segregation

WT phenotype
Bulk WT

mutant phenotype
Bulk M

NGS sequencing

BACKCROSS OR OUTCROSS ?



Micro-Tom
mutant

X

Polymorphic
genotype

F2 segregating population

Crossing mutant with polymorphic plant material
⇒ *large phenotypic diversity in F2 population*

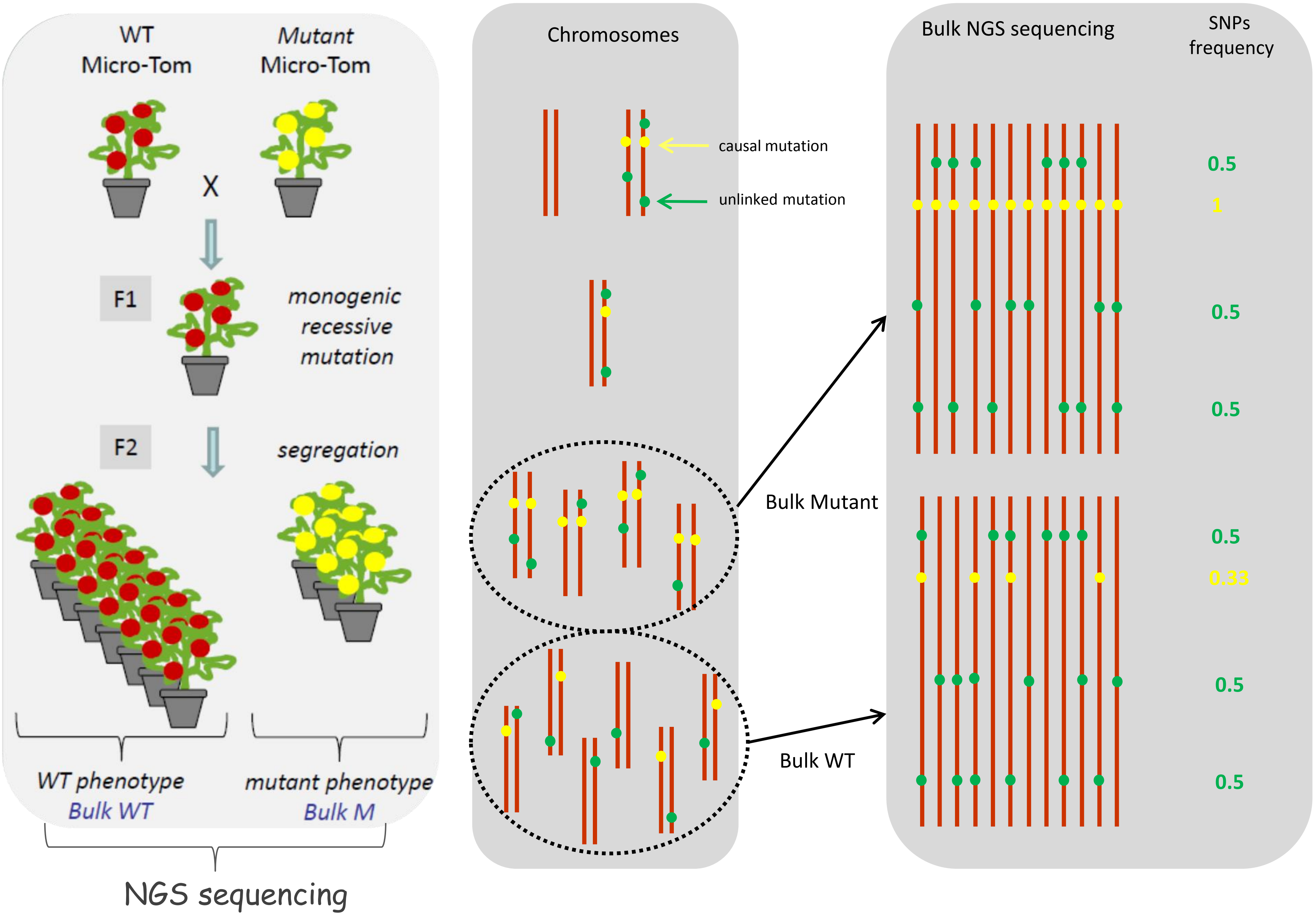
HOW MANY BACKCROSSES?

EMS mutations = source of genetic variability

used as marker

HiSeq 2000 (Illumina)
Paired-end reads -2*100bp
1 Bulk /lane
30-40Gb (~30-40X)

NGS MAPPING



WT
Micro-Tom

Mutant
Micro-Tom

X

F1

monogenic recessive mutation

F2

segregation

WT phenotype
Bulk WT

mutant phenotype
Bulk M

NGS sequencing

Chromosomes

causal mutation

unlinked mutation

Bulk Mutant

Bulk WT

Bulk NGS sequencing

SNPs frequency

0.5
1
0.5
0.5

0.5
0.33
0.5
0.5

PROOF OF CONCEPT…. YELLOW MUTANT

Micro-Tom

***Yellow colour* mutant**

Alignement against Heinz genome	Bulk Yellow	Bulk Red
Nb of reads paired in sequencing	377 312 055	471 503 833
Nb reads properly paired	345 502 360	376 536 347
Nb of samtools variants (A)	1 738 770	1 738 770
A without INDELS (B)	1 545 418	1 538 200
B & DP > 5 (C)	1 502 011	1 495 883
C & DP < 250 (D)	1 495 322	1 488 836
D & DP4/DP > 0.9 (E)	1 070 666	1 318 587
E without « parental » variants	57 594	72 050

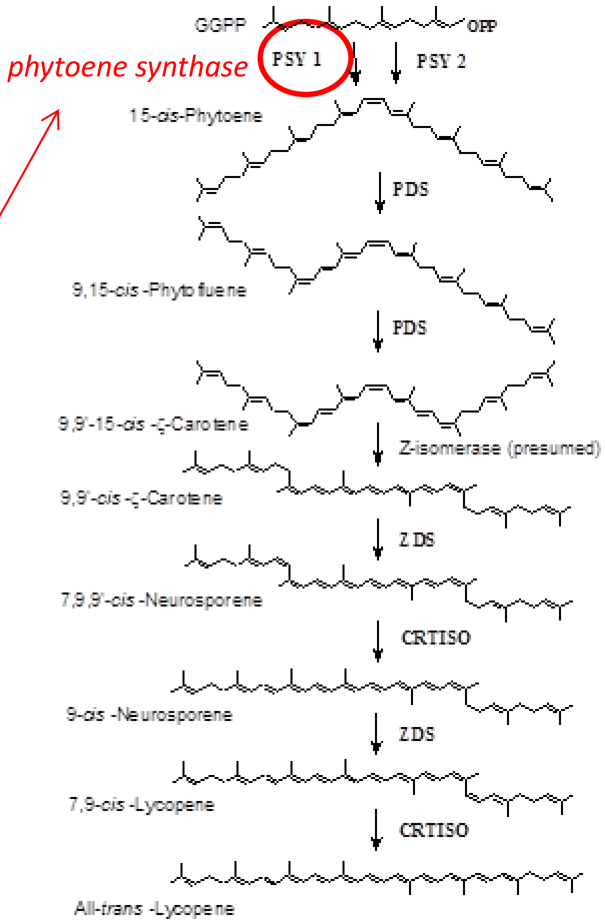
PROOF OF CONCEPT.... YELLOW MUTANT

Micro-Tom ***Yellow colour mutant***

Monogenic recessive mutation

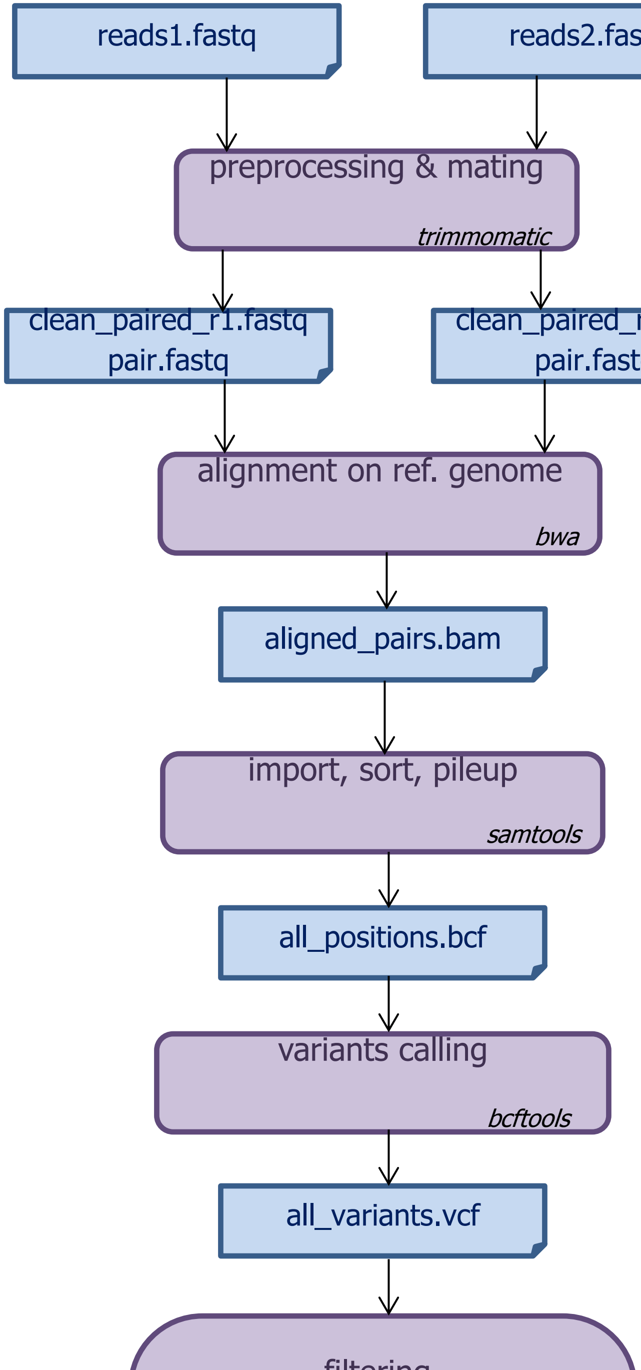
Bulk Yellow / Bulk Red	% of variant reads ≥ 97%
33% ± 1	No result
33% ± 3	 1 mutation (8.7 cM)
33% ± 5	 2 mutations (8.6 cM and 8.7cM)

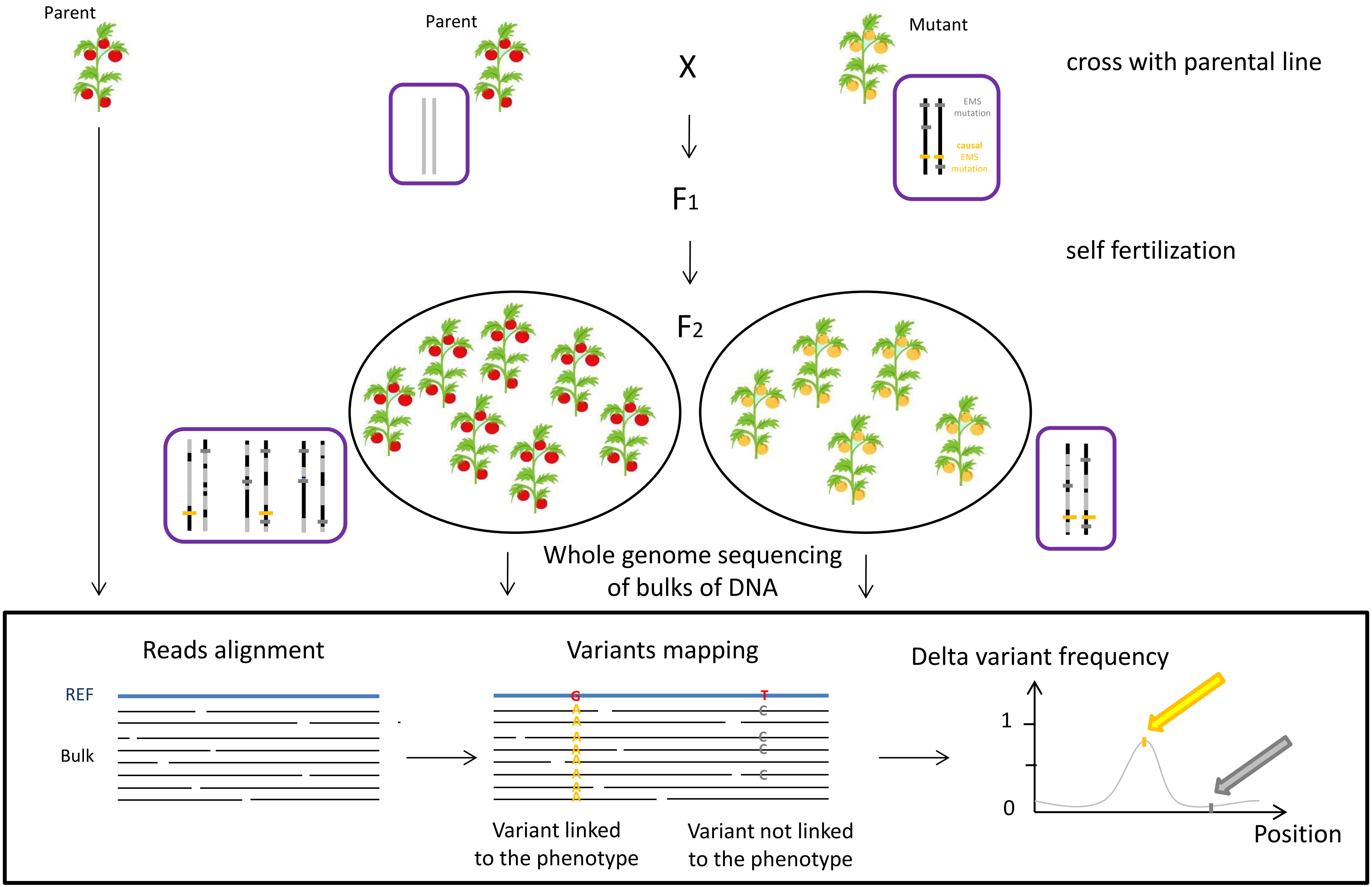
Carotenoid biosynthesis



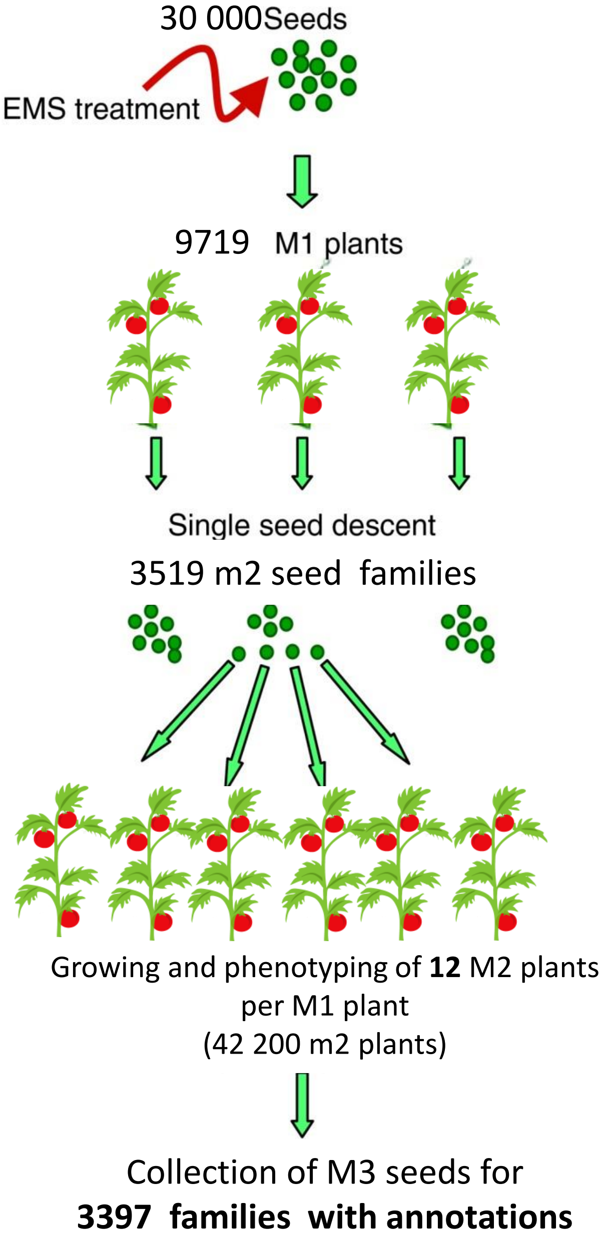
Biologie du Fruit

Christophe Rothan
Cécile Bres
Daniel Just
Virginie Garcia
Martine Lemaire-Chamley
Joana Jorly
Jean-Philippe Mauxion
Isabelle Atienza
Pierre Baldet
Céline Bournonville
Carine Ferrand
Kentaro Mori
Johann Petit


reads1.fastq
reads2.fastq
preprocessing & mating
trimmomatic
clean_paired_r1.fastq
pair.fastq
clean_paired_r2.fastq
pair.fastq
alignment on ref. genome
bwa
aligned_pairs.bam
import, sort, pileup
samtools
all_positions.bcf
variants calling
bcftools
all_variants.vcf
filtering


Parent
Parent
X
Mutant
EMS mutation
causal EMS mutation
cross with parental line
F1
self fertilization
F2
Whole genome sequencing of bulks of DNA
Reads alignment
REF
Bulk
Variants mapping
G
A
T
C
Variant linked to the phenotype
Variant not linked to the phenotype
Delta variant frequency
1
0
Position

EMS MicroTom mutant collection



30 000Seeds

EMS treatment

9719 M1 plants

Single seed descent

3519 m2 seed families

Growing and phenotyping of **12** M2 plants per M1 plant

(42 200 m2 plants)

Collection of M3 seeds for

3397 families with annotations

385 plants = 4 m²

Florida Basket *(dwarf, Fusarium resistance)*

MicroTom x

Ohio 4013 *(small leaf, small fruit, cold resistance)*

Moneymaker and L. minutum

from Scott & Harbaugh 1989