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GENOMICS LAB
fruitgenomicslab.com

Using genetics to mitigate berry cracking: What do we know so far?

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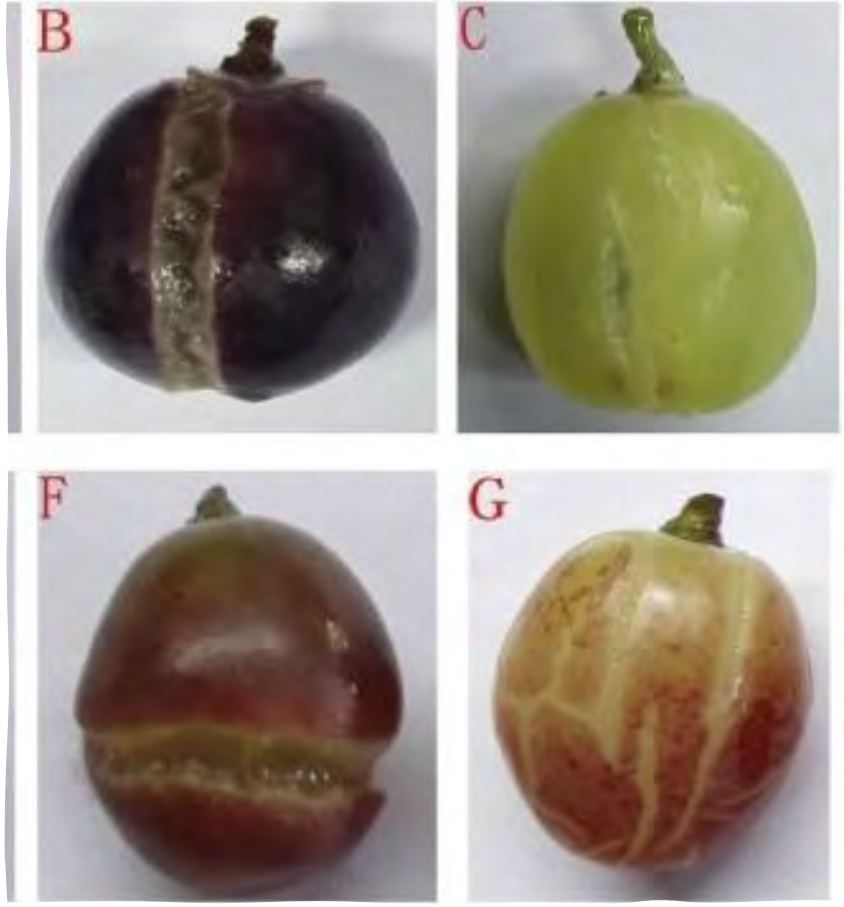
SATI Table Talk
Paarl 26/07/2024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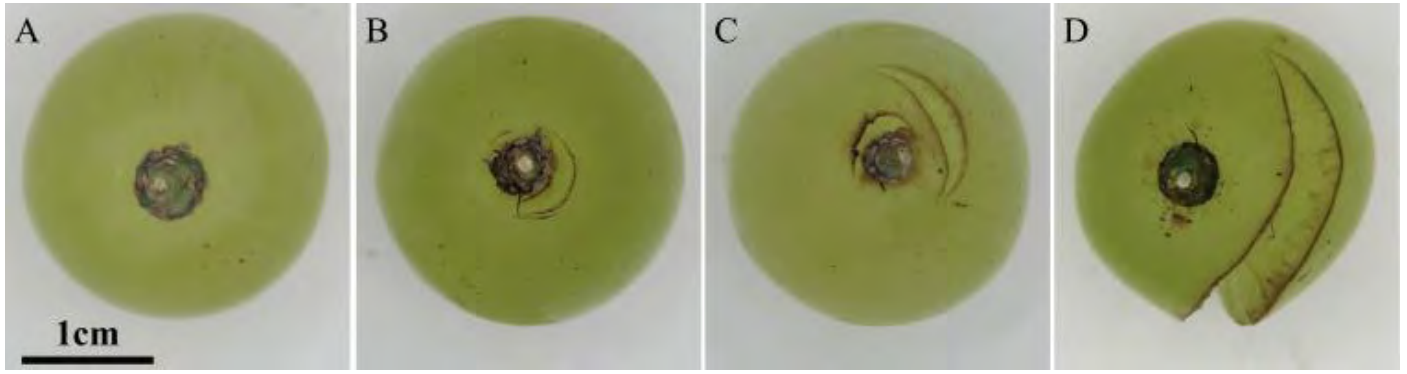
A
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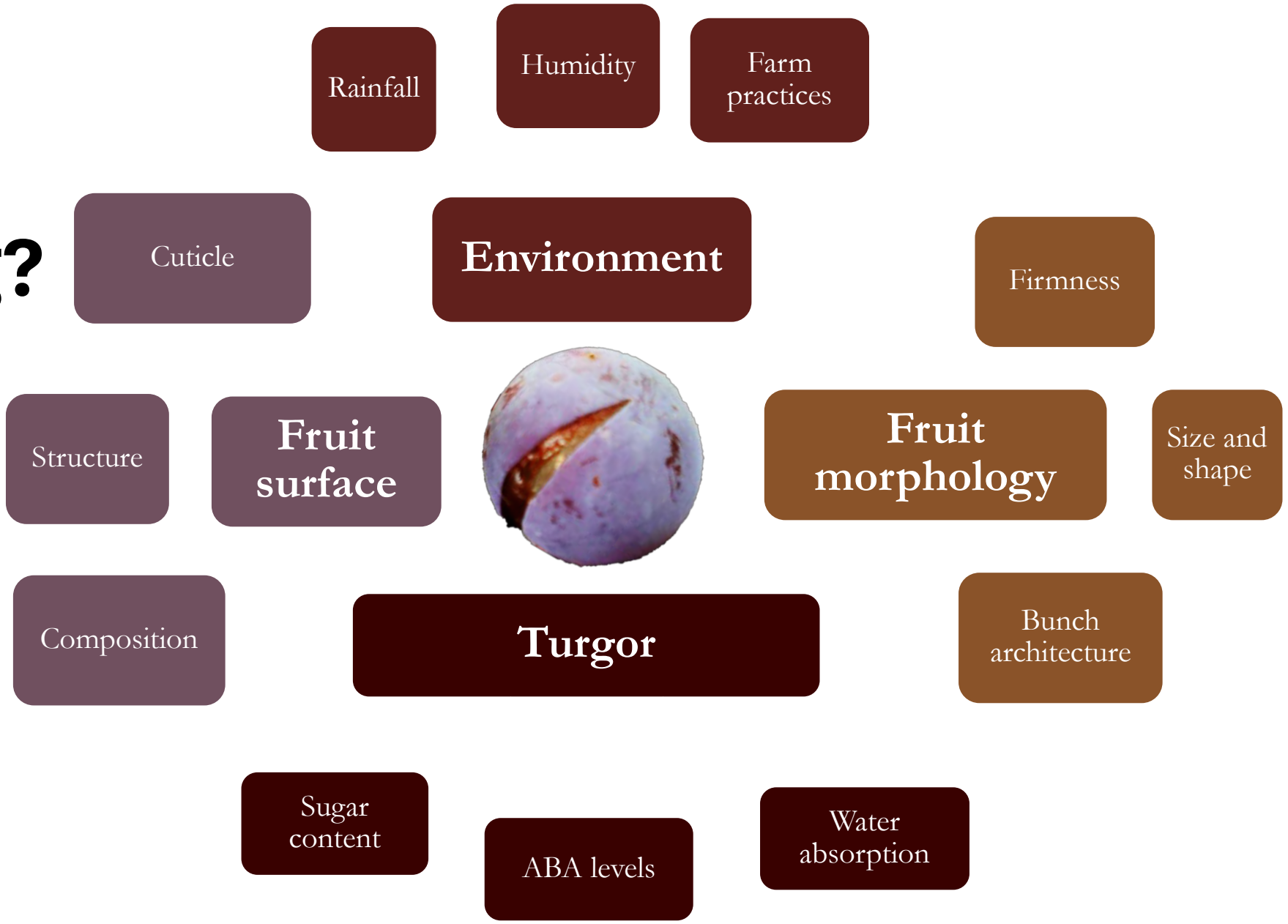
What causes fruit cracking?

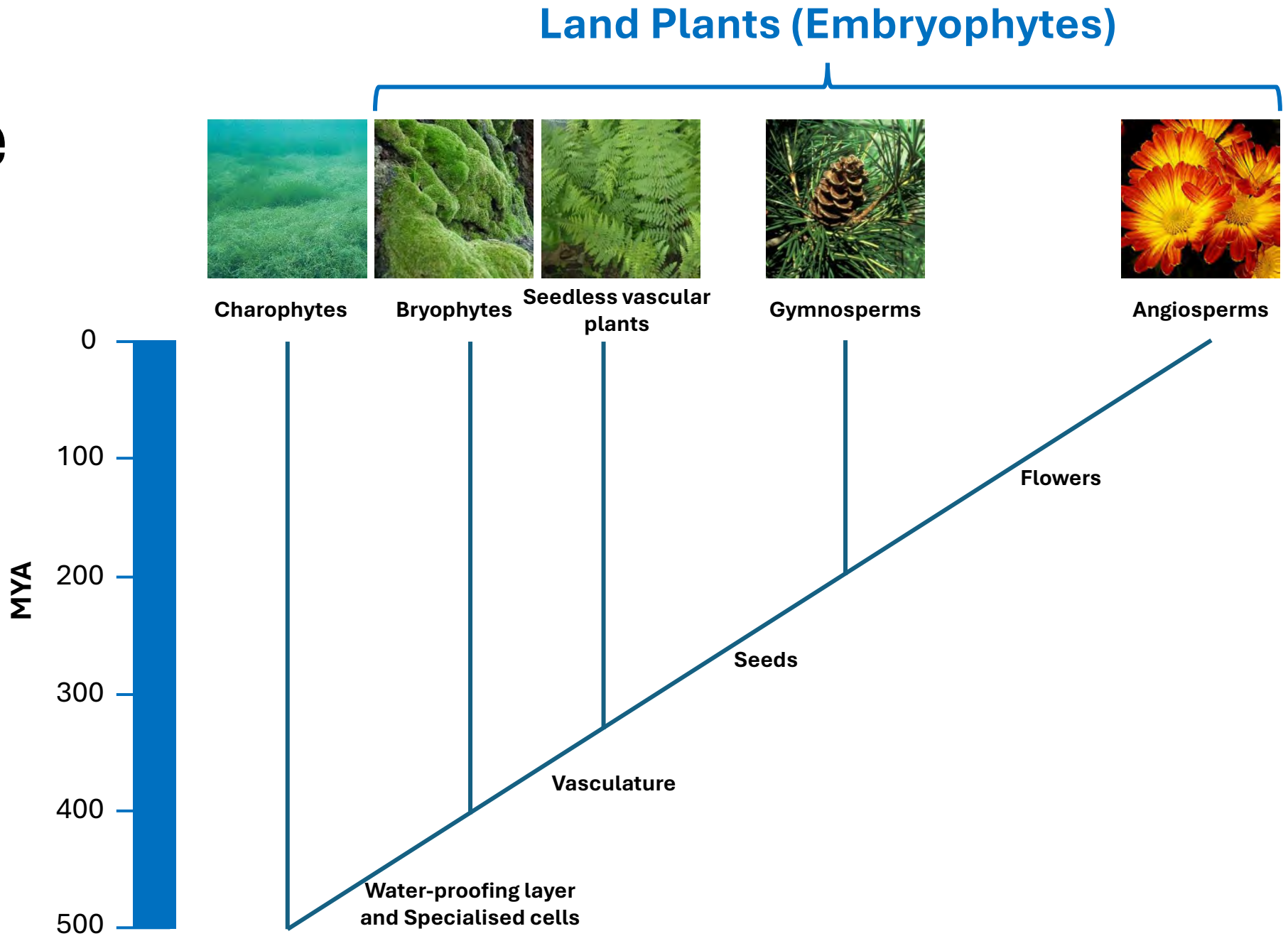


Giménez *et al*., 2015

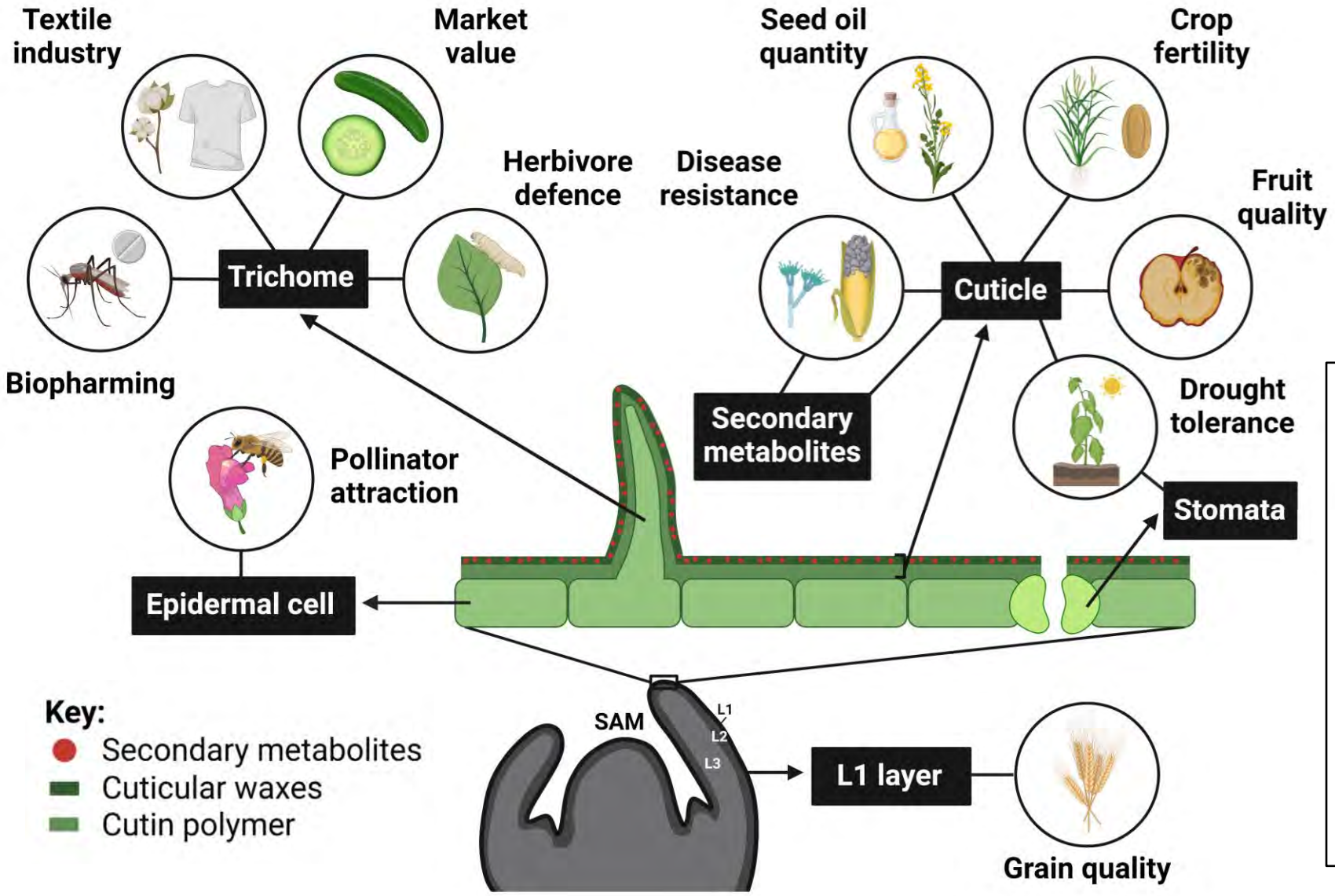
Plant cuticle and surface and the evolution of plants

Nothing in Biology Makes Sense except in the Light of Evolution

- Theodosius Dobzhansky (1973)



Plant Surface



Plant surface:

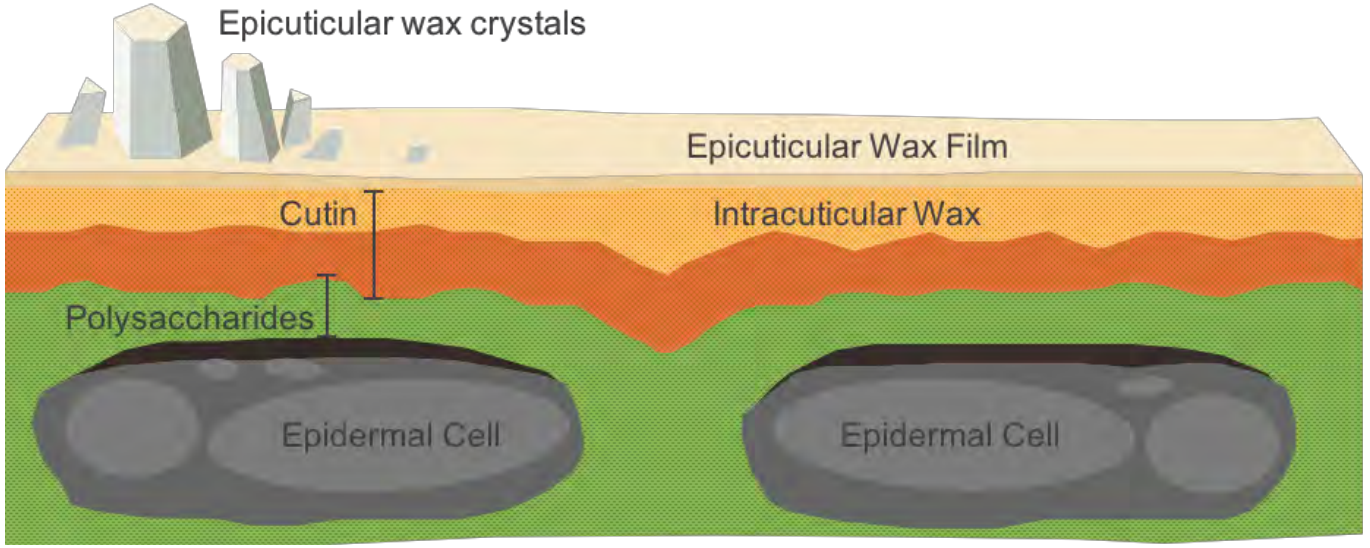
Different epidermal **cell types**: **stomata** + **trichomes**

Epidermal cells **synthesise** + **deposit** waxy layer: **cuticle**

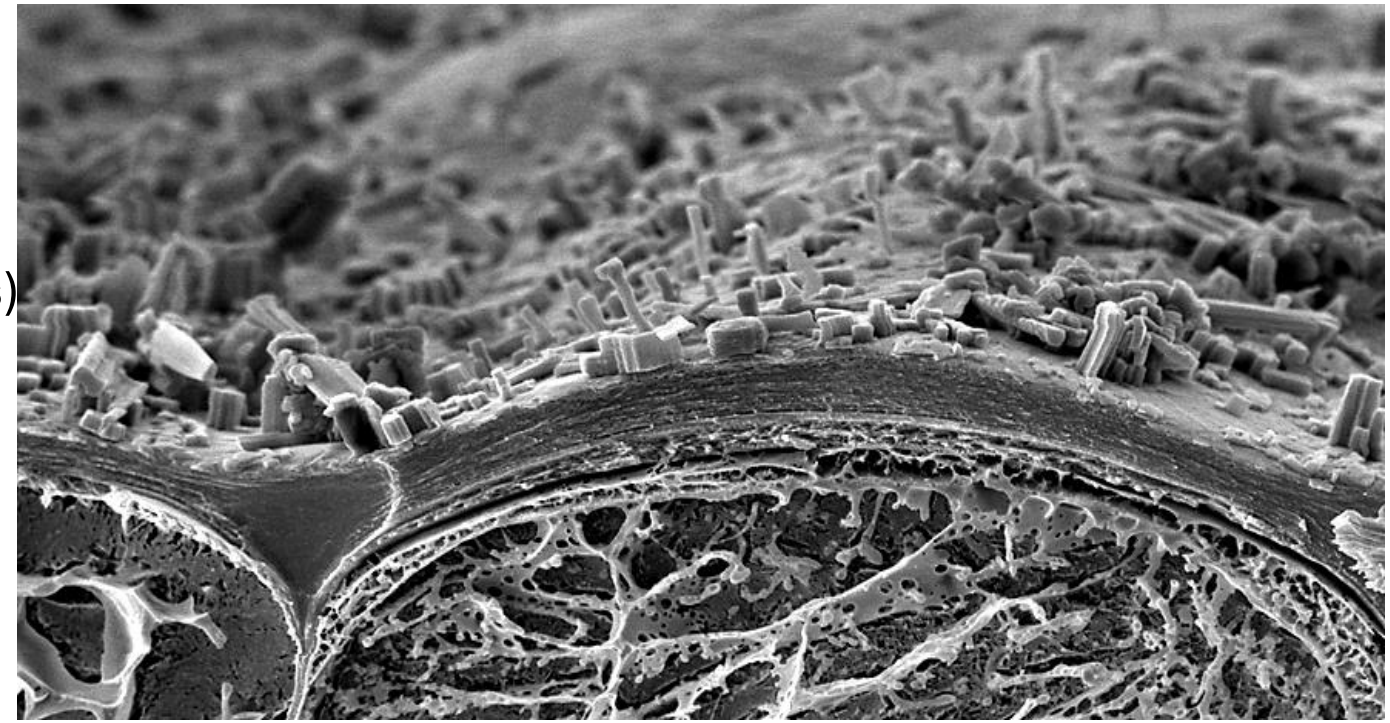
Together **contribute** important **crop traits**

Jolliffe *et al.*, 2023

Plant cuticle structure



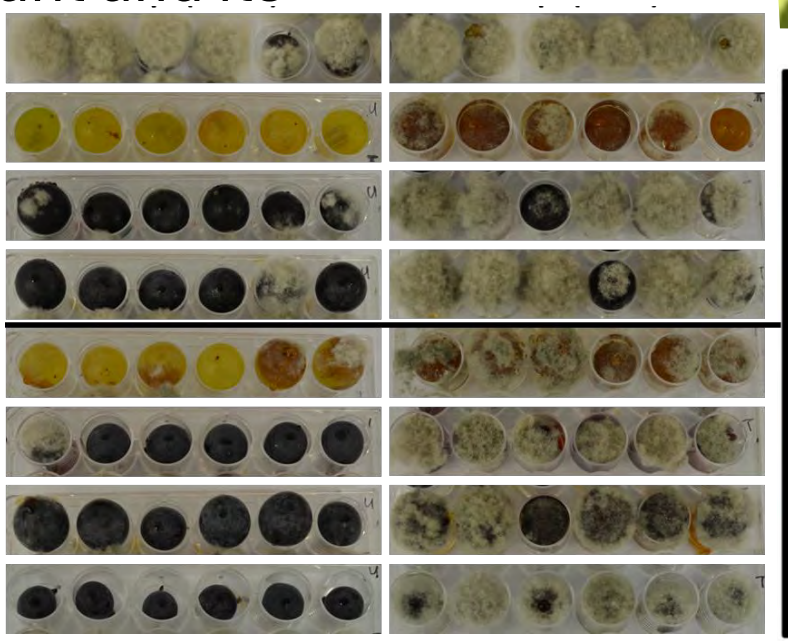
- **CUTIN polymer matrix**
  - C_{16} and C_{18} fatty acids
  - glycerol
- **WAXES**
  - Very long chain fatty acids (VLCFAs) up to C_{34}
  - Intra- and epicuticlur
- **TRITERPENOIDS**

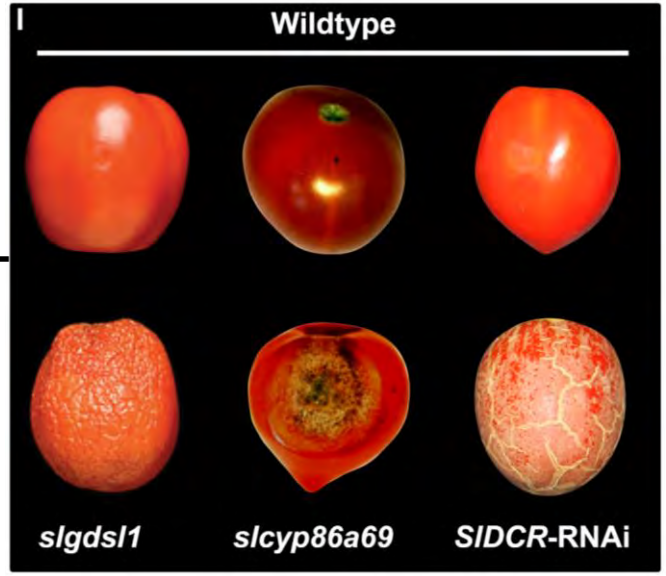


Arabidopsis Inflorescence Stem Surface (Freeze Fraction-SEM)

Grape berry cuticle

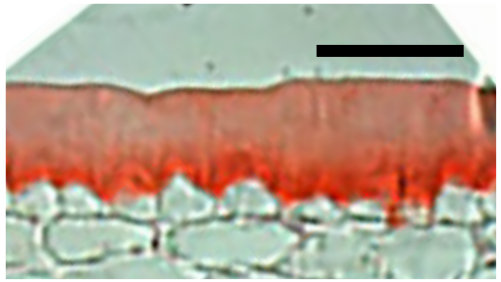
- Cuticle is specialised in organs and species
- Fruit cuticles must tolerate **rapid fruit expansion** during ripening **and high turgor pressure**
- Why study grape berry cuticular waxes?
  - Interaction point between the plant and its environment
  - Regulates non-stomatal water loss
  - Defence against fungal attack
  - Influences fruit cracking



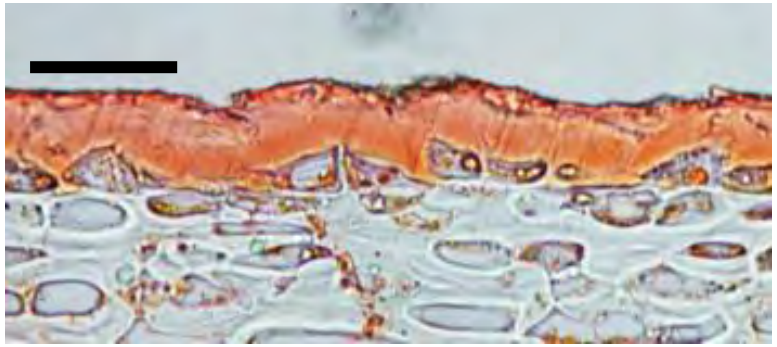


Grape berry cuticle vs other fruit

Tomato



Olive, Diarte *et al* 2019

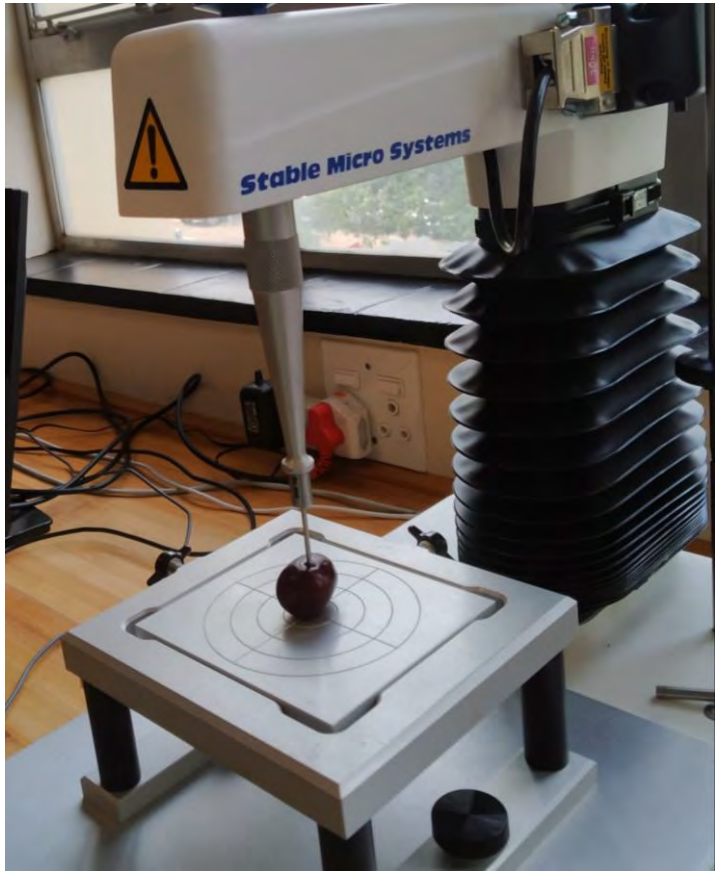


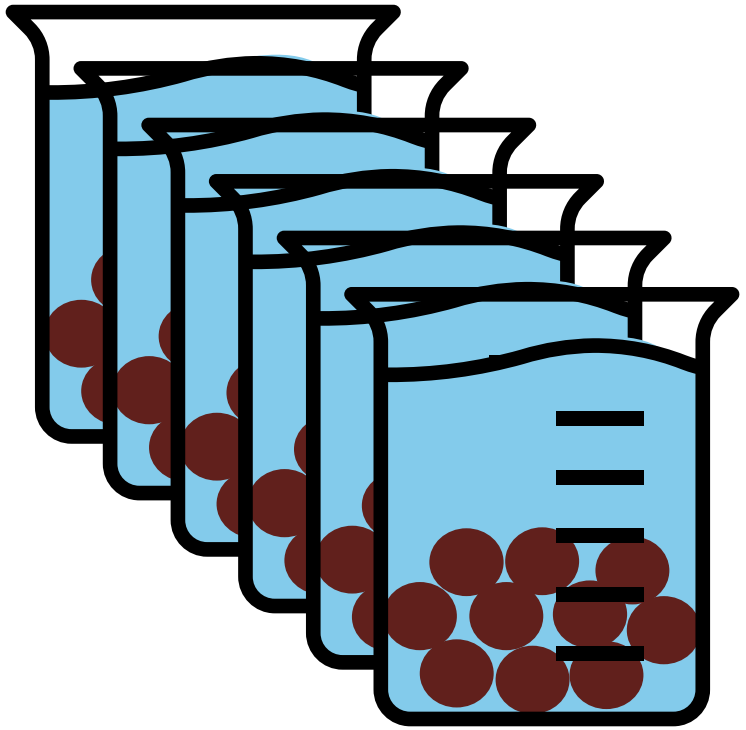
Apple

Grape

Evaluate cuticle tensile properties in relation to cracking

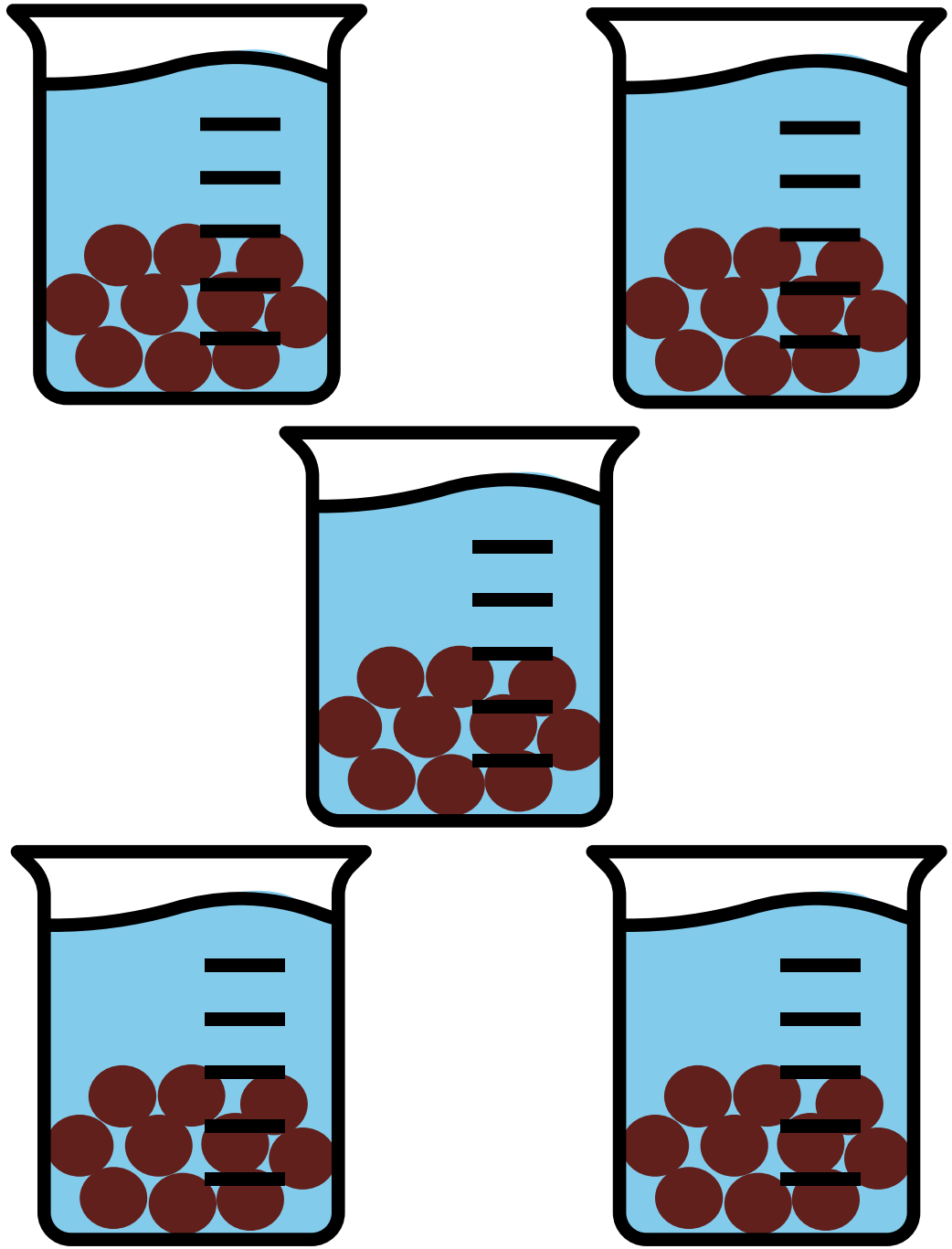
- Mechanical tension assays
- Induce cracking





Berry cracking score

- 5 reps per cultivar
- Ten berries per rep
- Submerged in dH_2O + 0,1% Tween
- Score number of berries cracked after 24 hrs
- Measured mass at 0 hrs and 24 hrs

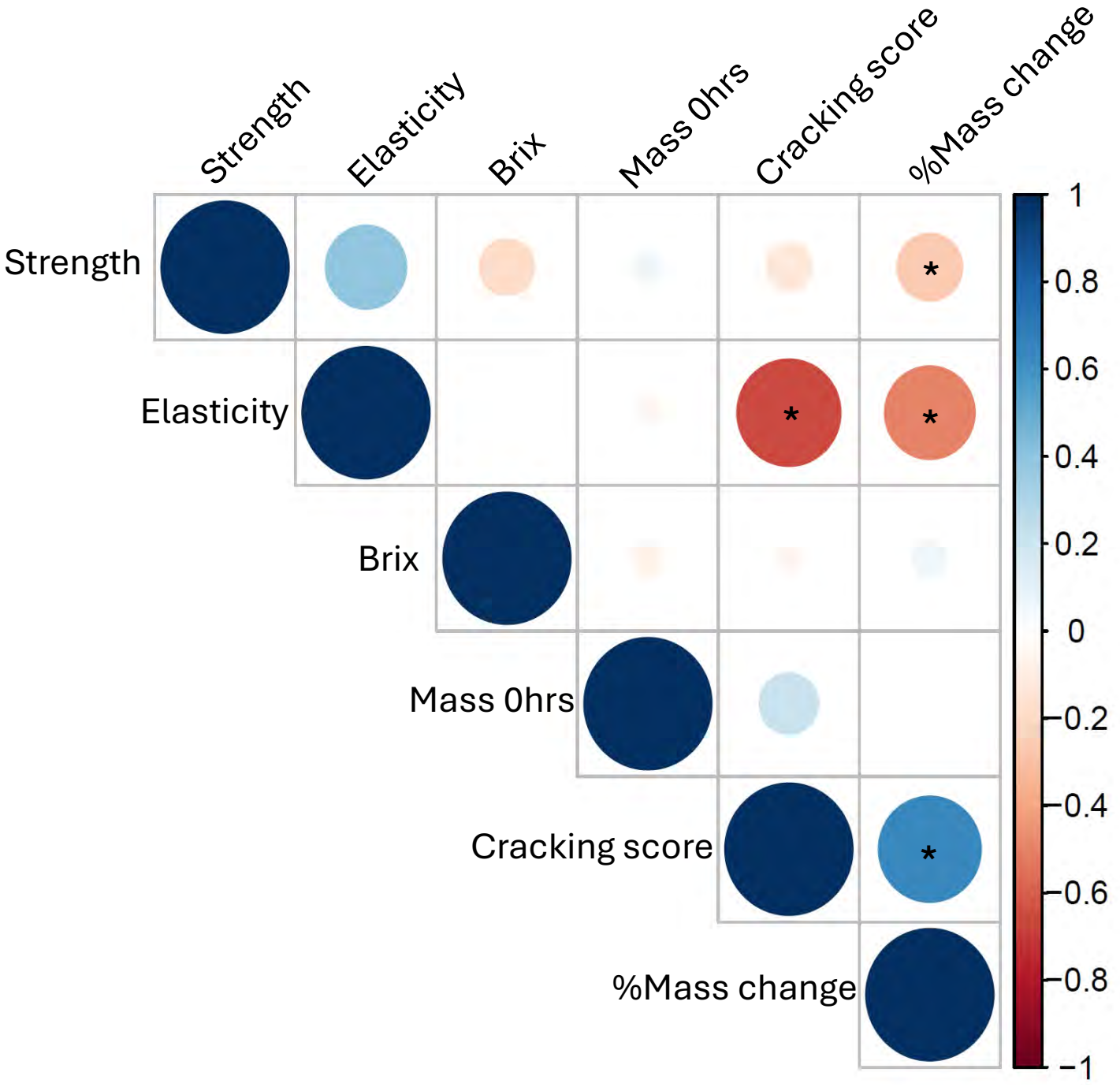


Compression measurements

- Used a food texture analyser
- Ten (10) berries
- Measure distance (mm) and force (N)
- Distance = skin elasticity
- Force = skin strength

Berry cracking

- Elasticity vs strength
- 73 table grape cultivars measured for cracking score
- **Elasticity** and **permeability** play a role in cracking score
- Skin strength and berry size plays little role in cracking



Plant growth regulator treatment

- Four PGR treatments in 2023 and two in 2024
- Three reps per cultivar per treatment
- Two sprays
  - Veraison
  - Veraison + 1 week
- Sampled at harvest ripeness

ABA
ProTone™ SG

GA3
ProGibb® 40%

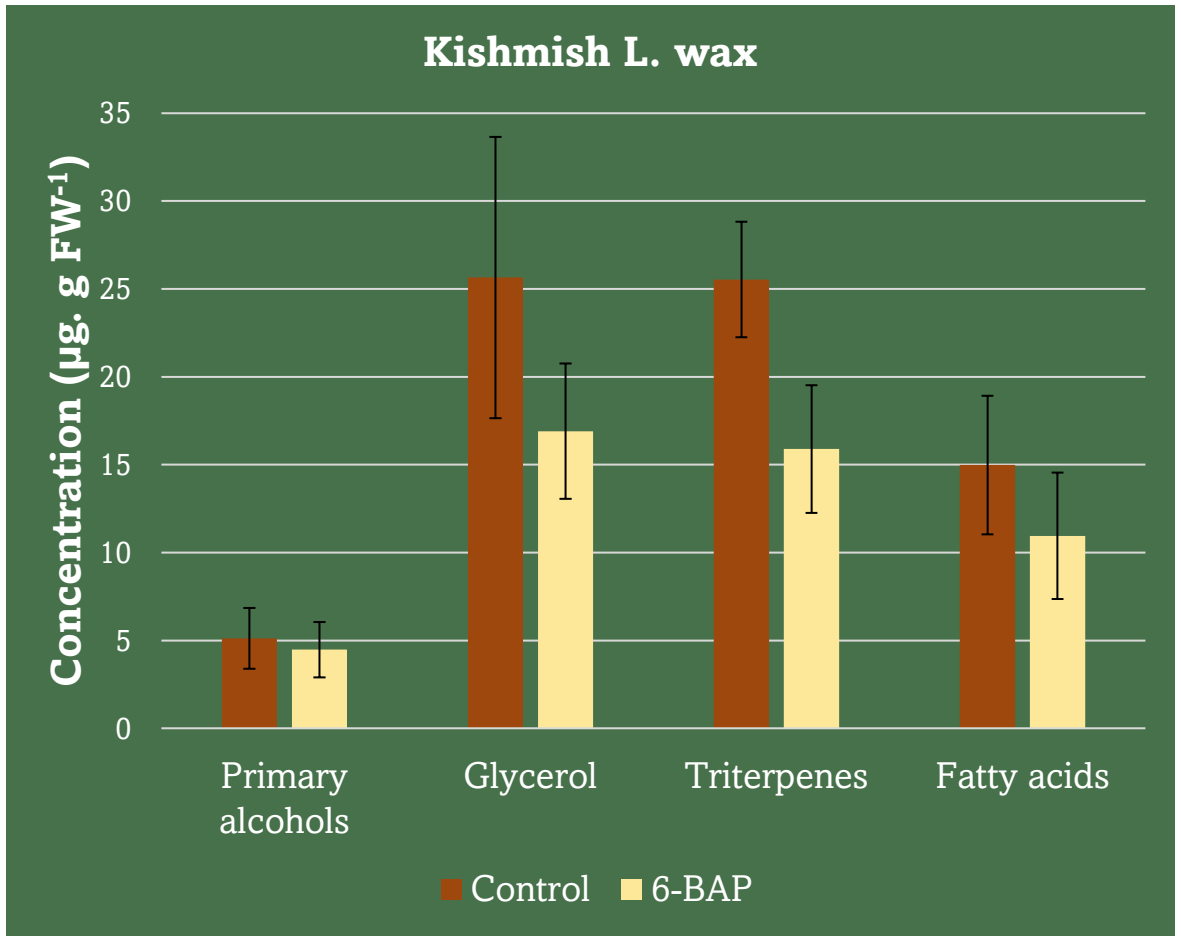
Cytokinin
MaxCel (6-Benzyl adenine; 6-BAP)

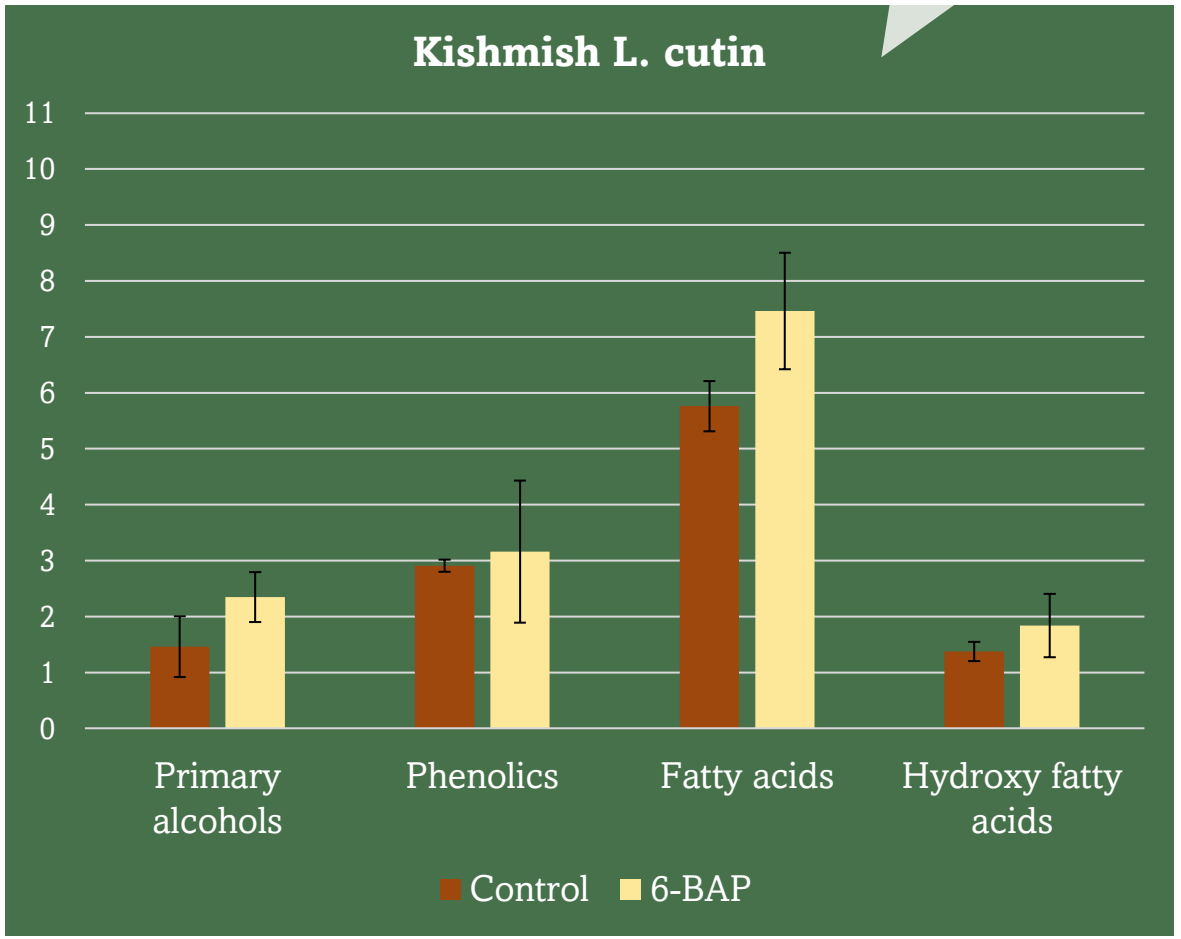
Cytokinin
SITOFEX® 10EC (Forchlorfenuron; CPPU)

Control

Cuticle composition

Overall decrease in waxes and increase in cutin content





Berry Cracking

- Elasticity is genotype dependent
  - Appears to show correlation with wax content
- Hormone treatment shows **cultivar dependant results**
  - Altered surface wax accumulation and cracking

Can we identify the genetics for specific wax monomer formation?

- Knowledge of individual genes in the pathway not known
- QTL analysis in a mapping population

QTL mapping for Cuticular Waxes

Mapping population

Deckrot x G1 - 7720

Created by Ms Phyllis Burger
ARC – Nietvorbij, Stellenbosch

QTL mapping for Cuticular Waxes

Mapping population

Deckrot

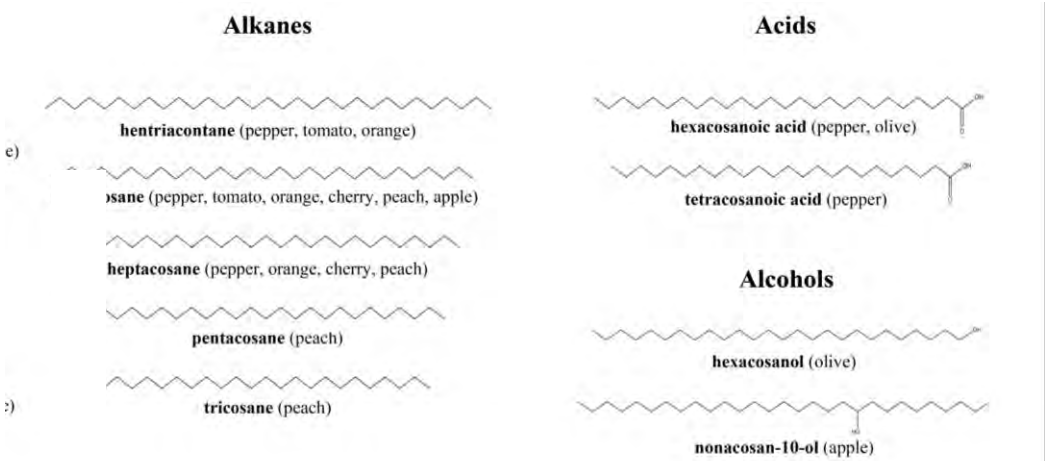
Wine grape
Red flesh
Seeded
Small berries
Compact bunch
Neutral aroma
Waxy (Glaucous)
…

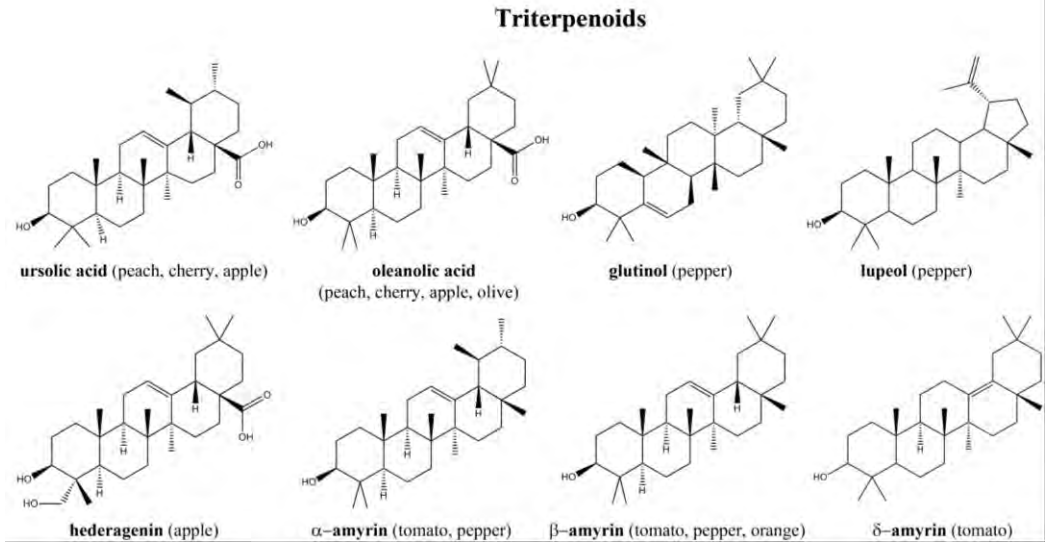
G1 - 7720

Table grape
White flesh
Seedless
Large berries
Loose bunches
Aromatic
Glossy
…

QTL mapping for Cuticular Waxes

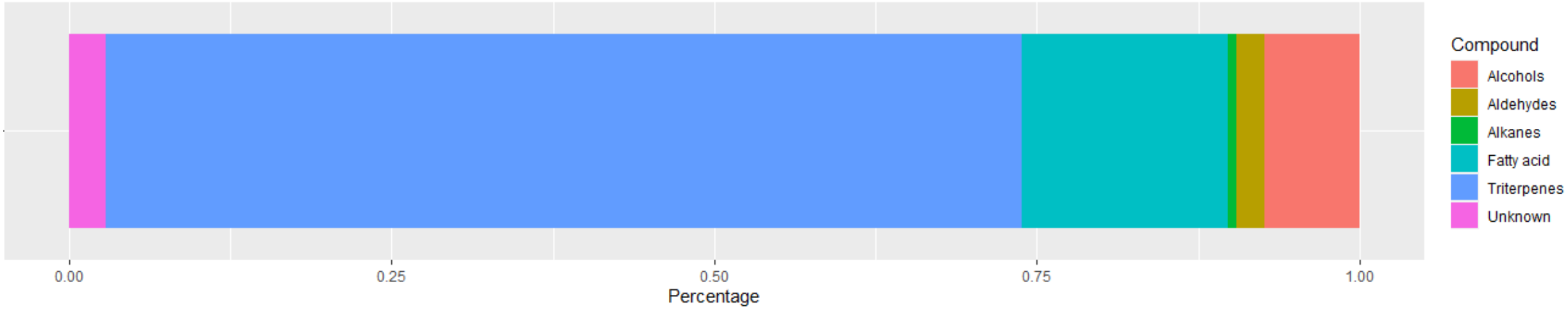
- Very long chain fatty acids (VLCFAs)
  - Fatty acids, fatty alcohols, alkanes aldehydes, others
- Intra- and epicuticular
- Specialised metabolites:
  - Triterpenoids
  - Phenolics



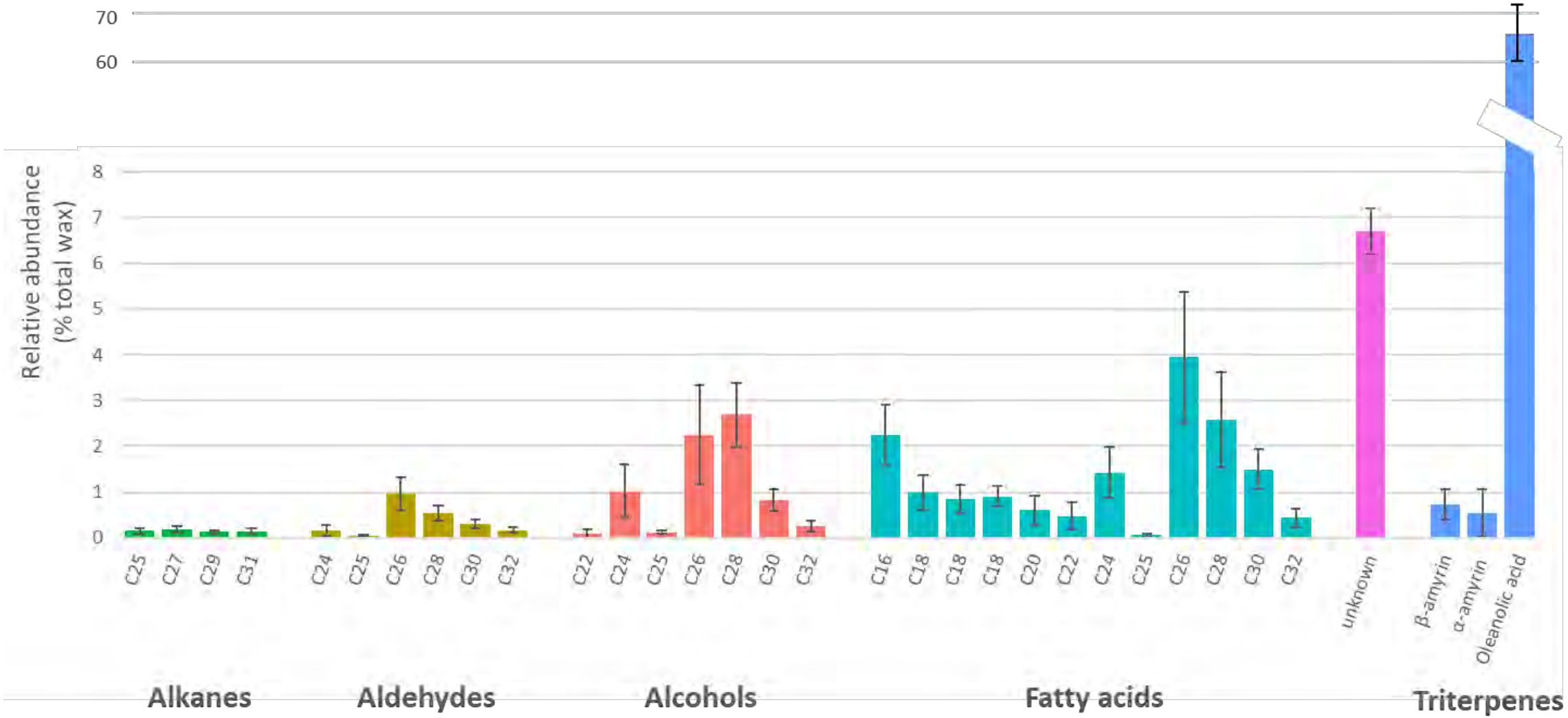


QTL mapping for Cuticular Waxes

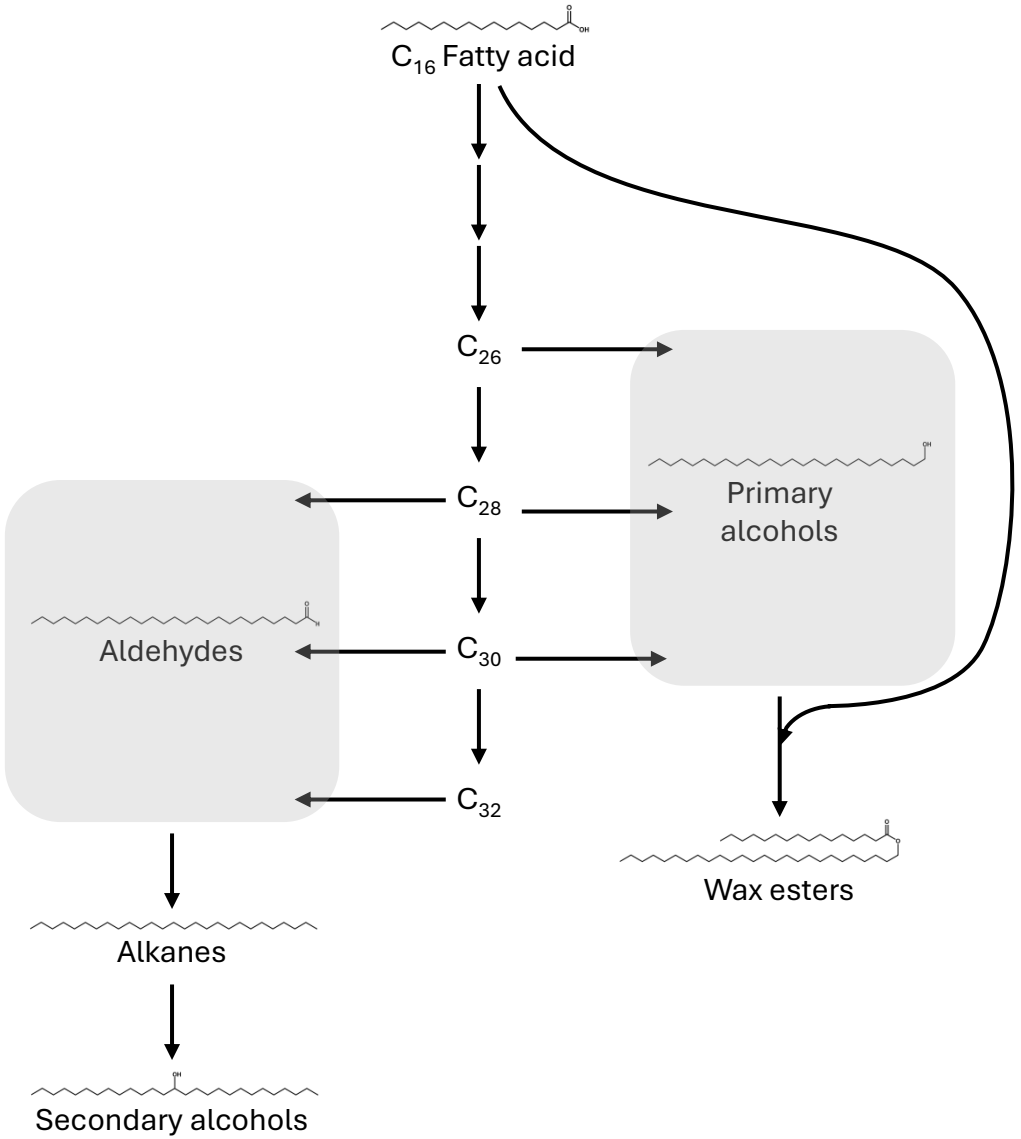
- Cuticular wax quantification via Gas Chromatography-Mass Spectrophotometry (GC-MS)
- Over 30 different wax monomers identified in the mapping population
- Across 90 progeny



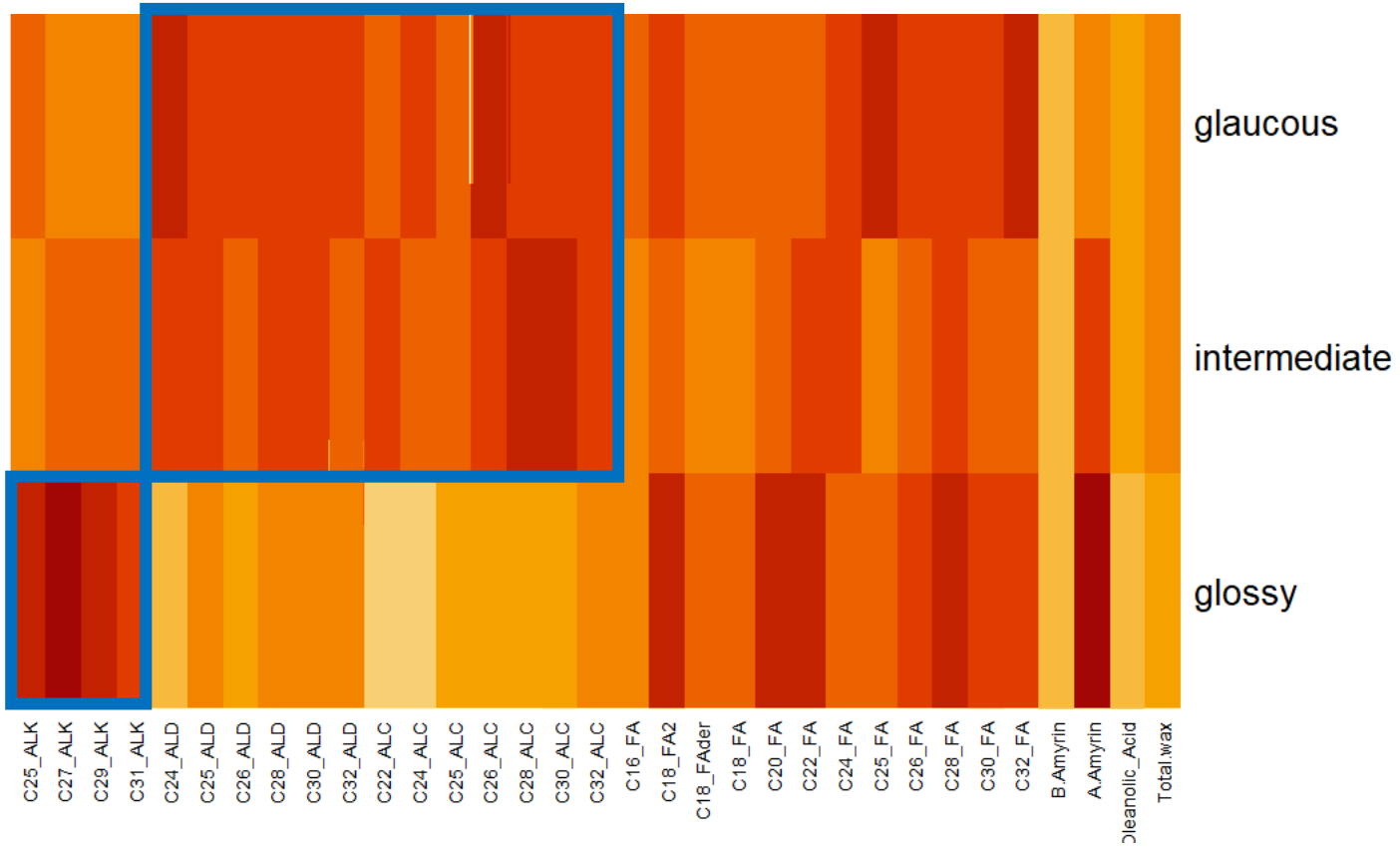
QTL mapping for Cuticular Waxes



Cuticular Wax biosynthesis pathways



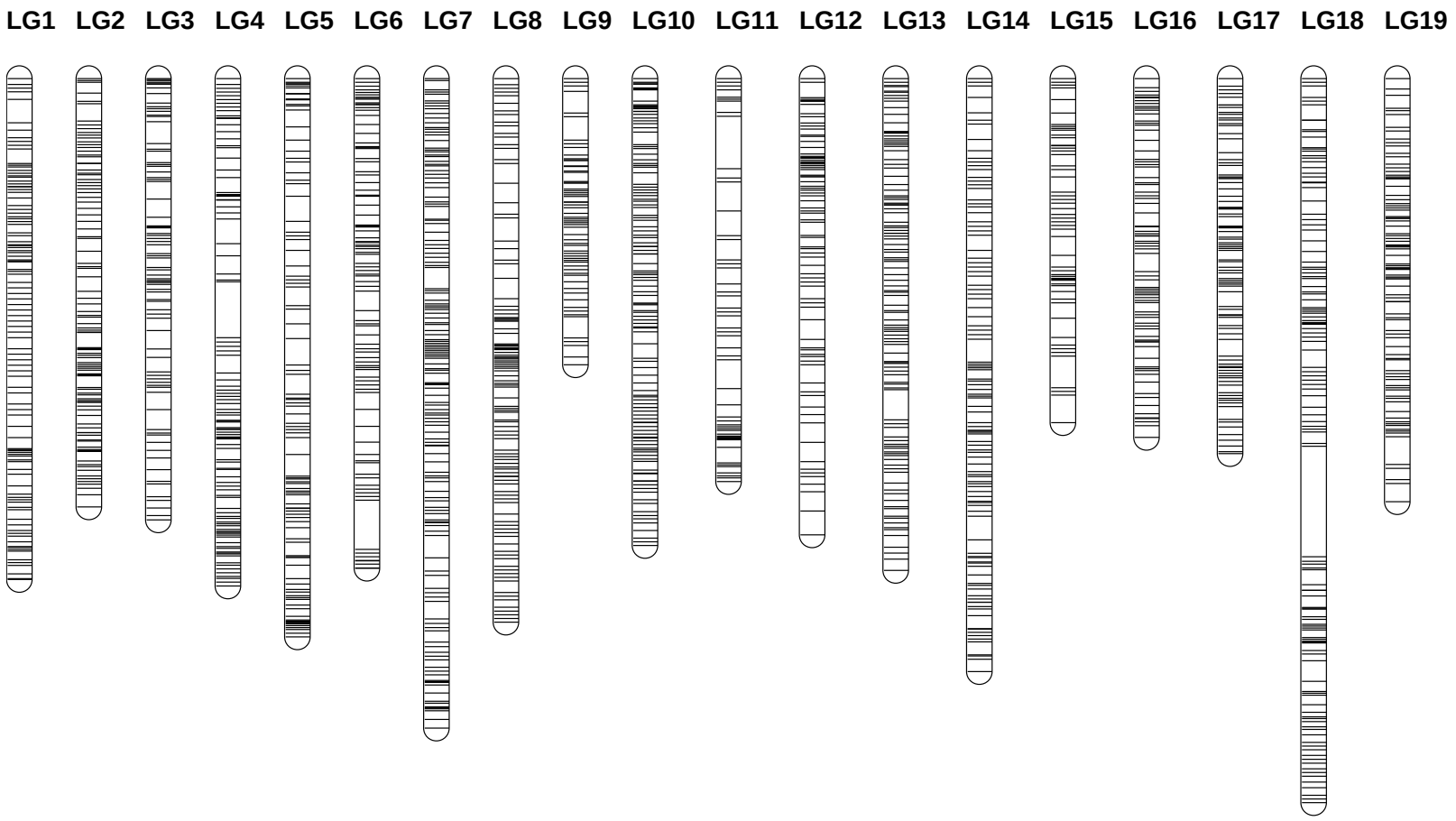
A “waxy” phenotype does not mean more wax



- Increased ratio of aldehydes and alcohols to alkanes leads to glaucous appearance

QTL mapping for Cuticular Waxes

- Genetic map: Illumina SNP chip (Vervalle et al., 2023)
- 31 QTLs identified for numerous waxes
- Includes 3500 genes (PN v3)



Hypothesis:

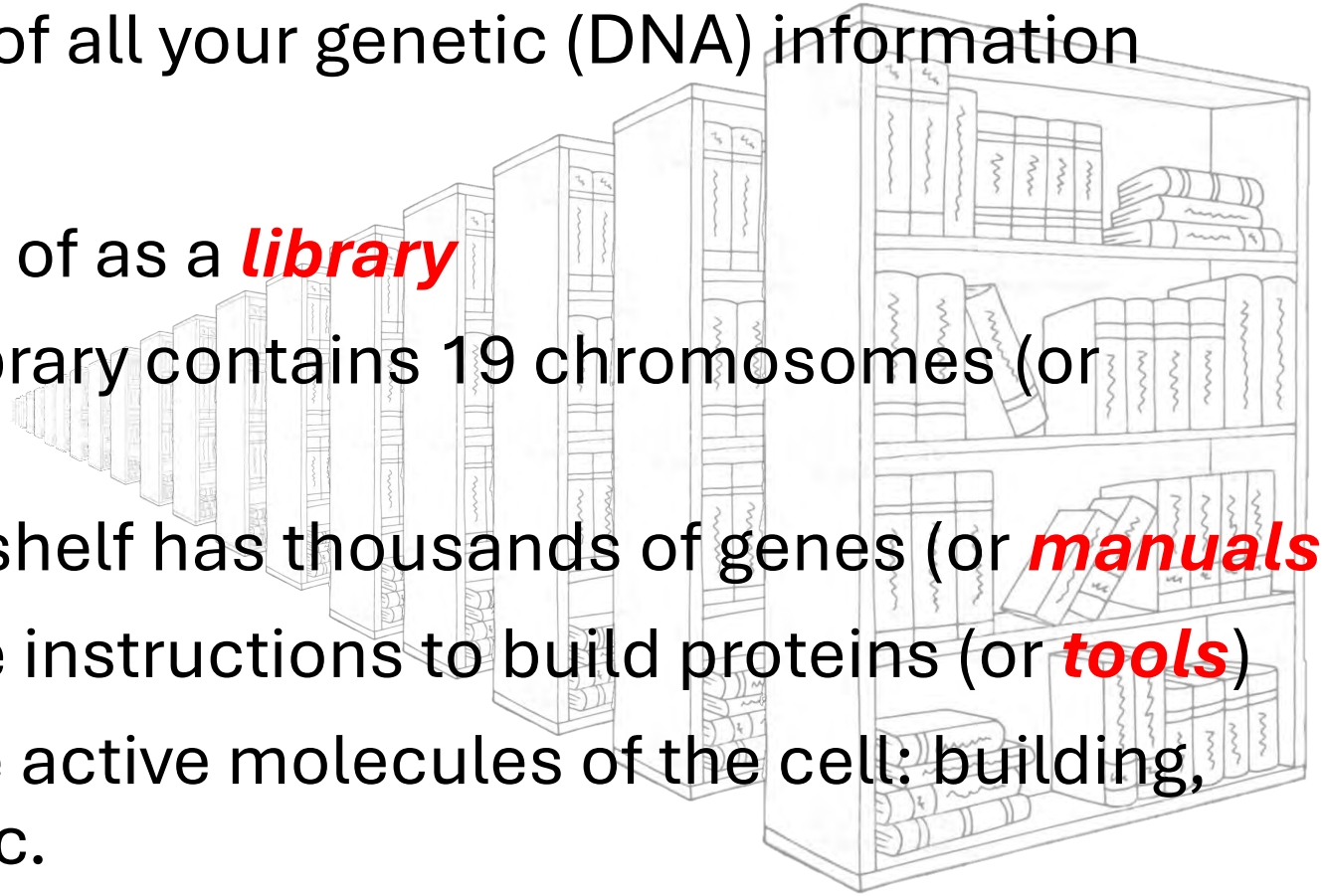
Genetics provide stronger more reliable resistance to cracking than viticultural practices

- ***Do you agree?***

- ***So, how do we improve the genetics of grapevine?***

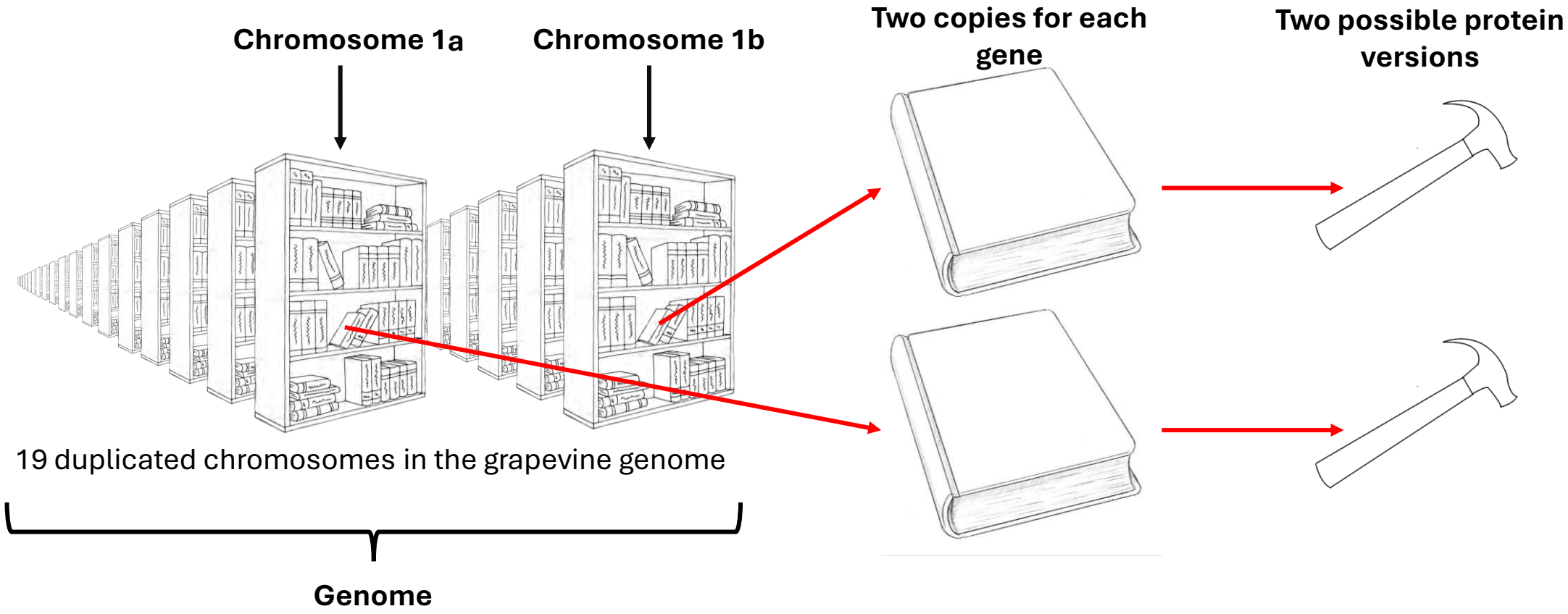
Breeding in the “Genomics Era”

- Your genome is the store of all your genetic (DNA) information



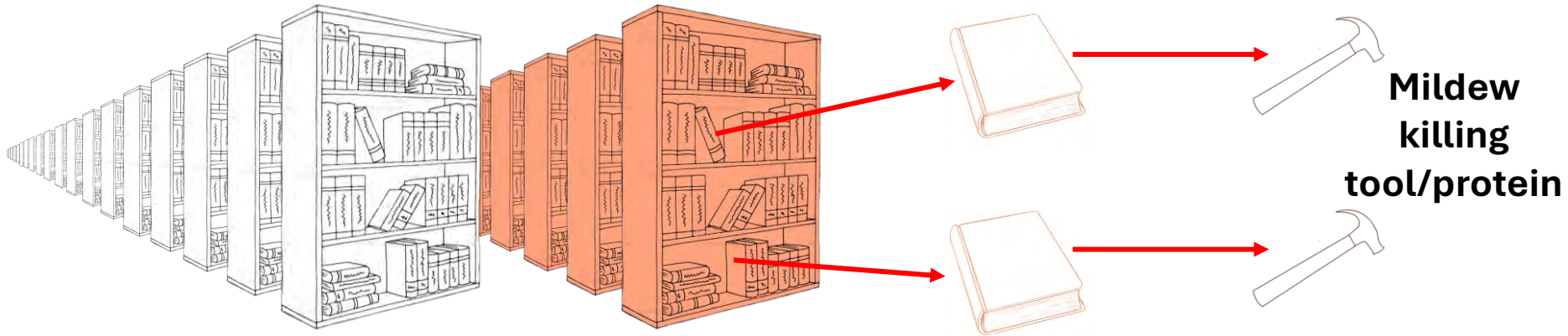
- A genome can be thought of as a ***library***
- The grapevine genome/library contains 19 chromosomes (or ***bookshelves***)
- Each chromosome/bookshelf has thousands of genes (or ***manuals***)
- These genes/manuals are instructions to build proteins (or ***tools***)
- The proteins/tools are the active molecules of the cell: building, modifying, processing, etc.

What is a genome?



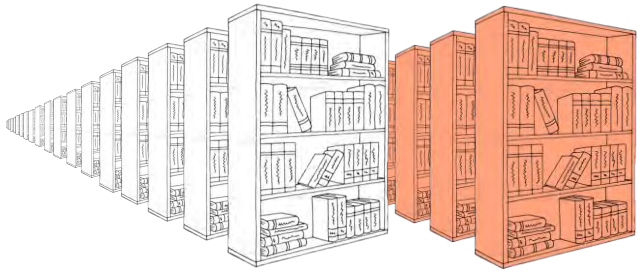
What happens when you make a cross?

- Each parent passes half its genetic material onto the progeny
- In the case of grapevine, crossing with a wild species to integrate an *R* gene, brings with it half the genetic material of the wild species
- By definition crossing with wild species to integrate *R* genes results in a grapevine with undesirable traits (in terms of wine production)

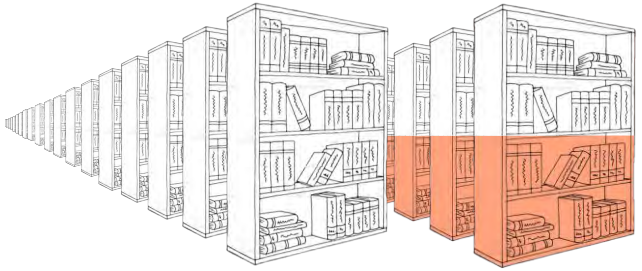


Getting rid of undesirable traits

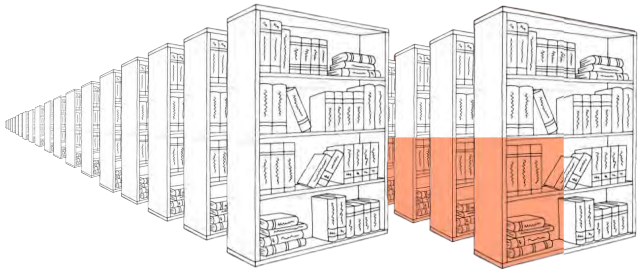
- Multiple generations (back-crossing)



First generation



Second generation



Third generation

Etc…

Time is a key constraint

Genome editing

A tool for **precise** modification of the genome of a cell/organism

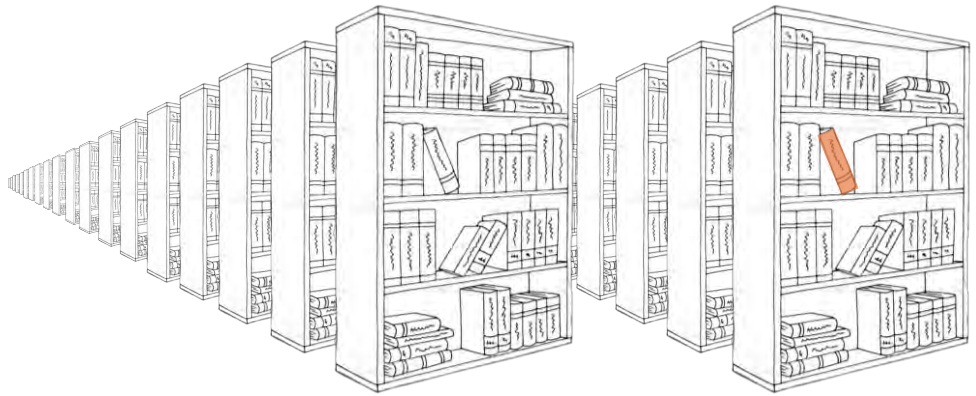
- meganucleases
- zinc finger nucleases (ZFNs)
- transcription activator-like effector nucleases (TALENs)

- **CRISPR/Cas** (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR-associated protein (Cas))

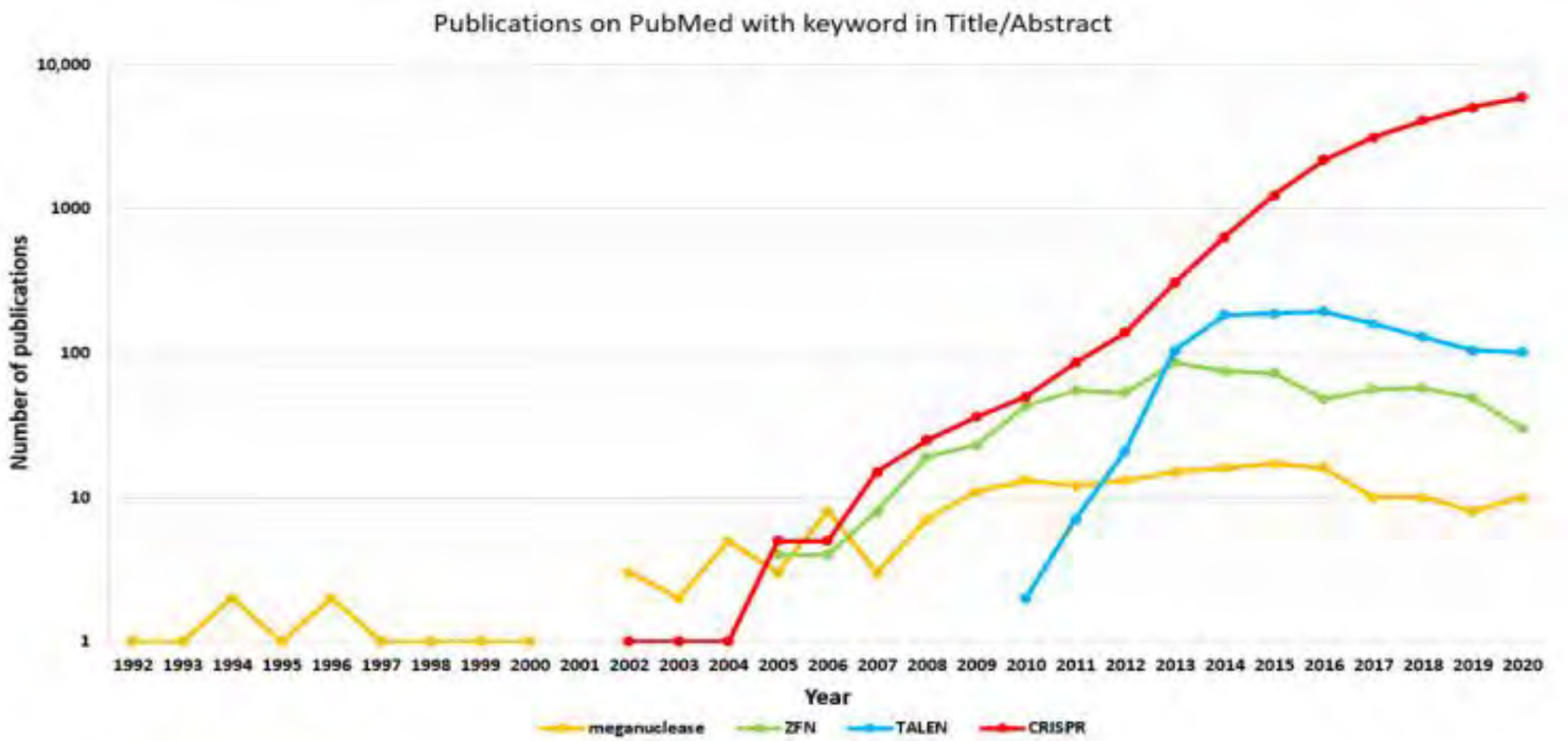
Genome editing

A tool for **precise** modification of the genome of a cell/organism

Implementing CRISPR/Cas genome editing we can target genes we want to change and keep the rest of the genome intact



Genome editing



Already on commercial markets

Soybean

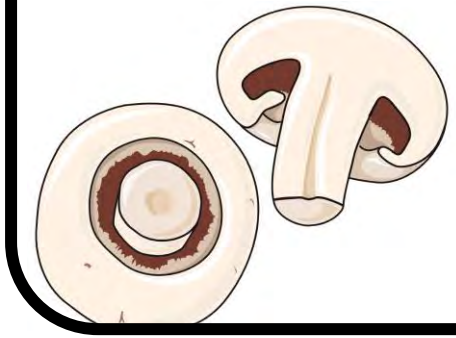
Improved oil content

Canola

Reduced oil acidity

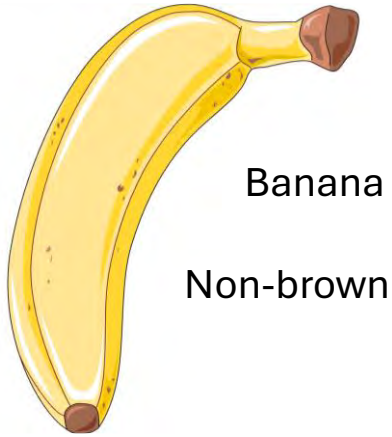
Mushroom

Non-browning



Tomato

Increased nutrition



Banana

Non-browning

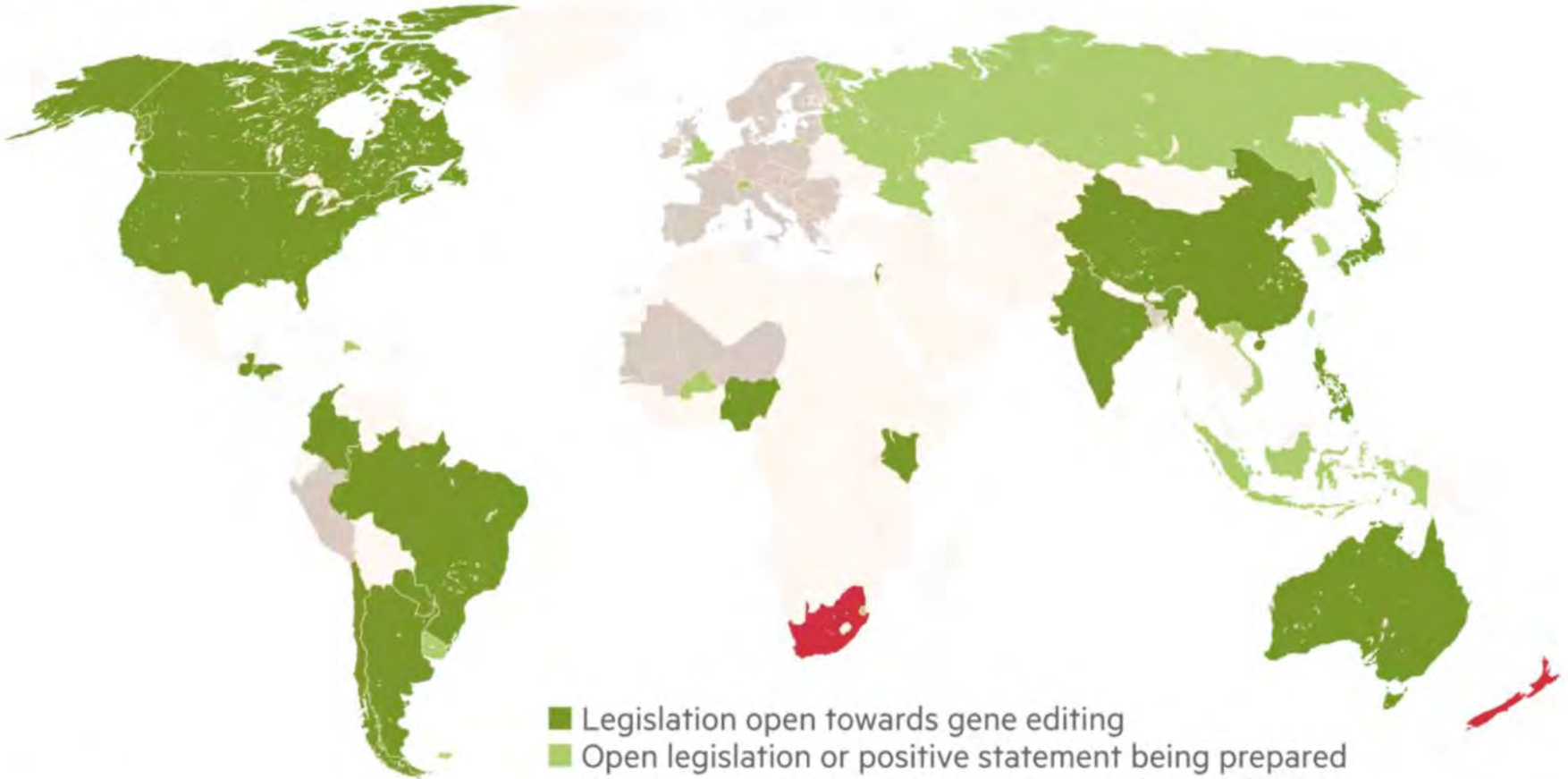
Maize

Waxiness trait

Regulatory landscape

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT
forward together sonke siya phambili saam vorentoe

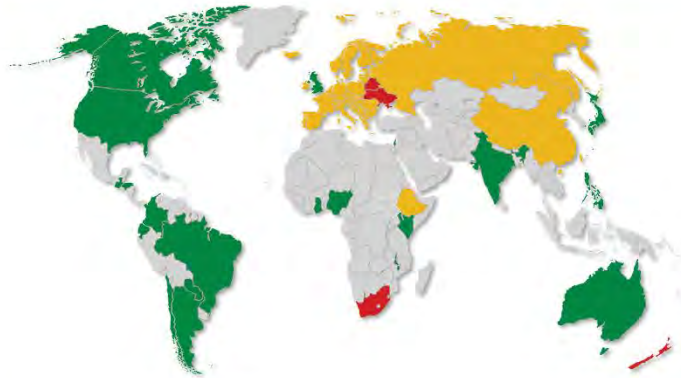
Global regulatory landscape with GMOs



Source: Plant Physiology
© FT

GLOBAL REGULATORY LANDSCAPE FOR GENE-EDITED CROPS

Established regulatory criteria for new breeding innovations in different world regions in the past decade



Countries where products are likely to be regulated as conventional new varieties after recent regulatory policy updates.

Countries where there are noticeable policymaking discussions over proposals to treat SDN1 as conventional new varieties.

Countries where SDN1 products should be treated as GMO according to court interpretations based on old regulations.

NORTH AMERICA

US & CANADA AMONG FIRST COUNTRIES WITH CONCRETE REGULATORY DECISIONS ON NEW BREEDING INNOVATIONS

SOYBEANS PRODUCING HIGH-OLEIC SOYBEAN OIL SOLD AS CALYNO

FIRST COMMERCIALIZED GENE-EDITED CROP IN THE US IN 2019 DEVELOPED USING TALENs

EUROPE

EU PROPOSAL ON NEW GENOMIC TECHNIQUES RELEASED IN JULY 2023

UK'S PRECISION BREEDING BILL

INTRODUCED IN MAY 2022; BECAME A LAW IN MARCH 2023 AFTER RECEIVING ROYAL ASSENT

INTRODUCES SCIENCE-BASED AND STREAMLINED REGULATORY SYSTEM TO FACILITATE RESEARCH

AFRICA

4 COUNTRIES WITH ESTABLISHED GUIDELINES ON NEW BREEDING INNOVATIONS:

NIGERIA (FEBRUARY 2022)
KENYA (MARCH 2022)
MALAWI (AUGUST 2022)
GHANA (OCTOBER 2023)

LATIN AMERICA

8 COUNTRIES WITH ESTABLISHED CRITERIA OF NEW BREEDING INNOVATIONS:

BRAZIL • CHILE • COLOMBIA • ECUADOR GUATEMALA HONDURAS • PARAGUAY ARGENTINA

ARGENTINA PIONEER REGULATION ISSUED IN 2015

GENE-EDITED NON-BROWNING POTATO DEVELOPED USING CRISPR RELEASED IN 2018

ASIA AND THE PACIFIC

AUSTRALIA, JAPAN, PHILIPPINES, AND INDIA

ISSUED IMPLEMENTING REGULATIONS AND SOME APPROVED THEIR FIRST GENE-EDITED PRODUCTS

JAPAN

STARTED SALE OF GENE-EDITED HIGH GABA TOMATO IN 2021

PHILIPPINES

REDUCED BROWNING GENE-EDITED BANANA DETERMINED AS NON-GMO IN 2023

FIRST GENE-EDITED PRODUCT TO GO THROUGH THE PHILIPPINES' GENE EDITING REGULATORY PROCESS

ISAAA Inc.

For more information, visit:
www.isaaa.org

Sources:
ISAAA. 2021. Breaking Barriers with Breeding: A Primer on New Breeding Innovations for Food Security. *ISAAA Brief* No. 56.
ISAAA Biotech Updates. https://www.isaaa.org/kc/cropbiotechupdate/

Updated January 24, 2024

SDN1: site-directed nuclease
GMO: genetically modified organism
TALENs: transcription activator-like effector nucleases
CRISPR: clustered regularly interspaced short palindromic repeats
GABA: gamma-aminobutyric acid

isaaa.org isaaa.org isaaa_org isaaa isaaavideos

Conclusions

- **Berry surface waxes are worth studying**: impact **post harvest**, **disease resistance**, **appearance and quality**
- What can we do to **encourage legislative change**?
  - Should we do anything?
- What traits are important to target?
- **Cracking** is complicated **and controlled by may genes**, but we are making progress
- Can we use this knowledge for **grapevine improvement**?

Acknowledgements

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science & innovation
Department:
Science and Innovation
REPUBLIC OF SOUTH AFRICA
Making sure it's possible

NRF
National
Research
Foundation

SATI
SOUTH AFRICAN TABLE
GRAPE INDUSTRY

SOUTH AFRICA WINE
discover diversity in a glass

Stellenbosch
UNIVERSITY · IYUNIVESITHI · UNIVERSITEIT

Thank you

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