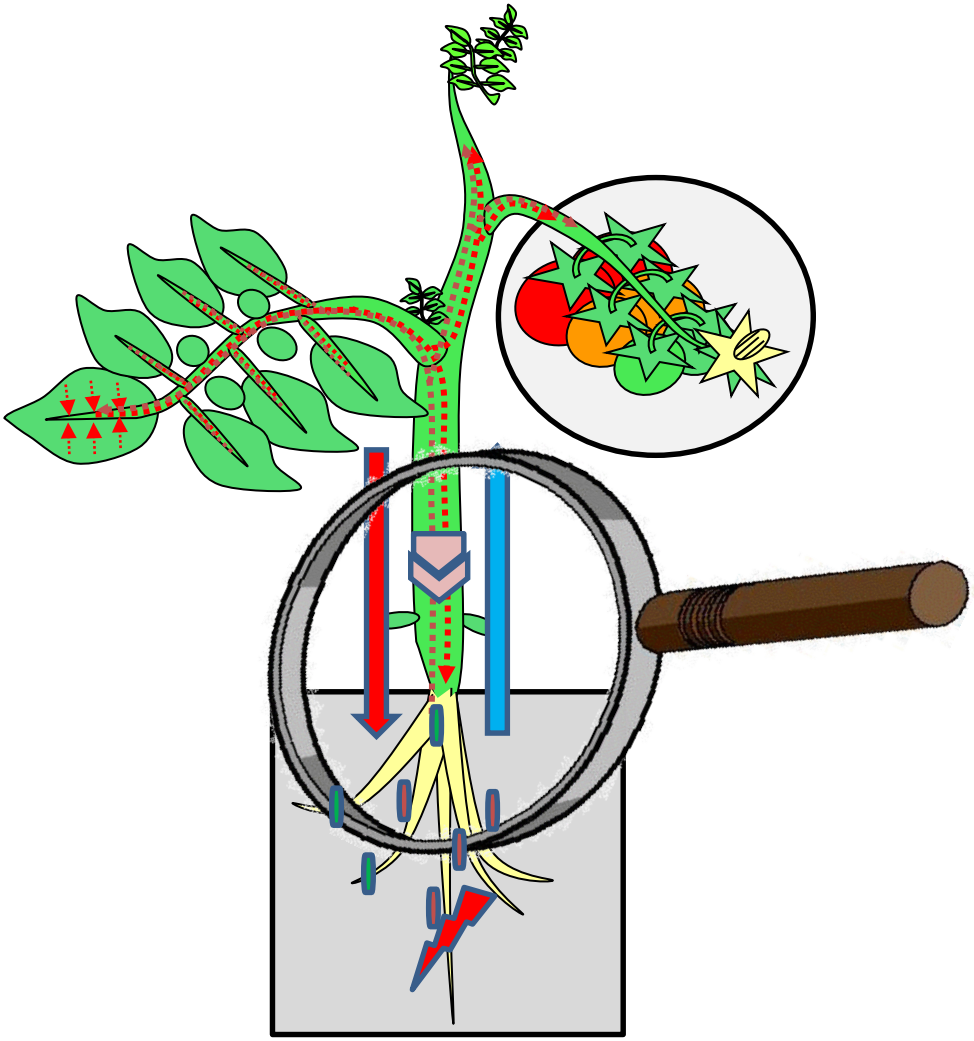
COST Action FA 1204 “Vegetable Grafting” WG2

Rootstock-scion signalling: factors that mediate scion performance and graft compatibility



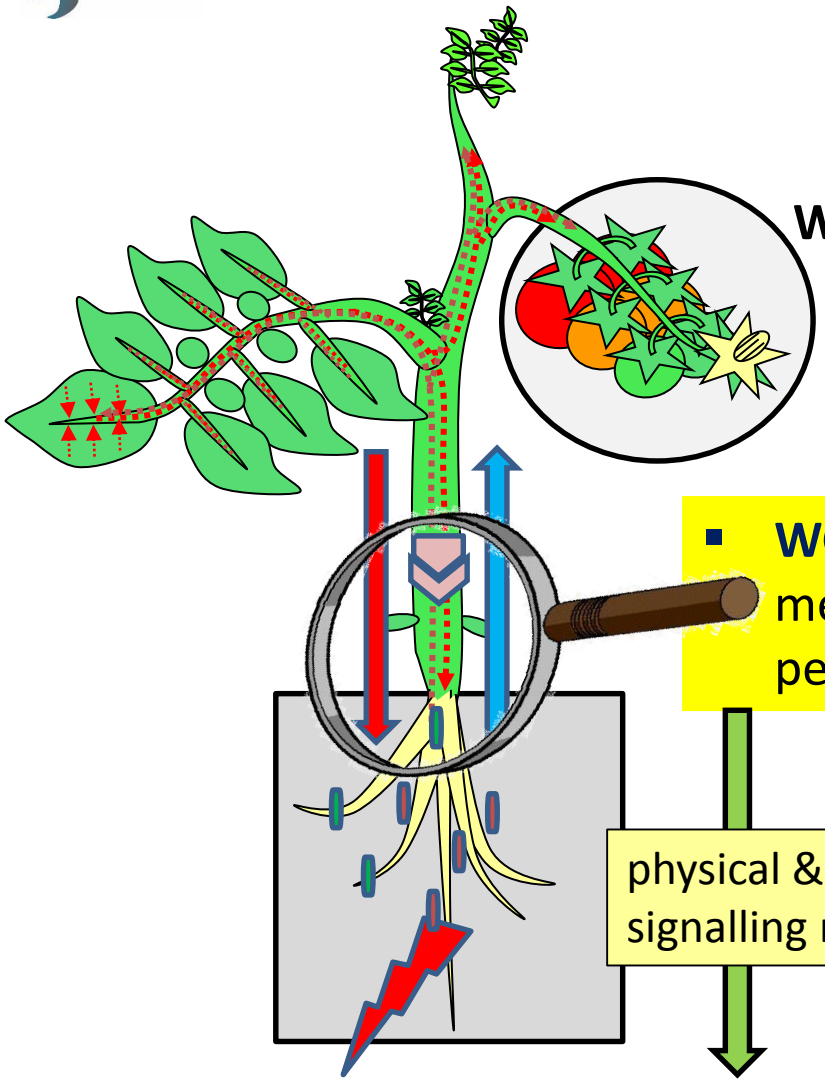
Jan Henk Venema — university of groningen

Ana Pina — Universidad Zaragoza

Ian C. Dodd — Lancaster University

COST Action FA 1204 "Vegetable Grafting"

Aims and deliverables of WG2



WG4: fruit production and quality

regulators scion and fruit development

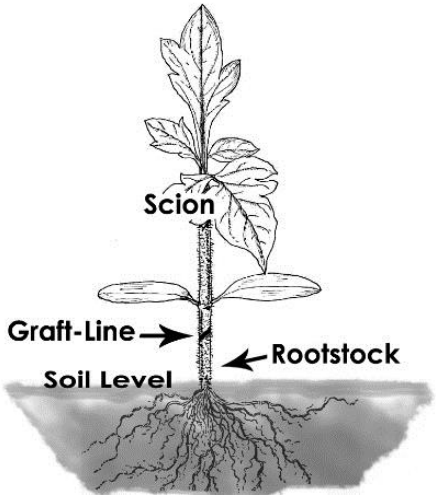
- **WG2:** defining the mechanisms of rootstock-mediated improvements in vegetable crop performance and graft compatibility

physical & chemical signalling mechanisms

rootstock selection tools

WG3: biotic and abiotic stresses

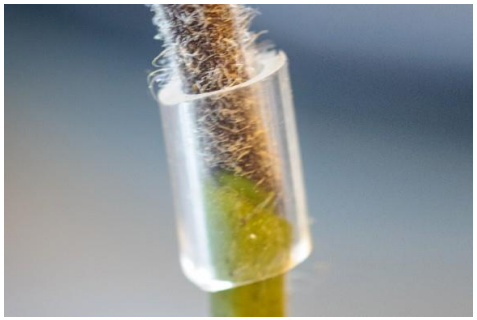
WG1: rootstock breeding



Working fields WG2

I. **Rootstock-scion signalling**

II. Graft compatibility



Aim I:

elucidating root-derived signals that facilitate improved rootstock / scion performance

Root signals that mediate scion performance:

- Xylem: increase or decrease of an existing signalling compound
- Phloem: via reduction or increase in root demand (sink strength)

Signalling mechanisms:

- chemical: ions, phytohormones, proteins, peptides, assimilates
- molecular: small RNAs (epigenetic mechanism)
- physical: hydraulic and electrical signals, ROS and Ca^{2+} waves

Exciting progress in metabolomics and proteomics for sap profiling

<u>Metabolomics:</u>

U-HPLC-(HR)MS/MS technology

Xylem sap: 800 metabolites (MW 90-500)

<u>Proteomics:</u>

iTRAQ-labelling

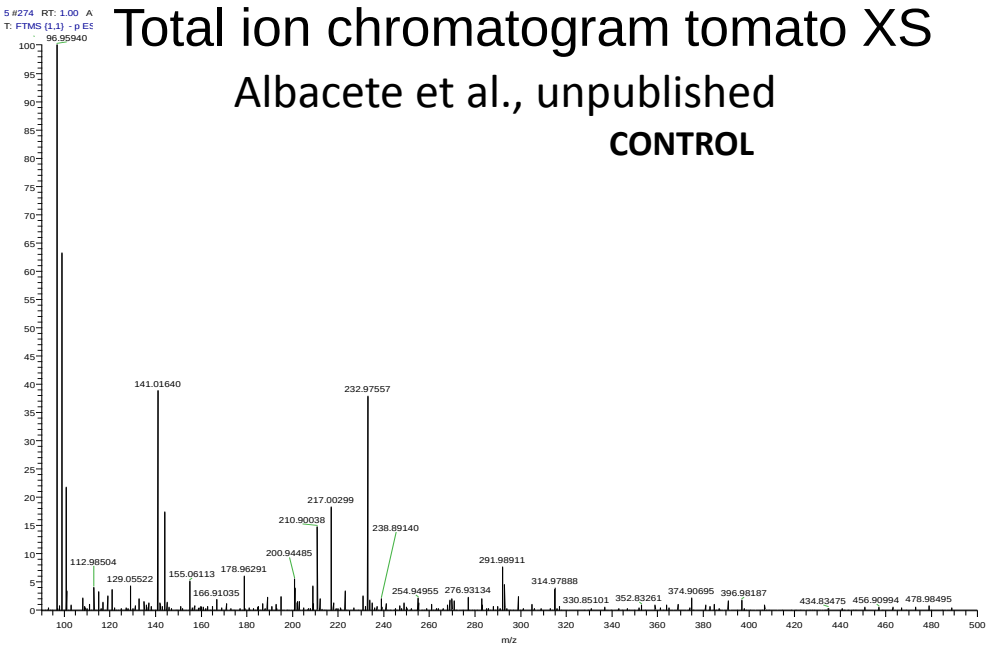
Fractionation SCX chromatography

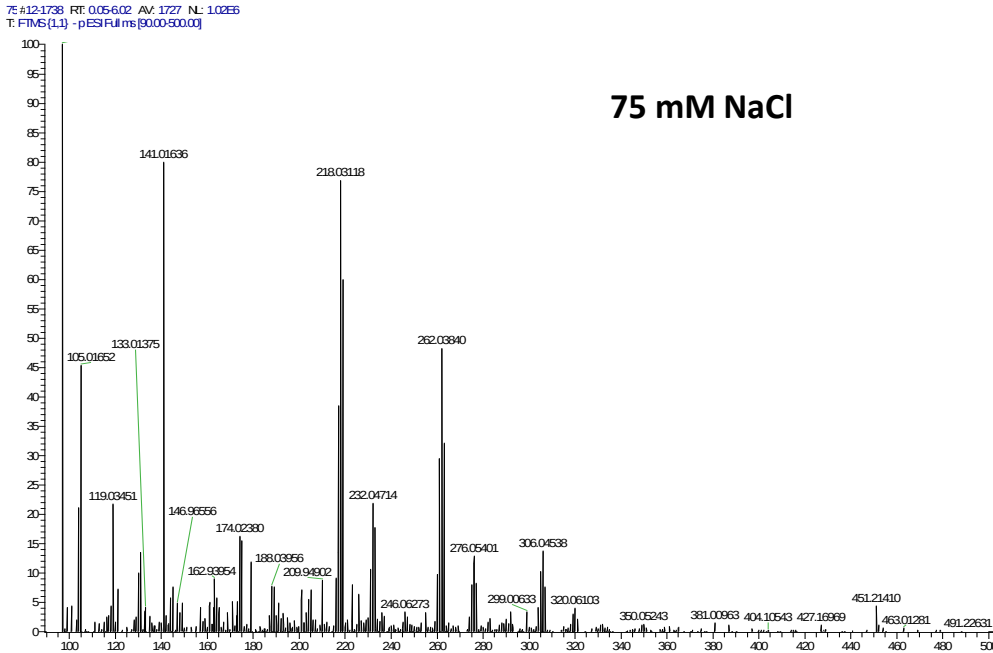
LC-ESI-MS/MS analysis by Q Exactive

Phloem sap: 750 proteins

Total ion chromatogram tomato XS

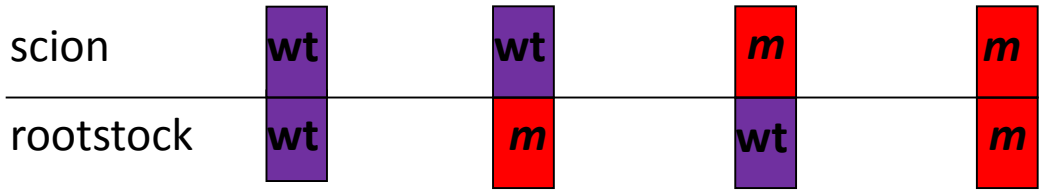
Albacete et al., unpublished





Experimental design

Reciprocal grafting experiments between **wild type** (**wt**) and a ***signalling mutant*** (***m***)



4 different rootstock-scion combinations

Focus on role of phytohormones in abiotic stress signalling in tomato

ABA: *notabilis*, *flacca, sitiens* (deficient); *sp5* and *sp12* (overproducers)

Ethylene: *Never ripe* (partly insensitive)

Jasmonic acid: *JL5* (deficient)

Cytokinin: *IPT* (overproducer), *CKX2-MM* (deficient)

Auxin: *dgt* (partly insensitive)

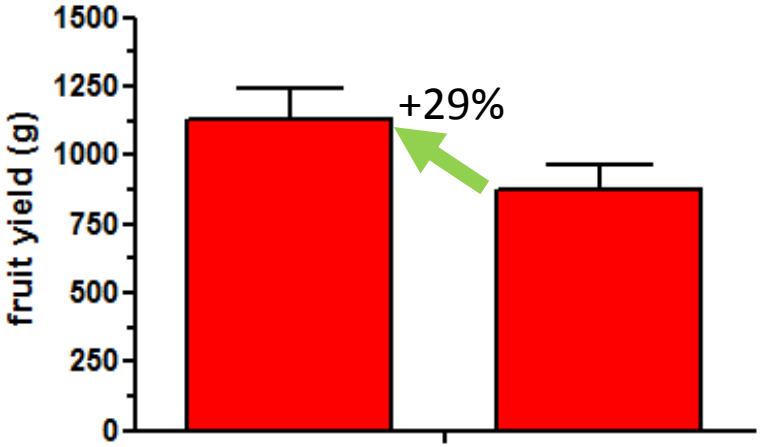
Salicylic acid: *nahG* (deficient)

Root-supplied cytokinins improve shoot growth and fruit yield under salt stress

Ghanem et al. 2011; *Journal of Experimental Botany* 62, 125-140

100 mM NaCl for 22 days

75 mM NaCl for 3 months



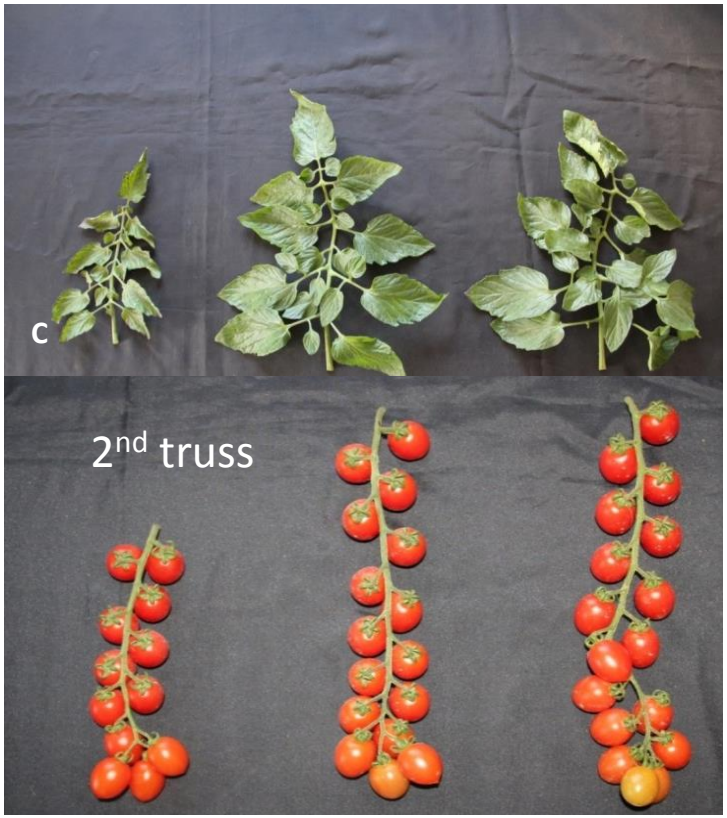
Wild-Type scion

CK-overproducing rootstock

ABA-overproducing roots improve shoot growth and fruit yield under salt stress

Albacete et al. 2015; *Journal of Experimental Botany* 66, 2211-2220

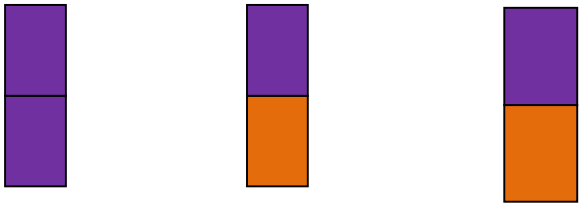
30 mM NaCl for 100 days



SD/AC **SD/sp12** **SD/sp5**

Wild-Type (WT) scion

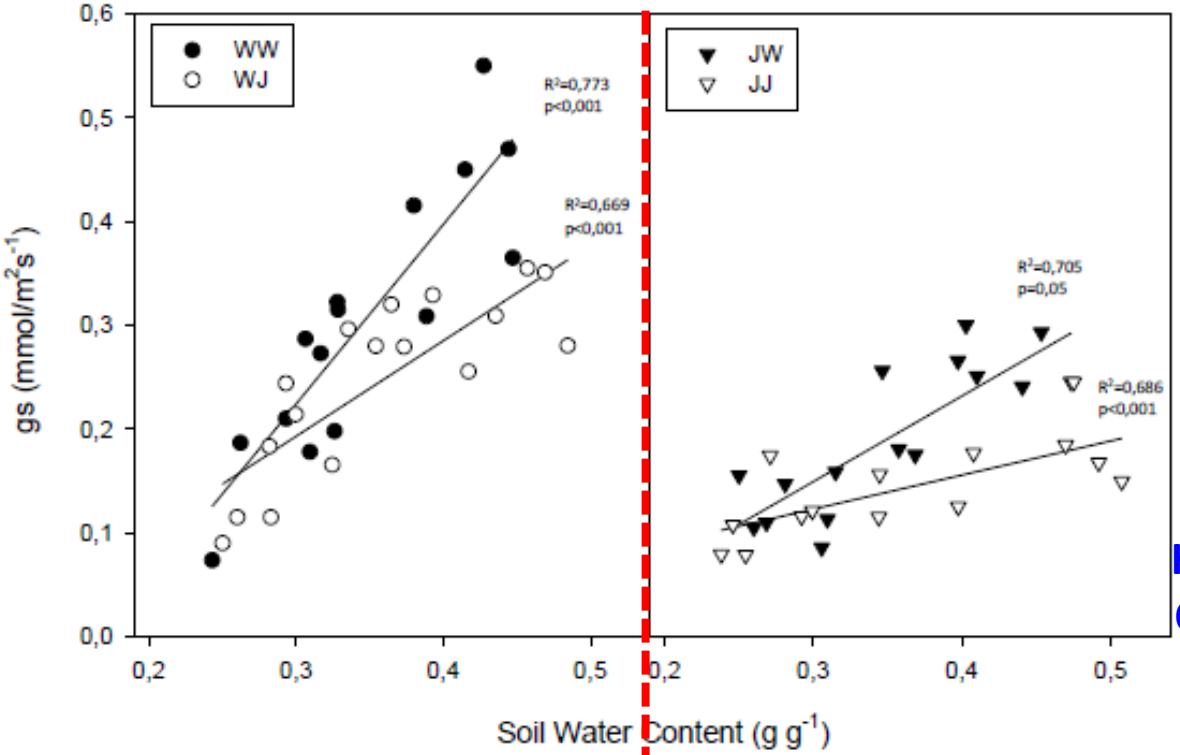
ABA-overproducing rootstock



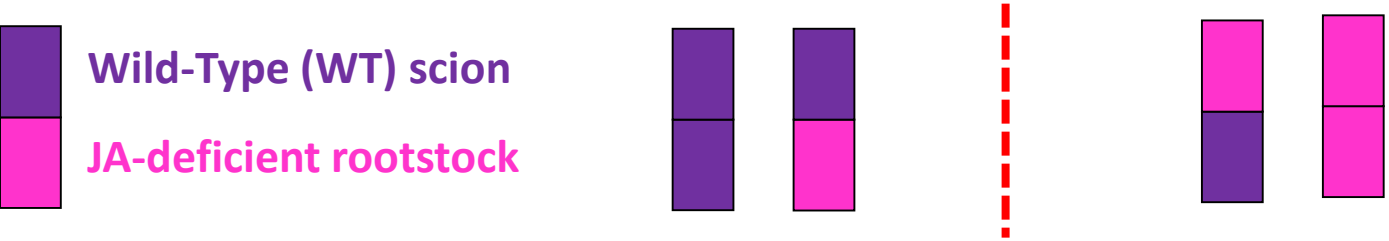
Root-supplied JA is needed to maintain stomatal conductance as the soil dries (independent of Ψ_{leaf})

de Ollas et al.; unpublished (COMING UP !)

LOWER STOMATAL CONDUCTANCE OF WT SCIONS WITH JA-DEFICIENT ROOTSTOCK

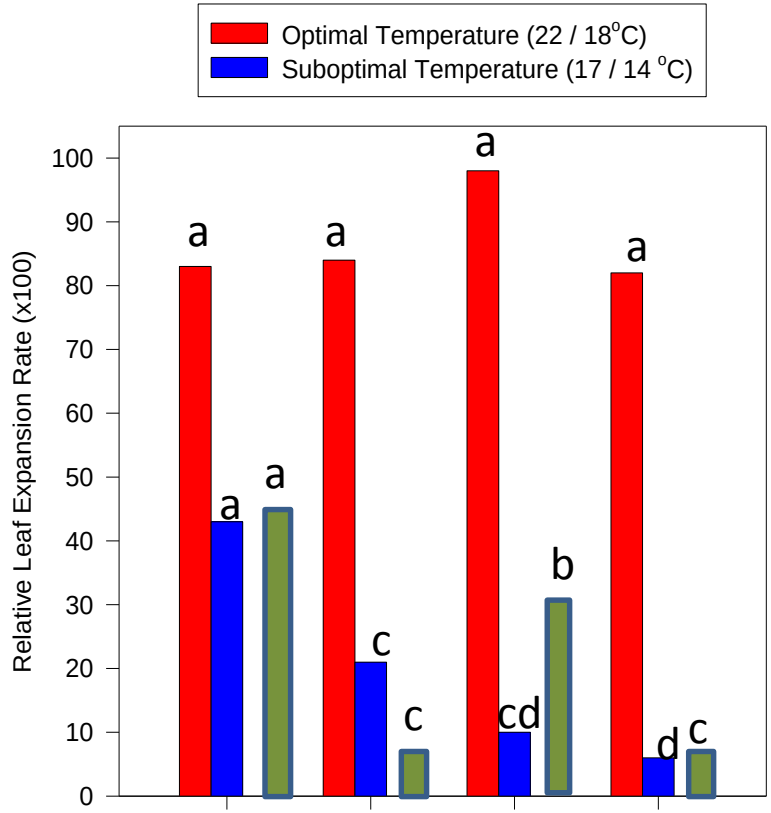


HIGHER STOMATAL CONDUCTANCE OF *JL5* SCIONS WITH WILD-TYPE ROOTSTOCK



Root-supplied ABA contributes to prevent leaf growth inhibition at low temperature

Ntatsi et al. 2013; *Scientia Horticulturae* 149, 28-38



Leaf [ABA]

SIMILAR LEAF GROWTH INDEPENDENT OF ROOTSTOCK AND SCION GENOTYPE AT OPTIMAL TEMPERATURE

ABA DEFICIENCY IN ROOTSTOCK OR SCION DECREASES LEAF GROWTH AT LOW TEMPERATURE

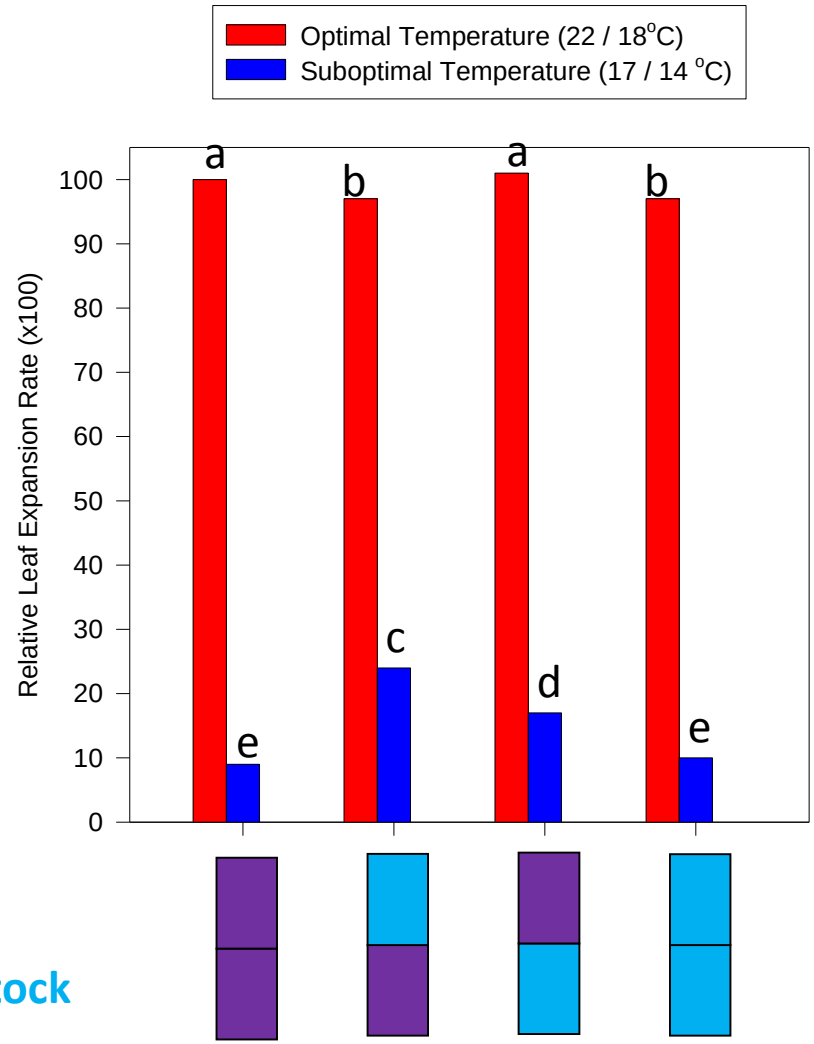
Wild-Type scion

ABA-deficient rootstock

Rootstock partial ethylene insensitivity increases leaf growth at low temperature

Ntatsi et al. 2013; *Scientia Horticulturae* 149, 28-38

COMPARABLE LEAF GROWTH INDEPENDENT OF ROOTSTOCK AND SCION GENOTYPE UNDER OPTIMAL TEMPERATURE

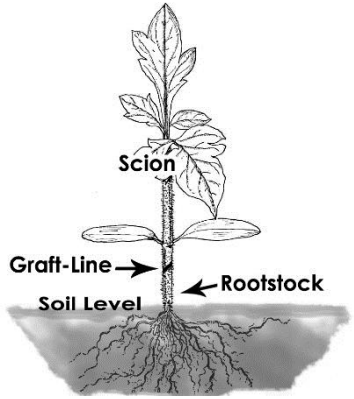


ETHYLENE INSENSITIVITY IN ROOTSTOCK OR SCION INCREASES LEAF GROWTH AT LOW TEMPERATURE

Wild-Type scion

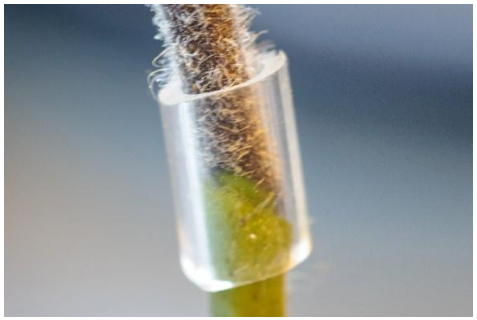
C_2H_4-insensitive rootstock

Working fields WG2



I. Rootstock-scion signalling

II. Graft compatibility



Aims II:

1. To identify the anatomical / physiological /molecular markers for graft incompatibility

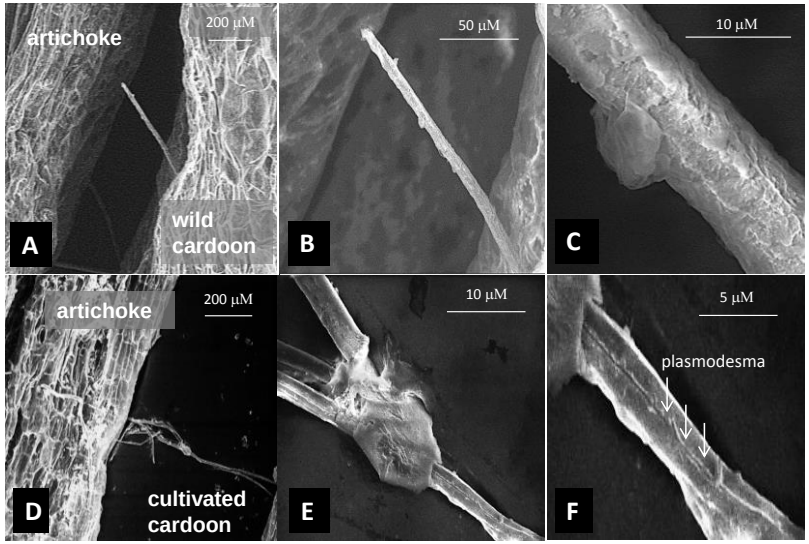
2. To develop a test to predict (early) compatibility succes

Mechanisms involved in graft compatibility

Research tools

- Scanning Electronic Microscopy
- ROS analyses
- Gene expression studies
- CFI - Chlorophyll Fluorescence Imaging
- Electrical impedance measurements

Physiological mechanisms

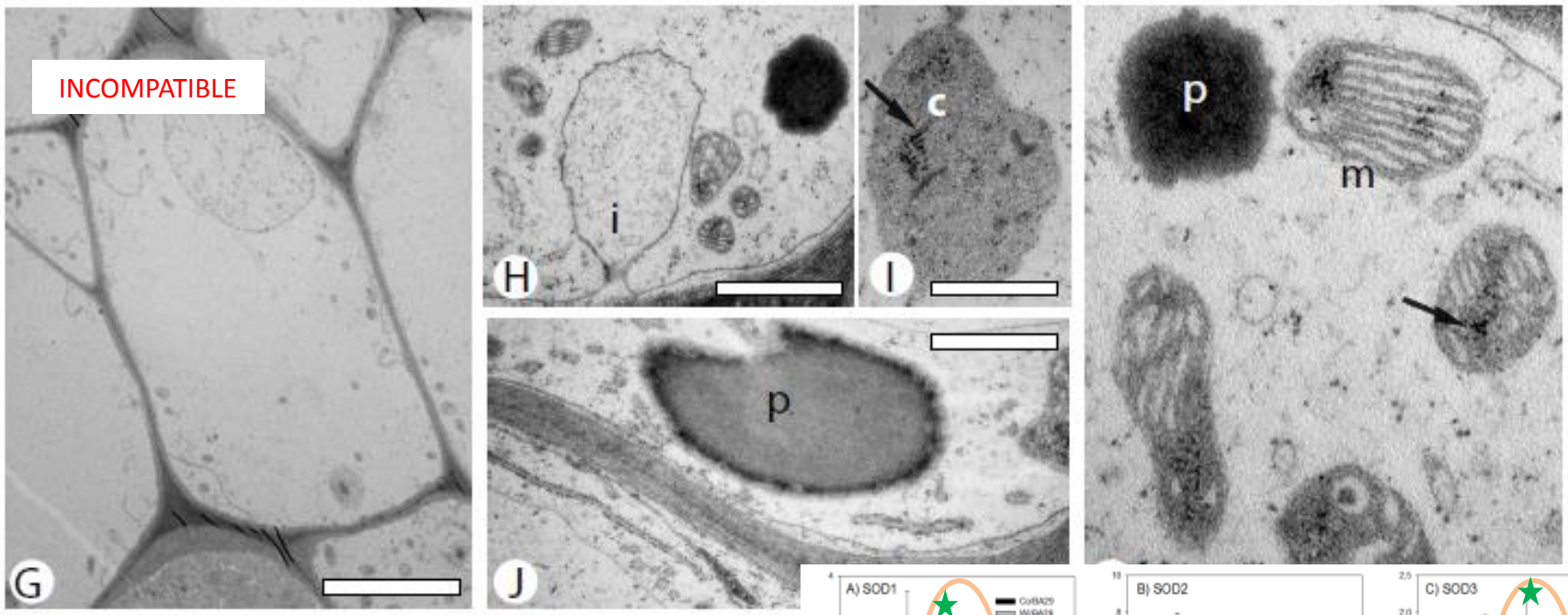


SEM (SE-VP) images of interconnections between artichoke/wild cardoon [A-C] and artichoke/cultivated cardoon [D-F] grafting combinations, after 3 d from grafting.

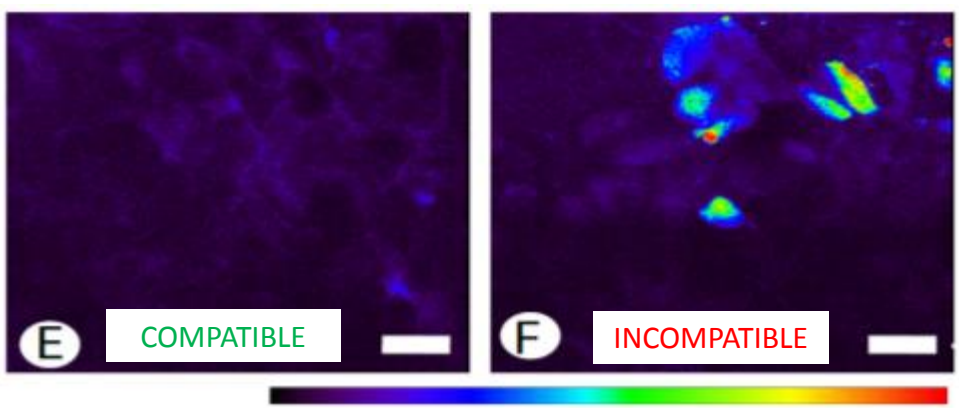
- High graft compatibility was found between globe artichoke (scion) and cardoon (rootstock). This has a direct application to avoid *Verticillium* wilt in artichoke crops and may help to identify the underlying rootstock-scion signals.

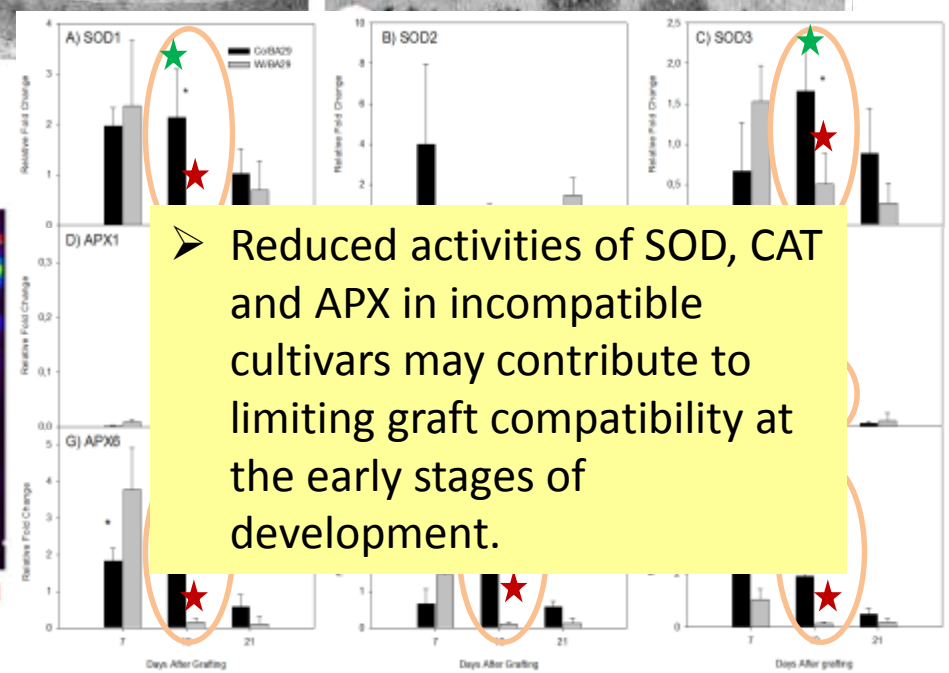
Trinchera et al. 2013; *Journal of Plant Physiology*, 170: 1569– 1578

Physiological mechanisms



- **ROS analysis**





- Reduced activities of SOD, CAT and APX in incompatible cultivars may contribute to limiting graft compatibility at the early stages of development.

Irisarri et al. 2015; *Journal of Plant Physiology* 176, 25–35

Molecular mechanisms

Genes differentially expressed during graft union formation.

Metabolic pathways candidates for physiological failure in graft-incompatible rootstock-scion combinations

Morphological changes

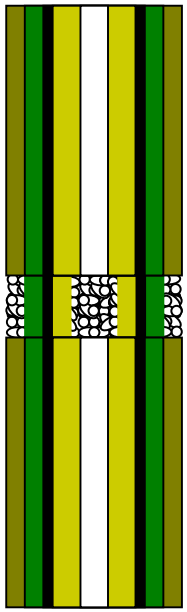
Formation of brown necrotic layer (hours)

↓

First callus cells appear (14 days)

↓

Successful graft union formation (28 days)



Mechanisms involved

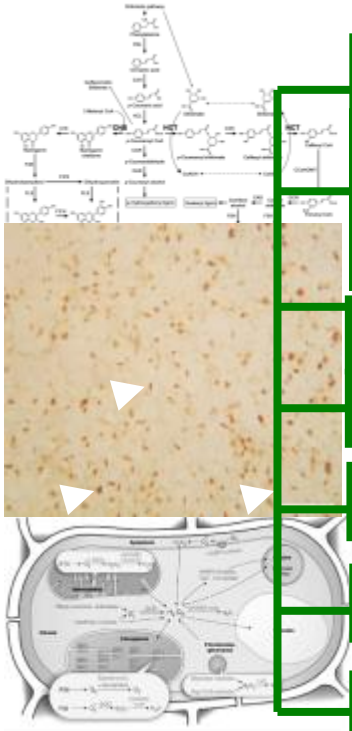
Secondary metabolism
Jasmonate signalling
Biotic stress
Response to stimulus
PR protein expression

↓

Polyamine oxidase

↓

Secondary metabolism
Receptor kinases (S-locus)
Down-regulation of protease inhibitors
Differentialy expression of carbohydrate metabolism



- Stress responses
- Catabolism and byosinthesis of the L-phenylalanine
- Programme cell death processes
- Oxidoreductase activity
- Cell wall catabolism
- Cell wall modification
- Glucosyltransferase activity

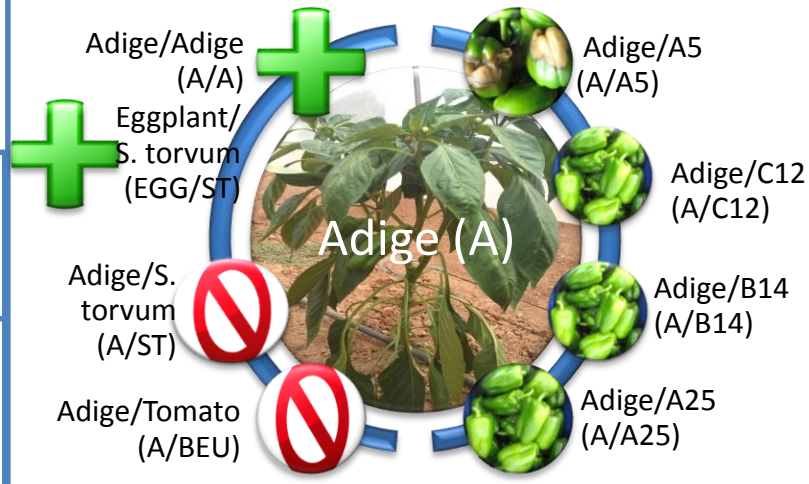
↕

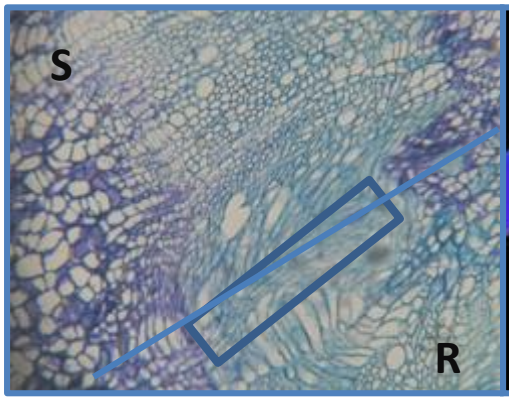
Complex agronomic trait – polygenic trait

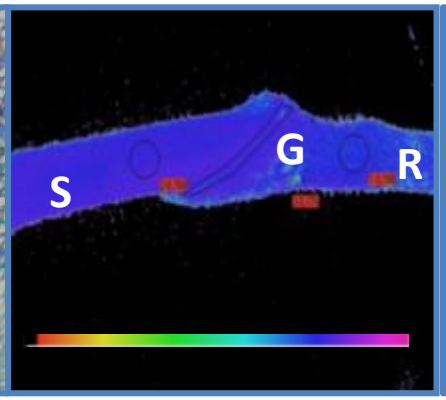
Cookson et al. 2013; *Journal of Experimental Botany*, 64 (10), pp. 2997–3008

CFI - Chlorophyll Fluorescence Imaging

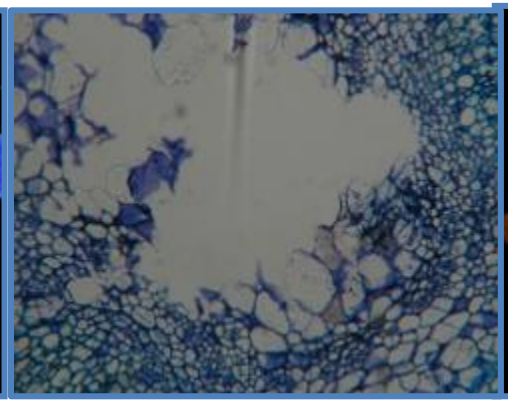
Evaluate the potencial of CFI	• To predict compatibility/ incompatibility
Connecting values of CFI	• To histological studies
Demonstrate if CFI reflects	• Morphological • Anatomical development

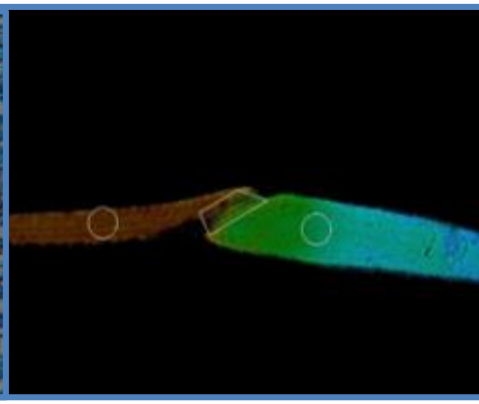






- Strong unions — Highest Fv/Fm values





- Weak unions — Lowest Fv/Fm values

Penella et al. 2016; submitted

Conclusions

Identification of root(stock)-derived signals for improved rootstock / scion performance: **take advantage of new metabolomics & proteomics techniques on vascular sap collected in reciprocal grafting experiments under different conditions.**

Key-mechanisms involved in graft (in)-compatibility:

- **cell-to-cell communication,**
- **oxidative stress tolerance in both graft partners**
- **stress induced genes**

Deliveries

Rootstock breeders: selection of stress-tolerant rootstocks (biomarkers) based on: **production of cytokinin (salt), jasmonic acid (drought) and ABA (multi stresses) sensitivity for ethylene (cold)**

Nurseries / Growers: compatibility test of rootstock and scion genotypes based on: **ultrastructural studies, gene expression analysis, and a quick and non-invasive technique for screening (in)-compatible unions.**