FUNCTIONAL CHARACTERISATION OF CUTICLE-RELATED GRAPEVINE GENES

J. Jolliffe[1,2], J. Lashbrooke[1], L. Vittani[2,3], L. Dalla Costa[2], M. Malnoy[2], S. Pilati[2] and C. Moser[2]

[1]South African Grape and Wine Research Institute, Stellenbosch University, Stellenbosch, 7600, South Africa, [2]Research and Innovation Centre, Edmund Mach Foundation, San Michele all'Adige, 38010, Italy, [3]C3A - Centro Agricoltura Alimenti Ambiente, Edmund Mach Foundation, San Michele all'Adige, 38010, Italy.

BACKGROUND

The epidermis of aerial plant tissues is covered by a waxy cuticular layer which acts to protect the plant against abiotic and biotic stresses, such as nonstomatal water loss and pathogen infection, as well as contributes towards postharvest fruit quality traits such as colour, texture and shelf life. Despite its economic significance, there are still considerable knowledge gaps concerning cuticle biosynthesis and its regulation in grapevine. However, in a recent study done by Leida *et al.* (2016), *VviERF045* was identified as a potential negative cuticle regulator since its overexpression caused impaired cuticle development. The aim of this study is to establish a greater understanding of *VviERF045*'s role in cuticle formation by identifying and characterising its putative regulatory pathway members.

METHODOLOGY

1 Identification of *VviERF045* regulatory pathway members

- **Select** putative cuticle-related genes from *VviERF045*-OE RNAseq data
- **Clone** promoter regions (> 1kb) of selected genes
- **Perform** dual-luciferase assay via agroinfiltration of *N. benthamiana*

2 Functional characterisation of *VviERF045* regulatory pathway members

Overexpress genes in **tomato** (cv. Ailsa Craig)

Overexpress/knockout genes in **grapevine** (cv. Sugraone)

Phenotyping: cuticle staining, SEM, stomatal and trichome density etc.

RESULTS

1

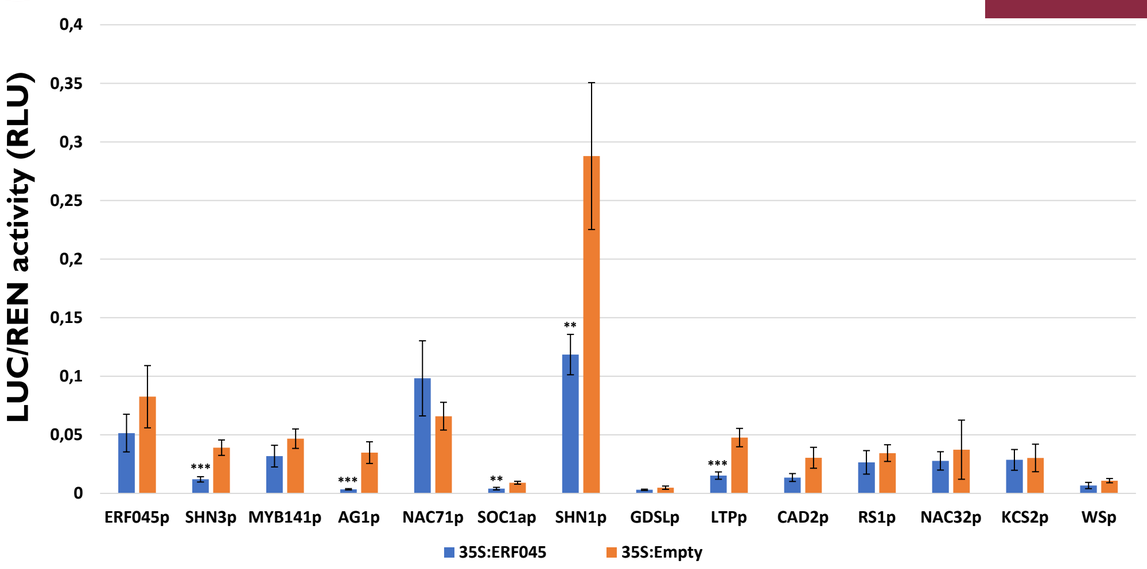

Fig. 1 Results of the dual-luciferase assay indicate that *VviERF045* represses the transcription of *VviSHN3*, *VviAG1*, *VviSOC1a*, *VviSHN1* and *VviLTP*. Asterisks represent significant differences between the 35S:Empty-promoter and 35S:ERF045-promoter combinations according to Student's *t*-test (***$P < 0.01$, **$P < 0.05$). Error bars represent SD of n=5.

2

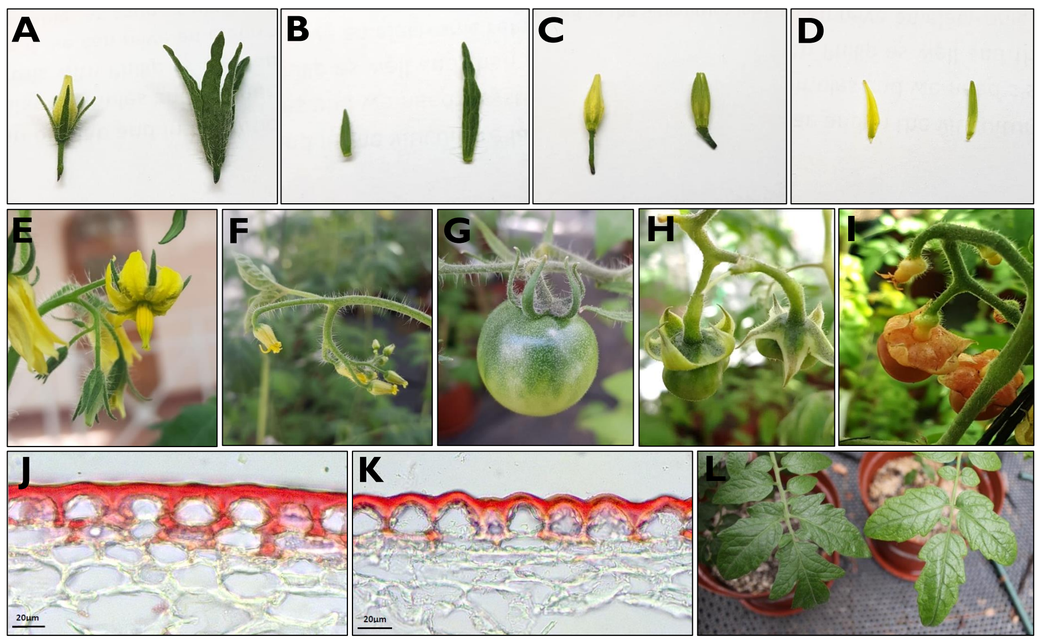

Fig. 2 Preliminary phenotypic analyses of overexpressing tomato (cv. Ailsa Craig) lines. Sepals enlarged (**A-B**) and petals converted into sepal-like structures (**C-D**) in *VviSOC1a*-OE (right) compared to WT (left) flowers at similar developmental stages. Sepals converted into carpel-like organs in *VviAG1*-OE lines (**E-I**), encapsulating flowers (**F**) in comparison to WT (**E**), turning fleshy during green stages of fruit development (**G**= WT; **H**= *VviAG1*-OE) and ripening along with fruit (**I**). Cuticle thickness reduced and epidermal conical cell shape more pronounced in Sudan IV-stained tissue sections of *VviMYB141*-OE immature green fruit (**K**) compared to control (**J**). (**L**) *VviSHN3*-OE plants (right) displaying a typical shiny surface compared to WT (left).

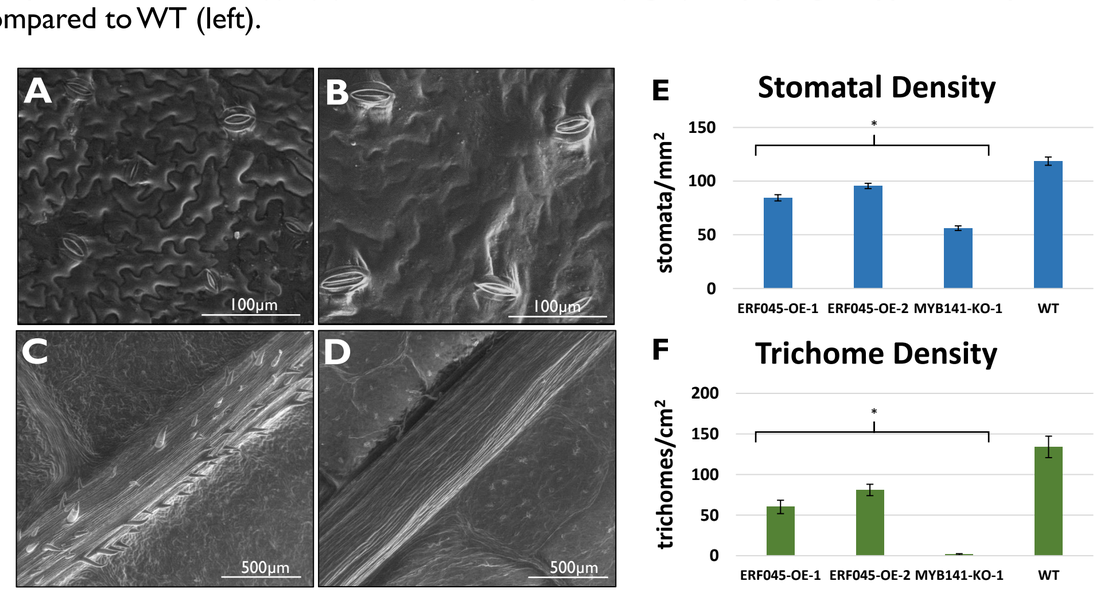

Fig. 3 Alterations to pavement cell shape, trichome density and stomatal density in knockout and overexpressing grapevine (cv. Sugraone) lines. (**A-D**) ESEM images illustrating flatter pavement cells on abaxial leaf surfaces of *VviMYB141*-KO plants (**B**) compared to WT (**A**), and an absence of abaxial vein trichomes (**C**= WT; **D**= *VviMYB141*-KO). (**E-F**) Statistically significant reduction (*$P < 0.01$) in stomatal and trichome densities, according to Student's *t*-test, for *VviERF045*-OE lines (n = 3) and *VviMYB141*-KO lines (n = 2).

CONCLUSIONS

- **Dual-luciferase assay** identified ***VviSOC1a***, ***VviAG1***, ***VviSHN1***, ***VviSHN3*** and ***VviLTP*** as downstream **repressed** targets of ***VviERF045***.
- Transgenic **phenotypes** suggest **involvement** of selected **genes**:
  - Floral organ identity (***VviSOC1a*** and ***VviAG1***)
  - Fruit ripening (***VviAG1***)
  - Cuticle deposition (***VviMYB141*** and ***VviSHN3***)
  - Conical cell formation (***VviMYB141***)
  - Trichome and stomatal density (***VviERF045*** and ***VviMYB141***)

REFERENCES

- Leida, C., Dal Rì, A., Dalla Costa, L., Gómez, M.D., Pompili, V., Sonego, P., Engelen, K., Masuero, D., Ríos, G. and Moser, C., 2016. Insights into the role of the berry-specific ethylene responsive factor *VviERF045*. *Frontiers in Plant Science*, *7*, p.1793.