Synthesis of Grapevine Chimeras

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Project: Developement of Grapevine Synthetic Chimeras

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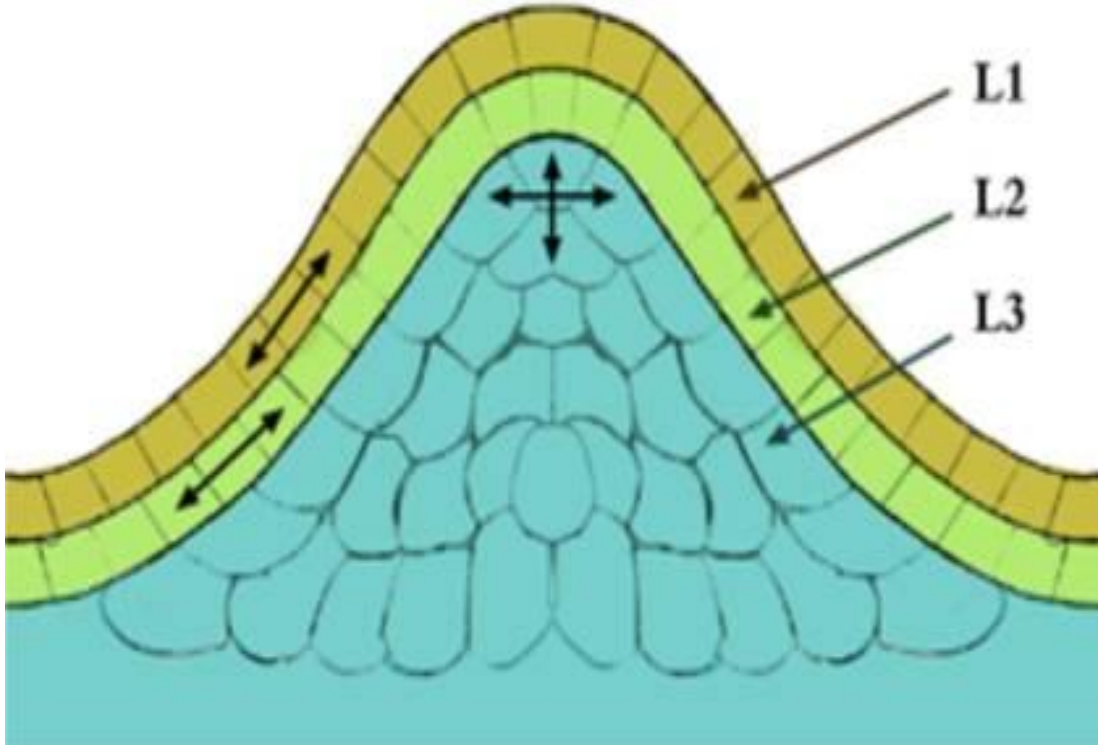
Plant chimeras

- plants which contain tissues of at least two genotypes in different cell layers
- *Tunica-corpus* model– higher plants have 2 or 3 cell layers in apical meristem
- Cells in the outermost layer(s) divide only anticlinally

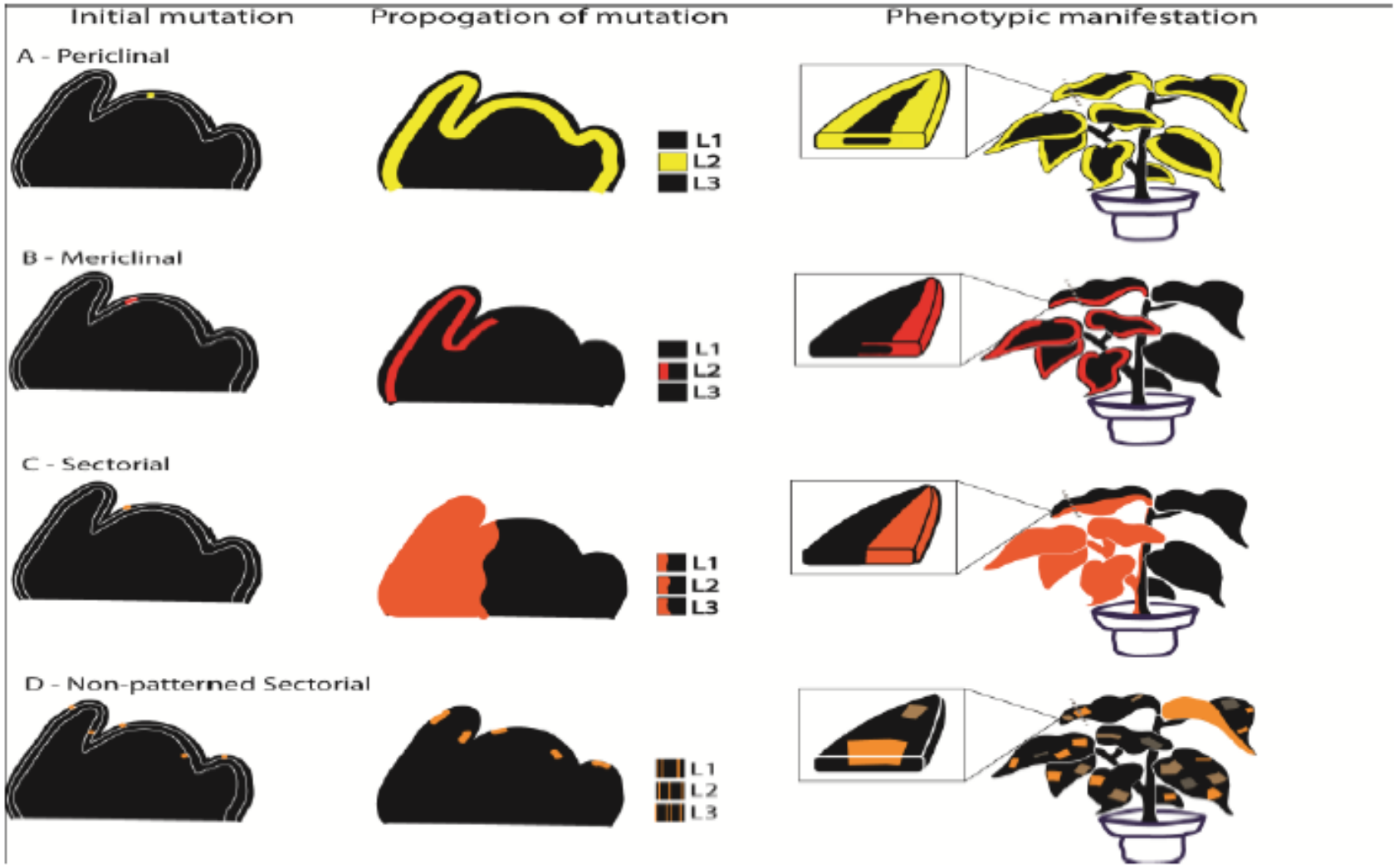
- Cell layers of apical meristem preserve their integrity - future organs and tissues

- **Grapevine – (in most cases) two layers - L1 i L2**

- L1 – epidermal tissue (leaf, berry…)
- L2 - other tissues – inner part of leaf, berries, adventive roots…



Types of chimeras



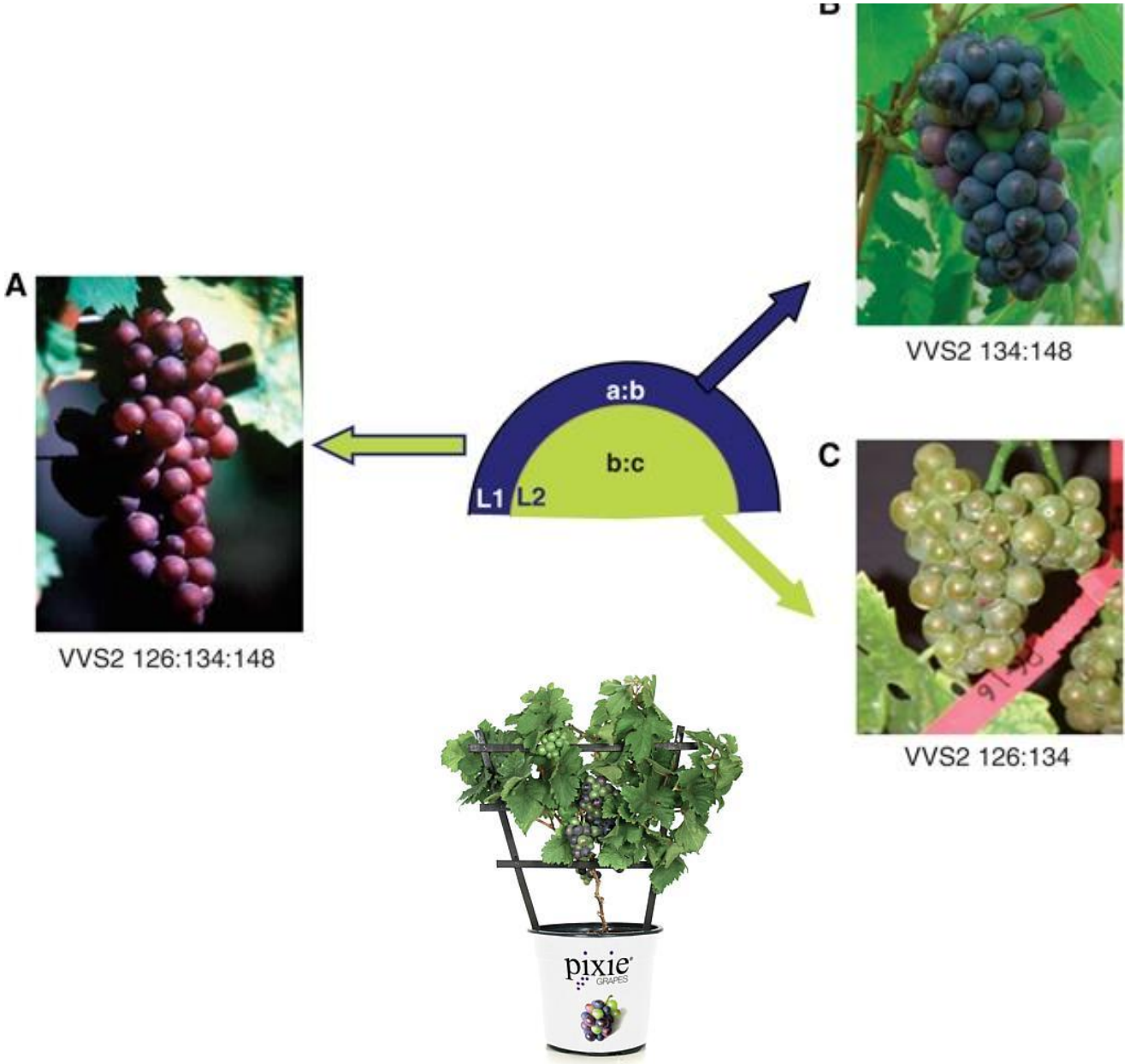
Margaret H. Frank, Daniel H. Chitwood, Plant chimeras: The good, the bad, and the 'Bizzaria', Developmental Biology, Volume 419, Issue 1, 2016, Pages 41-53,

Grapevine chimeras

- Manny cultivars (clones) are chimeras – mutations in one of the cell layers
- Pinot gris, Pinot meunier, some Chardonnay clones, Plavac mali sivi

- They can be separated using somatic embryogenesis

- Eg. - npr. Pixie (*dvarf vine, microvine*) L1 layer mutation from Pinot meunier



Pelsy, F. (2010). Molecular and cellular mechanisms of diversity within grapevine varieties. *Heredity*, *104*(4), 331-340.

Synthetic chimeras – chimeral breeding

- Developed from two different genotypes
  - Cultivars (or even species)

- Different methods used:
  - **Graft-chimeras** - Adventive shoots from mixed callus developed after grafting - *in-vivo*

  - Known samples of graft-chimeras.:
    - *Citrus medica i C. aurantium; - Bizzaria*
    - *Laburnum anagyroides i Cytisus purpureus;*
    - *Crataegus monogyna i Mespilus germanica;*
    - *Cassava (Manihot esculenta + Manihot fortalezensis)*

  - ***In-vitro*** **-** Shoots developed from callus after micrografting or contact cultivation of two genotypes

Only one reseach in case of grapevine

- Verdisson (1998., 1999.) – Champagne (France) – Chardonnay + Pinot noir
- *In-vitro* – mixed callus from grafted nodal and internodal segments – organogenesis – no results preserved (personal communication)

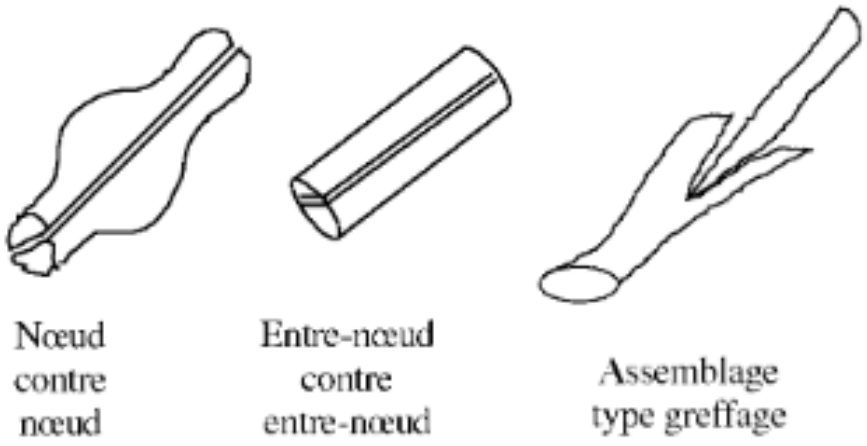


Fig.1 - Types d'assemblages réalisés entre le Chardonnay 7535 et le Pinot noir 7613

Fig.1 - Different types of assemblages performed

Potential for grapevine breeding

- Alternative method for grapevine breeding

- Combination of existing genotypes
- Resistant genotype as protection for non-resistant

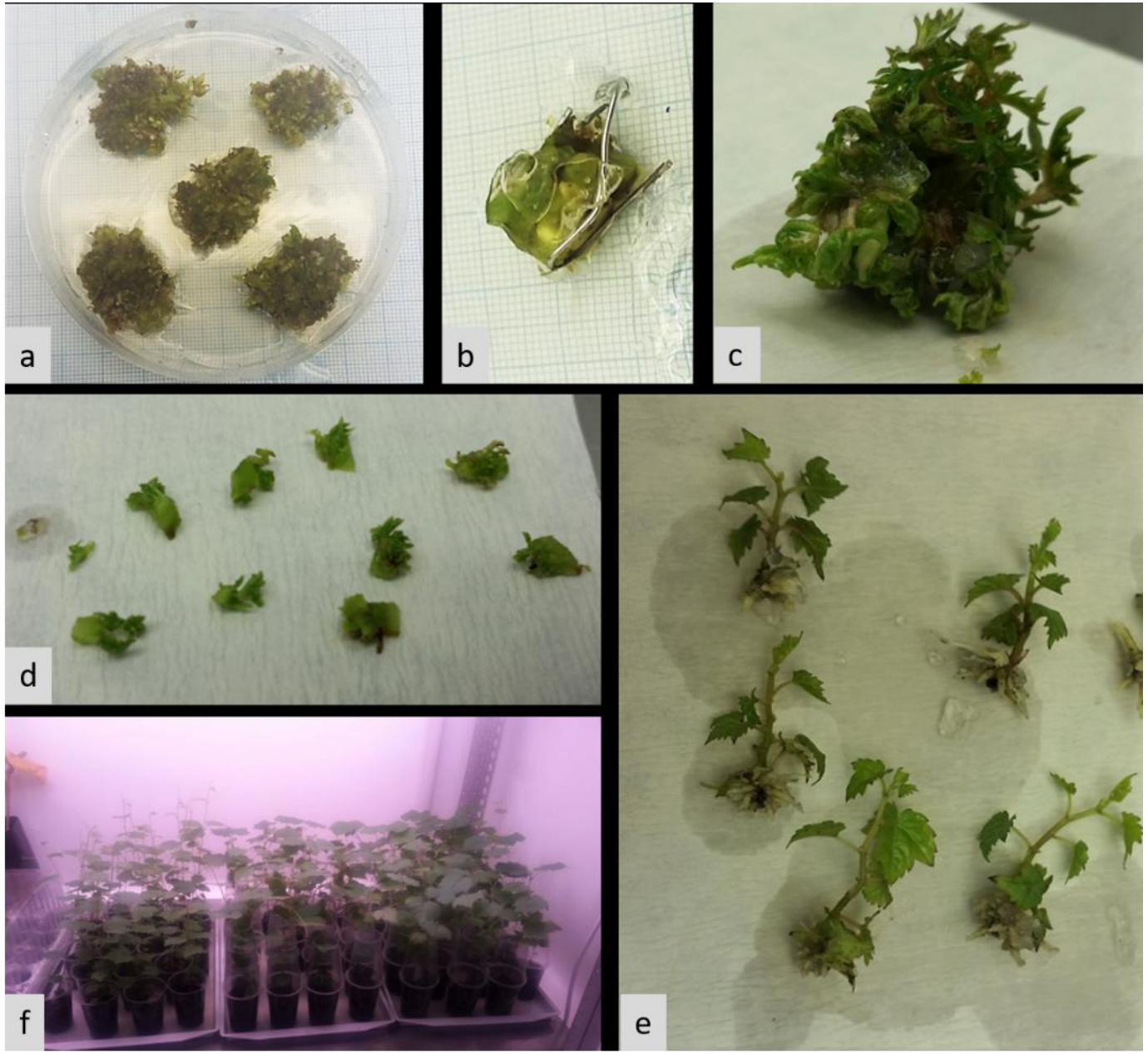
- „hand in glove”

Our approach

- In – vitro Meristematic Bulk Tissue - MBT (Mezzeti i sur. 2002.) grafting
- High regeneration capacity of MBT
- (Alternative for somatic embryogenesis for genetic transformation)

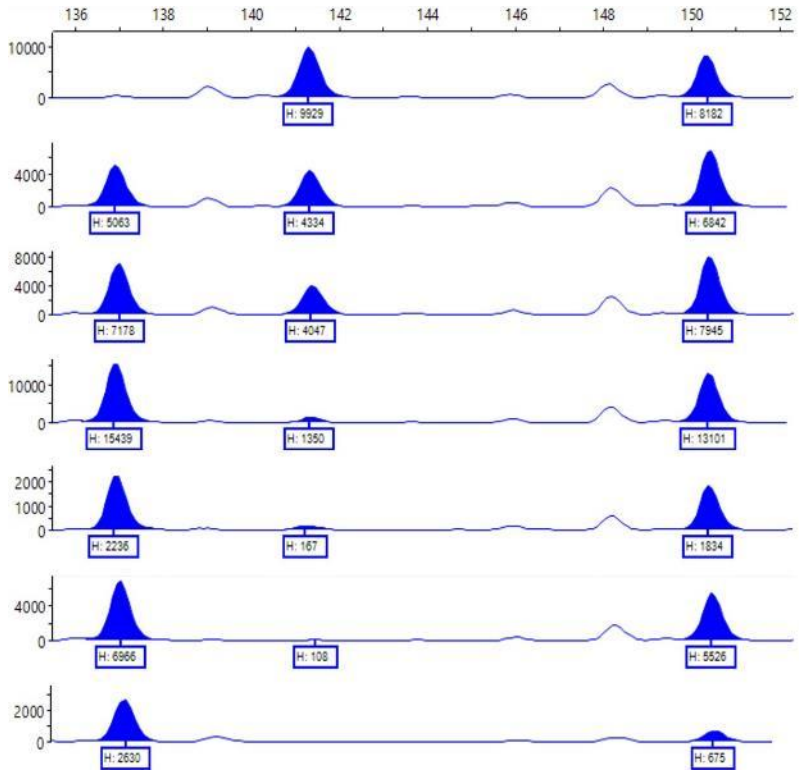
- Development of mixed MBT from two genotypes

- Regeneration of plants



Detection of chimeral plants

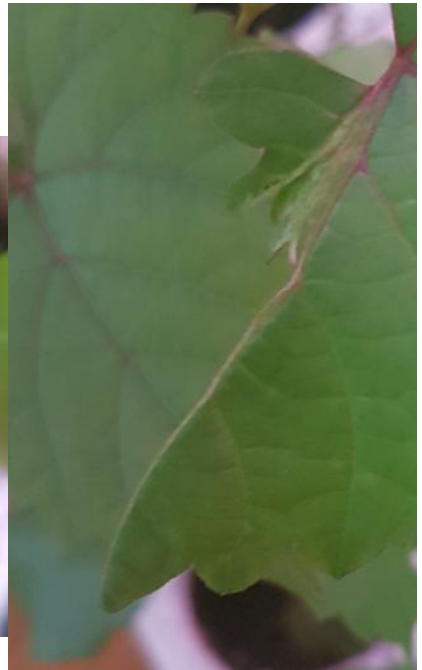
- Phenotyping
- Genotyping – SSR markers
  - Detection and quantification



Results

- Cabernet sauvignon + Babić
  - 118 plants regenerated grafted MBT
  - 6 plants with different types of mosaics on leaves – heterogenic phenotype
  - 15 plants corresponding to Babić's phenotype
  - Others Cabernet sauvignon

- Cabernet sauvignon + Chardonnay
  - 142 plants regenerated
  - All plants with uniform phenotype corresponding to Chardonnay



Results

- Cabernet sauvignon + Babić
  - 6 plants with heterogenic phenotype – SSR profile of both genotype detected
  - 1 additional plant with uniform phenotype displayed profile of both genotypes.
- Cabernet sauvignon + Chardonnay
  - Only one plant displayed profile of both genotypes
  - Uniform phenotype

Field evaluation in prgress

Conclusion

MBT grafting shows promising results in development of grapevine synthetic chimeras

Ongoing research

- Interspecific chimeras development (Vitis vinifera + other Vitis sp.)
- Stability and organization of chimeras
- Reorganization of sectorial to periclinal chimeras

Thank you for your attention