

# In vitro early detection of grapevine virus-induced graft incompatibility in Syrah/R110: the involvement of GRSPaV

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## 01 INTRODUCTION

In viticulture, grafting is used to propagate European grapevines (*Vitis vinifera*) susceptible to phylloxera (*Daktulosphaira vitifolia* Fich.) using resistant American rootstocks. However, graft incompatibility manifests in short-term graft failure or long-term decline affecting grape yield and the vineyard longevity.

In this work, we:

- Assess the suitability of *in vitro* systems as early detection methods for grapevine incompatibility
- Provide evidences for the involvement of *Grapevine Rupestris Stem Pitting associated Virus* (GRSPaV) in Syrah/Ritcher-110 (110R) graft incompatibility,

using certified homografted (i.e., a graft between the same genotype) and heterografted (i.e., a graft between two genotypes) *in vitro* grapevine plants, with known compatibility response when grafted onto 110R rootstock (*V. berlandieri* x *V. rupestris*)

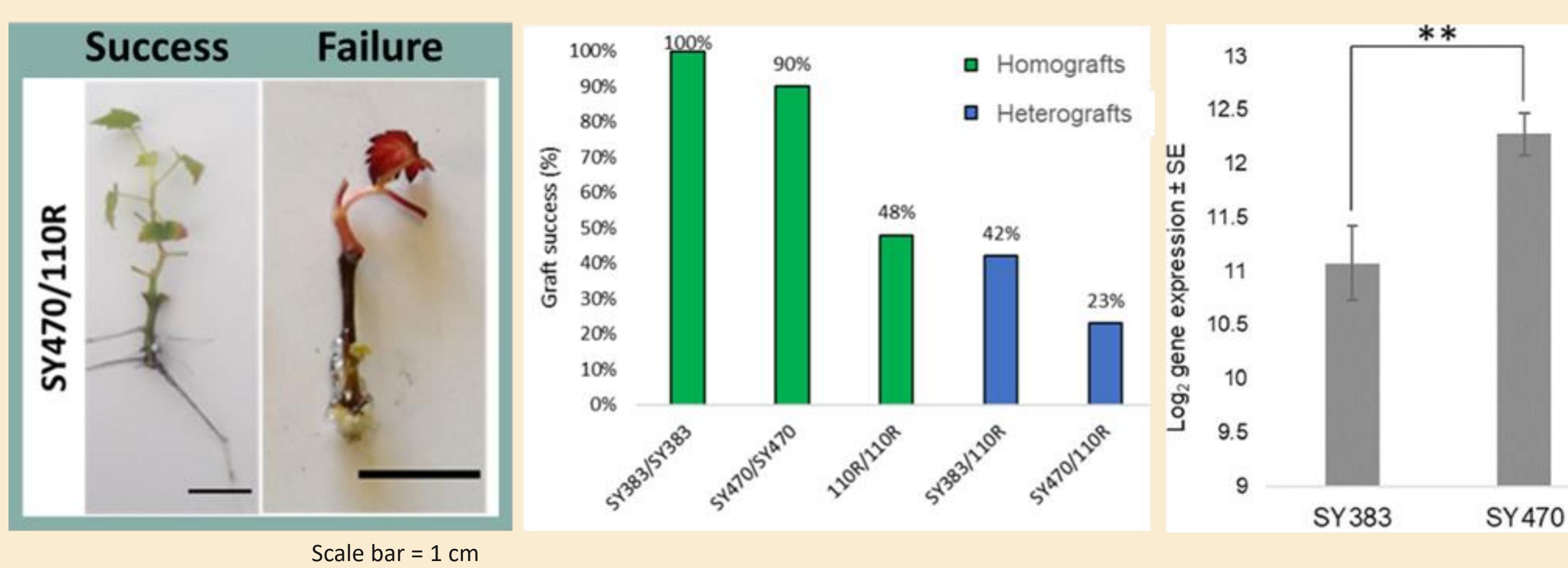
### Graft Incompatibility Classifications:

- Translocated incompatibility** when associated with starch accumulation and phloem degeneration,
- Localized incompatibility** when characterized by weakness of the graft union,
- Virus-induced incompatibility** when due to viral infections.

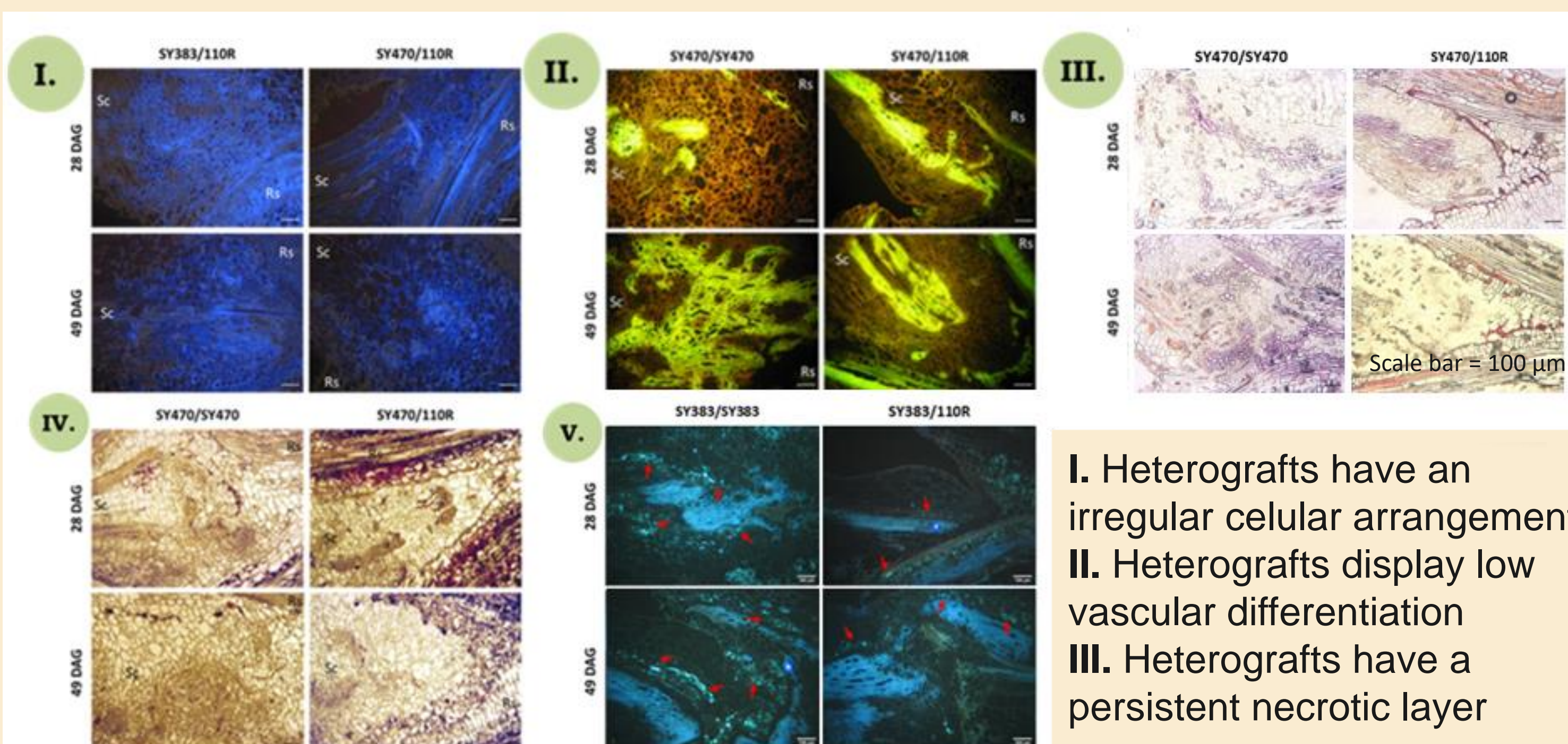
## 03 RESULTS AND DISCUSSION

### 3.1 Graft success and histochemistry

- Failing heterografts display a viral phenotype
- Levels of GRSPaV correlate with graft success



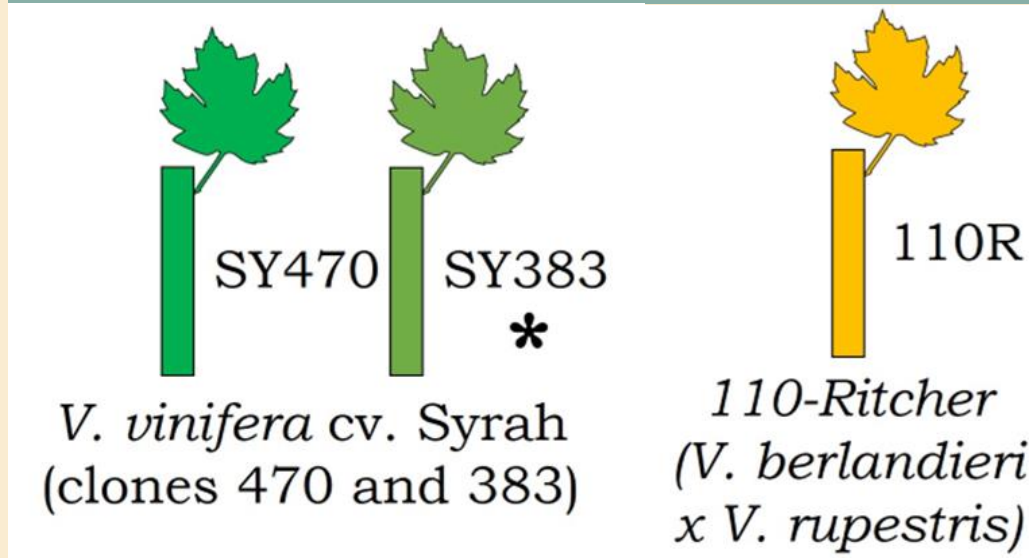
- Successful heterografts display translocated graft incompatibility:



- Heterografts display starch accumulation
- Heterografts are depleted in callose indicating development of new phloem cells

## 02 MATERIALS AND METHODS

### 2.1. Plant material



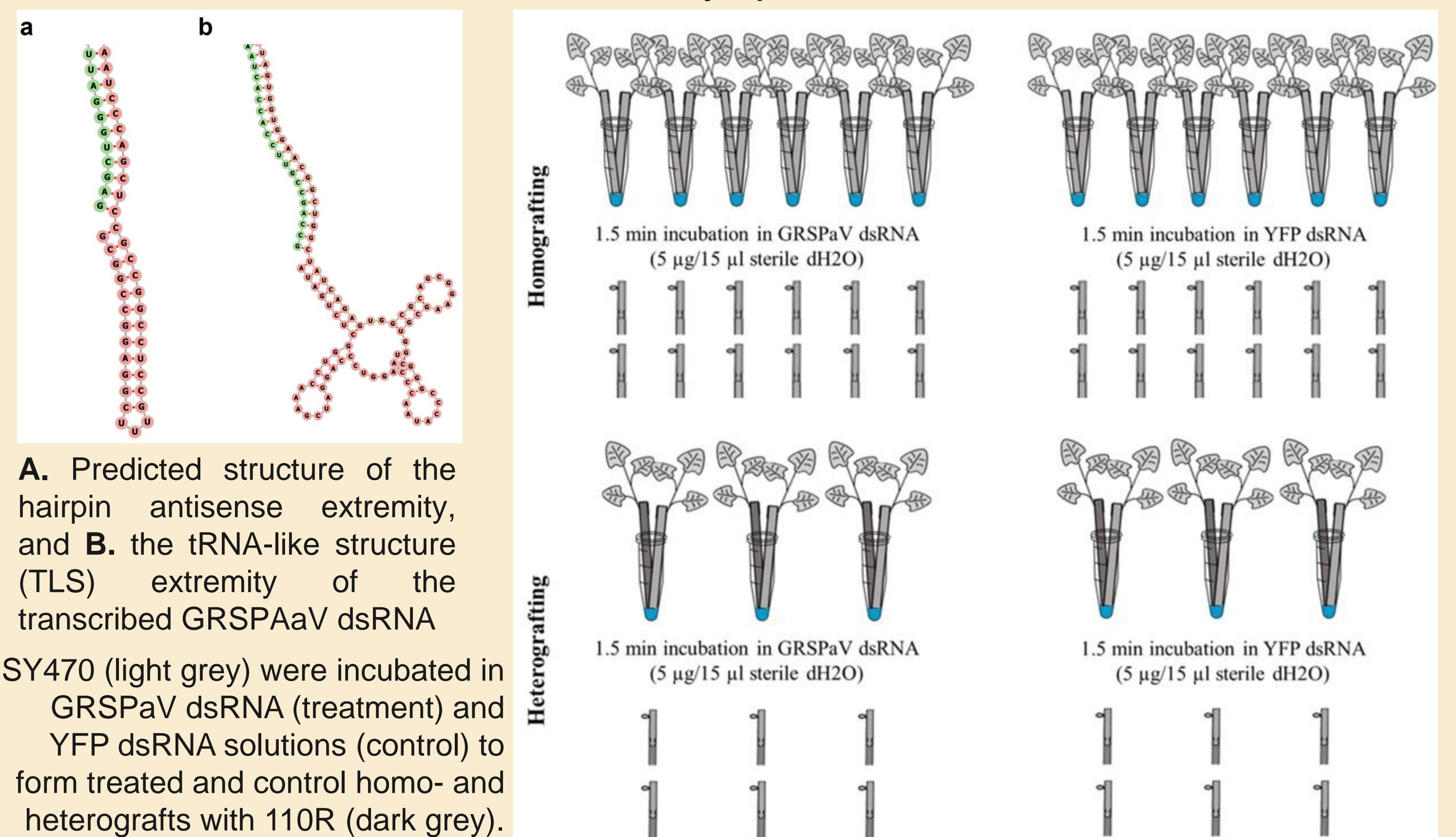
- The absence of the EU certification mandatory viruses was confirmed by ELISA.
- Micrografts of SY383/SY383, SY470/SY470, 110R/110R, SY383/110R, and SY470/110R were performed.

### 2.2 Graft success and histochemistry evaluation

- Grafts were considered successful when scion growth and/or rooting of the rootstock was observed at 49 days after grafting (DAG).
- Histochemical analysis of the graft unions at 28 and 49 DAG with: Calcofluor to stain cellulose; Acridine orange for differentiating xylem; Phloroglucinol-HCl for lignin; Potassium iodine (I<sub>2</sub>KI) for starch; Aniline blue for callose.

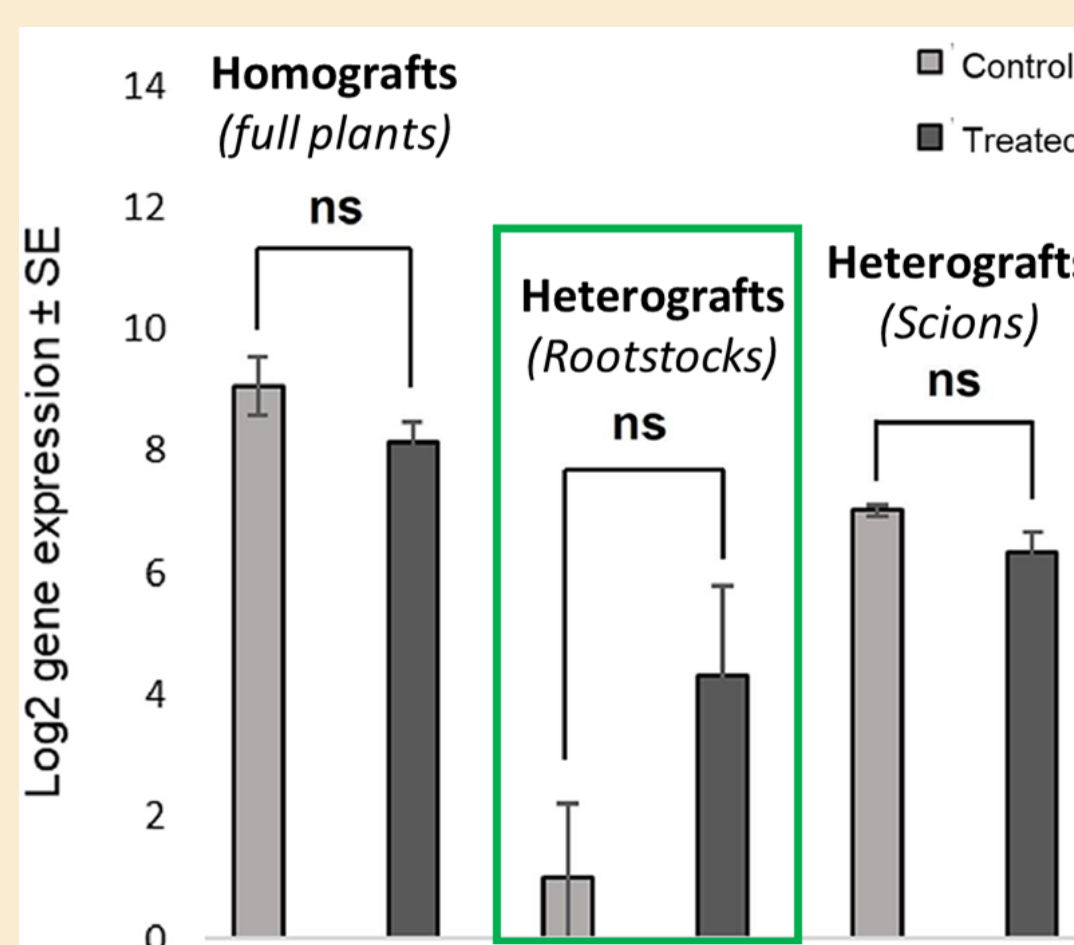
### 2.3 GRSPaV dsRNA construction and delivery

One of the most representative GRSPaV transcript variants found in SY470 served as a template to produce a dsRNA construct targeting the viral coat protein and was delivered in SY470 homo- and heterografts just before grafting. At 49DAG, graft success and GRSPaV levels were assessed by qRT-PCR.



SY470 (light grey) were incubated in GRSPaV dsRNA (treatment) and YFP dsRNA solutions (control) to form treated and control homo- and heterografts with 110R (dark grey).

### 3.2 Rescue of Syrah/110R incompatibility after GRSPaV silencing



- Graft success was rescued in GRSPaV dsRNA-treated grafts
- More viral transcripts translocated scion-to-rootstock in GRSPaV silenced heterografts.

49 DAG Graft success	
Homografts control (YFP dsRNA-treated)	80%
Homografts treated (GRSPaV dsRNA-treated)	100%
Heterografts control (YFP dsRNA-treated)	67%
Heterografts treated (GRSPaV dsRNA-treated)	100%

Incompatibility seems due to the rootstock hypersensitivity to scion-derived viruses

## 04 CONCLUSIONS

We propose the involvement of GRSPaV in Syrah/110R incompatibility and alert that grapevine incompatibility is a virus-induced phenomenon that can arise even in certified plants.