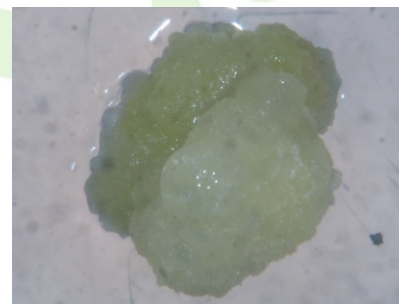
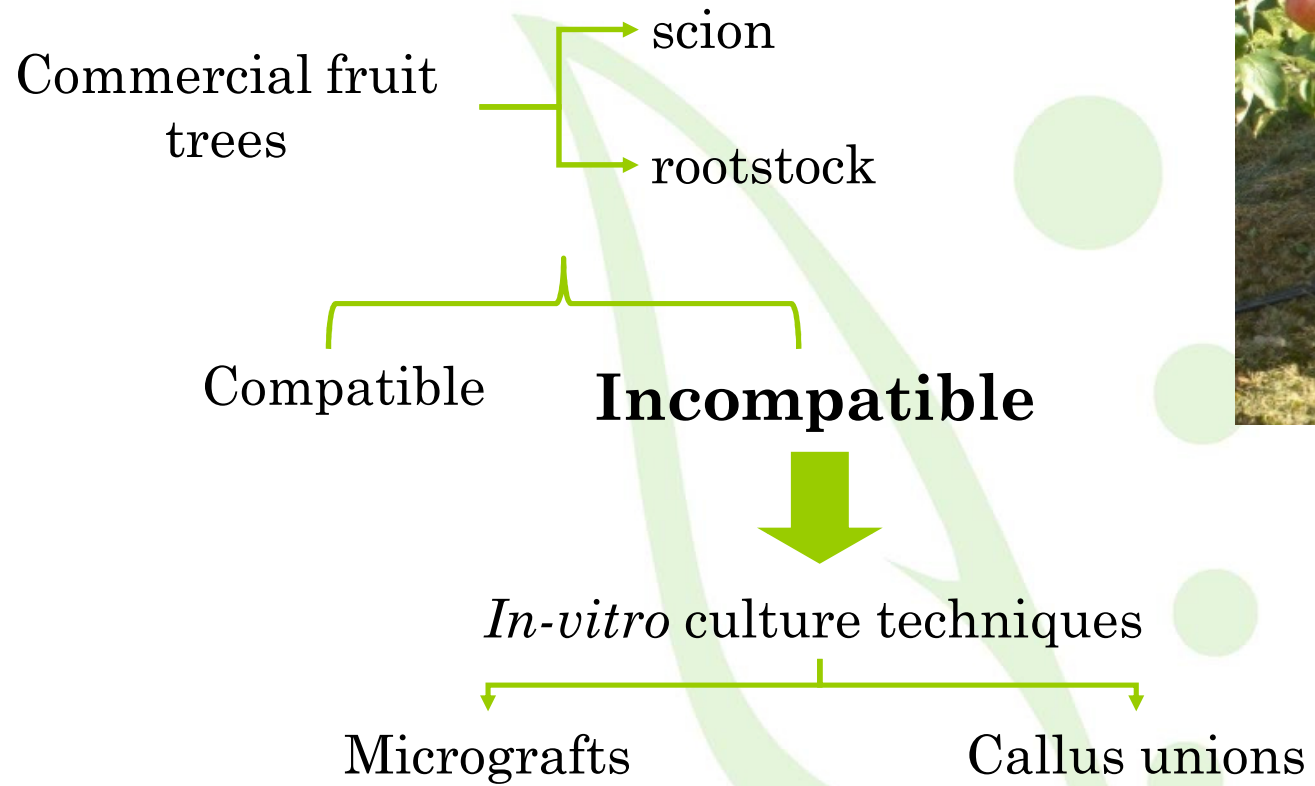
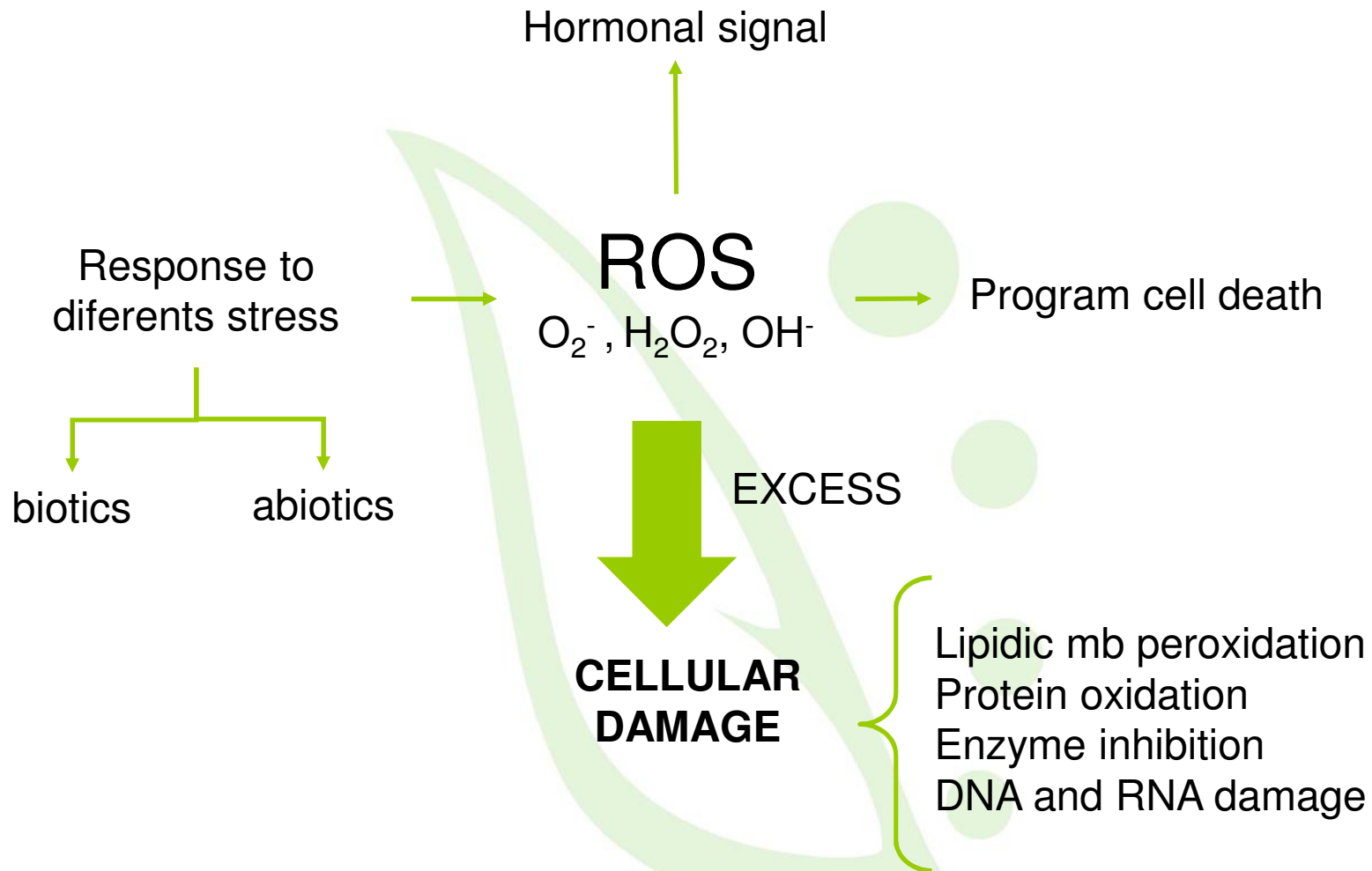


OXIDATIVE STRESS ASSOCIATED WITH ROOTSTOCK-SCION INTERACTIONS

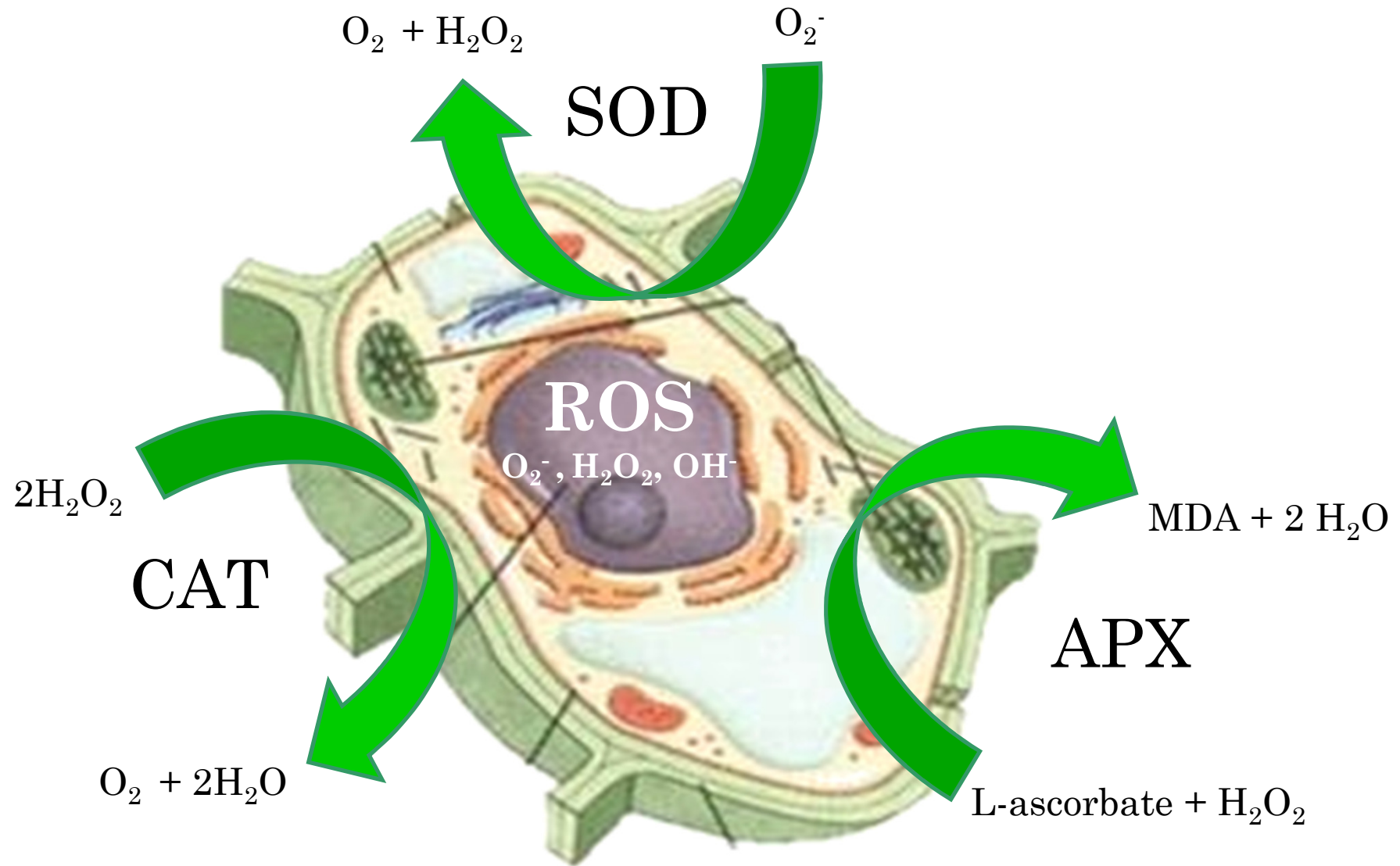
Patricia Irisarri, Helle Juel Martens, Pilar Errea, Ana Pina
Fruit-tree Department
Agrifood research and Technology Centre of Aragón (CITA)
Zaragoza, Spain

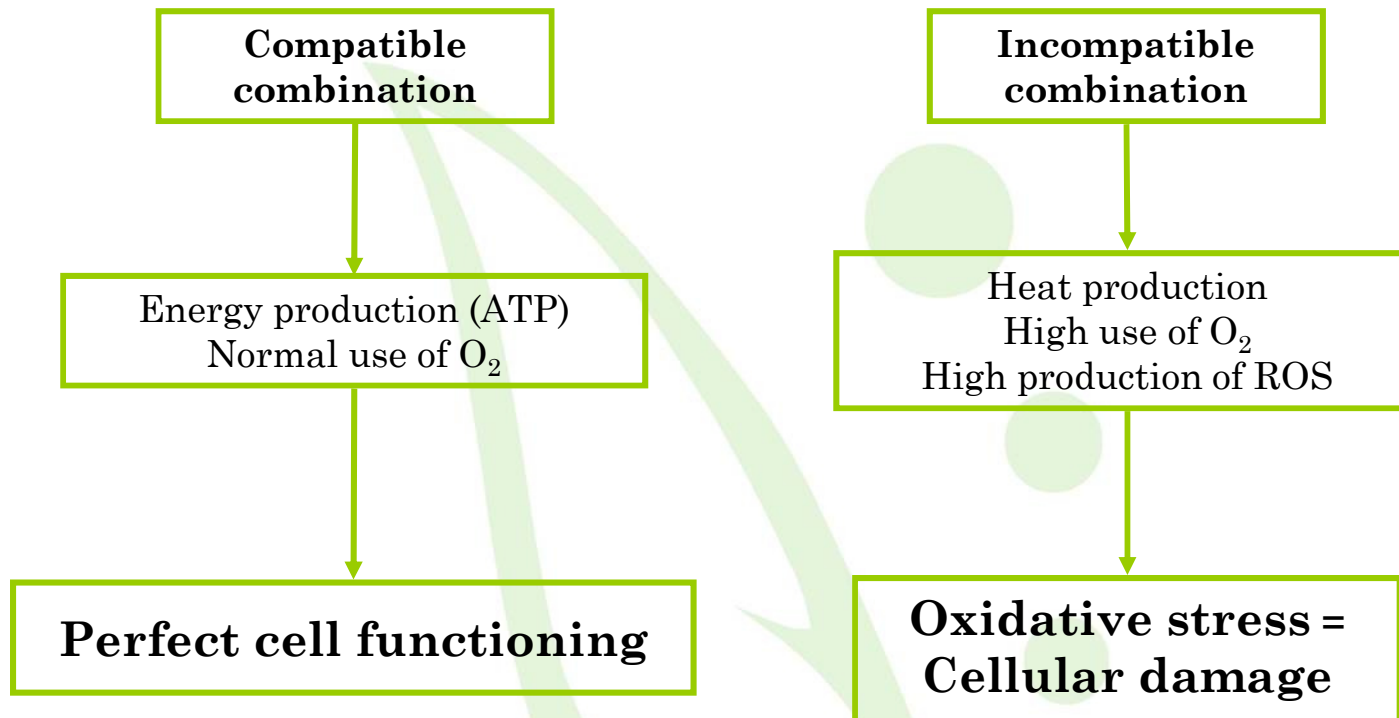
INTRODUCTION





All stress situations disturb cellular metabolism and the unbalance production / elimination of ROS lead to its accumulation and impose conditions of oxidative stress.





The oxidative stress as a mechanism that could trigger the degradation of tissues and cells in the incompatible graft union, due to a high accumulation of ROS.

OBJETIVE

To study different antioxidant enzymes in combinations of pear / quince with different degrees of compatibility at the early stages of development.

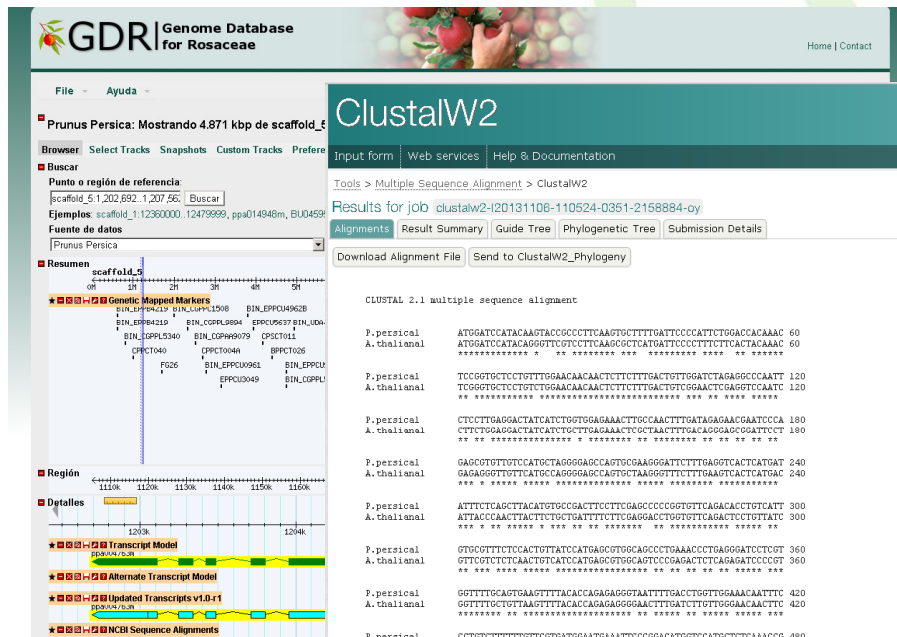
- ❖ **Determination of gene expression and activity of antioxidant enzymes**
 - ❖ Superoxide dismutase (SOD)
 - ❖ Catalase (CAT)
 - ❖ Ascorbate peroxidase (APX)

- ❖ ***In vivo* ROS detection**

MATERIAL and METHODS

In silico analysis

Antioxidant enzymes are the most important components in the scavenging system of ROS and mostly they exist in some plant species as a family of genes



ClustalW2

Input form | Web services | Help & Documentation

Tools > Multiple Sequence Alignment > ClustalW2
Results for job clustalw2-i20131108-110524-0351-2158884-oy

Alignments | Result Summary | Guide Tree | Phylogenetic Tree | Submission Details

Download Alignment File | Send to ClustalW2_Phylogeny

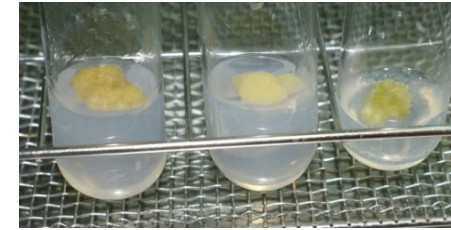
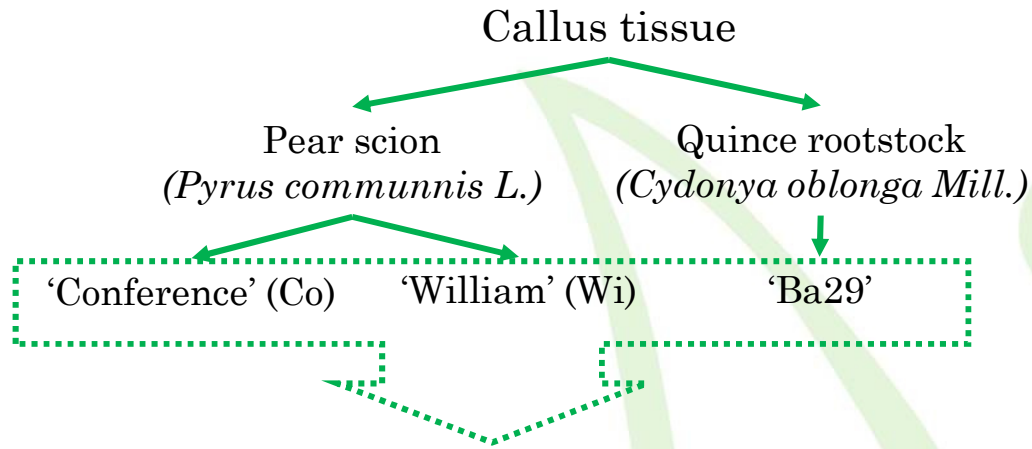
CLUSTAL 2.1 multiple sequence alignment

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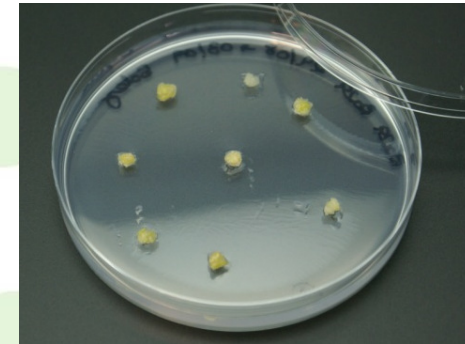
Assembled for these sequences → specific primers were designed



MATERIAL and METHODS

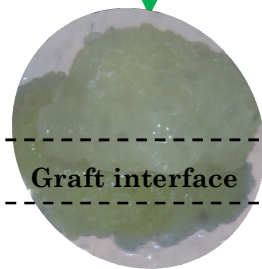


Co Wi Ba29



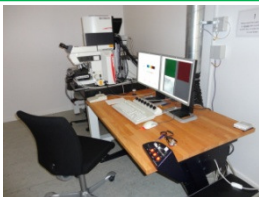
Homografts	Heterografts	Control
Co/Co	Co/BA29 (+)	Ungrafted
Wi/Wi	Wi/BA29 (-)	Wounded tissue
BA29/BA29		Co, Wi, BA29

1,3,5,7,10 and 21DAG



Graft interface

In vivo ROS detection



Protein extraction

Enzymatic activity measure



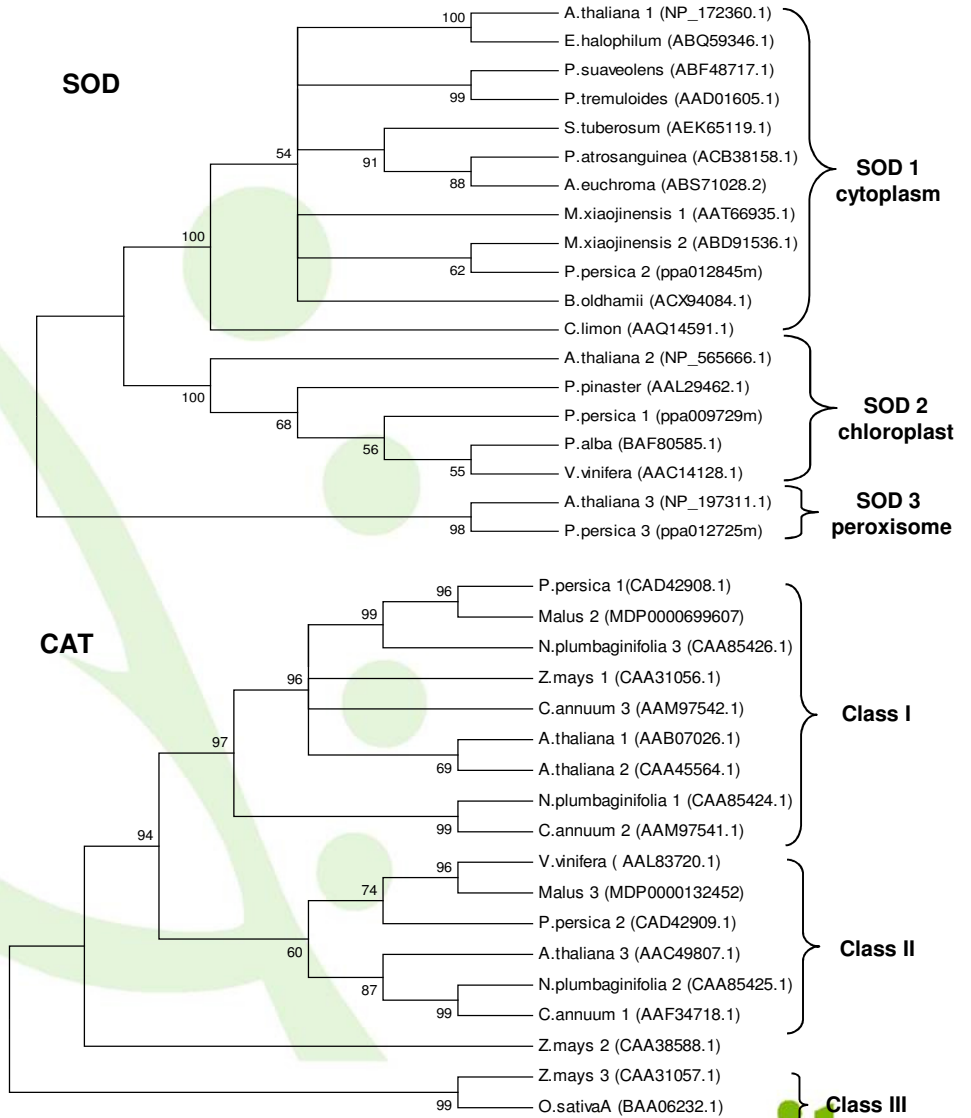
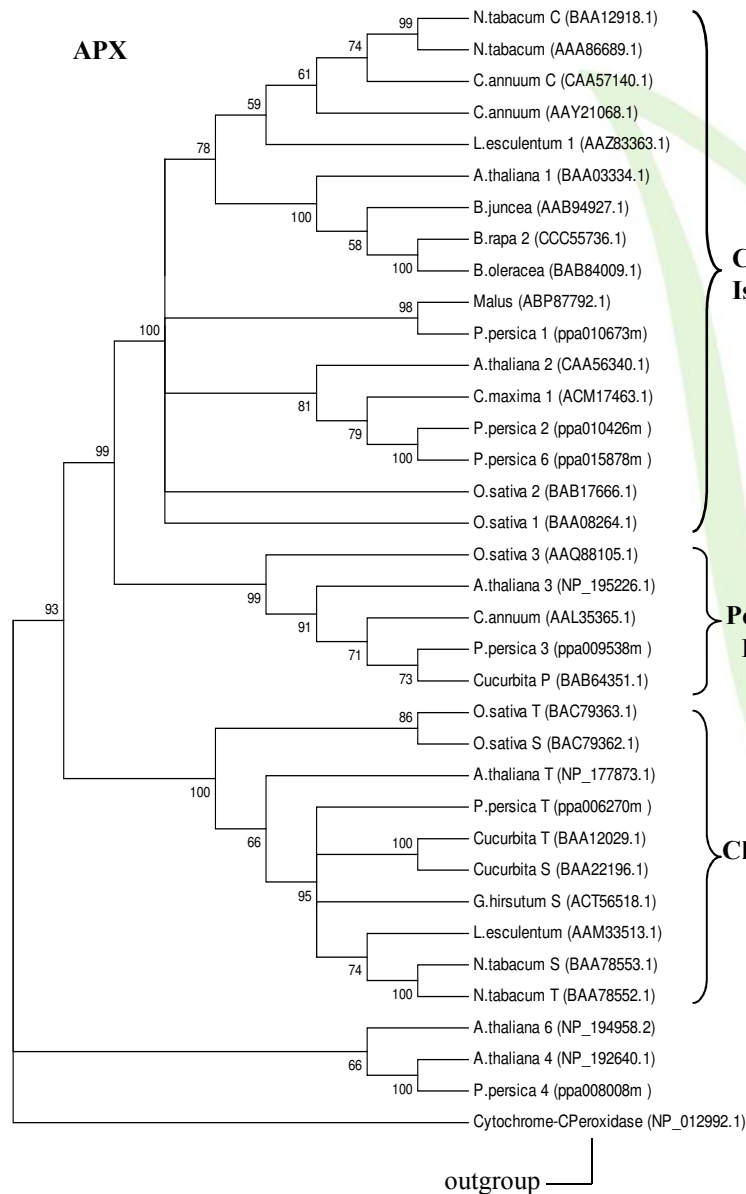
RNA extraction

qRT-PCR

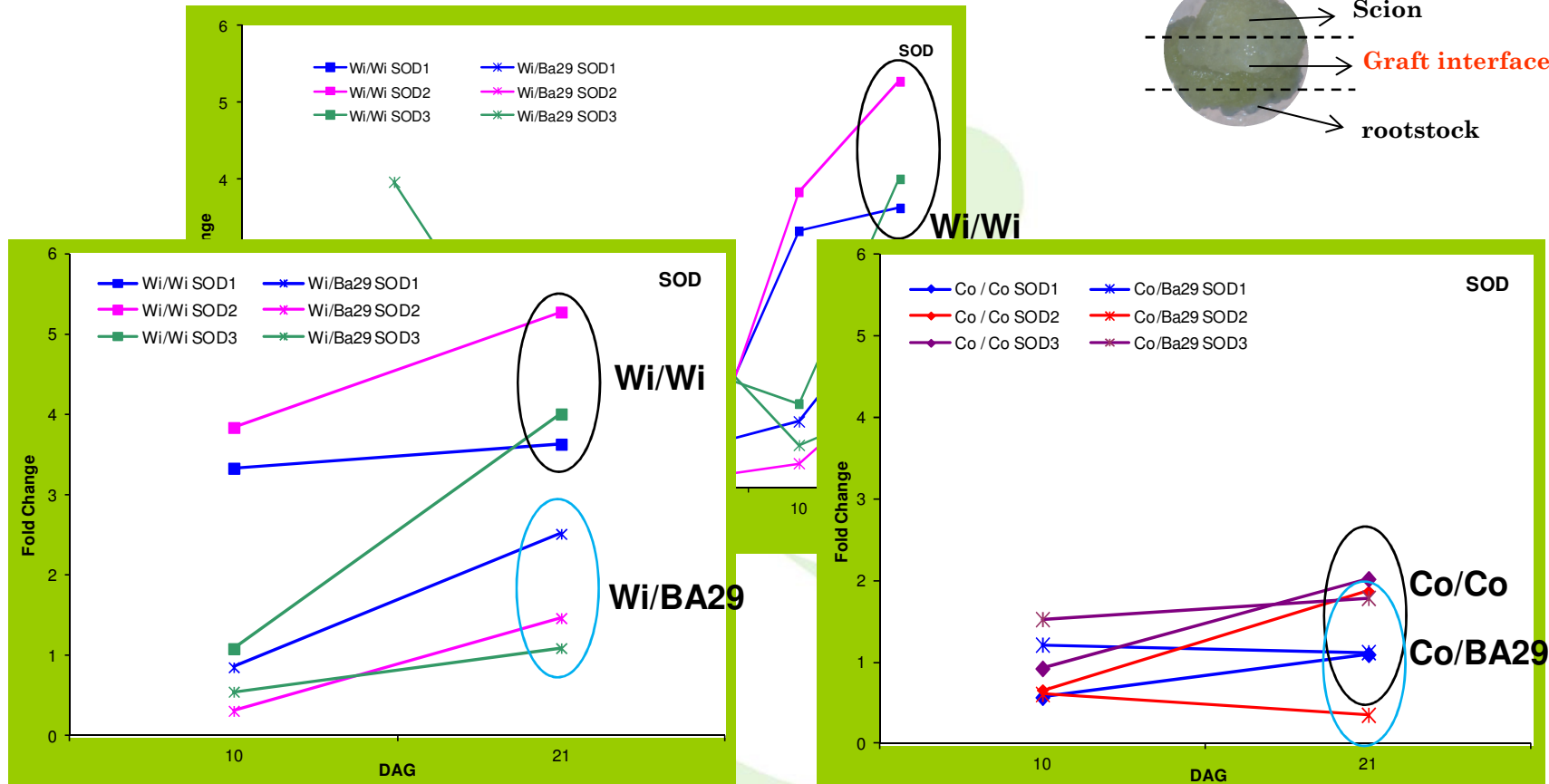
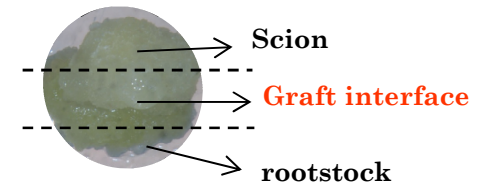


RESULTS

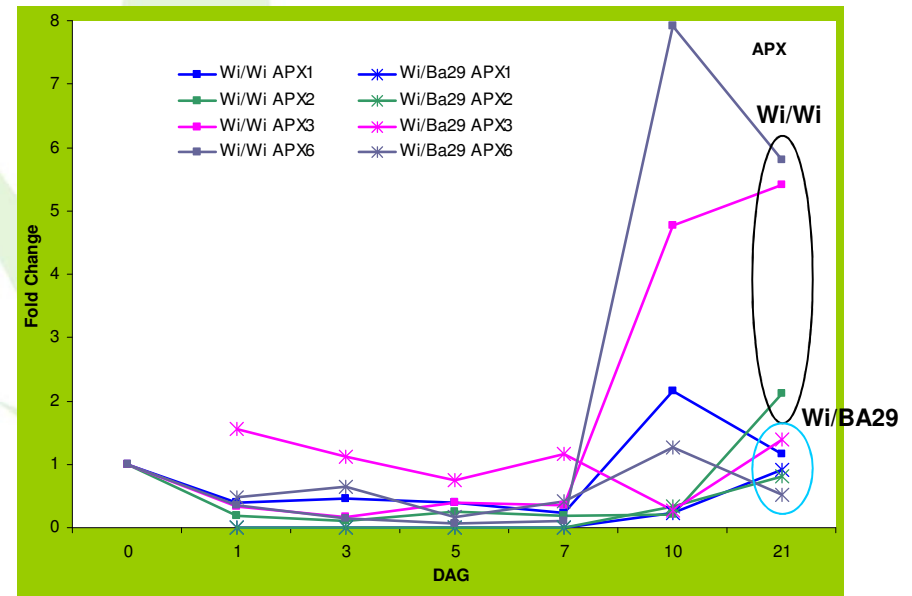
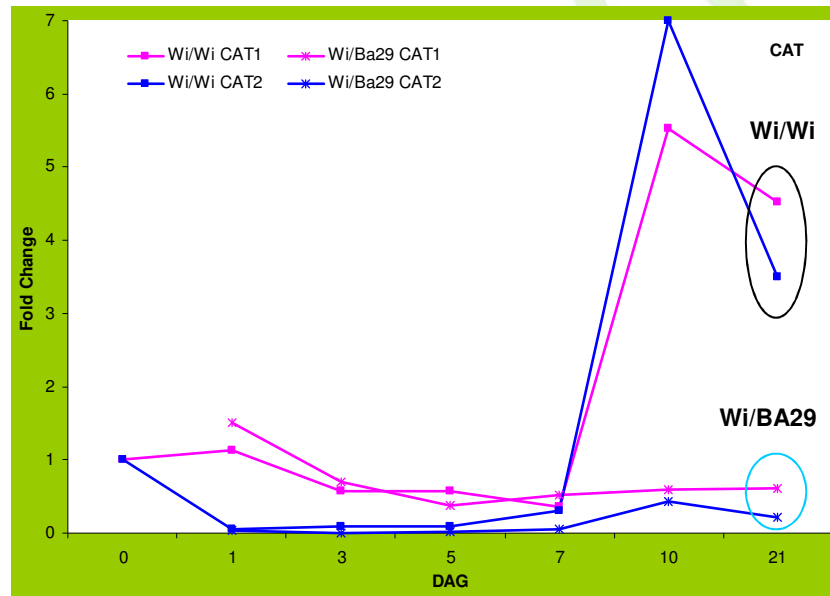
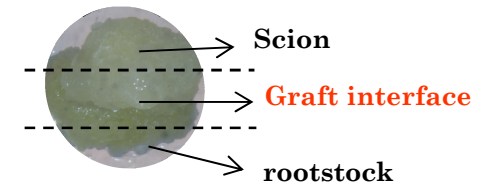
Gene expression



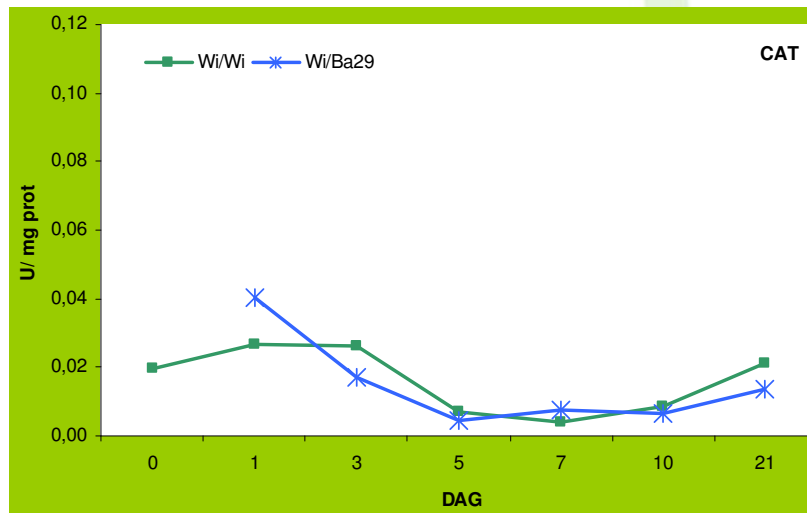
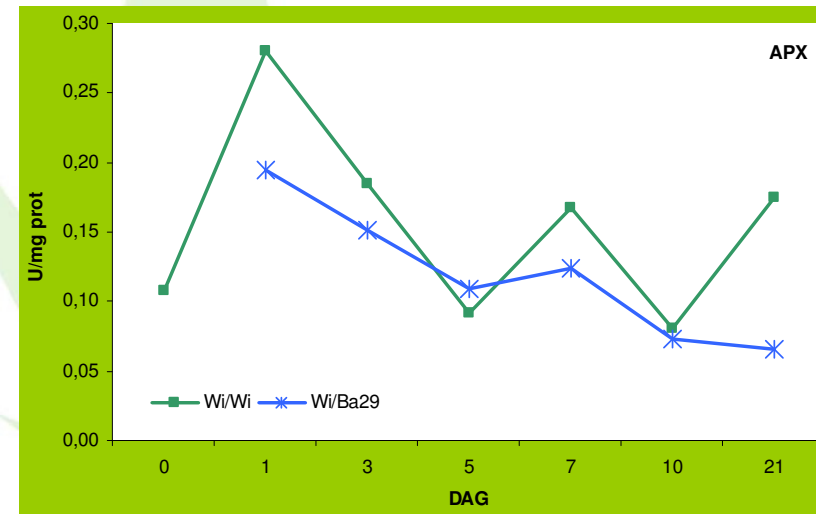
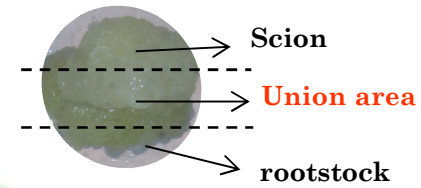
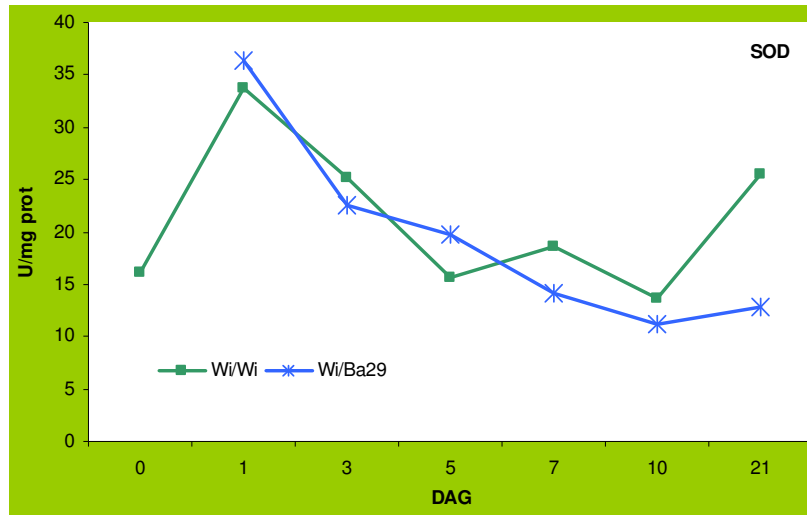
Gene expression



Gene expression



Enzyme activity



The activity was not correlated with the gene expression.

It is expected that some posttranscriptional mechanisms are playing an important role in this biological process.

BA29 (stock)

CM-H2DCFDA

PI

In vivo ROS detection

buffer without staining

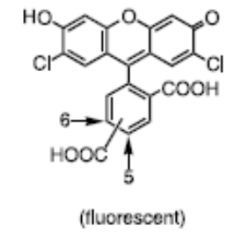
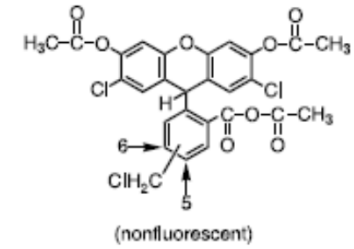
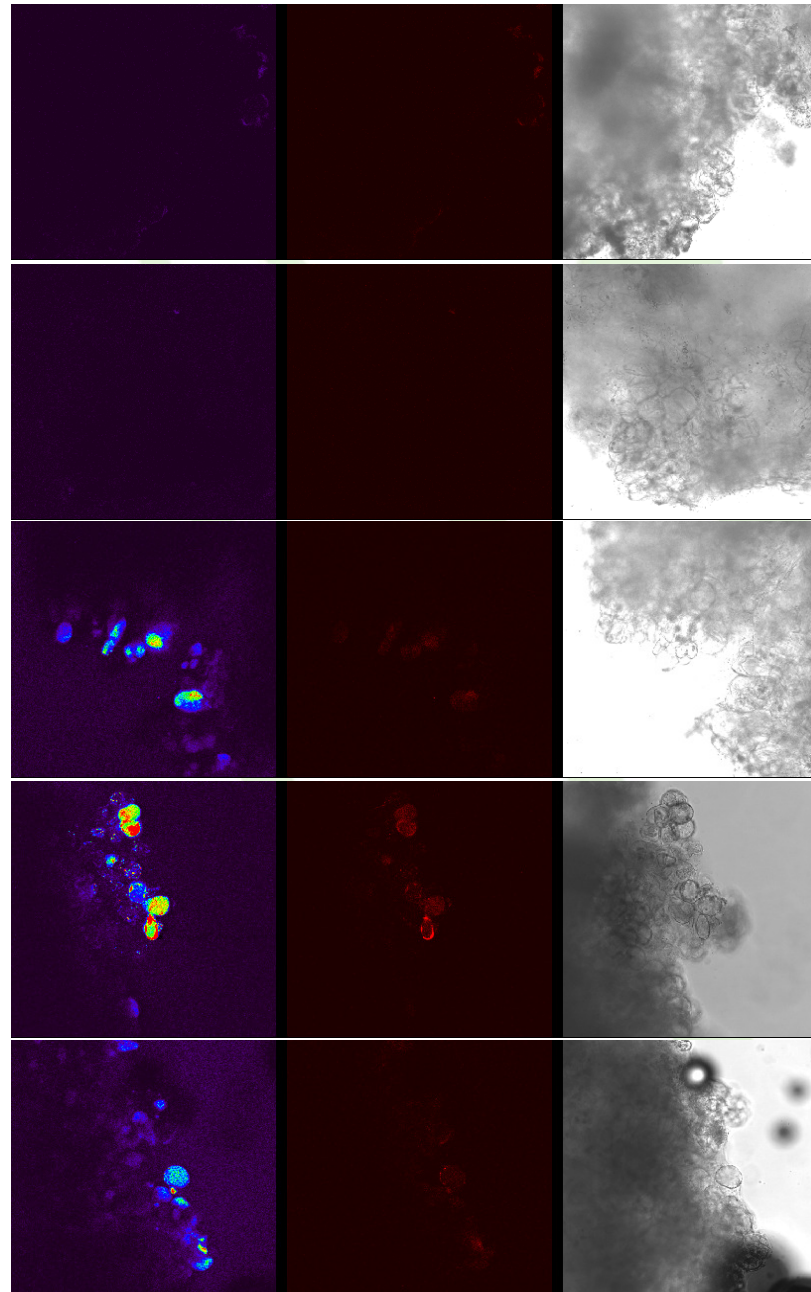
Without stress

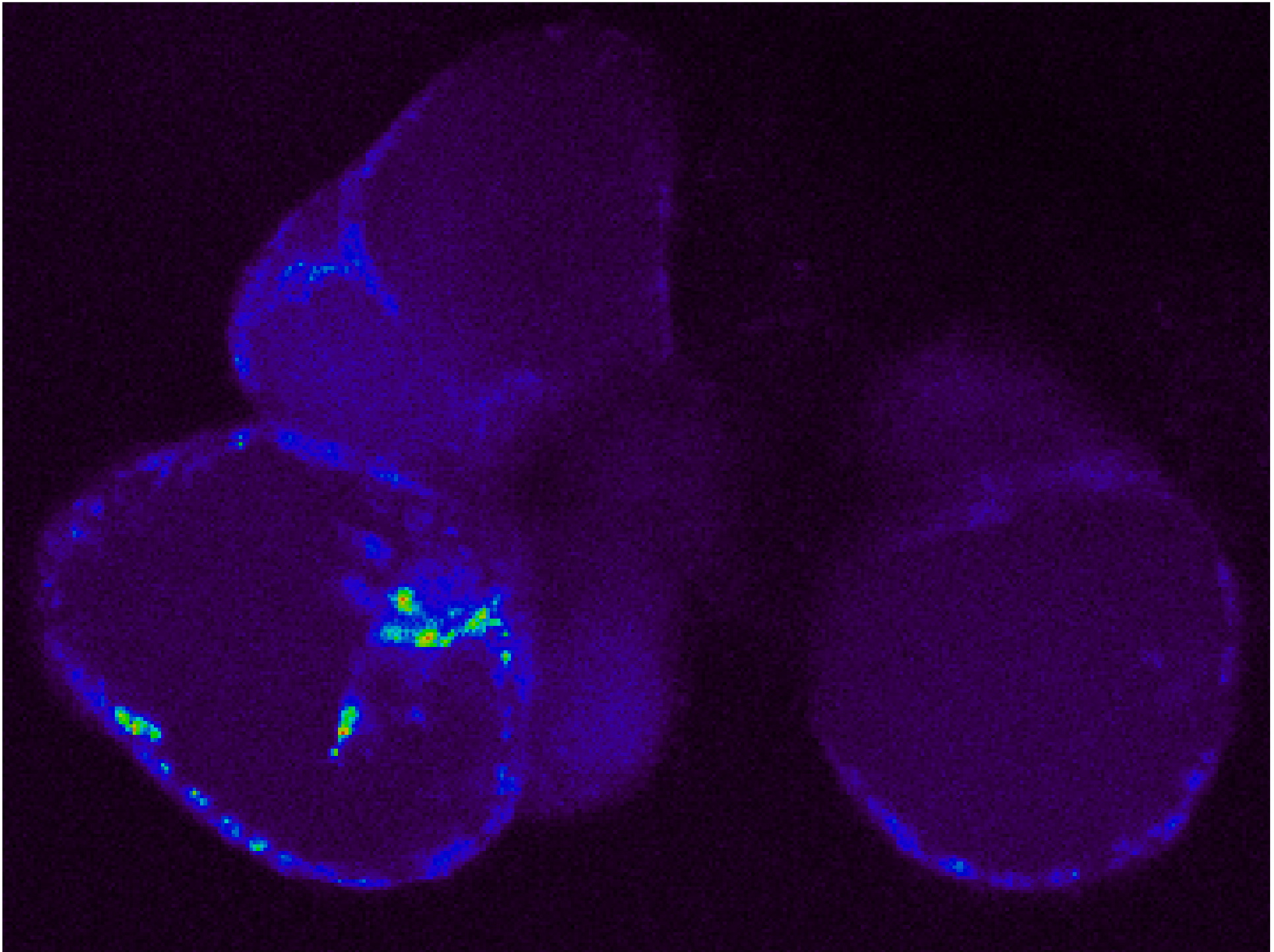
Wound

Hot water 60°C

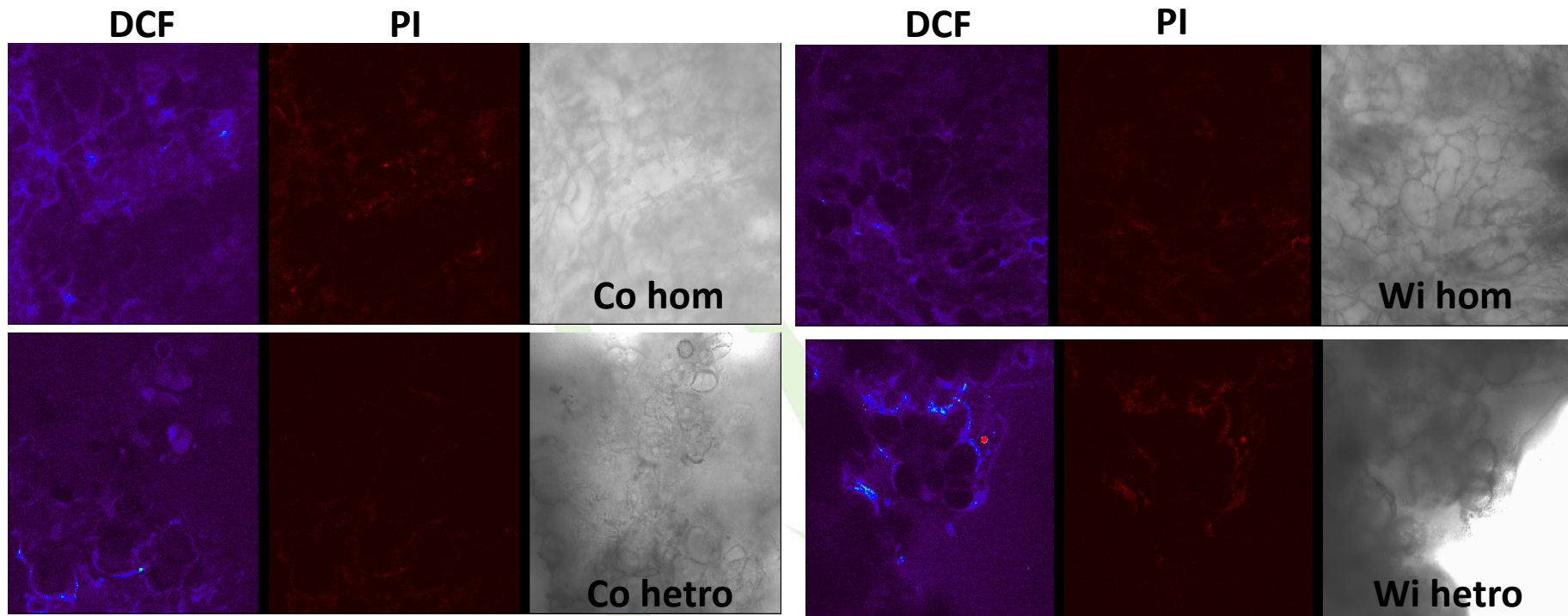
H₂O₂ 2mM

With stain





In vivo ROS detection



Incompatible combinations showed a slight increase in the accumulation of ROS two weeks after grafting.

CONCLUSIONS

- Gene expression analyses showed significant differences in the expression of antioxidant enzymes between homografts and heterografts at 10 and 21 DAG for the Wi genotype (incompatible cultivar).
- The scion-rootstock incompatible combination (Wi/BA29) displayed a lower antioxidant activity and could not counterbalance the negative effects of the oxidative stress.
- The low gene expression and antioxidant activity in the incompatible combination might produce an accumulation of ROS.
- More studies are needed to determine the role of ROS in the processes of graft (in-)compatibility, and its possible involvement in PCD and toxic effects on the cells.

Acknowledgements

Thank you for your attention

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