CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIA DE ARAGÓN

OXIDATIVE STRESS ASSOCIATED WITH ROOTSTOCK-SCION INTERACTIONS

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INTRODUCTION

Commercial fruit trees → scion, rootstock

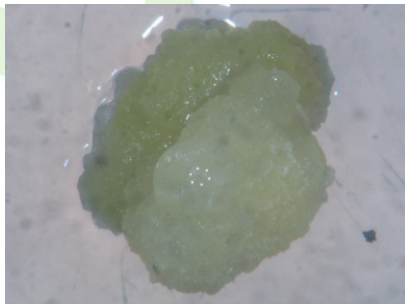
Compatible

Incompatible

In-vitro culture techniques

Micrografts

Callus unions



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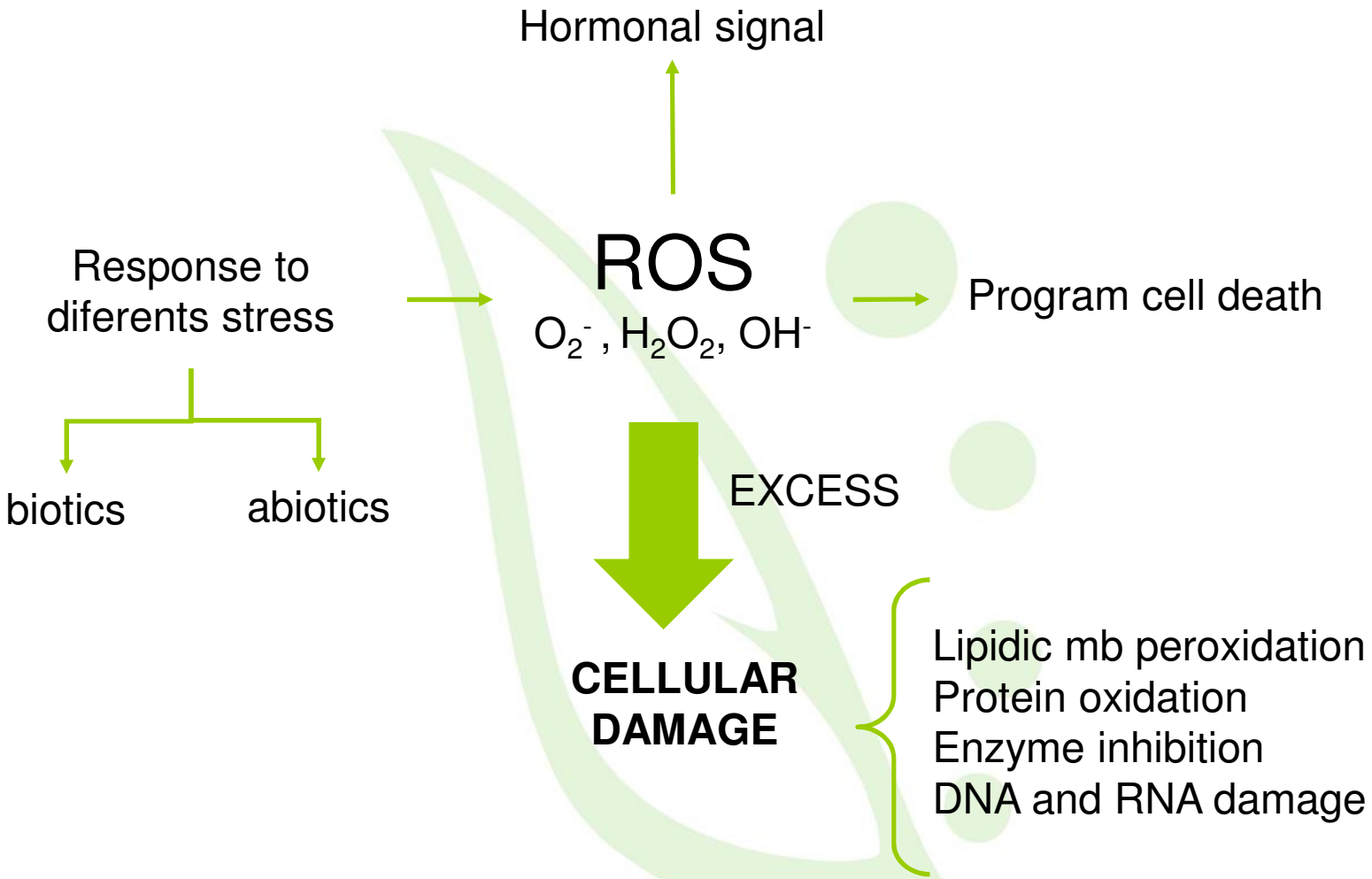

All stress situations disturb cellular metabolism and the unbalance production / elimination of ROS lead to its accumulation and impose conditions of oxidative stress.

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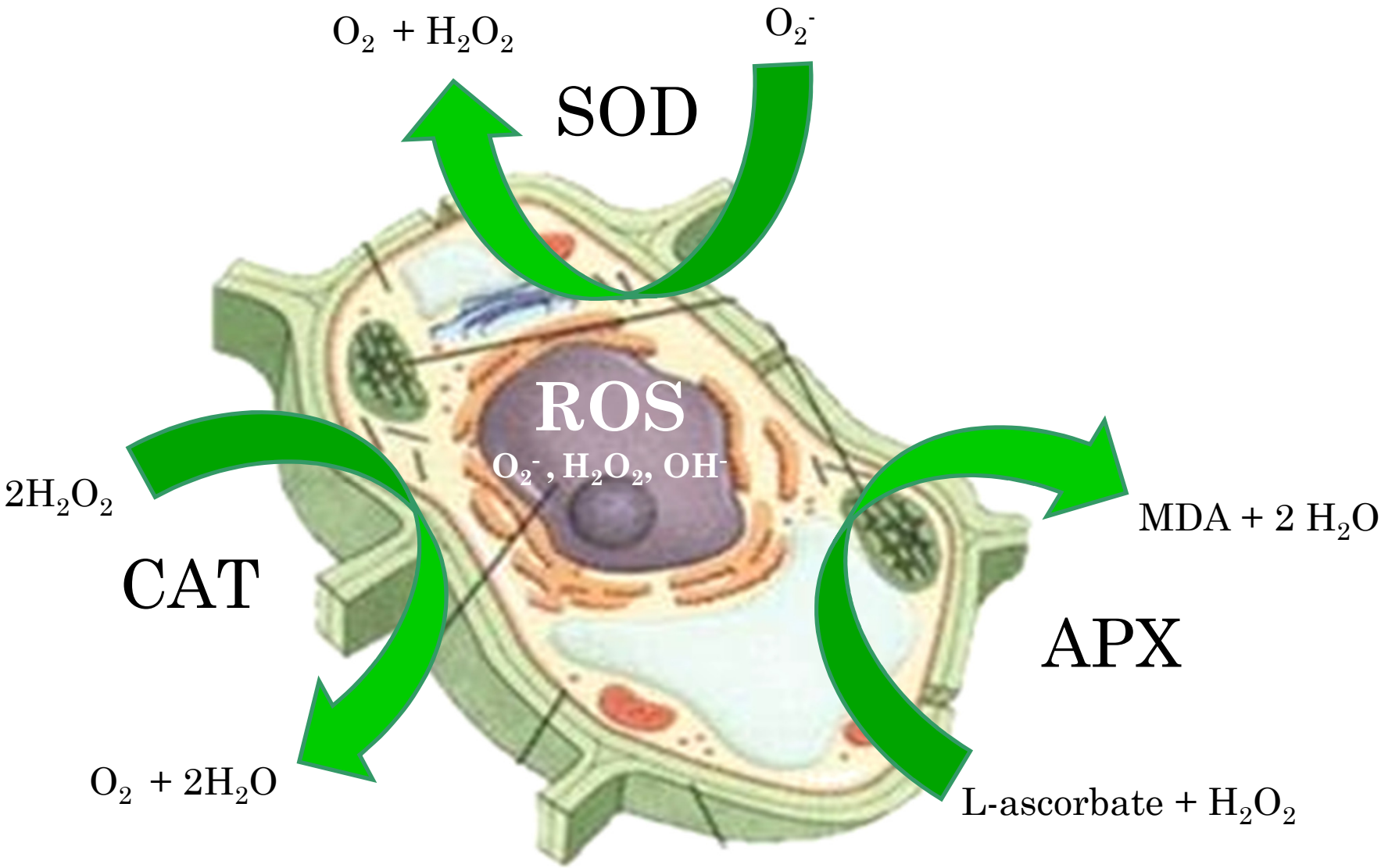

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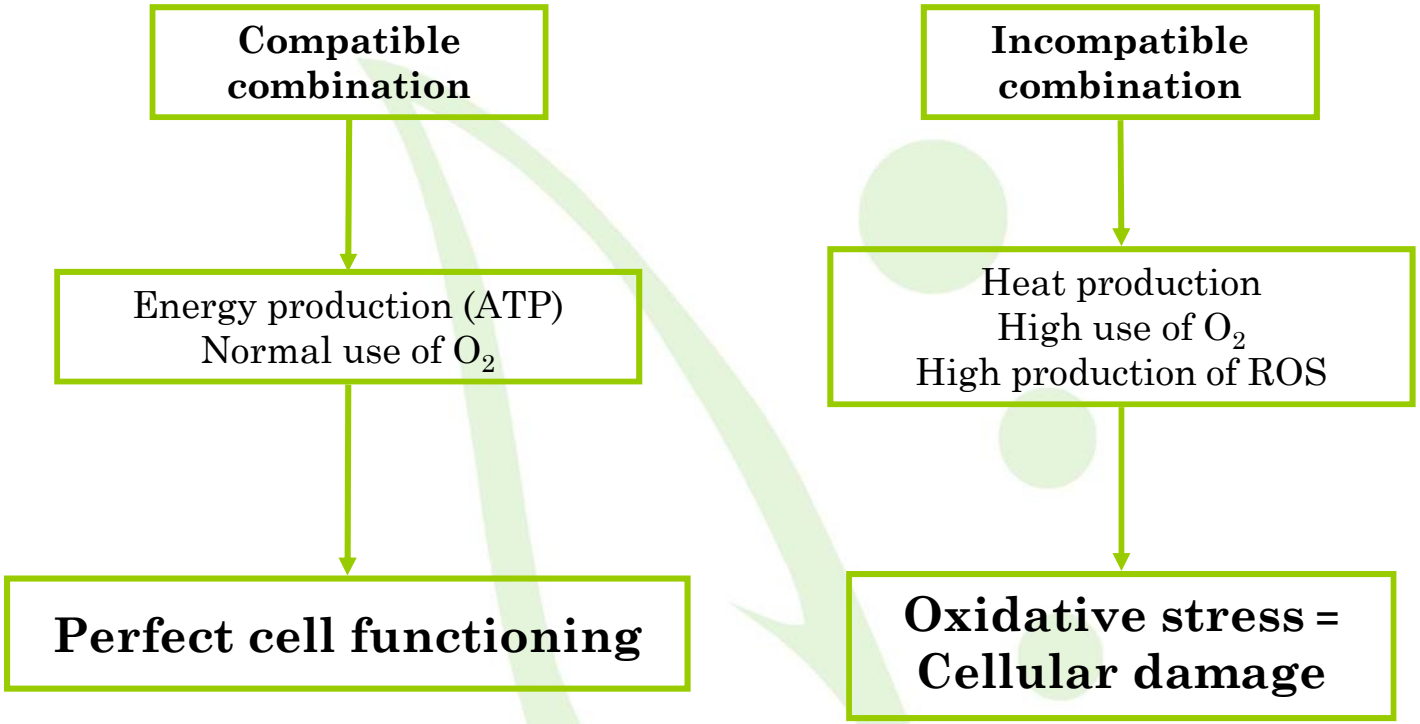

The oxidative stress as a mechanism that could trigger the degradation of tissues and cells in the incompatible graft union, due to a high accumulation of ROS.

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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**

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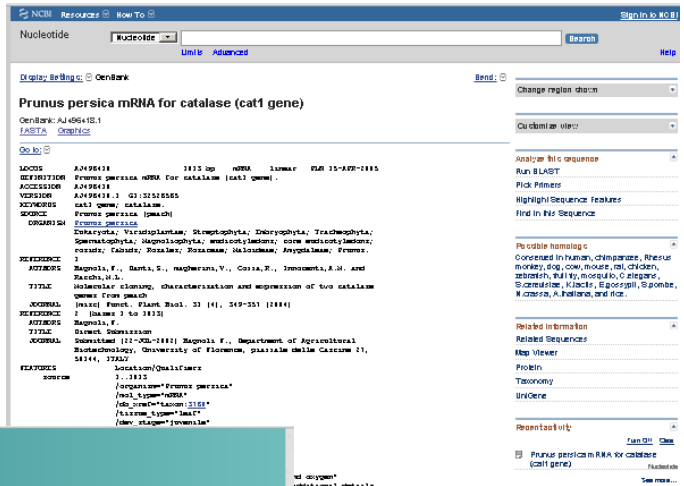
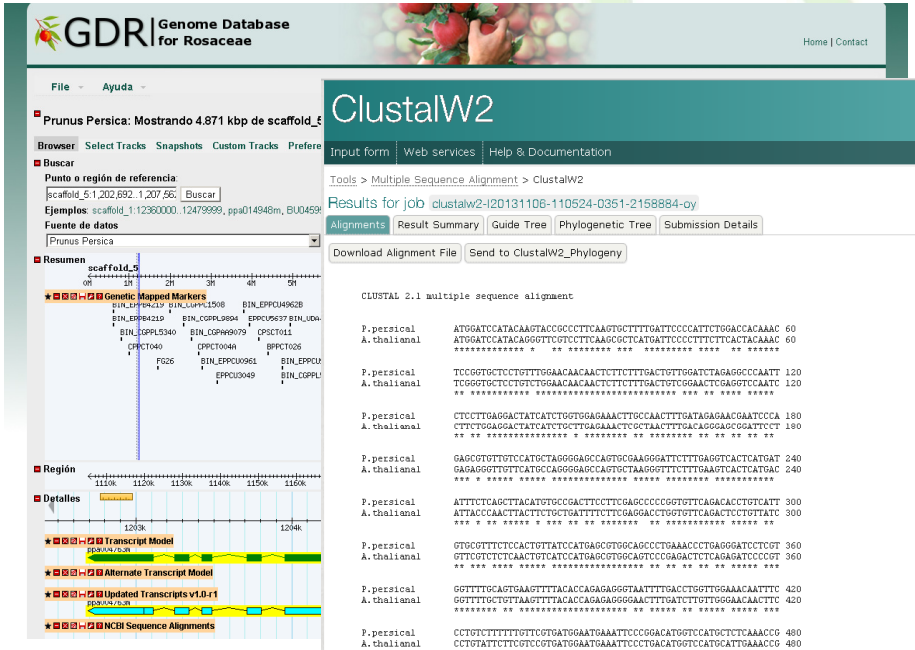
MATERIAL and METHODS

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





Assembled the sequences → specific primers were designed

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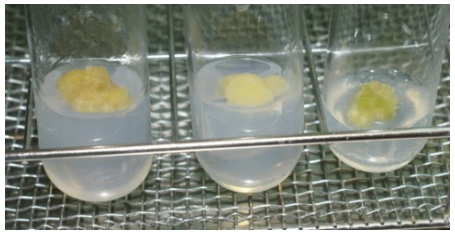
MATERIAL and METHODS

Callus tissue

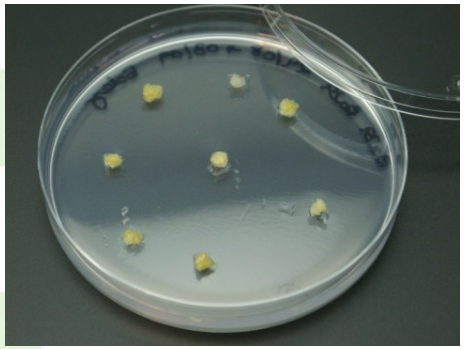
Pear scion
(Pyrus communnis L.)

Quince rootstock
(Cydonya oblonga Mill.)

'Conference' (Co) 'William' (Wi) 'Ba29'

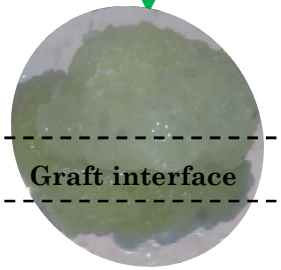


Co Wi Ba29



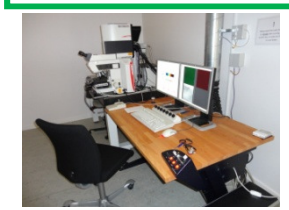
Homografts	**Heterografts**	**Control**
Co/Co	Co/BA29 (+)	Ungrafted
Wi/Wi	Wi/BA29 (-)	Wounded tissue
BA29/BA29		Co, Wi, BA29

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



Protein extraction → Enzymatic activity measure

***In vivo* ROS detection**



RNA extraction → qRT-PCR

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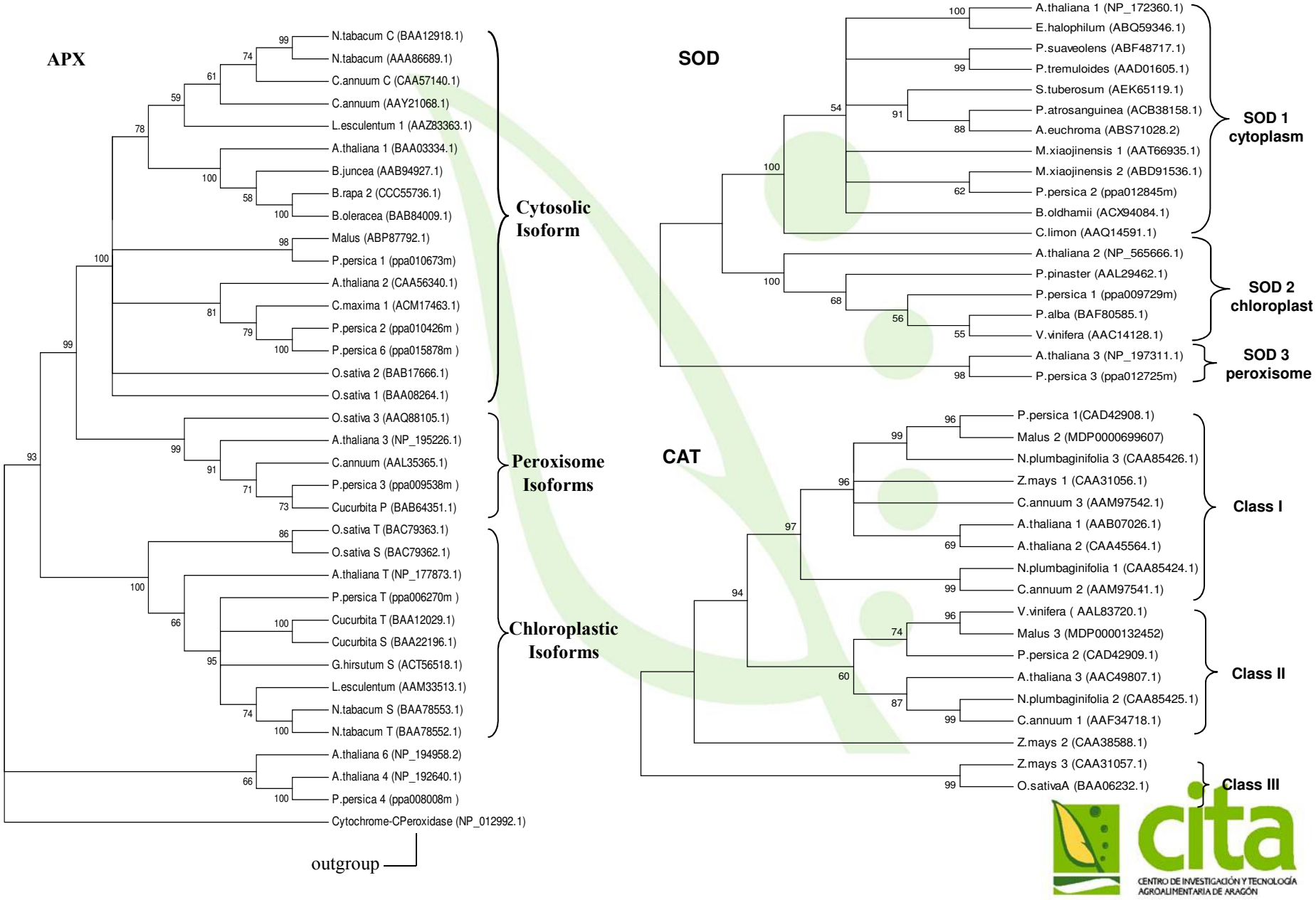
RESULTS

Gene expression



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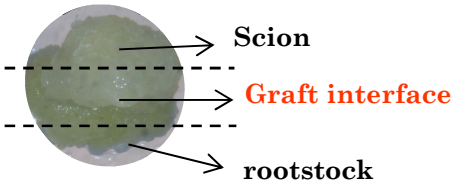
Gene expression



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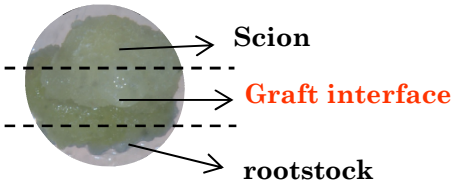
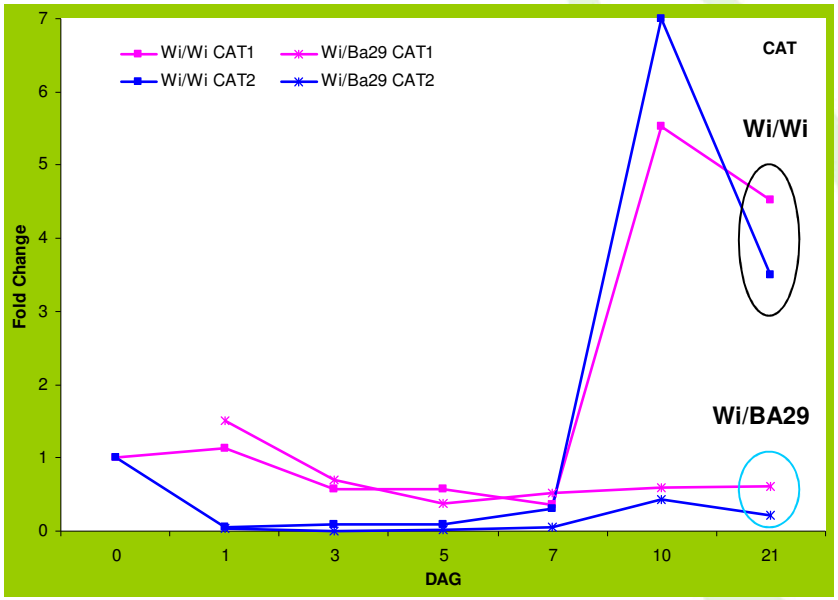
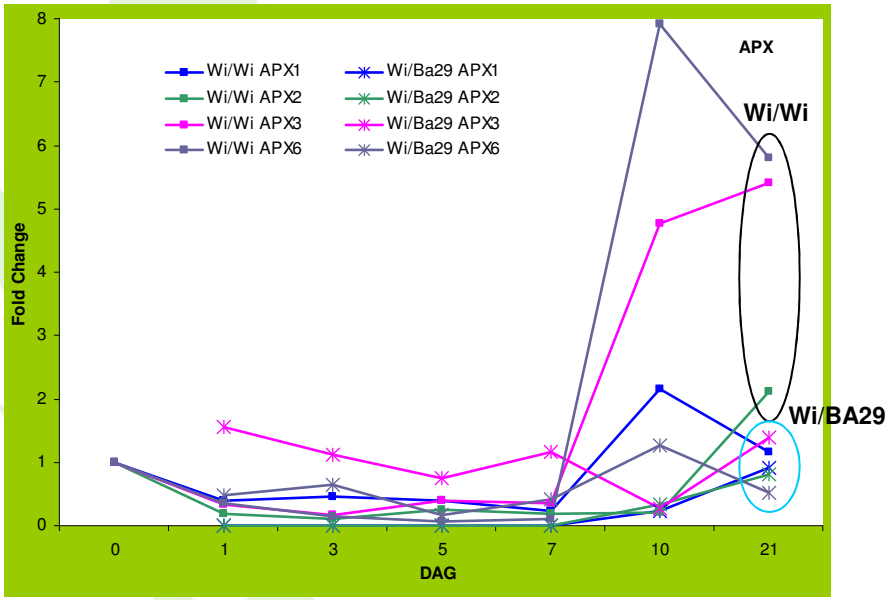
Gene expression







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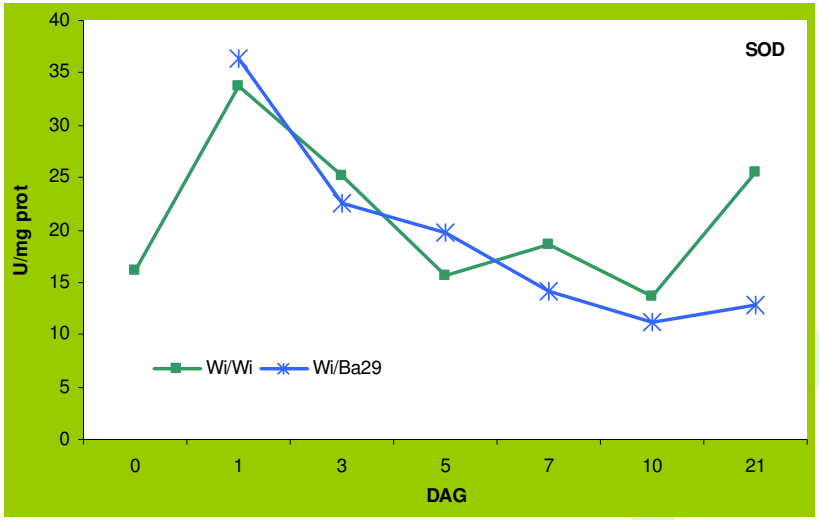
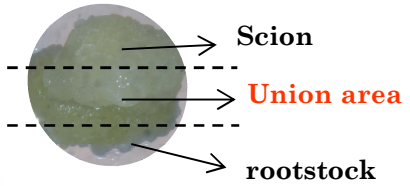
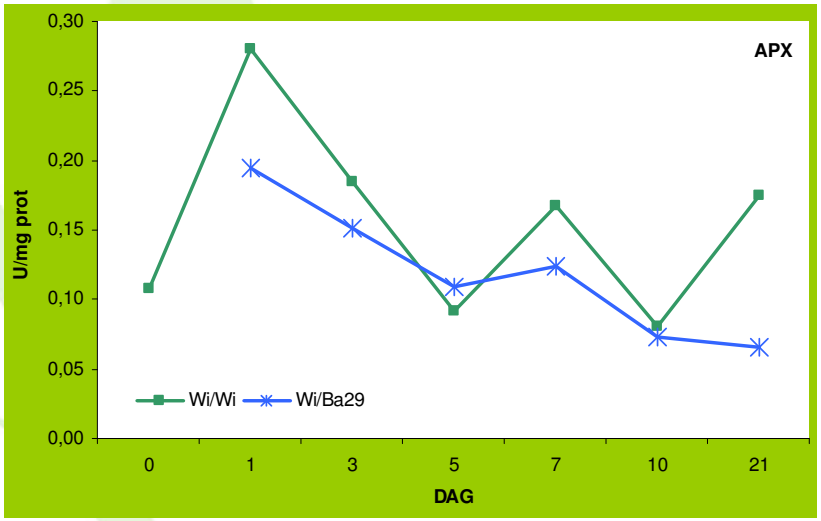
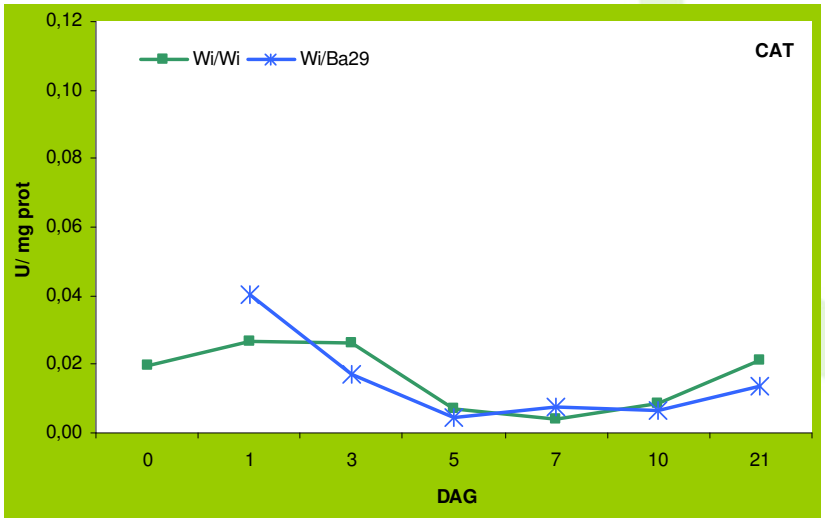
Enzyme activity









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.

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In vivo ROS detection

BA29 (stock)

CM-H2DCFDA

PI

With stain

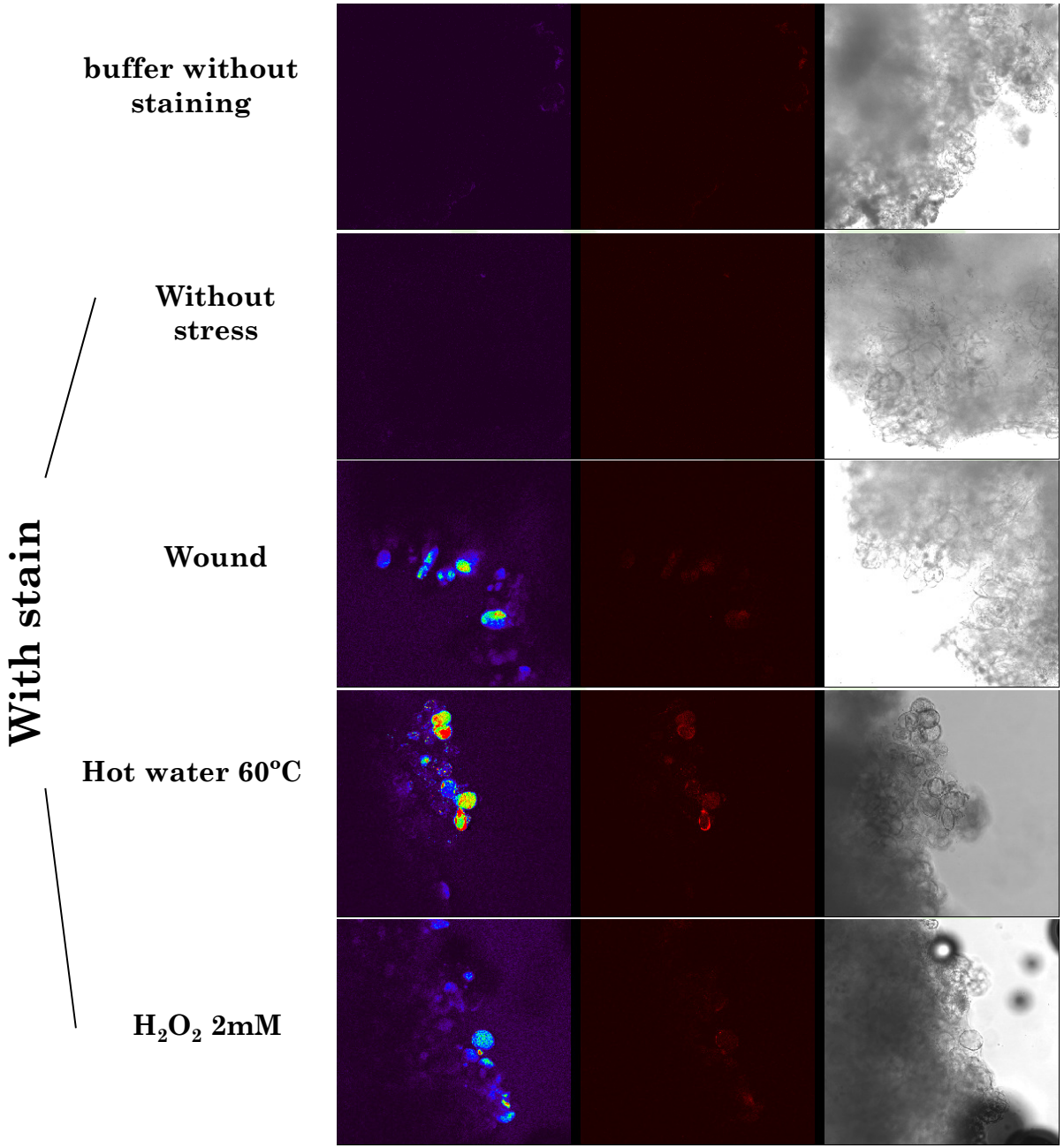
buffer without staining

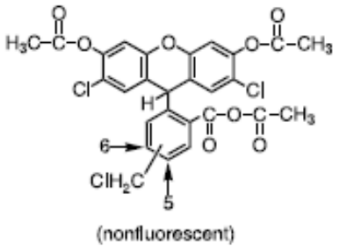
Without stress

Wound

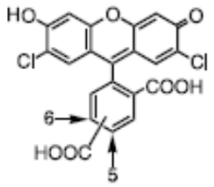
Hot water 60°C

H_2O_2 2mM





(nonfluorescent)



(fluorescent)

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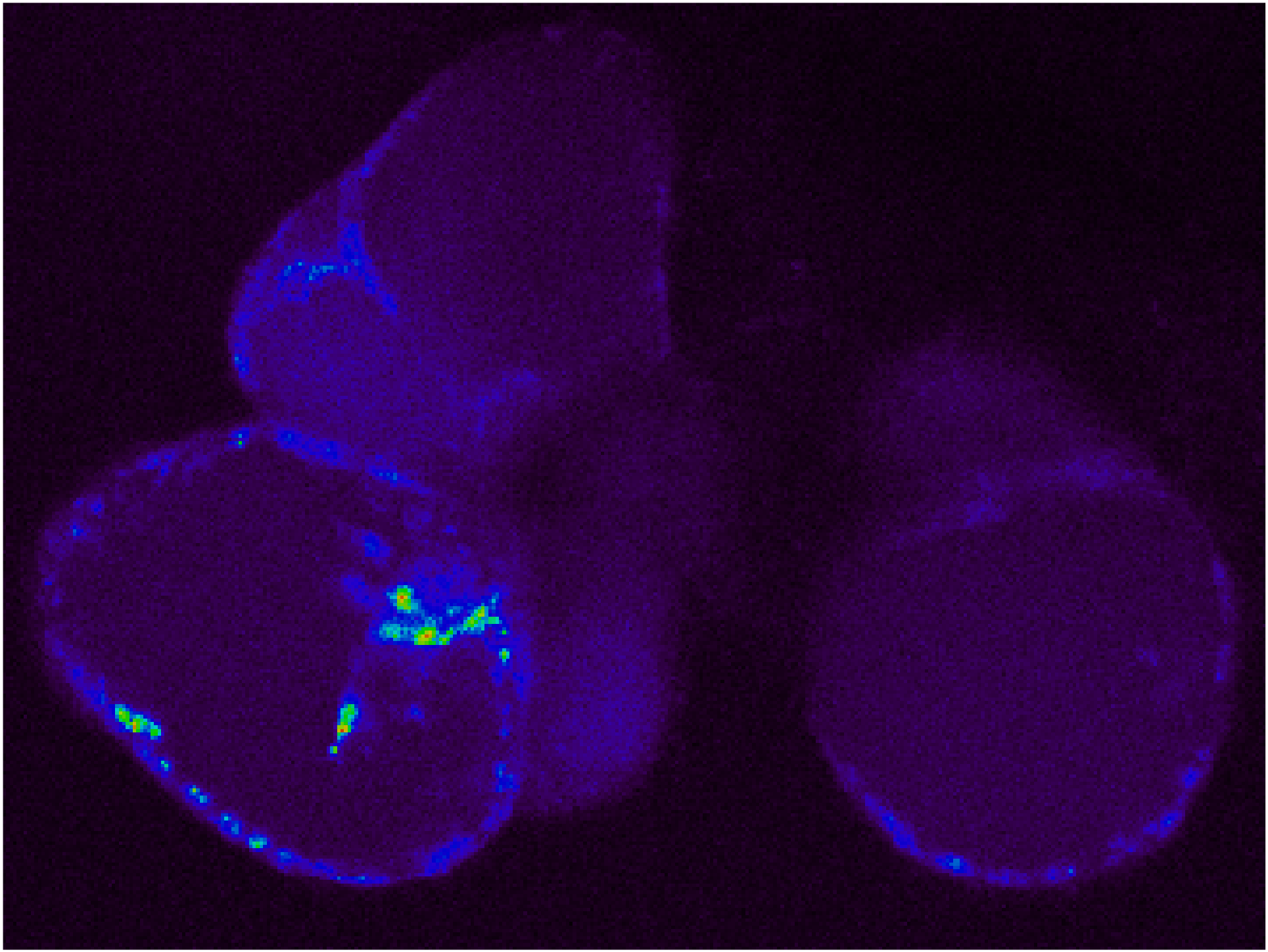
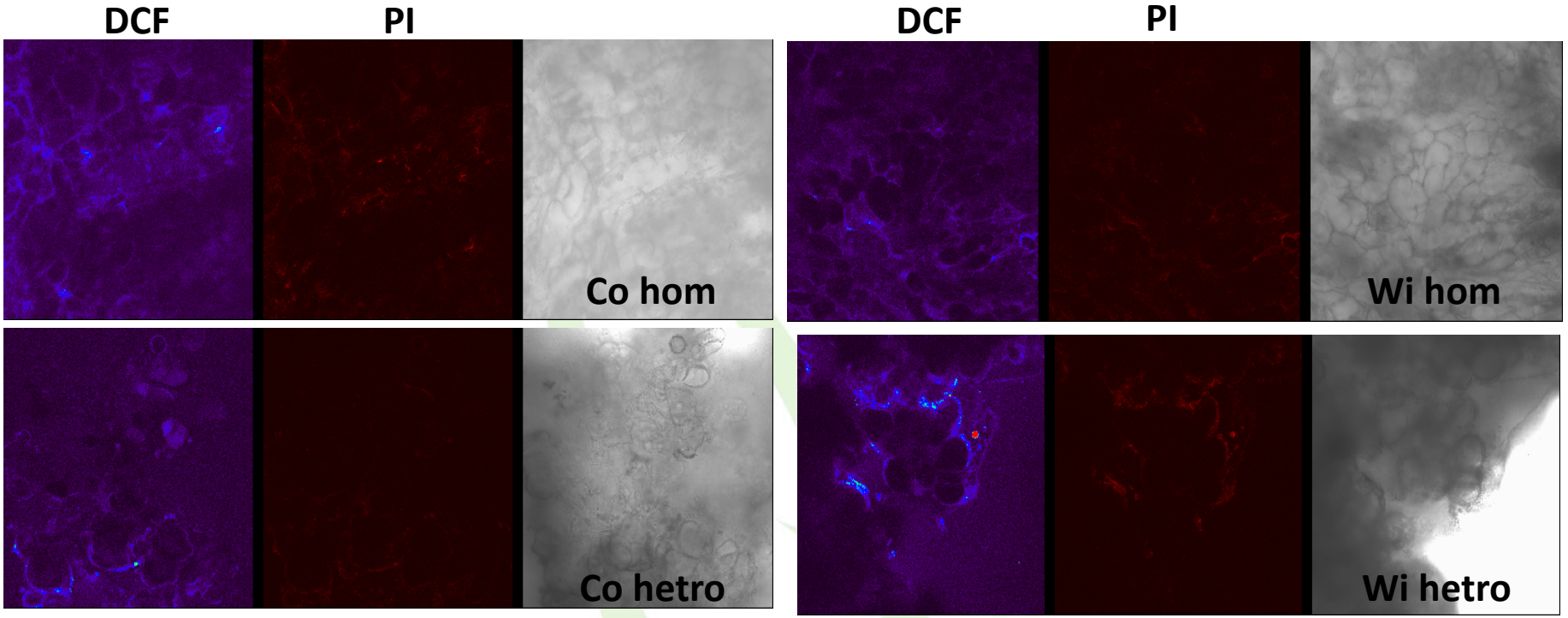


In vivo ROS detection



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

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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.

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Acknowledgements

Thank you for your attention

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