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The valuable grape marc in crop protection

Razik Benouaret, Pascale Goupil

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HAL Authorization

Razik Benouaret and Pascale Goupil

Laboratoire PIAF, UMR 547-UBP/INRA, Université Blaise Pascal, Campus universitaire des Cézéaux, 24, avenue des Landais, 63177 Aubière cedex, France,
e-mail : rbenouaret@yahoo.fr; Pascale.GOUPIL@univ-bpclermont.fr

Introduction

Activation of plant defense response against diseases provides a powerful management tool in phytoprotection. Many different substances could elicit plant defence, and there is an increasing demand for searching and identifying new ones. Currently, fast emerging natural phytosanitary products are known as plant defence inducers (PDIs).

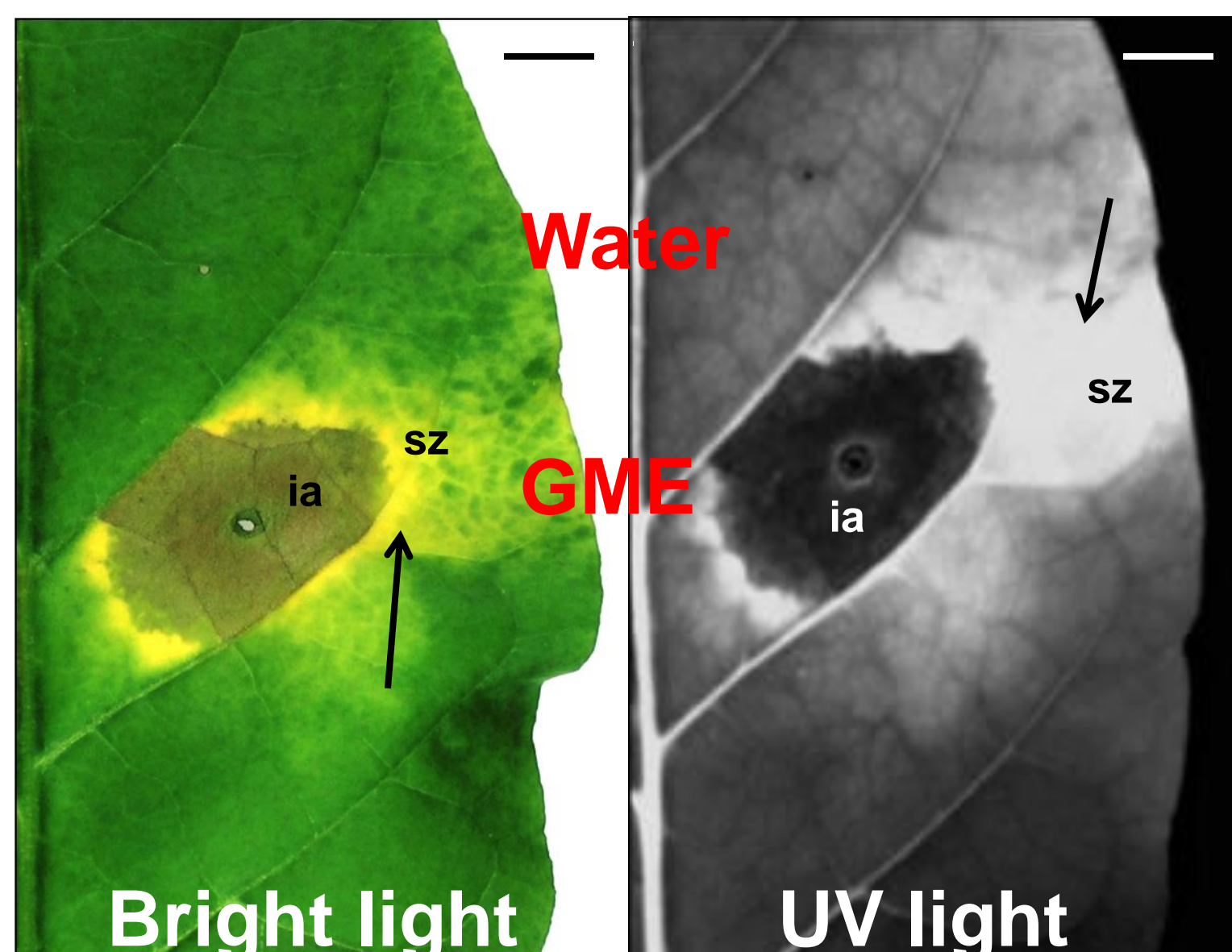
Our group reports the elicitor activity of red grape marc extract (GME). GME is a wine by-product that contains a large proportion of polyphenols with anthocyanins. On application to tobacco leaves, it elicits a variety of defence reactions such as local injury, biochemical changes and systemic molecular response with upregulation of PR proteins. Additionally, we demonstrate that this extract induces resistance against *Phytophthora parasitica* on tobacco plants.

Results

Technical assistance: Céline SAC and Dominique MARCON

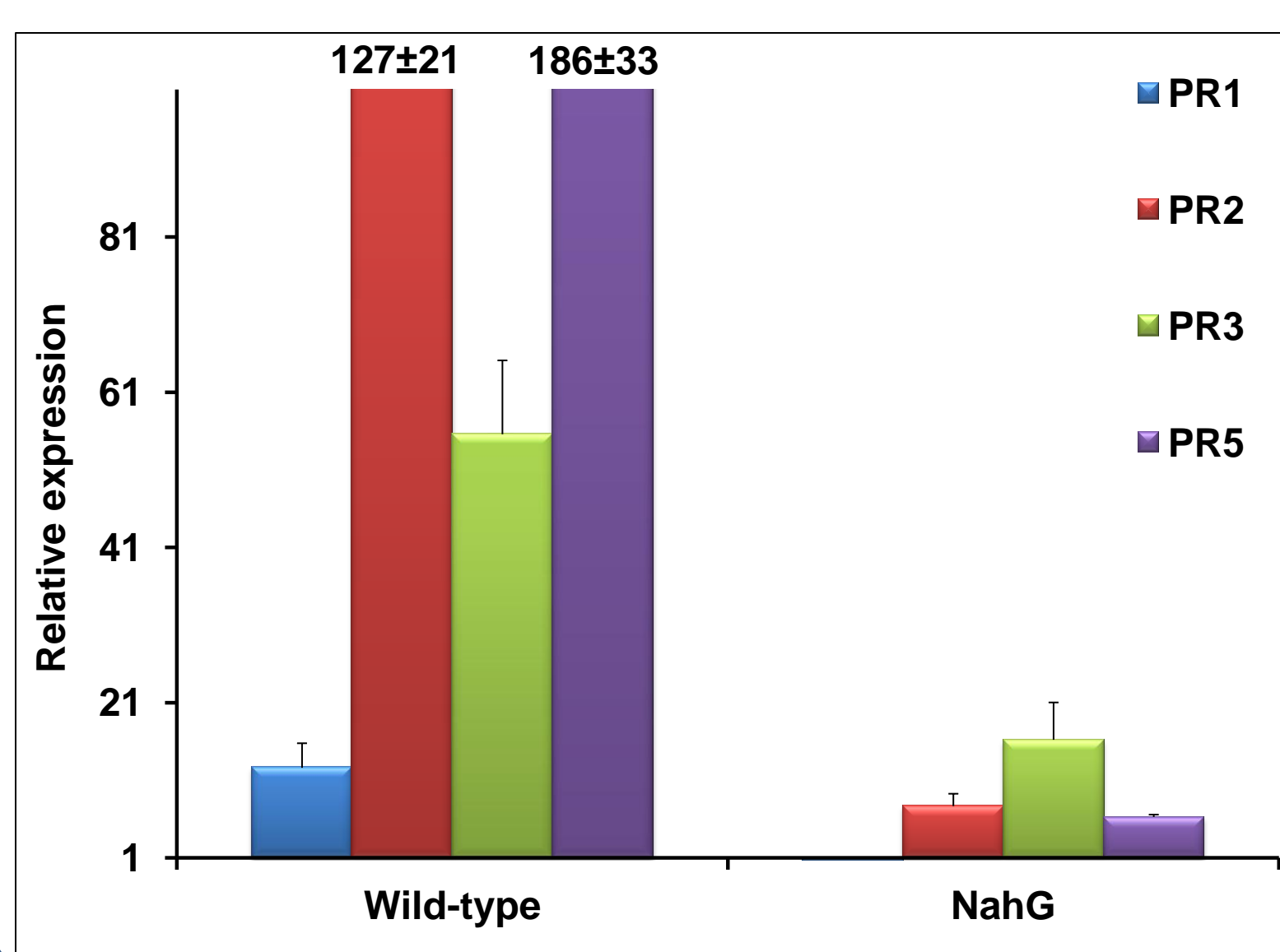
PDI potential

GME mediates local tissue injury and PR expression in tobacco leaves



Macroscopic symptoms induced on tobacco leaves by water or 0.7% (v/v) GME infiltration. Leaves were observed under bright light field or UV light at 4 days post-infiltration. (ia) infiltrated area (sz) surrounding chlorotic zone. Bars 1 cm. Arrows point necrotic or fluorescent areas.

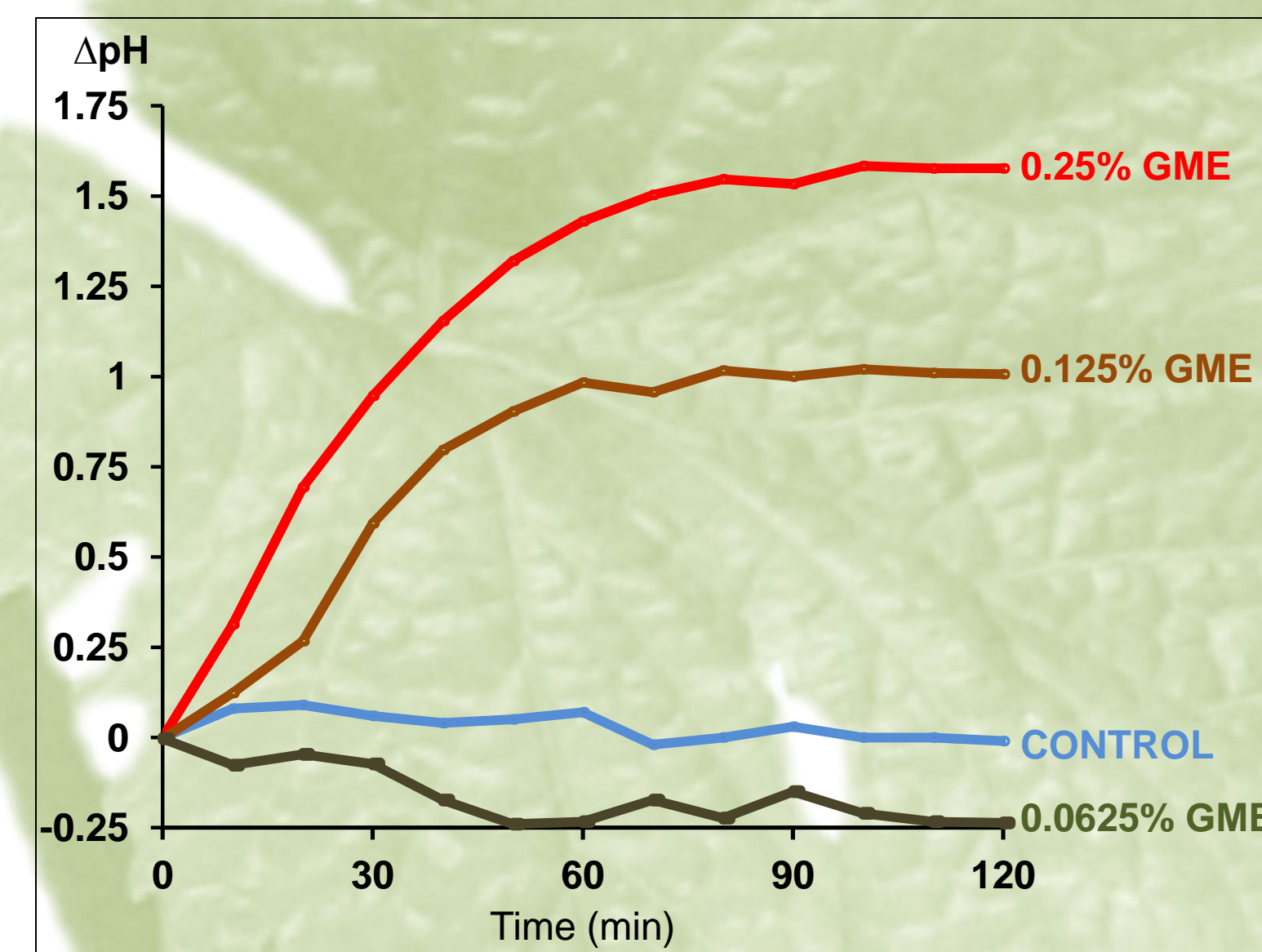
- GME induced HR-like lesions into tobacco leaves. After infiltration, the surrounding leaf tissues spread out autofluorescent compounds.



Expression of PR1, PR2, PR3 and PR5 in wild-type tobacco and NahG tobacco plants leaves infiltrated with 0.7% GME. The amount of transcript encoding genes was quantified by real-time PCR. Values are expressed relative to the control (water treatment) values. Two treated plants were pooled for each experiment, and the results are the means of duplicate experiments. Bars represent the mean values ± SE.

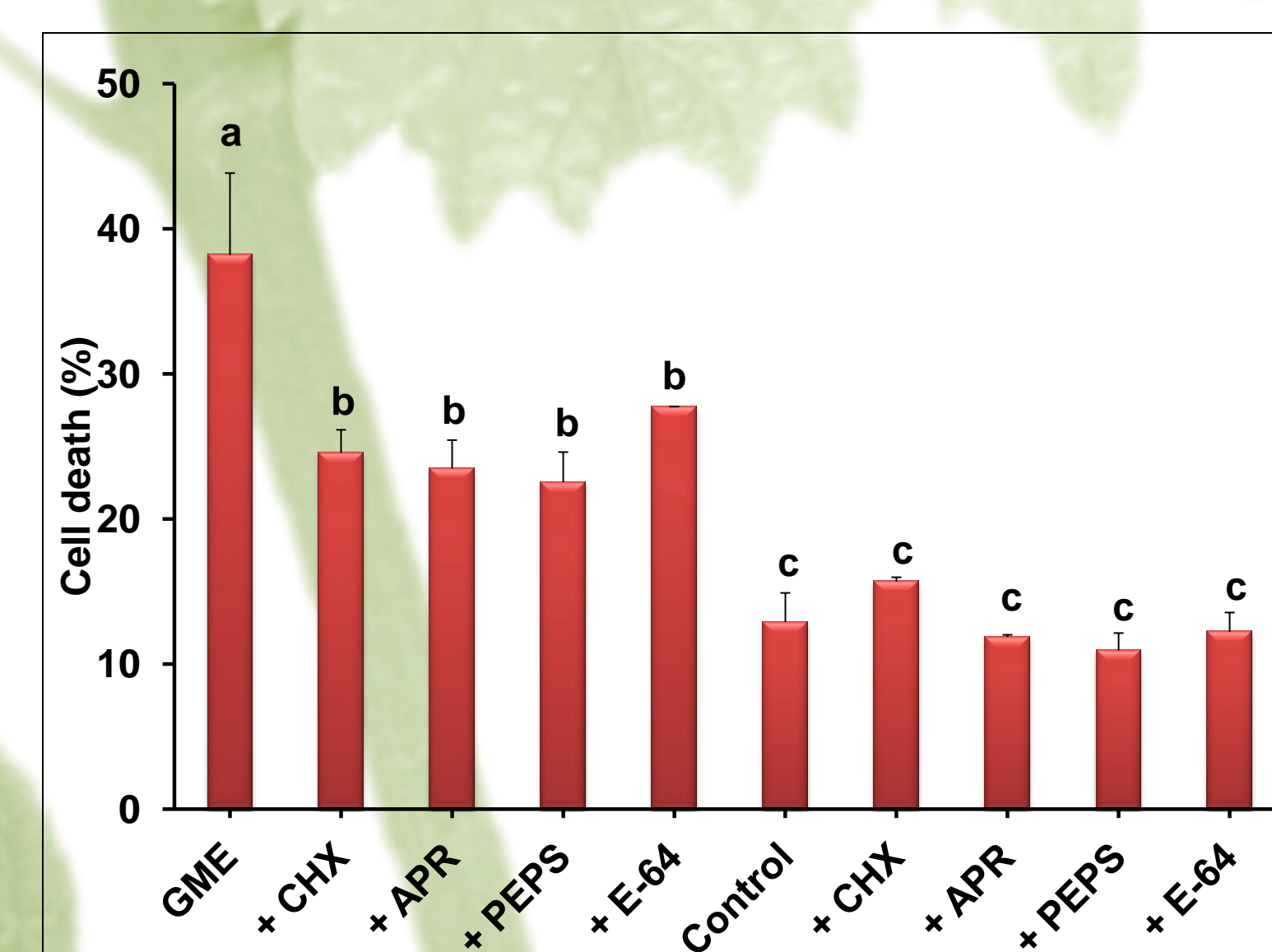
- GME induced gene expression of PR1, PR2, PR3 and PR5. The defense gene transcript accumulation was dramatically affected in the NahG tobacco plants harboring the bacterial gene for salicylate hydroxylase.

GME shows elicitor activity on suspension-cultured cells of tobacco



Extracellular alkalization in tobacco BY-2 suspension cells on addition of different concentrations of GME. The data are from representative experiments out of a total of five.

- The BY-2 cells reacted to GME (0.25% and 0.125%) with a long-sustained pH rise in their growth medium.



Effects of cycloheximide and protease inhibitors on GME-induced cell death. Chemicals were added 30 min prior to cell death measurement of BY-2 treated with water (Control) or 0.25% GME for 48 h. The final concentration of each chemical is 50 μM cycloheximide (+CHX), 0.2 μM aprotinin (+APR), 1 μM pepstatin (+PEPS), 1.5 μM E-64 (+E-64). Letters next to bars indicate statistically significant differences between samples, according to one-way ANOVA followed by LSD Significant test (p ≤ 0.05).

- Cycloheximide and protease inhibitors suppressed partially GME-induced cell death.

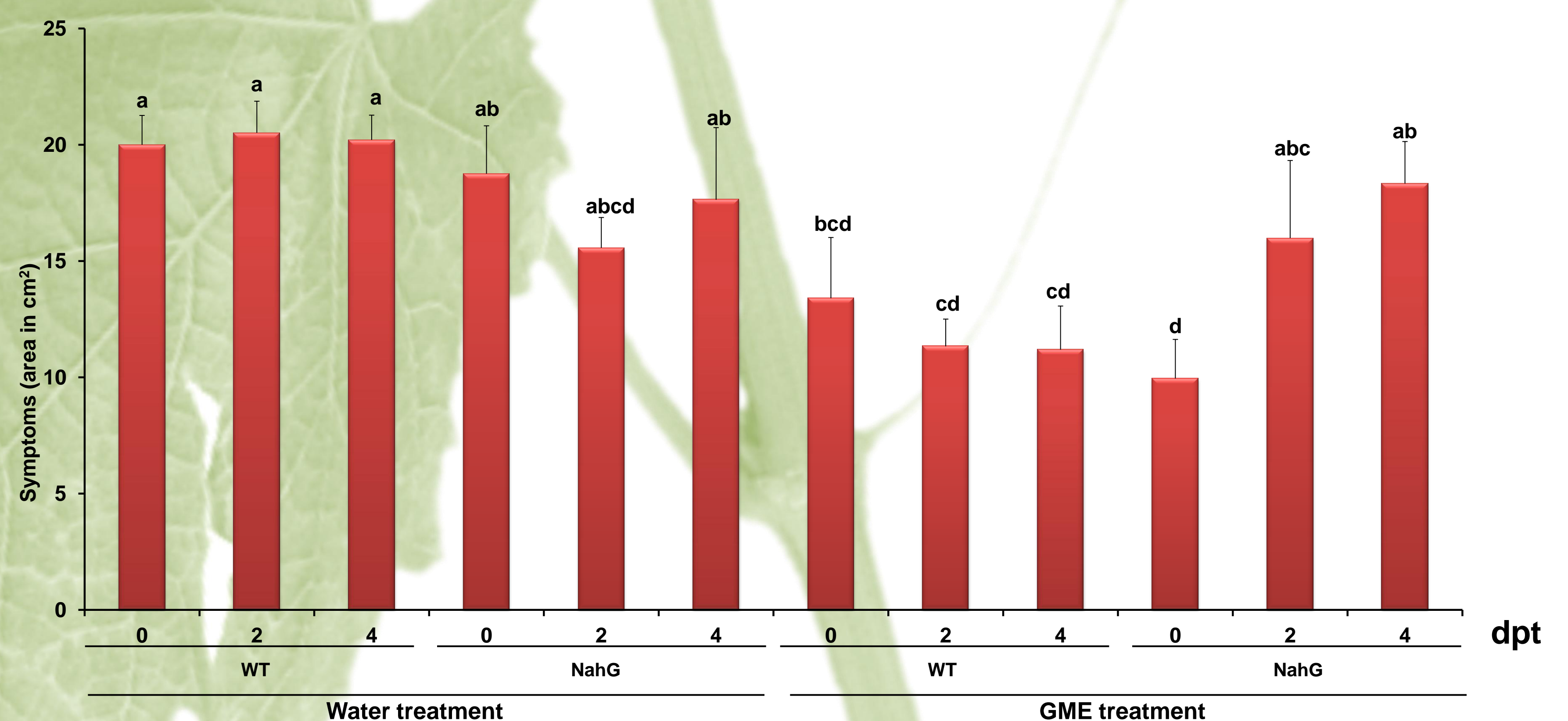
Plant protector

GME induces resistance to *Phytophthora parasitica* infection of tobacco plants

Leaf protection induced by GME on tobacco plants expressing or not expressing the *nahG* gene. Leaves from 8-week-old tobacco plants treated with water or GME were inoculated with zoospores of *P. parasitica* at 0, 2 or 4 days post-treatment (dpt).

The invaded areas were measured 3 days after inoculation. Each bar represents the standard error of four replicates from two different experiments. A replicate corresponds to eight inoculated areas on four leaves from two plants. Letters next to bars indicate statistically significant differences between samples, according to one-way ANOVA followed by LSD Significant test (p ≤ 0.05).

- GME induced a significant protection of tobacco leaves towards the Oomycete *P. parasitica*. This level of protection is not shown in GME-treated NahG tobacco leaves.



Conclusions

- The marc extract of red grape cultivars reveals plant defence inducer properties. Treatment with grape marc extract efficiently induced hypersensitive reaction-like lesions and PR transcript accumulation.
- GME induced extracellular alkalization of BY-2 cells characterized by long-sustained pH rise. The inhibitory effect of cycloheximide on GME-induced cell death indicates that GME triggered an active process requiring protein synthesis. Also, proteolytic events may take part in the mechanisms leading to cell death.
- GME triggers high level of SAR gene expression in wild type tobacco plants compared to NahG plants. Moreover, the NahG plants display enhanced sensitivity to *Phytophthora parasitica*. These results suggest the potential role of SA in GME-induced defense responses and protection to pathogen.