Induced resistance: a strategy for the control of grape downy mildew?

Assay systems to test potential inducers

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Abstract

A desirable goal not only in organic viticulture is the application of ecologically harmless active agents for the control of fungal diseases. A potentially successful approach to inhibit growth and propagation of the pathogens is the induction of the plant's defence mechanisms through application of suitable compounds. Screening to identify potential elicitors might be facilitated by using model systems. Therefore a glucanase-promoter/reporter system in transgenic grape cell culture was established, to analyze potential inducers of PR-protein transcripts.
In assays with floating leaf-discs structural analogs of the highly effective 3-aminobutyric acid (BABA) and other amino acids were tested with regard to the inhibition of *Plasmopara* sporulation. Except BABA however no other comparably effective compounds could be found that also work in intact plants until now.

Keywords

Vitis, Plasmopara, cell culture, PR-protein, glucanase, luciferase, resistance, beta-amino butyric acid

Introduction

One important objective in organic viticulture is to control fungal diseases by using active agents that are ecologically accepted. Integrated viticulture expected as future standard in Germany aims at a pathogen control that takes care of the ecology of the vineyard. The high demand on quality of the crop of grapes requires intense plant protection measures against the causative organisms of grape powdery- and downy mildew (*Uncinula necator* resp. *Plasmopara viticola*). *U. necator* is an ascomycete whereas *P. viticola* belongs to the fungal-like oomycetes. Both pathogens were introduced to European vineyards from North America in the second half of the 19th century. In their natural habitats the pathogens coexist with their native hosts, American *Vitis* species, which have developed a high degree of resistance during evolution. Original European *Vitis vinifera* varieties however usually show a high susceptibility towards these pathogens, so that there is a high risk of epidemics without an extensive spraying of fungicides.
These diseases may lead to intense yield losses and consequently endanger the economic viability of wine farms saving on natural resources. Copper salt solutions as peronosporacide (against downy mildew) are used in ecological viticulture. The application of this substance however might be prohibited because of its potential eco-toxicity.

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The hesitant approach of integrated viticulture is mainly due to the enhanced risk of crop shortfall after reduced pesticide treatments. For that reason is it important to develop effective but ecological harmless methods for downy mildew control. A potentially efficient way of controlling not only *P. viticola* but also other pathogens might be the induction of natural plant resistance mechanisms.
Besides breeding of new resistant varieties, the use of artificial or naturally occurring compounds to induce the plant's own defence responses are important alternatives for plant protection. Plants can defend themselves from pathogen infection through a wide variety of mechanisms that can be either local or systemic, constitutive or inducible. Similar plant responses can also be triggered by certain natural and synthetic chemical compounds, designated as elicitors, that act as ligands for receptors in the plant plasma membrane or cytosol. Pathogen respectively elicitor recognition at the site of infection initiates cellular and possibly systemic signaling processes that activate defense responses like generation of reactive oxygen species (ROS), formation of cell wall appositions, activation of defense genes e.g. encoding for pathogenesis-related (PR) proteins, resulting in rapid establishment of local resistance. Potentially a delayed systemic acquired resistance (SAR) is induced. The phenomenon of SAR has been frequently shown in annual plants such as *Arabidopsis*, tomato and cucumber (Sticher *et al.*, 1997). In perennial plants such as grapevine however investigations are still rare.

Glucanase promoter/luciferase reporter assay system

In order to investigate potential resistance inducers, different experimental approaches were made: One approach was to create a PR-gene expression assay. Therefore a b-1,3-glucanase cDNA as a typical PR-gene from *V. vinifera* was cloned. This glucanase as GFP-fusion protein was found to be localized in the cytoplasm of protoplasts and therefore might be extracellularly secreted, to attack the polyglucane containing cell wall of the growing oomycete. The glucanase transcript levels from leaves infected with *P. viticola* zoospores revealed a strong upregulation 2-3 d after inoculation. As this glucanase shows a clear infection dependent mRNA accumulation we isolated and sequenced the corresponding promoter. This promoter was used for the establishment of a stably transformed glucanase-promoter/luciferase-reporter suspension cell culture from the American bush grape (*Vitis rupestris*). Compounds that act as potential elicitors of PR-gene activation can efficiently be detected in this luminometric assay system (For further information see posters viticulture; Seibicke *et al.*). Additionally this cell culture can be used in detecting compounds that elicit the earliest responses of the cells to the elicitors, namely changes in plasma membrane permeability, resulting in alkalization of the culture medium by proton influx (Ebel and Scheel, 1991).

Leaf-disc and whole plant experiments to test naturally occuring potential inhibitors of growth and sporulation of *P. viticola*.

In these experiments based on the results of Asselin *et al.* (1985) mainly proteinogenic and non-proteingenic amino acids were tested in different concentrations in buffered solution. Leaf discs from susceptible 'Mueller-Thurgau' floating with

the lower epidermis uppermost on the liquid surface were inoculated with droplets of a zoospore suspension. After a 4 d incubation period sporangiophore formation depending on the concentration of active ingredient was evaluated. DL-BABA (more exact the D- (R) enantiomer), which was already found to be universally active in inducing resistance against a broad spectrum of pathogens in quite a few plants species (Cohen, 2001, Jakab *et al.*, 2001) fully inhibited sporulation most effectively at concentrations above 250M. Different substances with structural analogy to BABA (e.g. beta-amino acids), were tested and found to have no or much lower efficiency on inhibition of sporangiophore formation. This supports the assumption of the existence of a highly specific receptor for BABA.
L-Arginine showing no structural similarity with BABA was found to be comparably effective as BABA in leaf-disc experiments.
Compounds found to inhibit growth and sporulation of *P. viticola* in leaf disc experiments were tested by spraying greenhouse-grown potted grafted vine. Arginine however was of low efficiency compared to BABA in the experiment using the intact plant.
Besides testing low molecular weight substances, harpin preparations (hrpN from *Erwinia amylovora* [messenger], hprZ from *Pseudomonas syringae*) were tested under greenhouse conditions. Preliminary results obtained with these proteins however show no or only low efficiency under our experimental conditions.

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