Induction of Phenylpropanoid Resistance Factors in Grapevine

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In vitro plantlet of grapevine 'Nero'

Introduction

Phenylpropanoids such as stilbenes [1] and proanthocyanidins [2] are known to play an important role for the defence against various pathogens in plants. Understanding the mechanisms of induced resistance by studying changes in contents and biosynthetic activity can lead to more effective strategies of protecting plants.

In vitro plants of grapevine cultivar 'Nero' were treated with the extract of the epiphytic yeast *Aureobasidium sp*. Samples of plantlets treated with yeast extract and with yeast growth medium (control) were collected after 24 h, 48 h and 72 h.

Gene expression

The gene expression of important genes in the phenylpropanoid pathway was studied by semi-quantitative PCRs. Two cDNAs from each sample (24 h and 48 h after treatment) were subjected to PCR analyses in two repetitions. The expression of phenylalanine ammonia lyase (*pal*) and stilbene synthase (*sts*) was significantly increased by the yeast treatment (see fig. 2), whereas the expression of chalcone synthase (*chs*) and anthocyanidin reductase (*anr*) was reduced. Of two distinctly different leucoanthocyanidin reductases (*lar1* and *lar2*), *lar1* was reduced and *lar2* unchanged.

Enzyme activity

Enzyme assays with ^{14}C-labelled substrates were used to measure the enzyme activities of key enzymes of flavonoid and stilbene biosynthesis, namely PAL, CHS and STS. ANR-assays were performed with cyanidin as substrate. For these analyses, two samples of four plantlets each were taken 24 h after treatment, both from yeast-treated and control plantles. Enzyme assays were repeated three times. Enzyme activity assays showed a slight increase in PAL activity following the yeast treatment, a marked increase in STS activity but no significant change in CHS and ANR activity (see fig. 3).

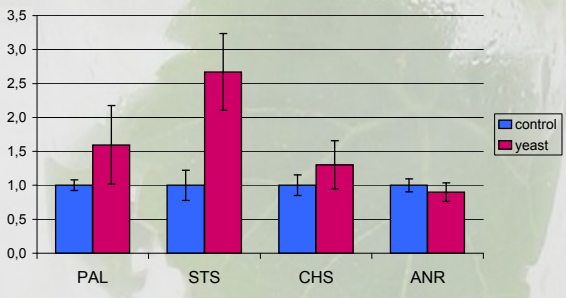

Fig. 3: Enzyme activities (two mixed samples of four plantlets each, three assays; mean relative to control ± stdev)

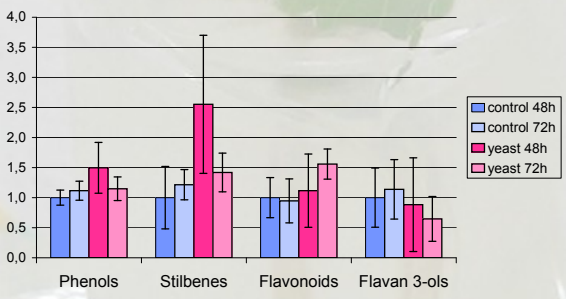

Fig. 4: Relative amounts of relevant phenolic compounds (five mixed samples of five plantlets each; mean relative to control 48 h ± stdev)

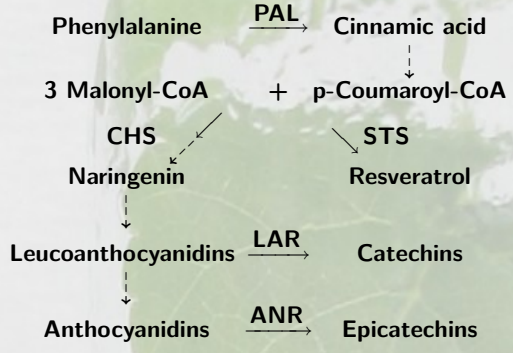

Fig. 1: Flavonoid and stilbene biosynthesis (simplified)

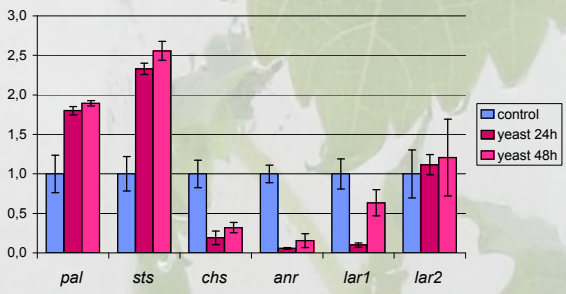

Fig. 2: Gene expression (mixture of two plantlets, two cDNAs each, two PCRs; mean relative to control ± stdev)

Phenolic compounds

The phenolic compounds of the samples were analysed by reverse phase HPLC. Treating the *in vitro* plants with extracts of yeast led to an induction of the phenylpropanoid pathway. In particular, contents of stilbenes, such as piceid and other resveratrol derivatives, were much higher in treated plants (see fig. 4). The overall content of phenolic compounds was also slightly increased. Flavan 3-ols, namely the monomers catechin and epicatechin and the dimers B2 and B3, are the products of a biosynthetic pathway competing for the substrate coumaroyl-CoA with that of stilbenes. The content of flavan 3-ols in yeast-treated plantlets tended to be slightly lower than in the control plants.

Discussion

It could be shown that the extract of an epiphytic yeast was able to induce the biosynthesis of stilbenes as known phytoalexins in *in vitro* grapevine plants. The overall phenylpropanoid biosynthesis was also induced. In a preliminary study, *Botrytis* inoculation of yeast-treated and control plantlets tended to result in a reduced infection after yeast treatment. This experiment needs to be repeated because of a very low rate of infection even in control plants. In further studies the transfer of these results to field-grown grapevine plants and therefore a possible application of the yeast extracts as plant resistance inductors will be examined.

References

[1] Jeandet P. et al. (2002) J. Agric. Food Chem. 50: 2731-2741.
[2] Treutter D. (2005) Plant Biol. 7: 581-591.

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