Induction of phenylpropanoid accumulation in *Vitis* vinifera callus cultures by an epiphytic yeast

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Cryptococcus laurentii, an epiphytic yeast, was used for biocontrol of postharvest diseases of different fruits including grapes (Lima et. al., 1998; 1999). It is generally assumed (Filonow, 1998; Zhang et al. 2003) that the yeast may inhibit the growth of many postharvest pathogens by site exclusion and by sugar competition.
Additional, *Cryptococcus laurentii* is able to reduce the germination of *Botytis cinerea* conidia (Filonow, 1999).
The present study aimes to investigate the influence of the epiphytic yeast *Cryptococcus laurentii* isolated from apple leaves on the secondary methabolism of callus cultures of *Vitis vinifera* in order to elucidate its potential for activating the accumulation of defensive compounds.

In the experimental system callus from *Vitis vinifera* „Regent“ was cultivated on modified MS-medium (Murashige and Skoog 1962). *Cryptococcus laurentii* was cultivated in liquid medium on a shaker (100 U/min) for 48 h and subsequently autoclaved to mortify the yeast cells. After centrifugation of the yeast suspension the supernatant was added to the culture medium of grapevine callus. Samples were harvested after 5, 10, 24 and 48 h. The phenylpropanoids were measured by HPLC and identified by UV-spectroscopy and thin layer chromatography.
Treatment with yeast suspension induced an accumulation of stilbenes and oligomeric hydroxycinnamic acids after 10 h (Fig. 1). The content of most compounds increased up to 48 h after treatment. Some stilbenes constitutively existing in the callus increased after treatment (Fig.1 A). Other stilbenes such as resveratrol were newly synthesized (Fig. 1 B).
Nitrogen deficiency also led to an increase of stilbenes and of oligomeric hydroxycinnamic acids. Nitrogen excess produced analogue reactions as nitrogen deficiency, but to a lower extent. Nitrogen deficiency and excess may be stress conditions for the callus cells which was confirmed by a reduced growth in these variants. The interaction between N-nutrition, secondary metabolism and resistance was already discribed for apple (Rühmann and Treutter, 2003; Rühmann et al. 2002).

The cells of *Vitis vinifera* react to this unspecific stress with an accumulation of the new compounds. The effect of yeast treatment on the accumulation of phenylpropanoids was affected by further stress conditions such as N deficiency and N excess.

Hydroxycinnamic acids are important constructions of the cell wall, representing the first barrier during pathogen attack. Furthermore, stilbenes are not only playing an important role in the defense against pathogens, but are also discussed as bioactive compounds in human nutrition.

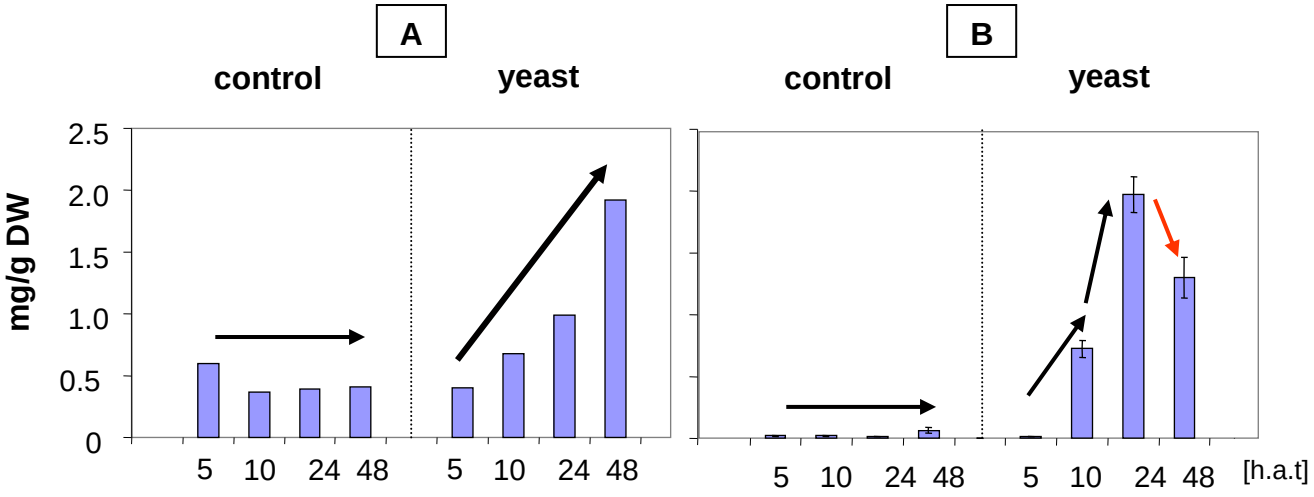

Fig. 1. stilbenes of callus from Vitis vinifera "Regent" at different hours after treatment (h.a.t.) (A) total content of stilbenes without resveratrol (B) resveratrol

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