# Phytohormones Effect on Resveratrol Production by *Vitis vinifera* cell cultures

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**Abstract**

This study was conducted to optimize phytohormones combination and concentration on resveratrol production of *Vitis vinifera* callus cultures. TDZ was so effective for callus proliferation and resveratrol production and can be expected as a stimulus to the cells which keep the ability of producing resveratrol. We optimized the hormone combination of NAA 0.5 mg/L and TDZ 2 mg/L to stimulate resveratrol production very effectively. And callus under MS medium with the optimized phytohormones combination was treated by fungal elicitor, *Botrytis*.

## Introduction

Phytoalexins are organic metabolites that are produced by plants in response to fungal infection or abiotics such as heavy metal ions or UV light (Bailey, 1982). In grapevines, the response to stresses as fungal infection and UV irradiation, includes the production of a simple stilbene, resveratrol (*trans*-3,5,4'-trihydroxystilbene) and its glucoside with biosynthetically related compounds viniferins and pterostilbene. Resveratrol is a compound that is found in the skin and seeds of grapes and is currently being tested as an anti-cancer compound. Especially, Jang *et al* reported that resveratrol reduced the numbers of tumors in mice treated with the tumor promoter TPA (Jang *et al.* 1997) and it displayed a wide range of anti-cancer properties.

When *Vitis vinifera* cells are grown in a resveratrol-inducing medium, they can accumulate high levels of resveratrol, while showing good growth (Decendit and Merillon 1996; Decendit *et al.* 1996; Waffo Teguo et al. 1996). Exogenous phytohormones are normally required for tissue culture initiation and to promote growth *in vitro*. Choice of hormones also has a profound effect on the profile of secondary metabolites accumulation in plant cell cultures. Further systematical investigations are needed to determine the role of hormones in resveratrol production. Therefore, this study is aiming at determining whether phytohormones could be used to stimulate *in vitro* production of resveratrol. We investigated phytohormone effects on resveratrol production of *Vitis* callus, which plays an important role to optimize the hormone concentration to maximize resveratrol productivity.

## Materials and Methods

*Callus induction and subculture*

*V. vinifera* L. plantlet were kindly donated by Dept. of Horticulture Science, Korea University.

Callus initiated from the plantlet was maintained on a culture medium containing Murashige and Skoog salts, 3% sucrose, 1.0 $mgl^{-1}$ NAA(naphthalenacetic acid), 5.0 $mgl^{-1}$ BA($N^6$-bemzyladennine) and 0.8% agar. One year after callus initiation, callus was transferred on MS medium with 81 combinations of 2 auxins (NAA and IBA (indole-3-butyric avid)) and 2 cytikinins (TDZ(1-phenyl-(1,2,3-thiadiazol-5-yl)-urea) and BA).

*Quantification of resveratrol*

Resveratrol was extracted from dried cells overnight with methanol. Analysis of resveratrol was performed by HPLC(*Waters, Massachusetts, USA*) on a reversed phase C18 column (*LiChroCART*® *Merck*) and $CH_3CN/H_2O$ eluant system(60:40). The chromatogram was monitored between 200 and 600 nm using a photodiode array detector.(Fig.1) Resveratrol contents were estimated from a calibration curve prepared with authentic standards purchased form Sigma.

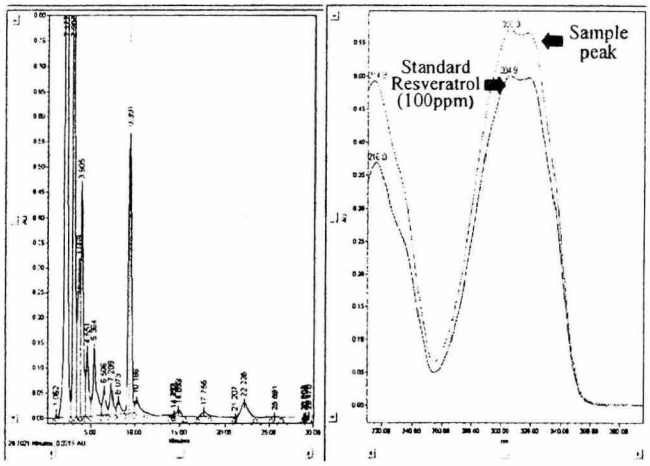


Fig.1 HPLC analysis result of *Vitis* callus extracts

*Elicitor Treatment*

Fungla elicitor was prepared from *Botrytis spp.* According to Facchini(1998). A section ($1cm^2$) of mycelia cultured on potato dextrose agar was grown in 50 ml B5 medium, including supplements but lacking phytohormones, on a gyratory shaker (120rpm) at 24°C in the dark for 6 days. Mycelia and remaining medium were homogenized at maximum speed for 10 min, autoclaved (121°C) for 20 min, and subsequently centrifuged under sterile conditions with the supernatant serving as elicitor. Elicitor treatments were initiated by the addition of 250μl of fungal homogenate to a callus colony.

**Results and Discussion**

The type and concentration of phytohormones available to cultured cells is probably the most important factor influencing their potential for secondary product synthesis (P. Morris, *et al.*, 1986). A range of hormone combinations and concentrations should be examined for optimum effect on secondary product formation. To study the effect of phytohormones on resveratrol accumulation, grape callus was grown on MS medium containing 81 combinations of 2 auxins (NAA and IBA) and 2 cytokinins(TDZ and BA). (Table 1) TDZ showed much better effect on

callus growth than BA as cytokinin effect. TDZ was used alone in several orchid species mainly to induce shoot regeneration for mass propagation (Chen and Piluek 1995; Ernst 1994; Nayaketal. 1997) or combined with auxin to induce embryogenic calli from rhizomes.

Table1. Variable exogenous phytohormones combinations added to Vitis callus medium (+++++ excellent growth; ++++ good; +++ moderate; ++ slow; + bad)

| | TDZ 0.0 | TDZ 0.1 | TDZ 0.5 | TDZ 1.0 | TDZ 2.0 |
|---|---|---|---|---|---|
| NAA 0.0 | + | ++++ | ++++ | ++++ | +++ |
| NAA 0.1 | +++ | +++++ | +++++ | +++++ | +++++ |
| NAA 0.5 | ++++ | +++++ | +++++ | +++++ | +++++ |
| NAA 1.0 | + | +++++ | +++++ | +++++ | ++++ |

| | BA 1.0 | BA 2.0 | BA 3.0 | BA 5.0 | BA 10.0 |
|---|---|---|---|---|---|
| NAA 0.0 | ++ | + | ++ | ++ | + |
| NAA 0.1 | +++ | +++ | +++ | +++ | +++ |
| NAA 0.5 | ++++ | +++++ | +++ | +++ | +++ |
| NAA 1.0 | +++++ | +++ | ++++ | ++ | ++ |

| | TDZ 0.0 | TDZ 0.1 | TDZ 0.5 | TDZ 1.0 | TDZ 2.0 |
|---|---|---|---|---|---|
| IBA 0.01 | + | ++++ | ++++ | +++++ | +++++ |
| IBA 0.1 | ++++ | +++++ | +++++ | +++++ | +++++ |
| IBA 0.5 | +++ | +++++ | +++++ | +++++ | +++++ |
| IBA 1.0 | +++ | +++++ | +++++ | +++++ | +++++ |

| | BA 1.0 | BA 2.0 | BA 3.0 | BA 5.0 | BA 10.0 |
|---|---|---|---|---|---|
| IBA 0.01 | +++++ | ++ | ++ | ++ | + |
| IBA 0.1 | +++ | +++ | ++ | ++ | ++ |
| IBA 0.5 | ++++ | +++ | +++ | +++ | +++ |
| IBA 1.0 | ++++ | +++ | +++ | +++ | +++ |

Nakano *et al.* (1997) reported a callus induction media containing 10 μM 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) with 10 μM TDZ were suitable for inducing subsequent adventitious embryogenesis from leaf explants of *V. vinifera* Koshusanjaku. However, we found that TDZ with NAA or IBA had no effect on regeneration from the callus and further NAA/TDZ combination showed the best growth and proliferation. Fast proliferation of callus and suspended cells is very important factor for increasing secondary metabolite productivity through reactor culture. And even the average resveratrol productivity of the combination of NAA 0.5mg/L and TDZ 2.0mg/L (N0.5T2) was 600μg/gDCW.(Fig.2) Therefore we optimized the phytohormones condition for producing resveratrol as N0.5T2. In order to investigate the response of Vitis callus to stress of fungal elicitor, we treated Vitis callus with *Botrytis* (data not

shown), which stimulated resveratrol production.

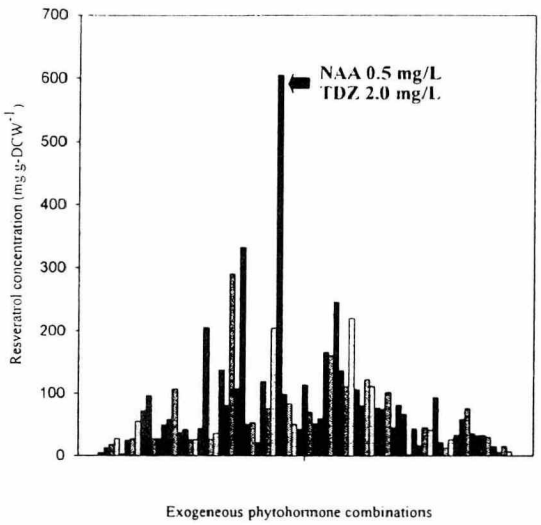


Fig.2 Optimization of phytohormones combination and concentration for maximizing resveratrol productivity

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