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Effect of indole-3-butyric acid on the recovery of phytoplasma-infected grapevine

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Abstract

Phytoplasma-infected and healthy grapevines were treated on the leaves with indole-3-butyric acid. The effect of IBA was examined by testing the grapevine for presence of phytoplasma 16S rDNA and by measuring stress parameters before and after the treatment. Vines treated with IBA showed signs of recovery, but high incidence of natural recovery was also observed among untreated vines in the same experiment. In naturally-recovered untreated plants, higher levels of H_2O_2 were measured, while in IBA-treated plants recovery was not correlated with higher H_2O_2 levels after the treatment. Other biochemical parameter measurements, lipid peroxidation and total phenolics, did not show difference between the groups of vines tested.

Key words: IBA, phytoplasma, grapevine, hydrogen peroxide.

Introduction

Phytoplasmas cause numerous economically relevant diseases that affect hundreds of plant species including grapevine (*Vitis vinifera* L.). So far, research attempts have been made in order to understand natural-recovery phenomenon of phytoplasma-infected grapevines (Musetti *et al.*, 2007), and different methods and techniques have been applied in the attempts to eliminate phytoplasmas from their hosts (Romanazzi *et al.*, 2009). Previous research also revealed that supplement of plant growth regulator, IBA, causes recovery of phytoplasma-infected periwinkle shoots grown in vitro and elimination of '*Candidatus* Phytoplasma asteris' reference strain HYDB (Ćurković- Perica *et al.*, 2007, Ćurković-Perica, 2008; Leljak-Levanić *et al.*, 2010). The aim of this research was to test the effect of IBA phytoplasma-infected vineyard-grown grapevine.

Materials and methods

In July 2008 leaves of *Vitis vinifera* L. cv. Chardonnay from the University vineyard Jazbina (Zagreb, Croatia) were taken to determine the presence of phytoplasmas in vines that exhibited symptoms of grapevine yellows. Presence of phytoplasmas was confirmed by nested PCR. Sixteen plants that were positive in 2008 were chosen for the treatment in 2009. Plants that have never showed symptoms, and in which the presence of phytoplasma was not confirmed, served as negative controls. Phytoplasma-positive grapevines were randomly divided into four different treatment groups. Vines were treated by spraying the leaves with 1 L of IBA solution at three different concentrations (0.5, 1.0, 1.5 g/L). Positive controls (four symptom-expressing, phytoplasma positive vines) were treated with water and negative controls were treated with 1.0 g IBA/L or water. All the treated groups of grapevines, including positive and negative controls consisted of four vines. Leaf samples were collected before the treatment (May 2009) and two times after the treatment (July and August 2009), and immediately stored at -20°C. Leaf veins were cut out and used for isolation of total nucleic acid. The rest of the leaf material was ground in liquid nitrogen and used for the measurement of stress parameters.

For phytoplasma detection total nucleic acid isolation was performed according to Ćurković Perica *et al.* (2007). Direct PCR, first nested and second nested PCR assays were performed using primer pairs R16F1/R0, R16F2n/R2 and R16(I)F1/R1, respectively. Nested PCRs were performed with the samples collected in July 2008 and August 2009 (table 1). Concentration of hydrogen peroxide was determined according to the method of Mukherjee and Choudhari (1983). The level of lipid peroxidation, expressed as malonyldialdehyde (MDA), was determined according to the modified method of Heath and Packer (1968). Total phenolics were determined according to the modified method of Slinkard and Singleton (1997).

Results and discussion

Škorić *et al*. (1998) showed that phytoplasma detection later in the growing season of grapevine is more accurate than in spring. Therefore, presence/absence of phytoplasma was determined in symptom-expressing and symptom-free vines, respectively, by nested PCR in July 2008. Symptoms of grapevine yellows included discoloration and necrosis of leaf veins and leaf blades, downward curling of leaves, lack or incomplete lignification of shoots, necrosis of shoots and shriveling of berries. Based on the results in 2008, vines were selected for the treatment in May 2009. Concentrations of IBA used for treatments and presence/absence of symptoms and phytoplasma in tested vines are listed in table 1.

Table 1. Presence of symptoms and phytoplasma detection on IBA-treated grapevines before and after the treatment.

vine number	treatment (g of IBA/vine)	symptoms and phytoplasma detection before the treatment	symptoms after the treatment	phytoplasma detection by PCR after the treatment
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	1.0	-	-	-
6	1.0	-	-	-
7	1.0	-	-	-
8	1.0	-	-	-
9	-	+	-	-
10	-	+	+	+
11	-	+	+	+
12	-	+	-	-
13	0.5	+	-	-
14	0.5	+	-	-
15	0.5	+	-	-
16	0.5	+	-	-
17	1.0	+	+/-	+/-
18	1.0	+	-	-
19	1.0	+	+	+
20	1.0	+	-	-
21	1.5	+	+/-	+/-
22	1.5	+	+/-	+/-
23	1.5	+	+	+
24	1.5	+	-	-

(+) = symptomatic/phytoplasma 16S rDNA detected;
(-) = asymptomatic/absence of phytoplasma 16S rDNA;
(+/-) = one shoot with symptoms, one asymptomatic, phytoplasma 16S rDNA not detected in asymptomatic part of the vine.

High incidence of natural recovery in the vineyard which also affected vines that served as positive untreated controls in the experiment made explanation of the results difficult; two vines although symptomatic and phytoplasma positive in 2008, were symptomless and phytoplasma-negative in summer of 2009. Those recovered untreated vines had higher H_2O_2 levels than symptomatic vines and this is consistent with finding of Musetti *et al*. (2007). High incidence of recovery was also obvious among IBA-treated vines, but recovery was not correlated with higher H_2O_2 levels in those vines. Total phenolics and MDA contents measured in tested vines correlated neither with infection nor with recovery. MDA increased in all tested groups of vines through the growing season.

Because natural recovery was observed within the positive control group of vines treated with water and the percentage of IBA-treated completely recovered vines was not significantly higher than the percentage of naturally-recovered vines, further research is required to elucidate the possible role of IBA in grapevine recovery.

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References

ĆURKOVIĆ-PERICA M., 2008.- Auxin-treatment induces recovery of phytoplasma-infected periwinkle.- *Journal of Applied Microbiology*, 109: 1826-1834.

ĆURKOVIĆ-PERICA M., LEPEDUŠ H., ŠERUGA MUSIĆ M., 2007.- Effect of indole-3-butyric acid on phytoplasmas in infected *Chatarantus roseus* shoots grown *in vitro*.- *FEMS Microbiology Letters*, 268: 171-177.

HEATH R. L., PACKER L., 1968.- Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation.- *Archives of Biochemistry and Biophysics*, 125(1): 189-198.

LELJAK-LEVANIĆ D., JEŽIĆ M., CESAR V., LUDWIG-MÜLLER J., LEPEDUŠ H., MLADINIĆ M., KATIĆ M., ĆURKOVIĆ-PERICA M., 2010.- Biochemical and epigenetic changs in phytoplasma-recovered periwinkle after indole-3-butyric acid treatment.- *Journal of Applied Microbiology*, 109: 2069-2078.

MUKHERJEE S. P., CHOUDHARI M. A., 1983.- Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings.- *Physiologia Plantarum*, 58: 116-170.

MUSETTI R., MARABOTTINI R., BADIANI M., MARTINI M., SANITÀ DI TOPPI L., BORSELLI S., BORGO M., OSLER R., 2007.- On the role of H_2O_2 in the recovery of grapevine (*Vitis vinifera* cv. Prosecco) from flavescence dorée disease.- *Functional Plant Biology*, 34(8): 750–758.

ROMANAZZI G., D'ASCENZIO D., MUROLO S., 2009.- Field treatment with resistence inducers for the control of grapevine bois noir.- *Journal of Plant Pathology*, 91: 677-982.

ŠKORIĆ D., ŠARIĆ A., VIBIO M., MURARI E., KRAJAČIĆ M., BERTACCINI A., 1998.- Molecular identification and seasonal monitoring of phytoplasma infecting Croatian grapevines.- *Vitis*, 37(4): 171-175.

SLINKARD K., SINGLETON V. L., 1977.- Total phenol analyses: automation and comparison with manual methods.- *American Journal of Enology and Viticulture*, 28: 49-55.

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