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Technical Report

Influence of grapevine flower treatment with gibberellic acid (GA_3) on indole-3-acetic acid (IAA) contents of white wine

Martin S. Pour Nikfardjam, Krisztián Gaál and Péter Teszlák

FVM Research Institute for Viticulture and Oenology
H-7634 Pécs, Pázmány Péter u. 4
E-mail: martinpn@axelero.hu

'Riesling' and 'Sauvignon' grapevine flowers were treated at full bloom with an aqueous solution with 20 µg/l gibberellic acid (GA_3). The resulting wines were analysed for indole-3-acetic acid contents by means of HPLC with fluorescence detection. Compared to the untreated control, wines from the GA_3 treated variants showed higher IAA contents; 'Riesling': control: 34.8 µg/l, GA_3: 86.6 µg/l; 'Sauvignon blanc': control: 37.7 µg/l, GA_3: 54.2 µg/l. Since values above 50 µg/l have been reported to be sufficient for the formation of the untypical aging off-flavour (UTA), further research should be conducted to reveal the impact of GA_3 treatment of grapevine flowers on the UTA formation potential in white wine.

Keywords: Vitis vinifera, flower treatment, gibberellic acid, indole-3-acetic acid, untypical aging flavour

The tryptophan metabolite indole-3-acetic acid (IAA) is regarded an important potential precursor of 2-aminoacetophenone (AAP), an aroma compound, which is associated with the so-called „untypical aging off-flavour" (UTA) in *Vitis vinifera* L. white wines and generally occurs after a few months of storage (Rapp et al., 1993; Dollmann et al., 1996; Hoenicke et al., 2002). The off-flavour is described by various aroma descriptors, such as „acacia blossom, naphthalene, furniture polish, wet wool or fusel alcohol". Depending on wine flavour, UTA can be organoleptically recognised at concentrations as low as 0.5 µg/l AAP (Rapp et al., 1993).

Gibberellins are an important class of natural growth regulators in plants. They are products of the diterpenoid pathway and their formation is initiated by cyclisation of the common C_{20} precursor geranylgeranyl diphosphate (GGPP) (Graebe et al., 1965; Hedden and Proebsting, 1999). GA_3 has been widely used in table grape production and its effects on grape quality have been intensively studied (Guelfat-Reich and Safran, 1973; Ben-Tal, 1990; Korkas et al., 1999). Generally, GA_3 application reduced berry set, increased berry weight, and improved juice quality. It also led to increased petiole lengths, more rigid pedicels, less firm berry skin, reduced the number of seeds, and enhanced shoot growth.

While still not being generally used in grape-wine production, there is increasing interest in the wine producing community to use GA_3 for thinning purposes, e.g. in Germany (Bader, 2004). Thinning would decrease the risk of diseases, such as *Botrytis cinerea* and bunch rot, and would also reduce workload for viticulturists. More recent research has shown, that GA_3 treatment leads to higher polyphenol and anthocyanin contents, respectively (Teszlák et al., 2005). Research performed on bayberries (*Myrica rubra* Bieb.) showed that GA_3 spraying of bayberry flower buds leads to inactivation of the enzymes phenylalanine lyase (PAL) and polyphenol oxidase (PPO), which could explain the higher polyphenol contents in wines made from GA_3 treated grapes. But GA_3 treatments also led to higher contents of IAA in bayberries (Li et al., 2003). Christoph et al. (1999) reported on increased 2-AAP contents of (model) wines after addition of IAA. Thus, an increased IAA content of grape berries evoked through GA_3

could further increase the peril of 2-AAP formation and would militate against GA_3 treatment of UTA sensitive white wine cultivars. To our knowledge no data exist on the influence of GA_3 treatment of grapevine flowers on IAA contents of white wines.

Materials and Methods

Chemicals

All reagents used were of analytical grade unless otherwise stated. Gibberellic acid (GA_3) and Tween 20® were from Fluka (Buchs, Switzerland). Deionised, nanopure water was from Szkarabeusz (Pécs, Hungary). Acetonitrile (gradient grade) was from Merck (Darmstadt, Germany).

Grapes

Grapes were grown in our own vineyards on the south-facing slopes of the Mecsek Hills in Pécs (latitude: 46°07' N, longitude: 18°17' E, 180 to 200 m above sea level). The soil is Ramann-type brown duff on red sandstone. The vines were not irrigated. The 5-year-old 'Riesling' and 'Sauvignon blanc' grapevines were grown according to the Lenz-Moser-system. In each case 40 grapevines were selected from each cultivar, of which 20 were kept untreated and 20 were treated with GA_3 as follows.

GA_3 treatment

Grapevine flowers were sprayed to the drip point at full bloom with an aqueous 20 mg/l GA_3 solution (containing 0.2 % Tween 20®). The control group remained untreated.

Winemaking technology

The grapes were harvested manually at physiological ripeness, destemmed and crushed with Cantinetta C.D.A. TR (Nuova Zambelli, 36043 Camisano Vicentino (VI), Italy). After crushing the grapes received 20 mg/kg SO_2. After destemming and crushing the mashes were immediately pressed by means of a hydraulic press and the resulting must was divided in three plastic containers (5 litres) resulting in three replicates for each cultivar and treatment. They then were inoculated with yeast culture (Lalvin® EC 1118, Lallemand, Rexdale, Canada) and fermented for one week. After fermentation was completed, the wines were racked off the lees, received 50 mg/l SO_2, and were then stored at 14 °C in the dark until analysis.

HPLC analysis

Analysis of IAA was performed as described by Bonerz et al. (in preparation) with a Perkin Elmer (Wellesley, USA) 200 Series HPLC system consisting of degasser, autosampler, pump, column oven, and fluorescence detector (FLD). IAA was best detected by means of a fluorescence detector with an extinction wavelength of 225 nm and an emission wavelength of 365 nm, respectively. Quantification limit was 0.2 µg/l.

Sample preparation

Before HPLC analysis all samples and standards were filtered (Minisart RC 15® 0.45 µm, Fa. Sartorius, Göttingen, Germany). A volume of 20 µl was injected into the HPLC.

Mobile phase

The gradient consisted of two eluents: solvent A: water/phosphoric acid (99.5/0.5; v/v); solvent B: acetonitrile/water/phosphoric acid (50/49.5/0.5; v/v/v). The flow rate was 1.0 ml/min. Separation of components was achieved by following gradient: The concentration of A was kept constant at 100 % for 2 min, then the concentration of B was increased within 5 min to 20 %, then further increased to 40 % within 18 min, followed by a holding time of 6 min. Within 4 min solvent B was increased to 80 % and then within 5 min to 100 %, followed by a holding time of 2 min. Equilibrium time to original conditions was 15 min. All samples were analysed in duplicate.

Column

An end-capped RP-18 column (ChromSep, LiChrospher® 250 x 4.6 mm, 5 µm, Fa. Varian, Budapest, Hungary) was used and kept at 30 °C.

Results and Discussion

Figure 1 shows a typical HPLC/FLD-chromatogram of a white wine made from GA_3 treated grapes. Treatment

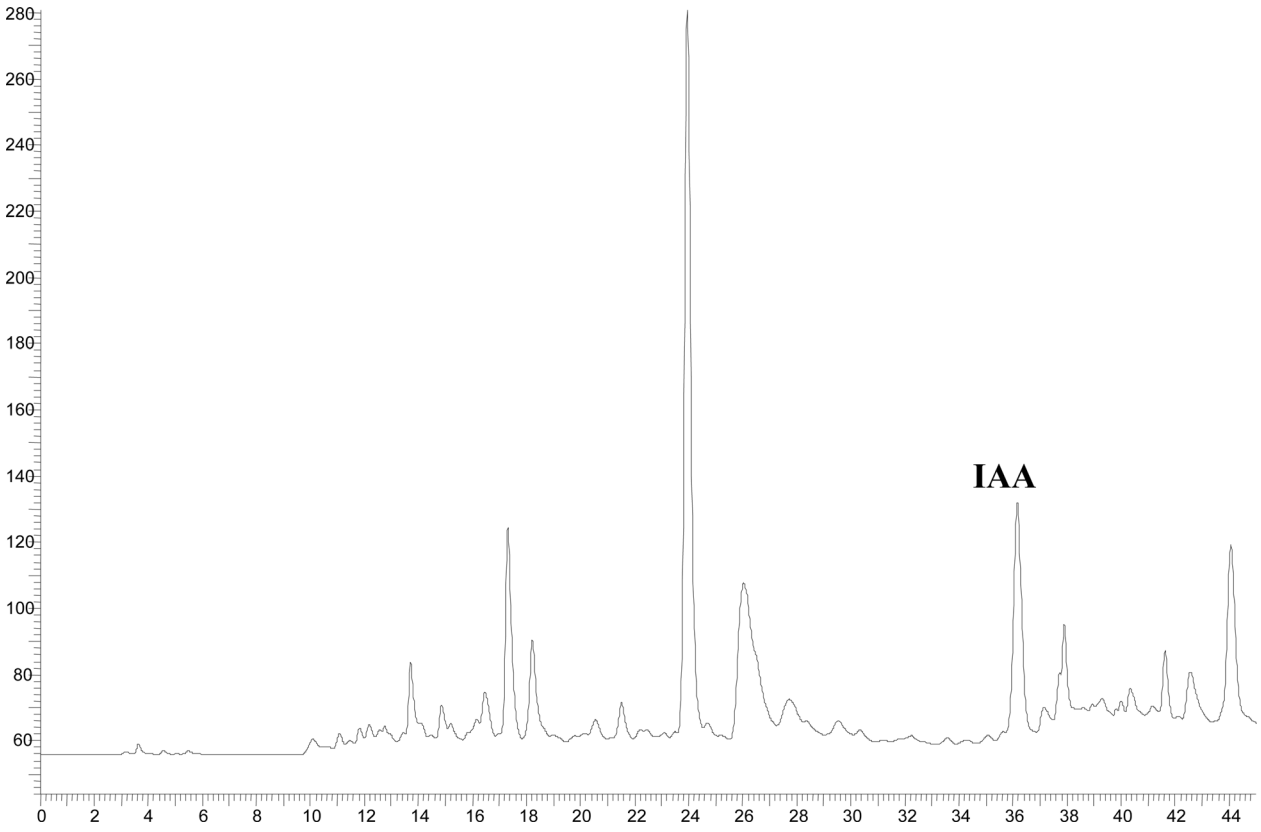

Fig. 1: HPLC-fluorescence chromatogram (extinction: 225nm, emission: 365nm) of a 'Riesling' wine

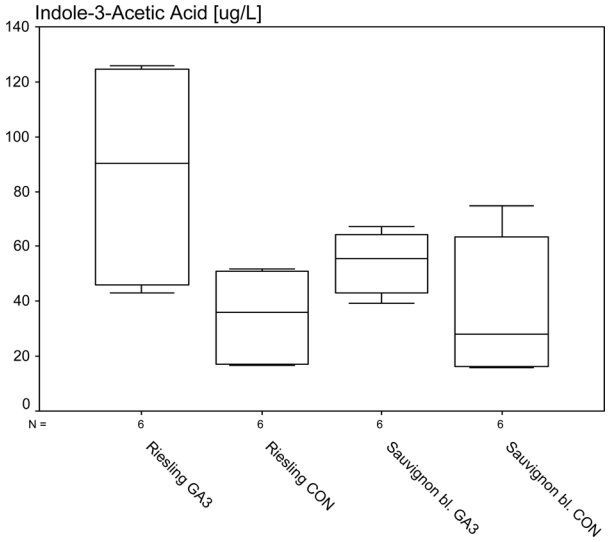

Fig. 2: IAA content (µg/l) of white wines made from GA_3 treated (GA3) and untreated (CON) grapes

of grapevine flowers with 20 mg/l GA_3 led to significantly higher ($p < 0.05$) contents of IAA in 'Riesling' wine (Fig. 2). While 'Sauvignon blanc' seemed to be less sensitive to the GA_3 treatment, 'Riesling' showed a much higher response. Mean IAA content of 'Sauvignon' blanc was: control: 37.7 µg/l; GA_3-treatment: 54.2 µg/l (+ 43.8 %). Mean IAA content in 'Riesling' was: control: 34.8 µg/l; GA_3-treatment 86.6 µg/l (+ 148.9 %).

Our results on IAA contents of white wines made from untreated grapevines are thus in accordance with the data recently published by LINSENMEIER et al. (2004), who found IAA concentrations between 3.7 and 46.6mg/l with a mean of 21.6 µg/l in 'Riesling' wines of the vintages between 1994 and 1999. MATTIVI et al. (1999) found similar concentrations in 'Chardonnay' wines ranging from 13 to 58 µg/l.

CHRISTOPH et al. (1998) regarded IAA values above 50 µg/l as critical, since according to their results IAA concentrations above 50 µg/l might already lead to a formation of organoleptically active 2-AAP concentra-

tions of around 1 µg/l. While IAA values in our wines from untreated grapevines were within the concentration range published in literature, the concentrations found in GA_3 treated wines were higher and were 8 % ('Sauvignon blanc') and 73 % ('Riesling'), respectively, higher than the critical value of 50 µg/l introduced by CHRISTOPH et al. (1998). Thus, our results implicate that GA_3 treatment of grapevine flowers increases IAA contents in white wine and might lead to higher UTA off-flavour formation during storage. Furthermore, our biological measurements on the physiological performance of the grapevines, especially on apoplastic water content ($A_{WSD\%}$), indicated water stress conditions in GA_3 treated 'Riesling', while 'Sauvignon blanc' and the respective control vines were practically free of water stress (data not shown). This would support our hypothesis that a) 'Riesling' is more sensitive to GA_3 treatment and b) that GA_3 treatment could lead to more UTA off-flavour because water stress of grapevines is regarded a key factor in UTA formation (RAPP et al., 1993; CHRISTOPH et al., 1998; HOENICKE et al., 2002).

Yet, since IAA is not the only component discussed as a potential precursor of the UTA off-flavour and not all researchers found a direct correlation between IAA content and UTA potential (HOENICKE et al., 2002), further trials have to reveal if wines made from GA_3 treated grapevines inevitably develop more 2-AAP or other substances associated with UTA. These studies are already on the way and results will be available in the near future.

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