https://doi.org/10.17221/70/2020-HORTSCI

The effect of defoliation on the 3-isobutyl-2-methoxypyrazine biosynthesis in wine grapes

Yujuan Lei[1,2], *Zhansheng Ma*[1], *Pengbao Shi*[1], *Yuxia Sun*[3], *Ping Wang*[4], *Xueqiang Guan*[3]*

[1]*College of Food Science & Technology, Hebei Normal University of Science and Technology, Qinhuangdao, P.R. China*
[2]*Hebei Yanshan Agricultural Characteristic Industry Research Institute, Qinhuangdao, P.R. China*
[3]*Shandong Academy of Grape/Winegrape and Wine Technological Innovation Center of Shandong Province, Jinan, P.R. China*
[4]*College of Food Engineering, Shihezi University, Shi Hezi, P.R. China*

**Corresponding author: guanxq90@126.com*

Citation: Lei Y.J., Ma Z.N., Shi P.B., Sun Y.X., Wang P., Guan X.Q. (2022): The effect of defoliation on the 3-isobutyl-2-methoxypyrazine biosynthesis in wine grapes. Hort. Sci (Prague), 49: 117–120.

Abstract: Field studies were conducted on *Vitis vinifera* cv. 'Cabernet Sauvignon' to evaluate the effects of the defoliation time (pre- and post-flowering and veraison) and severity on the 3-isobutyl-2-methoxypyrazine (IBMP) concentration and expression levels of the key related genes in grape berries. The IBMP concentration in the grapes decreased significantly at harvest after all the defoliation treatments. The earlier and heavier the defoliation treatment, the lower the IBMP concentration. The relative expression level of VvOMT3 in the berry skin was closely positively correlated with the IBMP accumulation in all the treatments. Early defoliation coupled with a 1-week delay in the harvest time may be an effective management strategy to control the IBMP concentration in grapes.

Keywords: defoliation; 'Cabernet Sauvignon'; 3-Isobutyl-2-methoxypyrazine; biosynthesis

The "herbaceous and vegetal tastes" of wines made from some grape (*Vitis vinifera*) varieties have troubled wine makers for a long time. 3-Alkyl-2-methoxypyrazines (MPs) are a main source of these sensory characteristics (Mozzon et al. 2016). To date, seven MPs have been identified in grapes and wines, and the most important one is 3-isobutyl-2-methoxypyrazine (IBMP), which is responsible for the green pepper aroma of some grape varieties, because its concentration in grapes often markedly exceeds the sensory detection threshold (Sidhu et al. 2015).

IBMP mainly exists in the grape berry skins (72.0%), but it is also present in the seeds (23.8%). It remains relatively unchanged during fermentation and is marginally affected by winemaking techniques (Roujou-de-Boubée et al. 2002). Thus, an effective way to decrease the IBMP concentration in wine is to decrease the IBMP contentin the grape.

This work explored appropriate defoliation strategies to decrease the IBMP concentration and the expression levels of the key related genes in grape berries.

Supported by the Natural Science Foundation of Hebei Province, China, Project No. C2021407016; the Hebei Yanshan Agricultural Characteristic Industry Technology Research Institute post-performance subsidy, China, Project No. 205676154H; the major agricultural application innovation project of the Shandong Province "Research and application of root restriction and rain sheltered cultivation techniques for grape in saline-alkali soil".

Table 1. Different defoliation treatments

Treatments	Time	Method
DF-pre-F-H	pre-flowering	removing the 1st, 3rd and 5th leaves from the base of the shoot
DF-pre-F-W		removing six leaves from the base of the shoot
DF-post-F-H	post-flowering	removing the 1st, 3rd and 5th leaves from the base of the shoot
DF-pre-F-W		removing six leaves from the base of the shoot
DF-V-H	veraison	removing the 1st, 3rd and 5th leaves from the base of the shoot
DF-V-W		removing six leaves from the base of the shoot
CK		vines untouched with the complete maintenance of all canopy leaves

Veraison refers to approximately 5% of the cluster in the vineyard and 5% of the berries per cluster having begun to colour

During 2016–2017, seven defoliation treatments were carried out in a comercial 'Cabernet Sauvignon' vineyard (Table 1). The vines were trained onto a unilateral cordon with a vertical shoot positioning trellis system. All the defoliation treatments were performed manually in the cluster zone and maintained until harvest. Each treatment had three independent replicates, and each replicate consisted of ten vines (approximately 14 shoots per vine), arranged in a randomised block design. The different defoliation times were determined in accordance with the Modified Eichhorn-Lorenz (E-L) system (Coombe 1995). "Pre-flowering" was determined as approximately occurring eight and six days before anthesis in 2016 and 2017, respectively; "Post-flowering" was determined as approximately occurring 10 and 12 days after anthesis (DAA) in 2016 and 2017, respectively; and "Veraison" occurred at 62 and 65 DAA in 2016 and 2017, respectively.

Within the study, grape berry samples were collected at 31, 62, 76, 101 and 132 DAA in 2016, and at 25, 65, 95, 114 and 138 DAA in 2017. Three biological replicates of each sample were taken, and 250 berries were collected randomly per replicate.

The analysis showed that the berry weight (weight per berry) increased along with the grape development until 65 DAA, and then, the weight gain stopped. There were no significant differences ($P \geq 0.05$) in the berry weights among the different defoliation treatments during the experimental period (Table 2). The total soluble solids (TSS) contents of the grapes increased along with the berry ripening, which increased significantly after the DF-pre-F-H treatment, from 65 DAA until harvest (by 0.4 to 0.5 °Brix) compared with the CK at the same time points ($P < 0.05$) (Table 2).

The IBMP content was determined in the grape berries in accordance with a previously reported method with slight modifications (Koch et al. 2010; Sidu et al. 2015). The IBMP concentrations in the grapes at harvest (132 DAA) in 2016 were 14.24, 8.18, 14.68, 13.90, 17.39 and 16.07 ng/kg after the DF-pre-F-H, DF-pre-F-W, DF-post-F-H, DF-post-F-W, DF-V-H and DF-V-W treatments, respectively. These values were significantly decreased, by 33.83% ($P < 0.05$) to 68.87% ($P < 0.01$), compared with those in the CK (26.28 g/kg) at the same time points. In 2017, the levels decreased to below the quantitation limit (2.00 ng/

Table 2. The effect of the defoliation on the TSS content and berry weight in 2017

Treatments	Days after anthesis (DAA)									
	25		65		95		114		138	
	TSS	BW	TSS	BW	TSS	BW	TSS	BW	TSS	BW
DF-pre-F-H	2.4 ± 0.1^{a}	0.8 ± 0.0^{a}	10.9 ± 0.2^{a}	1.1 ± 0.2^{a}	15.5 ± 0.1^{a}	1.2 ± 0.1^{a}	18.8 ± 0.1^{a}	1.2 ± 0.0^{a}	20.5 ± 0.2^{a}	1.2 ± 0.0^{a}
DF-pre-F-W	2.2 ± 0.1^{a}	0.8 ± 0.1^{a}	10.6 ± 0.3^{b}	1.2 ± 0.1^{a}	15.2 ± 0.4^{b}	1.2 ± 0.1^{a}	18.4 ± 0.2^{b}	1.2 ± 0.2^{a}	20.6 ± 0.2^{a}	1.2 ± 0.2^{a}
DF-post-F-H	2.3 ± 0.2^{a}	0.7 ± 0.0^{a}	10.4 ± 0.4^{b}	1.2 ± 0.1^{a}	15.1 ± 0.3^{b}	1.2 ± 0.1^{a}	18.5 ± 0.2^{b}	1.2 ± 0.0^{a}	20.3 ± 0.5^{b}	1.2 ± 0.1^{a}
DF-pre-F-W	2.4 ± 0.1^{a}	0.7 ± 0.0^{a}	10.5 ± 0.3^{b}	1.2 ± 0.1^{a}	15.1 ± 0.4^{b}	1.1 ± 0.1^{a}	18.5 ± 0.3^{b}	1.2 ± 0.0^{a}	20.1 ± 0.3^{b}	1.2 ± 0.2^{a}
DF-V-H	2.5 ± 0.1^{a}	0.7 ± 0.0^{a}	10.4 ± 0.3^{b}	1.1 ± 0.1^{a}	15.2 ± 0.2^{b}	1.1 ± 0.1^{a}	18.4 ± 0.3^{b}	1.1 ± 0.0^{a}	20.0 ± 0.2^{b}	1.2 ± 0.1^{a}
DF-V-W	2.5 ± 0.1^{a}	0.7 ± 0.1^{a}	10.5 ± 0.2^{b}	1.2 ± 0.0^{a}	15.1 ± 0.4^{b}	1.2 ± 0.1^{a}	18.3 ± 0.2^{b}	1.2 ± 0.0^{a}	20.2 ± 0.4^{b}	1.1 ± 0.2^{a}
CK	2.3 ± 0.1^{a}	0.8 ± 0.0^{a}	10.5 ± 0.3^{b}	1.2 ± 0.1^{a}	15.1 ± 0.2^{b}	1.1 ± 0.1^{a}	18.4 ± 0.4^{b}	1.2 ± 0.0^{a}	20.0 ± 0.3^{b}	1.2 ± 0.1^{a}

BW – berry weight (g/berry); TSS – total soluble solids content (°Brix); for treathments description see Table 1

The data are the mean of three replicates and their standard error (SE) kg) at harvest (138 DAA) after the different defoliation treatments, except for the DF-V-F-H treatment (6.68 ng/kg), but the levels had still significantly decreased by 48.69% ($P < 0.01$) compared with the CK (13.02 ng/kg) at harvest (Figure 1).

The IBMP concentrations of the grapes decreased significantly after all the defoliation treatments, especially after the pre-flowering defoliation treatment. These results were similar to those of previous studies, which suggested that the early season basal leaf removal was an effective management strategy to reduce the IBMP accumulation in grape berries (Martin et al. 2016). However, the IBMP contents of the grapes at harvest after the pre-flowering or post-flowering treatments were still above the quantitation limit in 2016, whereas the levels were below the quantitation limit in 2017. This might be because the harvest time was delayed for approximately one week in 2017, which further suggests that a delayed harvest time could effectively reduce the IBMP content in the grape berries (Bindon et al. 2013).

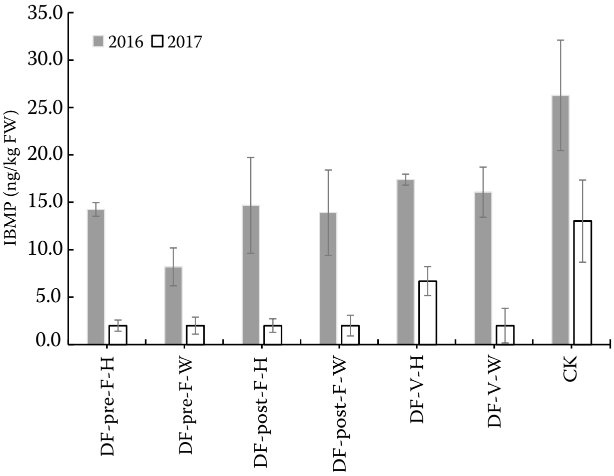

Figure 1. Effect of the defoliation on the IBMP content in the 'Cabernet Sauvignon' grapes in 2016 and 2017
For treatments description see Table 1

To further investigate the impact of the defoliation on the IBMP metabolism, the relative expression levels of VvOMT1 and *-3* in the berry skins were evaluated in two consecutive years in accordance with the method reported by Lei et al. (2019). The VvOMT3 expression level in the berry skins mirrored the IBMP accumulation in the grape berries in the two consecutive years (Figure 2C, D). This result was similar to that of a previous study in which VvOMT3 had a high affinity for the 3-alkyl-2-hydroxypyrazines (HPs) precursors of MP, which played

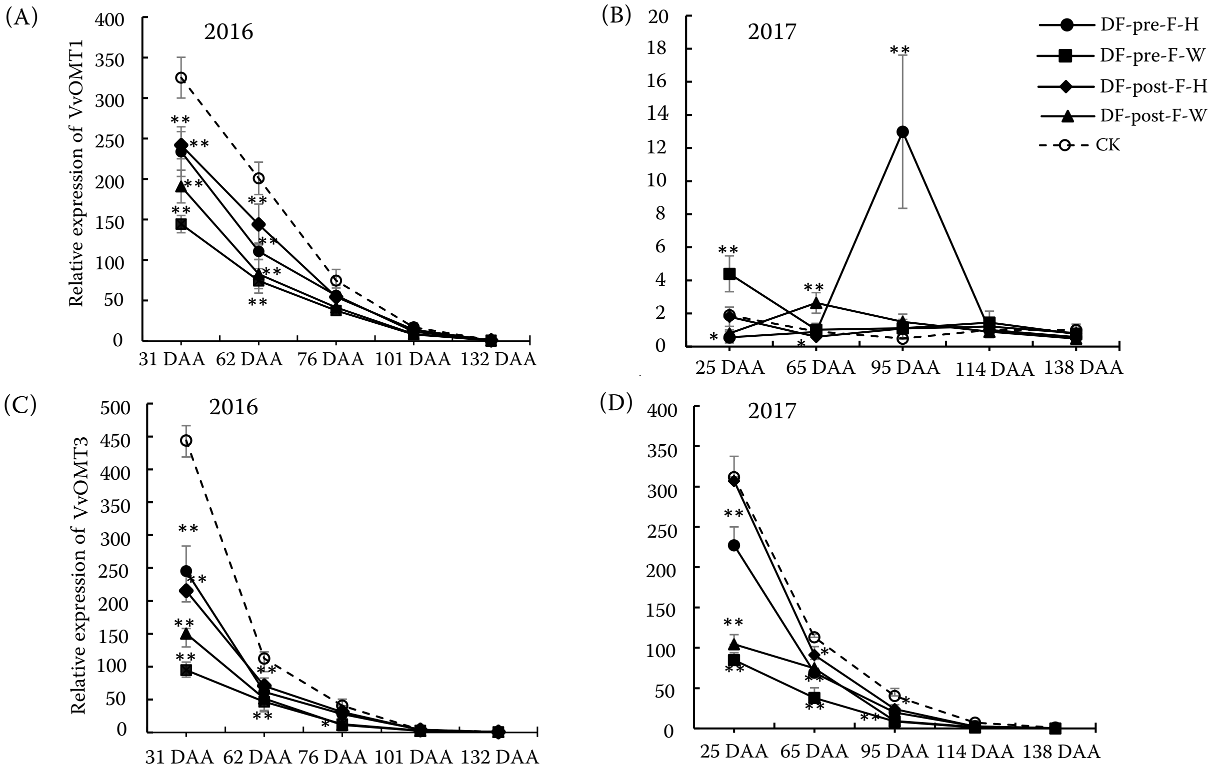

Figure 2. Effect of the different defoliation treatments on the key gene expression levels (A, B – VvOMT1 and C, D – VvOMT3) in the 'Cabernet Sauvignon' grape berry skins in 2016–2017
For the treatments description see Table 1

https://doi.org/10.17221/70/2020-HORTSCI

an important role in the MP biosynthesis in grape berries (Dunlevy et al. 2013).

The VvOMT1 expression level in the berry skins showed a continuous decreasing trend in 2016, which was synchronous with the IBMP accumulation in the grape berry (Figure 2A). However, in 2017, it did not share the same trend with the IBMP accumulation in the grape berry (Figure 2B). This might be because the methylation activity of VvOMT1 preferred the flavonol quercetin to HPs (Dunlevy et al. 2010) owing to the climate or other influencing factors. This warrants further study.

In conclusion, the pre- and post-flowering defoliation treatments significantly decreased the IBMP concentration at harvest, and the earlier and heavier the defoliation treatment, the lower the IBMP concentration. In addition, if the weather conditions permit doing so, delaying the harvest time for approximately one week could result in a greater decrease in the IBMP concentrations of the grapes.

REFERENCES

Bindon K., Varela C., Kennedy J., Holt H., Herderich M. (2013): Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. 'Cabernet Sauvignon' Grape and wine chemistry. Food Chemistry, 138: 1696–1705.

Coombe B.G. (1995): Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research, 1: 100–110.

Dunlevy J.D., Dennis E.G., Soole K.L., Perkins M.V., Davies C., Boss P.K. (2013): A methyltransferase essential for the methoxypyrazine-derived flavor of wine. Plant Journal, 75: 606–617.

Dunlevy J.D., Soole K., Perkins V.M., Dennis E.G., Keyzers R.A., Kalua C.M., Boss P.K. (2010): Two O-methyltransferases involved in the biosynthesis of methoxypyrazines: Grape-derived aroma compounds important to wine flavor. Plant Molecular Biology, 74: 77–89.

Koch A., Doyle C.L., Matthews M.A., Williams L.E., Ebeler S.E. (2010): 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. Phytochemistry, 71: 2190–2198.

Lei Y.J., Xie S., Chen H.Z., Guan X.Q., Zhang Z.W. (2019): Behavior of 3-isobutyl-2-methoxypyrazine biosynthesis related to proposed precursor and intermediate in wine grape. Food Chemistry, 217: 609–616.

Martin D., Grose C., Fedrizzi B. (2016): Grape cluster microclimate influences the aroma composition of 'Sauvignon blanc' wine. Food Chemistry, 210: 640–647.

Mozzon M., Savini S., Boselli E., Thorngate J.H. (2016): The herbaceous character of wines. Italian Journal of Food Science, 28: 190–207.

Roujou-de-Boubée D., Cumsille A.M., Pons D., Dubordieu D. (2002): Location of 2-methoxy-3-isobutylpyrazine in 'Cabernet sauvignon' bunches and its extractability during vinification. American Journal of Enology and Viticulture, 53: 1–5.

Sidhu D., Lund J., Kotseridis Y., Saucier C. (2015): Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. Critical Reviews in Food Science and Nutrition, 55: 485–502.

Received: May 5, 2020
Accepted: February 8, 2022