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Preliminary study: The effect of gibberellic acid and girdling application on two dual-purpose grape varieties in a cool climate

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SUMMARY

In our 2023 experiment, Nero and Muscat Pölöskei grapevines were treated with gibberellic acid (12 mg L^{-1}) during flowering, followed by stem-end girdling at the onset of berry development. Photosynthetic activity was monitored regularly. Assimilation rate (A, µmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mol H_2O m^{-2} s^{-1}) were measured under natural light. Sugar content and acidity were assessed via refractometry, while berry dimensions and texture (e.g., berry hardness [Bh, mN]) were recorded. Gibberellin treatment increased sugar content compared to both control and girdled samples. However, girdling significantly enhanced sugar accumulation in Muscat Pölöskei compared to untreated samples. Gibberellin also induced berry elongation, increasing the longitudinal axis in Nero (19%) and Muscat Pölöskei (24%). Girdling temporarily reduced photosynthetic activity, which recovered post-healing. The elongated berries due to gibberellin treatment led to a looser cluster structure, which is beneficial for Nero, which is prone to fungal diseases due to dense clusters.

Keywords: *gibberellic acid; girdling; gas-exchange*

INTRODUCTION

Grapes rank among the most ancient cultivated crops worldwide. Their fruits are enjoyed fresh as table grapes, dried for long-term storage, or transformed through fermentation into wine, spirits, vinegar, and various health-promoting pharmaceutical products (Pilati et al., 2017). The fruit ripening process has been thoroughly researched to enhance fruit quality and, more recently, preserve it amid changing climatic conditions. (Pilati et al., 2017). Climatic conditions are broadly recognized as critical in grape production and quality. Although it is widely recognized that climate plays a crucial role in the different phenological stages of grapevines, daily weather variations also exert a significant influence (Anastasiou et al., 2022). Air temperature during the ripening period is crucial for grape maturation, including the development of aroma and color (Koshita et al., 2011).

Hungary's relatively cool climate and limited vegetation period, in contrast to Mediterranean regions, present greater difficulties for cultivating table grapes (Roebroek et al., 2020). For late-maturing grape varieties, the onset of early frosts in October can sometimes interfere with the completion of the ripening process (Tóth, 2020). Additionally, high humidity in late August and early September makes grapes vulnerable to various fungal diseases (Bernáth et al., 2021), and the use of chemical defenses may be limited by the withdrawal periods of these products in food production. Producers must also consider consumer expectations regarding key grape quality parameters, such as sweetness, texture, and size (Chironi et al., 2017). Girdling involves the removal of a bark ring from the trunk or branches, disrupting the downward movement of photosynthates (sugars and nutrients) in the phloem while allowing the upward flow in the xylem to remain unaffected (Goren et al., 2010; Lukácsy & Zanathy, 2011). This practice leads to larger berries, improved fruit set, and accelerated maturation. Similarly, the gibberellic acid treatment promotes berry enlargement and cluster elongation, as was described in several studies (Dokoozlian & Peacock, 2001; Brar et al., 2008).

MATERIALS AND METHODS

Experimental design

The experiment was conducted in the northwestern part of Hungary, specifically in Abasár, a region within the Mátra vineyard area, using two dual-purpose Hungarian-bred grape varieties. Nero (Vitis interspecific crossing) is an early ripening cultivar attributed with compact clusters containing deformed dark blue, oval-shaped berries (22 × 17 mm, 3.2 g). The variety is susceptible to rot because of its compactness. Muscat Pölöskei (hereafter referred to as Pölöskei) is a favorable, intensely muscat-flavored, middle-ripening interspecific Vitis variety. Its clusters are loosened, and the berries are greenish-yellow and slightly oval-shaped.

The vines were planted between 2008 and 2010 with 3 m × 0.9 m row spaces. All vines were cane-pruned in a vertical shoot position with 5–8 buds on a horizontal trellis. The crop load was set at two clusters per shoot (Tóth et al., 2024).

The experiment was set in a randomized block design (three blocks per treatment, ten plants per block). The vines were in similar conditions and had the same crop load in all blocks. Gibberellic acid was sprayed at the beginning of the anthesis and ten days after on both varieties (18.7 m/m %, 0.012 g l^{-1}) (Ferrara et al., 2014), as it was in the product description (Florgib, Fine Agrochemicals). During the initial stage of berry development, cane girdling was carried out using a double-bladed device that excised a 4 mm-wide strip of bark from the cane (pea-sized phase

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BBCH 75) (Gökbayrak et al., 2013; Soltekin et al., 2016).

Berry sampling

Sampling was conducted when the grape cultivars reached their appropriate maturity. In each block, ten bunches per treatment were collected (two per plant), yielding a total of 30 bunches per treatment (Tóth et al., 2024). In this study, 1–2 berries were manually collected from each grape cluster, with care taken to retain the pedicels and ensure consistent sampling positions across bunches. Each berry was visually inspected, and only healthy, intact samples were included. Fifty berries were used per treatment for texture measurements. An additional 100 berries were selected at random to determine average berry weight and berry axis dimensions. This sample size was chosen to enhance the reliability and representativeness of the results. (Tóth et al., 2024; Zsófi et al., 2015). The remaining grape clusters from each treatment group were crushed and pressed to produce juice for further analytical evaluations. Total soluble solids content was measured in triplicate for each treatment. At a temperature of 20 °C, total soluble solids (°Bx) were assessed using a portable refractometer (OIV, 2022).

Gas exchange measurement

Leaf gas exchange parameters, including transpiration rate (E, mol H_2O m^{-2} s^{-1}) and net assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), were measured using a portable CIRAS-1 infrared gas analyzer (PP Systems, Hitchin, UK) equipped with a round Parkinson-type leaf cuvette (2.5 cm²) as described by Pálfi et al. (2022). Four measurement sessions were carried out in July following the cane girdling treatment. The timing was adjusted to local weather conditions and conducted on clear, windless days between 12:00 and 14:00, when the photosynthetically active radiation (PAR) exceeded 1000 µmol m^{-2} s^{-1}, in accordance with the methodology of Cataldo et al. (2021) and Mattii & Orlandini (2005). Leaf temperature remained consistent across treatments during the process. Data was collected on 5-6 plants per treatment at each sampling interval. Mature, intact leaves fully sun-exposed with a random selection of dry leaf surfaces were used within the mid-canopy zone to ensure consistency in sampling height and light exposure. (2 leaves per plant). The reference CO_2 concentration in the gas analyzer was adjusted to match the ambient atmospheric levels, which varied between 380 and 400 ppm in both years. The reference H_2O concentration was 70% of the ambient H_2O, with an airflow of 200 cm³/min through the leaf chamber (Cataldo et al., 2021; Mattii & Orlandini, 2005; Pálfi et al., 2022).

Texture analysis

The mechanical analysis of grape berries was carried out using a TA.XTplus Texture Analyzer (Stable Micro Systems, Surrey, UK), operated with Exponent 6.1.1.0 software. To assess berry hardness (BH, mN), a P/35 cylindrical probe was used to compress the berries to 25% of their original diameter at a speed of 1 mm s^{-1}. Prior to testing, the berries were removed with pedicels and placed on the analyzer platform. The method was based on the general principles described by Letaief et al. (2008), with adjustments adapted to the present experiment.

Berry skin hardness test was evaluated via puncture testing using a P/2N needle, also at a speed of 1 mm s^{-1}. The probe was inserted to a depth of 3 mm into the lateral side of the berry (Letaief, Rolle, Zeppa, et al., 2008). From the puncture test, skin break force (Fsk, N) was calculated.

Skin thickness (Spsk, mm) was determined using a P/2 probe (2 mm diameter) at a constant speed of 0.2 mm s^{-1}. A small piece of skin (~0.25 cm²), removed from the berry's side and carefully separated from the pulp, was placed flat on the platform for measurement. The procedure was adapted from existing methods to fit the requirements of this study (Río Segade et al., 2011).

Statistical analysis

Data analysis was conducted using GraphPad Prism software (version 6.0; GraphPad Software Inc., La Jolla, CA, USA). Statistical differences among treatments were evaluated by one-way analysis of variance (ANOVA), and individual comparisons were performed using unpaired t-tests. A significance level of $p \leq 0.05$ was applied throughout.

RESULTS AND DISCUSSION

Analytical parameters

The sugar content (°Bx) showed a statistically more advanced maturity in the two treatments for the Pölöskei grape variety. For the Nero grape variety, girdling resulted in higher sugar content than the control and gibberellin treatments. Similar to our findings, other studies have also observed increased sugar content due to specific treatments, indicating that the treated samples are in a more advanced stage of maturity (*Table 1*). Several studies have confirmed that girdling is an effective method for improving the quality parameters of grapes, depending on the application timing. By disrupting the phloem transport, the treatment causes the accumulation of assimilates and signaling compounds above the girdled zone (Gökbayrak et al., 2013; Goren et al., 2010; Soltekin et al., 2016), which can enhance ripening dynamics and lead to an earlier harvest (Soltekin et al., 2016; Tóth, et al., 2022a; Tóth et al., 2022b).

Berry parameters

References suggest that both gibberellin treatment (Ferrara et al., 2014) and girdling (Gökbayrak et al., 2013; Lukácsy et al., 2021; Tóth et al., 2022a; Zabadal, 1992) could increase berry size. In our experiments, the berry weight varied as follows: gibberellin-treated samples of the Pölöskei variety had the highest berry weight (5.18 g), followed by the girdled samples (4.32 g), with the control samples having the lowest weight (3.81 g). There was no detectable difference in berry weight for the Nero variety between the girdled (3.38

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g) and gibberellin-treated (3.31 g) samples. However, both treatments had a higher weight than the control (2.93 g) (*Table 1*).

A similar trend was observed when comparing the axes of the samples (*Table 1*). The latitudinal and longitudinal axes of the treated Pölöskei berry samples were longer than those of the control berries. The longitudinal axis of the gibberellin-treated Pölöskei cultivar was 13% longer than that of the control berries, while the latitudinal axis was 23% longer. For the girdled Pölöskei berries, the longitudinal axis was 5.5% longer than the control berries, and the latitudinal axis was 8% longer. However, there was also a significant difference between the two treatments. In the Nero grape variety, the width and length of gibberellin-treated berries were 4% greater than those of the control samples. In the girdling treatment, only the longitudinal axis increased significantly (6%) compared to the control.

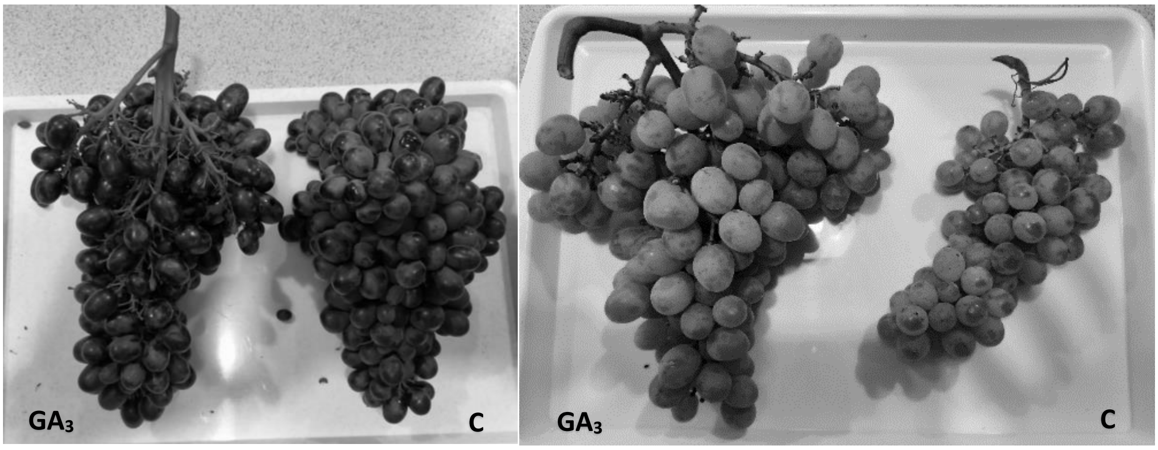
Gibberellic acid application during anthesis has been shown to reduce berry set, particularly in seedless cultivars (Dokoozlian & Peacock, 2001), and to increase berry size after berry set by elongating the stems (Soltekin et al., 2016; Tóth et al., 2022b). This treatment not only enlarges the berries and clusters but also decreases bunch compactness, thickens the berry skin, significantly enhances the microclimate within clusters, and reduces the risk of Botrytis infection and bunch rot (Reynolds & de Savigny, 2004). Notably, when comparing the size of the berry peduncles between the gibberellin-treated and control treatments for both cultivars in our experiment. When measuring the berry peduncles of the gibberellin-treated and control samples in our experiment, a 29% elongation was observed in the Pölöskei variety and a 22% elongation in the Nero variety (*Table 1*, *Figure 1*).

Table 1. **Total soluble solids (°Bx) of the grape juices and berry parameters in 2023**

	TSS (°Bx)	Berry weight (g)	Longitudinal axis (mm)	Latitudinal axis (mm)	Peduncle (mm)
			Muscat Pölöskei		
GA_3	18.20 ± 0.10 a	5.81 ± 0.16 a	24.50 ± 0.18 a	20.58 ± 0.20 a	10.87 ± 0.41 a
G	18.83 ± 0.35 a	4.32 ± 0.09 b	21.46 ± 0.15 b	19.21 ± 0.16 b	n.s.
C	17.00 ± 0.21 b	3.81 ± 0.13 c	19.86 ± 0.20 c	18.20 ± 0.24 c	8.40 ± 0.31 b
			Nero		
GA_3	17.80 ± 0.31 a	3.31 ± 0.08 a	21.41 ± 0.20 a	16.46 ± 0.15 a	7.87 ± 0.26 a
G	18.63 ± 0.07 a	3.38 ± 0.06 a	21.84 ± 0.17 a	16.04 ± 0.18 ab	n.s.
C	17.17 ± 0.03 b	2.93 ± 0.10 b	20.51 ± 0.28 b	15.80 ± 0.20 b	6.45 ± 0.43 b

GA_3: Gibberellin treatment, G: Girdling treatment, C: Control. Mean values with different letters within a column indicate significant differences ($p<0.05$) between the treatments (n=50) ± standard error.

Figure 1. **Gibberelin treated (GA_3) and control (C) bunches of Nero (left) and Muscat Pölöskei grape variety (right)**



Textural analysis

Berry hardness values were measured to be 18% higher for berries from gibberellin-treated samples of the Pölöskei variety and 24% higher for girdling-treated samples than the control samples (*Table 2*). In contrast, lower berry hardness values were recorded for the Nero variety in the treated samples compared to the control (717 mN), with the gibberellin-treated samples at 684 mN and the girdling-treated samples at 618 mN (*Table 2*). The results for the Pölöskei variety are consistent with our previous research, where girdling resulted in more complex berries (Tóth et al., 2024;

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Tóth et al., 2022b). Presumably, the wounding-induced stress response increases abscisic acid levels, which induces stomatal closure above the girdled area. This stomatal closure also reduces water loss in this stage (Düring, 1978; Ezzahouani & Williams, 2001; Castellarin et al., 2016), which may result in more complex berries. Softer skin was detected in the girdled samples of both grape varieties compared to the gibberellin-treated berries. For the Pölöskei variety, the gibberellin-treated berry samples had thinner skin compared to the control samples. The thinner berry skin induced by gibberellin treatment correlates with the results of other studies (Reynolds & de Savigny, 2004). There were no other remarkable differences between the treatments in berry thickness.

Table 2. **Results of the berry texture analysis of different treatments in 2023**

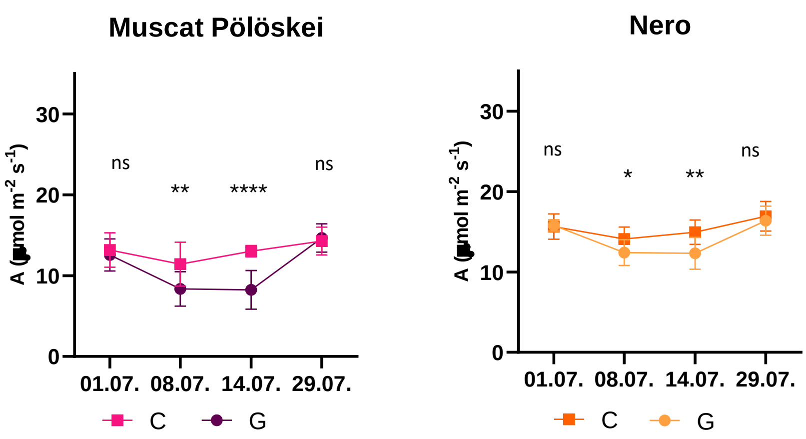
	Berry hardness (mN)				Berry skin hardness (mN)				Berry skin thickness (mm)			
					Muscat Pölöskei							
GA_3	918.4	±	23.1	a	526.4	±	8.9	a	0.157	±	0.01	b
G	9673	±	35.1	a	496.4	±	12.2	b	0.188	±	0.01	a
C	778.5	±	15.3	c	514.6	±	9.9	ab	0.188	±	0.00	a
					Nero							
GA_3	683.8	±	21.2	ab	332.3	±	10.3	a	0.228	±	0.01	a
G	618.1	±	22.9	b	305.8	±	14.4	b	0.243	±	0.01	a
C	716.9	±	28	a	355.8	±	11.2	a	0.238	±	0.01	a

GA_3: Gibberelin treatment, G: Girdling treatment, C: Control. Mean values with different letters within a column indicate significant differences ($p<0.05$) between the treatments ($n=50$) ± standard error. GraphPad 8.0.1 software performed statistical analyses using an unpaired T-test.

Gas exchange

The trend of changes in photosynthesis can also be observed on the gas exchange analysis graph, although there is no statistical difference at all time points. The assimilation rate (A) (*Figure 2*) was significantly higher at both the second and third-time points in the girdled plants (Pölöskei: control 13.2, girdled 8.25; Nero: control 14.9, girdled 12.3), while the transpiration rate (E) (*Figure 3*) was significantly lower in the treated samples compared to the control vines (Pölöskei: control 4.6, girdled 3.4; Nero: control 5, girdled 4.1) at third-time measurement (*Figure 3*). The gas exchange investigations also confirm that changes occur in photosynthetic activity. Previous studies suggest that sugar accumulation due to girdling can cause osmotic stress, negatively affecting photosynthesis (Hartmann & Trumbore, 2016; López et al., 2015). Our research findings supported the previous results: the photosynthetic activity of the girdled vines decreased during the wound healing period and then returned to the pre-treatments (López et al., 2015; Proietti & Tombesi, 1990; Setter et al., 1980).

Figure 2. **Assimilation rate of the girdled (G) and control (C) vines**



The asterisk indicated the differences according to t-test (* $p \leq 0.05$, ** $p < 0.01$, and **** $p < 0.0001$, ns – non significant)

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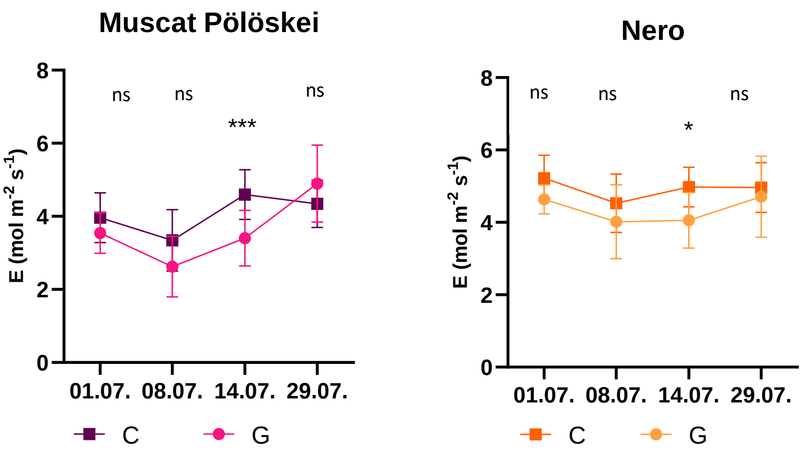
Figure 3. **Transpiration rate of the girdled (G) and control (C) vines**



The asterisk indicated the differences according to t-test (* $p \leq 0.05$, *** $p < 0.001$, ns – non significant)

CONCLUSIONS

Gibberellin increases the longitudinal axis of the berries, resulting in a looser cluster structure. This is considered a beneficial effect in varieties more susceptible to fungal diseases (e.g., Nero) due to the tightness of the cluster. At the same time, Muscat Pölöskei, which already has marketable properties due to its flavor, has a significant berry increase, which is a positive change for both consumers and producers.

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