

CHANGES IN YIELD AND QUALITY CHARACTERISTICS OF *VITIS VINIFERA* L. CV. MUSCAT DE HAMBURG UNDER THE INFLUENCE OF GIBBERELIC ACID (GA₃)

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INTRODUCTION

Plant growth and development is controlled besides genetic factors by certain endogenous substances belonging to plant hormones group (Davies, 2004). Phytohormones are a class of organic substances that at low concentrations affect physiological processes of growth, differentiation and development of plants (Kende and Zeveaart, 1997; Hooykaas *et al.*, 1999). Photosynthetic pigments, represented by chlorophylls (a and b) and carotenoids pigments (carotenes and xanthophylls), are essential compounds in light energy conversion (Davies, 2004). *Vitis vinifera* L. leaves contain high levels of photosynthetic pigments, their biosynthesis varying with the stage of leaf maturation and plant phenophase. Chlorophyll and carotenoids pigments content in vines was reported to be in the range of 1.05-1.58 mg/g f.w., respectively 0.33 to 0.65 mg/g f.w. (Burzo *et al.*, 2005; Acatrinei and Andor, 2006).

GA₃ applied to table grape varieties leads to a higher accumulation of sugars and a decrease of total acidity in berries (Rusjan, 2010). Grapes appearance is also improved by the existence of larger and more uniform coloured berries once with the stimulation of anthocyanins biosynthesis (Peacock, 1999).

Implementation of some less expensive cultivation technology that provide benefits both to producers and recipients, and the presence on the market of quality vine products in adequate quantities and at reasonable prices for the final consumer remains an actual problem for Romania.

MATERIAL AND METHODS

Experimental researches were focused on cosmopolitan variety Muscat de Hamburg grown in Iasi vineyard area, Ampelographic Collection of Faculty of Horticulture, belonging to the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, Romania.

Muscat de Hamburg is a variety with normal hermaphrodite flowers, whose pollen has very low capacity of germination, being necessary to provide pollinators in plantations. Grapes are large (300-500 g), with secondary branches. Berries are spherical,

slightly oval, 15-20 mm thick, with uniform blue-black colour and strong Muscat flavour. The variety has medium vegetation period (165-170 days), strong vegetative growth and good fertility (50-60% fertile shoots) (Țârdea and Rotaru, 2003). Rootstock used was Berlandieri×RipariaKober 5 BB. Planting distances were 2.2/1.2 m, half-high leading form, bilateral cordon with cutting in fructification rings. Soil maintenance was "black field" and maintenance operations of vines were specific to industrial vineyard ecosystem. To perform the treatments was used gibberellic acid GA₃ (purity 99%) (Merck, Germany). The observations were conducted in 2011 and 2012 vegetative cycles. Scheme of experience was the following: control sample V_m - H₂O d., V₁ - 25 ppm GA₃, V₂ - 50 ppm GA₃, V₃ - 100 ppm GA₃. The experience was organized in three repetitions, with ten stocks in each plot. Applying of GA₃ was performed by spraying the inflorescences in the flowering phenophase when 70% of corollas were fallen. Extraction of leaf photosynthetic pigments was conducted with acetone (99%) two weeks after the GA₃ treatment. Chlorophylls and carotenoids were quantitatively determined by spectrophotometry according to Lichtenhaler (1987).

Intensity of photosynthesis and additional parameters were performed using LCi 600 device.

Physical measurements were assessed after grape harvesting, followed by achieving the alcoholic extracts (0.1% HCl in 96% ethanol) for determination of anthocyanins and total phenolic content of berries. Plant material/solvent ratio was 1:30. The containers were stored in the dark at room temperature (18±2°C), 2 hours. The extracts obtained were stored at -20°C. For determining the total amount of anthocyanins was used pH differential method: A (absorbance) = (A_{520nm}-A_{700nm}) pH 0.68 - (A_{520nm}-A_{700nm}) pH 3.5 (Lee *et al.*, 2005).

Total phenolics were determined using Folin-Ciocalteu reagent, method OIV-MA-AS2-10, by spectrometry at λ = 750 nm (OIV, 2012). Results were expressed as gallic acid equivalent (g GAE · 100 g⁻¹). Research aimed to highlight the influence of biostimulating treatments on some physiological, physical and chemical parameters of vines varieties.

Table 1. The main physiological parameters recorded at Muscat de Hamburg leaves under GA₃ influence

Variant	PAR ($\mu\text{mol}/\text{m}^2/\text{s}^{-1}$)		Ci (cm^3/m^3)		E ($\text{mmol}/\text{m}^2/\text{s}^{-1}$)		Gs ($\text{mmol}/\text{m}^2/\text{s}^{-1}$)		A ($\mu\text{mol}/\text{m}^2/\text{s}^{-1}$)		A/E	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
V _m	108.00	129.00	358.00	344.17	2.00	2.53	0.12	0.14	1.06	1.75	0.58	0.70
V ₁	278.00	134.00	292.00	316.67	2.71	2.73	0.15	0.17	5.18	4.32	2.01	1.58
V ₂	515.00	492.00	277.33	274.17	3.55	4.49	0.16	0.24	5.70	8.26	1.68	1.84
V ₃	211.00	154.00	274.80	315.00	1.00	2.00	0.05	0.10	2.51	2.60	2.55	1.40

Note: PAR - photosynthetic active radiation; Ci - CO₂intracellular; E - evapotranspiration; Gs - stomatal conductance; A - rate of photosynthesis

RESULTS AND DISCUSSIONS

Physiological parameters of Muscat de Hamburg under the influence of GA₃ treatments are presented in Table 1. Photosynthetic active radiation (PAR) recorded in middle leaves of the vine stocks showed a very high value compared to the control, with a maximum at V₂-50 ppm (515.00 $\mu\text{mol}/\text{m}^2/\text{s}^{-1}$, in 2011). At the same experimental variant was observed an increase of evapotranspiration and an increase by five to eight times of the photosynthesis rate (A) compared with control, as a physiological response of plant to the application of biostimulating substances such as gibberellins.

Synthesis of photosynthetic pigments in leaves of Muscat de Hamburg was stimulated by the application of reduced doses of gibberellic acid. The highest values of chlorophyll a (2.82 mg/g, in 2012), chlorophyll b (2.11 mg/g, in 2012) and carotenoids (0.74 mg/g, in 2012) were reported at V₁-25 ppm. Increasing concentrations of GA₃ led to a significant decrease in the individual quantities of photosynthetic pigment (Table 2).

Ratio chlorophyll a/chlorophyll b was constant, being placed within the range of 1.3 to 1.4.

Table 2. The content of photosynthetic pigments in leaves of Muscat de Hamburg under GA₃ influence

Variant	Chlorophyll a		Chlorophyll b		Carotenoids	
	2011	2012	2011	2012	2011	2012
V _m	2.19	2.56	1.60	1.87	0.60	0.71
V ₁	2.74	2.82	1.98	2.11	0.72	0.74
V ₂	1.92	1.90	1.37	1.33	0.56	0.56
V ₃	1.72	1.72	1.32	1.30	0.50	0.50

In addition, ratio chlorophyll/carotenoids presented very high values at variant V₁, low doses of stimulator favoring the accumulation of chlorophyllian pigments, in detrimental of carotenoids pigments biosynthesis.

Regarding the total photosynthetic pigments, was noted that it has evolved in opposite sense with dose of gibberellin applied, decreasing with the increasing of GA₃ concentrations (Table 3).

Table 3. Total content of photosynthetic pigments in Muscat de Hamburg leafs and the relationships established between them

Variant	Chlorophyll a / Chlorophyll b		Chlorophylls / Carotenoids		Total pigments ($\text{mg}\cdot\text{g}^{-1}\text{f.w.}$)	
	2011	2012	2011	2012	2011	2012
V _m	1.37	1.37	6.34	6.28	4.39	5.13
V ₁	1.38	1.33	6.57	6.64	5.43	5.67
V ₂	1.40	1.43	5.93	5.80	3.85	3.80
V ₃	1.31	1.32	6.10	6.02	3.54	3.51

At Muscat de Hamburg cultivar the increase of grapes weight per vine stock was inversely correlated with the concentration of the biostimulating substance applied, in both years of study. Therefore, at high doses of GA₃ (50 and 100 ppm) production was lower than at the variant treated with low concentrations (25 ppm). At the same time, the number of normal developed berries in clusters was almost similar at all treated variants (70), but superior to the control variant (Table 4). Berry index was slightly higher in 2012 compared with 2011, indicating a lower weight of berries compared to the first year of study.

Table 4. Physical and structural features of Muscat de Hamburg grapes under the influence of GA₃ treatments

Characteristics	V _m		V ₁		V ₂		V ₃	
	2011	2012	2011	2012	2011	2012	2011	2012
Number of grapes/ wine stock	13.00	12.00	15.00	11.90	14.00	12.00	14.00	11.00
Grape weight (g)	368.36	377.15	412.59	410.38	405.12	400.04	395.78	402.56
Number of berries/rachis	75.00	68.00	82.00	73.00	86.00	69.00	79.00	68.00
Berry weigh (g)	4.39	4.35	4.84	4.20	4.27	4.85	5.10	5.03
100 berries weigh (g)	469.80	346.35	466.76	422.71	470.64	453.63	393.69	456.11
Rachis weight (g)	7.31	6.93	8.38	7.35	7.84	7.18	7.86	6.95
Number of seeds/berry	2.00	2.00	2.00	2.50	2.00	3.00	2.50	2.00
Skin weight (g)	0.61	0.35	0.43	0.47	0.63	0.40	0.68	0.49
Pulp weight (g)	3.69	3.87	4.31	3.65	3.54	4.30	4.32	4.46
Seeds weight (g)	0.09	0.10	0.09	0.08	0.09	0.14	0.10	0.07
Structure index	50.39	53.42	49.24	54.83	51.67	54.71	50.35	56.92
Berry index	22.77	22.98	20.66	23.80	23.42	20.61	19.60	19.88
Compozition index	5.27	8.06	8.19	6.65	4.90	7.88	5.57	7.90
Production/vine stock (kg)	4.78	4.52	6.18	4.88	5.67	4.80	5.50	4.42

Table 5. Fertility and productivity characteristics of Muscat de Hamburg under the influence of GA₃

Characteristics	V _m		V ₁		V ₂		V ₃	
	2011	2012	2011	2012	2011	2012	2011	2012
Merchandise production percentage (%)	68	73	72	74	67	74	70	73
Percentage of fertile shoots (%)	50.00	47.61	56.6	55.00	53.33	50.00	53.33	50.00
Coefficient of relative fertility	0.66	0.57	0.76	0.59	0.73	0.60	0.73	0.55
Coefficient of absolute fertility	1.33	1.20	1.35	1.08	1.37	1.20	1.37	1.10
Relative productivity index	243.11	214.97	313.56	242.12	295.73	240.02	288.91	221.40
Absolute productivity index	489.91	452.58	556.99	443.21	555.01	480.04	542.21	442.81
Number of inflorescences/vine stock	13.00	12.00	15.00	11.90	14.00	12.00	14.00	11.00
Average production/hectare (t/ha)	14.34	13.56	18.54	14.64	17.01	14.40	16.50	13.26

Unlike literature citations referring to the reduction of seeds number in berries under the influence of gibberellins (Nicolaescu *et al.*, 2009), at Muscat de Hamburg variety cultivated in Iasi vineyard was not induced the seedless phenomenon in berries. In generally, number of seeds and their weight average presenting similar values, whatever it was the concentration of gibberellic acid applied on inflorescences.

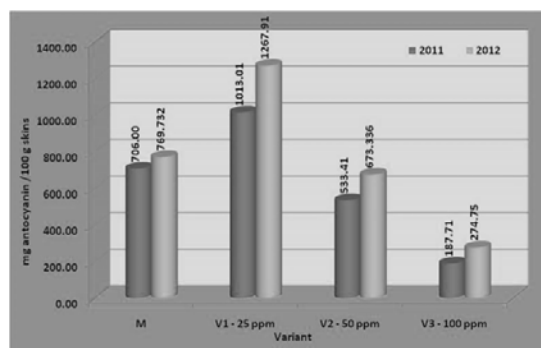
In 2012 the number of inflorescences per vine stock was under 12, lower than in 2011, for all treated variants, leading to a lower grape production and thereby influencing negatively the productivity indices (Table 5).

At most all characteristics of fertility and productivity could be noticed the positive influence of biostimulating treatments, control sample presenting lower values compared to the treated variants. Following the conducting of treatments with low doses of stimulator (V₁-25 ppm) was observed an increase of calculated production (18.5 t/ha, in 2011) and in the percentage of merchandise production (over 72%) correlated with an increase in the number of inflorescences per vine stock and the percentage of fertile shoots (56.6%, in 2011).

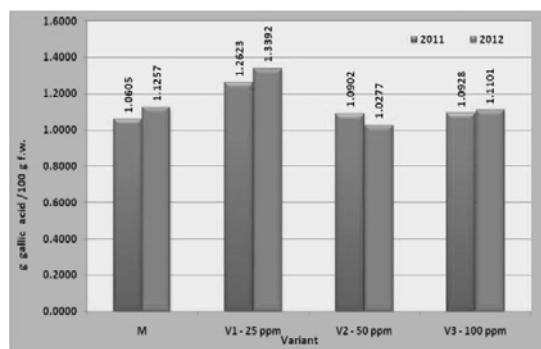
Soluble sugars content of the grapes presented an accumulation tendency in same time with the increasing of GA₃ concentrations, reaching 187 g/L in 2011, at the variant V₂-50 ppm. In 2012, the trend was similar to the previous year, changes occurring being attributable to the specific climatic conditions of the harvest year. Titratable acidity of grapes was influenced by stimulating treatments, the values of treated grapes were lower than those of the control variant (7.54 g tartaric acid/L) (Table 6). Variant V₁-25 ppm which presented the most significant yield of production per hectare had slightly lower values of sugars content than V₂ variant but with an equilibrated acidity, lower than the control sample.

Gluco-acidimetric ratio was higher in 2012 compared with 2011, because of the higher accumulation of sugars in berries.

Anthocyanin content of Muscat de Hamburg grapes was negatively correlated to the dose of gibberellic acid applied, having the maximum value at variant treated with 25 ppm GA₃ (1267.91 mg/100 g skins), thereby completing the high productivity of plants under the influence of low biostimulator concentrations (Fig. 1).

Fig. 1. Anthocyanin content of Muscat de Hamburg grapes under the influence of GA₃

Same trend of accumulation at low concentrations of gibberellin was observed for the phenolic compounds. The most important amount of phenolic compounds was recorded at V₁-25 ppm, 1.2623 g GAE/100 g f.w. in 2011 and reaching up to 1.3392 g GAE/100 g f.w. in 2012 (Fig. 2).

Fig. 2. Total phenolic content of Muscat de Hamburg grapes under the influence of GA₃

Higher additional quantities of anthocyanins and phenolic compounds determined with the application of biostimulating treatments of low concentrations (25 ppm), contributing to an increase of berries colour uniformity and therefore an increase in the nutritional value of grapes, along with the optimisation of the financial resources necessary to purchase bioactive substances and their application on plants.

Table 6. Sugar content and acidity of Muscat de Hamburg grapes under the influence of GA₃

Characteristics	V _m		V ₁		V ₂		V ₃	
	2011	2012	2011	2012	2011	2012	2011	2012
Sugars (g/L)	180.50	206.20	181.80	213.00	187.50	220.00	184.00	195.80
Titrateable acidity (g tartaric acid /L)	7.54	7.10	5.33	6.20	4.84	5.90	5.02	6.20
Sugars/acidity ratio	23.94	29.04	34.11	34.35	38.74	37.29	36.65	31.58

CONCLUSIONS

Applying biostimulating substances such as gibberellins in low concentrations (25 ppm), on inflorescences of Muscat de Hamburg cultivated in Iasi vineyard, Romania, led to an improvement of fertility and productivity features, increasing grapes production per hectare and the percentage of marketed production. Also, an intensification of the photosynthesis rate in leaves was observed. Lower GA₃ concentrations resulted in an equilibrated sugars/acid ratio, a higher accumulation of anthocyanins and phenolic compounds in berries, contributing to improving of berry colour uniformity and subsequently to the commercial aspect of grapes.

ABSTRACT

The aim of the work was to determine the optimal dose of gibberelic acid (GA₃) to be applied to achieve a higher yield and a harmonization of quality features at the grapevine cultivar for table grapes Muscat de Hamburg grown in Iasi vineyard, NE of Romania, in two years of harvest. Treatments were performed by spraying the inflorescences with 25 ppm, 50 ppm and 100 ppm GA₃. The optimal dose of GA₃ applied was 25 ppm, resulting in a higher yield and quality of grapes. Lower GA₃ doses resulted in an equilibrated sugars/acid ratio, a higher accumulation of anthocyanins and phenolic compounds in berries, improving the commercial aspect of grapes by increasing color intensity and uniformity. The treatment led to an increase in production of over 18% and to a percentage of marketed production up to 74%. This variety develops a tolerance at high concentrations of GA₃. The foliar photosynthetic activity was more intense causing an increasing intracellularly CO₂ concentration.

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