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(Original Article)

Effect of Ascorbic, Citric, and Abscisic Acids Spraying on Yield and Anthocyanin Concentration of Ruby Seedless Grape Cultivar under Assiut Climatic Conditions

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Abstract

This investigation was implemented during two successive seasons (2020 and 2021) on Ruby Seedless grapevines cultivated at the Experimental Orchard of Assiut University, Faculty of Agriculture. This work was designed to investigate the effectiveness of Ascorbic, Citric, and Abscisic acids on yield and anthocyanin concentration in the Ruby Seedless grape cultivar under Assiut climatic conditions. The obtained results revealed that all the treatments significantly increased the total yield/vine as compared to the control (untreated vines). Importantly, the highest yield weight was acquired from the Ascorbic acid (AA) at 600 ppm + Citric acid (CA) at 600 ppm + Abscisic acid (ABA) at 600 ppm treatment. In addition, the treatment of AA at 600 ppm + CA at 600 ppm + ABA at 600 ppm gave the highest value of anthocyanin content.

Keywords: *Ruby Seedless, Vitis vinifera, Ascorbic acid, Citric acid, Abscisic acid, Yield, Color.*

Introduction

In Egypt, grapes (*Vitis vinifera* L.) are classified as the first fruit crop in the area and production all around the world.it is the second major fruit crop following citrus (El-Kenawy, 2017). According to the ministry of agricultural statistics (2020), the total cultivated area devoted to grapes in Egypt reached 187358 feddans and the fruiting area 133811, producing about 1,183,968 tons with an average of 8.848 ton\feddan. Ruby Seedless cultivar is considered one of the most important table grapes in local and international markets (Belal, 2015). Grapes have a high nutritional value. The berry contains a high quantity of antioxidants that prevent viral and fungi infections, coronary heart disease, degenerative neurological diseases and colon and prostate cancer. As well as, grapes containing vitamins A, C, B6, folate and critical minerals including potassium, calcium, iron, phosphorus, magnesium and selenium (Champa, 2015).

The high weather temperatures in Egypt, especially in the Assiut region has a negative effect on the berry coloring. Accordingly, the grape producers in such region are using various practices to improve the coloring.

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Abscisic acid (ABA) is a phytohormone discovered in the 1960s that affects plant growth in abiotic stresses as well as seed maturity or dormancy (Baumann, 2010). Studies show that exogenous ABA application to grapes increases anthocyanin accumulation in order to improve grape color and quality (Jeong *et al*. 2004, Peppi *et al*., 2006, Xi *et al*., 2013 and Reynolds *et al*., 2016). Ascorbic acid and citric acid are considered antioxidants that have action in enhancing the biosynthesis of carbohydrates, proteins, plant pigments, cell division and cell elongation. They are also safe for humans and the environment (Elade, 1992). Furthermore, antioxidants play a crucial role in improving the biosynthesis of natural hormones, photosynthesis, nutrient uptake, plant defense against biotic and abiotic stresses and the biosynthesis of plant pigments and sugars (Rao *et al*., 2000). Previous research demonstrated that using antioxidants was beneficial in enhancing growth, yield and fruit quality (Fayed, 2010, Mostafa *et al*., 2011 and Nerway, 2011).

Therefore, this work was designed to investigate the effectiveness of Ascorbic, Citric and Abscisic acids on yield and anthocyanin concentration at the Ruby Seedless grape cultivar under Assiut climatic conditions.

Materials and Methods

This investigation was implemented during two successive seasons (2020 & 2021) on Ruby Seedless grapevines cultivated at the Experimental Orchard of Assiut University, Faculty of Agriculture. The vines were 28 years old, grown at 2 x 2 m apart in clay loam soil, and were trained by the spur pruning system by leaving 60 buds per vine (20 spurs x 3 buds\spur). The experimental grapevines were arranged in a randomized complete block design with 8 treatments and 4 replicates, and each replicate was presented by 4 vines.

The following treatments were used

1- Spraying Ascorbic acid (AA) at 400 ppm

2- Spraying Ascorbic acid(AA) at 600 ppm

3- Spraying Citric acid(CA) at 400 ppm

4- Spraying Citric acid (CA)at 600 ppm

5- Spraying Abscisic acid (ABA)at 400 ppm

6- Spraying Abscisic acid(ABA) at 600 ppm

7- Spraying Ascorbic acid(AA) at 600 ppm + Citric acid (CA)at 600 ppm + Abscisic acid (ABA)at 600 ppm

8- Control (untreated vines).

The spraying solutions were applied directly to the clusters with a handheld sprayer until run off. The treatments were applied on two dates: the 1st date at the beginning of June and the 2nd date at the beginning of the veraison stage. Ruby Seedless clusters were hand harvested from each treatment at a commercial maturity stage in both seasons and weighed to determine the yield as kg per vine.

The estimated yield weight/vine and cluster weight

At harvest, the crop was collected to estimate the total weight vine. All the cluster numbers on each vine were counted. The invalid clusters were excluded and only the sound clusters were weighted and then the average cluster weight was calculated. The calculated average cluster weight was multiplying in the total number of clusters/vine to estimate the total yield weight (kg/vine).

A sample of clusters was taken from each replicate and directly transported to the laboratory of the Pomology Department, Faculty of Agriculture, to estimate the total anthocyanin content. Anthocyanin (mg/100 g fresh wt.) content was determined according to the method of Onayemi *et al*. (2006). Total anthocyanin in mg / 100 g fruit skin = (A535 X V X 100) / (98.2 X W), where V = total volume of extract in ml, W = weight of fresh sample (in grams).

Statistical analysis

The collected data for the seasons 2020 and 2021 were tabulated and statistically analyzed following Snedecor and Cochran (1980), and the differences between the treatment means were tested using LSD values at a 5% level of probability.

Table 1. Effect of Ascorbic acid (AA), Citric acid (CA), and Abscisic acid (ABA) on yield weight(kg\vine) of Ruby Seedless grape cultivar during 2020 and 2021 seasons

Treatments	Yield weight (kg/vine)		
	2020	2021	Mean
Ascorbic acid 400 ppm	15.65	12.91	14.28
Ascorbic acid 600 ppm	14.30	13.49	13.90
Citric acid 400 ppm	15.48	12.77	14.13
Citric acid 600 ppm	14.74	13.28	14.01
Abscisic acid 400 ppm	15.35	12.70	14.03
Abscisic acid 600 ppm	14.64	12.32	13.48
Ascorbic acid 600 ppm + Citric acid 600 ppm + Abscisic acid 600 ppm	15.79	13.61	14.70
Control	11.37	10.30	10.84
L.S.D 0.05	0.23	0.30	0.23

Results

Yield weight (kg/vine)

The presented data found in Table 1 and Fig.1 showed that all the treatments significantly increased the total yield/vine as compared to the control (untreated vines). Importantly, the highest yield weight (14.7 kg/vine) was acquired from the AA at 600 ppm + CA at 600 ppm + ABA at 600 ppm treatment, which led up to 35.6% increase over the control (10.84 kg/vine as an average of the two consecutive seasons), followed by each of AA at 400 ppm, CA at 400 ppm, ABA at 400 ppm, CA at 600 ppm and then AA at 600 ppm treatment. They recorded 14.28, 14.13, 14.03, 14.01 and 13.90 kg/vine with an increment of 30.4, 29.4, 29.2 and 28.2% over the control, respectively, while the lower significant impacts were

ABA at 600 ppm treatment, which gave 13.48 kg/vine. However, the control vines recorded the lowest value of yield weight.

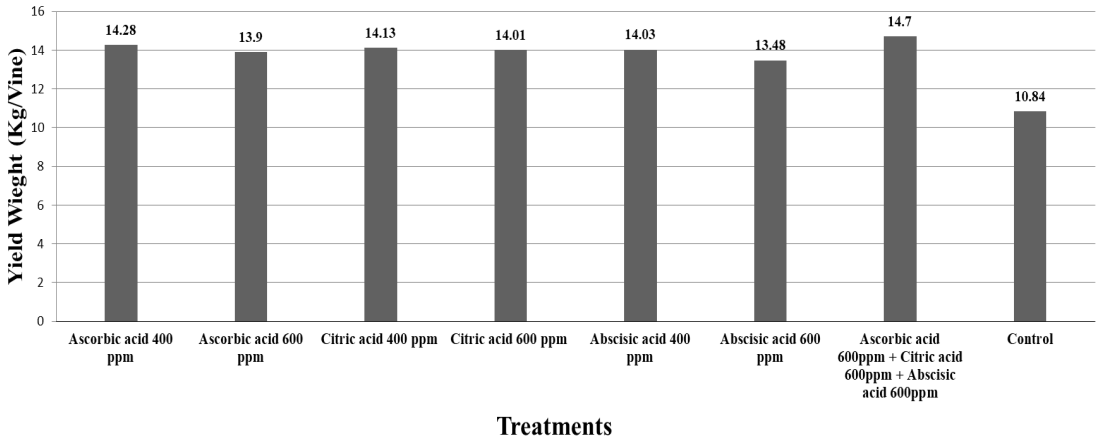

Fig. 1. Effect of Ascorbic acid (AA), Citric acid (CA) and Abscisic acid (ABA) on yield weight of Ruby Seedless grape cultivar (two seasons average data)

Table 2. Effect of Ascorbic acid (AA), Citric acid (CA), and Abscisic acid (ABA) on total anthocyanin content of Ruby Seedless grape cultivar during 2020 and 2021 seasons

Treatments	Total anthocyanin (mg/100 g)		
	2020	2021	Mean
Ascorbic acid 400 ppm	38.75	21.15	29.95
Ascorbic acid 600 ppm	35.50	18.20	26.85
Citric acid 400 ppm	41.34	23.14	32.24
Citric acid 600 ppm	37.25	21.75	29.50
Abscisic acid 400 ppm	43.16	27.65	35.41
Abscisic acid 600 ppm	47.59	28.15	37.87
Ascorbic acid 600 ppm + Citric acid 600 ppm + Abscisic acid 600 ppm	52.00	31.01	41.51
Control	24.99	15.77	20.38
L.S.D 0.05	2.63	2.59	1.80

Total anthocyanins (mg/100 g)

It is clear from the obtained data presented in Table 2 and Fig. 2 that there was a significant increase in total anthocyanin content (mg/100 g) in the skin of grape berries as compared to the untreated vines throughout the two studied seasons. Moreover, the treatments of ABA exceeded the other treatments in their influence on the total anthocyanin content, followed by CA at 400 ppm, AA at 400 ppm, CA at 600 ppm, and AA at 600 ppm treatments as a general average for the two years of study. In addition, the treatment of AA at 600 ppm + CA at 600 ppm + ABA at 600 ppm gave the highest average value of anthocyanin content (41.51 mg/100 g) with an increment percentage of 103.7% over the control, followed by the ABA at 600 ppm and ABA at 400 ppm treatments, which gave an average of 37.87 and 35.41 mg/100 g with an increment of 85.82 and 73.75% over the control, respectively. Likewise, CA at 400 ppm, AA at 400 ppm, CA at 600 ppm, and AA at 600 ppm treatments gave an average of 32.24, 29.95, 29.50, and 26.85 mg/100

g with an increment of 58.19, 46.96 and 44.75% over the control, respectively. On the other side, the control treatment gave the lowest value of total anthocyanin content.

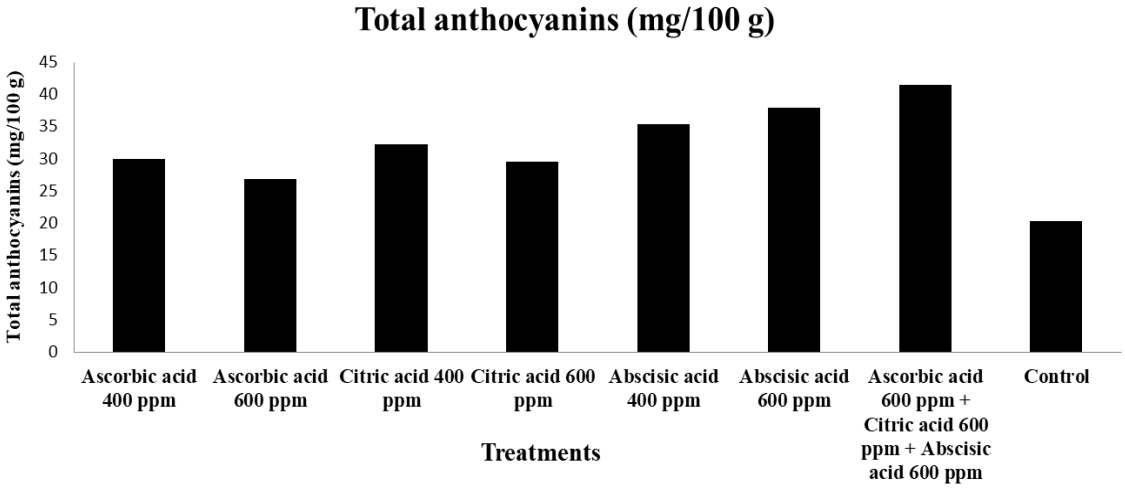

Fig. 2. Effect of Ascorbic acid (AA), Citric acid (CA), and Abscisic acid (ABA) on total anthocyanin content of Ruby Seedless grape cultivar (two seasons average data)

Discussion

Yield

Grape quality is of prime importance for local consumption and /or exportation. There are great challenges facing grape growers in upper Egypt in improving grape quality due to the harsh climatic conditions.

Investigators have been trying to improve grape quality by using various methods. For instance, spraying the growth regulators antioxidants vitamins, etc. Abscisic acid has been used for a long time for various purposes, e.g., anthocyanins and reducing sugars. The present study raveled that abscisic acid increased yield weight and cluster weight. In this study, the results showed a clear relationship between both the increases in yield weight and the increase in berry weight driven by water retention in the berries (Nakagawa *et al*. 1980).

Since the current study was conducted in a very hot area, a larger transpiration rate from the vines is to be predicted than if they had been planted in a zone with colder or more temperate temperatures. As a result, applying ABA to vines grown in hot climates has a greater impact than applying it to vines grown in cold climates. These findings are consistent with what has been said. Quiroga *et al*. (2009) discovered that ABA boosted yield and cluster weight and came to the conclusion that ABA encouraged photoassimilates to be allocated to the berries. Our findings were consistent with what was reported by Peppi and Fidelibus (2008).

These results are in accordance with those reported by Cantin *et al*. (2007), Peppi and Fidelibus (2008), (Lurie *et al*. 2009), (Quiroga *et al*., 2009), Gu *et al*. (2011), (Ferrara *et al*., 2013), (Leao *et al*., 2014), (Xi *et al*., 2013 and Luan *et al*.,

2014), (Woolard *et al*., 2016), (Neto *et al*., 2017) (Tecchio *et al*., 2017) and (Koyama *et al*. 2018).

Ascorbic acid or Citric acid and Ascorbic acid combined with Citric acid and Abscisic acid led to an increase in the yield weight. The results related to the effect of AA and CA on productive weight and cluster weight have been confirmed by many workers Ali(2000), Mansour *et al* (2000), Attia (2002), Khiamy (2003), Ahmed and Abd El-Hameed (2004), Nashed (2006), Ibrahi and Asmaa (2006), Farahat (2008), Abd El-Kariem (2009) Abada and Abd El-Hameed (2010) and Ahmed *et al*. (2010).

Anthocyanin

ABA has replaced exogenous ethephon as a way to improve grape color and hasten to ripening. The primary cause of such is that the application of ethephon may result in berry softening, which lowers the value of exported grapes (Peppi *et al*., 2007).

When compared to the control, the ABA-containing treatments in this study significantly enhanced the skin anthocyanin concentrations of Ruby Seedless. Peppi and Fidelibus (2008) showed that adding ABA to berries improved their color and anthocyanin concentration. Additionally, they observed that fruit exposed to 600 ppm ABA had a darker and more reddish hue than untreated berries.

Researchers found similar results with other grape cultivars where exogenous ABA administration improved coloring and increased anthocyanin concentration in the berries. (Lacampagne *et al*., 2010; Gu *et al*., 2011; Ruiz-Garcia *et al*., 2013; Xi *et al*., 2013; Reynolds *et al*., 2016; Zhu *et al*., 2016 and Ju *et al*., 2016). The abovementioned studies came in line with the results of the current study.

Sandhu *et al*. (2011) and Jeong *et al*. (2004) showed that ABA treatment increased anthocyanins accumulation in grape skins and increased mRNA accumulation of a putative regulatory gene for grape anthocyanin biosynthesis as well as all of the examined anthocyanin biosynthesis enzyme genes.

Conclusion

On the light of obtained results of this study, it could be concluded that the best treatment was Ascorbic acid (600 ppm) + Citric acid (600 ppm) + Abscsic acid (600ppm).

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تأثير رش أحماض الأسكوربيك والستريك والأبسيسيك على المحصول وتركيز الانثوسيانين في صنف العنب الروبي سيدلس تحت الظروف المناخية لأسيوط

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الملخص

تم أجراء هذا البحث خلال موسمين متتالين 2020-2021 على كروم العنب الروبي سيدلس المزروعة بمزرعة أبحاث كلية الزراعة جامعة اسيوط. ولقد صمم هذا العمل لدراسة مدي فاعلية كل من أحماض الأسكوربيك والستريك والأبسيسيك على وزن المحصول وتركيز الانثوسيانين في صنف العنب الروبي سيدلس تحت الظروف المناخية لمحافظة اسيوط. وقد أظهرت النتائج المتحصل عليها أن جميع المعاملات قد اوضحت زيادة معنوية في المحصول الكلى لكرم العنب الروبي سيدلس مقارنة بالكنترول (كروم العنب الروبي سيدلس غير المعالج) وتم الحصول على أعلى وزن محصول من معاملة حمض الاسكوربك (AA) 600 جزء في المليون + حمض الستريك (CA) 600 جزء في المليون + حمض الابسسك (ABA) 600 جزء في المليون. بالإضافة إلى ذلك، أعطت معاملة حمض الاسكوربك (AA) 600 جزء في المليون + حمض الستريك (CA) 600 جزء في المليون + حمض الابسسك (ABA) 600 جزء في المليون أعلى قيمة لمحتوى الأنثوسيانين.