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(Original Article)

Effect of Foliar Application of Gibberellic acid, Salicylic acid and Ammonium nitrate on Berry Quality of Ruby Seedless Grape Cultivar

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Abstract

Experiment was carried out throughout two successive seasons of 2020 and 2021 on Ruby Seedless grapevines grown at the Experimental Orchard of Assiut University, Faculty of Agriculture. The study was performed to evaluate the influence of Gibberellic acid (GA_3), Salicylic acid and Ammonium nitrate on berry quality of Ruby Seedless grape cultivar during 2020 and 2021 seasons. Data revealed that, the application of Salicylic acid (SA) and Ammonium nitrate increased TSS% while GA_3 reduced it compared with the control. All treatments decreased TA% except SA in the 1st season increased it. In 1st season although there was an increase in reducing sugars due to different treatments comparing with the control, the differences were not significant in most of the treatments. The significant effect was only found in the NH_4NO_3, while during 2nd season significantly increased it with an exception of (SA+GA_3). SA gave the highest values for anthocyanins either in the 1st season (61.85 mg/100gm) or two seasons average all the treatments (43.44 mg/100gm).

Keywords: *Ruby Seedless- Grape-Gibberellic acid (GA_3)- Ammonium Nitrate- Salicylic acid (SA)*

Introduction

Grape is considered as one of the most important fruit crops in the world. It is grown extensively in Europe and other regions all over the world. It occupies the third position after citrus and mango according to the cultivated area and the magnitude of fruit production. It is considered as an important source of antioxidants such as vitamins, phenols, flavonoids, anthocyanins, dietary glutathione and endogenous metabolites. Therefore, grapes are contributing significantly to the health benefits. (Anastasiadi *et al*., 2010).

Most of grape cultivars grown in Egypt belong to the table grapes and all of them are european cultivars. Seedless table grapes are the most popular cultivars in the Egyptian vineyards for both exportation and local marketing. According to the Ministry of Agriculture Statistics 2020, the total area devoted for grapes attained 187358 feddans including 133811 feddans as fruitful vines producing about 1183968 tons with an average of 8.848 tons/feddan.

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"Ruby Seedless" grape cultivar as well called “King Ruby” is a cross between "Emperor” and “Provano 75” (Olmo *et al*., 1981). "Ruby Seedless" is a late maturing cultivar, intermediate berry size, oval berries, red to purple colour, very susceptible to powdery mildew and bunch rot, ripens in mid to late August (Harry *et al*., 1991). The grape berries are consumed in fresh forms as a table fruit and in the processed form as wine, raisin and fresh juice.

The prevalent problems encounter the Ruby Seedless grape cultivar under Assiut conditions are cluster compactness, poor coloring and shot berries.

With a view to improve the grape quality and to increase the berry size, plant growth regulators are usually applied such as Gibberellic acid. Gibberellic acid (GA_3) applied to the clusters before full bloom to elongate the main cluster stem in order to overcome the cluster compactness (Omer and El Morsey, 2000 and Omer and Girgis, 2005 and Mohamed *et al.,* 2019). But it has some hazards as decreasing fertility and delaying maturity (Jawanda *et al*., 1974)

Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature. It plays some important roles in the regulation of plant growth development and enhances plant vigor under biotic and abiotic stresses (Hayat *et al*., 2010). SA plays an essential role in controlling berry quality such as color, flavor, astringency and bitterness (Chamkha *et al*., 2003), and enhance berry size (Marzouk and Kassem, 2011).

Nutrients play an important role in a life cycle of a plant. Nitrogen (N) is known to be essential nutrient for plant development because of its role in proteins, nucleic acids, and enzymes synthesis (Hassan *et al*., 2010; Kandil *et al*., 2010). It is used in form Ammonium Nitrate. or potassium nitrate as aground of fertilizers or on foliar application.

Materials and Methods

The current study was conducted during two successive seasons of 2020 and 2021 on Ruby Seedless grape cultivar grown at the Experimental Orchard of Faculty of Agriculture, Assiut University. The experiment was conducted on thirty years old Ruby Seedless grapevines. Vines were cultivated at 2 x 2 m apart. in a clay soil. The vines were trained according to the head training system was applied by leaving the total bud load of 60 buds/vine (16 fruiting spurs x 3 buds + 6 replacement spurs x 2 buds/vine). the total number of clusters/vines was fixed to 40±5 clusters.

Twenty-eight vines pruned as ahead training system were chosen for this study and all the vines received the same agriculture managements. The experiment consisted of seven treatments (7treatments× 4 replications) arranged in a randomized complete blocks design.

Treatments

1- Spraying with Gibberellic acid (GA_3) at 10 ppm.
2- Spraying with salicylic acid at 3 mM.

3- Spraying with Ammonium Nitrate at 300 ppm.
4- Spraying with Gibberellic acid (GA_3) at 10ppm + Spraying with salicylic acid at 3 mM.
5-Spraying with Gibberellic acid (GA_3) at 10 ppm + Spraying with Ammonium Nitrate at 300 ppm.
6- Spraying with Gibberellic acid (GA_3) at 10 ppm + Spraying with salicylic acid at 3 mM + Spraying with Ammonium Nitrate at 300 ppm.
7- Control spraying with (tap water).

A surfactant super film at 0.1% was added to the spraying solution to reduce the surface tension. The treatment consisted of four replicates. The vines were sprayed using a hand sprayer in the early morning. The clusters were sprayed with GA_3 when they reached a length of 8-10 cm. while the other spraying compounds (Ammonium nitrate - Salicylic acid) were sprayed once before it reaches the coloring stage in late June or early July.

The second spraying was executed, when the coloring reached 10% of the berries (veraison stage).

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).

Two clusters were taken from each vine and transferred to the fruit department laboratory to determine the following characteristics.

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Total soluble solids percentage (TSS %)

Total soluble solids percentage (TSS%) was measured by using a hand refractometer.

Total acidity percentage (TA %)

Total acidity was determined by titrating 5 ml of diluted juice against 0.1 N NaOH solution and phenolphthalein as an indicator. TA was calculated as grams of tartaric acid (TA) per 100 ml of berry juice and TA % according to the following equation AOAC (2005)

$$\text{Total acidity \%} = \frac{\text{Ml (s)NaOH} \times \text{standard solution of NaOH (N)} \times 75}{1000 \times \text{The volume of used juice}} \times 100$$

Where: Standard solution of NaOH (N) = 0.1 N , 75 = the equivalent weight of tartaric acid, The volume of used juice = 5 ml.

TSS/acid ratio was then calculated.

Total Anthocyanin (TAC)

The anthocyanin pigment was prepared through the process described by Onayemi *et al.* (2006). Were 20 ml of ethanol 85%+ HCL1.5 M (by volume) solution were added to gram of berry skin samples. The sample were covered and kept in deep freeze overnight. The extracts were completed to 50 ml of the solvent and then measured at wavelength 530 nm by spectrophotometer (Unico 1200 - USA). Results are expressed as mg anthocyanin per 100 g of fresh samples. Total anthocyanin was then calculated according to the following equation (Lees and Francis, 1971)

$$\text{Total Anthocyanin (TAC) mg/100g fruit skin} = (A535 * V * 100) / (98.2 * W)$$

Where: A535= absorbance of solutions was measured at a wavelength of 530 nm, **V** = total volume of extract in ml, **W** = weight of fresh sample in grams.

Reducing sugars percentage

Reducing sugars percentage in the juice was measured according to Lane and Eynon procedure AOAC (1985).

Statistical analysis

Statistical analysis was done using a randomized complete block design (RCBD) according to Mead et al., (1993) with four replications for each treatment and one vine per each. The analysis of variance (ANOVA). As well as the combined analysis of two seasons of study was done by SAS software program and the treatment means were compared by using the revised L.S.D. values at 5% level of the probability (Steel and Torrie, 1980).

Result

Total soluble solids percentage (TSS %)

The percentage of TSS as affected by GA_3, SA and NH_4NO_3 is presented in Table 1. During the 1st season T3 (NH_4NO_3) significantly exceeded the control. While the rest of treatments had not a significant effect. The increment percentage of T3was 6.02 % over the control. In 2nd season all the tested treatments were not significant although all the treatments were higher than the control. The most effective treatment in this respect was T6 which recorded the highest value (18.15). The increment percentage of T6 was 8.68 % over the control. The average of two seasons showed that, T3 and T6 gave the highest percentage of TSS compared with control. While the rest of treatments had not a significant influence. Such treatment T3 increased Total soluble solids (TSS %) by 5.59% over the control.

Total acidity percentage

The presented date found in Table 1 showing the effect of GA_3, SA and NH_4NO_3 on Total acidity of Ruby Seedless grape cultivar. During 1st season, the total acidity percentage significantly decreased due to applications of T1, T3, T4 and T5. The rest of treatments did not have a significant influence. During 2nd season of study, most of the treatments significantly decreased the Total acidity with an exception of T1 and T6. Two seasons average data showed that all treatments decreased the acidity compared with the control.

Table 1. Effect of Gibberellic acid (GA_3), Salicylic acid (SA) and Ammonium nitrate (NH_4NO_3) on Total soluble solids (TSS%), Total acidity% and Total soluble solids/Acid ratio of Ruby Seedless grape cultivar during 2020 and 2021 seasons

Treatment	Total soluble solids%			Total acidity%			TSS/TA ratio		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
T1	18.20	16.75	17.48 c	0.32	0.37	0.35 b	56.79	46.21	51.50 c
T2	19.65	17.00	18.33 ab	0.38	0.32	0.35 b	52.28	53.96	53.12 c
T3	20.60	17.55	19.08 a	0.31	0.31	0.31 c	67.75	56.61	62.18 a
T4	19.30	17.30	18.30 abc	0.29	0.30	0.30 c	66.26	58.62	62.44 a
T5	19.40	16.90	18.15 bc	0.30	0.33	0.32 c	64.28	52.02	58.15 b
T6	20.00	18.15	19.08 a	0.34	0.37	0.36 ab	58.95	48.77	53.86 c
T7	19.43	16.70	18.07 bc	0.37	0.39	0.38 a	53.00	42.83	47.92 d
L.S.D 0.5	**1.13**	**NS**	**-**	**0.04**	**0.03**	**-**	**4.20**	**3.62**	**-**

T1= GA_3 (10 ppm), T2= SA (3 Mm), T3= NH_4NO_3 (300 ppm), T4= SA (3Mm) + GA_3 (10 ppm), T5= NH_4NO_3 (300 ppm) + GA_3 (10 ppm), T6= SA (3 Mm) + NH_4NO_3 (300 ppm) + GA_3 (10 ppm), T7= Control.

Total soluble solids/acid ratio

Result in Table1 showed that influence of GA_3, SA and NH_4NO_3 on - total soluble solids/acid ratio of Ruby Seedless grape cultivar. The presented date demonstrated that most the treatments significantly increased on the ratio of TSS and acidity in the 1st season of study with an exception of T1and T2. The highest value was T3 (67.75). The increment percentage of T3 was 27.83 % over the control. The ratio of TSS and acidity was significantly affected by all the treatments with an exception of T1 during 2nd season. The best treatments in this respect were T4 followed by T3 and then T2 (58.62, 56.61 and 53.96, respectively). Such treatments increased Total soluble solids/acid ratio by 36.87%, 32.17% and 25.99% over the control, respectively. The combined analysis over the two years of study revealed that all the treatments exceeded the control respecting the Total soluble solids/acid ratio. The best treatments in this respect were T4 followed by T3 and then T5. The increment percentage of these treatments was 30.30%, 29.76% and 21.35% over the control, respectively.

Reducing sugars percentage

Result in Table 2 showed that effect of GA_3, SA and NH_4NO_3 on - reducing sugars of Ruby Seedless grape cultivar. In 1st season Although there was an increase in reducing sugars due to difference treatments comparing with the control, the differences were not significant in most the treatments. the significant effect was only found in the T3 (NH_4NO_3). The highest value was T3 (18.02). The increment percentage of this treatment was 19.10 over the control. During 2nd season all the treatments significantly increased with an exception of T4. The best treatments in this respect were T1 followed by T2 and then T6. The increment percentage of this tests was 18.45%, 18.03% and 15.28% over the control, respectively. Two seasons average date demonstrated that, all the treatments was significantly increased it comparing the control the reducing sugars ranged between 14.12 to 15.70%, while the control gave the lowest percentages. Such treatments increased reducing sugars of T3, T1 and T2 15.78%, 12.75% and 11.65% over the control, respectively.

Table 2. Effect of Gibberellic acid (GA_3), Salicylic acid (SA) and Ammonium nitrate (NH_4NO_3) on Anthocyanins (AN) (mg/100gm) and Reducing sugars (RS)% of Ruby Seedless grape cultivar during 2020 and 2021 season

Treatment	Reducing sugars%			Anthocyanins(mg/100gm)		
	2020	2021	Mean	2020	2021	Mean
T1	16.39	14.19	15.29 ab	24.34	23.80	24.07 f
T2	16.13	14.14	15.14 ab	61.85	24.80	43.33 a
T3	18.02	13.38	15.70 a	43.36	24.37	33.87 b
T4	16.02	12.21	14.12 cd	36.10	27.43	31.77 d
T5	15.94	13.37	14.66 bc	38.39	26.79	32.59 c
T6	16.13	13.81	14.97 abc	32.88	21.92	27.40 e
T7	15.13	11.98	13.56 d	24.99	23.91	24.45 f
L.S.D 0.5	**1.38**	**1.26**	**-**	**0.92**	**1.36**	**-**

T1= GA_3 (10 ppm), T2= SA (3 Mm), T3= NH_4NO_3 (300 ppm), T4= SA (3Mm) + GA_3 (10 ppm), T5= NH_4NO_3 (300 ppm) + GA_3 (10 ppm), T6= SA (3 Mm) + NH_4NO_3 (300 ppm) + GA_3 (10 ppm), T7= Control.

Total anthocyanins content of Ruby Seedless grapes

The percentage of (TAN content) as affected by GA_3, SA and NH_4NO_3 is presented in Table2. During the 1st season all the treatments significantly exceeded the control except T1 (GA_3). The best treatment in the respect was T2 which recorded the highest value (61.85 mg/ 100 g). The second treatments were T3 followed by T5. The increment percentage of T2 was 147.50 % over the control. During 2nd season some the treatments were non-significant and some significantly increased compared to the control. The best treatment in the respect was T4 (SA+GA_3) which recorded the highest anthocyanins content (27.43 mg/ 100 g) followed by T5. The increment percentage of this treatments was 14.72% and 12.05% over the control, respectively. Two seasons average date demonstrated that, most the treatments was significantly increased it comparing with the control. The highest value was T2 (SA) followed by T3. Such treatments increased total anthocyanins content by 77.22% and 38.53% over the control, respectively.

Discussion

The application of GA_3 combined with SA and NH_4NO_3 led to a significant increase in the TSS and TSS/TA in the juice comparing with the control. Our results showed also that the application of GA_3 alone to grape had not any significant effect on TSS. This may be due to the delaying effect of GA_3 on fruit maturity (Zahedi *et al*., 2013). Our results concerning the effect of GA_3 on TSS have been confirmed by many workers (Kaplan, 2017; Khalil, 2020 and Ibrahim *et al*., 2021).

These results came on line with the other papers reported by Alrashdi *et al*., 2017 and Ali *et al.,* 2014), where they found that SA had not a significant effect on TSS. On the other side, (Ahmed *et al.,* 2018 and Bassiony and Mahmoud, 2019) they found that SA had a significant effect on TSS.

NH_4NO_3 increased slightly on TSS of the grape cultivar. These results accordant with those reported by Huang et al., 2008 and Swathi *et al*., 2019. While others revealed that NH_4NO_3 had no effects on TSS. For instance, (Chater and Garner, 2019 and Aly *et al.,* 2020).

Concerning acidity GA_3 alone or combined with SA or NH_4NO_3 decreased the total acidity. Our results concerning the effect of GA_3 and other additions on acidity have been confirmed by workers Eleonora *et al.,* 2018 and Radwan *et al.,* 2019), while they found that GA_3 application of grape cultivars increased TA% (Ibrahim *et al*., 2021). On the other side, during our study GA_3 combined with SA and NH_4NO_3had not a significant effect on TA% compared with the control. The application of NH_4NO_3 individually led to a significant decrease of TA% in both seasons. Our results concerning the effect of Ammonium nitrate on TA% have been confirmed by workers (Wahab *et al.,* 2016 and Beerappa *et al.,* 2019). While others revealed that NH_4NO_3 significant increase TA% (Pathania *et al*., 2018 and Safa *et al.,* 2020). All the Treatments increased the TSS/acid ratio. For instance, Bassiony and Mahmoud, 2019; Aly *et al.,* 2020.

Under the conditions of the current study, it could be found that GA_3 alone or combined with NH_4NO_3 and/or combined with SA and NH_4NO_3 slightly increased the reducing sugars%. Where the results of the current study came on line with those reported by Marzouka and Kassem, 2011 and Radwan *et al.,* 2019. On the other hand, Abada *et al.* (2015), Ibrahim *et al*., (2021) and El-Akkad *et al.,* (2021) found that GA_3 decreased reducing sugars%.

The present study raveled that SA increased the concentration of reducing sugars. Reducing sugars was not significantly affected in 1st year and increased in the following year. The positive effects of SA application on reducing sugars have been noticed by Ahmed *et al.,* 2018; Nanthakumar *et al*., 2021.

The application of NH_4NO_3 led to a significant increase in reducing sugars. Our results also came on line with that reported by, Solhjooa *et at*., 2017 and Beerappa *et al.,* 2019. On the other hand, (Pathania *et al*., 2018) found that NH_4NO_3 decreased reducing sugars.

Gouda, 2016 and Khalil, 2020 demonstrated that GA_3 application decreased berry color and decrease anthocyanin contents. The reduce of anthocyanin content by GA_3 could be the result of their effect on phenylalanine ammonia-lyase (PAL) activity, which is regarded as the primary enzyme in the synthesis of anthocyanin (Lee *et al*., 1996). The abovementioned studies came on line with the results of the current study. Salicylic acid is one of the phenolic compounds that are important secondary metabolites in grape berries and play an essential role in determining grape berry quality as well as berries characteristics such as colour, astringency, flavour, and bitterness (Chamkha *et al.,* 2003). Under the conditions of the current study, it could be found that SA alone significantly increased the anthocyanins in grape skin in the 1st season, while in 2nd led to slight increase compared with the control. The positive effects of SA application on grape quality have been noticed by Bassiony and Mahmoud, 2019 and Nanthakumar *et al.,* 2021. Probably due to the effect of SA on the expression and activity of PAL and other enzymes attached to the biosynthesis of these metabolites. After veraison, phenylalanine ammonia-lyase activity mostly focuses on the production of anthocyanin specifically in red cultivars than other phenolics. Thus, enhancement in phenylalanine ammonia-lyase activity causes increase in Anthocyanin. Consequently, berries have better appearance and nutritional values. Champa *et al.,* 2015 and Chen, 2006. Evaluation of the M3G content, one of the essential anthocyanins in the grape berries extract, also demonstrated increased production when SA concentration was raised. This may also suggest that probably has a specific effect on the genes that control the production of this anthocyanin compound in grape berries, and the output of anthocyanins in large amounts does not solely depend on the activity of phenylalanine ammonia-lyase. Enhancement in PAL activity after SA treatments in grape berries was previously reported by Asghari and Aghdam, 2010 and Champa *et al.,* 2015. As reported positive influence of exogenous SA treatment on flavonoid, phenolic and anthocyanin enhancement. Dokhanieh *et al.,* 2013 and Ahn *et al*., 2014 In grapes, positive effects of SA application on the production of phenylpropanoids, flavonols, and anthocyanins have been reported together with enhancement of gene expression related to the synthesis of flavonoids. Dokhanieh *et al.,* 2013 and Giménez *et al.,* 2017. It was also found that ammonium nitrate had the same effects of salicylic on the anthocyanin content in both seasons. But to a lesser degree. These results were in agreement with Solhjooa *et at*., 2017 and Safa *et al.,* 2020. While Szot and Wieniarska, 2012. Revealed that NH_4NO_3 had no effects on anthocyanin content.

Conclusion

The present study confirmed that the use Salicylic acid and Ammonium nitrate alone or combined with GA_3 could enhance fruit quality. For this reason, it could be concluded that a foliar application with Salicylic acid at 3 Mm, Ammonium nitrate at 300 ppm alone or combined with GA_3 at 10 ppm before cluster reaches the coloring stage and when the coloring reached 10% of the berries for improving the chemical properties of grapes.

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تأثير الرش الورقي بحامض الجبريليك، حامض الســـاليســـيليك ونترات الأمونيوم على جودة الحبات في صنف العنب الروبى سيدلس

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الملخص

أجريت هذه الدراســة بمزرعة كلية الزراعة البحثية جامعة أســيوط خلال موســمي2020، 2021 على كرمات العنب الروبي اللا بذري بغرض دراسة تأثير رش حمض الجبريليك، حامض الســاليســيليك ونترات الأمونيوم على جودة الحبات. حيث تم الرش بحامض الجبريليك عند تركيز (10جزء فى المليون) عند وصول طول العنقود من 8-10 سم. ورش حامض الساليسيليك ونترات الأمونيوم بتركيز (3ملليمول و300جزء في المليون) على التوالي مرتين. المرة الأولي قبل بداية التلوين مباشرة والمرة الاخري عند وصول نسبة التلوين 10-15% من العناقيد.

وقد أظهرت النتائج ما يلي

أدت جميع المعاملات إلى زيادة في نســبة المواد الصــلبة الذائبة الكلية مقارنة بالكنترول والرش بالجبريلين منفردا وكانت أفضــل المعاملات هي الرش بنترات الامونيوم منفردا والرش بحمض السلسليك ونترات الامونيوم والجبريلين معا.

أدت جميع المعاملات إلى نقص معنوي في الحموضــة الكلية لعصــير الحبات باســتثناء حامض الساليسيليك زاد من نسبة الحموضة في الموسم الأول. أدت جميع المعاملات الي زيادة النسبة بين المواد الصلبة الذائبة الكلية والحموضة TSS/ acid ratio مقارنة بالكنترول وكانت أفضل المعاملات هي الرش بحمض الساليسيليك والجبريلين معا.

في الموســم الأول على الرغم من وجود زيادة في نســبة الســكريات المختزلة في مختلف المعاملات مقارنة بالتحكم الا انه لا يوجد اختلافات معنوية في معظمها. حيث وجد التأثير معنوي فقط عند الرش بنترات الأمونيوم، بينما خلال الموســم الثاني أو متوســط موســمين، كانت جميع المعاملات معنوية باستثناء المعاملة المركبة (حمض الجبريليك + حامض الساليسيليك).

أوضحت جميع المعاملات زيادة معنويه في محتوى قشر الحبات من صبغة الانثوسيانين مقارنة بالكنترول والرش بالجبريلين منفردا حيث أدى الرش بالجبريلين منفردا إلى رداءة تلوين حبات العنب ولم يكن هناك فرق معنوى بين الجبريلين والكنترول. حيث أعطي حامض الساليسيليك أعلى قيم للأنثوسيانين في الموسم الأول بمقدار (61.85 مجم / 100 جرام) وزاد متوسط الموسمين بمقدار (43.33 مجم / 100 جرام).

من نتائج التجربة يمكن التوصية بأهمية الرش بنترات الامونيوم وحمض الساليسيليك منفردا أو مختلطة مع الجبريلين للحصــول على خصــائص عناقيد وحبات جيده للعنب الروبى ســيدلس وتحسين التلوين والتغلب على مشكلة رداءة تلوين الحبات عند الرش بالجبريلين منفردا.