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COLOURING IMPROVEMENT OF CRIMSON SEEDLESS GRAPES BY USING PHENYLALANINE, COUMARIC ACID AND ABSCISIC ACID

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ABSTRACT

This investigation was conducted for two successive seasons (2017 & 2018) in a private vineyard located at El-Nubaria region, El-Behira governorate, Egypt to study the effect application of phenylalanine, coumaric acid and abscisic acid on bunch quality attributes of Crimson Seedless grape cultivar. The chosen vines were six-year-old, grown in a sandy soil, spaced at 1.5 X 3 meters apart and irrigated by the drip irrigation system. The vines were cane-pruned during the third week of January with bud load of 100 buds/vine so as to leave (10 canes X 10 buds/cane) with trellised by Gable supporting system. Seven treatments were carried out by spraying the clusters as follows: tap water (Control), phenylalanine at 250ppm, phenylalanine at 500ppm, coumaric acid at 500ppm, coumaric acid at 1000ppm, abscisic acid at 300ppm and abscisic acid at 400ppm. Clusters were sprayed twice: the first spray at veraison stage (20% berry colour), while the second spray was done two weeks later.

The results showed that spraying with phenylalanine, coumaric acid and abscisic acid at the higher concentration significantly had the best values in improving fruit quality attributes compared to those at the lower concentration of these ones in both seasons. Spraying with phenylalanine followed by coumaric acid followed by abscisic acid attained the best results in terms of improving fruit quality attributes by increasing average berry weight, size and dimensions as well as enhancing berry maturity by increasing total soluble solids, total soluble solids/acid ratio, total sugars and anthocyanin in berry skin and decreasing total acidity in berry juice of Crimson Seedless grapes.

From obtained results, it can be said that spraying with phenylalanine at 500ppm was the most effective in improving fruit quality attributes for Crimson Seedless grapes.

INTRODUCTION

Crimson Seedless (*Vitis vinifera* L.) grape is a late season, attractive, crisp and red Seedless grape cultivar. In Egypt, some of the main problems associated with the production of this cultivar

are related to its lack of adequate colour intensity, probably as a consequence of summertime high temperatures, which inhibit the accumulation of anthocyanins **(Spayd *et al*., 2002).**

Anthocyanins accumulate in berries at the beginning of the véraison stage of berry development, its are synthesized by the phenylpropanoid pathway; several structural genes and encoding enzymes of this pathway have been well described **(Mattivi *et al*., 2006).**

The anthocyanin biosynthetic pathway is a major branch of the general phenylpropanoid pathway that starts with phenolic compounds. Among these compounds; phenylalanine and coumaric acid, which are the precursors of important secondary metabolites including anthocyanins; the synthesis of anthocyanins begins with the conversion of phenylalanine through cinnamic acid and coumaric acid to p-coumaryl-coA which is converted through many steps in the presence of some enzymes such as phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumaroyl CoA: ligase (4CL), chalcone synthase (CHS), chalcone-flavanone isomerase (CHI), flavanone-3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glycose:flavonoid 3-O-glycosyltransferase (UFGT) to anthocyanin compound **(Xie *et al*., 2011 and Jaakola, 2013).** Few studies have indicated that the exogenous application of phenylalanine and coumaric acid has improved fruit quality attributes **(Farag *et al*., 2012 and El-Sayed 2013).**

The plant hormone abscisic acid (ABA) has traditionally been considered as the main triggering signal for the onset of ripening berries, and its appear to be implicated in the regulation of flavanols and anthocyanins synthesis in grapes berries as well as increased the synthesis of secondary metabolism compounds, including phenols **(Koyama *et al*., 2010; Ferrero *et al*., 2018 and Yamane *et al*., 2018).** Moreover, abscisic acid involve in various biochemical and physiological processes in grape berries, which directly improve the berry colour development **(Neto *et al*., 2017).** Various studies have suggested that the exogenous application of abscisic acid increases TSS, TSS/acid ratio and berry skin content of anthocyanin of table grape cultivars **(Jeong *et al*., 2004; Peppi *et al*., 2006, Cantin *et al*., 2007; Omran, 2011; Mohamed *et al*., 2015 and Olivares *et al*., 2017).**

This investigation aims to study the exogenous application of phenylalanine, coumaric acid and abscisic acid (ABA) on bunch quality attributes of Crimson Seedless grape cultivar.

MATERIALS AND METHODS

This investigation was conducted for two successive seasons (2017 & 2018) in a private vineyard located at El-Nubaria region, El-Behira governorate, Egypt to study the effect application of phenylalanine, coumaric acid and abscisic acid (ABA) on bunch quality attributes of Crimson Seedless grape cultivar.

The chosen vines were six-year-old, grown in a sandy soil, spaced at 1.5 X 3 meters apart and irrigated by the drip irrigation system. The vines were cane-pruned during the third week of January with bud load of 100 buds/vine so as to leave (10 canes X 10 buds/cane) with trellised by Gable supporting system.

Sixty-three vines were chosen, each three vines acted as a replicate and each three replicates were treated by one of the used treatments.

Clusters were sprayed twice: the first spray at veraison stage (20% berry colour), while the second spray was done two weeks later for all conducted treatments as follows:

1. Tap water (Control)
2. Phenylalanine at 250ppm
3. Phenylalanine at 500ppm
4. Coumaric acid at 500ppm
5. Coumaric acid at 1000ppm
6. Abscisic acid at 300ppm
7. Abscisic acid at 400ppm

Abscisic acid (ABA) was used as trademark (ProTone®) SL; ABA 10%).

The non-ionic surfactant Tween 80 at 0.05% (v/ v) was added to all treatments to reduce the surface tension and to increase the contact angle of sprayed droplets.

The following parameters were evaluated and recorded as follows:

Representative random samples of six clusters/vine were harvested at maturity when TSS reached about 16-17% according to **Tourky *et al*., (1995).**

The following characteristics were determined:

1. Physical characteristics of berries:

- Average berry weight (g).
- Average berry size (cm^3).
- Average berry dimensions (length and diameter) (cm).
- Average berry firmness (g/cm^2) by 1fra Texture analyzer instrument.

2. Chemical characteristics of berries:

- Total soluble solids (%) by hand refractometer.

- Total titratable acidity (%) as tartaric acid **(A.O.A.C. 1985).**
- TSS /acid ratio.
- Total sugars (%) according to **Malik and Singh (1980).**
- Total anthocyanins of the berry skin (mg/100g fresh weight) according to **Husia *et al*., (1965).**

Experimental design and statistical analysis:

The complete randomized block design was adopted for the experiment. The statistical analysis of the present data was carried out according to **Snedecor and Cochran (1980).** Averages were compared using the new L.S.D. values at 5% level.

RESULTS AND DISCUSSION

1. Physical characteristics of berries:

As shown in Table (1&2), all berry physical characteristics i.e. berry weight, size, length and diameter were significantly affected by two different concentrations of phenylalanine, coumaric acid and abscisic acid applications as compared to control in both seasons.

Highest significant berry weight and size were obtained by spraying with phenylalanine followed by coumaric acid followed by abscisic acid, whereas significantly the lightest ones were attributed to the control in both seasons. The application of phenylalanine, coumaric acid and abscisic acid at the higher concentration significantly resulted in the highest values compared to those at the lower concentration in both seasons.

These results are in concordance with those obtained by **El-Sayed (2013)** on Crimson Seedless grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly increased berry weight and size. As for the effect of cumaric acid, **Farag *et al.*, (2012)** showed that the exogenous application of Coumaric acid resulted in a significant increase in average berry weight of Crimson Seedless grape cultivar.

With respect to berry dimensions, using the highest concentration of phenylalanine, coumaric acid and abscisic acid applications resulted in the highest values as compared to the lowest concentrations of these ones. Spraying with phenylalanine significantly attained the highest values followed by spraying with coumaric acid followed by abscisic acid, while control had the least significant values in both seasons.

Concerning berry firmness, it was mentioned that berry firmness was statistically insignificant among all conducted treatments in both seasons.

The obtained results are in agreement with those achieved by **Omran (2011)** on Red Globe grapes; **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes and **Olivares *et al*., (2017)** on Crimson Seedless grapes pointed out that berry firmness remained unaffected by ABA treatment. As for the effect of phenylalanine**, El-Sayed (2013)** found that firmness of Crimson Seedless grape was not affected by phenylalanine application.

Table (1): Effect of phenylalanine, coumaric acid and abscisic acid on berry weight and size of Crimson Seedless grape in 2017 and 2018 seasons

Characteristic / Treatment	Average berry weight (g)		Average berry size (cm^3)	
	2017	2018	2017	2018
Control	3.43	3.47	3.02	3.09
250ppm phenylalanine	3.76	3.79	3.43	3.48
500ppm phenylalanine	3.85	3.87	3.54	3.58
500ppm coumaric acid	3.63	3.66	3.27	3.32
1000ppm coumaric acid	3.73	3.75	3.39	3.42
300ppm abscisic acid	3.52	3.56	3.13	3.19
400ppm abscisic acid	3.58	3.62	3.21	3.26
new L.S.D. at 0.05=	0.08	0.07	0.09	0.08

Table (2): Effect of phenylalanine, coumaric acid and abscisic acid on berry length, diameter and firmness of Crimson Seedless grape in 2017 and 2018 seasons

Characteristic / Treatment	Average berry length (cm)		Average berry diameter (cm)		Average berry firmness (g/cm^2)	
	2017	2018	2017	2018	2017	2018
Control	2.23	2.24	1.57	1.64	257.5	264.8
250ppm phenylalanine	2.41	2.43	1.73	1.79	229.2	231.4
500ppm phenylalanine	2.47	2.48	1.76	1.81	223.3	226.2
500ppm coumaric acid	2.38	2.39	1.67	1.75	243.5	244.1
1000ppm coumaric acid	2.39	2.41	1.71	1.78	238.6	237.8
300ppm abscisic acid	2.31	2.33	1.65	1.71	251.3	257.5
400ppm abscisic acid	2.33	2.36	1.66	1.73	249.2	253.7
new L.S.D. at 0.05=	0.03	0.04	0.02	0.01	N.S.	N.S.

2. Chemical characteristics of berries:

Data depicted in Table (3&4) clearly revealed that all berry chemical characteristics expressed total soluble solids (TSS), acidity, TSS/acid ratio, total sugars and anthocyanin content of berry skin were

significantly affected by two different concentrations of phenylalanine, coumaric acid and abscisic acid applications as compared to control in both seasons.

Table (3): Effect of phenylalanine, coumaric acid and abscisic acid on TSS, acidity and TSS/acid ratio of Crimson Seedless grape in 2017 and 2018 seasons

Characteristic / Treatment	TSS (%)		Acidity (%)		TSS/acid ratio	
	2017	2018	2017	2018	2017	2018
Control	16.91	16.97	0.56	0.59	30.20	28.76
250ppm phenylalanine	17.84	17.88	0.41	0.43	43.51	41.91
500ppm phenylalanine	17.97	18.03	0.38	0.41	47.29	43.98
500ppm coumaric acid	17.67	17.68	0.46	0.48	38.41	36.83
1000ppm coumaric acid	17.72	17.75	0.43	0.46	40.89	38.59
300ppm abscisic acid	17.33	17.42	0.49	0.54	35.37	32.26
400ppm abscisic acid	17.49	17.57	0.47	0.51	37.21	34.45
new L.S.D. at 0.05=	0.11	0.13	0.02	0.01	2.07	2.01

Table (4): Effect of phenylalanine, coumaric acid and abscisic acid on total sugars and total anthocyanins of Crimson Seedless grape in 2017 and 2018 seasons

Characteristic / Treatment	Total sugars (%)		Total anthocyanin (mg/100g F.W.)	
	2017	2018	2017	2018
Control	19.23	19.29	59.87	62.73
250ppm phenylalanine	19.77	19.91	74.41	76.57
500ppm phenylalanine	19.86	19.98	76.65	79.23
500ppm coumaric acid	19.57	19.71	71.67	74.38
1000ppm coumaric acid	19.63	19.79	73.34	75.69
300ppm abscisic acid	19.47	19.58	69.23	71.34
400ppm abscisic acid	19.54	19.67	70.58	73.13
new L.S.D. at 0.05=	0.08	0.05	1.93	1.87

Highest significant juice TSS percentage was obtained by spraying with phenylalanine followed by coumaric acid followed by abscisic acid, whereas significantly the least percentage was attributed to the control in both seasons. The application of phenylalanine, coumaric acid and abscisic acid at the higher concentration significantly resulted in the highest percentage compared to those at the lower concentration in both seasons.

These results are in harmony with those obtained by Omran (2011) on Red Globe grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly increased TSS percentage in berry juice. As for the effect of phenylalanine, **El-Sayed (2013)** found that TSS percentage of Crimson Seedless grape was increased by phenylalanine

application.. Concerning the effect of cumaric acid, **Farag *et al.*, (2012)** showed that the exogenous application of Coumaric acid resulted in a significant increase in TSS percentage of Crimson Seedless grape.

With respect to acidity percentage, using the highest concentration of phenylalanine, coumaric acid and abscisic acid applications resulted in the least percentage as compared to the lowest concentrations of these ones. Spraying with phenylalanine significantly attained the least percentage followed in an ascending order by spraying with coumaric acid followed in an ascending order by spraying with abscisic acid, while control had the highest significant percentage in both seasons.

The obtained results are in agreement with those achieved by **Omran (2011)** on Red Globe grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly decreased acidity percentage in berry juice. As for the effect of cumaric acid, **Farag *et al.*, (2012)** showed that the exogenous application of Coumaric acid resulted in a significant reduction in juice acidity of Crimson Seedless grape.

Concerning TSS/acid ratio, it was mentioned that TSS/acid ratio was increased by all conducted treatments in both seasons. Significantly, the highest TSS/acid ratio was obtained by spraying with phenylalanine followed by coumaric acid followed by abscisic acid, whereas significantly the least one was attributed to the control in both seasons. The application of phenylalanine, coumaric acid and abscisic acid at the higher concentration significantly resulted in the highest values compared to those at the lower concentration in both seasons.

These results are in concordance with those obtained by **Omran (2011)** on Red Globe grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly increased TSS/acid ratio in berry juice. Concerning the effect of cumaric acid, **Farag *et al.*, (2012)** showed that the exogenous application of Coumaric acid resulted in a significant increase in TSS/acid ratio of Crimson Seedless grape.

As for total sugars percentage, using the highest concentration of phenylalanine, coumaric acid and abscisic acid applications resulted in the highest percentage as compared to the lowest concentrations of these ones. Spraying with phenylalanine significantly attained the highest percentage followed by spraying with coumaric acid followed by spraying with abscisic acid, while control had the least significant percentage in both seasons.

The obtained results are in agreement with those achieved by **Omran (2011)** on Red Globe grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly increased total sugars percentage in berry juice. Concerning the effect of cumaric acid, **Farag *et al*., (2012)** showed that the exogenous application of Coumaric acid resulted in a significant increase in total sugars percentage of Crimson Seedless grape.

With respect to total anthocyanins, it was mentioned that total anthocyanins was increased by all conducted treatments in both seasons. Significantly, the highest total anthocyanins magnitude was obtained by spraying with phenylalanine followed by coumaric acid followed by abscisic acid, whereas significantly the least one was attributed to the control in both seasons. The application of phenylalanine, coumaric acid and abscisic acid at the higher concentration significantly resulted in the highest significant magnitude compared to those at the lower concentration in both seasons.

The positive effect of abscisic acid application at veraison stage on anthocyanin content of berry skin can be attributed to enhance the accumulation of mRNA of several genes involved in phenols and anthocyanin biosynthesis in grapes as described by **Jeong *et al*., (2004).** These results are in harmony with those obtained by **Omran (2011)** on Red Globe grapes and **Mohamed *et al*., (2015)** on Flame Seedless, Red Globe and Crimson Seedless grapes pointed out that S-ABA (ProTone) treatment significantly increased anthocyanin content in berry skin.

As for the positive effect of phenylalanine on anthocyanin accumulation in fruit skin could be attributed to their influence on anthocyanin biosynthesis since phenylalanine is the primer compound in the biosynthesis pathway of anthocyanin **(Xie *et al*., 2011).** The availability of phenylalanine leads to more activity of phenylalanine ammonium lyase enzyme, which reflects the accumulation of anthocyanin in the skin of coloured fruits **(Kanellis and Roubelakis-Angelakis, 1993).** These results are in line with those obtained by **El-Sayed (2013)** found that anthocyanin content in berry skin of Crimson Seedless grape was increased by phenylalanine application..

Concerning the positive influence of coumaric acid on anthocyanin formation, it could be attributed to enhance the biosynthesis of such pigment since coumaric acid is one of the important building blocks toward the biosynthesis of anthocyanin **(Jaakola, 2013).** The obtained

results are in agreement with those achieved by **Farag *et al*., (2012)** who showed that the exogenous application of coumaric acid resulted in a significant increase in anthocyanin content in berry skin of Crimson Seedless grape.

In conclusion, from the forgoing results, it could be concluded that spraying with phenylalanine at 500ppm was the most effective in improving fruit quality attributes for Crimson Seedless grapes.

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تحسين التلوين فى العنب الكريمسون باستخدام الفينايل ألانين وحمض الكيومارك وحمض الأبسيسك

أشرف رضا على فرج

قسم بحوث العنب – معهد بحوث البساتين – مركز البحوث الزراعية بالجيزة – مصر

أجرى هذا البحث لمدة موسمين متتاليين (2017 ، 2018) بإحدى المزارع الخاصة بمنطقة النوبارية التابعة لمحافظة البحيرة لدراسة تأثير إضافة الفينايل ألانين وحمض الكيومارك وحمض الأبسيسك على خصائص جودة عناقيد عنب الكريمسون سيدلس ، وكان عمر الكرمات 6 سنوات نامية فى تربة رملية، ومزروعة على مسافة 1,5 X 3 متر، وتروى بنظام الرى بالتنقيط ، كما تم تقليم الكرمات تقليما قصبيا فى الأسبوع الثالث من شهر يناير مع ترك حمولة عدد براعم 100 عين لكل كرمة وذلك بترك (10 قصبات X 10 براعم / قصبة) تحت نظام التدعيم وفقا لنظام الجيبل. وقد تم إجراء سبعة معاملات من خلال رش العناقيد على النحو التالى: الرش بالماء (الكنترول)، الرش بالفينايل ألانين بتركيز 250 جزء فى المليون، الرش بالفينايل ألانين بتركيز 500 جزء في المليون، الرش بحمض الكيومارك بتركيز 500 جزء في المليون، الرش بحمض الكيومارك بتركيز 1000 جزء فى المليون، الرش بحمض الأبسيسك بتركيز 250 جزء في المليون، الرش بحمض الأبسيسك بتركيز 500 جزء في المليون، وقد تم إجراء الرش في موعدين: الرشة الأولى عند مرحلة بداية التلوين (20٪ تلوين)، بينما كانت الرشة الثانية بعد أسبوعين من الرشة الأولى.

أشارت نتائج الدراسة إلى أن الرش بمركبات الفينايل ألانين وحمض الكيومارك وحمض الأبسيسك عند التركيز الأعلى لكل منهم قد أعطت أفضل القيم مقارنة عند التركيز الأقل لكل

منهم في كلا الموسمين، كما سجلت معاملات الرش بالفينايل ألانين تليها الرش بحمض الكيومارك تليها الرش بحمض الأبسيسك أفضل النتائج من حيث تحسين خصائص جودة الثمار والمتمثلة فى زيادة كل من متوسط وزن وحجم وأبعاد الحبة، وكذلك تحسين نضج الحبات وذلك من خلال زيادة المواد الصلبة الذائبة الكلية ، نسبة المواد الصلبة الذائبة الكلية / الحموضة ، السكريات الكلية ومحتوى الأنثوسيانين فى قشر الحبات وتقليل نسبة الحموضة الكلية فى عصير حبات عنب الكريمسون سيدلس.

من النتائج التي تم الحصول عليها ، يمكن القول بأن رش العناقيد بمركب الفينايل ألانين بتركيز 500 جزء فى المليون كان الأكثر فعالية من حيث تحسين خصائص جودة الثمار لعناقيد عنب الكريمسون سيدلس.