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Effect of Preharvest Application of ProTone, Methionine and Oleic acid as Alternative Materials to Ethephon for Enhancing Berry Coloration and Quality of "Flame Seedless" Table Grapes

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Abstract

In this experiment, ten-years-old "Flame Seedless" grape-vines at El-Nubaria region, Behira governorate, Egypt, were preharvest sprayed during 2016 and 2017 seasons with water only with surfactant (control), 400 mg/ L ethephon, 100 mg/ L ProTone (active ingredient of Abscisic acid 10%), 100 mg / L methionine, 400 mg/ L oleic acid, ProTone plus methionine, ProTone plus oleic acid and ProTone plus methionine and oleic acid, in order to investigate their influence on berry coloration, berry quality of grapes and the possibility of using these materials as alternatives to ethephon treatment. The results revealed that ethephon, ProTone, methionine and oleic acid-treated grapes increased berry anthocyanin contents, TSS and decreased berry acidity and chlorophylls. On the other hand, the formulation containing ProTone, methionine and oleic acid possessed higher anthocyanin contents and reduced berry shattering in addition to weight loss percentage as compared with ethephon and control treatments. This study recommended using the formulation containing ProTone plus methionine and oleic acid at véraison (10- 15% berry coloration) as an alternative to ethephon treatment for enhancing berry coloration, berry quality and overcome some adverse effects of ethephon such as increased berry shattering and berry weight loss, which reflects on berry appearance.

Keywords: *Abscisic acid, anthocyanin, grapes, methionine, oleic acid, berry shattering, ethephon.*

1. Introduction

"Flame Seedless" is considered as one of the most promising and important grape cultivars in Egypt, due to its earliness, high production, good quality and the ability of exporting and marketing. Improving color density, uniformity and hasten maturity of grapes is important for grape growers which reflects on the net income.

Since 1970, ethephon has been used to improve color and hasten ripening of red grapes (Weaver and Pool, 1971). On the other hand, there are some adverse effects such as soft berries, loose berries, lower postharvest quality and recently, the restriction were set by European Food Safety Authority (EFSA) representative in determination Maximum Residue Levels (MRLs) of ethephon (EFSA, 2009; Lombard *et al*., 2004).

Egyptian growers must use ethylene carefully because of the restriction set by EFSA for maximum allowed residue levels of ethephon as a coloring agent. Therefore, there is a need to investigate the use of alternative materials to hasten and improve table grape qualities especially berry coloration such as Abscisic Acid (ABA), methionine and oleic acid.

Abscisic acidis a plant growth regulator which regulated the development of pigments in red grape cultivars that give them the distinctive color (Katayama-Ikegami *et al*., 2016). There are positive effects of S-ABA such as improving colors of grapes without negatively impacting berry or cluster quality (Cantín *et al*., 2007: Peppi *et al*., 2007). Besides, low toxicity and no MRLs limitation. In the past, the application of ABA was expensive for grape growers, but recently ABA production methods have improved sufficiently to use by growers in viticulture (Peppi *et al*., 2006).

Oleic acid is unsaturated fatty acid reported to advance fruit coloration and maintaining fruit quality of some fruit species such as apricotsand plums (Farag *et al*., 2012: Farag and Attia, 2016).

Methionine is considered the main precursor of ethylene biosynthesis in higher plants through a pathway involving S-Adenosyl-Methionine (SAM) and l-Amino-cyclopropane-l-Carboxylic Acid (1-ACC) (Adams and Yang, 1979).

Thus, the objectives of this study were to investigate the effect of ABA, oleic acid, methionine and combinations as alternatives to ethephon to enhance berry coloration of "Flame Seedless" table grapes and to overcome the negative effects of ethephon on berry quality.

2. Materials and Methods

2.1. Experimental vines and vineyard layout

Ten-years-old "Flame Seedless" grape-vines (*vitis vinifera* L.) grown on own roots, during 2016 and 2017 seasons were selected for conducting this investigation. The description of experimental vines and vineyard layout was shown in Table 1:

Table (1):

Study location	Irrigation system	Soil type	Distance between vines	Training system	Trellis system	Dormex application	Crop load	Gibberellic acid (GA_3) treatments
Private orchard at El-Nubaria region, Behira governorate, Egypt.	Drip irrigation	Sandy soil	2* 3.5m	Spur (2buds) plus cane pruning (5- 6 buds)	Gable	5% at the beginning of Jan.	25- 30 bunch/ vine.	1-bunch stretch (5ppm GA_3applied at 6-8 cm bunch length). 2-berry thinning (5ppm GA_3 applied at 50% and 80% flowering). 3-berry enlargement (30ppm GA_3 applied at 4-6 mm berry diameter and 40 ppm GA_3 applied at 6- 8 mm.

The experiment was arranged in a completely randomized block design (RCBD). There were eight treatments replicated three times, consisting of the control (water only with surfactant), 400mg/L ethephon (2-chloroethylphosphonic acid (an ethylene releasing compound), 100 mg/L ProTone (active ingredient of ABA 10%), 100 mg/L methionine (100%), 400mg/L oleic acid (65%), ProTone plus methionine, ProTone plus oleic acid and ProTone plus methionine and oleic acid. The treatments were applied twice during the season at 10- 15% berry coloration (13, 17 May during 2016 and 2017 seasons, respectively) and after seven days later with hand sprayer until run off and 0.5 cm/ L Tween 80 were added to all treatment solutions.

2.2. Physical and chemical characteristics

Two bunches from each replication was picked at harvest (7, 4 June during 2016 and 2017 seasons, respectively) and berries were removed from each bunch to determine the following properties: weight of 50 berries (g), berry diameter (cm) and berry length (cm).on the other hand, in juice of the berries, total soluble solids (TSS %) were determined by hand refractometer, titratable acidity% (as tartaric acid) was determined according to AOAC (1985), the ratio between TSS/acidity was calculated. Berry skin chlorophyll a and b (mg/100g) were determined according to Lichtenthaler and Wellburn (1985), also, berry skin anthocyanin (mg/100g) was determined according to Fuleki and Francis (1968).

2.3. Shelf life assessment

To investigate the effect of different treatments on berry shatter and berry weight loss, a sample of two bunches from each replicate was kept at ambient temperature (20+2°C) for seven days. Berry shatter was evaluated for each replicate at the end of seven days:

Berry shatter %= weight of free berries from each replicate/ total bunch weight*100, and weight loss also was calculated:

Weight loss %= initial bunch weight-final bunch weight/ initial bunch weight *100.

2.4. Statistical analysis

The data was analyzed using SAS software (SAS, 2000). The least significant differences (LSD) at 5% probability level according to Sendecor and Cochran (1980) were used to separate treatments means.

3. Results and Discussion

The effect of ProTone (Abscisic acid), oleic acid and methionine on some berry physical characteristics of "Flame Seedless" grapes at harvest was reported in Table 2. The results indicated that oleic acid treatment increased slightly the berry weight as compared with control treatment. On the other hand, ethephon treatment slightly decreased the berry weight as compared with control treatment. The data in Table 2 revealed that there was no significant alteration in berry length due to all the used treatments except the decrease obtained with ethephon treatment. In addition, berry diameter was not significantly affected by various applied treatments in both seasons except with the application of oleic acid resulted in a significant increase in berry diameter in the first season compared with the control. These results agree with the work of Cantín *et al.* (2007) on "Crimson Seedless" grapes and Farag and Attia, (2016) on plums. Roberto *et al.* (2013) found that preharvest application of ethephon at 500 ppm seven days before véraison and ABA at 200 or 400 ppm twice at seven days before véraison and fifteen days before harvest of 'Rubi' grapes did not affect berry physical characteristics such as berry length, diameter and berry weight. The effect of ethephon treatment strongly depends on berry development stage at the time of application (Davies *et al.*, 2009). The decrease in berry weight reported in this study could be attributed to the ethylene released from ethephon which reduced leaf activity and the accumulation of carbohydrates by leaves.

Table 2. Effect of Abscisic acid (ProTone), oleic acid and methionine on some berry physical characteristics of "Flame Seedless" grapes at harvest during 2016 and 2017 seasons.

Treatments	weight of 50 berries (g)		Berry length (cm)		Berry diameter (cm)	
	2016	2017	2016	2017	2016	2017
Control (water only)	198.85b	201.21bc	1.76ab	1.79bc	1.69bc	1.79a
Ethephon 400 mg/ L	191.99c	198.08d	1.68c	1.77d	1.66c	1.76bc
ProTone 100 mg/ L	201.72ab	202.22bc	1.75ab	1.79bc	1.73ab	1.77abc
Methionine 100 mg/ L	201.24ab	200.86bcd	1.73b	1.79bc	1.76a	1.75c
Oleic acid 400 mg/ L	207.02a	211.45a	1.79a	1.81ab	1.76a	1.78ab
ProTone + Methionine	200.88ab	199.87cd	1.79ab	1.79bc	1.74ab	1.77abc
ProTone+ Oleic acid	204.17ab	203.49b	1.80a	1.81a	1.75a	1.78ab
ProTone+ Methionine+ Oleic acid	204.39ab	200.58cd	1.78ab	1.79bc	1.73ab	1.77abc
L.S.D.0.05	6.85	2.80	0.054	0.014	0.055	0.023

Values, within a column with same letter(s) were not significantly different by L.S.D ($p < 0.05$).

The data in Table 3 showed the effect of preharvest treatments on some berry chemical characteristics of "Flame Seedless" grapes at harvest. The data illustrated that all applied preharvest treatments increased TSS % as compared with untreated control in 2016 and 2017, seasons. Moreover, the formulation containing ProTone plus methionine and oleic acid had the highest TSS % as compared by individual treatments. On the other hand, total acidity percentage in grape juice was significantly reduced with the formulation containing ProTone plus methionine and the formulation containing ProTone plus methionine and oleic acid in a consistent manner in both seasons of study. However, preharvest application of oleic acid resulted in a similar juice acidity to that found in the control. Meanwhile, the TSS to acidity ratio was significantly affected with some treatments. The formulation containing ProTone plus methionine and oleic acid possessed higher TSS/ acidity ratio as compared with control and all other treatments. On the other hand, oleic acid treatment had the same TSS/ acidity ratio as compared with control treatment. The increase in TSS, TSS/ acidity ratio and decreased acidity of "Flame Seedless" grape berries found in this study agreed with the findings of Farag *et al*. (2012) on apricots and Farag and Attia (2016) on plums. Preharvest application of ethephon at 250 ppm applied at 15- 20% berry coloration increased the berry TSS and TSS/ acidity ratio and lowered berry acidity of "Crimson Seedless" grapes (Hezema, 2012).

Table 3. Effect of Abscisic acid (ProTone), oleic acid and methionine on some berry chemical characteristics of "Flame Seedless" grapes at harvest during 2016 and 2017 seasons.

Treatments	TSS (%)		Acidity (%)		TSS/ acidity ratio	
	2016	2017	2016	2017	2016	2017
Control (water only)	15.67c	16.00f	0.997a	1.027ab	15.82d	15.63d
Ethephon 400 mg/ L	17.17b	17.10d	0.927ab	0.923e	18.56bcd	18.59b
ProTone 100 mg/ L	17.60b	17.60c	0.997a	0.980cd	17.73bcd	18.05c
Methionine 100 mg/ L	17.73b	17.97b	0.953ab	1.010bc	18.78bc	17.88c
Oleic acid 400 mg/ L	17.00b	16.67e	1.02a	1.050a	16.85cd	15.87d
ProTone + Methionine	17.23b	17.50c	0.857bc	0.993c	20.29b	17.69c
ProTone+ Oleic acid	17.43b	17.73bc	0.963ab	0.960d	18.17bcd	18.57b
ProTone+ Methionine+ Oleic acid	18.77a	18.33a	0.793c	0.913e	23.80a	20.15a
L.S.D.0.05	0.781	0.288	0.113	0.030	2.770	0.503

Values, within a column with same letter(s) were not significantly different by L.S.D ($p < 0.05$).

The data in Table 4 showed that, there was a significant increase in berry anthocyanin content by all the used treatments in both seasons of study as compared with control, except for the oleic acid-treated grapes in the first season. Meanwhile, the application of ProTone in combination with methionine or oleic acid causes similar anthocyanin content to ethephon treatment in the first season, but possessed higher anthocyanin content to ethephon treatment in the second season. With regard to berry chlorophyll a content at harvest, the data in Table 4 indicated that all the used treatments were able to reduce chlorophyll a in berry skin. The application of ProTone plus methionine possessed lower chlorophyll a content that similar to ethephon treatment. Similar trend of result was obtained with chlorophyll b, the greatest content was found in the control berries. Moreover, the incorporation of oleic acid with ProTone treatment had inconsistent reduction in chlorophyll b (Table 4). The positive effect of ethephonon berry anthocyanin contents was previously reported (Fidelibus *et al*., 2010: Ferrara *et al*., 2013; Brighenti *et al*., 2017). Furthermore, ABA treatment increased berry anthocyanin content. The positive effects of ABA might be attributed to the stimulation of the production of UDP-glucose-flavonoid 3-O-glucosyl-transferase which enhancing the conversion of anthocyanidins into anthocyanins (UFGT-enzyme), stimulating the uptake and storage of sugars by berries and increased the activity of both soluble and cell wall acid invertases (Boss *et al*., 1996 a, b; Davies *et al*., 2009; Pan *et al*., 2005). Moreover, preharvest application of methionine increased the berry anthocyanin pigments as compared with control treatment. The role of the amino acid methionine in enhancing berry anthocyanin contents might be attributed to its role in ethylene biosynthesis pathway that is reflected on enhancing berry coloration in an indirect manner. The positive effect of oleic acid on berry coloration could be attributed to its moderate stimulation of ethylene production (Farag *et al*., 2012). On the other hand, all the used treatments lowered berry skin chlorophylls. The above trend of results whether for chlorophyll a or b agreed with those reported by Farag *et al*. (2012) and Farag and Attia (2016). Ethephon stimulated the progressive loss of chlorophylls in apples (Huybrechts *et al*., 2003). Katayama-Ikegami *et al*. (2016) revealed that ABA treatment at 200 and 400 ppm around véraison resulted in the up-regulation of genes encoding enzymes responsible for both general flavonoid and anthocyanin biosynthesis.

Table 4. Effect of Abscisic acid (ProTone), oleic acid and methionine on skin berry pigments of "Flame Seedless" grapes at harvest during 2016 and 2017 seasons.

Treatments	Anthocyanin (mg/ 100g)		Chlorophyll a (mg/ 100g)		Chlorophyll b (mg/ 100g)	
	2016	2017	2016	2017	2016	2017
Control (water only)	28.09d	26.65g	1.21a	1.23a	1.26a	1.28a
Ethephon 400 mg/ L	34.67a	34.72c	0.77c	0.723d	0.857c	0.810bc
ProTone 100 mg/ L	32.40b	33.68d	0.83c	0.803c	0.913bc	0.827bc
Methionine 100 mg/ L	31.39bc	31.27e	1.01b	1.04b	0.963bc	0.750c
Oleic acid 400 mg/ L	29.75cd	29.79f	0.94b	1.04b	1.02bc	0.873bc
ProTone + Methionine	34.67a	34.72bc	0.757c	0.733d	0.960bc	0.817bc
ProTone+ Oleic acid	36.01a	35.88ab	0.753c	0.783c	1.087ab	0.953b
ProTone+ Methionine+ Oleic acid	36.56a	36.67a	0.770c	0.807c	0.883bc	0.770c
L.S.D.0.05	1.97	1.021	0.106	0.038	0.219	0.157

Values, within a column with same letter(s) were not significantly different by L.S.D ($p < 0.05$).

The data in Table 5 provided evidence that the highest percentage of berry shattering was obtained with ethephon treatment ranging between 10.95 to 11.71% and the lowest percentage was achieved by oleic acid treatment ranging between 3.96 to 5.73% in the two studied seasons, followed by ProTone plus oleic acid and ProTone plus methionine and oleic acid. The data also indicated that there was no significant difference between ProTone application and control treatment on berry shattering percentage in 2016 and 2017, seasons. It seemed that the addition of oleic acid with ProTone in one formulation minimized the berry shattering. Regarding to weight loss percentage, the data in Table 5 illustrated that the minimum weight loss percentage was obtained by oleic acid treatment and the maximum weight loss percentage was obtained by ethephon treatment in 2016 and 2017, seasons. The positive role of oleic acid on reducing berry shattering and weight loss percentage after seven days on shelf might be attributed to its influence on the properties of cellular membranes such as membrane permeability, fluidity and rigidity (Maxfield and Tabas, 2005), which reflects on the extent of berry grapes shelf life. preharvest application of oleic acid at 400 ppm on "Canino" apricots either at pit-hardening or at 15-20% fruit coloration, enhanced the fruit quality at harvest and after one week on shelf life at room temperature especially, fruit weight loss and storability (Attia, 2009). On the other hand, ethephon negatively affected berry shattering and increased weight loss as a result of accelerating senescence and increasing cell wall enzymes activity (Pressey, 1977). The negative quality effects of ethephon on berry grapes were previously reported (Lombard *et al*., 2004; Weaver and Pool, 1971).

Table 5. Effect of Abscisic acid (ProTone), oleic acid and methionine on berry shattering (%) and weight loss (%) of "Flame Seedless" grapes after 7 days on room temperature during 2016 and 2017 seasons.

Treatments	Berry shattering (%)		Weight loss (%)	
	2016	2017	2016	2017
Control (water only)	8.95b	7.57b	4.72bc	5.07bc
Ethephon 400 mg/ L	11.71a	10.95a	5.69a	6.45a
ProTone 100 mg/ L	8.96b	7.57b	4.59bc	5.06bc
Methionine 100 mg/ L	8.10c	7.58b	4.70bc	5.06bc
Oleic acid 400 mg/ L	5.73e	3.96e	3.38d	2.98e
ProTone + Methionine	9.03b	7.90b	5.01b	5.72ab
ProTone+ Oleic acid	7.18d	6.35d	4.32c	4.15d
ProTone+ Methionine+ Oleic acid	7.25d	6.99c	4.45c	4.48cd
L.S.D.0.05	0.537	0.572	0.443	0.834

Values, within a column with same letter(s) were not significantly different by L.S.D ($p < 0.05$).

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تأثير المعاملة قبل الجمع بالبروتون، الميثيونين وحامض الأوليك كمواد بديلة للايثيفون لتحسين لون وجودة ثمار العنب صنف فليم سيدلس

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قسم البساتين (فاكهة)، كلية الزراعة- جامعة دمنهور - مصر

الملخص:

فى هذة التجربة، تم رش شجيرات العنب صنف فليم سيدلس عمر عشر سنوات بمنطقة النوبارية، محافظة البحيرة-مصر قبل الجمع خلال موسمى ٢٠١٦، ٢٠١٧ بالمعاملات الأتية: كنترول (ماء مع مادة ناشرة)،٤٠٠ ملجم/لتر ايثيفون،١٠٠ ملجم/ لتر بروتون (المادة الفعالة حامض الأبسيسيك ١٠%)، ١٠٠ ملجم/لتر ميثيونين، ٤٠٠ ملجم/لتر حامض أوليك، بروتون مع ميثيونين، بروتون مع حامض الأوليك و بروتون فى وجود كلا من الميثيونين وحامض الأوليك وذلك لدراسة تاثير هذة المواد على تلوين وجودة ثمار العنب وامكانية استخدام هذة المواد كبديلة لمعاملة الايثيفون. وتوضح النتائج ان معاملة ثمار العنب بالايثيفون أدت الى زيادة كلا من محتوى الحبات من صبغة الأنثوسيانين، المواد الصلبة الذائبة الكلية، نسبة الفرط والفقد فى الوزن. وعلى الجانب الأخر، أدت الى نقص الحموضة ومحتوى الحبات من الكلوروفيل. أدت المعاملة بالبروتون الى زيادة محتوى الحبات من صبغة الأنثوسيانين ونقص نسبة الفرط والفقد فى الوزن. أدت المعاملة بالميثيونين الى زيادة محتوى الحبات من صبغة الأنثوسيانين والمواد الصلبة الذائبة الكلية بينما أدت الى نقص الكلوروفيل. أدت المعاملة بحامض الأوليك الى زيادة وزن الحبات، المواد الصلبة الذائبة الكلية وتقليل نسبة الفرط والفقد فى الوزن. أدت التركيبة المحتوية على كلا من البروتون والميثيونين وحامض الأوليك الى زيادة محتوى صبغة الأنثوسيانين فى الحبات، وتقليل نسبة الفرط بالاضافة الى تقليل نسبة الفقد فى الوزن وذلك مقارنة بكل من معاملة الايثيفون والكنترول.

وتوصى هذة الدراسة باستخدام التركيبة المحتوية على البروتون مع كل من الميثيونين وحامض الأوليك عند بداية تلوين الحبات (١٠- ١٥ % تلون للحبات) كبديل لمعاملة الايثيفون لتحسين لون وجودة حبات العنب وللتغلب على بعض التاثيرات السلبية للايثيفون مثل زيادة نسبة الفرط والفقد فى الوزن، والذى ينعكس على مظهر الحبات.