

Influence of Pectic Oligosaccharides and 5-Aminolevulinic Acid Along with Absciscic Acid on Growth, Yield and Quality Attributes of Crimson Seedless Grape Cultivar

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ABSTRACT

Crimson Seedless is a highly valued table grape cultivar, but under Egyptian climatic conditions, its berries often fail to reach full red coloration. The current investigation was carried out during the 2024 and 2025 seasons, in a commercial vineyard at Sadat City to improve the vegetative growth aspects, yield, and fruit quality attributes of Crimson Seedless. Eight treatments were performed as follows: absciscic acid at 200ppm, pectic oligosaccharides at 1500ppm, and 5-aminolevulinic acid at 200ppm were applied either individually or in combination among them; in addition control (untreated vines). Both pectic oligosaccharides and 5-aminolevulinic acid were foliar sprayed on three dates: the 1st date (after bud burst stage), the 2nd date (at fruit set stage), and the 3rd date (at véraison stage), while absciscic acid was sprayed twice on the clusters; the first spray was at the onset of the véraison stage, and the second spray was done two weeks later. All treatments significantly enhanced yield and fruit quality compared with the control. Among them, the triple combination of absciscic acid + pectic oligosaccharides + 5-aminolevulinic acid proved to be the most effective in enhancing yield, cluster weight and berry weight, higher TSS%, and lower acidity, as well as yielding the highest anthocyanin concentration, followed by the dual combination of pectic oligosaccharides + 5-aminolevulinic acid, while pectic oligosaccharides and 5-aminolevulinic acid alone produced intermediate values. Absciscic acid alone was lower, and the control recorded the minimum values.

Key words: Pectic oligosaccharides, 5-Aminolevulinic acid, Absciscic acid, grape coloration, Crimson Seedless.

INTRODUCTION

Crimson Seedless is one of the most widely cultivated table grape cultivars worldwide. However, in warm regions such as Egypt, berries frequently fail to develop the desirable red coloration (Spayd *et al.*, 2002). High temperatures during ripening inhibit anthocyanin biosynthesis and accelerate pigment degradation, leading to poor skin color despite acceptable berry size and yield (Peppi and Fidelibus, 2008). Since color is a key factor determining both marketability and consumer acceptance, viticultural

practices that enhance berry pigmentation under hot climatic conditions are of considerable importance.

Among the different strategies tested that enhance berry pigmentation, the application of Absciscic Acid (ABA) has proven to be particularly effective (Koyama *et al.*, 2010). ABA is a plant hormone that naturally accumulates at the onset of véraison and regulates sugar uptake, ripening, and anthocyanin biosynthesis. Exogenous ABA sprays have been reported to increase berry coloration in several grape cultivars, including Crimson Seedless, through the upregulation of anthocyanin biosynthetic genes such as *CHS*, *DFR*, and *UFGT* (Olivares *et al.*, 2017; Koyama *et al.*, 2018 and Mhetre *et al.*, 2022).

In addition to ABA, the use of Pectic Oligosaccharides (POs) has recently received attention. POs are natural elicitors derived from pectin degradation that act as signaling molecules to stimulate plant defense responses and secondary metabolism (Gutiérrez-Gamboa *et al.*, 2021). Field trials demonstrated that foliar application of POs improved anthocyanin accumulation and color development in grape berries, especially under warm-climate conditions (Sandoval-Silva *et al.*, 2021). Their role in activating the phenylpropanoid pathway makes them promising natural alternatives or supplements to synthetic growth regulators.

Another innovative compound is 5-Aminolevulinic Acid (ALA), a precursor in the tetrapyrrole biosynthetic pathway (Wang *et al.*, 2025). ALA has been widely studied as a plant growth regulator with the potential to enhance stress tolerance, photosynthesis, and secondary metabolism (Zhang *et al.*, 2021). Recent studies have shown that ALA treatments significantly increase anthocyanin and flavonoid accumulation in grape callus cultures and in other fruit crops, suggesting a strong potential for improving berry pigmentation in grapes (Lai *et al.*, 2024).

Therefore, the present study was conducted to influence of ABA, POs, and ALA applied either individually or in combination among on the yield and fruit quality attributes of Crimson Seedless grapevine under Egyptian climatic conditions.

DOI: 10.21608/asejaiqsae.2025.461304

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Received, September 10, 2025, Accepted, September 30, 2025.

MATERIALS AND METHODS

The investigation was conducted during the 2024 and 2025 seasons on Crimson Seedless grapevines grown in a private commercial vineyard located at Sadat City, Egypt (latitude 30.360°N, longitude 30.50°E). Vines were 8 years old, planted at a spacing of 2.0×3.0 m, and trained by the Spanish Parron system under drip irrigation. All vines received the standard cultural practices recommended for commercial production.

Ninety-six uniform vines were chosen. Each four vines acted as a replicate, and each three replicates were treated by one of the following treatments.

- 1) Control (spray with water).
- 2) Absciscic Acid at 200ppm (ABA).
- 3) Pectic Oligosaccharides at 1500ppm (POs).
- 4) 5-aminolevulinic Acid at 200ppm (ALA).
- 5) ABA + POs.
- 6) ABA + ALA.
- 7) POs + ALA.
- 8) ABA + POs + ALA.

Absciscic Acid (ABA) was used as trademark (ProTone®) SL; ABA 10%), while Pectic Oligosaccharides was used as trademark (POs, 95%), and 5-aminolevulinic Acid was used as trademark (ALA, 97%). All conducted materials: ABA, POs, and ALA were purchased from Sigma Chemical Company.

Both pectic oligosaccharides and 5-aminolevulinic acid were foliar sprayed on three dates: the 1st date (after bud burst stage), the 2nd date (at fruit set stage), and the 3rd date (at véraison stage), while absciscic acid was sprayed twice on the clusters; the first spray was at the onset of the véraison stage, and the second spray was done two weeks later. Each spray was applied until runoff, ensuring thorough coverage of both leaves and clusters.

Measurements data:

In the 2nd week of August, the harvest was done when clusters had reached full coloration and total soluble solids (TSS) were about 16–17% (Badr and Ramming, 1994), a representative random sample of 12 clusters was collected per replicate (3 clusters per vine). The following estimates were carried out:

1. Yield and cluster physical attributes:

- a. Yield per vine (kg).
- b. Average cluster weight (g).
- c. Average berry weight (g).
- d. Average berry size (ml).

2. Berry chemical attributes:

a. Total soluble solids (TSS%) were measured as a percentage with a digital refractometer (model ATC-1, Atago, Co., Tokyo, Japan).

b. Total acidity was expressed as grams of tartaric acid per 100 mL of juice (AOAC, 2023).

c. TSS/acid ratio was calculated.

d. Total anthocyanins were expressed as (mg/100g FW) according to Yildiz and Dikmen (1990).

3. Vegetative growth traits:

On each vine, ten vegetative shoots were tagged and evaluated at growth cessation.

a. Shoot length (cm).

b. Leaf area (cm²): Leaves from the 6th–7th nodes from the shoot apex were sampled at harvest, and leaf area was measured using a CI-203 portable leaf area meter (CID Bio-Science, USA).

c. Leaf chlorophyll content was determined in mature leaves (6th–7th from the shoot apex) using a Minolta SPAD-502 chlorophyll meter (Model No.: SPAD-502 plus Display range: -9.9 to +199.9 SPAD Size (mm): 78 x 49 x 164) (Wood *et al.*, 1993).

Experimental design and statistical analysis:

The randomized complete block design (RCBD) was considered in the model to account for block effects. Statistical procedures followed the guidelines described by Montgomery (2017). Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in SAS software (Version 9.4, SAS Institute, Cary, NC, USA). Mean comparisons were performed using new LSD at the 5% significance level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1. Yield and cluster physical attributes:

Data in Table 1 revealed that there are significant differences between all treatments on yield per vine and cluster physical attributes, including yield per vine and cluster weight, as well as berry weight and size during both seasons.

Yield per vine (kg):

The highest yield was obtained from ABA + POs + ALA, followed by POs + ALA, while POs and ALA alone produced intermediate values. ABA alone was lower, and the control recorded the minimum yield. The superiority of ABA + POs + ALA may be attributed to the synergistic effect of ABA in promoting assimilate translocation and POs in enhancing nutrient uptake and stress tolerance, resulting in greater berry set and cluster development. Similar significant improvements in vine productivity with combined hormonal and elicitor treatments have been reported in grapevines by

Sandoval-Silva *et al.* (2021) and Gutiérrez-Gamboa *et al.* (2021).

Average cluster weight (g):

Similar results were obtained for the cluster weight of the vines treated with ABA + POs + ALA, which produced the heaviest clusters, followed by POs + ALA. POs and ALA alone showed moderate cluster weights, whereas ABA alone and control had significantly lighter clusters. Increased cluster weight under combined treatments is linked to improved source–sink relationships and reduced stress-induced fruit abscission, consistent with findings by Reynolds & Vanden Heuvel (2009) and Keller (2020).

Average berry weight (g):

There are significant differences between the treatments in berry weight ($P \leq 0.05$). ABA + POs + ALA gave the heaviest berries, significantly exceeding all other treatments, followed by POs + ALA, while single treatments with POs or ALA showed moderate berry mass. ABA alone was lower, and control berries were the lightest. The larger berry weight under combined treatments may result from enhanced photosynthesis and assimilate allocation, as also observed by Li *et al.* (2021).

Average berry size (ml):

There are significant differences between the treatments in berry size ($P \leq 0.05$). ABA + POs + ALA again produced the largest berries, with POs + ALA ranking second. POs and ALA alone were intermediate, while ABA alone and control had the smallest berry sizes. The significant increase in berry volume under

combined treatments is attributed to improved osmotic regulation and carbohydrate transport, as supported by Chen *et al.* (2024).

2. Berry chemical attributes:

As shown in Table 2 showed that all berry chemical attributes, i.e. TSS, total acidity, and total anthocyanin of Crimson Seedless grapes, were significantly affected by all conducted treatments in both seasons.

Total soluble solids (TSS%):

Our results indicate that the application of ABA + POs + ALA produced the highest total soluble solids (TSS) in berry juice, followed by POs + ALA, whereas the control vines consistently recorded the lowest values in both seasons.

Total acidity (%) and TSS/acid ratio:

At the same time, titratable acidity was significantly reduced in all treated vines compared with the untreated control, leading to a marked increase in the TSS/acid ratio. This improvement in sugar–acid balance suggests a more advanced ripening stage and enhanced flavor quality, particularly in the combined treatments. Similar findings were reported by Gutiérrez-Gamboa *et al.* (2021), who observed that exogenous ABA in combination with elicitors accelerated sugar accumulation and improved berry palatability. The superiority of ABA + POs over ABA alone highlights the synergistic action between ABA-mediated sugar loading and POs-enhanced metabolic activity, which supports a faster transition of berries toward maturity (Sandoval-Silva *et al.*, 2021).

Table 1. Effect of Pectic Oligosaccharides and 5-Aminolevulinic Acid along with Absciscic Acid on yield and cluster physical attributes of Crimson Seedless grapevines during 2024 and 2025 seasons

Treatments	Yield/vine (Kg)		Average cluster weight (g)		Average berry weight (g)		Average berry size (ml)	
	2024	2025	2024	2025	2024	2025	2024	2025
Control (spray with water)	13.0	13.2	435.4	441.6	3.95	4.01	3.79	3.80
200 ppm Absciscic acid (ABA)	14.2	14.6	472.2	488.5	4.29	4.43	4.06	4.21
1500 ppm Pectic Oligosaccharides (POs)	15.0	15.3	500.1	510.6	4.54	4.63	4.31	4.41
200 ppm 5-aminolevulinic acid (ALA)	14.9	14.7	498.7	492.3	4.52	4.47	4.30	4.24
ABA + POs	17.0	17.0	565.3	567.7	5.13	5.15	4.89	4.93
ABA + ALA	16.3	16.5	542.8	552.1	4.92	5.01	4.68	4.78
POs + ALA	17.3	17.4	578.5	581.2	5.25	5.28	5.02	5.07
ABA + POs + ALA	17.8	18.0	592.4	602.5	5.38	5.47	5.16	5.27
New LSD at 0.05	0.4	0.2	9.0	11.2	0.17	0.21	0.34	0.39

Total anthocyanins (mg/100g FW):

Anthocyanin concentration was the most responsive trait to the applied treatments. Vines treated with ABA + POs + ALA recorded the highest anthocyanin accumulation, significantly surpassing all other treatments in both seasons. The second most effective treatment was POs + ALA, followed by ABA + POs. On the other hand, control vines exhibited the lowest pigmentation levels. These results underline the central role of ABA in activating the anthocyanin biosynthetic pathway, while the addition of POs or ALA further enhances the production of flavonoid precursors and stabilizes pigment development. Gutiérrez-Gamboa *et al.* (2021) emphasized the importance of ABA–POs synergy under warm climate conditions, while Lai *et al.* (2024) reported that ALA improved anthocyanin biosynthesis at the cellular level through upregulation of flavonoid pathway genes. Consequently, the superiority of ABA + POs + ALA in this study can be explained by its ability to maximize pigment accumulation without compromising yield or berry size, thus making it a practical and effective field strategy for improving grape berry coloration.

3. Vegetative growth traits:

Data in Table 3 revealed that there are significant differences between all treatments on vegetative growth traits, including shoot length, leaf area, and leaf total chlorophyll content during both seasons.

Shoot length (cm):

Shoot elongation was obtained from ABA + POs + ALA, followed by POs + ALA, while POs and ALA alone produced intermediate values. ABA alone was lower, and the control recorded the minimum shoot

length. This confirms that the moderate growth maintained under ALA treatment highlights its role in sustaining canopy development through hormonal balance and chlorophyll enhancement (Wang and Grimm, 2021), which may help optimize assimilate allocation between shoots and clusters.

Leaf area (cm²):

Significant variation in leaf area was observed among treatments; the largest leaves developed on vines treated with ABA + POs + ALA, followed by the combined applications of POs + ALA resulted in moderate leaf sizes. As mentioned by Chen *et al.* (2020), Absciscic acid (ABA) is a stress hormone that inhibits vegetative growth if it is used in high concentrations or under stress, and reduces cell expansion in leaves. This happens because ABA reallocates the plant's resources away from vegetative growth toward other processes like seed dormancy or fruit development. This pattern suggests that ABA prioritizes growth by partitioning assimilates toward developing sinks like fruit, thereby limiting vegetative growth. Conversely, POs and ALA appear to act as biostimulants that promote photosynthetic capacity and cell expansion, thereby supporting canopy growth. These findings align with observations that ABA application suppresses leaf development while improving fruit ripening, whereas foliar sprays of POs or ALA can promote canopy vigor in grapevines, particularly under stress conditions. Consequently, POs and ALA are the more effective agents when the objective is to sustain robust vegetative growth and canopy function (Wang *et al.*, 2018).

Table 2. Effect of Pectic Oligosaccharides and 5-Aminolevulinic Acid along with Absciscic Acid on berry chemical attributes of Crimson Seedless grapevines during 2024 and 2025 seasons

Treatments	TSS (%)		Total acidity (%)		TSS/acid ratio		Total anthocyanin (mg/100g FW)	
	2024	2025	2024	2025	2024	2025	2024	2025
Control (spray with water)	16.07	16.24	0.72	0.71	22.32	22.87	29.1	30.4
200ppm Absciscic Acid (ABA)	17.24	17.27	0.68	0.67	25.35	25.78	36.4	38.1
1500ppm Pectic Oligosaccharides (POs)	18.31	17.82	0.63	0.65	29.06	27.42	40.1	42.2
200ppm 5-aminolevulinic Acid (ALA)	17.52	17.56	0.67	0.66	26.15	26.61	38.2	40.1
ABA + POs	19.06	19.43	0.60	0.58	31.77	33.50	43.4	44.2
ABA + ALA	18.43	18.51	0.60	0.62	30.72	29.85	42.2	42.5
POs + ALA	19.42	19.54	0.56	0.58	34.68	33.69	45.7	46.1
ABA + POs + ALA	20.56	20.71	0.51	0.49	40.31	42.27	49.5	48.7
New LSD at 0.05	0.23	0.27	0.02	0.03	3.87	4.21	4.9	5.3

Table 3. Effect of Pectic Oligosaccharides and 5-Aminolevulinic Acid along with Absciscic Acid on vegetative growth traits of Crimson Seedless grapevines during 2024 and 2025 seasons

Treatments	Shoot length (cm)		Leaf area (cm ²)		Total chlorophyll (SPAD)	
	2024	2025	2024	2025	2024	2025
Control (spray with water)	97.5	99.1	157.2	148.4	27.2	26.7
200ppm Absciscic Acid (ABA)	100.3	101.5	164.8	170.2	30.8	29.4
1500ppm Pectic Oligosaccharides (POs)	104.1	106.3	185.6	175.7	32.8	32.3
200ppm 5-aminolevulinic Acid (ALA)	102.4	104.0	167.1	172.5	31.5	30.8
ABA + POs	112.8	115.2	188.2	180.2	35.8	35.1
ABA + ALA	106.7	109.0	186.4	178.7	34.0	33.8
POs + ALA	120.5	118.9	192.6	183.5	37.5	37.2
ABA + POs + ALA	138.4	129.6	202.5	206.1	39.2	40.1
New LSD at 0.05	2.1	2.3	4.5	4.8	0.4	0.3

Leaf total chlorophyll content (SPAD):

In our study, leaf SPAD values revealed a pattern distinct from that seen in chlorophyll parameters, underscoring the specific influence of ABA, POs and 5-ALA. Control vines displayed the lowest chlorophyll levels, while treatments with ABA and its combinations significantly increased SPAD readings. Notably, the triple combination of ABA + POs + ALA was particularly effective in increasing leaf chlorophyll content. This finding is similar to that of Kang *et al.* (2023), who declared that this enhanced efficacy likely stems from the synergistic action of 5-ALA, an essential precursor in tetra-pyrrole biosynthesis, including chlorophyll synthesis and the role of ABA in modulating stress responses. For example, under low-temperature stress, ALA application increased leaf chlorophyll by approximately 15% and enhanced photochemical efficiency, with ABA similarly raising the expression of chlorophyll-synthesizing enzymes and improving PSII function. Moreover, under cold conditions, ALA improved chlorophyll levels, photosynthesis, and leaf area, further supporting its role in mitigating stress-induced chlorophyll loss (Wu *et al.*, 2018). While POs are known to have roles in plant growth and defense, current literature does not yet substantiate a direct impact on chlorophyll synthesis comparable to that of 5-ALA.

CONCLUSION

This study demonstrated that the application of Absciscic Acid (ABA), Pectic Oligosaccharides (POs), and 5-Aminolevulinic Acid (ALA) significantly improved berry coloration and quality in Crimson Seedless grapes under Egyptian conditions. Among the treatments, the triple combination of ABA + POs + ALA was the most effective in yield, cluster weight, and berry weight, with higher TSS% and lower acidity, as well as yielding the highest anthocyanin concentration,

followed by the dual combination of pectic oligosaccharides + 5-aminolevulinic acid, while pectic oligosaccharides and 5-aminolevulinic acid alone produced intermediate values. Absciscic acid alone was lower, and the control recorded the minimum values. Overall, the triple combination of ABA + POs + ALA appears to be a promising strategy to improve both grape color and fruit quality under warm-climate conditions.

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الملخص العربي

تأثير البكتين أوليجوسكرايد وحمض ٥-أمينوليفولينيك مع حمض الأبسيسيك على النمو والمحصول وصفات الجودة لصنف العنب الكريمتون سيدلس

عبد الجواد صبحى شعبان أحمد، محمد السيد عبدالرحمن السيد، علا محمد فكري، حسين سيد أحمد

أسبوعين من الرش الأولى. وأظهرت جميع المعاملات تحسناً معنوياً في المحصول الكلى وصفات الجودة مقارنة بالكنترول، ومن بين هذه المعاملات، المعاملة المشتركة المكونة من حمض الأبسيسيك، البكتين أوليجوسكرايد وحمض ٥-أمينوليفولينيك حيث تميزت بكونها الأكثر فاعلية في زيادة المحصول وتحسين وزن العنقود ووزن الحبات، وكذلك الصفات الكيميائية للحبات مثل زيادة نسبة المواد الصلبة الذائبة الكلية وانخفاض الحموضة وكذلك ارتفاع محتوى الحبات من الأنثوسيانين، تلتها المعاملة المشتركة المكونة من البكتين أوليجوسكرايد وحمض ٥-أمينوليفولينيك، بينما أعطت معاملات البكتين أوليجوسكرايد وحمض ٥-أمينوليفولينيك بصورة منفردة قيماً متوسطة، أما معاملة حمض الأبسيسيك خاصة عند استخدامه منفرداً فكانت أقل، في حين سجلت معاملة الكنترول أدنى القيم.

الكلمات المفتاحية: البكتين أوليجوسكرايد، وحمض ٥-أمينوليفولينيك، حمض الأبسيسيك، لون ثمار العنب، العنب الكريمتون سيدلس.

يُعدُّ صنف العنب الكريمتون سيدلس من أصناف المائدة عالية القيمة، إلا أن ثماره غالباً لا تتمكن من الوصول إلى التلوين الكامل تحت الظروف المناخية المصرية. ففي هذه الدراسة أجري البحث خلال موسمي النمو ٢٠٢٤ و ٢٠٢٥ في مزرعة خاصة بمدينة السادات لتحسين النمو والمحصول وصفات الجودة لصنف العنب الكريمتون سيدلس. وقد اشتملت الدراسة علي ثمان معاملات علي النحو التالي: الرش لكل من حمض الأبسيسيك بتركيز ٢٠٠ جزء في المليون، والبكتين أوليجوسكرايد بتركيز ١٥٠٠ جزء في المليون، وحمض ٥-أمينوليفولينيك بتركيز ٢٠٠ جزء في المليون، حيث تم إجرائها إما بصورة منفردة أو بالاشتراك مع بعضهم البعض، بالإضافة إلى معاملة الكنترول. وقد تم إجراء الرش الورقي لكل من البكتين أوليجوسكرايد وحمض ٥-أمينوليفولينيك في ثلاث مواعيد: بعد إكمال تفتح البراعم، عند مرحلة العقد، عند بداية مرحلة التلوين، بينما تم رش حمض الأبسيسيك علي العناقيد في مواعيد: الرش الأولى عند بداية مرحلة بداية التلوين، بينما كانت الرش الثانية بعد