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MITIGATION OF EXCESSIVE PROTON CONCENTRATIONS OF "CRIMSON" GRAPES BY THIDIAZURON AND BENZYLADENINE

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ABSTRACT: The present study was conducted during the two consecutive seasons 2018 and 2019 on "Crimson Seedless" table grapes to mitigate the excessive ProTon concentration by some cytokinin use. The following treatments were used: control, thidiazuron at 50ppm (TDZ), abscisic acid at 250ppm (ABA) preceded by TDZ at 50ppm, ABA at 500ppm preceded by TDZ at 50ppm, benzyl adenine at 50ppm (6-BA), ABA at 250ppm preceded by 6-BA at 50ppm, ABA at 500ppm preceded by 6-BA at 50ppm, ABA at 250ppm and ABA at 500ppm.Treatment of TDZ and 6-BA were applied at berry set while ABA treatment was applied at 10-15% berry coloration. The results showed that pre-harvest application of TDZ or 6-BA increased cluster weight, 100 berry weight, pedicel weight, berry acidity, berry and leaves chlorophylls and decreased berry coloration, TSS, total sugars. The application of ABA at 10-15% berry coloration enhanced berry anthocyanin content, TSS, Total sugars and lowered acidity and chlorophyll. The present study recommended using either TDZ or 6-BA at 50ppm followed by ProTon at 500ppm to mitigate the excessive ProTon concentration on "Crimson Seedless" grapes.

Key words: Crimson Seedless, Grapes, Mitigation, Pre-harvest, ABA, TDZ.

INTRODUCTION

Grape culture in Egypt is very important and highly profitable when the suitable cultural practices are followed, Grapevine culture has been expanding especially in the arid regions in Egypt. According to FAO data, the grape growing area in Egypt has been increasing from 59765 hectares in 2000 to 73351hectar in 2019 which produced about 179888 tons in 2000 to reach 221709 tons in 2019 **(FAO data)**. Most producers and growers are focusing on certain colored cultivars at ripening such as "Crimson Seedless'' and "Flame Seedless" However, grape growers complain about some specific problems that are common especially under arid conditions such as need to improve berry set and to reduce berry shatter under field conditions. They also face the problem of small berry size and weight and their need to enhance anthocyanin production and color uniformity. The relatively high temperature during the day and the night led to the inhibition of the enzyme activity called phenylalanine ammonium lyase (PAL). Most grape growers lack the knowledge to overcome the problems they face under field conditions. Most grape producers count on the use of gibberellic acid starting from the early stages of flowering and fruit set. They even exaggerate in the number of applications of GA_3. Some producers are not aware of the adverse effects of excess GA_3 content in the vegetative growth on inhibiting the floral induction and differentiation that occur while intended to improve the quality of the current season with the abuse of GA_3. They negatively affect the next year's crop. In this study, we tried a new approach by utilizing some cytokinins such as thidiazuron and benzyl adenine at relatively low concentrations either alone or followed by ProTon at low and high concentrations to improve the physical and chemical properties of ''Crimson seedless'' berries. It is well known that the use of cytokinins increases the sink strength **(Body *et al.* 2013 and Meldau *et al.* 2012)** that is actually needed for a seedless fruit such as "Crimson". Meanwhile, the diffused and stored thidiazuron or 6-BA will not adversely affect the floral induction for the next season crop. The increase of sink strength is important for increasing carbohydrate partitioning from mature leaves to various sinks in the grapevine such, as new leaves, young shoots, berries, buds and

the root system. In addition, certain cytokinin such as thidiazuron inhibits the activity of some enzymes that break down the natural cytokinin's in plant and they also inhibit the respiration rate of fruits **(Giehl *et al.*, 2010)**. Thus, the objectives of this study were to mitigate the excessive ProTon concentration by low concentration of a cytokinin, to improve "Crimson Seedless'' berry quality without an adverse effect on the canopy and to provide grape growers and producers with a production system that could be adopted under field conditions.

MATERIALS AND METHODS

Nine-year-old trees of table grapes cv. "Crimson Seedless" grown in a private orchard in the El Nubaria region, Behira governorate, Egypt. Plants were spaced at 2 x 3m. grown on their own roots using an overhead trellis system and watered with drip irrigation. Vines were cane pruning. Standard vineyard operations such as plant fertilization, pest control, and other crop management were carried out according to local practices. There were nine treatments replicated four times and one replicate corresponded to one vine, while the control was treated only by water spray, thidiazuron (N-Phenyl-N′-l,2,3-thidiazol-5-ylurea) at 50 ppm (TDZ), ProTon (abscisic acid) at 250 ppm (ABA) preceded by TDZ at 50 ppm, ABA at 500 ppm preceded by TDZ at 50 ppm, benzyl adenine at 50 ppm (6-BA), ABA at 250 ppm preceded by 6-BA at 50 ppm, ABA at 500 ppm preceded by 6-BA at 50 ppm, ABA at 250 ppm and ABA at 500 ppm. Treatments of TDZ and 6-BA were applied at berry set (3-4mm berry diameter) and ABA treatments were applied at 10 -15% berry coloration (Veraison). All treatments were applied with a hand-held sprayer until runoff and 0.5 ml/ L Tween 80 were added to all treatment solutions. The experiment layout was designed as a factorial arranged in randomized complete block design (RCBD). All data were assessed by analysis of variance (ANOVA) using SAS 9.3 software **(SAS, 2000)**. Means were compared using the least significant difference (LSD) test at $p< 0.05$. Two bunches from each replication were picked at harvest (18, 25 Jul. and 19, 26 Aug. during the 2018 and 2019 seasons, respectively) and berries were removed from each bunch to determine the following properties: weight of 100 berries (g), the volume of 100 berries (cm^3), cluster weight (g). and pedicel weight(gm) and length(cm). On the other hand, in berries juice, total soluble solids % (TSS) were determined by a hand refractometer, total sugars determined according to **(Egan *et al.*, 1987)**, and titratable acidity % (as tartaric acid) was determined according to **A. O. A. C (1985)**, the ratio between TSS/acidity was calculated. Chlorophyll a,b in the leaf , berry skin chlorophyll a, b and carotene (mg/100g) were determined according to **(Lichtenthaler and Wellburn, 1985)**, and Berry skin anthocyanin (mg/100g) was determined according to **Fuleki and Francis (1968)**.Vitamin C was determined according to **Egan *et al.* , (1987)**.

The electrical conductivity (EC) of the leaf and the bunch stems were determined by a conductivity meter.

RESULTS

Physical characteristics

The effect of various used treatments before harvest on the cluster weight of " Crimson seedless " grapes was reported in Table 1. The data revealed that thidiazuron treatment at 50 ppm resulted in the greatest cluster weight in both seasons as compared with the control and other treatments including the individual treatment of benzyl adenine at 50 ppm. Meanwhile, the two ProTon concentrations, each of them resulted in reducing the cluster weight more than the control. However, such weight with ProTon (250ppm) was significantly greater than that obtained with ProTon at 500ppm in both seasons. Moreover, when ProTon at either 250ppm or at 500 ppm was preceded by TDZ, a significant increase was obtained in cluster weight as compared with the individual use of ProTon. On the other hand, the application of 6-BA alone resulted in greater cluster weight than that obtained with ProTon alone at 250 ppm or 500 ppm. However, when 6-BA treatment preceded ProTon at 250 ppm or 500 ppm it caused a significant increase in cluster weight relative to the use of ProTon alone. In addition, the two ProTon concentrations resulted in lower cluster weight than the control in both seasons, while such negative impact was mitigated by the advanced treatment with TDZ or 6-BA.

The response to berry weight to various applications was reported in Table 1 and expressed as the weight of 100berries. The data showed that the TDZ treatment resulted in the highest berry weight when compared with the control and many other treatments. Moreover, 6-BA individually caused a significant increase in berry weight relative to the control. Meanwhile, ProTon alone either at 250ppm or 500ppm resulted in a slight increase in berry weight in the first season only as compared with control. However, when ProTon at 250 ppm was preceded by TDZ at 50 ppm, a significant and consistent increase occurred in berry weight. However, ProTon at 500 ppm was preceded by 6-BA, there was an increase in berry weight only in the first season. Similarly, when ProTon at 500ppm was preceded by TDZ at 50 ppm a significant increase was obtained in berry weight as compared with the use of ProTon at 500 ppm alone. Thus, it could be concluded that TDZ was able to mitigate the adverse effect of ProTon on berry weight in a more consistent manner than that obtained with 6-BA when preceded by ProTon treatment.

Changes in berry size expressed as the 100-berry size of the "Crimson seedless" grape as influenced by

various used treatments were also reported in Table1. The data indicated that many similar trends to that found in berry weight were also found in berry size. For instance, the greatest magnitude of increase in berry size was also obtained by the application of TDZ in both seasons. Furthermore, the application of ProTon that followed TDZ had a greater berry size than the application of ProTon alone whether at 250ppm or 500 ppm. Meanwhile, similar influence when 6-BA followed the application of ProTon at 250ppm or 500 ppm by alleviating the adverse effect of ProTon on fruit size when applied alone. However, the magnitude of the influence of TDZ was greater than that obtained with6-BA the influence on berry size. Moreover, the sole application of BA to the "Crimson seedless" cluster resulted in greater berry size than that obtained with the control in both seasons. It could be concluded that both TDZ and 6-BA were effective in enhancing berry size and on the alleviation of the adverse effect of ProTon on berry size but with a greater influence by TDZ when compared with 6-BA.

Changes in pedicel weight in response to pre-harvest treatments of "Crimson seedless" grapevines were reported in Table 1. The data indicated to a significant increase by TDZ as compared with the control. Meanwhile, 6-BA treatment caused a significant increase in pedicel weight relative to the control but less than that obtained with TDZ in the two seasons. Moreover, ProTon alone at 500 ppm caused a significant reduction of pedicel weight when compared with the control. However, ProTon at 250 ppm resulted in lower pedicel weight than the control in the first season but was similar to the control in the second season. when TDZ was followed by PoTon at 250 ppm or 500ppm it caused a reduction of pedicel weight compared to the TDZ alone. However, when 6-BA treatment was followed by ProTon at 500ppm the pedicel weight was reduced relative to BA alone while when 6-BA preceded ProTone at 250 ppm, the pedicel weight was reduced only in the first season.

The effect of pre-harvest treatments of "Crimson seedless" grapevines with used treatments on pedicel length was reported in Table 1. The data revealed that TDZ caused a significant increase in such length in both seasons as compared with the control. The greatest increase in pedicel length was found in ProTon at 250 ppm when was preceded by 6-BA while ProTon at 500ppm when preceded by 6-BA did not enhance pedicel length significantly compared with 6-BA alone. On the other hand, when TDZ preceded the application of ProTon at 250 ppm, no significant change occurred relative to pedicel length with TDZ alone. Moreover, there was no significant difference especially in the first season between TDZ alone or when it was followed by ProTon at 500 ppm. Furthermore, ProTon alone either at 250 ppm or 500 ppm caused a significant increase in pedicel length as compared with the control in a consistent in both seasons.

Table 1. Effect of thidiazuron (TDZ), Benzyl adenine (6-BA) and ProTon on physical characteristics of "Crimson seedless" grapes during 2018 and 2019 seasons

Treatments	Cluster weight (g)		100berry weight (g)		100berry size (cm^3)		Pedicle weight (g)		Pedicle length (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	433.7 g	396 e	313.24 h	291.0 c	302.00 h	302.0 f	0.478 f	0.503 e	0.583 d	0.608 e
TDZ (50ppm)	657.12 a	632.8a	403.07 a	370.0 a	388.87 a	405.0 a	0.830 a	0.838 a	0.706b c	0.657 cd
ProTon (250ppm) preceded by TDZ	637.6 b	621.6 b	394.47 c	388.0 a	377.75 c	401b	0.746 c	0.737 b	0.701b c	0.648d e
ProTon (500ppm) preceded by TDZ	628.8 c	622.3 b	398.15 b	392.0 a	381.25 b	404.0 ab	0.726 c	0.736 b	0.685 c	0.693 bc
6-BA(50ppm)	524.5 d	491.8 c	342.52 e	331.7 b	333.5 e	337.0 d	0.652 d	0,701b c	0.727a b	0.731 ab
ProTon (250ppm) preceded by 6-BA	516.0 e	479.0 d	351.81 d	321.0 bc	341.0 d	341.0 c	0.608 e	0.685 c	0.747 a	0.735 a
ProTon (500ppm) preceded by 6-BA	493.0 f	481.0 d	342.48 e	325.0 bc	332.12 e	343.0 c	0.591 e	0.632 d	0.757 a	0.732 ab
ProTon (250ppm)	396.0 i	396.0 e	316.47 g	297.0 bc	306.87 g	311.0 e	0.393 g	0.473 e	0.756 a	0.727 ab
ProTon (500ppm)	412.8 h	388.0 f	320.59 f	299.0 bc	311.87 f	312.0 e	0.357 h	0.396 f	0.753 a	0.723 abc
LSD at 0.05	0.219	0.198	0.018	0.020	0.246	0.287	0.145	0.174	0.213	0.235

*Values within a column of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 levels.

CHEMICAL CHARACTERISTICS

The effect of pre-harvest treatments used in this investigation on total soluble solids (TSS) in the berry juice was reported in Table 2. The data indicated to a significant increase in the TSS of ''Crimson seedless'' grapes juice by ProTon at 500 ppm and by ProTon at 250 ppm relative to the control and many other treatments. Moreover, TDZ alone resulted in a reduction of TSS relative to the control in both seasons. In a similar trend, 6-BA alone resulted in the reduction of TSS. However, in both cases, the magnitude of the actual reduction was slight, furthermore, when TDZ was followed by the application of ProTon at either 250 ppm or 500 ppm there was a significant increase in TSS relative to the application of TDZ alone. In addition, when BA preceded ProTon at 250 ppm or at 500 ppm, there was a significant increase in TSS relative to the application of 6-BA alone. Thus, the application of TDZ or 6-BA followed by the application of ProTon was able to regain the TSS values and magnitude of the adverse effect of both cytokinins in TSS values at harvest.

Regarding the changes in juice acidity at harvest of "Crimson seedless" grapes berries, the data in Table 2 indicated to the highest increase occurred in TDZ. Treated berries in both seasons However, when TDZ application preceded ProTon at 250ppm or 500 ppm, juice acidity was significantly decreased. Meanwhile, 6-BA treated berries had a significant increase in juice acidity relative to the control. especially in the second season. However, a significant reduction of juice acidity occurred when 6-BA was followed by either ProTon at 250 ppm or 500 ppm in a consistent manner thus, the undeserved acidity tasted by either TDZ or 6-BA was overcome by the application of ProTon either at 250 ppm or 500 ppm in both seasons. Meanwhile, the least acidity value was obtained with ProTon alone at 500 ppm.

The influence of used treatments before harvest on the TSS to acidity ratio of "Crimson seedless'' grape berries was shown in Table 2. The data showed that the greatest ratio was obtained with the application of ProTon at 500 ppm followed by ProTon at 250 ppm in both seasons. However, when each ProTon treatment was preceded by 6-BA at 50 ppm, there was a significant reduction in the TSS to acidity ratio. Moreover, when the TDZ treatment preceded ProTon, The TSS to acidity was increased significantly as compared with TDZ alone. The least ratio of TSS to acidity was obtained with the sole application of TDZ at 50 ppm in both seasons. Thus, the individual application of the two cytokinins varied in their influence on the TSS to acidity ratio since 6-BA alone resulted in a greater ratio than TDZ at harvest. However, the application of each one following ProTon led to a significant increase in the value of the TSS to acidity ratio.

The effect of pre-harvest applications on the percentage of total sugars at harvest was reported in Table2. The data indicated that the greatest total sugars were obtained with the application of ProTone alone at 500ppm. The most effective treatment was ProTon at 250 ppm as compared with the control and all other used treatments. Meanwhile, with the individual treatment of TDZ, total sugars were significantly reduced and were lower than that of the control. That was also the case with the application of 6-BA as compared with the control Meanwhile, the low concentrations of used cytokinin were equally effective in total sugars percentage in that properly especially the first season with a slight difference in the second season. However, with TDZ preceded ProTon at 250 ppm or at 500ppm, there was a significant increase in total sugars. similarly, when 6-BA preceded the ProTon treatments at 250 ppm or500 ppm, a significant increase occurred in the total sugar percentage.

The effect of pre-harvest applications of ProTon on "Crimson seedless'' berries anthocyanin content was reported in Table 2. It was evident that ProTon at 500 ppm resulted in the greatest increase in the anthocyanin content as compared with the control and the rest of the treatments. However, ProTon at 250 ppm was able to enhance anthocyanin formation in "Crimson seedless'' berries in a significant manner in both seasons. Moreover, in the individual treatments of the two cytokinins either TDZ or 6-BA, each of them was able to give an equal amount of anthocyanin to each other and similar anthocyanin content to that of the control. The amount of anthocyanin formed with TDZ, or BA ranged from 19-20gm/100gm while with ProTon at 500ppm ranged between 24-25 mg/100g when TDZ preceded ProTon at 250 ppm or 500 ppm there was a significant increase in anthocyanin content as compared with TDZ alone. Thus, the use of each cytokinin, meaning TDZ or 6-BA was able to benefit the''Crimson seedless'' vine especially some physical properties as shown before while didn't harm berry coloration. The advanced application of either TDZ or 6-BA was also helpful in raising the total sugars and the TSS to acidity ratio.

Table 2. Effect of thidiazuron (TDZ), Benzyl adenine (6-BA) and ProTon on chemical characteristics of "Crimson seedless" grapes during 2018 and 2019 seasons

Treatments	TSS %		Acidity %		TSS/acidity (ratio)		Total sugars %		Anthocyanin (mg/100gm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	14.7 e	14.47 e	1.2 b	1.25 c	12.25 h	11.52 f	15.46 g	15.18 ef	19.65 e	20.53 e
TDZ (50ppm)	14.47 f	14.03 f	1.23 a	1.39 a	11.76 i	10.08 h	15.26 h	14.53 g	19.33 e	20.36 e
ProTon (250ppm) preceded by TDZ	15.26 d	14.95 d	0.98 d	1.22 d	15.57f	12.27 e	15.76 f	15.46 ed	21.24 d	21.83 d
ProTon (500ppm) preceded by TDZ	15.57 c	15.46 c	0.90 f	1.14 e	17.3 c	13.52 d	16.43 d	16.02 c	22.33 c	22.80 c
6-BA (50ppm)	14.40 f	14.32 ef	1.07 c	1.33 b	13.45 g	10.71 g	15.28 h	15.11 f	19.61 e	20.30 e
ProTon (250ppm) preceded by 6-BA	15.60 c	15.07 d	0.94 e	1.13 e	16.59 e	13.36 d	16.16 e	15.63 d	21.23 d	21.66 d
ProTon (500ppm) preceded by 6-BA	16.07 b	15.47 c	0.94 e	1.07 f	17.09 d	14.57 c	16.82 c	15.92 c	23.97 b	24.60 b
ProTon (250ppm)	16.22 b	16.62 b	0.89 f	0.796 g	18.22 b	20.94 b	17.35 b	17.30 b	24.17 b	25.27 a
ProTon (500ppm)	16.56 a	17.31 a	0.79 g	0.743 h	20.96 a	23.39 a	17.75 a	17.76 a	24.75 a	25.63 a
LSD at 0.05	0.219	0.198	0.018	0.020	0.246	0.287	0.145	0.174	0.213	0.235

*Values within a column of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 levels.

The response of "Crimson seedless'' grape berries in terms of the changes of carotene influenced by various used treatments showed that the highest carotene content in the berry skins was obtained with ProTon at 500 ppm when applied alone, followed in magnitude by the individual application of ProTon at 250 ppm in both seasons such carotenes were reduced when ProTon at 250 ppm or 500 ppm preceded by the application of 6-BA. However, TDZ alone resulted in a slight reduction of carotenes as compared with the control. In addition, when ProTon at 500 ppm was preceded by TDZ carotene content was as equal as its content in the case of using proton alone at 500 ppm. When ProTon at 250 ppm was preceded by TDZ, its carotene content was smaller than the combination of ProTon at 500 ppm plus TDZ during both seasons. Thus, the high concentration of ProTon at 500 ppm was the most effective in controlling the berry carotene content.

The effect of various used treatments on the chlorophyll content of the berries at harvest indicated that TDZ -treated clusters had the greatest chlorophyll a content even greater than that of the control. Meanwhile, ProTon at 250 ppm or 500 ppm resulted in lower chlorophyll a than the control. However, when TDZ preceded the application of ProTon at 500 ppm, chlorophyll a was raised as compared with ProTon alone. In addition, 6-BA- treated berries had greater chlorophyll a than the control, but when was combined with ProTon at 250 ppm or 500 ppm there was a reduction in chlorophyll a content in the berry skins. Thus, the reduction of chlorophyll a obtained by the application of ProTon was counteracted by combining other TDZ or 6-BA with ProTon.

The data in Table 3 showed the response of chlorophyll b in "Crimson seedless'' grapes to various pre-harvest treatments. The data indicated that the greatest amount was obtained with TDZ individual treatment in both seasons as compared with the control and other treatments. However, when TDZ was applied just before ProTon at 250 ppm or at 500 ppm, chlorophyll b in the berries was significantly reduced at harvest time. On the other hand, chlorophyll b was also increased by the application of 6-BA but was still lower than that obtained with TDZ alone at harvest. When 6-BA preceded the application of PoTon at 250 ppm or at 500 ppm, a further reduction occurred in the content of chlorophyll b in the berry as compared to its content in 6-BA-treated berries. In addition, both ProTon treatments caused a significant reduction of chlorophyll b, As the ProTon concentration increased to 500 ppm, it was more effective in reducing chlorophyll b than when applied at 250 ppm in both seasons.

The changes in chlorophyll a in the leaf response to various treatments were reported in Table 3. The data showed leaves had the highest chlorophyll a when treated with TDZ at 50 ppm consistently in both seasons. Moreover, 6-BA- treated leaves also had a marked increase in chlorophyll a as compared with the control. However, TDZ application resulted in

significantly greater chlorophyll a in the leaf than 6-BA treated leaves. Meanwhile, when TDZ was followed by the applications of ProTon whether at 250 ppm or 500 ppm chlorophyll a was significantly reduced. In addition, when ProTon at 250 ppm or 500 ppm, each of them was preceded by 6-BA, there was a reduction in chlorophyll a of the leaves but only in the first season. Moreover, the highest reduction in chlorophyll a was found with ProTon alone at 250 ppm or 500 ppm, especially in the first season.

The effect of various preharvest applications to "Crimson seedless'' vines and their influence on chlorophyll b in the leaf was shown in Table 3. The data emphasized again the role of cytokinin in delaying leaf senescence as found with the remarkable content of chlorophyll b in the leaf with the individual application of TDZ, followed by the application of 6-BA alone compared with the control. Meanwhile, the highest magnitude of chlorophyll b reduction was obtained with the application of ProTon at 500 ppm then ProTon alone at 250 ppm especially in the first season when ProTon at 500 ppm preceded by TDZ, chlorophyll b was also reduced but to less extent as compared with applying ProTon alone. A Similar trend was obtained when ProTon at 250 ppm was preceded by TDZ. Moreover, there were similar trends when ProTon at 250 ppm or 500 ppm was preceded by 6-BA in the two seasons.

Table 3. Effect of thidiazuron (TDZ), Benzyl adenine (6-BA) and ProTon on pigments characteristics of "Crimson seedless" grapes during 2018 and 2019 seasons

Treatments	Carotene (mg/l)		Chlorophyll a (mg/l)		Chlorophyll b (mg/l)		Chlorophyll a leaf(mg/l)		Chlorophyll b leaf (mg/l)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	0.62 h	0.639 g	0.425 g	0.502 de	0.426 c	0.516 d	1.06 c	1.03 b	1.12 d	1.17 b
TDZ (50ppm)	0.63 f	0.645 f	0.518 a	0.595 a	0.459 a	0.620 a	1.15a	1.27 a	1.17a	1.23 a
ProTon (250ppm) preceded by TDZ	0.67 e	0.680 e	0.474 c	0.509 d	0.429 c	0.546 c	0.935 d	1.09 c	1.07 e	1.15 b
ProTon (500ppm) preceded by TDZ	0.71 a	0.715 b	0.460 e	0.495 e	0.412 d	0.509 e	0.929 e	1.08 b	1.06 f	1.12 b
6-BA (50ppm)	0.63 g	0.637 g	0.498 b	0.576 b	0.445 b	0.597 b	1.11b	1.11 b	1.14 b	1.17 b
ProTon (250ppm) preceded by 6-BA	0.68 d	0.686 d	0.467 d	0.526 c	0.403 e	0.520 d	0.849 g	1.09 b	1.12 c	1.15 b
ProTon (500ppm) preceded by 6-BA	0.69 c	0.702 c	0.433f	0.494 e	0.385f	0.501 f	0.880 f	1.07 b	1.03 g	1.13 b
ProTon (250ppm)	0.71 b	0.713 b	0.384 h	0.455 f	0.344 g	0.476 g	0.743 h	0.645 c	0.810 h	0.798 c
ProTon (500ppm)	0.71 a	0.721 a	0.371 i	0.438 g	0.337 h	0.462 h	0.714 h	0.613 c	0.762 i	0.774 c
LSD at 0.05	0.002	0.002	0.002	0.006	0.005	0.003	0.003	0.086	0.003	0.033

*Values within a column of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 levels.

The response of "Crimson seedless" grapes to various pre-harvest treatments was reported in Table 4. The data indicated to a significant increase in vitamin c by the application of ProTon alone at 500 ppm followed in magnitude by ProTon at 250 ppm. With both concentrations, they caused a significant increase over the control. Moreover, TDZ alone resulted in a significant reduction in vitamin c as compared with the control. The reduction of vitamin c by TDZ was more than that occurred by the 6-BA in the first season only, but they had a similar influence on vitamin c in the second season. When TDZ alone was compared with its application before ProTon, it was found that the application of ProTon at 500 ppm preceded by TDZ had slightly higher vitamin c as compared with TDZ alone only during the first season. Meanwhile, the combined treatments of ProTon at 250 ppm or 500 ppm plus 6-BA resulted in higher vitamin c than the use of 6-BA alone.

Regarding the changes in electrolyte leakage expressed by the electrical conductivity (EC) of bunch stems, it was evident from Table 4 that ProTon at 500 ppm caused the greatest EC as compared with the control and all other treatments. that leakage magnitude was followed by that caused by ProTon alone at 250 ppm, such increase in EC were alleviated by the earlier treatment of either TDZ or 6-BA. Such cytokinin when used separately resulted in a significant reduction of the bunch stem EC in a

consistent manner the EC of the control was greater than obtained with TDZ or with 6-BA alone in both seasons. Such trends agree with the concept that the use of cytokinins resulted in delaying tissue senescence which means reducing in leakage of electrolytes from tissues.

The data regarding the changes in the electrical conductivity (EC) of "Crimson seedless'' vines leaf as a result of various treatments were reported in Table 4. The data emphasized again the influence of ProTon at 500 ppm on enhancing the EC of leaf tissues in both seasons. A Similar trend of results was also obtained with ProTon at 250 ppm but with less magnitude especially in the first season. The EC of the control and the TDZ alone were similar in both seasons. While the EC of the leaf caused by 6-BA was smaller than the control only during the first season. Moreover, the application of ProTon either at 250 ppm or 500 ppm when preceded by either TDZ or by 6-BA caused a significant reduction in the leaf EC when compared with the sole application of ProTon in both seasons.

Table 4. Effect of thidiazuron (TDZ), Benzyl adenine (6-BA) and ProTon on Vitamin C and electrical conductivity of "Crimson seedless" grapes during 2018 and 2019 seasons

Treatments	Vitamin C (mg/100ml)		EC stem (%)		EC leaf (%)	
	2018	2019	2018	2019	2018	2019
Control	1.67d	1.70bc	3.33d	3.10d	3.02f	2.70c
TDZ (50ppm)	1.51g	1.58d	2.91f	2.91e	2.62f	2.52c
ProTon (250ppm) preceded by TDZ	1.51g	1.52d	3.41d	3.10d	3.28e	3.06b
ProTon (500ppm) preceded by TDZ	1.54f	1.57d	3.61c	3.17cd	3.50d	3.22b
6-BA (50ppm)	1.62e	1.53d	3.12e	2.98e	2.91g	2.61c
ProTon (250ppm) preceded by 6-BA	1.69d	1.65c	3.61c	3.13cd	3.56d	3.15b
ABA (500ppm) preceded by 6-BA	1.74c	1.73b	3.71c	3.23c	3.81c	3.08b
ProTon (250ppm)	1.79b	1.80a	4.23b	3.75b	4.48b	4.08a
ProTon (500ppm)	1.86a	1.83a	4.62a	4a	4.75a	4.28a
LSD at 0.05	0.0293	0.0387	0.1019	0.1078	0.0717	0.1324

*Values within a column of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 levels.

DISCUSSION

The utilization of some plant growth regulators to enhance berry quality and coloration especially for "Crimson seedless" berries since there has been an expansion in growing this cultivar under the arid condition in the desert in newly cultivated lands such culture suffers from many stressful conditions that adversely affect their quality and storability .The warm temperature during the day and night hours hinders The formation of anthocyanin biosynthesis since the heat inhibits the activity of phenylalanine ammonium lyase(PAL) that is needed for anthocyanin production **(Laanset and margna, 1972)**. The producers and growers of grapes have been focusing on the use of ethephon (Ethrel) which needs several sprays to increase its efficacy. That frequent applications of ethephon can also enhance berry shatter and reduce their shelf life whether at ambient temperature, or during refrigeration. ProTon (ABA) has been recently used to enhance grape ripening and to increase of ProTon concentration could have many undesired affection additions to the high cost since this compound is completely imported and has a hydrophilic nature which adversely affects its diffusion across the hydrophobic structure of the cuticle of "Crimson seedless" berries. This study provided evidence about the possibility of employing relatively new growth regulators, namely the thidiazuron and benzyl adenine to mitigate the excessive ProTon use on "Crimson seedless" grapes. The use of cytokinins on grapes was reported before especially thidiazuron along with GA_3 or CPPU with GA_3 (**Susila *et al.* 2013: Shin *et al.* 2019 and Tyagi *et al.* 2021)**. The excessive use of gibberellins isn't desired since it causes many problems to the grape producer mainly it can inhibit the floral induction in the current season which reduces next year crop **(Lenhan and Whiting, 2006)**. The use of TDZ and 6-BA in this study was beneficial since these cytokinins increase the sink strength of many parts of the grapevine such as the berries and the data indicated to a high increase in the 100berry weight by TDZ followed in order by 6-BA. The significant increase in the EC of the berry stems was mitigated by the application of TDZ or 6-BA that preceded the ProTon treatment. Furthermore, each of TDZ or 6-BA

alone didn't have a significant effect on anthocyanin in the berry. However, each of them when preceded the application of ProTon, that was the trend with total sugars, TSS and TSS to acidity ratio since they were not affected by TDZ or 6-BA alone at harvest but when preceded the application of ProTon even at 250 ppm, there was a significant increase the three characteristics in a consistent way in both seasons. Thus, this study provided a new approach to use the cytokinins, either TDZ at (50 ppm) or 6-BA at (50ppm) and also benefit from the outcome and advantage of their applications in delaying leaf senescence as the chlorophylls a and b were high especially with the thidiazuron (TDZ) and benzyl adenine and as appeared from the reduction in the leaf electrical conductivity (EC leaf).

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RESEARCH ARTICLE

Mitigation of excessive proton concentrations of "Crimson" grapes by thidiazuron and benzyladenine

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