

EFFECT OF LOW CONCENTRATIONS OF BRASSINOLOIDS ALONG WITH PROTON ON "Crimson" BERRIES COLORATION, FRUIT QUALITY AND SHELF LIFE

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ABSTRACT: Grapevines "Crimson" suffer from the problem of poor coloration under their growth conditions in many Egyptian farms, mainly due to high temperature day and night especially at maturity. This study aimed at using low concentrations of Brassinoloid (0.5, 1.0, or 2 ppm) alone or in combination with ABA (Commercially known as ProTon) (200 ppm). Spray was done twice at veraison and one week later. The results revealed that Brassinoloid (Br) at low concentration resulted in better quality of "Crimson" grapes. The combination of Br at 0.5 ppm plus ProTon had the best outcome when compared with the control or with other treatments especially on enhancing anthocyanin formation and improving the shelf life of treated berries.

Key words: Grapevines, "Crimson", Brassinoloid, ProTon, Fruit Quality, Berry Coloration, Shelf life.

INTRODUCTION

Grape cultivation and production are expanding in Egypt whether by increasing the cultivated area or adopting new profitable cultivars. The growing area has been expanding in Egypt mostly in the newly reclaimed areas in the desert under hostile condition. The grapevine cultivated areas was 59765 feddans in 2000 with an increase that reached to 72517 feddans in 2019 and the productive trees are 1595380 according to the published statistics of (FAO, 2020).

Under arid conditions especially during the growing seasons, grapevines and their cluster face many problems such as the need to control fruit set, the need for safe treatments to reduce berry shatter, and to control berry size and the highly demanded request of farmers to enhance berry coloration and uniformity by safe treatments of mature berries especially of cultivars such as "Flame" and "Crimson". There has been the use of the ethylene forming compounds such as Ethrel or ethephon (Roberto *et al.*, 2012; Petoumenou and Patris, 2021; Al-Obeed, 2011), which had many accompanying problems such as irregular coloration with leaf burn and yellowing. Such effects passively impact on the differentiation of flower buds that occurs in the same season, and subsequently reducing of the next season flowering and yield. In

addition, Ethrel resulted in increasing berry shatter before harvest. In addition, Ethrel has been found to cause rapid softening and accelerate fruit tissue senescence. In many cases, it also induces fruit abscission as well as its hydrophilic nature that reduces its ability to diffuse through the fruit cuticle (Peppi *et al.*, 2006, 2007; Peppi and Fidelibus, 2008).

More recently, there has been an expansion in adopting commercial product, namely ProTon, or abscisic acid. This compound was successful in enhancing berry coloration (Lee *et al.* 1997; Peppi *et al.* 2006, 2007). However, ProTon application under Egyptian condition faces the problems of the relatively high price especially with the need of more than one spray during the same season. The availability of some new compounds that can be used with ProTon could be a smart way to reduce the expenses of ProTon application to grapevines.

Brassinoloid (Br) is relatively a new natural compound that provides its efficacy on enhancing ripening of grapes under field conditions (Clouse, 2002; Symons *et al.* 2006; Xi *et al.* 2013; Champa *et al.*, 2015; Işci and Gökbayrak 2015; Xu *et al.* 2015 and Ghorbani *et al.* 2017). Brassinoloid also reduced berry shatter of grape berries and reduced gray mold of grape berries. This compound

enhanced anthocyanin biosynthesis in sweet cherries while maintained fruit firmness (Mandava and Wang, 2015).

Brassinosteroids (BRs) constitute a new group of plant hormones that has been given different designations such as "New Class of natural plant hormones" (Clouse and Sasse, 1998; Khripach *et al.*, 1999), polyhydroxylated steroidal plant hormone (Fariduddin *et al.*, 2014) and a new and unique class of plant growth regulators (Sirhindi, 2013). BRs represent a group of hormones first isolated from pollen extracts of *Brassica napus* L. (Mitchell *et al.*, 1970). More than 70 BRs have been extracted from different plant species, out of which only three most biologically active forms are extensively used in physiological studies namely Brassinoloid, 24-epiBrassinoloid and 28-homoBrassinoloid (Esposito *et al.*, 2011).

BRs have a great potential to confer resistance of plants against various biotic and abiotic stresses, such as salinity (Ali *et al.*, 2007), water stress (Vardhini and Rao, 2002), temperature extremes and heavy metals (Hayat *et al.*, 2007; Ali *et al.*, 2008; Yusuf *et al.*, 2012). In addition, BRs play many physiological roles in plant, starting from seed germination to harvest or seed maturation (Ali *et al.*, 2005; Sirhindi, 2013; Leubner-Metzger, 2001). External application of 24-epiBrassinoloid (an analogue of Brassinosteroids) increases the accumulation of phenolic compounds such as anthocyanins, which are secondary metabolites that help to determine berry color. Recent studies have shown that external application of 24-epiBrassinoloid during veraison significantly increased sugar and total anthocyanin accumulations at harvest, but reduced total acidity (Luan *et al.*, 2013; Xi *et al.*, 2013; Xu *et al.*, 2015).

Thus, there is a potential use of Br to enhance the intensity and uniformity of the "Crimson" color. The safety potential and positive responses to Br whether used during flowering, fruit development, or even to mitigate abiotic stress and delay tissue senescence in addition to increasing leaf chlorophylls and enhancing the rate of leaf photosynthesis. All the above characteristics of Br directed us to use it at low concentrations in combinations with ProTon to achieve the following:

- 1- To utilize the combination of Br and ProTon at low concentration to enhance berry coloration and quality characteristics of "Crimson" grape berries, as well as improving their shelf life.
- 2- To reduce the amount of needed ProTon when used alone on grapevines under field conditions.
- 3- To provide grape growers with a production system that could be successfully adopted under field conditions.

MATERIALS AND METHODS

This research was conducted in El-Nour farm, Bader district, Beheira governorate, Egypt. During the two successive seasons 2019 and 2020, Thirty-Two uniform vines, spaced at 2 x 3 m, free from various physiological and visible pathological disorders were selected for investigation. The studied treatments were arranged in randomized complete block design (RCBD) with 4 replications per treatment. Vines were similar in vigor, health, fruit load and irrigated with drip irrigation system. The vines were trained on an overhead trellis with four main arms and were pruned on a spur-cane system, each cane contained six to seven buds. Brassinoloid was applied at three concentrations (0.5, 1.0, 2.0 ppm) and ABA (ProTon 10 %) applied at one concentration (200 ppm) at two stages of growth and berry development at veraison and one week later. The treatments were: control, Br (0.5, 1.0, 2.0, 0.5 ppm) + ProTon (200 ppm), Br (1.0ppm) + ProTon (200 ppm), Br (2.0 ppm) + ProTon (200 ppm), and ProTon alone (200 ppm).

Physical properties

These parameters were measured in the two seasons in two stages, the first one was directly after the harvest on August 17, during 2019 and 2020 respectively. While the second parameters were after the shelf life duration of (2 weeks) on September 1, during 2019 and 2020 respectively.

- 1- Cluster weight (g): A sample of one cluster from each replicate was weighed in grams.
- 2- Weight loss (%): The difference between cluster weight before shelf life and after this divided by initial weight.
- 3- Berry weight (g): The average weight of 100 berries per replicate was determined directly after harvest.
- 4- Berry size (cm³): was determined by using graduated cylinder of 1000 ml containing tap water was used to measure the average berry volume and measuring the water drifting as an indicator of berries size.
- 5- Rachis (cluster stem) weight (g): The average weight of cluster stem without the berries directly after harvest.

Chemical constituents

- 1- Total soluble solids (TSS %): it was measured in berry juice by using hand refractometer.
- 2- Total acidity (%): was assessed as tartaric acid by titration using five milliliters of the berries juice of each sample and titrated against sodium hydroxide solution of a known normality using phenolphthalein as an indicator (A.O.A.C., 1985). The results of

these calibration were converted to percent of titrable acidity using the following equation:

Percent of titrable acidity = $\{N. NaOH \times ml. NaOH \times 0.075^* \times 100\} / ml. Juice \text{ used}$

*0.075= Milliequivalent weight of malic acid

3- TSS / acid ratio was calculated as a ratio between TSS (%) and acidity (%).

4- Vitamin C (%): vitamin C was estimated by titration with (2,6 dichlorophenyl indophenol) on 5ml of fresh juice using acidic indicator (**Egan *et al.*, 1987**).

5- Total sugars: were assessed by using the phenol sulfuric acid method (**Smith, 1956**). The standard curve of glucose was used to calculate the concentration (mg. per gm. Fresh weight of fruit tissue).

6- Chlorophylls a, b, and Beta- Carotene: Chlorophylls a, b and Beta- Carotene were separated in a way of (**Lichtenthaler and Wellburn, 1985**) as follows: half gram of grape rind was used to 20 ml of 85% acetone. The optical density of constant volume was measured at a wavelength of 662 nm, for chlorophyll A, 644 nm, for chlorophyll B and at 440 nm, for carotene using spectrophotometer.

The following equation were used:

$Chl.A = 9.784 \times E.662 - 0.99 \times E.644 = \text{mg/L.}$

$Chl.B = 21.426 \times E.644 - 4.65 \times E.662 = \text{mg/L.}$

$Carotene = [(4.695 \times E.440)] - [0.268 \times (Chl. A + Chl. B)] = \text{mg/L.}$

where, E = It denoted to optical density at the wavelength.

7- Anthocyanin was estimated according to the method of (**Fuleki and Francis, 1968**) as follow: one gram of fresh peel was extracted by using 20 ml of the extraction solution (80% (v/v) ethyl alcohol 95% + 20% HCl 1.5N). The mixture was left for the extraction of anthocyanin for 2 weeks, one ml of this filtrate was used to estimate the optical density at 535 nm, using spectrophotometer. The blank was just the above used extraction solution.

Statistical analysis

The obtained data was analyzed as a factorial experiment in randomized completely block design (RCBD). Data were analyzed using SAS (200) program. The means were compared according to the least significant difference (LSD) at 0.05 levels (**Sendecor and Cochran, 1980**).

RESULTS

Physical Characteristics

The effect of various treatments on cluster weight of "Crimson" grape berries at harvest was reported in Table 1. The data showed there was a significant increase in cluster weight by many treatments as compared with the control in both seasons in a consistent manner. The greatest increase in cluster weight was obtained with the combination of Br at 0.5 ppm plus ABA (ProTon) at 200 ppm. Furthermore, the other two combinations, namely, Br (1.0 ppm) plus ProTon (200 ppm) as well as the combination of Br (2.0 ppm) plus ProTon (200 ppm) were equally effective on increasing cluster weight significant way in both seasons but with lower magnitude. Meanwhile, the individual application of Br at 0.5 ppm resulted in greater cluster weight at harvest than that obtained with the application of Br at either 1.0 ppm or 2.0 ppm. The last two concentration of Br were similar in their influence on cluster weight especially in the first season. Moreover, ProTon alone (200 ppm) was able to increase the cluster weight significantly as compared with control in both seasons but did not vary much from Br alone at 0.5 ppm and led to cluster weight higher than that obtained with Br (1.0 ppm) and Br (2.0 ppm).

The report of berry size of "Crimson" to various applications as found at harvest was reported in Table1. The data indicated that the greatest berry size (as found with the 100-berry size) was obtained with the combination of Br (0.5 ppm) plus ProTon (200 ppm) in both seasons relative to the control and the other two combinations namely the ones that contained Br (1.0 ppm) plus ProTon and the second one that had Br (2.0 ppm) plus ProTon. Meanwhile, these last two combinations were statistically similar in their influence on berry size in both seasons. ProTon application, however, had similar effectiveness to that obtained with the two combinations of Br (1.0 ppm or 2.0 ppm) plus ProTon on fruit size especially in the second season. Moreover, the individual application of each Br was able to cause a signification of Br at 0.5 ppm or at 1.0 ppm while Br at 2.0 ppm resulted in a similar berry size to that obtained with the control.

Changes in berry weight of treated "Crimson" grape berries at harvest as effected by the used applications and the Control were shown in Table1. Again, the superior influence was obtained with the combination of Br (0.5 ppm) plus ProTon (200 ppm) when compared with the control and with all other treatments in both seasons. Except Br at 1.0 ppm plus ProTon in the second season. Moreover, the sole application of Br at 0.5 ppm was effective on increasing berry weight as compared with the control. However, Br individually at 1.0 ppm or at 2.0 ppm, each had a similar influence on berry

weight to that of the control except Br at 1.0 ppm in the first season that gave higher berry weight than that of the control. Meanwhile, the sole treatment of ProTon (200ppm) resulted in higher berry weight than that obtained with the control. In addition, ProTon alone resulted in berry weight greater than that obtained with Br alone at 1.0 ppm or at 2.0 in a consistent manner in both seasons.

The influence of various used preharvest applications on cluster stem weight (Fruitless cluster) was also reported in Table 1. The data revealed that highest stem weight was found with the application of Br (0.5 ppm) plus ProTon (at 200

ppm) as compared with the control and with all other treatments. Meanwhile, the other two combinations of Br (1.0 ppm or at 2.0 ppm) plus ProTon (200 ppm) had similar effect on cluster stems weight especially in the second season. The sole application of Br at either 0.5 ppm or at 1.0 ppm or at 2.0 ppm had similar influence to that found by the control except with Br at 0.5 ppm during the second season that had significantly greater weight of cluster stems than the control. Moreover, the individual application of ProTon resulted in cluster stems weight greater than that of the control and then that found with Br alone at 2.0 ppm.

Table 1. Effect of preharvest applications of brassinoloids at low concentrations along with ProTon or individually on physical characteristics of "Crimson" grape clusters and berries at harvest (2019 and 2020 seasons)

Treatments	Cluster weight (g)		100 Berry size (cm ³)		100 Berry weight (g)		Rachis weight (g)	
	2019	2020	2019	2020	2019	2020	2019	2020
Control	292.50 f	286.25 e	248.75 f	247.50 e	280.00 f	277.50 d	7.25 d	6.50 e
Brassinoloids (0.5ppm)	305.00 d	303.75 c	258.75 ed	255.00 cd	295.00 d	288.75 c	8.00 cd	7.50 cd
Brassinoloids (1.0ppm)	298.75 e	298.75 d	255.00 e	251.25 de	288.75 e	281.25 d	7.75 d	7.25 cde
Brassinoloids (2.0ppm)	297.50 ef	287.50 e	250.00 f	247.50 e	282.50 f	278.50 d	7.50 d	6.75 de
Brassinoloids (0.5ppm) +ProTon (200ppm)	325.00 a	318.75 a	280.00 a	272.500 a	311.25 a	310.00 a	11.00 a	10.00 a
Brassinoloids (1.0ppm) +ProTon (200ppm)	313.75 b	311.25 b	267.50 b	261.250 b	306.25 b	306.25 a	9.75 b	9.50 ab
Brassinoloids (2.0ppm) +ProTon (200ppm)	311.25 bc	308.75 b	263.75 cb	258.75 bc	300.00 c	297.50 b	8.75 c	8.75 b
ProTon (200ppm)	307.50 cd	303.75 c	260.00 cd	257.50 bc	297.50 dc	292.50 b	8.75 c	7.75 c

*Values within a column of the similar letter (s) were not significantly different according to the least significant difference (LSD) at 0.05 levels

Chemical Characteristics

Changes in TSS of the berry juice in response to various treatments of "Crimson" grape vines were shown in Table 2. The data revealed that the greatest TSS was found with the combination of Br (0.5 ppm) plus ProTon (200 ppm) as compared with the control and with all other treatments. Meanwhile, the combination of Br (1.0 ppm) followed in magnitude when was combined with ProTon (200 ppm). However, the third combination of Br (2.0 ppm) plus ProTon (200 ppm) was also effective on increasing the TSS significantly in a similar magnitude to the above combination (when Br was 1.0 ppm) plus ProTon. Meanwhile, the individual application of ProTon resulted in a significant increase in TSS when compared with the control but such increase in TSS was at harvest less than what was achieved with

the combinations treatments. On the other hand, the sole use of Br at either 0.5 or at 1.0, or at 2.0 ppm was able to give a significant increase in TSS when compared with the control. However, the sole use of ProTon resulted in a significant increase in TSS when compared with the sole use of Br except the use of Br at 0.5 ppm in the first season.

The effected of Preharvest treatments on vitamin C content in the juice of "Crimson" grape berries at the harvest time was reported in Table 2. The data illustrated that the highest vitamin C was obtained with the combination of Br (0.5 ppm) plus ProTon (200 ppm) as compared with the control. However, the other two combinations, namely, Br (1.0 ppm or 2.0 ppm) each of them plus ProTon caused a significant increase in vitamin C content relative to the control but with a similar magnitude

that the first combination. Moreover, the individual application of Br (2.0 ppm) resulted in a similar vitamin C to that of the control. In addition, the individual application of Br (2.0 ppm) resulted in a similar vitamin C to that of the control. In addition, the individual application of Br at the lower concentration (0.5 or 1.0 ppm) was able to cause a significant increase in vitamin C as compared with the control in the two seasons. Moreover, the individual application of ProTone resulted in a significant increase in vitamin C relative to the control.

The effect of used preharvest applications on juice acidity of "Crimson" grapes was reported in Table 2. The data revealed that the control berries had the highest acidity in the two seasons. On the contrary, the lowest juice acidity was obtained with the combination of Br (0.5 ppm) plus ProTone (200 ppm). The rest of the combinations (Meaning Br 1.0 ppm or 2.0 ppm) each plus ProTone (200 ppm) had a similar effect on juice acidity than that of the control. Furthermore, the individual treatment of the two later combinations (namely, Br 1.0 or 2.0 ppm), each plus ProTone at the same concentrations of ProTone alone. Thus, it did not make a difference to apply ProTone alone or ProTone plus Br at 1.0 ppm or at 2.0 ppm. However, when Br was applied at 0.5 ppm plus ProTone at the same concentration, there was a significant reduction in juice acidity.

The influence of various used preharvest application total sugars of "Crimson" berries was

reported in Table 2. The data showed that the greatest percentage of total sugars was found again with the combination of Br at 0.5 ppm plus ProTone at 200 ppm. Meanwhile, the control berries had the lowest total sugars in their juice. The sole treatment of ProTone at 200 ppm had higher total sugars than control but significantly lower total sugars than the combination of Br at 0.5 ppm Plus ProTone at 200 ppm and similar to the combination. with regard to the effect of the individual application of Br at 0.5, 1.0 or 2.0 ppm, all had a significant increase in total sugars but with a slight increase in the actual amount of sugars.

The effect of pre-harvest applications on the TSS/Acidity Ratio of "Crimson" grapes was investigated. Where the data showed that the highest TSS/Acidity Ratio was obtained with the combination of Br (0.5 ppm) in addition to the ProTone (1.0 ppm) in comparison with the control and with all other treatments. It also showed that both of the other two combinations, namely Br (1.0 ppm or 2.0 ppm) each in addition to the ProTone resulted in a significant increase in the TSS/Acidity Ratio compared to the control, but by a lower percentage than the first combination. Also, the single application of the ProTone led to a significant increase in the TSS/Acidity Ratio when compared to the control, Although the single application of Br (1.0 ppm and 0.2 ppm) had a low effect on the previous formulations, but they led to an increase in the TSS/Acidity Ratio compared to the control in both seasons.

Table 2. Effect of preharvest applications of brassinoloids at low concentrations along with ProTone or individually on some chemical characteristics of "Crimson" grape berries at harvest (2019 and 2020 seasons)

Treatments	TSS%		Acidity%		TSS /Acid ratio		Vitamin C%		Total sugars (mg/ g.F.W. of fruit)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	17.18 f	17.50 f	0.95 a	0.88 a	18.18 f	19.86 f	1.73 d	1.60 e	14.31 f	14.59 f
Brassinoloids (0.5ppm)	18.23 cd	18.35 d	0.90 bc	0.82 cd	20.42 c	22.35 c	2.27 c	2.22 cd	15.19 cd	15.29 d
Brassinoloids (1.0ppm)	18.05 d	18.20 e	0.92 b	0.830 c	19.73 d	21.97 c	2.25 c	2.18 d	15.04 d	15.17 de
Brassinoloids (2.0ppm)	17.75 e	18.08 e	0.94 a	0.86 b	18.86 e	21.15 d	1.82 d	1.73 e	14.79 e	15.06 e
Brassinoloids (0.5ppm) +Proton (200ppm)	19.80 a	19.90 a	0.85 d	0.78 f	23.37 a	25.65 a	2.87 a	2.82 a	16.50 e	15.58 a
Brassinoloids (1.0ppm) +Proton (200ppm)	18.73 b	18.88 b	0.88 c	0.80 ef	21.43 b	23.75 b	2.73 ab	2.50 b	15.61 b	17.73 b
Brassinoloids (2.0ppm) +Proton (200ppm)	18.63 b	18.75 bc	0.88 c	0.80 de	21.32 b	23.37 b	2.59 b	2.46 bc	15.52 b	15.63 bc
Proton (200ppm)	18.30 c	18.63 c	0.89 c	0.80 cde	20.77 c	23.11 b	2.34 c	2.34 bcd	15.25 c	15.52 c

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

The response of the anthocyanin content in "Crimson" berries at harvest to various treatments was reported in Table 3. The data indicated a significant increase in anthocyanin obtained by many treatments. The greatest increase was found with the combination of Br (0.5 ppm) plus ProTone (200 ppm) followed in magnitude by the second combination that contained Br (1.0 ppm) plus ProTone (200 ppm). Moreover, there was a significant increase with the application of ProTone alone (200 ppm). Meanwhile, the sole applications of the three Br concentrations, each of them was capable of inducing higher content of the anthocyanin in the berry skin of "Crimson" grapes especially in the first season when compared with the control. Thus, the brassinoid alone was able to stimulate anthocyanin production even in a non-climacteric fruit such as grapes. Moreover, the third combination, namely, Br (2.0 ppm) plus ProTone also caused a significant increase in anthocyanin production by the harvest time. It is very considerable to find that the highest increase of anthocyanin reaches to about 77% by the application of Br (0.5 ppm) plus ProTone as compared with the control.

The effect of preharvest treatments to "Crimson" grape berries on carotene content was shown in Table 3. The data showed that the greatest carotene content was again obtained with the combination of Br at 0.5 ppm ProTone (200 ppm) in a consistent manner in both seasons. Moreover, as Br concentration in the combination was increased the concentration of carotene was reduced. However, in all cases, the outcome was positive on the carotene content in the fruit skin. Furthermore, the sole application of Br either at 0.5, or 1.0, or 2.0 ppm proved its effectiveness causing a significant increase in the berry carotene when compared with the control in the two seasons.

Changes in chlorophyll a in "Crimson" berries in response to preharvest treatments was shown in Table 3. The data revealed that the highest chlorophyll a content was found in the control berries. Moreover, the application of Br alone resulted in a significant increase in chlorophyll a content especially Br at 2.0 ppm followed by Br at 1.0 ppm whereas Br at 0.5 ppm resulted in chlorophyll a similar to other single Br applications only during the second season. Moreover, the application of the combination of Br (0.5 ppm) plus ProTone (200 ppm) resulted in lowest chlorophyll a as compared with the control and with other treatments. Moreover, the individual application of ProTone at 200 ppm was also effective on reducing chlorophyll a but to lesser extent than its combination with any of the Br concentrations.

Regarding the changes in chlorophyll b in response to various treatments of "Crimson" grapes, the data reported in the Table 3 showed that the control berries had the highest chlorophyll b among all used treatments. Meanwhile, the combination of Br (0.5 ppm) plus ProTone (200 ppm) had the lowest values of chlorophyll b in the two seasons. The sole application of ProTone was still able to reduce chlorophyll b but to lower extent than the combinations containing Br at 1.0 ppm or 2.0 ppm, each in addition to ProTone at the same concentration. Meanwhile, the single application of Br at 0.5 ppm was able to reduce chlorophyll b to a level less than the control. Moreover, Br alone at 1.0 ppm and at 2.0 ppm caused a reduction of chlorophyll b but to levels higher than that obtained with Br at 0.5 ppm.

The effect of preharvest treatment of "Crimson" Grape vines on the shelf life of berries was reported in Table 4 the data revealed that the highest weight loss was found with the control berries in both seasons while the least weight loss was found with the combination of Br (0.5 ppm) plus ProTone (200 ppm). Moreover, the individual application of ProTone (200 ppm) resulted in similar weight loss to that found with the combination of Br (2.0 ppm) plus ProTone (200 ppm). However, there was a significant reduction in weight with the application of Br (1.0 ppm) plus ProTone (200 ppm). Furthermore, with the individual application of Br, as the concentration of Br consistent manner. Thus, it was preferred to reduce weight loss by applying a low concentration Br at 0.5 ppm mixed with ProTone at 200ppm.

Changes in TSS of "Crimson" berries after storage were reported in Table 4. Such clusters were treated first before harvest and storage as shown in the same Table. The data indicated that the greatest TSS was obtained with the combination of Br (0.5 ppm) plus ProTone at (200 ppm) in both seasons. Moreover, the use of Br at either 1.0 or 2.0 ppm, each of them plus ProTone (200 ppm) had similar effect on the TSS percentage which was greater than that obtained with the control. Moreover, the individual treatment of the Br at 0.5 ppm or at 1.0 ppm had a similar influence on TSS in both seasons. However, when Br was individually applied at 2.0 ppm, there was a reduction in TSS only in the first season in a similar magnitude to that found with the control.

The changes in juice acidity following storage of treated clusters of "Crimson" grapes were shown in Table 4. the data showed that the control berries had the highest juice acidity whereas the lowest acidity was found with the treatment of Br (0.5 ppm) plus ProTone in both seasons. The application of ProTone alone resulted in juice acidity similar to that

obtained with the combination of Br (2.0 ppm) plus ProTon in both seasons. Moreover, the application of Br (1.0 ppm) plus ProTon resulted in similar acidity to that found with the combination of Br at 2.0 ppm plus ProTon. Meanwhile, the individual

treatments of brassinoloids resulted in the reduction of juice acidity as compared with the control without a significant difference between Br at 1.0 ppm and 2.0 ppm.

Table 3. Effect of preharvest applications of brassinoloids at low concentrations along with ProTon or individually on pigment concentration in the "Crimson" grape berries at harvest (2019 and 2020 seasons)

Treatment	Anthocyanin (mg/100g)		Carotene (mg/L)		Chlorophyll A (mg/L)		Chlorophyll B (mg/L)	
	2019	2020	2019	2020	2019	2020	2019	2020
Control	18.90 g	18.82 g	0.93 h	1.01 h	3.77 a	3.79 a	3.09 a	2.29 a
Brassinoloids (0.5ppm)	26.04 d	21.94 e	1.22 e	1.30 e	3.45 d	3.45 c	2.50 d	1.84 c
Brassinoloids (1.0ppm)	25.18 e	20.24 f	1.12 f	1.23 f	3.47 c	3.47 c	2.95 c	1.86 c
Brassinoloids (2.0ppm)	22.15 f	20.18 f	1.05 g	1.15 g	3.50 b	3.51 b	3.05 b	1.96 b
Brassinoloids (0.5ppm) +ProTon (200ppm)	33.08 a	32.60 a	1.79 a	1.77 a	3.01 g	3.02 e	1.79 h	1.12 g
Brassinoloids (1.0ppm) +ProTon (200ppm)	31.89 b	31.17 b	1.68 b	1.71 b	3.14 f	3.22 d	2.08 g	1.22 f
Brassinoloids (2.0ppm) +ProTon (200ppm)	27.75 c	26.40 c	1.60 c	1.64 c	3.21 e	3.23 d	2.13 f	1.35 e
ProTon (200ppm)	27.23 c	25.10 d	1.31 d	1.59 d	3.43 d	3.44 c	2.20 e	1.76 d

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

Table 4. Some characteristics of "Crimson" grapes after storage are influenced by the application of pre-harvest brassinoloids at low concentrations along with ProTon or individually during the 2019 and 2020 seasons

Treatment	Weight loss %		TSS %		Acidity %		TSS/Acidity %		Vitamin C %	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	4.756 a	4.68 a	20.28 d	20.40 d	1.07 a	0.94 a	19.04 f	21.68 e	1.55 d	1.18 g
Brassinoloids (0.5ppm)	3.42 d	3.38 d	21.15 c	21.30 bc	0.97 cd	0.87 c	21.86 cd	24.60 c	2.25 c	1.89 d
Brassinoloids (1.0ppm)	3.50 c	3.60 c	21.13 c	21.25 bc	0.99 bc	0.91 b	21.43 d	23.53 d	2.20 c	1.69 e
Brassinoloids (2.0ppm)	4.67 b	4.59 b	20.40 d	21.08 c	1.00 b	0.93 ab	20.39 e	22.76 d	1.64 d	1.46 f
Brassinoloids (0.5ppm) +ProTon (200ppm)	2.66 g	2.60 g	21.68 a	21.70 a	0.93 e	0.80 e	23.42 a	27.31 a	2.75 a	2.78 a
Brassinoloids (1.0ppm) +ProTon (200ppm)	3.16 f	3.20 f	21.43 b	21.50 ab	0.94 de	0.82 de	22.95 ab	26.46 ab	2.69 ab	2.46 b
Brassinoloids (2.0ppm) +ProTon (200ppm)	3.31 e	3.30 e	21.35 b	21.40 b	0.95 ed	0.84 dc	22.43 bc	25.49 bc	2.53 b	2.37 bc
ProTon (200ppm)	3.38 de	3.31 e	21.20 c	21.33 bc	0.96 de	0.86 c	22.18 bcd	24.95 c	2.32 c	2.32 c

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

The response of "Crimson" berries to used treatments and storage was reported in Table 4. The data showed that the highest Vitamin C content was obtained with the combination of Br (0.5 ppm) plus ProTon. In addition, the other combinations of Br at 1.0 ppm or at 2.0 ppm each of them plus ProTon had significantly higher vitamin c than the control while both combinations wee similar to each other meaning there was no significant difference between both the control fruits had similar Vitamin C content to use of Br (2.0 ppm) especially in the first seasons. The individual treatment of ProTon (200 ppm) attained a significant increase in Vitamin C relative to the control but less than the three used combinations of Br plus ProTon.

Changes in TSS/Acidity Ratio for "Crimson" berries after storage were evaluated in Table 4. These clusters were processed before harvesting and storage as shown in the Table. Note that the highest TSS/Acidity Ratio when using the combination of Br (0.5 ppm) plus a ProTon at 200 ppm. Also, the individual use of Br at 0.5, 1.0 and 2.0 ppm each showed a similar effect on TSS/Acidity Ratio compared to the control, but to a lesser degree than what was achieved with the combination treatments or individual application of ProTon, as it led to a significant increase in the TSS/Acidity Ratio When compared with the control, however, this increase in total TSS/Acidity Ratio was also less than what was achieved with the combination treatments in both seasons.

The effect of various applied treatments on total sugars of "Crimson" grapes at harvest was reported in Table 5 .The data revealed that the greatest increase in such sugars was obtained with the combination of Br (0.5 ppm) plus ProTon (200 ppm) in consistent manner in both seasons .Moreover, the application of Br (1.0 ppm) plus ProTon (200 ppm) followed in order the above treatment in terms of increasing total sugars and it gave a similar magnitude of increase to that obtained with Br (2.0 ppm) plus ProTon. Meanwhile, the individual application of ProTon (200 ppm) resulted in significantly lower total sugars than that obtained when it was involved in a combination with Br where at 0.5 or at 1.0 or at 2.0ppm .However , the sale application of Br at 0.5 or at 2.0 ppm was able to cause a significant increase in total sugars in "Crimson" grapes as compared with the control during the two season .Moreover, Br at 2.0 ppm alone resulted in a significant increase of total sugars only in the second season. Thus, increasing Br concentration did not mean obtaining better response of the grape berry whether applied alone or in combination with ProTon as shown in Table 5.

The influence of the application of Br alone at different concentrations or in combination with ProTon at the same concentration was shown in Table 5. The data confirmed again the highest anthocyanin content was obtained in the skins of "Crimson" berries that were treated with the combination of Br at 0.5 ppm plus ProTon at 200 ppm in both seasons. However, when this combination included Br at 1.0 ppm or Br at 2.0 ppm, the resulting anthocyanin content was significantly reduced as compared with the first mentioned combination. Meanwhile, all the three combinations caused a better anthocyanin content than the control in both seasons. Furthermore, the sole treatment of Br at 0.5 or 1.0 or 2.0 ppm all caused a significant increase in the anthocyanin content consistently in both seasons as compared with the control. Lower the Br concentration, better the content of anthocyanin in the berry skins.

With regard to the response of "Crimson" grapes to various treatments on carotene content in the berry skins, the data in Table 5 showed that there was almost a typical pattern and trend to that found with anthocyanin since again the greatest content of carotenes was obtained with the combination of Br at 0.5 ppm followed by the combination of Br at 1.0 ppm plus ProTon the third combination of Br at 2.0 ppm plus ProTon, all the three combinations had in order greater carotene content than the control in both seasons. The application of ProTon alone resulted in significantly lower carotenes than each of the three combinations of ProTon plus Br except ProTon alone in the second season that had similar carotenes to that found with ProTon plus Br (2.0 ppm). Moreover, the sale application of Br at 0.5 or at 1.0ppm resulted in a significant increase in berry carotene as compared with the control. However, when such Br was at 2.0 ppm. It resulted in a significant increase in carotene only in the second season.

The chlorophyll content of "Crimson" berries was determined after storage for several treatments in Table 5. The data showed a decrease in chlorophyll a content that was obtained when using a mixture of Br (0.5 ppm) plus ProTon (200 ppm) compared to the control group and the other treatments. And that the highest content of chlorophyll was found in the control berries. While the application of Br alone led to a significant increase in the content of chlorophyll a, especially Br, at 2.0 ppm, followed by Br at 1.0 ppm, while the use of Br at 0.5 ppm resulted in chlorophyll production similar to Br single applications only during the second season. The individual ProTon at a rate of 200 ppm is the lowest, but to a lesser degree

than its combination with any of the Br concentrations.

The changes in chlorophyll b in the results of Table 5 in response to the different treatments of "Crimson" grapes showed that the highest content of chlorophyll b appeared in the control group, and the lowest percentage in the mixture between Br (0.5 ppm) plus ProTon (200 ppm) was the lowest values during the two seasons. Followed by the

formulations that contain BR at its different concentrations of 0.1 or 0.2 ppm in addition to the ProTon at a concentration of 200 ppm. The single application of the ProTon at the rate of 200 ppm showed a decrease in chlorophyll b content but less than the previous formulations. And the individual applications of Br showed an increase Significant in chlorophyll b content, especially at 2.0 ppm, but at lower rates than the control.

Table 5. Some characteristics of "Crimson" grapes after storage are influenced by the application of pre-harvest brassinoloids at low concentrations along with ProTon or individually during the 2019 and 2020 seasons

Treatment	Total sugars (mg/ g. F.W. of fruit)		Anthocyanin (mg/100 g)		Carotene (mg/L)		Chlorophyll A (mg/L)		Chlorophyll B (mg/L)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	16.90 d	17.06 e	18.60 g	18.07 g	0.86 g	0.91 g	3.34 a	3.57 a	2.10 a	2.25 a
Brassinoloids (0.5ppm)	17.63 c	17.73 bc	26.00 d	21.40 e	1.01 e	1.27 d	3.18 d	3.34 d	1.84 d	1.80 d
Brassinoloids (1.0ppm)	17.61 c	17.63 cd	25.03 e	20.18 f	0.95 f	1.22 e	3.24 c	3.41 c	1.87 c	1.92 c
Brassinoloids (2.0ppm)	17.00 d	17.56 d	22.08 f	20.03 f	0.87 g	1.11 f	3.26 b	3.45 b	2.04 b	2.17 b
Brassinoloids (0.5ppm) +ProTon (200ppm)	18.06 a	18.19 a	33.05 a	32.25 a	1.47 a	1.79 a	0.70 h	2.87 g	1.02 h	1.04 g
Brassinoloids (1.0ppm) +ProTon (200ppm)	17.85 b	17.85 b	30.87 b	31.56 b	1.35 b	1.67 b	1.90 g	3.14 f	1.06 g	1.08 f
Brassinoloids (2.0ppm) +ProTon (200ppm)	17.79 b	17.84 b	26.94 c	27.10 c	1.28 c	1.57 c	1.93 f	3.21 e	1.20 f	1.28 e
ProTon (200ppm)	17.67 c	17.77 bc	26.76 c	26.16 d	1.17 d	1.56 c	2.15 E	3.33 d	1.76 e	1.29 e

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

DISCUSSION

This study provided evidences about the positive roles of applying Brassinoloids at low concentrations on grape berries before harvest. It was found in this study that the individual applications of Brassinoloids (Br) especially at 0.5 ppm or at 1.0 ppm was able to increase cluster weight, 100- berry size and 100- berry weight as well as TSS and total sugars of "Crimson" berries in a consistent manner in both seasons. Moreover, the sole treatments of the three used concentrations of the brassinoid resulted in a significant increase in the anthocyanin content and carotenes of the berry skins of "Crimson" grapes as compared with the control. Such positive effects of brassinoids on some physical and chemical characteristics agreed with the findings of previous studies reported worldwide by (Ali *et al.*, 2007; Sirhindi, 2013; Fariduddin *et al.*, 2014).

Moreover, the individual application of ABA at 200 ppm was able to increase the reported berry characteristics in a significant manner with different magnitudes of increase when compared with the combination treatments. Such ABA (200 ppm) is the same as the commercial product called ProTon. The literature reported the need of grapevines and their berries to a dose of ABA since it was reported that there was a decline of natural ABA by the last period of the second stage of the double sigmoid curve of the grape berry growth as reported by (Giribaldi *et al.*, 2010; Jeong *et al.*, 2004; Koyama *et al.*, 2010; Wheeler *et al.*, 2009 and Adams-Phillips *et al.*, 2004).

Moreover, the combinations of brassinoid plus ABA had a synergistic influence on many important berry characteristics of "Crimson" berries in this study especially with the application of (Br) at 0.5 ppm plus ABA at 200 ppm. However, the increase

of (Br) to 2.0 ppm plus ABA (200 ppm) did not result in further increase of the reported physical characteristics whether the cluster weight, 100- berry weight or size or the cluster – stem weight. Meanwhile, (Br) alone at 2.0 ppm caused a significant increase in total sugars, TSS and anthocyanin content. Thus, based on the consistent results, it could be concluded that the best synergism of this study was found with the combination of (Br) at 0.5 ppm plus ABA (200 ppm) which is important to reduce the high cost at ProTon in the market with better performance and higher magnitude of increases in many cluster and berry characteristics. Boosting cluster weight, berry weight and size, in addition to total sugars, Total soluble solids of the juice, carotene content and anthocyanin in the berry skin in a greater magnitude of increase than other combinations or other individual treatments is a unique finding of this research that could be utilized by grape producers in the field.

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RESEARCH ARTICLE

Effect of Low Concentrations of Brassinoloids Along with Proton on "Crimson" Berries Coloration, Fruit Quality and Shelf Life

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