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Effect of Application of β-aminobutyric Acid on Maintaining Quality of Crimson Seedless Grape and Controlling Postharvest Diseases under Cold Storage Conditions

[1]M.A. EL-Metwally, [2]M.E. Tarabih and [2]E.E. EL-Eryan
[1]Department of Mycological Research, Institute of Plant Pathology Research,
[2]Department of Fruit Handling, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

Abstract: The effect of application β-Aminobutyric Acid (BABA) on Crimson Seedless grape vine was studied in 2012 and 2013 seasons, as an alternative to traditional chemical compounds that are harmful to the environment to control the pre and postharvest diseases. The vines were sprayed with three levels of BABA (200, 250, 300 ppm) two weeks before harvest. The linear growth and dry weight of *Botrytis cinerea* isolated from Crimson Seedless grape were greatly influenced by the increase in BABA concentration up to 300 ppm. In both seasons, prolonging the marketing stage resulted in decreased in disease infection with the increase in concentrations, at 300 ppm concentration, BABA gave the maximum reduction in disease infection caused by *Botrytis cinerea*, in addition to the improvement of fruit quality at harvest and during cold storage (for 60 days at 0±1°C with 90-95% RH), as well as 3 days marketing period at room temperature. Clusters treated with 300 ppm BABA developed less decay shatter and total loss. Moreover, it was more effective in rising SSC, titratable acidity, total sugar and anthocyanin accumulation in the skin of berry during cold storage conditions and marketing period compared with the other treatments. While, BABA 250 ppm showed high berry firmness and adherence strength.

Key words: Crimson Seedless grape, berry decay, berry shatter, β-aminobutyric acid, cold storage, shelf life

INTRODUCTION

Grapevine (*Vitis vinifera*) is the most widely cultivated fruit crop in the world and the most important in economic terms. 'Crimson Seedless' is a new late-season table grape recently developed by scientists at the USDA-Fruit Genetics and Breeding Research Unit, Fresno, California, USA. When properly mature, the fruit of Crimson Seedless is bright red and has excellent eating characteristics; its berries are firm and crisp and have good flavor. Grapevine is affected by several fungal and diseases such as grey mold (*Botrytis cinerea*), powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*). Gray mold (*Botrytis cinerea*) is the main postharvest decay problem of table grapes and limits their shelf-life.

*Botrytis cinerea*is responsible for the gray mold disease on more than 200 host plants. This necrotrophic ascomycete displays the capacity to kill host cells through the production of toxins, reactive oxygen species and the induction of a plant-produced oxidative burst. Thanks to an arsenal of degrading enzymes, *B. cinerea*is then able to feed on different plant tissues. Recent molecular approaches, for example on characterizing components of signal transduction pathways, show that this fungus shares conserved virulence factors with other phytopathogens but also highlight some *Botrytis*-specific features. The discovery of some first strain-specific virulence factors, together with population data, even suggests a possible host adaptation of the strains. The availability of the genome sequence now stimulates the development of high-throughput functional analysis to decipher the mechanisms involved in the large host range of this species (Choquer *et al*., 2007).

The teleomorph (sexual form) of *Botrytis cinereais anascomycete*, *Botryotinia fuckeliana*, also known as *Botryotinia cinerea*. A total of 356 field isolates of *B. fuckeliana* were collected from grapes (*Vitis vinifera*) growing at four locations in Champagne (Trepail, Bar-sur-Seine, Bour-sault and Plumecoq). The isolates were collected from three varieties of grape (Chardonnay, Pinot Noir and Pinot Meunier) and three organs (berry, leaf and stem). Two hundred fifty-nine of the 356 isolates were collected from separate lesions, sometimes on the same plant. The remaining 97 isolates were separate isolations from the same berries

Corresponding Author: M.A. EL-Metwally, Department of Mycological Research, Institute of Plant Pathology, Agricultural Research Center, Giza, Egypt

(Giraud *et al*., 1997). Sulfur dioxide (SO_2) is traditionally used as an antimicrobial postharvest, in packages or storage rooms. Thus, practices such as postharvest fumigation with SO_2 and carbon dioxide (CO_2) have a beneficial effect in preserving quality attribute for table grape (Mitcham and Leesch, 2004). One of the problems associated with SO_2 fumigation of grapes is the constant potential for injury to the berries andrachis. Injured tissue first shows bleaching of color, followed by sunken areas where accelerated water loss has occurred. Another problem with SO_2 fumigation of grapes is the level of sulphite residue remaining at time of final sale. Concerns about harm to human health from SO_2 encourage evaluation of alternatives. Considerable research has been conducted to find alternatives to SO_2 treatments for grey mould control.

A new technology for plant disease control is based on the activation of the plant's own defense system with the aid of low molecular weight synthetic molecules.

β-aminobutyric acid (BABA), a non-protein amino acid and a derivative of carboxylic acid. It has a carboxyl group at the first carbon atom and an amino group positioned at the third carbon atom, thus the name (DL)\-3-aminobutyric acid (β-aminobutyric acid) (Fig. 1). BABA is able to potentiate signal transduction pathways in different plants against a broad spectrum of microbial pathogens (Conrath *et al*., 2002; Ton *et al*., 2005).

The first line of defense a pathogen will encounter during plant infection consists of structural preformed barriers such as the cuticle and cell wall. The cuticle layer represents the first barrier for the pathogen to penetrate directly through the cell wall (Kerstiens, 1996; Zimmerli *et al*., 2000) observed that the protective effect of BABA is due to a potentiation of natural defense mechanisms against biotic and abiotic stresses. Higher concentrations of BABA are required on leaves in contrast to small doses that are required when applied to the root system (Cohen and Gisi, 1994).

Tavallali *et al*. (2008) reports that BABA induced resistance in orange peel tissue against infection of *Penicillium italicum* in a concentration-dependent manner and the optimum concentration was 40 mM. Therefore, these data suggest that BABA probably acts as a plant growth regulator which is most effective when applied at an optimal concentration and not as a fungicide

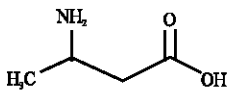

Fig. 1: Structure of β-aminobytyric acid

that usually becomes continually more effective as the dose increases. Thus, the main mode of action of BABA in reducing decay development on the fruit surface was through the activation of pathogen resistance mechanisms and not any possible direct antifungal effects.

However, Fischer *et al*. (2009) found direct effects on fungi, since BABA inhibited the mycelial growth of *Botrytis cinerea* and affected *Saccharomyces cerevisiae* growth in a concentration dependent manner. Application of BABA in fruit possesses promising results in the control of postharvest diseases as an alternative to traditional methods.

Recently it has become clear that BABA also can cause major alterations in plant amino acid balance, induce stress-responsive energy sensor protein kinases, induce anthocyanin accumulation and reduce vegetative growth in Arabidopsis (Wu *et al*., 2010).

The objective of the present study was to evaluate the inhibitory effect of BABA for maintaining quality and controlling decay of Crimson Seedless grape caused by grey mould (*Botrytis cinerea*), in the laboratory and nature after cold storage and through marketing.

MATERIALS AND METHODS

Isolation and identification of the pathogen: *Botrytis cinerea* was isolated from naturally infected Crimson Seedless grape. These isolates were the most aggressive one in our collection and produced the largest lesions on inoculated fruits. These fungi were purified and maintained on Potato Dextrose Agar (PDA) and stored at 4°C, with periodic transfers through citrus fruits to maintain its aggressiveness. Seedless grape were ready for examination under a stereoscopic binocular microscope (6-50 ×) for the presence of fungi and to study their habit characters. When necessary the compound microscope was used for confirming the identification after having examined the morphology of conidia and conidiophores. Fungi presented on infested seeds were identified by means of comparison with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as publication of Ellis (1971).

Effect of BABA on growth of fungi isolated from Crimson Seedless grape and disease infection percentage

Linear growth: BABA was tested *in vitro* on the linear growth of the pathogenic fungi. Different concentrations

were added to 10 mL of sterilized PDA before solidification and then poured in sterile Petri-dishes. After solidification, the plates were inoculated with fungal disc (5 mm) in the center of the plate and incubated at 27±1°C. Three plates for each particular treatment for each fungus were used as replicates; three plates were prepared to serve as control for each fungus. Linear growth was observed daily and diameter of fungal colonies were recorded when plates of any treatment were filled with the fungal growth.

Dry weight: One hundred mL of liquid PD medium in 250 mL Erlenmeyer flasks were amended with different concentrations of the tested compounds after autoclaving. Each flask was inoculated using two discs of 0.6 mm in diameter of fungal culture, then incubated at 20±2°C for 7 days. Control flasks contain no concentrations of these compounds. Three replicates were used for each concentration. At the end of incubation period, the mycelium was filtered off and washed several times with distilled water, then dried in an oven at 80°C for 48 h till constant weight (El-Morsy, 1993).

Field study: The present investigation was carried out during the two successive seasons of 2012 and 2013 on five years old Crimson Seedless grapevines grown in a private vineyard located at Aga city, Dakahlia, Governorate. The vines were grown in clay loam soil and planted at 1.5×2.5 m apart and trained according to cane pruning system. All vines received the recommended regular fertilizer and other horticulture practices. Thirty-six vines of nearly similar vigor and bud load were chosen for the spraying applications according to completely randomized block design with three replicates each one was represented by three vines. Two weeks before harvest, clusters of the selected vines were sprayed as follows:

- Control (spray cluster with tap water)
- Spray clusters with BABA 200 ppm
- Spray clusters with BABA 250 ppm
- Spray clusters with BABA 300 ppm

The surfactant super film as a wetting agent was added at the rate of 40 cm/100 L water to all spraying solutions in order to obtain best penetration results.

Harvest time was adjusted when the berry skin was full red color and SSC percentage in berry juice of the control treatment reached 18-19% in the two seasons. Clusters were picked at morning and immediately transported to the laboratory. Samples from each replicate were taken to study the effect of each treatment on clusters and berries quality at harvest time to determine the initial properties.

For storage study, clusters of all treatments were sorted to remove any infected and berry damaged then clusters were packed using perforated plastic bags. All bags with clusters were weighted and every six bags were put in ventilated carton box. The total numbers of carton boxes were 48 for all treatments. Clusters of each treatment were placed in 12 carton box, each box containing 6 perforated plastic bags (6 clusters). All boxes were stored 30 days at 0±1°C with 90-95% RH, then 3 carton boxes for each treatment were taken 10 days intervals to determine clusters and berry characteristics.

Disease infection: It was determined according to the following equation:

$$\text{Disease infection (\%)} = \frac{\text{No. of nutural infected fruits}}{\text{No. of total fruits}} \times 100$$

For shelf life study, after 60 days of cold storage three carton boxes for each treatment were held 3 days at room temperature conditions as shelf life at 20±2°C with 70-75% relative humidity to determine the following parameters.

Clusters and berries measurements

Cluster weight loss (%): It was calculated according to the following equation:

$$\text{Cluster weight loss (\%)} = \frac{\text{Initial cluster weight} - \text{weightat sampling date}}{\text{Initial cluster weight}} \times 100$$

Berry decay (%): It was calculated according to the following equation:

$$\text{Berry decay (\%)} = \frac{\text{Weight of decayed barries}}{\text{Initial cluster weight}} \times 100$$

Berry shatter (%): It was determined according to the following equation:

$$\text{Berry shatter (\%)} = \frac{\text{Weight of barriy shatter}}{\text{Initial cluster weight}} \times 100$$

Total loss (%): It was calculated according to the following equation:

Total loss (%) Cluster weight loss (%)+Decayed (%)+Shatter berries (%)

Berry adherence strength: It was measured as (gf) by using PHSH-PULL (Dynamometer Modle DT101).

Berry firmness: It was measured as (g cm^{-2}) by using PHSH-PULL (Dynamometer Model DT101) with 3/16 inch plunger.

Soluble Solids Content (SSC): It was measured in berry juice by using a Carl-Zeiss hand refractometer according to (AOAC, 2005).

Total titratable acidity: Titratable acidity of berry juice was determined in terms of anhydrous tartaric acid percentage according to (AOAC, 2005).

SSC/acid ratio: It was calculated by dividing the value of SSC over the value of titratable acidity of each sample.

Total anthocyanin content: It was measured in berry skin at 535 nm using spectrophotometer according to Hsia *et al.* (1965). The content of total anthocyanin in berry skin was calculated using the following equation:

$$\text{Total anthocyanin content (mg/100 g)} = \frac{\text{Total absorbance 100 g}^{-1}\text{ skin}}{98.2\ (E)}$$

The E value for 1% solution at 535 nm is equal to 98.2. Therefore, the absorbance of a solution containing 1 mg is equal to 98.2 according to (Ranganna, 1979).

Total sugar: The extract was prepared by taking 0.5 g of fresh berries and extracting the same with 80% ethanol by centrifuging three times. The supernatant was collected and measured quantity of distilled water was added to it and heated until all ethanol got evaporated. Then volume of sample was made up to 150 mL by adding distilled water. The total sugar was estimated using anthrone's reagent (Ranganna, 1979). The 1 mL of alcoholic extract was taken in a test tube and chilled. After a while 4 mL of anthrone's reagent was carefully run down the walls of the test tube. The test tubes were thereafter immersed in ice water. The tubes were brought to ambient temperature and boiled in water bath for 10 min. After proper cooling, the absorbance was measured at 625 nm.

Statistical analysis: Data of both seasons of the study were designed and analyzed as completely randomized block design by using analysis of variance (ANOVA), with two factors; time and temperature. Differences among treatment means were statistically analyzed by using the least significant differences test (LSD) at p = 0.05%, means separation using the CoStat program.

RESULTS AND DISCUSSION

This study was to estimate the effect of postharvest treatments by BABA on fruits quality and decreasing decay of Crimson Seedless grape fruits during storage.

Effect of BABA on growth of fungi isolated from Crimson Seedless grape and disease infection percentage: Data in Table 1 show the effect of in BABA on linear growth and dry weight of *Botrytis cinerea* isolated from Crimson Seedless grape. It was noticed that the reduction in linear growth and dry weight were correlated to the increase in BABA concentrations. BABA at 300 ppm treatment completely inhibited the linear growth and dry weight of *Botrytis cinerea*. This result is in agreement with the finding of Latifa *et al.* (2011) on citrus who reported complete inhibition of mycelia growth of *Penicillium itelicum* which was generally associated with complete inhibition of sporulation by organic acids and salts.

Data in Table 2 showed the effect of BABA on disease infection of Crimson Seedless grape during cold storage conditions through 60 days at 0±1°C with 90-95% RH and 3 days as marketing at room temperature. In both seasons, prolonging the marketing stage resulted in decreased in disease infection with the increase of BABA concentrations. BABA at 300 ppm treatment gave complete reduction in disease infection caused by *Botrytis cinerea*.

Some fruits, apples among them, are usually stored after harvest. During cold storage, losses of economic importance are produced by several decays due to fungal rot. *Penicillium expansum* and *Botrytis cinerea* are well-known postharvest pathogens. They produce blue and gray rots, respectively (Calvo *et al.*, 2007).

Table 1: Effect of BABA on linear growth and dry weight (g) of *Botrytis cinerea* isolated from Crimson Seedless grape

Treatments	Linear growth (cm)	Dry weight (g)
Control	9.0000	1.2600
BABA 200 ppm	4.0400	0.8200
BABA 250 ppm	2.5900	0.4900
BABA 300 ppm	0.0000	0.0000
LSD (0.05)	1.1535	0.0999

Table 2: Effect of BABA on disease infection percentage by *Botrytis cinerea* of Crimson Seedless grape stored for 60 days at cold storage during 2012 and 2013 seasons

	Season											
	2012						2013					
Treatments	0	15	30	45	60	Marketing (3 days)	0	15	30	45	60	Marketing (3 days)
Control	0.00	5.99	20.19	43.17	51.21	54.33	0.00	5.07	16.88	34.88	43.35	46.78
BABA 200 ppm	0.00	3.96	12.90	16.42	19.73	22.64	0.00	3.32	9.87	13.93	17.51	18.08
BABA 250 ppm	0.00	3.13	7.31	9.69	11.62	13.41	0.00	2.67	5.83	7.03	9.69	12.30
BABA 300 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD (5%)												
Treatment	0.8087						0.7992					
Time	0.3270						0.3221					
Treatment/time	0.6539						0.6441					

Table 3: Effect of pre-harvest BABA applications on Crimson Seedless grape characteristics at harvest during 2012 and 2013 seasons

	Berry adherence (gf)		Berry firmness (g cm^{-2})		Soluble solids content (%)	
Treatments	2012	2013	2012	2013	2012	2013
Control	830.00	818.00	820.00	805.00	18.460	18.160
BABA 200 ppm	868.00	858.00	832.00	826.00	19.200	18.900
BABA 250 ppm	853.00	848.00	836.00	830.00	19.400	19.100
BABA 300 ppm	892.00	887.00	840.00	840.00	19.600	19.260
LSD (5%)	7.18	10.56	7.14	7.96	0.382	0.635
	Acidity (%)		Anthocyanin (mg/100 g fw)		Total sugar (%)	
Treatments	2012	2013	2012	2013	2012	2013
Control	0.631	0.621	96.600	101.400	16.50	16.060
BABA 200 ppm	0.630	0.621	101.300	105.600	17.00	16.500
BABA 250 ppm	0.631	0.624	105.630	107.660	17.20	17.000
BABA 300 ppm	0.634	0.626	109.000	110.000	17.30	17.000
LSD (5%)	0.004	0.006	0.475	0.411	0.31	0.492

This result is in agreement with the finding of Troncoso-Rojas and Tiznado-Hernández (2007) on fruits and vegetables by chemical alternatives to conventional fungicides for postharvest disease control should be natural or synthetic compounds with known and minimal toxicological effects on mammals and the environment. The origin of these alternatives includes classifications such as food additives and substances listed as "Generally Regarded as Safe" (GRAS) by the United States Food and Drug Administration, natural compounds obtained from plants, animals or microorganisms, some volatiles and essential oils, phenolic compounds, plant extracts, peptides, alkaloids, lectins, antibiotics, propolis, latex or chitosan and other chemicals such as calcium polysulfide or ammonium molybdate.

Fungicide application is now the most efficacious method for controlling plant diseases caused by oomycetes and fungi. As legislation is limiting and reducing their use, this is strongly stimulating studies for the identification of additional and environmentally friendly approaches in the control of their associated diseases. Among these, induced resistance offers the prospect of long-lasting, broad-spectrum disease control through activation of the resistance defense machinery of the plant itself. At least three types of systemic induced resistance have been described that are effective against biotrophic and necrotrophic oomycetes and fungi: Systemic acquired resistance induced systemic resistance and β-aminobutyric-acid-induced resistance. The phytohormones salicylic acid, ethylene, jasmonic acid and abscisic acid are involved in the pathways that characterize these forms of resistance. Through crosstalk between the induced resistance pathways, it is likely that plants can minimize energy costs and create a flexible signaling network that allows fine tuning of the plant defense response to invaders. Possible resistance mechanisms to oomycetes and fungi during induced resistance are described here and some examples of the application of induced resistance for the control of field and postharvest diseases of plants are reported and briefly discussed (Buonaurio *et al*., 2009).

Effect on characteristics of Crimson Seedless grape at harvest: Regarding to the effect of pre-harvest BABA applications on Crimson Seedless grape characteristics at harvest during the two seasons of 2012 and 2013 data are presented in Table 3.

Berry adherence gram-force (gf): With regard to the effect on berry adherence, the data in Table 3 reveal that, all applied treatments gave significant effect in this respect during both seasons. However, berry adherence was significantly higher by sprayed clusters, BABA 300 ppm (892 and 887 gf) in the two seasons. While, BABA at 200 ppm application increased the berry adherence (868 and 858 gf) in both seasons than those applied with BABA at 250 ppm (853 and 848 gf) during both seasons.

Berry firmness (g cm^{-2}): It is clear from Table 3 that BABA treatments gave significant differences on the berry firmness at harvest than the untreated clusters during both seasons Furthermore, BABA (300 ppm) gave higher berry firmness (840 g cm^{-2}) than the other treatments used during both seasons. BABA at 250 ppm increased the firmness (836 and 830 g cm^{-2}) in both seasons than those applied with at 200 ppm (832 and 826 g cm^{-2}) during both seasons.

Soluble solids content (SSC) (%): Regarding the effect of pre-harvest BABA applications on SSC (%) of Crimson Seedless grape, data from Table 3 revealed that all treatments gave no significant values of SSC (%) in berry juice during both seasons. BABA at 300 ppm gave somewhat higher values of SSC in berry juice (19.60 and 19.26%) during the two seasons under the study. The data also disclose that, BABA 250 ppm application increased SSC (19.40 and 19.10%) than 200 ppm BABA (19.20 and 18.90%) in both seasons.

Titratable Acidity (TA%): Data from Table 3 demonstrate that no significant differences between BABA treatments or control on the value of juice acidity at harvest during both seasons. While, BABA at 300 ppm gave a higher value of total acidity in fruit juice (0.634 and 0.626%) than all treatments used during the two seasons under the study.

Total anthocyanin content: With regard to the effect on anthocyanin content, the data also in Table 3 reveal that, all treatments used gave a somewhat increment in the values of anthocyanin in berry skin than the control in both seasons. However, BABA at 300 ppm application gave higher significant anthocyanin content (109.0 and 110.0 mg/100 g fw) during both seasons, respectively. Also, clusters sprayed with BABA at 250 ppm increased anthocyanin content (105.63 and 107.66) than BABA 200 at ppm (101.30 and 105.60 mg/100 g fw) during both seasons under the study. From the obtained data it is obvious that a good relation between soluble solids and anthocyanin content, since, the treatment which increased soluble solids produced clusters with higher anthocyanin content.

Total sugar (%): The data also in Table 3 disclose that, all treatments increased the values of total sugars insignificantly than the untreated clusters during the two seasons under the study. While, BABA at 300 ppm application gave higher value of total sugar (17.30 and 17.0%) during both seasons, respectively. Also, clusters sprayed with BABA at 250 ppm increased sugar content (17.20 and 17%) than at 200 ppm (17 and 16.50%) during both seasons under the study.

Effect on characteristics of Crimson Seedless grape under cold storage and through marketing at room temperature:

Cluster weight loss (%): Data from Tables 4 and 5 showed that the percent of clusters weight loss significantly increased gradually with the progress of storage period and during marketing. The data also disclose that all applied treatments significantly reduced the percent of loss in fruit weight than the control under cold storage or held at room temperature. Since, the percent of loss in fruit weight at the untreated clusters were 5.36 and 5.73 after 60 days of cold storage and it were 8.33 and 8.07 after three days as marketing in both seasons, respectively.

The lowest significant values of weight loss percentage were recorded by applications of BABA at 300 ppm ranged 3.91 and 4.11 after 60 days of cold storage and 4.12 and 4.81 after 3 days as marketing in the two seasons, respectively.

Grapes are non-climacteric fruits with a relatively low physiological activity and are subject to serious water loss and softening after harvest which can result in stem browning, berry shatter, wilting, shriveling of berries (Crisosto *et al*., 2002).

Water loss is strictly a physical factor related to the evaporative potential of the surrounding air. The higher the evaporative potential of the air surrounding the fruit, the more water is lost from the fruit. This relationship may be expressed directly as the vapor pressure deficit a term which indicates the combined influence of temperature and relative humidity (Ngcobo *et al*., 2012).

Berry decay (%): It is clear from Tables 3 and 4 that the most treated fruits did not present any decayed berries till

Table 4: Effect of pre-harvest BABA applications on weight loss, decay Shatter and total loss of Crimson Seedless grapes under cold storage during 2012 and 2013 seasons

	Storage period (days)									
	Weight loss (%)					Decay (%)				
Treatments	0	15	30	45	60	0	15	30	45	60
Season 2012										
Control	0.000	1.44	2.90	4.83	5.36	0.000	0.61	2.63	3.00	4.48
BABA 200 ppm	0.000	1.31	2.83	3.91	4.93	0.000	0.00	0.81	1.14	1.22
BABA 250 ppm	0.000	1.12	2.17	3.24	4.65	0.000	0.00	0.95	0.81	1.21
BABA 300 ppm	0.000	0.99	2.16	2.71	3.91	0.000	0.00	0.00	0.62	0.93
Mean	0.000	1.21	2.51	3.67	4.71	0.000	0.15	1.09	1.39	1.96
LSD (5%)										
Treatment	0.034					0.013				
Time	0.036					0.013				
Treatment/time	0.073					0.026				
Season 2013										
Control	0.000	1.27	3.15	4.65	5.73	0.000	0.00	2.00	2.50	4.96
BABA 200 ppm	0.000	1.11	2.70	3.78	5.12	0.000	0.00	0.73	1.99	2.13
BABA 250 ppm	0.000	1.05	2.25	3.45	4.36	0.000	0.00	0.58	1.15	1.74
BABA 300 ppm	0.000	1.01	1.91	3.02	4.11	0.000	0.00	0.38	1.03	1.38
Mean	0.000	1.11	2.50	3.72	4.83	0.000	0.00	0.92	1.67	2.55
LSD (5%)										
Treatment	0.018					0.014				
Time	0.019					0.015				
Treatment/time	0.038					0.031				
	Shatter (%)					Total loss (%)				
Treatments	0	15	30	45	60	0	15	30	45	60
Season 2012										
Control	0.000	0	1.64	3.12	5.17	0.000	2.05	7.17	10.96	15.01
BABA 200 ppm	0.000	0	0.81	2.12	4.12	0.000	1.31	4.45	7.17	10.27
BABA 250 ppm	0.000	0	0.40	1.66	3.17	0.000	1.12	3.52	5.72	9.04
BABA 300 ppm	0.000	0	0.11	1.00	2.11	0.000	0.99	2.27	4.33	6.95
Mean	0.000	0	0.74	1.97	3.64	0.000	1.37	4.35	7.04	10.32
LSD (5%)										
Treatment	0.012					0.059				
Time	0.016					0.039				
Treatment/time	0.033					0.078				
Season 2013										
Control	0	0	2.12	4.01	6.87	0.000	1.27	7.28	11.17	17.56
BABA 200 ppm	0	0	1.18	2.91	5.12	0.000	1.11	4.62	8.69	12.38
BABA 250 ppm	0	0	1.00	2.33	4.02	0.000	1.05	3.83	6.93	10.13
BABA 300 ppm	0	0	0.65	2.11	2.61	0.000	1.01	2.96	6.17	8.10
Mean	0	0	1.24	2.84	4.65	0.000	1.11	4.67	8.24	12.04
LSD (5%)										
Treatment	0.023					0.025				
Time	0.015					0.031				
Treatment/time	0.031					0.063				

15 days of cold storage except control clusters in the second season. Thus, the percent of decayed berries for the untreated clusters were 4.48 and 4.96% after 60 days of cold storage but it reached about 7.08 and 6.31% through marketing in both seasons.

Furthermore, clusters treated with BABA at 300 ppm gave a lower percent of decayed berries after 60 days of cold storage (0.93 and 1.38%) but it were 3.80 and 3.21% during marketing in both seasons, respectively. Zhang *et al.* (2011) instituted that the effects of BABA on blue mould caused by *Penicillium expansum* in apple fruit stored at 25°C were investigated. BABA provided an effective control and strongly inhibited spore germination, germ and tube elongation of *P. expansum in vitro*. Using Propidium Iodide (PI) staining combined with fluorescent microscopy, it was found that the plasma membrane of BABA-treated *P. expansum* spores was damaged and the leakage of protein and sugar was significantly higher in BABA-treated mycelia than in the control. BABA treatment induced a significant increase in the activities of chitinase, β-1, 3-glucanase and peroxidase in apple fruit. These findings suggest that the effects of BABA on blue

Table 5: Effect of pre-harvest BABA applications on weight loss, decay, Shatter and total loss of Crimson Seedless grape 3 days at marketing in room temperature after 60 days of cold storage during 2012 and 2013 seasons

	Weight loss (%)		Decay (%)		Shatter (%)		Total loss (%)	
Treatments	2012	2013	2012	2013	2012	2013	2012	2013
Control	8.330	8.070	7.080	6.310	7.530	8.64	20.980	23.030
BABA 200 ppm	6.130	6.130	4.880	4.430	5.190	6.15	15.940	16.450
BABA 250 ppm	6.000	5.380	4.370	4.170	5.060	5.22	15.700	15.040
BABA 300 ppm	4.120	4.810	3.800	3.210	3.630	3.12	11.560	11.150
LSD (5%)	0.108	0.058	0.072	0.038	0.068	0.04	0.165	0.049

mould in apple fruit may be associated with the direct fungitoxic property against the pathogens and the elicitation of defense-related enzymes in fruit.

Berry shatter (%): Berry shatter is mainly triggered by mechanical damage occurring during harvesting, packaging and transportation. As shown in Tables 4 and 5 the berry shatter increased significantly with the advanced in storage period. In all the cases, excessive water loss leads to berry shatter. However, clusters sprayed with BABA at 300 ppm evaluated low level of berry shattering (2.11-2.61%) closely followed by clusters treated with BABA at 250 ppm (3.17-4.02%) after 60 days of cold storage during both seasons, respectively. Whereas, berry shatter was very high in control clusters presented 5.17 and 6.87%, respectively.

Additionally, BABA 300 at ppm reduced the percent of berry shattering (3.63 and 3.12%) significantly than all treatments used or the control, while the untreated ones gave 7.53 and 8.64% after 3 days during marketing in both seasons, respectively.

There appear to be three types of berry shatter; physiological, pathological and mechanical. The first is associated with the thickening and hardening of the pedicel and production of an abscission layer. The increase of berry shatter during shelf life has been recommended the pedicle and stalk of cluster behave in a climacteric process showing respiration and ethylene peaks (Fayed, 2010).

Total loss in cluster (%): Total loss in cluster is mainly due to the cluster loss weight, berry shattering and decayed berries which are presented in Tables 3 and 4 from these data, it is clear that the total loss in cluster gradually increased as storage period advanced.

Moreover, all the applied treatments reduced the percent of total loss in cluster than the untreated clusters. Yet, the percent of total loss in untreated clusters were 15.01 and 17.56% under 60 days of cold storage and reached 20.98 and 23.03% after three days at marketing in the both seasons, respectively.

BABA at 300 ppm reduced the total loss in clusters than the other treatments used either at 60 days from cold storage or during marketing at the same time. Yet, the percentage of total loss in clusters due to use BABA at 300 ppm was about 6.95 and 8.10 after 60 days of cold storage but reached about 11.56 and 11.15% during marketing, respectively through the two seasons. Since, this treatment was more effective for reducing the percentage of decayed and shattering berries. Moreover, BABA at 250 ppm application was more effective in reducing the percent of total loss in clusters compared with BABA at 200 ppm during both seasons. The percentage of total loss due to this treatment was about 9.04 and 10.13% after 60 days of cold storage and reached 15.70 and 15.04% during marketing in both seasons, respectively.

A possible explanation that such a high concentration of BABA is needed to elicit disease resistance is probably that only a small portion of the compound is actually absorbed by the tissue and also that of the entire BABA compound mixture only a small portion of its R-anantiomer isoform was found to be active in inducing pathogen resistance (Cohen, 2001).

Soluble Solids Content (SSC) (%): Regarding to the effect of pre-harvest BABA applications on SSC (%) of Crimson Seedless grape data from Tables 6 and 7 revealed that the percent of SSC in berry juice was gradually increased as a storage period advanced either at cold storage or during marketing. Since, all treatments gave somewhat higher values of SSC in berry juice than the untreated fruit during the two seasons under the study.

The data also disclose that, BABA at 300 ppm increased SSC (%) (20.50 and 20.30%) than the other treatments used or the control during cold storage, while reached 20.90 and 20.70 during marketing, respectively through the two seasons.

Khan *et al.* (2012) confirmed that increase in TSS may be related with enzymes which are presented when amino acids enhanced the synthesis of different proteins, acids and sugars. This explanation is in the line

Table 6: Effect of pre-harvest BABA applications on SSC, titratable acidity and total sugar of Crimson Seedless grape under cold storage during 2012 and 2013 seasons

	Storage period (days)														
	Soluble solids (%)					Acidity (%)					Total sugar (%)				
Treatments	0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
Season 2012															
Control	18.46	18.90	18.50	18.00	19.00	0.631	0.640	0.644	0.661	0.671	16.50	16.70	16.53	16.50	16.90
BABA 200 ppm	19.20	19.20	19.60	19.70	20.06	0.630	0.643	0.646	0.663	0.670	17.00	17.00	17.00	17.30	18.00
BABA 250 ppm	19.40	19.50	19.43	19.66	20.36	0.631	0.650	0.666	0.683	0.700	17.20	17.53	17.53	17.60	18.26
BABA 300 ppm	19.60	19.70	19.86	19.90	20.50	0.634	0.656	0.673	0.690	0.709	17.30	17.33	17.00	17.90	18.30
Mean	19.16	19.32	19.35	19.31	19.98	0.631	0.647	0.657	0.674	0.687	17.00	17.14	17.01	17.32	17.86
LSD (5%)															
Treatment	0.112					0.002					0.184				
Time	0.166					0.002					0.180				
Treatment/time	0.332					0.004					0.361				
Season 2013															
Control	18.16	18.40	18.66	18.60	18.83	0.621	0.633	0.639	0.650	0.667	16.06	16.26	16.33	16.30	16.63
BABA 200 ppm	18.90	18.93	19.33	19.26	19.70	0.621	0.645	0.661	0.669	0.680	16.50	16.60	16.60	16.73	16.96
BABA 250 ppm	19.10	19.10	19.13	19.96	20.20	0.624	0.648	0.669	0.681	0.703	17.00	17.13	17.10	17.60	18.00
BABA 300 ppm	19.26	19.40	19.50	19.53	20.30	0.626	0.652	0.674	0.689	0.700	17.00	17.00	17.13	17.80	18.00
Mean	18.85	18.95	19.15	19.34	19.75	0.623	0.644	0.660	0.672	0.687	16.64	16.75	16.79	17.10	17.40
LSD (5%)															
Treatment	0.185					0.001					0.227				
Time	0.156					0.002					0.222				
Treatment/time	0.312					0.002					0.445				

Table 7: Effect of pre-harvest BABA applications on SSC, titratable acidity and total sugar of Crimson Seedless grape 3 days at marketing in room temperature after 60 days of cold storage during 2012 and 2013 seasons

	Soluble solids (%)		Acidity (%)		Total sugar (%)	
Treatments	2012	2013	2012	2013	2012	2013
Control	19.33	19.00	0.694	0.680	17.20	16.90
BABA 200 ppm	20.00	20.00	0.696	0.691	18.20	17.03
BABA 250 ppm	20.70	20.53	0.718	0.710	18.50	18.20
BABA 300 ppm	20.90	20.70	0.724	0.716	18.80	18.50
LSD (5%)	0.613	0.475	0.004	0.004	0.512	0.512

with using foliar application of green algae extract on superior grapes which significantly improved TSS synthesis (Abd El Moniem and Abd-Allah, 2008).

Titratable Acidity (TA) (%): The acidity level is a very important quality factor in table grapes and those used for wine production. Consumer acceptance of table grapes and grape juice is strongly influenced by the sweetness to acid balance.

Data from Tables 6 and 7 demonstrate that the most treatments used gave a somewhat increment of total acidity in berry juice than the control after 60 days of cold storage.

Application of BABA at 300 ppm increased the percent of titratable acidity in berry juice than the other treatments or the untreated ones especially in the first season that presented 0.709% after 60 days of cold storage and ranged 0.724 and 0.716% three days during marketing. Whereas, BABA at 250 ppm treatment produce a higher value of this trend at cold storage in the second season realized 0.703%. On the other hand, all treatments used gave a higher value of titratable acidity in berry juice than the control clusters through marketing conditions.

All treatments increased acidity in fruits due to amino acids enhanced synthesis of different acids. This finding is in agreement with exogenous application of BABA which increased acidity of treated fruits. Similarly, foliar applications of mixture of amino acids and *Ascophylum nodosum* (Seaweed) amino acids enhanced acidity in Perlette grapes (Khan *et al*., 2012).

Total sugar (%): Considering to the effect on total sugar, data in Tables 6 and 7 reveal that the values of total sugar were progressively decreased by the storage period advanced from harvest till 60 days either at cold storage or during marketing at room temperature.

With regard to the effect of these treatments on total sugar the data reveal that, clusters treated with BABA 300 ppm produced a higher value of total sugar at cold storage since the values averaged about 18.30 and 18.0% while during marketing application gave a higher value of this trend (18.80 and 18.50%) under the two seasons, respectively.

BABA at 250 ppm application increased the percent of total sugar in cluster juice (18.26 and 18.0%) than those treated with BABA at 200 ppm (18.0 and 16.96%) after 60 days of cold storage during both seasons.

Table 8: Effect of pre-harvest BABA applications on berry adherence, berry firmness and anthocyanin content of Crimson Seedless grapes under cold storage during 2012 and 2013 seasons

	Storage period (days)														
	Berry adherence (gf)					Berry firmness (g cm^{-2})					Anthocyanin (mg/100 g fw)				
Treatments	0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
Season 2012															
Control	830	794	764	690	653	820	773	733	694	644	96.60	90.03	83.40	71.33	77.16
BABA 200 ppm	868	809	782	715	682	832	793	760	699	667	101.30	94.70	90.00	88.20	85.00
BABA 250 ppm	853	820	796	751	706	836	788	770	710	683	105.63	98.40	93.50	90.10	89.30
BABA 300 ppm	892	848	819	788	726	840	780	756	702	673	109.00	102.73	98.00	93.90	90.50
Mean	861	818	790	736		832	784	755	701	667	103.13	96.46	91.22	85.88	85.49
LSD (5%)															
Treatment	2.87					4.67					0.206				
Time	3.33					2.85					0.224				
Treatment/time	6.66					5.71					0.448				
Season 2013															
Control	818	781	760	682	647	805	763	713	678	624	101.40	98.33	91.63	84.83	80.30
BABA 200 ppm	858	800	778	707	677	826	770	739	694	656	105.60	97.30	93.50	90.06	89.53
BABA 250 ppm	848	814	795	747	701	830	793	741	705	686	107.66	100.00	97.40	94.30	90.73
BABA 300 ppm	887	844	809	776	713	840	790	731	691	670	110.00	103.90	99.63	97.60	93.83
Mean	853	810	786	728	684	825	779	731	692	659	106.16	99.88	95.54	91.70	88.60
LSD (5%)															
Treatment	3.50					2.86					0.337				
Time	3.78					2.69					0.248				
Treatment/time	7.56					5.38					0.497				

Table 9: Effect of pre-harvest BABA applications on berry adherence, berry firmness and anthocyanin content of Crimson Seedless grape 3 days at marketing in room temperature after 60 days of cold storage during 2012 and 2013 seasons

	Berry adherence strength (gf)		Berry firmness (g cm^{-2})		Anthocyanin (mg/100 g fw)	
Treatments	2012	2013	2012	2013	2012	2013
Control	618.00	592.00	584.00	570.00	66.50	72.100
BABA 200 ppm	644.00	624.00	594.00	586.00	72.00	81.700
BABA 250 ppm	657.00	639.00	624.00	615.00	83.70	83.660
BABA 300 ppm	697.00	680.00	619.00	595.00	83.00	85.930
LSD (5%)	7.27	5.87	7.90	4.67	5.46	0.324

It was reported that the accumulation of sugars in grapes preceding the rise in anthocyanin content, thus a strong correlation is found between the accumulation of certain amount of sugars in the berries and formation of anthocyanin (Pirie and Mullins, 1980). Furthermore, it has been found that the percentage of sugar has a triggering effect on anthocyanin accumulation.

Berry adherence strength (gf): It evident from Tables 8 and 9 that berry adherence was gradually reduced significantly as storage period advanced. Also, all sprayed treatments improved berry adherence than the control. However, berry adherence was significantly higher at clusters sprayed with BABA at 300 ppm that attained 726 and 713 gf till 60 days under cold storage since reached 697 and 680 gf during marketing through both seasons, respectively.

While, the decrement value of berry adherence was found of control clusters which reached 653 and 647 gf after 30 days under cold storage but arranged 618 and 592 gf during marketing through both seasons, respectively.

BABA at 250 ppm offered higher berry adherence (706 and 701 gf) after 60 days under cold storage and 3 days during marketing (657 and 639 gf) than BABA 200 ppm in both seasons under the study.

BABA enhanced the activities of antioxidant enzymes and non-enzymatic antioxidants. Accumulation of ABA transcription and signaling factors in BABA-treated Arabidopsis might prime it for ABA accumulation (Jakab *et al*., 2005). According to Zimmerli *et al.* (2001), BABA primes Arabidopsis plants to respond quicker and stronger to biotic and abiotic stresses. Recent studies revealed that BABA enhances mRNA Accumulation of Abscisic Acid (ABA) and ethylene early signaling intermediates.

Berry firmness (g cm^{-2}): Data in Tables 8 and 9 showed that all treatments had the highest effects on berry firmness comparing with control. Since, berry firmness was gradually decreased during marketing as storage period advanced. However, the reduction in berry firmness was higher during marketing at room temperature than at cold storage. Since, the values of

berry firmness of the control clusters were 644 and 624 g cm^{-2} after 60 days under cold storage but reached 584 and 570 g cm^{-2} during marketing through both seasons respectively.

Meanwhile, BABA 250 ppm application presented higher berry firmness than all treatments used or the control at 60 days of cold storage (770 and 741 g cm^{-2}) or during marketing at room temperature which ranged 624 and 615 g cm^{-2} in both seasons. BABA 300 ppm treatment gave a higher significant increment in berry firmness (673 and 670 g cm^{-2}) after 60 days of cold storage or 3 days during marketing (619 and 595 g cm^{-2}) than clusters treated with BABA 200 ppm application (667 and 656 g cm^{-2}) till 60 days under cold storage and 3 days during marketing (594 and 586 g cm^{-2}) in both seasons.

Firmness of BABA is increased by increasing Ca^{2+} content. Probably, BABA activates ABA signaling and ABA stimulates the increases in cytosolic Ca^{2+} by inducing both Ca^{2+} influx from the extracellular space and Ca^{2+} release from intracellular stores. ABA-induced increases in the activities of a can be prevented by the antioxidant enzymes pretreatments with the Ca^{2+} chelator indicating the involvement of Ca^{2+} in ABA-induced antioxidant defense (Jakab *et al*., 2005).

Total anthocyanin content: Tables 8 and 9 showed the changes in total anthocyanin content in the skin of Crimson Seedless grape were gradually reduced from harvest till 60 days either at cold storage or 3 days at room temperature as marketing after cold storage. Also, all treatments applied increased the content of anthocyanin in berry skin than the control under cold storage or at room temperature.

Moreover, BABA at 300 ppm application significantly increased the content of anthocyanin in berry skin (90.50 and 93.83 mg/100 g fw) after 60 days under cold storage and 3 days during marketing (83.70 and 85.93 mg/100 g fw) than the other treatments used or the control during both seasons.

However, control clusters presented lower values of anthocyanin in berry skin (80.30 and 77.16 mg/100 g fw) after 60 days of cold storage or 3 days during marketing (66.50 and 72.10 mg/100 g fw) through the both seasons.

Anthocyanins are colored pigments, thus the manipulation of anthocyanin production in grapes, is potentially a mean of influencing the visual perception of color in the fruit (Mori *et al*., 2005). Also, anthocyanins are plant secondary metabolites providing pigmentation to flowers, fruits, seeds and leaves. Guidoni *et al.* (2002) reported that anthocyanin concentration of grapes is an important fruit quality parameter for marketing. Anthocyanins contribute to grape quality by effecting both color intensity and color quality.

Anthocyanin considers one of the important flavonoids classes. Red and black grapes owe their attractive color to their anthocyanin pigments. Moreover, anthocyanin levels and the other flavonoid classes in grape skin are parameters available for evaluating grape quality.

It has been shown, BABA stimulate anthocyanin biosynthesis by regulating the expression of CHS (chalcone synthase) and DFR (dihydroflavonol-4-reductase) (Wu *et al*., 2010).

CONCLUSION

Based on the results of this study, it can be concluded that, the foliar applications of BABA had more pronounced effect on berry quality of Crimson Seedless grape than the control during cold storage at 0±1°C with 90-95% RH and through marketing at room temperature. Spraying 300 ppm BABA gave the best results for reducing total loss weight percentage, keeping the quality of berry during cold storage and during marketing at room temperature compared with the other treatments used or the control. Application of BABA in table grape possesses promising results in the control of grey mold as an alternative to SO_2 treatments.

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