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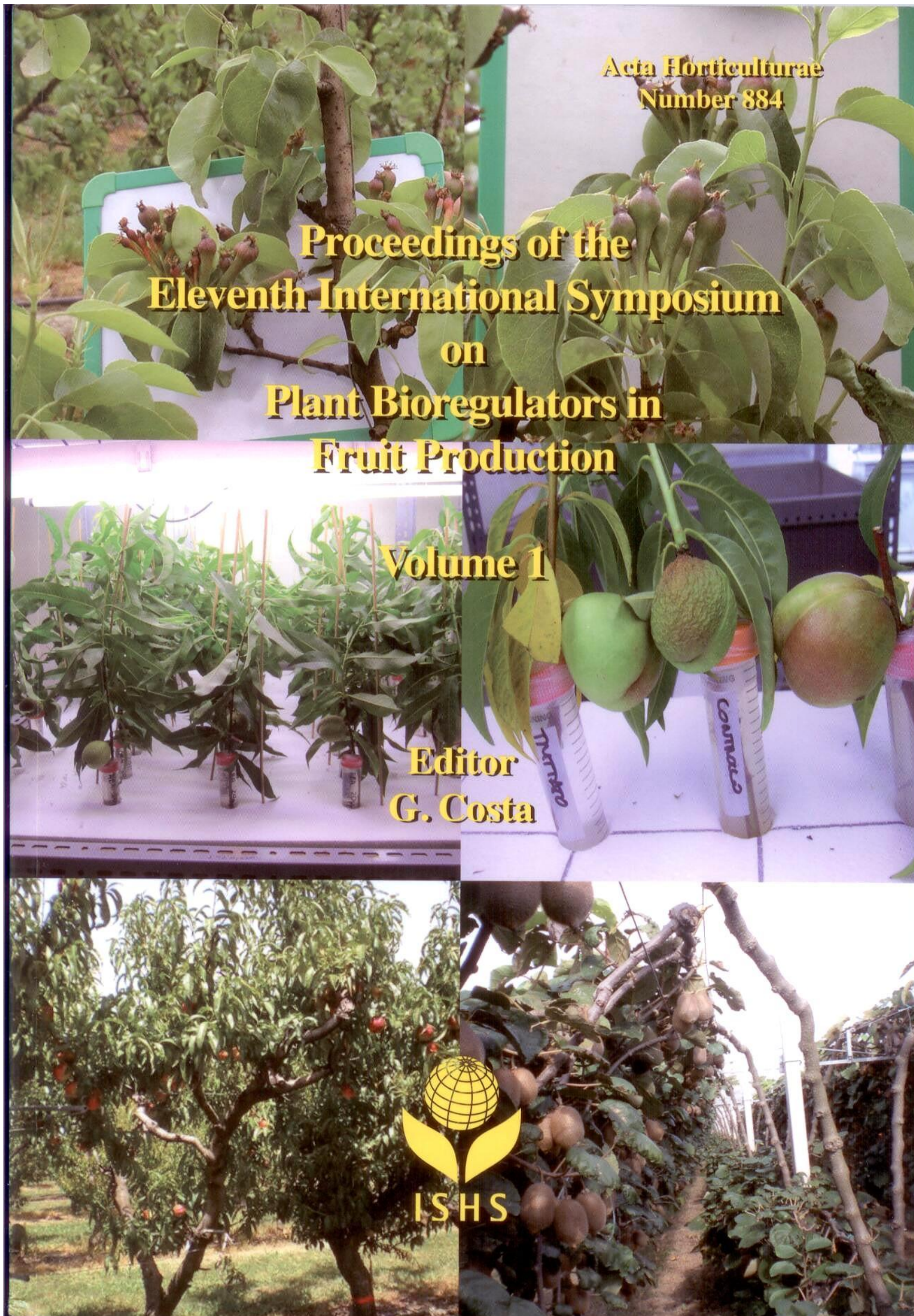

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Application of Ethephon and ABA at 40% Veraison Advanced Maturity and Quality of 'Beidaneh Ghermez' Grape

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Abstract

The market value of grape 'Beidaneh Ghermez', which is one of the most popular cultivar of table grapes in Iran, is dependant upon its desirable appearance (red color). Its color is relatively poor in Zanjan province, Iran. The research was conducted to study the effects of of ethephon and abscisic acid (ABA) at four dosages (0, 100, 200, 300 ppm) at 20 to 30% verasion of berries on skin color quality of 'Beidaneh Ghermez' during two seasons (2007-2008). Application of ABA was more effective than ethephon for enhancing the color and maintaining quality of fruits. The ABA application significantly increased berry total anthocyanin content. Total berry skin smart anthocyanin (extracted and absorbance at 520 nm) was associated with skin color. ABA-treated grapes were characterized by superior appearance both in berries and clusters, compared to ethephon-treated and un-treated control grapes. Grape treated with ABA showed higher firmness and soluble solids concentrations than the control. Firmness decreased with increasing ethephon dosages (up to 200 ppm). Berry size (berry weight) increased by ethephon application. Ethephon application enhanced fruit ripening, which is very important to avoid rain cracking.

INTRODUCTION

'Beidaneh Ghermez' is the most important and vastly planted cultivar of table grape (*Vitis vinifera* L.) in Zanjan province, Iran. This cultivar is a popular red table seedless grape, but fruits often fail to develop adequate red color. Poor berry color at the time of harvest, uneven ripening, inability of the growers to delay harvest because of early autumn rains are some problems that make 'Bidaneh' production less profitable. Various reasons for inferior color development in red table grapes have been reported (Yahuaca et al., 2006). Cultural practices that improve yield (berry size), such as irrigation, nitrogen application, shading, girdling and GA_3 application, reduce the color even more (Avenant and Avenant, 2006; Peppi et al., 2006; Cantín et al., 2007). For example, shading significantly reduced anthocyanin concentration in 'Emperor' grapes (Wicks and Kliewer, 1983). Red grapes fail to achieve the desired level of red color, in part due to high temperatures which inhibit the accumulation of anthocyanins (Spayd et al., 2002), the class of pigments that impart red color to grape berries (Peppi et al., 2006). Applications of the plant growth regulators (PGRs) gibberellic acid (GA_3) and forchlorfenuron (CPPU), which may be needed to increase berry size, can further inhibit coloring. Chervin et al. (2004) reported that ripening of grape delayed by application of 1-methylcyclopropene, a specific inhibitor of ethylene receptors. But, application of ethephon (2-chloroethylphosphonic acid) optimize the color of 'Crimson Seedless' grapes (Spayd et al., 2002). Leão and De Assis (1999) confirmed ethephon improved ripening and maturity, better fruit color, and hasten maturity of grapes. Similarly, ABA treatment improved the color of 'Flame Seedless' and 'Redglobe' grapes (Peppi et al., 2006).

Many studies have shown that ethephon increased phenylalanine-ammonia-lyase (PAL) activity in table grapes which is accompanied by increased color development. Ethephon treatments have also been shown to enhance gene expression for enzymes involved in anthocyanin biosynthesis such as UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT) with concomitant increases in anthocyanin accumulation in *Vitis*

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vinifera 'Cabernet Sauvignon' (El-Kereamy et al., 2003). Higher anthocyanin levels at harvest in ethylene-treated 'Cabernet Sauvignon' grapes were due to increased synthesis of anthocyanins, namely Mv-gluc (El-Kereamy et al., 2003). Therefore, it is important to determine whether the quality (red akin color) of 'Beidaneh Ghermez' grapes treated (at 20 to 30% of berry veraison with different concentrations) of ethephon and ABA, differed from that of grapes untreated control.

MATERIAL AND METHODS

Experiment was carried out with *Vitis vinifera* L. 'Beidaneh Ghermez' in a 15-year old commercial vineyard in Khoram-dareah, in Zanjan province, Iran, over two growing seasons (2007 to 2008). Ten vines were used per experimental unit within the row, but only the five central vines were used for determinations. The five replicates were arranged in a randomized complete block design. Grapevines in this experiment were trained to quadrilateral cordons, supported by an open gable trellis, and spur-pruned.

Ethephon (active ingredient of Ethrel®, a registered product of Aventis Acros Organics-174, France™), and abscisic acid (ABA) were applied at four dosages (0, 100, 200, and 300 ppm) were applied at a spray volume of 800 L ha^{-1} to bunches using a backpack pressure sprayer with hand lances to ensure thorough wetting. A standard buffering wetting agent, Bladbuff® 5, was added at recommended rates. Applications were made weekly for four weeks and commenced at the acid maximum (approximately one week before beginning of color or veraison).

Fruits from all treatments were harvested only once a year at the end of September. At harvest, all the harvested clusters were counted and weighed, and then each cluster was inspected and berries that were green-colored, or had other quality defects, were recorded. Number of and average weight of 100 berries (g), each cluster (g) and total yield (kg $vine^{-1}$) were recorded. Fifty berries were randomly sampled, and berry weight and berry quality attributes total soluble solid (TSS) was determined with a hand-held refractometer (Atago N1α). pH was measured with a pH meter (Horiba F-22) and titatable acidity was determined by titration with 0.1 N NaOH. The flesh firmness (kg cm^{-2}) was measured with an Effegi penetrometer (8 mm Plunger) in fruit samples consisting of 40 berries per treatment at harvest. Thirty berries were randomly selected from each sample after harvesting and frozen at -25°C until extraction. Two 10 mm skin disks were removed from the top and bottom of each berry using a cork borer and forceps, taking care not to include any pulp. The disks were placed in a glass bottle containing 20 ml acidified methanol (containing 1% hydrochloric acid v/v). These were extracted at room temperature 23±2ºC in darkness for one day, during which the bottles were gently shaken by hand every eight hours. After this period the contents were mixed for 5 s on a vortex mixer and allowed to settle for 40 min. The absorbance of a 5 ml aliquot was determined at 520 nm (total anthocyanins) and 280 nm (total phenols) using a spectrophotometer (LKB Biochrom Ultrospec II E Model 4057). The surface color of berries was measured according to Cantín et al. (2007). It was used by a reflectance colorimeter (CR-200, Minolta Inc., Tamsere, Japan), using the CIELAB color system. From these data, the color index of red grapes (CIRG; Carreño et al., 1995) was calculated as CIRG = $(180-h^{\circ})/(C^{*}+L^{*})$, where L^{*} is the lightness and corresponds to a black-white scale (0, black; 100, white), h° is the hue angle on the color wheel, and C^{*} is the chroma, a measure of the intensity of color, which begins at zero (achromatic) and increases in intensity. Three equidistant color measurements were made around the equator of each berry. Treatment bunches were collected and weighed separately at harvest. For analysis, 40 berries were collected at random from each bunch, 20 berries were frozen at -20ºC for later analysis of anthocyanins and the remaining 20 berries were weighed and then crushed by hand to extract the juice. All data were subjected to analysis of variance (ANOVA) procedures and means were separated, using Duncan multiple range test at P=0.05, using SAS-PC (ver. 6.12) software.

RESULTS

Application of ABA and ethephon significantly increased the anthocyanin absorption (A 520 nm), which was an indication of ripening/maturity and inducing better fruit color. Data with regard to yield and berry quality were collected during two seasons. As there were no seasonal interactions (regarding same dosages), the results for both ABA and ethephon over two seasons were sufficiently similar to be presented as combined averages and are presented in Tables 1 and 2. Significantly higher fruit yields, and heavier berries were recorded in response to foliar application of ethephon (Table 1). High yield was associated with larger berries, which resulted in greater fruit weight per bunch under 300 ppm ethephon, followed by 200 ppm ethephon, compared to ABA treatments and untreated vines (Table 1). The number of loose berries increased with applications of higher than 200 ppm, (Table 2). It is notable that grapes treated with ABA had the higher the CIRG color index than other treatments. With respect to color, C^* was the only variable that differed among treatments; fruits treated with 300 ppm ABA had a lower C^* than other treatments (Table 2). The greater C^*, the less pure color (Cantin et al., 2007). Based on the CIRG color index, all grapes could be classified as pink-colored ($2<CIRG<4$) (Carreño et al., 1995).

Irrespective of growth season, different dosages of PGRs did not affect on sugar accumulation (TSS/°Brix) (Table 2), however, berry firmness, and berry visual appearance were improved (Fig. 2). Grapes treated with 300 ppm ABA were harvested at the lowest TSS, compared to non-treated grapes (Table 2). Grapes treated with 300 ppm ABA colored quickly and higher than grapes treated with ethephon (Table 2). The ABA-application had the most significant effect on total anthocyanins concentration. All dosages produced a significantly higher total anthocynins than the ethephon treated grapes. Dosages of 300 ppm produced the highest amount of total anthocynins (Fig. 1), however, increasing ABA dosages tended to increase the occurrence of loose berries and berry crack (data not shown). Exogenous applications of ABA increased the total anthocyanins of grape skins (Fig. 1). Grapes having high skin anthocyanin content will appear more red-colored, than grapes having low anthocyanin content (Table 2). ABA application reduced the excess vegetative growth and it caused a better light penetration to vine and more uniform maturation (data not shown). Direct effects of ABA on fruit maturation, coloration, and bunch properties, are of significant importance for maintaining quality at high yields.

DISCUSSION

Endogenous ethylene is of questionable effect in grape maturation since its production rapidly declines after anthesis and remains at low levels throughout fruit ripening (Chervin et al., 2004). Therefore, application of exogenous fruit-ripening (ABA and ethephon) stimulates coloration and maturation of cultivar 'Beidaneh Ghermez' grape. PGRs have being used successfully to improve color of many *Vitis vinifera* cultivars (Wicks and Kliewer, 1983; Peppi et al., 2006). The effects of PGRs on fruit maturity and composition are well documented in literature but the results are variable, it depends on cultivar and environmental condition. It is important to remember that different table grape cultivars will, however, differ in their response to different dosages of PGRs due to their unique genetic, rootstock (which will affect growth vigor and thus response to PGRs) and cultivation differences. Crop load, climate and vigor also have an influence on the efficacy of the treatment. Application of ABA and ethylene for 'Beidaneh Ghermez' at 20 to 30% of verasion improved coloration.

It can be inferred that ABA and ethephon may be related to anthocyanin formation by expression of UDP-glucose flavonoid 3-O-glucosyltransferase (*UFGluT*) anthocyanin biosynthetic gene (Cantin et al., 2007). On the other hand, no changes in juice quality (TSS or acidity) have been noted, and no or little change in pH, but total yield and weight per berry have been increased due to their application (Tables 1 and 2). The ripening response observed in this study is therefore in agreement with literature to date (Wicks and Kliewer, 1983; Price et al., 1995).

The most desirable results, based on quality berry color and other berry quality, were obtained with ABA, rather than ethephon applied at two weeks after acid maximum (30 to 40% color). This is later than results obtained by Jensen et al. (1975) on 'lame Seedless' who obtained best results with an application, with a dosage of 225 mg/L ethephon. Timing of treatments closer to harvest did, however, increase the risk of quality defects such as berry crack, soft berries and loose berries. The development of a model to accurately predict the natural development of anthocyanins in a specific cultivar, and to prescribe the necessary PGRs dosage and timing would be of great benefit.

This research has shown a grape ripening-dependent effect on the response of anthocyanin accumulation to PGRs application. For red table grapes, the response of anthocyanin accumulation have been shown to be positive sensitive to ABA and less positive response to ethephon application in terms of anthocyanin accumulation (Fig. 1). This indicates that it is sensitive to the application of growth regulators, such as ethephon and potentially ABA (Cantín et al., 2007) which can be used for the commercial enhancement of skin color properties.

Neither ABA, nor ethephon had significant effect on the total soluble solids or titratable acidity (Lee et al., 1997; Peppi et al., 2006). In any case, all of the fruit surpassed minimum quality standards. Berry compression force decreased in ethephon treated (Table 1), which it caused undesirable softening of grapes (Fig. 2). This is not consistent as it has been observed in some works (Jensen et al., 1975; Peppi et al., 2006). Many non-treated grapes never achieved adequate color, and good visual appearance, so treatment with ABA or ethephon increased market values. The excellent visual appearance (as an indication of the highest market value) was observed for grapes treated with 300 ppm ABA, due to grater red color intensity, and cluster turgidity. Probably ABA delays berry senescence. However, there are some defects in fruit retention force (berry shattering). Application of ABA advanced harvest date because it rapidly improved grape color, which is very important to prevent from fruit decay due to early autumn rains in the region. On the whole, treatment with ABA did not reduce grape quality in any way. Thus, treatment with ABA is an effective alternative to treatment with ethephon to improve the color of 'Beidaneh Ghermez' grapes.

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<u>Tables</u>

Table 1. Effects of various dosages of PGRs (ppm) on yield compartment, firmness, and loss berry in 'Beidaneh Ghermez' grape in Zanjan province, Iran[a].

Firmness (kg P/cm^2)	Cluster wt. (g)	100 berries wt. (g)	Yield (kg/vine)	Doseges (ppm)	Treatment
0.23	278	92.5	18.5	0	ABA
0.25	283	93.3	18.9	100	"
0.27	288	90.0	19.2	200	"
0.27	291	87.0	19.4	300	"
0.24	281	91.6	18.7	0	ethephon
0.22	287	93.5	19.1	100	"
0.21	275	99.5	18.3	200	"
0.19	263	102.5	17.5	300	"
0.05	18.5	5.2	1.4		LSD

[a]Each value is average over two years (2007-2008). Mean separation within columns by Duncan's multiple range test at P=0.05. Main effects are shown when the interactions were nonsignificant.

Table 2. Effects of various dosages of PGRs (ppm) on skin color (CIRG-index), and juice quality in 'Beidaneh Ghermez' in Zanjan province, Iran[a].

Treatment	Doseges (ppm)	Berry color L^*	C^*	h^*	CIRG index	Juice-TSS (°Brix)	Juice pH
ABA	0	34.8	11.4	24.7	3.36	17.65	4.28
"	100	35.2	10.5	25.2	3.39	17.10	4.53
"	200	34.7	9.8	23.8	3.51	16.40	4.65
"	300	33.6	8.3	24.5	3.71	24.5	4.70
ethephon	0	34.5	11.6	22.5	3.42	17.70	4.10
"	100	35.0	10.9	25.2	3.47	17.30	4.20
"	200	34.5	9.9	23.1	3.53	17.80	4.50
"	300	34.6	9.7	23.7	3.53	16.90	4.55
LSD		1.32	1.02	0.52	0.07	1.45	0.54

[a]Each value is average over two years (2007-2008). Mean separation within columns by Duncan's multiple range test at P=0.05; [b]The color index was calculated as CIRG = $(180-h°)/(C^*+L^*)$, where L^* is the lightness (0, black; 100, white), $h°$ is the hue angle on the color wheel, and C^* is the chroma, a measure of the intensity of color, which begins at zero (achromatic) and increases in intensity.

Figures

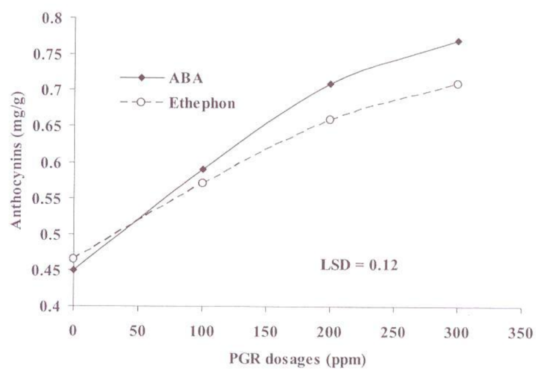

Fig. 1. Influence of PGRs (ABA, and Ethephon) dosages (0, 100, 200, and 300 ppm) on total anthocyanins (absorbance of a 5 ml aliquot at 520 nm) of 'Beidaneh Ghermez' in Zanjan province, Iran. Each value is average over two years (2007-2008).

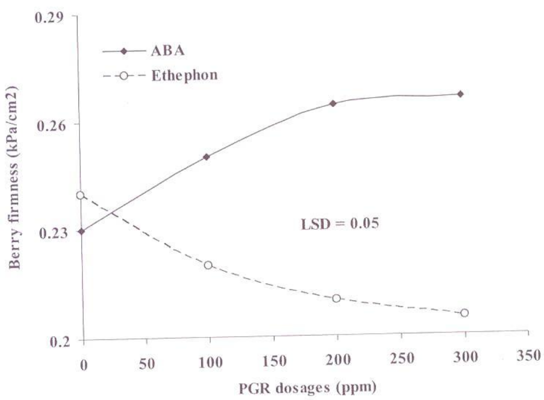

Fig. 2. Influence of PGRs (ABA, and Ethephon) dosages (0, 100, 200, and 300 ppm) on berry firmness (KPa/cm^2) of 'Beidaneh Ghermez' in Zanjan province, Iran. Each value is average over two years (2007-2008).