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Enhancing Color Formation of Flame Seedless Grapes Using Safe Compounds or Low Dose of Ethephon.

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ABSTRACT

Enhancing color intensity and uniformity of 'Flame' seedless grapes is demanded by grape producers. Safe compounds such as putrescine, phenylalanine, thiamin, and urea at low concentrations were either used alone or in various combinations as grape ripening agents and were compared with ethephon at a relatively low concentration (100 ppm). Ethephon, phenylalanine, thiamin, and the combination of urea plus putrescine, or urea plus phenylalanine and thiamin caused a significant increase in anthocyanin production. There was a synergistic effect on enhancing anthocyanin development when urea was combined with phenylalanine plus thiamin. Higher color uniformity for the bunch was obtained with ethephon or urea plus thiamin especially in the second season. Ethephon caused a significant reduction in juice acidity especially in the first season. After cold storage, phenylalanine alone or the formulation of urea plus putrescine caused a significant delay in the loss of fin-nness in both seasons.The addition of urea to phenylalanine caused a significant reduction in the berry firmness as compared with phenylalanine alone. This study provided evidence that it is possible to enhance anthocyanin production and bunch coloring uniformity by safe compounds and there is a possibility of improving berry coloration by low concentration of ethephon.

Key Words: Anthocyanin, coloration, maturation,firmness, storage, phenylalanine, thiamin,urea.

INTRODUCTION

Color is very important attribute especially for pigmented grape varieties. Appearance of grapes is mainly determined by color. There is a minimum color requirements that vary with varieties and grade standard. The grade standard designates the percentage of berries on the cluster that must show a certain minimum color intensity and coverage (Nelson, 1979, kanellis and Angelakis, 1993).

There is a great opportunity for Egypt to increase the export of grapes especially to some European countries, if ripening is manipulated in such a way that makes grapes ready for harvesting early in the season. Meanwhile, these grapes must meet the requirements in terms of color and quality. Flame seedless is a desired grape cultivar that needs improvement in color intensity and uniformity. Plant growth regulators and in particular ethylene and abscissic acid cause an increase in anthocyanin accumulation (Jensen *et al.*, 1975; Lee *et al.*, 1979; Tomana *et al.*, 1979; Kataoka *et al.*, 1982).In general, spray applications of ethephon (an ethylene-releasing compound) enhances color development in pigmented cultivars, increases soluble solids, reduces acidity. Ethephon, however, at high concentrations increases abscission of berries and reduces firrrmess in some varieties whether on the shelf or after a period of cold storage (Szyjewicz *et al.*, 1984). Moreover, grape producers are complaining about the high cost of ethephon especially with large scale production where they use 1500-2000 ppm. In studies of grape coloration, ethephon was also used at high concentration such as 1000 ppm (Lee and Lee, 1982; Dokoozllan *et al.*, 1995). In other cases, ethephon had to be applied twice at 100 ppm to obtain positive results (Hartmann, 1988).

Moreover, It was found that urea at low concentration could stimulate ethylene production of some fruit tissues (Farag, 1989). The same author found that phenylalanine and thiamin was also found to stimulate ethylene production in apple and cranberry fruits. Thus, urea, phenylalanine, thiamin or their combination were used in this study and compared with ethephon at low concentration. Increasing number of grape growers are also interested in organic grapes where they use natural compounds.

The objective of this research was to investigate the possibility of using some natural-safe compounds as graperipening agents, focussing on their effects on color intensity and uniformity and their storage life. The study also investigated the possibility of using one application of ethephon at relatively low concentration to achieve the same goals and reduce the cost.

MATERIALS AND METHODS

This research was conducted during two successive seasons 1994 and 1995 on Flame seedless grapes that were grown at Mariut district near Alexandria, Egypt. Vines were produced from flame stem cuttings. Vines were five years old, uniform, healthy, grown in calcareous soil on wire trills system and under drip irrigation from Nile water canals. Grape bunches distributed on five vines per replication were sprayed to the run off on July 3 and 4 in the two mentioned seasons respectively using a hand sprayer. Ten grape bunches at 10-15% coloration were sprayed by each treatment. Harvest was done ten days after spray. The treatments included: water, urea at 50 mM, urea at similar concentration but incorporated with either putrescine at 2.5 mM or phenylalanine (200 ppm) or thiamin (200 ppm), Urea (50 mM) plus phenylalanine and thiamin (both at 200 ppm). Ethephon was sprayed at 100 ppm to represent the reduced concentration treatment. In addition to the previous treatments, phenylalanine (200 ppm) or thiamin (200 ppm) were sprayed alone. The surfactant Aqua-Zorb was added to all treatments at 0.I %, v/v as a wetting agent and was also used alone. At harvest, fruits of the whole bunch were evaluated for their color uniformity according to an established color score (0-25%, >25-<50% >50-<75%, >75-<100%, and 100% coloration were rated as 1,2,3,4, and 5 respectively), total soluble solids of the juice using hand refractometer, titratable acidity of the juice against 0.1 N sodium hydroxide (A.O.A.C5 1984), total anthocyanin in the berry juice using the method of Fuelki and Francis (1968). Bunches were stored in plastic crates without wraping for 5 weeks at zero' C and 90-95% relative humidity (10 bunches per treatment), then evaluated for their total soluble solids,titratable acidity, firminess using pressure tester of grapes, and berry retention using the retention force meter (10 berries distributed randomly on each bunch).

The completely randomized design with 10 bunches per treatment as ten replications was used. Statistical analysis and the least significant difference among means at 5% level were performed using the SAS (1983) computer software.

RESULTS AND DISCUSSION

The data indicated that there was a significant increase in anthocyanin content of the fruit during the first season as a result of several treatments such as urea plus putrescine, ethephon, thiamin and the combination of urea plus phenylalanine and thiamin when compared with the water control (Table1). Similar trend was obtained for anthocyanin content in the second season (Table 2). There was a synergistic effect on enhancing anthocyanin development when thiamin and phenylalanine were combined with urea.The value of anthocyanin content with the combination of the three mentioned chemicals was significantly higher than using urea plus phenylalanine or urea plus thiamin alone. Thiamin treatment, however, was significantly better than phenylalanine in enhancing anthocyanin development m both seasons. The incorporation of putrescine along with urea led to asignificant increase in anthocyanin content when compared with urea alone in the first season only (Tables 1and 2).

Higher color uniformity was obtained with spraying either ethephon, urea plus putrescine, urea plus phenylalanine, or the combination of the three chemicals namely urea, phenylalanine and thiamin, especially in the second season (Tables 1 and 2).

Total soluble solids values at harvest were similar for various treatments in both seasons (Tables I and 2).

Treatments such as urea or urea plus putrescine or ethephon resulted in a significant reduction in total acidity of the juice at harvest only dun'ng the first season. However, other treatments such as thiamin, phenylalanine, urea plus thiamin, or the combination of these three chemicals (urea, thiamin, and phenylalanine) caused a significant increase in titratable acidity of the juice at harvest in both seasons when compared with the control (Tables 1 and 2).

With regard to fruit characteristics after 5 weeks of cold storage, the data indicated that phenylalanine alone or the formulation of urea plus putrescine caused a significant delay in the loss of firmness in both seasons as compared with the control. The addition of urea to phenylalanine caused a significant reduction in the berry firmness as compared to phenylalanine alone in both seasons (Tables1 and 2).The formulation of urea plus thiamin and phenylalanine caused a significant reduction in berry firmness only in the first season as compared with the control

Values of total soluble solids were not statistically different for many treatments after cold storage in both seasons except with these of urea-treated fruits which had significantly higher TSS than the control in both seasons. Furthermore, urea plus putrescine led to increased TSS compared with the control, especially in the first season. Similar trend of results was obtained with thiamin-treated fruits which tended to have higher TSS than the control after cold storage (Tables 1 and 2).

Total acidity of the juice was not generally affected after 5 weeks of cold storage. Higher acidity was obtained with urea alone in both seasons or its combination with phenylalanine in the first season only. Furthermore, thiamin treatment caused a significant reduction in acidity as compared with the control in the first season after cold storage.

Berries retention force after this period of cold storage had a similar trend in both seasons. As the data indicated that berries of all treatments had significantly higher retention values when compared with the control except urea plus putrescine (Tables 1 and 2).

The enhanced berry coloration of Flame seedless grapes by ethephon has been previously reported (Jensen *et al.*, 1975; Kataoka *et al.*, 1982; Hartmann, 1988; Han *et al.*, 1996). One application of ethephon at 100 ppm also enhanced color development and reduced S02 damage of grapes (Hartmann, 1988). Our results agree with the above reports. Ethephon spray also causes a reduction in juice acidity of pigmented grapes (Lee and Lee, 1982; Szyjewicz *et al.*, 1984; Tiku *et al.*, 1988; Al-Juboory *et al.*, 1990 which was also found in this study especially in the first season. Ripening uniformity was also improved by ethephon application especially in the second season which agrees with the finding of Panwar *et al.*, 1995.

Phenylalanine is the pn'mer compound in the pathway of anthocyanin biosynthesis.It requires the activity of the enzyme phenylalanine ammonia-lyase(PAL). Anthocyanins are the major pigments of grape cultivars (Kanellis, 1993). A correlation was found between PAL activity in the skin of colored grapes and the accumulation of anthocyanin (Kataoka *et al.*, 1983; Hrazdlna *et al.*, 1984; Han *et al.*, 1996). Few reports are available about the utilization of this natural compound, phenylalanine, to enhance anthocyanin development in fruit tissues (Ezz, 1996). The increase in anthocyanin production and the reduction in acidity by phenylalanine agree with the finding of Ezz, 1996. The present study provides an evidence that there 's a potential of using phenylalanine as a fruit ripening enhancer with better performance when it was combined with thiamin and urea at low concentrations. It also shows the possibility of using lower concentration of ethephon than that used by grape growers. However, the use of natural compounds such as thiamin, and phenylalanine could be more adopted especially with the trend of organic agriculture.

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Table 1. Some fruit characteristics, at harvest and after cold storage, of Flame grapes as influenced by urea, putrescine Phenylalanine, thiamin, or ethephon during 1994 season.

Treatments	At harvest				After cold storage			
	Uniformity Score	TSS %	Acidity %	Anthocyanin Content (mg/100 g)	TSS %	Acidity %	Firmness (g/cm^2)	Retention Force (g/cm^2)
Control	2.0	12.9	0.88	5.56	12.96	0.83	183.0	49.0
Urea	1.5	14.1	0.80	6.10	15.22	0.93	180.0	69.0
Urea+PUT.	2.4	13.5	0.80	7.97	15.12	0.82	210.0	52.0
Urea+Ph.ala	2.0	12.4	0.89	5.29	14.04	0.93	161.0	87.0
Ethephon	2.4	13.5	0.60	8.37	14.12	0.82	156.0	101.0
Urea+thiam.	1.7	13.9	0.92	4.52	14.34	0.85	159.0	97.0
Thiamin	1.6	12.8	0.90	7.81	14.82	0.77	147.0	68.0
Ph.ala.	2.0	13.0	0.94	6.07	14.36	0.82	212.0	155.0
Urea+Ph.ala +thiam	2.9	12.5	0.98	14.85	13.58	0.87	145.0	159.0
Aqua-Zorb	1.7	13.6	0.87	6.47	14.50	0.93	137.0	238.0
LSD	0.91	1.10	0.02	0.73	1.82	0.04	26.0	14.0

PUT: putrescine; Phen.ala: phenylalanine; Thiam: thiamin.

Table 2. Some fruit characteristics, at harvest and after cold storage, of Flame grapes as influenced by urea, putrescine Phenylalanine, thiamin, or ethephon during 1995 season.

Treatments	At harvest				After cold storage			
	Uniformity Score	TSS %	Acidity %	Anthocyanin Content (mg/100 g)	TSS %	Acidity %	Firmness (g/cm^2)	Retention Force (g/cm^2)
Control	1.4	14.2	0.82	4.45	13.26	0.81	152.0	44.0
Urea	1.7	13.7	1.31	6.89	15.76	0.84	238.0	88.0
Urea+PUT.	2.4	13.4	0.86	6.19	15.40	0.88	255.0	62.0
Urea+Ph.ala	2.4	12.6	0.81	4.33	14.20	0.82	176.0	121.0
Ethephon	3.7	13.2	0.81	5.50	14.38	0.80	178.0	108.0
Urea+thiam.	2.6	14.0	0.90	4.18	15.12	0.80	145.0	125.0
Thiamin	1.1	11.96	0.99	9.56	13.84	0.80	178.0	131.0
Ph.ala.	2.2	13.94	0.94	5.75	15.36	0.83	248.0	161.0
Urea+Ph.ala +thiam	1.6	12.69	0.92	8.51	14.68	0.79	147.0	188.0
Aqua-Zorb	1.8	13.0	0.91	6.09	14.02	0.88	160.0	78.0
LSD	1.05	1.8	0.02	1.04	2.31	0.02	13.0	18.0

PUT: putrescine; Phen.ala: phenylalanine; Thiam: thiamin.