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Effect of Pre-Harvest Sprays of Ascorbic Acid, Calcium Chloride and Ethephon on Fruit Quality of Grapes (*Vitis vinifera* L.)

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ABSTRACT

The present investigation was conducted to evaluate the effect of pre-harvest sprays of ascorbic acid, calcium chloride and ethephon on hastening maturity and fruit quality of Flame Seedless grapes. The pre-harvest sprays of ascorbic acid (750, 1000 and 1250 ppm) and calcium chloride (0.25, 0.50 and 1.0 %) were given at fruit-set and veraison stage. Ethephon @ 400ppm was sprayed at veraison stage on vines with 75 per cent crop load. The treatment combination of flower bud thinning + gibberellic acid (GA3) 40 ppm was also included for comparison along with water sprays as control. Each treatment was replicated thrice, in which one vine served as a unit treatment. The time of ripening was advanced by 5 d in the treatment 75 per cent crop load + ethephon 400ppm as compared to control, while in the treatment flower bud thinning + GA3 ripening was advance by 3 d. The same treatment i.e. flower bud thinning + GA3 resulted in significantly higher yield (33.54 kg/vine), maximum bunch weight, bunch length and bunch breadth. However, the fruit quality with respect to higher total soluble solids (TSS), lower acidity, higher sugars and anthocyanin content was better in 75 per cent crop load + Ethephon 400 ppm. Also the yield in treatment was at par with flower bud thinning + GA3. Thus, considering yield as well as quality parameters, the treatment 75 per cent crop load+ 400ppm Ethephon was found to be the best.

Key words: Ascorbic acid, Calcium chloride, Ethephon, Flame Seedless, Gibberellic acid.

INTRODUCTION

Although, the grapes are commercially grown in the tropical regions of India, but an early ripening variety can be successfully grown in the regions of northern India too. The grapes grown in this region have higher productivity than grown in commercial region of India. However, very short period (April–June) is available for the ripening of berries in this region which limits the choice of varieties and only early maturing varieties can be grown successfully. Perlette is a leading cultivar of this region which occupies 90 per cent of total area under grapes. To restrict the monoculture of this cultivar recently, coloured Flame Seedless grapes, has been recommended in northern India for commercial cultivation for table purpose (Anonymous, 2009). Flame Seedless is a complex hybrid, whose parents include Sultanina, Cardinal, Malaga and Muscat d' Alexandric, and was developed in early 1960s by John Weinberger in California (Vander Merwe *et al*, 1991). Uneven colour and small berry size are the primary quality problems in this variety. Previously, various attempts have been made to improve fruit quality of Flame Seedless grapes with use of pre-harvest sprays of chemicals like GA3, ethephon and calcium chloride (Ramteke *et al*, 2002, Fatma and Aisha 2005). Therefore, the present study was conducted to ascertain the effect of ascorbic acid, calcium chloride and ethephon applied as foliar sprays on maturity and fruit quality of Flame Seedless grapes.

MATERIALS AND METHODS

Experimental details

The present investigation was conducted on 12 yr old vines of Flame Seedless in Fruit Research Farm of Department of Fruit Science at Punjab

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Agricultural University, Ludhiana. The different treatments used were ascorbic acid @750ppm (T1), 1000ppm (T2), 1250ppm (T3); calcium chloride @ 0.25% (T4), 0.5% (T5),1.0% (T6), flower bud thinning + GA3 40 ppm (T7), 75 % crop load + ethephon 400 ppm (T8) and control, water spray (T9).

Each treatment was replicated thrice, in which one vine served as a unit treatment. Thus, Twenty seven uniform vines distributed in the vineyard were selected for this investigation. Application of chemicals was done twice; at fruit-set (pea size) and veraison stage (colour break stage). In treatment T7 which comprised of flower bud thinning + GA3 dips, flower bud thinning is done one week before flowering thereby leaving 100–120 flower buds per panicle followed by GA3 dips when fruit was pea size. For treatment T8 (of 75% crop load), 90-98 bunches were retained on the vines while rest were removed immediately after bunch emergence.

The harvesting was done manually in first fortnight of June. After harvesting, fruits were brought to laboratory at Department of Fruit Science, P.A.U Ludhiana for analysis of various physico-chemical properties.

Evaluation of maturity, yield and physical properties

Date of ripening was recorded as and when vines reached commercial maturity i.e. the checked berries reached maturity stage (16-17 % TSS). Number of bunches were counted at the time of harvest. The bunch weight, length and breadth were measured from 10 randomly selected bunches from each vine and means were worked out. Mean bunch weight was multiplied with counted bunches to record yield. Fifty berries were randomly sampled from ten bunches for the determination of physical and chemical properties. The weight (g) of 50 randomly selected berries was taken with weighing balance and average berry weight for each treatment was calculated. The berry size (cm) and berry pedicel diameter (mm) were taken with digital caliper and averages for each treatment were calculated. Berry firmness was measured using a Texture Analyzer (TA+HDi ® Stable Micro Systems, UK) equipped with a HDP/90 platform and 5 kg load cell. The measurement was made on the equatorial position of the berry with 4 mm probe at a test speed of 1 mm/s to a constant compression distance of 1 mm. The readings were expressed as resistance force of the skin or flesh in grams (Rolle et al, 2011).

Evaluation of chemical properties

The soluble solids (TSS) content of the fruit was measured with hand refractometer and correction at 20o C was applied and the results were expressed in percent (AOAC, 2000). The acidity (%) of the fruit was estimated by titrating a known volume of juice against N/10 sodium hydroxide. TSS: acid ratio was calculated by dividing the values of TSS with the corresponding values of titratable acidity. Reducing and total sugars were determined as per AOAC (2005).

Total anthocyanin content of berries was determined as described by Ranganna (1986) with the extraction solvent ethanolic HCL and absorbance was noted at 535 nm wavelength by spectrophotometer. For anthocyanin estimation, 5g of sample was taken and macerated using motar and pestle with small amount of ethanolic HCL (made by 85 parts 95 % ethanol and 15 parts of 1.5 N HCL) and final volume was made 100 ml with ethanolic HCL and kept overnight at 4oC. Following morning the mixture was filtered through Whatman's No.1 filter paper and residue on the filter paper was washed repeatedly with ethanolic HCL and volume was made up to 100 ml with the same solvent. It was again filtered through fine millipore and 10 ml of aliquot was taken and diluted up to 20 ml with ethanolic HCL. It was kept in dark for 2h after that absorbance was measured at 535 nm wavelength by spectrophotometer. The obtained data were statistically analyzed by Randomized Block Design (RBD) described by Singh et al (1998).

Table 1. Effect of pre-harvest treatments on time of ripening, yield, bunch weight and bunch size of Flame Seedless grapes.

Treatments	Time of ripening	Yield (kg/vine)	Bunch weight (g)	Bunch length (cm)	Bunch breadth (cm)
T1-Ascorbic acid 750 ppm	5/6	29.74	248.62	22.08	11.95
T2 -Ascorbic acid 1000 ppm	7/6	28.56	235.64	21.41	11.89
T3- Ascorbic acid 1250 ppm	8/6	26.42	223.87	20.51	11.75
T4- Calcium chloride (0.25%)	3/6	28.68	263.16	22.37	12.97
T5- Calcium chloride (0.5%)	6/6	25.42	260.42	22.21	12.77
T6-Calcium chloride (1.0 %)	9/6	24.50	254.41	22.14	12.49
T7- Flower bud thinning + GA3 (40ppm)	4/6	33.54	345.57	23.42	14.52
T8 - 75 % crop load + Ethephon (400 ppm)	2/6	31.05	282.57	20.16	12.07
T9- control	7/6	28.29	220.52	19.62	10.98
CD (p=0.05)	--	2.66	2.10	1.95	1.72

RESULTS AND DISCUSSION

Maturity, yield and physical properties

It was observed that treatment T8 (75% crop load + ethephon 400 ppm) and treatment T7, (flower bud thinning + GA3 40ppm) hastened the harvest maturity in Flame Seedless as compared to control (Table 1). Data indicated that the time of ripening was advanced by 5d in T8 compared to control whereas, in treatment T7, it was advanced by 3d. The advancement of maturity was due to ethylene which accelerated berry ripening. Singla et al (1992) reported that ethephon 750 ppm advanced ripening by 7 d in Early Muscat and by 8 d in cv. Gold. Advancement in maturity in treatment T8 may be attributed to the reduced crop load per vine. Cheema et al (2003) found that cluster-thinning advanced maturity by nine days in Perlette.

The yield was the highest (33.54 kg/vine) in treatment T7 and at par with treatment T8 (Table 1). The results were in conformity with findings of Gowda *et al* (2006). The pre-harvest sprays of ascorbic acid (750 ppm) and calcium chloride (0.25%) increased the fruit yield as compared to control but the differences were non- significant.

All the treatments increased bunch weight compared to control (Table 1). Treatment T7 resulted in higher bunch weight (345.57g) followed by T8 (282.57g) which was significantly higher than obtained with application of calcium chloride and ascorbic acid. The increase in bunch weight was probably attributed to the availability of more food materials due to decreased crop load and increased cell division with GA3 application. GA3 has been used for seedless grape production to increase berry and bunch weight, and cause thinning of clusters (Omran and Girgis, 2005). Abu-Zahira and Salameh (2012) also found that application of GA3 or GA3+ girdle resulted in heavier berries as well as increased in bunch weight.

The data regarding bunch size revealed that all the treatments reduced the bunch compactness as evident from increased bunch length and breadth over control (Table 1). The maximum bunch length (23.42 cm) was recorded in treatment T7 followed by T 4 (22.37 cm) and T5 (22.21 cm). The treatments in which ascorbic acid was sprayed, bunch length ranged between 20.51 to 22.0 cm and was minimum (19.62 cm) in control vines. Maximum bunch breadth (14.52 cm) was recorded in treatment T7. Similar results with pre-harvest application of gibberellic acid on increasing cluster size in Thompson and Belgrade Seedless grapes has been reported by Dimovaska et al (2011).

The data (Table 2) revealed that the berry weight was the maximum (2.72 g) in treatment T7

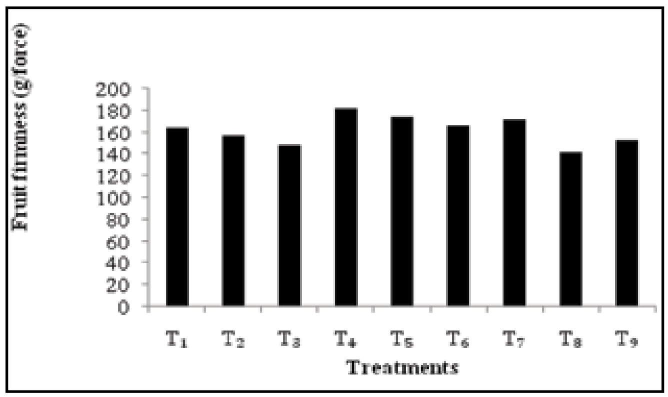
Table 2. Effect of pre-harvest treatments on berry weight, berry size and pedicel length of Flame Seedless grapes.

Treatments	Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry pedicel diameter (mm)
T1- Ascorbic acid (750 ppm)	2.21	1.35	1.28	2.32
T2- Ascorbic acid (1000 ppm)	2.16	1.27	1.20	2.27
T3- Ascorbic acid (1250 ppm)	2.09	1.24	1.17	2.24
T4- Calcium chloride (0.25%)	2.34	1.42	1.34	2.59
T5- Calcium chloride (0.5%)	2.24	1.39	1.32	2.42
T6-Calcium chloride (1.0 %)	2.18	1.38	1.31	2.38
T7- Flower bud thinning + GA3 (40ppm)	2.72	1.75	1.67	3.18
T8- 75 % crop load + Ethephon (400 ppm)	2.58	1.44	1.36	2.35
T9- control	1.98	1.33	1.26	2.19
CD (5%)	0.32	0.25	0.20	0.66

followed by treatment T8 (2.58 g) and treatment T4 (2.34g) which was significantly higher than control. Results were in agreement with findings of Zabadal and Dittmer (2000) and Roberto (2002). The observed increase in berry weight with 75 per cent crop load + ethephon 400 ppm might be due to more availability of photosynthates to the left over bunches.

Grapes subjected to flower bud thinning + GA3 (40 ppm) produced larger berries (higher berry length and breadth compared to control. These results comply with those reported by Casanova *et al* (2009) and Kalpan (2011) in grapes. This effect takes place through larger berry growth rate, early uptake of glucose, sucrose and fructose and increased of absolute berry water content.

The perusal of data (Table 2) revealed that the berry pedicel diameter was recorded maximum (3.18 mm) with treatment T7 followed by T4 (2.59 mm) and T5. Casanova *et al* (2009) found that application of GA3 @160 mg/l increased berry peduncle length and berry pedicel diameter. The maximum fruit firmness (182.0 g force-1) was recorded in T4 followed by treatment T5 which had the fruit firmness of 174.0 g force-1. However, the treatment T8 resulted in lowest value of fruit firmness (142.0 gforce-1) as compared to other treatments (Fig.1). The cation Ca2+ promotes the stability of the cell wall by chelating the free carboxylic groups of galacturic units and cross linking the pectic polysaccharide chains forming a firmer tighter structure.



T_1- Ascorbic acid (750 ppm), T_2- Ascorbic acid (1000 ppm), T_3- Ascorbic acid (1250 ppm), T_4- Calcium chloride (0.25%), T_5- Calcium chloride (0.5%), T_6-Calcium chloride (1.0 %), T_7- Flower bud thinning + GA_3 (40ppm), T_8- 75 % crop load + Ethephon (400 ppm), T_9- control

Fig.1 Effect of pre-harvest treatments on fruit firmness of Flame Seedless grapes.

Chemical Properties

Total Soluble Solids (TSS), Titratable Acidity (TA) and TSS/TA ratio were significantly affected by pre-harvest sprays of ascorbic acid, calcium chloride, gibberellic acid and ethephon (Table 3). The TSS were recorded maximum (19.46%) with treatment T8 followed by treatment T7 (18.98 %). Increase in TSS with ethephon application was in line with the findings of Sharma and Jindal (1983)

Table 3. Effect of pre-harvest treatments on TSS, acidity and TSS.

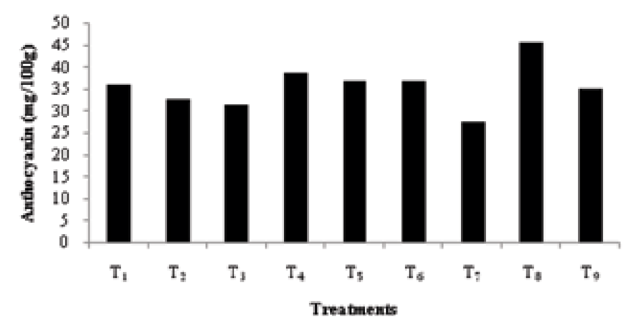
Treatments	TSS (%)	Acidity (%)	TSS/acidity
T1- Ascorbic acid (750 ppm)	16.87	0.81	20.83
T2-Ascorbic acid (1000 ppm)	16.62	0.86	19.33
T3- Ascorbic acid (1250 ppm)	16.48	0.89	18.52
T4- Calcium chloride (0.25%)	17.96	0.70	25.66
T5- Calcium chloride (0.5%)	17.67	0.72	24.54
T6 -Calcium chloride (1.0%)	16.72	0.76	22.00
T7- Flower bud thinning + GA3 (40ppm)	18.98	0.71	26.73
T8-75 % crop load + Ethephon (400 ppm)	19.46	0.68	28.61
T9-control	16.32	0.92	17.74
CD (5%)	1.40	0.07	1.82

who reported an increase in TSS by ethrel application in Beauty Seedless grapes. The data (Table 3) revealed that all the treatments significantly reduced the acidity over the control. Minimum (0.68%) acidity was recorded with treatment T8, followed by treatment T7 which recorded 0.71 per cent value of acidity and the maximum acidity (0.92%) was recorded in control. Reduction in acidity as a result of Ethephon application could be due to effect of this chemical in increasing membrane permeability which permits acids, stored in cell vacuoles, to respire at a faster rate.

TSS/Acidity ratio is considered as a reliable maturity index for grapes and sharp increase in TSS/TA ratio indicates the onset of ripening. It was evident from the data given in Table 3 that all the pre-harvest treatments significantly influenced the TSS/acid ratio. Maximum TSS/ acid ratio (28.61) was recorded with treatment T8, followed by the treatment T7.

Anthocyanin content

The data presented in Fig. 2 indicated that all the pre-harvest treatments resulted in significant increase in anthocyanin content as compared to control. The treatment T8 recorded significantly higher (45.36 mg/100g) anthocyanin content followed by treatments T4 (38.54 mg/100g). The treatment in which ascorbic acid was sprayed recorded anthocyanin ranged between 31.47 to 35.72 mg/100g. The minimum anthocyanin content (27.29 mg/100g) was recorded in treatment T7. Kitamura *et al* (2005) found that controlling crop load resulted in proper colouration of 'Aki Queen' fruits through the regulation of anthocyanin concentration in grapes.

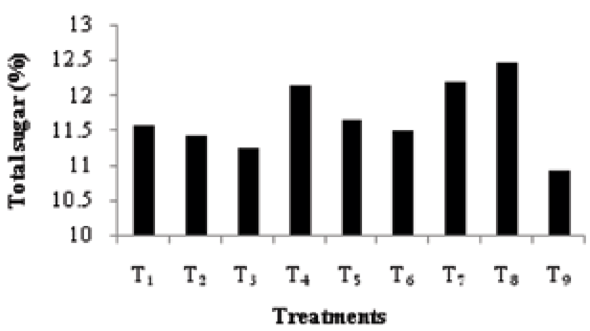


T1- Ascorbic acid (750 ppm), T2- Ascorbic acid (1000 ppm), T3- Ascorbic acid (1250 ppm), T4- Calcium chloride (0.25%), T5- Calcium chloride 0.5%), T6-Calcium chloride (1.0 %), T7- Flower bud thinning + GA3 (40ppm), T8- 75 % crop load + Ethephon (400 ppm), T9- control

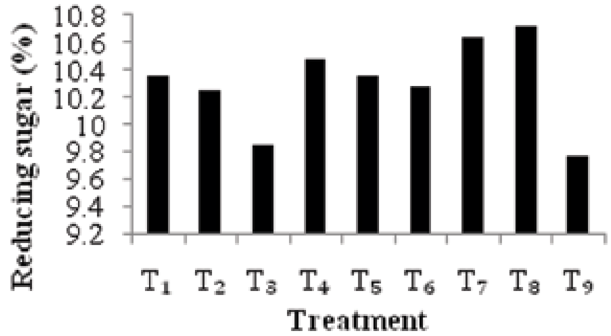
Fig. 2: Effect of pre-harvest treatments on anthocyanin of Flame Seedless grapes.

Total and reducing sugars

Effect of pre-harvest treatments on reducing and total sugars are shown in Fig. 3A and 3B. Maximum (12.45 %) total sugar was recorded (Fig. 3A) with treatment T8, followed by treatment T7, (12.19 %). The treatment in which ascorbic acid and calcium chloride were sprayed, total sugar ranged between 11.25 to 12.14 per cent. The control registered lower total sugar (10.92%) as compared to other treatments. The Fig. 3B showed that the maximum (10.72) reducing sugar percentage was registered



T1- Ascorbic acid (750 ppm), T2- Ascorbic acid (1000 ppm), T3- Ascorbic acid (1250 ppm), T4- Calcium chloride (0.25%), T5- Calcium chloride (0.5%), T6-Calcium chloride (1.0 %), T7- Flower bud thinning + GA3 (40ppm), T8- 75 % crop load + Ethephon (400 ppm), T9- control



T1- Ascorbic acid (750 ppm), T2- Ascorbic acid (1000 ppm), T3- Ascorbic acid (1250 ppm), T4- Calcium chloride (0.25%), T5- Calcium chloride (0.5%), T6-Calcium chloride (1.0 %), T7- Flower bud thinning + GA3 (40ppm), T8- 75 % crop load + Ethephon (400 ppm), T9- control

Fig. 3A&3B. Effect of pre-harvest treatments on total and reducing sugar of Flame Seedless grapes.

with treatment T8. Gaser et al (1998) reported that ethrel (500 ppm) and hand thinning increased the TSS and sugar content. Ahmad and Zargar (2005) found that Ethephon treatment (500ppm) with or without girdling at veraison stage increased TSS and accumulation of sugars in cv. Perlette.

CONCLUSION

It can be concluded that retaining 75 per cent crop load + pre-harvest spray of ethephon @ 400 ppm at verasion stage could be an effective treatment for advancement of ripening along with improvement in fruit quality of Flame Seedless grapes.

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