J. Hortl. Sci.
Vol. 16(2) : 177-184, 2021

Original Research Paper

Optimization of GA_3 concentration for improved bunch and berry quality in grape cv. Crimson Seedless (*Vitis vinifera* L)

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ABSTRACT

Crimson Seedless is a coloured grape, gaining popularity in India for its attractive colour, bunch and berry quality with better shelf life. In cultivation of any seedless grape variety, application of GA_3 at different stages is very much essential to produce good quality berries and bunches. However, this variety is highly sensitive to excess application which adversely affects bunch quality. Thus, there is a need to standardize mild dose of GA_3 for rachis elongation which will help to reduce bunch compactness to a greater extent. Hence, an experiment was initiated to standardize concentration of GA_3 for rachis elongation of Crimson Seedless grapes. Three different concentrations of GA_3 (viz., 5 ppm, 7.5 ppm, and 10 ppm) were sprayed during pre bloom stage and compared with unsprayed control. Among different treatments, pre-bloom spray of GA_3@5 ppm could produce less compact bunches with highest average bunch weight, berry weight, berry length and TSS. However, bunches sprayed with 7.5 ppm and 10 ppm GA_3 could also produce good quality bunches, average berry weight with TSS. Because of severe coiling of rachis at 7.5 ppm and 10 ppm GA_3 spraying, bunches were too straggly compared to spraying of 5 ppm GA_3. The control bunches without GA_3 produced very compact clusters with less average bunch weight, berry weight, berry diameter and berry length.

Keywords: Crimson Seedless, cluster compactness, fruit quality, GA_3, grapes and rachis elongation

INTRODUCTION

Grape cultivation in India is highly remunerative owing to its high foreign exchange with maximum net returns to grape growers. Thompson Seedless is the preferred variety by growers and more than 70% of the area under grape cultivation is occupied by Thompson Seedless and its clonal selections like Tas-A-Ganesh, Sonaka, Manik Chaman *etc*. Though Thompson Seedless is the internationally accepted table grape across the globe, in recent years many new green and colored varieties are dominating in the export market. The important varieties are Crimson Seedless, Fantasy Seedless, Red Globe, Autumn Royal *etc*. Due to change in the international export market scenario, the area under coloured grape varieties is steadily increasing in mild tropical climatic regions of India especially in southern India. The important cultivars grown there are Flame Seedless, Sharad Seedless (Syn: Kishmish Cheyrni) and its clonal selections, Red Globe, Crimson Seedless etc. Though most of the cultural practices are similar to that of Thompson Seedless, their response is different for growth regulator application and canopy management practices. Coloured grape variety Crimson Seedless is gaining importance in recent years due to their superior quality with respect to bunch and berry parameters and extended shelf life.

Gibberellic acid (GA) is commonly used in grape cultivation to improve size of berries and length of clusters. Though grapevine cultivars shows large variation in response to applied GA, the reasons for such variations are unclear. This variation in response of different varieties to GA_3 might be possible due to variation in GA signalling components and/or availability of bioactive GA (Acheampong *et al.,* 2017). Unlike seeded varieties of grapes, berries of the small stenospermic grape varieties like Thompson Seedless, Flame Seedless, and Crimson Seedless etc. will have lower concentration of GA as they carry rudimentary seed traces due to abortion of endosperm following fertilization (Cheng *et al.,* 2013). Hence, external application of GA_3 is routinely followed to stimulate development of berries in stenospermic varieties for commercial acceptance of berry size in addition to flower thinning and rachis elongation (Weaver, 1965; Harrell and Williams, 1987). Thompson Seedless grapes require quite higher concentration of GA_3 which is to be applied at different stages of cluster development to attain desirable bunch and berry qualities (Chadha and Shikhamany, 1999). Without the knowledge on concentration of GA_3 to be applied to Crimson Seedless, some of growers used similar concentrations as used for Thompson Seedless which resulted in adverse effects on bunch and berry quality parameters. However, application of higher concentration of GA_3 at different stages of berry development in Crimson Seedless grapes is found to be toxic and not advisable. Higher concentration of GA_3 results in excessive berry thinning (straggly clusters) and shot berry formation, as well as an unacceptable reduction in fruitfulness in the following year (Dokoozlian *et al.,* 2000). Higher concentration of GA_3 sometimes causes lignifications and contortion of the rachis (Aguero *et al.,* 2000). Iqbal *et al.* (2011) suggested that GA rates @ 20 g/ ac effective for berry sizing are detrimental to the productivity and fruit quality of Crimson Seedless. Hence, there was a need to optimize the concentration of $GA_3$to elongate rachis which can improve the overall bunch and berry quality parameters. Higher concentration of GA_3 used arbitrarily was found to have adverse effect wherein it caused severe coiling of rachis. Under tropical climatic conditions of India, no information is available on concentration of GA_3 to be used to improve rachis elongation in Crimson Seedless grapes. Hence, the present investigation was taken up to standardize the concentration of GA_3 to be sprayed at pre-bloom stage to improve bunch and berry characters.

MATERIALS AND METHODS

This study was undertaken at the experimental vineyard of ICAR - Indian Institute of Horticultural Research (ICAR - IIHR) located at Hessaraghatta, Bengaluru during three consecutive years 2016-17 to 2018-19. It is situated at an elevation of 890 meters above sea level, 12^{0} 68' North latitude and 77^{0}38' East latitude. Four year old vines of cv. Crimson Seedless grafted on Dogridge rootstock and trained on to 'Y' trellis were utilized for imposition of treatments. The spacing followed was 3.3m × 2.0m. Throughout the experiment regular soil management and plant protection practices were followed in compliance with the schedule developed for successful grape cultivation in the region. Similar to the practices followed in most of the tropical grape growing countries, the vines were pruned twice in a year once after harvest of previous crop which is popularly known as foundation pruning. This pruning usually coincides with summer season and is done to encourage canes with fruitful buds. Again on these developed canes, one more pruning was done retaining 5-6 buds per cane, encouraging cluster development which is usually called as fruit pruning. Different concentrations of GA_3 *viz.*, 5 ppm (5 mg/L), 7.5 ppm (7.5 mg/L) and 10 ppm (10 mg/L) were sprayed at panicle emergence stage (23-28 days after pruning, EL stage 15) along with one treatment as control (water spray). The stock solution of GA_3 was prepared just before spraying, by dissolving 1g of GA_3 in 5 ml absolute alcohol and make up the volume to 1 litre using distilled water. From this stock solution desired concentrations were made with suitable dilutions. The experiment was laid out as randomized block design with 4 treatments and seven replications. Each treatment consisted of six vines. In each replication 20 clusters were tagged to record all the bunch and berry quality parameters. Berry physiochemical analysis was performed immediately after harvest. Average berry weight, berry diameter and berry length were measured as per the standard procedures using electronic balance and measuring scale. Cluster compactness was calculated using number of berries per bunch and total length of rachis and first five rachillae. Berry total soluble solids (TSS) was measured using temperature compensated refractometer calibrated at Room Temperature of 25^{o}C. Titratable acidity was measured using titration method where in 10 ml of grape juice was titrated against 0.1 N sodium hydroxide using phenolphthalein as indicator. Peel anthocyanin concentration was estimated as per the procedure reported by Fuleki, (1969) using spectrophotometer and quantity of anthocyanin in the sample was calculated using cyanidin hydrochloride as standard and expressed as mg/100g fresh weight. Total phenol content in grape juice was estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) as per the method developed by Singleton and Rossi, (1965). Total sugar was estimated by the method developed by Somyogi, (1952) and expressed in g/100gFW.The average of three years observations were used for statistical analysis. SPSS for Windows version 9.0 and Microsoft Excel 2003 were used to carry out statistical analysis and graphical data presentation.

RESULTS AND DISCUSSION

Significant differences among the treatments were recorded for rachis length in response to different concentrations of GA_3 applied. The clusters treated with GA_3 @ 5 ppm recorded highest total rachis length of 124.90 cm followed by those treated with GA_3 @ 7.5 ppm which recorded rachis length of 89.52cm (Table 1). The least length of the rachis (55.68cm) was recorded in untreated control. Though higher rachis length of more than 124.90 cm was recorded when GA_3 was applied at 10 ppm, there was severe coiling of rachis which affected the bunch quality at later stages of berry development with respect to shape, appearance, lignified rachis etc. Statistically significant differences among the treatments were recorded for bunch compactness. GA_3 at 5 ppm recorded the less bunch compactness (0.94 berries / cm of rachis length) among all the treatments resulting in development of loose cluster, while in treatment where no GA_3 application was applied, it recorded maximum bunch compactness (2.59 berries/cm of rachis length) resulting in very tight clusters. Though GA_3 @ 7.5 and 10 ppm could produce loose clusters, their bunch shape was not desirable due to coiling of rachis. Application of GA_3 at different concentrations has brought significant changes in cluster morphological parameters like rachis length, length of internodes, rachis weight etc. Rachis elongation is the most essential phenomenon to produce loose grape bunches. Application of GA_3 has brought significant changes in rachis length compared to control clusters and which might be due to lot of biochemical events which takes place at cellular level. There was negative correlation (-0.743) between the total rachis length and cluster compactness (Fig 1) which means, more the rachis length the number of berries per unit length is less indicating loose clusters. The bunch morphological

Table 1. Influence of different concentration of GA_3 on bunch characters of grape cv. Crimson Seedless (mean of three years)

Treatments	Total length of Rachis (cm)	Total number of berries per bunch	Bunch compactness (no. of berries/cm of rachis)	Bunch weight (g)
GA_3 at 5ppm	124.90	102.40	00.94	507.42
GA_3 at 7.5ppm	089.50	110.42	01.26	482.04
GA_3 at 10ppm	132.90	119.11	01.11	499.55
Control	055.60	140.75	02.59	442.56
SEM ±	009.80	010.52	00.18	037.20
CD(P=0.05)	029.50	NS	00.54	NS

*NS: Non Significant

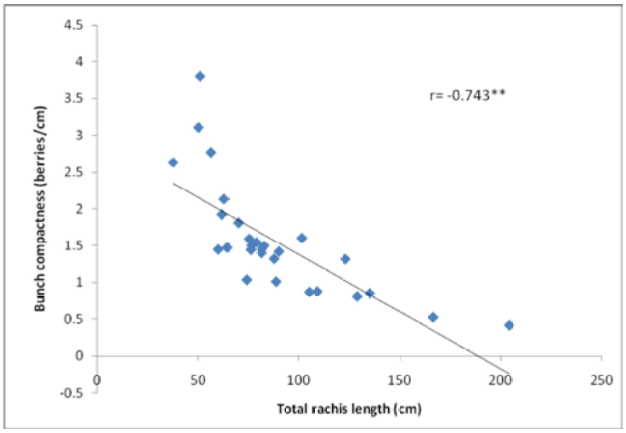

Fig. 1. Correlation between total rachis length and cluster compactness in grape cv. Crimson Seedless

**Correlation is significant at the 0.01 level (P<0.01) parameters of the present experiment are in accordance with established reports on the application of GA_3 for improved berry and bunch characters (Looney and Wood, 1977; Molitor *et al.,* 2012; Weaver, 1958; Weaver, 1975). The rachis elongation is a complex process which requires enhanced carbon metabolism of sugar accumulation by phloem area expansion. The increased rachis elongation in our studies might be due to over expression of some proteins involves in these processes which belong to biological processes like generation of precursor metabolites, cellular protein metabolic process, responses to abiotic stimulus and protein processes (Ghule *et al.,* 2019a). The process of rachis elongation in response to applied GA_3 has been studied extensively at different levels *viz.,* phenotypic, physiological and transcriptomes (Domingos *et al.,* 2016; Upadhyay *et al.,* 2018). Most of these studies have indicated cell wall loosening and cell enlargement as the key physiological processes which are essential for rachis elongation to make grape clusters less compact. Schopfer (2001) and Liszkay *et al,* (2004) in their studies reported that hydroxyl radicals generated via Fenton reaction with H_2O_2 as the substrate which helps in cell wall loosening and cell enlargement. Similarly some of the proteins associated with cell biogenesis like IRX15-LIKE like proteins which are involved in secondary wall participate in xylan biosynthesis as they are major hemicelluloses in secondary cell walls of most of dicotyledonous plants (Brown *et al.,* 2011). Similarly, the process of cell wall elongation and wall loosening involves significant alterations in the properties of cell wall polysaccharides. Nunan *et al.* (2001) predicted the activation of some of the enzymes that participate in cell wall modification. In our study also, the protein EOCPF 1 (β galactosidase BG1) belonging to carbohydrate, monosaccharide, and galactose metabolism might have played a key role in elongating the cell wall which usually exists with other proteins, viz. pectin methylesterase, polygalacturonase, and xyloglucan endotransglycosylase.

Though no difference was recorded for total bunch weight in response to application of different concentrations of GA_3 which is a factor of number of berries per cluster, GA_3 at 5 ppm recorded maximum bunch weight (507.48g) among the all treatments while treatment without GA_3 application recorded the least bunch weight (442.54g). But, application of GA_3 brought a significant difference in individual berry weight wherein GA_3 @ 5 ppm registered maximum berry weight (4.93g) followed by GA_3@ 7.5ppm (4.85g). The least average berry weight was recorded in untreated control T_4 (3.98g). Some of the mechanisms proposed for GA_3 action are increased activity of soluble invertase (Pérez and Gómez, 2000) and subsequent change in water potential of berries and modulation of aquaporin genes by GA_3 (Espinoza *et al.* 2009) to increase the water content of berries during berry growth. Recent proteome and transcriptome-based analyses *(*Cheng *et al.,*2015*;* Wang *et al.,* 2012) have also shown GA_3-mediated modulation of several genes involved in cell expansion and cell wall modification which might be responsible for the increase in berry size and volume. In a study to see the effect of GA_3 on berry sizing in Thompson Seedless grapes, Ghule *et al,* (2019 b) reported the increased size of berries in GA_3 applied bunches and was attributed to increase level of peroxidase as early response and suppressed level of catalase and glutaredoxin as late response and concluded that berry enlargement might have influenced by expression of antioxidant enzymes such as catalase and peroxidise which was also suggested by Wang *et al.* (2017).

No significant difference was recorded for berry quality parameters like berry diameter, Total soluble solids etc (Table 2). However, titratable acidity was found to be highest in control vines (0.52%) while the least acidity (0.41%) was recorded in clusters treated with 5 ppm GA_3. Observations on anthocyanin concentration are presented in Table 3. Significant differences among the treatments were recorded. Among all treatments bunches treated with GA_3 @ 7.5ppm (247.914mg/100g) registered maximum anthocyanin concentration (Table 3) followed by GA_3@ 5ppm T_1 (177.327 mg/100g). The least anthocyanin concentration was recorded in bunches with no GA_3 application i.e., T_4 (167.143 mg/100g). The highest anthocyanin concentration in treatment with 7.5 ppm GA_3 might be due to its lower total sugar concentration which has exhibited negative correlation (r= -0.413, Fig 2) and vice versa in treatments with GA_3 @ 5 ppm and 10 ppm**.** The sugar conversion into anthocyanin biosynthesis is reported by few workers in different flowers and fruit crops as reported by Ozer *et al.* (2012). Our findings are in accordance with that of Peppi *et al.* (2006), where the application of gibberellic acid (GA_3) was effective at increasing the

Table 2. Influence of different concentration of GA$_3$ on berry quality parameters of grape cv. Crimson Seedless (mean of three years)

Treatments	50 berry weight (g)	Average berry weight (g)	Berry diameter (mm)	Berry length (mm)	TSS (^{0}B)	Acidity (%)
GA$_3$at 5 ppm	246.41	4.92	17.31	25.82	18.52	0.24
GA$_3$at 7.5 ppm	242.51	4.81	17.30	25.43	17.57	0.32
GA$_3$at 10 ppm	233.44	4.63	17.43	24.64	17.66	0.41
Control	199.35	3.92	16.82	22.77	18.21	0.51
SEM ±	8.843	0.17	0.27	0.47	0.40	0.054
CD (P = 0.05)	26.27	0.53	NS	1.40	NS	0.16

*NS: Non Significant

Table 3. Influence of different concentration of GA$_3$ on berry quality parameters of grape cv. Crimson Seedless (mean of three years)

Treatments	Anthocyanin concentration (mg/100g)	Total phenols (mg/100g)	Total sugars (g/100g)
GA$_3$ at 5ppm	177.30	112.70	18.20
GA$_3$ at 7.5ppm	247.90	172.60	15.90
GA$_3$ at 10ppm	173.70	217.60	16.00
Control	167.10	155.10	17.40
SEM ±	016.50	023.73	00.38
CD(P=0.05)	049.40	071.06	01.13

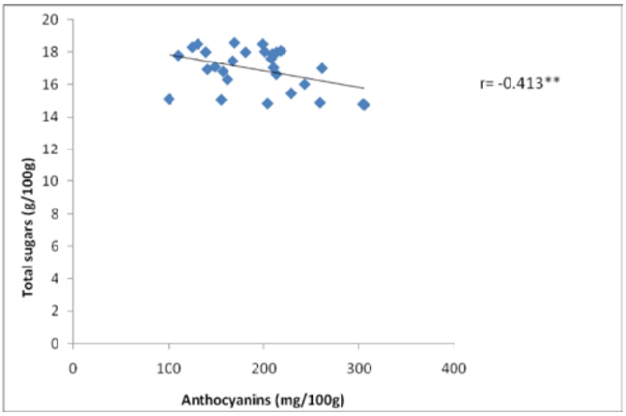

Fig. 2. Correlation between anthocyanins and total sugars in grape cv. Crimson Seedless

**Correlation is significant at the 0.01 level (P<0.01)

anthocyanins content of grape variety Flame Seedless. The use of higher concentrations of GA$_3$ (over 50 ppm) leads to a reduction in the content of anthocyanins in berries (Rusjan, 2010) and this in turn has an adverse effect on the organoleptic properties of varieties with red and blue color of the skin intended for consumption in fresh condition.

Significant differences among the treatments were recorded with respect to total phenol content wherein, bunches treated with GA$_3$ @ 10ppm (217.605 mg/100g) registered maximum total phenols followed by GA$_3$ @ 7.5ppm T$_2$ (172.664mg/100g). The least total phenol was recorded in clusters treated with GA$_3$ @ 5ppm T$_1$ (112.752mg/100g). GA$_3$ (highest 3 concentrations) and CPPU treatments (highest 2 concentrations) significantly increased the total phenol content of the grapes after cold storage Avenant *et al* (2017). Increased phenol content of 'Regal Seedless' was correlated with an increased astringent taste (Fraser, 2007), with serious negative implications regarding consumer preferences and market access. Application of higher concentration of GA3 might not only reduce the physical appearance of cluster with respect to lignifications of rachis but also reduce the chemical properties with respect to reduced sugar content and more phenolic compounds as evidenced in present study which is in accordance with the findings of Avenant *et al.* (2017).

Significant differences among the treatments were recorded for total sugars. Among all treatments bunches treated with GA_3 at 5 ppm (18.211g/100g) registered maximum total sugars followed by bunches without GA_3 application (17.444g/100g). The least total sugars was recorded in bunches treated with GA_3 at 7.5 ppm (15.914g/100g). The increase in reducing, non-reducing and total sugars might be ascribed to the conversion of starch and acids into sugars in addition to continuous mobilization of sugars from leaves to berries (Singh *et al.,* 1993). Singh and Khanduja, (1977) further reported that the application of GA_3 in Pusa Seedless showed increased sugars and decreased acidity content. Application of GA_3 at rachis elongation stage might have stimulated internal synthesis of GA_3 in young berries which might have increased the sink drawing ability leading to more accumulation of sugars in treated berries than in control. The phloem loading capacity is increased or stimulated by application of GA_3 in many crops which helps in better translocation of photosynthates synthesized in leaves to young berries via phloem vessels. Application of GA_3 modifies phloem loading, phloem area and increased expression of sugar transporters to enhance carbon metabolism (Murcia *et al.,* 2016). A ten-fold increase in some of the genes involved in sugar transport and metabolism was observed in Malbec grapes compared to control. A positive correlation was observed between photosynthesis and stomatal conductance in GA_3 treated vines (Murcia *et al.,* 2016). Berry growth is stimulated due to increase in rate of cell division as well as cell elongation (Dokoozlian and Peacock, 2001). Plant hormones have strong effects on berry growth and development (Guerios *et al.,* 2016) among them, GAs take part in a critical function in berry sizing and enlargement (Weaver and McCune, 1960). In the last few years, the effect of exogenous GA_3 application on grape berry growth and cell enlargement has been studied by several researchers; however, the basic mode of action of GA_3 to produce maximum berry size is not very clear.

GA_3 applications may also have negative effects on grapevine, including excessive reduction of the number of berries per cluster, the production of grassy or herbaceous flavors in the fruit, a reduction in tissue winter hardiness and a reduction in node fruitfulness. These phytotoxic effects of GA tend to become more pronounced in the seeded varieties. Considering the above findings from the present study and other supported results from different workers, it might be summarized that GA_3 at 5 ppm might be optimum for bringing about desirable changes in bunch morphology in Crimson Seedless. Super or suboptimal level of GA_3might result in adverse effect on bunch characters.

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(Received on 21.08.2021, Revised on 11.10.2021 and Accepted on 17.11.2021)