Improvement berry color skin profile by exogenous cyanocobalamin treatment of ‘Crimson seedless’ grapevines

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 8 June 2017
Accepted 14 June 2017
Available online 27 June 2017

ABSTRACT

The experiment was conducted to study the effect of cyanocobalamin (B12) treatments (0, 3, 6, and 9 mM B12) on Vitis vinifera L. ‘Crimson seedless’ which conducted during two seasons 2014 and 2015. The study aims to regenerate berry color during growth and preserve it during shelf-life at room temperature for four days. The results showed that B12 treatments were significantly effective in reducing weight loss. Berry shatter, rachis browning index, while it preserved another quality parameter high such as berry firmness, separation force, total phenol content (TPC), total sugar content (TSC), total anthocyanin content (TAC), B-Carotene, ascorbic acid (AA) and color hue angle during shelf-life for four days. The previous results were significantly observed with B12 at 9 mM compared to control and other B12 concentrations. However, total solid content (SSC%), titratable acidity (TA%), and SSC/TA ratio were significantly affected by B12 at 9 mM up to end the shelf-life period. In contrast, the lowest values of total chlorophyll (Chl_a+b) content during shelf-life compared with other B12 concentrations. Therefore, cyanocobalamin (B12) is an effective vitamin for improving or generating berry color at harvest time and maintaining cluster quality of ‘Crimson seedless’ grapes during shelf-life (marketing).

1. Introduction

‘Crimson Seedless’ is a late maturing red seedless grape cultivar with firm berries. It ripens in mid-September and can be stored on vines until mid-November. Also, it possible the storage grapes until early winter under Egyptian climate [1]. The color of red grape berries is an important factor for the market acceptance of crimson table grapes but it remains poorly colored, especially when vines grown in regions or seasons with high or fluctuation temperature [2]. Sometimes, ‘Crimson seedless’ grapes develop red color on some berries while others remain green and unripe or some cluster had different berry color stages [3]. Anthocyanin is reflected the berry skin color in crimson seedless grape. It accumulates in berries at the beginning of the véraison stage of berry development. Continually, accumulation during berry development related to abscisic acid (ABA) metabolism by which berry skin anthocyanin content increased then color appeared [4]. Generally, there are five anthocyanin substituents that found in crimson skin grapes such as malvidin-3-glucosides, delphinidin-3-glucosides, peonidin-3-glucosides, cyanidin-3-glucosides, and petunidin-3-glucosides [5]. The cyanidin-3-glucoside and peonidin-3-glucoside are major two substituents that were responsible for color appearing. Then they acylated derivatives of these anthocyanins higher content in fresh grape skins [6]. Many studies focused on the application of plant growth regulation (PGRs), abscisic acid (ABA), auxins, cytokinins, ethylene and gibberellic acid (GA3). These hormones all have different functions and peak at different stages during vine and berry development as they are responsible for the regulation of growth and ripening can further inhibit coloring [7].

Cobalamin, also called vitamin B12, is a water-soluble vitamin [8]. Also, it found in the plant cell organs such as cytosol, plastids, and mitochondria [9]. Higher plants neither synthesize nor require vitamin B12 because they contain cobalamin-independent methionine synthase (Met) [10]. Methionine synthase catalyzes the final reaction of the Met biosynthetic pathway in two steps, the first step, is catalyzed by the enzyme cystathionine-γ-synthase (CγS) to form cystathionine from the substrates cysteine and O-phosphohomoserine. It is important to note that O-phospho-homoserine is also the immediate precursor of threonine so that methionine synthesis and threonine synthesis compete for a common substrate. The reaction catalyzed by CγS is followed by the conversion of cystathionine to homocysteine by the enzyme cystathionine-β-lyase. In the last step, a methyl group is transferred to plants from N5-methyl-tetrahydrofolic acid to homocysteine by a vitamin-B12-independent methionine synthase to yield Met [11]. In plants, Met serves as a precursor for a variety of metabolic processes, including protein synthesis, as the prime methyl donor for a large number of biological methylation, polyamine synthesis, and ethylene synthesis. Since methionine synthase is also required for
both the regeneration and the de novo biosynthesis of Met. It is the convergence point for two major biochemical domains in cellular metabolism. The Met biosynthetic pathway and the one-carbon cycle as to photosynthesis capacity [11]. Recently, the application of cyanocobalamin to many fruit in order to improve fruit quality during shelf life and to reduce fruit losses such as during cold storage of mango to alleviate chilling injury incorporation with ascorbic acid [12] also extend shelf life of kaki fruit [13,14], ‘Thompson seedless’ grapevines [15], and increasing storability and marketing of guava [16] and productivity and quality attributes of ‘Williams’ banana cultivar [17].

To enlarge the knowledge of cyanocobalamin (Vit.B12) to evaluate the effect of B12 at different concentrations to improve berry skin color on ‘Crimson’ grapes during handling or marketing. So, the study aims to exploit the physiological roles of cyanocobalamin (B12) in a plant cell for enhancing and improving coloration of berry skin on ‘Crimson during shelf-life.

2. Materials and methods

2.1. Plant materials and experimental setup

Cluster samples were harvested on November 2014 and 2015 from trees 10 years old growing in the sandy soil of a commercial orchard. It was located in Sadat city, Egypt. Crimson seedless vines 36 were treated by three doses of cyanocobalamin at 0, 3, 6 and 9 mM. Vitamin B12 or cobalamin in Cyanocobalamin form (1255.3 molecular weight) is water-soluble vitamin and was purchased from El-Gomhoreya Com. suppliers, EGY, purity 98%. Each treatment contained 9 vines which distributed on three replicates. The doses were applied three times (at the véraison stage, after véraison 14 days and before harvest 14 days at sunset due to the sensitivity of vitamin to sunlight. Upon arrival in pomology department lab, the 200 clusters were picked at the soluble solid content (SSC 17% in orchid). Samples were divided into two main batches. The first batch was composed of 120 clusters fruits. 30 clusters per treatment were distributed in three replicates, for non-distractive measurements such as rachis browning index and color hue angle. Distractive measurements were measured on four treatments, each treatment composed 20 fruit clusters in three replicates. Two fruit clusters of each replicate were picked every day for measure chemical analysis up to 4th day of shelf life at ambient conditions at 25 ± 1°C and air humidity average during shelf-life period 57 ± 2) (imitating marketing).

2.2. Physical quality analysis

Berry firmness was recorded using fruit texture Effegi-penetrometer supplemented with a plunger 2 mm diameter penetrator and separation force was measured using a hook instead of a plunger. Firmness and separation force of berries were expressed as an N. The Berry shattering percentage and rachis browning index was recorded as described by [15]. Water loss percentage was recorded [15]. The color was recorded according to [18], and thereafter, all images were analyzed by using software ImageJ Ver. 1.43u USA to get RGB signals to calculate hue angle of clusters as reported to [19].

2.3. Chemical quality analysis

Quality elements were determined, berries were randomly removed from several cluster samples and were divided into three replicates to measure soluble solid content (SSC%) using Carleizss hand refractometer, acidity as tartaric acid (TA) was determined by titration with 0.1 N NaOH and ascorbic acid content (vitamin C) was measured by titrimetric method using 2,6-dichlorophenol indophenol and 6% oxalic acid as substrate according to [20], SSC/TA ratio was calculated as defined maturity index. Total phenol (TP) in the treated fruits were measured spectrophotometrically using the Folin–Ciocalteu reagent with gallic acid as standard [21]. The phenols were measured at the wavelength 750 nm. The results were reported as mg of gallic acid equivalents (GAE) 100 g-1 FW. β-carotene and chlorophyll a and b was spectrophotometrically determined by modified methods [22]. The extraction method was modified by using N,N-dimethylformamide (DMF) instead of acetone. Samples were stored at 4 °C for 16 h to allow the DMF to extract the pigments from the sample. Finally, samples were centrifuged for 5 min at 16,000 rpm, and then samples were determined for wavelengths 452 nm for β-Carotene. It was expressed in mg 100 g-1 FW. Chlorophyll a and b were measured at wavelength 663.8 and 646.8 nm and it presented in mg 100 g-1 FW. Anthocyanin was determined spectrophotometry at wavelength 535 nm and it presented in mg 100 g-1 FW [23]. Total sugars were measured by using phenol 18% and sulphuric acid 96% and the absorbance was recorded with spectrophotometer at 490 nm as it described by [24].

2.4. Statistical analysis

Data for evaluating of physical and chemical analysis were analyzed using two ways incomplete block randomize (ANOVA). The means were compared using the least significant differences (L.S. D.) at p < 0.05 level of probability. The statistical software package GenStat ver. 11 (Lawes Agricultural Trust, Rothamsted Experimental station, UK) was used.

3. Results and discussion

3.1. Physical quality attributes

Table 1 shows a significant at P < 0.05 when B12 concentrations were considered. Considering the different B12 concentrations, it is clear that the B12 treatment (9 mM) is more effective to improve physical quality attributes compared to the other B12 treatments. The water loss is approximately stable during shelf life when vine treated with high B12 dose during shelf life time. It was recorded at 2nd day 2.89% and reached to 10.76% at the 4th day of shelf life compared to control vines (5.79% up to 22.48%). At harvest time, there was no evidence for BS before the 2nd day and for RBI 3rd day of shelf life time. In this case, B12 at 9 mM delays BS up to a 2nd day (5.66%) and increase gradually up to a 4th day (30.13%) compared to other treatments. While RBI was detected at 4th day (2.20 = more than slight browning incidence), while control treatment has severed brown (4.06). However, the hue angle value is a quite dark red (h° 16.96), when vines treated with high B12 concentration compared to other treatments at harvest time. Continuously, the h° decrease more rapidly up to end shelf life period.

Taken as a whole these results show that the physical quality attributes can be affected differ. It seems plausible that B12 control water loss during shelf life. Basically, berries have a somewhat, thick epidermis which covered with waxes layer on berries surface, acting as a protective layer against dehydration [15]. It could be that the highest B12 treatment at 9 mM increases waxes during berries development. Moreover, the responses of berries to B12 treatments could be related to that B12 keeps enhancing ascorbic acid, β-carotene and α-Tocopherol synthesis in berries tissues [25]. Since the last vitamines are considered as antioxidants which play a role to scavenge active oxygen species during shelf-life [8,26]. The important roles of these vitamines are to maintain the right functions of the cell membrane of cells/tissue of berries. How-
Table 1
Effect of preharvest cyanocobalamin (B12) application on cluster weight losses, berry shattering %, rachis browning index, Hue angle (ho), Berry firmness (N) and separating force (N) of ‘Crimson Seedless’ grapes during four-day shelf-life at 2014 and 2015 seasons.

| Treatments | D1 | D2 | D3 | D4 |
|------------|----|----|----|----|
| Water loss % | Berry shuttering % | Treatments |
| 0 | 0.00 | 5.79a | 11.95a | 22.48a | 0.00 | 4.48a | 20.21a | 44.06a | 52.30a |
| 3 | 0.00 | 4.17b | 9.12b | 18.08b | 3 | 1.63c | 12.09c | 33.89c | 48.17c |
| 6 | 0.00 | 3.66c | 7.97c | 13.14c | 6 | 1.10c | 9.32c | 27.14c | 36.07c |
| 9 | 0.00 | 2.89d | 7.22d | 10.76d | 9 | 1.00d | 5.66d | 23.11d | 30.13d |
| LSD | 0.717 | 4.885 | 6.576 | LSD | 0.81 | 1.64 | 3.70 | 7.89 |

Rachis browning index

| Treatments | Hue angle (h°) | Treatments |
|------------|---------------|------------|
| 0 | 1.00 | 1.17a | 2.61a | 4.06a | 0 | 108.87a | 105.60a | 95.93a | 85.64a |
| 3 | 1.00 | 1.07b | 1.67b | 3.04b | 3 | 68.13b | 65.08b | 62.14b | 59.13b |
| 6 | 1.00 | 1.00c | 1.28c | 2.95c | 6 | 47.88c | 46.00c | 43.48c | 40.26c |
| 9 | 1.00 | 1.00d | 1.00d | 2.20d | 9 | 16.96d | 15.55d | 13.33d | 10.67d |
| LSD | 0.06 | 0.23 | 0.16 | LSD | 2.19 | 4.56 | 3.82 | 8.20 |

Berry firmness (N)

| Treatments | Berry separation forces (N) | Treatments |
|------------|-----------------------------|------------|
| 0 | 7.69e | 7.27e | 6.06e | 4.92e | 0 | 6.67e | 5.89e | 4.42e | 4.25e |
| 3 | 8.48f | 7.95f | 6.97f | 5.92f | 3 | 7.00f | 6.64f | 5.17f | 4.49f |
| 6 | 8.62g | 8.27g | 7.34g | 6.38g | 6 | 7.45g | 7.08g | 5.61g | 5.04g |
| 9 | 13.08h | 8.74h | 7.91h | 7.08h | 9 | 7.68h | 7.43h | 5.96h | 5.56h |
| LSD 5% | 0.61 | LSD 5% | 0.35 |

Means in a column are significantly different at (P < 0.05) according to the LSD. Each value represents mean of 3 replicates during two seasons of 2014 and 2015. The superscript letters, differ (P < 0.05) according to the LSD of two way using two ways incomplete block randomize according to Duncan’s test.

3.2. Chemical quality attributes

Table 2 depicts the variation of soluble solid content (SSC), titratable acidity (TA%), and SSC/TA ratio and total sugars (TSC), ascorbic acid (AA) and total phenol content (TP) as a function of shelf life time (days) at four preharvest B12 applications. In fact, the previous parameters show a significant interaction P ≤ 0.001 when the shelf time and B12 concentrations were considered. The main significant effect is observed at harvest time with vines treated with high B12 concentration (9 Mm) compared to other treatments. Initially, SSC, SSC/TA ratio, TSC, AA and TP content were observed to be higher than other B12 treatments, while TA% decreased at harvest time. It is most likely that the increases in chemical parameters content might be affected by high concentration of B12. It could be that B12 enhance activation of carbohydrate enzymes synthesis and keeping them in an active status in a plant cell in equilibrium [30]. Therefore, the activation of Calvin cycle, the pentose phosphate pathway and glycolysis might be activated by applying high B12 concentration [8], resulting in increasing in total sugars [14].

3.3. Skin berry pigments: β-carotene (β-Car), total Chlorophylla (Chla) and anthocyanin (TAC)

Table 3 shows the changes of berry skin pigments content as a function of shelf life time (days). A significant interaction P ≤ 0.001 between shelf life time and B12 concentrations. Berry skin pigments decreased with increasing shelf life time up to end of the experiment. Pre-harvest B12 treatment at 9 mM presented more β-Car (2.28 mg 100 g−1 FW) and TAC (36.11 mg 100 g−1 FW), on the contrary, the total Chla content 0.66 mg 100 g−1 FW at harvest
time was observed. The changes in β-Car and TAC during shelf life are noticed owing to the continued synthesis of β-Car and TAC which occurred for a limited period at all B12 treatments at 2nd day of shelf life. Therefore, in β-Car and TAC which were then followed by a decrease up to end shelf life time.

Total chlorophyll responds differently to B12 treatments. The reductions of Chlab is evenly distributed across all components, however, with B12 at 9 mM treatment, the decreasing of Chlab suggests a breakdown of Chl containing chlorophyll binding protein such as the LHC (light harvest center) of photosystem I or II [31]. While β-Car behave quite differently which increases up to 2nd day and then decrease up to end experiment time. It could be due to the effect of high concentration of B12 (9 mM) activates many metabolic processes are enhanced which are diverted towered the lipid-soluble vitamin A [32]. Also, the increases TAC at 2nd day of shelf life can be related to activating major precursors of anthocyanin are responsible for increasing TAC such as cyanidin, peonidin and the acylated derivatives in berry skin and pulp [3,6]. Continuously, the degradation of chlorophyll might be due to senesce of berry and deterioration during shelf life period, thereafter, TAC was more appearance [2].

### Table 2
Effect of preharvest cyanocobalamin (B12) application on SSC, TA%, SSC/AT ratio, total sugar content (TSC), ascorbic acid (AA mg 100 g⁻¹ FW) and Total phenol content (TP mg 100 g⁻¹ FW) of ‘Crimson Seedless’ grapes during four-day shelf-life at 2014 and 2015 seasons.

| Treatments | D1 | D2 | D3 | D4 | Shelf-life time (days) |
|------------|----|----|----|----|------------------------|
| SSC%       |    |    |    |    |                        |
| Treatments | 0  | 17.00a | 18.00b,c,d | 17.33d,e | 17.66f,e             |
|            | 3  | 17.67c,d | 18.33d,e | 18.00f,c,d | 18.00f,c,d        |
|            | 6  | 18.00c,d | 18.67f,g | 17.00f,c,d | 18.33d,e         |
|            | 9  | 19.00c,d | 18.66f,g | 17.67f,e | 18.33d,e          |
| LSD 5%     | 0.89 |    |    |    |                        |

| SSC/TA ratio |    |    |    |    |                        |
| Treatments | 0  | 22.08i | 24.00j,k | 23.42i | 26.36f             |
|            | 3  | 26.37j,k | 27.77l,m | 26.56i | 29.51k         |
|            | 6  | 29.51i | 31.64a,b | 29.82c,d | 33.33e          |
|            | 9  | 35.19g,h | 34.56i | 41.09k | 35.25c          |
| LSD 5%     | 1.90 |    |    |    |                        |

| Ascorbic acid content (AS mg 100 g⁻¹ FW) |    |    |    |    |                        |
| Treatments | 0  | 1.96h | 1.66i,j | 1.29c | 1.10g             |
|            | 3  | 2.11c | 2.00d | 1.58e | 1.35f         |
|            | 6  | 2.85a,b | 2.47c,d | 2.26f,g | 2.07h       |
|            | 9  | 3.96a,b | 3.73c,d | 3.41e,f | 3.00g      |
| LSD        | 0.18 |    |    |    |                        |

| Total phenol content (TPC mg 100 g⁻¹ FW) |    |    |    |    |                        |
| Treatments | 0  | 0.83d | 0.94c | 0.94c | 0.80d             |
|            | 3  | 0.78a,b | 0.82b,c | 0.80d | 0.62c         |
|            | 6  | 0.85a,b | 0.78e,f,g | 0.73c | 0.60d       |
|            | 9  | 0.64a,b | 0.63e,f | 0.54b | 0.48c        |
| LSD        | 0.05 |    |    |    |                        |

### Table 3
Effect of preharvest cyanocobalamin (B12) application on β-Carotene (β-Car), Total chlorophyll a and b (Chlab), and Total anthocyanin content (TAC) of ‘Crimson Seedless’ grapes during four-day shelf-life at 2014 and 2015 seasons.

| Treatments | D1 | D2 | D3 | D4 | Shelf-life time (days) |
|------------|----|----|----|----|------------------------|
| β-Carotene content (CAR mg 100 g⁻¹ FW) |    |    |    |    |                        |
| Treatments | 0  | 1.30b | 1.96d | 1.59e | 1.12f             |
|            | 3  | 1.99d | 2.15c | 1.85c | 1.34d         |
|            | 6  | 2.11c | 2.30b | 1.99c | 1.54c       |
|            | 9  | 2.28b | 2.53a,b | 2.11c | 1.69d      |
| LSD        | 0.14 |    |    |    |                        |

| Chlorophyll (Chlab mg 100 g⁻¹ FW) |    |    |    |    |                        |
| Treatments | 0  | 1.12a | 0.94b | 0.80d | 0.60c             |
|            | 3  | 0.94b | 0.84c | 0.77d | 0.64c        |
|            | 6  | 0.85c | 0.78e,f,g | 0.73c | 0.60d       |
|            | 9  | 0.64a,b | 0.63e,f | 0.54b | 0.48c      |
| LSD        | 0.05 |    |    |    |                        |

| Total anthocyanin content (TAC mg 100 g⁻¹ FW) |    |    |    |    |                        |
| Treatments | 0  | 16.43b,c | 17.80e,f | 14.69d | 10.43g             |
|            | 3  | 19.95c,d | 20.52e,f,g | 16.58e,f | 12.14h         |
|            | 6  | 25.84a,b | 30.66h,i | 19.55e,f | 15.76h       |
|            | 9  | 36.11a,b | 37.70a,b | 23.66d | 15.50h      |
| LSD        | 2.98 |    |    |    |                        |

Means in a column are significantly different at (P < 0.05) according to LSD. Each value represents mean of 3 replicates during two seasons of 2014 and 2015. The superscript letters, differ (P < 0.05) according to the LSD of two way using two ways incomplete block randomize according to Duncan’s test.
4. Rachis browning index

Rachis browning index: rachis quality of bunches has been investigating extensively among producers and exporters because of its high impact on the cluster freshness that determines consumers. Table 1 shows a significant at P < 0.05 when the B12 concentrations were considered as a factor. It is clear that the treatments at 9 mM are more effective to reduce cluster rachis browning compared to other treatments. At harvest time, there was no evidence for RBI up to 3 days of shelf life time. In this case, RBI was detected only at 4th day. It was minimized RBI round slight browning incidence (2.20) compared to other control fruit (4.06 severity symptoms) and B12 treatments (3 mM, 3.04 and 6 mM, 2.95). It is clear that B12 treatment at 9 mM has a good potential beneficial against rachis browning of detached grape clusters. It could be due to activating enzymatic carbohydrate synthesis during berry development [30]. Therefore, the activation processes such as Calvin cycle, the pentose phosphate, and Glycolysis pathway might constitute the increase sugar content in berry development. So, total sugars increased (Glucose and fructose) which it could be a precursor toward to increase AA synthesis in berry juice [8]. The increases of TP might be explained that the increases of AA berry content by which maintaining the amount of TP as antioxidant properties also, cyanocobalamin may modulate the active oxygen species [26].

5. Conclusion

It might be concluded that the application of cyanocobalamin (B12), especially preharvest treatment at 9 mM had a positive impact in improving anthocyanin pigment content of berries and maintaining cluster quality during shelf life. Moreover, it minimizes the cluster/rich browning incidence by preserving phenolic compounds from oxidation during four days of shelf life. It is due to increase ascorbic acid synthesis by increasing metabolic carbohydrates during berry development. Hence, the preharvest application of B12 at 9 mM can be applied as an effective method for improving anthocyanin pigment and postharvest quality attributes of 'Crimson seedless' at harvest time and during marketing.

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