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RESEARCH PAPER

Pre-harvest foliar application of calcium chloride improves berry quality and storage life of table grape cvs. 'perlette' and 'kings's ruby'

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Key Message: This study was performed to evaluate the pre-harvest foliar application of calcium chloride on two grapes varieties "perlette and king's ruby". It was confirmed that calcium chloride improved long term storage and berry quality of these grapes varieties.

Abstract: Grapes being the most perishable fruit crop need certain pre-harvest measures to improve berry quality. Therefore, current study was conducted for the improvement of early bearing 'Perlette' and 'King's Ruby' table grape cultivars by applying pre-harvest sprays of calcium chloride ($CaCl_2$) concentrations. Table grape cvs. 'Perlette' and 'King's Ruby' were sprayed at 15 days' interval from fruit set to maturity with different concentrations of $CaCl_2$ (0, 0.5%, 0.75% and 1.0%) to evaluate the photosynthetic activity and postharvest biochemical quality. Pre-harvest sprays of 0.5% and 0.75% maintained increased net photosynthetic rate (*A*) in 'King's Ruby' and 'Perlette' respectively; whereas transpiration rate (*E*) and stomatal conductance (*gs*) was significantly higher with 0.75% treatment in both cultivars. Pre-harvest foliar sprays of 0.5% and 0.75% $CaCl_2$ significantly improved berry quality with increased berry length and diameter. Postharvest biochemical quality of 'Perlette' and 'King's Ruby' was considerably improved with higher TSS, TA, ascorbic acid contents, sugars, total phenolic contents and antioxidants. These results clearly depicted that pre-harvest application of different $CaCl_2$ concentrations improved growth, development, physico-chemical and antioxidative attributes of table grape cvs. 'Perlette' and 'King's Ruby' under long term storage conditions at 0.5 ± 0.5 °C and 90 % RH for four weeks.

Keywords: Calcium chloride, Cold storage life, King's Ruby, Perlette, Photosynthesis, Quality

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Introduction

Grapes (*Vitis vinifera* L.) are the world's most popular fruit and belong to the family vitaceae. Production of grapes is more than 8 million metric tons worldwide, whereas, area under grape production in Pakistan is 14409 hectares with 57920 tonnes' production annually (FAOSTAT, 2017). Among all provinces, Balochistan tops with 70% contribution of total grape production; although, grapes are also produced in parts of Khyber Pakhtunkhwa and Punjab (Safdar, 2013). However, yield and quality of grapes is associated with a number of biotic and abiotic stresses that cause decrease in yield and overall berry quality (Ahmed et al., 2004). Management practices like selection of early maturing cultivars such as 'Perlette' and 'King's Ruby' or pre-harvest foliar application of certain chemicals that could increase yield and quality deserves crucial importance. The bottlenecks associated with quality of these cultivars such as cv. 'Perlette' is characterized by thin skin and compact bunch that makes it susceptible to diseases caused by *Botrytis cinerea* (Ahmed et al., 2004). While, grape cv. 'King's Ruby' is a red coloured variety characterized by problems like uneven colour development and susceptibility to grey mold disease caused by *Botrytis cinerea*.

Previously, sulfur dioxide (SO_2) fumigation used to be employed as a tool to improve grape quality and protection against botrytis infection (Droby & Lichter, 2004); however, its use has been restricted due to certain health hazards (Pretel et al., 2006). Alternative safe approaches that can reduce fungal infection, improve yield, quality and postharvest life are of imperial importance. Calcium (Ca) being a fundamental macronutrient is essential for growth and development processes and is considered to improve various fruit quality attributes of fruits (Barker & Pilbeam, 2015; Eryani-Raqeeb et al., 2009); besides, inorganic salt of Ca^{2+} such as calcium chloride ($CaCl_2$) have also been reported to alleviate the harmful effects of abiotic stresses (Upadhyaya et al., 2011, Xu et al., 2013). Previous reports regarding Ca^{2+} application show its drought tolerant properties with increased net photosynthesis rate in grape cv. 'Kyoho' (Zheng et al., 2010); whereas, in another study $CaCl_2$ increased photosynthesis in *Zoysia japonica* (Xu et al., 2013). While, postharvest application of $CaCl_2$ conserved quality and storage life of blueberries (Sabir et al., 2019).

On the other hand, $CaCl_2$ also exhibited antifungal characteristics as it had effectively controlled decay of grapes berries (Nigro et al., 2006; Chervin et al., 2009) and improved 'Thompson Seedless' grapes quality by developing uniform colour associated with early maturity and enhanced resistance against *B. cinerea* (Chardonnet et al., 1997). While, foliar application of $CaCl_2$ has been found to control physiological disorders of fruit with improved fruit quality (Bakshi et al., 2005). Some scientists argue that Ca^{2+} accumulate in grape berries during the process of growth and development (Rogiers et al., 2000) till ripening stage (Cabanne and Doneche, 2001; Cabanne & Doneche, 2003). Therefore, adequate availability of Ca^{2+} is essential for improved grape quality as formerly cvs. 'Venus' and 'Nigara Rosada' exhibited reduced berry abscission, fungal infection and disease incidence that ultimately resulted in higher yield (Danner et al., 2009; Tecchio et al., 2009; Devi & Kumari, 2015). Moreover, postharvest $CaCl_2$ treatment reduced berry drop index, decay incidence and maintained quality of 'Isabel' grapes under modified atmosphere storage condition (Silva et al., 2012).

Above review clearly illustrates the importance of $CaCl_2$ application for improving fruit quality. However, very little is known about the influence of pre-harvest sprays of $CaCl_2$ treatments on photosynthesis activity along with its effect on yield and postharvest quality of grape cvs. 'Perlette' and 'King's Ruby'. Therefore, a current experiment was undertaken to investigate the influence of pre-harvest foliar application of $CaCl_2$ on photosynthesis, yield and postharvest quality of grape cvs. 'Perlette' and 'King's Ruby'.

Materials and Methods

Plant material

An experiment was carried out at a well maintained vineyard located at Rawat 33° 40' N, 73° 10' E) Islamabad. A total of 30 vines for each cultivar i.e. 'Perlette' and 'King's Ruby' having four years of age grown in a uniform environment, protected from disease and pests were selected simultaneously for this study. The layout system used in orchards was rectangular, having vine to vine 8′ and row to row 10′ distance. Selected vines were given uniform cultural practices throughout the experiment. Each vine was used as a treatment unit in a block and treatments were replicated three times. Bunches of grapes, free from disease or disorder were selected and sprayed with different concentrations of calcium chloride (0, 0.5%, 0.75% and 1.0%) at 15 days' interval from fruit set to maturity. The experiment was conducted in two parts where first part (pre-harvest) was comprised of photosynthetic [photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), sub-stomatal CO_2 concentration (Ci)] and growth parameters (berry length, berry diameter and yield $vine^{-1}$) after 48 hours of each spray. Data for the first part of the experiment (pre-harvest section) was subjected to two factor factorial arrangements ($CaCl_2$ concentrations and cultivars) under RCBD and replicated three times. In the second part (postharvest section), grape bunches were harvested at the ripening stage with 17-20^{o}Brix total soluble solids. Each treatment comprised of 60 bunches with 20 bunches per replicate which were shifted to the Postharvest Laboratory of the Department of Horticulture, PMAS-AAUR for biochemical quality and storage life evaluation. At arrival misshapen, injured and disease berries/bunches were sorted out. Bunches were washed followed by drying at room temperature to remove any infectious spores and dust particles. Data was recorded for fresh organoleptic, physico-chemical, phenolic and antioxidative attributes on zero day; whereas, remaining bunches were packed in corrugated cardboard boxes for further postharvest evaluation under long term cold storage conditions at 0.5 ± 0.5 °C and 90 % RH for four weeks. Grapes bunches were removed from cold storage at weekly interval for various postharvest analyses and data were subjected to three-factor factorial arrangements (chemical concentrations, cultivars and storage period) for statistical analysis.

Part 1 Pre-harvest parameters

Photosynthesis rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO_2 concentration (Ci)

Photosynthesis rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO_2 concentration (Ci) was recorded after 48 hours of each spray from one fully expanded leaf after fruit set of each tagged vine with the help of IRGA (Infra-Red Gas Analyzer, Type LCA-4, USA) by the method outlined in detail by Long & Bernacchi (2003).

Berry length, berry diameter and yield $vine^{-1}$

Bunch was divided into 3 sections lengthwise and 10 berries from each section were randomly picked to determine the berry length. Measuring scale was used to calculate the average berry length and expressed as millimeter (mm). While, berry diameter was calculated using an electronic digital caliper and expressed as millimeter (mm). Whereas, yield of each vine was calculated right after harvest and expressed in kilogram (kg).

Part 2 Post-harvest parameters

Weight loss and decay incidence

Weight loss was calculated in percentage using electronic balance (ELB 1200, Shimadzu, Japan) at each removal by subtracting the initial weight at harvest from the weight of berries at the time of removal from the cold store at weekly intervals. While, decayed berries were observed visually at weekly intervals and decay percentage was calculated by counting decayed berries out of healthy berries.

Biochemical fruit quality analysis

Total soluble solid (TSS) (^{o}Brix), titratable acidity (TA) (%) and ascorbic acid (mg/100g)

Soluble solids (TSS) of grape juice were evaluated using a digital refractometer (Atago, ATC-1, Tokyo, Japan). Titratable acidity (TA) of grape juice was quantified by titrating against 0.1 N NaOH till light pink color. Ascorbic acid contents at the time of harvest well as at each removal from cold storage were estimated using the method given by Hans (1992).

Total phenolic contents (TPC) (mg/g) and total antioxidants (%)

Total phenolic contents (TPC) were determined using Folin-Cicalteau reagent by the method adopted by Singleton et al. (1999), with slight alterations, based on colorimetric oxidation/reduction reaction of phenols and expressed as mg/g. Radical scavenging activity was analyzed using the DPPH method as drafted by Brand-Williams (1995).

Sugars (%)

Reducing sugar, non-reducing and total sugar percentage from grape berries was estimated as per the method of Hortwitz (1960).

Berry colour (*L, *a**, *b**) and organoleptic evaluation**

Colour of bunches was measured with a chromameter (CR-300, Minolta) and presented as *L**, *a** and *b** (chroma). *L** represents lightness (-) to darkness (+), a* represents greenness (-) to redness and b* represents yellow (-) to blue (+). Whereas, organoleptic evaluation for taste, texture and flavour was carried out by using Hedonic scale method given by Peryam & Pilgrim (1957). A panel of five judges in the range of 22-45 years was chosen on the basis of their consistency and reliability of judgment. Panelists scored the difference between samples by scoring numbers to each sample like 1: dislike extremely, 2: dislike moderately, 3: neither like nor disliked, 4: like moderately and 5: like extremely.

Statistical analysis

Experimental data was subjected to analysis of variance (ANOVA) using Statistix 10 for windows software. Current investigation was conducted in two parts i.e. pre-harvest and postharvest. Experimental data for pre-harvest part was subjected to two factors factorial arrangement ($CaCl_2$ concentrations and cultivars) under randomized complete design block (RCBD); whereas, experimental data for postharvest part was subjected to three factor factorial arrangements (chemical concentrations, cultivars and storage period) under completely randomized design (CRD). Treatment differences were determined using the least significant differences test (Fisher's LSD) at $P \leq 0.05$ (Steel et al., 1997).

Results

Photosynthesis rate (*A*), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO_2 concentration (Ci)

Photosynthesis (A) did not differ significantly in both cultivars up to 30 days of fruit setting. However, significant variation in net photosynthesis rate was observed among different $CaCl_2$ treatments from 30 to 60 days of fruit set. All $CaCl_2$ treatments exhibited higher photosynthetic rate than control in both cultivars after 60 days of fruit set. Overall, grape cv. 'Perlette' maintained 20.44% higher photosynthetic rate; whereas, cv. 'King's Ruby' treated with 0.5% $CaCl_2$ exhibited about 17.31% higher photosynthetic rate than control treatment (Fig. 1A, B). All $CaCl_2$ treatments significantly affected transpiration rate (*E*) in both cultivars. Transpiration rate showed an increasing trend up to 45 days after fruit set and decreased later on as time of harvesting approached. After 60 days of fruit set, grape cvs. 'Perlette' as well as 'King's Ruby' treated with 0.75% $CaCl_2$ resulted in 35.76% and 32.21% higher transpiration rate, respectively, as compared to control (Fig. 1C, D). Stomatal conductance was significantly influenced by foliar sprays of $CaCl_2$ up to 45 days after fruit set; although cultivars and $CaCl_2$ treatments significantly affected stomatal conductance from 45-60 days after fruit set. All $CaCl_2$ treatments maintained significantly higher stomatal conductance than control. Maximum stomatal conductance (445.75 and 468.0) was observed in 0.75% $CaCl_2$ treatment that was 32.69% and 31.67% higher in grape cvs. 'Perlette' and 'King's Ruby', respectively (Fig. 1E, F). Sub-stomatal CO_2 concentration did not show any significant differences among $CaCl_2$ treatments. Whereas, the difference between both cultivars was also non-significant up to 45 days after fruit set. However, from 45-60 days after fruit set, cv. 'Perlette' exhibited significantly higher sub-stomatal CO_2 concentration (about 3.1%) than cv. 'King's Ruby' (Fig. 1G, H).

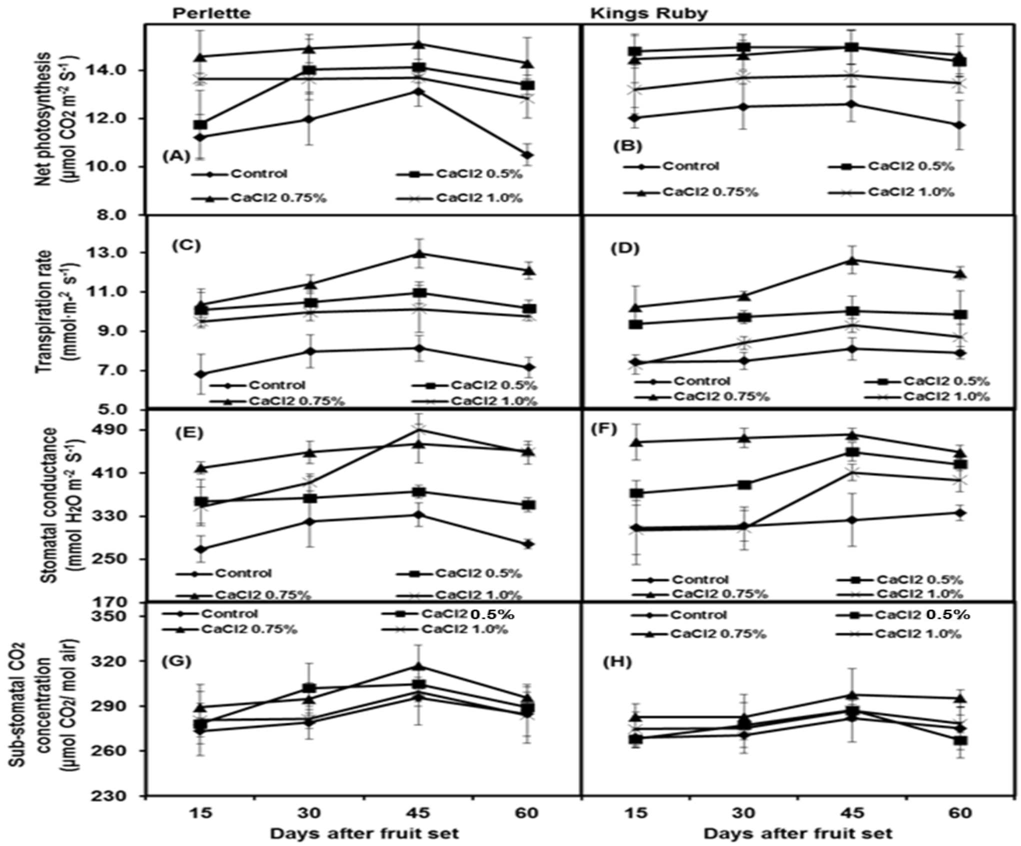

Fig. 1 Effect of pre-harvest foliar application of $CaCl_2$ at different intervals after fruit set on photosynthetic efficiency of grape cvs. ‘Perlette’ and ‘King’s Ruby’

Berry length (mm), berry diameter (mm) and yield vine^{-1} (kg)

Foliar application of $CaCl_2$ significantly increased berry length of both cultivars i.e. cvs. ‘Perlette’ and ‘King’s Ruby’. Vines of grape cv. ‘Perlette’ sprayed with 0.75% $CaCl_2$ showed maximum berry length (16.02 mm) which was 18.14% more than control berries. Whereas, ‘King’s Ruby’ exhibited maximum berry length (15.79 mm) with 0.5% $CaCl_2$ treatment; showing about 13.82% increase in berry length, as compared with control. On the other, hand non-significant differences among cultivars were observed for berry length; although, ‘King’s Ruby’ maintained relatively higher berry length than ‘Perlette’ grape berries (Fig. 2A). Foliar application of $CaCl_2$ significantly affected berry diameter; however, 0.5% $CaCl_2$ and 0.75% $CaCl_2$ treatments showed at par results for berry diameter with increased berry diameter than other treatments. Maximum berry diameter in ‘Perlette’ berries (15.3 mm) was recorded in 0.5% $CaCl_2$ and 0.75% $CaCl_2$ sprayed berries showing about 22.27% increased berry diameter than control. While, maximum diameter (15.1 mm) of ‘King’s Ruby’ berries was resulted with 0.5% $CaCl_2$ which was about 7.43% more than control (Fig. 2B). Pre-harvest application of $CaCl_2$ increased vine yield; however, the difference among treatments was non-significant. While, the difference among cultivars was significant as ‘King’s Ruby’ exhibited significantly higher yield vine^{-1} (about 42.1%) as compared with cv. ‘Perlette’ (Fig. 2C).

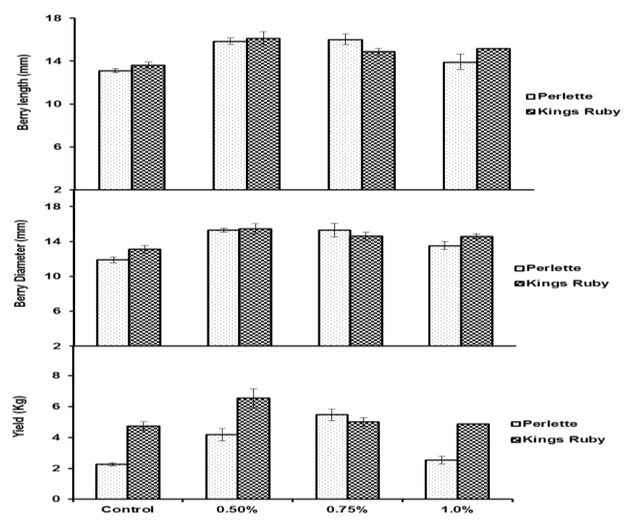

Fig. 2 Effect of pre-harvest foliar application of $CaCl_2$ on berry length, berry diameter, and yield of grape cvs. ‘Perlette’ and ‘King’s Ruby’.

Berry weight loss and decay incidence

Foliar application of $CaCl_2$ significantly affected weight loss, as all $CaCl_2$ treatments exhibited reduced berry weight loss than control. Among all treatments 0.5% $CaCl_2$ concentrations resulted in significantly reduced berry weight loss in both cultivars throughout the cold storage period, as compared with control. Berries of cv. 'Perlette' sprayed with 0.75% $CaCl_2$ resulted in 59.31% less weight loss; whereas, berries of cv. 'King's Ruby' exhibited 48.96% less weight loss, as compared with control after 28 days of cold storage. Moreover, both cultivars showed significant differences for weight loss; as cv. 'King's Ruby' exhibited 86.68% reduced fruit weight loss than grape cv. 'Perlette' (Fig. 3A, B). Similarly, $CaCl_2$ concentrations, cultivars and storage period significantly influenced decay incidence of grape berries. All $CaCl_2$ treated vines exhibited considerably less decay incidence than control; however, berries treated with 0.5% $CaCl_2$ exhibited 37.53% and 64% less decay incidence in grape cvs. 'Perlette' and 'King's Ruby' than that of control after 28 days of cold storage. Grape cv. 'King's Ruby' maintained significantly less decay incidence (about 70.73%) than 'Perlette' grapes (Fig. 3C, D).

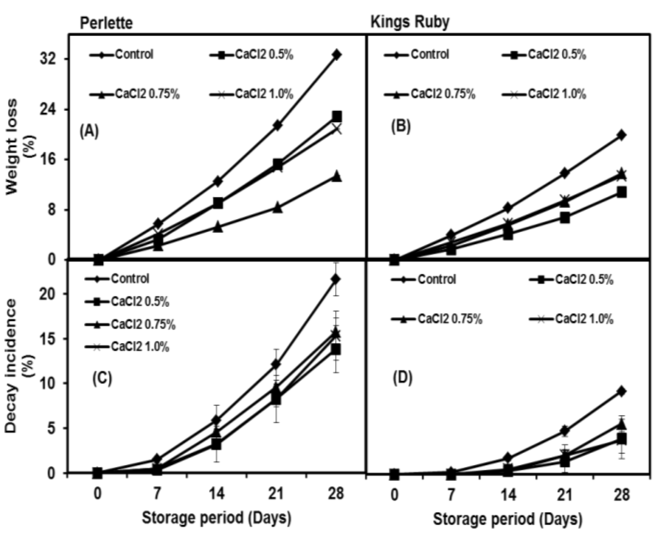

Fig. 3 Effect of pre-harvest foliar application of $CaCl_2$ on Weight loss and decay incidence of grape cvs. 'Perlette' and 'King's Ruby' under cold storage conditions.

TSS, TA, ascorbic acid, TPC and antioxidants

TSS and TA of berries were significantly affected by $CaCl_2$ concentrations, cultivars and storage period. In both cultivars, TSS was increased under cold storage conditions regardless of treatments and cultivars; however, $CaCl_2$ treatments maintained higher TSS than control treatment. Grape cv. 'Perlette' sprayed with 0.75% $CaCl_2$ exhibited more TSS (about 14.6%) than control treatment after 28 days of cold storage period. Whereas, 'King's Ruby' berries sprayed with 0.75% $CaCl_2$ showed more TSS (9.3%) than control berries. Overall, cv. 'Perlette' exhibited 3% higher TSS than 'King's Ruby' cultivar of grapes (Fig. 4A, B). On the other hand, TA decreased under cold storage conditions regardless of $CaCl_2$ concentrations and cultivar differences. Grape cv. 'Perlette' showed 12.22% higher TA in berries treated with 0.5% $CaCl_2$; whereas, 15.86% more TA was observed in 0.75% $CaCl_2$ treated 'King's Ruby' berries, as compared with control. While, cv. 'King's Ruby' berries maintained 9.19% more TA than 'Perlette' berries (Fig. 4C, D).

Ascorbic acid contents of both grape cultivars showed a decreasing trend with the advancement of storage period regardless of $CaCl_2$ treatments; however, vines sprayed with $CaCl_2$ treatments showed relatively less decrease in ascorbic acid contents, as compared with control. About 13.1% higher (3.79) ascorbic acid contents were observed in cv. 'Perlette' berries sprayed with 0.75% $CaCl_2$ and about 9.87% more ascorbic contents (2.25) were recorded in 'King's Ruby' berries sprayed with 0.75% $CaCl_2$ than control. While, ascorbic acid contents of 'Perlette' and 'King's Ruby' did not vary significantly (Fig. 5A, B). Foliar application of $CaCl_2$ significantly affected TPC and antioxidants. Decreasing trend was observed in TPC and antioxidants levels with the progression of storage period regardless of $CaCl_2$ treatments. However, decline in TPC and antioxidants was considerably low in $CaCl_2$ treated berries, as compared to control. Grape vines sprayed with 0.75% $CaCl_2$ resulted in 33.87% and 36.16% less decrease in TPC of cvs. 'Perlette' and 'King's Ruby' berries, as compared with control. While, 0.5% $CaCl_2$ treatment resulted in 24.96% and 20.9% less decrease in antioxidants of cvs. 'Perlette' and 'King's Ruby' berries, respectively, as compared with control (Fig. 5C, D, E, F).

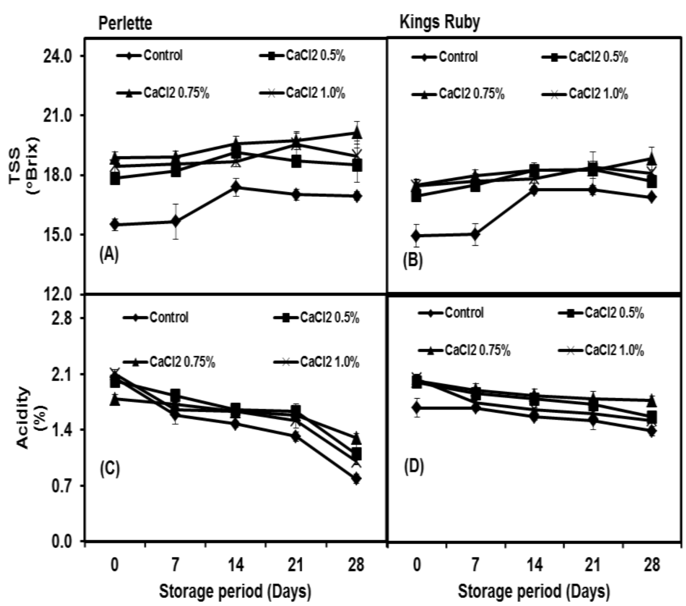

Fig. 4 Effect of pre-harvest foliar application of $CaCl_2$ on total soluble solids (TSS) and titratable acidity (TA) of grape cvs. 'Perlette' and 'King's Ruby' under cold storage conditions.

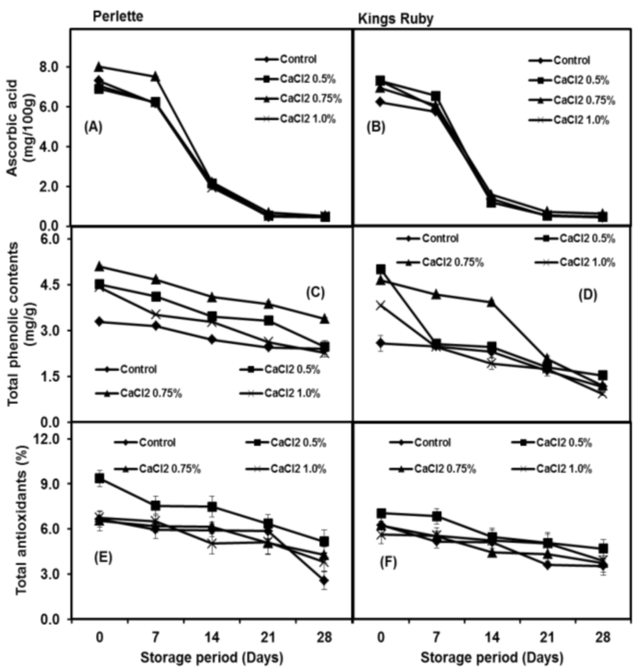

Fig. 5 Effect of pre-harvest foliar application $CaCl_2$ on ascorbic acid contents, total phenolic contents, and total antioxidants of cvs. ‘Perlette’ and ‘King’s Ruby’ under cold storage conditions.

Reducing, non-reducing and total sugars (%)

Sugar contents of grape cvs. ‘Perlette’ and ‘King’s Ruby’ berries were significantly affected by pre-harvest foliar application of $CaCl_2$. Reducing sugar showed an increasing trend; whereas, a decreasing trend in non-reducing sugars was observed during the entire cold storage period. Grape cv. ‘Perlette’ sprayed with 0.75% $CaCl_2$ maintained about 20.56% and 8.37% more reducing sugar as well as non-reducing sugar after 28 days of cold storage, as compared with control. Similarly, ‘King’s Ruby’ berries sprayed 0.75% $CaCl_2$ resulted in 11.61% and 11.48% more reducing sugar as well as non-reducing sugar, respectively, as compared with control. Moreover, grape cv. ‘Perlette’ maintained 5.61% and 68.51% more reducing sugar and non-reducing sugar, respectively, as compared with cv. ‘King’s Ruby’ under cold storage conditions (Fig. 6A, B, C, D). While, total sugars increased during cold storage regardless of cultivar differences, $CaCl_2$ treatments and storage period. Grape cv. ‘Perlette’ and ‘King’s Ruby’ sprayed with 0.75% $CaCl_2$ maintained about 11.85% and 13.12% more total sugars percentage, respectively, as compared with control. While, ‘Perlette’ berries maintained about 2.1% more total sugar percentage than cv. ‘King’s Ruby’ (Fig. 6E, F).

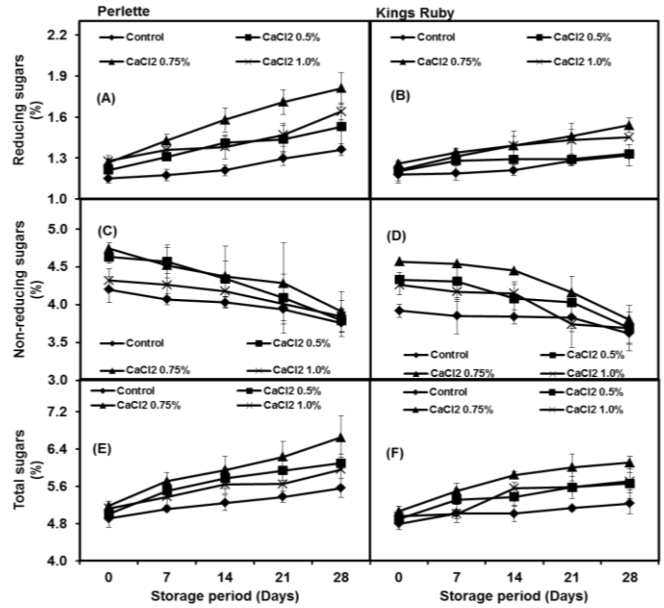

Fig. 6 Effect of pre-harvest foliar application of $CaCl_2$ on reducing, non-reducing, and total sugars of grape under cold storage conditions

Berry colour (*L, *a**, *b**)**

Overall, berry colour development was significantly affected by foliar application of $CaCl_2$, cultivar differences and storage period. Colour values (*L**, *a**, *b**) showed variations in treatments and cultivars in this experiment. Berry lightness (*L**) did not show significant differences among treatments; however, difference among cultivars was significant. Berry lightness (*L**) was significantly higher after harvest up to 21 days followed by decline in lightness in both cultivars i.e. ‘Perlette’ and ‘King’s Ruby’. Grape cv. ‘Perlette’ berry showed about 41.1% more lightness (*L**) than ‘King’s Ruby’. Similarly, application of $CaCl_2$ exhibited a non-significant effect on green to red (*a**) colour development. Berries of cv. ‘King’s Ruby’ maintained substantially higher *a** value than ‘Perlette’ berries. On the other hand, foliar application of $CaCl_2$ on grape vines significantly affected blue to yellow (*b**) colour development. Grape cv. ‘Perlette’ fruit exhibited a significantly higher value of *b** parameter (about 89.27%), as compared with cv. ‘King’s Ruby’ (Table 1).

Table 1 Effect of different concentrations of $CaCl_2$ on $L*$ colour coordinate under cold storage conditions

Cultivar		Treatment	Storage period				
			0	7	14	21	28
Perlette	$L*$	Control	50.3^{c-f}	54.2^{b-e}	54.4^{b-e}	56.0^{a-c}	47.4^{f}
		$CaCl_2$ 0.5%	49.4^{ef}	53.8^{b-e}	55.7^{a-d}	57.9^{ab}	55.3^{a-d}
		$CaCl_2$ 0.75%	50.7^{c-f}	54.2^{b-e}	57.0^{ab}	60.4^{a}	54.1^{b-e}
		$CaCl_2$ 1.0 %	52.7^{b-f}	53.1^{b-f}	53.2^{b-e}	53.2^{b-e}	50.3^{d-f}
Kings Ruby		Control	29.2^{j-m}	30.3^{h-m}	32.6^{g-m}	34.8^{g-j}	27.4^{m}
		$CaCl_2$ 0.05%	28.9^{k-m}	29.3^{j-m}	31.6^{g-m}	33.5^{g-l}	28.4^{lm}
		$CaCl_2$ 0.75%	30.2^{h-m}	30.6^{g-m}	34.4^{g-k}	34.7^{g-j}	31.1^{g-m}
		$CaCl_2$ 1.0%	29.6^{i-m}	35.1^{g-i}	36.0^{gh}	36.3^{g}	28.3^{lm}
Perlette	$a*$	Control	-10.7^{gh}	-10.3^{f-h}	-9.8^{f-h}	-9.7^{f-h}	-8.8^{f-h}
		$CaCl_2$ 0.5%	-10.9^{h}	-9.7^{f-h}	-9.4^{f-h}	-8.9^{f-h}	-8.5^{f-h}
		$CaCl_2$ 0.75%	-9.8^{f-h}	-8.7^{f-h}	-8.6^{f-h}	-8.3^{f-h}	-8.0^{fg}
		$CaCl_2$ 1.0 %	-9.0^{f-h}	-9.0^{f-h}	-8.2^{f-h}	-8.2^{fg}	-7.9^{f}
Kings Ruby		Control	7.7^{c-e}	9.6^{a-e}	9.7^{a-d}	9.9^{a-c}	10.1^{a-c}
		$CaCl_2$ 0.05%	7.0^{e}	10.2^{a-c}	10.3^{a-c}	10.7^{a}	11.0^{a}
		$CaCl_2$ 0.75%	7.0^{de}	8.0^{b-e}	8.7^{a-e}	10.2^{a-c}	10.4^{ab}
		$CaCl_2$ 1.0%	8.0^{b-e}	9.0^{a-e}	9.2^{a-e}	9.5^{a-e}	9.6^{a-e}
Perlette	$b*$	Control	24.1^{a}	24.5^{a}	23.0^{a}	22.3^{a}	22.1^{a}
		$CaCl_2$ 0.5%	23.8^{a}	24.8^{a}	24.4^{a}	23.9^{a}	23.0^{a}
		$CaCl_2$ 0.75%	24.8^{a}	25.3^{a}	25.1^{a}	24.2^{a}	23.1^{a}
		$CaCl_2$ 1.0 %	24.6^{a}	25.3^{a}	24.2^{a}	22.5^{a}	22.5^{a}
Kings Ruby		Control	4.47^{b-d}	3.56^{b-f}	2.91^{b-g}	0.81^{e-g}	0.04^{g}
		$CaCl_2$ 0.05%	5.07^{b-d}	4.04^{b-e}	3.33^{b-g}	1.61^{d-g}	0.16^{fg}
		$CaCl_2$ 0.75%	6.40^{b}	4.89^{b-d}	4.77^{b-d}	3.34^{b-g}	2.35^{c-g}
		$CaCl_2$ 1.0%	5.60^{bc}	3.51^{b-g}	2.99^{b-g}	2.11^{c-g}	0.58^{e-g}

Means not sharing a same letter are significantly different at $p \leq 0.05$.

Taste, aroma, flavour and texture

Pre-harvest foliar application $CaCl_2$ significantly affected organoleptic characteristics of both cultivars of grapes. All $CaCl_2$ treatments maintained higher values of taste and aroma, as compared with control. Pre-harvest foliar application of 0.75% $CaCl_2$ resulted in 18.48% higher score for taste in case of 'Perlette' berries; whereas, 19.51% higher values for taste were observed in 0.5% $CaCl_2$ sprayed 'King's Ruby' vines as compared with control. Aroma values of 'Perlette' and 'King's Ruby' cultivars sprayed with 0.5% $CaCl_2$ were about 25.24% and 13.33% higher than control berries, respectively, after 28 days of cold storage. Foliar sprays of $CaCl_2$ improved flavour of grape cvs. 'Perlette' and 'King's Ruby' berries than untreated berries. Grape cv. 'Perlette' sprayed with 0.5% $CaCl_2$ maintained about 14.58% higher score for flavour as compared with control; whereas, grape cv. 'King's Ruby' sprayed with 0.75% $CaCl_2$ maintained about 23.86% better flavour than control treatment. Deterioration in texture of grape berries of both cultivars was observed with the progression of cold storage, irrespective of treatments. The texture of control berries deteriorated more rapidly than that of $CaCl_2$ treatments. Pre-harvest foliar application of 0.75% $CaCl_2$ resulted in 25.68% higher values for texture in cv. 'Perlette'; whereas, in case of 'King's Ruby' 0.5% $CaCl_2$ exhibited 16.52% higher values of texture after 28 days of cold storage. Loss of texture under cold storage conditions in grape cv. 'King's Ruby' was significantly less (about 11.60%), as compared to cv. 'Perlette' berries (Table 2).

Discussion

Ca^{2+} maintains the integrity of the cell wall by making a chelate with free carboxylic groups of galacturic units; thus plays a key role in berry firmness and shelf life (Chardonnet et al., 1997). Results of present study indicated that photosynthetic parameters of grape vines i.e. photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal conductance (Ci) were improved by pre-harvest treatment with $CaCl_2$ (Fig. 1). Ca^{2+} being a component of $CaCl_2$ exhibits the ability to regulate as well as stabilize cell membrane phospholipids and proteins (Kim et al., 2009; Upadhyaya et al., 2011). Photosynthetic pigments deteriorate under drought conditions accompanied with instability of pigment-protein complexes (Xu et al., 2013). Previously, similar observations were found in zoysiagrass, where $CaCl_2$ pretreatment resulted in higher chlorophyll contents under drought stress (Xu et al., 2013). Ca^{2+} increases photosynthetic performance by maintaining the osmotic strength of cytoplasm in plants (Yang et al., 2016). Such improvements in photosynthetic parameters improved berry quality as berry length, berry diameter and overall yield was increased in $CaCl_2$ pre-treated grape berries compared with non-treated berries as shown in Fig. 2. Our results are in line with the findings of El-

Sayed (2013) who reported increased berry diameter, length and yield in $CaCl_2$ treated 'Crimson Seedless' grapes. Likewise, comparative findings were reported by Colapietra & Alexander (2006); Peppi et al. (2008) for increase in weight of 'Flame seedless' grape bunches. However, Kumar et al. (2017) reported non-significant increase in grape yield treated with 0.25% $CaCl_2$.

Table 2 Effect of different concentrations of $CaCl_2$ on organoleptic characteristics of grape cvs. 'Perlette' and 'King's Ruby' under cold storage conditions

Cultivar		Treatment	Storage period				
			0	7	14	21	28
Perlette	Taste	Control	6.3^{b-d}	7.7^{a-c}	7.7^{a-c}	6.3^{b-d}	4.3^{d}
		$CaCl_2$ 0.5%	7.7^{a-c}	8.3^{ab}	8.3^{ab}	6.3^{b-d}	6.3^{b-d}
		$CaCl_2$ 0.75%	7.7^{a-c}	8.3^{ab}	8.3^{ab}	8.3^{ab}	7.0^{a-c}
		$CaCl_2$ 1.0 %	7.7^{a-c}	8.3^{ab}	8.3^{ab}	7.7^{a-c}	7.0^{a-c}
Kings Ruby		Control	7.0^{a-f}	7.7^{a-e}	7.7^{a-e}	5.7^{EF}	5.0^{f}
		$CaCl_2$ 0.05%	8.3^{a-c}	9.0^{a}	9.0^{a}	7.7^{a-e}	7.0^{a-f}
		$CaCl_2$ 0.75%	7.7^{a-e}	9.0^{a}	9.0^{a}	7.0^{a-f}	6.3^{c-f}
		$CaCl_2$ 1.0%	7.7^{a-e}	8.3^{a-c}	7.7^{a-e}	6.3^{c-f}	5.7^{ef}
Perlette	Aroma	Control	5.7^{c-e}	5.7^{c-e}	5.0^{de}	5.0^{de}	4.3^{e}
		$CaCl_2$ 0.5%	7.0^{a-c}	7.7^{ab}	7.0^{a-c}	6.3^{b-d}	6.3^{b-d}
		$CaCl_2$ 0.75%	7.7^{ab}	7.0^{a-c}	7.0^{a-c}	5.7^{c-e}	5.7^{c-e}
		$CaCl_2$ 1.0 %	7.0^{a-c}	7.0^{a-c}	7.0^{a-c}	7.0^{a-c}	5.7^{c-e}
Kings Ruby		Control	6.3^{b-d}	7.0^{a-c}	6.3^{b-d}	5.7^{c-e}	5.0^{de}
		$CaCl_2$ 0.05%	7.7^{ab}	7.7^{ab}	6.3^{b-d}	7.0^{a-c}	6.3^{b-d}
		$CaCl_2$ 0.75%	7.7^{ab}	8.3^{a}	7.0^{a-c}	6.3^{b-d}	5.7^{c-e}
		$CaCl_2$ 1.0%	7.7^{ab}	7.7^{ab}	7.7^{ab}	5.7^{c-e}	5.7^{c-e}
Perlette	Flavour	Control	7.7^{a-c}	7.0^{b}	5.7^{d-f}	5.7^{d-f}	5.0^{e-f}
		$CaCl_2$ 0.5%	8.3^{ab}	7.7^{a-c}	7.0^{b-d}	7.0^{b-d}	6.3^{c-e}
		$CaCl_2$ 0.75%	7.7^{a-c}	7.7^{a-c}	7.0^{b-d}	6.3^{c-e}	5.7^{d-f}
		$CaCl_2$ 1.0 %	8.3^{ab}	7.0^{b-d}	7.0^{b-d}	6.3^{c-e}	5.7^{d-f}
Kings Ruby		Control	7.0^{b-d}	7.0^{b-d}	6.3^{c-e}	5.0^{ef}	4.3^{f}
		$CaCl_2$ 0.05%	8.3^{ab}	8.3^{ab}	8.3^{ab}	7.0^{b-d}	6.3^{c-e}
		$CaCl_2$ 0.75%	9.0^{a}	8.3^{ab}	8.3^{ab}	7.0^{b-d}	6.3^{c-e}
		$CaCl_2$ 1.0%	8.3^{ab}	8.3^{ab}	7.7^{abc}	5.7^{d-f}	5.7^{d-f}
Perlette	Texture	Control	6.3^{c-e}	6.3^{c-e}	5.7^{def}	5.0^{e-g}	3.7^{g}
		$CaCl_2$ 0.5%	8.3^{ab}	8.3^{ab}	7.0^{bcd}	6.3^{c-e}	5.0^{e-g}
		$CaCl_2$ 0.75%	7.7^{a-c}	7.7^{a-c}	7.7^{abc}	7.0^{b-d}	6.3^{c-e}
		$CaCl_2$ 1.0 %	7.0^{b-d}	7.0^{b-d}	7.0^{bcd}	6.3^{c-e}	5.7^{d-f}
Kings Ruby		Control	8.3^{ab}	8.3^{ab}	7.0^{bcd}	5.7^{d-f}	4.3^{fg}
		$CaCl_2$ 0.05%	9.0^{a}	9.0^{a}	8.3^{ab}	7.7^{a-c}	6.3^{c-e}
		$CaCl_2$ 0.75%	9.0^{a}	9.0^{a}	8.3^{ab}	7.0^{b-d}	6.3^{c-e}
		$CaCl_2$ 1.0%	8.3^{ab}	8.3^{ab}	7.0^{b-d}	6.3^{c-e}	5.7^{d-f}

Means not sharing a same letter are significantly different at $p \leq 0.05$.

Persistent weight loss of grape berries in current study can be attributed to loss of water via transpiration and respiration. While, pre-treatment of $CaCl_2$ improved fruit quality by decreasing weight loss and decay incidence (Fig. 3A, B) (Madani et al., 2014). Ca^{2+} being a key nutrient improves fruit quality (El-Badawy, 2012) by reducing softening and senescence of fruits (Barker & Pilbeam, 2015). Ca^{2+} applications might have increased the integrity of the cell wall; thereby, resulted in reduced weight loss of grape berries. Previously, pre- and post-harvest applications of Ca^{2+} significantly reduced weight loss in papaya (Mahmud et al., 2008), peach (El-Badawy, 2012), plum (Kirmani et al., 2013) and crimson seedless grapes (Elwahab et al., 2014). Our results for decay percentage (Fig. 2C, D) are in similarity with the findings of Serrano et al. (2004) where Ca^{2+} pre-treatment increased firmness of peach fruit due to accumulation of cellular Ca^{2+} that effectively reduced decay incidence. In another study postharvest $CaCl_2$ immersion increased firmness of 'Barhi' dates (Abd Elwahab et al., 2019). Ca^{2+} accumulates in the middle lamella of cell wall; thus, making the cell wall more resistant to decay causing organisms (Tobias et al., 1992). Comparative findings were also reported by Al-Qurashi & Awad (2013) where decay incidence of El-Bayadi table grapes was reduced by pre-harvest Ca^{2+} application.

Consumer acceptance relies upon sugar contents and organic acids that are responsible for taste and is highly affected during cold storage (Sen et al., 2016). Current investigation showed an increasing trend in TSS during cold storage and maximum value of TSS was recorded in grape berries pre-treated with $CaCl_2$ (Fig. 4A, B). Increased TSS of grape berries during cold storage may be attributed to breakdown of starch into simple sugars (Gallo et al., 2014), while moisture loss from fruiting tissue during cold storage might also have contributed towards increment of TSS (Javed et al., 2016). Influence of Ca^{2+} in increasing TSS of the litchi fruits was previously reported by Hasan & Jana (2000). Our results are in agreement with the findings of (Badawy et al., 2019) where preharvest spray of 0.5% $CaCl_2$ increased TSS of the pomegranate fruit; likewise, comparable with the findings of Al-Qurashi & Awad (2013) where pre-harvest foliar application of $CaCl_2$ increased TSS of El-Bayadi table grapes. Likewise, $CaCl_2$ combined with sodium alginate improved TSS along with a shelf of sapota fruit (Neeru & Anshu, 2019). Principle organic acid present in grapes is tartaric acid that was declined during cold storage regardless of treatments; although, reduction in acidity was significantly less in $CaCl_2$ pre-treated grape berries of both cultivars as compared to control treatment (Fig. 4C, D). Higher TA in $CaCl_2$ pre-treated berries could be attributed to increased cell wall; while steady reduction in TA under cold storage could be related to continuous metabolic activities of berries that resulted in consumption of organic acid and similar observations were reported apricot and pomegranate aril (Yousefi et al., 2015; Shaarawi et al., 2016). Previously, reduction in acidity of under cold storage was also found in ‘Muskule’, ‘El-Bayadi’ and ‘Isabel’ table grape berries treated with $CaCl_2$ (Sabir et al., 2010; Al-Qurashi & Awad, 2013). While, pre-harvest foliar application of $CaCl_2$ retained postharvest ascorbic acid contents of both grape cultivars. However, loss in ascorbic acid contents during cold storage could be the result of the oxidation process. Previously, $CaCl_2$ application retained higher ascorbic acid contents due to delayed oxidation process in raspberries and strawberries (Turmanidze et al., 2016). Likewise, our results showed that untreated berries exhibited higher respiration rate and ethylene production resulting in rapid fall in ascorbic acid contents as compared to Ca^{2+} treated fruits.

Investigations of current study revealed steady decline in phenolic contents along with total antioxidants of both grape cvs. ‘King’s Ruby’ and ‘Perlette’ regardless of treatment applied; while, $CaCl_2$ application resulted in retention of more phenolic contents (Fig. 5C, D, E, F). TPC and antioxidants are well correlated in most cases (Balik et al., 2009). Polyphenols vary with berries skin color, cultivar, species, and environment as well as postharvest management skills (Yang et al., 2009); though, they are naturally present in ample amount in grapes in the form of flavonoids, anthocyanin and phenolic contents (Nile et al., 2013). Our results are comparable with the investigations of Al-Qurashi & Awad (2013); Imlak et al. (2017) where $CaCl_2$ treated ‘El-Bayadi’ and ‘Thompson Seedless’ table grapes exhibited higher phenolic compounds than control fruit. Increase in phenolic contents as a result of preharvest $CaCl_2$ application have also been reported in sweet cherry (Ozturk et al., 2019). While, steady decrease in phenolic contents under cold storage conditions (Fig. 5C, D) were also supported by Pinheiro et al. (2009) who observed decline in total phenolic contents and anthocyanin in ‘Benitaka’ grape cultivar under cold storage. Likewise, Turmanidze et al. (2016) reported higher antioxidants in $CaCl_2$ treated raspberries and strawberries.

A large percentage of grapes are known to be pooled with sugars, mainly glucose and fructose that are reducing sugars and are involved in cell respiration and synthesis and sucrose that is non-reducing. In current study reducing sugars and total sugars increased; while, a declining trend was observed in non-reducing sugars. Increase in reducing and total sugars under prolonged cold storage resulted in greater sugar composition that could be attributed to rapid conversion of complex starch molecules into simpler sugars (Gallo et al., 2014). Such changes in sugar contents of pre-treated $CaCl_2$ ‘Thmopson’ grapes have also been reported by Imlak et al. (2017). While, improved berry colour with $CaCl_2$ application as presented in Table 1 could be related to anthocyanin biosynthesis signaled by Ca^{2+} (Vitrac et al., 2000). Our results comply with the findings of Sudha & Ravishankar (2003) where Ca^{2+} increased anthocyanin level in carrot. Recently, a study conducted by Scavroni et al. (2018) also showed comparative results for berry colour of ‘Rubi’ table grapes treated with combined application of $CaCl_2$ and ethephon. Our results are comparable with the findings of Anantheswaran et al. (1996) where $CaCl_2$ application made strawberry fruit aesthetically brighter as Ca^{2+} stabilizes vacuole membranes.

Consumer acceptability is of foremost importance when it comes to visual quality. Pre-harvest $CaCl_2$ treatments significantly affected visual as well as sensory attributes of grape berries stored under cold conditions (Table 2). A study elaborated that pre-harvest Ca^{2+} sprays improve tissue firmness of horticulture produce (Siddiqui & Bangerth, 1995; Gerasopoulos et al., 1996). Application of Ca^{2+} protected sensory quality of grapes berries by penetrating into the cell wall resulting in resistance against infections and thereby improved firmness and external appearance as previously observed in apple and strawberry fruits (Wang et al., 1993; Jouki & Khazaei, 2012). Comparable results were reported by Sabir & Sabir (2017) where pre-harvest Ca^{2+} application improved visual quality of ‘Thompson Seedless’ grapes.

Conclusion

Evidently pre-harvest foliar application of all $CaCl_2$ treatments significantly improved berry quality of both grape cultivars. Foliar application of 0.5% and 0.75% exhibited higher photosynthesis that resulted in increased berry length, diameter and yield. Whereas, post-harvest quality was also better in 0.5% and 0.75% $CaCl_2$ treated berries with improved physico-chemical as well as antioxidative attributes.

Author Contribution Statement: Muhammad shafique and Irfan Ali conducted the experiments and collected the data. Nadeem Akhtar Abbasi supervised the research study. Muhammad Shafique wrote the manuscript. Abdul Ahad Qureshi and Ishfaq Ahmed Hafiz analyzed the data.

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