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RESEARCH REPORT

Rachis browning in grapes: temperature influence, ethylene and respiration rates, and evaluation techniques

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Abstract

Rachis browning is a key postharvest challenge affecting the visual quality and marketability of table grapes (Vitis vinifera L.), yet its physiological basis remains poorly understood, particularly across different cultivars. This study investigated the relationship between rachis browning, endogenous ethylene production, and respiration in two table grape varieties—'Autumn Crisp' and 'Sweet Globe'—under cold (−0.5 °C) and ambient (20 °C) storage. Grapes were evaluated over 12 days for quality parameters, weight loss, gas exchange (CO_2 and ethylene), and rachis browning, assessed visually and through digital image analysis. Overall, storage at 0.5 °C effectively reduced browning and ethylene production compared to 20 °C. However, varietal differences were pronounced: 'Sweet Globe' exhibited higher rachis browning than 'Autumn Crisp' at 0.5 °C despite similar ethylene and respiration levels, indicating variety-specific susceptibility. At 20 °C, increased ethylene and respiration coincided with more severe browning, particularly in 'Autumn Crisp'. A strong correlation was found between visual and image-based assessments, though discrepancies emerged under high-stress conditions. The results suggest that while ethylene and respiration are associated with rachis browning, they do not fully explain the varietal differences observed, emphasizing the need to consider cultivar-specific physiological responses. Furthermore, image-based evaluation offers a more consistent method for quantifying browning severity. This study advances our understanding of rachis browning physiology in grapes and highlights the importance of tailored postharvest strategies and objective assessment tools to extend shelf life and maintain visual quality.

Keywords Ethylene · Table grapes · Image analysis · Rachis browning · Respiration · Storage

1 Introduction

Table grapes (*Vitis vinifera* L.) are an economically important fruit, valued for their flavor, convenience, and nutritional benefits. However, they are highly perishable and face several postharvest challenges, including quality degradation due to dehydration, browning, and fungal infections (Sonker et al. 2016). These issues are further complicated by varietal differences in responses to storage conditions, such as temperature and humidity, which are critical for mitigating dehydration and preventing rachis browning (Hamie et al. 2022; Owoyemi et al. 2024). Rachis browning is a particularly important issue, as it significantly reduces visual appeal and directly affects consumer acceptance and marketability.

The causes of rachis browning are multifactorial and closely linked to postharvest storage conditions. Maitaining cold temperatures (typically between −1 °C and 2 °C) and high relative humidity (90–95%) is essentidal to delay

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dehydration and preserve overall fruit quality. Certain grape varieties are more prone to dehydration and browning; for example, thicker-skinned varieties like 'Thompson Seedless' are more resistant to moisture loss and physical damage compared to more delicate varieties like 'Krissy' (Hamie et al. 2022). Previous research has shown that modified atmospheres and humidity-controlled storage can effectively reduce browning and maintain grape quality. However, these approaches must be tailored to each grape variety's unique characteristics for optimal results (Romero et al. 2020).

This quality deterioration has substantial economic implications for the grape industry. Browning symptoms can lead to rejection of export shipments, reduced shelf-life, and fruit waste, ultimately impacting profitability for producers and distributors. Lichter et al. (2011) emphasized that a green rachis is perceived by consumers as a sign of freshness, and its browning is a major cause of rejection. Similarly, Hamie et al. (2022) identified rachis browning as a key factor in postharvest quality loss in 'Krissy' grapes, while Owoyemi et al. (2024) reported that deviations from optimal storage temperature increased browning severity and led to commercial losses. These findings underscore the importance of understanding the physiological mechanisms behind rachis browning to develop effective, cultivar-specific postharvest strategies.

While grapes are classified as non-climacteric fruits, with ripening largely independent of ethylene, some studies report a transient increase in endogenous ethylene during critical processes such as anthocyanin accumulation, sugar production, and acidity reduction, which clearly suggests its involvement in these processes (Chervin et al. 2004). Additionally, recent studies link ethylene to rachis browning. Application of synthetic plant growth regulators that inhibit ethylene perception have been shown to delay rachis browning (Li et al. 2015; Zou et al. 2023), indicating that ethylene may play a direct role in the progression of browning symptoms.

Respiration has also been implicated in the browning process. For example, Lichter et al. (2011) found that enhanced rachis respiration in 'Superior' grapes stored at 20 °C under high humidity conditions was associated with increased browning. Similarly, other studies have reported that treatments which reduced respiration also decreased rachis browning (Zhang et al. 2018; Ban et al. 2023), further supporting the connection between respiratory activity and browning progression. Moreover, enzymatic browning due to oxidative stress, specifically actions of polyphenol oxidase (PPO) and peroxidase (POD) enzymes, is a well-established pathway for tissue discoloration and has been described in grapes and other fruit crops (Rathjen and Robinson 1992; Nokthai et al. 2010; Rosales et al. 2013; Kaya and Bağci 2021; Romero et al. 2022). Chlorophyll degradation, which directly impacts rachis color and overall tissue senescence, is also recognized as a key pathway in browning processes (Ni et al. 2016; Li et al. 2023b; Zou et al. 2023). While these factors were not the focus of the current study, they are important components of rachis senescence and merit further investigation.

Despite these insights, the variety-specific relationship between rachis browning, endogenous ethylene production, and respiration under cold storage remains poorly understood. To date, no systematic comparison has been conducted between different table grape cultivars to elucidate how these physiological factors interact and contribute to cultivar-specific browning responses. Furthermore, while both visual and image-based assessment methods exist, their integration and comparative validation under different storage conditions has not been thoroughly investigated, representing a significant methodological gap.

Therefore, this study represents the first systematic comparison of rachis browning between 'Autumn Crisp' and 'Sweet Globe' table grape varieties, integrating both traditional visual assessment and advanced image-based analysis methods. The dual evaluation approach provides a methodological advance that enables more objective and standardized assessment of browning severity. By investigating the relationship between rachis browning, ethylene production, and respiration across two distinct cultivars under contrasting storage temperatures, this work establishes a foundation for developing cultivar-specific postharvest strategies and standardized evaluation protocols.

2 Materials and methods

2.1 Plant material and experimental design

This study was conducted on two table grape varieties, *Vitis vinifera* L. 'Autumn Crisp' and 'Sweet Globe.' L. 'Autumn Crisp' and 'Sweet Globe'. A total of 80 kg of each cultivar was harvested at commercial maturity from a commercial vineyard in Murcia, Spain (MOYCA). Bunches were randomly collected from multiple vines across different rows to ensure representative sampling. Grapes were harvested at a commercial vineyard in Murcia, Spain, without pre-cooling, and immediately placed into a cooled van maintained at 0–4 °C. Transport from the vineyard to the IRTA (Institute of Agrifood Research and Technology) Fruitcentre in Lleida took less than 6 h. Upon arrival, grapes were promptly placed in cold storage to minimize postharvest physiological changes prior to experimental evaluations.

Upon arrival, grapes were randomly divided into two experimental sets: (i) immediate post-arrival evaluation, and

(ii) storage under two conditions—cold storage at −0.5 °C and ambient shelf-life conditions at 20 °C, both at 88–90% relative humidity. The −0.5 °C temperature was selected to reflect industry protocols (–1 to 0 °C) while minimizing freezing risk, since the rachis freezing point is reported at −2 °C (Crisosto and Crisosto 2020). Storage was conducted in controlled chambers at IRTA.

The experimental design followed a completely randomized structure. The experimental unit consisted of three grape bunches (approximately 1 kg total) per replicate, and four biological replicates were used per treatment. All samples were obtained from a single harvest batch and the experiment was not repeated across multiple harvests. While this may limit the study's generalizability, it is important to emphasize that this represents the first systematic comparison of rachis browning between 'Autumn Crisp' and 'Sweet Globe' table grape varieties. For each replicate, samples were pooled prior to analysis. Evaluations were performed at 0, 2, 5, 8, and 12 days of storage and included overall quality assessments (grape bunch mass, total soluble solids [TSS], and titratable acidity [TA]), gas measurements (ethylene and CO_2), and rachis browning (visual and image-based analysis).

2.2 Overall quality analysis

Overall quality assessments were performed using pooled samples from each replicate, which consisted of three grape bunches (approximately 1 kg in total). Four biological replicates were evaluated per treatment and time point. To assess grape bunch mass, each replicate was weighed using a precision balance before further processing.

For grape berry quality analysis, destemmed berries were used. A total of 60 berries per replicate were randomly selected (20 berries from each of three bunches). Total soluble solids (TSS) were measured in juice extracted from 30 of these berries using a digital hand-held refractometer (Atago, Tokyo, Japan) and expressed in degrees Brix (°Brix). Titratable acidity (TA) was determined by titrating 10 mL of grape juice with 0.1 N sodium hydroxide (NaOH) to a pH endpoint of 8.2, using phenolphthalein as an indicator. Results were expressed as grams of tartaric acid equivalents per liter.

2.3 Visual and image-based rachis browning analysis

Rachis browning was assessed using both subjective visual scoring and objective digital image analysis. Visual scoring of rachis browning was performed by five independent evaluators, following the scale proposed by Lichter et al. (2011), ranging from 1 (no browning) to 5 (severe browning), based on the extent and intensity of discoloration observed on the rachis. This method allowed for a rapid and consistent qualitative assessment across replicates. Inter-rater reliability was quantified using intraclass correlation coefficients from a two-way mixed-effects model with absolute agreement—ICC(2,1) for a single rater and ICC(2,5) for the mean of five raters—with 95% confidence intervals computed overall and stratified by variety and temperature. Agreement between the mean visual score (linearly rescaled to 0–100%) and the image-based percentage was examined using Bland–Altman analysis to estimate mean bias and 95% limits of agreement. For image-based analysis, rachises were photographed using a standardized setup under controlled lighting and uniform background conditions. The total rachis area was segmented from the background, and a color threshold filter was applied to isolate green areas, representing healthy tissue. Browning severity was then quantified as the percentage of non-green pixels, following the method adapted from Hamie et al. (2022), providing a continuous and objective measure of discoloration. Each replicate consisted of three rachises, which were individually imaged and analyzed. Both visual and image-based assessments were conducted at each storage time point across all treatments to evaluate browning progression over time.

2.4 Gas measurements

For gas exchange analysis, whole grape bunches were used. One bunch from each replicate was placed in a 2 L glass flask sealed with a silicon stopper and incubated for 2 h at room temperature (~20 °C). After incubation, a 1 mL headspace gas sample was extracted using a gas-tight syringe and injected into a gas chromatograph (GC 6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and an alumina column (F1 80/100, 2 m × 1/8″ OD × 2.1 mm ID, Teknokroma, Barcelona, Spain), following the method of Lindo-García et al. (2020). Ethylene production was calculated and expressed as microliters per kilogram per hours ($\mu L\ C_2H_4 \cdot kg^{-1} \cdot h^{-1}$). Respiration rate ($mg\ CO_2 \cdot kg^{-1} \cdot h^{-1}$) was determined in parallel flasks by measuring headspace gas using an O_2/CO_2 analyzer (CheckPoint O2/CO2, PBI Dansensor, Ringsted, Denmark). All gas measurements were conducted on four independent replicates per treatment and time point.

2.5 Data visualization and statistical analysis

All statistical analyses were performed using StataMP 14.1 for Mac (StataCorp, College Station, TX, USA). Data from the randomized design were analyzed using four biological replicates per treatment, based on pooled samples as described above. Inter-rater reliability of visual scores was

estimated using a two-way mixed-effects ICC with absolute agreement [icc score id rater, mixed absolute], reported as ICC(2,1) and ICC(2,5) with 95% confidence intervals, computed overall and by variety, temperature, and their combination. Agreement between the mean visual score (rescaled to 0–100%) and the image-based percentage was evaluated using Bland–Altman analysis (difference = visual − image; average = (visual + image)/2), with mean bias and 95% limits of agreement calculated for the full dataset and by the same strata. Normality and homoscedasticity of residuals were verified using Shapiro-Wilk and Levene's tests. Depending on data distribution, parametric tests (e.g., one-way or two-way ANOVA) or non-parametric tests (e.g., Kruskal–Wallis) were applied, followed by appropriate post hoc comparisons (e.g., Tukey's HSD or Dunn's test). Statistical significance was set at $p < 0.05$. Graphs and data visualizations were generated using DataGraph 5.4 (Visual Data Tools, Chapel Hill, NC, USA).

3 Results

3.1 Quality differences between cultivars across storage

Quality was assessed in terms of total soluble solids (TSS) content, acidity (g malic acid · L^{-1}), and weight loss, measured at day 0 and subsequent storage time points under both conditions (Table 1; Fig. 1). For *Autumn Crisp*, TSS and acidity at day 0 were higher than common values for this variety, but after 2 days of storage at both storage temperatures, the grape berries exhibited TSS and acidity values similar to those generally found. For both storage temperatures, significant fluctuations in both TSS and acidity were observed over time, with the clear pattern of increasing TSS and decreasing acidity. In contrast, *Sweet Globe* exhibited values at harvest that aligned more closely with what was expected. Under −0.5 °C storage, TSS showed a trend toward an increase over time, reaching its maximum value at day 12. At 20 °C, TSS content in *Sweet Globe* increased significantly by day 5 and then the values did not increase or decrease significantly with higher storage times. Regarding the acidity levels, there was not a clear trend throughout storage at -0.5 °C for *Sweet Globe*, but at 20 °C there was a tend to a reduction in acidity. Weight loss was recorded throughout the trial, showing a general trend of limited dehydration for all varieties and storage conditions, ranging from 0.5 to 4% at each evaluation day (Fig. 1). It was observed that for both varieties, the weight loss was higher at 20 °C when compared to -0.5 °C. Furthermore, *Autumn Crisp* showed higher variability in weight loss at day 12. However, the weight loss was still relatively small (3.65 ± 1.38%), indicating limited dehydration despite the observed variability.

Table 1 Total soluble solids (TSS, %), acidity (g acid malic · L^{-1}), and rachis state according to lichter et al. (2011) of berry grapes after transport and under two storage temperature (−0.5 and 20 °C) in 4 different time points (2, 5, 8 and 12d). Letters indicate significant differences in storage time across each storage temperature for each variety

Variety	T (ºC)	Storage time (d)	Total Soluble Solids (%)	Acidity (g malic acid · L^{-1})	Rachis browning (1–5 scale, Lichter et al. (2011)	Rachis browning (image-based, %)
Autumn Crisp	0–4	0	19.68 ± 1.21	4.40 ± 0.58	1.25 ± 0.50	0.97 ± 0.81
	-0.5	2	19.83 ± 0.28 ab	2.60 ± 0.50 a	1.50 ± 0.58 a	0.68 ± 0.53 a
		5	19.14 ± 1.60 b	2.80 ± 0.40 ab	1.50 ± 0.58 a	4.55 ± 1.01 b
		8	20.68 ± 1.23 a	3.28 ± 0.25 c	2.00 ± 0.00 a	10.93 ± 1.92 c
		12	19.45 ± 0.23 b	3.09 ± 0.23 bc	2.75 ± 0.50 b	14.65 ± 1.97 d
	20	2	19.64 ± 0.91 ab	3.17 ± 0.31 ab	3.00 ± 0.00 a	20.52 ± 3.05 a
		5	19.85 ± 0.25 ab	3.13 ± 0.86 ab	3.00 ± 0.00 a	35.23 ± 1.68 b
		8	19.02 ± 1.63 b	3.24 ± 0.07 b	3.25 ± 0.50 a	63.6 ± 6.04 c
		12	20.63 ± 0.48 a	2.67 ± 0.38 a	4.00 ± 0.00 b	98.33 ± 1.11 d
Sweet Globe	0–4	0	14.27 ± 1.74	3.06 ± 0.35	2.00 ± 0.82	1.32 ± 0.43
	-0.5	2	15.49 ± 2.23 b	2.39 ± 0.26 b	2.25 ± 0.50 a	6.36 ± 1.81 a
		5	15.61 ± 0.81 ab	2.48 ± 0.15 ab	3.75 ± 0.96 b	16.15 ± 1.3 b
		8	15.48 ± 1.13 b	2.66 ± 0.24 a	4.25 ± 0.50 bc	28.67 ± 5.86 c
		12	16.30 ± 0.81 a	2.56 ± 0.12 ab	4.75 ± 0.50 c	46.91 ± 2.77 d
	20	2	15.02 ± 1.13 b	2.78 ± 0.09 a	3.25 ± 0.50 a	14.66 ± 1.67 a
		5	16.72 ± 1.66 a	2.74 ± 0.25 a	4.00 ± 0.82 ab	37.28 ± 2.18 b
		8	16.43 ± 1.07 a	2.33 ± 0.23 b	4.75 ± 0.50 bc	46.91 ± 6.81 c
		12	16.15 ± 0.48 a	2.50 ± 0.27 ab	5.00 ± 0.00 c	93.18 ± 0.61 d

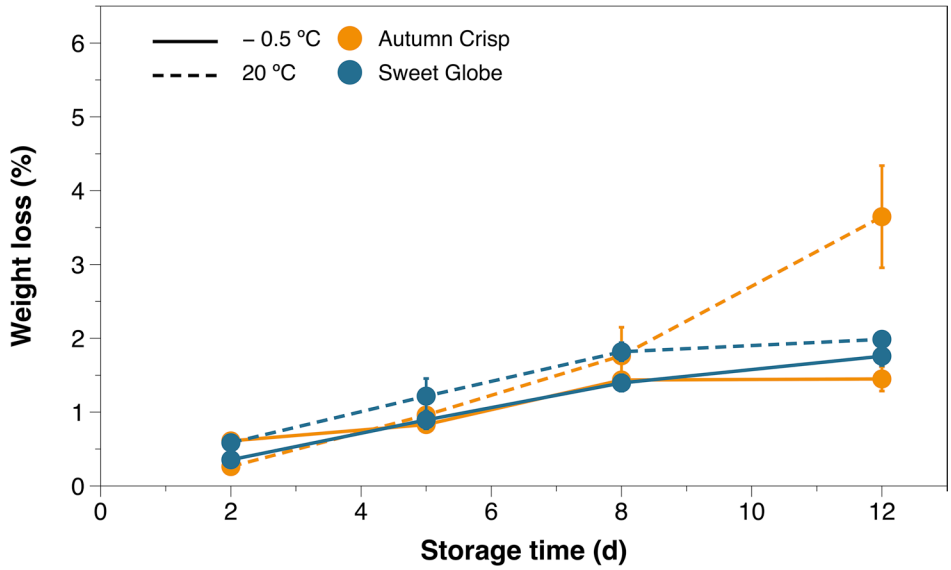

Fig. 1 Weight loss (%) of *Autumn Crisp* and *Sweet Globe* grapes during storage at two temperatures (-0.5 °C and 20 °C) over 12 days. Solid lines represent storage at -0.5 °C, while dashed lines represent storage at 20 °C. Data is shown as mean ± standard error ($n = 4$)

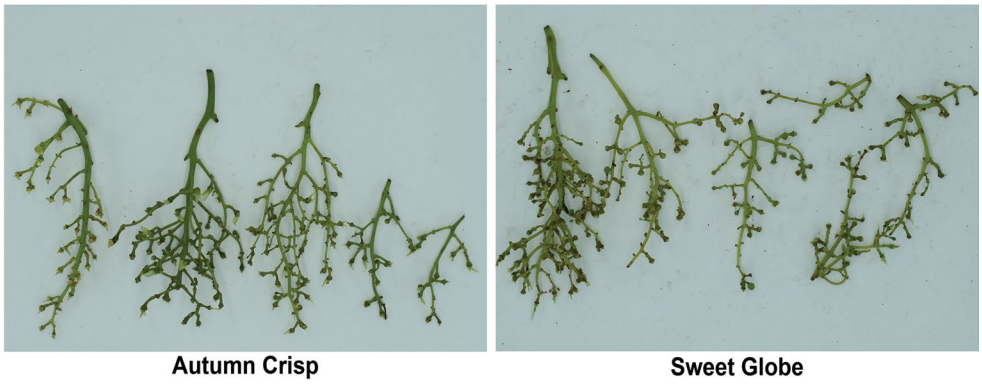

Fig. 2 Representative picture of the rachis of autumn crisp and sweet globe varieties at day 0

3.2 Visual and image-based rachis browning: effects of cultivar and storage temperature

The evolution of rachis browning was an important aspect of the trial, as it directly impacts grape visual quality and shelf life. Hence, it was monitored throughout the trial using both visually and through image-based analysis (Figs S1 and S2), and in general the rachis of *Autumn Crisp* is darker than that of *Sweet Globe*. It is important to note that at day 0, image-based analysis showed no significant differences between varieties, while visual evaluation indicated browner rachises in Sweet Globe, likely due to its inherently browner pedicels, the structure that attaches an individual grape or flower (Fig. 2). To document the consistency of visual scoring, Inter-rater agreement for visual scoring was good for a single rater and excellent for the 5-rater mean [overall ICC(2,1) = 0.79, 95% CI 0.69–0.86; ICC(2,5) = 0.95, 95% CI 0.92–0.97], with higher reliability in Autumn Crisp than Sweet Globe and lower reliability at 20 °C than at 0 °C; nevertheless, ICC(2,5) remained ≥ 0.84 across all except Sweet Globe 20 °C, which was still acceptable (0.76, 95% CI 0.44–0.91, Fig. S3). Under − 0.5 °C storage, both evaluation methods agreed that *Autumn Crisp* exhibited less browning that *Sweet Globe* (Table 1; Fig. 3). However, at 20 °C, the methods yielded contradictory results. For both storage temperatures, there were significant changes in the rachis over time (Table 1), as well as differences between the varieties and storage temperatures at each time point (Fig. 3).

That said, it is worth noting that both rachis browning evaluation methods were contradictory when assessing grapes stored at 20 °C. When grapes were stored at -0.5 °C, both evaluation methods were in agreement: *Autumn Crisp* showed less browning than *Sweet Globe* (Table 1; Fig. 3). However, at 20 °C, the results diverged. Visual evaluation suggested that *Autumn Crisp* was less brown during first days of storage, situation that was reversed at days 8 and 12 (Table 1; Fig. 3A), and image-based analysis showed similar trends, with the rachis of *Autumn Crisp* showing significantly more browning at days 8 and 12 compared to *Sweet Globe* (Fig. 3B). Moreover, the differences between varieties at the same temperature were greater in the visual evaluation than in the image-based analysis at day 2, and this pattern was also observed when comparing varieties at different temperatures. This is supported by Fig. S4, where

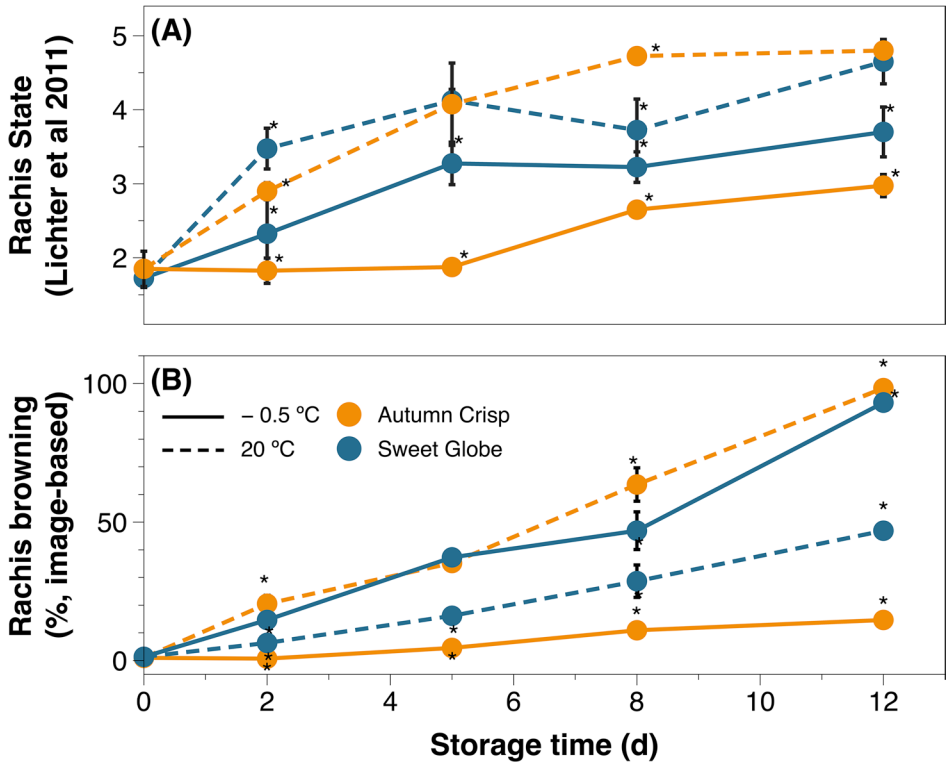

Fig. 3 Rachis browning (%) of Autumn Crisp and Sweet Globe grapes during storage at two temperatures (-0.5 °C and 20 °C) over 12 days. (**A**) Visual scoring results according to Lichter et al. (2011), and (**B**) Image-based analysis. Solid lines represent storage at -0.5 °C, and dashed lines represent storage at 20 °C. Data are presented as mean ± standard deviation ($n = 4$). Asterisks denote significant differences between varieties and storage temperatures at each evaluation time (Pairwise comparison test following Welch's ANOVA, adjusted $p < 0.05$)

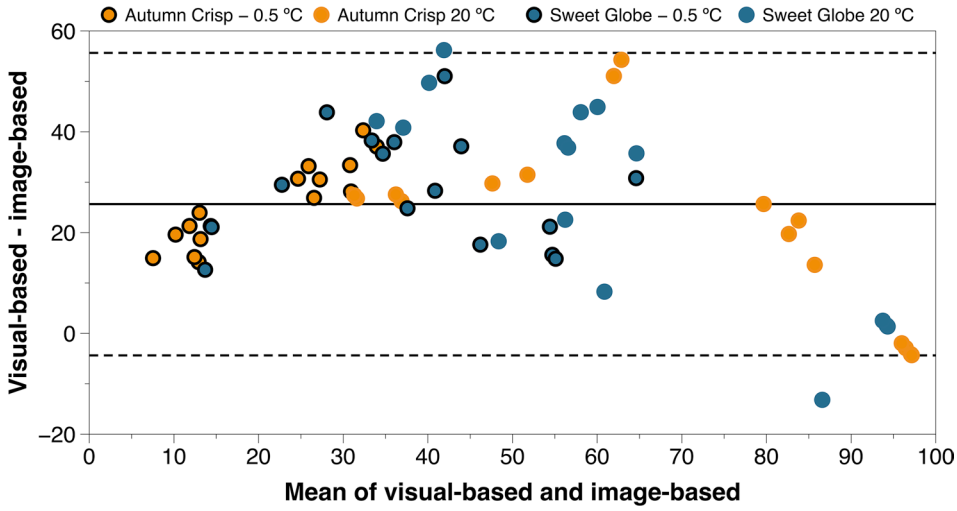

Fig. 4 Bland–Altman plot comparing the mean visual score (rescaled to 0–100%) with the image-based rachis browning percentage across varieties and storage temperatures. Each point represents one sample; the y-axis shows the difference (visual − image) and the x-axis the average of the two methods. The solid horizontal line denotes the mean bias, and dashed lines indicate the 95% limits of agreement (mean ± 1.96 SD). Points are colored by variety; markers with a black outline indicate 0 °C, and markers without an outline indicate 20 °C

it can be seen that the dispersion is wider for the visual evaluation. Moreover, to formally compare methods, a Bland–Altman analysis was conducted (Fig. 4), which showed a positive mean bias of visual versus image-based measurements and 95% limits of agreement encompassing most observations; bias decreased at high deterioration, consistent with ceiling effects of the 1–5 visual scale.

The relationship between visual and image-based analysis methods was explored using correlation analysis, as shown in Fig. 5. For both varieties and storage temperatures, there was a strong positive correlation between the two methods, with the degree of rachis browning increasing alongside higher rachis state scores. Moreover, the correlation was strongest for *Autumn Crisp* at -0.5 °C ($R^2 = 0.9527$), likely because the rachis state values were confined to a narrower range (up to 2.98), making the relationship between the two methods more consistent. However, for *Sweet Globe* at -0.5 °C, the correlation was weaker ($R^2 = 0.826$), reflecting a broader range of rachis states. At 20 °C, correlations were slightly weaker for both varieties (*Autumn Crisp*: $R^2 = 0.8146$; *Sweet Globe*: $R^2 = 0.619$), likely due to greater variability in rachis deterioration over the extended evaluation period. Despite the strong correlations, some discrepancies between the methods were observed, particularly at higher

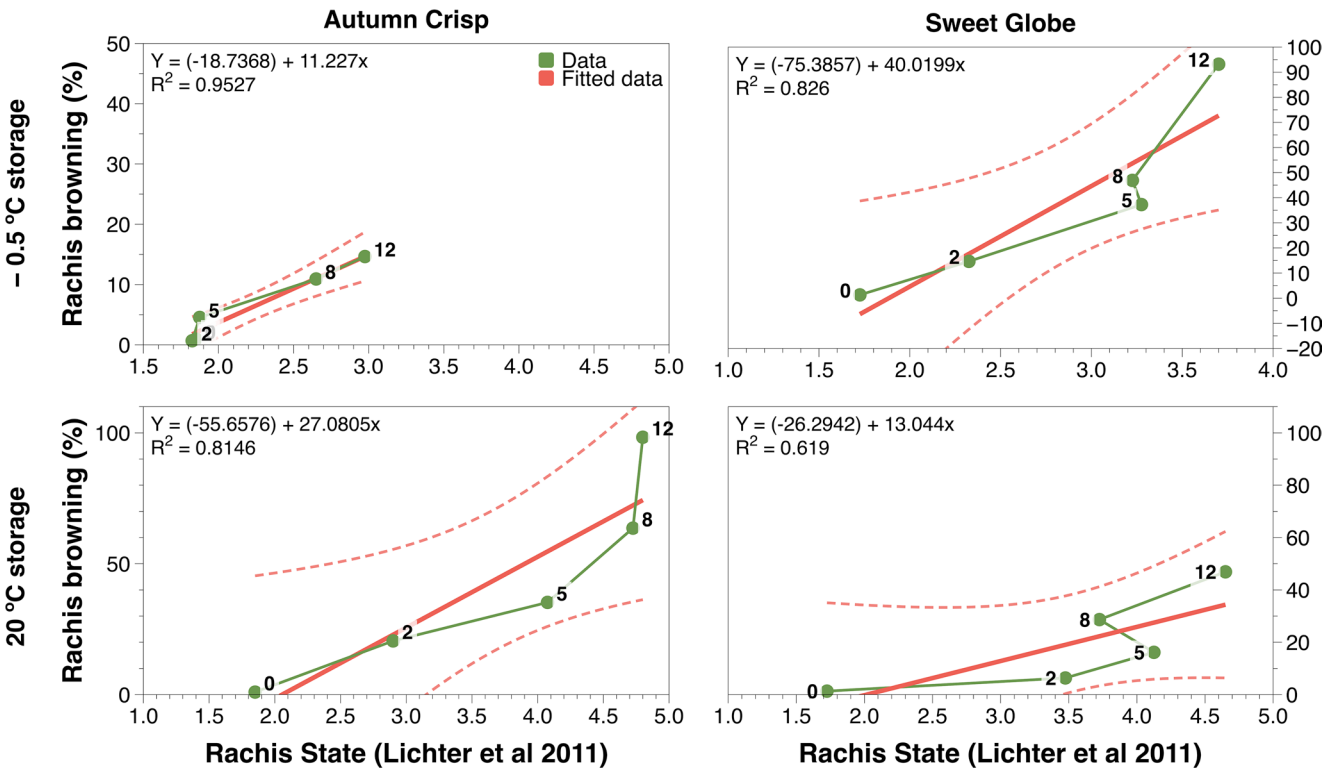

Fig. 5 Correlation between visual (1–5 scale) and image-based analysis (0-100%) of rachis browning for *Autumn Crisp* and *Sweet Globe* grapes stored at -0.5 °C and 20 °C. The green line represents the progression of rachis browning over time, with points indicating the evaluation times (days: 0, 2, 5, 8, and 12; $n=4$); the numbers next to the points correspond to the respective day of assessment. Red solid lines represent the linear regression fit, and red dashed lines indicate the 95% confidence intervals. The linear regression equation and the coefficient of determination (R^2) are shown for each correlation analysis

rachis state scores, which corresponded to more advanced stages of deterioration. Consistent with the Bland–Altman plot in Fig. 4, this suggests that while the two methods generally align, their sensitivity differs at high severity, with the visual scale showing saturation effects at extreme browning.

3.3 Gas exchange in table grapes: varietal and temperature effects

Rachis browning has been associated with high CO_2 levels, and recently ethylene has also been proposed to play a role in this process. To investigate these factors, we measured the respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) and ethylene production (µL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$) in both varieties under the two storage temperatures at five evaluation time points (Fig. 6A and B). Both varieties exhibited similar trends, with respiration rates peaking at day 2 for grapes stored at 20 °C (Fig. 6A). At this time point, significant differences were observed between all storage conditions and varieties. By day 5, the respiration rates of both varieties were comparable for each storage temperature, with differences only evident between the two storage temperatures. At day 8, the respiration rate was significantly higher in all treatments compared to *Autumn Crisp* stored at -0.5 °C. At day 12, it stabilized, maintaining comparable values across all measurements. Ethylene production in grapes stored at 20 °C followed a pattern similar to respiration rate (Fig. 6B), with an increase beginning at day 2 and peaking at day 5, consistent with the CO_2 production peak at day 2 (Fig. 6A). Ethylene production was significantly higher at 20 °C than at -0.5 °C at days 2, 5, and 8, irrespective of the variety. However, at day 12, a significant difference was observed for *Sweet Globe* stored at 20 °C, which exhibited higher ethylene production compared to all other measurements. At -0.5 °C, both varieties exhibited almost identical ethylene production levels. However, rachis browning, as assessed by image-based analysis, was significantly higher in Sweet Globe compared to *Autumn Crisp* starting from day 2. Moreover, evaluations at days 8 and 12 showed higher ethylene production in *Sweet Globe*, with ethylene production being significantly higher at day 12 as well. This increase in ethylene, however, was not correlated with a higher rachis browning in *Sweet Globe* at that same time point as rachis browning at 12 days was significantly higher in *Autumn Crisp*.In contrast, when visual evaluation was used, the higher ethylene production in *Autumn Crisp* at day 12 did not correlate with the observed rachis browning severity (4.00 ± 0.00) compared to *Sweet Globe* (5.00 ± 0.00).

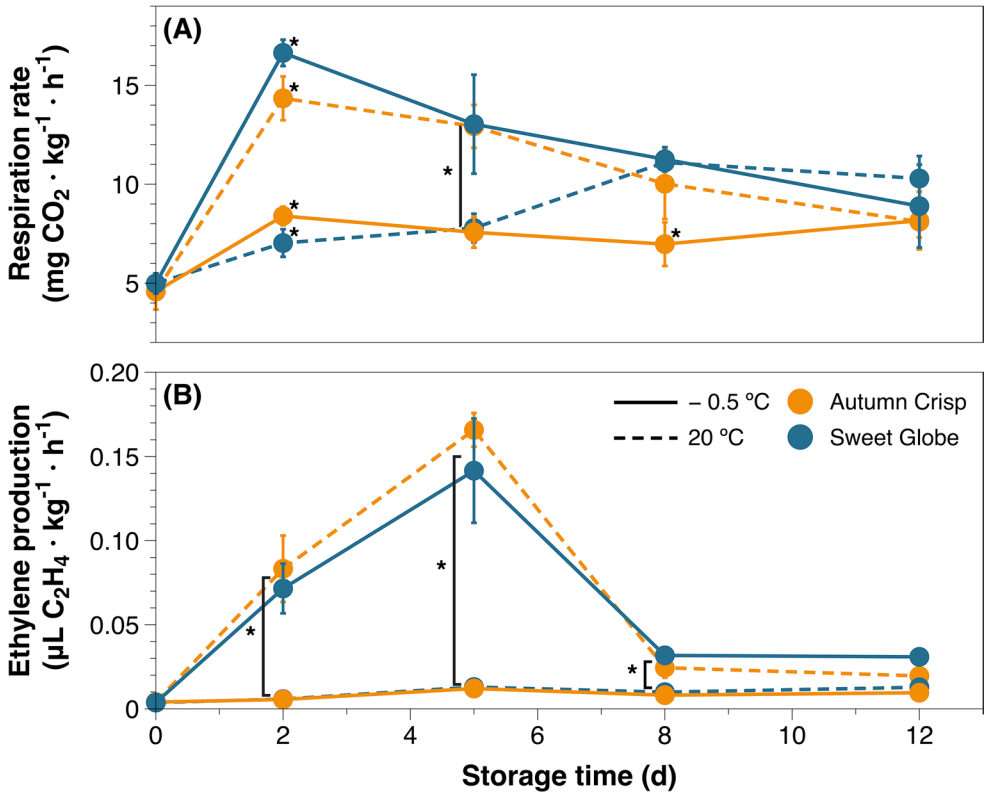

Fig. 6 Respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) (A), and (B) ethylene production (µL $C_2H_4 \cdot kg^{-1} \cdot h^{-1}$) of *Autumn Crisp* and *Sweet Globe* grapes during storage at two temperatures (-0.5 °C and 20 °C) over 12 days. Solid lines represent storage at -0.5 °C, while dashed lines represent storage at 20 °C. Data is shown as mean ± standard deviation (n = 4). Asterisks denote significant differences between varieties and storage temperatures at each evaluation time (Dunn's test following Kruskal–Wallis, adjusted $p < 0.05$)

4 Discussion

In this study, we observed that different storage conditions had a limited impact on overall grape quality parameters, including total soluble solids (TSS), acidity, and weight loss. In general, ethylene production and respiration rates did not directly correlate with rachis browning, as the two grape varieties with similar values for both parameters exhibited distinct degrees of rachis browning when stored at low temperatures. However, it is noteworthy that *Sweet Globe* displayed more pronounced rachis browning, both visually and image-based, at 0.5 °C on days 8 and 12 which correlated with a higher respiration rate. Specifically, we found that rachis browning was less pronounced at -0.5 °C compared to 20 °C, and that *Autumn Crisp* had a lower degree of rachis browning than *Sweet Globe* under cold storage.

While this study provides valuable insights into the roles of ethylene production and respiration rate in rachis browning, it does not include direct measurement of oxidation-reduction enzymes such as polyphenol oxidase (PPO), peroxidase (POD), or superoxide dismutase (SOD), which are established contributors to tissue browning through pathways of oxidative stress (Nokthai et al. 2010; Romero et al. 2022; Li et al. 2023aa). This omission reflects a deliberate focus on gas exchange physiology across two contrasting cultivars and storage temperatures, prioritizing reproducible and comparable metrics under logistical constraints. Although enzyme activity data would have enriched the mechanistic understanding of browning processes, the current approach allows for a clear characterization of varietal differences in physiological responses. Notably, the observed discrepancies in rachis browning at similar ethylene and respiration levels suggest the involvement of cultivar-specific biochemical pathways, potentially including enzymatic browning and antioxidant systems (Kaya and Bağci 2021). Future studies integrating enzyme activity profiles could build upon these findings to more fully elucidate the interplay between gas exchange and oxidative metabolism in browning development.

Another physiological factor that may contribute to rachis browning is chlorophyll degradation. Although not measured in this study, chlorophyll breakdown is closely associated with visual discoloration and senescence in plant tissues, and has been specifically identified as a main component of rachis browning in grapes (Ni et al. 2016). Zou et al. (2023) demonstrated that the ethylene response factor VvERF111 regulates chlorophyll degradation in grape rachis by binding to VvCLH1 promoter, directly linking ethylene signaling to browning symptoms. Additionally, Li et al. (2023) showed that VvERF75 accelerates chlorophyll degradation during grape ripening through transcriptional activation of chlorophyll degradation genes. The absence of chlorophyll quantification in our study limits the ability to correlate visual browning scores with biochemical markers

of senescence, particularly given that chlorophyll degradation may explain some of the varietal differences observed independent of ethylene/respiration patterns.

Ethylene is well established as a key regulator in climacteric fruits, but its role in non-climacteric fruit like grapes has often been overlooked. However, recent studies suggest that ethylene may be more involved in berry ripening and other physiological processes than previously thought (Böttcher et al. 2013a, b; Leida et al. 2016; Wang et al. 2022). In grapes, the rachis is of particular interest because its ethylene production rate is higher than that of the berries themselves (Ye et al. 2017). Rachis browning is a complex, multifactorial issue influenced by storage conditions, and its impact on visual quality directly affects consumer acceptance and marketability. Several studies have investigated this phenomenon, showing that treatments with ethylene action inhibitors or sulfur dioxide can reduce rachis browning in grapes (Zhang et al. 2022; Li et al. 2023c). Moreover, a recent study suggests that ethylene may be directly involved in rachis browning, as *VvERF111* (ethylene response factor 111) has been shown to positively regulate chlorophyll degradation in grape rachis (Zou et al. 2023). Consistent with this, we found that grapes stored at 20 °C exhibited the highest respiration rates and ethylene levels, which, as previous studies have suggested, were associated with higher rachis browning in both varieties (Lichter et al. 2011; Zhang et al. 2018; Ban et al. 2023). However, at -0.5 °C, where ethylene levels remained low and similar between *Autumn Crisp* and *Sweet Globe*, the two varieties still exhibited distinct degrees of rachis browning. This aligns with findings by Li et al. (2015), who reported that three grape varieties (*Thompson*, *Mystery*, and *3003*) had similar rachis browning indexes after three days of cold storage, despite differing respiration rates and ethylene production levels, suggesting variety-specific responses in rachis browning development.

Traditionally, rachis browning has been assessed visually, a method prone to subjectivity and inconsistencies, especially when differentiating between various browning levels on a 1–5 scale because. While an experienced evaluation team may mitigate this issue, visual assessments remain difficult to standardize across studies. As a result, image-based analysis is increasingly being used to provide a more objective evaluation of this symptom (Lichter et al. 2011; Bahar et al. 2017; Hamie et al. 2022). We observed that visual assessments tend to be less reliable, particularly when the rachis browning is more intense, e.g. in high Tº storage condititition, and it approaches and index of 5, at which point further deterioration is minimal and scoring variability is reduced and all rachis browning is evaluated at 5 when in fact not all the rachis is browning. In fact, when evaluating the rachis browning index (1–5 scale) or browning percentages separately, greater dispersion was observed in visually assessed scores compared to image-based scores, which likely explains the discrepancies between the two methods. To confirm this, we conducted a correlation analysis between the two approaches and found that, in general, visual assessments tended to overestimate browning. This effect was most pronounced for *Autumn Crisp* stored at -0.5 °C, where the correlation was strongest. This was likely because the rachis remained in relatively good condition, resulting in lower visual scores (up to 2.75 on average). However, when the rachis browning severity increased, as seen in grapes stored at 20 °C, the correlation weakened as these rachis had a more intense browning which leads to reduce variability in rachis browning index scoring.

Beyond refining the method used to assess rachis browning, it is also essential to consider varietal differences, as intervarietal variability plays a significant role in all aspects of grape quality (Romero et al. 2020). Some varieties, particularly those more prone to dehydration, tend to experience more severe rachis browning. For example, the variety *Krissy* has been shown to exhibit high dehydration and increased browning (Hamie et al. 2022). However, in the same study, no correlation was found between rachis water content and browning, as different varieties displayed similar water loss rates despite exhibiting different degrees of browning. In our study, we did not measure rachis water loss, but the weight loss did not correlate with rachis browning. Both *Autumn Crisp* and *Sweet Globe* showed similar weight loss values yet differed in rachis browning severity. In fact, it is curious that *Autumn Crisp* had significanlty lower rachis browning than *Sweet Globe* at -0.5 °C but had significantly higher rachis browning at 20 °C.

To advance understanding of cultivar-dependent rachis browning, future research should adopt a more comprehensive biochemical approach that addresses several critical knowledge gaps identified in this study. Integration of detailed assays for oxidation-reduction enzymes (polyphenol oxidase, peroxidase, and superoxide dismutase) alongside the physiological parameters measured here would provide mechanistic insight into the enzymatic browning pathways that may explain varietal differences independent of ethylene/respiration responses. Additionally, chlorophyll quantification should be included to correlate visual browning scores with biochemical markers of senescence and provide a more complete understanding of the pigment degradation processes underlying cultivar-specific browning responses. Expanding these investigations to include antioxidant systems and markers of oxidative stress may help clarify varietal tolerance mechanisms, while genetic analyses of cultivar-specific responses could provide insight into the heritability of rachis senescence traits. Furthermore, expanding the scope to include more grape varieties and longer storage durations under commercial conditions would

inform targeted postharvest strategies and improve shelf-life prediction models, while standardizing non-destructive imaging tools across studies could enhance consistency in quality assessment.

5 Conclusions

This study presents the first systematic comparison of rachis browning physiology between 'Autumn Crisp' and 'Sweet Globe' table grape varieties, demonstrating that browning susceptibility is influenced by both storage temperature and cultivar-specific responses. The integration of visual and image-based assessment methods represents a methodological advance, revealing that objective image analysis provides more consistent quantification of browning severity, particularly during early browning assessment and also under high-stress storage conditions. These findings establish important groundwork for understanding varietal differences in rachis senescence while simultaneously providing a step toward developing cultivar-specific postharvest strategies and standardized evaluation protocols. Notably, at −0.5°C, despite similarly low ethylene levels, 'Sweet Globe' showed more pronounced rachis browning than 'Autumn Crisp', highlighting factors beyond ethylene regulation. The effectiveness of cold storage in mitigating rachis browning underscores the importance of rapid cooling and maintaining a consistent cold chain during handling and transportation.

Future studies should explore the biochemical and molecular pathways involved in rachis browning, particularly the roles of oxidative enzymes such as polyphenol oxidase and peroxidase, as well as chlorophyll degradation. Investigating antioxidant systems and markers of oxidative stress may also help clarify varietal tolerance mechanisms. Additionally, genetic analyses of cultivar-specific responses could provide insight into the heritability of rachis senescence traits. Expanding the scope to include more grape varieties and longer storage durations under commercial conditions would further inform targeted postharvest strategies and improve shelf-life prediction models. Standardizing non-destructive imaging tools across studies could also enhance consistency in quality assessment.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that they have no financial conflicts of interest or personal relationships that could have influenced the work reported in this paper.

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