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Evaluation of methods for determining rachis browning in table grapes

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ABSTRACT

Rachis browning is an important quality parameter of table grapes which can limit the development of novel storage technologies. Previous research has shown the feasibility of using image analysis by photography or scanning to measure rachis quality. Here, these two methods were compared, and rachis browning was also evaluated by auto-fluorescence. Two table grape cultivars, Mystery and Superior, were stored under different conditions and durations and the three objective methods were employed, in addition to a subjective evaluation. As shown in the past, weight loss was poorly correlated to rachis browning. Correlation coefficients between image analyses by photography and scanning were 0.88 and 0.98 for 'Mystery' and 'Superior', respectively, suggesting that the two methods are interchangeable. Auto-fluorescence compared to photographic image analysis yielded correlation coefficients of 0.73 and 0.90 for 'Mystery', which had a low range of browning, and 'Superior' which had a wide range of browning, respectively. For 'Superior' the lower median of browning had a low correlation to auto-fluorescence whereas the high median range was highly correlated. In general, all methodologies asserted that 3 d of shelf life is a feasible time frame for both cultivars under the conditions tested. A web-based application http://www.agri.gov.il/en/blogs/chapter.aspx?peopleId=21&chapterId=472 was established to upload images and retrieve rachis-browning data.

1. Introduction

Rachis browning is the most important physiological disorder of table grapes post-storage, while the primary pathological spoilage problem is decay caused by *Botrytis cinerea* (Lichter, 2016). To the consumer, a green rachis is an indication of freshness and hence a brown rachis can be a major cause of consumer rejection and fruit waste. Many studies have been dedicated to decay control: the current commercial technology is based on various forms of SO_2 application (Nelson, 1985). Apart from its very effective control of decay, SO_2 bleaches the rachis and prevents its browning. However, the SO_2 technology has three major problems: it is perceived as unsafe, it can cause bleaching of berry tissue, mainly around the pedicel and, if improperly used, may also produce an undesirable aftertaste. Thus, there have been numerous attempts to find alternatives to this technology (Lichter et al., 2006; Romanazzi et al., 2012). However, most of these studies have focused on preventing decay, with varying degrees of efficacy and potential usage, but little attention has been paid to controlling rachis browning.

From a biological perspective, rachis browning belongs to a wide range of postharvest disorders that are associated with loss of cell integrity and subsequent enzymatic browning, and potentially non-enzymatic browning as well (Lichter, 2016). Water loss is considered a major factor in browning, but chlorophyll degradation, wounding, programmed cell death, ethylene-mediated browning and other biological processes may also play a role (Crisosto et al., 2001; Hall et al., 2011; Lichter et al., 2011; Balic et al., 2012; Li et al., 2015).

One of the obstacles to developing efficient means of rachis-browning control is the lack of a robust and accurate methodology to measure browning. While subjective evaluation is the fastest method, it is the least reliable as it is conveyed with many variations in scale and low uniformity (Lichter, 2016). Cluster or rachis weight loss is the most simple objective measure but its correlation to browning may be low (Lichter et al., 2011). Photographic image analysis of the whole rachis has proven effective at quantifying browning, but it requires a dedicated space and uniform lighting conditions. Scanning with standard equipment has also been reported as a tool to measure browning but its efficacy has not been compared to those of other methods (Balic et al., 2012). Lastly, whole-tissue autofluorescence, which has been successfully employed to measure berry properties in table grapes and many other crops (Ghozlen et al., 2010; Bahar et al., 2012), was used to measure differences in rachis browning (Li et al., 2015), albeit without

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Table 1
Fruit characteristics of 'Mystery' and 'Superior' after harvest. Cluster weight was measured for all clusters. Other parameters were measured in 3 replications of 20 berries.

	n	Mystery	Superior
Cluster weight (g)	140, 240	463 ± 74	482 ± 69
Berry weight (g)	20 × 3	6.30 ± 0.07	5.98 ± 0.25
Berry diameter (mm)	20 × 3	20.8 ± 0.1	20.1 ± 0.3
Firmness (g mm^{-1})	20 × 3	222 ± 2	393 ± 7
TSS (%)	20 × 3	17.8 ± 0.2	15.0 ± 0.3
Acidity (%)	20 × 3	0.47 ± 0.01	0.55 ± 0.00

Table 2
Storage conditions in this study.

	Mystery		Superior	
	Temperature (°C)	RH (%)	Temperature (°C)	RH (%)
Cold storage	2.1 ± 0.2	90.8 ± 3.6	2.2 ± 0.2	94.0 ± 0.3
Optimal shelf life	17.4 ± 0.1	92.2 ± 3.7	17.9 ± 0.1	93.4 ± 1.6
Non-optimal shelf life	27.0 ± 0.2	51.7 ± 1.1	25.0 ± 0.0	53.2 ± 0.1

Table 3
Terms used in this study and their description.

Term	Description
Subjective evaluation	
Pedicels	Relative coverage of brown (non-green) area of pedicels, laterals or main stems in the range of 0 (all green) to 1 (all brown)
Laterals (side branches)	
Main stem	
Image evaluation	
Trained individual[a]	Subjective evaluation of the image by a trained individual
Untrained individual[b]	Subjective evaluation of the image by an untrained individual
Objective parameters	
BR50_photo	Camera image processed by the browning index algorithm at hue angle threshold of 50
BR50_scan	Image acquired by a standard table scanner and processed using the BR50 algorithm
Primary auto-fluorescence signals	
FRF_UV	InfraRed (Far-red) fluorescence excited with UV light
FRF_B	InfraRed fluorescence excited with blue light
RF_R	Red fluorescence excited with red light
FRF_R	InfraRed fluorescence excited with red light
Auto-fluorescence ratios	
SFR_R	FRF_R/RF_R. Index of chlorophyll
FLAV	Log (FRF_R/FRF_UV). Index of compounds which absorb at 375 nm
FER_RG	FRF_R/FRF_G. Index of anthocyanins)
ANTH_RB	Log (FRF_R/FRF_B). Index of anthocyanins

[a] Trained individual graded more than 300 samples prior to image evaluation.
[b] Untrained individual graded 10 to 20 samples prior to image evaluation.

proper validation.

The objectives of the current study were to validate the use of scanning to measure rachis browning and to determine whether rachis auto-fluorescence can also be reliably used to measure this parameter.

2. Materials and methods

2.1. Fruit source

The experiment was performed on two cultivars of *Vitis vinifera* L.: 'Mystery' harvested on 5 Jul 2015 from a vineyard in Moshav Pedaya (lat. 31°51′, long. 34°53′), and 'Superior Seedless' ('Sugarone') harvested on 2 Aug 2015 from a vineyard in Moshav Lachish (lat. 31°33′, long. 34°51′). The planting system was a Y-shaped trellis with planting distances of 3 × 1 m. In general, the shoots were trained to 15 buds with 6 to 7 shoots per vine. Irrigation was by drippers placed 50 cm apart. The fruit were harvested during the commercial harvest period and were transported within 2 h of harvesting by air-conditioned van to the Department of Postharvest Science at the Volcani Center. Basic parameters of the clusters and berries were determined (Table 1) as previously described (Bahar et al., 2012).

2.2. Experimental setup

The clusters were sorted for uniform size and appearance, cleaned of damaged berries, weighed and put in 1-kg punnets which were placed in cardboard boxes. The boxes were placed in cold storage or under shelf-life conditions (Table 2) and at each time point, 10 clusters were analyzed. The clusters were weighed to measure weight loss. Berries were removed from the cluster using a clipper and rachis browning was evaluated subjectively. Each individual rachis was photographed, scanned and analyzed for auto-fluorescence (Section 2.2). The diameter of the main stem was measured in the middle of the cluster.

2.3. Measurements of rachis browning

2.3.1. Subjective evaluation

Subjective evaluation of rachis browning was carried out by the first author throughout the experiments. He evaluated the surface area of the main stems, lateral branches and pedicels for non-green parts: completely green stems, laterals and pedicels scored a value of 0 while completely brown parts scored a value of 1. Relative coverage of brown area was scored in intervals of 0.1 representing 10% coverage units. Average browning of the stems, laterals and pedicels was calculated in addition to the average of the three parts.

Two people also rated each photograph of the rachis for overall browning coverage (photography was performed as outlined in Section 2.3.2). One person was a highly trained evaluator (the first author) while the other was briefly trained with about 30 photos of different levels of browning under the guidance of the corresponding author.

2.3.2. Image analysis by photography and scanning

Photographic image analysis was performed as described previously (Lichter et al., 2011) on whole rachis. Briefly, immediately after clipping off the berries, the rachis was placed on a white A4 sheet and photographed. The photography room was equipped with a dual halogen light source and reflectors (SLS-DL1000, Tokina, Hong Kong). A camera (Sony DSC-R1) was positioned 50 cm above the image on an adjustable camera stand. Photos were taken in RAW format with an image size of 3888 × 2592 pixels, a lens aperture of F11, and a shutter speed of 0.1 s; the sensor sensitivity was equivalent to ISO 160 and the image was recorded in the sRGB color space. Prior to analysis, images were processed by the image data convertor SR software version 1.0 (Sony) by setting the white balance to a color temperature of 3000 K and saving the image in JPEG format at high-quality compression yielding images of about 7 Mb.

Immediately after photography, the rachis was scanned with a standard flatbed G4010 scanner (Hewlett Packard, Palo Alto, CA). Images were saved in TIF format at an output resolution of 300 points per inch in "millions of colors" (24 bit color resolution − 8 bits per channel) and "sharpen" modes. Scan preferences were set to "automatically adjust black and white threshold" and "use enhanced color (vivid)", and image size ranged from 5 to 10 Mb (cropped to contain the rachis).