Scientia Horticulturae 220 (2017) 243–249

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Quality, antioxidant compounds, antioxidant capacity and enzymes activity of 'El-Bayadi' table grapes at harvest as affected by preharvest salicylic acid and gibberellic acid spray

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ARTICLE INFO

Keywords:
Grapes
Salicylic acid
GA_3
Quality
Resveratrol
Antioxidants
Enzymes

ABSTRACT

Berry quality, antioxidant compounds, antioxidant capacity and enzymes activity of 'El-Bayadi' table grapes cultivar at harvest as affected by preharvest spray of salicylic acid (SA, 4.0 mM) and gibberellic acid (GA_3, 30 mg/L) were evaluated during 2014 and 2015 seasons. GA_3 spray increased berry weight, length and width compared to SA and control treatments. SA spray decreased berry length and width compared to control. Both cluster weight and length increased by SA and GA_3 compared to control. TSS content was not affected by treatments, but was higher in 2015 than 2014 season. Titratable acidity was higher at GA_3 and SA treatments than control and was higher in 2014 than 2015 season. TSS/acid ratio was lower at SA than GA_3 and control treatments. Berry firmness was lower at SA than GA_3 and control treatments and was higher in 2014 than 2015 season. Total phenols and flavonoids concentrations were higher at SA and GA_3 treatments than control. *trans*-resveratrol concentration increased, while *trans*-piceid decreased by SA spray compared to GA_3 and control treatments. Total phenols concentration was higher in 2015 than 2014 season, in contrast to total flavonoids and *trans*-resveratrol. Vitamin C concentration increased by GA_3 spray compared to SA and control and was higher in 2015 season than 2014. Peroxidase (POD) polyphenoloxidase (PPO), polygalacturonase (PG) and xylanase activities increased by GA_3 spray compared to SA and control. While, SA spray decreased both POD and PPO activities compared to control. Both SA and GA_3 spray lowered antioxidant capacity (higher DPPH IC_{50} values) than control. Nitrogen, phosphorus and potassium, and protein concentrations were higher at SA and GA_3 treatments than control. In conclusion, preharvest spray of SA and GA_3 could be used to improve the overall quality 'El-Bayadi' table grapes.

1. Introduction

Generally, the attractiveness of fruit to consumers is determined not only by regular quality attributes such as size, color, texture, sugar and acidity levels but also by their contents of health-promoting phytochemicals. Grapes contain considerable amounts of bioactive antioxidants such as phenolic compounds (phenolic acids, flavonols, anthocyanins, flavanols, and stilbenes) and vitamins that largely contribute to both fruit quality and, via consumption, to human health (Xia et al., 2010; Zhou and Raffoul, 2012). Thus, the quantitative and qualitative characteristics of phenolics are important for quality of fresh grapes and grape products (Gomez-Cordoves and Gonzalez-Sanjose, 1995). Resveratrol, its 3-glucopyranoside piceid, and their *cis* isomers are natural plant phenolics, representing the major active compound of stilbene phytoalexins that mainly occur in grapes, berries, and other dietary constituents and is presumed to be involved in defense system against plant pathogens and metabolic diseases in human (Adrian et al., 1997; Xia et al., 2010; Zhou and Raffoul, 2012). The induction of resveratrol and other phenolics biosynthesis and/or maintaining their level during storage is desirable for improving both postharvest disease control and

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http://dx.doi.org/10.1016/j.scienta.2017.04.005
Received 18 December 2016; Received in revised form 29 March 2017; Accepted 5 April 2017
Available online 23 April 2017

nutraceutical properties of grapes (Sanchez-Ballesta et al., 2006). Therefore, it is important to study factors that affect the accumulation of natural antioxidant compounds with the aim of further improving the relevant fruit attributes. SA is a phenolic acid considered as a plant hormone that involved in plant responses to abiotic stress and regulation of plant growth and development (Raskin, 1992). Several studies have showed that either pre or postharvest application of SA induced the systemic acquired resistance (SAR) to pathogens and reduced decay in strawberries (Babalar et al., 2007), peaches (Wang et al., 2006), sweet cherries (Xu and Tian, 2008). SA induced both phenylalanine ammonia-lyase (PAL) mRNA accumulation and activity as well as increased phenolics accumulation in harvested 'Cabernet Sauvignon' grapes (Chen et al., 2006). Preharvest spray of SA (100 mg/L) at pea and verasion stages increased yield, and improved quality at harvest and during storage of 'Thompson seedless' grapes (Marzouk and Kassem, 2011). Preharvest spray of SA (1.5 or 2.0 mM) at pea and verasion stages improved quality at harvest and extended postharvest life of 'Flame Seedless' grapes (Champa et al., 2015). GA_3 is being widely applied in grapes production at different concentrations during berry development and growth to increase yield, and improve quality characteristics of both clusters and berries of especially seedless cultivars (Rizk-Alla and Meshrake, 2006; Zoffoli et al., 2008; Marzouk and Kassem, 2011). In Saudi Arabia, the table grapes cultivated area reached about 13282 ha producing 149847 tons in year 2013 (FAO, 2013). In this respect, 'El-Bayadi', a white seeded, is the main table grape cultivar in Taif region representing about 90% of total table grapes produced in this region (Al-Qurashi and Awad, 2013). There is relatively much published information on the impact of different plant growth regulators such as GA_3 and SA on yield and regular quality of grapes but little on their effects on antioxidants accumulation (such as flavonoids and stilbenes), antioxidant capacity and enzymes activity, especially on the locally produced cultivars. This study, therefore, aim to evaluate the effect of SA and GA_3 spray on berry quality, antioxidant compounds, antioxidant capacity, and antioxidant and hydrolytic enzymes activity of 'El-Bayadi' table grapes cultivar.

2. Materials and methods

2.1. Plant materials and experimental procedure

During 2014 and 2015 seasons, uniform vines were selected in a commercial drip irrigated vineyard of 'El-Bayadi' table grape in Taif region, Saudi Arabia. The experimental design was a randomized complete block with three replicates/treatment and each replicate contained three vines. The vines were either treated by salicylic acid (SA) (Fisher Scientific, USA) at 4.0 mM or gibberellic acid (GA_3) (Central Drug House (P) Ltd, India) at 30 mg/L solutions as foliar spray. Both clusters and leaves were covered by the spray solutions until runoff with a plastic hand sprayer (Matabi Style 1,5 Sprayer-1L, Goizper, Spain) in the early morning. SA was sprayed four times starting at pea stage (30–40 days from fruit set), and then every 20 days with the last application at version stage (about 3–4 weeks before commercial harvest). While, the spraying schedule for GA_3 was twice at pea and version stages. A control treatment in which vines sprayed with water and surfactant was included. A non ionic wetting agent (Tween 20 surfactant) at 0.01% was included in all foliar applications. The grape vines received the regular cultural practices. At harvest, samples of four clusters from each replicate/treatment were randomly collected, kept in perforated carton, and directly transferred to the horticulture laboratory at King Abdulaziz University, Jeddah for quality and biochemical measurements.

2.2. Cluster and berry physical characteristics

Cluster weight (g), length and breadth (cm) were recorded in the four clusters for each replicate/treatment and the mean was calculated. Berry weight (g) was calculated by tacking the mean values of 100 berries randomly selected from the four clusters per replicate/treatment. While berry length and width were calculated by tacking the mean values of 30 berries randomly selected from the four clusters per replicate/treatment.

2.3. Firmness, TSS, titratable acidity and vitamin C measurements

Berry firmness was recorded independently in each of 30 berries (randomly collected from each replicate) by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter that measure the compression force required to penetrate the berry and the results expressed in Newton. A homogeneous sample was prepared from these 30 berries per replicate for measuring TSS, titratable acidity and vitamin C. TSS content was measured as percentage in berry juice with a digital refractometer (Pocket Refractometer PAL-3, ATAGO, Japan). Titratable acidity was determined in distilled water diluted juice (1: 2) by titrating with 0.1 N sodium hydroxide up to pH 8.2, using automatic titrator (HI 902, HANNA Instrument, USA) and expressed as percentage of tartaric acid. Vitamin C concentration was measured by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results expressed as mg/100 mL juice (Ranganna, 1979).

2.4. Extraction and quantification of trans-resveratrol and its glycoside trans-piceid

Extraction and quantification of *trans*-resveratrol and *trans*-piceid were carried out according to Romero-Perez et al. (2001) with modifications. Two grams of frozen berry skin (randomly collected from 30 berries/replicate) were homogenized with 25 mL of ethanol/water (80:20 v/v) using a homogenizer and maintained at 60 °C for 30 min. The extract was filtered through a Whatman inor-ganic 15 µm and concentrated to 3 mL by rotary evaporation (in vacuo) at room temperature (20 °C ± 2). The concentrated extracts were filtered through CA Syringe filters 0.2 µm and injected into a high-performance liquid chromatography (Shimadzu, Japan) coupled with ultraviolet-visible diode array detector (HPLC-UV–VIS-DAD) for *trans*-resveratrol and *trans*-piceid quantification. The system was equipped with a Tracer Agilent ZORBX Eclipse plus C18 Analytical column (4.6 × 150 mm), 5 µ particle size. The column temperature was kept at 30 °C. The mobile phase consisted of A and B where solvent A was glacial acetic acid in water mixture (0.1 glacial acetic acid:70 water v:v) and solvent B 29.9 aceto- nitrile/acetic acid, with a flow rate of 1.0 mL/min. Injection volume was 20 µL. Detection was performed at a 310 nm wavelength and run time was 15 min. Retention time was about 2 and 4.5 min for *trans*-piceid and *trans*-resveratrol, respectively. Quantification was based on the peak area. The chromatogram peaks of individual compounds were identified by comparing their retention times with the retention times of pure standards. *trans*-resveratrol standard was purchased from Baoji Guokang Bio-Technology Co., Ltd (Baoji, China). *trans*-piceid standard was purchased from Sigma Chemical Co., St. Louis, MO. (USA). Integrated peaks were calculated by comparison with standard solutions of known concentration and the results expressed as mg kg^{-1} on a fresh weight (FW) basis.

2.5. Preparation of the methanol extract for total phenols, flavonoids and antioxidant activity determinations

Two grams of berries skin tissue (randomly collected from 30 berries/replicate) were extracted by shaking at 150 rpm for 12 h with 20 mL methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract that will be used for total phenols and flavonoids and antioxidant activity estimations.