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The effects of genotype and growing conditions on antioxidant capacity, phenolic compounds, organic acid and individual sugars of strawberry

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ABSTRACT

In this study, the genotypic and environmental effects for bioactive compounds in strawberries were partitioned. 13 strawberry genotypes from diverse breeding programs were selected. The genotypes were grown in three growing conditions: greenhouse (GH), plastic tunnel (PT) and open-field (OF) for two growing seasons. The results indicated that the genotypes were significantly different for most of the characteristics tested except the ferric reducing ability assay (FRAP) and Trolox-equivalent antioxidant capacity assay (TEAC) in the second growing season, while the growing conditions were only significant for total phenolic content (TPC) and fructose and total sugar content in the first growing season. Genotype had 71% and 72% of the total variance for total monomeric anthocyanin contents (TMA), while it had only 12% and 13% of the variance for TPC in the first and second year of the experiment. Genotype effect was larger than that from the growing conditions for most of the bioactive component variables in the experiment indicated that breeding for bioactive components may be successful.

1. Introduction

In recent years, there has been an increasing interest in determining antioxidant properties of red fruits, due to their rich dietary sources of antioxidant phenolics and anthocyanins (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002). Epidemiological studies suggested that consumption of red fruit juices such as grape, different berry juices and pomegranate correlate with reduced risk of coronary heart disease, stroke, certain types of cancers and ageing (Malik & Mukhtar, 2006). For this reason, it is believed that the consumption of fruit and vegetables which are rich in bioactive compounds may increase the resistance against such diseases (Sandra, 2004). The beneficial effects of fruit and vegetables are becoming increasingly appreciated. Amongst the fruit species, antioxidant activities of berries are very important as a potential cancer chemo preventive (Seeram, 2008). Strawberries are a common and important fruit in the Mediterranean diet because of their high content of essential nutrients and beneficial phytochemicals, which seem to have relevant biological activity in human health (Giampieri et al., 2012). Recent studies have focused on the nutrient and phytochemical contents of the strawberry and on factors affecting the composition of this fruit. These studies have included genotype, harvest time, influence of the degree of maturity, climatic factors, post-harvest storage, plant materials such as (berries, fruits, vegetables, herbs, cereals, tree material, plant sprouts, and seeds), geographic origin, environmental factors such as light exposure, cultural system and storage temperature, cultivated and wild form, genotype, cultural system (covered with black polyethylene mulch and without mulch) and integrated pest management and organic farming (Aaby, Mazur, Arnfinn, & Grete, 2012; Capocasa, Scalzo, Mezzetti, & Battino, 2008; Crespo, Bordonaba, Terry, & Carlen, 2010; Diamanti et al., 2012; Fernandes, Domingues, de Freitas, Delerue-Matos, & Mateus, 2012; Giampieri et al., 2012; Jin, Wang, Wang, & Zheng, 2011; Pincemail, Kevers, Tabart, Defraigne, & Dommes, 2012; Tulipani et al., 2011). Although the variation amongst the genotypes was extensively studied for the bioactive compounds in strawberries, the variation caused by the environmental effect has drawn less attention. In this study, the objective was to test several strawberry genotypes representing a large genetic base across years and under different growing conditions that mimic the standard production methods in regions with Mediterranean climate, to calculate the relative proportions of bioactive compounds genetic and environmental variation.

2. Materials and methods

TMA, TPC, total antioxidant capacity (AOC) (determined by two methods FRAP and TEAC, organic acids and individual sugars were

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studied in a diverse array of strawberry cultivars originated from several breeding programmes.

2.1. Plant material

The goal was to capture a large genotypic variance across cultivars. Therefore, 13 genotypes from diverse breeding programmes were selected. The genotypes included: "Sweet Charlie" (University of Florida), "Camarosa", "Gaviota" and "Whitney" (University of California), "Cigouletta" and "Marlatte" (Breeder CIREF, France), "Alba" and "MT 99 121 9" (CRA-Fruit Research Unit of Forlì, Italian national program, Italy), "Type 3", "Type 5", "Ebru", "Kaşka" and "Osmanlı" (Çukurova University, Turkey). The genotypes were grown in three growing conditions: GH, PT and OF for two growing seasons during 2007–2008 and 2008–2009. GH is 10 m wide, 25 m long, and 4.5 m high (in the centre), providing about 250 m^2 total area a structure. PT is also similar GH. To maintain stable ventilation, retractable sidewalls are used that extend the length of both sides of structure both GH and PT. GH was covered with a 3 mm glass. PT was covered with a single layer of 0.25 mm and two annual plastic-house trade polyethylene materials. Plants were planted in raised beds (60 cm wide hills) at 1.3 m spacing into black polyethylene mulch. The black mulch was applied to reduce weed pressure and increase soil temperature. Beds were irrigated with a drip irrigation system under the plastic mulch. Runner tips were obtained from the nursery at the end of June and were rooted in pots under a mist propagation system for approximately 40 days and then transplanted to the planting locations in mid-August. The pots (6 cm in diameter and 10 cm in height) were filled with a mixture of sand and farmyard manure (2:1 ratio). Greenhouse-rooted plug plants were planted in mid-August 2007 and mid-August 2008. The planting was conducted as double row with 30 × 25 cm distances. The experiment was a completely randomized factorial arrangement with three environments (GH, PT and OP) and 13 cultivars using 4 replications. Each replication consisted of 18 plants, which totaled 72 plants per cultivar per replication. The GH was heated to keep the temperate above the 4 °C as needed whilst PT was unheated. Moreover, low tunnels (40 cm height) were built on raised beds within the PT to protect plants against frost damage. Bumble bee hives were placed during the flowering season to increase pollination. In addition to the initial fertiliser application, (20:20:20 + Fe NPK fertilizers) was applied biweekly by drip irrigation throughout the growing season. Of fertilisation was not manure in the winter months.

2.2. Extraction

Sampling was conducted at the peak of the harvest season in each treatment. The fruit were harvested when 75% of the fruit had the typical colour formation of the cultivar. 500 g of fruit was sampled and placed at −20 °C until the analysis. Four replicates were utilised for each analysis. Later, samples were thawed at room temperature and then homogenized in a food processor. Chemical analysis was completed within 30 days of storage.

2.3. Analytical procedures

2.3.1. Total monomeric anthocyanins (TMA)

TMA were estimated by a pH differential method (Giusti & Wrolstad, 2005), using a UV–VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using A = (A533–A700) pH 1.0 – (A533–A700) pH 4.5 with a molar extinction coefficient of 31,600. Results were expressed as mg Pg-3-glk/kg fresh weight (FW) basis.

2.3.2. Determination of total phenolic content (TPC)

TPC content was measured according to the Singleton and Rossi (1965) procedure. Fruit samples were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 1 h in room conditions the dark. Three parallel extracts were made for each cultivar. Then, extract, Folin–Ciocalteu's phenol reagent and water was incubated for 8 min followed by the addition of 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV–VIS spectrophotometer at 750 nm. Gallic acid was used as standard. Standard curve was used to determine the concentration of total phenolics. For this purpose 0, 50, 100, 200, 400, 800 and 1600 mg/L of gallic acid concentration was used as a quality assurance. The results were expressed as mg gallic acid equivalent in kg fresh weight basis (GAE/kg FW).

2.3.3. The total antioxidant capacity (AOC)

AOC was estimated by two standard procedures FRAP and TEAC assays as suggested by Ozgen, Reese, Tulio, Miller, and Scheerens (2006). Ferric Reducing Ability of Plasma (FRAP) was determined according to the method of Benzie and Strain (1996). The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid (1000:3.3 v/v), and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay in duplicate, 2.97 mL of FRAP reagent and 30 μL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm in a spectrophotometer. For the standard trolox equivalent antioxidant capacity (TEAC) assay, ABTS was dissolved in acetate buffer and prepared with potassium persulfate, as described (Ozgen et al., 2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Ozgen et al., 2006). For the spectrophotometric assay, 2.98 mL of the ABTS·solution and 20 μL of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm. *Trolox* were used as positive control and linear regression curves were drawn. For this purpose 1–20 μL to 1 mM trolox and appropriate water was used to bring volume to 20 μL. Results were expressed as mmol trolox equivalent in kg fresh weight basis (TE/kg FW).

2.4. Extraction of organic acids and individual sugars for HPLC

Fruit slurries (5 g) were diluted with purified water or metaphosphoric acid (2.5%) solutions for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45-μm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use. All the samples and standards were injected three times each and mean values were calculated.

2.4.1. Chromatographic conditions

The HPLC analyses were carried out using a Perkin Elmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (Perkin Elmer series-200) (Waltham, Massachusetts, USA). Procedures for separation and determination of organic acids were modified from Shui and Leong (2002). The separation was carried out on a SGE wakosil C18RS 5 μm column (250 × 4.6 mm I.D.). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using a pH 2.5 sulphuric acid solution (solvent A), and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 μL; column temperature, 30 °C; and detection wavelength, 215 nm.