

Agronomic characteristics and phytochemical profiles of advanced June-bearing strawberry lines for the northern Canadian climate

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Eleven advanced strawberry lines ('SJ01110', 'SJ04402', 'SJ0618', 'SJ0663', 'SJ0693', 'SJ06912', 'SJ081437', 'SJ851811', 'K0412', 'LL022010' and 'V151') were evaluated for their yield, fruit quality, total phenolic content, total antioxidant capacity, and phenolic composition, and were compared with a commercial cultivar ('Wendy'). The results showed that 'SJ0693' had excellent soluble solids content, mid-level titratable acidity, low weight loss, and the best firmness among all the cultivars. Higher total antioxidant capacity was found in 'SJ0693', according to ferric reducing antioxidant power and oxygen radical absorbance capacity assays, an indication that 'SJ0693' is a promising new cultivar for the fresh market. In addition to rich individual phenolics, 'SJ0618' had the highest total antioxidant capacity, which was significantly different from the other genotypes, suggesting the potential use of this line as parent material in breeding or as a functional food ingredient. There was a strong relationship between total antioxidant capacity and total phenolic content, according to Folin–Ciocalteu, ferric reducing antioxidant power, and oxygen radical absorbance capacity assays. This study confirms that anthocyanins are major phenolic compounds contributing to the main antioxidant power of strawberries.

Key words: *Fragaria × ananassa* Duch., breeding, fruit attributes, phytochemical profile, selection, yield

Introduction

Lately, there has been growing appreciation for the role that horticultural products play in preventing and reducing pathological conditions such as heart disease, cancer, and stroke (Joshiyura et al. 2001, Johnsen et al. 2003, Hung et al. 2004). Fruits and vegetables are regarded as good sources of natural antioxidants, particularly thanks to the presence of high contents of polyphenolic compounds, which potentially protect the human body against damage and delay senescence induced by oxidative stress (Cook and Samman 1996, Samman et al. 2003, Manach et al. 2005, Williamson and Manach 2005).

Berries, especially strawberry (*Fragaria × ananassa* Duch.), contain high antioxidant levels that are two to 11 times the levels found in apple, peach, pear, grape, tomato, orange, or kiwifruit (Wang et al. 1996, Scalzo et al. 2005b). That difference could be ascribed to the high levels of nutrients, such as dietary fibre, fructose, minerals, vitamin C, folate, and polyphenolic phytochemicals, that strawberry contains (Bailey and Gregory Iii 1999, Proteggente et al. 2002, Scalzo et al. 2005a, Battino et al. 2009). There is growing *in vitro* and *in vivo* evidence that dietary intake of strawberry positively affects human health. For example, total antioxidant capacity (TAC) in plasma increases significantly after strawberry consumption (Tulipani et al. 2011). Some studies reported that one cup of fresh strawberries (149 g) can contain high levels of phenolic compounds (300 mg), including 5 mg of quercetin. The consumption of this level of quercetin was related to protection against lung cancer (Knekt et al. 1997). In addition to these benefits to human health, the various phenolics in strawberry not only increase disease resistance and product shelf-life but also affect taste, colour, and flavour (Tomás-Barberán and Espín 2001, Lesschaevé and Noble 2005, Tao et al. 2010). The high amounts of proanthocyanins observed in strawberry had some correlation with resistance to grey mould (*Botrytis cinerea* Pers. ex Fr.) and improved fruit preservation (Tao et al. 2010).

Compositional concentration and changes may vary strongly among strawberry cultivars depending on their genetic background and rely on other factors, such as cultural practices (conventional, organic), development stage, growing conditions (climate, temperature), and post-harvest management and processing, most of which may be upgraded to improve their quality. Substantial changes in the ellagic acid (EA) content were observed among strawberry cultivars, varying from 43 to 464 $\mu\text{g g}^{-1}$ fresh weight (FW) (Maas et al. 1991). Strawberries grown organically showed markedly higher TAC than fruits from conventional agriculture (Jin et al. 2011). As fruits mature, the anthocyanin level rises (Wang and Lin 2000). Higher amounts of anthocyanins and aroma compounds were found in strawberries kept at higher temperatures during storage (Ayala-Zavala et al. 2004). Although numerous factors affect antioxidant potential and the content of bioactive constituents, genotype is still the most crucial determinant of post-harvest quality, because of its phytochemical content, fruit firmness, shelf-life, and disease resistance. Therefore, much more attention has been devoted in recent years to exploring new strawberry cultivars with desired nutrient benefits.

Connor et al. (2002) suggested improving antioxidant power by means of selection in breeding programs based on the evaluation of the heritability of antioxidant power (0.43), total phenolics (0.46), and total anthocyanins (0.56) in blueberry progenies. In 1955, an Agriculture and Agri-Food Canada strawberry breeding program began in Quebec to develop cold hardiness and high-quality selections. In Quebec, several studies have already considered strawberries in terms of their horticultural characteristics and chemical composition (Rekika et al. 2005, Khanizadeh et al. 2008, Wang et al. 2010). Presently, a limited number of cultivars have been characterized as having good performance, such as high yield and large fruits rich in antioxidants, in northern Canadian climates. Therefore, there is a great deal of interest in better exploiting differences and variations in the potential health-promoting effects of new strawberry selections with high yield.

The main aim of the present study was to analyze 11 advanced strawberry lines for their agronomic attributes and phytochemical profiles and compare them with those of one commercial cultivar ('Wendy'), and to attempt to provide theoretical data for the possible release of new strawberry cultivars.

Materials and methods

Plant materials

One strawberry cultivar ('Wendy'), and 11 advanced strawberry selections ('SJ01110', 'SJ04402', 'SJ0618', 'SJ0663', 'SJ0693', 'SJ06912', 'SJ081437', 'SJ851811', 'K0412', 'LL022010' and 'V151') were cultivated in 2012 in an experimental field located in L'Acadie, QC, Canada (long 73°35' W lat 45°32' N). A randomized complete block design was established for the study, with three replicates per genotype. Each experimental plot was 2 × 0.6 m and supplied with drip irrigation. The strawberries were picked in a 1m long section in the middle of each plot. Fruits were harvested under standard ripening conditions from each plot two to three times per week, from the beginning of June until mid-July, over the complete production season. The harvested fruits were immediately placed in a cooler and then brought to the laboratory, where 30 fruits of each genotype were used for weight loss and firmness analyses. A composite sample was made with all remaining fruits which was divided into four 150 g sub-samples, rapidly cut into four pieces, and then frozen in liquid nitrogen. Thereafter, they were kept at -80 °C until extractions for chemical composition analysis were performed.

Chemicals

Acetone, methanol (MeOH), gallic acid, NaHCO_3 , Folin–Ciocalteu (FC) reagent, sodium acetate, acetic acid, HCl, 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, FeCl_3 , NaOH 0.1 N, NaH_2PO_4 , $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, fluorescein disodium, 2,2'-azobis (2-aminidopropane) dihydrochloride (AAPH), Trolox, pelargonidin-3-glucoside (P3G), and kaempferol-3-glucoside were purchased from Sigma-Aldrich (Oakville, ON, Canada). Quercetin-3-glucoside (Q3G) and EA were obtained from Apin Chemicals Ltd (Abingdon, UK), and cyanidin-3-glucoside (C3G) was obtained from Polyphenols Laboratories AS (Sandnes, Norway). All reagents were of analytical grade. The H_2O used in the experiment was double-distilled by a NanoPure system (Dubuque, IA, USA).

Soluble solids content and titratable acidity

About 50 g of thawed frozen fruits from each replicate were blended using a Supreme Juicerator (Acme Juicer Mfg. Co., New Hartford, CT, USA). The juice obtained was used for the soluble solids content (SSC), pH, and titratable

acidity (TA) measurements. The SSC was measured using a refractometer (AR200 digital refractometer; Reichert Inc., Depew, NY, USA), and the results were reported as degrees Brix ($^{\circ}$ Brix). The pH and TA were measured based on the assay reported by Khanizadeh et al. (2009). Briefly, 2 ml of strawberry juice was diluted with 18 ml of H₂O (1:9 v/v), the pH was measured, and then the solution was titrated with NaOH 0.1 N up to pH 8.1 using a pH meter (Accumet AB15 Basic pH meter; Thermo Fisher Scientific Inc., Waltham, MA, USA). The TA was expressed as percent citric acid equivalent, according to the volume of NaOH added during titration.

Horticultural characteristics

Total yield and average fruit weight were measured at every harvest. A universal testing machine (LRX; Lloyd Instruments Ltd., Hampshire, UK) with a flat tip and a deflection limit of 12 mm, at a speed of 25 mm min⁻¹, was used to measure firmness, and the results were expressed as peak force (newtons, N). The determination of weight loss began right after harvest: five randomized fruits per replicate were placed on Whatman #1 filter paper in open petri dishes and were observed at room temperature (23 °C) for 5 d. The percent weight loss was calculated as previously reported by Wang et al. (2010), as follows:

$$\text{Weight loss (\%)} = (x - y) \times 100/x$$

where x is the weight on day 1, and y is the weight on day 5.

Total antioxidant capacity

The TAC of all samples was estimated with ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) methods. For each replicate, two 5 g samples of frozen fruits were transferred into two 50ml tubes, one with 25 ml of 50% MeOH for FRAP, and one with 15 ml of 50% acetone for ORAC. The samples were then homogenized using a Polytron homogenizer (Brinkman Instruments Inc., Westbury, NY, USA) at 17,500 rpm for 1 min. Then, the mixtures were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatants were transferred into 1.5ml Eppendorf tubes and kept at -20 °C until analysis.

The FRAP assay was performed in accordance with the modified method of Benzie and Strain (1996), using FeSO₄·7H₂O for the standard curve. The FRAP solution was prepared daily by mixing 10 mM TPTZ with 40 mM HCl, 20 mM FeCl₃·6H₂O, H₂O, and 300 mM acetate buffer (pH 3.6), in the ratio of 20:20:24:200 (v/v/v/v). Then, 0.06 ml of the standard or extract was mixed with 2 ml of the FRAP solution and incubated at 37 °C for 4 min. The absorbance readings at 593 nm were measured with a UV spectrophotometer (Fisher Scientific, Ottawa, ON, Canada). The TAC value of the samples was expressed as micromoles of FeSO₄·7H₂O equivalent per gram FW ($\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O equiv. g}^{-1}\text{ FW}$). The ORAC assay was conducted in accordance with the procedure previously reported by Ou et al. (2001), with slight modifications, using a Synergy 2 multi-mode microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) equipped with an automated injector. Briefly, 150 μl of fluorescein (4×10^{-9} M) was added into the wells of a 96well microplate, followed by 25 μl of the Trolox solution as the control standard, 25 μl of 75 mM phosphate buffer (pH 7.4) as the blank, and 25 μl of diluted sample. After 30 min of incubation at 37 °C, the reaction was initiated through the addition of AAPH (153 mM) as a source of free radicals. Fluorescence was monitored and read at 2min intervals, with the emission at 520 nm and excitation at 485 nm. Data were calculated according to the regression equation between a series of Trolox standard curves, and the net area under the curve was expressed in micromoles of Trolox equivalent per gram FW ($\mu\text{mol TE g}^{-1}\text{ FW}$).

Total phenolic content

The total phenolic content (TPC) was analyzed using the FC method reported by Slinkard and Singleton (1977), with slight modifications. The supernatant was the same as for the FRAP assay. A working solution containing 1.58 ml of H₂O and 0.1 ml of FC reagent was mixed with 0.02 ml of the standard or extract and left at room temperature for 8 min. Then, 0.3 ml of 7.5% Na₂CO₃ was added to the solution, which was incubated for 30 min in a water bath set at 40 °C. Thereafter, absorbance readings were taken at 765 nm. Concentrations of samples exceeding the highest point (1 mg ml⁻¹) of the linear range of the standard curve were diluted with 50% MeOH before the final assays. Gallic acid was used as the standard, and data were expressed in milligrams of gallic acid equivalent per 100 g of fresh weight (mg GAE 100 g⁻¹ FW).

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was used to separate and quantify the phytochemicals in the strawberries, based on the study published by Tsao and Yang (2003). For the extraction, 3 g of frozen fruits, for each replicate, were blended in 15 ml of acetone using a Polytron homogenizer (Brinkman Instruments Inc., Westbury, NY, USA) at 17,500 rpm for 1 min. The mixture was filtered through Whatman #3 filter paper covered with fibre-glass wool and evaporated under vacuum by means of a Laborota 4000 rotary evaporator (Heidolph Instruments Inc., Elk Grove Village, IL, USA) set at 35 °C. The concentrated sample was rehydrated three times with 5 ml of H₂O acidified with 3% formic acid after each evaporation, and then filtered through a C₁₈ Sep-Pak cartridge (Waters Ltd., Mississauga, ON, Canada), which was first activated with 5 ml of MeOH followed by 6 ml of H₂O and 7 ml of 3% formic acid. Then, 3 ml of acidified MeOH was used to recover anthocyanins and other phenolics. All extracts were filtered through a 0.45µm Acrodisc syringe filter (Gelman Laboratory Inc., Ann Arbor, MI, USA). 10µl fresh extracts of each sample were injected (Zheng et al. 2007).

The HPLC system (model 240; Varian Inc., California, USA) was composed of a quaternary pump, an inline degasser, a column oven (model 500; Varian Inc.), and a diode array detector (model 335; Varian Inc.), along with a thermostatic autosampler (model 410; Varian Inc.). All compounds were separated with a 300 × 4.6 mm, 5 µm, Polaris C₁₈A column (Varian Inc.) coupled to a guard column (Metaguard Polaris C₁₈A, 4.6 mm, 5 µm; Varian Inc.). The temperature of the column was kept at 40 °C. The elution solvents consisted of H₂O (A) and MeOH (B), both acidified with 2.5% formic acid. The system was run as follows: 15% to 30% B in 15 min, 30% B for 5 min, 30% to 80% B in 5 min, 80% B for 17 min, 80% to 100% B in 0.5 min, 100% B for 0.75 min, and 100% to 0% B in 1 min. There was a 15min equilibration after each sample run. The flow rate was kept constant at 1.0 ml min⁻¹ throughout the total run time of 51.25 min. Detection of the different groups of phenolics was performed at 280, 365, 503, and 519 nm simultaneously. Phenolic compounds were quantified as follows: EA as EA (280 nm), flavonols as kaempferol3-glucuronide (K3Gr), Q3G, and quercetin3-glucuronide (Q3Gr) (365 nm), and anthocyanins as P3G and Pelargonidin3-rutinoside (P3R) (503 nm) and as C3G (519 nm). The peaks were identified by comparing their retention times and spectra with those of the standards. The system was operated by the Varian Star Workstation software (version 6.41; Varian Inc.), and all data were expressed in micrograms per gram FW (µg g⁻¹ FW).

Statistical analysis

All measurements were performed in triplicate. The analysis of variance of the data was performed using the GLM procedure of SAS software package (1989). Mean values were compared according to the least significant difference (LSD) test. Correlation coefficients were calculated by the CORR procedure of SAS (1989). A difference of $p < 0.05$ was regarded as statistically significant.

Results and discussion

Total phenolic content and total antioxidant capacity

Data for TPC (Table 1), as measured by the FC method, varied significantly. The highest TPC were found in 'SJ0618' (420.6 mg 100 g⁻¹) followed by 'SJ0693' (353.7 mg 100 g⁻¹), more than or almost double the values for 'SJ01110', 'Wendy', and 'SJ04402', which had the lowest levels (176.5, 183.3, and 192.5 mg 100 g⁻¹, respectively). The amounts TPC for the remaining genotypes were intermediate, ranging from 215.5 mg 100 g⁻¹ in 'SJ0663' to 308.6 mg 100g⁻¹ in 'SJ081437'. The values obtained were within the range of previous findings by Pincemail et al. (2012) for 12 strawberry cultivars but contrasted with the results reported by Khanizadeh et al. (2008), which were much lower. The differences might be explained by different genotypes considered and production years.

For determining TAC, data from a single method always offer an incomplete view of the antioxidant activities. Moreover, the chemical complexity of fruits, such as the many types of polyphenolics present, can mean scattered results, depending on the method used. For these reasons, the use of several assays would be very valuable in research, given the complementary results that they would provide (Sacchetti et al. 2005). In this study, the TAC of strawberry extracts was measured according to the FRAP and ORAC methods. In general, the data showed a similar trend for the two methods, with the exception that the FRAP values seemed, under statistical analysis, to emphasize significant inter-genotype differences more than the ORAC values. Using FRAP, 'SJ0618' was found to have the highest TAC level (46.3 µmol g⁻¹), followed by 'SJ0693' and 'SJ081437' (41.4 and 39.6 µmol g⁻¹, respectively), whereas the lowest values were observed for 'SJ01110', 'Wendy', and 'SJ04402' (23.8, 26.5, and 26.9 µmol g⁻¹, respectively). The order was the same as for TPC. According to ORAC, 'SJ0618' again had the highest level

(64.8 $\mu\text{mol g}^{-1}$), followed by ‘SJ0693 (52.0 $\mu\text{mol g}^{-1}$), whereas ‘SJ06912’ had the lowest level (26.4 $\mu\text{mol g}^{-1}$). Other genotypes were ranked differently by the two methods, producing an intermediate level with significant differences within the other genotypes, varying from 27.7 $\mu\text{mol g}^{-1}$ in ‘SJ0663’ to 33.1 $\mu\text{mol g}^{-1}$ in ‘V151’ using FRAP and from 28.7 $\mu\text{mol g}^{-1}$ in ‘SJ04402’ to 44.4 $\mu\text{mol g}^{-1}$ in ‘SJ851811’ using ORAC. The report by Tulipani et al. (2008) described large differences in the antioxidant activities of strawberries. Such differences were also detected in the present study. A highly positive correlation was found between the two methods ($r = 0.75749$, $p < 0.0001$), confirming the previous results of Aaby et al. (2005) and Rupasinghe et al. (2012). In addition, it is worthwhile to note that there are markedly positive correlations between TPC and TAC using FRAP ($r = 0.96032$, $p < 0.0001$) or ORAC ($r = 0.81430$, $p < 0.0001$), indicating that higher TPC led to stronger activity for scavenging oxygen radicals, thus improving the nutritional parameters of the fruits. Previous studies demonstrated the same high correlation between TPC and FRAP (Capocasa et al. 2008, Khanizadeh et al. 2008, Tulipani et al. 2008). A strong correlation between TPC and ORAC was also reported by Aaby et al. (2005) and Rupasinghe et al. (2012). Cai et al. (2004) demonstrated that phenolics are the primary antioxidant components of vegetables, medicinal plants, fruits and spices. There is substantial evidence that phenolic compounds play a protective role against several disturbances (ultraviolet radiation, pathogens, etc.), thus preserving the quality of fresh fruit during storage and extending shelf-life by delaying fruit senescence (Connor et al. 2002, Ehsani-Moghaddam et al. 2006, Schijlen et al. 2006, Ehsani-Moghaddam et al. 2008). Polyphenols, which are important antioxidants with high reactive activity, exhibit positive effects on human health in terms of anticarcinogenic and antiatherogenic activities (Middleton Jr and Kandaswami 1992, Nakayama 1994, Decker 1995).

Table 1. Agronomic characteristics and antioxidant capacities of advanced strawberry lines compared to a commercial cultivar (‘Wendy’)

Genotype	Yield	Average weight	SSC ¹	TA ²	pH	Firmness	Weight loss	TPC ³	FRAP ⁴	ORAC ⁵
	(g m ⁻²)	(g)	(°Brix)	(%)		(N)	(%)			
SJ011-10	1350.0 ^{cd}	9.44 ^a	7.70 ^d	0.84 ^{ef}	3.37 ^{de}	12.00 ^{cd}	44.8 ^a	176.5 ^h	23.8 ^f	32.2 ^{efg}
SJ04402	1518.4 ^{cd}	7.38 ^{bc}	6.63 ^g	0.66 ^h	3.45 ^{cd}	9.01 ^f	38.9 ^b	192.5 ^{gh}	26.9 ^{ef}	28.7 ^{gh}
SJ0618	1298.4 ^{cd}	6.23 ^{cd}	7.93 ^d	0.98 ^{bc}	3.42 ^d	11.19 ^{cde}	35.5 ^{bcde}	420.6 ^a	46.3 ^a	64.8 ^a
SJ0663	2100.0 ^{abc}	9.01 ^{ab}	7.27 ^f	1.18 ^a	3.18 ^g	12.29 ^c	33.4 ^e	215.5 ^{fg}	27.7 ^{de}	32.9 ^{efg}
SJ0693	2893.4 ^a	9.29 ^{ab}	9.33 ^a	0.95 ^{cd}	3.53 ^{bc}	15.53 ^a	33.8 ^{de}	353.7 ^b	41.4 ^b	52.0 ^b
SJ06912	2589.2 ^{ab}	9.55 ^a	7.63 ^{de}	0.98 ^{bc}	3.40 ^{de}	12.34 ^c	38.0 ^{bcd}	242.4 ^{ef}	33.0 ^c	26.4 ^h
SJ081437	1728.4 ^{bcd}	7.60 ^{abc}	8.70 ^b	0.75 ^g	3.68 ^a	13.98 ^b	43.9 ^a	308.6 ^c	39.6 ^b	42.8 ^c
SJ851811	1916.7 ^{bc}	6.91 ^{cd}	6.37 ^g	0.77 ^{fg}	3.41 ^d	10.24 ^{ef}	35.8 ^{bcde}	262.3 ^{de}	32.4 ^c	44.4 ^c
K0412	1263.3 ^{cd}	8.20 ^{abc}	8.85 ^b	1.15 ^a	3.32 ^{ef}	10.68 ^{de}	34.5 ^{cde}	234.4 ^{ef}	31.7 ^c	35.3 ^{def}
LL022010	902.5 ^d	6.22 ^{cd}	7.10 ^f	0.88 ^{de}	3.44 ^d	9.85 ^{ef}	38.2 ^{bc}	224.8 ^f	31.2 ^{cd}	37.0 ^{de}
V151	824.2 ^d	4.95 ^d	7.30 ^{ef}	1.04 ^b	3.29 ^f	9.78 ^{ef}	44.1 ^a	277.1 ^d	33.1 ^c	40.0 ^{cd}
Wendy	1615.0 ^{cd}	7.77 ^{abc}	8.30 ^c	0.82 ^{efg}	3.55 ^b	12.41 ^c	39.0 ^b	183.3 ^h	26.5 ^{ef}	31.2 ^{fgh}
LSD _{0.05}	924.4	2.00	0.34	0.09	0.08	1.49	4.3	29.3	3.6	4.9

Data are averages from three replicates. Values in the same column followed by different superscripts are significantly different ($p < 0.05$).

¹Soluble solids content expressed as °Brix (% FW).

²Titrateable acidity expressed as percent citric acid equivalent (% citric acid equiv.).

³Total phenolic content expressed as milligrams of gallic acid equivalent per 100 g fresh weight (mg GAE 100 g⁻¹ FW).

⁴Ferric reducing antioxidant power expressed as micromoles of Fe₂SO₄·7H₂O equivalent per gram fresh weight ($\mu\text{mol Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O g}^{-1}$ FW).

⁵Oxygen radical absorbance capacity expressed as micromoles of Trolox equivalent per gram fresh weight ($\mu\text{mol TE g}^{-1}$ FW).

Yield and fruit quality

Way et al. (1983) observed that even though several factors affect crop yields, genetic differences are still the main one that strongly influences them. High variability among genotypes was found for fruit yields in the present study (Table 1). Selection ‘SJ0693’ was the most productive (2893.4 g m⁻²), followed by ‘SJ06912’, ‘SJ0663’, ‘SJ851811’, and ‘SJ081437’ (2589.2, 2100.0, 1916.7, and 1728.4 g m⁻², respectively), whereas ‘V151’ and ‘LL022010’ were the least productive (824.2 and 902.5 g m⁻², respectively). Selections ‘SJ0693’, ‘SJ06912’, ‘SJ0663’, ‘SJ851811’, and ‘SJ081437’ were harvested the earliest, at beginning of June, whereas the others were harvested in mid-June, suggesting that higher profitability can be provided to producers by means of early-maturing genotypes. The selection ‘V151’ had relatively lower productivity, because some plants died during the winter of 2011, a phenomenon that

indicates that this selection is not very resistant to cold weather. The largest fruit was found in 'SJ06912' (9.55 g), whereas 'V151' had the smallest fruit (4.95 g). There was a positive correlation between yield and average fruit weight, similar to the finding by Zatylny et al. (1996) in raspberry. Generally smaller fruits have higher TAC or TPC content due a higher ratio of peel to total fresh weight (Connor et al. 2005). However, in the present study we could not clearly establish such a relation. Firmness has a profound impact on the sensory quality of strawberries (Gunness et al. 2009) and is also closely associated with shipping and shelf-life. The firmest fruits were detected in 'SJ0693' (15.53 N), whereas 'SJ04402' had the softest fruits (9.01 N). The highest weight loss was found in 'SJ01110', 'V151', and 'SJ081437' (44.8%, 44.1%, and 43.9%, respectively), whereas 'SJ0663' and 'SJ0693' had the least weight loss (33.4% and 33.8%, respectively).

Sensory fruit quality derives from the interaction of different tastes and aromas from many chemical compounds. In general, the main criteria for strawberry selection are high sweetness and high acidity. Citric acid is the primary component of organic acids, and combined with sugars, it contributes most to fruit flavour. High pH impacts the perception of sweetness (Gunness et al. 2009). The highest pH value was found in 'SJ081437' (3.68), whereas the lowest was found in 'SJ0663' (3.18). The SSC of the fruits differed strongly among the genotypes. Selection 'SJ0693' had the highest concentration, in contrast to 'SJ851811' and 'SJ04402', which had the lowest concentrations. The range of SSC varied from 6.37% to 9.33%, and a similar range was found in the study by Capocasa et al. (2008). Fruits of 'SJ0663' and 'K0412' had the highest TA content, at 1.18% and 1.15%, respectively, whereas 'SJ0440-2' had the lowest content, at 0.66%. The other values fell within a narrow range, from 0.75% in 'SJ081437' to 1.04% in 'V151'. Fruits with high sugar and low acid levels, low sugar and high acid levels, or low sugar and low acid levels have an unpleasant taste (Kader 1991). Selection 'SJ0693' had the highest SSC and a moderate TA content, indicating that it has good sensory quality for the fresh market, whereas 'K0412' had high SSC and high TA levels, making it the most suitable for the processing industry (Kader 1991).

Phenolic composition (HPLC)

Phenolic compounds are consumed mainly as flavonoids and phenolic acids, which account for 60% and 30% of such compounds, respectively (Scalbert and Williamson 2000), and are not only linked to overall sensory-organoleptic attributes, such as colour, astringency, bitterness, and taste, in many fruits, vegetables, red wines, and juices (Tomás-Barberán and Espín 2001, Lesschaeve and Noble 2005, Haslam 2007) but also have a positive effect on human health because of the nutritional value of phenolics as antioxidants (Quideau et al. 2011). For the fruits in the present study, the composition of phenolic compounds was assayed, and they were quantified as EA derivatives, flavonols, and anthocyanins by HPLC. The significant variations in every phenolic compound detected in this investigation were similar to prior findings regarding strawberry (Rekika et al. 2005).

Anthocyanins, a group of flavonoids, comprise the most pivotal group of water-soluble plant pigments and play a decisive role in the formation of red strawberry colour. Three dominant anthocyanin compounds, namely P3G, P3R, and C3G, were identified and quantified. Their levels differed strongly among the genotypes tested (Table 2). The highest P3G and P3R levels were found in 'SJ0618' (575.9 and 36.3 $\mu\text{g g}^{-1}$, respectively), followed by 'SJ081437' (433.0 and 25.1 $\mu\text{g g}^{-1}$, respectively). Selection 'SJ081437' (45.8 $\mu\text{g g}^{-1}$) had the highest C3G level, followed by 'LL022010' (41.4 $\mu\text{g g}^{-1}$) and 'SJ0663' (41.0 $\mu\text{g g}^{-1}$). In contrast, 'SJ01110' had the lowest P3G, P3R, and C3G levels (177.1, 0.5, and 11.3 $\mu\text{g g}^{-1}$, respectively). It was therefore concluded that P3G was the primary anthocyanin. The P3R and C3G contents were lower, similar to those in the studies done by Goulas and Mangaranis (2011), Kajdžanoska et al. (2011), Fernandes et al. (2012), and Tarola et al. (2013). The total anthocyanins content was also systematically different between the genotypes investigated, with levels in the range of 188.8 to 659.8 $\mu\text{g g}^{-1}$, similar to the results obtained by Padula et al. (2012). The genotype with the highest anthocyanin content was 'SJ0618', followed by 'SJ081437', whereas 'SJ01110' contained the fewest anthocyanins. There was a positive correlation between anthocyanins and TAC using ORAC ($r = 0.59366$, $p < 0.0014$) and using FRAP ($r = 0.47926$, $p < 0.0114$), in agreement with several researchers who reported that crops with higher amounts of anthocyanins had higher antioxidant power based on ORAC (Zheng et al. 2007, Wang and Millner 2009), and in contrast with a previous study reporting no relationship between anthocyanins and FRAP (Khanizadeh et al. 2008). The antioxidant activity of anthocyanins is probably one of their most remarkable biological attributes and is linked to a chain of health benefits (Wang et al. 1997, Murkovic et al. 2000, Kong et al. 2003). Many studies demonstrated that anthocyanins are powerful antioxidants due to their phenolic hydroxyl groups attached to their ring structures, providing protective effects against free radical damage and inhibiting the oxidation of low-density lipoprotein (Yoshiki et al. 1995, Rice-Evans et al. 1996, Wang et al. 1997, Heinonen et al. 1998).

Table 2. Phytochemical composition ($\mu\text{g g}^{-1}$ fresh weight) of advanced strawberry lines compared to a commercial cultivar ('Wendy')

Genotype	EA	K3Gr	Q3Gr + Q3G	Total flavonols	P3G	P3R	C3G	Total anthocyanins
	(280 nm)	(365 nm)	(365 nm)		(503 nm)	(503 nm)	(519 nm)	
SJ01110	14.0 ^{cd}	4.0 ^e	19.8 ^f	23.8 ^e	177.1 ^f	0.5 ^e	11.3 ^e	188.8 ^h
SJ04402	20.1 ^{ab}	12.0 ^{ab}	72.4 ^a	85.4 ^a	304.9 ^e	13.3 ^d	32.3 ^{cd}	350.6 ^e
SJ0618	20.2 ^{ab}	13.2 ^a	73.6 ^a	86.8 ^a	575.9 ^a	36.3 ^a	36.7 ^{bc}	659.8 ^a
SJ0663	8.1 ^e	5.2 ^{fg}	23.8 ^{ef}	29.0 ^{fg}	289.1 ^e	20.0 ^c	41.0 ^{ab}	328.7 ^{ef}
SJ0693	12.9 ^{cde}	9.0 ^{cd}	41.7 ^{cd}	50.7 ^{de}	276.3 ^e	13.4 ^d	23.3 ^{ef}	312.9 ^f
SJ06912	9.8 ^{de}	6.3 ^{ef}	27.0 ^{ef}	33.3 ^{fg}	209.5 ^f	9.2 ^d	13.5 ^e	238.1 ^e
SJ081437	21.7 ^a	10.2 ^{bc}	72.0 ^a	82.4 ^{ab}	433.0 ^b	25.1 ^b	45.8 ^a	503.9 ^b
SJ851811	14.2 ^{cd}	7.7 ^{de}	34.3 ^{de}	42.0 ^{ef}	384.8 ^{cd}	18.6 ^c	28.0 ^{de}	431.3 ^{cd}
K0412	9.5 ^{de}	6.7 ^{ef}	45.5 ^{cd}	52.2 ^{de}	300.8 ^e	20.2 ^c	15.4 ^e	336.3 ^{ef}
LL022010	22.1 ^a	7.7 ^{de}	50.5 ^{bc}	58.2 ^{cd}	407.7 ^{bc}	22.0 ^{bc}	41.4 ^{ab}	456.3 ^c
V151	15.3 ^{bc}	9.0 ^{cd}	41.7 ^{cd}	50.4 ^{de}	356.3 ^d	21.1 ^{bc}	21.6 ^f	399.1 ^d
Wendy	9.7 ^{de}	8.0 ^{de}	62.3 ^{ab}	70.3 ^{bc}	358.8 ^d	12.0 ^d	25.0 ^{ef}	395.8 ^d
Mean	14.8	8.2	47.0	55.4	339.5	17.7	27.9	383.5
Percent (%)		14.9	84.9	100.0	88.5	4.6	7.3	100.0
LSD _{0.05}	5.4	1.8	12.7	14.0	38.6	4.3	6.1	37.5

Data are averages from three replicates. Phenolic compounds were identified as follows: ellagic acid as ellagic acid (EA); flavonols as the total of kaempferol3-glucuronide (K3Gr), quercetin3- glucuronide (Q3Gr), and quercetin3-glucoside (Q3G); and anthocyanins as the total of pelargonidin3-glucoside (P3G), pelargonidin-3-rutinoside (P3R), and cyanidin3-glucoside (C3G). Values in the same column that are followed by different superscripts are significantly different ($p < 0.05$).

Ellagic acid, a naturally occurring phenolic compound, is found in a number of plant species (Daniel et al. 1989). The literature shows that EA has high free-radical scavenging activity (similar to that of flavan3-ols and gallic acid) (Zafrilla et al. 2001) and offers a potential protective reaction against chemicals that trigger cancers (Okuda et al. 1989, Maas and Galletta 1991). Ellagitannins are the main form of EA (Määttä-Riihinen et al. 2004) and include in particular sanguin H6, the primary ellagitannin, which was found to be the dominant contributor (30%) to the antioxidant power of raspberries (Mullen et al. 2002). Sanguin H6 was also found in strawberry leaves (Haddock et al. 1982) and fruits (Määttä-Riihinen et al. 2004). Based on the data derived from the present study (Table 2), 'LL022010' and 'SJ081437' had the highest levels, because their fruits were rich in EA (22.1 and 21.7 $\mu\text{g g}^{-1}$, respectively), followed by 'SJ0618', 'SJ04402', and 'V151' (20.2, 20.1, and 15.3 $\mu\text{g g}^{-1}$, respectively), whereas the lowest content was observed in 'SJ0663' (8.1 $\mu\text{g g}^{-1}$). Ellagic acid contents were detected at various levels depending on species, cultivars, and plant organs (Maas et al. 1991, Wang et al. 1995). Rekika et al. (2005) reported a variation in EA between 33.96 and 14.31 $\mu\text{g g}^{-1}$ in strawberries, which was higher than in this study.

Flavonols include highly bioactive compounds, such as kaempferol and quercetin derivatives, which are potential scavengers of reactive oxygen species (Larson 1988). In particular, one of the main quercetin metabolites, Q3Gr, protects human plasma against oxidative modification (Kawai et al. 2008). In the present study, the major flavonols were found to be K3Gr as well as Q3Gr and Q3G (Table 2). Selection 'SJ0618' had the richest quercetin and kaempferol contents, whereas 'SJ01110' had the poorest. The quercetin contents were higher than the kaempferol contents, in agreement with the study by Wang and Millner (2009). The quercetin and kaempferol contents differed statistically among the different genotypes tested, varying from 73.6 to 19.8 $\mu\text{g g}^{-1}$ and 13.2 to 4.0 $\mu\text{g g}^{-1}$, respectively. In contrast, Häkkinen and Törrönen (2000) found fairly small variations in quercetin and kaempferol concentrations between six different Finnish strawberry cultivars. The present results may possibly be explained by the different genotype backgrounds coupled with the geographic location and the analytical methods employed. The total flavonol amounts were also significantly different between the genotypes tested, ranging from 23.8 to 86.8 $\mu\text{g g}^{-1}$. Hertog et al. (1992) reported that the flavonol level in food could be recommended to be considerable if it exceeds 50 mg kg^{-1} . On the basis of that threshold, 'SJ0618', 'SJ04402', 'SJ081437', 'Wendy', 'LL022010', 'K0412', 'SJ0693', and 'V151' had the richest flavonol contents, whereas the other genotypes had the poorest contents.

Conclusion

This study reported the impacts of strawberry genotypes on fruit quality attributes, total antioxidant power, and phytochemical profile. The advanced line 'SJ0693' was the most productive and had higher antioxidant capacity and improved fruit quality attributes, with the best SSC, intermediate TA, low weight loss, good firmness, and larger fruits. These findings clearly demonstrate the potential value of 'SJ0693' as a promising new cultivar for the fresh market. It should also be pointed out that selection 'SJ0618' had the highest antioxidant activity, using both ORAC and FRAP, and differed significantly from the other genotypes. In addition, 'SJ0618' was rich in every phenolic compound and can thus be considered valuable parent material for breeding programs, may offer enhanced nutritional benefits for consumer health, or could be used as a functional food ingredient in manufacturing processes.

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