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Effects of site and genotype on strawberry fruits quality traits and bioactive compounds

Carine Cocco[a], Sabina Magnani[b], Maria Luigia Maltoni[b], Irene Quacquarelli[b], Mattia Cacchi[c], Luis Eduardo Corrêa Antunes[d], Luigi Filippo D'Antuono[c], Walther Faedi[b] and Gianluca Baruzzi[b,*]

[a]*University of Pelotas, Department of Crop Science, Faculty of Agronomy Eliseu Maciel, Pelotas, RS, Brazil*
[b]*Council for Agricultural Research and Economics, Fruit Crop Research Unit of Forlì (CRA-FRF), Forlì, Italy*
[c]*University of Bologna, Department of Agri-Food Science and Technology, Cesena, Italy*
[d]*Embrapa Clima Temperato, Pelotas, RS, Brazil*

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Abstract. Site effects on the main active and putative health-promoting compounds of strawberry were investigated using 5 strawberry genotypes. The plants were grown at three locations in Italy (Verona and Cesena in the North and Scanzano Jonico in the South) of differing latitude, environmental conditions (temperature) and crop management practices (planting date, plant type, harvest duration, yield per plant and per day) influencing fruits quality traits. At each site, fruits for analysis were picked at mid harvest (50% of total estimated yield) during the peak April-May marketing season, a mid-harvest window when consumers can choose fruits from both northern and southern districts. Yield per plant and fruits total soluble solids, titratable acidity, flesh firmness, skin colour, antioxidant activity, ascorbic acid, total phenols, total anthocyanins and phenolic compounds were determined. Genotype × site × climatic factors and cultivation technique interaction significantly affected yield per plant and almost all fruits quality traits. Given the longer harvest period and, hence, lower yield per day in the South, the fruits of this site were sweeter and of higher ascorbic acid and anthocyanin contents than that grown at the two North sites; the Verona fruits registered the highest acidity and antioxidant capacity. Fruits size and colour were unaffected by site. Soluble solids and ascorbic acid were negatively correlated to plant yield per day. A significant negative correlation between total antioxidant capacity, total polyphenols and fruits size was found. The main anthocyanin (pelargonidin-3-glucoside) was correlated to both total anthocyanin and total antioxidant capacity. Our overall data show that site-specific environmental conditions, especially in regard to the length of the climate-induced harvest window, and crop management practices affected fruits quality traits.

Keywords: Antioxidant capacity, genotype × environment × cultivation interaction, *F.* × *ananassa*, sensory traits

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) fruits have a high content of antioxidant compounds, especially polyphenols and vitamins, which can potentially contribute to nutrition [12]. The key factors affecting antioxidant capacity and the content of bioactive compounds include genotype, crop management techniques and environmental conditions [3, 6, 8, 16]. Studies indicate that organically grown strawberry have a higher total antioxidant capacity than conventionally cultivated ones [15, 23]. It has also been posited that environmental stresses like high temperatures can induce high levels of secondary metabolites. In fact, recent trials of strawberry grown in northern European districts show that low temperatures reduce antioxidant potential [17]. Low temperatures during fruits development

*Corresponding author: Gianluca Baruzzi, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di Ricerca per la Frutticoltura - Forlì (CRA-FRF), Via La Canapona, 1 bis – Magliano, 47100 FORLI' (FC), Italy. Tel.: +39 0543 89566; Fax: +39 0543 89077; E-mail: gianluca.baruzzi@entecra.it.

also lower the content of such bioactive compounds as anthocyanins and ellagitannins while increasing that of ascorbic acid [30].

While strawberry is grown in Italy from North to South, the main districts are located in the regions of Basilicata and Campania in the latter and of Emilia-Romagna and Veneto in the former. These locations differ considerably in regard to daily mean temperature, soil profile, planting date and density, cultivar, plastic tunnel type, and field practices. Winter planting system is adopted in the South. Bare-root plants can be planted in early October and can crop continuously for as long as 4-5 months from January to May. By comparison, summer planting is the rule in the North, where picking dates are mostly concentrated in 3-4 weeks over April-May. In Verona large cold-stored plants, "A+" type with crown diameter of 14–16 mm, are planted in the latter half of August: the first pick begins 50 days later, and thereafter the plants overwinter and produce a second crop in spring. In Cesena cold-stored "A" type plants (crown diameter of 12 mm) are planted in late July for harvest the following May.

While the varying planting density in the three areas results in similar yields per hectare, yield per plant is higher in the North. Since plant stress is also greater in the North due to the short, concentrated cropping season, fruits quality traits can also differ considerably between the two areas. Then too different cultivars types are grown in these areas: those of low chilling requirement in the South and of high chill in the North, with adaptability to 'fall culture' in Verona. Since the peak marketing season is April-May, consumers can choose fruits from either or both areas depending on their purported preferences regarding fruits origin and their reputed effects on quality and sensory attributes.

The aim of the present study was to compare the fruits traits of Verona and Cesena in the North and of Scanzano Jonico in the South in a trial that tested five genotypes at all three sites.

2. Material and methods

2.1. Trial sites and cultivation practices

The trials were run in 2011-2012 at Verona, 45°45'N/11°00'E, Cesena, 44°14'N/12°15'E, and Scanzano Jonico, 40°25'N/16°42'E, hereinafter designated as V, C and SJ. The districts are different to environmental conditions, soil profile, planting density and harvest date and length of picking season (Table 1). Air temperature (°C) was measured at all the sites from September 2011 to May 2012, and the average mean, minimum and maximum temperature (T) per month and site were calculated from the recorded daily minimums (T_{min}) and maximums (T_{max}) temperature.

The plants at all sites were grown under tunnel-culture practices in accordance with the standards of Integrated Production Management Guidelines of the Emilia-Romagna Regional Authority [14]. The SJ site planted bare-root fresh plants on 5 October 2011: the tunnel was covered three weeks later and picking ran from early March to late May. The C site planted standard cold-stored A-type plants, with rhizome diameter of 12 mm, on 25 July 2011: the tunnel was covered in mid-March before bloom and picking ran from early May to early June. The V site planted large cold stored "*A* +" plants, with rhizome diameter of 16 mm, on 20 August 2011: the tunnel was covered in late September, the first crop picked from early October to mid-November, and the post-overwinter crop was picked from mid-April to mid-May. All sites featured double rows on raised beds covered with black polyethylene mulch and spaced 1.2–1.4 m (centre-to-centre); planting density ranged from 4.5 to 7.4 plants per m^2 (Table 1).

The trials were run using five June-bearing strawberry (*Fragaria* × *ananassa* Duch.) genotypes: 'Jonica', 'Nora' and 'Pircinque' and 2 advanced selections ('CE 51' and 'CE 56') from a CRA-FRF breeding programme based in southern Italy. The genotypes were chosen for their low chilling requirement and adaptability to all three sites. All the plants were produced in the same nursery; four replicated, fully randomized plots of 30 plants per genotype were trialled per site.

2.2. Yield and average berry weight (fruit size)

Fully red coloured fruits were harvested and total yield was recorded at each picking. Harvest frequency, i.e. the interval between one picking and the next, varied from 4 to 8 days depending on the seasonal weather pattern at each site. Total yield per plant and per hectare was calculated by summing the values of each picking. Marketable

Table 1
Daily temperature from September 2011 to May 2012, soil profile, planting date and density at the three trial sites

	Site 1: Scanzano J.	Site 2: Cesena	Site 3: Verona
Altitude	40 m	38 m	42 m
Latitude	40°25'N	44°14'N	45°45'N
Average daily temperature (Sept to May)	13.7	10.4	11.4
Min (°C)	8.8	5.4	6.2
Max (°C)	18.7	15.9	16.5
Average daily temperature (30 days before sampling)	13.6	12.2	13.1
Min (°C)	8.9	6.2	7.2
Max (°C)	19.7	18.3	18.7
Soil pH	8	7.8	7.8
Soil texture	18% Sand	24% Sand	65% Sand
	42% Silt	37% Silt	19% Silt
	40% Clay	39% Clay	16% Clay
Soil organic matter (%)	2.1	1.8	1.8
Planting time	October 4th	July 25th	August 24th
Planting density (plants/m^2)	7.4	4.5	6.5
Harvest start (cultivar average)	March 1th	May 3rd	April 18th
Harvest duration, days (cultivar average)	93	22	29

yield was defined as intact fruits having a diameter of >22 mm; malformed, rotten and fruits of smaller diameter were recorded as discard. Average berry weight was calculated by $(\sum[fp])P^{-1}$, where f is average berry weight per harvest, p the marketable yield per plant per harvest, and P the sum of marketable yield of all pickings ($\sum p$). The length of the harvest window (harvest duration) is given as the sum of days from the start to end of picking. Yield per day was calculated by dividing marketable yield by the number of the days of the harvest duration.

2.3. Fruit sampling

Fruits samples for testing were picked at mid-harvest, i.e. 50% of the total estimated crop, on approximately 20 April at SJ, 3 May at V and 15 May at C. Secondary or tertiary fruits were selected for uniform size, fully red colour and lack of damage. Twenty ripe fruits of the first sample batch were used within 10 hours of picking for determining flesh firmness, total soluble solids, skin colour, titratable acidity and ascorbic acid. A second batch was collected, freeze dried and ground with pestle and mortar to a fine homogeneous powder for determining total antioxidant capacity, total polyphenols, total anthocyanins; phenolic profile detection was performed by HPLC-DAD after Andreotti et al. [2].

2.4. Flesh firmness and skin colour

Flesh firmness was measured on 20 fruits using an Ametek digital penetrometer with a 6 mm diameter probe (star-shaped plug). External fruits colour was measured at two opposite points on the equatorial region of each undamaged fruit using a Minolta Chromameter reflect II with an 8 mm window providing the three colour coordinates: L^*, a^* and b^*. Colour data are reported as L^* (brightness), Hue angle degree indicating colour shade (Hue = arctan [b^* a^{*-1}] where 0° = red-purple; 90° = yellow) and chroma [$C^* = (a^{*2+}b^{*2})^{1/2}$] indicative of colour intensity or saturation. The instruments were calibrated with standard white ($Y = 93.96$; $x = 0.3138$; $y = 0.3214$).

2.5. Total soluble solids and titratable acidity

Twenty fruits were chopped and homogenized prior to assay. A small fraction of filtration juice was measured with an Atago digital refractometer PR-32 Alpha (LaboandCo, Torino, Italy) to determine total soluble solids; data are reported in °Brix.

Titratable acidity was determined using a 702 SM Titrino automatic titrator Metrhom Swiss. A 5 g sample of juice was diluted with 25 ml distilled water and titrated with 0.1 N sodium hydroxide solution (NaOH) to pH 7.0. The data are reported as mEq of NaOH per 100 g fresh weight (FW).

2.6. Ascorbic acid

Ascorbic acid content was determined by the Merckquant® Ascorbic Acid Test (Reflectoquant®, Merck) using test strips dipped in strawberry juice after Camin et al. [4]. Results are expressed in mg per 100 g FW.

2.7. Total phenolic content, total anthocyanin content and total antioxidant capacity

Extracts were prepared after Diamanti et al. [9], slightly modified: 250 mg of freeze-dried fine homogeneous powder were enriched with 10 mL methanol, stirred, performed for 5 min in ultrasonic bath at 4°C and centrifuged for 30 min at 4,500 rpm. The supernatant was collected in vial and stored at –20°C for subsequent analysis.

Total phenolic content (TPH) was determined via the Folin-Ciocalteu method as gallic acid equivalents (GAE) after Slinkard and Singleton [31]. Absorbance was measured after two hours using a uv-1601-spectophotometer at 750 nm; the results are expressed as mg GAE per 100 g FW.

Total Anthocyanin concentration (TACY) was determined using a pH differential method [13]. The assay was based on the peculiar anthocyanin colour change that depends on a pH shift [22]. The samples were diluted (1:20) with KCl 0.025 mol/L (pH 1.0) and sodium acetate 0.4 mol/L (pH 4.5); the absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5, respectively, using $A = [(A_{510}–A_{700})_{pH1.0} – (A_{510}–A_{700})_{pH4.5}]$ with a molar extinction coefficient of pelargonidin-3-glucoside of 15600. The results are reported as mg of pelargonidin-3-glucoside equivalent (Pg-3-gluc) per 100 g FW.

Total antioxidant capacity (TAC) was determined by ABTS assay after Re et al. [29]. The radical cation solution was generated overnight with potassium persulfate. The sample reading was carried out after 3 min using a uv-1601-spectophotometer at 734 nm; the results are expressed as mmol Trolox equivalent (TE) per 100 g FW.

2.8. Phenolic content by HPLC (high performance liquid chromatography)

The phenolic profile was determined after Andreotti et al. [2]. Samples of phenolic compounds from 0.1 g of freeze-dried fruits were extracted by 1 ml methanol, stirred for 30 sec, performed for 30 min in ultrasonic bath at 4°C and centrifugated for 30 min at 12,500 rpm. The supernatant was filtrated with millipore 0.2 μm filters, collected in vial and stored at –20°C for analysis using an Agilent 1100 HPLC system with a photodiode array detector (DAD) and ZORBAX SB-C18 column-Waters Rapid Resolution (3.0 × 50 mm, 1.8 μm particle size), kept at 35°C in a solution of 0.1 M H_3PO_4 (solvent A) and methanol (solvent B) as mobile phase. Solvent gradient was 5% B in A at the beginning of analysis; 50% B in A at 8 min; 100% B at 13 min; and 100% B (isocratic) at 15 min, followed by column preparation for the next analysis, for a total analysis time of 20 min. The flow rate was 0.5 ml/min and chromatograms were recorded at 280, 320, 350 and 510 nm for simultaneous monitoring. Total phenolic compounds were divided into five groups and quantified using external standards: anthocyanins as pelargonidin-3-glucoside (Pg-3-gluc) and cyanidin-3-glucoside (Cya-3-gluc) at 510 nm, flavonols as quercetin-3-gluoside (350 nm), ellagitannins as ellagic acid (280 nm), hydroxycinnamic acids as chlorogenic acid (320 nm) and flavan-3-ols as catechin (280 nm). The phenolic compound count is reported as μg per g of FW.

2.9. Statistical analysis

The data were processed by two-way analysis of variance (ANOVA), including the effects of site and genotype and their interaction. Significant differences among means were evaluated by the LSD multiple range test. The coefficient of variation (CV, %) was calculated for each trait. Connections between traits were analyzed by Pearson correlation coefficient (r; $p < 0.05$). All analyses were performed using STATGRAPHICS Centurion (Statpoint Inc. USA).

3. Results and discussion

3.1. Plant yield and fruit size

Site and genotype significantly affected yield per plant. In general C plants showed the highest and SJ the lowest. SJ yield per hectare was similar to that of V because of the former's higher planting density (Table 2). The shortest harvest period was registered on average at C (22 days). Tunnel protection at V for the fall picking induced an extended period of differentiation, both in fall and in spring, that determined a 29-day harvest window in the latter period. As expected, SJ's 93-day picking window was the longest.

On average yield per day depended on genotype and notably differed across sites, ranging from 54.2 to 41.9 g per day in C, 24.3 to 11.3 in V and 6.3 to 3.8 in SJ. Genotypes posted differing results at the three sites as to both gram per plant and ton per hectare: at SJ 'Pircinque' was the most productive, and no significant differences were detected among the other genotypes; at C 'Nora' proved the most productive, and at V 'Jonica' outcropped all the others.

In general average berry weight showed no significant site-related differences. While other studies [17, 21] report larger fruits size in areas characterized by lower temperatures, this was not found in our trial for the not enhanced

Table 2
Strawberry marketable yield per plant and per hectare, harvest duration, plant yield per day and average fruit weight of different genotypes and production sites in 2012

Genotypes (G)	Production Site (S)	Yield (g/plant)	Yield (t/ha)	Harvest duration (days)	Plant yield per day (g/day)	Average fruit weight, (g)
'CE 51'	Scanzano J.	370 g	27.4 g	92 b	4.0 e	29.3 a
	Cesena	1089 b	49.0 ab	21 ef	51.9 a	23.4 c
	Verona	443 ef	28.8 fg	28 d	15.8 d	21.0 d
'CE 56'	Scanzano J.	350 g	25.9 g	91 b	3.8 e	23.8c
	Cesena	837 cd	37.7 e	20 f	41.9 b	24.3 c
	Verona	317 gh	20.6 h	28 d	11.3 d	24.0 c
'Nora'	Scanzano J.	389 g	28.8 fg	93 ab	4.2 e	22.2 c
	Cesena	1139 a	51.3 a	21 ef	54.2 a	18.7 d
	Verona	493 ef	32.0 f	31 c	15.9 d	20.5 d
'Pircinque'	Scanzano J.	594 e	43.9 cd	94 a	6.3 e	25.9 b
	Cesena	971 c	43.7 c	22 e	44.1 b	26.8 b
	Verona	384 g	24.9 gh	30 c	12.8 d	30.3 a
'Jonica'	Scanzano J.	409 efg	30.3 f	95 a	4.3 e	24.4 bc
	Cesena	1045 bc	47.0 c	23 e	45.4 b	22.0 cd
	Verona	704 d	45.8 c	29 cd	24.3 c	23.3 c
	G	***	***	***	***	**
	S	**	***	***	***	ns
	G × S	*	***	***	***	***

Different letters in the same column indicate statistically significant differences via LSD test $P < 0.05$. Significant parameters are indicated as follows: ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

temperature differences between sites. While Krüger et al. [20] reported berry-size variation for genotypes in different areas, this did not hold true for our tested genotypes. The biggest berry size was found in 'CE 51' at SJ and 'Pircinque' at V and the lowest in 'Nora' at all sites. The coefficient of variation (CV) of 'CE 51' was high at 17%, and 'CE 56' showed a very stable fruit size at 1% CV across sites.

3.2. Standard quality traits

On average the fruits at C were significantly firmer than those at the other two sites, and those at V the least firm: 625 g and 441 g, respectively (Table 3). The sandy soil of V (Table 1) required a greater supply irrigation which negatively influenced the fruits flesh firmness. The firmest fruits by site and by genotype were 'CE 56' at SJ, 'Jonica' at C and 'Pircinque' at V. 'CE 56' and 'Jonica' fruits showed the highest CV among sites (33%) for this trait; 'Nora' fruits showed the lowest firmness values at SJ and C with a stable 6% CV over sites.

In general the highest value of total soluble solids content (TSS) was recorded in SJ fruits and the lowest in C's. The higher pre-harvest temperature influenced TSS. SJ showed the highest temperature followed by V and C. Plant production per day proved to be negatively correlated to this trait ($r = -0.86^{*}$): SJ registered the lowest daily yield due to having the longest harvest period by site and the highest fruit sugar content, whereas C posted the highest yield per plant but lower TSS over its notably shorter harvest window. The greater plant stress at C and V due to their higher yield per day in comparison to SJ resulted in lower TSS. Several genotypes at C produced more than 300 g per plant at the peak harvest period (data not reported). All the genotypes showed varying TSS by site. The highest values at SJ were found for the two selections ('CE 51' and 'CE 56') and 'Nora', at C for 'Pircinque' and at V for 'Jonica'. Note too that 'Pircinque' had the highest average TSS value at 6.8° Brix and the lowest CV (11%). The lowest TSS values at SJ registered for Jonica' and 'Pircinque', at C for the two selections and 'Nora' and at V for 'Nora'.

Table 3
Flesh firmness, total soluble solids, titratable acidity and colour traits (brightness, chroma and Hue) of different genotypes and production sites in 2012

Genotypes (G)	Site (S)	Flesh firmness (g)	Total soluble solids (°Brix)	Titratable acidity TA (mEq/100 g FW)	Brightness (L^{*})	chroma C^{*}	Hue
'CE 51'	Scanzano J.	527 cd	8.3 a	10.1 a	41.6 bc	51.4 a	33.8 b
	Cesena	490 cde	5.0 i	7.2 f	40.2 cd	49.6 bc	31.5 b
	Verona	470 def	6.7 ef	10.4 a	40.7 cd	51.9 a	33.0 b
'CE 56'	Scanzano J.	563 bc	8.0 ab	9.6 abc	43.4 a	50.2 ab	33.7 b
	Cesena	736 a	5.0 i	8.7 cd	39.9 d	50.4 ab	33.8 b
	Verona	367 g	6.8 e	9.7 ab	42.7 ab	51.5 a	35.4 a
'Nora'	Scanzano J.	483 de	8.1 ab	9.0 bcd	36.9 f	46.0 d	27.0 e
	Cesena	491 cde	5.5 h	8.5 de	40.6 cd	49.0 bc	31.6 c
	Verona	402 fg	6.2 fg	9.9 a	37.9 ef	47.2 cd	30.7 c
'Pircinque'	Scanzano J.	492 cde	7.4 cd	6.1 g	36.7 f	41.9 e	28.3de
	Cesena	634 b	5.9 gh	7.2 f	37.1 ef	43.0 e	29.5 d
	Verona	516 cde	7.0 de	8.5 de	34.8 g	38.9 f	26.5 ef
'Jonica'	Scanzano J.	524 cde	7.3 cd	7.6 ef	36.8 f	47.9 cd	31.5 c
	Cesena	777 a	5.0 i	6.9 fg	37.3 ef	47.6 cd	31.1 c
	Verona	451 df	7.6 bc	7.7 ef	38.4 e	48.1 bd	35.5 a
	G	***	ns	***	***	***	***
	S	***	***	***	ns	ns	ns
	G × S	***	***	***	***	***	***

Different letters in the same column indicate statistically significant differences via LSD test $P < 0.05$. Significant parameters are indicated as follows: ns, not significant; *** $P < 0.001$.

In general the highest titratable acidity values were found in V and the lowest in C fruits. In other trials conducted by authors in V and C, the V fruits showed higher titratable acidity in comparison to C, due to higher fertilizer and irrigation supply. 'CE 51', 'CE 56' and 'Nora' fruits showed the highest acidity at all sites and 'CE 56' registered the most stable CV (5%). The lowest value at SJ were found for 'Pircinque' and at C and V for 'Jonica'.

While fruits brightness, chroma and Hue were on average unaffected by location, a significant genotype × site interaction was detected for fruits colour differences. The highest L* value at SJ was recorded for 'CE 56' fruits, at C for 'CE 51', 'CE 56' and 'Nora' and at V for 'CE 56' again; the lowest was found for 'Pircinque' at all three sites. The highest chroma values were posted by 'CE 51' and 'CE 56' at all three sites; the lowest were again found for 'Pircinque' at all three sites. The highest Hue values at SJ were found for the two selections, at C for the two selections and 'Nora' and at V for 'CE 56' and 'Jonica'; the lowest values at all three sites were found for 'Pircinque' and in SJ also 'Nora'. Fruits colour traits of all genotypes were more stable by site in comparison to other parameters. The lowest CV was recorded for 'CE 51' at 2%, 3% and 3% respectively for L*, chroma and Hue, and the highest for 'Nora' (5%, 4% and 8%).

3.3. Antioxidant properties

Ascorbic acid (AA) is one of the main bioactive compounds of strawberry fruits and a main determinant of their antioxidant capacity [8, 32]. Our AA content ranged from 33 to 48 mg per 100 g FW (Table 4). In general the fruits at C showed a significantly lower value than at the other two sites. While this finding deviates in part from that of Josuttis et al. [16], who detected higher AA content in fruit from cooler regions, it is in line with Crespo et al. [8], who report a negative correlation between daily production (g per day) and AA content (average of varieties: $r = -0.51^{*}$). Indeed, the high yield per plant found at C matched the lowest AA content. 'Nora' and CE51 at SJ,

Table 4
Ascorbic acid (Vitamin C), total phenolics content (TPH), total anthocyanins (TACY) and total antioxidant capacity (TAC) of different genotypes and production sites in 2012

Genotypes (G)	Site (S)	Ascorbic acid (mg/100 g FW)	Total phenolics content (mg GAE/100 g FW)	Total anthocyanins (mg Pg/100 g FW)	Total antioxidant capacity (mmolTE/100 g FW)
'CE 51'	Scanzano J.	44.50 ab	105.11 g	21.95 e	1.30 g
	Cesena	33.50 de	139.79 de	19.95 ef	1.30 g
	Verona	40.75 bc	143.30 d	27.13 d	1.76 b
'CE 56'	Scanzano J.	42.25 b	91.17 h	11.08 h	1.19 h
	Cesena	42.50 b	145.72 cd	14.17 g	1.47 d
	Verona	40.75 bc	143.83 d	15.93 fg	1.50 d
'Nora'	Scanzano J.	48.25 a	118.49 f	26.42 d	1.65 c
	Cesena	37.00 cd	154.42 bc	21.52 e	1.33 fg
	Verona	43.00 b	165.57 a	29.82 c	1.85 a
'Pircinque'	Scanzano J.	42.25 b	87.11 h	43.29 a	1.50 d
	Cesena	37.75 c	148.10 cd	26.21 d	1.38 ef
	Verona	43.50 b	103.56 g	33.99 b	1.30 g
'Jonica'	Scanzano J.	33.00 e	117.15 f	26.81 d	1.28 g
	Cesena	33.50 de	131.15 e	24.96 d	1.43 de
	Verona	43.50 b	162.79 ab	17.68 f	1.76 b
	G	***	***	***	***
	S	***	***	***	***
	G × S	***	***	***	***

Different letters in the same column indicate statistically significant difference via LSD test $P < 0.05$. Significant parameters are indicated as follows: $^{***}P < 0.001$.

'CE 56' at C and 'Pircinque' and 'Jonica' at V showed high AA content. 'CE 56' recorded a very stable content over sites (2% CV), and 'Jonica' showed varying results, with a CV of 16% and high AA content at V but low AA at C and SJ.

Strawberry fruits contain considerable amounts of phenolic compounds of putative health-promoting effects. Total polyphenolic content (TPH) ranged from 87 to 163 mg GAE per 100 g FW, with the highest contents registered, on average, at V and C and the lowest at SJ. These results appear to support those of Josuttis et al. [16], who detected lower TPH in southern cultivation areas, and to be linked to climatic factors, particularly temperature in the pre-harvest period (30 days before harvest). Our TPH values negatively correlated to daily mean temperature 30 days before sampling ($r = -0.71^{**}$). Even though it showed variation by site (16% CV), 'Nora' registered the highest TPH among genotypes at all sites and 'Pircinque' at SJ and V and 'CE 56' and 'CE 51' at SJ the lowest value. A significant negative correlation between TPH and fruit weight was found ($r = -0.55^{*}$), in agreement with Anttonen et al. [3]. Since phenolics are mainly concentrated in fruits skin [18], the big-sized fruits had a lower surface-to-pulp ratio and, hence, lower TPH than did the smaller-sized.

Total anthocyanins (TACY) ranged from 11 to 43 mg Pg-3-gluc per 100 g FW. Site differences were significant, in disagreement with Crespo et al. [8]. In general SJ fruits had the highest TACY, supporting the findings of Josuttis et al. [16] and Diamanti et al. [9], who report the highest count in areas with higher temperature before harvest. 'Pircinque' showed the highest and 'CE56' the lowest values across sites and genotypes. TACY was negatively correlated to colour traits L*, chroma and hue ($r = -0.69^{*}$, -0.67^{*} and -0.66^{*} respectively), indicating that high TACY corresponded to darker fruit of more saturated colour.

Total antioxidant capacity (TAC) is taken as the cumulative action and synergic interaction of all the antioxidant compounds and, hence, represents overall free radical scavenging capacity. TAC ranged from 1.19 to 1.85 mmol TE per 100 g FW. On average, the highest TAC values were registered at V, the northernmost site. These results are also in agreement with Josuttis et al. [16], who found the highest TAC in fruit of European northern areas characterized by lower temperature before harvest. 'Nora' showed the highest TAC at SJ and V and 'CE 56', 'CE 51' and 'Pircinque' the lowest value respectively in SJ, C and V; 'Pircinque' was the most stable (CV = 7%). It is well known that phenolic compounds are closely associated with TAC: we found a significant correlation between TAC and TPH ($r = 0.53^{**}$), in agreement with others [5, 25, 32]. As found for TPH, TAC too was negatively correlated to pre-harvest temperature ($r = -0.48^{*}$). A significant negative correlation between TAC and fruit size was also registered ($r = -0.53^{*}$), denoting a higher antioxidant capacity in smaller fruits.

3.4. HPLC analysis of phenolics

The data in Table 5 provide an overview of the phenolic composition and the contribution of each compound to antioxidant activity. The five classes identified are anthocyanins, hydroxycinnamic acids, ellagitannins, flavan-3-ols and flavonols, and their quantitative range of variation supports previous findings [1, 7, 8, 10, 11, 15, 16, 19, 24, 35].

Pelargonidin–3–glucoside (Pg-3-gluc) and cyanidin-3-glucoside (Cya-3-gluc) were the main anthocyanins. Pg-3-gluc was predominant, with more than 90 % of the total on average, and the widest range of values, from 87 to 298 μg per g FW, findings that highlight the wide variability of this anthocyanin. While Pg-3-gluc content registered the highest average value in fruits at V, the highest value overall was found in 'Pircinque' and 'Nora' and the lowest in 'CE 56' fruit across sites. 'Pircinque' also showed the most stable values among sites (13% CV). Pg-3-gluc content was more variable by cultivars than by site, indicating that strawberry anthocyanin content is affected more by genotype than by growing conditions, as previously reported [6, 16].

Predictably, Pg-3-gluc was correlated to both TACY ($r = 0.66^{*}$) and TAC ($r = 0.51^{*}$), in agreement with other findings [27, 32]. Crespo et al. [8] did not find a significant correlation between TAC and individual anthocyanins, a fact indicating that anthocyanins are not the only important TAC determinants. Moreover, a significant negative correlation between fruit colour parameters and Pg-3-gluc content was found ($r = -0.55^{*}$, -0.58^{*} and -0.60^{*} for L*, chroma and Hue, respectively), indicating that a high Pg-3-gluc count corresponded to darker and less bright colour. Similar results are reported by Crescente-Campo et al. [7] for organic fruits with reddish colour and higher pelargonidin content than for conventionally grown fruits. Pineli et al. [26] pointed out that 'Camino Real' fruits had a redder colour than 'Oso Grande"s because of the higher pelargonidin content.

Table 5
Concentrations of phenolic compounds (μg/g FW) determined by HPLC of different genotypes and production sites in 2012

Genotypes	Site (S)	Anthocyanins		Hydroxy-cinnamic acids	Ellagitannins	Flavan-3-ols	Flavonols
(G)		Pg-3-gluc	Cya-3-gluc				
'CE 51'	Scanzano J.	127.40 e	5.85 b	67.63 i	85.04 f	89.81 e	38.65 a
	Cesena	135.84 de	3.83 d	57.59 i	13.93 i	42.31 f	20.29 c
	Verona	179.31 c	5.06 bc	61.97 i	36.03 h	103.25 de	25.91 b
'CE 56'	Scanzano J.	87.45 f	1.36 f	286.77 c	258.34 b	183.09 b	12.04 de
	Cesena	86.84 f	n.f.	360.20 b	142.94 d	43.95 f	20.46 c
	Verona	118.81 e	n.f.	451.22 a	281.79 a	128.23 cd	3.72 g
'Nora'	Scanzano J.	240.19 b	13.46 a	262.26 de	158.73 d	89.17 e	26.27 b
	Cesena	134.09 de	4.84 c	241.05 e	47.06 gh	63.58 f	5.27 fg
	Verona	298.43 a	0.92 f	197.96 f	63.57 fg	152.22 c	9.63 ef
'Pircinque'	Scanzano J.	240.15 b	0.97 f	280.54 cd	187.64 c	288.76 a	7.70 efg
	Cesena	254.26 b	1.34 f	187.71 f	67.81 ef	40.96 f	9.15 ef
	Verona	198.58 c	0.75 fg	203.42 f	119.70 e	41.46 f	2.83 g
'Jonica'	Scanzano J.	135.41 de	2.89 e	129.24 h	74.15 ef	104.98 de	16.99 cd
	Cesena	116.71 e	n.f.	144.18 gh	31.92 h	50.46 f	10.18 ef
	Verona	155.01 d	1.38 f	154.38 g	57.75 fg	41.76 f	7.69 efg
	G	***	***	***	***	***	***
	S	***	***	***	***	***	***
	G × S	***	***	***	***	***	***

Different letters in the same column indicate statistically significant difference via LSD test $P < 0.05$. Significant parameters are indicated as follows: $^{***}P < 0.001$. n.f., not found.

Cya-3-gluc contents were also variable and affected by site and genotype. The highest value of 13.46 μg per g FW was recorded by 'Nora' at SJ, the site that also posted the highest average value. Cya-3-gluc was not detected in 'CE 56' at C and V or in 'Jonica' at C. 'CE 51' had the most stable content across sites (CV = 21%).

As with anthocyanins, the count of other detected phenolic compounds was more affected by genotype than by site, as reported elsewhere [3, 34]. The hydroxycinnamic acid content ranged from 58 and 451 μg per g FW. On average the fruits at V showing the highest average contents. Genotypes differed significantly: 'CE 56' had the highest count at all sites, albeit evincing high site-linked variability (CV = 23%), and 'CE 51' the lowest content, but with low variability across-site (CV = 8%). Ellagitannin contents ranged from 14 to 282 μg per g FW. On average the fruits at C had the lowest values and those at SJ the highest. The correlationship between the content of ellagitannins and yield per day was $r = -0.53^{*}$. 'CE 56' showed the highest and most stable (CV = 33%) counts on average and 'CE 51' and 'Jonica' the lowest at all sites. Flavan-3-ols contents ranged between 41 and 289 μg per g FW. In general the highest contents were at SJ, in particular for 'Pircinque' and 'CE 56', respectively scoring 289 and 183 μg per g FW. No significant differences across genotypes were found at C. At V 'Nora' and 'CE 56' fruits showed the highest contents. Flavan-3-ol contents also correlated to yield per day ($r = -0.53^{*}$). Flavonol contents ranged from 2.8 to 38.7 μg per g FW. Fruit at SJ had the highest counts, except for 'CE 56' and those at V the lowest, except for 'CE 51', for all genotypes. 'CE 51' had the highest contents across sites.

No correlation was found between phenolic classes evaluated by HPLC and TPH assays, in agreement with Tulipani et al. [32]. Nor was any correlation found also between phenolic classes by HPLC and TAC. Similar results are reported for strawberry by Rekika et al. [28] and Meyers et al. [25], although others report opposite results for other berries species [27, 33].

4. Conclusions

Our overall data show that the differing environmental conditions and field management practices specific to trial site (plant type, planting date, harvest time, cultural technique) affected fruits quality traits. The long picking period in the South resulted in lower plant stress and higher fruits sugar and ascorbic acid contents. In the North, particularly at V where crop management allows a double cropping cycle, the greater plant stress induced lower fruits sugar content but a higher count of health-related compounds.

Genotype performance also differed by site. 'CE 51' had medium yield and medium-high average fruits weight, bright pale colour and medium-firm, sweet flesh of well-balanced acidity at SJ. 'CE 56' evinced outstanding fruits firmness, good acidity and very bright orange-red colour at SJ. 'Nora' had high yield at C and low average fruits weight and firmness but high AA, TPH and TAC contents at SJ. 'Pircinque' had high yield at SJ, average fruits weight and sweetness at C, and average flesh firmness, AA and TACY with low acidity and deep red colour at V. 'Jonica' showed high yield, sweetness and AA at V and firm flesh at C.

Genotype had a prominent effect on the content of bioactive compounds in relation to site, as reported by others [5, 8, 16, 34]. It is thus important to test cultivars in different environments for several years in order to draw guidelines about potential ways of enhancing the content of bioactive compounds in fruits.

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