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Phenols and Ascorbic Acid in Black Currants (*Ribes nigrum* L.): Variation Due to Genotype, Location, and Year

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Supporting Information

ABSTRACT: Black currant berries contain many biochemical compounds with proven or potential human health benefits. We studied the content of total and single polyphenols, ascorbic acid, soluble sugars, and titratable acidity for two advanced selections and three cultivars of black currant at two distant locations in Sweden (south: 56°06′N; north: 65°21′N) over a 3 year period. Regression analyses revealed the effect of genotype to be considerably larger than that of location and year. However, significant effects of location, year, and interactions were also revealed. A principal component analysis nevertheless separated the genotypes. The content of ascorbic acid, total phenols, total anthocyanins, and soluble sugars was highest in berries from the south, whereas the content of phenolic acids and titratable acidity was highest in berries from the north. The results show that selection of cultivars and production sites are important for cultivation of high-quality black currant raw material for health-promoting products.

KEYWORDS: *anthocyanins, ascorbic acid, cultivar, environment, flavonols, functional food, HPLC, organic growing, phenols, polyphenols*

INTRODUCTION

There is an increasing interest in the inclusion of fruits, berries, and derived products in the human diet, primarily for the health benefits.[1−3] Black currant (*Ribes nigrum* L.) is an economically important soft fruit crop in temperate zones of Europe, Russia, northern Asia, New Zealand, and to a lesser extent, North America. The plant is hardy and reliably high-yielding, suitable for growing in extreme climatic conditions, such as close to the artic circle in northern Scandinavia. For the year 2012, the estimated annual black currant production worldwide was 166 300 tonnes with 158 600 tonnes in EU (IBA, 2012; http://www.internationalblackcurrantassociation.com). The fruit of the black currant, black currant berries, are favored for their organoleptic properties such as distinctive color and intense flavor, which is due to phenolic compounds such as anthocyanins, and the presence of sugars, acids, and volatile compounds.[4,5] Black currants are primarily cultivated for juice and beverage production and also processed for jams, jellies, purées, teas, as functional food products, and to some extent, it is consumed fresh.[6] The berries have significant antioxidant activity in part attributed to the relatively high content of ascorbic acid (vitamin C).[7] The content of ascorbic acid in commercial cultivars ranges from 130−200 mg/100 mL fresh juice, but even higher levels (over 350 mg/100 mL) have been detected in some breeding materials.[8] However, the antioxidant activity is also attributed to the high levels of phenolic compounds. The most important compounds are the anthocyanins, with an average content of approximately 250 mg 100 g^{-1} in fresh fruits.[9] In addition to anthocyanins, black currants also contain significant amounts of hydroxycinnamic acids, flavan-3-ols and flavonols, with potential health-promoting properties.[10−14]

There is convincing evidence about the positive contribution of black currants on human health, including effects on vascular function.[13−16] Due to its health-promoting properties, black currants could be an important fruit in the daily diet. In addition, black currants are also a potential raw material for various end-user requirements.

The quality and composition of different bioactive compounds in black currant berries may be influenced by a number of factors such as genotype, climate and growth conditions, cultivation practices, degree of ripening when harvested, and storage conditions.[10,17−22] For example, it has been reported that black currants grown at higher latitudes in Finland have higher content of sugars and citric acid and lower content of malic acid, quinic acid, ascorbic acid, total flavonols, total anthocyanins, and total phenolic compounds when compared to the ones grown at lower latitudes.[19,22] However, in other fruits (e.g., bilberries and strawberries) the berries of plants grown in northern latitudes contained higher levels of phenolic compounds, soluble solids, and titratable acidity in comparison to the ones grown in the south.[23,24]

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It is essential for the processing and functional food sector that the quality of the berry is affected by environmental conditions as little as possible. Furthermore, there is a demand for berries with high content of various bioactive compounds and improved sensory characteristics. Hence, from a breeding/cultivation viewpoint, increased knowledge of phenotypic and genetic data on fruit quality of genotype, location, and yearly variation is essential. Such knowledge makes it possible to breed and select for suitable cultivars in terms of quality and nutritional stability during cultivation in different climates.

The aim of the current study was to investigate the role of genotype (G), location (L), and year (Y) on a wide range of biochemical characteristics, which included ascorbic acid (AsA), single phenolic compounds, total phenolics (TP), total anthocyanins (TA), soluble solids (SS), and titratable acidity (TTA) in black currant berries from three cultivars and two advanced selections grown by organic field management principles in the north and south of Sweden over a 3 year period.

MATERIALS AND METHODS

Plant Material. The black currant plant material used in the present study was represented by two advanced selections, JHI 8944−13 (James Hutton Institute, Scotland) and BRi 9504-2-227 (Swedish University of Agricultural Sciences, Balsgård), and three cultivars Ben Finlay (James Hutton Institute, Scotland), Poesia (Russia), and Titania (Sweden). The plants were grown in organic field trials at Balsgård, in the south of Sweden (56°06′N, 14°10′E), and Öjebyn, in the north of Sweden (65°21′N, 21°23′E) . These two locations are located nearly 1102 km (the way the bird flies) apart. The bushes were planted in a randomized block design with five blocks (rows) and one plant (genotype) per plot in 2006. The planting distance was 4 m between rows and 2 m between plants. For fertilization, pelleted organic manure was provided two or three times per year (at a total amount during the season of 500 kg Biofer 6-3-12 per ha, Gyllebo Gödning, Sweden), and an approved pest spray (Raptol, Biobasiq Sverige AB, Sweden) was applied on plants as frequently as needed to combat aphids. The berries were sampled at full ripeness on the basis of organoleptic properties in July at Balsgård and in August at Öjebyn during three successive years from 2008−2010. Approximately 500 g of berries were picked from each of the five plants per genotype and immediately stored at −20 °C until analysis.

Reagents and Chemicals. Acetonitrile (isocratic grade, purity >99.8%), methanol (HPLC grade, purity >99.8%), Folin−Ciocalteu reagent, 85% ortho-phosphoric acid (H_3PO_4), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), sodium acetate (CH_3COONa), sodium hydroxide (NaOH), and potassium chloride (KCl) were obtained from Merck KGaA (Darmstadt, Germany). Formic acid (pro analysi, purity 98−100%), sodium carbonate (Na_2CO_3), ascorbic acid, gallic acid, chlorogenic acid, and quercetin were purchased from Sigma (St. Louis, MO). Delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, neo-chlorogenic acid, myricetin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, and isorhamnetin-3-*O*-glucoside were purchased from Extrasynthese (Genay, France). Ultrapure water was obtained by use of the Elgastat Prima UHQPS (Buckinghamshire, England). Ethanol (purity 99.9%) and *meta*-phosphoric acid (MPA) were purchased from VWR (Fontenay-Sous-Bois, France).

Extraction Procedure. For extraction of phenolic compounds, total anthocyanins and total phenols, two subsamples of 50 g from each plant were lyophilized for 1 week and ground to a fine powder using a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany). Extraction of phenolic compounds and total anthocyanins followed the protocol by Vagiri et al.[25] For each sample, about 50 mg was weighed in 2 mL tubes, extracted, and stabilized by 1.5 mL 10% formic acid and 5% acetonitrile in water. The samples were vortexed (Janke & Kankel, Staufen, Germany) and extracted in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) for 20 min at ambient temperature. The extracts were centrifuged at 13 000 rpm for 10 min, and the supernatants were stored at −20 °C until analyzed. Extraction was performed in duplicate.

The extraction of total phenols was based on Vagiri et al.[25] with modifications. Briefly, about 50 mg was weighed from each of the two lyophilized subsamples and extracted with 1 mL of 50% ethanol containing 0.05 M H_3PO_4. The samples were sealed using aluminum foil and placed in an orbital shaker (Forma Scientific, Inc., Marietta, OH) in darkness at 4 °C for 20 h for extraction and thereafter centrifuged at 13 000 rpm for 10 min. The extraction was performed in duplicates.

For extraction of ascorbic acid (AsA), approximately 50 g of fresh frozen berries was homogenized in a blender. For each sample, about 1 g of the homogenate was weighed in triplicate and stabilized with 9 mL of 2% MPA. The samples were extracted in an ultrasonic bath for 10 min and later centrifuged at 4500 rpm for 10 min. The supernatant was filtered through a 0.45 μm filter (Sarstedt, Nümbrecht, Germany) into HPLC vials and analyzed.

Total Anthocyanins. The total anthocyanin (TA) content was measured at the day of extraction by the pH differential method.[26] Briefly, 25 μL of the extracted sample was mixed with 2 mL of KCl buffer at pH 1.0 and 2 mL of CH_3COONa buffer at pH 4.5. After 15 min, the absorbance was recorded at 516 and 700 nm, respectively, using a UV-2101PC (Shimadzu, Kyoto, Japan) spectrophotometer.

The absorbance (A) of the diluted sample was calculated as follows:

$$A = (A_{516} - A_{700})_{\mathrm{pH1.0}} - (A_{516} - A_{700})_{\mathrm{pH4.5}}$$

The TA content in the sample was calculated as cyanidin-3-*O*-glucoside equivalents according to the formula: TA content (mg/L) = $(A \times \mathrm{MW} \times \mathrm{DF} \times 1000)/(\varepsilon \times 1)$

A molecular weight (MW) of 449.2 was used for cyanidin-3-*O*-glucoside, DF is the dilution factor, and ε (26 900) is the molar absorption of cyanidin-3-*O*-glucoside. The result was expressed as mg/g dry weight (DW) of the sample. Analyses were performed as duplicates for each sample.

Total Phenolics. The total phenolic (TP) content was determined on the same day of extraction using the Folin−Ciocalteu method.[27] Briefly, 0.5 μL of the extracted sample, 100 μL of ethanol (5% v/v), 200 μL of Folin−Ciocalteu reagent, and 2 mL 15% Na_2CO_3 solution was added, followed by addition of 1 mL of ultrapure water. The solutions were mixed in the cuvette by use of a pipet and allowed to equilibrate at room temperature for 2 h. The absorbance was recorded at 765 nm using a UV-2101PC (Shimadzu, Kyoto, Japan) spectrophotometer. Gallic acid was used as the standard, and the results, corrected for AsA interference, were expressed as milligrams of gallic acid (GA) per gram of dry weight (DW) of plant material. The analyses were performed in duplicate.

Table 1. Relative Amount of Variance (in % Based on R^2 Values from Regression Analyses) Explained by the Studied Sources for the Analyzed Compounds (A) Monomeric Anthocyanins, (B) Flavonols, and (C) Phenolic Acids, Total Phenols, Total Anthocyanins, Ascorbic Acid, Soluble Solids, and Titratable Acidity (for Abbreviations, See Material and Methods)

A. monomeric anthocyanins (relative variance, %)				
source	del-glu	del-rut	cya-glu	cya-rut
genotype	0.2	3.5	19.3	13.9
location	2.4	1.3	2.1	1.2
year	18.2	4.5	6.7	7.7
genotype × location × year	23.4	11.7	23.8	19.8

B. flavonols (relative variance, %)						
source	myr-mal-glu	que-rut	que-glu	que-mal-glu	kae-glu	iso-glu
genotype	5.4	14.3	0.4	0.1	34.9	45.5
location	9.4	10.6	5.0	18.5	1.1	2.2
year	4.5	6.2	2.4	6.6	2.7	12.4
genotype × location × year	22.8	29.0	7.0	29.3	36.0	53.1

C. phenolic acids, total phenols, total anthocyanins, ascorbic acid, aoluble solids, and titratable acidity (relative variance, %)							
source	neo-chl	chl	TP	TA	AsA	SS	TTA
genotype	15.5	23.1	60.4	1.7	50.0	39.6	7.7
location	7.1	1.9	6.8	6.3	17.1	14.0	1.9
year	0.2	0.7	6.3	13.5	9.3	0.2	0.7
genotype × location × year	24.7	25.1	68.7	21.4	71.2	57.5	9.8

Soluble Solids. The soluble solids (SS) of the samples were measured using a PR-100 Digital Refractometer (Atago, Tokyo, Japan). For these analyses, purée were pressed out of thawed berries. Three replicate measurements were done for each sample, and results were expressed as °Brix. The SS values were used as an estimate of the sugar content in this study.

Titratable Acidity. The content of organic acids in the samples was measured as titratable acidity (TTA) using an automatic titrator equipped with an ABU-91 auto buret and a SAC-80 sample changer controlled by TIM-90 titration manager (Radiometer Analytical, Inc., Copenhagen, Denmark). Briefly, 1 mL of slurry was diluted with ultrapure water to a total volume of 10 mL and titrated with 0.1 M NaOH to an end point of pH 8.4. The analyses were performed in triplicate, and the results were expressed as 0.1 M NaOH per mL of juice.

Ascorbic Acid. The ascorbic acid (AsA) analysis was performed on a Shimadzu (Kyoto, Japan) HPLC system (SIL-10A autosampler, SCL-10AVP control unit, LC-10AD pump, SPD-10AV VP UV−vis detector unit) controlled by Class-VP software (6.13 SP2). The isocratic mobile phase consisted of 0.05 M NaH_2PO_4 and H_3PO_4 (8.5% v/v), and the pH of the eluent was adjusted to 2.8. The separation was performed using a Restek (Bellefonte, PA, USA) column (150 × 4.6 mm, 5 μm particle size), operated at 30 °C (Column Chiller, Sorbent AB) with a guard column at a flow rate at 1 mL min^{-1}. Detection was carried out at a wavelength of 254 nm with an injection volume of 10 μL and total run time of 12 min. Analyses were performed in triplicate, and results were expressed as mg/100 g FW.

Single Phenolic Compounds. The single phenolic compounds were analyzed as previously described by Vagiri et al.[25] In brief, the samples were analyzed by an HPLC-DAD system (Shimadzu, Kyoto, Japan) with a LC-20AB model dual pump, a SPD-M10A model diode array detector equipped with a Waters 717 plus autosampler linked to a Shimadzu SCL-10A model system controller. The eluent consisted of formic acid/water (7:93 v/v; mobile phase A) and acetonitrile/methanol/water (90:5:5 v/v/v; mobile phase B) with gradient elution as follows: The linear gradient starting with 0−2 min, 8% B; 2−21.5 min, 8−16% B; 21.5−51.5 min, 16−23% B; 51.5−56.5 min, 23−40% B; and 56.5−61.5 min, back to 8% B followed by re-equilibration of the system for 2 min with initial conditions. The linear binary gradient was set to a flow rate of 1.2 mL min^{-1}. The injection volume of samples was 20 μL, and the total run time was 63.5 min. The column used was a Synergie hydro RP-80A column (250 × 4.60 mm inside diameter, 4 μm particle size), protected with a C-18 guard cartridge (4 × 3.0 mm), both from Phenomenex (Værløse, Denmark). The column temperature was set at 24 °C using a Shimadzu CTO-10AS thermostatically controlled column compartment. The chromatographic data was collected using Class VP software (Shimadzu 5.0). The acquisition wavelength was set in the range of 260−550 nm, and chromatograms were recorded at 320 nm (hydroxycinnamic acids), 360 nm (flavonols), and 520 nm (anthocyanins). The analyses were performed in duplicates, and the results were expressed as μg/g dry weight (DW). Because a standard was not available for myricetin malonlyglucoside, it was quantified in equivalents to myricetin.

Statistical Analysis. A general linear model (GLM) analysis was followed by a Duncan post-hoc test ($p < 0.05$) to evaluate the effects of various sources (G, Y, L) and their interactions. Further, regression analyses were performed using least-squares estimation, and the coefficients of determination (R^2) from the regression analyses were used to determine the relative amount of variance of the dependent variable y (the evaluated compounds) explained by the explanatory variables (the sources). Pearson correlation coefficients were calculated to reveal the relationship between the metabolites. All statistical analyses mentioned above were carried out using SAS software, version 9.2 (SAS Institute Inc., Cary, NC). Principal component analyses (PCA) were done using Minitab software, version 16.1 (State college, PA).

Abbreviations for Phenolic Compounds. Del-glu (delphindin-3-*O*-glucoside); del-rut (delphindin-3-*O*-rutinoside); cya-glu (cyanidin-3-*O*-glucoside); cya-rut (cyanidin-3-*O*-rutinoside); anthsum (sum of monomeric anthocyanins); myr-mal-glu (myricetin malonylglucoside); que-rut (quercetin rutinoside); que-glu (quercetin glucoside); que-mal-glu (quer-

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Table 2. Means (Averages of Locations and Years) for (A) Monomeric Anthocyanins, (B) Flavonols, (C) Phenolic Acids, and (D) Total Phenols, Total Anthocyanins, Ascorbic Acid, Soluble Solids, and Titratable Acidity for Different Genotypes

A. monomeric anthocyanins (μg/g DW)[a]

genotype	del-glu	del-rut	cya-glu	cya-rut	sum
Ben Finlay	2534 b	4561 c	1173 b	4509 b	12 777 ab
JHI 8944-13	2195 c	3795 d	1807 a	4974 a	12 772 ab
BRi 9504-2-227	1272 d	6158 a	479 c	4955 a	12 863 b
Poesia	3066 a	4641 c	1182 b	4660 ab	13 550 a
Titania	2260 c	5326 b	558 c	3630 c	11 774 b

B. flavonols (μg/g DW)[a]

genotype	myr-mal-glu	que-rut	que-glu	que-mal-glu	kae-glu	iso-glu	sum
Ben Finlay	92 b	217 b	147 d	8 d	140 b	41 a	715 c
JHI 8944-13	70 c	267 a	337 a	94 c	191 a	42 a	1001 a
BRi 9504-2-227	51 d	257 a	218 c	112 b	95 c	24 b	757 c
Poesia	147 a	249 a	305 b	134 a	66 d	21 c	922 b
Titania	88 b	127 c	160 d	80 d	61 d	14 d	530 d

C. phenolic acids (μg/g DW)[a]

genotype	neo-chl	chl	sum
Ben Finlay	203 a	449 b	652 b
JHI 8944-13	121 b	1016 a	1137 a
BRi 9504-2-227	205 a	227 c	432 c
Poesia	101 c	217 c	318 d
Titania	51 d	248 c	299 d

D. total phenols (mg/g GA DW), total anthocyanins (mg cya-glu/g DW), ascorbic acid (mg/100 g FW), soluble solids (°Brix), and titratable acidity (mL 0.1 M NaOH)

genotype	TP[a]	TA[a]	AsA[b]	SS[b]	TTA[b]
Ben Finlay	31.8 a	11.6 a	310 a	12.6 e	9.0 c
JHI 8944-13	22.0 b	10.6 b	219 b	13.3 d	8.7 d
BRi 9504-2-227	21.0 c	9.9 c	166 d	15.4 c	12.6 a
Poesia	21.8 c	11.4 a	190 c	16.7 a	10.2 b
Titania	19.2 d	10.5 b	148 e	15.9 b	10.1 b

[a]Mean based on two replicate analyses for each sample. [b]Mean based on three replicate analyses for each sample. Different letter/letters within each column indicate significant differences between the cultivars at $p < 0.05$ using a Duncan post-hoc test. (For abbreviations of compounds, see the Materials and Methods section.)

cetin malonyl glucoside); kae-gluc (kaempferol glucoside); iso-glu (isorhamnetin glucoside); flavsum (sum of flavonols); neo-chl (neo chlorogenic acid); chl (chlorogenic acid); phlacdsum (sum of phenolic acids).

RESULTS AND DISCUSSION

Influence of Genotype, Location, and Year. Genotype (G) was found to be a significant source of variation for all the analyzed compounds. Also, location (L) and year (Y) as well as the interactions in all possible combinations significantly contributed to the variation for most of the compounds (Appendix 1). The phenolic compounds observed in this study have also previously been detected in black currants.[10,12,28−30] Detailed information on the content of monomeric anthocyanins (Appendix 2a), flavonols (Appendix 2b), phenolic acids, TA, TP, AsA, SS, and TTA (Appendix 2c) for specific genotypes at every location and year is also presented. Similar to previous studies,[31,32] R^2 values from regression analyses were used to report the relative contribution to the variance of the compounds by the sources. In general, from 7 to over 70% of the variance of the analyzed compounds were explained by G, Y, and L (Table 1). For the four monomeric anthocyanins (del-glu, del-rut, cya-glu, and cya-rut) and T, about 20% (11.7−23.8%) of the variance was explained by G, Y, and L (Table 1A). Y was the most important source for variation of del-glu, del-rut, and TA, while G was the most important source for cya-glu and cya-rut (Table 1A and C). G, Y, and L only explained 7% of the variance for que-glu, while the other flavonols were accounted for by greater than 20% (22.8−53.1%). G was the most important source for que-rut, kae-glu, and iso-glu, while L was most important for myr-mal-glu, que-glu, and que-mal-glu (Table 1B). Around 25% of the variance in phenolic acids and 60% of the variance in TP content was accounted for by the studied sources, among which G was the most important (Table 1C). The studied sources accounted for a high proportion of the variance (71.2 and 57.3%) in AsA and SS, while only for 9.8% of the variance in TTA, although G was the most important source to the variation for all variables (Table 1C).

Previous studies have elucidated the effects of genotype on the content of various phenolic compounds, TP, TA, and AsA in black currants where content varied widely among the germplasm studied.[17,19−22,33,34] In blueberries, variation in phenolic composition among genotypes was much greater than that found between the years, and the interaction of genotype and environment was also found to be significant.[35] However, several environmental factors attributed to growing location can also affect the content of bioactive compounds. For instance, Anttonen and Karjalainen[10] found that the cultivation site was a major factor affecting the composition of phenolic compounds rather than cultivation technique in black currants. In another study conducted on potatoes, it was found that the content of

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Table 3. Means (Averages of Genotypes and Years) for (A) Monomeric Anthocyanins, (B) Flavonols, (C) Phenolic Acids, and (D) Total Phenols, Total Anthocyanins, Ascorbic Acid, Soluble Solids, and Titratable Acidity at Different Locations

A. monomeric anthocyanins (μg/g DW)[a]

location	del-glu	del-rut	cya-glu	cya-rut	sum
south	2440 a	5237 a	922 b	4457 a	13 056 a
north	2092 b	4623 b	1118 a	4619 a	12 451 b

B. flavonols (μg/g DW)[a]

location	myr-mal-glu	que-rut	que-glu	que-mal-glu	kae-glu	iso-glu	sum
south	103 a	252 a	214 b	120 a	96 b	28 a	812 a
north	78 b	194 b	254 a	82 b	119 a	27 a	754 b

C. phenolic acids (μg/g DW)[a]

location	neo-chl	chl	sum
south	108 b	352 b	459 b
north	160 a	481 a	642 a

D. total phenols, total anthocyanins, ascorbic acid, soluble solids, and titratable acidity

location	TP[a] (mg/g GA DW)	TA[a] (mg cya-glu/g DW)	AsA[b] (mg/100 g FW)	SS[b] (°Brix)	TTA[b] (mL 0.1 M NaOH)
south	25.0 a	11.3 a	226 a	15.7 a	10.1 b
north	22.6 b	10.2 b	170 b	14.0 b	10.5 a

[a]Mean based on two replicate analyses for each sample. [b]Mean based on three replicate analyses for each sample. Different letter/letters within each column indicate significant differences between the locations at $p < 0.05$ using a Duncan post-hoc test. (For abbreviations of compounds, see the Materials and Methods section.)

different bioactive compounds was more affected by growing season, genotype, and location than by cultivation technique.[36] In strawberries, higher temperatures have been shown to increase the content of flavonols and anthocyanins.[37] The activation of flavonoid biosynthesis by solar radiation was found in blueberries.[38] Also, soil conditions such as moisture deficit led to a lower activity of phenylalanine ammonia lyase (PAL) that consequently lowered the synthesis of phenolic compounds in tea.[39] Hence, it is to be noted that even though a genotype is known to accumulate high levels of specific compounds, the final content can also be dependent on several environmental parameters.

Variation between Genotypes (Cultivars and Selections). When the data were combined for all years and locations, genotype was the most important source to the variation of the analyzed compounds (Table 2). In the case of single phenolic compounds, the selection JHI-8944−13 had the highest amount of cya-glu, cya-rut, que-glu, kae-glu, and chl, whereas BRi-9504−2−227 had the highest amount of del-rut and neo-chl. The cultivar Poesia had the highest amount of del-glu but the lowest amount of del-rut. The compound que-rut was highest in JHI-8944-13, BRi-9504-2-227, and Poesia, and iso-glu was highest in Ben Finlay and JHI-8944-13. The cultivar Titania had lower levels of que-rut and iso-glu than any of the other four genotypes. The TP content varied from 19.2−31.8 mg/g GA DW with Titania and BRi-9504-2-227 having the lowest content and Ben Finlay having the highest content. TA content varied from 9.9−11.6 mg cya-glu/g DW, the lowest in BRi-9504-2-227 and highest in Ben Finlay and Poesia. The SS values ranged from 12.6−16.7, the lowest value was observed in Ben Finlay and the highest in Poesia. In terms of TTA content, there was considerably less variation among the genotypes with BRi-9504-2-227 having the highest content (12.6 mL 0.1 M NaOH per mL juice) and lowest observed in JHI 8944-13 (8.7 mL mL 0.1 M NaOH per mL juice). The AsA content varied significantly among all the genotypes, the highest content was observed in Ben Finlay (310 mg/100 g FW) and the lowest in Titania (148 mg/100 g FW) with an approximately 2-fold difference.

In terms of compositional differences, among the monomeric anthocyanins (Table 2A), del-rut and cya-rut showed highest amounts comprising 29.7−47.8% and 30.8−38.9% of the total monomeric anthocyanins, respectively, followed by del-glu (9.8−22.6%) and cya-glu (3.7−14.1%). Similar amounts of monomeric anthocyanins have been reported by other authors,[40] where cya-rut accounted for 33−38%, del-rut by 27−34%, and cya-glu by 8−10%. Among the flavonols (Table 2B), que-glu was the most abundant comprising 20.5−33.6%, followed by que-rut (23.9−26.6%) and iso-gluc (2.6−4.2%) of the total flavonols. Chl comprised 68.2−89.3% among the detected phenolic acids (Table 2C).

Several previous studies have reported up to a 4- and 6-fold difference in AsA values among germplasm tested.[20,21,41] A 2-fold variation in the content of AsA observed in this study is therefore not surprising but underlines the possibility and importance of selecting proper cultivars for different purposes.

TA and TP content are partly linked with berry size and peel thickness in black currants—the bigger the berry size the less accumulation of TP and the thicker the peel, the more anthocyanins.[28] Berry size is also a genotype characteristic and the fact that the cultivar Ben Finlay in our study had highest content of TA and TP may be due to the fact that this cultivar also has small berries with a thick peel. Previously, up to a 2.1- and 3.4-fold variation for TP and TA, respectively, have been reported among different black currant germplasm,[21,28] which is larger than the variation determined in our plant material with a limited number of genotypes.

Thus, the results from the present study corroborate that the genetic background is a very important factor determining the composition and content of bioactive compounds in black currant fruits. The variation between genotypes shows that it is possible to enhance the content of health-promoting compounds by proper cultivar choice. In the present investigation, the cultivar Ben Finlay showed a commonly described feature of genotypes with small berries: high

accumulation of TP, TA and a thick peel. The genotypes JHI 8944-13 and Poesia differed from the other investigated genotypes in a less previously well-documented manner, with high contents of several of the investigated compounds. Thus, it is possible to improve black currant quality by breeding and to develop cultivars with a multiple combination of superior quality traits.

Variation between Locations (Latitudes). Despite the fact that location was the main determinant only for a few of the investigated compounds (Table 3), significant differences in amounts were noticed between the locations for all compounds except cya-rut and iso-glu. For phenolic acids, cya-glu, que-glu, kae-glu, and TTA (Table 3), the content was higher in berries from the north than in berries grown in the south. Previous investigations have reported a lower content of sum of monomeric anthocyanins, sum of flavonols, and higher content of phenolic acids in black currant samples from the north compared to those from the south.[22] In the present study, contents of del-glu, del-rut, cya-rut, anthsum, myr-mal-glu, que-rut, que-mal-glu, flavsum, TP, TA, AsA, and SS were higher in berries from the south than those from the north. The high contents of AsA and SS in berries from southern Sweden in comparison to the north was in contrast to earlier studies by Zheng et al.[19] who observed higher contents of these compounds in berries grown in the north of Finland in comparison to the south. The reason might be due to climatic differences especially between southern Sweden and southern Finland, but it may also be attributable to different genetic background of the plant material studied (a possible maladaptation), the degree of maturity of the berries at harvest, and soil qualities.[42] The reason for variation in the content of phenolic compounds is likely due to differences in both genetic and environmental factors such as temperature, day length, precipitation, soil nutrient composition, humidity, and solar radiation.[43] These factors are known to have a direct or indirect constraint on plant photosynthesis, thereby affecting the content of bioactive compounds.[44]

Among the weather variables, temperature and radiation have previously been shown to influence the content of del-glu, del-rut, and myr-mal-glu in black currants.[22] In our study, the difference between the locations in terms of anthsum, TA, TP, AsA, and SS content might be due to higher average temperatures (Figure 1) recorded in the south than in the north of Sweden during the months of harvest. The findings on locational variations in our study is in agreement with previous studies, in which high average temperature during growth season increased AsA and sugar content.[20]

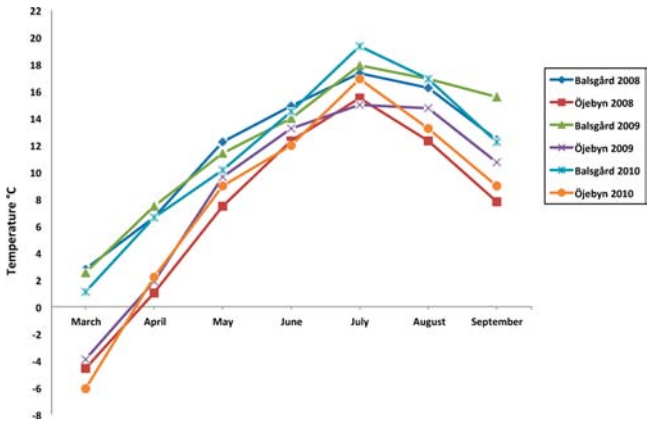

Figure 1. Average monthly temperatures from March to September for Balsgård in the south (56°06′N, 14°10′E) and Öjebyn in the north (65°21′N, 21°23′E) of Sweden for 3 years (2008−2010).

In the present study, there was no significant difference between the locations for the content of cya-rut and iso-glu, and for TTA, the content did not vary much (although there was a significant difference) between the locations in comparison to other compounds. This indicates that irrespective of growth location, the composition of these compounds would be stable. Currently, in black currant breeding, cultivars with enhanced content of anthocyanins are in demand. This is especially requested for the delphinidin levels from the juice processors and functional food industry due to an increasing interest in the color stability of the juice and benefits on human health.[8,13,15] Certain flavonols and phenolic acids are known to contribute to the mouth-drying astringency and puckering taste in black currant berries.[45] In the present study, it could be inferred that black currants grown in lower latitudes (south) with high content of del-glu, del-rut, AsA, and SS and a lower level of titratable acidity (TTA) and phenolic acids, may have higher vitamin C content and better sensory and quality characteristics than those grown at higher latitudes (north) under Scandinavian environmental and weather conditions. Hence, from a breeding point of view, the effects of location on the composition of bioactive compounds should be considered in selecting the cultivation site and plant material for commercial production and various end-users.

Variation between Years (Seasons). Overall, the highest contents of monomeric anthocyanins and flavonols were found in 2010 (Table 4). The highest content for TP, TA, and AsA was found in both 2010 and 2009. The contents of neo-chl and phlacdsum were higher in 2009; chl was higher in both 2008 and 2010. The lowest contents of all the above compounds, except que-glu, kae-glu, and chl, were found in 2008. The highest content for kae-glu was found in both 2008 and 2009. Over the 3 years, the TTA content was not altered by the yearly influences, although a slight increase in 2008 could be observed.

Previous studies have observed a year to year variation on the content of AsA and phenolic compounds in black currants due to influence of environmental factors.[19,20,23] In accordance with these studies, the annual variation shown in this study can be speculated as a climatic effect that may be attributed to variations in solar radiation, temperature, humidity, and irrigation. Additionally, other factors such as the variation in the availability of relative amounts of soil nutrients and the degree of foliar diseases could have effected the composition of bioactive compound analyzed in this study.

Principal Component Analysis (PCA). PCA was used to reveal any pattern among the samples and the berry metabolites of plants grown in the north and south of Sweden. Berries were sampled over a period of 3 years for all genotypes except for Ben Finlay that did not set sufficient standards for fruit in 2008. Three principal components were required to capture 69.1% of the total variance with PC1 explaining 34.8% and PC2 explaining 20.7% (Figure 2). It is evident that all factors (genotype, growing location, and year) have an impact on the biochemical content and influence grouping. A clear clustering was however obtained for all genotypes studied. The overlapping of clusters is reflecting similarities in composition. For example, it could be noticed that location influences the cultivar Ben Finlay with a clear north and south separation in

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Table 4. Means (Averages of Genotypes and Locations) for (A) Monomeric Anthocyanins, (B) Flavonols, (C) Phenolic Acids, and (D) Total Phenols, Total Anthocyanins, Ascorbic Acid, Soluble Solids, and Titratable Acidity in Different Years

A. monomeric anthocyanins (μg/g DW)[a]

year	del-glu	del-rut	cya-glu	cya-rut	sum
2008	1844 c	4430 c	940 b	4383 b	11 598 c
2009	2273 b	4925 b	989 ab	4429 b	12 617 b
2010	2617 a	5357 a	1119 a	4778 a	13 872 a

B. flavonols (μg/g DW)[a]

year	myr-mal-glu	que-rut	que-glu	que-mal-glu	kae-glu	iso-glu	sum
2008	85 b	204 b	230 b	89 b	114 a	24 b	746 b
2009	84 b	217 b	220 b	93 b	99 b	26 b	740 b
2010	101 a	243 a	252 a	117 a	110 a	32 a	855 a

C. phenolic acids (μg/g DW)[a]

year	neo-chl	chl	sum
2008	82 b	441 a	523 b
2009	219 a	380 b	599 a
2010	96 b	433 a	529 b

D. total phenols, total anthocyanins, ascorbic acid, soluble solids, and titratable acidity

year	TP[a] (mg/g GA dw)	TA[a] (mg cya-glu/g DW)	AsA[b] (mg/100 g FW)	SS[b] (°Brix)	TTA[b] (mL 0.1 M NaOH)
2008	22.9 c	10.0 c	161 c	14.4 c	10.4 a
2009	23.7 b	10.8 b	206 b	14.9 b	10.2 b
2010	24.7 a	11.3 a	217 a	15.3 a	10.2 b

[a]Mean based on two replicate analyses for each sample. [b]Mean based on three replicate analyses for each sample. Different letter/letters within each column indicate significant differences between the years at $p < 0.05$ using a Duncan Post-hoc test. (For abbreviations of compounds, see the Materials and Methods section.)

years 2009 and 2010. It is evident that the amounts of neo-chl, cya-rut, que-mal-glu, que-rut, AsA, and TA contribute to the lack of clustering. There is however a tight clustering of BRi 9504-2-227, indicating that regardless of year and location, the content of metabolites remain similar, suggesting a strong genotypic effect. A similar pattern to BRi 9504-2-227 was observed in the clustering of JHI 8944-13, but there seems to be a yearly effect as plants grown in the north for the years 2008 and 2010 cluster a bit further from the rest. This is due to the contribution of kae-glu and chl for 2008 and iso-glu, cya-glu, and TP for 2010. For Poesia, a location effect similar to Ben Finlay was observed, but it was driven by different compounds contributing to the separation. Thus, there is a clear north and south separation observed regardless of yearly effect with del-rut, SS, que-mal-glu, and myr-mal-glu contributing to the separation. In the case of Titania, a complex pattern was observed where an effect of year is noticed regardless of the location, especially during 2008 and 2010, with TTA slightly influencing the results during 2008 and SS contributing to the samples collected in 2010. This could be explained by differences in the content of del-rut during 2009.

Correlation Coefficients among Bioactive Compounds. Significant associations ($p < 0.005$) were observed between several of the bioactive compounds (Appendix 2). Cya-glu correlated positively with del-glu ($r = 0.461$) and negatively with del-rut ($r = -0.418$). Cya-rut correlated positively ($r = 0.482$ and 0.446) with del-rut and cya-glu, respectively. Myr-mal-glu showed a strong positive correlation ($r = 0.832$) with del-glu, while que-rut correlated positively with cya-rut ($r = 0.674$). In addition, significant correlations among the flavonols were also observed. Furthermore, correlations between the sums of anthocyanins and flavonols ($r = 0.531$) and sums of phenolic acids and flavonols ($r = 0.484$) were observed. Also, TA and TP showed positive correlation with each other ($r = 0.394$) and correlated with most of the metabolites studied. AsA strongly correlated with TP ($r = 0.862$), corroborating the fact that AsA is a powerful antioxidant and a major contributor to antioxidant activity in black currants (and also known to interfere with the Folin−Ciocalteu reagent used in the analysis of total phenols).[46] TTA surprisingly correlated positively with SS ($r = 0.348$). Generally, sugar and acid content are an indicator for fruit flavor, and TTA and SS are used in black currant cultivation as a measure of acid and sugar content.[10] The positive correlation implies that it could be difficult to reduce contents of TTA and still have a high level of SS.

TA, TP, and AsA showed negative correlations ($r = -0.331$, $r = -0.51$, and $r = -0.491$, respectively) with TTA. Interestingly, it therefore seems possible to combine a high content of TP and AsA with a low content of TTA.

The correlations observed between the phenolic compounds in this study can be expected as a result of the common phenylpropanoid (shikimate) metabolic pathway. The phenylalanine transforms to an activated form of cinnamic acid, (4-coumarol CoA), which combines with malonyl-CoA (flavonoid pathway) from which all anthocyanins and flavonols derive.[47] In blueberries, the expression of different genes involved in the biosynthesis of phenolic compounds has been reported.[48] However, for black currants, the information about biosynthesis of phenolic compounds is limited. The correlations observed in this study between different compounds is important in relation to the breeding of new genotypes with high or low levels of specific bioactive compounds, and further investigations aimed at clarifying the biosynthesis of these compounds in black currants should be undertaken.

In conclusion, the effect of genotype was larger than that of location and year for the content of most of the bioactive compounds studied. In terms of latitudinal differences, the black currant plants grown in northern Sweden had a higher content of phenolic acids, cya-glu, que-glu, kae-glu, and TTA

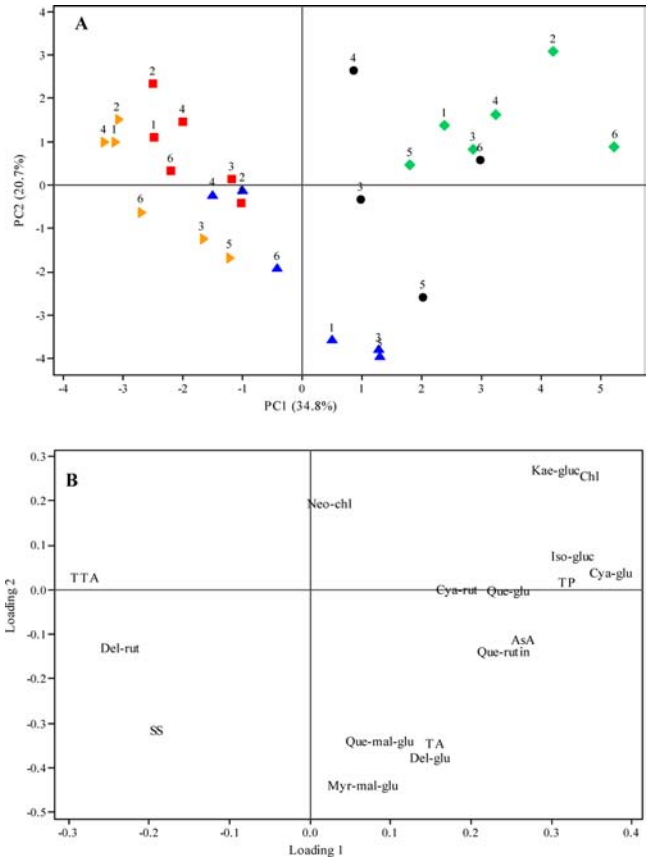

Figure 2. Principal component analysis score (A) and loading plots (B) based on berry chemical analyses of different black currant genotypes (black circles, Ben Finlay; red squares, BRi 9504-2-227; green titled square, JHI 8944-13; blue triangle, Poesia; yellow titled triangle, Titania), grown in the south and north of Sweden and sampled during 3 years. (Abbreviations used: 1 = 2008/south, 2 = 2008/north, 3 = 2009/south, 4 = 2009/north, 5 = 2010/south, 6 = 2010/north. (For abbreviations of compounds, see the Materials and Methods section).

than the ones grown in southern Sweden. However, the content of del-glu, del-rut, cya-rut, sum of monomeric anthocyanins, myr-mal-glu, que-rut, que-mal-glu, sum of flavonols, TP, TA, AsA, and SS were higher in berries from the south than those from the north. Moreover, a significant yearly variation was observed in the composition of the compounds analyzed. Significant differences in the content of various compounds were also observed between the studied black currant genotypes. Because berries of Ben Finlay, JHI 8944-13, and Poesia were high in most of the measured compounds, these genotypes, although not sufficiently competitive to be recommended for commercial growing, would be ideal as parents for use in breeding programs aimed at developing black currant cultivars for Scandinavian conditions with enhanced phytochemical content. These results provide a sound basis for the development of breeding strategies, as well as for selecting cultivars with high phytochemical content and for production of specific berry material for the food and health industry.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables for mean squares from GLM analyses of variance, content of monomeric anthocyanins, flavonols, phenolic acids, TA, TP, AsA, SS, and TTA for specific genotypes at every location and year and Pearson correlation coefficients for analyzed characteristics of black currants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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