# Effects of Latitude and Weather Conditions on Proanthocyanidins in Blackcurrant (*Ribes nigrum*) of Finnish Commercial Cultivars

Wei Yang[†#], Xueying Ma[†#], Oskar Laaksonen[†], Wenjia He[†], Heikki Kallio[†], Baoru Yang[†‡*]

† Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland

‡ Institute of Quality, Safety of Agro-Products and Testing Technology, Shanxi Academy of Agricultural Sciences, Taiyuan 030031, China

#Author contributed equally to this work.

*Author for correspondence

Professor Baoru Yang

Food Chemistry and Food Development

University of Turku

Email: baoru.yang@utu.fi

Tel: +358452737988

## ABSTRACT

Blackcurrants of three Finnish commercial cultivars 'Mortti', 'Ola' and 'Melalahti' cultivated in southern and northern Finland were compared on the basis of the content and composition of proanthocyanidins (PAs). Seventeen B-type PA oligomers (degree of polymerization 2-5 and 7) were detected by hydrophilic interaction liquid chromatography and electrospray ionization mass spectrometry. Total PAs, dimers, trimers and tetramers were quantified. Among the three cultivars, 'Ola' had the highest contents of both total PAs and PA oligomers. 'Melalahti' was separated from both 'Mortti' and 'Ola' by PA profiles in the partial least squares discriminant analysis model. All three cultivars revealed distinct responses to latitude and weather conditions. The content of total PAs showed a positive correlation to latitude in 'Ola' and 'Melalahti'. Among the meteorological variables, high temperature and radiation correlated negatively with total PAs, while only specific variables showed a correlation with PA oligomers.



Keywords: blackcurrant, cultivars, HILIC, latitude effect, proanthocyanidins, weather conditions

## INTRODUCTION

Proanthocyanidins (PAs), also named condensed tannins, are oligomers or polymers of flavan-3-ols synthesized *via* the flavonoid biosynthesis pathway. The most common flavan-3-ols are (epi)catechins, (epi)gallocatechins and relatively rare (epi)afzelechins, forming procyanidins (PCs), prodelphinidins (PDs), and propelargonidins (PPs), respectively. In proanthocyanidins, flavan-3-ol units are linked through interflavan bonds. B-type PAs contain a single C4–C6 or C4–C8 carbon–carbon bond, whereas A-type PAs have additionally ether C2–O–C7 or C2–O–C5 bonds.[1] B-type PAs are more common in foods of plant origin. However, A-type PAs are present also in some foods, such as avocado, bilberry, cranberry, crowberry, lingonberry, peanut, etc.[2,3]

As the second most abundant natural group of phenolic compounds after lignin, PAs widely exist in a variety of plant tissues and organs, and play an important role in plant physiology.[4] The compounds maintain seed dormancy and integrity, and participate in plant protection against biotic and abiotic stresses.[5-7] PAs have been reported to exert also several other physiological activities including antioxidant, antimicrobial and antiviral properties.[8,9] In recent years, epidemiological studies also indicate an association between consumption of PAs and a reduced risk of oxidative stress–related diseases, such as cardiovascular diseases, type 2 diabetes, and specific cancers.[10-12] The health-promoting properties of PAs may be attributed to their oligomers with the degree of polymerization (DP) between 2-4 (DP 2-4) or colonic microbial metabolites derived from PAs of higher DP values.[13] In addition to their beneficial health effects, PAs also play an essential role in sensory properties of astringency and bitterness in fruits, wine and tea [14,15], where the composition of the monomeric subunits, type of stereochemistry in linkages, and DP will further affect the intensity sensation.[15-18]

Like most flavonoids, PAs accumulate in plants as defense agents against environmental stress, such as drought, frost, UV-irradiation.[19] Genetic background e.g. cultivar is the main determinant of PA profiles in plants.[20] Besides the genetic background, environmental factors may also affect the synthesis and accumulation of PAs.[21,22] Light exclusion by shading decreased PA concentration and the proportion of epigallocatechin subunits, as well as the mean degree of polymerization.[23] Gesell et al. verified that MdMYB9 gene encodes a positive regulator of proanthocyanidin synthesis in leaves of 'Royal Gala' apple (*Malus domestica*) in response to light stress treatment. The concentration of PAs in juniper (*Juniperus communis*) needles and sea buckthorn berries (*Hippophaë rhamnoides* ssp. *mongolica*) clearly increased at high latitudes.[20,24] Specific weather conditions in high latitudes, such as high precipitation and moderate humidity were considered to be favorable conditions for elevating the content of PAs, however, the temperature sum and total radiation were negatively correlated with PAs.[25] These abiotic stresses influence the biosynthetic pathways of PAs, controlling the expression of proteins involved in flavonoid transportation and accumulation by multiple regulatory genes, such as transcription factors family genes MYB and MYC.[26,27]

Blackcurrant (*Ribes nigrum* L.) is an important berry crop and raw material for the food industry. Different cultivars of *Ribes nigrum* are widely cultivated in Europe, Asia and the North America. Over 95% of the global production takes place in Europe. In 2017, the European harvested area of currants was 113,514 hectares leading to a total production of

563,657 tons fresh berries, of which blackcurrant dominated.[28] Most blackcurrants are processed into juice concentrates, and the rest is consumed as fresh and frozen berries, as well as extracts for food supplements and fragrance industry. Blackcurrants are considered to have potential health benefits, due to the high content of bioactive components such as vitamin C, anthocyanins, proanthocyanidins and polyunsaturated fatty acids.[29] Blackcurrant has been shown to have a range of bioactivities including anti-inflammatory, anti-oxidant and anti-bacterial effects, as well as the therapeutic potential of cardiovascular and nervous-related diseases.[30,31]

Although the total content of PAs in blackcurrant has been reported to be as high as 400 mg/100 g (Fresh Weight, FW), the limited PAs studies are mostly directed towards total PAs in juices.[2] Only a few reports on PA oligomers in commercial cultivars from North America and Finland could be found, and data on the impact of environmental factors on PA profiles of blackcurrant is still lacking.[17,32] Despite the limited information on PAs, a number of research has been published on impact of environmental factors on most other phenolic compounds, sugars, fruit acids, ascorbic acid, and volatile compounds in blackcurrant.[33-36] Therefore, research related to PAs is necessary for the overall understanding of phenolic metabolites in blackcurrant. In the present study, PAs in three commercial cultivars of Finnish blackcurrant cultivated at two latitudes were studied in a period of 3 to 5 years. The effects of growth latitude and weather conditions on PA content and composition were investigated. The research improves the understanding of the impact of environmental factors on PAs that are important for both the bioactivities and sensory properties of blackcurrants. The research also contributes to creating an overall profile of secondary metabolites in berry crops using blackcurrant as one of the model species. The results provide guidance for breeding and commercial cultivation as well as industrial utilization of blackcurrant.

# MATERIALS AND METHODS

**Plant Materials.** Blackcurrants of three Finnish commercial cultivars 'Mortti', 'Ola' and 'Melalahti' were cultivated in the test fields of LUKE (Natural Resources Institute Finland) located in Piikkiö (southern Finland, 60°23′ N, 22°33′ E) and Apukka (northern Finland, 66°34′ N, 26°01′ E). The cultivar 'Melalahti' is an old landrace from Paltamo (Kajaani, Finland), and 'Mortti' and 'Ola' originated from the crossings ('Wellington XXX' × 'Öjebyn') and ('Wellington XXX' × 'Lepaan musta'), respectively.[37] Twelve bushes of each cultivar were evenly planted into four field blocks and all field blocks in south and north were set up in an identical way. The sample information and harvest date are summarized in **Supplementary Table 1.** Blackcurrants were harvested when optimally ripe based on color, flavor, and structure as determined by experienced horticulturists. The berries were picked randomly from the bushes of each block, mixed well and pooled for each cultivar. Berries were dispensed into a 500 mL sealed plastic box, frozen immediately at −20 °C and stored at −20 °C until analysis. For dry weight (DW) measurement, ca. 5 g of blackcurrants was weighed accurately, cut by scalpel which rinsed by water, dried to a constant weight at 105 °C, cooled in a desiccator, and weighed.

**Sample Preparation and Purification.** Extraction and purification of PAs were modified from the previous method of PA analysis of sea buckthorn.[25] About 7 g of blackcurrants were weighed accurately in duplicate, homogenized with an Ultraturrax T25 at 7000 rpm (IKA, Staufen, Germany), extracted three consecutive times with a 20 mL mixture of acetone, water and acetic acid (80:19.5:0.5, v/v) by sonication for 15 min. After centrifugation at 4420 × $g$ for 10 min, the supernatants were combined and evaporated to remove acetone, the remaining aqueous phase was degreased with petroleum ether (15 mL× 2) and filtered through a 0.20 μm regenerated cellulose (RC) filter (15 mm inner diameter, Phenomenex, Torrance, CA). Then the samples were purified with column chromatography. The size and packings of the column were the same as described in the previous study.[25] The column was eluted sequentially with 200 mL water (Fraction I), 200 mL methanol in water (20:80, v/v, Fraction II), 150 mL acetone in water (70:30, v/v, Fraction III), 100 mL methanol, and 100 mL water. The fractions were evaporated to dryness (40 °C) and re-dissolved in 1 mL methanol for analyses. In order to understand the composition of the purified fractions, the crude extract and fractions were analyzed using a Nexera ultrahigh performance liquid chromatograph with diode array detection (UHPLC-DAD) system (Shimadzu Corporation, Kyoto, Japan). A Phenomenex Aeris peptide XB-C18 (3.6 μm, 150 × 4.60 mm) column was used for separation, the liquid chromatography conditions were the same as used in the previous anthocyanin analyses.[38]

**Proanthocyanidin Analysis.** Identification of blackcurrant PAs was carried out by a method combining hydrophilic interaction liquid chromatography and electrospray ionization mass spectrometry (HILIC–ESI–MS). The instruments and liquid chromatography conditions were the same as described previously.[20] The ESI conditions were as follows: capillary voltage, 3.5 kV; cone voltage, 35 V; extractor voltage, 7 V; source temperature, 120 °C; desolvation temperature, 300 °C. The ESI source was operated in the negative ion mode by scanning from 500 to 1500 *m/z*. Quantitative analysis of PA oligomers and the total PAs was carried out by the HILIC–ESI in selective ion recording mode (SIR) method and spectrophotometric Brunswick Laboratories 4-dimethylaminocinnamaldehyde (BL-DMAC) method, respectively.[20] A commercial procyanidin B2 was used as an external standard. The concentrations of PAs in samples were calculated as procyanidin B2 equivalent and expressed as mg/100g in DW.

**Meteorological Data.** The meteorological data during 2011–2017 were provided by the Finnish Meteorological Institute. Data for Piikkiö and Apukka were recorded at the weather station in Yltöinen (60°23′N, 22°33′E, 6 m) and the weather station at Rovaniemi Airport (66°33′ N, 25°50′ E, 195 m), respectively. The meteorological parameters used in this study and their abbreviations were summarized in **Supplementary Table 2.**

**Statistical Analyses.** All the samples were analysed in duplicate. Statistical analyses and multivariate models were performed using SPSS 16.0.1 (SPSS, Inc., Chicago, IL), JMP Pro 14 (SAS Institute, Cary, NC) and Unscrambler X 10.5 (CAMO Software, Oslo, Norway). The one-way analysis of variance (ANOVA) and independent *t*-test were performed to compare the differences in PAs in different cultivars and growth locations. Partial Least Squares Discriminant Analysis (PLS-DA) was used to explain the PA profiles among the three cultivars (all samples, n=48), and differences between the growth locations within each cultivar samples (n=16). Principal Component Analysis (PCA) was used to investigate the correlations between PA profiles and meteorological data variables. The correlations between specific meteorological variables and PA contents were carried out by bivariate correlation analysis.

## Results and Discussion

**Purification of Proanthocyanidins by Column Chromatography.** The crude extract and elution fractions (Fraction I, Fraction II, and Fraction III) were analyzed by UHPLC-

DAD. The chromatograms of crude extract and Fraction III are presented in **Supplementary Figure 1**. The non-tannin substances were mainly eluted in Fraction I and Fraction II. Fraction I contained primarily low-molecular weight phenolic compounds, carbohydrates and anthocyanins, and Fraction II consisted of mainly partially acylated anthocyanins, flavonols and their glycosides. Practically all PAs were recovered in Fraction III with hardly any traces of PAs detected in other fractions. This is confirmed by the UV absorption spectra, which showed absorption maxima around 280 nm, and no absorption maxima were shown in wavelength exceeding 300 nm.

**Identification of Proanthocyanidins.** The PA fractions (Fraction III) were further analyzed by HILIC–ESI–MS. The total ion spectra within 15.40 min (from 3.80 to 19.20 min, based on the UV absorption spectra) in full-scan spectra were used to recognize the PA oligomers (**Figure 1**). The composition of PA oligomers in blackcurrant extract is summarized in **Table 1**. The $[M - H]^{-}$ ions of PAs with DP from 2 to 5 and $[M - 2H]^{2-}$ ions of PAs with DP 7 were detected by comparing the corresponding exact molecular weight of PAs. All the 17 PA oligomers belonged to B-type PAs with (epi)catechin and/or (epi)gallocatechin as the subunits. No A-type PAs were detected. Three dimers (Dim-1, *m/z* 577.61; Dim-2, *m/z* 593.62; Dim-3, *m/z* 609.71), four trimers (Tri-1, *m/z* 865.48; Tri-2, *m/z* 881.51; Tri-3, *m/z* 897.63; Tri-4, *m/z* 913.72), five tetramers (Tet-1, *m/z* 1153.83; Tet-2, *m/z* 1170.07; Tet-3, *m/z* 1186.19; Tet-4, *m/z* 1202.26; Tet-5, *m/z* 1217.75) and two pentamers (*m/z* 1522 and 1505) were presented as single-charged molecular ions. Three heptamers (*m/z* 1049, 1057 and 1065) were found as double-charged molecular ions. It is worth noting that only two pentamers were detected with low intensity in single-charged ions, and no hexamers or PAs with DP value above 7 were detected in either single-charged or double-charged molecular ions.

**Content and Composition of Proanthocyanidins.** The contents of PA dimers (Dim-1, Dim-2 and Dim-3), trimers (Tri-1, Tri-2, Tri-3 and Tri-4) and tetramers (Tet-1, Tet-2, Tet-3, Tet-4 and Tet-5) were determined by HILIC–ESI–MS-SIR analysis, and the total content of PAs was obtained by BL-DMAC analysis based on colorimetric method. The sum of dimers, trimers and tetramers was considered as the total content PA oligomers, which is summarized in **Table 2** according to cultivars and growth locations. Among all the samples, the content of total PAs, dimers, trimers and tetramers were in the range of 700-2078, 0.10-0.36, 0.11-0.55, 0.05-0.29 mg/100g (DW), respectively. The oligomers quantified in this study accounted only for a small portion (0.02-0.02 %) of the total PAs. Dim-3, Tri-3, Tri-4, Tet-4, Tri-4 and Tet-5 dominated by (epi)gallocatechin presented a high portion in the contents of PA oligomers.

**Comparison among Cultivars.** The content and composition of PAs were compared between the cultivars 'Mortti', 'Ola' and 'Melalahti'. As shown in **Table 2**, 'Ola' had the highest contents of almost all PAs compared to the other two cultivars ($p$ < 0.05). The contents in 'Mortti' and 'Melalahti' were quite similar. Dim-3 and Tet-1 were found higher in 'Melalahti', while the contents of Tri-1 and Tri-2 were higher in 'Mortti' ($p$ < 0.05). A PLS-DA model was created to explain the variation of PA components between the three cultivars (**Figure 2A**). The cultivars were only partially separated, mainly due to the overlapping 'Mortti' and 'Ola' (with two factors, $R^2$ = 0.59, $Q^2$ = 0.53). The first factor sorted out 'Ola', which was located on the right of the scores plot due to the higher content of PAs compared to the other cultivars. 'Melalahti' was well separated from both 'Mortti' and ‘Ola’ along both factor 1 and factor 2 ($Q^2$ value around 0.7), whereas ‘Mortti’ and ‘Ola’ were only partially separated ($Q^2$ value around or below 0.5). Analogous results were described in previous studies on phenolic and volatile compounds in the same cultivars.[33,35] ‘Mortti’ (‘Wellington XXX’ × ‘Öjebyn’) and ‘Ola’ (‘Wellington XXX’ × ‘Lepaan musta’) share the same genetic background from ‘Wellington XXX’, which may explain the common compositional characteristics.

**Comparison between Samples from South and North.** The three black currant cultivars separately farmed in the southern (60°23′ N, 22°33′ E) and northern (66°34′ N, 26°01′ E) Finland were used to study the effects of latitude on the PA profiles. An independent *t*-test was performed to determine any significant differences between the southern and northern samples within each cultivar. As shown in **Table 2**, significant differences were found in all of the three cultivars in the north-south comparison. The content of total PAs was about 1.5 fold higher in ‘Ola’ and ‘Melalahti’ berries from north ($p < 0.05$), while no significant difference was observed in ‘Mortti’. Most of the PA oligomers accumulated more in ‘Mortti’ and ‘Melalahti’ in southern Finland ($p < 0.05$), but less in ‘Melalahti’ in south ($p < 0.05$).

A PLS-DA model was created to discriminate the samples cultivated at the two different latitudes (Y-data, n = 2) using the PA content variables (X-data, n = 17) (**Supplementary Figure 2**). Overall, the three cultivars from different locations were not fully separated along Factors -1 or 2 (three factors, $R^2$ 0.65, $Q^2$ 0.53). The PA content variables located on the upper left corner in the correlation loadings plot corresponded mainly with the northern ‘Ola’ samples with high contents of PAs. Influence of the genetic background of the cultivars was stronger than the impact of growth location, resulting in poor separation in the PLS-DA model within all the cultivars. Based on this, independent PLS-DA models were established within individual cultivars (**Figure 2B, 2C and 2D**). In these models the northern and southern samples were completely separated within each cultivar according to the PA profiles. In 'Mortti', the southern berries located mainly on the right of the scores plot along Factor-1 (with two factors; $Q^2$ 0.84), were characterized by higher levels of all PAs excluding total PAs and Tet-5, which were close to the central axis (**Figure 2B**). However, the opposite situation occurred in 'Ola' (with two factors; $Q^2$ 0.78), where the northern samples mainly located on the right of the plot containing higher levels of PAs (excluding Tet-1 and Tri-2) (**Figure 2C**). 'Melalahti' samples cultivated at different locations were greatly separated along Factor-1 with one validated factor ($Q^2$ 0.91). The northern and southern berries were characterized by high levels of total PAs and PA oligomers, respectively (**Figure 2D**).

The impact of latitude on the flavonoid contents has been well summarized in a variety of plants.[21] Content of anthocyanins in bilberry (*Vaccinium myrtillus*), bog bilberry (*Vaccinium uliginosum*) and pomegranate (*Punica granatum*)[39-42], flavonol glycosides in sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) and bog bilberriy[39,43], phenolic compounds in juniper needles (*Juniperus communis*) [24] and total PAs in sea buckthorn (ssp. *rhamnoides*) [25] were found to be higher in samples from higher latitude. These results were consistent with current results concerning the total PAs and oligomeric PAs in 'Ola', and the total PAs in 'Melalahti'. However, in our previous studies with the same cultivars of blackcurrant, all the cultivars grown at higher latitude (northern Finland) had lower contents of total flavonols, total anthocyanins, and total phenolic compounds.[35] In the current study, low levels of oligomeric PAs were also found in 'Mortti' and 'Melalahti' cultivated at higher latitude. Similar results were obtained for the contents of PA oligomer in sea buckthorn of varieties 'Terhi' and 'Tytti' cultivated in southern and northern Finland.[20] Flavonols, anthocyanins and PAs share the same upstream pathways in flavonoid biosynthesis using the same key enzymes for coordinated expression, hence, it might be speculated that these compounds may be influenced by growth latitude in a similar manner. Unlike these flavonoids, high latitude has been shown to promote accumulation of volatiles, malic acid, quinic acid, and vitamin C in blackcurrants.[33,36] It is worth to notice that the studies described above were all based on the northern hemisphere, especially in the Nordic countries.

**Effects of Weather Conditions on PA Composition.** The results of the previous section indicated that the accumulation of PAs was affected by the growth locations. The distance between the two growth locations in southern and northern Finland is around 700 km with latitude difference between 60°N in the south and 66°N in the north, which will cause changes in weather conditions. To further explain the correlation between PAs profiles and weather conditions, selected meteorological parameters (related to the parameters of temperature, radiation, precipitation and humidity) were recorded (**Supplementary Table 2**) and used for multivariate analysis.

PCA models were created to investigate correlations between the PA contents (n=17) of cultivars (n=24×2) and the meteorological variables (n=95). In the loadings plots, variables closed to each other and located between the ellipses were considered as having a positive correlation, while those located distant from each other were considered as being negatively correlated. In PCA models including all three cultivars (not shown), all the PA variables were located around 'Ola' indicating positive correlations. However, they did not correlate well with the weather variables. The situation was similar to the results in latitude comparison due to the high content of PAs in ‘Ola’, again indicating the decisive role of the genetic background in PAs accumulation in the berries.

In order to exclude the influence of the genetic background, separated PCA models (temperature, n=39; radiation, n=12; precipitation, n=12 and humidity, n=32) were created for individual cultivars (**Figure 3A, 3B and 3C**). In the model of ‘Mortti’ (**Figure 3A**), all PA variables were grouped around the meteorological variables of southern Finland with positive correlations, but comparing with PA oligomers, total PAs were closer to central axis with lower correlation. In the loadings plots, the variables of temperature near 2017 (MaxiTJul, TJul, MaxiTApr and SUMTgs) associated positively with all PA variables along PC-2. PA dimers located close to most radiation variables on the lower side of the plot on the PC-2, whereas total PAs and other PA oligomers were on the opposite side of the component close to Radiation in growth season (SUMRMay and SUMRJun). Most PA variables showed positive correlation with precipitation in the last week before harvest (PreW) and humidity in June (HuJun) along the first component (50% and 45%), but correlated negatively with precipitation and days of medium humidity (DHu30to40gh and DHu40to50gh) during the period from May to July. In the models of ‘Ola’ (**Figure 3B**), almost all PA variables (except Tet-1) associated positively with the meteorological variables near 2017, which were characterized by low temperature, low radiation, low precipitation, and medium humidity. Tet-1, again, located at the central axis along both PC-1 and PC-2 with lower correlations with the weather variables studied. In the loadings plot of radiation, total PAs, Tet-5 and Tri-1 located on the lower side of the plot along PC-2 and showed less dependence on the radiation variables. In the PCA model of ‘Melalahti’ are shown in **Figure 3C**. The most PA oligomers located on the right side of the plots and associated positively with the southern meteorological variables, which were characterized by high temperature, high radiation, precipitation in February and before harvest and medium humidity. However, the total PAs located on the opposite side of the plots along PC-1**,** and correlated negatively with the previous meteorological variables.

Overall, PAs in the three cultivars showed different responses to the weather conditions, due to the difference in genetic backgrounds. Most PAs in 'Mortti' showed a positive correlation with meteorological variables in southern Finland, while PA variables in 'Ola' were negatively correlated with them. In 'Melalahti', total PAs and PA oligomers were positively correlated with the meteorological variables in northern and southern Finland, respectively.

In order to understand the common correlation between PAs and weather variables in all the cultivars (n=24×2) and meteorological variables (n=95), bivariate correlation analysis was applied (**Supplementary Table 4**). The total PAs and Tet-2 showed negative correlation with most of the meteorological variables, especially with temperature and radiation-related variables, while Dim-1 showed positive correlation. Most of the PA oligomeric variables correlated positively to humidity during last week before harvest (Huweek) and negatively to radiation sum during growth season until harvest (ΣTgh) (significant at the 0.01 or 0.05 level). Dim-3 showed no correlation with any meteorological variables. The remaining PA variables were related to specific meteorological variables. It is worth noting that PA oligomers related only to a few meteorological variables compared to total PAs.

Among the 95 meteorological variables, twelve meteorological variables, (HDgh, ΣTMon, TMon, MaxTmon, TAug, TSep, PreFeb, PreMar, ΣRJan,ΣRSep, ΣRMon and DHu30to40gh) were selected to further verify the correlation with total PAs using modeling types determine analysis (**Figure 4**). In correlation analysis, almost all points were within the 0.95 bivariate normal ellipse (Green lines), and the two variables were significantly correlated at a 95% confidence level. Number of hot days during the growth season (HDgh), high temperature of the last month before harvest (ΣTMon, TMon and MaxTmon), temperature in last two months before harvest (TAug, TSep), radiation sum of January, September, and the last month before harvest (ΣRJan, ΣRSep and ΣRMon), and precipitation in February showed significant negative correlations with the content of total PAs ($R^2$ from 0.185 to 0.336, $p < 0.01$). However, the total content of PAs showed a positive correlation with PreMar and DHu30to40gh ($R^2$ 0.182 and 0.221, respectively, $p < 0.01$).

Typically, environmental factors such as weather and soil conditions influence production of the secondary metabolites in plants. As a part of the plant defense mechanism, secondary metabolites are self-regulated against environmental stress such as low temperature and drought. Plants have a higher photosynthetic rate at lower temperatures to provide more carbon sources for synthesizing secondary metabolites.[44] Moreover, some key enzymes of flavonoids biosynthesis such as PAL (phenylalanine ammonia lyase), C4H (cinnamate 4-hydroxylase), CHS (chalcone synthase) and FLS (flavonol synthase), of which gene expression was increased in a variety of plants under low temperature stress.[45-47] This can partly explain the negative correlation between total PAs content and temperature in the current study. Light conditions at high latitudes are characterized by high level of UV radiation, long day-time but low total solar irradiance. Yang et al. have reported that the major phenolic compounds of three currant cultivars correlate negatively with high radiation,[48] which is in agreement with the results in the current study. Furthermore, also long photoperiod has been shown to promote the accumulation of anthocyanins and flavonols in blackcurrants.[49] Generally, plants exposed to high UV radiation, especially to UV-B, are susceptible to DNA damages. One of the protective mechanisms is to synthesize UV-absorbing pigments such as flavonoids in the vacuoles of the epidermal cell layers to minimize penetration of UV-B to the deeper photosynthetically active cell layers.[50] Research on the effects of precipitation and humidity on metabolites in currants has been limited. In previous studies of our group, the contents of sugars and acids were negatively associated with low humidity variables in blackcurrant. Vitamin C and *p*-coumaroylquinic acid, again, were positively correlated with these variables.[36,48] Lower contents of anthocyanins in 'Red Dutch' as well as flavonol glycosides and hydroxycinnamic acid conjugates in 'White Dutch' were associated with Precipitation in March.[35] High humidity variables around harvest appeared to be positively related to volatile substances in blackcurrant.[33] In the current study, total PAs showed positive correlation with precipitation in March, lower humidity during the growth season (DHu30to40gh and DHu40to50gh). The correlation of metabolite in currants with precipitation and humidity variables is less significant compared to their association with temperature and radiation.

As a summary, seventeen PA oligomers values of DP 2 -5 and 7 were detected in blackcurrants of three Finnish commercial cultivars 'Mortti', 'Ola' and 'Melalahti', which belonged to B-type PAs with (epi)catechin and/or (epi)gallocatechin as subunits, and no

A-type PAs was detected. PA dimers, trimers, tetramers and the total PA were quantified and calculated as procyanidin B2 equivalent. Genetic background and growth environment influenced the PA content and composition in blackcurrants. Among the three cultivars, ‘Ola’ had the highest contents of both total PA and PA oligomers. The content of total PAs of ‘Ola’ and ‘Melalahti’ correlated positively to latitude, while no significant correlation was observed in ‘Mortti’. ‘Mortti’ and ‘Melalahti’ cultivated at lower latitude had higher levels of PA oligomers, but ‘Melalahti’ from southern Finland contained less. Although the genetic background determined that the three cultivars showed different response to weather conditions, total PAs correlated negatively with high temperature and radiation. Most PA oligomers showed correlation with specific variables.

## Acknowledgment

We are grateful to Professor Emeritus Risto Tahvonen; Kati Hoppula, M.Sc.; research technicians Alpo Heinonen, Jorma Hellsten, and Kaisa Soppela; laboratory technician Sari Välitalo; and trainee Juho Haveri-Heikkilä at the Natural Resources Institute Finland (Luke) for providing the blackcurrant, Ella Aitta for her contribution to samples preparation, and Bingyu Sun for her contribution to cover art.

## Supporting Information Description:

**Supplementary Table 1.** Information of Blackcurrant Samples

**Supplementary Table 2.** The Abbreviations of Meteorological Parameters.

**Supplementary Table 3.** Correlation Coefficients between PA composition and Meteorological Variables.

**Supplementary Figure 1.** The chromatograms of crude extract of blackcurrant and Sephadex-purified Fraction III measured at 280 nm, 360 nm and 520 nm.

**Supplementary Figure 2.** PLS-DA models for all the three cultivars ($n = 24 \times 2$) classified according to growth latitude (South samples, Violet icons; North samples, Brown icons) with the PA contents (variables; $n = 17$). Abbreviations of the compounds refer to Table 1.

## References


1. Huemmer, W.; Schreier, P. Analysis of proanthocyanidins. *Mol Nutr Food Res* **2008,** *52,* 1381-1398.

2. Hellström, J.K.; Törrönen, A.R.; Mattila, P.H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009,** *57,* 7899-7906.

3. Gu, L.; Kelm, M.A.; Hammerstone, J.F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R.L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003,** *51,* 7513-7521.

4. Gu, L.; Kelm, M.A.; Hammerstone, J.F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R.L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004,** *134,* 613-617.

5. Mierziak, J.; Kostyn, K.; Kulma, A. Flavonoids as important molecules of plant interactions with the environment. *Molecules* **2014,** *19,* 16240-16265.

6. Lepiniec, L.; Debeaujon, I.; Routaboul, J.; Baudry, A.; Pourcel, L.; Nesi, N.; Caboche, M. Genetics and biochemistry of seed flavonoids. *Annu.Rev.Plant Biol.* **2006,** *57,* 405-430.

7. Debeaujon, I.; Leon-Kloosterziel, K.M.; Koornneef, M. Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. *Plant Physiol.* **2000,** *122,* 403-414.

8. Smeriglio, A.; Barreca, D.; Bellocco, E.; Trombetta, D. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *Br. J. Pharmacol.* **2017,** *174,* 1244-1262.

9. Daglia, M. Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* **2012,** *23,* 174-181.

10. Bladé, C.; Aragonès, G.; Arola ‐ Arnal, A.; Muguerza, B.; Bravo, F.I.; Salvadó, M.J.; Arola, L.; Suárez, M. Proanthocyanidins in health and disease. *Biofactors* **2016,** *42,* 5-12.

11. Nunes, M.A.; Pimentel, F.; Costa, A.S.; Alves, R.C.; Oliveira, M.B.P. Cardioprotective properties of grape seed proanthocyanidins: An update. *Trends Food Sci. Technol.* **2016,** *57,* 31-39.

12. Zamora-Ros, R.; Forouhi, N.G.; Sharp, S.J.; Gonzalez, C.A.; Buijsse, B.; Guevara, M.; van der Schouw, Y.T.; Amiano, P.; Boeing, H.; Bredsdorff, L.; Fagherazzi, G.; Feskens, E.J.; Franks, P.W.; Grioni, S.; Katzke, V.; Key, T.J.; Khaw, K.T.; Kuhn, T.; Masala, G.; Mattiello, A.; Molina-Montes, E.; Nilsson, P.M.; Overvad, K.; Perquier, F.; Redondo, M.L.; Ricceri, F.; Rolandsson, O.; Romieu, I.; Roswall, N.; Scalbert, A.; Schulze, M.; Slimani, N.; Spijkerman, A.M.; Tjonneland, A.; Tormo, M.J.; Touillaud, M.; Tumino, R.; van der, A.D.L.; van Woudenbergh, G.J.; Langenberg, C.; Riboli, E.; Wareham, N.J. Dietary

intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations. *J. Nutr.* **2014,** *144,* 335-343.

13. Ou, K.; Gu, L. Absorption and metabolism of proanthocyanidins. *J. Funct. Foods* **2014,** *7,* 43-53.

14. Casassa, L.F.; Harbertson, J.F. Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *Annu Rev Food Sci Technol.* **2014,** *5,* 83-109.

15. Peleg, H.; Gacon, K.; Schlich, P.; Noble, A.C. Bitterness and astringency of flavan‐3‐ol monomers, dimers and trimers. *J. Sci. Food Agric.* **1999,** *79,* 1123-1128.

16. Cala, O.; Pinaud, N.; Simon, C.; Fouquet, E.; Laguerre, M.; Dufourc, E.J.; Pianet, I. NMR and molecular modeling of wine tannins binding to saliva proteins: revisiting astringency from molecular and colloidal prospects. *FASEB J.* **2010,** *24,* 4281-4290.

17. Laaksonen, O.; Salminen, J.; Mäkilä, L.; Kallio, H.; Yang, B. Proanthocyanidins and their contribution to sensory attributes of blackcurrant juices. *J. Agric. Food Chem.* **2015,** *63,*5373-5380.

18. Soares, S.; Mateus, N.; De Freitas, V. Interaction of different polyphenols with bovine serum albumin (BSA) and human salivary *α*-amylase (HSA) by fluorescence quenching. *J. Agric. Food Chem.* **2007,** *55,* 6726-6735.

19. Treutter, D. Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* **2006,** *4,* 147-157.

20. Yang, W.; Laaksonen, O.; Kallio, H.; Yang, B. Proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins with special reference to influence of genetic background and growth location. *J. Agric. Food Chem.* **2016,** *64,* 1274-1282.

21. Jaakola, L.; Hohtola, A. Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell Environ.* **2010,** *33,* 1239-1247.

22. Winkel-Shirley, B. Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol.* **2002,** *5,* 218-223.

23. Koyama, K.; Ikeda, H.; Poudel, P.R.; Goto-Yamamoto, N. Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. *Phytochemistry* **2012,** *78,* 54-64.

24. Martz, F.; Peltola, R.; Fontanay, S.; Duval, R.E.; Julkunen-Tiitto, R.; Stark, S. Effect of latitude and altitude on the terpenoid and soluble phenolic composition of juniper (*Juniperus communis*) needles and evaluation of their antibacterial activity in the boreal zone. *J. Agric. Food Chem.* **2009,** *57,* 9575-9584.

25. Yang, W.; Laaksonen, O.; Kallio, H.; Yang, B. Effects of latitude and weather conditions on proanthocyanidins in berries of Finnish wild and cultivated sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*). *Food Chem.* **2017,** *216,* 87-96.

26. He, F.; Pan, Q.; Shi, Y.; Duan, C. Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules* **2008,** *13,* 2674-2703.

27. Ambawat, S.; Sharma, P.; Yadav, N.R.; Yadav, R.C. MYB transcription factor genes as regulators for plant responses: an overview. *Physiol Mol Biol Pla.* **2013,** *19,* 307-321.

28. FAOSTAT Database Results. http://www.fao.org. **2017**

29. Cyboran, S.; Bonarska-Kujawa, D.; Pruchnik, H.; Żyłka, R.; Oszmiański, J.; Kleszczyńska, H. Phenolic content and biological activity of extracts of blackcurrant fruit and leaves. *Food Res. Int.* **2014,** *65,* 47-58.

30. Gopalan, A.; Reuben, S.C.; Ahmed, S.; Darvesh, A.S.; Hohmann, J.; Bishayee, A. The health benefits of blackcurrants. *Food & function* **2012,** *3,* 795-809.

31. Karjalainen, R.; Anttonen, M.; Saviranta, N.; Stewart, D.; McDougall, G.; Hilz, H.; Mattila, P.; Törrönen, R. A review on bioactive compounds in black currants (*Ribes nigrum* L.) and their potential health-promoting properties. *Acta Hortic.* **2008,** 301-307.

32. Wu, X.; Gu, L.; Prior, R.L.; McKay, S. Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. *J. Agric. Food Chem.* **2004,** *52,* 7846-7856.

33. Marsol-Vall, A.; Kortesniemi, M.; Karhu, S.T.; Kallio, H.; Yang, B. Profiles of volatile compounds in blackcurrant (*Ribes nigrum*) cultivars with a special focus on the influence of growth latitude and weather conditions. *J. Agric. Food Chem.* **2018,** *66,* 7485-7495.

34. Vagiri, M.; Ekholm, A.; Öberg, E.; Johansson, E.; Andersson, S.C.; Rumpunen, K. Phenols and ascorbic acid in black currants (*Ribes nigrum* L.): Variation due to genotype, location, and year. *J. Agric. Food Chem.* **2013,** *61,* 9298-9306.

35. Zheng, J.; Yang, B.; Ruusunen, V.; Laaksonen, O.; Tahvonen, R.; Hellsten, J.; Kallio, H. Compositional differences of phenolic compounds between black currant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. *J. Agric. Food Chem.* **2012,** *60,* 6581-6593.

36. Zheng, J.; Yang, B.; Tuomasjukka, S.; Ou, S.; Kallio, H. Effects of latitude and weather conditions on contents of sugars, fruit acids, and ascorbic acid in black currant (*Ribes nigrum* L.) juice. *J. Agric. Food Chem.* **2009,** *57,* 2977-2987.

37. Aaltonen, M.; Antonius, K.; Hietaranta, T.; Karhu, S.; Kinnanen, H.; Kivijärvi, P.; Nukari, A.; Sahramaa, M.; Tahvonen, R.; Uosukainen, M. Suomen kansallisten

kasvigeenivarojen pitkäaikaissäilytysohjeet: hedelmä-ja marjakasvit. **2006,** Myynti Maa- ja elintarviketalouden tutkimuskeskus(MTT) https://jukuri.luke.fi/handle/10024/463545

38. Yang, W.; Kortesniemi, M.; Ma, X.; Zheng, J.; Yang, B. Enzymatic acylation of blackcurrant (*Ribes nigrum*) anthocyanins and evaluation of lipophilic properties and antioxidant capacity of derivatives. *Food Chem.* **2019,** *281,* 189-196.

39. Latti, A.K.; Jaakola, L.; Riihinen, K.R.; Kainulainen, P.S. Anthocyanin and flavonol variation in bog bilberries (*Vaccinium uliginosum* L.) in Finland. *J. Agric. Food Chem.* **2009,** *58,* 427-433.

40. Lätti, A.K.; Riihinen, K.R.; Kainulainen, P.S. Analysis of anthocyanin variation in wild populations of bilberry (*Vaccinium myrtillus* L.) in Finland. *J. Agric. Food Chem.* **2008,** *56,* 190-196.

41. Åkerström, A.; Jaakola, L.; Bång, U.; Jäderlund, A. Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of *Vaccinium myrtillus* L.(bilberries). *J. Agric. Food Chem.* **2010,** *58,* 11939-11945.

42. Borochov-Neori, H.; Judeinstein, S.; Harari, M.; Bar-Ya'akov, I.; Patil, B.S.; Lurie, S.; Holland, D. Climate effects on anthocyanin accumulation and composition in the pomegranate (*Punica granatum* L.) fruit arils. *J. Agric. Food Chem.* **2011,** *59,* 5325-5334.

43. Ma, X.; Laaksonen, O.; Zheng, J.; Yang, W.; Trépanier, M.; Kallio, H.; Yang, B. Flavonol glycosides in berries of two major subspecies of sea buckthorn (*Hippophaë rhamnoides* L.) and influence of growth sites. *Food Chem.* **2016,** *200,* 189-198.

44. Zobayed, S.; Afreen, F.; Kozai, T. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiol Biochem* **2005,** *43,* 977-984.

45. Wang, G.; Cao, F.; Wang, G.; El-Kassaby, Y.A. Role of Temperature and Soil Moisture Conditions on Flavonoid Production and Biosynthesis-Related Genes in Ginkgo (*Ginkgo biloba* L.) Leaves. *Nat Prod Chem Res* **2015,** *3:1.*

46. Azuma, A.; Yakushiji, H.; Koshita, Y.; Kobayashi, S. Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* **2012,** *236,* 1067-1080.

47. Wang, Z.; Sha, Y.; Yu, X.; Liang, Y. Determination of flavonol glycosides in tea samples by ultra-high performance liquid chromatography-photodiode array detection-tandem mass spectrometry. *Se Pu* **2015,** *33,* 974-980.

48. Yang, B.; Zheng, J.; Laaksonen, O.; Tahvonen, R.; Kallio, H. Effects of latitude and weather conditions on phenolic compounds in currant (*Ribes* spp.) cultivars. *J. Agric. Food Chem.* **2013,** *61,* 3517-3532.

49. Woznicki, T.L.; Aaby, K.; Sønsteby, A.; Heide, O.M.; Wold, A.; Remberg, S.F. Influence of controlled post-flowering temperature and daylength on individual phenolic compounds in four black currant cultivars. *J. Agric. Food Chem.* **2016,** *64*,752-761.

50. Hideg, É; Strid, Å The effects of UV-B on the biochemistry and metabolism in plants. *UV-B Radiation and Plant Life: Molecular Biology to Ecology.CAB International* **2017,** 90-110.

## Figure captions

**Figure 1.** Total ion spectrum of purified PAs fraction (Fraction III) within 15.40 min operated by HILIC-ESI-MS.

**Figure 2.** PLS-DA models for (A) all the three cultivars (n = 24 × 2) classified according to cultivars (‘Melalahti’, green triangles; ‘Ola’, red circles; and ‘Mortti’ blue squares); (B) ‘Mortti’ (n = 8 × 2), (C) ‘Ola’ (n = 8 × 2) and (D) ‘Melalahti’ (n = 8 × 2) classified according to growth latitude (‘Mortti’, squares; ‘Ola’, circles; ‘Melalahti’, triangles; North samples, Violet icons; South samples, Brown icons) with the PA contents (variables; n = 17). Abbreviations of the compounds refer to Table 1.

**Figure 3**. PCA plots of the correlations between weather conditions and combined PA contents in (A) ‘Mortti’, (B) ‘Ola’ and (C) ‘Melalahti’. Abbreviations of the compounds refer to Table 1 and the meteorological parameters refer to Supplementary Table 2.

**Figure 4.** The correlation between twelve meteorological variables (HDgh, ΣTMon, TMon, MaxTmon, TAug, TSep, PreFeb, PreMar, ΣRJan, ΣRSep, ΣRMon and DHu30to40gh) and the contents of total PAs. Abbreviations of the meteorological parameters refer to Supplementary Table 2.

**Table 1.** The composition of PA oligomers in blackcurrant extract obtained by HILIC-ESI.

| DP [a] | Abbr. | Molecular formula | Number of subunits [b] | | Exact mass | Detected mass | |
|---|---|---|---|---|---|---|---|
| | | | (E)C | (E)GC | | $[M-H]^-$ | $[M-2H]^{2-}$ |
| 2 | Dim-1 | $C_{30}H_{26}O_{12}$ | 2 | 0 | 578.14 | 577.61 | |
| 2 | Dim-2 | $C_{30}H_{26}O_{13}$ | 1 | 1 | 594.14 | 593.62 | |
| 2 | Dim-3 | $C_{30}H_{26}O_{14}$ | 0 | 2 | 610.13 | 609.71 | |
| 3 | Tri-1 | $C_{45}H_{38}O_{18}$ | 3 | 0 | 866.21 | 865.48 | |
| 3 | Tri-2 | $C_{45}H_{38}O_{19}$ | 2 | 1 | 882.20 | 881.51 | |
| 3 | Tri-3 | $C_{45}H_{38}O_{20}$ | 1 | 2 | 898.20 | 897.63 | |
| 3 | Tri-4 | $C_{45}H_{38}O_{21}$ | 0 | 3 | 914.19 | 913.72 | |
| 4 | Tet-1 | $C_{60}H_{50}O_{24}$ | 4 | 0 | 1154.27 | 1153.83 | |
| 4 | Tet-2 | $C_{60}H_{50}O_{25}$ | 3 | 1 | 1170.26 | 1170.07 | |
| 4 | Tet-3 | $C_{60}H_{50}O_{26}$ | 2 | 2 | 1186.25 | 1186.19 | |
| 4 | Tet-4 | $C_{60}H_{50}O_{27}$ | 1 | 3 | 1202.25 | 1202.26 | |
| 4 | Tet-5 | $C_{60}H_{50}O_{28}$ | 0 | 4 | 1218.25 | 1217.75 | |
| 5 | | $C_{75}H_{62}O_{34}$ | 1 | 4 | 1505.31 | 1505.29 | |
| 5 | | $C_{75}H_{62}O_{35}$ | 0 | 5 | 1522.31 | 1522.41 | |
| 7 | | $C_{105}H_{86}O_{47}$ | 2 | 5 | 2098.43 | | 1049.41 |
| 7 | | $C_{105}H_{86}O_{48}$ | 1 | 6 | 2114.43 | | 1057.09 |
| 7 | | $C_{105}H_{86}O_{49}$ | 0 | 7 | 2130.42 | | 1065.33 |

[a]DP=degree of polymerization; [b] (E)C=(epi)catechin, (E)GC=(epi)gallocatechin.

**Table 2.** Proanthocyanidin contents in blackcurrant (mean ± standard deviation, mg/100 g DW).

| Samples | Dim-1 | Dim2 | Dim-3 | Tri-1 | Tri-2 | Tri-3 | Tri-4 | Tet-1 | Tet-2 | Tet-3 | Tet-4 | Tet-5 | Dimers | Trimers | Tetramers | PA oligomers* | Total PAs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Comparison of cultivars*** | | | | | | | | | | | | | | | | | |
| Mortti (n=8×2) | 0.01±0.00a | 0.03±0.01a | 0.12±0.04a | 0.02±0.01b | 0.05±0.02b | 0.09±0.03a | 0.09±0.02a | 0.005±0.00b | 0.01±0.01a | 0.02±0.00a | 0.03±0.01a | 0.04±0.01a | 0.16±0.05a | 0.25±0.07a | 0.11±0.03a | 0.52±0.14a | 1173.34±258.6a |
| Ola (n=16) | 0.02±0.01b | 0.04±0.01b | 0.21±0.05c | 0.03±0.01c | 0.06±0.02b | 0.16±0.05b | 0.16±0.04c | 0.006±0.00b | 0.03±0.01b | 0.05±0.01b | 0.06±0.02b | 0.06±0.02b | 0.27±0.06b | 0.42±0.1b | 0.21±0.05b | 0.89±0.20b | 1465.05±372.17b |
| Melalahti (n=8×2) | 0.01±0.01a | 0.02±0.01a | 0.15±0.04b | 0.01±0.01a | 0.03±0.01a | 0.07±0.02a | 0.12±0.05b | 0.002±0.00a | 0.02±0.02a | 0.02±0.02a | 0.04±0.02a | 0.04±0.01a | 0.19±0.05a | 0.23±0.08a | 0.11±0.04a | 0.53±0.15a | 1072.24±288.32a |
| ***Comparison of cultivar 'Mortti'*** | | | | | | | | | | | | | | | | | |
| South (n=5×2) | 0.01±0.00b | 0.03±0.01b | 0.14±0.03b | 0.02±0.01 | 0.06±0.02 | 0.1±0.02b | 0.1±0.02b | 0.01±0.00b | 0.02±0.01b | 0.02±0.00b | 0.04±0.01b | 0.04±0.01 | 0.19±0.03b | 0.28±0.06b | 0.12±0.03b | 0.60±0.10b | 1183.51±320.92 |
| North (n=3×2) | 0.01±0.00a | 0.02±0a | 0.09±0.01a | 0.01±0.00 | 0.04±0.02 | 0.07±0.02a | 0.07±0.01a | 0.005±0.00a | 0.01±0.00a | 0.02±0.00a | 0.02±0.00a | 0.03±0.02 | 0.11±0.01a | 0.19±0.05a | 0.09±0.02a | 0.39±0.08a | 1156.37±121.20 |
| ***Comparison of cultivar 'Ola'*** | | | | | | | | | | | | | | | | | |
| South (n=5×2) | 0.02±0.01 | 0.04±0.01 | 0.19±0.04a | 0.02±0.00a | 0.06±0.02 | 0.14±0.04a | 0.15±0.04 | 0.01±0.00 | 0.02±0.01a | 0.04±0.01a | 0.05±0.02a | 0.05±0.01a | 0.25±0.06a | 0.38±0.09 | 0.17±0.04a | 0.80±0.17a | 1215.49±154.92a |
| North (n=3×2) | 0.02±0.00 | 0.05±0.01 | 0.25±0.02b | 0.03±0.01b | 0.06±0.03 | 0.2±0.03b | 0.18±0.05 | 0.01±0.00 | 0.03±0.01b | 0.06±0.01b | 0.08±0.02b | 0.08±0.01b | 0.31±0.03b | 0.47±0.10 | 0.26±0.03b | 1.05±0.15b | 1880.99±200.37b |
| ***Comparison of cultivar 'Melalahti'*** | | | | | | | | | | | | | | | | | |
| South (n=5×2) | 0.02±0.00b | 0.03±0.00b | 0.18±0.04b | 0.01±0.01b | 0.04±0.00b | 0.08±0.01b | 0.15±0.04b | 0.002±0.00b | 0.01±0.00 | 0.03±0.02b | 0.04±0.02b | 0.04±0.01b | 0.22±0.04b | 0.29±0.02b | 0.13±0.04b | 0.64±0.04b | 912.14±75.01a |
| North (n=3×2) | 0.004±0.00a | 0.01±0.00a | 0.11±0.01a | 0.01±0.00a | 0.02±0.01a | 0.04±0.01a | 0.07±0.01a | 0.001±0.00a | 0.03±0.03 | 0.01±0a | 0.02±0a | 0.03±0.01a | 0.13±0.01a | 0.14±0.02a | 0.09±0.03a | 0.35±0.05a | 1339.09±320.23b |

Significant differences ($p < 0.05$) are marked as a, b; *PA oligomers are sums of Dimers, Trimers and Tetramers.

Fig.1

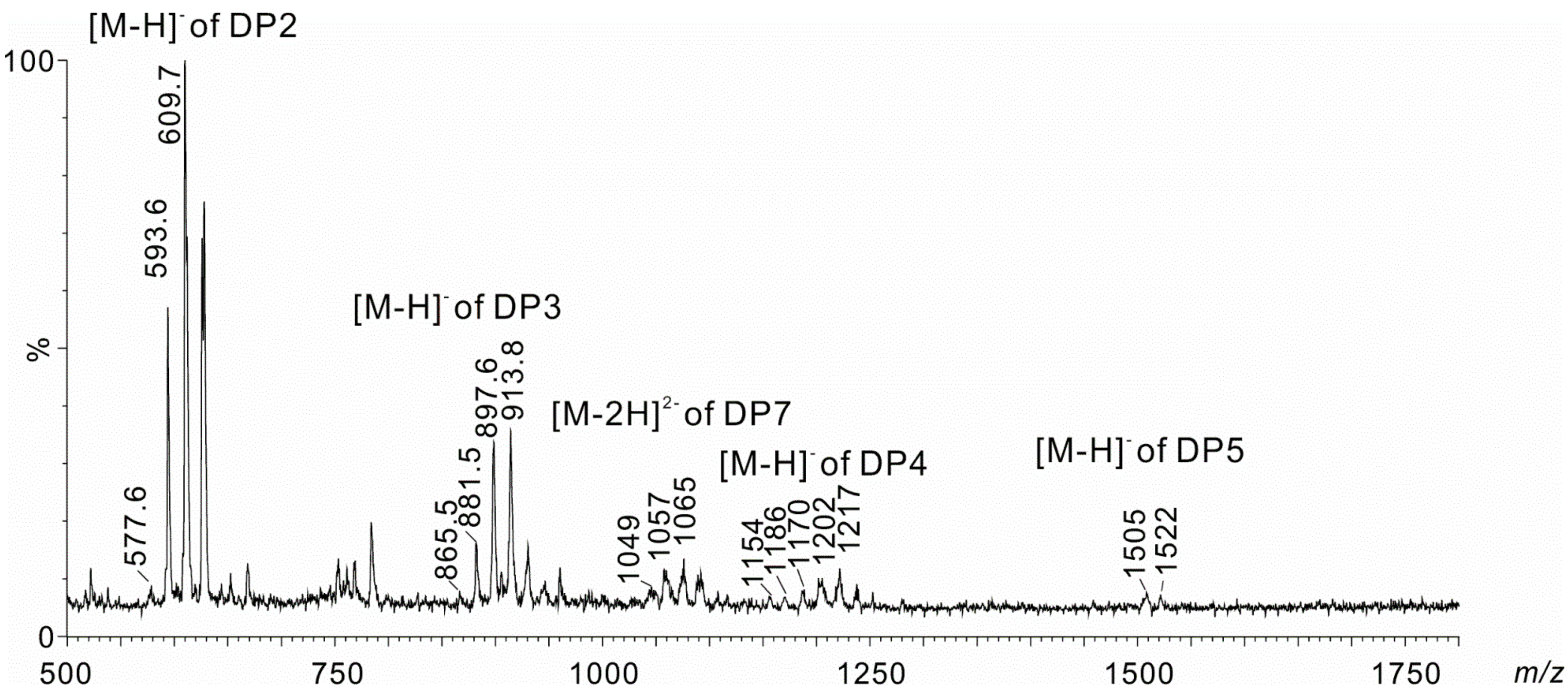
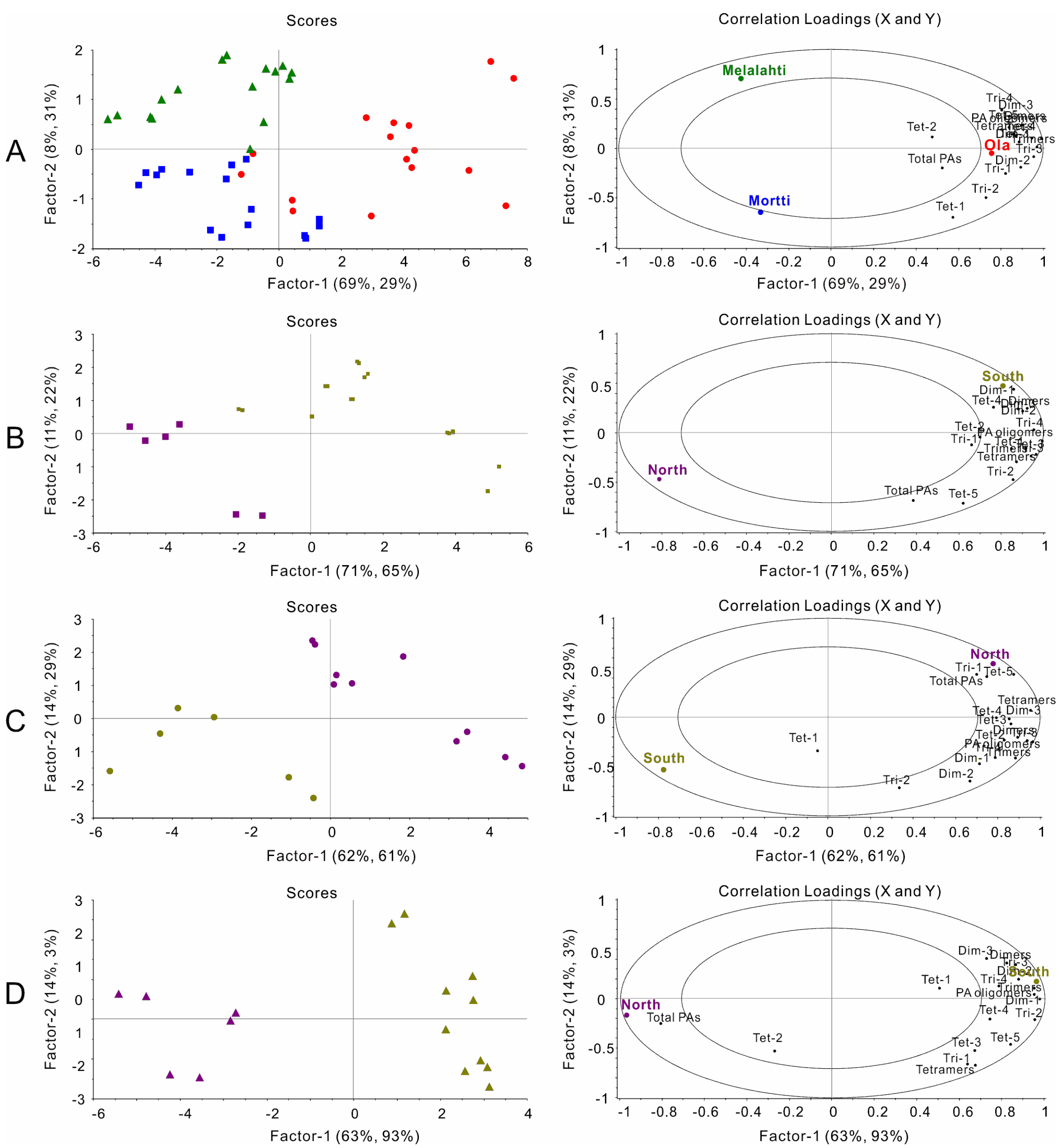

[M-H]- of DP2
[M-H]- of DP3
[M-2H]2- of DP7
[M-H]- of DP4
[M-H]- of DP5
609.7
593.6
577.6
913.8
897.6
881.5
865.5
1049
1057
1065
1154
1186
1170
1202
1217
1505
1522
100
%
0
500
750
1000
1250
1500
1750
m/z

Fig.2



Scores
Correlation Loadings (X and Y)
A
Factor-2 (8%, 31%)
Factor-1 (69%, 29%)
Melalahti
Mortti
Ola
B
Factor-2 (11%, 22%)
Factor-1 (71%, 65%)
South
North
C
Factor-2 (14%, 29%)
Factor-1 (62%, 61%)
North
South
D
Factor-2 (14%, 3%)
Factor-1 (63%, 93%)
North
South

Fig.3

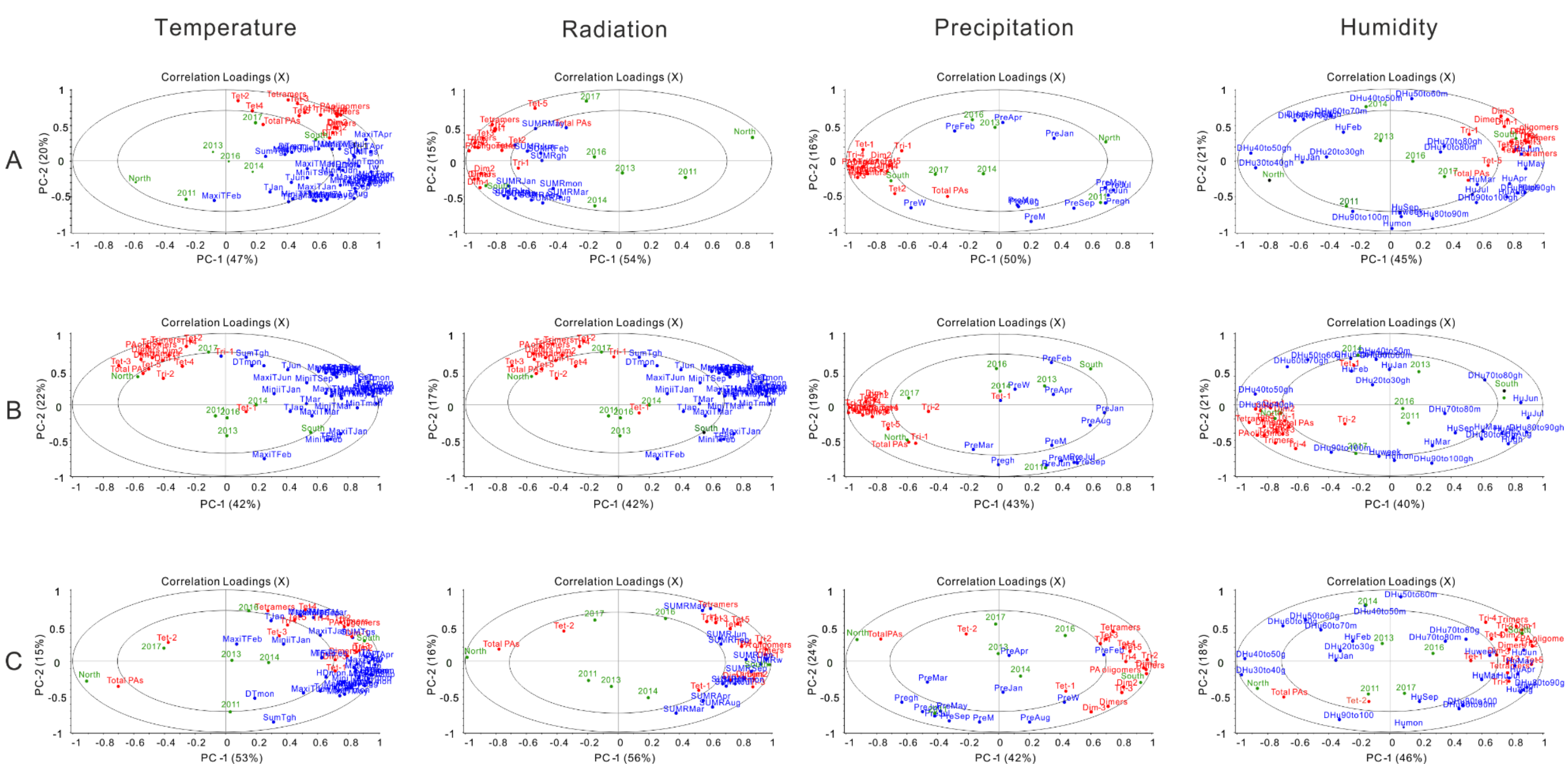
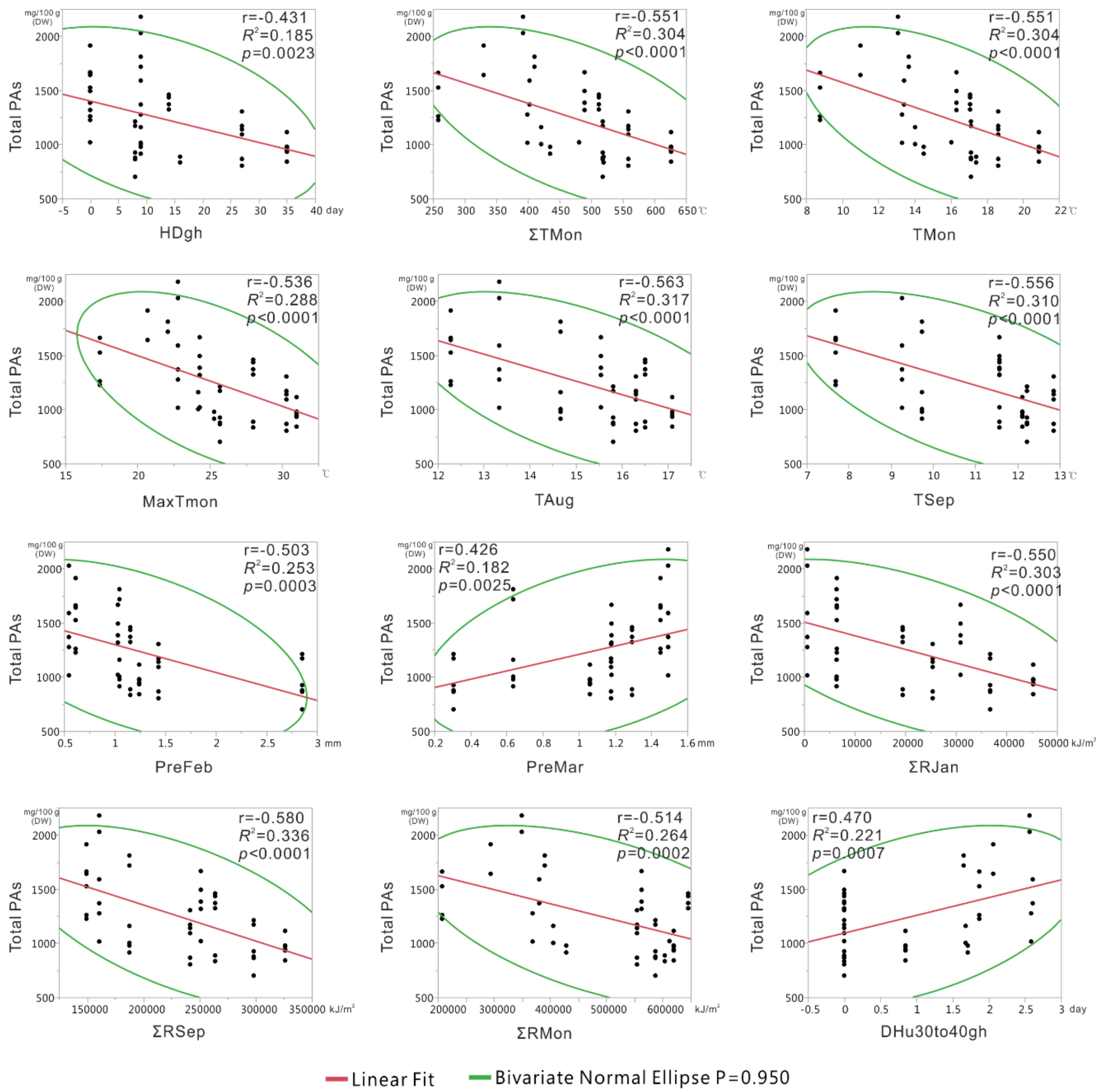
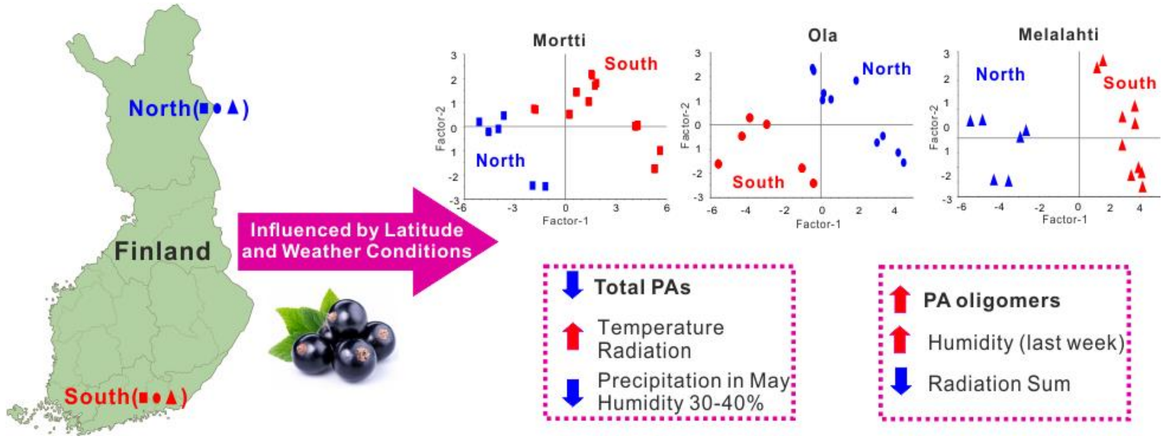

Temperature
Radiation
Precipitation
Humidity
A
B
C
Correlation Loadings (X)
PC-1 (47%)
PC-1 (54%)
PC-1 (50%)
PC-1 (45%)
PC-1 (42%)
PC-1 (42%)
PC-1 (43%)
PC-1 (40%)
PC-1 (53%)
PC-1 (56%)
PC-1 (42%)
PC-1 (46%)
PC-2 (20%)
PC-2 (15%)
PC-2 (16%)
PC-2 (21%)
PC-2 (22%)
PC-2 (17%)
PC-2 (19%)
PC-2 (21%)
PC-2 (15%)
PC-2 (16%)
PC-2 (24%)
PC-2 (18%)

Fig.4



r=-0.431
$R^2$=0.185
$p$=0.0023
Total PAs
HDgh
r=-0.551
$R^2$=0.304
$p$<0.0001
ΣTMon
r=-0.551
$R^2$=0.304
$p$<0.0001
TMon
r=-0.536
$R^2$=0.288
$p$<0.0001
MaxTmon
r=-0.563
$R^2$=0.317
$p$<0.0001
TAug
r=-0.556
$R^2$=0.310
$p$<0.0001
TSep
r=-0.503
$R^2$=0.253
$p$=0.0003
PreFeb
r=0.426
$R^2$=0.182
$p$=0.0025
PreMar
r=-0.550
$R^2$=0.303
$p$<0.0001
ΣRJan
r=-0.580
$R^2$=0.336
$p$<0.0001
ΣRSep
r=-0.514
$R^2$=0.264
$p$=0.0002
ΣRMon
r=0.470
$R^2$=0.221
$p$=0.0007
DHu30to40gh
Linear Fit
Bivariate Normal Ellipse P=0.950

579 Table of contents




580