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ARTICLE

Geographical and Climatic Dependencies of Green Tea (*Camellia sinensis*) Metabolites: A ^{1}H NMR-Based Metabolomics Study

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The effects of climatic conditions on green tea metabolites in three different growing areas of Jeju Island, South Korea, were investigated through global metabolite profiling by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Pattern recognition methods, such as principal component analysis (PCA) and orthogonal projection on latent structure–discriminant analysis (OPLS-DA), revealed clear discriminations of green teas from the three different growing areas. Variations of theanine, isoleucine, leucine, valine, alanine, threonine, glutamine, quinic acid, glucose, epicatechin (EC), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), and caffeine levels were responsible for the discriminations. Green teas grown in an area with high temperature, long sun exposure time, and high rainfall had higher levels of theanine but lower levels of isoleucine, leucine, valine, alanine, EC, EGC, EGCG, and caffeine than those grown in areas with relatively low temperature, short sun exposure time, and low rainfall. These results indicate that high temperature, long sun exposure, and high preciptation stimulate theanine synthesis in green tea during the spring season. This study highlights how metabolomics coupled with multivariate statistical analysis can illuminate the metabolic characteristics of green tea associated with climatic variables, thereby allowing for the assessment of quality strategy in green tea production.

KEYWORDS: ^{1}H NMR; green tea; metabolite; metabolomics; climate

INTRODUCTION

Green tea is one of the most popular drinks in the world. It has been characterized as a natural medicine containing great amounts of tea polyphenols, caffeine, theanine, and vitamins (*1*). The major tea polyphenols are catechins and their derivatives, epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG); theanine is the most abundant green tea amino acid. These tea chemical metabolites affect green tea quality and are known to possess various biological activities. General metabolites of the green tea leaf depend on several variables including the genetic strain of the green tea tree, climate conditions, soil, growth altitude, and plucking season as well as geographical factors such as temperature, sun exposure time, rainfall, and soil. These factors could be explained by the "terroir", which originated from grape-growing and winemaking (*2*−*4*). There are some studies on the relationship between tea chemical compositions and growing conditions. Catechin, gallic acid, and caffeine levels in green tea determined by HPLC varied in five different countries of origin (*5*) and in different growing areas in Japan (*6*). Recently, variations in the catechins and polyphenols of oolong tea samples from different seasons and altitudes were also reported (*7*). Therefore, it is very likely that growing and climate conditions as well as plucking time influence the metabolic diversity of green tea leaves. However, a direct relationship between tea metabolites and geographic factors, in particular, between global metabolome and real climatic variables, has not been well studied to date.

Recently, ^{1}H NMR-based global metabolic profiling has been used to gain better understanding of food quality and fermentative mechanisms. Over 180 commercial teas were characterized, and Japanese green tea quality was evaluated by ^{1}H NMR-based global metabolic profiling (*8*, *9*). In addition, ^{1}H NMR-based metabolomics provided information on the geographical dependencies of grapes and wine metabolome (*3*, *10*) and evidence of vintage effect on grape wine (*11*). Therefore, the ^{1}H NMR-based metabolomic approach is considered to be a useful tool to investigate the relationship between green tea metabolome and climatic variables. In the present study, we characterized the green tea metabolites in three different growing areas of Jeju Island, South Korea, through ^{1}H NMR-based global metabolite profiling and investigated the association of metabolic variations with meteorological data from the three tea gardens. This metabolomic study revealed that metabolic mechanisms in green tea leaves were strongly influenced by climatic conditions.

MATERIALS AND METHODS

Origin of Green Tea. Fresh green tea (*Camellia sinensis* var. Yabukita) leaves cultivated in the Hannam (33° 19′ 00.06″ N, 126° 40′

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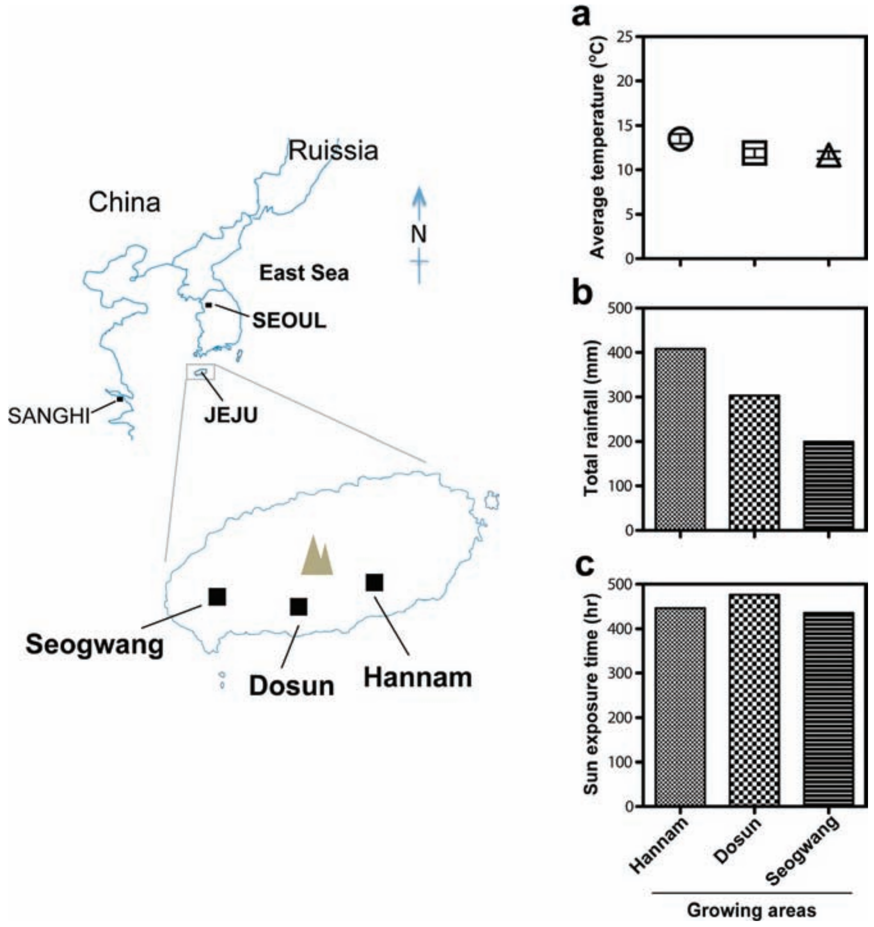

Figure 1. Geographical green tea growing areas (left) and climate conditions (right) with average temperature (°C), total rainfall (mm), and total sun exposure time (h) of the three growing areas in the southernmost region of Jeju-do, South Korea. The standard deviation for average temperature is calculated from the daily average temperature.

45.57″ E), Dosun (33° 16′ 22.79″ N, 126° 28′ 58.86″ E), and Seogwang (33° 18′ 17.67″ N, 126° 17′ 42.97″ E) areas of Jeju Island of South Korea were harvested or plucked on May 6, 2009. Tea gardens in each region belong to the AmorePacific Co. (Seoul, South Korea). The company controls the green tea from cultivation to production of commercial products through standard protocols. Fresh green tea samples were collected from each growing area, steamed, pan-fried, and dried in a tea-rolling dryer. The green tea samples were stored in different boxes and at 4 °C until extraction. Data for average temperature (°C) and total rainfall (mm) from March 1 to May 6, 2009, were obtained from each tea garden, whereas total sun exposure time (hours) was from local branches of the Korean Meteorological Administration.

Extraction of Green Tea. For each growing area, 10 green tea samples selected randomly from the different storage boxes were ground in a blender for 15 s and sieved using a stainless sieve mesh (1 mm). Deionized water (1 mL) was added to 50 mg of ground green tea in a 1.5 mL Eppendorf tube. The mixture was extracted in a shaking water bath at 60 °C at a constant rate for 30 min and then centrifuged at 25 °C and 13000 rpm for 15 min. Supernatants were lyophilized for ^{1}H NMR spectroscopic analysis.

^{1}H NMR Spectroscopic Analyses of Green Tea Extracts. The lyophilized green tea samples were dissolved in 600 μL of 0.1 M phosphate buffer (pH 7.0 in D_2O/H_2O = 90:10, v/v) and centrifuged at 13000 rpm for 15 min. Supernatants (540 μL) were mixed with 60 μL of 0.5 mM 3-(trimethylsilyl)-[2,2,3,3-$^2H_4$]-propionic acid sodium salt (TSP). The mixture (550 μL) was transferred into 5 mm NMR tubes. D_2O and TSP provided a field frequency lock and a chemical shift reference (^{1}H, δ 0.00), respectively. ^{1}H NMR spectra were acquired on a Varian INOVA-500 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) operating at 499.98 MHz, ^{1}H frequency, and a temperature of 298 K, using a 5 mm HCN triple-resonance indirect probe. A NOESYPRESAT pulse sequence was applied to suppress the residual water signal. For each sample, 64 transients were collected into 32K data points using a spectrum width of 8012.6 Hz with a relaxation delay of 1.0 s, an acquisition time of 2.5 s, and a mixing time of 400 ms. A 1.0 Hz line-broadening function was applied to all spectra prior to Fourier transformation (FT). Signal assignment for representative samples was facilitated by two-dimensional (2D) total correlation spectroscopy (TOCSY), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum correlation (HSQC), spiking experiments, and comparisons to the literature (*8*, *12*).

Multivariate Data Analyses. All NMR spectra were phased and baseline corrected with VnmrJ software 2.1B (Varian Inc.) and then converted to ASCII format. The ASCII format files were imported into MATLAB (R2008a, The Mathworks, Inc., Natick, MA). Probabilistic quotient normalization of the spectra using the median spectrum to estimate the most probable quotient was carried out after total integral normalization to avoid dilution effects of samples and effects of metabolites in massive amounts on changes in the overall concentration of samples (*13*). The spectra were then aligned by the recursive segment-wise peak alignment (RSPA) method to reduce variability in the peak position (*14*). Regions corresponding to water (4.68−4.8 ppm) and TSP (−0.5 to 0.7 ppm) were removed prior to normalization and spectrum alignment. The resulting data set was then imported into SIMCA-P version 12.0 (Umetrics, Umeå, Sweden) for multivariate statistical analysis. Principal component analysis (PCA), an unsupervised pattern recognition method, was initially performed to examine intrinsic variation in the data set. Furthermore, a supervised pattern recognition method, orthogonal projection on latent structure−discriminant analysis (OPLS-DA) (*15*), was used to extract maximum information on discriminant compounds from the data using MATLAB (The MathWorks, Inc.) with scripts developed in-house at Imperial College London. For visualization purpose, the OPLS coefficient indicating variables or metabolites responsible for the

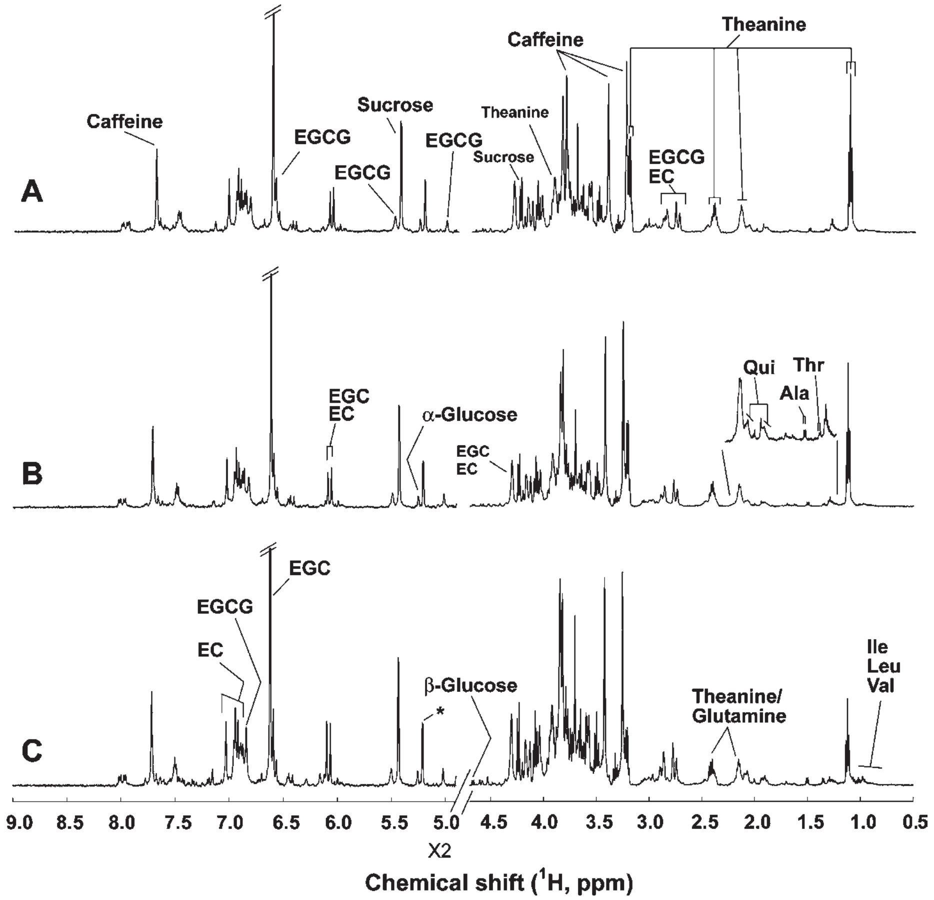

Figure 2. Representative ^{1}H NMR spectra of green tea harvested at the three different growing areas of Hannam (**A**), Dosun (**B**), and Seogwang (**C**). Ile, isoleucine; Leu, leucine; Val, valine; Ala, alanine; Thr, threonine; Qui, quinic acid; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate.

differentiation or discrimination in the model were back transformed as described by Cloarec et al. (*16*). Validation of the model was conducted using 7-fold cross-validation and permutation tests 200 times. Q^2 values generated from the permutation test were compared to the Q^2 values of the real model. If the maximum value Q^2_{max} from the permutation test was smaller than the Q^2 of the real model, the model was regarded as a predictable model. R^2 was used to evaluate possible overfitting of the model. Hence, the quality of the models is described by Rx^2 and Q^2 values. Rx^2 is defined as the proportion of variance in the data explained by the models and indicates goodness of fit, and Q^2 is defined as the proportion of variance in the data predictable by the model and indicates predictability.

Chemicals. All chemical reagents were of analytical grade. All standard reagents, D_2O (99.9%), and TSP (97%) were purchased from Sigma (St. Louis, MO).

RESULTS

Average temperature (°C) and total rainfall (mm) from March 1 to May 6, 2009, were obtained directly from each tea garden. The average temperature was calculated from the daily average temperature during the period. Unfortunately, there were no data of total sun exposure time (h) from the tea gardens, and therefore we used total sun exposure time data from the local branches of the Korean Meteorological Administration located at each city corresponding to the three different tea-growing areas during the same period. Average temperature, total rainfall, and total sun exposure time at the three different tea-growing areas of Hannam, Dosun, and Seogwang are shown in **Figure 1**. The Hannam area had the highest average temperature and total rainfall, whereas the Seogwang area had the lowest average temperature and total rainfall as well as the shortest sun exposure time; the Dosun area had a lower temperature and rainfall but longer sun exposure time compared to the Hannam area. These different climatic conditions could contribute to variations in green tea metabolites or chemical compositions.

Figure 2 shows representative ^{1}H NMR spectra of green tea extracts obtained from the three different growing areas of Hannam, Dosun, and Seogwang. A total of 14 green tea metabolites, including theanine, quininc acid, glutamine, isoleucine, leucine, valine, threonine, alanine, caffeine, sucrose, α- and β-glucose, epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC), were identified by ^{1}H NMR spectroscopy. Visual inspection of the ^{1}H NMR spectra revealed marked differences in theanine, catechins of EGC, EC, and EGCG, caffeine, and amino acids.

To provide comparative interpretations and visualization of these metabolic differences in green tea according to the different growing areas, pattern recognition methods, principal component analysis (PCA) and orthogonal projection on latent structure−discriminant analysis (OPLS-DA), were applied to the NMR spectrum data set.

PCA and OPLS-DA score plots showed clear differentiations among the green tea samples from different growing areas (**Figure 3**), indicating that tea metabolites were strongly influenced by the environmental conditions at each area.

To identify the tea metabolites responsible for each differentiation, OPLS-DA models were generated in pairwise comparisons (**Figure 5**). All OPLS-DA models were constructed with one PLS component and one orthogonal component. Although the model between the Hannam and Dosun areas was relatively weak

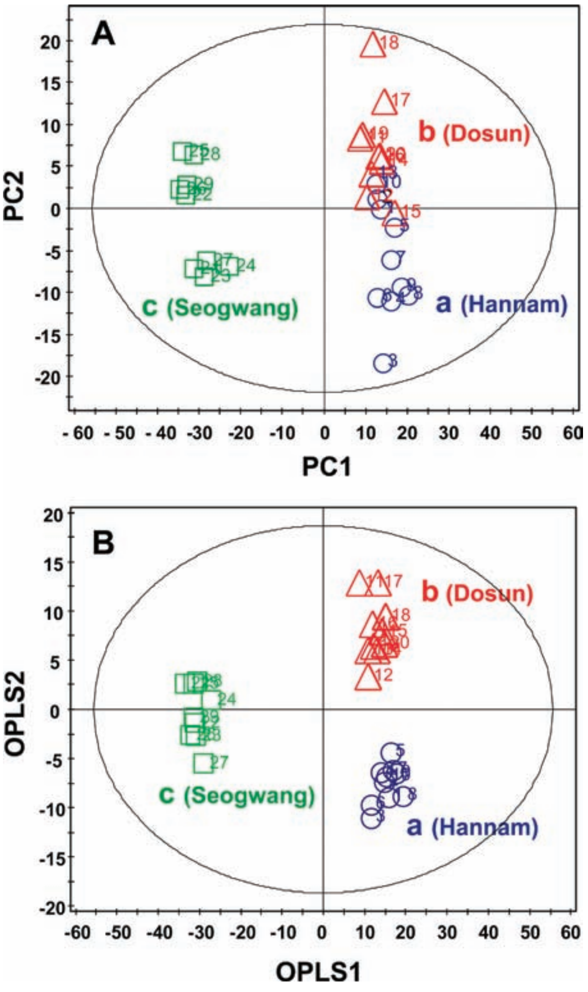

Figure 3. PCA (**A**) and OPLS-DA (**B**) score plots derived from ^{1}H NMR spectra of green tea extracts from different growing areas, demonstrating a clear geographical dependence of tea metabolites.

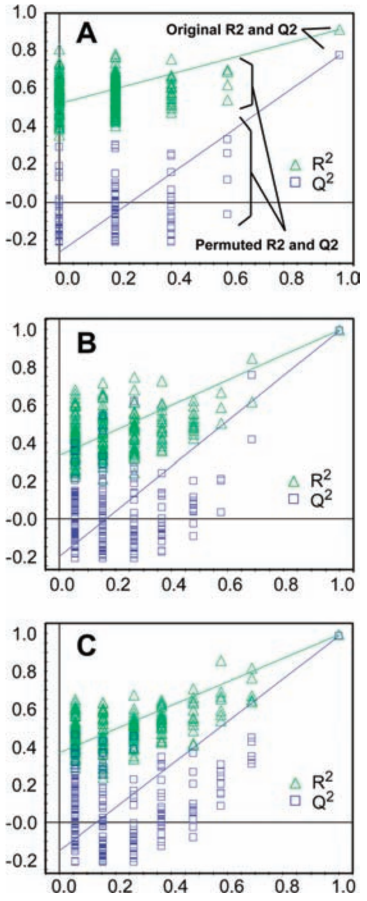

Figure 4. Permutation tests in PLS-DA model with the same predictive and orthogonal components as the OPLS-DA model for pairwise comparison between two classes, indicating great predictability (Q^2) and goodness of fit (R^2) of models between Hannam versu Dosun (**A**), Dosun versu Seogwang (**B**), and Hannam versus Seogwang (**C**).

compared to the models between the Hannam and Seogwang areas and between the Dosun and Seogwang areas, the model was still predictable, as indicated by corresponding R^2X (49.5% in Hannam vs Dosun, 77.8% in Dosun vs Seogwang, and 79.2% in Hannam vs Seogwang), Q^2Y (80.8% in Hannam vs Dosun, 99.4% in Dosun vs Seogwang, and 6.72×10^{-13} in Hannam vs Seogwang), and P values (2.92×10^{-5} in Hannam vs Dosun, 1.59×10^{-15} in Dosun vs Seogwang, and 79.2% in Hannam vs Seogwang). Moreover, although some sample models between the Hannam and Dosun areas were overlapped in the PCA (**Figure 3A**), all samples were clearly separated in the OPLS-DA model (**Figures 3B** and **5A**). This indicated that noncorrelated variation in X variables (metabolites) to Y variables (growing areas) or variability in X that is orthogonal to Y variables was removed, resulting in maximum separation in the OPLS-DA model. Furthermore, permutation tests were also performed in the PLS-DA model to validate each OPLS-DA model. All Q^2_{max} and R^2 values were higher in the permutation test than in the real model, revealing great predictability and goodness of fit (**Figure 4**).

Clear differentiations were observed in the OPLS-DA score plots derived from the ^{1}H NMR green tea spectra between the Hannam and Dosun areas (**Figure 5A**), between the Dosun and Seogwang areas (**Figure 5B**), and between the Hannam and Seogwang areas (**Figure 5C**). Furthermore, metabolites responsible for the differentiation could be found in the corresponding OPLS loading or coefficient plots (**Figure 5D−F**). The direction of the ^{1}H NMR resonances in the OPLS loading plot represents the relative differences in metabolites between classes. For example, the upper section of the OPLS loading plot represents higher levels in green tea obtained from the Dosun area compared to the Hannam area, whereas the lower section denotes lower levels (**Figure 5D**). The colors on the OPLS loading plot are associated with the significance of metabolites responsible for the differentiation between classes. In the present study, the correlation coefficients were considered to be significant when >0.5, which corresponded to the critical value of a correlation coefficient at $P < 0.05$ verified by Student's *t* test with integral areas of individual metabolites.

Differentiation between green teas grown in Hannam and Dosun areas was caused by higher levels of isoleucine, alanine, sucrose, EGCG, and EC and lower levels of glutamine, quinic acid, and an unknown compound in green tea from the Dosun area, compared to green tea from the Hannam area, as shown in the OPLS-DA loading plot (**Figure 5D**). An OPLS-DA loading plot for a model differentiating green tea grown in the Seogwang area from green tea grown in the Dosun area revealed that levels of isoleucine, leucine, valine, threonine, quinic acid, EC, EGCG, and caffeine were higher in green tea grown in the Seogwang area, whereas levels of theanine and sucrose were lower (**Figure 5E**). In addition, differentiations between green teas grown in the Hannam and Seogwang areas were due to higher levels of theanine and lower levels of isoleucine, leucine, valine, threonine, alanine,

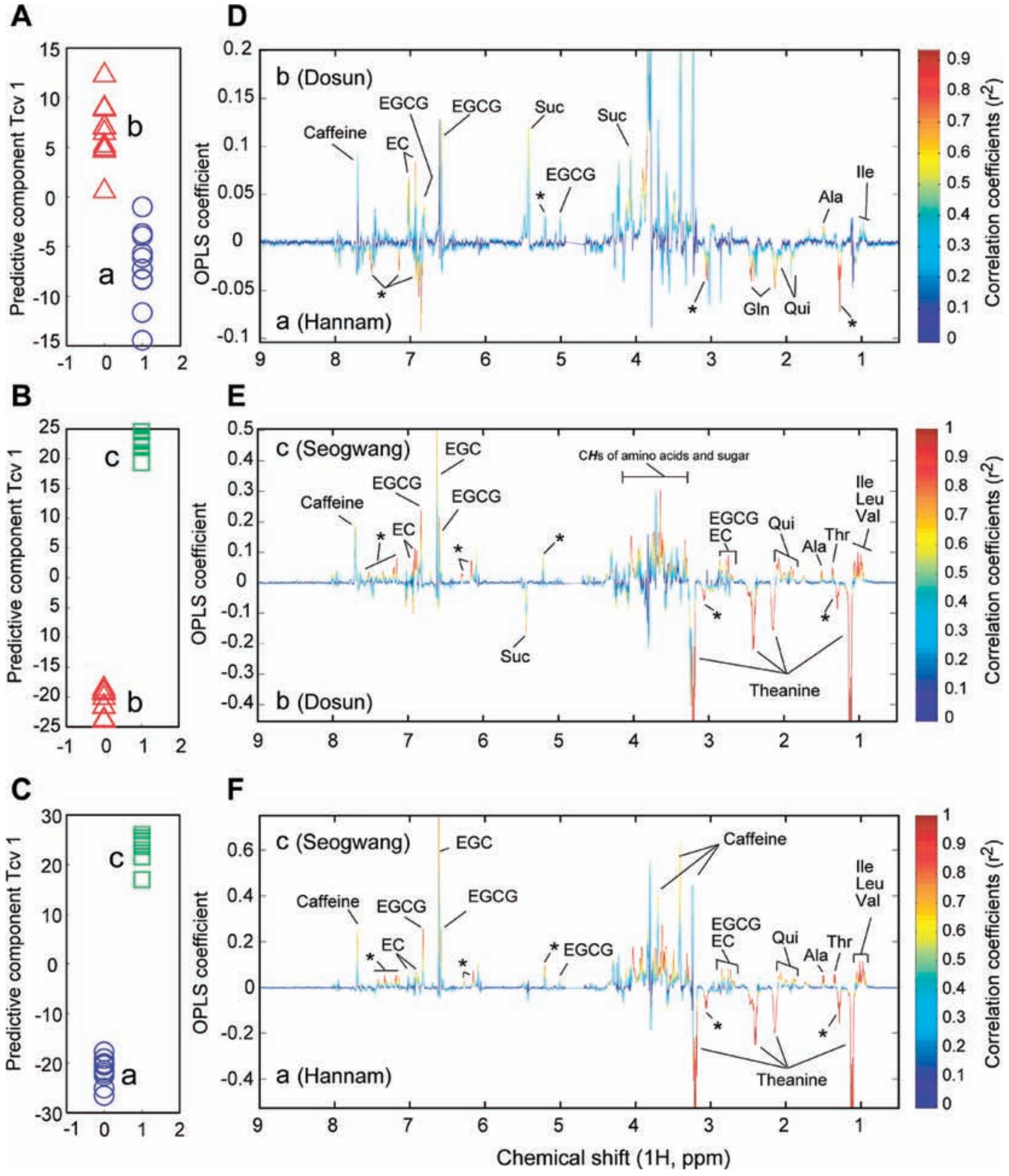

Figure 5. OPLS-DA scores (**A**−**C**) and loading (**D**−**F**) plots derived from ^{1}H NMR green tea spectra, providing a pairwise comparison among green tea extracts obtained from three different growing areas of Hannam (a), Dosun (b), and Seogwang (c), regions of Jeju-do, the southernmost island of South Korea. The color code in the loading plot corresponds to the correlation coefficients of the variables. Gln, glutamine; Qui, quinic acid; Ala, alanine; Ile, isoleucine; Leu, leucine; Val, valine; Thr, threonine; Suc, sucrose; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate. Asterisks (∗) denote unknown compounds produced during tea fermentation.

quinic acid, EC, EGCG, EGC, and caffeine in Hannam tea than in Seogwang tea (**Figure 5F**).

DISCUSSION

It is likely that elemental compositions of teas vary with tea varieties and geographical origins (*17*, *18*). Moreover, Fernández et al. (*19*) reported that profiles of green tea catechins, gallic acid, and alkaloids were different according to their country of origin, and Chen et al. (*7*) revealed that the catechin contents of oolong tea were significantly affected by plucking season and altitude of the growing area. However, to date most studies have focused on variations of metal contents or phenolic compounds in green tea according to growing area by targeted analysis in particular and have not examined a direct association with climatic variables. The principal climatic parameters of sun exposure time, temperature, and rainfall are the most important factors affecting not only the growth but also the metabolic activity of grapevines and vary considerably by year and region, thereby giving the enological notions of vintage and terroir in grapevines and grape wines (*10*, *11*, *20*). Therefore, association of variation in green tea metabolome with climatic conditions at different tea-growing areas was expected. The present study showed metabolic dependencies of theanine, catechins, amino acids, and caffeine in green tea according to three different tea-growing areas through global profiling of tea metabolites, based on metabolomics with ^{1}H NMR spectroscopy. We were able to demonstrate a strong association between metabolic variations in green tea and climatic variables.

Theanine. Amino acids contribute to the full-bodied flavor and sweetness of tea. Of these amino acids, >60% are L-theanine

(γ-glutamyl-L-ethylamide), which is responsible for the exotic taste of green tea. L-Theanine is synthesized from glutamic acid and ethylamine by theanine synthetase (EC 6.3.1.6) in all parts of seedlings (*21*). Ethylamine is derived from alanine by alanine decarboxylase. In general, theanine is known to be degraded when exposed to light and, consequently, converted to catechins. Green tea grown in shady conditions contains high theanine and low catechin levels compared to that grown in unshaded conditions (*22*). Theanine synthesis in green tea increases during the spring season. The fact that theanine content decreases after the specific plucking season time is mainly due to degradation or conversion by light to catechins (*23*). Moreover, large amounts of theanine found in green tea with very young leaves picked earlier also indicates that theanine contents vary with plucking positions and seasons and are influenced by synthesis rather than degradation during the spring season (*23*). Unfortunately, to date, seasonal changes in theanine levels in green tea have not been published. However, our own data on changes in amino acid during green tea cultivation revealed that total amino acid content did not change significantly by May 10 but decreased markedly after May 13 (data not shown). In addition, it is clear that decreases in theanine content are accompanied by a decrease in total amino acids in green tea (*24*). Therefore, it is likely that theanine synthesis in green tea could increase during the spring season between April and May in South Korea and then decrease after May in the areas evaluated in this study mainly due to degradation or conversion by light to polyphenolic compounds such as catechins. Details on the metabolic behaviors of theanine content in green tea according to plucking seasons will be published following completion of an ongoing study.

In the present study comparing theanine levels, the lowest levels of theanine were observed in the green tea from the Seogwang area, which had the lowest mean temperature and total rainfall (**Figures 1** and **5**). Moreover, although average temperature and total rainfall during the period from March 1 to May 6 were lower in Dosun than in Hannam, theanine levels in green tea were not significantly different between those areas. As compared to the sun exposure time between the areas of Dosun and Hannam during the same period, obtained from local branches of the Korean Meteorological Administration, the time was longer in Dosun than in Hannam. Therefore, theanine levels or synthesis in green tea could be influenced by sun exposure time as well as temperature and total rainfall. That is, the longer sun exposure time found in the Dosun area may cause no significant difference in theanine levels from those in the Hannam area where mean temperature and total rainfall were higher.

Furthermore, although average temperature in the Seogwang area was not different from that in the Dosun area, theanine levels were markedly lower in green tea grown in the Seogwang area compared to that in the Dosun area, perhaps resulting from lower total rainfall and shorter sun exposure time in the Seogwang area than in the Dosun area. These results suggest that theanine synthesis in green tea increases with increasing temperature, sun exposure time, and precipitation during the spring season. However, this suggestion is not consistent with reports that theanine content in green tea leaves increased under shaded conditions (*22*), indicating different growing conditions in artificial shaded and geographical environments. In addition, plucking season or time could play an important role in theanine content in the artificial shaded conditions because active synthesis of theanine in green tea tree would occurr during the spring season and be degraded at a later season. To date, most studies did not consider the effects of plucking season on theanine or global metabolites in green tea. Therefore, our results indicate that plucking season should be considered in the future when the effects of cultivation conditions on green tea plant metabolism or metabolic variations are investigated.

Amino Acids. It is well-known that the amino acid content in green tea leaves harvested in spring is higher than that of later seasons (*25*), which demonstrates that the younger the green tea leaves are, the higher the level of amino acids they contain. In the present study, the highest levels of amino acids including isoleucine, leucine, valine, alanine, and threonine in green tea grown in the Seogwang area indicate that temperature, rainfall, and sun exposure time affect the synthesis of amino acid, leading to a high accumulation of amino acids in green tea as temperature, rainfall, and sun exposure time decrease. In particular, the highest level of alanine found in green tea grown in the Seogwang area could be directly associated with the lowest level of theanine in green tea grown in the same area, because alanine is converted to ethylamine, which is a precursor in theanine synthesis (*21*). This reverse relationship between alanine and theanine demonstrates that theanine synthesis is closely associated with climatic variables as described previously. Therefore, most retardation of theanine synthesis could be found in green tea grown in the Seogwang area as a result of low temperature and rainfall and short sun exposure time. On the other hand, high temperature and rainfall and long sun exposure time may lead to the stimulation of theanine synthesis in green tea, resulting from retardation of alanine conversion to theanine. Furthermore, high levels of glutamine and low levels of alanine in green tea from Hannam compared to green tea from Dosun may indicate a potential for stimulating theanine synthesis, because glutamine is a precursor of glutamic acid necessary for theanine synthesis.

Catechins. Catechins are a group of polyphenols found in green tea and belong to compounds generally known as flavonoids, which have a $C_6-C_3-C_6$ carbon structure and are composed of two aromatic rings. Catechins are a main component of astringency and a key determinant of tea quality, along with caffeine and several amino acids including theanine. Catechin content varies in varieties or cultivars (*26*).

Kito et al. (*27*) have shown that the *N*-ethyl carbon of theanine was converted to the phloroglucinol nucleus of catechins in tea shoots and that conversion was controlled by light. The production of catechins in the tea plant increases when exposed to sunlight but is retarded in shaded conditions (*28*). In addition, it has been revealed that carbon in the *N*-ethyl group of theanine was found to be incorporated into catechins (*27*), indicating a reverse relationship between theanine and catechin contents. These phenomena are related to the activity of phenylalanine ammonialyase (PAL, EC 4.3.1.5), a key enzyme in phenylpropanoid synthesis, which is followed by biosynthesis to catechins by several enzymes (*25*, *29*). It has been reported that the levels of EGCG, EGC, and EC in old leaves were higher than in young green tea leaves (*30*) and that the biosynthesis of the flavan-3-ols, EC, ECG, EGC, and EGCG, in young tea leaves was also enhanced by light (*31*), which pointed out the importance of the plucking position of tea leaves. The first to third tea leaves used in the present study were mainly included during the plucking time at each growing area. However, in the present study, the levels of catechins of EC, EGC, and EGCG were clearly higher in green tea grown in the Seogwang area, where temperature and sun exposure time were lowest among the three different growing regions. Furthermore, total rainfall was markedly lower in the Seogwang area, at 199.9 mm compared to 303 mm in Dosun and 408 mm in Hannam (**Figure 1**). In particular, although the temperature between the Seogwang and Dosun areas was not significantly different and the Seogwang area showed shorter sun exposure time than the Dosun area, clear differences in catechin levels in green tea between the two regions indicated that total rainfall could affect the catechin contents in the green tea. In addition, higher levels of catechins in green tea grown in the Dosun area, which had lower total rainfall compared to the Hannam area, may indicate that catechin synthesis could be stimulated as total rainfall decreases, if we assume that the sunlight effects between the Dosun and Hannam areas on catechin synthesis would be similar because temperature and sun exposure time were reversed between these two areas (**Figure 1**). This assumption is very likely because theanine levels between the two areas were similar (**Figure 5D**).

Caffeine. Caffeine (1,3,7-trimethylxanthine) is a trimethyl derivative of purine-2,6-diol, produced in young leaves, and continues to accumulate gradually during the maturation of the tea plant (*1*). Caffeine biosynthesis contains four steps consisting of three methylation reaction and one nucleosidase reaction (*32*). The first step is the conversion of xanthosine to 7-methyxanthosine, the second step is the hydrolysis of 7-methylxanthosine, and the last two steps are the conversion of 7-methylzanthine to caffeine through theobromine by *N*-methyltransferase (*32*). It has been reported that caffeine biosynthesis increases in green tea leaves from April to June in Korea and Japan, demonstrating that caffeine contents are higher in old leaves compared to young leaves (*30*, *33*−*35*). In addition, sunlight does not have any significant effect on caffeine biosynthesis (*36*). Moreover, caffeine is mainly catabolized to xanthine through theophylline and 3-methylzanthine, but it is very slowly degraded (*32*). Unfortunately, to date, a relationship between climate conditions and caffeine synthesis in green tea has not been reported. According to the present study, the metabolic behavior of caffeine was very similar to that of catechins in green tea. That is, caffeine synthesis was highly stimulated in green tea grown in the Seongwang area but delayed in the Hannam area (**Figure 5**), indicating the dependence mainly on total rainfall as discussed with catechins. The physiological behavior of caffeine synthesis in green tea according to plucking season is an area of interest for future study.

In conclusion, this study demonstrates that the green tea metabolome was clearly dependent on growing area and hence influenced by climatic conditions such as sun exposure time, precipitation, and temperature. Furthermore, this metabolomic approach coupled with global metabolite profiling could be useful for understanding tea plant metabolism and for assessing tea quality.

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