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High-quality green tea leaf production by artificial cultivation under growth chamber conditions considering amino acids profile

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The current study focused on the tea plant (*Camellia sinensis*) as a target for artificial cultivation because of the variation in its components in response to light conditions. We analyzed its sensory quality by multi-marker profiling using multicomponent data based on metabolomics to optimize the conditions of light and the environment during cultivation. From the analysis of high-quality tea samples ranked in a tea contest, the ranking predictive model was created by the partial least squares (PLS) regression analysis to examine the correlation between the amino-acid content (X variables) and the ranking in the tea contest (Y variables). The predictive model revealed that glutamine, arginine, and theanine were the predominant amino acids present in high-ranking teas. Based on this result, we established a cover-culture condition (i.e., a low-light intensity condition) during the later stage of the culture process and obtained artificially cultured tea samples, which were predicted to be high-quality teas. The aim of the current study was to optimize the light conditions for the cultivation of tea plants by performing data analysis of their sensory qualities through multi-marker profiling in order to facilitate the development of high-quality teas by plant factories.

[**Key words:** *Camellia sinensis*; Plant factory; Metabolomics; Quality of green tea; Prediction model]

The development of light-emitting diode (LED) technology has led to its increased use for various purposes, including domestic use. In addition, in the fields of agronomy and plant physiology, the narrowband-emitting property of LEDs has been utilized in many studies investigating the influence of light conditions on plant growth (1,2). The production of crops by factories in a controlled environment (i.e., plant factories) has been one of the goals of such studies. Plant factories aspire for crops that have a unique advantage over outdoor-grown crops, such as pesticide-free production, stable production, and/or the enhancement of a specific component. Knowledge on the influence of environmental light on plants and the optimal conditions for cultivation, including the spectrum and amount of light irradiation, plays a key role in the development of plant factory technology for the future.

Research on medicinal plants targeting a particular organism is targeted to develop methodologies for obtaining beneficial component variations by controlling light conditions. Medicinal plants consist of bioactive components, and light conditions are examined in order to maximize these components. Nishimura et al. (3) reported that an increase in the medicinal properties of St. John's wort (*Hypericum perforatum* L.) was observed by adjusting the quality of light. Likewise, Afreen et al. (4) reported that the exposure to ultraviolet (UV)-B radiation during the later stages of the cultivation process of *Glycyrrhiza uralensis* increases the concentration of glycyrrhizin in the roots by nearly 1.5-fold when compared to that in the control.

Research on the relationship between environmental light during cultivation and the sensory qualities of crops (e.g., taste, flavor, preference) has produced limited results, to date. It could be assumed that the sensory qualities of crops are subjective and differ widely among individuals or the sensory attributes are complex in nature involving many components. In contrast to medicinal plants, it is difficult to determine the optimum culture conditions for maximizing the sensory qualities of crops because an increase in a particular component is not necessarily linked to the qualitative development of these crops. However, the development of sensory qualities of crops is necessary to produce special high-quality crops in plant factories. Thus, in cultured crops, it is necessary to develop a feedback system on the basis of sensory analyses in order to determine the optimum light conditions.

In this study, we aimed to reveal the relationship between crop sensory quality and light conditions in cultured crops by using multi-marker profiling of multicomponent data based on metabolomics to identify the components (and their compositions) that contribute to the sensory quality of crops. We cultivated the tea plant (*Camellia sinensis* L.) and attempted the following to obtain high-quality teas that are comparable to tea contest winners: (i) analyses of high-quality teas, (ii) the development of artificial culture techniques for tea plants, and (iii) the evaluation of artificially cultured (AC) teas. The green tea plant has been traditionally cultivated under shade conditions in Japan. Thus, its reactive properties, with regard to component variations and sensory

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attributes under variable light conditions, are well-known (5,6). Furthermore, the quality of green tea has been evaluated by professional tea tasters. Therefore, the results of sensory evaluations for tea, which are important for data analysis, are reliable. For these reasons, tea plant cultivation was considered as an appropriate model for the current study.

Previously, free amino acids and L-theanine have been used as evaluation indices for green tea. Amino acids are well-known substances that influence taste; in essence, they directly affect the taste and quality of green tea. L-Theanine, which constitutes 50–60% of the free amino acids, is considered a primary umami component. The contents of free amino acids and L-theanine have been reported to be highly correlated with the quality of green tea (6), but this correlation is weaker in higher-quality teas (7–11). In the current study, since we targeted high-quality teas, we required another index for our assessment; hence, we focused on the amino-acid profile of the tea plant. Although many studies have investigated individual amino acids in the tea plant (7,8,12), to the best of our knowledge, there is limited information on amino-acid profiling and only some studies have suggested the importance of other amino acids over L-theanine in the quality of green tea (13), to date.

Pongsuwan et al. (14) proposed a method for the determination of the quality of the Japanese green tea (Sencha), which is based on its metabolite profile using gas chromatography (GC)/mass spectrometry (MS) for high-quality teas ranked in a tea contest. The results have shown that multivariate data analysis makes it possible to predict the ranking of a tea from the observed contents of its components. Pongsuwan's method has indicated that conducting a multicomponent analysis (i.e., multi-marker profiling), rather than targeting a single component, can explain the variations in high-quality teas, which cannot be differentiated by conventional approaches. Several authors have used multi-marker profiling to evaluate food quality. For instance, Tarachiwin et al. (15) revealed the usefulness of multi-marker profiling in the prediction of watermelon quality using ^{1}H NMR spectrometry, and Cho et al. (16) used 1H NMR spectrometry and principal component analysis (PCA) to discriminate between different grades of mushrooms. According to these reports, multi-marker profiling, which explains the property of a sample by assessing multicomponent properties, is a powerful method for the analysis of sensory attributes involving the complex composition of a crop's components.

In the current research, we first identified high-quality teas by analyzing the amino-acid content in the Japanese green tea (Tencha; unground Matcha), which was ranked in a tea contest, and attempted to assess the specific amino-acid composition of high-quality teas by using multi-marker profiling techniques.

Second, we developed artificial culture techniques for tea plants, under simulated open tea plantation conditions. The leading cultivar, Yabukita, was selected because of the large amount of data available for that cultivar (although cultivars of high-quality green teas such as Gyokuro and Tencha were also recommended). Light conditions in the culture were determined based on quality and quantity. Furthermore, the light conditions used in this study were designed to mimic the conditions of an open field and/or result in the production of a high-quality tea.

In the third stage of evaluation, we analyzed the grown AC tea and compared it with the ranked teas; we also attempted to elucidate the relationship between the light conditions in the culture and the sensory quality of the teas.

MATERIALS AND METHODS

Tea samples In a tea contest in Japan, the quality of green tea (Tencha) and its ranking was determined by the total scores (200-point scale) given for leaf appearance (40 points), smell (65 points), color of the brew (20 points), taste (65 points), and leaf color in hot water (10 points), as judged by professional tea tasters. In this study, we analyzed 18 ranked tea samples from a tea contest in the year 2012 (Table S1).

The AC tea samples were prepared through the process of artificial culture, as described below. After harvest, raw tea leaves were immediately blanched and dried by using a microwave oven and stored at −80°C until analysis.

Sample preparation and HPLC analysis The amino-acid content and composition of tea samples were measured by using the high-performance liquid chromatography (HPLC) method of Takayanagi et al. (17). All samples were ground into a powder; 0.1 g of each sample was extracted in 100 mL of purified water at 80°C for 30 min. After the addition of an internal standard, 0.4 mg of norvaline, the extraction liquid was mixed with an *o*-phthalaldehyde (OPA) solution, which consisted of 0.143 g of OPA dissolved in 10 mL of 0.1 M borate buffer and 0.25 mL of 2-mercaptoethanol. The OPA solution was stored at 5°C and renewed weekly. Fifteen μL of the mixture of the extraction and the OPA solution was injected into the HPLC immediately after mixing, and amino acids derivatives were separated on an ODS column (4.6 × 150 mm, 5 μm particle size; GL Science Inc., Tokyo) eluted with a gradient of solvent A (12% v/v ethanol, pH 6.0, adjusted with citric acid) and solvent B (50% v/v ethanol, pH 6.0, adjusted with citric acid). The gradient was linearly from 100% A to 100% B in 60 min, and the flow rate was 1 mL/min. The eluant was monitored in a fluorometer (λex = 340 nm, λem = 455 nm), and the following 17 free amino acids were detected: aspartic acid, glutamic acid, asparagine, serine, glutamine, arginine, threonine, theanine, alanine, tyrosine, γ-aminobutyric acid, methionine, valine, phenylalanine, isoleucine, leucine, and lysine. The standard solution for the preparation of the calibration curves, which contained a known concentration of each amino acid, was measured simultaneously, and the amino acids in the tea were quantified by comparing the peak areas with the standard.

Multivariate analysis The results from the HPLC analysis of the tea contest samples were converted into matrix data and then used for multivariate data analysis. The partial least squares (PLS) regression model, which examined the correlation between the amino-acid composition (X variables) and quality ranking (Y variables) for green tea, was created using Aloutput software (18). The efficiency and reliability of the PLS regression model were verified by percent variation, explained by the model goodness (R2Y) and the predictive parameter (Q2).

Data results for the AC tea samples were also converted and applied to the ranking predictive model created from the analysis of the tea contest samples, and the ranking for the AC tea was predicted.

Culture conditions Tea seedlings, one-year-old rooted cuttings of the cultivar Yabukita, were transplanted into hydroponic conditions in a glasshouse. The composition of the nutrient solution was determined according to the method described by Konishi et al. (19); it contained the macronutrients (mM) NH_4–N (2.1), NO_3–N (0.7), P (0.1), K (1.0), Ca (0.7), and Mg (1.0) and the micronutrients (μM) Fe (6.3; as EDTA salt), B (9.3), Mn (18.2), Zn (1.5), Cu (0.4), Mo (0.5), and Al (400). After a 3-week culture in 1/4 strength nutrient concentration, the seedlings were cultured in a full-strength solution. The culture solution was constantly aerated and fully renewed once every two weeks. Seedlings were acclimated to the hydroponic condition for at least seven weeks following transplantation and then used for the artificial culture experiment.

Seedlings were grown in a growth chamber (LPH-220SPC, Nippon Medical & Chemical Instruments Co., Ltd., Osaka). At the start of the cultivation process, the seedlings were in the dormancy condition (Table 1). For the experiment, there were 15 plants per section across 3 sections. The 3 sections consisted of: (i) an artificial culture under a high shading rate (AC-HS; final light intensity; 16 μmol m^{-2} s^{-1}, white light with supplemental far red); (ii) an artificial culture under a low shading rate (AC-LS; final light intensity; 160 μmol m^{-2} s^{-1}, white light with supplemental

TABLE 1. Culture conditions in each period.

		Dormancy period	Budbreak period	Cover-culture period
Length		6 weeks	Until new secondary leaf opening	40 days
Light condition				
AC-HS	Source[a]	W + FR + FL	W + FR + FL	W + FR
	PPFD[b]	240	320	160→16
	PFD of FR[b]	100	100	100→12
AC-LS	Source[a]	W + FR + FL	W + FR + FL	W + FR
	PPFD[b]	240	320	160
	PFD of FR[b]	100	100	100
AC-BR	Source[a]	B + R + FL	B + R + FL	B + R
	PPFD[b]	240	480	160→48
	PFD of FR[b]	0	0	0
Day length		10 h/14 h	12 h/12 h	12 h/12 h
Temperature		7°C/5°C	22°C/10°C (AC-BR; 25°C/15°C)	22°C/10°C (AC-BR; 25°C/15°C)

[a] W, white LED; FR, far red LED; B, blue LED; R, red LED; and FL, fluorescent light.
[b] Photosynthetic photon flux density (PPFD) and photon flux density of far red (PFD of FR) were measured at 10 cm above the canopies. The unit is μmol m^{-2} s^{-1}.