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Quantifying carotenoid contents has many applications in agriculture, ecology, and health science. Hyperspectral reflectance has been one of the promising tools for this purpose. However, previous studies were based on measurements under relatively low light–stress conditions. Therefore, assessing its robustness by using measurements under various levels of stress is required. In this study, the measurements of reflectance and carotenoid contents were carried out with four shading treatments including open–0 %, 35 %, 75 %, and 90 % shading to generate various chlorophyll/carotenoid ratios. Then the performances of fifteen published hyperspectral indices and PROSPECT–D inversion were evaluated based on our dataset for estimating leaf carotenoid contents. According to the ratio of performance to deviation, R_{NIR}/R_{510}, $R_{720}/R_{521}–1$, and PROSPECT–D inversion were applicable for this purpose, although calibration of the absorption coefficients was required for PROSPECT–D. Using them, root mean square percentage errors of 4.53–5.46 % were achieved. Given that total chlorophyll/carotenoid ratios could be a good indicator for evaluating environmental stress in plants, PROSPECT–D, which also estimates total chlorophyll and anthocyanin contents, could be a strong tool for controlling the qualities of shade grown tea.

Keywords: carotenoid; hyperspectral; PROSPECT–D; reflectance; shading treatment; tea

1. Introduction

Carotenoid pigments, which include two carotenes and five xanthophyll cycle pigments, are important photosynthetic pigments and play important roles for plant survival. Carotenoid compositions are determined by the developmental stage, tissue type, and environmental stimuli (Cazzonelli *et al.*, 2009). Furthermore, carotenoid rich foods decrease the risk of developing certain types of cancer (Giovannucci *et al.*, 1995, Wright *et al.*, 2003) and atherosclerosis (Klipstein–Grobusch *et al.*, 2000, Abbott *et al.*, 2003). Therefore, quantifying carotenoid contents has many applications in agriculture, ecology, and health science.

Although ultraviolet and visible (UV–VIS) spectroscopy or high performance liquid chromatography (HPLC) (Thayer and Bjorkman, 1990) have been used for assessing carotenoid contents, these approaches require destruction of samples, and they are time consuming and expensive. On the other hand, hyperspectral remote sensing offers some non–destructive methods that could be alternatives. Moreover, field spectroscopy integrated with hyperspectral remote sensing has been applied in forestry, vegetation, and environmental monitoring (Prasad *et al.*, 2015).

Two approaches including the numerical inversion of radiative transfer models (RTM) and hyperspectral indices have been widely used to estimate carotenoid contents. However, the similarity between chlorophyll and carotenoid over the wavelengths of 400–700 nm sometimes makes it difficult to estimate carotenoid concentrations from hyperspectral reflectance (Féret *et al.*, 2011). As a result, the methods for remote estimation of carotenoid contents are still not well developed compared with those for chlorophyll content (Yi *et al.*, 2014). Therefore, we evaluated these approaches for assessing carotenoid contents.

The PROSPECT model (Jacquemoud and Baret, 1990) is one of the most famous RTMs and has been widely used for simulating reflectance from given vegetation properties (Hernandez–Clemente *et al.*, 2014, Gu *et al.*, 2016, Hunt *et al.*, 2016, Sonobe and Wang, 2017) and retrieving chlorophyll, carotenoid (Féret *et al.*, 2008, Hernandez–Clemente *et al.*, 2014), or dry matter content (Romero *et al.*, 2012). Furthermore, PROSPECT–D, which makes it possible to simulate leaf optical properties through a complete lifecycle, has been released and is superior to the previous version for the estimation of pigment content, especially carotenoid contents (Féret *et al.*, 2017).

Hyperspectral indices have also been widely used to estimate the growth conditions of vegetation and are a synthesis of leaf area index (LAI), coverage, chlorophyll content, biomass, and photosynthetically active radiation (Zou *et al.*, 2015, Huang *et al.*, 2017). To assess carotenoid contents, several hyperspectral indices have been developed, and most of them are based on a small peak of reflectance around 470–530 nm, which is called the ‘green peak’(Chappelle *et al.*, 1992, Blackburn, 1998, Gitelson *et al.*, 2002, Gitelson *et al.*, 2006, Hernandez–Clemente *et al.*, 2012, Fassnacht *et al.*, 2015). Besides them, some indices were proposed to assess the epoxidation state (EPS) of xanthophyll cycle pigments, which are also included in the carotenoid (Gamon *et al.*, 1992, Hernandez–Clemente *et al.*, 2011, Sonobe and Wang, 2016), and some of them have been used for estimating carotenoid contents (Gitelson *et al.*, 2002, Zhou *et al.*, 2017).

Generally, these studies were based on measurements under relatively low light–stress environments except for the studies focusing on EPS. However, the highest quality green tea is cultivated using shading treatments, which changes the balance between chlorophyll content and carotenoid contents, and shading has sometimes led to early mortalities in tea plats. Although Féret *et al*. (2008) reported that the coefficients of linear regression models for estimating chlorophyll *a* content from carotenoid contents were 2.99 (for LOPEX dataset) to 3.45 (for HAWAII dataset), those of the dataset used in this study ranged from 3.61 to 6.35 due to environmental stress. Even though some assumptions in the previous studies for estimating carotenoid contents are not valid for assessing the carotenoid contents of shade grown tea, a knowledge of carotenoid contents from remote sensing is required because the ratio of chlorophyll to carotenoid could be a good indicator for evaluating environmental stress in plants.

The purpose of this analysis is to evaluate the potentials of PROSPECT–D inversion and hyperspectral indices for estimating the leaf carotenoid contents of shade grown tea.

2. Materials and Methods

2.1. Measurements and datasets

Tea leaf samples were collected from the Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organisation, Shimada, Japan. Shading treatment which is conducted for the top grades (Figure 1) makes tea leaves synthesise higher levels of chlorophyll and amino acids. Therefore, tea leaves were shaded for approximately 2 weeks before harvesting to produce high quality green tea. Four shading treatments (open–0 % shading, 35 % shading, 75 % shading, and 90 % shading) were conducted using a Dio Chemicals shading net #410 (35 % shading), #1210 (75 % shading), and #1220 (90 % shading) (Dio Chemicals, Ltd., Japan) from 21 April to 11 May. Figure 2 represents the weather conditions during the experiment. Averages of daily temperature were 12.5–19.2 °C and daily precipitations were 0–17.5 mm during the experiment (Japan Meteorological Agency, 2017).

<Figure 1>

<Figure 2>

The spectral reflectance and carotenoid contents of leaves were measured on 11 May. A FieldSpec spectrometer (Analytical Spectral Devices Inc., USA) was used with a leaf clip for acquiring reflectance data. This devise is composed of three detectors including VNIR, SWIR 1, and SWIR 2. However, some inherent variation in detector sensitivities can cause differences in the spectral drifts at two wavelength locations (1000 and 1800 nm). These drifts were corrected by applying the splice correction function of ViewSpec Pro Software (Analytical Spectral Devices Inc., USA) (Prasad *et al.*, 2015). On the other hand, a dual–beam scanning ultraviolet–visible spectrophotometer (UV–1280, Shimadzu, Japan) was utilised, and Wellburn's method (Wellburn, 1994) was applied for quantifying carotenoid contents. The equations used in this method for quantifying chlorophyll *a* (Chl*a*, μg ml^{-1}), chlorophyll *b* (Chl*b*, μg ml^{-1}), and carotenoid (Car, μg ml^{-1}) in dimethyl–formamide extracts are as follows:

$$\mathrm{Car} = (1000.00A_{480.0} - 1.12\mathrm{Chl}a - 34.07\mathrm{Chl}b)/245.00 \quad (1)$$

$$\mathrm{Chl}a = 12.00A_{663.8} - 3.11A_{646.8} \quad (2)$$

$$\mathrm{Chl}b = 20.78A_{646.8} - 4.88A_{663.8} \quad (3)$$

where *A* is the absorbance and the suffixes are the wavelength (nm).

The hyperspectral reflectance and carotenoid contents of sixty samples were measured, and then a stratified random sampling approach was applied to select 28 samples (7 samples × 4 treatments) as the training samples. The remaining 32 samples (8 samples × 4 treatments) were used to perform the accuracy assessment.

2.2. Hyperspectral indices for assessing carotenoid

In this study, the fifteen reported hyperspectral indices (Table 1) were evaluated for their correlations with carotenoid contents based on the aforementioned four datasets.

The ratio of reflectance at 760 nm to 500 nm (Chappelle) (Chappelle *et al.*, 1992) was developed based on the measurements from soybean leaves, whereas R_{NIR}/R_{510} (Gitelson1), CRI_{550}, and CRI_{700} (Gitelson *et al.*, 2002) were based on the experiment using juvenile, mature, and senescent leaves of Norway maple (*Acer platanoides*) and horse chestnut (*Aesculus hippocastanum*). Furthermore, Gitelson *et al.* (2002) evaluated their advantages by comparing Chappelle with PRI (Gamon *et al.*, 1992), Datt, which is expressed as $R_{672}/(R_{550} \times R_{708})$ (Datt, 1998), and Blackburn's indices including Blackburn1, which is expressed as R_{800}/R_{470}, and Blackburn2, which is expressed as $(R_{800}–R_{470})/(R_{800}+R_{470})$ (Blackburn, 1998). Furthermore, Gitelson *et al*. (2006) developed the two indices $(1/R_{510–520}–1/R_{560–570}) \times R_{NIR}$ and $(1/R_{510–520}–1/R_{690–710}) \times R_{NIR}$ using anthocyanin–free juvenile, mature, and senescent leaves. Hernandez–Clemente *et al*. (2012) proposed the ratio of reflectance at 515 nm to 570 nm (Hernandez–Clemente) using measurements from a conifer forest and simulation data.

Besides them, angular vegetation indices (AVI) were considered, and three AVIs and merged vegetation indices were developed by Fassnacht *et al*. (2015) based on the dataset obtained from Norway maple (*Acer platanoides*), horse chestnut (*Aesculus hippocastanum*), and European beech (*Fagus sylvatica*). Zou *et al*. (2017) proposed the Carotenoid index (CARI) based on the ANGERS dataset (Féret *et al.*, 2008) and experimental data acquired in field experiments in China.

<Table 1>

2.3. Model inversion

Inversion of PROSPECT–D was carried out as another approach for quantifying leaf carotenoid concentrations using hyperspectral data. In the PROSPECT model, a leaf is assumed to be a stack of plates composed of absorbing and diffusing constituents, and leaf optical properties are simulated from pigment content (chlorophyll, carotenoid, and anthocyanin for PROSPECT–D), leaf dry mass, leaf water mass, and effective number of leaf layers. To estimate these parameters, model inversion was conducted. Inversion of PROSPECT–D was applied using MATLAB and Statistics Toolbox Release 2016a (MathWorks, Inc., USA). The source codes (PROSPECT–D_Matlab.rar) were downloaded from the portal site (Institut de physique du globe de Paris, 2017), and the code for inversion of PROSPECT–5 was modified for application of PROSPECT–D.

The absorption coefficient of this model was conducted according to the calibration algorithm of Féret *et al*. (2008) using the training data to avoid potential systematic bias and error propagation in the inversion process.

2.4. Statistical criteria

For revealing the wavelengths in which significant differences were observed among the four shading treatments, stepwise linear discriminant analysis was applied (Draper, 1998). This technique has been applied for selecting suitable wavelengths to be included in a multiple regression model with a combination of forward and backward stepwise regression (Sonobe and Wang, 2016). The addition or removal of wavelengths was determined by a significance level of 5% in this study.

Regression models were generated based on linear or exponential regression using the training data. Next, the regression models were applied for the test data, and their performances were evaluated.

The ratio of performance to deviation (RPD, Equation (1)) (Williams, 1987) was applied to evaluate the performances of the hyperspectral indices, and the indices were classified into three categories (Chang *et al*., 2001): Category A (RPD > 2.0), Category B (1.4 ≤ RPD ≤ 2.0), and Category C (RPD < 1.4). RPD was calculated using equation (4):

$$\mathrm{RPD} = {}^{\mathrm{SD}}/_{\mathrm{SEP}} \qquad (4)$$

where SEP is the standard error of prediction, which is calculated as the root mean square error, and SD is the standard deviation of the carotenoid contents. The indices ranked as Category A or B were assumed to have the potential to estimate carotenoid contents.

Besides RPD, the root mean square percentage error (RMSPE, Equation (5)) and the determination coefficient (R^2) were also calculated to evaluate the fit between the index values and carotenoid contents.

$$\mathrm{RMSPE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{\hat{y_i}-y_i}{y_i}\right)^2} \qquad (5)$$

where n is number of samples, y_i is the measured value, and $\hat{y_i}$ is the estimated value.

3. Results and Discussion

3.1. Carotenoid contents after each treatment

Table 2 summarises the carotenoid contents after the different shading treatments. The carotenoid contents ranged from 6.16 to 11.29 µg cm^{-2}, and there were no significant differences among the treatments (p > 5.0%, based on the Tukey–Kramer test). However, significant differences were confirmed against the chlorophyll contents except for the treatments of 90 % shading and 35 or 75 % shading. The shading treatments contributed to increasing the chlorophyll *a* content of leaves to harvest more light and nitrogen (Suzuki and Shioi, 2003). However, excessive shading can sometimes prevent leaves from producing chlorophyll, and the opposite tendencies have been reported (Rozali *et al.*, 2016). Indeed, hakuyocha (white leaf) green tea is produced in Japan by completely shading the leaves. Thus, 90 % shading might have prevented the leaves from synthesising chlorophyll pigments during 21 April to 11 May, and this treatment made this tendency obscure.

Figure 3 represents the relationships between carotenoid contents and total chlorophyll content (*a*) or chlorophyll *a* content (*b*). Positive correlations were confirmed between them, and their R^2s were increased with levels of shading.

For the relationship between carotenoid contents and chlorophyll *a* content, their confidents increased with levels of shading except for the relationship between 0 % and

35 % shading. Whereas the coefficients of linear regression models for estimating chlorophyll *a* content from carotenoid contents was 2.99 (for LOPEX dataset) to 3.45 (for HAWAII dataset) (Féret *et al.*, 2008), those of our measurements ranged from 3.61 to 6.35. As a result, we concluded that various combinations of chlorophyll and carotenoid contents were generated by the four shading treatments.

<Table 2>

<Figure 3>

3.2. Spectral reflectance of different treatments

The mean reflectance of each treatment is shown in Figure 4. Generally, the reflectance after 0 % shading was the highest, and that of 75 % shading was the lowest over 400 – 900 nm. In particular, the green peak of shading of 0 % was significantly higher compared with the other treatments. Although clear trends related to shading treatments were not confirmed regarding reflectance, the four shading treatments could be identified with an overall accuracy of 65 % using the reflectance values at 564 nm and 701 nm with a stepwise linear discriminant analysis.

<Figure 4>

3.3. Accuracy validation

The accuracies from the test data of the 15 indices including the RPD, RMSPE, and R^2 are presented in Table 3. Gitelson1 and CARI were classified as 'A' category, and their estimated values are compared in Figure 5 with the measured values. CARrededge, Fassnacht1, and Fassnacht2 were categorised as 'B' based on RPD, and the results revealed that they also have the potential for assessing carotenoid contents; however, they were inferior to Gitelson1 and CARI.

Although Gitelson1, CRI_{550}, and CRI_{700} were developed from the same dataset, the differences in performance for our dataset were obvious. Figure 6 shows the correlations between reflectance at each wavelength and carotenoid contents. The reflectance near 510 nm was negatively correlated with carotenoid contents (r = –0.893, p < 0.1%), whereas the reflectance at 760–800 nm had no significant correlation, and it was reported to be almost constant by Gitelson *et al*. (2002). Thus, the reflectance at 760–800 nm could be a reference wavelength to clarify the change of reflectance at 510 nm. These features also caused the high performance of CARI, but the correlation coefficient of reflectance at 521 nm (r = –0.755, p < 0.1%) was inferior to that of reflectance at 510 nm.

<Table 3>

<Figure 5>

<Figure 6>

Next, the performance of PROSPECT–D inversion was evaluated. The absorption coefficients for carotenoid pigments are shown in Figure 7, and the calibrated values were smaller than their original values. Figure 8 shows the relationship between estimated and measured carotenoid contents of the test data. Although a high R^2 was confirmed between the measured carotenoid contents and that estimated by the original PROSPECT–D, the calibration of absorption coefficients made the RMSPE value smaller (from 9.86 % to 4.90 %) and RPD value larger (from 1.05 to 2.23). Although the statistics of the calibrated PROSPECT–D were inferior to those of Gitelson1, there were no significant differences in the estimated values among Gitelson1, CARI, and calibrated PROSPECT–D (p > 5.0% based on a Tukey–Kramer test). Furthermore, they were categorised as ‘A’ according to RPD, and the results revealed that they could be good indicators for assessing carotenoid contents.

Carotenoid content could be a good indicator for assessing stress of green tea because carotenoid is involved in photoprotection and light collection in photosynthesis (DemmigAdams *et al.*, 1996) and they help to protect unsaturated fatty acids, phospholipids and galactolipids from damage (Edge *et al.*, 1997). However, a combination use of content of chlorophyll, which also absorbs sunlight and synthesizes carbohydrates from CO_2 and H_2O is more useful for evaluating environmental stress in plants (Hendry and Price, 1993).Therefore, PROSPECT–D inversion was more of an ideal choice because it also estimated total chlorophyll content with a high accuracy (RMSPE = 6.73 %). However, chlorophyll pigments consist of two main types, namely chlorophyll *a* and *b*, and chlorophyll *a*/*b* ratio can also be influenced on the change of the physiological state of the plant (Kouril *et al.*, 1999). Chlorophyll *a*/*b* ratios increase sharply in a linear manner at low light intensity, but increase gradually and linearly at higher light intensities (Leong and Anderson, 1984), and the ratio is positively correlated with the ratio of PSII cores to light harvesting chlorophyll–protein complex (LHCI) (Terashima and Hikosaka, 1995). Therefore, assessing individual influence on leaf reflectance could improve the models.

<Figure 7>

<Figure 8>

4. Conclusions

The relationship between carotenoid contents and fifteen published hyperspectral indices has been assessed using the measurements from shade grown tea, which resulted in various combinations between chlorophyll and carotenoid contents. Among the indices, Gitelson1 and CARI had the highest performances, achieving the RMSPEs of 4.53 and 5.46 %, respectively, and they were categorised as ‘A’ based on RPD.

Inversion of PROSPECT–D was also considered for quantifying leaf carotenoid concentrations in this study. Although calibration of the absorption coefficients was required, it could estimate carotenoid contents with an RMSPE of 4.90 %, and it was categorised as ‘A’ based on RPD. Thus, both approaches were applicable for estimating carotenoid contents of shade grown tea from hyperspectral data, although not all hyperspectral indices were applicable.

Although the dataset used in this study included measurements under high stress environments, these treatments are normal for green tea production. Thus, the applicable approaches can help to improve our ability to monitor agricultural fields.

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Disclosure statement

No potential conflicts of interest are reported by the authors.

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Table

Table 1. Published hyperspectral indices.

Index	Formula	Reference
Chappelle	R_{760} / R_{500}	Chappelle *et al*. (1992)
PRI	(R_{531} – R_{570}) / (R_{531} – R_{570})	Gamon *et al*. (1992)
Blackburn1	R_{800} / R_{470}	Blackburn (1998)
Blackburn2	(R_{800} – R_{470}) / (R_{800} + R_{470})	Blackburn (1998)
Datt	R_{672} / (R_{550} × R_{708})	Datt (1998)
Gitelson1	R_{NIR} / R_{510}	Gitelson *et al*. (2002)
CRI550	1 / R_{510} – 1 / R_{550}	Gitelson *et al*. (2002)
CRI700	1 / R_{510} – 1 / R_{700}	Gitelson *et al*. (2002)
CARrededge	(1 / $R_{510-520}$ – 1 / $R_{690-710}$)R_{NIR}	Gitelson *et al*. (2006)
CARgreen	(1/$R_{510–520}$–1/$R_{560–570}$)R_{NIR}	Hernandez–Clemente *et al*. (2012)
Hernandez–Clemente	R_{515} / R_{570}	Hernandez–Clemente *et al*. (2012)
AVIcar	AVI2(R_{410}, R_{530}, R_{550})	Hernandez–Clemente *et al*. (2012)
Fassnacht1	scale(AVIcar) + scale(Chappelle)	Fassnacht *et al*. (2015)
Fassnacht2	scale(AVIcar) + scale(CARred–edge)	Fassnacht *et al*. (2015)
Carotenoid index (CARI)	R_{720} / R_{521} – 1	Zhou *et al*. (2017)

Note. The $R_{wavelength}$ indicate the reflectance at this wavelength. The use of scaled index values from 0–1(scale) and the angular vegetation index (AVI) were proposed by Fassnacht *et al*. (2015). Gitelson *et al*. (2006) defined NIR as the reflectance at 760–800 nm.

Table 2. Carotenoid content (μg cm^{-2}) for each treatment.

Treatment	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum
0% shading	7.37	8.25	8.51	8.53	8.86	9.86
35% shading	7.30	8.54	8.86	9.07	9.43	11.29
75% shading	7.68	9.10	9.36	9.35	9.68	10.27
90% shading	6.16	8.27	8.80	8.78	9.70	10.22
All	6.16	8.34	8.86	8.93	9.46	11.29

Table 3. Performance of 15 published indices for test data (n=32).

Index	Regression Type	RPD	RMSPE (%)	RMSE (μg cm^{-2})	R^2
Chappelle	Linear	1.15	8.84	0.85	0.489
PRI	Linear	1.04	10.25	0.93	0.125
Blackburn1	Linear	1.00	10.38	0.97	0.101
Blackburn2	Linear	1.02	10.10	0.95	0.161
Datt	Linear	1.06	10.17	0.92	0.257
Gitelson1	Exponential	2.33	4.53	0.42	0.833
CRI550	Linear	0.98	10.32	0.99	0.085
CRI700	Linear	0.99	10.34	0.98	0.088
CARrededge	Exponential	1.62	6.53	0.60	0.689
CARgreen	Linear	0.98	10.49	0.99	0.034
HernandezClemente	Linear	1.03	10.40	0.94	0.139
AVIcar	Exponential	1.14	9.53	0.85	0.343
Fassnacht1	Linear	1.73	6.63	0.56	0.739
Fassnacht2	Exponential	1.96	5.53	0.50	0.751
CARI	Exponential	2.06	5.46	0.47	0.782

Figures

Figure 1. Shading treatments conducted in this study.

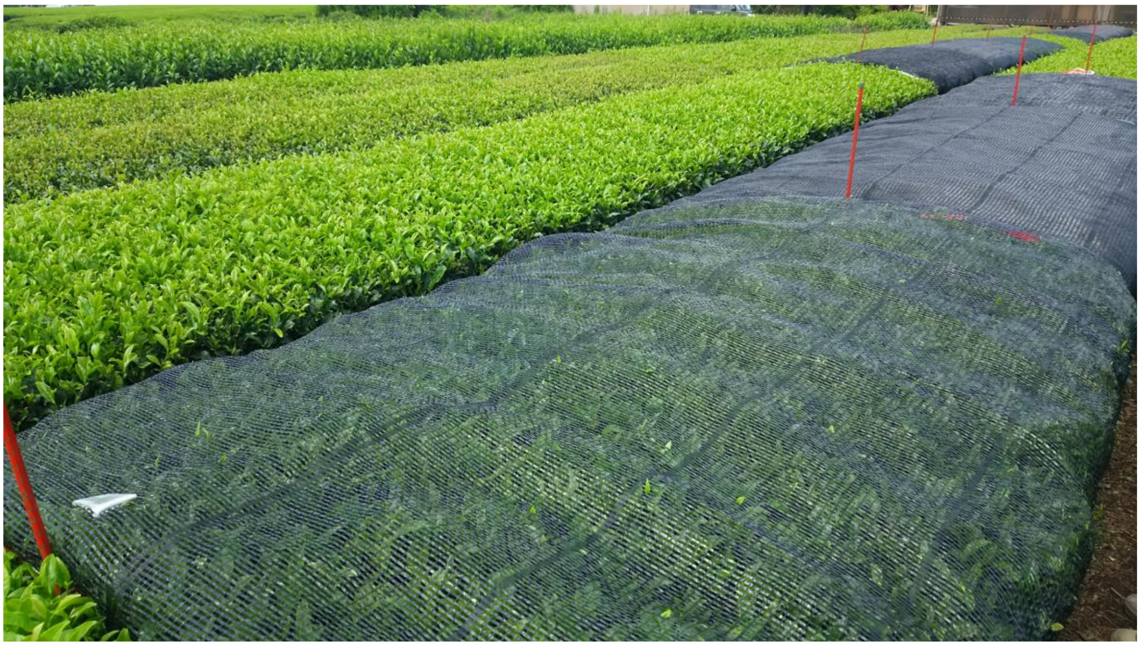


Figure 2. Weather conditions during the experiment.

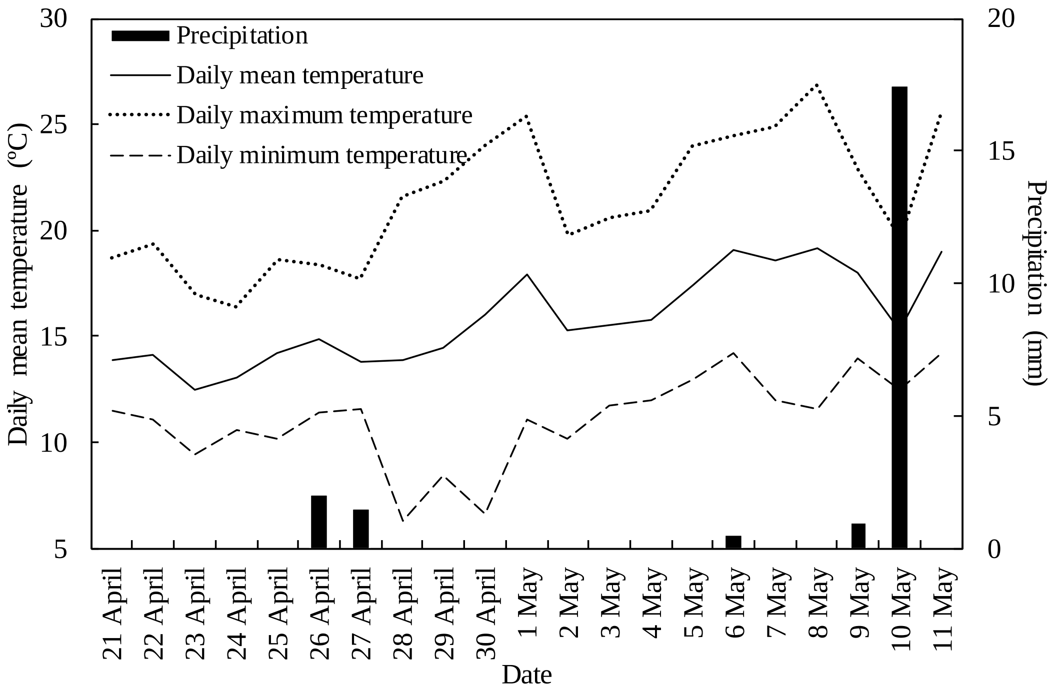
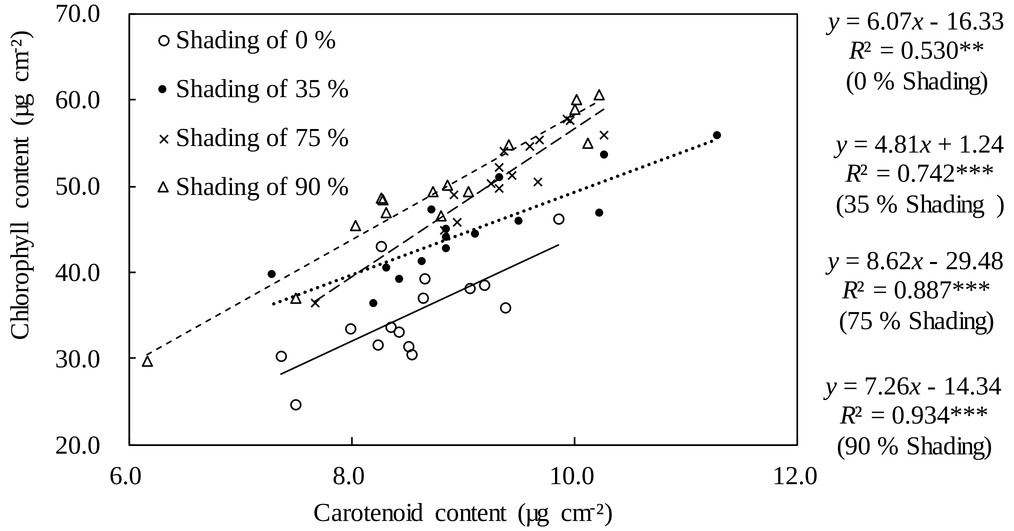


Figure 3. Relationship between carotenoid content and total chlorophyll content (*a*) or chlorophyll *a* content (*b*). *** indicates $p < 0.1\%$ and ** indicates $p < 1\%$.

(*a*)



(*b*)

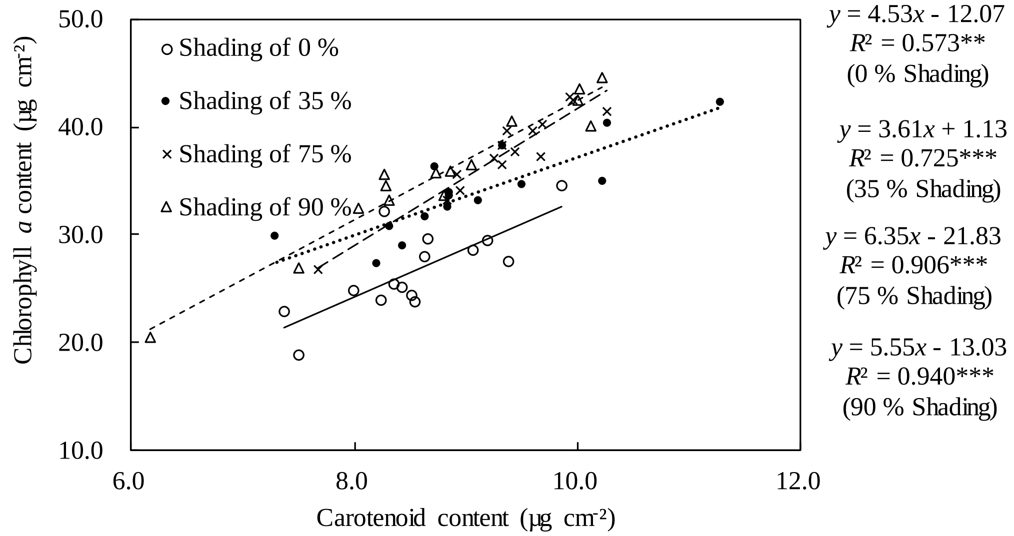


Figure 4. Mean reflectance spectra of different shading treatments.

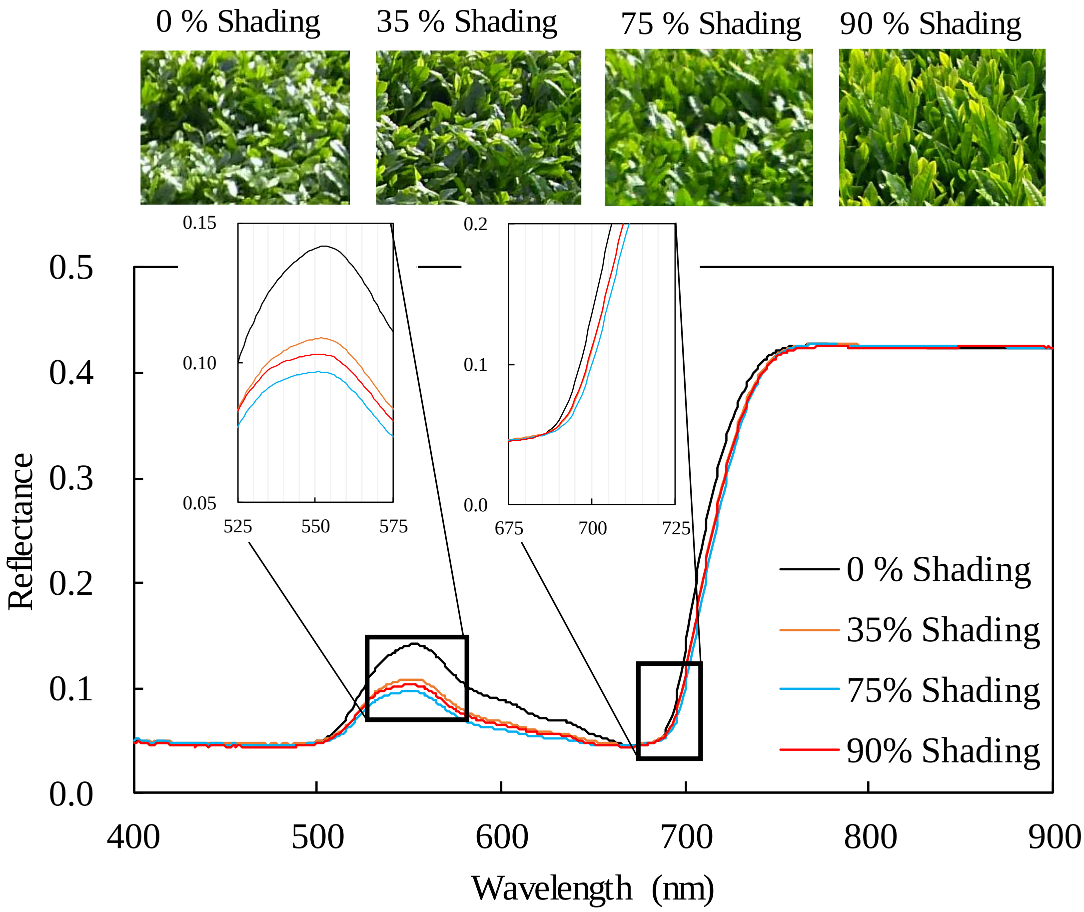


Figure 5. Relationships between estimated and measured carotenoid contents using Gitelson1 and CARI. *** indicates p < 0.1%

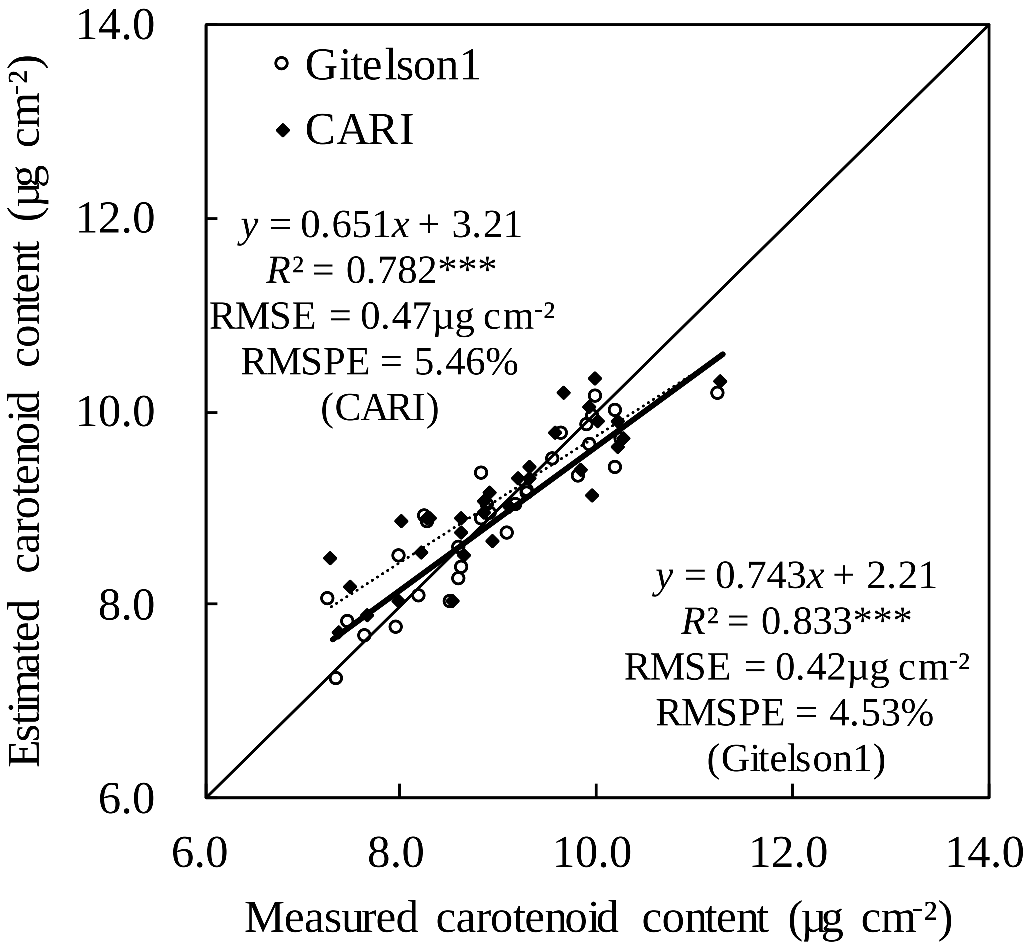


Figure 6. Correlations coefficient between reflectance at each wavelength and carotenoid content.

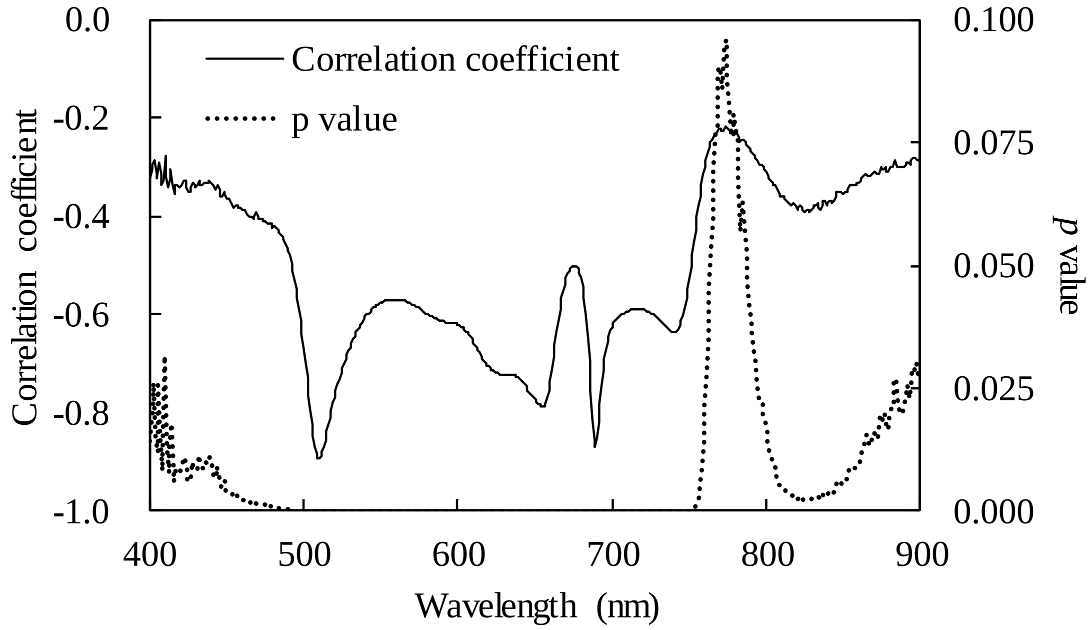


Figure 7. Absorption coefficients for carotenoid pigments.

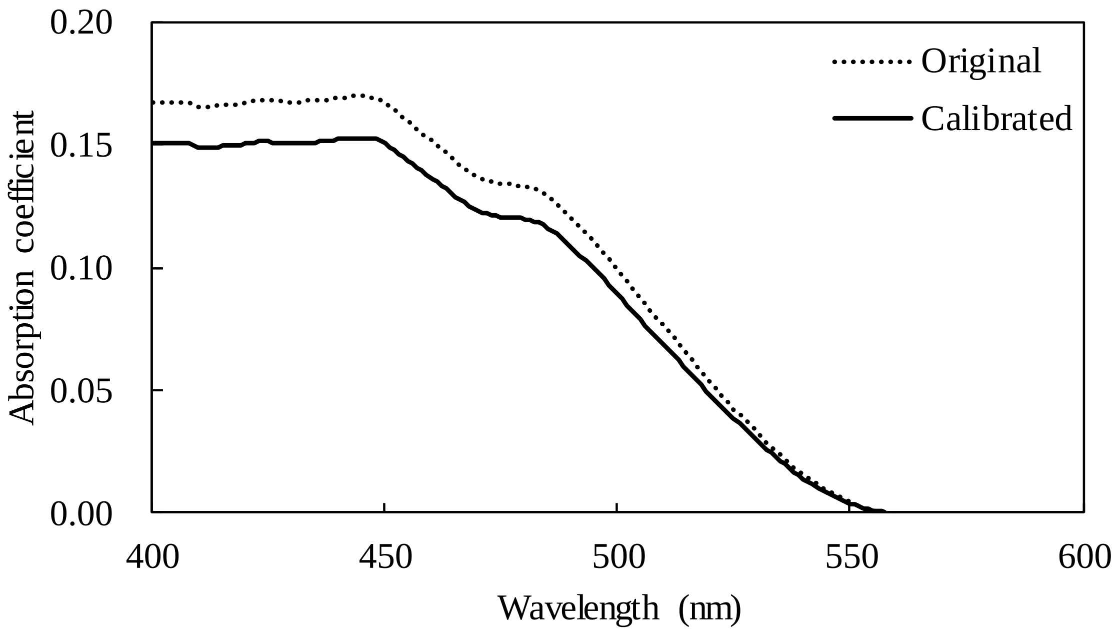


Figure 8. Relationship between estimated and measured carotenoid contents using original and calibrated PROSPECT–D. *** indicates $p < 0.1\%$