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Non-destructive determination of leaf chlorophyll content in two flowering cherries using reflectance and absorptance spectra

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Abstract:

Quantifying leaf chlorophyll is a key technique in tree vigor assessment. Although many studies on non-destructive and in-field determination by spectroscopy have been conducted, for accurate determination it is reasonable to develop species-specific chlorophyll indices because leaf spectra can vary independently of chlorophyll content due to leaf surface and structural differences among species. The present study aimed to develop optimal reflectance and absorptance indices for estimating leaf chlorophyll content of *Cerasus jamasakura* (Siebold ex Koidz.) H. Ohba var. *jamasakura* and *Cerasus* ×*yedoensis* 'Somei-yoshino' and to examine their performance by comparing with 46 published chlorophyll indices and SPAD. For 96 and 100 leaf samples, measurements by a spectroradiometer with a leaf-clip attachment and the SPAD-502 chlorophyll meter were made, and chlorophyll content was determined by extraction with *N*, *N'*–dimethylformamide. Then the optimal leaf chlorophyll indices were systematically developed by testing eight types of indices. As a result, we confirmed that the optimal chlorophyll indices performed better than any of the published leaf chlorophyll indices or SPAD, about two-times better RMSE than SPAD, and found that the newly proposed index type, difference and ratio combination type, may be a useful form of chlorophyll content estimation. We also found that absorptance indices achieved equivalent results to reflectance indices despite the hypothesis that absorptance measurement is direct and has more potential. Among the published indices, the reflectance ratio index of Datt (1999) and the red edge chlorophyll index of Ciganda et al. (2009) were effective to estimate leaf chlorophyll content of both flowering cherries.

Key words: leaf pigments, hyperspectral remote sensing, derivative spectra, SPAD, *Cerasus jamasakura* (Siebold ex Koidz.) H. Ohba var. *jamasakura*, *Cerasus* ×*yedoensis* 'Somei-yoshino'

INTRODUCTION

Flowering cherries are the most popular ornamental trees which herald the arrival of spring in Japan. Cherry blossom festivals, or *Hanami*, are a special feature of Japanese life. During the *Hanami*, all ages spend time outdoors, enjoying the beauty of the cherry blossoms by day and by night, with their family, friends, and workmates (Primack and Higuchi, 2007). Flowering cherries have been the subject of numerous poems and songs and depicted in paintings and textiles for more than a thousand years. Because of their great popularity and cultural significance, the tree vigor assessment has been the most important issue for their management (Masuda and Iwase, 1989; Satomura et al., 2005; Kume and Hioki, 2006).

Quantifying leaf chlorophylls is a key technique in tree vigor assessment because leaf chlorophyll content is an indicator of the physiological status of a plant. For trees chlorophylls a and b (hereafter Chl a and Chl b) have a dominant control upon the amount of solar radiation that a leaf absorbs, therefore, chlorophyll content in a canopy controls photosynthetic potential and primary production of a tree (Lieth, 1973; Larcher, 2004). Chlorophylls are also responsive to the status of plant nutrition, especially of nitrogen (Taiz and Zeiger, 2006), and decrease under various types of stresses (Carter 1993; Carter and Knapp, 2001).

Traditionally, chlorophyll content is determined by extraction with an organic solvent and subsequent measurement on a spectrophotometer (Arnon, 1949; Porra, et al.1989). However, the method is often disadvantageous because it is destructive and time consuming. More recently, an alternative approach based on spectral characteristics of a leaf has been developed and enables non-destructive, rapid and in-field determination of chlorophyll content.

The SPAD–502 (Minolta Camera Co., Japan), a commercially-available chlorophyll meter, is one of the most common non-destructive method to determine the level of leaf chlorophyll content (Castelli, et al., 1996; Azia and Stewart, 2001; Wang et al., 2004; Netto et al., 2005; Pinkard et al., 2006; Uddling et al., 2007) and therefore nitrogen status of plants (Wang et al., 2004; Netto et al., 2005; Pinkard et al., 2006). Although the SPAD chlorophyll meter is successfully applied in many scientific researches, a few limitations are known, for example, that the correlation between chlorophyll content and SPAD value is weaker in some species (Uddling et al., 2007), or that the accuracy of the instrument considerably diminishes above the chlorophyll level of 300 mg m^{-2} in grapevine leaves (Steele et al., 2008). Therefore, the prediction accuracy has to be examined before the instrument is applied to a leaf of a target species.

Alongside developments in hyperspectral data acquisition, there has been an increasing intensity of research focused on developing techniques for analyzing vegetation spectra in order to quantify pigment concentrations (Blackburn, 2007). However, to extract pigment information the range of other factors which also influence vegetation reflectance spectra must be taken into account (Blackburn, 2007). Leaf reflectance can vary independently of pigment concentrations due to differences such as leaf thickness and surface characteristics (e.g. hairs and waxes) (Sims and Gamon, 2002; Levizou et al., 2005). Thus relationships between spectra and chlorophyll content must be determined for each species of interest if more accurate quantification is required.

Studies on estimating leaf chlorophyll content from absorptance spectra in field are extremely limited. Richardson et al. (2002) discussed that it could be hypothesized that instruments that estimate chlorophyll content by directly measuring the amount of radiation absorbed should be able to give better estimates of chlorophyll content than those relying on reflectance measures. However, they have not assessed the performance of absorptance spectra but only of two hand-held chlorophyll meters, CCM-200 (Opti-Sciences Inc., U.S.A.) and SPAD-502 that are regarded as chlorophyll transmittance meters. Therefore, the ability of absorptance spectra should be more investigated.

In the present study we aimed to find the optimal indices for estimating leaf chlorophyll content of two flowering cherries in field by measuring reflectance or absorptance spectra and to examine performance of the optimal species-specific chlorophyll indices by comparing with published chlorophyll indices and SPAD.

METHODS

Sample collection

In the present study we focused on two flowering cherries; *Cerasus jamasakura* (Siebold ex Koidz.) H. Ohba var. *jamasakura* (or known as *Prunus serrulata* Lindl. var. *spontanea* (Maxim.) E. H. Wilson or *Prunus jamasakura* Siebold ex Koidz.) and *Cerasus ×yedoensis* 'Somei-yoshino' (or known as *Prunus ×yedoensis* Matsum. cv. Yedoensis).

C. jamasakura is a most familiar wild cherry tree, known in English as the Japanese mountain cherry and in Japanese as the yama-zakura. It is a deciduous tree and typically found in the foothills of western Japan, often in secondary forests. It is also extensively cultivated as ornamental plants. The five-petaled flowers are pale pink and petal about 1.1–1.9 cm long. The young leaves are brownish-red to red in color, presenting a harmonious landscape with the flowers. The mature leaves are 8–12 cm long and 3–4.5 cm wide, upper surface with sparse hairs when young, lower surface glaucous.

C. ×yedoensis is the most common flower cherry, known in English as the Potomac cherry or the Yoshino cherry blossom tree and in Japanese as the Somei-yoshino, and considered to be derived from a hybrid between *Cerasus speciosa* (Koidz.) H. Ohba and *Cerasus spachiana* Lavallée ex E. Otto f. *ascendens* (Makino) H. Ohba. It is a deciduous tree and widely cultivated across the country. Although it is usually considered that it has no variation because it is vegetatively propagated by grafting, individual variations of *C. ×yedoensis* are observed in terms of flowering period and petal color (Iwasaki, 1990a; Iwasaki, 1990b). The five-petaled flowers are pale pink and petal about 1.5 cm long. The abundant pink flowers appear before the leaves. The mature leaves are 7–11 cm long and 4–6 cm wide, both surfaces glabrous or with sparse hairs, lower surface pale green.

We collected a total of 96 and 100 leaf samples for *C. jamasakura* and *C.* ×*yedoensis* on July 24 and 28, 2008, respectively, from four famous cherry-tree sites, Lake Biwa Canal in Okazaki and Yamashina areas, Maruyama Park and Path of Philosophy in Kyoto City, Japan. Sample leaves were preliminarily selected by visual evaluation of leaf color to cover wide range of chlorophyll content levels. After the collection, the samples were immediately treated to keep water and were stored under cool and dark condition.

Spectral Measurements and Chlorophyll Content Determination

Within a few hours after the collection, all the samples were processed in a laboratory as described below. Reflectance and absorptance spectra of leaves at wavelength from 325 to 1,075 nm were measured using the FieldSpec HandHeld spectroradiometer with the 10-mm-spot-size Plant Probe and Leaf Clip attachments (Analytical Spectral Devices Inc., U.S.A.). The spectral sampling interval is 1.6 nm, and the spectral resolution is about 3.5 nm half-bandwidth. The output from the device is a spectrum of 1-nm interval by interpolation. The spectroradiometer attachments allow easily measuring leaf reflectance and transmittance, and thus absorptance spectra, using white and black background standards on the Leaf Clip head. Although the transmittance and absorptance spectra are approximate as transmittance spectrum is calculated from difference of measurements with the white and black background standards, we considered that it is more realistic for non-destructive measurement in field than using an integrating sphere. For each leaf measurement, scans repeated 15 times and the mean spectrum was recorded.

Additionally, three separate measurements with a commercial hand-held chlorophyll meter, the SPAD–502, was made on each leaf at the same position as the leaf spectra were measured. It weighs 225 g, has a 0.06-cm^2 measurement area, and calculates an index in SPAD units based on transmittance at around 650 and 940 nm (Markwell et al. 1995). The claimed accuracy of the SPAD–502 is ± 1.0 SPAD units. We used the mean of the three measurements for subsequent analysis.

Immediately after the SPAD measurements, one 15-mm-diameter disc was punched from the same position of each leaf, cut into halves and extracted with 2-mL *N*, *N*'–dimethylformamide (DMF) for one night at four degrees Celsius. Chlorophyll absorbance was measured with the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., U.S.A.) and chlorophyll concentrations were determined by the formulae of Porra et al. (1989).

Data Preparation

To remove noise of reflectance and absorptance spectra, the Savitzky-Golay convolution filter with a 17-point moving window and a 4th-degree polynomial was applied. Additionally, using the filter, first and second derivative spectra were directly derived (Savitzky and Golay, 1964). Derivative spectra were studied as they have distinctive information of detecting vegetation stress (Curran et al. 1990; Imanishi et al. 2004; Imanishi et al. 2007). Only the wavelength from 400 to 1,000 nm were analyzed for the subsequent analysis as the edges of measured spectra contain greater noise attributed to the sensor's nature.

Finding the species-specific optimal indices

We tested the following eight types of indices:

T1) single type	V_1
T2) difference type	$V_1 - V_2$
T3) ratio type	V_1/V_2
T4) normalized difference type	$(V_1 - V_2)/(V_1 + V_2)$
T5) ratio type with the third band	$(V_1 - V_3)/(V_2 - V_3)$
T6) normalized difference type with the third band	$(V_1 - V_2)/(V_1 + V_2 - 2 \times V_3)$
T7) area type	$\sum_{\lambda 1}^{\lambda 2} V_i$
T8) difference and ratio combination type	$(V_1 - V_2)/V_3$

where V_1, V_2 and V_3 represent values of reflectance or absorptance spectra or values of their first or second derivative spectra at different wavelengths, and λ_1 and λ_2 in T7 indicate different wavelengths ($\lambda_1 < \lambda_2$).

T8 is original in the present study and the others were obtained from the literature review on estimation of leaf chlorophyll content using optical remote sensing. While the first four are well-known basic types, T5 and T6 are recently recognized as modified types of T3 and T4, involving correction terms of the third band for removing constant additive effect on spectra due to leaf surface differences (Sims and Gamon, 2002). T7 is related to area between two wavelengths. Although it has not been studied in depth in the previous studies, this type seemed to have potential because it is usual that a part of spectrum increases or decreases during chlorophyll degradation and the use of several continuous bands seems more stable than the single type (T1). T8 was developed in the present study based on the idea that the combination of the two basic types, difference and ratio types, would produce better results and remove both additive and multiplicative effects on spectra that are possibly caused by individual leaf surface and structural differences or illumination change during spectral measurements.

For selecting optimal chlorophyll indices for Chl a+b, Chl a and Chl b of the two flowering cherries, all the combinations of wavelengths (bands) at 5-nm intervals were tested for the eight types of indices. Linear, second-degree polynomial and exponential functions were fitted to a data set of index value and laboratory-determined chlorophyll content, and 10-fold cross-validated root mean square errors (cvRMSE) were calculated. Then, we selected a pair of an optimal index and a fitting function that has the lowest cvRMSE. We also derived the cross-validated R squared (cvR^2) at the same time.

Three cases on availability of number of bands were assumed during the selection of optimal indices as it is sometimes important to reduce number of required bands, e.g. for developing a low-cost hand-held device: 1) All bands are available like hyperspectral data which can calculate derivative spectra as well, 2) only three bands available and 3) only two bands available like the hand-held chlorophyll meter SPAD-502.

In order to understand the mechanism that the selected indices explain variation of chlorophyll content, cvR^2 of components of the indices were analyzed. We defined a primary element as a component that separately has greater than or equal to 80 % of cvR^2 of the chlorophyll index whereas a secondary as a component that separately has less than 80 % of cvR^2 of the chlorophyll index.

Assessing performance of the published chlorophyll indices and SPAD

A total of 46 published chlorophyll indices were listed from the literature review (cf. Tables 3 and 4). All the published indices were based on reflectance and its derivative spectra and none of them utilized absorptance spectra. The performance of the published indices and SPAD were assessed by calculating 10-fold cvRMSE and cvR^2 by the same method as described above.

RESULTS

Sampled leaves

The chlorophyll content determined by the DMF extraction were ranged from 61.9 to 544.3 mg m^{-2} for Chl a+b, 52.1 to 436.9 mg m^{-2} for Chl a and 9.7 to 129.8 mg m^{-2} for Chl b in *C. jamasakura*, and from 191.6 to 663.6 mg m^{-2} for Chl a+b, 140.4 to 515.0 mg m^{-2} for Chl a and 37.6 to 151.1 mg m^{-2} for Chl b in *C.* ×*yedoensis*. The means and standard deviations of Chl a/b were, respectively, 3.8 ± 0.9 in *C. jamasakura* and 3.6 ± 0.6 in *C.* ×*yedoensis*. The Pearson's correlation coefficients between Chl a and Chl b were 0.933 in *C. jamasakura* and 0.908 in *C.* ×*yedoensis*.

Change of leaf spectra corresponding to chlorophyll content

Overall, the response of spectra to chlorophyll content was similar between the two flowering cherries (Figs. 1 and 2). In reflectance spectra (Figs. 1a and 2a), the response at the green peak around 550 nm seemed straightforward, i.e. increase of chlorophyll content led to decrease of reflectance. However, the change at near infrared region (NIR) was unstable. In first derivative spectra of reflectance (Figs. 1b and 2b), blue shift of red edge were observed: the peak at around 700 nm was shifted to shorter-wavelength side according to decrease of chlorophyll content. In second derivative spectra of reflectance (Figs. 1c and 2c), the height of peaks and depth of troughs generally increased corresponding to chlorophyll reduction. In absorptance spectra and its derivative spectra (Figs. 1d, 1e, 1f, 2d, 2e and 2f), the characteristics of spectral change in response to chlorophyll content were similar to reflectance spectra on the whole, but the direction was opposite, i.e. peaks in reflectance correspond to troughs in absorptance spectra and vice versa.

Cerasus jamasakura var. *jamasakura*

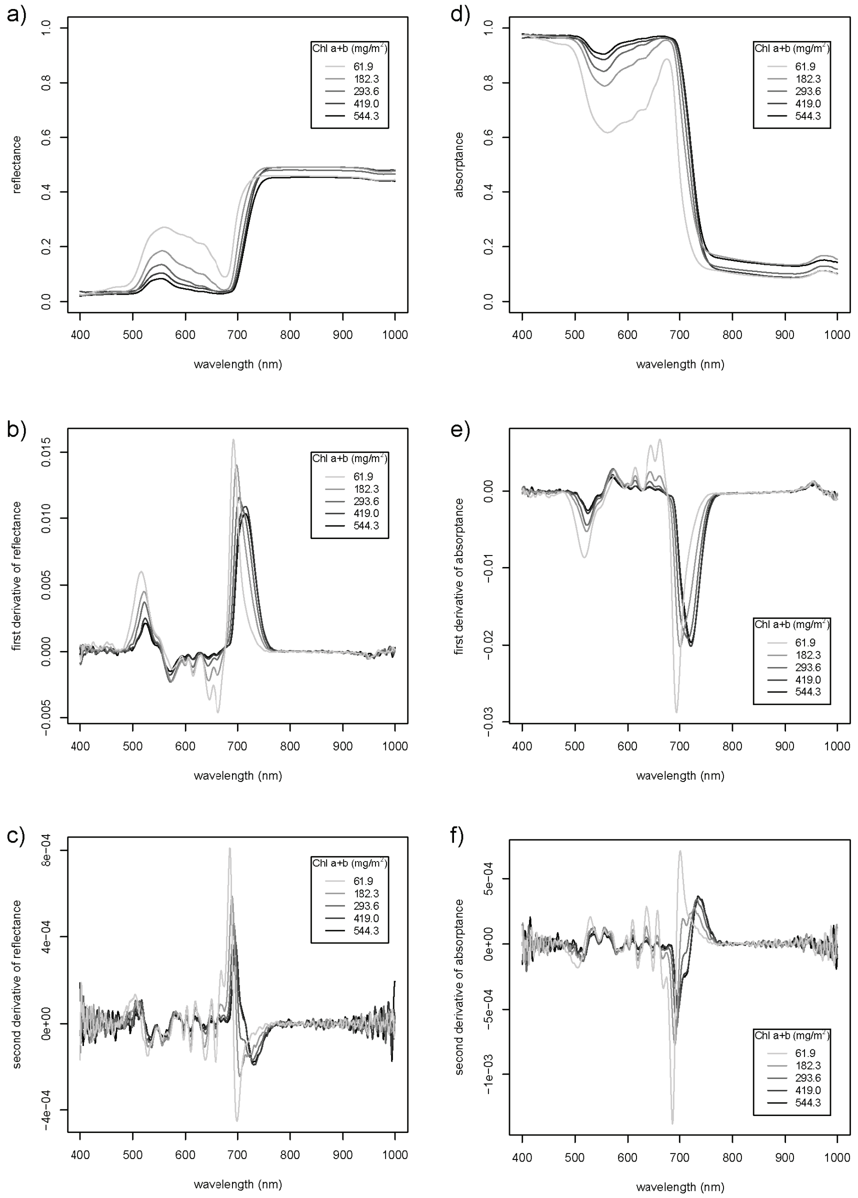

Fig. 1 Five representative leaf spectra of *C. jamasakura* corresponding to different levels of chlorophyll a+b content per area. a) reflectance spectra, b) first derivative spectra of reflectance, c) second derivative spectra of reflectance, d) absorptance spectra, e) first derivative spectra of absorptance, f) second derivative spectra of absorptance.

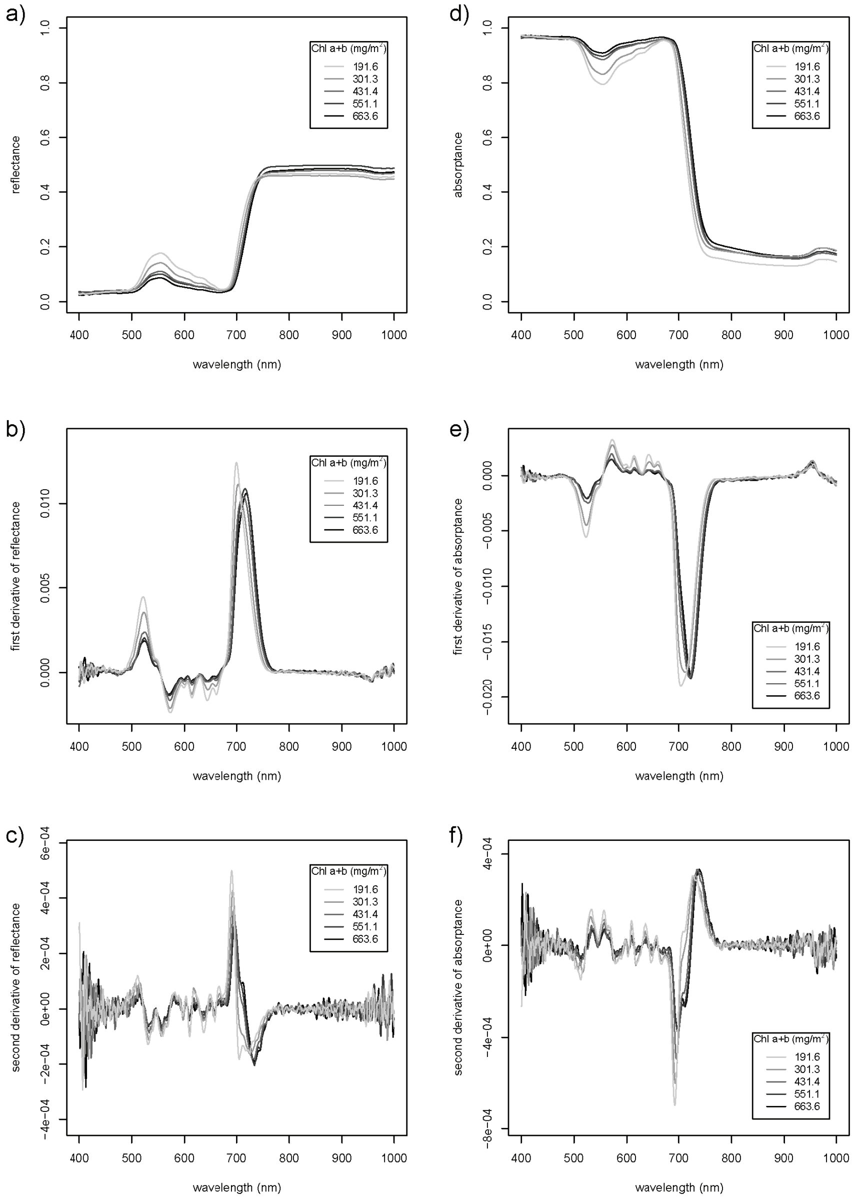
Cerasus ×*yedoensis* ‘Somei-yoshino’

Fig. 2 Five representative leaf spectra of *C.* ×*yedoensis* corresponding to different levels of chlorophyll a+b content per area. a) reflectance spectra, b) first derivative spectra of reflectance, c) second derivative spectra of reflectance, d) absorptance spectra, e) first derivative spectra of absorptance, f) second derivative spectra of absorptance.

The amplitude of peaks and troughs were smaller in *C. ×yedoensis* than *C. jamasakura*, and tended to decrease according to increase of chlorophyll content per area. The reflectance and absorptance at red region around 680 nm appeared saturated beyond about 300 mg m^{-2} of Chl a+b for both flowering cherries.

Performance of the specie-specific chlorophyll indices

The cvR^2 of the selected optimal chlorophyll indices were high, 0.963–0.861 in *C. jamasakura* and 0.903–0.774 in *C. ×yedoensis* (Tables 1 and 2). However, it was lower in Chl b than Chl a+b or Chl a, and in *C. ×yedoensis* than *C. jamasakura*. The performance of the optimal indices was equivalent between reflectance and absorptance spectra in terms of cvR^2 and cvRMSE (Tables 1 and 2, also see Figs 3 and 4). When the number of available bands increased from two to more bands, cvR^2 and cvRMSE were improved (Tables 1 and 2). The optimal indices using three bands were the same as those that can use all the bands, for estimating Chl a+b and Chl a of *C. jamasakura* in reflectance spectra (these are identical also between Chl a+b and Chl a), and Chl a+b and Chl a of *C. ×yedoensis* in absorptance spectra. The newly proposed type, difference and ratio combination type (T8) was selected in four (two in reflectance and two in absorptance) of the 12 cases that use three or more bands for *C. jamasakura*, and ten (four in reflectance and six in absorptance) of the 12 cases for *C. ×yedoensis*.

The wavelengths where primary elements were located were 615, 620 and 645 nm (unsaturated red region) and 745 nm (red edge region) in reflectance indices for *C. jamasakura*, 530 and 540 nm (green region), 635 nm (unsaturated red region) and 690, 695, 700, 715, 725 and 740 nm (red edge region) in absorptance indices for *C. jamasakura*, 570 and 585 nm (yellow region) and 705, 720 and 755 nm (red edge region) in reflectance indices for *C. ×yedoensis*, and 520, 540, 550 and 560 nm (green region) and 700, 710 and 765 nm (red edge region) in absorptance indices for *C. ×yedoensis* (Tables 1 and 2).

The chlorophyll indices that were only consisted of secondary elements, which means combination of elements significantly improved explanation of chlorophyll variation compared to single use of the elements, made use of wavelengths at 730 nm (red edge region) and 810 nm (NIR) in a reflectance index for *C. jamasakura*, and 565 and 575 nm (yellow region), 735 and 740 nm (red edge region) and 770, 830, 845 and 855 nm (NIR) in four reflectance indices for *C. ×yedoensis* (Tables 1 and 2). All the five reflectance indices always included a wavelength at NIR.

Performance of the published chlorophyll indices and SPAD

None of the published indices exceeded the performance of the optimal species-specific chlorophyll indices (Tables 3 and 4). The reflectance ratio index of Datt (1999), $(R_{850} - R_{710})/(R_{850} - R_{680})$ (hereafter Datt's RI), ranked first for Chl a+b and Chl a in *C. jamasakura* and ranked third for Chl a+b and Chl a for *C. ×yedoensis* among the tested published indices. The first derivative ratio index of Datt (1999), fR_{754}/fR_{704}, ranked first for Chl a+b, Chl a and Chl b in *C. ×yedoensis*, but ranked 34th for Chl a+b

Table 1 Performance of systematically-selected optimal chlorophyll index for leaves of *C. jamasakura*

	objective variable ($\mathrm{mg\ m^{-2}}$)	assumption on available bands	selected optimal chlorophyll index	cvR^2	cvRMSE	primary element(s)	secondary element(s)	the best fitted equation (x = value of the chlorophyll index)
reflectance	Chl a+b	all bands	$(R_{915}-R_{645})/(R_{620}-R_{645})$	0.963	22.2	R_{620}, R_{645}	R_{915}	$0.0955+11.1x-0.0577x^2$
		3 bands	$(R_{915}-R_{645})/(R_{620}-R_{645})$	0.963	22.2	R_{620}, R_{645}	R_{915}	$0.0955+11.1x-0.0577x^2$
		2 bands	R_{915}/R_{725}	0.955	24.3	R_{725}	R_{915}	$-1038.0+1064.3x$
	Chl a	all bands	$(R_{915}-R_{645})/(R_{620}-R_{645})$	0.961	17.7	R_{620}, R_{645}	R_{915}	$-0.236+8.74x-0.0465x^2$
		3 bands	$(R_{915}-R_{645})/(R_{620}-R_{645})$	0.961	17.7	R_{620}, R_{645}	R_{915}	$-0.236+8.74x-0.0465x^2$
		2 bands	$R_{645}-R_{615}$	0.953	19.3	R_{615}, R_{645}	–	$575.5e^{50.0x}$
	Chl b	all bands	$(fR_{455}-fR_{745})/fR_{590}$	0.890	8.9	fR_{745}	fR_{590}, fR_{455}	$22.6+20.3x-0.996x^2$
		3 bands	$(R_{950}-R_{400})/R_{725}$	0.889	8.9	R_{725}	R_{950}, R_{400}	$-235.4+256.7x$
		2 bands	R_{810}/R_{730}	0.883	9.2	–	R_{730}, R_{810}	$-387.1+389.1x$
absorptance	Chl a+b	all bands	$sA_{690}-sA_{655}$	0.960	22.8	sA_{690}	sA_{655}	$727.4e^{1842.7x}$
		3 bands	$(A_{700}-A_{695})/A_{400}$	0.960	23.0	A_{700}, A_{695}	A_{400}	$930.6e^{17.4x}$
		2 bands	$A_{700}-A_{695}$	0.957	23.7	A_{700}, A_{695}	–	$929.2e^{17.9x}$
	Chl a	all bands	$sA_{690}-sA_{635}$	0.964	17.0	sA_{690}, sA_{635}	–	$553.0e^{1636.9x}$
		3 bands	$(A_{700}-A_{695})/A_{400}$	0.960	17.8	A_{700}, A_{695}	A_{400}	$722.9e^{17.3x}$
		2 bands	$A_{700}-A_{695}$	0.959	18.1	A_{700}, A_{695}	–	$721.9e^{17.8x}$
	Chl b	all bands	$(sA_{740}-sA_{715})/(sA_{740}+sA_{715}-2\times sA_{835})$	0.878	9.3	sA_{715}, sA_{740}	sA_{835}	$34.9+23.1x-1.48x^2$
		3 bands	$(A_{925}-A_{695})/(A_{725}-A_{695})$	0.877	9.4	A_{695}, A_{725}	A_{925}	$-147.6+134.8x$
		2 bands	A_{540}/A_{530}	0.861	10.0	A_{540}, A_{530}	–	$8.84\times10^{-19}\cdot e^{47.0x}$

A primary element separately has greater than or equal to 80 % of cvR^2 of the chlorophyll index whereas a secondary element has less than 80 % of the cvR^2.

R_λ, fR_λ, sR_λ: Reflectance, and first and second derivatives of reflectance spectra at λ nm, respectively

A_λ, fA_λ, sA_λ: Absorptance, and first and second derivatives of absorptance spectra at λ nm, respectively

Table 2 Performance of systematically-selected optimal chlorophyll index for leaves of *C.* ×*yedoensis*

	objective variable (mg m^{-2})	assumption on available bands	selected optimal chlorophyll index	cvR^2	cvRMSE	primary element(s)	secondary element(s)	the best fitted equation (x = value of the chlorophyll index)
reflectance	Chl a+b	all bands	$(fR_{555} - fR_{755})/fR_{560}$	0.903	33.3	fR_{755}	fR_{560}, fR_{555}	$138.5 + 325.1x - 35.0x^2$
		3 bands	$(R_{885} - R_{705})/(R_{725} - R_{705})$	0.899	34.1	R_{705}	R_{725}, R_{885}	$-1046.3 + 908.1x$
		2 bands	R_{830}/R_{735}	0.882	36.8	–	R_{735}, R_{830}	$-1240.8 + 163.4x + 1149.8x^2$
	Chl a	all bands	$(fR_{555} - fR_{755})/fR_{565}$	0.903	25.8	fR_{755}	fR_{565}, fR_{555}	$116.4 + 386.5x - 77.0x^2$
		3 bands	$(R_{760} - R_{720})/(R_{725} - R_{720})$	0.901	26.1	R_{720}	R_{725}, R_{760}	$-250.5 - 15.4x + 50.0x^2$
		2 bands	R_{770}/R_{740}	0.881	28.6	–	R_{740}, R_{770}	$-3655.9 + 3719.2x$
	Chl b	all bands	$(fR_{570} - fR_{585})/fR_{720}$	0.819	11.1	fR_{570}, fR_{585}	fR_{720}	$370.5e^{15.7x}$
		3 bands	$(R_{575} - R_{565})/R_{845}$	0.808	11.4	–	R_{565}, R_{575}, R_{845}	$400.4e^{43.8x}$
		2 bands	$(R_{855} - R_{735})/(R_{855} + R_{735})$	0.770	12.5	–	R_{735}, R_{855}	$35.3e^{15.0x}$
absorptance	Chl a+b	all bands	$(A_{710} - A_{700})/A_{540}$	0.897	34.5	A_{710}, A_{540}, A_{700}	–	$1516.8e^{8.20x}$
		3 bands	$(A_{710} - A_{700})/A_{540}$	0.897	34.5	A_{710}, A_{540}, A_{700}	–	$1516.8e^{8.20x}$
		2 bands	A_{710}/A_{700}	0.893	35.1	A_{710}, A_{700}	–	$0.584e^{7.82x}$
	Chl a	all bands	$(A_{710} - A_{700})/A_{560}$	0.902	26.0	A_{710}, A_{560}, A_{700}	–	$1146.0e^{7.94x}$
		3 bands	$(A_{710} - A_{700})/A_{560}$	0.902	26.0	A_{710}, A_{560}, A_{700}	–	$1146.0e^{7.94x}$
		2 bands	A_{710}/A_{700}	0.900	26.2	A_{710}, A_{700}	–	$0.484e^{7.74x}$
	Chl b	all bands	$(fA_{765} - fA_{520})/fA_{825}$	0.802	11.6	fA_{520}, fA_{765}	fA_{825}	$149.3 + 9.72x + 0.250x^2$
		3 bands	$(A_{550} - A_{585})/A_{710}$	0.777	12.3	A_{710}, A_{550}	A_{585}	$234.7 + 3458.0x + 16504.5x^2$
		2 bands	A_{560}/A_{585}	0.774	12.4	A_{560}	A_{585}	$29738.8 - 64307.8x + 34826.3x^2$

A primary element separately has greater than or equal to 80 % of cvR^2 of the chlorophyll index whereas a secondary element has less than 80 % of the cvR^2.

R_λ, fR_λ, sR_λ: Reflectance, and first and second derivatives of reflectance spectra at λ nm, respectively

A_λ, fA_λ, sA_λ: Absorptance, and first and second derivatives of absorptance spectra at λ nm, respectively

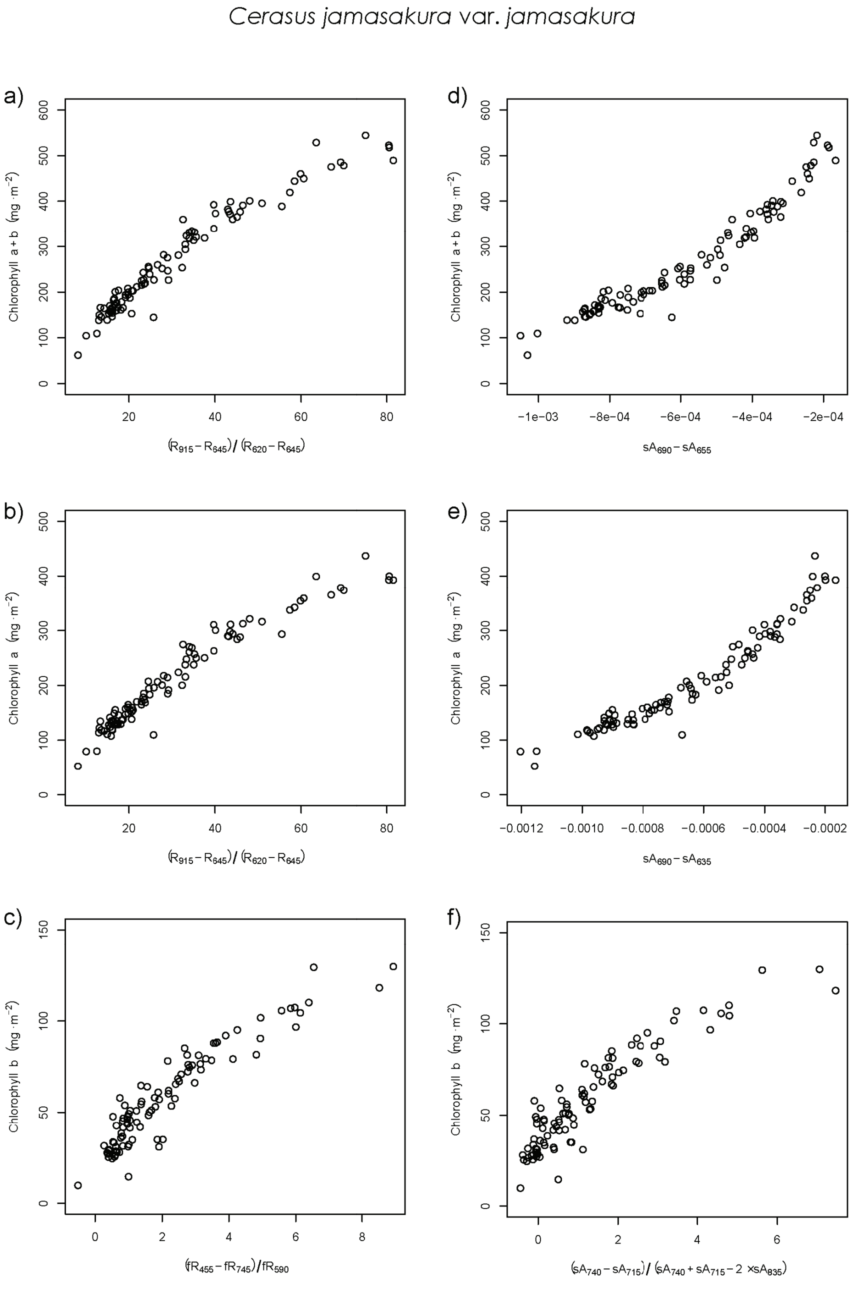

Fig. 3 Relationships between chlorophyll contents and the optimal chlorophyll indices of *C. jamasakura* that were systematically selected with the assumption that all the bands are available. Reflectance indices for a) Chl a+b, b) Chl a, c) Chl b and absorptance indices for d) Chl a+b, e) Chl a, f) Chl b.

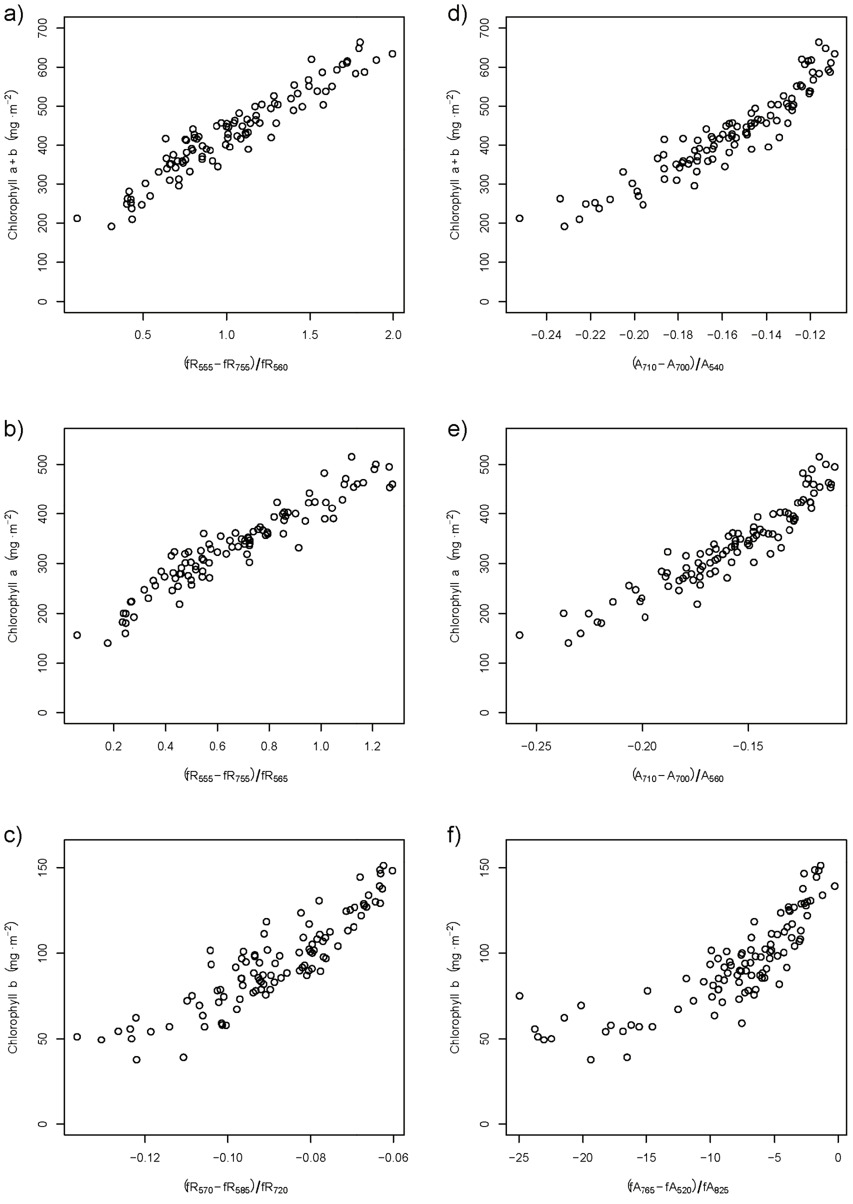

Fig. 4 Relationships between chlorophyll contents and the optimal chlorophyll indices of *C.* ×*yedoensis* that were systematically selected with the assumption that all the bands are available. Reflectance indices for a) Chl a+b, b) Chl a, c) Chl b and absorptance indices for d) Chl a+b, e) Chl a, f) Chl b.

Table 3 Performance of 46 published indices and SPAD in estimating leaf chlorophyll content of *C. jamasakura.* The list was sorted by the rank based on cvRMSE for Chl a+b.

Index	cvR^2			cvRMSE			Rank			Originally related to	Source
	Chl a+b	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b	Chl a	Chl b		
Datt's RI: $(R_{850}-R_{710})/(R_{850}-R_{680})$	0.952	0.947	0.866	25.3	20.6	9.8	1	1	11	Chl a+b, Chl a	Datt (1999)
Ciganda's RECI: $(R_{770-800}/R_{720-730})-1$	0.951	0.943	0.877	25.5	21.3	9.4	2	7	1	Chl a+b	Ciganda et al. (2009)
R_{750}/R_{705}	0.950	0.945	0.868	25.7	21.0	9.7	3	4	9	Chl a	Gitelson and Merzlyak (1994)
$(R_{706}-R_{\mathrm{rm}})/(R_{750}-R_{\mathrm{rm}})$	0.949	0.945	0.861	25.8	20.9	10.0	4	2	18	Chl a	Maccioni et al. (2001)
$(R_{750}-R_{705})/(R_{750}+R_{705})$	0.949	0.945	0.868	25.9	20.9	9.7	5	3	7	Chl a	Gitelson and Merzlyak (1994)
$(R_{780}-R_{710})/(R_{780}-R_{680})$	0.948	0.944	0.861	26.1	21.1	10.0	6	5	16	Chl a+b	Datt(1999), Maccioni et al. (2001)
$(R_{750-800}/R_{695-740})-1$	0.948	0.939	0.874	26.1	22.1	9.5	7	15	2	Chl a+b	Gitelson et al. (2003)
$\sum_{\lambda=705}^{750}(R_{\lambda}/R_{705}-1)$	0.948	0.941	0.860	26.1	21.6	10.0	8	13	20	Chl a	Gitelson and Merzlyak (1994)
$(R_{706}-R_{400-480})/(R_{750}-R_{400-480})$	0.947	0.942	0.858	26.4	21.6	10.1	9	12	26	Chl a	Maccioni et al. (2001)
$(R_{760-800}/R_{690-720})-1$	0.947	0.942	0.869	26.4	21.5	9.7	10	11	6	Chl a+b	Gitelson et al. (2006)
R_{750}/R_{700}	0.947	0.943	0.863	26.4	21.3	9.9	11	8	13	Chl a+b, Chl a	Gitelson and Merzlyak (1996), Lichtenthaler et al. (1996)
$(R_{749}-R_{720})-(R_{701}-R_{672})$	0.947	0.944	0.860	26.4	21.2	10.0	12	6	21	Chl a+b	le Maire (2004)
$(R_{750}-R_{445})/(R_{705}-R_{445})$	0.947	0.942	0.857	26.4	21.5	10.1	13	10	27	Chl a+b	Sims and Gamon (2002)
$(R_{750}-R_{705})/(R_{750}+R_{705}-2R_{445})$	0.946	0.943	0.857	26.6	21.3	10.1	14	9	28	Chl a+b	Sims and Gamon (2002)

$(fR_{722}-fR_{699})/(fR_{722}+fR_{699}-2fR_{502})$	0.943	0.938	0.864	27.3	22.3	9.9	15	16	12	Chl a+b	le Maire (2004)
$(R_{750-800}/R_{520-585})-1$	0.943	0.936	0.869	27.4	22.6	9.7	16	19	5	Chl a+b	Gitelson et al. (2003)
R_{792}/R_{697}	0.943	0.937	0.855	27.4	22.3	10.2	17	17	30	Chl a+b	le Maire (2004)
$R_{734-747}/R_{715-726}$	0.942	0.935	0.866	27.5	22.8	9.8	18	20	10	Chl a+b	Vogelmann et al. (1993)
$(R_{556}-R_{rm})/(R_{750}-R_{rm})$	0.942	0.937	0.859	27.6	22.5	10.1	19	18	25	Chl b	Maccioni et al. (2001)
$(R_{556}-R_{400-480})/(R_{750}-R_{400-480})$	0.942	0.935	0.859	27.6	22.8	10.0	20	24	24	Chl b	Maccioni et al. (2001)
R_{750}/R_{555}	0.942	0.935	0.870	27.7	22.8	9.7	21	23	4	Chl a	Gitelson and Merzlyak (1994)
R_{750}/R_{550}	0.942	0.933	0.870	27.7	23.1	9.7	22	26	3	Chl a+b, Chl a	Gitelson and Merzlyak (1996), Lichtentaler et al. (1996)
$\log(R_{800}/R_{550})$	0.941	0.932	0.868	27.9	23.2	9.7	23	28	8	Chl a+b	Buschmann and Nagel (1993)
$(R_{728}-R_{720})/(R_{728}+R_{720}-2R_{434})$	0.941	0.935	0.861	27.9	22.8	10.0	24	21	15	Chl a+b	le Maire (2004)
$(R_{728}-R_{434})/(R_{720}-R_{434})$	0.940	0.935	0.861	28.0	22.8	10.0	25	22	17	Chl a+b	le Maire (2004)
$(R_{542}-R_{rm})/(R_{750}-R_{rm})$	0.940	0.930	0.860	28.2	23.7	10.0	26	29	22	Chl a+b	Maccioni et al. (2001)
$R_{675}/(R_{650}\times R_{700})$	0.939	0.941	0.839	28.3	21.8	10.8	27	14	34	Chl b	Chappelle et al. (1992)
$(R_{542}-R_{400-480})/(R_{750}-R_{400-480})$	0.938	0.933	0.855	28.5	23.1	10.2	28	25	31	Chl a+b	Maccioni et al. (2001)
$(R_{792}-R_{697})/(R_{792}+R_{697})$	0.938	0.933	0.847	28.7	23.2	10.5	29	27	33	Chl a+b	le Maire (2004)
$fR_{710-720}/fR_{700-710}$	0.937	0.928	0.854	28.9	24.0	10.2	30	31	32	Chl a+b	Vogelmann et al. (1993)
fR_{725}/fR_{702}	0.936	0.929	0.859	29.0	23.8	10.0	31	30	23	Chl a+b	Kochubey and Kazantsev (2007)
$(fR_{722}-fR_{502})/(fR_{701}-fR_{502})$	0.932	0.926	0.856	29.8	24.3	10.2	32	32	29	Chl a+b	Le Maire (2004)
$\sum_{\lambda=705}^{750}(R_{\lambda}/R_{555}-1)$	0.932	0.924	0.862	30.0	24.6	9.9	33	33	14	Chl a	Gitelson and Merzlyak (1994)

fR_{754}/fR_{704}	0.928	0.922	0.861	30.7	24.9	10.0	34	34	19	Chl a+b, Chl a	Datt (1999)
Red Edge Position	0.917	0.916	0.821	33.1	26.0	11.3	35	35	36	Chl a+b	Horler et al. (1983)
R_{800}/R_{635}	0.908	0.900	0.826	34.7	28.3	11.2	36	36	35	Chl b	Blackburn (1998)
$(R_{800}-R_{635})/(R_{800}+R_{635})$	0.887	0.883	0.800	38.5	30.6	12.0	37	37	37	Chl b	Blackburn (1998)
$R_{672}/(R_{550}\times R_{708})$	0.875	0.878	0.772	40.6	31.2	12.8	38	38	38	Chl a+b, Chl a	Datt (1998)
SPAD	0.858	0.860	0.759	43.2	33.4	13.2	39	39	39	Chl a+b	–
$(fR_{RE}-fR_{G})/(fR_{RE}+fR_{G})$	0.801	0.791	0.745	51.2	40.8	13.5	40	40	40	Chl a+b	Penuelas et al. (1994)
fR_{RE}	0.754	0.752	0.657	56.9	44.5	15.7	41	42	41	Chl a+b	Gitelson et al. (1996)
R_{675}/R_{700}	0.753	0.764	0.640	57.1	43.4	16.1	42	41	42	Chl a	Chappelle et al. (1992)
sR_{712}/sR_{688}	0.673	0.675	0.608	65.6	50.9	16.8	43	43	43	Chl a+b, Chl a	Datt (1999)
$(R_{800}-R_{680})/(R_{800}+R_{680})$	0.563	0.551	0.557	75.8	59.9	17.8	44	45	44	Chl a	Blackburn (1998)
R_{800}/R_{680}	0.559	0.543	0.543	76.2	60.4	18.1	45	46	45	Chl a	Blackburn (1998)
$(R_{580}-2R_{624}+R_{668})/44^{2}$	0.540	0.564	0.472	77.8	59.0	19.5	46	44	46	Chl a+b	Adams et al. (1999)
R_{672}/R_{550}	0.473	0.476	0.408	83.3	64.7	20.6	47	47	47	Chl b	Datt (1998)

R_{λ}: Reflectance at λ nm

fR_{λ}: First derivative of reflectance spectra at λ nm

sR_{λ}: Second derivative of reflectance spectra at λ nm

fR_{G}: Maximum of the first derivative spectrum of reflectance in the green region at around 525 nm; in the present study we derived maximum between 500 and 550 nm

fR_{RE}: Maximum of the first derivative spectrum of reflectance in the red edge region; in the present study we derived maximum between 680 and 750 nm

Red Edge Position: Wavelength of maximum of the first derivative spectrum of reflectance in the red edge region; in the present study we derived wavelength of maximum between 680 and 750 nm

$R_{\lambda1-\lambda2}$: Mean reflectance from λ1 to λ2 nm

R_{rm}: Minimum of the reflectance spectrum in the red region near 675 nm; in the present study we derived minimum between 650 and 700 nm

Table 4 Performance of 46 published indices and SPAD in estimating leaf chlorophyll content of *C.* ×*yedoensis*. The list was sorted by the rank based on cvRMSE for Chl a+b.

Index	cvR^2			cvRMSE			rank			Originally related to	Source
	Chl a+b	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b	Chl a	Chl b		
$\mathrm{fR}_{754}/\mathrm{fR}_{704}$	0.886	0.883	0.764	36.1	28.3	12.7	1	1	1	Chl a+b, Chl a	Datt (1999)
Ciganda's RECI: $(\mathrm{R}_{770-800}/\mathrm{R}_{720-730})-1$	0.853	0.858	0.718	41.0	31.3	13.8	2	2	3	Chl a+b	Ciganda et al. (2009)
Datt's RI: $(\mathrm{R}_{850}-\mathrm{R}_{710})/(\mathrm{R}_{850}-\mathrm{R}_{680})$	0.838	0.841	0.709	43.1	33.1	14.0	3	3	4	Chl a+b, Chl a	Datt (1999)
$(\mathrm{fR}_{\mathrm{RE}}-\mathrm{fR}_{\mathrm{G}})/(\mathrm{fR}_{\mathrm{RE}}+\mathrm{fR}_{\mathrm{G}})$	0.838	0.830	0.738	43.2	34.3	13.3	4	9	2	Chl a+b	Penuelas et al. (1994)
$\mathrm{fR}_{725}/\mathrm{fR}_{702}$	0.832	0.834	0.700	43.9	33.8	14.3	5	7	9	Chl a+b	Kochubey and Kazantsev (2007)
$(\mathrm{fR}_{722}-\mathrm{fR}_{699})/(\mathrm{fR}_{722}+\mathrm{fR}_{699}-2\mathrm{fR}_{502})$	0.831	0.832	0.695	44.1	34.0	14.4	6	8	11	Chl a+b	le Maire (2004)
$(\mathrm{fR}_{722}-\mathrm{fR}_{502})/(\mathrm{fR}_{701}-\mathrm{fR}_{502})$	0.830	0.836	0.703	44.1	33.6	14.2	7	5	6	Chl a+b	le Maire (2004)
$(\mathrm{R}_{780}-\mathrm{R}_{710})/(\mathrm{R}_{780}-\mathrm{R}_{680})$	0.830	0.836	0.697	44.2	33.6	14.3	8	4	10	Chl a+b	Datt(1999), Maccioni et al. (2001)
$\mathrm{fR}_{710-720}/\mathrm{fR}_{700-710}$	0.825	0.835	0.703	44.9	33.7	14.2	9	6	7	Chl a+b	Vogelmann et al. (1993)
$\mathrm{R}_{672}/(\mathrm{R}_{550}\times\mathrm{R}_{708})$	0.820	0.829	0.700	45.4	34.3	14.3	10	10	8	Chl a+b, Chl a	Datt (1998)
$(\mathrm{R}_{728}-\mathrm{R}_{434})/(\mathrm{R}_{720}-\mathrm{R}_{434})$	0.817	0.824	0.690	45.8	34.8	14.5	11	11	13	Chl a+b	le Maire (2004)
$(\mathrm{R}_{750-800}/\mathrm{R}_{695-740})-1$	0.817	0.822	0.688	45.8	35.0	14.5	12	13	14	Chl a+b	Gitelson et al. (2003)
$(\mathrm{R}_{728}-\mathrm{R}_{720})/(\mathrm{R}_{728}+\mathrm{R}_{720}-2\mathrm{R}_{434})$	0.816	0.822	0.684	45.9	35.0	14.7	13	12	16	Chl a+b	le Maire (2004)
$(\mathrm{R}_{706}-\mathrm{R}_{\mathrm{rm}})/(\mathrm{R}_{750}-\mathrm{R}_{\mathrm{rm}})$	0.814	0.820	0.681	46.2	35.2	14.7	14	14	18	Chl a	Maccioni et al. (2001)
$\mathrm{R}_{734-747}/\mathrm{R}_{715-726}$	0.812	0.820	0.681	46.5	35.2	14.7	15	15	17	Chl a+b	Vogelmann et al. (1993)
$(\mathrm{R}_{542}-\mathrm{R}_{\mathrm{rm}})/(\mathrm{R}_{750}-\mathrm{R}_{\mathrm{rm}})$	0.812	0.818	0.706	46.5	35.4	14.1	16	16	5	Chl a+b	Maccioni et al. (2001)

$(R_{556}-R_{rm})/(R_{750}-R_{rm})$	0.808	0.813	0.691	46.9	35.9	14.5	17	17	12	Chl b	Maccioni et al. (2001)
$(R_{750}-R_{705})/(R_{750}+R_{705}-2R_{445})$	0.804	0.809	0.670	47.5	36.2	15.0	18	19	22	Chl a+b	Sims and Gamon (2002)
$(R_{706}-R_{400-480})/(R_{750}-R_{400-480})$	0.803	0.811	0.671	47.6	36.1	14.9	19	18	21	Chl a	Maccioni et al. (2001)
$(R_{750}-R_{445})/(R_{705}-R_{445})$	0.798	0.809	0.665	48.2	36.3	15.1	20	20	23	Chl a+b	Sims and Gamon (2002)
$(R_{749}-R_{720})-(R_{701}-R_{672})$	0.797	0.805	0.661	48.3	36.7	15.2	21	21	25	Chl a+b	le Maire (2004)
$(R_{542}-R_{400-480})/(R_{750}-R_{400-480})$	0.796	0.801	0.688	48.5	37.0	14.5	22	22	15	Chl a+b	Maccioni et al. (2001)
Red Edge Position	0.794	0.798	0.671	48.6	37.3	14.9	23	23	20	Chl a+b	Horler et al. (1983)
$(R_{556}-R_{400-480})/(R_{750}-R_{400-480})$	0.793	0.797	0.672	48.8	37.4	14.9	24	24	19	Chl b	Maccioni et al. (2001)
$(R_{760-800}/R_{690-720})-1$	0.777	0.781	0.649	50.7	38.8	15.4	25	25	26	Chl a+b	Gitelson et al. (2006)
$(R_{750}-R_{705})/(R_{750}+R_{705})$	0.770	0.771	0.636	51.5	39.7	15.7	26	26	27	Chl a	Gitelson and Merzlyak (1994)
R_{750}/R_{705}	0.762	0.770	0.632	52.3	39.8	15.8	27	27	29	Chl a	Gitelson and Merzlyak (1994)
sR_{712}/sR_{688}	0.747	0.743	0.661	53.9	42.0	15.2	28	30	24	Chl a+b, Chl a	Datt (1999)
$\sum_{\lambda=705}^{750}(R_{\lambda}/R_{705}-1)$	0.746	0.751	0.621	54.0	41.4	16.0	29	28	30	Chl a	Gitelson and Merzlyak (1994)
$\log(R_{800}/R_{550})$	0.744	0.747	0.634	54.2	41.7	15.8	30	29	28	Chl a+b	Buschmann and Nagel (1993)
R_{750}/R_{550}	0.731	0.733	0.609	55.6	42.8	16.3	31	32	34	Chl a+b, Chl a	Gitelson and Merzlyak (1996), Lichtenthaler et al. (1996)
R_{675}/R_{700}	0.728	0.743	0.618	55.9	42.1	16.1	32	31	31	Chl a	Chappelle et al. (1992)
R_{750}/R_{555}	0.728	0.728	0.614	55.9	43.3	16.2	33	33	33	Chl a	Gitelson and Merzlyak (1994)
R_{750}/R_{700}	0.711	0.717	0.575	57.6	44.2	17.0	34	36	37	Chl a+b,	Gitelson and

									Chl a	Merzlyak (1996), Lichtentaler et al. (1996)
$R_{675}/(R_{650} \times R_{700})$	0.710	0.719	0.569	57.7	44.0	17.1	35	34	38	Chl b	Chappelle et al. (1992)
$(R_{750-800}/R_{520-585}) - 1$	0.701	0.718	0.587	58.7	44.0	16.7	36	35	35	Chl a+b	Gitelson et al. (2003)
R_{672}/R_{550}	0.696	0.700	0.616	59.1	45.5	16.2	37	37	32	Chl b	Datt (1998)
$\sum_{\lambda=705}^{750}(R_\lambda/R_{555} - 1)$	0.689	0.686	0.583	59.8	46.5	16.8	38	38	36	Chl a	Gitelson and Merzlyak (1994)
$(R_{792} - R_{697})/(R_{792} + R_{697})$	0.662	0.677	0.540	62.3	47.2	17.7	39	39	40	Chl a+b	le Maire (2004)
R_{792}/R_{697}	0.659	0.674	0.535	62.6	47.4	17.8	40	40	41	Chl a+b	le Maire (2004)
SPAD	0.574	0.562	0.547	70.0	54.9	17.5	41	41	39	Chl a+b	–
R_{800}/R_{635}	0.470	0.487	0.374	78.1	59.5	20.6	42	43	42	Chl b	Blackburn (1998)
$(R_{800} - R_{635})/(R_{800} + R_{635})$	0.462	0.488	0.370	78.6	59.4	20.7	43	42	43	Chl b	Blackburn (1998)
fR_{RE}	0.170	0.180	0.079	97.7	75.1	25.0	44	44	44	Chl a+b	Gitelson et al. (1996)
R_{800}/R_{680}	0.057	0.035	0.030	104.1	81.5	25.7	45	46	46	Chl a	Blackburn (1998)
$(R_{800} - R_{680})/(R_{800} + R_{680})$	0.052	0.064	0.036	104.4	80.3	25.6	46	45	45	Chl a	Blackburn (1998)
$(R_{580} - 2R_{624} + R_{668})/44^2$	0.007	0.026	0.000	106.8	81.9	26.5	47	47	47	Chl a+b	Adams et al. (1999)

R_λ: Reflectance at λ nm

fR_λ: First derivative of reflectance spectra at λ nm

sR_λ: Second derivative of reflectance spectra at λ nm

fR_G: Maximum of the first derivative spectrum of reflectance in the green region at around 525 nm; in the present study we derived maximum between 500 and 550 nm

fR_{RE}: Maximum of the first derivative spectrum of reflectance in the red edge region; in the present study we derived maximum between 680 and 750 nm

Red Edge Position: Wavelength of maximum of the first derivative spectrum of reflectance in the red edge region; in the present study we derived wavelength of maximum between 680 and 750 nm

$R_{\lambda 1-\lambda 2}$: Mean reflectance from λ1 to λ2 nm

R_{rm}: Minimum of the reflectance spectrum in the red region near 675 nm; in the present study we derived minimum between 650 and 700 nm and Chl a and ranked 19th for Chl b in *C. jamasakura*. The red edge chlorophyll index of Ciganda et al. (2009), $(R_{770-800}/R_{720-730}) - 1$ (hereafter Ciganda's RECI), ranked second for Chl a+b and ranked first for Chl b in *C. jamasakura* and ranked second for Chl a+b and Chl a and third for Chl b in *C.* ×*yedoensis*.

The index value of SPAD-502 ranked 39th for Chl a+b, Chl a and Chl b in *C. jamasakura* and 41st for Chl a+b and Chl a and 39th for Chl b in *C.* ×*yedoensis* (Tables 3 and 4). The cvRMSE of SPAD were 43.2, 33.4 and 13.2 mg m^{-2} for Chl a+b, a and b in *C. jamasakura*, respectively, and 70.0, 54.9 and 17.5 mg m^{-2} for Chl a+b, a and b in *C.* ×*yedoensis*.

The cvR^2 were lower in Chl b than Chl a+b or Chl a, and in *C.* ×*yedoensis* than *C. jamasakura* (Tables 3 and 4). The ranks of Chl a+b and Chl a within an index were mostly similar, but the rank of Chl b was somewhat different from them. The published indices originally related to Chl b were not necessarily good indicators for estimating content of Chl b.

DISCUSSION

The species-specific indices developed in the present study were superior to any published indices or SPAD for estimating leaf chlorophyll content of *C. jamasakura* (Tables 1 and 3) or *C.* ×*yedoensis* (Tables 2 and 4). In the species-specific indices, the wavelengths in yellow and unsaturated red regions (570–645 nm in the present study) and red edge region (705–755 nm) were selected as primary elements in reflectance indices, and wavelengths in green region (520–560 nm) and red edge region (690–765 nm) were selected in absorptance indices (Tables 1 and 2). Therefore, these wavelengths were deemed especially important for estimating leaf chlorophyll content. These findings were in line with the previous studies on reflectance spectra (Carter, 1994; Gitelson and Merzlyak, 1994; Datt, 1998; Maccioni et al., 2001; Richardson et al., 2002; Blackburn, 2007).

On the other hand, violet (400 nm in the present study) and blue (455 nm) regions and NIR (beyond 770 nm) were selected only as secondary elements of the optimal indices and were ineffective in separate use because the spectral change corresponding to chlorophyll content was unstable at these regions (Figs 1 and 2, Tables 1 and 2). However, NIR was deemed effective in combination with yellow or red edge regions as the five reflectance indices were significantly improved by NIR (Tables 1 and 2). It is probable that NIR effectively removed effect of measurement errors such as illumination change because it is not directly related to chlorophylls' absorption and contains relatively smaller noise than violet or blue region in reflectance spectra as it shows high reflectivity in leaves.

The wavelengths in violet or blue region were utilized in the indices using three or more bands (Tables 1 and 2) and were likely to have supplementary function to reduce effect of individual difference of leaf surface because, for example, 445 nm is proposed as a measure to compensate for high leaf surface (specular) reflectance, which tends to increase reflectance across the whole visible spectrum (Sims and Gamon, 2002).

The newly proposed type, difference and ratio combination type (T8), seemed effective as it was selected as optimal chlorophyll indices in four of the 12 cases that use three or more bands for *C. jamasakura*, and ten of the 12 cases for *C. ×yedoensis* (Tables 1 and 2). This type is new in terms of usage of the third band: the third band was previously used only for cancelation of unwanted effects in the other two bands, i.e. $V_1 - V_3$ or $V_2 - V_3$ in T5 or T6, whereas T8, $(V_1 - V_2)/V_3$, combines difference and ratio types and uses the third band as an individual variable. Note that the numerator of T6 is simplified by combining terms. Although T8, from its form, is expected to remove both additive and multiplicative effects on spectra, it was unsure that it is always successful. The applicability of the new index type needs to be examined more.

The characteristics of spectral change in response to chlorophyll content were basically similar between reflectance and absorptance spectra on the whole, but the peaks and troughs were interchanged (Figs 1 and 2). The performance of reflectance and absorptance indices was almost equivalent for both flowering cherries (Tables 1 and 2). Although Richardson et al. (2002) hypothesized that absorptance should be able to give better estimates of chlorophyll content than reflectance, the difference between reflectance and absorptance indices were small in the present study (Tables 1 and 2).

Datt's RI and Ciganda's RECI were found useful to estimate leaf chlorophyll content of both flowering cherries among the published chlorophyll indices (Tables 3 and 4). Datt's RI was developed for estimating leaf chlorophyll content of several Eucalyptus species (Datt, 1999). Datt (1999) argued that this index effectively removes the additive and multiplicative scatter components because it kept identical correlation coefficients and RMSE regardless of applying the Multiple Scatter Correction. Datt's RI also yielded satisfactory results in the study of le Maire et al. (2004) that aimed to develop universal broad leaf chlorophyll indices. The result of the present study further supports the performance of Datt's RI.

Ciganda's RECI was developed from reciprocal reflectance (Gitelson et al., 2003). The constant, -1, was originally important to make the interception of the index against Chl a+b close to zero (Gitelson et al., 2003). Nevertheless, the form of Ciganda's RECI is actually similar to ratio type (T3) except the fact that it uses broader spectral bands. The ratio of reflectance at NIR and red edge region may be a successful type of indices because the two-band ratio-type optimal reflectance indices were all similar to Ciganda's RECI in this respect (Tables 1 and 2).

SPAD did not perform better than the optimal species-specific chlorophyll indices and the most published indices (Tables 3 and 4). The cvRMSE of SPAD were about two times greater than those of the optimal indices of the present study (Tables 1, 2, 3 and 4). The wavelengths selected in SPAD−502 may not be the best because it was initially designed for diagnosing the nitrogen status of rice plant (*Oryza sativa* L.) in Japan (Wang et al., 2004).

The estimation of leaf chlorophyll content was more difficult in *C. ×yedoensis* than *C. jamasakura* (Tables 1, 2, 3 and 4). It is probably because the chlorophyll content per area tended to be higher in *C. ×yedoensis* due to its leaf thickness. Smaller change in the amplitude of peaks and troughs on reflectance and absorptance spectra caused by the higher chlorophyll content (Figs 1 and 2) was thought to make estimation difficult. In fact, the reflectance and absorptance at 680 nm were saturated over about 300 mg m^{-2} of Chl a+b in the present study (Figs 1 and 2). A thicker leaf may lead to greater estimation error caused by the sieve effect, i.e. heterogeneous distribution of absorbing pigments, and the detour effect, i.e. increased probability of light absorption due to lengthening of light path through a leaf by internal reflection, refraction and scattering (Buschmann and Nagel 1993).

The estimation of Chl b was more difficult than Chl a+b or Chl a (Tables 1, 2, 3 and 4). The distinction between Chl a and Chl b by the chlorophyll indices was problematic. This was observed in the facts that the published indices originally related to Chl b were not necessarily good estimators of Chl b (Tables 3 and 4) and that the optimal reflectance indices for Chl a+b and Chl a of *C. jamasakura* were identical (Table 1). The possible reasons are that Chl a was contained about 3.6–3.8 times greater than Chl b in average and that correlation between Chl a and Chl b was high.

CONCLUSIONS

In the present study, we adopted a non-destructive spectral measurement in field, and systematically developed the optimal leaf chlorophyll indices for *C. jamasakura* and *C. ×yedoensis* by testing eight types of indices including a new one. As a result, we confirmed that the selected species-specific chlorophyll indices performed better than any of the published leaf chlorophyll indices or SPAD, about two-times better cvRMSE than SPAD, and found that the newly proposed type, difference and ratio combination type, may be a useful form of chlorophyll content estimation. We also found that absorptance indices achieved comparable results with reflectance indices despite the hypothesis that absorptance measurement is direct and has more potential. Among the published indices, Datt's RI and Ciganda's RECI were especially effective to estimate leaf chlorophyll content of both flowering cherries whereas SPAD was not a better indicator of chlorophylls than these indices. Difficulties in estimating chlorophyll content of thicker leaves and in distinction between Chl a and Chl b were also revealed.

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