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Chlorophyll and nitrogen determination in coconut using a non-destructive method

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ABSTRACT

Portable non-destructive meters are used to estimate foliar chlorophyll or nitrogen (N) concentrations for many crops. In this study, we calibrated chlorophyll meter (atLeaf) to estimate chlorophyll in coconut using data sets collected from wide-ranging field experiments involving plant type, cropping system, fertigation trial and soil type. The atLeaf chlorophyll meter provided good estimates for chlorophyll concentrations in coconut, with coefficients of determination greater than 0.81. The product is suitable for determining foliar chlorophyll concentration of coconut, which can serve as a generic indicator of moisture or nutrient stress. Detecting N deficiencies requires caution, because the wide range of foliage colors across genotypes does not reflect the relationship between chlorophyll index (CI) and N. Regardless, detection is good for a given genotype with different management options. The product has potential as a management tool for optimizing irrigation and fertilizer regimes in plantation crops.

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Chlorophyll index; chlorophyll meter; coconut; cropping system; nitrogen; plant type

Introduction

Coconut palm (*Cocus nucifera* L.) is an important crop to millions of inhabitants of more than 90 tropical countries. It occupies 12.5 m ha in the coastal lowlands of continental South Asia and is spread along the Indian and Pacific Ocean. In India, coconut is grown in a 1.9 m ha area covering 18 states and 3 union territories, which contribute 15.5 and 21% of the world's coconut area and production, respectively. Coconuts are commonly cultivated in alluvial, laterite, sandy loam, red sandy loam, and coastal sandy soils. These soils are present all along the coastal tract of the west and east coasts of peninsular India and are the predominant soil type (Subramanian et al., 2011). Coconut is grown as a rainfed crop and is exposed to frequent droughts and high temperatures during the summer months; consequently, crop growth is affected and the gap between actual productivity and realizable productivity is wide (Thomas et al., 2013). In a tree crop like the coconut, monitoring crop growth and plant health at regular intervals is difficult. A quick technique to enable frequent monitoring and early detection of stress will assist in timely correction for the deficiencies and narrow the yield gap.

Foliar chlorophyll content is a good indicator of plant stress and plant health because of its effects on photosynthesis and growth (Datt, 1999). Environmental [(drought and high temperatures) and nutrient (particularly nitrogen (N)] stresses commonly cause loss of leaf chlorophyll content leading to poor photosynthesis, growth, biomass, and economic yield. Because chlorophyll is mostly made up of

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N-containing enzymes and other organic compounds, stress-restricted uptake of N causes early senescence, which is commonly expressed with loss of chlorophyll content and loss of green leaf area (Hebbar et al., 2014). The ability of a plant to maintain chlorophyll content and green leaf area for a longer duration under stress will allow plants to remain photosynthetically active for a longer period. Therefore, determining chlorophyll content and leaf N can help evaluate the effects of environmental stress, nutrition, and crop management practices to ultimately improve growth and yield potential.

Destructive methods of estimating leaf chlorophyll and leaf N are accurate but time-consuming and expensive. Portable, non-destructive meters are available and have been used successfully with many crop species to estimate foliar chlorophyll or N (Schaper and Chacko, 1991; Castelli et al., 1996; Loh et al., 2002; Abdelhamid et al., 2003). These meters also measure chlorophyll or N on the same leaf over time (Yamamoto et al., 2002), which allows tracking of senescence and determination of the duration of green leaf area. These meters calculate a chlorophyll index (CI) based on reflectance or absorbency at particular wavelengths and need to be calibrated for given species to estimate the actual chlorophyll or N content. The relationship between CI and chlorophyll or CI and N can be affected by species, genetic differences within species (Chapman and Barreto, 1997; Kim et al., 2002; Sandoval et al., 2002), leaf age and ontogeny (Thompson et al., 1996), and growing conditions (Campbell et al., 1990; Barraclough and Kyte, 2001). In a perennial crop like the coconut, it is difficult to measure the chlorophyll or N content at frequent intervals, so using portable chlorophyll meters would be helpful. However, the calibration curves for coconut have not been developed and are not available with any of the portable chlorophyll meters.

We investigated if a portable chlorophyll meter (atLeaf) could be used to estimate chlorophyll or N content of coconut leaves. The objectives were to determine (a) functionality and accuracy of atLeaf+ to estimate leaf chlorophyll and leaf N and (b) if the relationship differed between cultivars, fertilizer treatments, soil types, and crop management practices.

Materials and methods

Site and experimental treatments

Samples were collected from different experiments with established coconut orchards containing different treatments at the Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala, India. The orchards were located at latitude 12° 30′N, longitude 75° 00′E, and at an altitude of 10.7 m above mean sea level. Measurements were taken of different plant types (tall vs. dwarf; green foliage vs. colored foliage), soil types (sandy loam and sandy soil), cropping systems, fertigation, and other crop and nutrient management trials (Table 1). In all the experiments, the locally popular variety of coconut, West Coast Tall (WCT), was used, although the plant types included tall and dwarf varieties. Some of the experiments were in sandy loam, whereas others were in coastal sandy soil. Planting was done during the 1960s, except for the dwarf trial, which was planted in 1990. The various treatment combinations and their details used for the calibration of atLeaf+ are listed in Table 1.

The recommended package of practices was adopted for all treatments unless otherwise mentioned. Chemical fertilizers (500, 320, and 1200 g of N, phosphorus pentoxide (P_2O_5), and potassium oxide (K_2O), respectively, per palm per year) were applied to soil in the form of urea, Mussoorie phos, and muriate of potash. Fertilizers were applied in two splits: one-third (33%) in May–June (the beginning of monsoon season) and two-thirds (66%) in September–October (the receding of monsoon season). In the fertigation treatments, fertilizers were applied through drip irrigation from December to May in six equal splits per treatment requirements. With organic treatments, no chemical fertilizers were applied and biomass produced in the coconut orchard (such as coconut leaves), biomass of weed material, and biomass from intercrops was recycled through 30 kg vermicompost per palm during September. All the palms were irrigated by a drip irrigation system. Palms were irrigated at 66% open-pan evaporation (Eo) from December to May (during the non-rainy season).

Table 1. List of experiments and their details used for this research. Treatments differed in genotypes, soil types, nitrogen levels, fertigation, and other management aspects. CI, chlorophyll index; Chl, total chlorophyll; N, nitrogen.

				Year			
S. No	Experiments	Treatments	Soil type	Planting	Exp. start	Data	Genotype / varieties / hybrids
1	Plant type	Tall	Sandy loam	1990		CI, Chl	WCT, Gangabondam, WCTxPratap, PHOTxWCT, FIJIxWCT, WCTxFIJI, PHOTxFIJI, ADOTxWCT, WCTxADOT, WCTxWCT, PHOTxPHOT, FIJIxFIJI, ADOTxADOT
		Dwarf					MOD, MYD, COD, CGD, CRD
		Green foliage	Sandy loam	1990		CI, Chl, N	WCT, CGD, Gangabondam, Tiptur tall, PHOT, Java tall, Zangiber, Fiji tall
		Color foliage					MOD, MYD, COD, CRD
2	Soil type	Coastal sand		1968	—	CI, Chl	WCT
		Sandy loam		1967			
3	Fertigation trial	Control T1: 25% NPK (Drip) T2: 50% NPK(Drip) T3: 75% NPK(Drip) T4:100%NPK(Drip) T5: 100%NPK(soil)	Coastal sandy	1968	2004	CI, Chl, N	WCT
4	High-density multiple cropping systems	T1: 2/3rd RDF T2: 1/3rd RDF+GM +vermiwash T3: Fully organic	Red sandy loam	1965	2007	CI, Chl, N	WCT
5	Cropping systems	T1: Coconut T2: T1+pineapple T3:-T1+grass T4: T1+vegetables	Coastal sand	1968	2004	CI, Chl, N	WCT
6	Sandy soil management	T1: Intercropping T2: RDF+drip irrigation T3: Polythene mulch T4: Cowdung slurry+drip irrigation T5: 75% poultry manure+25% NPK T6: 100% poultry manure T7:Vermicompost+ biofertiliser	Coastal sand	1968	2007	CI, Chl	WCT

Sampling and measurements

Samples were collected during the month of February in 2013. Three plants (representing each of the treatments) were selected for the study. Two leaflets from each plant from the topmost expanded leaf were cut between 9:00 and 11:00 am and immediately analyzed. The chlorophyll index (CI) was measured using atLeaf (FT Green LLC, Wilmington, DE, USA). This meter measures optical density at two (660 and 940 nm) wavelengths to estimate relative chlorophyll content. The leaf sensor was placed on leaf mesophyll tissue only, avoiding veins. Measurements were made at a photon flux density of 800 to 1200 μmol m^{-2} s^{-1}. From each leaf, five measurements were taken and averaged to provide a single CI per leaf.

Immediately after measurement of the CI, the leaves were placed in plastic bags, placed in a cooler, and carried to a laboratory for further analyses. Chlorophyll was estimated immediately by the method described by Arnon (1949). The same leaf position from where the CI was measured was sampled and weighted for further analysis. Leaf samples (0.5 g) were cut and immersed in 10 ml of DMSO (dimethylsulfoxide) and incubated at 65° C for 4 h. The extract was assayed with a double beam spectrophotometer (Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA), to determine absorbency at wavelengths of 750, 663, and 645 nm and levels of chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl). The data were expressed on the basis of fresh leaf weight.

Nitrogen (N) concentration of the same leaf sample was measured in selected experiments. The samples were oven-dried at 70°C and powdered with a stainless steel Wiley mill. The finely ground 0.25g tissue samples were digested in a sulfuric acid and potassium sulfate (K_2SO_4) mixture using a selenium catalyst in a semimicro-Kjeldahl apparatus as described by Buresh et al. (1982).

Data analyses

There were a total of 6 samples for each measurement (two leaves from each of the three palms). Data were log-transformed and regression analysis was performed to test the relationship between CI and Chla, Chlb and total Chl in different experiments using SAS 9.2 (SAS Institute, Cary, NC, USA). The significant difference between the treatments was tested using two-way analysis of variance (ANOVA), and the treatments were compared using the Duncan test.

Results

The samples collected for this study from various experiments (e.g. plant type, soil type, management systems) showed wide variability in CI values and chlorophyll concentrations (Table 2). The CI value of dwarf genotypes ranged from 26 to 66, whereas the corresponding value in tall genotypes ranged from 51 to 68. The foliage of tall genotypes was green in color, but for most of the dwarfs, except CGD (Chowghat Green Dwarf), the foliage color was either yellow, orange, or yellow tinge. The chlorophyll concentration was significantly lower in colored foliage than in green foliage. The relationship between total Chl and CI was 0.56 for plant height (Table 3; Figure 1a) and 0.77 for foliage color (Figure 1b).

`Soil type also significantly influenced the CI of coconut. The CI value was 45.3 for varieties in coastal sandy soil and 58.9 for varieties in sandy loam soils (Table 2). There was a strong positive relationship between CI measured and Chla ($R^2 = 0.808$), Chlb ($R^2 = 0.608$), and total Chl ($R^2 = 0.684$) concentrations (Figure 1c).

Management

In a high-density multi-species cropping systems (HDMSCS) experiment involving different nutrient treatments under sandy loam soil, significantly higher chlorophyll was recorded following organic treatment, and the lowest chlorophyll was found in the inorganic treatment (Table 2). CI measured followed the same trend, which was higher in organic (57.7) compared with integrated nutrient management (INM) (54.6) or inorganic (51) treatment but was not statistically significant. A strong positive relationship was observed between CI and Chl ($R^2 = 0.722$; Table 3).

Fertigation trial

In a fertigation trial in sandy soil, the CI value was lower (43.7) in control plots (no fertilizer) (Table 2). Fertilizer application to the soil did not increase the CI significantly. On the other hand, fertilizer application through drip, either 75% (48.85) or 100% NPK (51.55), significantly increased the CI. The trend of total Chl across the treatments was also the same, but the increases were significant in all the treatments compared with the no-fertilizer control. This resulted in a relatively lower but significant relationship between the CI and Chl ($R^2 = 0.671$) (Table 3; Figure 2a).

Table 2. Mean chlorophyll index (CI), chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl) concentrations in different treatments measured in various experiments. Range is given for plant types. Mean with the same letter represented are not significant, and means with different letter represents significant difference. HDMSCS: high-density multiple cropping systems.

		CI				
Experiment	Treatment	Mean	Range	Chla	Chlb	Chl
Plant type	Dwarf	47.3b	26.0–66.1	0.641b	0.193b	0.835b
	Tall	58.8a	51.46–68.4	1.054a	0.422a	1.450a
	P < 0.05	<0.0001		<0.0001	<0.0001	<0.0001
	Green foliage	59.1a	52.7–66.4	0.937a	0.377a	1.327a
	Color foliage	44.6b	26.0–52.8	0.520b	0.151b	0.687b
	P < 0.05%	<0.0001		<0.0001	<0.0001	<0.0001
Soil type	Sandy loam	58.9a	53.6–66.1	0.858a	0.334a	1.349a
	Coastal sand	45.3b	36.1–50.5	0.516b	0.214b	0.732b
	P < 0.05	<0.0001		<0.0001	<0.0001	<0.0001
Fertigation	C	43.7c		0.442c	0.203b	0.649c
	T1	45.9bc		0.664b	0.314a	0.986b
	T2	46.5bc		0.769ab	0.340a	1.109ab
	T3	48.8ab		0.798ab	0.346a	1.143ab
	T4	51.5a		0.886a	0.384a	1.324a
	T5	45.6bc		0.690b	0.310a	1.026b
	P < 0.05	<0.0001		0.004	0.012	0.0041
HDMSCS	T1	51.1a		0.632b	0.271b	1.322b
	T2	54.6a		0.969a	0.373a	1.567a
	T3	57.7a		0.984a	0.398a	1.590a
	P < 0.05	0.12		0.0098	0.0064	0.0093
Cropping systems	T1	45.7c		0.688c	0.288d	0.978c
	T2	55.4a		1.053a	0.489a	1.444a
	T3	53.7b		0.866b	0.395b	1.282b
	T4	51.9b		0.728c	0.337c	1.066c
	P < 0.05	<0.0001		0.0005	0.0001	<0.0001
Sandy soil management	T1	54.9a		0.928a	0.346a	1.275a
	T2	47.7e		0.491c	0.232bc	0.924c
	T3	36.9f		0.398d	0.202cd	0.600d
	T4	46.5d		0.503c	0.203cd	0.906c
	T5	47.4cd		0.545bc	0.188d	0.734c
	T6	49.5bc		0.550bc	0.218bcd	0.768bc
	T7	51.7b		0.640b	0.246b	0.887b
	P < 0.05%	<0.0001		<0.0001	<0.0001	<0.0001

Cropping system

The CI value for coconut as monocrop treatment was 45.7. This value increased significantly with intercropping treatments. CI was highest in intercropping with pineapple (T2, CI 55.4), followed by grass (T3, CI 53.74), then vegetables (T4, CI 51.88). A similar trend followed for chlorophyll measurement except for T4, which was not significant from T1. The relationship between CI and Chl was strong and significant ($R^2 = 0.753$; Table 3; Figure. 2b).

In another experiment, sandy soil involving different management options recorded the highest CI value for intercropping in T1 (54.9). CI was the least for T3, the polythene mulch treatment (36.9). A similar trend was seen for chlorophyll content; it was significantly higher in T1 and was low in T3. The relationship between CI and Chl was significant ($R^2 = 0.807$; Table 3; Figure 2c).

Across all treatments, the CI values ranged from 26 to 68. The chlorophyll content ranged from 0.6 to 1.5. There was a strong positive correlation ($R^2 = 0.810$) between CI measured through atLeaf and chlorophyll content (Figure 3). It was also observed that Chla influenced the total Chl more than the Chlb (Table 2).

Leaf N concentrations varied from 0.86 to 1.58%. The relationship between CI and N across all treatments including all varieties was significant ($P = 0.0001$), but the coefficient of determination was about 0.42 (Figure 4a). The relationship improved to 0.68 when management options were considered for a given variety and the variety treatment was excluded from analyses (Figure 4b).

Table 3. Slope (a) and intercept (b) of the relationship between chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl) with chlorophyll index (CI) for different treatments under field conditions. The relationship takes the form of: ln CI = a+bxln (Chl). RMSE is the root mean square error. HDMSCS: high-density multiple cropping systems.

Experiment	Chlorophyll	a	b	R-square	Pr > F	RMSE
Tall vs. dwarf	Chl a	2.442	0.313	0.546	<.0001	0.054
	Chl b	2.608	0.256	0.593	<.0001	0.051
	Total	2.406	0.309	0.560	<.0001	0.053
Green vs. color	Chl a	2.340	0.433	0.726	<.0001	0.042
	Chl b	2.586	0.321	0.770	<.0001	0.038
	Total	2.296	0.421	0.771	<.0001	0.037
Soil	Chl a	2.379	0.393	0.808	<.0001	0.029
	Chl b	2.532	0.399	0.608	<.0001	0.041
	Total	2.361	0.343	0.684	<.0001	0.037
Fertigation	Chl a	2.566	0.406	0.588	<.0001	0.025
	Chl b	2.571	0.376	0.470	<.0001	0.033
	Total	2.552	0.431	0.671	<.0001	0.025
HDMSCS	Chl a	2.443	0.316	0.716	0.0005	0.024
	Chl b	2.538	0.369	0.655	0.0014	0.026
	Total	1.839	0.764	0.722	0.0005	0.024
Cropping systems	Chl a	2.195	0.580	0.745	<.0001	0.030
	Chl b	2.470	0.451	0.591	0.0005	0.038
	Total	2.097	0.584	0.753	<.0001	0.029
Sandy soil management	Chl a	2.268	0.545	0.849	<.0001	0.027
	Chl b	2.490	0.516	0.920	<.0001	0.050
	Total	2.149	0.587	0.807	<.0001	0.031

Discussion

A non-destructive atLeaf chlorophyll meter was used to determine the foliar chlorophyll and N concentrations in adult palm leaves. To validate its accuracy, leaf samples were collected from various experiments with different treatments: plant types (tall vs. dwarf and green foliage vs. colored foliage),

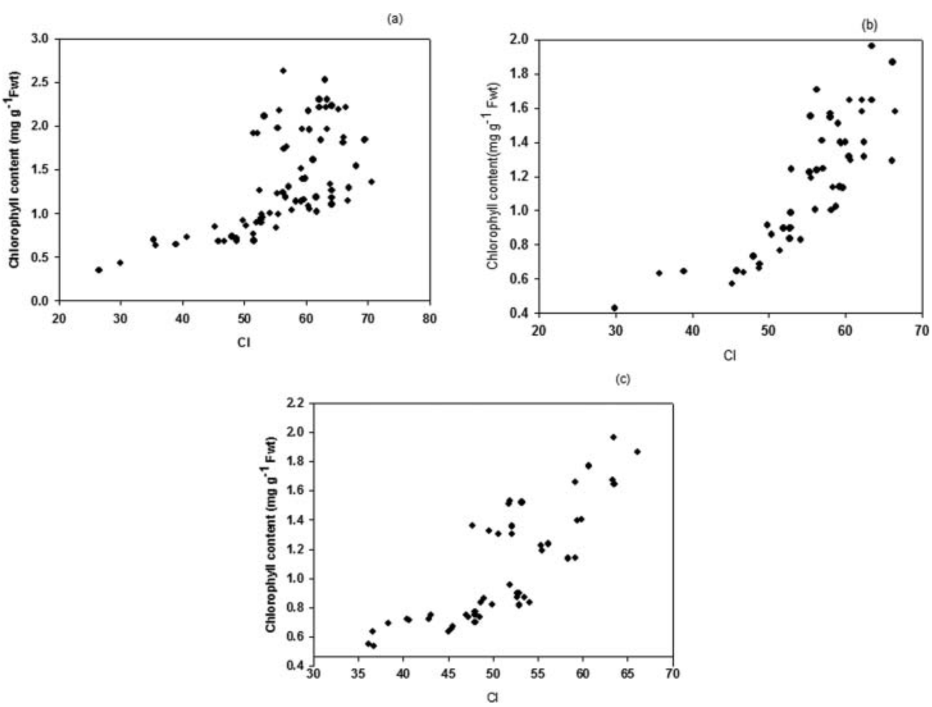

Figure 1. Relationship between total chlorophyll (Chl) and chlorophyll index (CI) for A) different plant types, tall vs. dwarf; B) different leaf color, green vs. color; and C) different soil types, laterite vs. sandy.

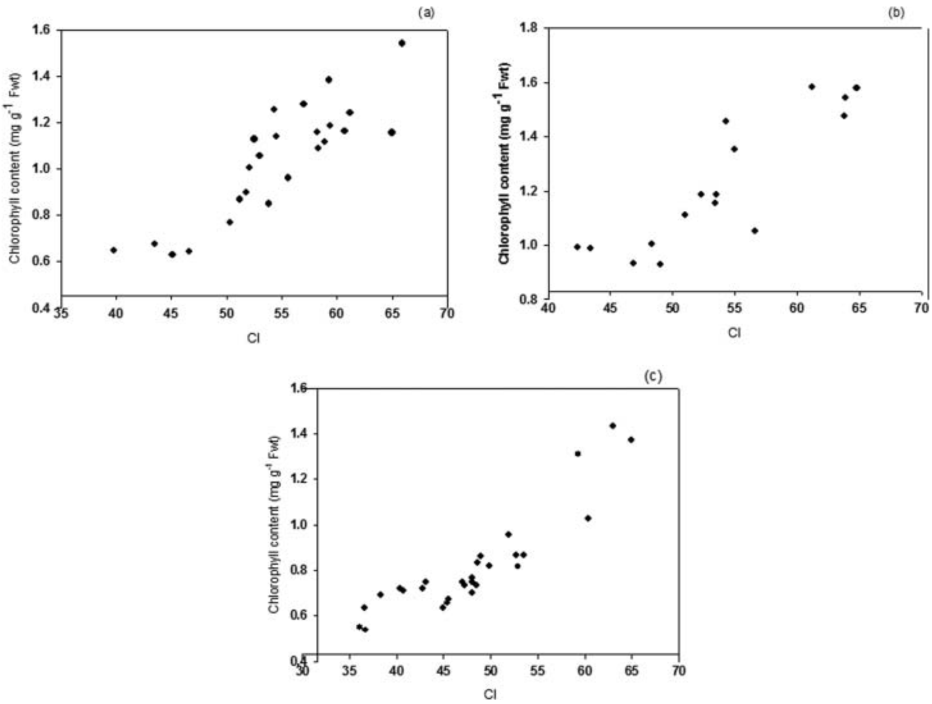

Figure 2. Relationship between total chlorophyll (Chl) and chlorophyll index (CI) with different agronomic practices: A) fertigation, B) cropping systems, and C) soil management practices.

soil types, cropping systems, fertigation trials, and sandy soil management. Chlorophyll content of different coconut genotypes varied widely; as expected, it was low in genotypes with colored foliage compared to those with green foliage. The atLeaf chlorophyll meter provided good estimates of chlorophyll in coconut, with coefficients of determination greater than 0.81. The relationship between chlorophyll and CI in many species is linear (Campbell et al., 1990; Schaper and Chacko, 1991; Barraclough and Kyte, 2001; Cate and Perkins, 2003). The curvilinear nature of the relationship for coconut suggested that the atLeaf overestimated chlorophyll at high CI. This also was observed for rice, wheat and

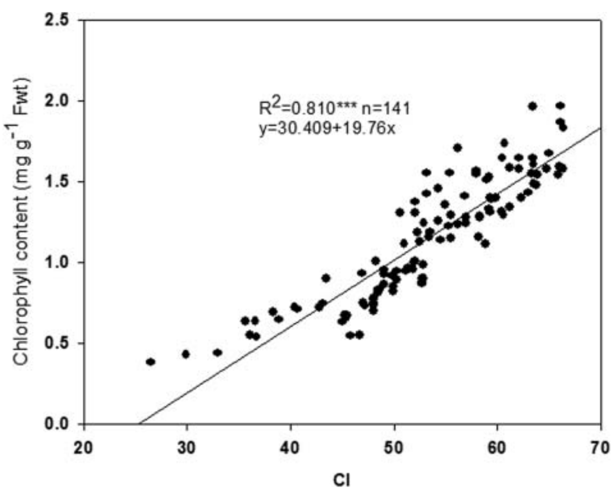

Figure 3. Relationship between total chlorophyll (Chl) and chlorophyll index (CI) across all treatments (fertigation, cropping systems, and soil management practices).

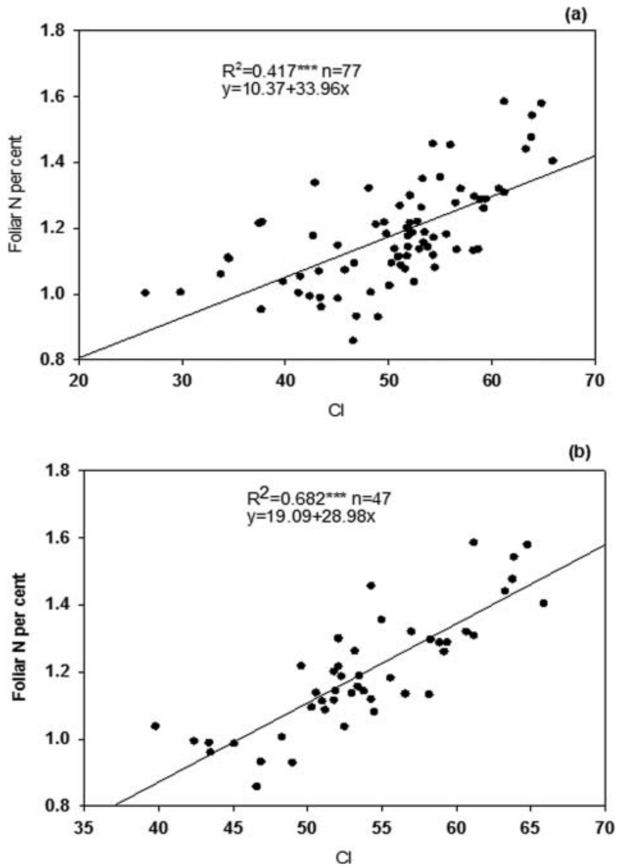

Figure 4. Relationship between foliar nitrogen (N) and chlorophyll index (CI) for A) all treatments and B) excluding genotype data (only various management data of WCT genotype were included).

soybean (Monje and Bugbee, 1992), and eucalyptus (Pinkard et al., 2006). These findings highlight the importance of sampling the extremes when developing calibration curves.

The relatively poor relationship between chlorophyll and CI for tall and dwarf genotypes observed in this experiment (R2 = 0.56) may have been a function of the differences in foliage color. Tall genotypes are generally green, whereas most of the dwarf genotypes used for the experiment had colored foliage (light orange or yellow tinged), except Chowghat green dwarf (CGD), which has green foliage. Genotypes with colored foliage have low chlorophyll content, so when the relationship between chlorophyll and CI of green and colored genotypes was analyzed, it was a good fit ($R^2 = 0.771$).

Environmental factors are known to affect leaf morphology, which in turn affects foliar optical properties and can be expected to influence CI (Monje and Bugbee, 1992). Processes like photosynthesis, stomatal conductance and transpiration, and growth and productivity are low when coconut is grown in coastal sandy soils (Dhanapal et al., 2013), mainly because of poor inherent physical and chemical properties of these soils. Coastal sandy soils are characterized by poor water retaining capacity, excessive infiltration (due to the porosity of sands), easy leaching, and low inherent fertility status. In this study, we observed that chlorophyll concentration of the leaf is significantly lower in sandy soil than in sandy loam soil. The atLeaf chlorophyll meter was able to capture these differences satisfactorily.

In the fertigation trial in coastal sandy soil, significantly higher CI and Chl concentrations were observed in coconut variety west coast tall (WCT) with fertigation through drip irrigation. Drip fertilizer was applied in more equal splits (six equal splits from December through May) than soil application (two equal splits). Frequent fertigation in sandy soil replenished the nutrient depletion zone at the vicinity of the root surface, enhanced uptake by the plants, and improved the leaf nutrient (N, P, and

K) concentrations (Subramanian et al. 2012). Coconut requires a continuous supply of nutrients for improved growth and productivity. Therefore, when it was grown in an organic treatment in an HDMSCS, which releases nutrients in a slow but continuous manner over a longer period of time, coconut had higher level of CI and Chl.

In the management strategies used to improve productivity in the coastal sandy soil trial, among the various treatment combinations tried, intercropping in the coconut interspaces with soil moisture conservation measures (husk burial) recorded higher levels of Chl and CI. Use of amendments such as husk or coir pith in the coconut basin improved root density and proliferation and enhanced the availability of moisture and nutrients (Subramanian et al., 2010, Dhanapal et al., 2013). The foliage of the coconut plants grown with a complete covering of soil with polythene mulch became yellow and had less chlorophyll (0.60 mg g^{-1} fresh wt), whereas intercropping with pineapple had high chlorophyll content (1.44 mg g^{-1} fresh wt). The same trend was observed for CI as determined by atLeaf chlorophyll meter. Similar observations were made in cropping system trial under coastal sandy soil.

The relationship between CI and foliar N was not strong, although strong relationships have been observed in other annual (maize, Blackmer and Schepers 1995; barley, Wienhold and Krupinsky 1999; winter wheat, Jifon et al. 2005) and perennial (apple, Neilsen et al. 1995; cottonwood, Moreau et al. 2004; European beech, Percival et al. 2008) species. Coconut genotypes with colored foliage have very low chlorophyll, but their N concentrations were similar to green leaf genotypes, which resulted in poor a relationship. Excluding the genotypes, consideration of only the management options for a variety improved the relationship between CI and N (0.682). The relationship between CI and N deserves to be corroborated on a broader cultivar spectrum with further experimentation to develop cultivar-specific CI and N predictive equations.

Conclusions

This study demonstrated the utility of the atLeaf chlorophyll meter to determine foliar chlorophyll and nitrogen concentrations in coconut leaves. It is suitable for determining foliar chlorophyll concentrations of coconut as a generic indicator of moisture or nutrient stress, but caution is required for it to be used directly to detect N deficiency of upper crowns. The wide range of foliage colors across the genotypes mismatches the relationship between CI and N in coconut, but detection was good for a given genotype with different management options. Thus, the atLeaf meter has potential as a management tool for optimizing irrigation and fertilizer regimes in plantation crops. Its usefulness in plantation management would be enhanced if a threshold CI could be identified below which growth and productivity will be decreased and remedial measures such as irrigation and fertilization may be warranted. More research to determine a relationship between foliar N and chlorophyll concentrations for coconut will facilitate the meter's use in developing efficient management practices for coconut.

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