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CHLOROPHYLL METER TECHNOLOGY: A PROMISING DIAGNOSTIC TOOL IN THE PRODUCTION OF CORN AND PATCHOI IN TRINIDAD.

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ABSTRACT: Fertilizer management is crucial to competitive food production, mainly because plant nitrogen (N) is a major limiting factor in crop productivity. As such, affordable and efficient modern technologies like the use of chlorophyll meters (CMs), portable instruments that are not only easy to use but also non-destructively measures the 'greenness' of leaves and by extension the health of the plant, are constantly being developed and redefined. The operation of CMs are based on the principle that sample readings from plants in a homogenous area out in the field, can be correlated to leaf N concentration and then to N fertilizer recommendations, instantaneously. Research in leading temperate centres have been generally promising, especially with respect to corn, *Zea mays* L. In Trinidad, laboratory diagnostic testing is underexploited by farmers, most likely due to a lack of understanding of the long term costs of improper plant nutrition management and its effect on soil productivity. The following research sought to validate whether CMs, particularly the YARA N-Tester, can be an appropriate alternative in the cultivation of corn and patchoi (*Brassica rapa* L. subsp. *chinensis*) under limited resources. Field and greenhouse trials were developed around traditional practices in an attempt to locally calibrate the instrument, utilising a range of fertilizer treatments in a complete randomized (block) design. The data collected and analysed from these experiments produced very significant relationships ($P < 0.05$) for both plant species with respect to N-Tester values (NTV) and leaf N concentrations (LNC). However, the strongest correlations obtained for corn (var. ICTA Farm) was found to be within a particular time frame and patchoi (var. Pak Choy White) while highly sensitive to differing N levels, its fresh marketable yield proved to be greatly influenced by leaf area and number. An increased experimental population and number of replications would better serve to produce more sound regression models.

Keywords: chlorophyll meter, nitrogen fertilizer management, plant nutrition, corn, patchoi.

Introduction

Food security, environmental degradation and climate change are intertwining issues that are exponentially growing with global population. Caribbean islands, like Trinidad and Tobago, are found to be most vulnerable to these processes, since their limited land area does not allow for economies of scale, which translates into higher than world prices for the production of primary agricultural products. Thus, it is not surprising that Caribbean agriculture has been experiencing declining production in recent years, a dilemma exacerbated by inadequate help provided to small farmers, use of inappropriate technology, the high level of imported inputs, paucity of local technical

skills, and the impact of regional and international organizations; which have led to an increasing dependence on foreign food (Ahmed 2001, 2-3).

There is a fervent need to enhance the ability of a soil to produce desired yield of crops under optimum management, otherwise known as soil productivity. One of the most effective ways to achieve this is via the management of soil fertility; a complex balance that seeks to supply plant nutrients in available forms and in sufficient quantities so as to facilitate efficient growth of specified crops without the pollution of the environment with excess nutrients (Osman 2013, 129). Great focus is paid to nitrogen (N) management, as it is an essential plant nutrient that is required in relatively copious amounts, 1.5 to 5 % plant dry weight (dw) and is most often the limiting factor in the growth and yield of non-leguminous plants; however, the availability of other nutrients such as phosphorous, potassium, calcium, magnesium, etc., must also be considered to ensure good plant health (Brady and Weil 2002, 544; Marschner 2012, 148-149 and Below 2002, 385).

In order to make the required quantities of N and other macromolecules and metabolites related to the vegetative and reproductive cycles of plants, the application of fertilizer is necessary to enhance crop productivity per unit area. In particular, it is more practical to ensure increased soil N levels via fertilization than the addition of organic matter because N mineralisation from the soil is normally too slow and variable to support desired crop production (Below 2002, 386). Furthermore, in order to ensure that crops continue to receive the required amount of N and guarantee the best yields, most farmers tend to liberally supply N to a crop (Trautmann, Porter and Wagenet 1989; Yadav, Peterson and Williams 1997 and Azam et al. 2002). Given that, the average current price of the locally popular fertilizer “Blaukorn” blend carrying only 12 % N is TT\$10 per kg, excessive N application can not only be detrimental to freshwater sources but also quite costly. Contrastingly, an undersupply would result in low productivity and nutrient mining. Since fertilization typically accounts for >20% of production costs, the development of fertilizer management strategies that encourage appropriate rates, timing and placement of fertilizer materials is essential to reduce input costs and increase profits. Thus, there is a need to develop local fertilizer guidelines based on yield response research for important crops (Eudoxie 2012, 20-21). In order to monitor the aforementioned nutrient levels in soil and plant tissues, diagnostic measures are continuously being developed and improved.

In Trinidad, there are several organizations that are capable of providing diagnostic services catering to soil fertility management, like laboratory soil and plant analysis, to farmers. Laboratory diagnostic methods, despite being time-consuming and destructive in nature, can provide precise information for appropriate fertility recommendations. Furthermore, in recent times, the government has been providing free diagnostic testing, although there is a major risk that results can be received too late to correct any deficiency present; while private institutions may provide the information needed in a more timely manner, the average cost per sample is TT\$650. Nevertheless, it is highly recommended that soil testing be conducted pre-planting to decipher application rates, as all essential nutrients except N can be determined using information provided.

However, other factors that might influence productivity must also be considered as such testing does not directly or indirectly relate soil nutrient concentrations to uptake and utilization. Thus, plant tissue analyses and growth monitoring are necessary, post-planting, to confirm whether the crop is able to access and utilize adequate amounts of nutrients, particularly N. Unfortunately, due to poor institutional support and the still largely traditional culture surrounding agriculture, such scientific measures are underexploited (Eudoxie 2012, 24-41).

Since the utilization of technology by farmers in tropical countries, like Trinidad and Tobago, is relatively sparse; it is important when designing or improving techniques to match them to site-specific problems taking into consideration both farmer's constraints and objectives and those of society (as it relates to issues of agricultural sustainability, soil biodiversity, carbon sequestration and watershed functions) in order to increase their likelihood of adoption. Farmers in the tropics, though they employ traditional practices, do not make decisions in isolation, but rather they think in a systematic fashion, incorporating a holistic view of their whole farm and the totality of the resources and assets available (Izac 2003, 13-14). Hence the 'adoptability' of any new technique by a farmer is highly dependent on tangible economic benefit. Currently, there are several studies being conducted in this area in the form of soil conservation practices, organic alternatives such as compost and manure, and geo-spatial information technologies. However, due to typical financial and land constraints of local farmers, chlorophyll meter technology was selected based on its practical potential in developing quick, in field, fertilization protocols for selected crops.

CMs, like the YARA N-Tester (\approx TT\$12,800.00 per unit), measure the chlorophyll content or the 'greenness' of leaves, a key indicator of plant health and are portable, lightweight, 'quick and easy' instruments, powered by two AA alkaline batteries. Its measurement principle is based on the ratio between light absorption at two wavelengths: 650 and 940 nm, as the former lies between the two primary wavelengths associated with chlorophyll activity (645 and 663 nm), while the latter serves as an internal reference to compensate for differences in leaf characteristic and biochemical factors (Waskom et al 1996, 546). The premise being that the user would be able to go from the generated weighted average of at least 30 compulsory N-Tester measurements (NTVs, ranging from 0 – 999), sampled from plants in a homogenous area out in the field; to leaf N concentration and then finally to a N fertilizer recommendation, instantaneously (Wood et al. 1992, 488; Waskom et al. 1996, 545-647; and YARA International 2005).

Hence, the main objective is to validate the use of the N-tester as an economical diagnostic tool in determining the N status of green corn and patchoi, which are currently sold an average annual price of TT\$18.10 and TT\$7.76 per kg; respectively (CSO 2012). Furthermore, this can only be achieved after the calibration of the instrument via quantitative correlations to leaf N concentration and the identification of the critical N-Tester value for the crop, above which fertilization is not needed; both with respect to the most appropriate phonological stage or time frame for which NTVs would

be best suited for predicting yield response to N fertilization in field conditions on River Estate loam.

Materials and Methods

Field Experiments: Two field experiments were conducted at the University of the West Indies field station (UFS), in Valsayn, on River Estate series. This soil, developed from micaceous phyllite alluvium, has free internal drainage, moderate acidity (\approx pH 6.0) and low cation exchange capacity (Brown and Bally 1966, 27-28). Each experiment involved one of two cultivars developed by the University of the West Indies: *Zea mays* L.; ICTA Farm corn (*indentata*) and UW7 sweet corn (*saccharata*), which were established in late October, 2008 and late March, 2009; respectively.

Site preparation first involved mechanical clearance of existing vegetation, then land was disc-ploughed, rotavated and beds formed. Though the width varied slightly along the length of the beds prepared, plant spacing involved 0.3m between plants with the average plot dimension of 21.7 m²; and 0.45m between plants and an average plot coverage of 22.3 m² for ICTA and UW7, respectively. Chemical pest control involved the use of a pre-emergent herbicide, 2-chloro-2-(6-diethyl-N-(methoxymethyl) acetanilide-1-yl) propan-1-ol (Lasso) after planting; and Alpha-cypermethrin (Fastac) and Lambda-cyhalothrin (Karate), alternately on a two week rotation after germination while weed management included the use of Paraquat dichloride (Gramoxone) between rows and plots and hand weeding between plants.

Each experiment consisted of four N rate-treatments and a no-fertilizer control, particularly 22.5, 45, 67.5 and 134.5 kg N/ha for the ICTA and 30, 60, 120 and 180 kg N/ha for the UW7, both utilizing a 12-12-17 +2 fertilizer blend (Blaukorn). All fertilizer treatments were applied as a single dose within four weeks after planting. Trenches, about 6 cm in depth, were dug along planting ridges in a straight band 10-15 cm from the plant, utilizing garden hoes; and pre-portioned fertilizer was placed as evenly as possible, along the length of each row of each plot, and then covered with soil.

All of the above were based on common practice at UFS. In particular, 10 – 14 days after planting corn, 120 kg N/ha is band applied followed by a mixture of 60 kg N/ha, 43.7 kg P/ha and 60 kg K/ha at first tassel (about 48 DAP) using the traditional carriers, sulphate of ammonia, triple superphosphate and muriate of potash.

N-Tester measurements (NTVs) and leaf samples were collected at 6.5, 8 and 9.5 weeks after planting (WAP); however for UW7, NTVs were taken at 4, 5, 6, 7, 8 and 10 WAP and leaf samples were only collected for weeks 5-7. At each sampling, a total of 30 measurements were conducted on the first fully developed leaf, otherwise known as the most recently developed leaf (MRDL) of selected plants located in the centre rows of each plot. N-Tester measurements were taken midway between the stalk and leaf tip, and midway between the midrib and leaf margin during early morning periods (Sawyer, Hawkins, Barker and Lundvall 2007, 1035). Sub-samples were removed from each plot; and 15 cm in length from the centre of each leaf; was cut, rinsed with distilled

water, and bagged accordingly. After eighty-eight (88) days and seventy-nine (79) days, for ICTA Farm corn and the sweet corn respectively, corn cobs were harvested from plants located about the centre of each plot and weighed for fresh yield determination.

Greenhouse Experiment: Experiment was conducted at Soils Greenhouse, University of the West Indies, St. Augustine. *Brassica rapa subsp chinensis* L. cv Pak Choy White seedlings were transplanted, late October, 2010; into styrofoam containers perforated at the base to allow for drainage before being filled with 800 g of soil, River Estate series. This sandy loam was extracted from a non-experimental location UFS, then air-dried and pulverized to pass through a quarter inch mesh (6.35 mm) sieve.

One healthy, two week old seedling was placed into a hole made in the dry soil of each pot. It was firmly positioned, ensuring full exposure of first leaves and immediately watered until saturation. Pots were evenly distributed (6 x 4) on a growing table (228 x 106.5 cm) and about 1 m off the ground, which was located to maximise light exposure.

Plants were initially watered once during the morning period at the base and then twice daily as they got larger, using watering cans. Soil was perforated as evenly as possible by hand around mid-season to allow for ease and better percolation of irrigation water. Pest management consisted of both chemical; Flubendiamide (Phoenix 20 WG), (Caprid 20 SL), Carboxamide-Strobilurin boscalid-Pyraclostrobin (Bellis 38 WG) and biological control, six pots of *Allium schoenoprasum* L. (Chive) placed evenly amongst experimental pots. Weeds were removed manually, as needed.

Treatments was composed of three N application rates (0.08, 0.17 and 0.25 g N/plant) and a no-fertilizer control, in a complete randomized design with six replicates. There was a single fertilizer application two weeks after transplanting (2 WAT). Although, the recommended rate ranges from 1.6 – 3 g N/plant at 7 day intervals over the space of three weeks. All treatments, other than the control, received a 12-8-16-3+TE fertilizer blend (Blaukorn), which was dissolved in water and applied to the base of plant carefully so as to avoid leakage.

Several NTVs were taken over time on the MRDL of each plant, while mid-season destructive sampling was conducted on representative plants within each treatment for laboratory analysis. Each leaf was rinsed with distill water and paper towels used to pat them dry before the main vein/petiole was cut out and the remaining leaf blade was then bagged into respective treatment groups. After forty-four days, plants were harvested (about 6 WAP), weighed and fresh marketable yield calculated on a per plant basis and then adjusted to a Mg/ha based on the minimum field plant density.

Laboratory Leaf N Analysis

Subsequent to oven-drying at 65° C for 24 hours, leaf samples were ground to pass through a 1 mm sieve and later analysed for N after first being digested in flasks utilizing the H₂SO₄-salicylic acid-H₂O₂ method and then regular Kjeldahl distillation method

utilizing Buchi's Distillation Unit B-324 (Walinga et al. 1995, PANA-A0/4 and PANA-A2/1-30; and Sparks et al. 1996, 1103-1105).

Statistical Analyses

Leaf N concentration (LNC), fresh crop yield and NTV data collected were analysed systematically using GenStat Discovery Edition 4, developed by VSN International. First a correlation matrix was established in order to obtain a summarized view of all the data and to identify possible main interactions. Two general forms of analysis of variance (ANOVA) were carried out, with treatment and blocks (if applicable) as factors: repeated measurements ANOVA on successive parameter data sets, in addition to a one way ANOVA performed to determine the effect of fertilizer treatment on yield. Simple linear regression was performed and fitted models were generated with appropriate coefficient of determination (R^2), which is a measure the proportion of the sample variation around the mean, as some studies have identified the relationship between CM readings and yield to be linear in nature (Zhang et al. 2008). Similar scatter plots associated with linear equations were also generated using Microsoft Excel 2007. However, as there exists no physiological reason to expect that the relationship would always be linear rather than curved (Markwell et al., 1995); nonlinearity was tested by the addition of the polynomial component, which is supported by studies done by Wood et al. 1992 and Dwyer et al. 1995. Choice of appropriate model was confirmed via visual inspection of the response variant fit (Hawkins et al. 2007, 1036). For yield response determinations, optimum N rates were calculated using a bulk density of 1.35 Mg per m³.

Results and Discussion

One-way ANOVA analysis on the fresh yield, given treatment and block factors generated a p-value of 0.024 for ICTA, which presents evidence of a difference among the population mean fresh cob yield for the five treatments and a p-value of 0.110 for UW7. Figure 1 (a-c) shows that not only does the four different but incremental N-rates and the no-fertilizer control have a very significant effect on yield but that the relationship is very strong producing an equation $y = -0.001x^2 + 0.213x + 4.4387$, R^2 of 0.99 (excluding the no fertilizer control) for ICTA and $y = -0.0002x^2 + 0.0578x + 14.943$, R^2 of 0.86 for UW7. Optimum rates of about 105 and 150 kg N/ha were observed, given initial soil available N of 95.3 and 88 kg N/ha for ICTA and UW7 maize cultivars, respectively. In addition, the quadratic trend displayed confirmed that fertilizer amendment recommendations for N deficient plants follow a rate of diminishing returns, as greater deficiencies would require greater amendments in order to optimize yield. Most likely a major portion of the variation is due to water availability, as the ICTA was completely rained with brief instances of some flooding and UW7 required some irrigation. In addition, the UW7 was subjected to stronger winds and disease prevalence that resulted in lower planting densities of particular plots at harvest.

There was very strong evidence ($P < 0.001$) to suggest that the mean population fresh yield was different among the three treatments and no-fertilizer control for the patchoi

var. Pak Choy White; which is graphically illustrated in Figure 1 d), producing an equation $y = -0.00002x^2 + 0.0281x + 2.5707$, R^2 of 1. Furthermore, classification using Fisher's LSD method showed that while the lowest N rate of 0.08 g N/plant produced a significant yield response compared to the no-fertilizer control; the fresh yield of 0.17 and 0.25 g N/plant though they were significantly even higher than the lowest rate, they were statistically similar to each other. Nevertheless, the optimum rate was found to be approximately 0.22 g N/plant, otherwise 557 kg N/ha.

It is immediately evident that rates administered simply based on common practice are excessive and profits could have been increased, on average, by approximately TT\$4375 and \$5265 per ha for maize and patchoi, respectively.

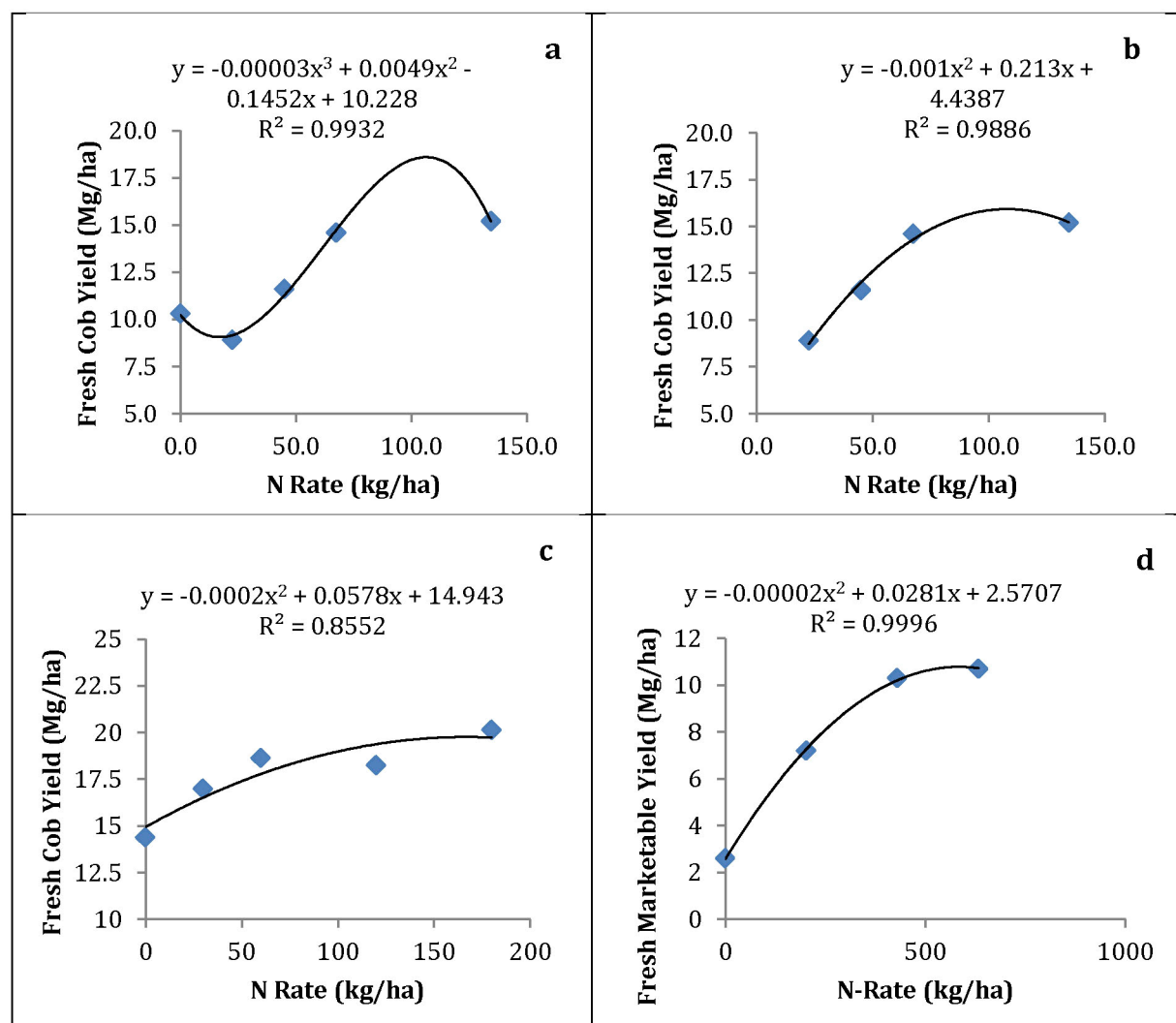


Figure 1. The nonlinear relationship between maize N-Rate and fresh cob. yield for a) ICTA Farm with control, b) ICTA Farm without control, c) UW7 Sweet and d) Pak Choy White, given a range of incremental treatments.

Furthermore for maize, there was evidence of a significant difference in the mean leaf N concentration among the five treatments, $P = 0.016$ and 0.005 ; and among the three sampling times, $P = 0.028$ and < 0.001 , for ICTA and UW7 respectively. In addition, there was very strong evidence of a difference in the mean critical N-Tester values among the five treatments ($P < 0.005$) as well as among the three sampling times ($P < 0.005$), controlling for replicates for both ICTA and UW7, respectively; which indicated that the whole data set was best separated. Subsequent regression analysis was found to be generally non-linear in nature. Data suggested that variability in maize is at its minimum during particular growth stages and may be confined to a narrow time span just before the onset of reproductive pillars; namely the stem elongation period just before first tassel, then at ear emergence and finally just before flowering; which generally corresponds with split application suggestions made by YARA International (YARA International 2005, 4). NTV related models were only generated at 3 WAP for patchoi var. Pak Choy White, which is a short-term crop; as it was established beforehand as the mid-season marker and subsequently confirmed that it had the best ability to predict fresh yield response, producing a R^2 value of 0.65. Corresponding laboratory testing produced data suggesting that there was a very significant relationship ($P < 0.001$) between NTV and macronutrient N concentration, although regression analysis was linear in nature (Lara and Gouveia, unpublished).

Since soil properties and environment vary considerably, calibration of the instrument and critical N determination is necessary to produce accurate yield response charts that farmers can utilize to make appropriate fertilizer amendments in a timely manner. However, the development of sound charts requires extensive fertilizer trials that take into account such variability. Nevertheless, an example of N sufficiency chart for maize and patchoi was developed from this study based on the significant data presented by quantitative ANOVA procedures, see Table 1; but further research has to be conducted to determine effective N amendments to be applied based on level of deficiency.

Table 1. Crop N Sufficiency Chart Correlating NTV units to Leaf N Concentration and N Level Interpretation for Maize and Patchoi.

Zea mays L. (at 8 WAP)			<i>Brassica rapa subsp chinensis</i> L. (at 3 WAP)		
NTV	Leaf N Concentration	N Level Interpretation	NTV	Leaf N Concentration	N Level Interpretation
<580	<2.1	Visibly Deficient	<640	<0.93	Visibly Deficient
580 – 639	2.1 – 2.75	Latent Deficiency	640 – 689	0.93 – 1.14	Latent Deficiency
640 – 655	2.76 – 2.95	Sufficient	690 – 709	1.15 – 1.24	Sufficient
>655	>2.95	Excess	>710	>1.25	Excess

Calibration of the instrument can be extremely time specific and would require further field trials for confirmation. Nevertheless, for maize, the traditional method of a second application at or just before first tassel seems to have merit as it corresponds to the most appropriate time for critical NTV determination. This suggests that there is enough time to optimize fertilizer N if it is deficient, which is supported by work done by Sawyer et al., who devised that there is a period of time during mid-to-late vegetative growth that CM measurements should be taken in order to make appropriate in-season N rate decisions and amendments (Sawyer et al. 2007, 1034). Furthermore, Scharf et al., found insignificant evidence to suggest that delayed fertilizer amendments, as late as V11, irrespective of N deficiency level would allow for irreversible yield loss as yield was still highly responsive to N application at this stage (Scharf, Wiebold and Lory 2002, 435).

Conclusions

Chlorophyll meters can provide instantaneous, on-site information in a non-destructive manner. Though CMs have been touted as a means to assess N availability and plant N status based on the close association between chlorophyll and leaf N concentration, the need for widespread calibration to determine different crop N status may not be practical. This is because leaf chlorophyll content has been found to vary by type of crop, cultivars within a crop, growth stage, leaf position, soil type and climate; these variables must be taken into consideration when interpreting the relative measurements of CMs. In addition, water stress, plant diseases, other nutrient deficiencies and/or other factors that cause plant stress can be monitored to assure accuracy. As a result, in order to standardize CM measurements across cultivars, locations and growth stages; it may be necessary to perform normalization procedures by comparing readings from well-fertilized reference plots with those from the test area (Below 2002, 287 and Panwar, Kumar & Chaudhary 2008, 330).

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