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Image Sensing, A Unique Tool for Early Detection of Nitrogen Deficiency of Tomato Grown in Hot and Humid Greenhouses

R. M. D. M. Bandara [a*], D. D. K. Koswatta [a], W. A. P. Weerakkody [a] and P. C. G. Bandaranayake [b]

[a] Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.
[b] Faculty of Agriculture, Agricultural Biotechnology Center, University of Peradeniya, Peradeniya, Sri Lanka.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Protected culture (PC) answers the major issues in conventional agriculture, and thus helps feed the rising world population. Meanwhile, soilless culture has become an integral part of the PC, for sake of catering to the plant nutrient requirements precisely. Therefore, innovative intellective diagnostic systems for diagnosing nutrient deficiency symptoms in protected culture is a timely need. Hence this experiment was conducted to test the effectiveness of "Image Sensing" as a diagnostic tool for nitrogen deficiency under semi-intensive greenhouse management in hot and humid weather. In this study N deficiency symptoms of tomato were detected by leaf color changes, identified through image sensing, and the deficiency was confirmed with respect to

**Corresponding author: Email: darshimb77@gmail.com;*

retardation of plant growth. Tomato plants were subjected to a series of N treatments by providing 200, 100, 50, 25, and 12.5 percent of the recommended N supply for soilless culture tomatoes. The results showed that morphology changes like stem thickness, leaf area, plant height, and leaf number were significantly reduced along with a reduction of N supply ($p<0.05$) beginning from 5th week after transplanting (WAT). Leaf images were processed in "ImageJ", software" to determine the green color intensity of leaves. Image analysis showed that there was a significant difference among treatments since the 3rd WAT. The leaf color chart was less effective for distinguishing leaf color at the early stages of N deficiency. The results revealed that diagnosis of N deficiency in tomato leaves could be effectively done by image sensing much earlier than the use of plant growth parameters or morphological changes. Hence, image sensing can be used as a more effective diagnostic tool for early detection of N deficiency of tomato cultivations in hot and humid greenhouses, that can be used to improve crop management, especially in large large-scale commercial practices.

Keywords: Image analyzing; leaf color; Nitrogen deficiency; plant growth parameters; protected culture.

1. INTRODUCTION

The agriculture sector faces the daunting challenge of providing enough food and other necessities for the growing world population, which is targeted to be nine billion by 2050. Fruit and vegetables is a vital food component in human diet, and recommended for maintaining the nutritional requirements across the population [1]. Meanwhile, protected culture or greenhouse crop cultivation is the most possible mode of production of perishable horticultural produce under all weather and edaphic conditions. Modern technology has paved the path to improved crop yields and produce quality over the last few decades all over the world. Protected culture technologies play a leading role in this context [2,3,4]. The technology basically provides controlled environment by creating a near optimum microclimate and soil environment for plant growth and thus maximizing productivity and production stability [5,6,7,8]. As a result, it can lead the market supply of horticultural produce, compared to the seasonal supply from the conventional open-field production [9,10,11]. Meanwhile, the overall impact of protected culture on crop growth and development of horticultural crops is highly contributed by hydroponics or soilless culture, besides its requirement for high degree of technical know-how and capital-intensive nature [12]. Hydroponically grown vegetables are usually fed with a combination of balanced soluble fertilizers (pre-mixed fertilizer) to meet their daily plant nutrient requirements mainly because of the practical ease [13,14].

Among popular greenhouse crop species, tomato is one of the most widely grown crops in the world as well as in Sri Lanka. Generally, tomatoes are an vital source of vitamins and minerals for the human diet while an important cash crop for smallholders and medium-scale farmers [1]. Albert's fertilizer is the most used complete fertilizer applied for hydroponics vegetable cultivation in Sri Lanka [15] and nitrogen is the most consumed plant nutrient by crop plants [16,17,18], despite its highly unstable nature in soil or hydroponics media. Therefore, undernourishment in N leads to a low rate of plant growth, followed by low- and poor-quality of yield [19]. Apart from this, over-supply may cause negative impacts in the form of reduced fruit set, soluble solid content (TSS), off-flavor, etc. [20,21,22]. Therefore, agronomists find difficulties in providing the optimum N nutrition for hydroponics tomato, targeting greater production and high market quality of produce. In case of greenhouse tomato cultivation, five growth stages with different plant nutrient requirements have been identified, namely seedling, vegetative, flowering, fruiting, and heavy fruiting states. [23] Most cultivation guides for greenhouse tomato, recommends a particular dosage of major plant nutrients (per plant or per liter) or a specific range of electrical conductivity (EC) of the fertigation solution (Weerakkody et al., 2005) depending on the daily weather. Further, considering the critical nature of the N requirement, critical N application rates have been identified for different growth stages of hydroponics tomato grown in drip fertigated coco peat culture in semi-intensive greenhouse conditions in humid tropics [20]. However, the uptake rates of N and other macro nutrients are highly variable depending on the daily weather and crop vigor [24]. Therefore, continuous monitoring of the plant nutrient status of the growing medium or early detection of deficiency/

toxicity symptoms of plants is an integral component of a successful plant nutrient management program in hydroponics [25]. At this juncture, intensive system monitoring on the plant nutrient status has attracted the attention of largescale commercial greenhouse crop producers.

The optional methods adopted are soil/ medium nitrogen analysis, leaf chlorophyll measurements and nitrate measurements in petiole sap using appropriate analytical or quick methods [26]. Other than that, there is a method to estimate composition chemically simplify sample preparation is near infrared microscopy (NIRM). The present investigation has assessed NIRM as a potential substitute for traditional. Analyzing tiny quantities of tomato (Solanum lycopersicum L.) leaf powder chemically to find out how much carbon and nitrogen are present. But the thing is this is a destructive and time-consuming method [27].

Indirect measurement of plant nitrogen status by using images of leaf color is a newly introduced quick and reliable method for this purpose. The close correlation of the intensity of green color of leaves with the N status of leaves has been established already [28]. However, other factors affecting the leaf color, such as varietal differences, variation of light intensity and plant health can be identified as limiting factors in this method. According to Xu et al. [29], usage of digital image processing has been identified as a useful diagnostic tool for identification of deficiency symptoms, compared to the capacity of the human eye. Real time monitoring of crop health status without compromising crop and environmental variables is possible with image sensing measurements [30]. To gain a better knowledge of the relationships between the microclimate and the physical conditions of the plants, the speaking plant approach and plant response-based sensing in general could be promising. To minimize both short-term and long-term productivity losses, early identification of plant stress is essential, especially in intensive production systems [18]. This enables the farmer to adopt appropriate remedial action on N deficiency well in time [29,31]. Further to this, nutrient deficiency symptoms in plants such as interveinal chlorosis, marginal chlorosis, uniform chlorosis, necrosis, etc. are easily detectable in leaves so that it they can be easily tracked by using color image analysis [32].

Considering all those facts, further improvements in image sensing as an effective and precise tool for early detection of nitrogen status of greenhouse tomato was examined in this study by using appropriate experimental protocols under tropical climatic conditions. The study considered the differences in morphology of the tomato canopy, by manipulating the nitrogen fertility of the vigorously growing tomato crop under greenhouse conditions.

2. MATERIALS AND METHODS

2.1 Experimental Setup

The experiment was conducted under greenhouse conditions at the University Experimental Farm at Meewathura, Peradeniya in Sri Lanka (belongs to the agro-ecological region, WM2b). The location is 500 m elevated from the sea level, at which the Mean minimum and maximum temperatures are approximately 28^{O} C and 19^{O} C, respectively while the mean annual rainfall 2000 mm. The Greenhouse was a fully automated greenhouse with an arch framed double cladded roof, made of UV-protected clear (keeping a 30 cm gap between two claddings) while the sides were covered with insect-proof net assisted with a large exhaust fan and with a misting system, controlled with IOT technology.

2.2 Experimental Design

Five nitrogen dosages (N treatments) having Excess N (T1), Optimum N/ recommended dosage (T2) and lower N levels (T3 –T5) were given to tomato, grown in drip fertigated cocopeat bag culture, keeping 10 replicates (plants) in a CRD. Per plant daily N dosages in T1, T2, T3, T4 and T5 were 20, 10, 5, 2.5, 1.25 mg, respectively at 0 – 2 weeks after transplanting (WAT). These were increased gradually according to the same ratio, following the fertilizer recommendations for different growth stages of tomato (Mawalagedera and Weerakkody, 2012) as illustrated in Table 1. The electric conductivity of the fertigation solution was monitored and the pH was regulated within 5.5 – 6.5. The treatments were applied two weeks after transplanting. The mean, EC of the irrigation water was 0.30 ds/m. Detailed fertigation dosages of different treatments are given.

Table 1. Application rate (dosage) of nitrogen for nutrient treatments

N Treatment	Dosage of Application (mg plant^{-1}/day^{-1})			
	0 -2 WAT	2 -4 WAT	4 -6 WAT	6 -8 WAT
T1	20	100	180	280
T2*	10	50	90	140
T3	05	25	45	70
T4	2.5	12.5	22.5	35
T5	1.25	6.25	11.25	17.5

Control (Recommended dosage)

2.3 Nursery Management and Transplanting

Nursery management followed farmers' practice with the use of coco peat medium and sterilizing (by autoclaving) before seeding. Then presoaked tomato seeds were sown in cells at the rate of one seed per cell. Trays were kept indoor with partial shade at the beginning while they were supplied with irrigation water daily and from the second week onwards a mild solution of Alberts fertilizer (CIC, Colombo) (1 g/ L) was applied daily. The nursery period was four weeks. Transplanting was done to coco-peat filled black color polybags (300-gauge) poly bags (volume: 38,500 cm $^{-2}$).

2.4 Management of Sowing and Emerging

The plants were placed with the inter-row spacing of 120 cm and intra-row spacing 90 cm. In order to provide all essential plant nutrients, the following fertilizer grade chemicals were used; Potassium Sulfate (K_2SO_4), Di phosphorus Pentoxide (P_20_5), Magnesium Sulfate ($MgSO_4$), Boric acid (H_3BO_3), Iron Chloride ($FeCl_3$), Manganese Sulphate ($MnSO_4$), Zinc Oxide (ZnO) and Copper Sulphate ($CuSO_4$), Ammonium Molebdate ($(NH_4)_6Mo_7O_{24}$). Other than that crop supporting, training and pruning, the other crop management practices were done. The watering schedule (common for all treatments.) for each growth stage is given in Table 2.

Table 2. Application rates of irrigation water at different growth stages

Plant growth stage (weeks after transplanting)	Water (ml plant^{-1} /day^{-1})
0 - 2	150 - 300
2 - 4	300 - 400
4 - 6	600 - 800
6 - 12	1000 - 1500

2.5 Morphological Measurements

Plant leaf dry weight was measured at 7 WAT while, leaf color (by using leaf color chart), plant height, leaf number, leaf area and stem thickness were measured weekly.

2.6 Image Analysis

Destructive samples were taken randomly from the upper canopy of test plants were subjected to photography by laying upside-up on a white background to ensure the highest contrast possible, making sure not to include any other object within the frame which may disturb the contrast between leaf color and the background. All photographs were taken under the same degree of illumination (using a fluorescent lamp of 100 watts) bulb and were fed to the software to measure color intensity. The software was ImageJ [33]

2.7 Statistical Analysis

Data analysis was done using SAS software. All the parametric data were analyzed through Proc. ANOVA (Analysis of Variance) procedure and mean separation was conducted following Duncan's multiple tests. All non-parametric data were analyzed by using the Chi-Square test. Software SAS (SAS Inc. 2015) was used for all statistical analysis of data.

3. RESULTS AND DISCUSSION

3.1 Plant Growth

Variable nitrogen treatments significantly affected the plant height, leaf area, leaf number and stem thickness in the 5th week after transplanting (WAT) onwards. Up to 5th week, there were no significant differences in these morphological characters among treatments.

The mean plant height of N deficient treatments (T3 – T5) were significantly lower than Control

(T2) and Over-dosage (T1) at 6th and 7th WAT (Fig. 1a). Similarly, N deficient treatments were significantly lower in leaf number and stem thickness at 6th and 7th WAT (Figs. 1b & 2b). Nitrogen treatments significantly affected the leaf area of tomato plants at 5 – 7 WAT. Leaf area of N deficient treatments were significantly lower than control treatment at 7th week but the differences were significantly lower than excess N supply (T1) at 6th and 7th WAT (Fig. 2a).

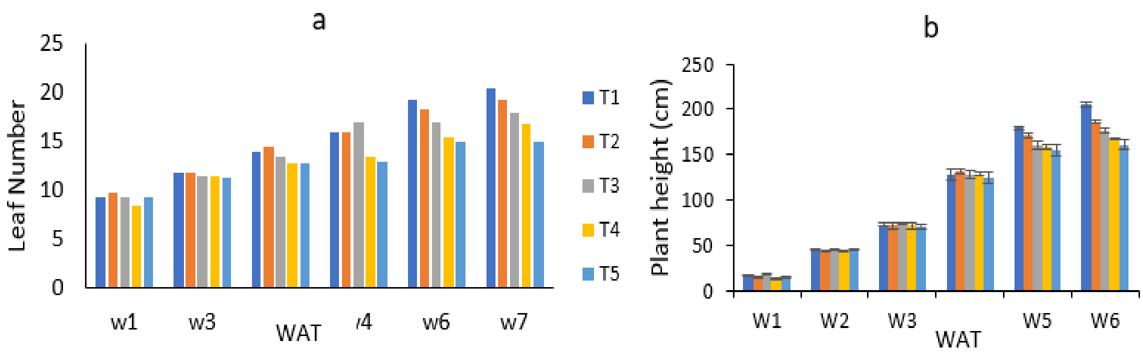

Fig. 1. Leaf number (a) and plant height (b) of tomato under different N treatments

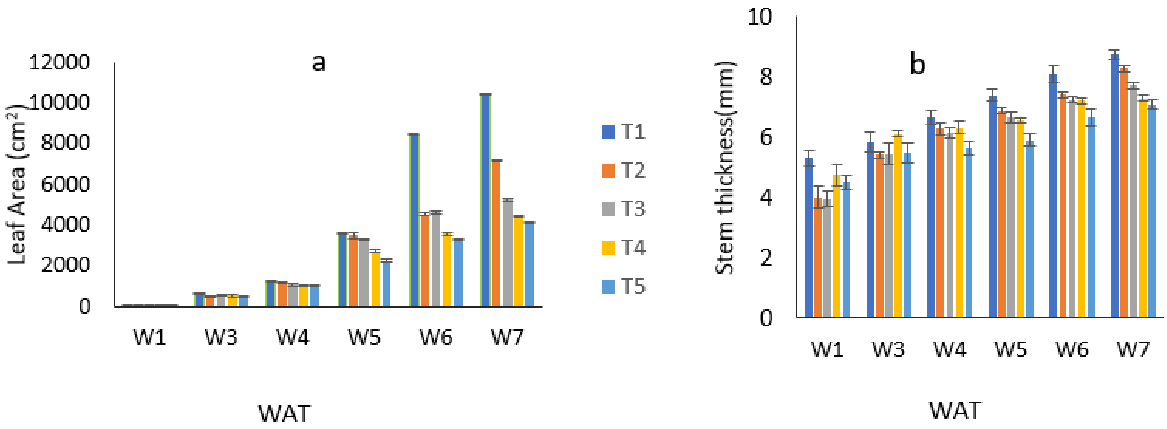

Fig. 2. Leaf area (a) and stem thickness (b) of tomato plants under different N treatments

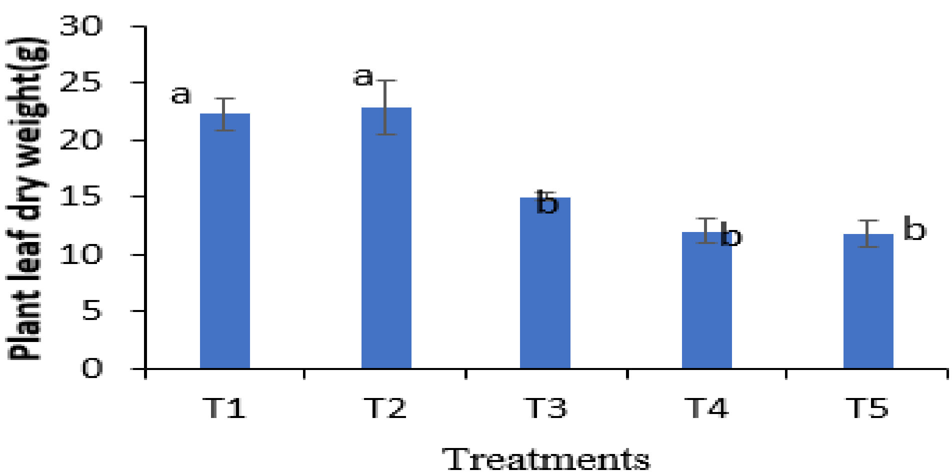

Fig. 3. Dry weight of tomato plant leaves under different N treatments

3.2 Leaf Color

leaf color was assessed (using Color Charts) to examine the relationship between nitrogen deficiency and leaf color, based on the knowledge of nitrogen usage in chlorophyll synthesis. Visual symptoms of N in vegetable crops are characterized by chlorosis progressing from light green to yellow. As reported by Tucker (1984). Visual symptoms of N deficiency are probably the most definitive of nutrient deficiency. According to Luna and García (2010), color image analysis provides an accurate and quick way for nitrogen estimation and can contribute for early detection of nitrogen deficiency in tomato seedlings [34,35]. The morphological characters and plant growth indices are sometimes not reliable to estimate the nitrogen status of tomato plants.

Considering leaf color chart results, in 4th, 5th and 6th WAT, colors of the T4 and T5 belonged to yellow green group and other colors were belong to green group. However, use of visible green color-based detection of the least deficient (12.5 %, lesser than the optimum) N in T3 could be done only at the 6th WAT (Table 3).

Meanwhile, the detection of the intensity of green colour of leaves with the use of Image Sensing software could detect the nitrogen deficiencies starting from the 3rd WAT. The lowest green color intensity (the highest value) could be detected in T4, and the highest green color intensity (the lowest value) was shown in T1 at 3rd WAT. Within T3 and T4 there was not significant difference among green color intensities (Table 4). However, as cited by Gloria et al. [31], green color (GC) of tomato leaves has no definite relationship with the N concentration of the nutrient solution [36-42].

Table 3. Detection of leaf colour of tomato along with plant growth

	Week 01	Week 03	Week 04	Week 05	Week 06
T1	Strong yellow green color	Strong yellow green color	Moderate olive green	Moderate olive green	Moderate olive green
T2	Strong yellow green color	Strong yellow green color	Moderate olive green	Moderate olive green	Moderate olive green
T3	Strong yellow green color	Strong yellow green color	Moderate olive green	Strong yellow green A	Brilliant yellow Green Yellow green group*
T4	Strong yellow green color	Strong yellow green color	Moderate yellow green Yellow green group*	Strong yellow green B Yellow green group*	Strong greenish yellow b Yellow green group*
T5	Strong yellow green color	Strong yellow green color	Moderate yellow green Yellow green group*	Strong yellow green B Yellow green group*	Strong greenish yellow Yellow green group*

** Contrasting colour detections*

Fig. 4. Gradual color variation of lower, same level leaves of tomato plants, from T1 -T5

Table 4. Variation of green color of tomato leaves among N treatments along with plant growth

Treatments	week 03	week 05	week 07
T1	56.71^{d}	67.23^{b}	62.06^{c}
T2	66.57^{c}	74.89^{b}	70.43^{c}
T3	74.59^{bc}	80.55^{a}	88.54^{b}
T4	83.69^{b}	90.14^{a}	93.4^{b}
T5	93.03^{a}	100.96^{a}	125.67^{a}

Note: Lower the value, higher the green color intensity of leaves

5. CONCLUSIONS

Low nitrogen supply (< 50%) affected most plant growth characteristics (e.g. plant height, stem thickness, leaf number, and leaf area) of tomatoes, but at a much later stage, 6 weeks after transplanting (WAT). Meanwhile, diagnosis of N deficiency of tomato leaves, even at the 50% below optimum level could be effectively done by digital detection of green color of the leaves with the use of image sensing much earlier (at 3 WAT) without waiting for visible morphological changes to appear (at 6 WAT). The option of using “Leaf color chart” for this purpose was found to be less effective and time-consuming. Therefore, the utility of “image sensing” for detection of N deficiency of tomatoes could be identified to develop an effective and efficient method for leaf color-based diagnosis of nutrient deficiencies in large-scale crop cultivations, particularly under hot and humid greenhouse conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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