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Original paper

Lettuce calcium deficiency detection with machine vision computed plant features in controlled environments

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ABSTRACT

Conventional greenhouse environmental conditions are determined by observation. However, destructive or invasive contact measurements are not practical for real-time monitoring and control applications. At the canopy scale, machine vision has the potential to identify emerging stresses and guide sampling for identification of the stressor. A machine vision-guided plant sensing and monitoring system was used to detect calcium deficiency in lettuce crops grown in greenhouse conditions using temporal, color and morphological changes of the plant. The machine vision system consisted of two main components: a robotic camera positioning system and an image processing module. The machine vision system extracted plant features to determine overall plant growth and health status, including top projected canopy area (TPCA) as a morphological feature; red–green–blue (RGB) and hue–saturation–luminance (HSL) values as color features; and entropy, energy, contrast, and homogeneity as textural features. The machine vision-guided system was capable of extracting plant morphological, textural and temporal features autonomously. The methodology developed was capable of identifying calcium-deficient lettuce plants 1 day prior to visual stress detection by human vision. Of the extracted plant features, TPCA, energy, entropy, and homogeneity were the most promising markers for timely detection of calcium deficiency in the lettuce crop studied.

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1. Introduction

Controlled-environment agriculture (CEA) is an integrated science and engineering approach to horticultural technology with the aim of enabling crop production in controlled environments that might otherwise be unfavorable for agriculture. Lettuces grown in CEA systems yield cleaner, more dependable food for consumption compared to open-field production. In CEA systems, plants can be grown hydroponically, enabling better control of pests and prevention of diseases. Greenhouses can also be deployed almost anywhere, enabling fresh produce to be grown year-round and closer to the consumer, reducing the need for long-distance transportation.

One technique that is commonly used for lettuce production is a floating hydroponic system (FHS). In this system, the plants float on top of a pond filled with a specific oxygenated nutrient solution at a controlled temperature and absorb the nutrients. This type of system conserves space and eliminates the necessity for constant watering, fertilizing, and pesticide spraying. The typical growing area for lettuce plants in this hydroponic system is approximately 38 plants m^{-2} for each harvest (Lettuce Handbook-Cornell, 2004). Assuming 40-day intervals from seed to harvest, there would be about 9 harvest cycles per year, resulting in a production rate of 342 plants m^{-2} $year^{-1}$. In contrast, open-field lettuce production yields roughly 6.5 plants m^{-2} for each harvest (Sanders, 2001; Jackson et al., 1996) and harvests are limited to growing seasons of about 6 months; about 5 harvests per year results in a production rate of only 32.5 plants m^{-2} $year^{-1}$. Thus, the FHS can offer a more efficient use of land and resources, generating higher-value produce in a smaller growing area.

A drawback to this hydroponic system is that the grower cannot access all areas of the growing space to monitor the status of the lettuce crop. One such issue that requires close monitoring is lettuce tip burn, an environmental condition that inhibits the transportation of calcium to young leaf regions. By the time symptoms are clearly visible, irreparable damage to the crop may have already occurred, lowering the overall marketable value.

Key components to any production system are increased efficiency, productivity and quality due to automation and mechanization of production, handling, and processing. The increased efficiency and productivity of CEA systems (with the use of smart technologies from mechanization, automation, and robotic appli-

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cations) has the potential to enable the U.S. to remain competitive in the global market and contribute to the U.S. economy (CSREES, 2007). Early detection and effective treatment of the potential problems, like lettuce tip burn, can improve resource use (Leinonen and Jones, 2004; Boissard et al., 2008; Jansen et al., 2010), plant-production efficiency (Ling et al., 1996) and plant quality (Linker and Seginer, 2003), thus leading to a sustainable, controlled-environment plant-production system.

In traditional greenhouse production, growing conditions are based on human observations or preset environmental parameters, instead of focused on the plants' specific needs at a given time. Contact sensing is typically used to determine a plant's physical characteristics, a process that is cumbersome, labor-intensive, and often destructive. Non-contact sensing with machine vision may be applied to determine the overall status of plants and to identify a plant's specific needs. This level of intelligent control in greenhouse cultivation would lead to more efficient use of resources and energy for production, and ultimately to improved plant quality, while lowering costs to the consumer.

Some of the machine vision applications needed for this type of monitoring have already been developed. For example, Hetzroni et al. (1994) used neural network and statistical classifiers to determine plant nutrient deficiency (iron, zinc and nitrogen) conditions by plant size, color and spectral features of individual lettuce plants. This study reported that nutrient deficiencies imposed on young lettuce plants were easily detectable, though symptoms were not easily predictable if deficiencies were imposed after about 2 weeks of normal growth. They reported a reduction of the green component in the treated group compared to the control group and an increase of the red component due to the yellowing of lettuce plant after imposed nitrogen deficiency. This report indicated that classification of the individual pixel to the classification of the whole plant is necessary for identification of plant status. Ling et al. (1996) used spectral and morphological characteristics of lettuce leaves to detect nutrient deficiency. This study showed that the reflectance of wave bands between 415 and 720 nm could be used as a signal wave band for machine vision implementation. A wave band closer to the visible was recommended because it gives a better signal strength. The study also suggested the possibility of using multiple signals based on water and nutrient stress detection in plants, using machine vision systems that could determine deviations from "normal" growth as an indicator of deficiencies and plant status. Meyer et al. (1992) used a machine vision system to detect single leaves and poinsettia foliage, and reported that a normalized difference index provided the best method of discriminating nitrogen-deficient from healthy plants. Low-nitrogen plants grown in greenhouses and growth chambers showed similar increases in red reflectance (0.7–0.75 μm) but had different levels of near-infrared reflectance due to differing amounts of plant canopy cover.

Previous efforts concerning machine vision and sensing have been successful in determining plant status by monitoring a single leaf (Seginer et al., 1992; Meyer et al., 1992; Shimizu and Heins, 1995; Revollon et al., 1998) or a single plant (Hetzroni et al., 1994; Kurata and Yan, 1996; Murase et al., 1997; Kacira et al., 2002; Changying and Guanghui, 2003). However, monitoring and sampling from the crop as a canopy would be more useful for larger-scale systems (Leinonen and Jones, 2004; Ushada et al., 2007; Hendrawan and Murase, 2009). In addition, in commercial settings, it is desirable to develop a real-time plant canopy health/growth and quality monitoring system with multi-sensor platforms. This could be achieved by a sensing system equipped with an artificial light source and a multi-sensor platform that moves over the canopy. Such a system could be used to detect deviations from normal growth/development and crop stress (e.g., nutrient deficiencies or diseases).

The objectives of the current study were (1) to develop a methodology using morphological, textural and temporal plant features with a machine vision system for the automated non-contact monitoring of plant health and growth and (2) to evaluate the ability of the developed methodology for early detection of tip burn associated with calcium deficiency in a greenhouse-grown lettuce crop.

2. Materials and methods

A machine vision-guided system for plant health and growth monitoring for use in controlled-environment agriculture production systems was developed (Story et al., 2008) (Fig. 1). The current paper reports the capability of this system's image processing and data interpretation module, and a methodology for early detection of calcium deficiency-induced tip burn in lettuce. The overall system consisted of a robotic camera positioning module, an image acquisition/processing module and a data analysis/storage module (Story et al., 2008).

2.1. Image acquisition system

An 8-bit CCD color camera (KP-D20AU, Hitachi, Tokyo, Japan) with a zoom lens (M6Z 1212-3S, Computar, Commack, NY) was attached to a robotic XY positioning system's cradle. An LED array (LDR2-90SW2, CCS, Kyoto, Japan) was also used attached to the camera to increase the light uniformity for the focused region of interest. This entire camera system was connected to a remote machine through an image grabber board (CronosPlus, Matrox, Quebec, Canada).

The functional flow of the machine vision system is as follows. First, a host computer retrieves a list of targeted locations from a database. This system then sends a signal to the remote machine vision system to position the image acquisition system at a designated location. After the camera is positioned over the center of the container (the focused region of interest), five sequential images are taken and averaged for analysis.

Image averaging was used to reduce the effect of random electronic noise and to reduce disturbances by wind or other external factors that would cause the plants to move. This averaged image was used as the original image to represent the plant canopy at that instantaneous moment. Therefore, a unified canopy of the plants within the container was analyzed for specific characteristics. The captured image dimension was 640 $\times$ 480 pixels and was analyzed as a raw bitmap image. The program for the plant health monitoring system was written with Microsoft's Visual Studio 2005 in the

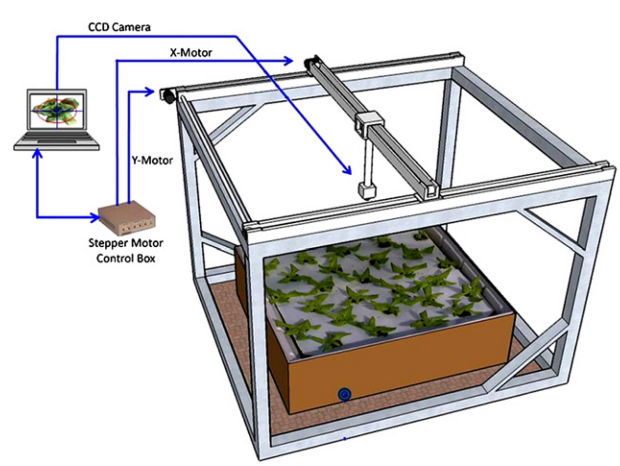

Fig. 1. Schematics of the machine vision-guided plant monitoring system.

VB.NET language. Some of the image processing tools used were from the AForge.NET library.

2.2. Image processing and pattern recognition

From the retrieved original image, the region of interest (the plant canopy) was extracted through an image segmentation process, and the plant foreground was extracted from the background. The resulting monochromatic image (of a white foreground on a black background) represented the plant's top projected canopy area (TPCA). The number of white pixels in the image represented the plant's area (morphological features).

Dynamically, the extracted blob image was converted so that all white areas were transparent, and this new image was overlaid to the original image. All black areas on the extracted blob image covered the original image's background. This allowed the plant-portion to become visible. This focused plant image was used to calculate the color features of the plant. All colored pixels were averaged together to identify the overall plant color and from that color, the following features were identified: red–green–blue (RGB), hue–saturation–luminance (HSL) and color brightness. The resulting color brightness value is a numerical representation of the color's brightness to human eyes (Bezryadin et al., 2007).

The gray-level co-occurrence matrix was used to capture the spatial dependence of gray-level values contributing to the perception of texture (Jain et al., 1995). Because the image texture is orientation dependent, four different matrices were calculated based on the different angles of pixel relativity (0°, 45°, 90°, and 135°). Each matrix was run through probability-density functions to calculate different textural parameters. After analyzing the color features of the focused image, the textural features were extracted. In one review, 21 textural parameters were identified (Zheng et al., 2006). However, another report indicated that only four textural parameters were useful in identifying plant health—entropy, energy, contrast, and homogeneity (Ushada et al., 2007).

2.3. Experimental setup for calcium deficiency induction

The plant-production system was constructed in a research center located at the Controlled Environment Agricultural Center at the University of Arizona (Tucson, AZ). The research greenhouse's dimensions were 14.6 m L × 7.3 m W × 2.0 m H. The ridge height was 2.7 m. The greenhouse was covered with a double polycarbonate glazing and equipped with a Pad and Fan evaporative cooling system. Desired climate set points were maintained by an automatic climate control system.

Environmental parameters were collected by a data logger (21X, Campbell Scientific, Logan, UT) and a National Instruments FieldPoint data acquisition system (FieldPoint, National Instruments, Austin, TX). Connected to the Campbell data logger, a LI-COR Quantum sensor (LI190SB, Campbell Scientific, Logan, UT) was placed at canopy height and a Campbell temperature and relative humidity probe (Vaisala HMP50 L, Campbell Scientific, Logan, UT) hung from the greenhouse roof, 1 m above the plant canopy. Connected to the National Instruments data acquisition system was the Vaisala carbon dioxide sensor and transmitter (GMT222, Vaisala Inc., Woburn, MA), which was fixed to the wall of the greenhouse at plant canopy level.

During the experiment, the greenhouse temperature was set to 25 °C for the day (14 h) and 20 °C for the night (10 h). The experiment consisted of 12 containers split into two groups. Each group had three control containers and three treatment containers. Each container held four lettuce plants (*Lactuca sativa* cv. Buttercrunch). Root-zone environments were maintained at a pH of 6.0, EC of 2.0 dS m^{-1}, and a temperature of 20 °C.

The treatment nutrient solution was deficient in calcium to induce tip burn. Deficiencies were induced by the removal of calcium chloride and the replacement of calcium nitrate with sodium nitrate. Initially, all 12 containers had the control nutrient solution for 6 days so that the machine vision system could detect similar trends with the plants. Then, calcium deficiency was induced in the treated groups. The experiment continued until all treatment plants had tip burn. Nutrient solutions were changed every 3 days to maintain proper nutrient levels in the root zone. One group of three treated and three untreated containers was brought into the laboratory for image acquisition and analysis. This occurred twice a day at 12-h intervals (6:00 am and 6:00 pm). We used these two groupings to determine whether transportation to the lab had an effect on the plants. The group that was brought into the lab was labeled Group 1 and the group that stayed within the greenhouse was labeled Group 2.

2.4. Methodology for early detection of stress

One objective of this study was to develop a methodology for early detection of calcium deficiency in a lettuce crop. In other words, the separation point identifying the onset of stress due to the calcium deficiency was calculated by identifying the mean difference between the treatment and control containers at each measured time for all collected parameters. Dual-segmented regression analysis was performed to identify where in time a change point was present between the nutrient-deficit group of plants and the healthy group of plants (Muggeo, 2003).

In detail, to detect the change point between the treatment group and control group, we examined the profile difference between the two groups for each parameter. The response was represented by:

$$y(t_i) = \mu_T(t_i) - \mu_C(t_i) \tag{1}$$

where μ_T and μ_C represent the average value of the specific parameter obtained from the treatment and control group at t_i time point of the experiment. The values of extracted plant parameters from the treatment group began to differ from the controls starting at some point in time. Thus, segmented regression analysis was employed for the profile difference to determine the onset of stress detection. In this method, the independent variable (time) was partitioned into intervals and a separate line segment was fitted to each interval. The change point, t_c, estimated the breakpoint between the two regression equations, determined by:

$$\begin{aligned} \hat{y} &= \beta_0 + \beta_1 t, \quad \text{when } t < t_c \\ \hat{y} &= \alpha_0 + \alpha_1 t, \quad \text{when } t \geq t_c \end{aligned} \tag{2}$$

Therefore, two regression lines were made to fit the observed data as closely as possible by minimizing the sum of squares of the differences between the observed response (y) and the calculated dependent variable ($\hat{y}$). This was implemented by R, an open source of statistical computations and analysis from the R-Project (www.r-project.org). In addition to the change point estimation, a confidence interval (95%) was measured about the change point to identify its proximity in relation to the developed trend line. At a certain confidence (e.g., 95%), the narrower the interval, the more accurate the estimation result.

3. Results and discussion

The experiment ran for a total of 15 days. The treatment started on the 6th day, and human visual detection of tip burn occurred on day 11.5. The average day temperature, night temperature, and day time/night time relative humidity values in the

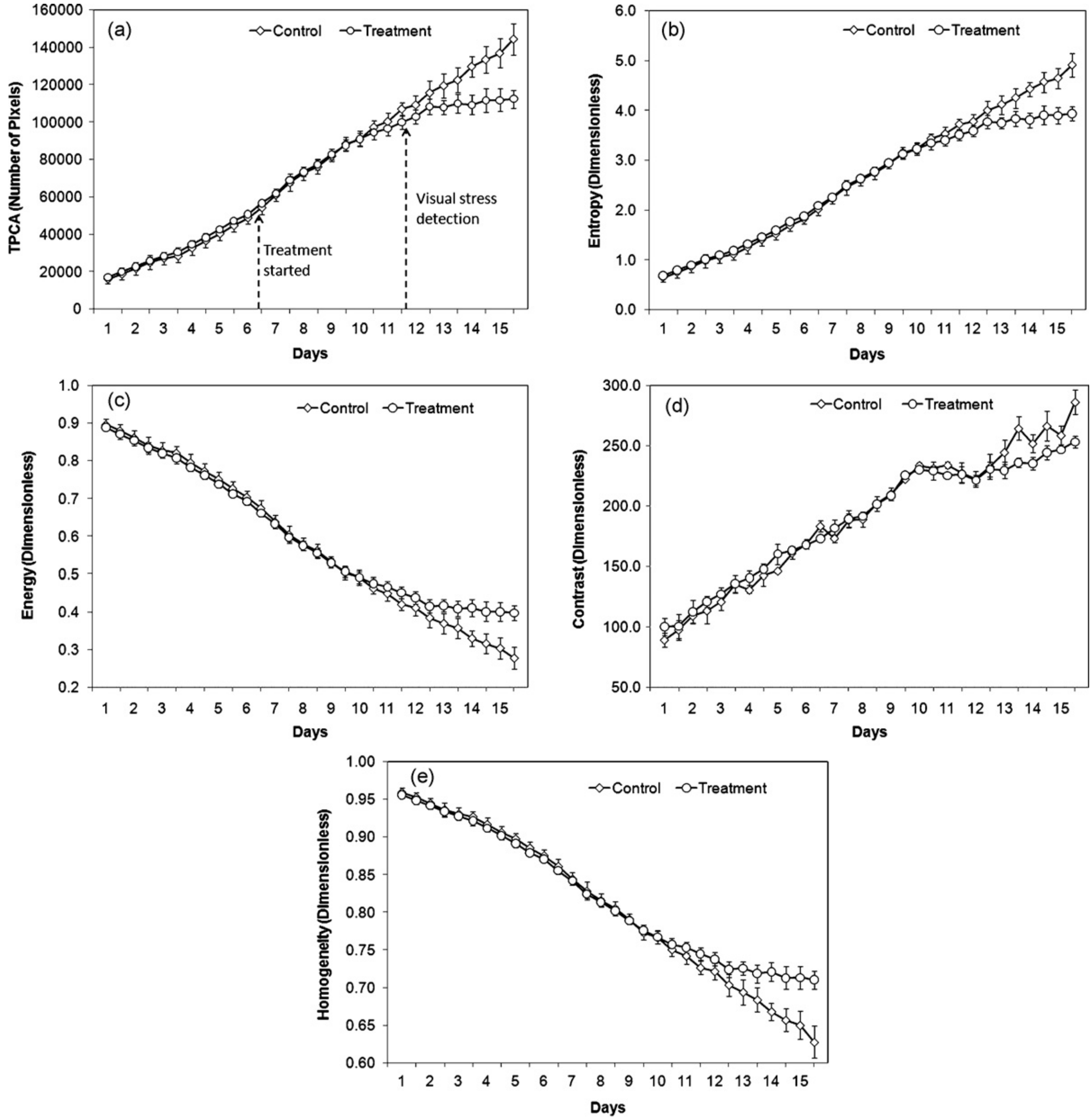

Fig. 2. Timeline of extracted plant features as lettuce plants experience calcium deficiency-induced tipburn (bars represent the standard deviation of the mean obtained from three containers in each group).

research greenhouse were $26.7 \pm 2.3\,^{\circ}C$, $22.1 \pm 1.7\,^{\circ}C$, $45.4 \pm 6.0\%$, 63.6%, and $63.6 \pm 7.1\%$, respectively, during the experimental period. The average photosynthetically active radiation (PAR) was $16.0 \pm 6.8\,mol\,m^{-2}\,day^{-1}$, and the average CO_2 concentration was 350 ± 7.0 ppm. The measured nutrient zone conditions electrical conductivity (EC) and nutrient temperature were $2.1 \pm 0.1\,dS\,m^{-1}$ and $20.1 \pm 1.2\,^{\circ}C$, respectively, during the experiment.

During the study, plants in the treatment group appeared lighter green (yellowish) due to the calcium deficiency. Other symptoms were marginal necrosis of the leaves and small, dark brown spots near the leaf margin. In detecting calcium deficiency with lettuce plants, one of the most noticeable visual signs is tip burn. Plants use calcium in the development of cell walls (Nance, 1973), and tip burn occurs when laticifer cells burst due to weak cell walls, which releases latex into the surrounding tissue (Barta and Tibbitts, 2000). Calcium is xylem-mobile, which means the nutrient moves in the direction of transpiration (Atkinson et al., 1992). If regions of the leaf are not transpiring, then calcium is not delivered to those leaf sections.

Fig. 2 illustrates the timeline of the extracted plant features (TPCA, entropy, energy, contrast and homogeneity) as averaged values obtained from the control and treatment containers. The plant monitoring system was capable of determining the overall canopy rate, represented by the TPCA feature (Fig. 2a). As the control and treatment plants were initially grown in a control nutrient solution, similar trends in both the treatment and control group plants were expected. However, as the treatment plants became calcium deficient, a gradual difference in TPCA value can be seen between the control, which continued to grow, and the treatment plants, which had a growth rate that decreased over time. This growth difference may be attributable to the lack of calcium, restricting the cellular wall structure and prohibiting the expansion of the plants' size. Similar effects of plant stress on the change of TPCA have been reported using the coefficient of relative variation of TPCA as a marker for water stress detection (Kacira et al., 2002).

The key assumption underlying the textural analysis of the canopy images in this study was that changes in the canopy texture and surface structure are external symptoms of the plant's internal

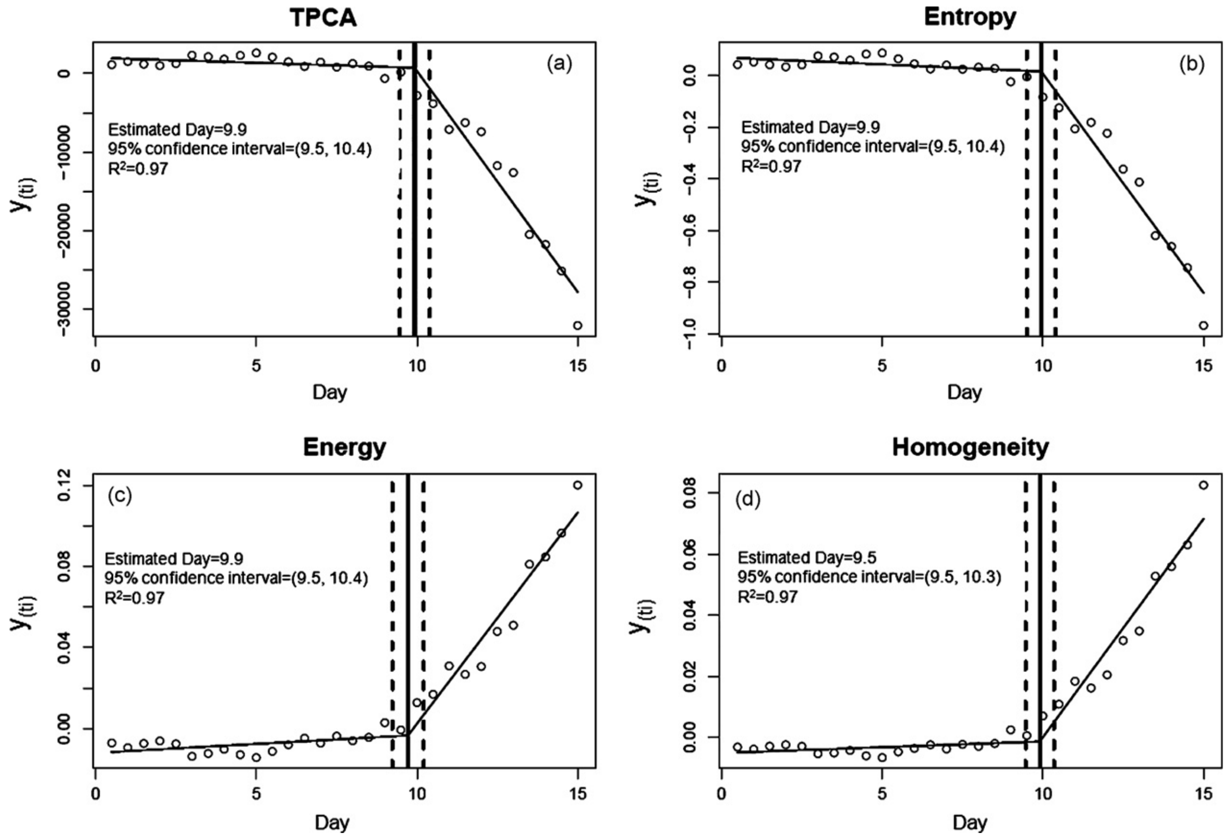

Fig. 3. Difference profile and segmented regression results for the four textural parameters for identification of the onset of stress induced by calcium deficiency on lettuce plants.

physiological status. Stress in plants is known to provoke changes on the surface, texture and the internal leaf structure (Penuelas and Filella, 1998). Textural features were therefore examined by probability-density functions on co-occurrence matrices of different relative angles.

In the textural analysis, entropy was defined as the randomness of gray-level distribution. As the control group plants were growing under optimal conditions, their leaves were healthy and colorful. This was detected by higher levels of entropy values from the canopy in the control group (Fig. 2b). As treatment plants became calcium deficient, the entropy value decreased due to a reduction in surface structure complexity.

Energy is the numerical value represented by the level of grayscale brightness. As the healthier plants in the control group became darker green in color, the energy value decreased over time (Fig. 2c). Similarly, as the treatment plants started to exhibit signs of calcium deficiency, the yellowish appearance in the leaves resulted in a lighter canopy color and raised the energy levels in the images of the treatment group plants.

Contrast is a measure of the local variations in an image. The control plants were darker but more colorful, which resulted in increased contrast values compared to the treatment plants, which were brighter but more uniform in color (Fig. 2d). We expected that the contrast value would be elevated if an image has high local variation. Similar phenomena were reported by Ushada et al. (2007) and Ondimu and Murase (2008), as contrast values increased when a sunagoke moss canopy became dry and more varied in color.

Homogeneity is the determination of the related gray-level pixel distribution amongst the surrounding pixels in the canopy image. As the control became colorful, with different shades of green, the related gray-level pixel distribution decreased over time. Conversely, the treated plants, being more unified in color due to calcium deficiency, had higher gray-level pixel distribution values (Fig. 2e).

We were interested in establishing a methodology for the machine vision system to determine the onset of stress detection as early as possible. This was achieved by statistical computations using dual-segmented regression analysis. For each of the collected parameters, the change point and regression lines were estimated. Among the plant features analyzed, TPCA and three textural parameters (entropy, energy, and homogeneity) identified the occurrence of the calcium deficiency earlier than human vision detection. The estimated change point for three of the four parameters was on day 9.9 (with 95% confidence interval [9.5, 10.4] and $R^2 = 0.97$) (Fig. 3a, b, and d). The change point of energy was found to be on day 9.7 (Fig. 3c).

Although the contrast parameter indicated a change on day 9.5 (data not shown), a wide confidence interval (7.5, 11.5) was observed, which casts doubt on its reliability as a timely stress detection parameter. Based on the statistical analysis performed, four parameters (TPCA, entropy, energy, and homogeneity) are promising as an early warning of stress due to calcium deficiency, indicated by a measurable difference between the treatment and control groups. From these four parameters, and the statistical approach used, the machine vision system could detect the onset of stress due to calcium deficiency (i.e., a deviation of treatment group plants from a control group) on the 10th day. This was a full day earlier than the detection of lettuce tip burn resulting from the calcium deficiency by human vision.

4. Conclusion

Plants' responses can be measured by sensors to determine their physical conditions and needs. To optimize the plant-production process, real-time monitoring of the physiological status of plants is necessary, and this information can be included in control processes. This approach can help to improve resource use efficiency in controlled-environment crop production systems.

In this study, we successfully developed an autonomous machine vision system for real-time monitoring of lettuce plant health and growth in controlled-environment plant-production systems. The monitoring system was capable of extracting plant morphological, textural and temporal features. The developed methodology was able to identify calcium-deficient lettuce plants 1 day earlier than the visual stress detection by human vision. Among the extracted plant features, TPCA, energy, entropy, and homogeneity were the most promising markers for timely detection of calcium deficiency in the lettuce crop studied. Though it is not feasible to rely on a single marker for stress detection, a detection approach using multiple markers is more reliable. The capability of the machine vision system for measuring plant stress and health can be improved by combining monitored parameters from the root zone, plant canopy and the aerial environment as a continuum. This study focused on maintaining uniform environmental conditions for both the control and treatment containers, and to induce tip burn only through calcium deficiency. Importantly, the analyzed data show trends that are similar to other characteristics of plant stress, and it will be of use to develop tools that identify different plant stresses from individual stressors.

In this research, image acquisitions were conducted in laboratory conditions to identify what type of markers could be utilized for the identification of plant stress, in this case, calcium deficiency-induced tip burn. In real greenhouse settings, this system could be applicable for nighttime plant monitoring, but during the day, non-uniform conditions, such as the variable nature of lighting, may be problematic. Therefore, it will be essential to revise the algorithms developed for image acquisition for this system to operate under daytime greenhouse settings.

Further studies are needed to develop a multi-sensor-based approach to better identify common stress symptoms that can occur due to several stressors. Combining this with an intelligent control technique, with a decision support system, can help to deal with the complexity of symptoms and control of the whole system. Finally, once the system is able to identify a particular stress, it must relay the plant status by means of a mark displayed on the plant canopy, specifying the area of interest to the grower. We aim to address these research directions in future studies.

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