

Article **Seedling Growth Stress Quantification Based on Environmental Factors Using Sensor Fusion and Image Processing**

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Abstract: Understanding the diverse environmental influences on seedling growth is critical for maximizing yields. The need for a more comprehensive understanding of how various environmental factors affect seedling growth is required. Integrating sensor data and image processing techniques offers a promising approach to accurately detect stress symptoms and uncover hidden patterns, enhancing the comprehension of seedling responses to environmental factors. The objective of this study was to quantify environmental stress symptoms for six seedling varieties using imageextracted feature characteristics. Three sensors were used: an RGB camera for color, shape, and size information; a thermal camera for measuring canopy temperature; and a depth camera for providing seedling height from the image-extracted features. Six seedling varieties were grown under controlled conditions, with variations in temperature, light intensity, nutrients, and water supply, while daily automated imaging was conducted for two weeks. Key seedling features, including leaf area, leaf color, seedling height, and canopy temperature, were derived through image processing techniques. These features were then employed to quantify stress symptoms for each seedling type. The analysis of stress effects on the six seedling varieties revealed distinct responses to environmental stressors. Integration of color, size, and shape parameters established a visual hierarchy: pepper and pak choi seedlings showed a good response, cucumber seedlings showed a milder response, and lettuce and tomato seedlings displayed an intermediate response. Pepper and tomato seedlings exhibited a wide range of growth stress symptoms, at 13.00% to 83.33% and 2.96% to 70.01%, respectively, indicating considerable variability in their reactions to environmental stressors. The suggested classification approach provides valuable groundwork for advancing stress monitoring and enabling growers to optimize environmental conditions.

Keywords: smart horticulture; seedling growth; plant growth stress; image processing; sensor fusion

1. Introduction

By 2050, the world population is expected to reach 9.1 billion, a 34% increase from current levels, and to support this larger, more urbanized, and economically prosperous population, a 70% increase in food production is necessary [\[1\]](#page-29-0). Agricultural technology advancements provide a solution to meet rising global food demand amid challenges like population growth and climate change [\[2\]](#page-29-1). Vegetables play an important role in ensuring food and nutrition security [\[3\]](#page-29-2). Their production not only presents a viable economic opportunity but also aids in alleviating rural poverty and unemployment in developing nations, forming a vital aspect of farm diversification strategies [\[4\]](#page-29-3). As a cost-effective

Citation: Islam, S.; Reza, M.N.; Ahmed, S.; Samsuzzaman; Cho, Y.J.; Noh, D.H.; Chung, S.-O. Seedling Growth Stress Quantification Based on Environmental Factors Using Sensor Fusion and Image Processing. *Horticulturae* **2024**, *10*, 186. [https://](https://doi.org/10.3390/horticulturae10020186) doi.org/10.3390/horticulturae10020186

Academic Editors: Most Tahera Naznin, Kellie Walters and Neil Mattson

Received: 25 January 2024 Revised: 15 February 2024 Accepted: 17 February 2024 Published: 18 February 2024

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source of essential vitamins and minerals, vegetables contribute significantly to maintaining good health [\[4,](#page-29-3)[5\]](#page-29-4).

High-quality seedling production refers to the process of cultivating seedlings that meet defined levels of performance, such as survival and growth, on a particular planting site [\[6\]](#page-29-5). The controlled environment in the seedling production facility is designed for the precise regulation of factors such as temperature, humidity, lighting, and nutrient supply, ensuring consistent and efficient crop growth (Huang et al., 2020) [\[7\]](#page-29-6). This controlled setting not only enables the production of high-quality crops but also reduces reliance on pesticides and minimizes bacterial contamination [\[8\]](#page-29-7). High-quality seedlings are essential for successful vegetable and floriculture crop cultivation, improving early crop establishment, final quality, uniformity, and yield and reducing production time [\[9\]](#page-29-8). The growth and development of high-quality seedlings can be significantly affected by environmental factors such as temperature, light intensity, water, and nutrients, with vegetable production being especially susceptible to these abiotic environmental stresses [\[10](#page-29-9)[–12\]](#page-29-10). Exposure to extreme temperature and light conditions can exacerbate these challenges, with susceptibility varying based on seedling conditioning and phenological stage [\[13\]](#page-29-11). Water loss consequences may extend over multiple growing seasons, impacting survival rates and growth [\[14\]](#page-29-12).

Plant/seedling growth stress poses a significant threat to agricultural productivity, leading to a substantial decrease in crop yield and quality [\[15](#page-29-13)[,16\]](#page-29-14). Early detection of seedling growth stress is crucial for enhancing crop production and ensuring agricultural sustainability. Stress can significantly impact the germination and early growth of seedlings. The major environmental factors that contribute to seedling stress include water availability (both drought and flooding), temperatures (heat and cold), light intensity (too much or too little), nutrient imbalances, and salinity [\[12\]](#page-29-10). These factors can hamper essential plant processes, leading to visible symptoms like wilting, discoloration, and stunted growth. Environmental stressors, such as temperature, light, water potential, and nutrient levels, can affect seed germination and pre-emergence seedling growth, leading to reduced crop establishment and yield [\[17\]](#page-29-15). Measuring seed vigor, which encompasses the ability of seeds to germinate and produce vigorous seedlings, is crucial for ensuring crop sustainability in changing climates [\[18\]](#page-29-16). Additionally, the genetic and physical quality of seedlings, influenced by early stress factors, plays a key role in determining the success of seedling production [\[19\]](#page-29-17). Quantifying and addressing early growth stress symptoms are essential for producing high-quality seedlings and improving crop establishment, uniformity, and yield [\[20\]](#page-29-18). Therefore, early stress quantification is necessary to identify and mitigate potential issues that could impact the overall success of seedling production.

Computer vision advancements have enabled the extensive application of image processing and machine learning in studying crop biotic and abiotic stress phenotypes [\[21\]](#page-29-19). Image analysis techniques, being non-invasive, offer significant potential for the automated detection of both types of stress in plants. This involves processing meticulously collected photographs to extract specific information [\[22,](#page-29-20)[23\]](#page-29-21). Various imaging techniques, such as digital, fluorescence, thermography, LIDAR, multispectral, and hyperspectral imaging, can be used to effectively identify and assess stress in crops [\[15\]](#page-29-13). These images provide valuable data on plant physical attributes like canopy area, leaf color, texture, size, and shape [\[24\]](#page-29-22). Commonly used color spaces include RGB, LAB, YCBCR, and HSV [\[25\]](#page-30-0). Descriptive elements like image contrast, homogeneity, dissimilarity, energy, and entropy contribute to texture analysis [\[26\]](#page-30-1). Sun et al. [\[27\]](#page-30-2) introduced a non-destructive method for diagnosing nutrient stress in paddy plants, specifically targeting nitrogen, phosphorus, and nitrogen, phosphorus, and potassium (NPK) deficiencies. Latte et al. [\[28\]](#page-30-3) detected nutrient deficiencies in paddy crops by analyzing leaf color during mid-growth.

Visual sensors play a significant role in controlled plant production facilities, enabling comprehensive crop monitoring and automation through image analysis [\[29\]](#page-30-4). Integrating visual sensors allows farmers to make informed decisions, optimize resources, and improve crop yields, supporting sustainable vertical farming practices [\[30\]](#page-30-5). Image sensor fusion and image processing offer a powerful approach to understanding and managing seedling growth stress. By combining image sensor data from diverse imaging sources and advanced image analysis and offering a comprehensive perspective on the environmental conditions impacting seedling health, subtle changes in plant health can be detected early [\[31\]](#page-30-6). This allows for timely adjustments to lighting, nutrients, or water, ensuring optimal growing conditions. Studies utilizing RGB image sensors have effectively assessed plant stresses, identifying issues like biotic stress in wheat [\[31\]](#page-30-6), nutrient deficiency in soybean [\[32\]](#page-30-7) and black gram [\[33\]](#page-30-8), and fungal blight in potato [\[34\]](#page-30-9).

Machine learning algorithms establish optimal decision boundaries in high-dimensional feature spaces, forming the foundation for accessible image analysis systems [\[35\]](#page-30-10). Traditional approaches in machine learning, proven to be versatile and effective, analyze crop phenotypes related to stress conditions [\[32,](#page-30-7)[36](#page-30-11)[,37\]](#page-30-12). Feature extraction provides quantified parameters facilitating classification by assigning objects to specific classes [\[38\]](#page-30-13). Image segmentation, based on color and texture features in RGB and grayscale intensity spaces, improves representativeness and ease of analysis [\[39\]](#page-30-14). Karadag et al. [\[40\]](#page-30-15) introduced a machine learning algorithm to differentiate healthy and fusarium-infected pepper plants. Support vector machine (SVM), which has been extensively applied in agriculture, proves effective for plant stress detection. SVM grades the severity of iron deficiency chlorosis in soybeans [\[41\]](#page-30-16). Prasad et al. [\[42\]](#page-30-17) devised an imaging system for plant leaf disease identification, incorporating disease spot detection, Gabor-wavelet-transform-based feature extraction, and SVM-based disease classification.

The ability to accurately measure and analyze seedling growth stress helps us to identify environmental factors that may impede healthy growth and development. Understanding these stressors is essential for implementing timely interventions and optimizing plant growth conditions. The potential of sensor fusion and image processing techniques for quantifying seedling growth stress caused by various environmental factors is immense. Utilizing these technologies enables improved understanding of plant stress responses, optimizing growing conditions for enhanced crop yields, and sustainability in agriculture and controlled horticulture. The objective of this study was to quantify the seedling growth stress symptoms of six seedling varieties under controlled conditions, intentionally subjecting them to variations in light, temperature, water, and nutrient levels.

2. Materials and Methods

2.1. Experimental Site Preparation and Seedling Growth Conditions

The experiments took place in a plant factory situated on the premises of the College of Agriculture and Life Science at Chungnam National University in Daejeon, Republic of Korea. In this experiment, 6 seedling varieties were sown in 45-hole trays for tomato and pepper, 128-hole trays for lettuce and pak choi, and 40-hole trays for cucumber and watermelon using horticultural soils. Eight-day-old pepper (variety: Cheongyang), tomato (variety: Defness), lettuce (variety: Cheongchima), pak choi (variety: Modom), cucumber (variety: Begdadagi), and watermelon (variety: Jhob) seedlings were grown in five small chambers to observe the environmental stress effects. Each seedling cultivation chamber was $1500 \times 1100 \times 2500$ mm (L \times W \times H). Within one chamber, a vertical frame with three beds accommodated three seedling trays on each bed. The overall size of the vertical frame was $980 \times 600 \times 2160$ mm (L \times W \times H) and the growing bed was $900 \times 600 \times 150$ mm $(L \times W \times H)$. To maintain optimal conditions, each of the five cultivation rooms was equipped with air conditioners, heaters, humidifiers, dehumidifiers, and solenoid valves for temperature, humidity, and carbon dioxide control. Two axial fans per bed circulated air in the cultivation room. Fluorescent lights were used for adequate seedling lighting on the cultivation beds. Figure [1a](#page-3-0),b illustrate the layout and configuration of the control room and cultivation chambers along with the actuator, fluorescent light, and seedling tray positions. sitions.

Figure 1. Experimental chamber layout and continuous water content measurement during the perimental period using a weight balance. (**a**) Layout and configuration of the cultivation chambers; experimental period using a weight balance. (**a**) Layout and configuration of the cultivation chambers; \overrightarrow{b} image of the custom fabricated cultivation chamber showing the positions of the actuators, orescent lights, and seedling trays; (**c**) weight measurement using the seedling tray containing soil fluorescent lights, and seedling trays; (**c**) weight measurement using the seedling tray containing soil and plants; and (**d**) continuous water content measurement during the experiment.

The study focused on four specific environmental parameters: temperature, light tensity, nutrients, and water supply. To investigate the effect of environmental stress, the intensity, nutrients, and water supply. To investigate the effect of environmental stress, the experiment maintained three levels of temperature, light intensity, nutrients, and water experiment maintained three levels of temperature, light intensity, nutrients, and water application. Table 1 shows the ambient environment parameters in all five controlled application. Table [1](#page-4-0) shows the ambient environment parameters in all five controlled chambers. The standard growth conditions for the six seedling varieties were derived chambers. The standard growth conditions for the six seedling varieties were derived from the literature, as detailed for lettuce [43], tomato [44], pepper [45], watermelon [46], from the literature, as detailed for lettuce [\[43\]](#page-30-18), tomato [\[44\]](#page-30-19), pepper [\[45\]](#page-30-20), watermelon [\[46\]](#page-30-21), cucumber [47], and pak choi [48]. cucumber [\[47\]](#page-30-22), and pak choi [\[48\]](#page-30-23).

Table 1. Ambient environment parameters for the experiments and control and stress conditions used in this study for six varieties of seedlings.

In a plant factory, effective water and nutrient application is vital for the healthy growth of seedlings. Table [1](#page-4-0) highlights two distinct types of water and nutrient application. For plant chambers 1, 2, 4, and 5, 1 L/tray/day of water and nutrient solutions with an electrical conductivity (EC) of 1.0 dS m^{-1} and pH of 6.0 were provided. In chamber 3, it was aimed to investigate the impact of water and nutrient stress on seedlings. This involved experimenting with three different levels of water and nutrient supply. Water levels were adjusted to 1, 0.75, and 0.50 L/tray/day for water stress trials, while nutrient solution levels were 1, 3, and 6 dS m⁻¹ for the nutrient stress trials. These variations enabled the examination of the effects of varying stress levels of water and nutrient availability. The selection of these supply levels was guided by established practices to ensure consistent nutrient delivery to the root zone, preventing both overwatering and underwatering of the seedlings. In this study, water mixed with commercial Hoagland nutrient solutions A and B (Daeyu Co., Ltd., Seoul, Republic of Korea) was applied to plant roots. All the essential and trace nutrient elements are combined in this commercial nutrient solution. The ingredients contained in each solution are shown in Table [2.](#page-5-0)

Table 2. Ingredients contained in nutrient solutions A and B.

To achieve the desired nutrient level, 5 mL each of nutrient solutions A and B were mixed with 5 L of water, and 1 L of this mixture was provided daily to each bottom irrigation tray, positioned beneath the seedling growing tray. The targeted nutrient level was maintained by checking the EC and pH meter every day while watering the seedlings. The seedling soil absorbed water through the bottom irrigation tray, facilitating water uptake by plants through the osmosis process. Irrigation was carried out at rates of 0.75 L/tray/day and 0.50 L/tray/day, representing 75% and 50% of the standard irrigation application, while the control group received 100% of the standard irrigation. To measure water stress, we continuously weighed the water in the seedling trays at 10 min intervals using a CAS weighing balance (Total Weighing Solution, Yangju, Republic of Korea) positioned beneath the seedling trays, as illustrated in Figure [1c](#page-3-0),d. Soil water content was calculated using Equation (1) based on weight basis method as follows:

$$
W_C = \frac{W_W}{W_W + W_M} \tag{1}
$$

$$
W_{C} = \frac{W_{W}}{W_{W} - \{W_{S} + W_{D} + W_{T} + (W_{P} \times N)\}}
$$
(2)

where W_C is the water content (%), W_W is the weight of water (g), W_M is the total weight of the materials (g), W_S is the weight of the tray including soil (g), W_D is the dry soil weight (g), W_T is the weight of the empty tray under the soil tray (g), W_P is the plant weight (g), and N is the number of plants in the tray.

Throughout the experiment, the remaining ambient environmental variables, such as humidity and $CO₂$ levels, were maintained at constant levels. The optimum relative humidity and $CO₂$ concentration were 60 \pm 5% and 600 to 800 ppm, respectively. Table [1](#page-4-0) also shows the control and stress conditions for the six seedling varieties used in this study.

2.2. Sensor Selection and Image Acquisition

The data collection process involved a multi-camera system, as shown in Figure [2.](#page-6-0) For top-view imaging of seedlings, an affordable, portable, and high-quality RGB camera (model: Raspberry Pi camera, Raspberry Pi Foundation, Cambridge, UK) was used. To capture thermal information, a thermal camera (Model: Seek Thermal Compact Pro, Seek Thermal Inc., Santa Barbara, CA, USA) was employed. Additionally, a depth camera (Model: Intel RealSense D435i, Intel Corporation, Santa Clara, CA, USA) was used for acquiring depth images. The specifications of these cameras are shown in Table [3.](#page-6-1) The cameras were strategically positioned vertically at a height of 0.36 m above the seedlings, ensuring optimal field-of-view (FOV) coverage (Figure [2\)](#page-6-0). Throughout the image capture process for each seedling bed, consistent lighting conditions were maintained. The Raspberry

Pi Camera Module V2 is a compact camera for Raspberry Pi single-board computers, capturing still images up to 8 megapixels (3280 \times 2464 pixels). Utilizing the Sony IMX 219 sensor, it ensures high-quality images with excellent color reproduction and low-light performance. Small and lightweight, with a standard field of view of 62.2[°] \times 48.8[°] and automatic image acquisition control, it was suitable for this study. The Intel RealSense D435i is an advanced depth camera designed for precise depth sensing, featuring a frame size of 1280×720 pixels and global shutter technology. With its stereoscopic depth sensing and integrated IMU, it offers a wide field of view of $87° \times 58°$ and a depth range of 0.3 to 3.0 m. Its compact design and seamless integration make it an ideal choice for this applications. The Seek Compact Pro is a compact and potent thermal imaging camera with a frame size of 320 \times 240 pixels, a field of view of 32° \times 32°, and a temperature sensing range of −40 to 330 °C. Equipped with automatic control and calibration systems, its pocket-sized design makes it perfect for installation in seedling chambers to facilitate data acquisition.

Figure 2. Image acquisition tool using RGB, thermal, and depth cameras with microcontrollers to **Figure 2.** Image acquisition tool using RGB, thermal, and depth cameras with microcontrollers to acquire top and side images of seedlings. acquire top and side images of seedlings.

Table 3. Specifications of RGB, thermal, and depth cameras used in this study.

Automation of the image capture process was achieved through the utilization of a microcontroller system (Model: Raspberry Pi 4B, Raspberry Pi Foundation, Cambridge, UK) equipped with an integrated display. This configuration facilitated seamless interfacing with the cameras, enabling the automated capture and storage of images. The system was designed for efficient remote monitoring and image acquisition tasks. For remote accessibility, a virtual network computing (VNC) viewer was implemented on the microcontroller system. This allowed for remote control through a graphical user interface (GUI) display, ensuring automatic initiation of the viewer upon device boot-up, as detailed by Islam et al. [\[49\]](#page-30-24). VNC, developed in the mid-1990s, is a remote desktop technology with open-source code licensed under the GNU General Public License. Commercial variations of VNC are also available. Images were saved in JPG format with a resolution of 3280×2464 pixels on a 128 GB micro SD memory card connected to the microcontroller. To mitigate the impact of camera jitter or potential unfocused images, three images were captured for each seedling bed. Subsequently, the average of these three images was com-puted and utilized for further analysis. Figure [3](#page-7-0) shows sample images captured during the experiments for all six varieties of seedlings.

Figure 3. RGB image acquisition for six seedling varieties using RGB camera from the top view: (a) cucumber seedlings, (b) pak choi seedlings, (c) lettuce seedlings, (d) pepper seedlings, (e) tomato seedlings, and (**f**) watermelon seedlings. seedlings, and (**f**) watermelon seedlings.

The study investigated the impact of various environmental stress effects on six varieties of seedlings. A total of 540 images from the six seedling varieties were acquired for analysis. Among these, 288 images were related to stressed seedlings, while 252 images represented non-stressed seedlings, serving as a control group. Four different types of stresses (temperature, light intensity, nutrient, and water) were considered for this experiment. For each stress type, 72 images were captured, resulting in a balanced distribution of stress samples. The severity of the stresses applied to the seedlings in terms of temperature, light intensity, nutrients, and water ranged from low to high levels. To ensure accurate analysis, all images of stressed seedlings were appropriately labeled based on reference guidelines describing the growth conditions and characteristics specific to each stress class.

2.3. Data Processing and Analytical Procedures

Figure [4](#page-8-0) shows the schematic diagram of the overall sensor fusion and image processing steps for stress quantification in this study. The schematic diagram illustrates the process of sensor fusion and image processing steps for stress quantification in seedling growth based on environmental factors.

Figure 4. The overall sensor fusion and image processing steps for the growth stress quantification in this study. this study.

Beginning with the data acquisition from RGB, thermal, and depth sensors capturing Beginning with the data acquisition from RGB, thermal, and depth sensors capturing various aspects of seedling growth and environmental conditions, the data then undergo various aspects of seedling growth and environmental conditions, the data then undergo integration and pre-processing to align and prepare them for analysis. This involves refining RGB imagery through image enhancement and feature extraction techniques, ensuring accurate representation of thermal data through temperature calibration, and generating depth maps for calculating seedling size during depth data processing. These integrated datasets are then represented visually for comprehensive analysis. The fusion data from different sensors was used to analyze seedling growth and environmental influences. Finally, stress quantification was obtained by analyzing the integrated data to identify color, shape, size, and canopy temperature patterns. Based on the pattern of healthy and stressed seedling data, seedling stress symptom levels (%) were calculated.

2.3.1. RGB Image Processing for Stress Symptom Features 2.3.1. RGB Image Processing for Stress Symptom Features

Image quality is crucial in image analysis, influencing accuracy and effectiveness. Low
se reality pages shall wasse in discoming datails due to issues like assessments and d Low image quality poses challenges in discerning details due to issues like overexposure, ows, debris, and focusing issues. High-quality images enhance the processing efficiency, shadows, debris, and focusing issues. High-quality images enhance the processing effi-reducing complexity and improving feature extraction [\[50\]](#page-30-25). The schematic flowcharts for ciency, reducing complexity and improving feature extraction [50]. The schematic the RGB image processing and leaf area (LA) estimation are shown in Figures [5](#page-9-0) and [6](#page-9-1) and illustrate the overall image processing and leaf area calculation steps. The initial and illustrate the overall image processing and leaf area calculation steps. ures 5 and 6 and illustrate the overall image processing and leaf area calculation steps. step involved histogram equalization to address light source variation. Subsequent preprocessing reduced noise and enhanced contrast in the image, comprising the target plant presenting comprise the image, comprise and background. Direct binarization faced challenges due to insufficient contrast, artifacts, and distortions [\[51\]](#page-31-0). Contrast-limited adaptive histogram equalization (CLAHE) was employed to overcome these issues, enhancing the image contrast by limiting amplification image quality poses challenges in discerning details due to issues like overexposure, shadand operating on small image areas called tiles [10]. Parameters like block size (BS) and clip limit (CL) governed improved image quality. tissue area per unit ground or trunk surface area of a plant $\frac{1}{2}$ and operating on small image areas called tiles [10]. Parameters like block size (BS) and
tissue area per unit ground the plant surface area plant in the plant of a plant of a plant in the plant of a
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ing shape and structure. From the contour area, the leaf area was calculated. This quanti-

Figure 5. Flow diagram of the overall RGB image processing and leaf area calculation.

Figure 6. RGB image preprocessing steps and leaf area calculation: (a) original image with histogram; (b) R, G, and B channel images; (c) enhanced image with histogram; (d) CLAHE enhanced $\overline{\text{image}}$; (e) converted HSV image; (f) masking applied to select green features; (g) segmented image; (**h**) broken contours; and (**i**) smooth contours. (**h**) broken contours; and (**i**) smooth contours.

CLAHE, applied separately to red (R) , green (G) , and blue (B) channels with a CL limit of 0.5, enhanced local contrast and improved background segmentation. Gaussian blur further reduced noise. To extract foreground objects, the blurred image in HSV format set upper and lower bounds for the green color range, creating a mask. Morphological operations removed noise and filled gaps, enhancing segmentation. For leaf area calculation, contour detection was employed to capture seedling shapes from a binary image. However, image quality issues and noise resulted in broken contours, complicating seedling detection and area calculation. To address this, contour smoothing was applied using the total arc length of each polygonal curve, simplifying complex contours while preserving shape and structure. From the contour area, the leaf area was calculated. This quantifies the number of pixels within the leaf region as LA, which is the total one-sided leaf tissue area per unit ground or trunk surface area of a plant [\[52\]](#page-31-1). LA provides essential insights into leaf size and morphology and valuable information about seedling stress conditions and growth.

2.3.2. Depth Image Processing for Stress Symptom Features 2.3.2. Depth Image Processing for Stress Symptom Features 2.3.2. Depth Image Processing for Stress Symptom Features

For plant height estimation, using the Intel RealSense depth camera images involved several key processing steps. Figure [7](#page-10-0) shows the flow diagram of the overall depth image processing to estimate plant height. Initially, the colorized depth image was loaded and the shortest and longest depth range for the camera were set to facilitate colorization. Subsequently, the depth map was restored using the colorized depth image, and the result-ing map was saved at its original size [\[53\]](#page-31-2). The restored depth map was then displayed, providing a visual representation of the plant and its surroundings. Pixel coordinates (u, v) were specified to extract depth values at specific points of interest on the image. The image metadata, including focal length (fx, fy) and principal point (ppx, ppy) , were input to facilitate accurate depth extraction. The sample pixel coordinates and their corresponding
death as has access astimated has also at the sample death of the conjude as allocation to see the depth values were estimated based on the specified points. These pixel coordinates were then converted into real-world coordinates using the provided image metadata. By extracting this spatial information, the distance from the camera to the plant was calculated in three-dimensional space, providing a quantitative measure of the plant height [\[54\]](#page-31-3). Figure 8 shows the depth image processing results in different steps. α in tunity were estimated based on the specified points. These pixel coordinates were were the converted into real-world coordinates using the provided in μ is μ and μ and extraction the this spatial information, the distance from the distance from the plant was calculated in β is μ and space dimensional space, providing a quantitative measure of the plant height $\left[\sigma_{\text{eff}}\right]$, μ_{g} , μ_{g} figure the depth image processing results in different steps.

Figure 7. Flow diagram of the overall depth image processing for plant height estimation.

Figure 8. Depth image processing for plant height estimation: (a) original color depth image, (b) raw depth data in three (R, G, B) channels, (c) combined depth map, and (d) restored depth map from the from the combined depth map and metadata.

2.3.3. Canopy Temperature Measurement 2.3.3. Canopy Temperature Measurement 2.3.3. Canopy Temperature Measurement

Canopy temperature detection from thermal leaf images is a multistep process that depends on converting gray values to temperature using a color bar as the re[fere](#page-31-4)[nce](#page-31-5) [55,56]. The thermal image, captured from a fixed position, registers changes in gray levels as

temperature fluctuations occur. Employing edge detection methods, the leaf area is isolated within the image. Within this defined leaf region, the gray pixel values represent the temperature of the leaves. By referencing the color bar and the established gray-to-temperature relationship, these gray values are subsequently converted into actual temperature measurements. This approach allows for the precise assessment of canopy temperature, making it a valuable tool for applications ranging from plant stress detection to microclimate anal-ysis in agricultural and environmental studies. Figure [9a](#page-11-0) shows the steps of the canopy temperature extraction method, and Figure [9b](#page-11-0)–e show the thermal image processing steps and temperature profiling using the image processing method.

Figure 9. Canopy temperature detection from the thermal images: (a) steps of thermal image processing, (b) original image, (c) grayscale image, (d) gray-pixel-to-temperature conversion, and temperature profile. (**e**) temperature profile.

2.4. Quantification of Stress Symptoms 2.4. Quantification of Stress Symptoms

A schematic diagram of the quantification of stress symptoms is shown in Figure [10.](#page-12-0) A schematic diagram of the quantification of stress symptoms is shown in Figure 10. Seedling stress quantification through the extraction of leaf length, width, area, plant Seedling stress quantification through the extraction of leaf length, width, area, plant height, and canopy temperature from images is a multifaceted process involving advanced image processing and quantitative analysis. Initially, high-resolution images of plants were captured, and image processing techniques were applied to extract the features as explained in previous sections. The integration of infrared imaging technology aids in the extraction of canopy temperature data, providing additional insights into the plant's physiological response to stress. The extracted numerical data, encompassing leaf dimensions, area, plant height, and canopy temperature, were meticulously organized into a structured dataset for further analysis. Stress quantification involves the labeling of plants as stressed or non-stressed based on domain knowledge, expert input, and additional horticultural data. Horticultural data encompass a wide range of information related to the cultivation and management of plants, particularly those cultivated for food, medicinal, ornamental, or landscaping purposes. These data are crucial for optimizing crop yields, ensuring plant health, and making informed decisions in horticultural practices.

 $\overline{}$ Descriptive statistical analyses (mean, standard deviation) were then applied to evaluate the significance of differences in extracted features between stressed and non-stressed groups. Subsequently, thresholds were determined to categorize stress levels, and the quantification of stress involved applying these thresholds to the dataset. The outputs were visualized through various graphical representations to facilitate a comprehensive understanding of the distribution of features across stressed and non-stressed seedlings. For the stress symptom quantification, we calculated all the features of seedlings such as leaf area, seedling height, and canopy temperature. Based on the calculated values

from both the healthy and stressed seedlings, the stress symptoms (%) of seedlings were estimated and calculated using Equation (1) as follows:

$$
S_s(\%)=\left|\frac{H_v-S_v}{H_v}\right|\times 100\tag{3}
$$

where Ss is the calculated value (%) representing the degree of stress in the seedlings, Hv represents the feature values of the healthy seedlings, and Sv represents the feature values of the stress-induced seedlings.

Figure 10. Schematic diagram of the quantification of stress symptoms using feature measurements **Figure 10.** Schematic diagram of the quantification of stress symptoms using feature measurements from the seedling images. from the seedling images.

\mathcal{D}_{sub} ses (mean, statistical analyses (mean, standard deviation) were then applied to evalue the applied to evalue to evalue the standard deviation of the standard deviation of the standard deviation of the standar **3. Results**

3.1. Stress Symptom Visualization Based on Seedling Color and Size

In visualizing stress effects on seedlings, a comprehensive approach, including color, size, and shape, provides a distinct representation of seedling health and response to stressors [57]. Vibrant and uniform colors signify health, while shifts towards yellows, oranges, and reds indicate increasing stress levels. Changes in plant size reflect growth patterns, with smaller sizes indicating potential stunted development under stress conditions. Altered plant morphology, such as variations in leaf shape, offers insights into stress responses [58]. By combining these elements, a visual hierarchy was created to depict the severity and nature of stress, enhancing the richness of the information. Interactive reatures and time-lapse visualizations further enabled dynamic exploration, allowing us
to gain a deeper understanding of the stress effects on plants over time. Figure [11](#page-13-0) shows the stress effect visualization based on color, size, and area changes for pepper, cucumber, tomato, watermelon, lettuce, and pak choi seedlings. Pepper and pak choi seedlings were more likely to be affected by stress than other seedlings and showed the most significant changes in color, size, and area under stress conditions. Watermelon seedlings were less **3. Results** in color, size, and area under stress conditions. Tomato seedlings and lettuce seedlings were *3.1. Stress Symptom Visualization Based on Seedling Color and Size* moderately affected by stress. This was evident from the fact that the tomato and lettuce $\frac{1}{2}$ stress effects on seedlings on seedlings on seedlings, and the stress equality construction of $\frac{1}{2}$ and $\frac{$ Lettuce seedlings were most affected by stress, which was evident from significant changes
in color $s_{\rm T}$. Vibrant and uniform colors significant and uniform colors significant while shifts towards yellows, $\frac{1}{2}$ features and time-lapse visualizations further enabled dynamic exploration, allowing us likely to be affected by stress than other seedlings and showed the least significant changes seedlings showed intermediate changes in color, size, and area under stress conditions. in color.

Figure 11. Growth stress effects on different seedling varieties based on color, shape, and size. **Figure 11.** Growth stress effects on different seedling varieties based on color, shape, and size.

3.2. Growth Features Based on Environmental Conditions and Growth Period 3.2. Growth Features Based on Environmental Conditions and Growth Period

Leaf area serves as a valuable indicator for detecting stress in plants, with a reduction Leaf area serves as a valuable indicator for detecting stress in plants, with a reduction in leaf area being a common response to various stresso[rs \[](#page-31-8)[59,6](#page-31-9)0]. This is because plants in leaf area being a common response to various stressors [59,60]. This is because plants are trying to conserve water and energy, and so they will reduce the amount of leaf surface area that is exposed to the enviro[nm](#page-31-9)[ent](#page-31-10) $[60,61]$. For stress quantification, leaf area was measured for each type of seedling, as shown [in](#page-14-0) Figure 12 [and](#page-23-0) Table A1. For each environmental condition, 15 leaf samples were collected from the images for each day and each seedling type. Then, average values were calculated for each day. Pepper, tomato, lettuce, and pak choi seedlings showed significant differences in leaf area, indicating heightened sensitivity to these stressors. Pepper seedlings, for instance, experienced alterations in leaf area attributed to stressors related to both nutrition and light conditions. In contrast, the leaf area of the tomato seedlings was primarily influenced by variations in light exposure. Lettuce seedlings, however, demonstrated sensitivity to a broader range of stressors, with their leaf area being affected by light, nutrient levels, and temperature fluctuations. Meanwhile, pak choi seedlings displayed a unique pattern of sensitivity, with their leaf area being influenced by variations in light, nutrient availability, and water conditions. Watermelon and cucumber seedlings showed relatively lower significance in terms of changes in leaf area. This comprehensive examination emphasizes the intricate and varied reactions of these seedlings to distinct environmental stressors, providing insight into the complex nature of their growth dynamics.

Figure 12. Comparison of seedling leaf area of six seedling varieties under different environmental **Figure 12.** Comparison of seedling leaf area of six seedling varieties under different environmental stress conditions in different growth periods. stress conditions in different growth periods.

Plant height is a key indicator for detecting stress in plants and vegetables. The stress Plant height is a key indicator for detecting stress in plants and vegetables. The stress tolerance index, which incorporates seedling height, serves as a crucial tool by which to tolerance index, which incorporates seedling height, serves as a crucial tool by which to assess seedling threshold potential against specific stress factors [23,62]. The influence of assess seedling threshold potential against specific stress factors [\[23,](#page-29-21)[62\]](#page-31-11). The influence of stress conditions, such as temperature, light, nutrients, and water supply, on the height of stress conditions, such as temperature, light, nutrients, and water supply, on the height of six seedling varieties was studied and the results are shown in Fig[ure](#page-15-0) 13 and Table $\mathsf{A2}.$ The results show that the heights of the pepper and lettuce seedlings were influenced by stress related to light exposure and temperature variations, while the heights of the tomato and pak choi seedlings predominantly responded to changes in light conditions. Additionally, cucumber seedlings exhibited a subtle impact on their height in response to temperature stress, indicating a nuanced sensitivity compared to the other seedlings. However, the watermelon seedlings did not display a significant alteration in plant height under the observed stress conditions. The morphological and physiological responses of plants to different stress conditions, including light intensity and temperature variations, can impact their growth parameters. The results of this study highlight the varying sensitivities of d ifferent seedlings to different stress conditions. This suggests that the optimal growing conditions for each type of seedling will depend on its specific needs and tolerances.

Figure 13. Comparison of seedling heights of six seedling varieties under different environmental **Figure 13.** Comparison of seedling heights of six seedling varieties under different environmental stress conditions in different growth periods. stress conditions in different growth periods.

Temperature stress is a major environmental stress that limits plant growth, metabolism, and productivity [\[63\]](#page-31-12). High-temperature stress is considered to be one of the major abiotic stresses for restricting crop production, while low-temperature stress can also affect plant growth [\[64\]](#page-31-13). The responses of plants to heat stress vary with the degree and duration of heat stress and the plant type. A detailed analysis of stress and canopy temperature effects on various seedlings has revealed distinct insights into the physiological responses of each plant type, as shown in Figure [14](#page-16-0) and Table [A3.](#page-29-23) Pepper seedlings exhibited noticeable changes in average canopy temperature when subjected to light and water stress, accompanied by an increased standard deviation. This heightened variability in response suggests a diverse reaction within the pepper seedlings. In contrast, tomato seedlings demonstrated a lings demonstrated a consistent response to stress conditions related to light, temperature, consistent response to stress conditions related to light, temperature, and water. However, a significant increase in standard deviation indicates amplified variability in their individual responses. Cucumber seedlings, while experiencing fluctuations in canopy temperature tions in canopy temperature during light stress, displayed a comparatively stable re-This implies a nuanced sensitivity of cucumber seedlings to specific environmental stressponse under stress conditions of cucumber securities to specific conditions. sors. For the watermelon, lettuce, and pak choi seedlings, a distinct pattern was observed,
sharing including the watermedia and pake choi seedlings, a distinct pattern was observed, Showing insignificant enanges in average canopy temperatures across an stress conditions.
This unique trend suggests a potentially different stress response mechanism for these and strength are trend suggests a potentially allevent success response incentifiable for these seedlings compared to their counterparts. The observed variations in average canopy different seedlings compared to their counterparts. The observed variations in average early f to distinct stressors, shedding light on the intricacies of their stress adaptation mechanisms. during light stress, displayed a comparatively stable response under other stress conditions. showing insignificant changes in average canopy temperatures across all stress conditions.

Figure 14. Comparison of seedling leaf canopy temperatures of six seedling varieties under different **Figure 14.** Comparison of seedling leaf canopy temperatures of six seedling varieties under different environmental stress conditions in different growth periods. environmental stress conditions in different growth periods.

3.3. Stress Quantification Based on Leaf Area Parameter 3.3. Stress Quantification Based on Leaf Area Parameter

In this study, the stress responses of six different seedling varieties (pepper, cucum-tomato, watermelon, lettuce, and pak choi) were quantified based on leaf area parameters ber, tomato, watermelon, lettuce, and pak choi) were quantified based on leaf area param-under four environmental conditions (temperature, light, nutrients, and water supply). eters under four environmental conditions (temperature, light, nutrients, and water sup-The stress factors considered include low and high light, nutrient levels, temperature, and water availability. Figure [15](#page-17-0) shows the stress symptoms (%) for six varieties of seedlings based on the leaf area parameter, where the measurements were considered on days $4, 9$, and 15 of the growth period to observe the seedlings' stress responses over time. In this study, the stress responses of six different seedling varieties (pepper, cucumber,

Across the observed time periods (days 4, 9, and 15), intriguing patterns emerged. Seedlings exhibited heightened sensitivity to high light, with watermelon and pak choi displaying the most significant increases in leaf area. High nutrient levels consistently led to larger leaf areas, particularly in lettuce and watermelon. Temperature variations also played a crucial role, with cucumber and pak choi responding prominently to high temperatures. The impact of water availability was complex, with watermelon and cucumber showing sensitivity to high water conditions, while lettuce and pak choi exhibited less pronounced responses. In the quantification of stress symptoms across the growth period from day 4 to day 15 for pepper, cucumber, tomato, watermelon, lettuce, and pak choi seedlings, a diverse range of stress (%) was observed. Pepper seedlings exhibited stress symptoms (%) from 13.00 to 83.33%, indicating considerable variability in their response to environmental

stressors. Cucumber seedlings showed a stress symptom (%) range of 1.59 to 41.67%, suggesting a relatively milder response compared to pepper. Tomato seedlings displayed stress symptoms (%) ranging from 2.96 to 70.01%, showing a wide spectrum of sensitivity to the environmental factors under consideration. Watermelon seedlings exhibited stress symptoms (%) ranging from 5.63% to 56.88%, indicating a moderate to high degree of susceptibility to stressors. Lettuce seedlings showed a stress percentage range of 5.80 to 73.71%, suggesting a high range of stress responses over the observed period. Pak choi seedlings displayed stress symptoms ranging from 4.99 to 63.53%, indicating a moderate level of susceptibility to stressors. Figure [16](#page-18-0) shows the average stress symptoms (%) induced in each type of seedling. These findings highlight the distinct stress response profiles of each seedling type, emphasizing the need for tailored management strategies to optimize their growth conditions and mitigate stress-induced symptoms.

Figure 15. Growth stress symptoms (%) for six varieties of seedlings based on the seedling leaf area **Figure 15.** Growth stress symptoms (%) for six varieties of seedlings based on the seedling leaf area parameter. parameter.

Average stress symptoms of different variety

Figure 16. Average growth stress symptoms (%) of different seedling varieties. **Figure 16.** Average growth stress symptoms (%) of different seedling varieties.

4. Discussion 4. Discussion

toms.

This study centered on the integration of advanced methodologies, specifically sen-This study centered on the integration of advanced methodologies, specifically sensor fusion and image processing techniques, to systematically evaluate the influence of various environmental factors on seedling stress within the context of a plant factory. Major stress-inducing conditions were investigated, including temperature fluctuations, light variations, nutrient deficiencies, and water supply irregularities. Six distinct varieties of seedlings were subjected to these stressors, and we aimed to precisely quantify the resulting
. stress symptoms.
 Γ ¹

The observed reduction in leaf area is a multifaceted response. As a consequence of stress-induced changes, smaller leaves are anticipated to maintain lower temperatures in light environments, effectively mitigating the risk of overheating [\[65\]](#page-31-14). This underscores the nght environments, encenvery mitigating the risk of overheating [65]. This underscores the adaptive nature of plants in modulating their leaf morphology to cope with environmental the adaptive nature of plants in modulating their leaf morphology to cope with environ-challenges [\[66\]](#page-31-15). Moreover, this study supports earlier research indicating a direct correlamental challenges [66]. Moreover, this study supports earlier research indicating a direct tion between growth rate and seedling leaf area, with individual leaf sizes influenced by daily temperature variations [\[67\]](#page-31-16). The study also aligns with previous research findings enced by differential variations [67]. The study also aligns with previous research intensiges suggesting a correlation between decreasing leaf size and diminishing water availabilfindings suggesting a correlation between decreasing leaf size and diminishing water ity [\[66\]](#page-31-15). This association underscores the intricate interplay between environmental factors and plant physiology, emphasizing the utility of leaf area as a measurable parameter for understanding and quantifying stress responses in diverse plant species. By employing techniques such as remote sensing or manual measurements, the assessment of leaf area variations provides valuable insights into the adaptive strategies employed by plants to contend with stress conditions. stress-induced changes, smaller leaves are anticipated to maintain lower temperatures in

When confronted with water scarcity, plants often demonstrate diminished growth marked by an overall reduction in plant height [\[68\]](#page-31-17). The stress induced by water scarcity triggers cell shrinkage, influencing cell elongation and consequently impacting the stature of the plant [\[69\]](#page-31-18). Insufficient availability of essential nutrients, particularly nitrogen, can lead to stunted growth and a decrease in overall plant height [\[70\]](#page-31-19). Extreme temperatures, whether excessively high or low, can exert substantial influence on plant height [\[71\]](#page-31-20). Elevated temperatures may prompt wilting and a decline in cell turgor pressure, thereby affecting the structural integrity of the plant, and extreme cold conditions can impede growth, resulting in a reduction in plant height [\[71\]](#page-31-20). Studies have shown that genotype and temperature strongly influence seedling growth, particularly in respect of height [\[72\]](#page-31-21). Elevated temperatures, coupled with water scarcity, can induce high-temperature stress, impacting seedling growth rates. Optimal seedling growth occurs at around 20 ◦C, with

temperatures beyond this range detrimentally affecting seedling height [\[73\]](#page-31-22). Light stress significantly impacts seedling height, influencing processes like plant food production, stem length, leaf color, and flowering [\[74\]](#page-31-23). Strong light combined with soil moisture constraints restricts seedling upward growth, affecting overall growth and survival [\[75\]](#page-31-24). While higher light intensity generally supports better seedling growth, excessive light can induce stress, exemplified by tip burn in lettuce seedlings [\[76\]](#page-31-25). Both insufficient and excessive light can result in detrimental light stress, adversely affecting plant growth and productivity [\[74\]](#page-31-23). Observing changes in plant height serves as a valuable indicator of stress impacts, aiding in the comprehensive assessment of plant health under diverse environmental conditions.

Different studies have shown the relationships between canopy temperature, stress conditions, and other plant characteristics. Leaf size can regulate leaf temperature via the thickness of the leaf boundary layer, where heat transfer is slower relative to the more turbulent air beyond the leaf [\[66\]](#page-31-15). Plant nutrition is vital in alleviating abiotic stress, including heat stress, by activating mechanisms like increased photosynthetic activity and reduced transpiration rates, and causing increases in canopy temperature [\[77\]](#page-31-26). Thermal infrared remote sensing is a widely used and effective method for detecting vegetation stress, and using the canopy temperature to track water stress is considered reliable for monitoring plant water status [\[78\]](#page-32-0). However, retrieving canopy component temperatures involves thermal infrared remote sensing problems, and the relationships between leaf temperature and water levels are not clear [\[79\]](#page-32-1). The interconnected impact of temperature, nutrient, light, and water stress on the seedling canopy temperature can be assessed by monitoring it, providing insights into the levels of stress experienced by the seedlings. Moreover, the influence of temperature and water stress on seed germination and seedling growth highlights their pivotal role in determining optimal conditions for seedling development. While this study focused on six specific seedling varieties, the observed trends regarding temperature, light, water supply, and nutrient impacts on seedling responses are consistent with similar studies on different species, indicating potential generalizability within certain bounds. However, further research with a broader variety of plant species is needed to validate this predictability.

Environmental factors such as temperature, light, water, and nutrients directly impact plant growth and health. Sensor fusion plays a crucial role in monitoring and understanding these relationships. By collecting and combining data from various cameras (RGB, thermal, and depth), it was possible to gain a more complete picture of the environmental conditions that the seedlings were experiencing. This data, coupled with image processing techniques, allowed us to precisely measure plant responses like leaf area, height, and canopy temperature. Color differences, canopy temperature variation, and seedling size monitoring provided more detailed information about the seedling growth conditions. Analyzing these sensor-derived measurements reveals how specific environmental stressors influence plant physiology, giving us insights into plant adaptation strategies and helping optimize growing conditions in plant factories. In essence, sensor fusion provided the environmental context, while image processing provided detailed measurements of the seedlings responses. By combining these two approaches, the researchers were able to gain a deeper understanding of the relationships between environmental factors and seedling stress conditions.

While sensor fusion and image processing offer valuable insights into plant stress responses, limitations were observed. Light variations affect image quality and processing accuracy, while external radiation sources can interfere with thermal and depth cameras, introducing data errors. The precision of image processing techniques in feature extraction requires careful evaluation. This research involved image processing techniques to assess key physiological parameters of the seedlings, including leaf area, seedling height, and canopy temperature. Future research in precision plant monitoring for stress detection could refine and extend the current findings by implementing controlled environments to isolate specific stressors. Moreover, advanced image processing and deep learning methods integrating additional sensors and utilizing larger datasets would offer a multidimensional

view of plant health. Finally, this could lead to real-time stress monitoring systems for optimized plant growth in commercial plant factories.

5. Conclusions

This study aimed to develop a seedling stress quantification model in six seedling varieties for early growth stages. Six varieties of seedlings, aged one week, were cultivated in controlled chambers with varying conditions: temperature (20, 25, and 30 ◦C), light intensity (50, 250, and 450 μ mol m $^{-2}s^{-1}$), nutrients (1, 3, and 6 dS m $^{-1}$), and water supply (1, 0.75, and 0.50 L/day). RGB, thermal, and depth camera sensors were used to capture canopy images from the top of the seedling beds automatically and daily for two weeks.

Stress quantification was performed with reference to major characteristics of the seedlings, such as leaf area, seedling height, and canopy temperature. The comprehensive visualization and quantification of stress effects on the six different seedlings revealed distinctive responses to various environmental stressors. The integration of color, size, and shape parameters provided slight differences, enabling us to establish a visual hierarchy. Pepper and pak choi seedlings exhibited high sensitivity to stress, while cucumber seedlings demonstrated a milder response, and lettuce and tomato seedlings displayed intermediate sensitivity. Pepper and tomato seedlings displayed a wide range of stress symptoms (%), at 13.00% to 83.33% and 2.96% to 70.01%, respectively, indicating considerable variability in their response to environmental stressors.

Sensor fusion and image processing provided valuable tools for plant stress analysis, but limitations like light variability and external radiation interference must be addressed and minimized. Future research should focus on controlled environments, advanced image processing techniques, integration of diverse sensors. This study on seedling stress quantification in seedling growth facilities through sensor fusion and image processing represents a significant step towards understanding and improving the efficiency of controlled environment agriculture. The integration of diverse data sources provides a more distinct and accurate assessment of seedling health, paving the way for informed decision making and sustainable agricultural practices.

Author Contributions: Conceptualization, S.I. and S.-O.C.; methodology, S.I. and S.-O.C.; software, S.I. and M.N.R.; validation, S.I., M.N.R., S.A., S., Y.J.C. and D.H.N.; formal analysis, S.A., S., Y.J.C. and D.H.N.; investigation, D.H.N. and S.-O.C.; resources, S.-O.C.; data curation, S.I., M.N.R., S.A. and S.; writing—original draft preparation, S.I.; writing—review and editing, M.N.R., D.H.N. and S.-O.C.; visualization, S.I., S.A., S. and Y.J.C.; supervision, S.-O.C.; project administration, S.-O.C.; funding acquisition, S.-O.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the Smart Farm Innovation Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Project No. 421035-04), Republic of Korea.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Comparison of leaf area using RGB image sensor of six seedling varieties with different stress conditions.

Table A1. *Cont.*

Number of sample, n = 15; Unit = pixels.

Table A2. Comparison of plant height using depth image sensor of six seedling varieties with different stress conditions.

Table A2. *Cont.*

Table A2. *Cont.*

Table A2. *Cont.*

Number of sample, n = 15; Unit = cm.

Water	high	high	high	normal	normal	normal	low	low	low
Max	26.9	29.6	30.8	26.7	30.4	32.0	29.2	31.3	33.4
Min	24.8	28.3	28.6	26.1	29.7	29.9	27.4	29.6	30.2
Avg	25.9	29.0	29.7	26.4	30.0	31.1	28.2	30.5	31.7
STD	0.8	0.4	0.7	0.2	0.2	0.7	0.6	0.6	

Table A3. *Cont.*

Number of sample, $n = 15$; Unit = Degree Celsius ($°C$).

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