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Phenotyping of Low-Temperature Stressed Pepper Seedlings Using Infrared Thermography

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Abstract

Purpose: This study was performed to evaluate the feasibility of using an infrared thermography technique for phenotype analysis of pepper seedlings exposed to a low-temperature environment. **Methods:** We employed an active thermography technique to evaluate the thermal response of pepper seedlings exposed to low-temperature stress. The temperatures of pepper leaves grown in low-temperature conditions (5°C, relative humidity [RH] 50%) for four periods (6, 12, 24, and 48 h) were measured in the experimental setting (23°C, RH 70%) as soon as pepper seedling samples were taken out from the low-temperature environment. We also assessed the visible images of pepper seedling samples that were exposed to low-temperature stress to estimate appearance changes. **Results:** The greatest appearance change was observed for the low-temperature stressed pepper seedlings that were exposed for 12 h, and the temperature from these pepper seedling leaves was the highest among all samples. In addition, the thermal image of low-temperature stressed pepper seedlings for 6 h exhibited the lowest temperature. **Conclusions:** We demonstrated that the leaf withering owing to the water deficiency that occurred under low-temperature conditions could induce an increase in temperature in plant leaves using the infrared thermography technique. These results suggested that the time-resolved and averaged thermal signals or temperatures of plants could be significantly associated with the physiological or biochemical characteristics of plants exposed to low-temperature stress.

Keywords: Exothermic response, Infrared thermography, Low temperature stress, Phenotype analysis, Red pepper seedling

Introduction

The Earth's climate is gradually changing, and plants or seeds must be developed, which are robust to environmental stresses, such as low temperature and water deficiency. Plants can be easily exposed to various environmental stresses, and it is known that low temperature is a key factor that can influence plant growth and productivity. During low-temperature stress and subsequent recovery periods, plants may develop several mechanisms to minimize potential damage. In addition, their response to low temperature is a highly complex process that involves physiological and biochemical modifications.

Plant responses to low temperature vary widely from one species to another. However, a common mechanism of plants in low temperatures is altered membrane lipid composition to protect their membrane stability and integrity (Badea et al., 2009). Furthermore, the cause of the change in membrane lipid composition was an increase in unsaturated fatty acids when plants were

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exposed to low temperature (Tasseva et al., 2004; De Palma et al., 2008). Tasseva et al. (2004) observed an increase in the level of unsaturated fatty acids when plants were grown at 4°C, and demonstrated that desaturation played a major role in the low-temperature tolerance mechanism of membrane lipids by revealing that tolerant plants exhibited more rapid accumulation of unsaturated fatty acids than did low-temperature-sensitive plants. Therefore, phenotype analysis is critical for the determination of low-temperature tolerance of plants, their adaptability to stressed environments, and their physiological/biochemical response to the stress.

A phenotype can be defined as the composite of an organism's characteristics, such as its morphology, development, physiological, or biochemical properties. 'Plant phenotyping' or 'phenomics' has been proposed as a novel discipline in biology and involves the gathering of high-dimensional phenotypic data at multiple levels of organization, to progress towards the full characterization of the plant (Houle et al., 2010). However, plant phenotypes are inherently complex because they result from the interaction of genotypes with a multitude of environmental factors. This interaction influences not only the development and growth of plants, which can be described by means of structural traits, but also plant functioning, described by means of physiological characteristics (Stijn et al., 2013).

The study of plant phenotyping has been developed by means of remote sensing methods, which enable high-throughput in the acquisition and processing of measurements, as well as possible interactions of different categories (Berger et al., 2010; Tardieu 2012). Some non-invasive and remote sensors were initially used for greenhouse and laboratory research to observe disease incidence, water and metabolic contents, evapotranspiration, and photosynthesis characteristics of the target (Bock et al., 2010; Furbank et al., 2011). Among these sensors, thermal infrared, and multispectral and hyperspectral imaging systems are considered the most promising tools because they show spatially high resolution data with high-throughput for the evaluation of agricultural products and bio-materials.

Recently, an infrared thermography technique has been widely used because the technique measures the temperature not only on the surface of objects, but also from subsurface or internal heat intrusions, and the heterogeneity of thermal properties within objects, which might serve as sensitive indicators of cell viability in living organisms (Baranowski et al., 2003; Hildebrandt et al., 2010). Moreover, this infrared thermography has been historically used to determine crop water stress for irrigation scheduling (Jackson et al., 1981). More recently, thermography has been used to compare evapotranspiration between genotypes at the canopy and plant level, and for the comparison and measurement of stomatal conductance in individual plants and leaves (Maes et al., 2012).

In this study, we constructed an infrared signal measurement system consisting of a far-infrared (7.5–14 μm) camera and computer system. We then measured the infrared thermal emission from pepper seedlings planted in low-temperature environments. In this method, pepper seedling samples were planted in a low-temperature environment (5°C and RH 50%) for four periods (6, 12, 24, and 48 h). Then, infrared thermal signals were measured to determine the variation in leaf temperature based on the duration of low-temperature stress using the thermal image processing methods to determine the temperature distribution of the pepper seedling leaves.

Materials and Methods

Sample materials

Thirty red pepper (*Capsicum annuum*) seedlings were planted in the greenhouse under normal conditions at a temperature of 20°C and RH of 80% for 10 days. From these, 20 seedlings, of which size and height were most similar, were selected as the sample group for low-temperature stress. Samples selected as the low-temperature stress group were kept in a low-temperature environment with a temperature of 5°C and relative humidity of 50%, and they remained there for four periods (6, 12, 24, and 48 h) as shown in Figure 1. Therefore, whole seedling samples were separated with four groups according to the period of cold duration and each group consisted of five pepper seedlings.

Measurements of infrared thermal images

Temperatures from pepper seedling leaves were measured using an infrared camera (VarioCAM, InfraTec GmbH, Germany) with 640 × 512-pixel resolution and sensitivity in the 7.5–14 μm spectral range as shown in Figure 2. The detector in the scanner unit was a micro bolometer, and the temperature sensitivity was 30 mK.

Figure 1. Cultivation of red pepper seedlings in a low-temperature environment.

Figure 2. Measurements of thermal emissions from pepper leaves.

The digitized thermal signals from the pepper seedling leaves were transferred to the computer, and were recorded to disk memory using commercial software (IRBIS3, InfraTec GmbH, Germany). The thermal signal measurement was performed under controlled laboratory environment conditions; temperature was 23°C and relative humidity 70%. The sampling rate and elapsed time for one measurement was 1 Hz and 300 s, respectively. Therefore, one measurement gathered 300 thermal images for a seedling. In addition, we measured the visible images of pepper seedling samples, which were exposed to low-temperature stress, to compare the changes in appearance of seedling leaves according to duration of low-temperature stress.

In this study, we employed an active thermography technique to evaluate the thermal response of pepper seedlings that were planted in a low-temperature environment. In an active approach, an external stimulus is required to generate relevant temperature differences not otherwise present. The temperatures of pepper seedling leaves that were planted in low-temperature conditions at 5°C and relative humidity of 50% during four periods (6, 12, 24, and 48 h) were measured under controlled laboratory conditions at a temperature of 23°C and relative humidity of 70% shortly after pepper seedling samples were taken out from the low-temperature environment. An external stimulus, during this study the abrupt rise in temperature, enables quantitative characterization, such as the nature of temperature acclimation of pepper seedlings. The infrared thermal images of a pepper seedling were measured at 1 Hz with a sampling rate for 300 s, and the resultant 300 thermal images of each sample were averaged into one thermal image. Measurement errors that might occur from surroundings and the measuring system were corrected using the constant thermal signals from flatted black color board, which was used as the background for measurements. The calibrated thermal image of each pepper seedling sample provided the correct temperature of pepper seedling leaves, and the thermal images of seedlings were used in further thermal image processing to analyze the temperature distribution for the seedling leaves.

Infrared image processing

We created thermal image analysis software using Matlab (ver. 8.0, MathWorks, Natick, MA, USA) to extract the region of interest and analyze the temperature distribution of the target leaves. Developed software used in the thermal image processing had the following features to produce accurate and reliable temperatures of the target samples, as shown in Figure 3.

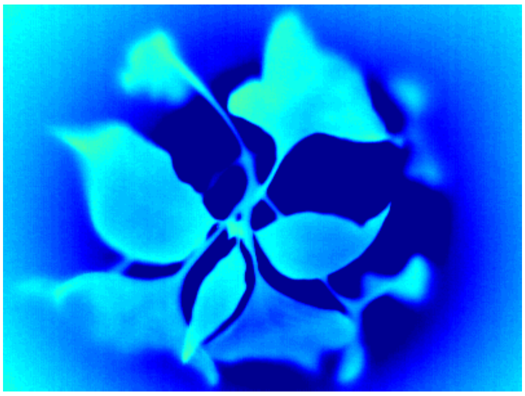
(i) Sequential image analysis

This provided an average image of thermograms and removed some noise components that occurred during measurements, so trend analysis could be performed, if required.

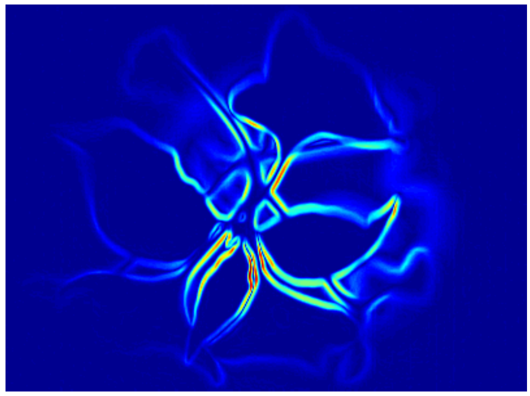
(ii) Extraction of the region of interest (seedling leaves)

This feature extracted the target leaves in the thermograms. It worked by segmenting leaves using the image watershed method following the detection of the edges of leaves using an image filtering method.

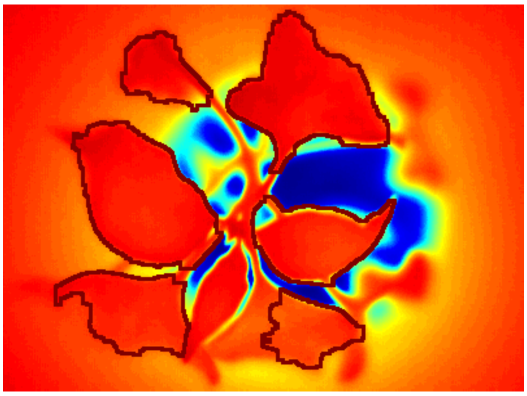
(iii) Temperature distribution analysis of the region of interest



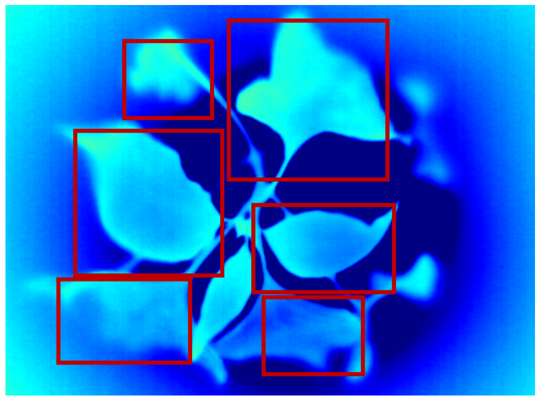
(a) image averaging



(b) edge detection



(c) leaf segmentation



(d) temperature distribution analysis

Figure 3. Sequential algorithms used in infrared image processing.

This feature permitted the measurement of average, maximum, and minimum temperature with standard deviations, and could provide a histogram of the temperature for the region of interest.

Results and Discussion

Appearance change caused by low-temperature stress

Figure 4 shows changes in appearance between non-stressed pepper seedlings (before) and low-temperature stressed pepper seedlings (after). All images of low-temperature stressed samples were acquired immediately after they were taken out of the low-temperature conditions at 5°C and relative humidity of 50%, where they had been kept for either 6, 12, 24, or 48 h. The greatest change in appearance was observed for the low-temperature stressed pepper seedlings that were in the cold environment for 12 h, followed by the low-temperature stressed pepper seedlings maintained for 24 h. However, the appearances 6- and 48-h low-temperature stressed pepper seedlings were fairly similar with those of normal samples that were not exposed to low-temperature stress.

Based on appearance changes, it seemed that some pepper seedling resource allowed plants to maintain its physiological and physical conditions during early exposure to low-temperature stress (Fig. 4 (a)), and increasing exposure time to low-temperature stress induced temporary or permanent damage to the plant (Fig. 4 (b)). Moreover, it was concluded that the pepper plants can acclimate themselves to the low-temperature stress after the required time of approximately 48 h, as shown in Figure 4 (d). Apparent appearance changes, such as withering, which were observed for the 12-h low-temperature stressed pepper seedlings (Fig. 4 (b)) was mainly caused by dehydration, which can easily happen because plants under low-temperature stress may suffer water deficiency. Therefore, the dehydration of the plant shown in Figure 4 (b) can be assumed to have been induced by a deficit of water, which is needed for biological activities of plants.

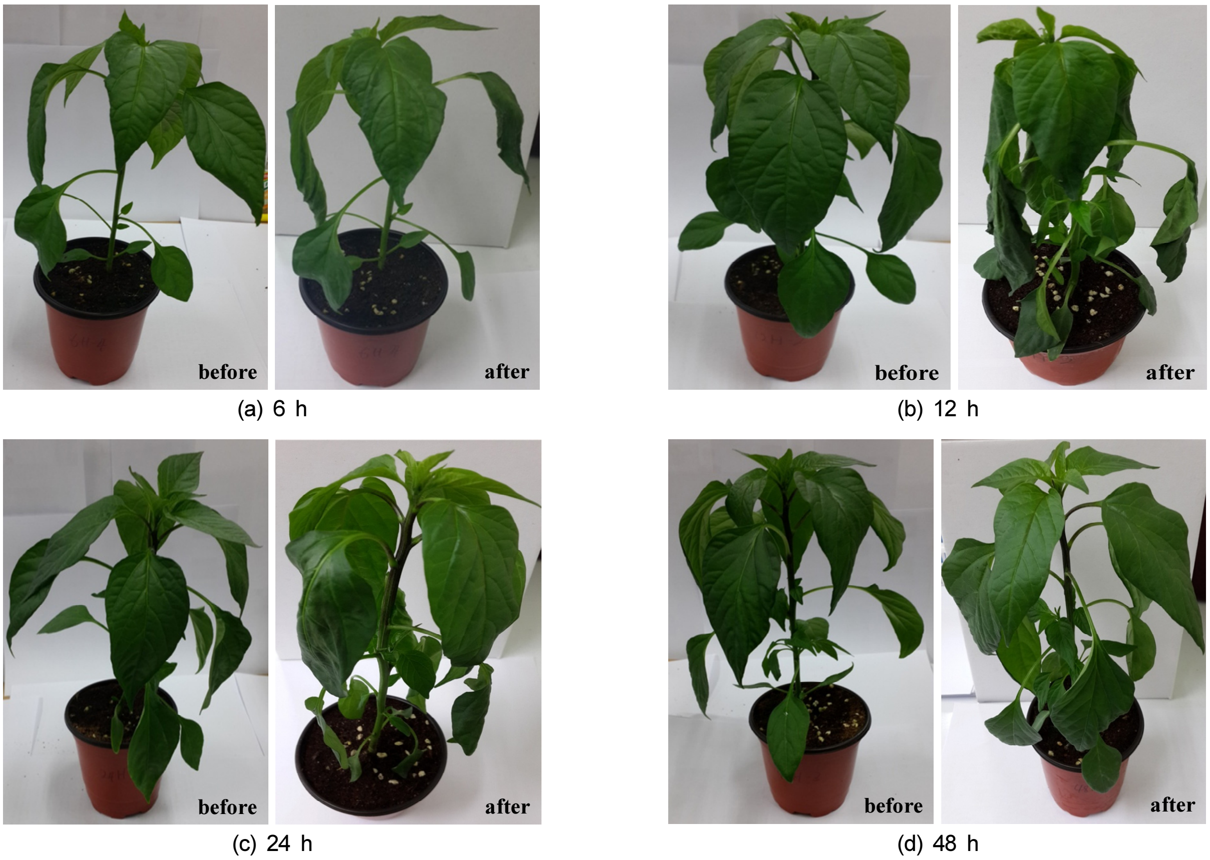


Figure 4. Comparison of the appearance of pepper seedlings according to storage time under low-temperature stress.

Exothermic characteristics caused by low-temperature stress

Several previous studies have indicated that leaf temperature increases under water deficit conditions (Ballester et al., 2013; Kim et al., 2013; Park et al., 2014). As mentioned above, we found the main cause of the change in appearance shown in Figure 4 (b) to be from a deficit of water needed by the plant. Therefore, we measured leaf temperature of the pepper seedlings that were exposed to low temperature to determine if a correlation existed between the pepper seedling exposed to low-temperature stress and its exothermic characteristics.

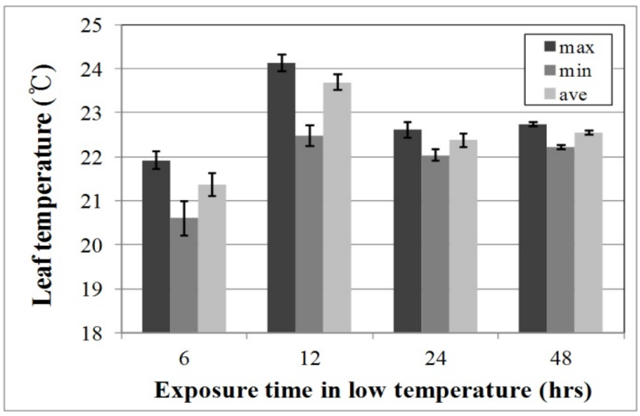


Figure 5. Temperature comparison of pepper seedling leaves after exposed to low-temperature stress.

Figure 5 shows the maximum, minimum, and average temperature of pepper leaves that were exposed to low-temperature stress according to the four durations (6, 12, 24, and 48 h), and the average temperature of the five pepper seedlings per group was analyzed for comparison. Temperature measurement was performed under experimental conditions (23°C and RH 70%) shortly after the pepper seedling samples were taken out from the low-temperature environment. As shown in Figure 5, average temperatures of pepper leaves exposed to low-temperature stress for 6, 12, 24, and 48 h were 21.36℃, 23.69℃, 22.37℃, and 22.54℃, respectively. Thus, the temperature from pepper leaves exposed to low temperature for 12 h was the highest. Based on these results, it was shown that the water deficit of plants manifested by leaf withering under low-temperature stress was induced by increased leaf temperature.

Figure 6 shows the representative thermal image of

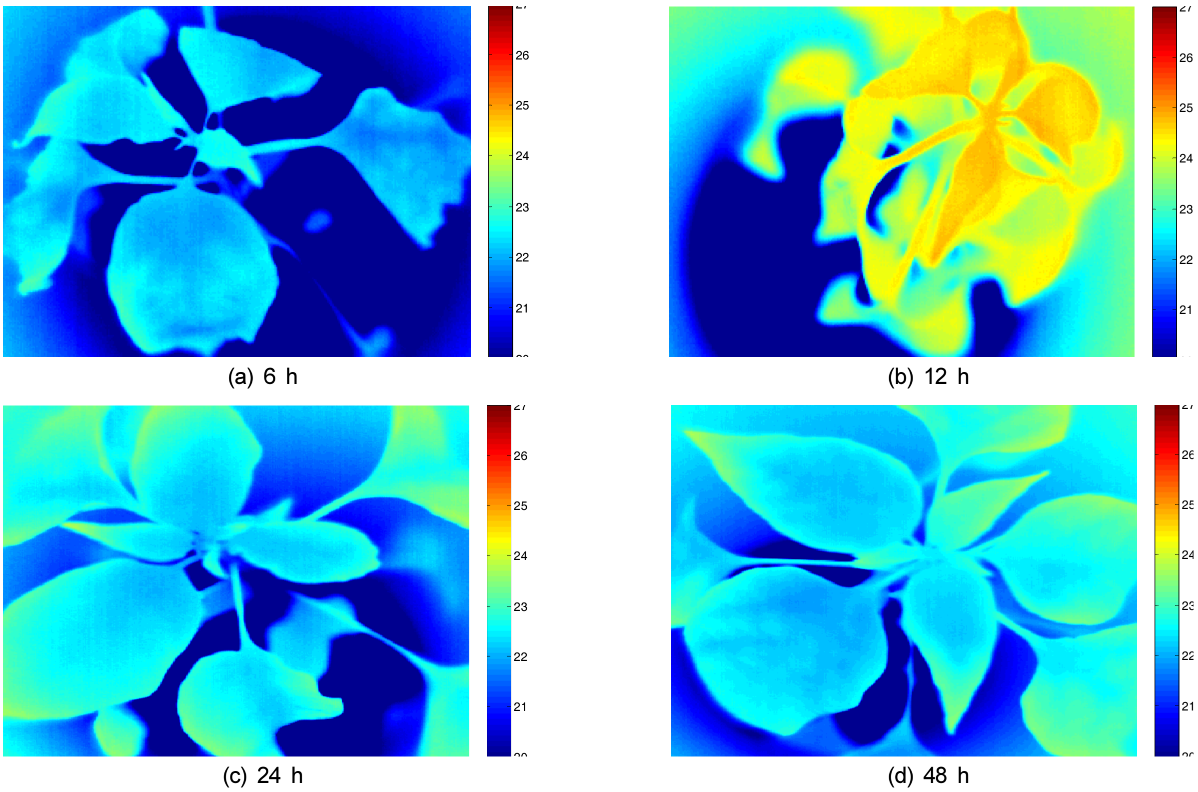


Figure 6. Exothermic characteristics of representative pepper seedling leaves according to storage time under low-temperature stress.

pepper seedlings exposed to low-temperature stress for four periods (6, 12, 24, and 48 h). Temperatures of pepper seedling leaves were measured using an infrared camera shortly after all pepper seedlings were taken out of the low-temperature environment. All images showed similar trends and the resultant leaf temperatures are shown in Figure 5. The thermal image of low-temperature stressed pepper seedlings for 6 h (Fig. 6 (a)) illustrated the coldest values, whereas the pixel values in the thermal image of low-temperature stressed pepper seedlings for 12 h (Fig. 6 (b)) were higher than those of other thermal images.

Conclusions

In this study, we showed the feasibility of an infrared imaging technique and thermal signal analysis method to analyze the thermal response of pepper seedlings exposed to low-temperature stress through a phenotype approach. Prominent withering was observed for the pepper seedlings exposed to the low temperature for 12 h, and the main reason for the withering state was a deficiency of water (i.e., dehydration), which is needed for the biological activities of plants. Plants such as pepper seedlings mainly consist of water, and they maintain their water content through the transport of water from their roots to supply vital nutrients and minerals, and water is indispensable for the functioning metabolism of their organs. For the transport of water, plants maintain the temperature most favorable for their metabolism through a number of thermoregulation functions. The most likely short-term thermoregulation method available to plants is transpiration, which is the active, controlled loss of water vapor through stoma. If a plant is exposed to low temperature for a specific time, it could induce damage such as withering, and the water uptake rate cannot match the potential transpiration rate and then stoma close to maintain the plant's water balance. This stoma closure causes transpiration to cease, which in turn leads to an increase in leaf temperature. The increase in leaf temperature under water deficit has been shown by previous studies.

Consequently, we demonstrated that the leaf withering state caused by a water deficiency occurred under low-temperature conditions and induced an increased temperature in the plant leaves, as detected by the infrared thermography technique. These results suggest that the time-resolved and averaged thermal signals or temperatures of plants could be significantly associated with the physiological or biochemical characteristics of plants exposed to low-temperature stress. We expect that this infrared thermography technique, including the thermal image measurement system and infrared image analysis software, can potentially be used in phenotype analysis in biological studies and the agriculture industry.

Conflict of Interest

The authors have no conflicting financial or other interests.

Acknowledgments

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