Optimization of Growth Environment in a Plant Production Facility Using a Chlorophyll Fluorescence Method

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Abstract

Chlorophyll fluorescence has been known as one of the indicators of photosynthetic status to various environmental stresses. The aims of this study were to assess the effects of environmental factors on lettuce chlorophyll fluorescent responses (Fv/Fm) and to develop an environment optimization model for lettuce growth using a simple genetic algorithm. High values of Fv/Fm were observed when environmental factors were 22–26ºC ambient temperature, 15–23ºC root zone temperature, 900–1,600 ppm CO_2 concentration, 0.4–1.3 m·s⁻¹ air current speed, and 65–85% relative humidity. As photosynthesis photon flux (PPF) increased over 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Fv/Fm values were decreased. Principle component analysis was used to estimate the combined effects of six environmental factors on lettuce growth. The developed model fitted observed Fv/Fm values with an average standard error of 1.2%. An optimal environment for lettuce growth was estimated by the model to be 22ºC ambient temperature, 20ºC root zone temperature, 1,578 ppm CO_2 concentration, 1.3 m·s⁻¹ air current speed, 216 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, and 75% relative humidity. The Fv/Fm value can be a good indicator of plant stress level and thus a useful parameter to optimize the environment for plant growth.

Discipline: Agricultural facilities
Additional key words: environmental stress, Fv/Fm, genetic algorithm, JAVA, principle component analysis

Introduction

Plant bio-information has been used to develop plant physiological models to optimize cultivation environments in controlled plant production systems[10,13]. Shimizu and Yamazaki[23] used morphological characteristics of leaves as parameters for a plant growth model. Dry and fresh weight, stem diameter, leaf area, and photosynthetic rate have been commonly used as indicators of plant growth. However, a precise measurement of photosynthesis requires expensive equipment and its responses to irregular plant physiological characteristics still need to be investigated[29]. These methods have disadvantages including damages to the plant, requirement of expensive equipment, and difficulties of measurements.

Chlorophyll fluorescence is one of the well-known indicators of photosynthetic stress status in various environments[22]. Chlorophyll fluorescence is often used to analyze spatial and visual photosynthesis without damage to the plant[2]. Fluorescence response can be a useful parameter for mathematical models for environment control[29]. Lichtenthaler[18] and Babani et al.[1] have used Fv/Fm ratio as an abiotic stress index.

Several studies used chlorophyll fluorescence responses to light stress[3], heat and chilling stress[20], and

water stress[21]. Willits and Peet[30] measured the chlorophyll fluorescence of tomato as a parameter of heat stress for a model. There have been a few studies on the chlorophyll fluorescence response method for plant growth environment. However, this method has not been applied to plant cultivation environment control.

The objectives of this study were to assess chlorophyll fluorescence (Fv/Fm) responses to microclimatic changes of ambient temperature, photosynthesis photon flux (PPF), relative humidity, CO_2 concentration, air current speed, and root zone temperature. The measured Fv/Fm values were regressed against each environmental factor. Using the regression equations and a simple genetic algorithm, an optimal environment for lettuce growth was predicted.

Materials and methods

1. Measurements of chlorophyll fluorescence and environmental variables

Chlorophyll fluorescence is the emitted light that is not used for the photosynthetic process. Therefore, the fluorescence change is a useful index to reflect the photosynthetic efficiency[5]. When exposed to a weak light after a few minutes residence in a dark place, a plant leaf emits an amount of Fo, dark or initial fluorescence. In this case, the light absorbed by chlorophyll is not available for photosynthesis and is emitted as fluorescence. The Fm value, maximum fluorescence, is the fluorescence emission when a dark-adapted leaf is exposed to saturated light. In general, the Fo value increases as plant stress increases, while the Fm value decreases. The ratio of Fo to Fm is commonly used to remove the dependence on the amount of chlorophyll, thickness, and age of the leaf since Fo and Fm values vary with these factors[16]. Maximum variable fluorescence, Fv, is the difference between Fm and Fo. The ratio, Fv/Fm, indicates a maximum potential of plant photosynthetic ability. Healthy plant leaves generally show an Fv/Fm value of 0.83 and a ratio below 0.83 means that plants are under stress[11,17].

Measurements of chlorophyll fluorescence started at day 11 after transplantation using a fluorometer (MINI-PAM, Walz, Germany) with the standard setting[26]. One environmental factor was controlled at a time in order to assess the individual effect on Fv/Fm. Table 1 shows the ranges of the environmental factors controlled.

Lettuce was acclimated for an hour every time a treatment changed. An additional 5 min was applied for the dark adaptation before the measurements of fluorescence response[25]. Four leaves of each lettuce head were chosen to measure fluorescence responses. The fluorescence response was measured twice for each leaf. After-

Table 1. Ranges of environmental variables

Environmental factor	Range
Ambient temperature (ºC)	10–36
Root zone temperature (ºC)	5–35
CO_2 (ppm)	400–2,000
Air current speed (m·s^{-1})	0.1–2.5
PPF (μmol·m^{-2}·s^{-1})	30–400
Relative humidity (%)	50–95

ward, the initial environment conditions were applied for 24 h in order for lettuce to recover from previous stress. Once lettuce fully recovered, another treatment was applied. This procedure was repeated for 30 d after transplantation. After a series of measurements for each environmental treatment, a new set of lettuce was prepared for the next environmental treatment. In total, 6 sets of lettuce were used for this study.

Leaf lettuce (*Lactuca sativa L.*) was grown in a growth chamber. Environmental factors of air temperature (T-type thermocouple, Omega, USA), PPF (PP-1, PP-system, UK), relative humidity (Hobo, Onset Computer, USA), CO_2 concentration (GMW-22, Vaisala, Finland), air current speed (Series 640, Dwyer, USA) and root zone temperature (T-type thermocouple, Omega, USA) were measured every 5 min and stored in a datalogger (DC100, Yokogawa, Japan). Fluorescent lamps (12 ea.), high-pressure sodium lamps (4 ea.), and high-pressure mercury vapor lamps (4 ea.) were used as light sources for the plant growth. Nutrient film (110 (W) × 50 (L) × 13 (D) cm) served as a platform for lettuce growth. Yamazaki solution (pH: 6.5 ± 0.5, EC: 1.2 ± 0.05 mS·cm^{-1}) supplied nutrients and was remade every 8 h[14]. After the transplantation of 10 lettuce heads, the environmental conditions were maintained for 10 days at 400 ± 30 μmol·m^{-2}·s^{-1} PPF (12-h photoperiod), 22 ± 2ºC air temperature, 65 ± 5% relative humidity, 300 ± 50 ppm CO_2 concentration, 0.1 ± 0.03 m·s^{-1} air current speed, and 20 ± 2ºC root zone temperature[19]. Chlorophyll fluorescence was measured after 2-h exposure to artificial light. For 11 days, the measured Fv/Fm ratio was maintained at 0.821 ± 0.005.

2. Statistical analysis and genetic algorithm

Chlorophyll fluorescence responses were regressed over each of the six environmental factors. Principle component analysis[12] was applied to evaluate correlations among the six environmental factors. Principal components were derived by the linear combination of variables as independent variables accounting for correlations among variables. A chlorophyll fluorescence response

model was developed in the form of a multi-variable function using the principal components.

A simple genetic algorithm was applied to find an optimal solution of the multi-variable function. Genetic algorithms are trial and error methods to optimize issues with complex objective functions employing the concepts of crossover and mutation from genetics and evolution[9]. A simple genetic algorithm has been applied to a number of optimization problems because of its powerfulness in finding a solution[6].

In an evolution process, individuals with better fitness to the environment have higher possibilities to survive. Two processes, crossover and mutation, come into play a role in reproducing the next generation. A population, a set of individuals, has to be sized first in order to apply a genetic algorithm to a chlorophyll fluorescence model. Each individual consists of a chromosome, which is a group of multiple genes. In this study, chromosomes consisting of six environmental factors made up of a population, which is the chlorophyll fluorescence response model. The number of chromosomes was assigned by 10 genes to find fast a dominant gene. The chromosome size of each environmental factor is presented in Table 2.

A random initialization technique generated an initial population in the combination of the six environmental factors. The chlorophyll fluorescence response model served as an objective function to test the fitness of each gene. The objective function governed the population evolution through the processes of reproduction, crossover and mutation. At the end of each evolution period,

fitness of the generation was tested in comparison with boundary conditions given in Table 3. An initial population with 50 chromosomes was allowed to evolve for 20 generations to find an optimal solution. Fig. 1 presents the overall schematic diagram for the development of a cultivation environment optimization model.

The computational process for optimization was coded in Java language (Java 2 standard development kit V 1.3.2, SUN, USA). The SAS software package (Version 8, SAS Institute, USA) was used for the statistical analyses of experimental data.

Results and discussion

1. Chlorophyll fluorescence response to environmental factors

Correlation among the environmental factors was analyzed using the Fv/Fm measurements at initial growth conditions (22 ± 2ºC ambient temperature, 20 ± 2ºC root zone temperature, 65 ± 5% relative humidity, 300 ± 50 ppm CO_2 concentration, 0.1 ± 0.05 m·s^{-1} air current speed, and 400 ± 30 μmol·m^{-2}·s^{-1} PPF). As shown in Table 4, low correlation coefficients indicate that environmental factors can be independent of one another.

In the ambient temperature treatment experiment, chlorophyll fluorescence was measured at every 2ºC interval as the temperature increased from 20ºC to 36ºC and decreased from 20ºC to 10ºC. The Fv/Fm value was 0.819 ± 0.005 in range between 22 and 26ºC ambient temperature, whereas it decreased at below 22ºC or above

Table 2. Chromosome size and number

Factor	Data degree	Start point	End point	Range	Size of chromosome	Fixed data range*
AT	1	0	40	40	63	0.635
RZT	1	0	40	40	63	0.635
RH	5	25	100	75	15	5
ACS	0.1	0	2.0	2	31	0.0645
CO_2	15	300	2,000	1,700	127	13.386
PPF	15	0	700	700	63	11.111

*: Range/Size of chromosome.
AT : Ambient temperature, RZT : Root zone temperature, CO_2 : CO_2 concentration.
ACS: Air current speed, PPF : Photosynthetic photon flux, RH : Relative humidity.

Table 3. Boundary conditions for optimizing environmental conditions

Factor	AT (ºC)	RZT (ºC)	CO_2 (ppm)	ACS (m·s^{-1})	PPF (μmol·m^{-2}·s^{-1})	RH (%)
Range	22–26	15–23	350–1,650	0.2–1.4	100–500	35–85

26ºC (Fig. 2). Eq. (1) presents the regression between the Fv/Fm value and air temperature ($R^2 = 0.92$).

$$Fv/Fm_{AT} = -0.000844185(AT)^2 + 0.0404832015(AT)$$
$$+ 0.3251895488 \qquad (1)$$

The highest Fv/Fm value was observed when the ambient temperature was 24ºC. The optimal ambient temperature for lettuce cultivation was reported to be 20 $\pm$ 2ºC[15], a temperature in which high Fv/Fm values were observed. Wang[27] reported that freezing stresses between 0 and 15ºC could be either reversible or irreversible, although normal ambient temperatures were reapplied[4].

Not only ultraviolet but visible light can cause plant stresses. The Fv/Fm value decreased as PPF increased above 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$, whereas the Fv/Fm value was 0.810 ± 0.002 at PPF between 30 and 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 3). A regression between Fv/Fm and PPF is given in Eq. (2) ($R^2 = 0.87$).
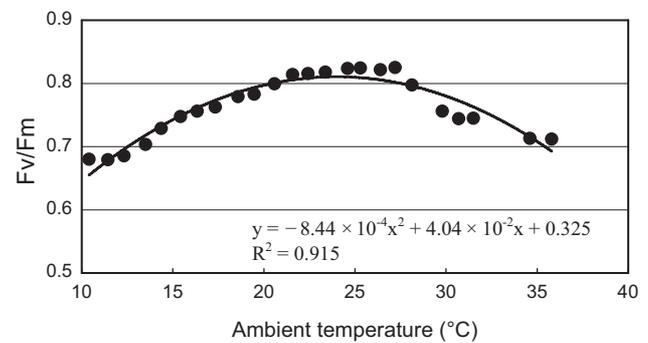
$$Fv/Fm_{PPF} = -0.0000324364(PPF) + 0.8097494887 \qquad (2)$$

The Fv/Fm value was 0.795 at the light level of saturation (PPF = 470 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of lettuce. Oxygen gen-
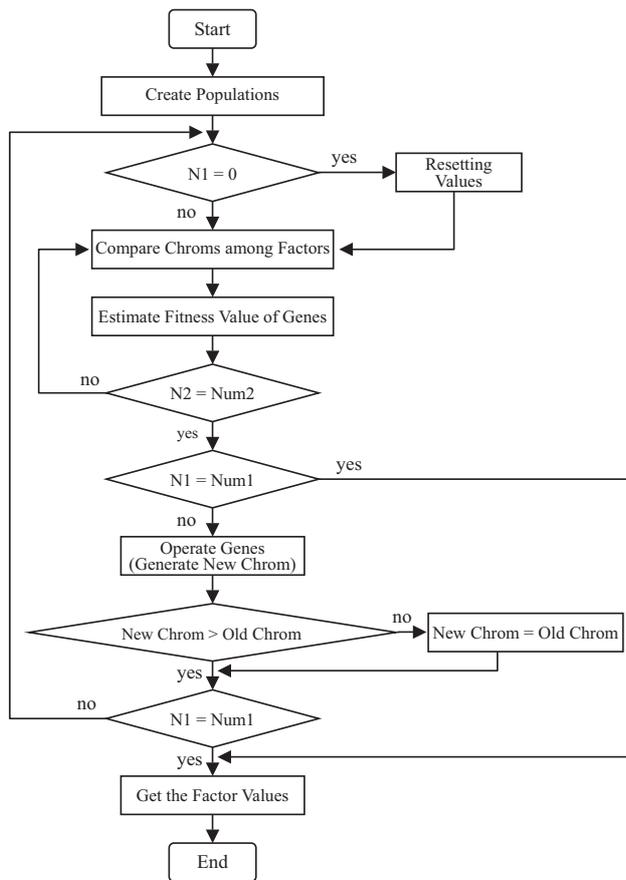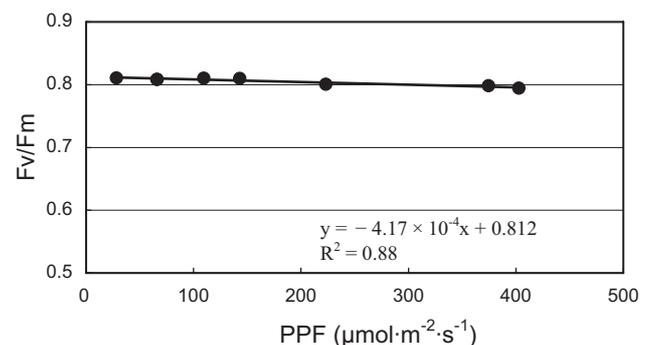

Fig. 1. **Flowchart for optimization of plant environment control**
N1: Current number of genes, N2: Current number of chromosomes, Num1: Number of chromosomes, Num2: number of genes.

y = $-8.44 \times 10^{-4}x^2 + 4.04 \times 10^{-2}x + 0.325$
$R^2 = 0.915$

Fig. 2. **Relationship between Fv/Fm and ambient temperature**

y = $-4.17 \times 10^{-4}x + 0.812$
$R^2 = 0.88$

Fig. 3. **Relationship between Fv/Fm and PPF**

Table 4. **Correlation coefficients among the environmental factors**

Factor	AT	RZT	CO_2	ACS	PPF	RH
AT	1.00000	−0.02696	−0.03786	0.02959	0.03418	0.02262
RZT	−0.02696	1.00000	0.02081	0.01896	−0.00400	0.17001
CO_2	−0.03786	0.02081	1.00000	0.17846	−0.05732	−0.02290
ACS	0.02959	0.01896	0.17846	1.00000	−0.03939	0.05764
PPF	0.03418	−0.00400	−0.05732	−0.03939	1.00000	−0.04160
RH	0.02262	0.17001	−0.02290	0.05764	−0.04160	1.00000

eration of lettuce was decreased by 35 to 40% after 2-h exposure to visible light of 325 μmol·m^{-2}·s^{-1} [24]. It seemed that light intensity as well as exposure time could cause plant stresses.

When CO_2 concentrations were controlled between 900 and 1,600 ppm, the Fv/Fm value was 0.813 ± 0.002. CO_2 concentrations out of this range resulted in decreased Fv/Fm values. Park and Lee[19] reported the optimal condition for lettuce growth was 1,000–2,000 ppm CO_2 concentration when PPF was greater than 200 μmol·m^{-2}·s^{-1}. This study also indicated a decrease in the Fv/Fm value when CO_2 concentration was out of the range of 900–1,600 ppm and suggested that the Fv/Fm value can serve as an indicator for environment control. A regression model (Eq. (3), $R^2 = 0.74$) between the Fv/Fm value and CO_2 concentration predicted the highest Fv/Fm value at 1,210 ppm CO_2 concentration (Fig. 4).

$$Fv/Fm_{CO2} = -0.0000000234(CO_2)^2 - 0.0000567595(CO_2) + 0.7792088389 \qquad (3)$$

The Fv/Fm value was 0.806 ± 0.003 at 65–85% relative humidity. It has been known that high or low relative humidity decreases the photosynthetic ability of plants[7]. This is because photosynthesis is reduced in the range of 10–20% relative humidity due to stomata closure and the decrease in intercellular CO_2 concentration levels. The maximum Fv/Fm value was predicted to be at 75% relative humidity (Fig. 5). A regression model between Fv/Fm and relative humidity is given in Eq. (4) ($R^2 = 0.73$). Fv/Fm values decreased when relative humidity was below 60% or above 85%, which suggests the Fv/Fm value can be an indicator for humidity control.

$$Fv/Fm_{RH} = -0.0000198754(RH)^2 + 0.00301791(RH) + 0.6906904622 \qquad (4)$$

The Fv/Fm value was 0.810 ± 0.003 at air current speeds between 0.4 and 1.3 m·s^{-1}, while it decreased above 1.3 m·s^{-1} (Fig. 6). A regression model showed the highest Fv/Fm value at 0.9 m·s^{-1} air current speed (Eq. (5)).

$$Fv/Fm_{ACS} = -0.013584327(ACS)^2 + 0.0227660957(ACS) + 0.7968448588 \qquad (5)$$

Kitaya et al.[15] reported that the net photosynthetic rate and transpiration rate significantly increased as the air current speed increased from 0.01 to 0.2 m·s^{-1}. The transpiration rate increased gradually with the increase of air current speeds from 0.2 to 1.0 m·s^{-1}, whereas the net photosynthetic rate remained constant at 0.5–1.0 m·s^{-1}.
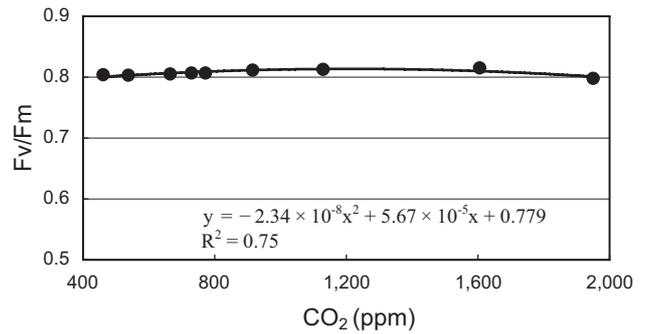

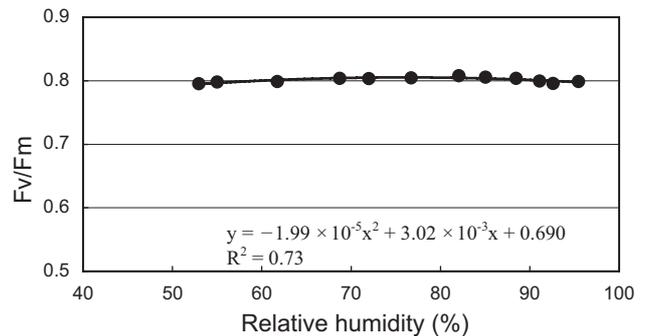**Fig. 4. Relationship between Fv/Fm and CO_2**

Fig. 5. Relationship between Fv/Fm and relative humidity

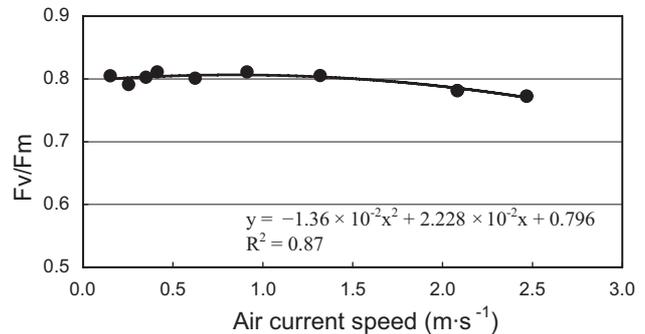**Fig. 6. Relationship between Fv/Fm and air current speed**

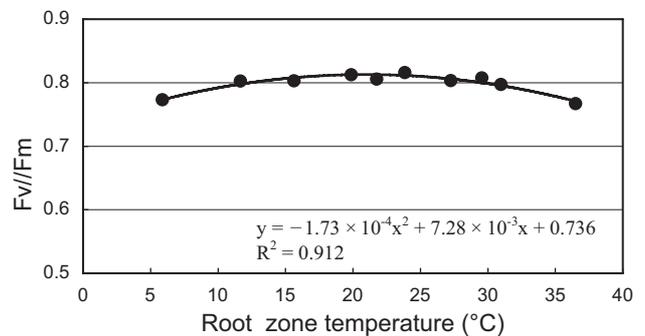**Fig. 7. Relationship between Fv/Fm and root zone temperature**

The Fv/Fm value was 0.809 ± 0.005 in the range of 12–23ºC root zone temperature (RZT) (Fig. 7). A regression model between Fv/Fm and RZT predicted an optimal RZT of 20ºC for lettuce growth (Eq. (6), $R^2 = 0.91$).

Table 5. Eigenvectors of measured Fv/Fm values

Factor	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6
AT	0.310031	0.604803	0.154923	−0.349728	−0.615815	−0.112049
RZT	−0.010160	0.352778	0.792585	0.164640	0.461040	−0.086576
CO$_2$	0.257798	−0.157072	0.142569	−0.589761	0.224641	0.696423
ACS	0.198054	−0.86639	−0.105189	0.396624	0.123957	−0.047444
PPF	−0.020619	0.614584	−0.560323	−0.138832	0.536436	−0.029354
RH	−0.168842	0.316022	−0.49549	0.571123	−0.233713	0.701307
Eigenvalue	2.36356441	1.38351051	1.12060559	0.74507042	0.27673273	0.11051635
Proportion	0.3939	0.2306	0.1868	0.1242	0.0461	0.0814

$$Fv/Fm_{RZT} = -0.0001730903(RZT)^2 + 0.0072890537(RZT)$$
$$+ 0.736501178 \qquad (6)$$

RZT is one of the most important factors affecting plant rooting[28]. He et al.[8] reported that the optimal range of RZT for lettuce growth was between 15 and 25ºC, and the photosynthesis increased by maintaining the RZT at 20ºC when the ambient temperature was under 38ºC.

2. Chlorophyll fluorescence response model using principle component analysis

Principle component analysis was used to estimate the combined effects of the environmental factors on lettuce growth. Environment conditions were randomly set within the ranges of environmental boundaries as given in Table 3. As a result, each of the six environmental variables showed significance in explaining the observed values of Fv/Fm ($P = 0.0001$).

As shown in Table 5, PRIN 1 showed the highest effect on the Fv/Fm value as compared to the other principal parameters. This parameter explains 39% of total population variation and was used to develop a chlorophyll fluorescence model (Eq. (7)).

The predicted Fv/Fm values by the model showed a good agreement with measured ones. Standard error was 1.2% with minimum and maximum errors of 0.2 and 4.7%, respectively (Fig. 8).

$$Fv/Fm_{Estimated} = 0.310031(Fv/Fm_{AT}) - 0.010160(FvFm_{RZT})$$
$$+ 0.257798(Fv/Fm_{CO2}) + 0.198054(Fv/Fm_{ACS})$$
$$- 0.020619(Fv/Fm_{PPF}) - 0.168842(Fv/Fm_{RH})$$
$$+ 0.384 \qquad (7)$$

3. Chlorophyll fluorescence response model with genetic algorithm

Optimal growth of lettuce resulted from 22ºC ambient temperature, 20ºC solution temperature, 1,578 ppm CO$_2$ concentration, 1.3 m·s^{-1} air current speed, 216 μmol·m^{-2}·s^{-1} PPF, and 75% relative humidity. This result
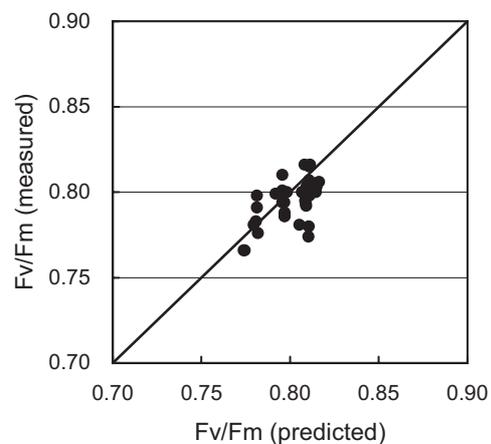
Fig. 8. Comparison of the measured and predicted Fv/Fm values

agreed with previous study results. Park and Lee[19] reported that the highest photosynthetic rate of lettuce was observed at 200 μmol·m^{-2}·s^{-1} PPF and 1,000–2,000 ppm CO$_2$ concentration. The relationship between photosynthetic rate and relative humidity varied with cultivation environment conditions. The higher the relative humidity is, the faster the air current must be to improve photosynthetic rate. Air current speeds of 0.5–0.8 m·s^{-1} resulted in the greatest photosynthesis at 65% relative humidity, whereas the highest photosynthetic rate resulted from 1.0–1.5 m·s^{-1} air current speeds at 85% relative humidity[31]. At the range of 24–40ºC ambient temperature, high Fv/Fm values were observed when root zone temperature was maintained at 20ºC as compared to the range from 23 to 40ºC. He et al.[8] reported better lettuce growth in the range of 15–25ºC ambient temperature.

Conclusion

The relationships of chlorophyll fluorescence responses between each environmental factor (ambient

temperature, PPF, relative humidity, CO_2 concentration, air current speed, and root zone temperature) and Fv/Fm values were analyzed. A chlorophyll fluorescence response model with the six environmental factors was developed using a principle component analysis and a simple genetic algorithm. The developed model predicted the observed Fv/Fm values. A simple genetic algorithm was incorporated into the chlorophyll fluorescence response model to predict an optimal environmental condition for lettuce growth. The optimal growth environment of lettuce was estimated to be 22ºC ambient temperature, 20ºC solution temperature, 1,578 ppm CO_2 concentration, 1.3 m·s^{-1} air current speed, 216 μmol·m^{-2}·s^{-1} PPF, and 75% relative humidity.

The chlorophyll fluorescence can be a good method to measure the plant physiological responses to environmental change without damage to the plant. The Fv/Fm value can be also a good indicator of plant status to environmental stress and can be a useful parameter to optimize the environment for plant growth.

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