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Respiration Rate and Quality of Spinach Cultivated in Night-cooling Greenhouse in Summer

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Respiration rate of spinach (*Spinacia oleracea* L.) cultivated in night-cooling greenhouse were measured and the changes in quality attributes were also determined during growth of spinach in summer. The result showed that there was lower level of respiration rate in night-cooling greenhouse than that in control, indicating that respiration rate was suppressed markedly in night-cooling greenhouse at night. The increase in the leaf length and area of spinach in night-cooling greenhouse was also much larger than that of control. Significant differences in height and leaf area ($p < 0.01$) were found between spinach planted in night-cooling greenhouse and control. It was also shown that there were significant differences ($p < 0.05$) in SPAD as a chlorophyll index and ascorbic acid content at harvest period. These results suggested that higher quality of spinach could be obtained from night-cooling greenhouse in summer.

INTRODUCTION

Plant growth is limited by the environment which is less than ideal it will become a limiting factor in plant growth. Most plant problems are caused by environmental stress, either directly or indirectly (Hans and Peter, 1992). Therefore, it is important to understand the environmental aspects that affect plant development and growth. Plants are grown within a controlled environment so that. One of the main tasks in greenhouse construction is to optimize the conditions for plant developments involving photosynthesis and respiration, generally during the off-season from normal outside field production. The greenhouse as a controlled environment agriculture (CEA) allows horticultural practices to be optimized and an increased variety of crops to be produced at the inappropriate environmental conditions of higher or lower temperatures. It effectively improves the environment in greenhouse and enables plants suited to the climates condition to be successfully grown. The greenhouse regulates the temperature, relative humidity (RH), and CO₂ concentration of the atmosphere inside. The vegetables are also grown in the greenhouse at different seasons of the year, especially for that the vegetables cannot be grown outdoors in summer climate.

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Spinach is one of the most important vegetables in Japan. But one factor of high temperature limits spinach production in summer (Kagawa, 1999). It is practiced less during the summer as the hot condition often lead to spinach growth irregularly and death (Tanaka *et al.*, 1993). Because spinach is classified as a hardy cool season crop (Toledo *et al.*, 2003). It is not suitable to grow under the environment of higher temperature, especially in summer. Ishibashi *et al.* (1978) and Tanaka *et al.* (1993) reported that better growth and quality of spinach cultivated were obtained in night-cooling greenhouse at temperature of 15°C by the refrigeration system in summer. But in control (cultivated in field), growth of spinach was irregular and most of them died due to higher temperature. However, little information of respiration rate and quality of spinach cultivated in night-cooling greenhouse was reported. The objectives of this work were to clarify the effects of lower night temperature on the respiration and quality of spinach sowed in greenhouse and develop new technique of high quality spinach production to meet the demand of consumer in summer. The changes in quality attributes were also determined during growth of spinach.

MATERIALS AND METHODS

Growing conditions

Spinaches (*Spinacia oleracea* L. cv Active) were sowed in pots (15 cm in diameter) containing a mixture of soil, peat, and sand (1:1:1). The pots were spaced 10 cm apart and were divided into two groups. One group (treatment) of spinach was grown under night temperature conditions of 15°C from PM 6:00 to AM 8:00 by refrigerator (200R-CLTA, Hitachi, Japan). Other group (control) of spinach was grown in greenhouse under normal condition. Spinach was fertilized with soluble liquid fertilizer (OK-F-1, Otuska Ltd, Japan) every 3 to 4 d during growth. Air and soil temperatures in greenhouse were measured by an aspirated type-T (copper-constantan) thermocouple at 1.5 m above ground and 5 cm below ground in the pot, respectively.

Respiration rate

Respiration rate was determined by using a respiratory system at 15°C for treatment and 25–27°C for control. The five pots of each treatment with growing spinach were randomly sampled and placed in respiratory room (diameter: 50 cm, height: 60 cm) and sealed. The fresh air that CO₂ in air was absorbed by 20% NaOH was flow through the room for 2 h. The well-distributed gas was obtained by fan in the room. When the gas concentration was stable inside, measurement was started. Gas samples in inlet, outlet were withdrawn by gas-tight 1.0 ml syringe. The gas concentrations were measured by injecting 1.0 ml gas sample into gas chromatography (GL Sciences GC-390, Tokyo Japan) equipped with thermal conductivity detector (TCD) and D2000 integrator (Hitachi, Ltd. Tokyo Japan). Helium was used as carrier gas and the flow rate was 30 ml·min⁻¹. The injector and column temperatures were 80 and 50°C, respectively. The respiration rate of spinach was calculated from following equations.

$$RR = \frac{(CO_2)_{out} - (CO_2)_{in}}{10^6} \times F \times \frac{273}{273 + T} \times \frac{44}{22.4} \times \frac{1000}{M}$$

Where RR : respiration rate ($\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$); $CO_2 \text{ in}$: CO_2 concentration in inlet of chamber ($\mu\text{l l}^{-1}$); $CO_2 \text{ out}$: CO_2 concentration in exit of chamber ($\mu\text{l l}^{-1}$); F : flow rate of fresh air (ml h^{-1}); T : temperature of chamber ($^{\circ}\text{C}$); M : mass of sample spinach (kg).

Ascorbic acid content

Ascorbic acid content was measured by high performance liquid chromatography (HPLC) equipped with Shim-pack SCR-101N column ($7.9 \text{ mm } \phi \times 30 \text{ cm}$) (Shimadzu Corp., Tokyo Japan). The column is packed with a cation exchanger resin which is sulfonated polystyrene-divinylbenzene copolymer. The mobile phase was mixed solution of 10 mM oxalic acid dihydrate, 15 mM NaOH and 1 mM EDTA at a flow rate of 1.0 ml min^{-1} . Temperature is 40°C and detector is RI. Spinach sample was extracted from excised slices of 5 g spinach with 5 times the volume of 5% metaphosphoric acid in a mortar with pestle. The homogenate was filtered through filter paper and centrifuged at 3,000 rpm for 15 min. The supernatant was taken as sample to measure the ascorbic acid content in spinach.

Dry matter

Dry matter was measured during growth. The condition of measurement was under 105°C for 24 h. Dry matter was determined and expressed as the percentage with respect to the initial fresh weight.

Leaf area

Leaf area was measured by weight method. The ratio of area and weight of copied graph paper was calculated. And then each leaf of spinach was placed on print paper, and then size and shape of spinach were drawn in the paper. The paper was copied, and then drew section was cut and weighed. The leaf area was determined based on the ratio from the result of measured weight of drew section of print paper. The area of spinach was calculated from following equations.

$$A_L = \frac{A}{M_1} \times M_2$$

Where A_L : area of spinach (cm^2); A : area of print paper (cm^2); M_1 : mass of print paper (g); M_2 : mass of copied drew section of print paper (g)

Chlorophyll index

The leaf color of spinach was determined by chlorophyll meter (Minolta, SPAD-502) during growth. Measurement was carried on 2 parts of each leaf. The value of SPAD was correlation with chlorophyll content as a chlorophyll index.

RESULTS AND DISCUSSION

Respiration rate

Changes in respiration rate of spinach in greenhouse during growth were shown in Fig. 1. Respiration rates were lower in night-cooling greenhouse than that of control at night during growth. In initial period, respiration rate increased gradually from 10.5 to 50.1 mg kg⁻¹ h⁻¹ and then decreased to 6.9 mg kg⁻¹ h⁻¹ at night-cooling greenhouse. For control, respiration rate increased and then decreased to 90.3 mg kg⁻¹ h⁻¹ after 8 d. And then increased sharply to 242.0 mg kg⁻¹ h⁻¹ and decreased to 110.0 mg kg⁻¹ h⁻¹. It showed that there was lower level of respiration rate in night-cooling greenhouse than that in control. Respiration rate was suppressed markedly in night-cooling greenhouse. It has been reported that respiration increases with temperatures increasing until temperature gets too high, then respiration decreases due to tissue deterioration and denatures proteins (unfolding of proteins). The environmental conditions can produce a stress on plants resulting in lower yields. In such cases the environment can be artificially modified, such as in greenhouses, to meet the crop requirements.

Each kind of crop grows and develops most rapidly at a favorable range of air temperatures. This is called the optimum air temperature range. For most crops the optimum functional efficiency occurs mostly between 12 and 24 °C. Most crops (especially vegetables) can be classified according to the temperature requirements of their optimum air temperature range. However, they are generally grouped into whether they require low or high air temperatures for growth. Temperature requirements are usually based on night temperature. Those that grow and develop below 18 °C are the cool season crops, which originated in temperate climates usually require low temperature.

High temperature results in increase rates of most biochemical functions within the plant, including respiration and enzymatic processes involved in growth. The plant gives

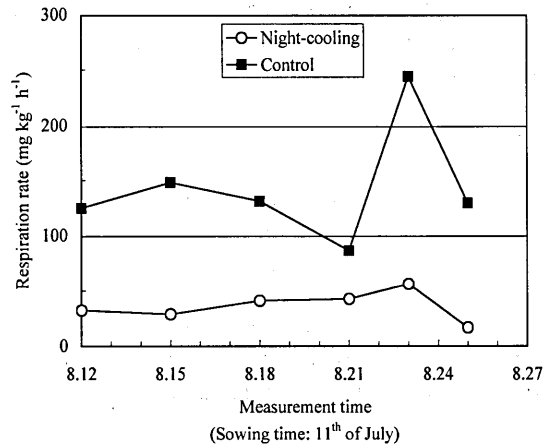


Fig. 1. Changes in respiration rate of spinach during growth in greenhouse.

off some CO_2 during respiration but it needs more for photosynthesis. Photosynthesis produces organic compounds from inorganic carbon by using the energy of sunlight (Massacci *et al.*, 1995). Above 35°C , the photosynthetic rate levels off, whereas the respiratory rate continues to increase linearly. Consequently, around 35°C growth ceases as net carbohydrate consumption through respiration exceeds net carbohydrate fixation through photosynthesis. In general, rates of biochemical processes slow down as the temperature drops. The energy stored in this sugar is released as ATP (adenosine triphosphate) during cellular respiration. At that high temperature the amount of glucose consumed exceeds the amount produced thereby ceasing growth. In this experiment, cultivated spinach is a kind of cool season crops, which usually require low temperature for development and growth.

Leaf length and area of spinach

Changes in the leaf length of spinach during growth were shown in Fig. 2. There was trend of increase in length of leaf during growth. In night-cooling greenhouse, increase in length of leaf was much larger than that of control. The similar result was also found in the changes in area of leaf during growth (Fig. 3). It showed that there was much higher value in night-cooling greenhouse than that of control. It indicated that night temperature affected the productivity and growth of spinach. If temperatures are high and day length is long, cool-season crops such as spinach will bolt rather than be in progress of desired growth. Adverse temperatures also cause stunted growth and poor quality. Temperature allows the plant to photosynthesize and respire during an optimum daytime temperature and to curtail the rate of respiration during a cooler night (Loreto *et al.*, 1994). This means that the products of photosynthesis are being stored much more than they are being used. For growth to occur, photosynthesis must be greater than respira-

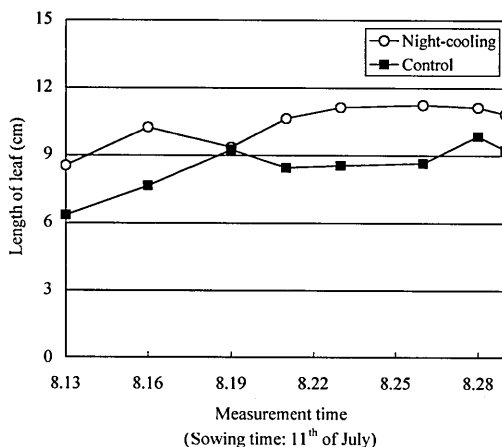


Fig. 2. Changes in the length spinach leaf during growth in greenhouse.

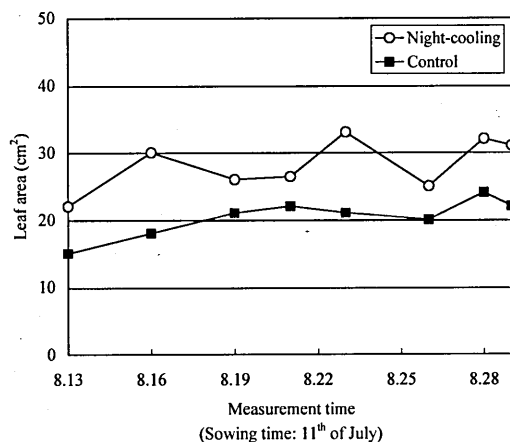


Fig. 3. Changes in leaf area of spinach during growth in greenhouse.

tion. Not all plants grow best in the same temperature range. For example, snapdragons grow best when nighttime temperatures are 12°C; the poinsettia prefers 17°C. Recently it has been found that roses can tolerate much lower nighttime temperatures than was previously believed. This has meant conservation in energy for greenhouse growers. However, in some cases a certain number of days of low temperatures are needed by plants to grow properly. The high temperature also takes placed increases in transpiration and results in wilt of spinach, and low temperatures reduce energy use and increase sugar storage during active growth.

SPAD green color

Changes in SPAD of spinach during growth were shown in Fig. 4. The value of SPAD was correlation with chlorophyll content as a chlorophyll index. In the night-cooling greenhouse, the value of SPAD was lower than that of control. But it showed that value of SPAD continued to increase until harvest and there was higher value of SPAD in the near harvest time. It was considered that biosynthesis of chlorophyll was slightly inhibited initial period of night-cooling greenhouse and then increased gradually. While in control, it showed the similar trend of increase in initial period, and decreased with increase in night temperature in summer. The production time is a bit longer and some reactions of biosynthesis of chlorophyll were carried out at suitable night temperature (Porra *et al.*, 1989). The spinach has a bit larger, deeper green leaves at initial period for control.

Ascorbic acid content

Changes in ascorbic acid of spinach during growth were showed in Fig. 5. In initial

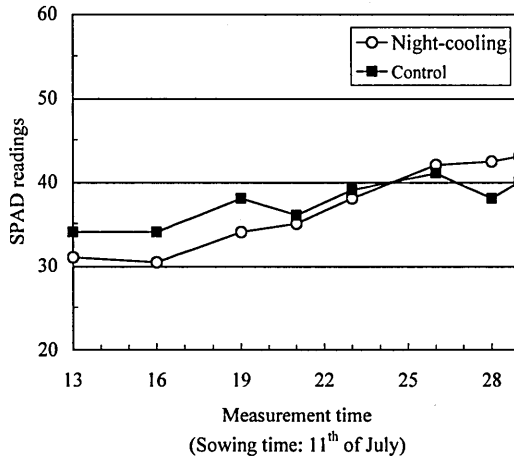


Fig. 4. Changes in SPAD of spinach during growth in greenhouse.

period, it showed the similar trend of increase, and then increased quickly for night-cooling greenhouse. While in control, ascorbic acid content decreased quickly and then increased, but the content was lower than that of treatment. It was considered that ascorbic acid in night-cooling greenhouse was oxidized less than control due mainly to inhibiting respiratory metabolism at lower night temperature. It indicated that higher quality of spinach could be obtained from cultivation in night-cooling greenhouse.

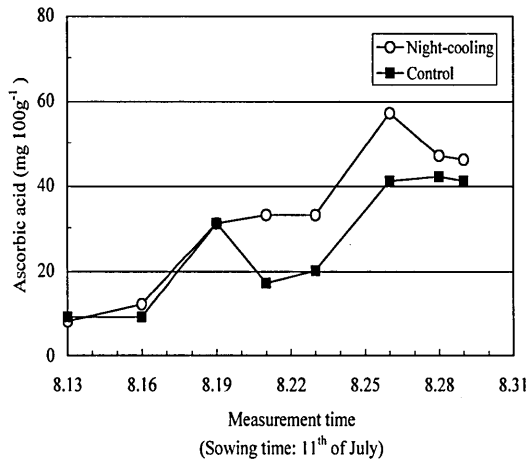


Fig. 5. Changes in ascorbic acid of spinach during growth in greenhouse.

Qualities of harvested spinach

The qualities of harvested spinach were shown in Table 1. Height and leaf area of harvested spinach were 23.2 cm and 406 cm², higher than that of control. Significant differences in height and leaf area ($p < 0.01$) were found between spinach planted in night-cooling greenhouse and control. It was also shown that there were significant differences ($p < 0.05$) in SPAD and ascorbic acid content as quality index. But values of leaf count and fresh weight in night-cooling greenhouse were lower than that of control. These results indicated that higher quality of spinach could be obtained from night-cooling greenhouse with large leaf area, deep green color and high ascorbic acid content in summer.

Table 1. Qualities of harvested spinach.

Items of measurement	Night-cooling	Control
Height of spinach (cm)	23.2	19.3**
Leaf area (cm ²)	406.2	354.3**
Leaf count (N)	14.0	16.0
Fresh weight (g)	21.1	21.2
Leaf/leaf stalk	2.3	2.0**
SPAD	42.0	39.8*
Ascorbic acid (mg 100g ⁻¹)	45.8	36.8*

** Significant differences between night-cooling and control value ($p < 0.01$)

* Significant differences between night-cooling and control value ($p < 0.05$)

CONCLUSIONS

The study shows that spinach can be grown successfully as a kind of cool season leafy vegetable in summer. Respiration rate was greatly reduced under night-cooling environmental condition in greenhouse. The higher values of leaf length and area of spinach in night-cooling greenhouse were obtained during growth and the relative higher value of SPAD as a chlorophyll index and ascorbic acid content were also achieved at harvest period. It was suggested that spinach could be cultivated in night-cooling greenhouse and better quality of spinach can be obtained in summer.

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