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Assessment of different growing conditions for enhanced postharvest quality and shelf-life of leaf lettuce (*Lactuca sativa* L.)

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Abstract Lettuce is the most important leafy vegetable. It is exclusively used as freshly raw form but sometime also as cooked. However, its quality depends on several pre- and postharvest factors. The effects of growing conditions and cultivars on the postharvest quality of leaf lettuce were investigated. In this experiment the interaction of variable factor; like growing conditions and fixed factor; cultivars are also observed. The leaf lettuce cultivars 'Cheongchima', 'Cheongchuckmyeon', 'Geockchima' and 'Geockchuckmyeon' were grown under favourable (natural condition) and unfavorable growing condition (with excess soil water and 50% shading) to evaluate their shelf-life and postharvest qualities. Plant height and the number of leaves were significantly varied in different growing conditions and by cultivars. In addition, fresh weight was affected by only growing condition, whereas leaf thickness was influenced by cultivars, growing conditions and the cultivars influenced the degree of fresh weight loss and respiration rate during postharvest storage. However, the postharvest storage qualities differed with cultivars and in combination with growing conditions. Leaf thickness and quality of leaf lettuce before and after harvest were also varied by cultivars and growing conditions. It could be concluded that postharvest leaf lettuce quality remains acceptable when growing under excess soil water with 50% shading.

Key words: Growing condition, shelf-life, postharvest quality, leaf lettuce.

INTRODUCTION

Leaf lettuce (*Lactuca sativa* L.) is an annual plant of the family *Compositae*. It is grown as leafy vegetables. Lettuce is normally used for salads, although it is also used with other foods, like wraps the meats, fish and so on. It is also grown for eating either as raw or cooked. Fresh lettuce leaves contain good amounts of folates and vitamin C. Among them folates is part of co-factors in the enzyme metabolism required for DNA synthesis and thereof, play a vital role in prevention of the neural defects in fetus during pregnancy (Agüero et al., 2008; Lettuce nutrition facts, 2016). Leaf lettuce can be grown year-round and is one of the most widely used fresh vegetables in Korea. Many studies of leaf lettuce have been focused on improving productivity, while postharvest qualities have not been coupled to production factors so far (Jang et al., 2007; RDA, 2007; Ryu et al., 2011).

The quality of fresh harvest leaf lettuce depends on number of factors such as genotypes, cultivation methods, soil conditions, growing season, and diseases (Park et al., 1999). In addition couple of postharvest factors like storage temperature, relative humidity, packaging materials, and packaging method are also determined the quality of leaf lettuce (Lee et al., 2008a). Currently, leaf lettuce is grown in open fields, greenhouses, and hydroponic culture (Lee et al., 2005) abundantly. But leaf lettuce has poor storage properties after harvest due to high respiration rates (Lee et al., 2007). Hence, most of the researches are concentrated on storage management for increasing its shelf-life. However, the effect of growing conditions on postharvest qualities of leaf lettuce has not been extensively investigated.

Leaf lettuce is cultivated year-round in Korea under plastic-film greenhouses (polyvinyl house). However, these plastic greenhouses are not well protected against cold and heavy rain compares to well-designed greenhouse. Therefore, this crop is easily influenced by the changes of outsides environment, that could directly influence the inside conditions of greenhouse. Thus, these changes can directly affect the growth performance and postharvest qualities. Along with uncertain rainy weather also reduces photosynthetic light intensity and sometime causes water logging in soil. The water logging conditions negatively affects the oxygenation status of the root environment, leading to wilting or shriveling (Lee, 1997). Therefore, this study focused on the effects of amount of excess watering and poor illumination conditions on postharvest qualities relevant to freshness and storability of leaf lettuce.

MATERIALS AND METHODS

Plant materials and experimental conditions

This experiment was conducted to assess the effects of growing environments and cultivars on the production and postharvest storage qualities of leaf lettuce. Four popular available leaf lettuce cultivars namely ‘Cheongchima’, ‘Cheongchuckmyeon’, ‘Geockchima’, and ‘Geockchuckmyoen’ were collected from Nongwoobio Seed Company, South Korea. Among them ‘Cheongchima’, ‘Cheongchuckmyeon’, are greenish type and ‘Geockchima’ and ‘Geockchuckmyoen’ are red colour type cultivars widely used by Korean people as wrapper of meat with different types of sauces. They were grown under conventional cultivation protocol and unfavorable environmental conditions. The unfavorable conditions were achieved by 28.4% excess soil water and 50% shading of the plants. The experiment was carried out in plastic greenhouses (polyvinyl house) at the National Institute of Horticultural & Herbal Science, Suwon, Korea. Firstly, the polyvinyl house was separated into two parts, one part was covered with black net intercepting 50% sunlight and other part had normal sunlight without interception. The experiment was conducted in the soil with 3.5% organic matter, 2.1% N, 445.7 mg kg^{-1} P2O5, 1.6 cmol kg^{-1} K, 7.7 cmol kg^{-1} Ca, and 2.2 cmol kg^{-1} Mg with the pH 6.5, EC 0.7 mS cm^{-1}. One month aged leaf lettuce seedlings (previously raised in nursery using multi-tray with 162 wholes) were transferred to experimental polyvinyl house. The seedlings were transplanted with 20 cm × 20 cm spacing (plant to plant × row to row) in well raised soil beds inside the polyvinyl house. In case of excess soil water, furrows were made in the bed with 20 cm apart to each other and 4 furrows/bed were made. The raised part of furrows was covered with black polythene and continuously irrigated into furrows using irrigation pump regulated by sensor with 28.4% excess soil water. Whereas, only subsistence watering was done in case of normal growing condition plants. Data recorded in leaf lettuce prior to harvest were plant height, number of leaves, shoot fresh weight, leaf thickness, crude fiber content, and leaf anatomy. Harvested plants were wrapped in plastic film package and stored in a retail showcase refrigerator at 5 °C. In addition, the postharvest parameters were measured on fresh weight loss, respiration rate, leaf color change, and external appearance.

Experimental design and application of treatments

This experiment was conducted following Split-Plot design with two factors and three replicates. The factors were genotypes (four cultivars), soil moisture and light interception (normal moisture with normal light condition and excess soil water with 50% shading). The excess soil moisture treatment was prepared by following the procedure of Lee (1997). In this method, soil water movement was prevented by piling up the soil wall (~30 cm thickness) and overlaying it with polyethylene film. Soil moisture was adjusted and maintained to 28.4% using a soil moisture control device (WT-1000 TDR, AgroNet, Seoul, Korea). The average soil moisture content under the control condition was 21.1%, whereas in excessive watering condition it was 28.4%. For the shade treatment, a high-density black polyethylene net was used to reduce 50% light. Because outside light intensity was on average 1.6 MJ/day during the cultural period.

Other growth factors were constant as standard cultivation procedures followed as Rural Development Administration (RDA, 2007), Korea. Seeds were sown on August 17th, seedlings were transplanted on September 9th, and crop was harvested on October 10th. Seedlings were planted in commercial nursery soil (Bio Bed Soil No. 1, Seminis Korea Inc., Seoul, Korea). Fertilizer was applied to achieve 200, 59 and 128 kg ha^{-1} nitrogen, phosphate and potassium, respectively. Leaf lettuce was planted with an inter-plant distance of 20 cm. The growth parameters of lettuce was recorded following standard criteria of Rural Development Administration (RDA, 2003), Korea. Crude fiber content was determined using a Fibertec System (M1020, Fosstecator, Höganäs, Sweden). Cross-sections of specimens were observed under light microscopy following the procedures of Luft (1961) and Chang (1973). Specimens were taken from below the leaf apex at position of 90% distance of leaf length from the base. 1,500-nm thick sections were stained with periodic acid Schiff and examined under a Carl Zeiss Axioscope binocular light microscope (Carl Zeis, Zena, Germany).

Shelf-life and postharvest qualities

Storage shelf-life was recorded by comparing the harvested plants of unsuitable environment and control (suitable) conditions. Three to ten upper leaves were harvested and used for determining shelf-life and postharvest qualities. Each lettuce sample (150 g) was packaged in a polypropylene film bag (32 × 22 cm, 0.05 mm) and stored at 5 °C in a refrigerator. Storage was terminated when samples had visibly deteriorated (yellowing/spoil). The fresh weights were measured before and after storage of the specimens to calculate weight losses. Four different packaged samples were weighed at one day intervals throughout the duration of storage. Respiration rates were measured by inserting 150 g leaf lettuce samples into gas-tight containers for 1 h at 5 °C under airflow of 1 mL min^{-1} and four replications were used for each sample. CO_2 production was recorded to estimate respiration rate. Measurements were done using an automated sampling flow-through system (Lee et al. 2007) equipped with gas chromatograph (HP6890, Hewlett-Packard, Palo Alto, CA, USA) fitted with stainless steel Porapak Q column (80–100 mesh, 1 m × 3.2 mm) and a thermal conductivity detector [150 °C, He (50 mL min^{-1})].

Surface color was measured with a chromameter (CR-300, Konica-Minolta, Tokyo, Japan). Color was recorded using CIE-Lab uniform color spacing procedures (Hunter Lab, 2001). Ten positions of each leaf of the four samples were measured and average values were used for recoding color changes. The total difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{\{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2\}} \quad (1)$$

where L^*_0, a^*_0, and b^*_0 represent readings at initial time (zero), and L^*, a^*, and b^* represent individual readings under a given storage condition. Total color changes were grouped as follows: 0–1.5 = detectable, 1.6–6.0 = appreciable, > 6.1 = noticeable.

A survey on external appearance changes was conducted referring the methods of Jeong et al. (1990), Lee et al. (2007), and Yang et al. (1991). Sensory properties, including external appearance and color change, were assessed by a three member's panel. Samples were scored on 0–6 scale with a five-step rating (6 = excellent, 4.5 = good, 3.0 = moderate, 1.5 = poor, and 0 = inferior).

Statistical analysis

Analyses were performed using the SAS software ver. 9.1 (SAS Institute, Cary, NC, USA). Data collected before plant harvest was subjected to split-plot analyses with three replicates. Postharvest data were subjected to analyse with four replicates. Analyses of variances (ANOVAs) were based on the Generalized Linear Model (GLM) initiated by the PROC GLM statement in SAS. LSD tests at 5% significance level were used to identify significant difference among pair of means, where ANOVA revealed a significant difference among means. The OriginLab software ver. 8.0, (OriginLab Co., Northampton, MA, USA) was used to generate graphical analyses and plots.

RESULTS AND DISCUSSION

Effects of growing conditions on growth parameters

Growth of leaf lettuce was varied with growing conditions and type of cultivars (Table 1). The growth of the four cultivars was affected by various growing conditions. Under the excessive soil moisture with shading treatments, plant height was increased but number of leaves, shoot fresh weight, and crude fiber content were decreased. Although, increased growth was observed in excess soil moisture with shading conditions, however the quality of the lettuces was decreased under that poor and unfavorable environment (Table 1).

Excess soil moisture with shading, and cultivars had significant effects on plant heights and numbers of leaves. Fresh weight was varied significantly with growing conditions but not by cultivars; leaf thickness varied significantly by cultivars but not with growing conditions. A significant interaction effect of growing conditions and cultivars on fiber content was observed (Table 1). 'Geockchima' plants were the tallest in excessive water with shading conditions, whereas the shortest plants was observed in

‘Cheongchuckmyeon’ (oak leaf type with green color) grown under control conditions. Plant height changed according to cultivars type, when the growing conditions were changed. However, plants grown under excess watering with shading conditions were taller than each of the cultivars grown under control conditions.

Table 1. Growth parameters of four lettuce cultivars subjected to excessive soil water and shading treatments and control conditions grown in a plastic greenhouse

Cultivar	Culture condition	Plant height (cm)	No. of leaves	Shoot fresh weight (g/plant)	Leaf thickness (mm)	Crude fiber (%)
Geockchima	Control	41.3	23.1	467.3	0.2	1.87
	Excessive soil water with shading	45.2	14.3	169.7	0.2	1.11
increase/decrease (%)		9.4	-38.1	-63.6	0.0	-40.6
Geockchuckmyeon	Control	35.0	22.3	467.3	0.4	0.90
	Excessive soil water with shading	37.2	11.0	132.0	0.5	0.82
increase/decrease (%)		6.2	-50.6	-71.7	25.0	-8.8
Cheongchima	Control	35.4	27.8	495.3	0.3	0.68
	Excessive soil water with shading	37.5	20.7	184.0	0.3	0.63
increase/decrease (%)		5.9	-25.5	-62.8	0.0	-7.3
Cheongchuckmyeon	Control	31.7	24.2	472.3	0.5	0.86
	Excessive soil water with shading	34.8	13.8	139.3	0.6	0.63
increase/decrease (%)		9.7	-42.9	-70.5	20.0	-26.7
Cultivar (A)		**	**	NS	**	**
Growing condition (B)		**	**	**	NS	**
A × B		NS	NS	NS	NS	**

NS, not significant at $P < 0.05$; *, ** Significant at $P < 0.05$ and 0.01, respectively.

The number of leaves also differed based on cultivars and growing conditions. In where, cultivar ‘Cheongchima’ had the highest number of leaves, followed by ‘Cheongchuckmyeon’ (oak-leaf type mixed with red color), ‘Geockchima’, and ‘Geockchuckmyeon’ in the plants grown under control conditions. The higher number of leaves was also produced in all cultivars grown under control conditions compare to excess water with shading conditions. Shoot fresh weight was differed little among cultivars when grown under control conditions; whereas, it was adversely affected by excess watering with shading (Table 1). Meanwhile, leaf thickness was varied among cultivars. ‘Cheongchuckmyeon’ had the thickest leaf, followed by ‘Geockchuckmyeon’, ‘Cheongchima’, and ‘Geockchima’. However, there was a non-significant effect of growing conditions on this trait. Although tendency was produced bit thicker leaves in excess watering with shading in cultivars ‘Geockchuckmyeon’ and ‘Cheongchuckmyeon’.

Shoot fresh weight was affected significantly by the growing conditions. It was severely affected than other parameters due to excess soil water with shading. Shoot fresh weight was led to about 63–72% decrease in excess soil water with shading compared to control for all cultivars (Table 1). Therefore, it might be concluded that shoot fresh weight was affected by growing conditions but not by the cultivars. The ‘Cheongchuckmyeon’ and ‘Geockchuckmyeon’ cultivars showed a greater decrease in shoot fresh weight than that of ‘Cheongchima’ and ‘Geockchima’. Morphologically similar cultivars showed similar trend of decreasing of fresh weight. The fresh weight of 'Cheongchuckmyeon' and 'Geockchuckmyeon' was decreased by 71% and 72%, respectively under excess water with shading treatment as compared to the control. Whereas, fresh weights of ‘Cheongchima’ and ‘Geockchima’ was decreased by 63% and 64%, respectively (Table 1).

Crude fiber content was significantly affected by all three factors: cultivar, growing conditions, and their interactions. Fiber is composed of long-chain polymers, including lignin and cellulose (Kwon, 1990; Choi et al., 2014; Kye, 2014). Fiber supports the plant structures in aerial environment. The plants grown in control condition contained more crude fibers than that of excess water with shading condition. Among the cultivars, the ‘Geockchima’ cultivar contained the highest crude fiber (Table 1). Lee et al. (2005) showed, in leaf lettuce cell osmotic solution, moisture, and cell structural fibers are affected by external environment. This experiment also showed crude fiber contains also changed due to cultural methods.

Growth was influenced by cultivars rather than growing conditions. Morphologically similar cultivars showed similar growth pattern. Thus, it was assumed that leaf lettuce cultivars of similar appearance have similar responses to the external environment. However, a leaf internal structure was changed due to presence of excessive soil water with shading (Fig. 1). Excessive soil water with shading increased plant height, but reduced both the number of leaves and plant biomass. Furthermore, in case of excess soil water and low light, intercellular spaces and bundle sheaths were widely spaced compared with those in the control conditions’ growing plants. In addition, the density of the leaf structures differed among the cultivars due to excess soil water with shading. Lettuce grown under excess soil moisture with shading showed reduced growth but increased size of individual cells. Loosen cell structure would be expected because of increase thickness of leaves as a result of presence excessive water inside cells. Lettuce grows well even under low light, but Nam (1996) reported that shading reduced the leaf thickness of lettuce. By contrast, we found that leaf thickness differed on growing conditions and also by cultivars. ‘Geockchima’ leaves showed little change in leaf thickness, either grown under excess water with shading or control condition. Simillarly, ‘Geockchuckmyeon’ leaves tended to be thicken in excess soil water with 50% shadingand also control conditions. Thus, it is clear that leaf thickness depends on cultivars rather than growing conditions.

Lettuce growth is not affected by shading down to 50% ambient illumination (Wolff & Coltman, 1989), but shading in combination with reduced nitrogen fertilization reduces growth rates in comparison with controls (Lee et al., 1998). Additionally, this experiment showed that shading can reduce growth in combination with other unfavorable conditions, like excessive soil water, in all cultivars. Lee et al. (1998 and 2005) also reported that growing conditions influence lettuce leaf morphology and finally effects on growth. Lee et al. (2008b) reported that the leaf thickness and volume of Chinese cabbage was affected by environmental conditions and postharvest salting on the characteristics of salted Chinese cabbage.

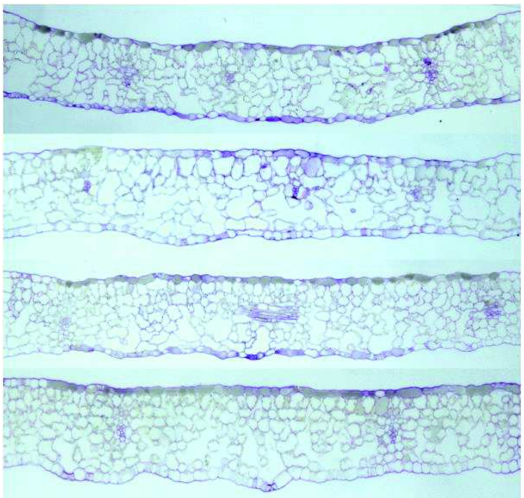

Figure 1. Cross-section micrographs of the lettuce cultivars 'Geockchima' and 'Geockchuckmyeon' under various culture conditions. (A) 'Geockchima' control, and (B) 'Geockchima' subjected to excess soil water with shading; (C) 'Geockchuckmyeon' control, and (D) 'Geockchuckmyeon' subjected to excess soil water with shading grown in a greenhouse.

Three leaf lettuce cultivars 'Geockchuckmyeon', 'Cheongchuckmyeon', and 'Cheongchima' responded differently to unfavorable environments. For example, leaf thickness of three of the four cultivars was changed differently with growing environments, whereas that was not significantly affected by excess soil water with shading in 'Geockchima' cultivar. Therefore, it could be marked that some cultivars are not susceptible to growing conditions. Our findings confirmed that excess soil water with shading affected growth, cell morphology and crude fiber content of leaf lettuce.

Effects of growing conditions on the postharvest qualities

Fresh weight loss ratio during postharvest storage at 5 °C was influenced by growing conditions and cultivars (Fig. 2). Weight loss ratio was increased in three cultivars except 'Geokchima' in case of plants grown under excess soil water with shading condition, which is confirmed by the findings of Lee et al. (2005). According to Yang et al. (1991), fresh weight loss is more affected by postharvest factors, such as packaging and storage conditions, than by growing conditions. However, growing conditions could become crucial if storage conditions are fixed.

Furthermore, the postharvest respiration rate was also influenced by growing conditions. In general, the shelf-life of harvested crops is reduced by high rates of respiration (Kader, 2002). Postharvest respiration rates were higher in three cultivars except 'Geokchima' grown under excess soil water with 50% shading than that of control (Fig. 3). The respiration rate of cultivar 'Cheongchuckmyeon' was double at 10 days of storage, while grown under excess soil water with 50% shading compare to control. The differences in respiration rate of cultivar 'Geockchima' between the excess water with 50% shading and control were negligible. In contrast, Lee et al. (2005) reported no significant effects of growing conditions on postharvest respiration rates. The effects of treatment on respiration rates may be due to changes of morphology caused by excessive soil water with 50% shading. We infer that an alteration of cell size due to growing environment causes difference in respiration rates. This putative relationship warrants further investigation. Moreover, the effect of cultivars are also be explored in more details.

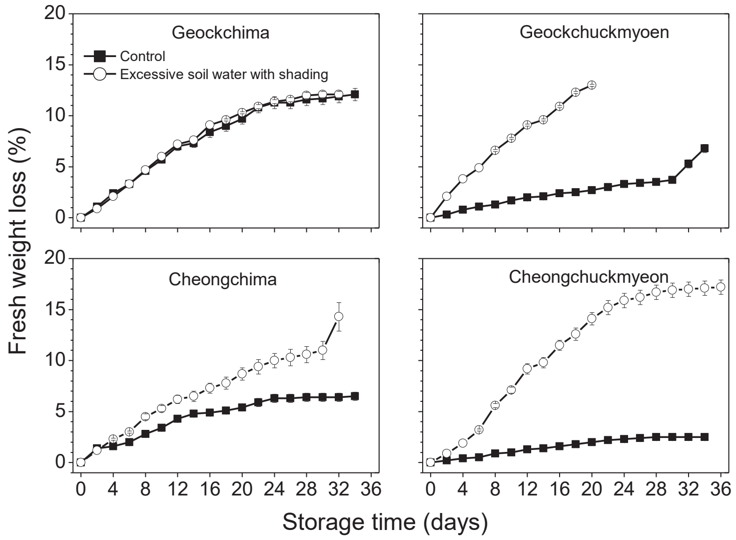

Figure 2. Effect of cultivation under excessive soil water and shading treatments, and control conditions, on fresh weight loss of the four lettuce cultivars stored at 5 °C. Values are means ± standard errors (n = 4).

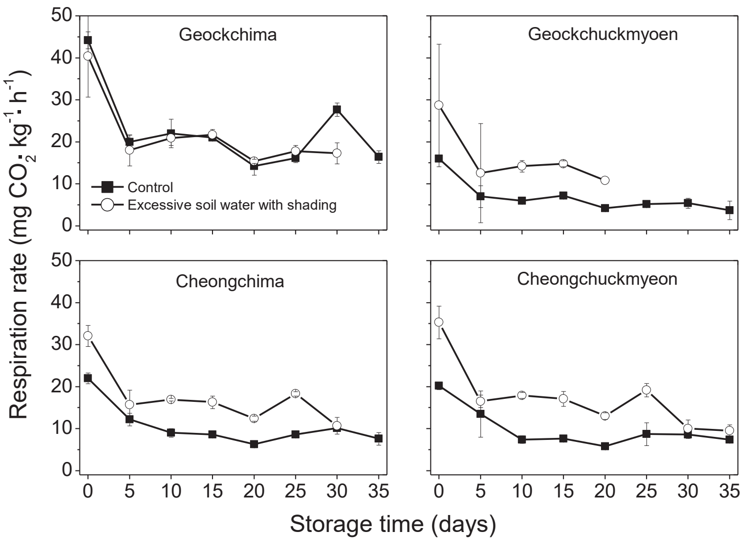

Figure 3. Postharvest respiration rates of four lettuce cultivars at 5 °C according to cultivation under excessive soil water, shading, or control conditions in a greenhouse. Values are means ± standard errors (n = 4).

The effects of growing conditions on changes in lettuce color during postharvest storage were assessed (Fig. 4) according to the method described in the manual Hunter Lab (2001). Total color change (ΔE) values of all lettuce samples were increased markedly with advancement of storage duration. Color changes were started apparently after 4days of storage. However, the effects of growing conditions were detectable in the 'Cheongchuckmyeon' and 'Cheongchima' cultivars. In these two cultivars, color changes were greater in control plants than those in the treatment groups. Total color change (ΔE) differed between the early and late periods of storage. However, color changes values had no significant difference between two growing conditions for the cultivars 'Geokchima' and 'Geockchuckmyeon' (Fig. 4). In this experiment, 'a' (greenness) values with excessive soil water and 50% shading was negative in compared to control. Although the differences of color change values was small. This experiment revealed that the postharvest quality of leaf lettuce could be reduced despite of grown under favorable control conditions. The degree of color change in the favorable control condition was larger than that resulting from excessive soil water with 50% shading, the absolute value of color change was remarkable in favorable control condition. Therefore, optimum storage conditions are necessary to ensure a good quality product, regardless of its initial condition. According to Lee et al. (2005), color changes in stored leaf lettuce are closely associated with changes in chlorophyll content, which is more influenced by cultivars than by growing conditions. The results reported here agree with this earlier work.

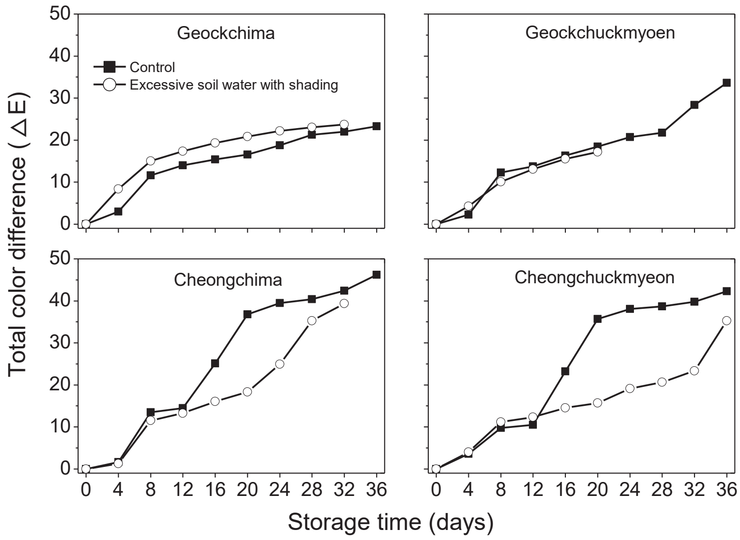

Figure 4. Effects of cultivation under excessive soil water, shading, or control conditions in a greenhouse on total color differences (ΔE) of the four lettuce cultivars stored at 5 °C. Total color difference: 0–1.5 = detectable, 1.6–6.0 = appreciable, > 6.1 = noticeable.

Qualitative measures of lettuce freshness judged by appearance and that was differed by cultivars and growing conditions (Fig. 5). Marketability represents by the time at up to which the product retain a value of 4. The marketability period for 'Geockchima' was 10 days for control condition growing product group, whereas it was 8 days for the product group grown under excess soil water with 50% shading condition.

The marketability period for ‘Geockchuckmyeon’ was 6 days in the control group and 4 days in the excess soil water with 50% shading group. The marketability period for ‘Cheongchima’ was 4 days in the control group and 8 days in the excessive soil water with 50% shading group. The marketability period for ‘Cheongchuckmyeon’ was 6 days in the control group and 12 days in the excess water with shading group. Hence, marketability was dependent on both cultivars and growing conditions. The marketability with large color changes was decreased more rapidly in ‘Cheongchima’ and ‘Cheongchuckmyeon’. The ‘Geockchima’ and ‘Geockchuckmyeon’ cultivars exhibited the least-marked color change, which was influenced by growing conditions (Fig. 4). Fig. 5 shows that the growing conditions had different effects on shelf-life depending upon the external appearance of cultivars. Cultivars ‘Geockchima’ and ‘Geockchuckmyeon’ had longer shelf-life when grown in control condition than those grown under excess soil water with 50% shading condition. Conversely, ‘Cheongchima’ and ‘Cheongchuckmyeon’ had shorter shelf-life when grown under excess water with 50% shading condition compared to control. It is assumed that there is a relationship of shelf-life with chlorophyll content of the cultivars (Agüero et al., 2008; Lee et al., 2008a). However, we cannot provide evidence of this due to lack of a pigment content analysis. The shelf-life of lettuce tended to be shorter in the presence of high chlorophyll (Lee et al., 2008a). However, broccoli florets tended to be increased in the presence of chlorophyll (Shi et al., 2016). This seems plausible, but requires further experimentation. These findings are in agreement with previous reports on vegetable quality is influenced by excess water supply, growing conditions, and combinations of other factors, including genetic factors and environmental conditions during storage (Beverly et al., 1993, Magwaza et al., 2017).

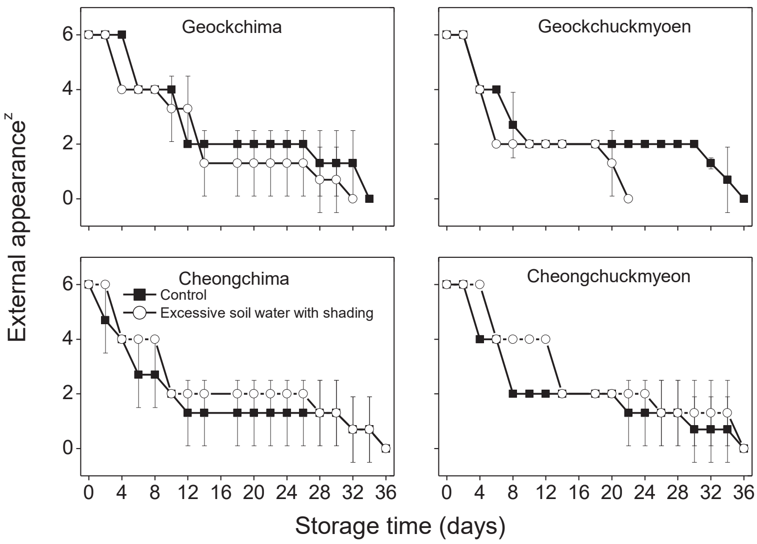

Figure 5. Effects of cultivation under excessive soil water, shading, and control conditions on the external appearance of the four lettuce cultivars grown in a greenhouse and stored at 5 °C. External appearance: 6 = very good, 4 = good, 2 = poor, 0 = very poor. Values are means ± standard errors (n = 4).

CONCLUSIONS

The aim of this study was to determine the effects of growing conditions, including excess soil water with 50% shading and cultivars on postharvest quality of leaf lettuce. The crossed design allowed separation of the effects of these two sets of factors and showed that conditions affected the postharvest qualities of the lettuce. Plant height, plant weight and number of leaves were affected by growing conditions and by cultivars; leaf thickness was affected by cultivars. Weight loss during storage was accelerated in three of the four cultivars (the exception was 'Geockchima'). Similarly, respiration rate was elevated in plants of three cultivars subjected to excess watering and 50% shading during cultivation. However, color of 'Cheongchima' and 'Cheongchuckmyeon' during storage was not markedly changed when plants grown in excess watering with 50% shaded compared to controls. It could be concluded that leaf lettuce should be grown in normal growing condition for getting higher yield, but for extending postharvest storage days with marketability; they should be grown under excess soil water with 50% shading conditions.

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