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**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE**

**Maximization of Yearly Production of Phytochemicals  
in Kale by Determining Adequate Cultivation Period  
in Plant Factories**

식물공장에서 적정 재배기간 결정을 통한 케일 내  
기능성 성분의 연간생산량 극대화

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UNDER THE DIRECTION OF DR. JUNG EEK SON  
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL  
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# **Maximization of Yearly Production of Phytochemicals in Kale by Determining Adequate Cultivation Period in Plant Factories**

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## **ABSTRACT**

To produce kale as a raw material for processed goods in plant factory, adequate cultivation period was determined in order to maximize yearly production of phytochemicals in this study. Two kale cultivars, ‘Manchoo collard’ and ‘Jangsoo collard’, were cultivated in plant factory modules. Every week from 2 to 7 weeks after transplanting, the plants were harvested and the growth, total phenolic compound, total flavonoid compound, antioxidant capacity, and glucosinolate concentration were analyzed. Yearly production was estimated with dry weight, phytochemical concentration, planting density, and cultivation cycle per year. In addition, the change in production with cultivation period was modeled. Production of total phenolic compound in ‘Manchoo collard’ was maximized with 37 days of one cultivation period and other phytochemical production with 42 days. The yearly production of phytochemicals in ‘Jangsoo collard’ showed the greatest when one cultivation period was 42 days. These results can be useful to make operational strategies in plant factories.

*Additional key words:* Phenolic compound, Flavonoid, Growth stage, Manchoo collard, Jangsoo collard, Glucobrassicin, Glucoiberin, Sinigrin

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## GENERAL INTRODUCTION

*Brassica* vegetables have lots of helpful phytochemicals for human health (Manchali et al., 2012). Among them especially kale (*Brassica oleracea* Acephala Group) contains high vitamin and mineral contents (Hertog et al., 1992) and lots of flavonoid compound (Bilyk and Sapers, 1985). In addition beneficial effects by consuming kale have been reported. High folic acid contents could decrease homocysteine concentration in blood so that cardiovascular disease could be prevented (Bendich, 2004; Gordon, 2012). Also antimutagenic effect (Wattenberg, 1977), decrease in cholesterol concentration in blood (Chung et al., 2005), and decrease in peroxide lipid concentration (Djoussé et al., 2004) were reported. For those health beneficial effect, kale is used for ingredient of green juice (Chung et al., 1999). Consuming kale juice also can contribute to reduce the risk of coronary diseases (Kim et al., 2008).

However quality of raw plant materials produced in fields or greenhouses can be affected by seasonal change, varying growth region, and different growth years (Yeh et al., 2007). It is hard to get reliable quality of product if materials have variations on their qualities. With plant factories (closed plant production systems) where growth environments are controlled, these variations during cultivation can be reduced and stable year-round production is available. In previous research, how environmental factors could affect the growth and phytochemicals of plants under controlled environment was reported. In the case of kale, effects of temperature

(Lefsrud et al., 2005), light intensity (Lefsrud et al., 2006), light quality (Lefsrud et al., 2008), and light period (Lefsrud and Kopsell, 2006) were studied.

It is important to control environmental factors in plant factories, however to determine adequate cultivation period is important as well from the point of year-round production. Therefore, this study was carried with kale cultivated in plant factory, and growth and phytochemical of kale analyzed with cultivation period so that adequate cultivation period to maximize yearly production could be determined.

## LITERATURE REVIEW

### *Phytochemicals in kale*

Consumption of vegetables and fruits that have plenty of antioxidants is reported to have health promoting effects like prevention of chronic disease (Finley et al., 2011; Wang et al. 2011). *Brassica* family especially is rich in phytochemicals which can promote human health (Jahangir et al., 2009) and antioxidants activity (Podsdek, 2007). Consuming *Brassica* vegetables is believed to prevent cancer and cardiovascular diseases (Beecher, 1994; van Poppel et al., 1999; Cohen et al., 2000; Wang et al., 2004). Antioxidants activity of *Brassica* attributes its phenolic compounds and some flavonoids (Galati and O'Brien, 2004). Also glucosinolates and their degradation products in *Brassica* are studied to possess anti-cancer activities (Traka and Mithen, 2009; Verkerk et al., 2009).

Among *Brassica* vegetables, kale (*Brassica oleracea* L. var. *acephala*) especially has plentiful phenolic and flavonoid compounds (Hertog et al., 1992; Cao et al., 1996) and glucosinolates (Ayaz et al., 2006). Most of glucosinolate and their hydrolysis showed anti-cancer effects, such as hydrolysis of sulforaphane (Li et al., 2011), glucobrassicin (Kim and Milner, 2005; Brew et al., 2009), and sinigrin (Smith et al., 1998). However, progoitrin can cause goiter (Liu et al., 2012). Therefore, not only total glucosinolate amount but also individual glucosinolate amount should be considered during cultivation (Ávila et al., 2014). Moreover, to

take fully advantage of these phytochemicals in vegetables, proper cultivation techniques should be adapted to increase its utility (Parr and Bolwell, 2000).

#### *Effects of environmental and cultivation condition*

Phytochemicals in plant are affected by environmental and cultivation conditions. For example, antioxidants concentration in vegetables is affected by environmental changes (Aires et al., 2011). Also, the glucosinolate contents in plants (Farnham et al., 2004) are effected by season and sulphur fertilization (Vallejo et al., 2003), light intensity and photoperiod (Charron and Sams, 2004), and soil and temperature (Velasco et al., 2007). For stable production of the phytochemicals in plants, environmental factors should be stably maintained. Plant factories with artificial lights can efficiently adjust the environmental factors regardless of outside conditions, and thereby the quality of plants can be controlled. In terms of time and space, cyclic cultivations from transplanting to harvest is suitable for efficient mass production in plant factories. In addition, growth stage of plants also affect the antioxidant potency (Šamec et al., 2011) and the glucosinolate contents in vegetables (Velasco et al., 2007). Therefore the cultivation period of one cycle is an important factor affecting total productivity in the plant factory.

#### *Determining adequate harvest time*

Studies on determining adequate harvest time were conducted with wormwood (Kim et al., 2013) and strawberry (Winardiantika et al., 2015) but these studies

carried on field so seasonal changes were main factors that affect its productivity. For life support system, adequate planting density and harvest time were determined to maximize the production of cowpea (Ohler et al., 1996). However, this study just focused on eatable fresh weight without nutritional effect. In transgenic plant for vaccine production, effects of light intensity and planting density were studied (Okamura et al., 2014b). In high planting density, production per plant was low but production per plant was high. Also, in optimal planting density that could avoid mutual shading, optimal harvest time was determined for maximizing yearly production in closed plant production system (Okamura et al., 2014a) but continuous model with cultivation cycle wasn't concluded. Therefore model to predict yearly production change with one cultivation period need to be developed.

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## **CHAPTER I**

# **Determination of Harvest time To Maximize Yearly Production of Antioxidants in Kale Cultivated in Plant Factories**

### **ABSTRACT**

Since kale (*Brassica oleracea* L. var. *acephala*) has plenty of phenolic compounds and flavonoids with high antioxidant capacity, lots of positive effects on human health by taking kale juice have been reported. For stable supply of kales not being affected by seasonal change or regional variation, more systematic cultivation methods such as plant factory are required. Plant factories can guarantee reliable productions by entire controls of indoor environments. The objectives of this study were to figure out the phase of the concentration or amount of major phytochemicals in kale and determine an optimal cultivation period for maximum yearly production. Two cultivars, 'Manchoo collard' and 'Jangsoo collard', were cultivated in a plant factory. The plants were thinned to avoid mutual shadings and harvested every week from 2 to 7 weeks after transplanting in order to measure the fresh weight and projected area. Whole harvested samples were freeze dried. Total phenolic compound, total flavonoid compound, and antioxidant capacity were analyzed. Yearly production was calculated with the formula: (dry weight × phytochemical concentration × planting density × cultivation cycle per year).

Continuous phase of yearly production was obtained by modelling and optimal cultivation period was determined for maximum yearly production. The fresh weights of both cultivars exponentially increased and the projected area hardly increased 35 days after transplanting (DAT). The TPC concentration showed fluctuations but the TFC concentration and antioxidant capacity slightly decreased and increased, respectively. The yearly production model of TPC in 'Manchoo collad' showed local maximum when one cultivation period was DAT 35 to 42. Considering other antioxidants, the yearly antioxidants production in kale will be optimal when one cultivation period is DAT 42.

***Additional key words:*** Flavonoid, Growth stage, Manchoo collard, Jangsoo collard, Phenolic compound

## INTRODUCTION

Consuming vegetables and fruits which are rich in antioxidants is reported to have health promoting effects like prevention of chronic disease (Finley et al., 2011; Wang et al. 2011). *Brassica* family especially has high antioxidants activity (Podsdek, 2007), consuming *Brassica* vegetables has anti-cancer effects (Beecher, 1994; van Poppel et al., 1999; Wang et al., 2004). Antioxidants activity of *Brassica* attributes its phenolic compounds and some flavonoids (Galati and O'Brien, 2004). Among *Brassica*, kale (*Brassica oleracea* L. var. *acephala*) is known for plentiful phenolic and flavonoid compounds (Hertog et al., 1992; Cao et al., 1996). To take fully advantage of these antioxidants in vegetables, proper cultivation techniques should be adapted to increase its utility (Parr and Bolwell, 2000).

However, antioxidants concentration in vegetables is affected by environmental changes (Aires et al., 2011), so it is difficult to stably supply of antioxidants in vegetables through current cultivation in field or greenhouse. If environmental factors are fully adjusted, more reliable productions can be expected. Plant factories with artificial lights, which are closed plant production systems, can fully adjust environmental factors regardless of outside conditions and efficient cyclic cultivations from transplanting to harvest are available for maximizing mass productions. Growth stage of plants also affect the antioxidant potency of vegetables (Šamec et al., 2011), therefore the cultivation period of one cycle is an important factor affecting total productivity in the plant factory.

The objectives are to figure out the phase of antioxidants concentration and their amounts in kale cultivated in a plant factory with cultivation day and to determine an optimal cultivation period maximizing the yearly antioxidants production.

## MATERIALS & METHODS

### *Plant materials and growth conditions*

Two kale cultivars, ‘Manchoo collard’ (*Brassica oleracea* Acephala Group Manchoo collard, Asia Seed Company, Seoul, Korea) and ‘Jangsoo collard’ (*Brassica oleracea* Acephala Group Jangsoo collard, Asia Seed Company, Seoul, Korea), were cultivated in a plant factory module of Seoul National University. The seeds were sowed in deep flow systems under fluorescent lamps at a photosynthetic photon flux density (PPFD) of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After normal leaves appeared, nutrient solutions developed by National Institute of Horticultural and Herbal Science (Choi et al., 2005) for *Brassica* was applied with an electrical conductivity (EC) of  $0.6 \text{ dS m}^{-1}$ . Two weeks after germination, kale sprouts were transplanted in the deep flow systems under light-emitting diodes (LEDs) (Red:Blue=8:2, PPFD=200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the same nutrient solutions with an EC of  $1.2 \text{ dS m}^{-1}$  were applied. For 16 hours of light period, temperature, relative humidity, and  $\text{CO}_2$  concentration were maintained at  $24^\circ\text{C}$ , 70%, and  $1000 \mu\text{mol mol}^{-1}$ , respectively. For 8 hours of dark period, only temperature was adjusted to  $20^\circ\text{C}$ . The plants were thinned to avoid mutual shadings because plant competition increases primary metabolites for rapid growth (Kozuka, 2005). From 2 to 7 weeks after transplanting, sampling was conducted every week and the samples were analyzed.

### *Growth analysis*

Images of the harvested kales were taken with a scale from vertical top view and image-analyzed with ImageJ 1.49 (Abràmoff et al., 2004; Schneider et al., 2012) to calculate projected area. Fresh weights were measured and also dry weight contents were measured after the whole leaves were freeze-dried.

### *Antioxidants analysis*

Because antioxidant concentration is different by leaf position and age, whole leaves were freeze-dried and milled and the samples were assumed to be uniform. Each sample of 100 mg was extracted with methanol 1 mL. Total phenolic compound was analyzed with Folin-ciocalteu procedure (Ainsworth and Gillespie, 2007). After incubated 48 hours in dark condition with room temperature, the samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 50  $\mu$ L was collected to 2 mL micro tube, and 10% Folin – cioalteu solution 750  $\mu$ L and distilled water 135  $\mu$ L were added. After vortexing, 600  $\mu$ L 700 mM  $\text{Na}_2\text{CO}_3$  was added and incubated 2 hours with room temperature. Absorbance in 765 nm was measured with a spectrophotometer (Photolab 6100vis, WTW, Germany). A standard curve was obtained with gallic acid and the results were expressed in mg gallic acid equivalent/g dry weight.

Total flavonoid compound was analyzed with Aluminum chloride colorimetric (Dewanto et al., 2002; Lee et al., 2012). After incubated 12 hours in dark condition with 4°C, samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 150

$\mu\text{L}$  was collected to 2 mL micro tube, and distilled water 135  $\mu\text{L}$  and  $\text{NaNO}_2$  45  $\mu\text{L}$  were added. After 5 min, 10%  $\text{AlCl}_3$  90  $\mu\text{L}$  was added and incubated for 6 min. 1 M  $\text{NaOH}$  300  $\mu\text{L}$  and distilled water 165  $\mu\text{L}$  were also added and after 5 min incubating, absorbance in 510 nm was measured with the spectrophotometer. A standard curve was obtained with catechin acid and the results were expressed in mg catechin equivalent/g dry weight.

Antioxidant capacity was analyzed with DPPH assay (Brand-Williams et al., 1995; Andarwulan et al., 2010). DPPH solution was prepared with methanol 100 mL and DPPH 24 mg. After incubated 48 hours in dark condition with room temperature, samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 150  $\mu\text{L}$  was collected to 2 mL micro tube, DPPH solution 1.35 mL was added. After 30 min incubating, absorbance in 517 nm was measured for samples and zero cell with the spectrophotometer. A standard curve was obtained with ascorbic acid and results were expressed in mg ascorbic acid equivalent antioxidant capacity/g dry weight.

#### *Calculation of yearly production estimation*

Measured dry weight and antioxidant concentration were used to calculate antioxidants per plant (=dry weight  $\times$  antioxidant concentration). In addition, a logistic model (Tsoularis and Wallace, 2002), which are often used for explanation of plant growth was tested.

$$y = \frac{1}{1/u + a*bt} \quad (\text{Eq. 1})$$

Also cultivatable cycles per year and planting density were used to calculate the number of plants in unit area per year (= cycle per year  $\times$  plant density). To check continuous phase of the number of plant by cultivation period, regression analysis was conducted. Models: linear, logarithmic, inverse, quadratic, cubic, power, compound, S-curve, growth, and exponential were tested.

Using antioxidants per plant and the number of plants per year, yearly antioxidant production per unit area could be estimated. Static analysis was conducted with SPSS (SPSS Statistics 23, IBM, USA).

## RESULTS & DISCUSSION

### *Plant growth*

Both cultivars of ‘Manchoo collad’ and ‘Jangsoo collad’ showed exponential increases in fresh weights (Fig. 1A) and dry weights (Fig. 1B) during cultivation. These results are corresponded with the exponential increases shown in the previous plant growth models (Cho and Son, 2005; Cho et al., 2015; Yan et al., 2004). Due to their rapid increases, the fresh and dry weight at DAT 42 was about 30 times of those at DAT 14, implying that the plant growth could be the most sensitive factor to estimate yearly production.

When thinning was applied to avoid mutual competition, projected areas of both cultivars increased till DAT 35 and were not changed a lot after that (Fig. 2). The fresh weight increased till DAT 42 but the projected area increased till DAT 35 by mutual competition of neighboring plants. The use efficiencies of cultivation area were the highest at DAT 35 in Manchoo collad and DAT 49 in Jangsoo collad.

### *Antioxidant concentration and amount per plant*

The TPC concentrations decreased with fluctuation during cultivation and the difference between the lowest and highest concentrations was about twice for both cultivars (Fig. 3A). The TFC concentrations of both cultivars were the highest at DAT 14 and slightly decreased to the half during cultivation (Fig. 3B). Similarly, decreases in TPC concentration during cultivation were also reported in kale (Lee and Oh, 2015) and lettuces (Lee et al., 2014) cultivated in plant factory. The

antioxidant capacity of both cultivars showed similar levels during cultivation (Fig. 3C). In overall, antioxidant concentrations were not changed during cultivation and the reason could be that the kale were cultivated in plant factory with stable environment control. Flavonoid contents in wormwood and antioxidant phytochemicals in strawberry were strongly affected by harvest time in greenhouse cultivation (Kim et al., 2013; Winardiantika et al., 2015).

After regression analysis, the model parameters and coefficients of determination of each antioxidant amount in Eq. 1 was obtained (Table 1). The  $R^2$  for TFC amount in 'Jangsoo collard' was the lowest value as 0.78 and the others showed higher than 0.8. All the antioxidant amounts per plant increased during cultivation (Fig. 4). Even though the antioxidant concentrations were not increased during cultivation, the antioxidant amounts per plant increased with a logistic model because of the increase in plant weight. In vaccine production using transgenic lettuces, Okamura et al. (2014a) reported that total soluble protein and target protein concentration decreased but the amounts per plant increased when cultivation period became longer. In both cases, plant dry weight was an important factor.

#### *Planting density and the number of plants per year.*

As the cultivation period became longer, the planting density exponentially decreased (Fig. 5A). Because the planting density directly affect the productivity by mutual shadings in restricted cultivation areas, the weight per plant was lower but

the weight per area was higher in higher planting density (Ohler et al., 1996; Cho and Son, 2005; Kim et al., 2013; Okamura et al., 2014b).

By using planting density and cultivation cycle, the number of plant per square meter in a year could be estimated (Fig. 5B). Among the models, Eq. 2 showed the highest R<sup>2</sup> (Table 2). As a cultivation period became shorter, the planting density and cultivation cycle increased and subsequently the number of plants increased. Assuming that the productivity is the same regardless of cultivation period, a shorter cultivation period, requiring more costs of seeds and labors, would be less advantageous from economic aspects. Similar results transgenic lettuce were discussed by Okamura et al.(2014b).

$$y = e^{a+b/t} \quad (\text{Eq. 2})$$

#### *Yearly productions per unit area*

Yearly productions of TPC, TFC, and antioxidant capacity showed two to threefold differences with cultivation period (Fig. 6). The TPC production in ‘Manchoo collard’ showed three different patterns with cultivation period per cycle (Fig. 6A). The first decrease occurred at DAT 14 to 16 because of decreasing planting density. The increase from DAT 17 was attributed to the exponential growth. The second decrease started around DAT 17 because of a few number of cultivation cycle due to the long cultivation period. Therefore, the yearly production of TPC in ‘Manchoo collard’ could be maximized at DAT 35 to 42 for harvest. However the amount of TPC in ‘Jangsoo collard’ and those of the TFC and

antioxidant capacity in both cultivars were saturated at DAT 42 to 49 or increased (Fig. 6B, 6C). The yearly production can be different with cultivar. Okamura et al. (2014a) reported that yearly vaccine production of transgenic lettuces rather decreased when cultivation period was longer than 30 days. Therefore, yearly productions of TPC in ‘Jangsoo collard’ and TFC, antioxidant capacity of both cultivars were expected to decrease following the trend of TPC in ‘Manchoo collard’ when one cultivation period would longer than 49 days.

## CONCLUSIONS

The phases of the concentration or amount of phytochemicals in kale were investigated and adequate cultivation periods for maximum yearly production were determined. The TPC and TFC concentrations for both cultivars decreased and the antioxidant capacity concentration was not significantly changed during cultivation. However, the increase in plant weight increased the amount of each antioxidant with logistic patterns. Considering planting density and cultivation cycles per year, the longer cultivation period did not always bring the more yearly-production. The adequate cultivation period was DAT 35 to 42 for TPC in 'Manchoo collard' and DAT 42 to 49 for the other antioxidants in both cultivars. These results can be applied to make operational strategies in plant factories.

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Table I-1. Parameters determined by regression analysis for antioxidant amounts per plant in Eq. 1 and their coefficients of determination.

Substance	Cultivar	u	a	b	R <sup>2</sup>
Total phenolic compound	Manchoo collard	350	4.36	0.79	0.91
	Jangsoo collard	730	3.12	0.83	0.83
Total flavonoid compound	Manchoo collard	280	2.53	0.85	0.84
	Jangsoo collard	260	2.01	0.85	0.78
Antioxidant capacity	Manchoo collard	400	2.37	0.84	0.87
	Jangsoo collard	500	7.88	0.82	0.90

Table I-2. Parameters determined by regression analysis for number of plants per year in Eq. 2 and their coefficients of determination.

Cultivar	a	b	R <sup>2</sup>
Manchoo collard	1.41	61	0.93
Jangsoo collard	1.27	64	0.97

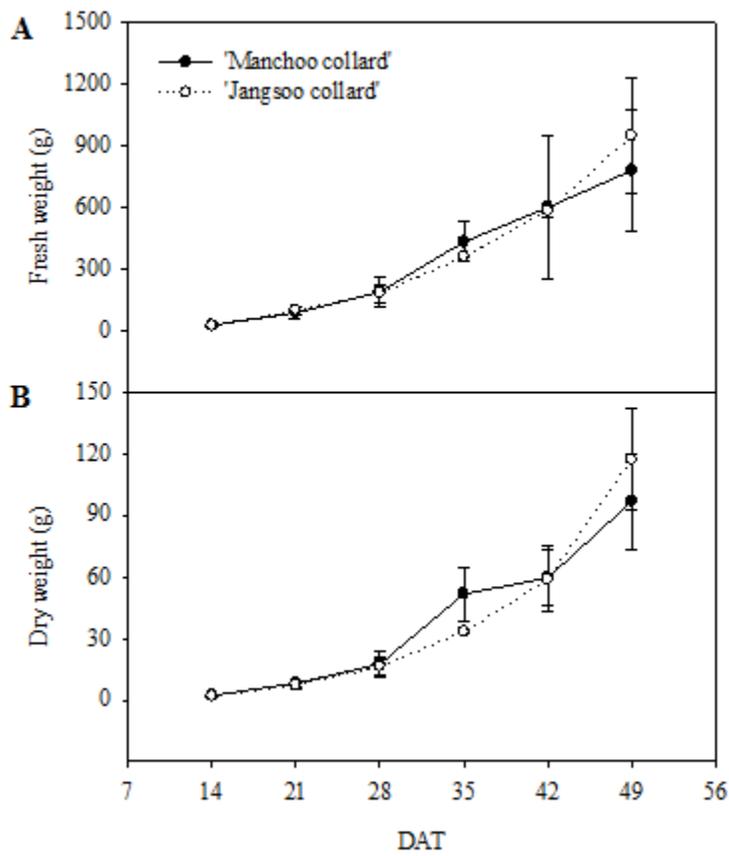


Fig. I-1. Exponential increases in fresh (A) and dry (B) weights of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

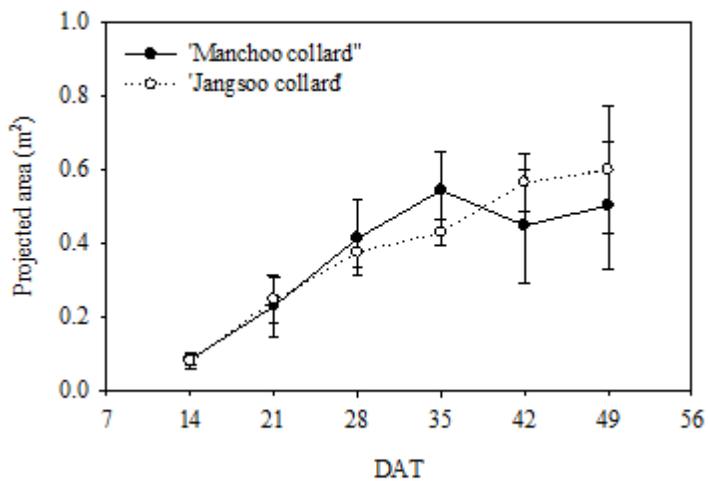


Fig. I-2. Increases in projected areas of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

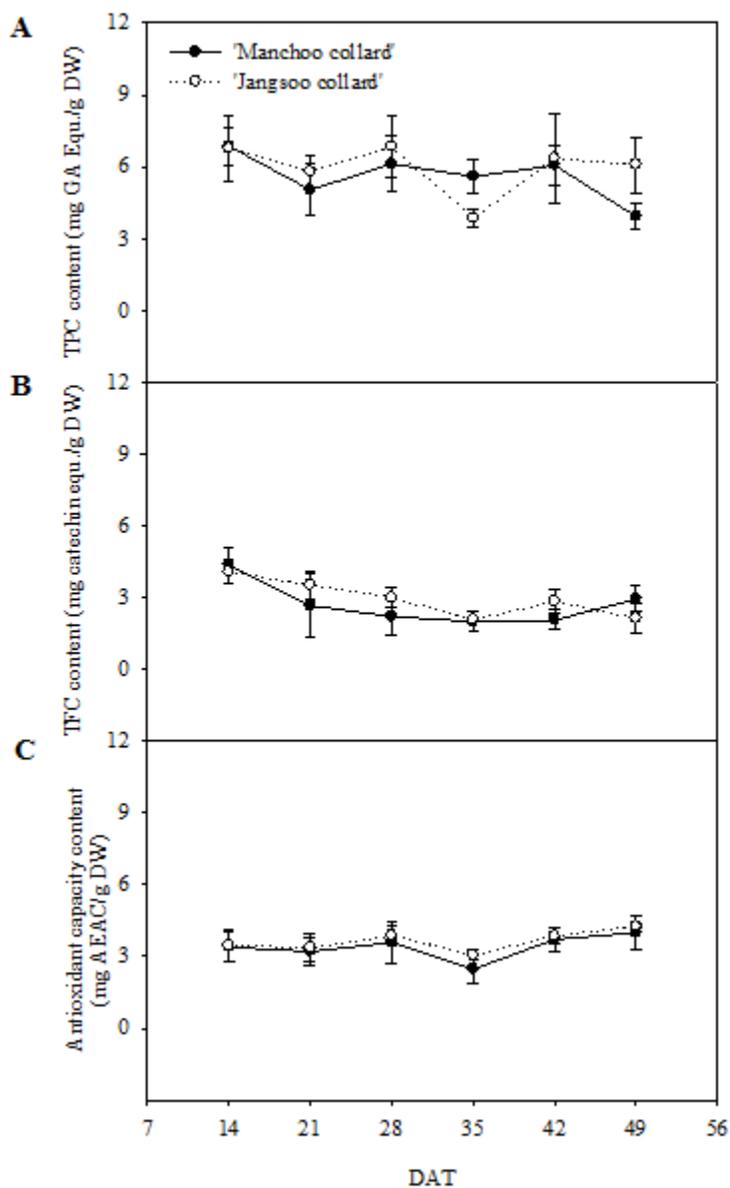


Fig. I-3. Changes in total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity (C) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

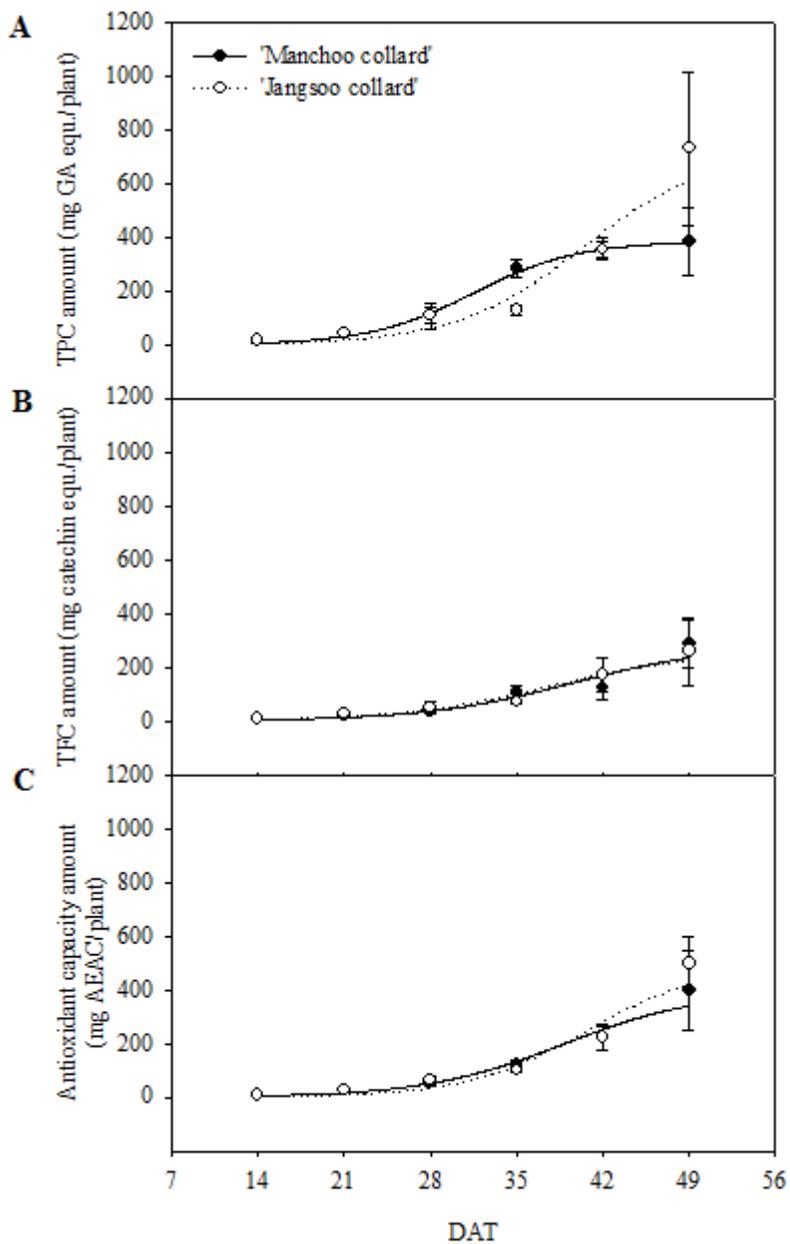


Fig. I-4. Change in total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity per plant (C) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

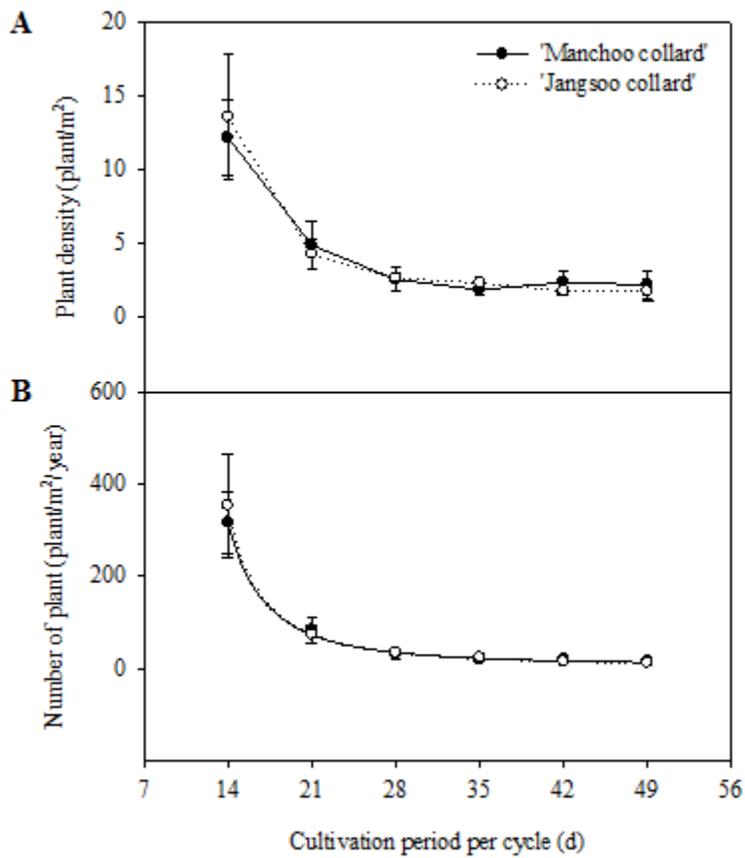


Fig. I-5. Changes in plant density (A) and the number of plants (B) of 'Manchoo collard' and 'Jangsoo collard' by cultivation period per cycle.

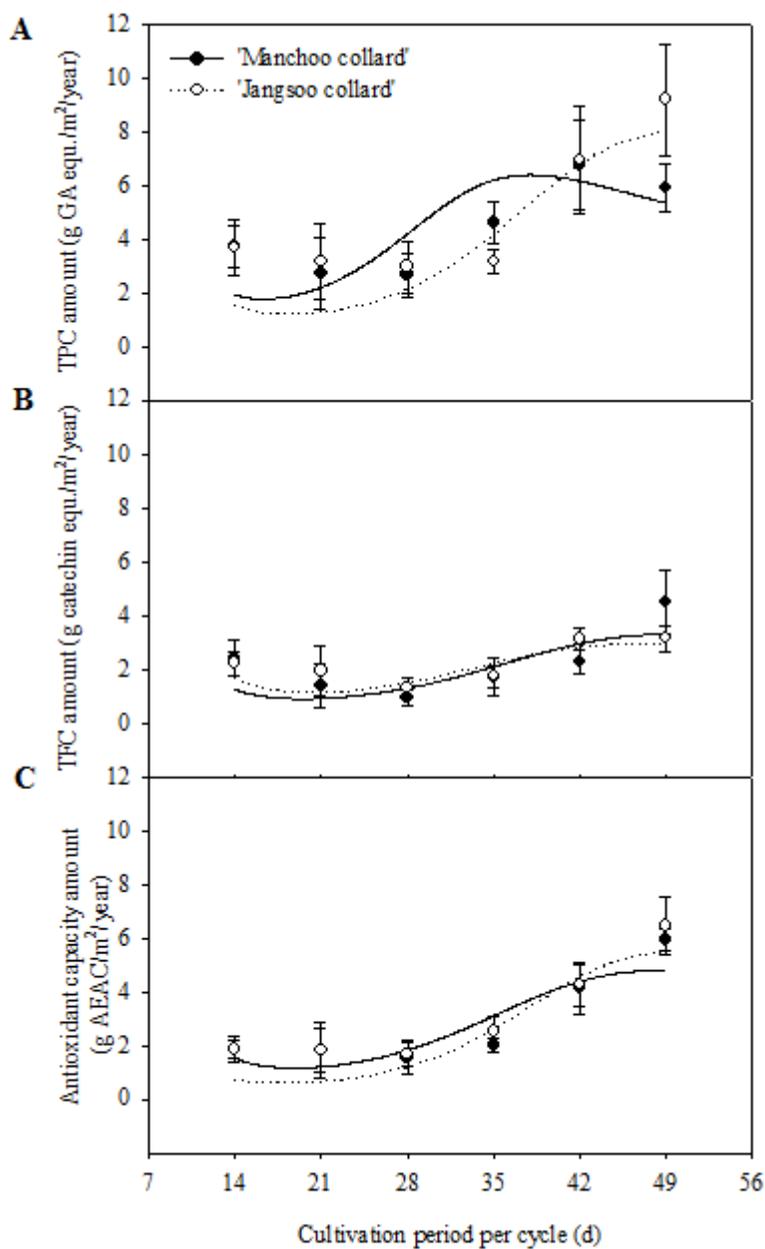


Fig. I-6. Changes in yearly productions of total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity (C) of 'Manchoo collard' and 'Jangsoo collard' by cultivation period per cycle.

## CHAPTER II

### Maximization of Yearly Production of Glucosinolate in Kale by Adequate Harvest Timing in Plant Factories

#### ABSTRACT

*Brassica* vegetables are known for its sulfur containing metabolite, glucosinolate. It has been reported that hydrolyzed glucosinolate has an anti-cancer effect. Kale (*Brassica oleracea* L. var. *acephala*) has high contents of glucosinolate. To grow and supply kales not affected by climate change, more efficient environment controls are required. Plant factory, a closed plant production system, could be one of the efficient alternatives. The objectives of this study were to analyze the glucosinolate concentration and its amount in kale and to determine an adequate cultivation period for maximizing its yearly production. Two cultivars, 'Manchoo collard' and 'Jangsoo collard' grown in a plant factory were harvested every week from 2 to 7 weeks after transplanting. In order to prevent mutual shadings among the plants, thinning was conducted. The plant growth and diameter of projected area were measured. Whole harvested samples were freeze dried. Glucosinolate were analyzed with HPLC. Yearly production was calculated as follows: dry weight  $\times$  glucosinolate concentration  $\times$  planting density  $\times$  cultivation cycle per year. A model for estimating yearly production with harvest time was

developed and an adequate harvest time was determined for maximizing the yearly production of glucosinolate. The dry weights of both cultivars exponentially increased and the projected diameter hardly increased until 35 days after transplanting. The total glucosinolate concentration increased with days after transplanting. And glucoiberin, sinigrin, and glucobrassicin were the major component of glucosinolate. Maximum total glucosinolate could be produced with 42 days of cultivation period and the content of glucoiberin was the highest of the glucosinolate.

***Additional key words:*** Glucobrassicin, Glucoiberin, Growth stage, Jangsoo collard, Manchoo collard, Sinigrin

## INTRODUCTION

Consumption of *Brassica* vegetables is believed to prevent cancer and cardiovascular diseases (Cohen et al., 2000). Because *Brassica* vegetables have a plenty of phytochemicals which can promote human health (Jahangir et al., 2009), glucosinolates and their degradation products are studied to possess anti-cancer activities (Traka and Mithen, 2009; Verkerk et al., 2009). Among *Brassica* vegetables, kale (*Brassica oleracea* L. var. *acephala*) is especially rich in glucosinolates (Ayaz et al., 2006). Most of glucosinolate and their hydrolysis showed anti-cancer effects, such as hydrolysis of sulforaphane (Li et al., 2011), glucobrassicin (Kim and Milner, 2005; Brew et al., 2009), and sinigrin (Smith et al., 1998). However, progoitrin can cause goiter (Liu et al., 2012). Therefore, not only total glucosinolate amount but also individual glucosinolate amount should be considered during cultivation (Ávila et al., 2014).

Also environmental and cultivation conditions affect the glucosinolate contents in plants (Farnham et al., 2004), such as season and sulphur fertilization (Vallejo et al., 2003), light intensity and photoperiod (Charron and Sams, 2004), and soil and temperature (Velasco et al., 2007). For stable production of the glucosinolates in plants, environmental factors should be stably maintained. Plant factories with artificial lights can efficiently adjust the environmental factors regardless of outside conditions, and thereby the quality of plants can be controlled. In terms of time and space, cyclic cultivations from transplanting to harvest is suitable for efficient mass

production in plant factories. In fact, glucosinolate contents in vegetables are changed with growth stage (Velasco et al., 2007), so a cultivation period (harvest time) is an important factor affecting total productivity in plant factories.

The objectives of this study were to analyze total and individual glucosinolates in kale with time and to determine an optimal cultivation period for maximizing the yearly glucosinolate production in plant factories.

## MATERIALS & METHODS

### *Growth conditions and analysis*

Manchoo collard (*Brassica oleracea* Acephala Group Manchoo collard, Asia Seed Company, Seoul, Korea) and Jangsoo collard (*Brassica oleracea* Acephala Group Jangsoo collard, Asia Seed Company, Seoul, Korea), were grown in a plant factory module of Seoul National University. The seeds were sowed in sponge cubes with deep flow technique (DFT) systems under fluorescent lamps. After normal leaves appeared, a modified recipe of nutrient solution for *Brassica* developed by National Institute of Horticultural and Herbal Science (Choi et al., 2005) was applied with EC 0.6 dS m<sup>-1</sup>. Two weeks after germination, kale seedlings were transplanted in the DFT systems under a light intensity of 200 μmol m<sup>-2</sup> s<sup>-1</sup> LEDs (Red:Blue=8:2) and applied the same nutrient solution with EC 1.2 dS m<sup>-1</sup>. During the 16 hours of photoperiod, temperature, relative humidity and CO<sub>2</sub> concentration were maintained at 24°C, 70%, and 1000 μmol mol<sup>-1</sup>, respectively. During dark period, temperature was adjusted to 20°C. The plants were thinned to prevent mutual shadings because mutual competitions among plants increase primary metabolites for rapid growth (Kozuka, 2005). The plants were sampled for analysis every week from 2 to 7 weeks after transplanting.

Images of the sampled plants were taken from vertical top view and calculated projected areas by using ImageJ 1.49 (Abràmoff et al., 2004; Schneider et al., 2012).

### *Sample preparation*

As glucosinolate concentrations are different by leaf position and age (Brown et al. 2003; Velasco et al. 2007), whole leaves were freeze-dried and milled to make them uniform. The glucosinolates were extracted and analyzed as previously described (Sun et al., 2011). Freeze-dried samples (100 mg) were extracted three times with 70% MeOH of 2 mL for 10 min in 80°C. The supernatant was collected after centrifugation (5 min, 3500 rpm). The aqueous extract was applied onto a DEAE–Sephadex A-25 (GE Healthcare, Piscataway, NJ) (70 mg) column (1 mL blue tip). The column was washed two times with 1 mL imidazole formate (6 M) and twice with 1 mL water. The glucosinolates were converted into their desulpho analogues by overnight treatment with 200  $\mu$ L of 1.67 mg mL<sup>-1</sup> sulphatase from *Helix pomatia* (Sigma Aldrich), and the desulphoglucosinolates were eluted with 2×0.75 mL water.

### *HPLC analysis*

HPLC analysis of desulphoglucosinolates was carried out by using an Alliance HPLC System (Model 2695, Waters, USA) equipped with an absorbance detector (Model 2996 PDA, Waters, USA). Samples (20  $\mu$ L) were separated at 30°C on a Waters Spherisorb C18 column using acetonitrile and water at a flow rate of 1.0 mL min<sup>-1</sup>. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min, followed by

isocratic elution with 20% acetonitrile for the final 10 min. Absorbance was detected at 226 nm.

### *Yearly production estimation*

Glucosinolate per plant was calculated by using dry weight and glucosinolate concentration as follows: dry weight  $\times$  glucosinolate concentration. In addition, regression analysis was conducted with a logistic model (Tsoularis and Wallace, 2002) to obtain continuous phase by cultivation day.

$$y = \frac{1}{1/u+a*b^t} \quad (\text{Eq. 1})$$

The number of plant per area in a year was calculated by using cultivatable cycles per year and planting density as follows: cycle per year  $\times$  plant density. To analyze the continuous phase of the number of plants by cultivation period, regression analysis was conducted. Various models such as linear, logarithmic, inverse, quadratic, cubic, power, compound, S-curve, growth, and exponential were compared., Yearly glucosinolate production per unit area could be estimated by using glucosinolate amounts per plant and the number of plant per year. Static analysis was conducted with SPSS (SPSS Statistics 23, IBM, USA).

## RESULTS & DISCUSSION

### *Plant Growth*

Dry weight of both cultivars increased exponentially with DAT (Fig. 1A) and relative water content of both cultivars tended to decrease (Fig. 1B). Previous researches of plant growth models showed similar results ( Cho and Son, 2005; Cho et al., 2015; Yan et al., 2004;). Due to rapid increase in dry weight during cultivation, the growth rate of plants sensitively affect the yearly production of target materials. By application of thinning to avoid mutual competition among neighboring plants, maximum projected diameters of both cultivars increased to about 80 cm at DAT 35. Therefore in plant factories where kales were cultivated for more than DAT 35, a planting density of 2 plants / m<sup>2</sup> could be enough to avoid mutual shadings (Fig. 2).

### *Glucosinolate concentration and amount per plant*

With HPCL analysis, 8 kinds of glucosinolate were analyzed (Fig. 3) and the total glucosinolate was calculated by summation of the 8 individual glucosinolates. Total glucosinolate, concentration of both cultivars increased about 3 times from DAT 14 to 42 and decreased slightly after that (Fig. 4). Similar results in total glucosinolate concentration were reported (Lee et al., 2015). Compared with the field cultivation results by Lee et al. (2015), total glucosinolate concentration of 'Manchoo collard' cultivated in plant factory was two times higher in the same

cultivation period. Therefore glucosinolate production was better in plant factories than fields. Among all the individual glucosinolates, glucoiberin, sinigrin, and glucobrassicin were major glucosinolates in both cultivars (Tables 1 and 2). Ratio of glucoiberin to total glucosinolate increased with DAT, while that of glucobrassicin decreased. This result explains that individual glucosinolate was affected by cultivar (Sun et al., 2011; Qian et al., 2015). Therefore cultivars suitable for cultivation purposes should be selected.

The parameters and coefficients of determination for the estimation models of total and each glucosinolate amounts were obtained by regression analysis (Table. 3). The models for total glucosinolate and glucoiberin showed higher  $R^2$  values with more than 0.9, while the models for sinigrin and glucoiberin showed lower  $R^2$  with 0.7 to 0.8. Observed and calculated amounts of glucosinolate increased with time but saturated (Fig. 5). With increase of cultivation period, the amount of glucosinolate per plant increased but its increasing rate decreased. However, in the both cultivars, individual glucosinolate amount varied with its concentration change. These trend was also reported in the vaccine production using transgenic lettuce (Okamura et al., 2014a). Total soluble proteins and target proteins per plant increased when cultivation period became longer followed by growth increase.

### *Planting density and the number of plants per year*

Optimum planting density to avoid mutual shadings was decreased with increase of maximum projected diameter of the plants (Fig. 2). Because the planting density directly affect the growth and total productivity of plants (Ohler et al., 1996; Cho and Son, 2005; Okamura et al., 2014b), in restricted cultivation areas, the weights per plant became lower but those per area became higher at higher planting density. The cultivation cycle per year was calculated by dividing one cultivation period into 365 days. With planting density and cultivation cycle, the number of plants in one square meter per year could be estimated. Among the models, S-curve model (Eq. 2) showed the highest regression coefficient (Table 4).

$$y = e^{a+b/t} \quad (\text{Eq. 2})$$

When one cultivation period becomes shorter, planting density and cultivation cycle increase. Therefore the increase in the number of plants causes much more cost for seeds and labors. Okamura et al. (2014b) recommended that when the productivity is not changed by plant density, the lower planting density has more advantages for economic reasons.

### *Yearly production per unit area*

Yearly productions of the total glucosinolate showed twofold differences with cultivation period (Fig. 6A). Yearly productions of the total glucosinolate increased till 42 days of cultivation period but were not changed after that. Okamura et al.

(2014a) mentioned that yearly vaccine productions of transgenic lettuces decreased at longer than 30 days of the cultivation period. Similarly, yearly productions of total glucosinolates in both cultivars were expected to decrease at longer than 49 days. Glucoiberin and sinigrin production of two cultivars showed similar trends but glucobrassicin production showed rather different trends (Fig. 6B, 6C). That was because the glucobrassicin concentration rapidly decreased with DAT. Therefore adequate cultivation period could be changed with target glucosinolate. In this experiment, 42 to 49 days of cultivation period could be adequate for maximum productions of the yearly total glucosinolate.

## CONCLUSIONS

The time changes in concentration or amount of glucosinolate in two cultivars of kale were analyzed and adequate cultivation periods for their maximum yearly productions were determined. The total glucosinolate concentrations for both cultivars increased with time. Glucoiberin, sinigrin, and glucobrassicin were found to be major glucosinolates in both cultivars. Glucosinoalate amount per plant increased following logistic growth model. Considering the planting density and cultivation cycles per year, the more yearly-production was not always achieved with the longer cultivation period. The adequate cultivation period was DAT 42 to 49 for maximizing total glucosinolate production in both cultivars in plant factories.

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Table. II-1. Changes in individual glucosinolate of ‘Manchoo collard’ with days after transplanting (DAT).

DAT	Gluciberin	Progoitrin	Sinigrin	Glucoraphanin	4-hydroxyl glucobrassicin	Glucobrassicin	4-methoxy glucobrassicin	Neo glucobrassicin
14	0.70±0.13 <sup>z</sup> (17)	0.24±0.09 (6)	1.03±0.27 (25)	0.10±0.04 (2)	0.04±0.01 (1)	1.83±0.41 (44)	0.19±0.05 (5)	0.05±0.02 (1)
21	1.19±0.38 (18)	0.34±0.12 (5)	1.79±0.75 (27)	0.17±0.08 (3)	0.04±0.03 (1)	2.59±0.83 (39)	0.34±0.04 (5)	0.10±0.02 (2)
28	1.82±0.60 (26)	0.38±0.13 (6)	1.85±0.70 (27)	0.20±0.10 (3)	0.14±0.03 (2)	2.11±0.34 (31)	0.29±0.08 (4)	0.09±0.03 (1)
35	2.76±0.59 (34)	0.71±0.18 (9)	2.25±0.62 (28)	0.61±0.16 (8)	0.09±0.01 (1)	1.33±0.28 (17)	0.25±0.03 (3)	0.05±0.02 (1)
42	3.67±0.80 (42)	0.74±0.01 (9)	1.65±0.60 (19)	0.76±0.05 (9)	1.01±0.20 (12)	0.68±0.05 (8)	0.15±0.04 (2)	0.04±0.00 (0)
49	3.16±1.26 (39)	0.65±0.10 (8)	1.98±0.45 (24)	0.74±0.20 (9)	0.58±0.12 (7)	0.85±0.14 (10)	0.14±0.07 (2)	0.01±0.00 (0)

<sup>z</sup>Mean±SD (ratio to total glucosinolate, %).

Table. II-2. Changes in individual glucosinolate of ‘Jangsoo collard’ with days after transplanting (DAT).

DAT	Glucobriferin	Progoitrin	Sinigrin	Glucoraphanin	4-hydroxyl glucobrassicin	Glucobrassicin	4-methoxy glucobrassicin	Neo glucobrassicin
14	0.77±0.16 <sup>z</sup> (19)	0.19±0.06 (5)	0.97±0.23 (24)	0.09±0.03 (2)	0.07±0.01 (2)	1.68±0.57 (42)	0.15±0.03 (4)	0.06±0.03 (2)
21	0.98±0.40 (17)	0.42±0.31 (7)	1.30±0.30 (22)	0.28±0.32 (5)	0.07±0.04 (1)	2.14±0.72 (37)	0.51±0.21 (9)	0.10±0.04 (2)
28	1.60±0.43 (27)	0.30±0.11 (5)	1.44±0.56 (24)	0.16±0.08 (3)	0.13±0.06 (2)	1.86±0.45 (31)	0.35±0.13 (6)	0.12±0.04 (2)
35	2.01±0.85 (25)	0.85±0.19 (11)	1.57±1.53 (20)	1.82±1.08 (23)	0.09±0.09 (1)	1.10±0.20 (14)	0.40±0.03 (5)	0.09±0.06 (1)
42	3.59±0.77 (39)	0.64±0.01 (7)	1.08±0.60 (12)	0.62±0.02 (7)	1.69±0.07 (19)	1.13±0.17 (12)	0.29±0.04 (3)	0.06±0.03 (1)
49	2.46±1.00 (36)	0.69±0.38 (10)	1.85±0.90 (27)	0.71±0.13 (10)	0.46±0.27 (7)	0.58±0.18 (8)	0.09±0.03 (1)	0.01±0.01 (0)

<sup>z</sup>Mean±SD (ratio to total glucosinolate, %).

Table. II-3. Parameters determined by regression analysis for glucosinolate amounts per plant and their coefficients of determination.

Substance	Cultivar	u	a	b	R <sup>2</sup>
Total glucosinolate	Manchoo collad	760	1.13	0.83	0.94
	Jangsoo collad	770	11.4	0.78	0.94
Glucoiberin	Manchoo collad	290	11.7	0.80	0.95
	Jangsoo collad	270	416	0.73	0.90
Sinigrin	Manchoo collad	190	2.32	0.85	0.81
	Jangsoo collad	200	15.6	0.82	0.79
Glucobrassicin	Manchoo collad	80	0.71	0.87	0.73
	Jangsoo collad	60	1.47	0.86	0.81

Table. II-4. Parameters determined by regression analysis for numbers of plant per yearly and their coefficients of determination.

Cultivar	a	b	R <sup>2</sup>
Manchoo collad	1.41	61	0.93
Jangsoo collad	1.27	64	0.97

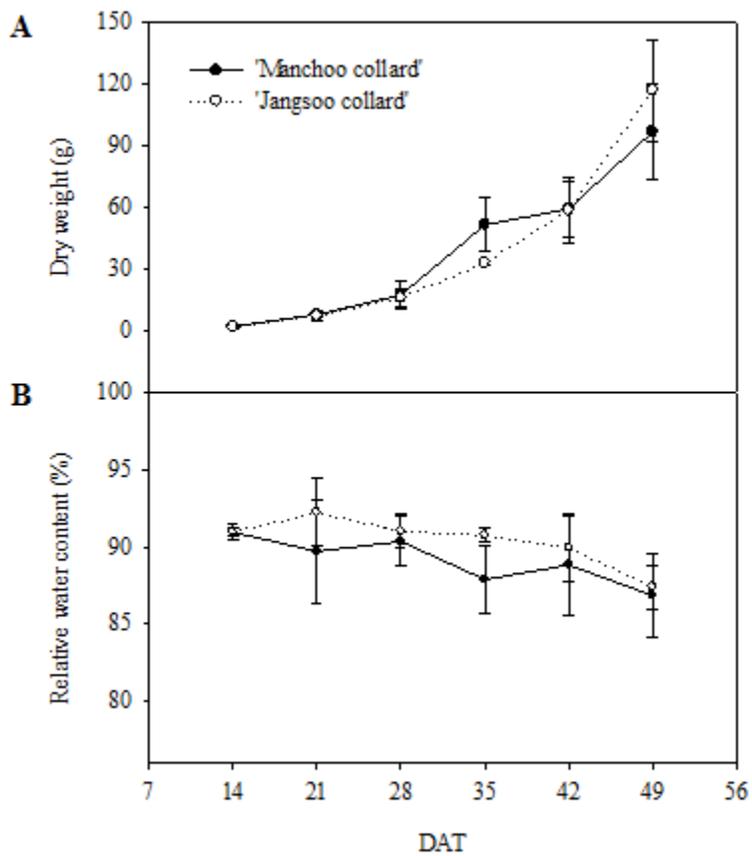


Fig. II-1. Exponential increases in dry weights (A) and their relative water contents (B) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

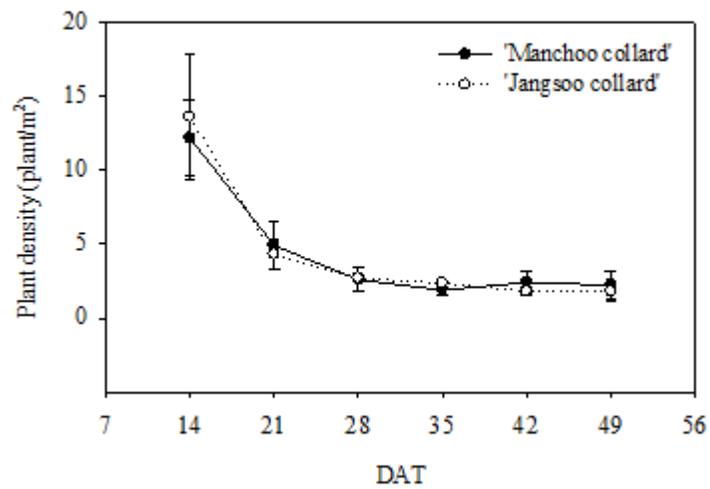


Fig. II-2. Decrease in optimum planting density to avoid mutual shadings of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

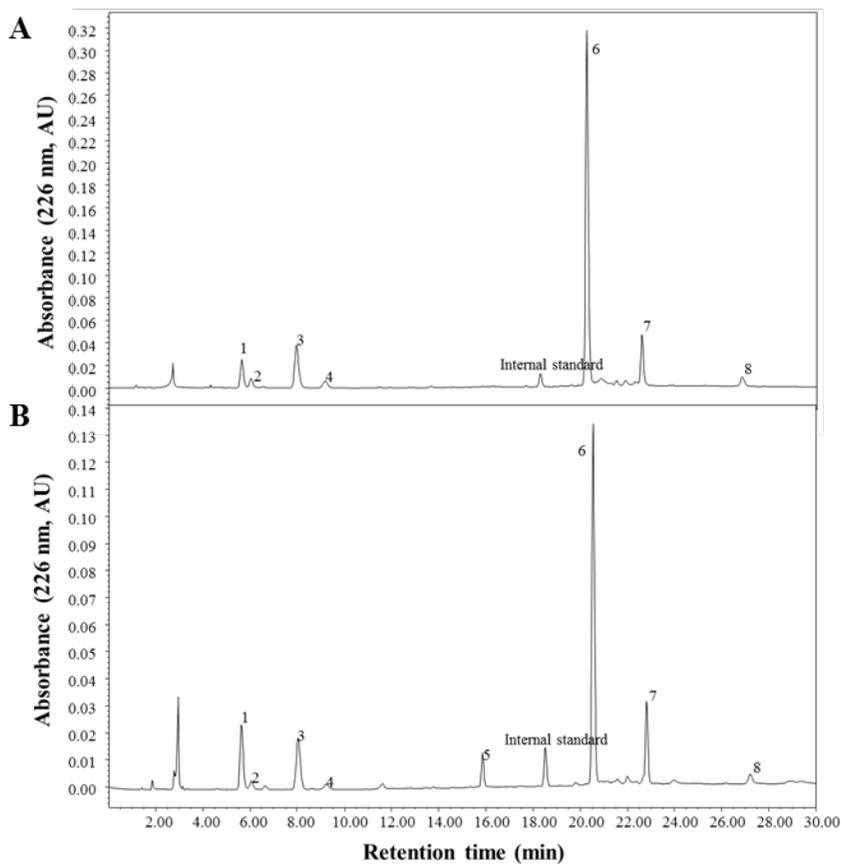


Fig. II-3. Chromatograms of ‘Manchoo collard’ (A) and ‘Jangsoo collard’ (B). Each number indicates that 1. glucoiberin, 2. progoitrin, 3. sinigrin, 4. glucoraphanin, 5. 4-hydroxyl glucobrassicin, 6. glucobrassicin, 7. 4-methoxy glucobrassicin, and 8. neo glucobrassicin

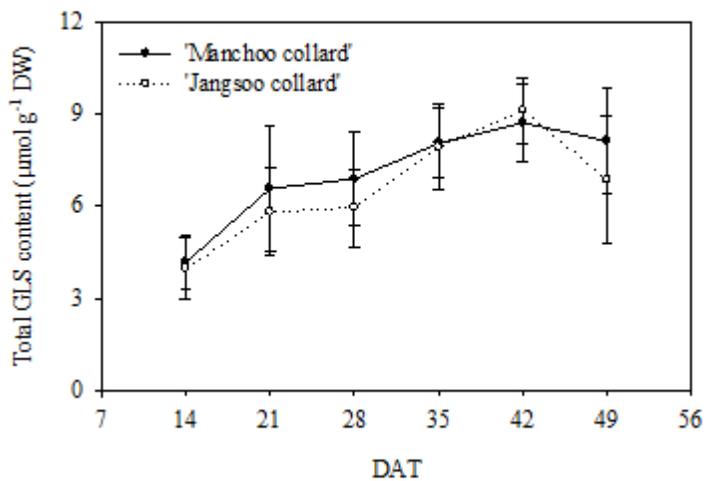


Fig. II-4. Changes in total glucosinolate content (GLS) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting.

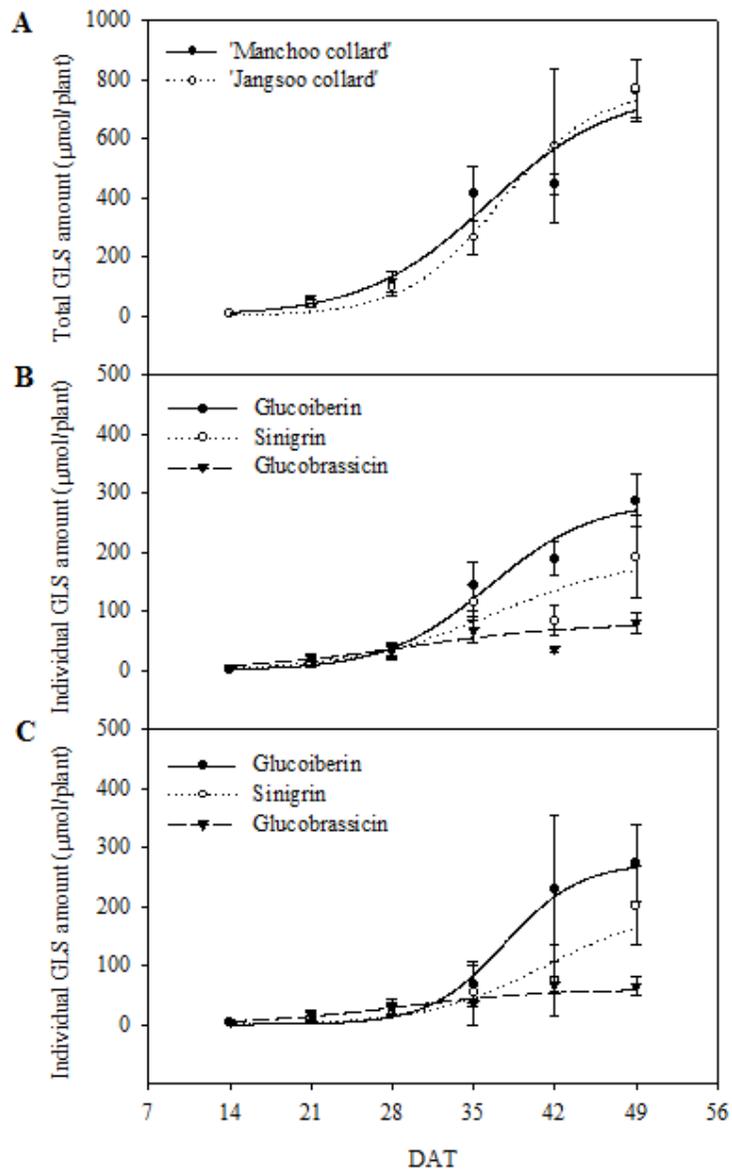


Fig. II-5. Changes in total glucosinolate (GLS) amounts per plant of both 'Manchoo collard' and 'Jangsoo collard' (A), three major GLS amounts per plant of 'Manchoo collard' (B), and 'Jangsoo collard' (C) with days after transplanting (DAT).

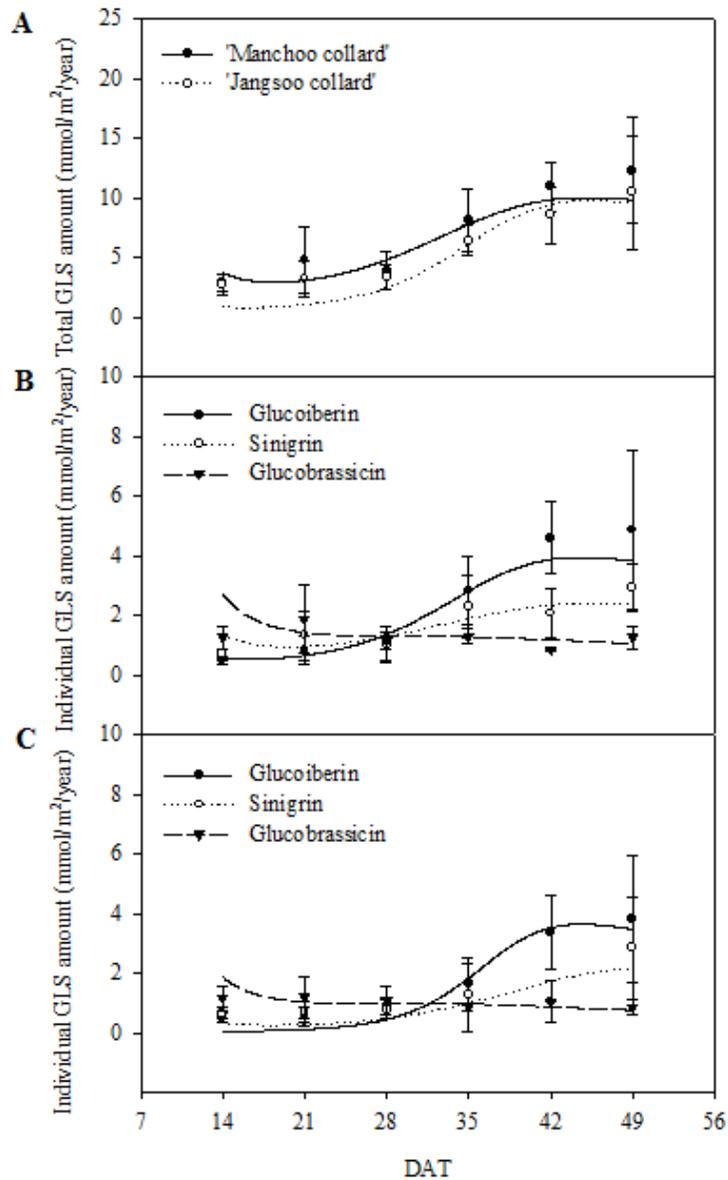


Fig. II-6. Changes in yearly amount of total glucosinolate (GLS) of 'Manchoo collard' and 'Jangsoo collard' (A), yearly amount of three major GLS of 'Manchoo collard' (B), and 'Jangsoo collard' (C) by the harvest time expressed as days after transplanting (DAT).

## CONCLUSIONS

The phases of the concentration or amount of phytochemicals in kale were investigated and adequate cultivation periods for maximum yearly production were determined. The TPC and TFC concentrations for both cultivars decreased and the antioxidant capacity concentration was not significantly changed during cultivation. The total glucosinolate concentrations for both cultivars increased with time. Glucoiberin, sinigrin, and glucobrassicin were found to be major glucosinolates in both cultivars. The increase in plant weight increased the amount of each antioxidant and glucosinolate with logistic patterns. Considering planting density and cultivation cycles per year, the longer cultivation period did not always bring the more yearly-production. The adequate cultivation periods were DAT 35 to 42 for TPC in 'Manchoo collard', DAT 42 to 49 for the other antioxidants in both cultivars, and DAT 42 to 49 for total glucosinolate in both cultivars in plant factories. In overall, 42 days of kale cultivation in plant factory should be proper for its antioxidants and glucosinolates amount. Although in this study, amount of the phytochemicals were considered to determine the adequate harvest time, other viewpoints, such as extraction efficiency or economical cost, need to be considered in further study.

## ABSTRACT IN KOREAN

본 연구에서는 식물공장에서 케일 재배 시, 이차대사산물의 연간생산량을 최대로 하는 수확시기를 결정하고자 하였다. ‘만추콜라드’와 ‘장수콜라드’ 품종을 식물 공장 모듈에서 재배하였다. 정식 후 2 주에서 7 주까지 매주 샘플링하며 생육조사와 총 페놀화합물, 총 플라보노이드, 글루코시놀레이트 농도와 항산화능을 분석하였다. 건물중, 기능성 물질 농도, 재식 간격, 연간 재배 가능 횟수를 이용하여 기능 성분의 연간 생산량을 추정하였고, 재배기간에 따른 생산량 변화를 모델화 하였다. 만추콜라드의 총 페놀화합물 생산량은 1 작기 37 일, 그 외의 물질은 42 일에서 최대값을 나타냈다. 장수콜라드는 전체적으로 45 일에서 최대값을 나타냈다. 본 결과는 식물공장의 연간생산량을 최대로 하는 경제적인 수확시기 결정에 활용될 수 있으리라 기대된다.

주요어: 페놀성 화합물, 플라보노이드, 생육 단계, 만추콜라드, 장수콜라드, 글루코브라시신, 글루코이베린, 시니그린

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**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE**

**Maximization of Yearly Production of Phytochemicals  
in Kale by Determining Adequate Cultivation Period  
in Plant Factories**

식물공장에서 적정 재배기간 결정을 통한 케일 내  
기능성 성분의 연간생산량 극대화

**BY**

**JI-SOO KIM**

**FEBRUARY, 2016**

**MAJOR IN HORTICULTURAL SCIENCE AND  
BIOTECHNOLOGY  
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COLLEGE OF AGRICULTURE AND LIFE SCIENCES  
SEOUL NATIONAL UNIVERSITY**

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UNDER THE DIRECTION OF DR. JUNG EEK SON  
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL  
OF SEOUL NATIONAL UNIVERSITY

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JI-SOO KIM

DEPARTMENT OF PLANT SCIENCE  
COLLEGE OF AGRICULTURE AND LIFE SCIENCES  
SEOUL NATIONAL UNIVERSITY

FEBRUARY, 2016

APPROVED AS A QUALIFIED THESIS OF JI-SOO KIM  
FOR THE DEGREE OF MASTER OF SCIENCE  
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CHANG HOO CHUN, Ph.D

# **Maximization of Yearly Production of Phytochemicals in Kale by Determining Adequate Cultivation Period in Plant Factories**

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## **ABSTRACT**

To produce kale as a raw material for processed goods in plant factory, adequate cultivation period was determined in order to maximize yearly production of phytochemicals in this study. Two kale cultivars, ‘Manchoo collard’ and ‘Jangsoo collard’, were cultivated in plant factory modules. Every week from 2 to 7 weeks after transplanting, the plants were harvested and the growth, total phenolic compound, total flavonoid compound, antioxidant capacity, and glucosinolate concentration were analyzed. Yearly production was estimated with dry weight, phytochemical concentration, planting density, and cultivation cycle per year. In addition, the change in production with cultivation period was modeled. Production of total phenolic compound in ‘Manchoo collard’ was maximized with 37 days of one cultivation period and other phytochemical production with 42 days. The yearly production of phytochemicals in ‘Jangsoo collard’ showed the greatest when one cultivation period was 42 days. These results can be useful to make operational strategies in plant factories.

*Additional key words:* Phenolic compound, Flavonoid, Growth stage, Manchoo collard, Jangsoo collard, Glucobrassicin, Glucoiberin, Sinigrin

Student Number: 2014-20022

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## GENERAL INTRODUCTION

*Brassica* vegetables have lots of helpful phytochemicals for human health (Manchali et al., 2012). Among them especially kale (*Brassica oleracea* Acephala Group) contains high vitamin and mineral contents (Hertog et al., 1992) and lots of flavonoid compound (Bilyk and Sapers, 1985). In addition beneficial effects by consuming kale have been reported. High folic acid contents could decrease homocysteine concentration in blood so that cardiovascular disease could be prevented (Bendich, 2004; Gordon, 2012). Also antimutagenic effect (Wattenberg, 1977), decrease in cholesterol concentration in blood (Chung et al., 2005), and decrease in peroxide lipid concentration (Djoussé et al., 2004) were reported. For those health beneficial effect, kale is used for ingredient of green juice (Chung et al., 1999). Consuming kale juice also can contribute to reduce the risk of coronary diseases (Kim et al., 2008).

However quality of raw plant materials produced in fields or greenhouses can be affected by seasonal change, varying growth region, and different growth years (Yeh et al., 2007). It is hard to get reliable quality of product if materials have variations on their qualities. With plant factories (closed plant production systems) where growth environments are controlled, these variations during cultivation can be reduced and stable year-round production is available. In previous research, how environmental factors could affect the growth and phytochemicals of plants under controlled environment was reported. In the case of kale, effects of temperature

(Lefsrud et al., 2005), light intensity (Lefsrud et al., 2006), light quality (Lefsrud et al., 2008), and light period (Lefsrud and Kopsell, 2006) were studied.

It is important to control environmental factors in plant factories, however to determine adequate cultivation period is important as well from the point of year-round production. Therefore, this study was carried with kale cultivated in plant factory, and growth and phytochemical of kale analyzed with cultivation period so that adequate cultivation period to maximize yearly production could be determined.

## LITERATURE REVIEW

### *Phytochemicals in kale*

Consumption of vegetables and fruits that have plenty of antioxidants is reported to have health promoting effects like prevention of chronic disease (Finley et al., 2011; Wang et al. 2011). *Brassica* family especially is rich in phytochemicals which can promote human health (Jahangir et al., 2009) and antioxidants activity (Podsdek, 2007). Consuming *Brassica* vegetables is believed to prevent cancer and cardiovascular diseases (Beecher, 1994; van Poppel et al., 1999; Cohen et al., 2000; Wang et al., 2004). Antioxidants activity of *Brassica* attributes its phenolic compounds and some flavonoids (Galati and O'Brien, 2004). Also glucosinolates and their degradation products in *Brassica* are studied to possess anti-cancer activities (Traka and Mithen, 2009; Verkerk et al., 2009).

Among *Brassica* vegetables, kale (*Brassica oleracea* L. var. *acephala*) especially has plentiful phenolic and flavonoid compounds (Hertog et al., 1992; Cao et al., 1996) and glucosinolates (Ayaz et al., 2006). Most of glucosinolate and their hydrolysis showed anti-cancer effects, such as hydrolysis of sulforaphane (Li et al., 2011), glucobrassicin (Kim and Milner, 2005; Brew et al., 2009), and sinigrin (Smith et al., 1998). However, progoitrin can cause goiter (Liu et al., 2012). Therefore, not only total glucosinolate amount but also individual glucosinolate amount should be considered during cultivation (Ávila et al., 2014). Moreover, to

take fully advantage of these phytochemicals in vegetables, proper cultivation techniques should be adapted to increase its utility (Parr and Bolwell, 2000).

#### *Effects of environmental and cultivation condition*

Phytochemicals in plant are affected by environmental and cultivation conditions. For example, antioxidants concentration in vegetables is affected by environmental changes (Aires et al., 2011). Also, the glucosinolate contents in plants (Farnham et al., 2004) are effected by season and sulphur fertilization (Vallejo et al., 2003), light intensity and photoperiod (Charron and Sams, 2004), and soil and temperature (Velasco et al., 2007). For stable production of the phytochemicals in plants, environmental factors should be stably maintained. Plant factories with artificial lights can efficiently adjust the environmental factors regardless of outside conditions, and thereby the quality of plants can be controlled. In terms of time and space, cyclic cultivations from transplanting to harvest is suitable for efficient mass production in plant factories. In addition, growth stage of plants also affect the antioxidant potency (Šamec et al., 2011) and the glucosinolate contents in vegetables (Velasco et al., 2007). Therefore the cultivation period of one cycle is an important factor affecting total productivity in the plant factory.

#### *Determining adequate harvest time*

Studies on determining adequate harvest time were conducted with wormwood (Kim et al., 2013) and strawberry (Winardiantika et al., 2015) but these studies

carried on field so seasonal changes were main factors that affect its productivity. For life support system, adequate planting density and harvest time were determined to maximize the production of cowpea (Ohler et al., 1996). However, this study just focused on eatable fresh weight without nutritional effect. In transgenic plant for vaccine production, effects of light intensity and planting density were studied (Okamura et al., 2014b). In high planting density, production per plant was low but production per plant was high. Also, in optimal planting density that could avoid mutual shading, optimal harvest time was determined for maximizing yearly production in closed plant production system (Okamura et al., 2014a) but continuous model with cultivation cycle wasn't concluded. Therefore model to predict yearly production change with one cultivation period need to be developed.

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## **CHAPTER I**

# **Determination of Harvest time To Maximize Yearly Production of Antioxidants in Kale Cultivated in Plant Factories**

### **ABSTRACT**

Since kale (*Brassica oleracea* L. var. *acephala*) has plenty of phenolic compounds and flavonoids with high antioxidant capacity, lots of positive effects on human health by taking kale juice have been reported. For stable supply of kales not being affected by seasonal change or regional variation, more systematic cultivation methods such as plant factory are required. Plant factories can guarantee reliable productions by entire controls of indoor environments. The objectives of this study were to figure out the phase of the concentration or amount of major phytochemicals in kale and determine an optimal cultivation period for maximum yearly production. Two cultivars, 'Manchoo collard' and 'Jangsoo collard', were cultivated in a plant factory. The plants were thinned to avoid mutual shadings and harvested every week from 2 to 7 weeks after transplanting in order to measure the fresh weight and projected area. Whole harvested samples were freeze dried. Total phenolic compound, total flavonoid compound, and antioxidant capacity were analyzed. Yearly production was calculated with the formula: (dry weight × phytochemical concentration × planting density × cultivation cycle per year).

Continuous phase of yearly production was obtained by modelling and optimal cultivation period was determined for maximum yearly production. The fresh weights of both cultivars exponentially increased and the projected area hardly increased 35 days after transplanting (DAT). The TPC concentration showed fluctuations but the TFC concentration and antioxidant capacity slightly decreased and increased, respectively. The yearly production model of TPC in 'Manchoo collad' showed local maximum when one cultivation period was DAT 35 to 42. Considering other antioxidants, the yearly antioxidants production in kale will be optimal when one cultivation period is DAT 42.

***Additional key words:*** Flavonoid, Growth stage, Manchoo collard, Jangsoo collard, Phenolic compound

## INTRODUCTION

Consuming vegetables and fruits which are rich in antioxidants is reported to have health promoting effects like prevention of chronic disease (Finley et al., 2011; Wang et al. 2011). *Brassica* family especially has high antioxidants activity (Podsdek, 2007), consuming *Brassica* vegetables has anti-cancer effects (Beecher, 1994; van Poppel et al., 1999; Wang et al., 2004). Antioxidants activity of *Brassica* attributes its phenolic compounds and some flavonoids (Galati and O'Brien, 2004). Among *Brassica*, kale (*Brassica oleracea* L. var. *acephala*) is known for plentiful phenolic and flavonoid compounds (Hertog et al., 1992; Cao et al., 1996). To take fully advantage of these antioxidants in vegetables, proper cultivation techniques should be adapted to increase its utility (Parr and Bolwell, 2000).

However, antioxidants concentration in vegetables is affected by environmental changes (Aires et al., 2011), so it is difficult to stably supply of antioxidants in vegetables through current cultivation in field or greenhouse. If environmental factors are fully adjusted, more reliable productions can be expected. Plant factories with artificial lights, which are closed plant production systems, can fully adjust environmental factors regardless of outside conditions and efficient cyclic cultivations from transplanting to harvest are available for maximizing mass productions. Growth stage of plants also affect the antioxidant potency of vegetables (Šamec et al., 2011), therefore the cultivation period of one cycle is an important factor affecting total productivity in the plant factory.

The objectives are to figure out the phase of antioxidants concentration and their amounts in kale cultivated in a plant factory with cultivation day and to determine an optimal cultivation period maximizing the yearly antioxidants production.

## MATERIALS & METHODS

### *Plant materials and growth conditions*

Two kale cultivars, ‘Manchoo collard’ (*Brassica oleracea* Acephala Group Manchoo collard, Asia Seed Company, Seoul, Korea) and ‘Jangsoo collard’ (*Brassica oleracea* Acephala Group Jangsoo collard, Asia Seed Company, Seoul, Korea), were cultivated in a plant factory module of Seoul National University. The seeds were sowed in deep flow systems under fluorescent lamps at a photosynthetic photon flux density (PPFD) of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After normal leaves appeared, nutrient solutions developed by National Institute of Horticultural and Herbal Science (Choi et al., 2005) for *Brassica* was applied with an electrical conductivity (EC) of  $0.6 \text{ dS m}^{-1}$ . Two weeks after germination, kale sprouts were transplanted in the deep flow systems under light-emitting diodes (LEDs) (Red:Blue=8:2, PPFD=200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the same nutrient solutions with an EC of  $1.2 \text{ dS m}^{-1}$  were applied. For 16 hours of light period, temperature, relative humidity, and  $\text{CO}_2$  concentration were maintained at  $24^\circ\text{C}$ , 70%, and  $1000 \mu\text{mol mol}^{-1}$ , respectively. For 8 hours of dark period, only temperature was adjusted to  $20^\circ\text{C}$ . The plants were thinned to avoid mutual shadings because plant competition increases primary metabolites for rapid growth (Kozuka, 2005). From 2 to 7 weeks after transplanting, sampling was conducted every week and the samples were analyzed.

### *Growth analysis*

Images of the harvested kales were taken with a scale from vertical top view and image-analyzed with ImageJ 1.49 (Abràmoff et al., 2004; Schneider et al., 2012) to calculate projected area. Fresh weights were measured and also dry weight contents were measured after the whole leaves were freeze-dried.

### *Antioxidants analysis*

Because antioxidant concentration is different by leaf position and age, whole leaves were freeze-dried and milled and the samples were assumed to be uniform. Each sample of 100 mg was extracted with methanol 1 mL. Total phenolic compound was analyzed with Folin-ciocalteu procedure (Ainsworth and Gillespie, 2007). After incubated 48 hours in dark condition with room temperature, the samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 50  $\mu$ L was collected to 2 mL micro tube, and 10% Folin – cioalteu solution 750  $\mu$ L and distilled water 135  $\mu$ L were added. After vortexing, 600  $\mu$ L 700 mM  $\text{Na}_2\text{CO}_3$  was added and incubated 2 hours with room temperature. Absorbance in 765 nm was measured with a spectrophotometer (Photolab 6100vis, WTW, Germany). A standard curve was obtained with gallic acid and the results were expressed in mg gallic acid equivalent/g dry weight.

Total flavonoid compound was analyzed with Aluminum chloride colorimetric (Dewanto et al., 2002; Lee et al., 2012). After incubated 12 hours in dark condition with 4°C, samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 150

$\mu\text{L}$  was collected to 2 mL micro tube, and distilled water 135  $\mu\text{L}$  and  $\text{NaNO}_2$  45  $\mu\text{L}$  were added. After 5 min, 10%  $\text{AlCl}_3$  90  $\mu\text{L}$  was added and incubated for 6 min. 1 M  $\text{NaOH}$  300  $\mu\text{L}$  and distilled water 165  $\mu\text{L}$  were also added and after 5 min incubating, absorbance in 510 nm was measured with the spectrophotometer. A standard curve was obtained with catechin acid and the results were expressed in mg catechin equivalent/g dry weight.

Antioxidant capacity was analyzed with DPPH assay (Brand-Williams et al., 1995; Andarwulan et al., 2010). DPPH solution was prepared with methanol 100 mL and DPPH 24 mg. After incubated 48 hours in dark condition with room temperature, samples were centrifuged with  $1.0 \times 10^4$  g for 10 min. The supernatant 150  $\mu\text{L}$  was collected to 2 mL micro tube, DPPH solution 1.35 mL was added. After 30 min incubating, absorbance in 517 nm was measured for samples and zero cell with the spectrophotometer. A standard curve was obtained with ascorbic acid and results were expressed in mg ascorbic acid equivalent antioxidant capacity/g dry weight.

#### *Calculation of yearly production estimation*

Measured dry weight and antioxidant concentration were used to calculate antioxidants per plant (=dry weight  $\times$  antioxidant concentration). In addition, a logistic model (Tsoularis and Wallace, 2002), which are often used for explanation of plant growth was tested.

$$y = \frac{1}{1/u + a*bt} \quad (\text{Eq. 1})$$

Also cultivatable cycles per year and planting density were used to calculate the number of plants in unit area per year (= cycle per year  $\times$  plant density). To check continuous phase of the number of plant by cultivation period, regression analysis was conducted. Models: linear, logarithmic, inverse, quadratic, cubic, power, compound, S-curve, growth, and exponential were tested.

Using antioxidants per plant and the number of plants per year, yearly antioxidant production per unit area could be estimated. Static analysis was conducted with SPSS (SPSS Statistics 23, IBM, USA).

## RESULTS & DISCUSSION

### *Plant growth*

Both cultivars of ‘Manchoo collad’ and ‘Jangsoo collad’ showed exponential increases in fresh weights (Fig. 1A) and dry weights (Fig. 1B) during cultivation. These results are corresponded with the exponential increases shown in the previous plant growth models (Cho and Son, 2005; Cho et al., 2015; Yan et al., 2004). Due to their rapid increases, the fresh and dry weight at DAT 42 was about 30 times of those at DAT 14, implying that the plant growth could be the most sensitive factor to estimate yearly production.

When thinning was applied to avoid mutual competition, projected areas of both cultivars increased till DAT 35 and were not changed a lot after that (Fig. 2). The fresh weight increased till DAT 42 but the projected area increased till DAT 35 by mutual competition of neighboring plants. The use efficiencies of cultivation area were the highest at DAT 35 in Manchoo collad and DAT 49 in Jangsoo collad.

### *Antioxidant concentration and amount per plant*

The TPC concentrations decreased with fluctuation during cultivation and the difference between the lowest and highest concentrations was about twice for both cultivars (Fig. 3A). The TFC concentrations of both cultivars were the highest at DAT 14 and slightly decreased to the half during cultivation (Fig. 3B). Similarly, decreases in TPC concentration during cultivation were also reported in kale (Lee and Oh, 2015) and lettuces (Lee et al., 2014) cultivated in plant factory. The

antioxidant capacity of both cultivars showed similar levels during cultivation (Fig. 3C). In overall, antioxidant concentrations were not changed during cultivation and the reason could be that the kale were cultivated in plant factory with stable environment control. Flavonoid contents in wormwood and antioxidant phytochemicals in strawberry were strongly affected by harvest time in greenhouse cultivation (Kim et al., 2013; Winardiantika et al., 2015).

After regression analysis, the model parameters and coefficients of determination of each antioxidant amount in Eq. 1 was obtained (Table 1). The  $R^2$  for TFC amount in 'Jangsoo collard' was the lowest value as 0.78 and the others showed higher than 0.8. All the antioxidant amounts per plant increased during cultivation (Fig. 4). Even though the antioxidant concentrations were not increased during cultivation, the antioxidant amounts per plant increased with a logistic model because of the increase in plant weight. In vaccine production using transgenic lettuces, Okamura et al. (2014a) reported that total soluble protein and target protein concentration decreased but the amounts per plant increased when cultivation period became longer. In both cases, plant dry weight was an important factor.

#### *Planting density and the number of plants per year.*

As the cultivation period became longer, the planting density exponentially decreased (Fig. 5A). Because the planting density directly affect the productivity by mutual shadings in restricted cultivation areas, the weight per plant was lower but

the weight per area was higher in higher planting density (Ohler et al., 1996; Cho and Son, 2005; Kim et al., 2013; Okamura et al., 2014b).

By using planting density and cultivation cycle, the number of plant per square meter in a year could be estimated (Fig. 5B). Among the models, Eq. 2 showed the highest R<sup>2</sup> (Table 2). As a cultivation period became shorter, the planting density and cultivation cycle increased and subsequently the number of plants increased. Assuming that the productivity is the same regardless of cultivation period, a shorter cultivation period, requiring more costs of seeds and labors, would be less advantageous from economic aspects. Similar results transgenic lettuce were discussed by Okamura et al.(2014b).

$$y = e^{a+b/t} \quad (\text{Eq. 2})$$

#### *Yearly productions per unit area*

Yearly productions of TPC, TFC, and antioxidant capacity showed two to threefold differences with cultivation period (Fig. 6). The TPC production in ‘Manchoo collard’ showed three different patterns with cultivation period per cycle (Fig. 6A). The first decrease occurred at DAT 14 to 16 because of decreasing planting density. The increase from DAT 17 was attributed to the exponential growth. The second decrease started around DAT 17 because of a few number of cultivation cycle due to the long cultivation period. Therefore, the yearly production of TPC in ‘Manchoo collard’ could be maximized at DAT 35 to 42 for harvest. However the amount of TPC in ‘Jangsoo collard’ and those of the TFC and

antioxidant capacity in both cultivars were saturated at DAT 42 to 49 or increased (Fig. 6B, 6C). The yearly production can be different with cultivar. Okamura et al. (2014a) reported that yearly vaccine production of transgenic lettuces rather decreased when cultivation period was longer than 30 days. Therefore, yearly productions of TPC in 'Jangsoo collard' and TFC, antioxidant capacity of both cultivars were expected to decrease following the trend of TPC in 'Manchoo collard' when one cultivation period would longer than 49 days.

## CONCLUSIONS

The phases of the concentration or amount of phytochemicals in kale were investigated and adequate cultivation periods for maximum yearly production were determined. The TPC and TFC concentrations for both cultivars decreased and the antioxidant capacity concentration was not significantly changed during cultivation. However, the increase in plant weight increased the amount of each antioxidant with logistic patterns. Considering planting density and cultivation cycles per year, the longer cultivation period did not always bring the more yearly-production. The adequate cultivation period was DAT 35 to 42 for TPC in 'Manchoo collard' and DAT 42 to 49 for the other antioxidants in both cultivars. These results can be applied to make operational strategies in plant factories.

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Table I-1. Parameters determined by regression analysis for antioxidant amounts per plant in Eq. 1 and their coefficients of determination.

Substance	Cultivar	u	a	b	R <sup>2</sup>
Total phenolic compound	Manchoo collard	350	4.36	0.79	0.91
	Jangsoo collard	730	3.12	0.83	0.83
Total flavonoid compound	Manchoo collard	280	2.53	0.85	0.84
	Jangsoo collard	260	2.01	0.85	0.78
Antioxidant capacity	Manchoo collard	400	2.37	0.84	0.87
	Jangsoo collard	500	7.88	0.82	0.90

Table I-2. Parameters determined by regression analysis for number of plants per year in Eq. 2 and their coefficients of determination.

Cultivar	a	b	R <sup>2</sup>
Manchoo collard	1.41	61	0.93
Jangsoo collard	1.27	64	0.97

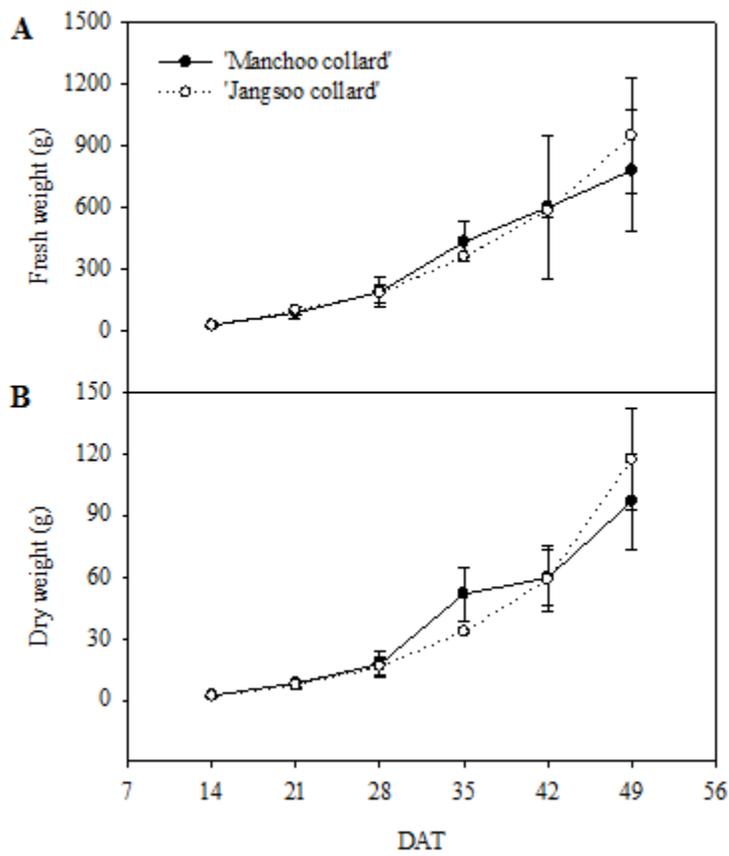


Fig. I-1. Exponential increases in fresh (A) and dry (B) weights of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

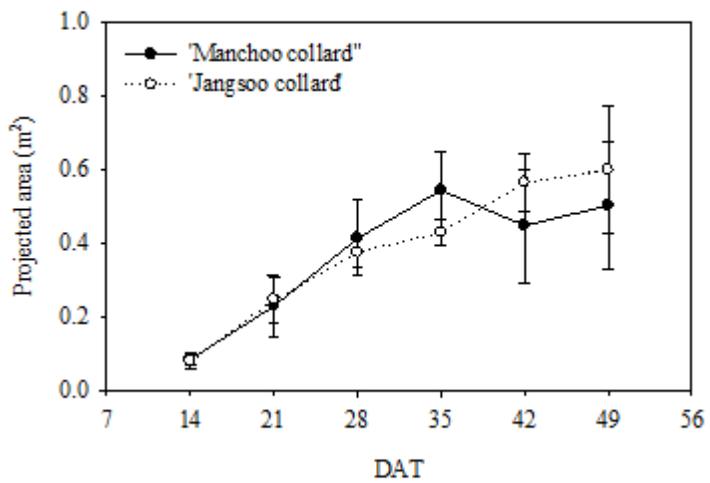


Fig. I-2. Increases in projected areas of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

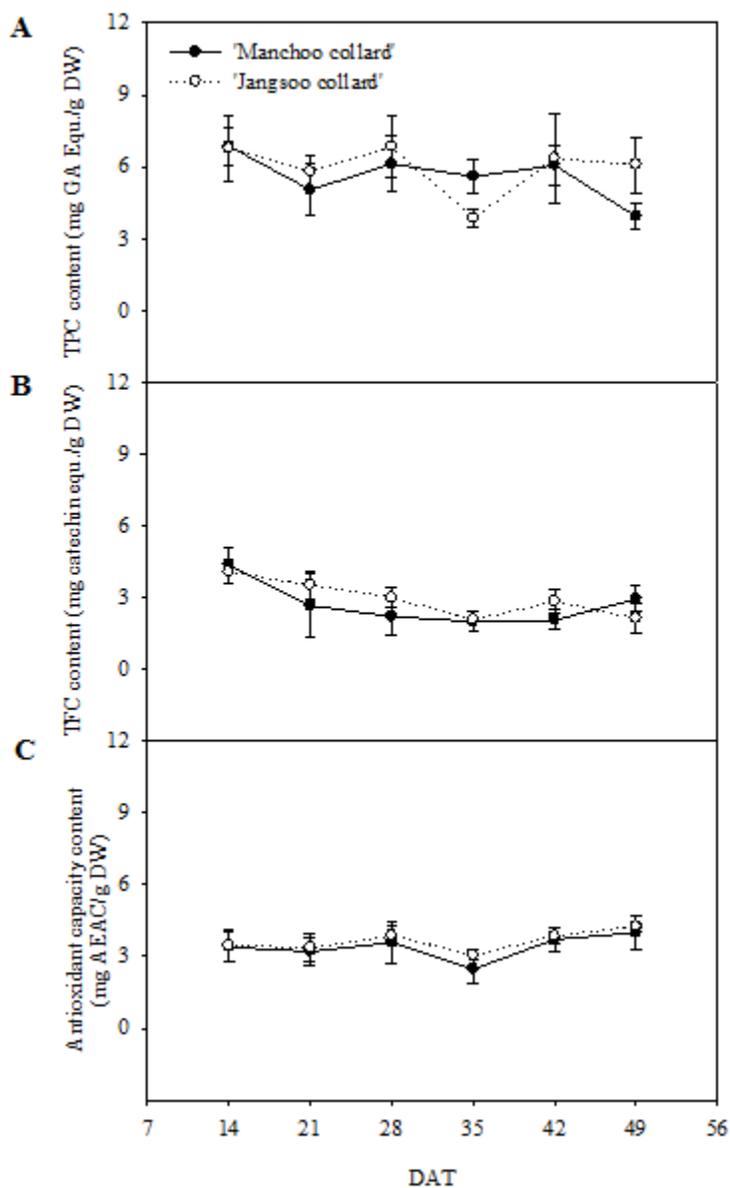


Fig. I-3. Changes in total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity (C) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

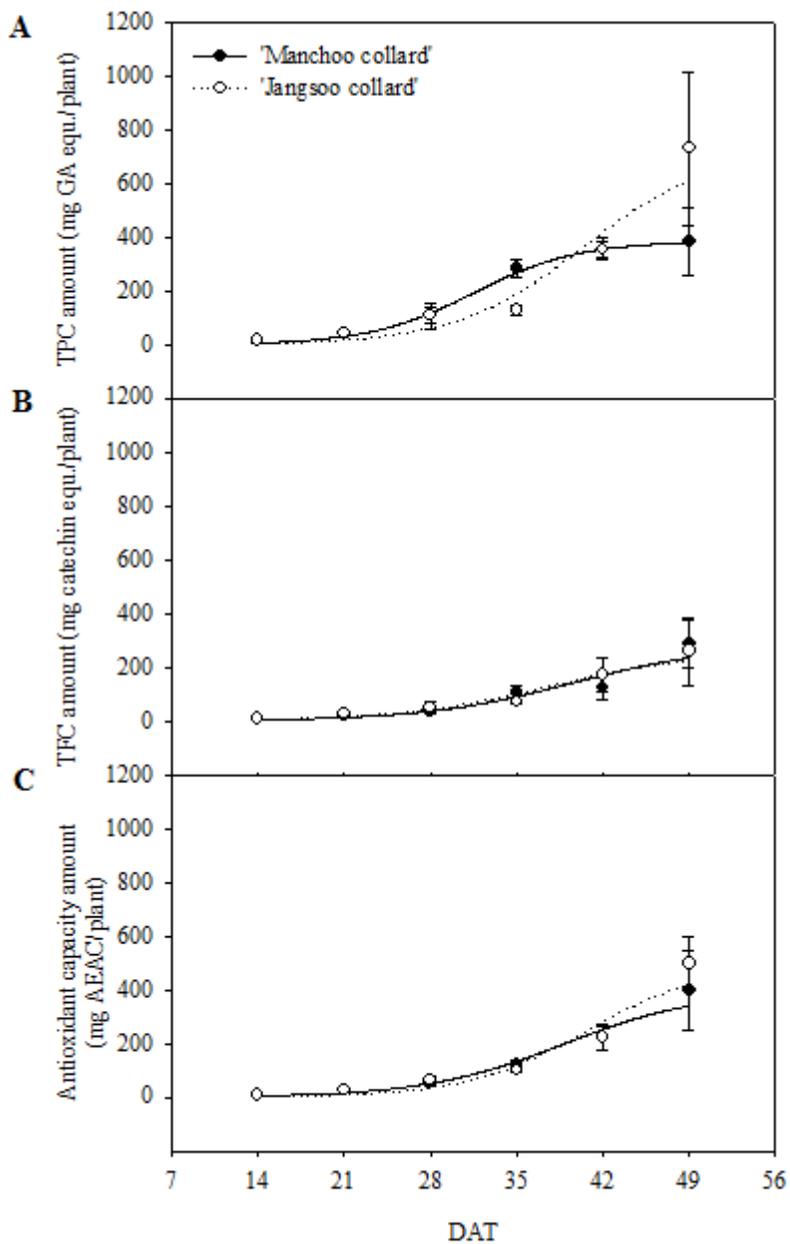


Fig. I-4. Change in total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity per plant (C) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

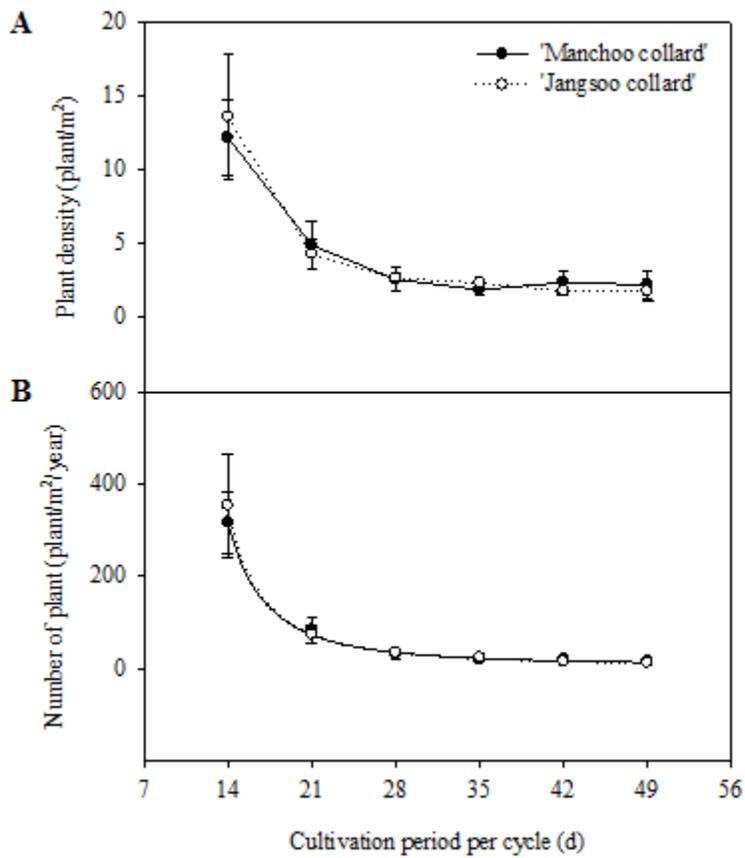


Fig. I-5. Changes in plant density (A) and the number of plants (B) of 'Manchoo collard' and 'Jangsoo collard' by cultivation period per cycle.

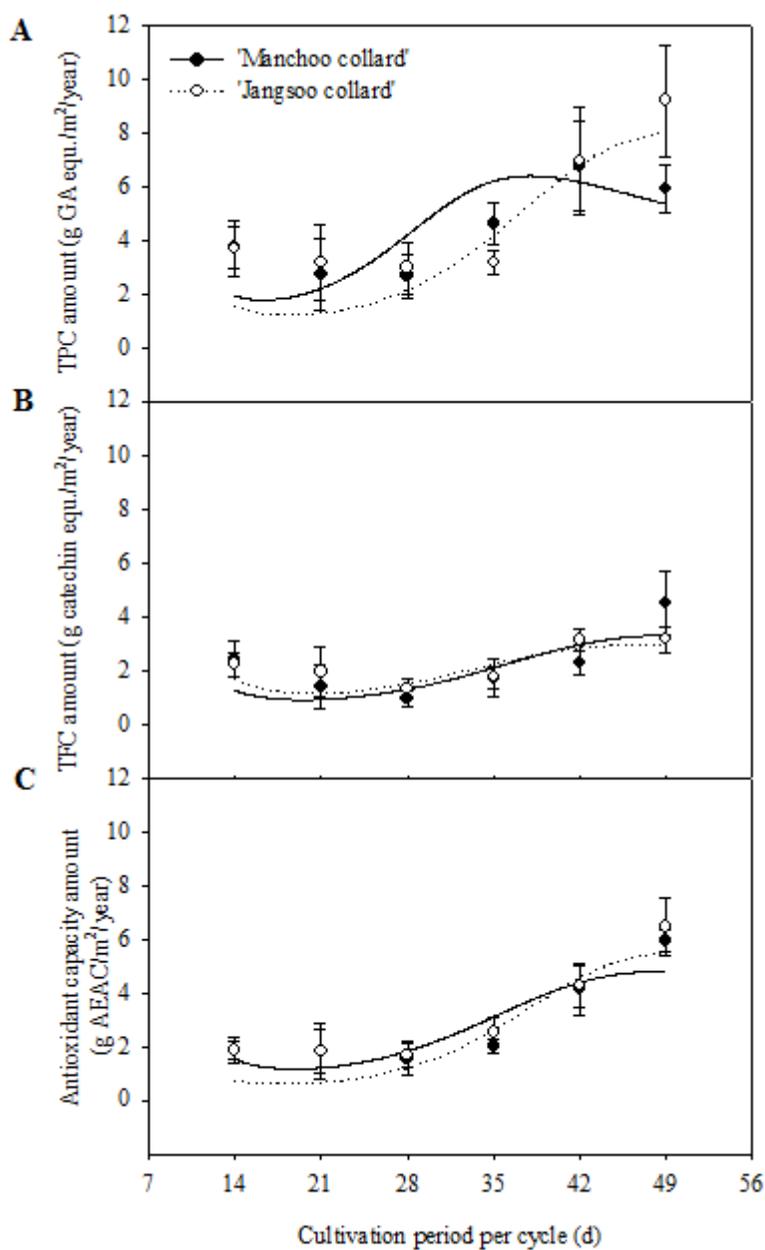


Fig. I-6. Changes in yearly productions of total phenolic compound (TPC, A), total flavonoid compound (TFC, B), and antioxidant capacity (C) of 'Manchoo collard' and 'Jangsoo collard' by cultivation period per cycle.

## CHAPTER II

### Maximization of Yearly Production of Glucosinolate in Kale by Adequate Harvest Timing in Plant Factories

#### ABSTRACT

*Brassica* vegetables are known for its sulfur containing metabolite, glucosinolate. It has been reported that hydrolyzed glucosinolate has an anti-cancer effect. Kale (*Brassica oleracea* L. var. *acephala*) has high contents of glucosinolate. To grow and supply kales not affected by climate change, more efficient environment controls are required. Plant factory, a closed plant production system, could be one of the efficient alternatives. The objectives of this study were to analyze the glucosinolate concentration and its amount in kale and to determine an adequate cultivation period for maximizing its yearly production. Two cultivars, 'Manchoo collard' and 'Jangsoo collard' grown in a plant factory were harvested every week from 2 to 7 weeks after transplanting. In order to prevent mutual shadings among the plants, thinning was conducted. The plant growth and diameter of projected area were measured. Whole harvested samples were freeze dried. Glucosinolate were analyzed with HPLC. Yearly production was calculated as follows: dry weight  $\times$  glucosinolate concentration  $\times$  planting density  $\times$  cultivation cycle per year. A model for estimating yearly production with harvest time was

developed and an adequate harvest time was determined for maximizing the yearly production of glucosinolate. The dry weights of both cultivars exponentially increased and the projected diameter hardly increased until 35 days after transplanting. The total glucosinolate concentration increased with days after transplanting. And glucoiberin, sinigrin, and glucobrassicin were the major component of glucosinolate. Maximum total glucosinolate could be produced with 42 days of cultivation period and the content of glucoiberin was the highest of the glucosinolate.

***Additional key words:*** Glucobrassicin, Glucoiberin, Growth stage, Jangsoo collard, Manchoo collard, Sinigrin

## INTRODUCTION

Consumption of *Brassica* vegetables is believed to prevent cancer and cardiovascular diseases (Cohen et al., 2000). Because *Brassica* vegetables have a plenty of phytochemicals which can promote human health (Jahangir et al., 2009), glucosinolates and their degradation products are studied to possess anti-cancer activities (Traka and Mithen, 2009; Verkerk et al., 2009). Among *Brassica* vegetables, kale (*Brassica oleracea* L. var. *acephala*) is especially rich in glucosinolates (Ayaz et al., 2006). Most of glucosinolate and their hydrolysis showed anti-cancer effects, such as hydrolysis of sulforaphane (Li et al., 2011), glucobrassicin (Kim and Milner, 2005; Brew et al., 2009), and sinigrin (Smith et al., 1998). However, progoitrin can cause goiter (Liu et al., 2012). Therefore, not only total glucosinolate amount but also individual glucosinolate amount should be considered during cultivation (Ávila et al., 2014).

Also environmental and cultivation conditions affect the glucosinolate contents in plants (Farnham et al., 2004), such as season and sulphur fertilization (Vallejo et al., 2003), light intensity and photoperiod (Charron and Sams, 2004), and soil and temperature (Velasco et al., 2007). For stable production of the glucosinolates in plants, environmental factors should be stably maintained. Plant factories with artificial lights can efficiently adjust the environmental factors regardless of outside conditions, and thereby the quality of plants can be controlled. In terms of time and space, cyclic cultivations from transplanting to harvest is suitable for efficient mass

production in plant factories. In fact, glucosinolate contents in vegetables are changed with growth stage (Velasco et al., 2007), so a cultivation period (harvest time) is an important factor affecting total productivity in plant factories.

The objectives of this study were to analyze total and individual glucosinolates in kale with time and to determine an optimal cultivation period for maximizing the yearly glucosinolate production in plant factories.

## MATERIALS & METHODS

### *Growth conditions and analysis*

Manchoo collard (*Brassica oleracea* Acephala Group Manchoo collard, Asia Seed Company, Seoul, Korea) and Jangsoo collard (*Brassica oleracea* Acephala Group Jangsoo collard, Asia Seed Company, Seoul, Korea), were grown in a plant factory module of Seoul National University. The seeds were sowed in sponge cubes with deep flow technique (DFT) systems under fluorescent lamps. After normal leaves appeared, a modified recipe of nutrient solution for *Brassica* developed by National Institute of Horticultural and Herbal Science (Choi et al., 2005) was applied with EC 0.6 dS m<sup>-1</sup>. Two weeks after germination, kale seedlings were transplanted in the DFT systems under a light intensity of 200 μmol m<sup>-2</sup> s<sup>-1</sup> LEDs (Red:Blue=8:2) and applied the same nutrient solution with EC 1.2 dS m<sup>-1</sup>. During the 16 hours of photoperiod, temperature, relative humidity and CO<sub>2</sub> concentration were maintained at 24°C, 70%, and 1000 μmol mol<sup>-1</sup>, respectively. During dark period, temperature was adjusted to 20°C. The plants were thinned to prevent mutual shadings because mutual competitions among plants increase primary metabolites for rapid growth (Kozuka, 2005). The plants were sampled for analysis every week from 2 to 7 weeks after transplanting.

Images of the sampled plants were taken from vertical top view and calculated projected areas by using ImageJ 1.49 (Abràmoff et al., 2004; Schneider et al., 2012).

### *Sample preparation*

As glucosinolate concentrations are different by leaf position and age (Brown et al. 2003; Velasco et al. 2007), whole leaves were freeze-dried and milled to make them uniform. The glucosinolates were extracted and analyzed as previously described (Sun et al., 2011). Freeze-dried samples (100 mg) were extracted three times with 70% MeOH of 2 mL for 10 min in 80°C. The supernatant was collected after centrifugation (5 min, 3500 rpm). The aqueous extract was applied onto a DEAE–Sephadex A-25 (GE Healthcare, Piscataway, NJ) (70 mg) column (1 mL blue tip). The column was washed two times with 1 mL imidazole formate (6 M) and twice with 1 mL water. The glucosinolates were converted into their desulpho analogues by overnight treatment with 200  $\mu\text{L}$  of 1.67  $\text{mg mL}^{-1}$  sulphatase from *Helix pomatia* (Sigma Aldrich), and the desulphoglucosinolates were eluted with  $2 \times 0.75$  mL water.

### *HPLC analysis*

HPLC analysis of desulphoglucosinolates was carried out by using an Alliance HPLC System (Model 2695, Waters, USA) equipped with an absorbance detector (Model 2996 PDA, Waters, USA). Samples (20  $\mu\text{L}$ ) were separated at 30°C on a Waters Spherisorb C18 column using acetonitrile and water at a flow rate of 1.0  $\text{mL min}^{-1}$ . The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min, followed by

isocratic elution with 20% acetonitrile for the final 10 min. Absorbance was detected at 226 nm.

### *Yearly production estimation*

Glucosinolate per plant was calculated by using dry weight and glucosinolate concentration as follows: dry weight  $\times$  glucosinolate concentration. In addition, regression analysis was conducted with a logistic model (Tsoularis and Wallace, 2002) to obtain continuous phase by cultivation day.

$$y = \frac{1}{1/u+a*b^t} \quad (\text{Eq. 1})$$

The number of plant per area in a year was calculated by using cultivatable cycles per year and planting density as follows: cycle per year  $\times$  plant density. To analyze the continuous phase of the number of plants by cultivation period, regression analysis was conducted. Various models such as linear, logarithmic, inverse, quadratic, cubic, power, compound, S-curve, growth, and exponential were compared., Yearly glucosinolate production per unit area could be estimated by using glucosinolate amounts per plant and the number of plant per year. Static analysis was conducted with SPSS (SPSS Statistics 23, IBM, USA).

## RESULTS & DISCUSSION

### *Plant Growth*

Dry weight of both cultivars increased exponentially with DAT (Fig. 1A) and relative water content of both cultivars tended to decrease (Fig. 1B). Previous researches of plant growth models showed similar results ( Cho and Son, 2005; Cho et al., 2015; Yan et al., 2004;). Due to rapid increase in dry weight during cultivation, the growth rate of plants sensitively affect the yearly production of target materials. By application of thinning to avoid mutual competition among neighboring plants, maximum projected diameters of both cultivars increased to about 80 cm at DAT 35. Therefore in plant factories where kales were cultivated for more than DAT 35, a planting density of 2 plants / m<sup>2</sup> could be enough to avoid mutual shadings (Fig. 2).

### *Glucosinolate concentration and amount per plant*

With HPCL analysis, 8 kinds of glucosinolate were analyzed (Fig. 3) and the total glucosinolate was calculated by summation of the 8 individual glucosinolates. Total glucosinolate, concentration of both cultivars increased about 3 times from DAT 14 to 42 and decreased slightly after that (Fig. 4). Similar results in total glucosinolate concentration were reported (Lee et al., 2015). Compared with the field cultivation results by Lee et al. (2015), total glucosinolate concentration of 'Manchoo collard' cultivated in plant factory was two times higher in the same

cultivation period. Therefore glucosinolate production was better in plant factories than fields. Among all the individual glucosinolates, glucoiberin, sinigrin, and glucobrassicin were major glucosinolates in both cultivars (Tables 1 and 2). Ratio of glucoiberin to total glucosinolate increased with DAT, while that of glucobrassicin decreased. This result explains that individual glucosinolate was affected by cultivar (Sun et al., 2011; Qian et al., 2015). Therefore cultivars suitable for cultivation purposes should be selected.

The parameters and coefficients of determination for the estimation models of total and each glucosinolate amounts were obtained by regression analysis (Table. 3). The models for total glucosinolate and glucoiberin showed higher  $R^2$  values with more than 0.9, while the models for sinigrin and glucoiberin showed lower  $R^2$  with 0.7 to 0.8. Observed and calculated amounts of glucosinolate increased with time but saturated (Fig. 5). With increase of cultivation period, the amount of glucosinolate per plant increased but its increasing rate decreased. However, in the both cultivars, individual glucosinolate amount varied with its concentration change. These trend was also reported in the vaccine production using transgenic lettuce (Okamura et al., 2014a). Total soluble proteins and target proteins per plant increased when cultivation period became longer followed by growth increase.

### *Planting density and the number of plants per year*

Optimum planting density to avoid mutual shadings was decreased with increase of maximum projected diameter of the plants (Fig. 2). Because the planting density directly affect the growth and total productivity of plants (Ohler et al., 1996; Cho and Son, 2005; Okamura et al., 2014b), in restricted cultivation areas, the weights per plant became lower but those per area became higher at higher planting density. The cultivation cycle per year was calculated by dividing one cultivation period into 365 days. With planting density and cultivation cycle, the number of plants in one square meter per year could be estimated. Among the models, S-curve model (Eq. 2) showed the highest regression coefficient (Table 4).

$$y = e^{a+b/t} \quad (\text{Eq. 2})$$

When one cultivation period becomes shorter, planting density and cultivation cycle increase. Therefore the increase in the number of plants causes much more cost for seeds and labors. Okamura et al. (2014b) recommended that when the productivity is not changed by plant density, the lower planting density has more advantages for economic reasons.

### *Yearly production per unit area*

Yearly productions of the total glucosinolate showed twofold differences with cultivation period (Fig. 6A). Yearly productions of the total glucosinolate increased till 42 days of cultivation period but were not changed after that. Okamura et al.

(2014a) mentioned that yearly vaccine productions of transgenic lettuces decreased at longer than 30 days of the cultivation period. Similarly, yearly productions of total glucosinolates in both cultivars were expected to decrease at longer than 49 days. Glucoiberin and sinigrin production of two cultivars showed similar trends but glucobrassicin production showed rather different trends (Fig. 6B, 6C). That was because the glucobrassicin concentration rapidly decreased with DAT. Therefore adequate cultivation period could be changed with target glucosinolate. In this experiment, 42 to 49 days of cultivation period could be adequate for maximum productions of the yearly total glucosinolate.

## CONCLUSIONS

The time changes in concentration or amount of glucosinolate in two cultivars of kale were analyzed and adequate cultivation periods for their maximum yearly productions were determined. The total glucosinolate concentrations for both cultivars increased with time. Glucoiberin, sinigrin, and glucobrassicin were found to be major glucosinolates in both cultivars. Glucosinoalate amount per plant increased following logistic growth model. Considering the planting density and cultivation cycles per year, the more yearly-production was not always achieved with the longer cultivation period. The adequate cultivation period was DAT 42 to 49 for maximizing total glucosinolate production in both cultivars in plant factories.

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Table. II-1. Changes in individual glucosinolate of ‘Manchoo collard’ with days after transplanting (DAT).

DAT	Gluciberin	Progoitrin	Sinigrin	Glucoraphanin	4-hydroxyl glucobrassicin	Glucobrassicin	4-methoxy glucobrassicin	Neo glucobrassicin
14	0.70±0.13 <sup>z</sup> (17)	0.24±0.09 (6)	1.03±0.27 (25)	0.10±0.04 (2)	0.04±0.01 (1)	1.83±0.41 (44)	0.19±0.05 (5)	0.05±0.02 (1)
21	1.19±0.38 (18)	0.34±0.12 (5)	1.79±0.75 (27)	0.17±0.08 (3)	0.04±0.03 (1)	2.59±0.83 (39)	0.34±0.04 (5)	0.10±0.02 (2)
28	1.82±0.60 (26)	0.38±0.13 (6)	1.85±0.70 (27)	0.20±0.10 (3)	0.14±0.03 (2)	2.11±0.34 (31)	0.29±0.08 (4)	0.09±0.03 (1)
35	2.76±0.59 (34)	0.71±0.18 (9)	2.25±0.62 (28)	0.61±0.16 (8)	0.09±0.01 (1)	1.33±0.28 (17)	0.25±0.03 (3)	0.05±0.02 (1)
42	3.67±0.80 (42)	0.74±0.01 (9)	1.65±0.60 (19)	0.76±0.05 (9)	1.01±0.20 (12)	0.68±0.05 (8)	0.15±0.04 (2)	0.04±0.00 (0)
49	3.16±1.26 (39)	0.65±0.10 (8)	1.98±0.45 (24)	0.74±0.20 (9)	0.58±0.12 (7)	0.85±0.14 (10)	0.14±0.07 (2)	0.01±0.00 (0)

<sup>z</sup>Mean±SD (ratio to total glucosinolate, %).

Table. II-2. Changes in individual glucosinolate of ‘Jangsoo collard’ with days after transplanting (DAT).

DAT	Glucobriferin	Progoitrin	Sinigrin	Glucoraphanin	4-hydroxyl glucobrassicin	Glucobrassicin	4-methoxy glucobrassicin	Neo glucobrassicin
14	0.77±0.16 <sup>z</sup> (19)	0.19±0.06 (5)	0.97±0.23 (24)	0.09±0.03 (2)	0.07±0.01 (2)	1.68±0.57 (42)	0.15±0.03 (4)	0.06±0.03 (2)
21	0.98±0.40 (17)	0.42±0.31 (7)	1.30±0.30 (22)	0.28±0.32 (5)	0.07±0.04 (1)	2.14±0.72 (37)	0.51±0.21 (9)	0.10±0.04 (2)
28	1.60±0.43 (27)	0.30±0.11 (5)	1.44±0.56 (24)	0.16±0.08 (3)	0.13±0.06 (2)	1.86±0.45 (31)	0.35±0.13 (6)	0.12±0.04 (2)
35	2.01±0.85 (25)	0.85±0.19 (11)	1.57±1.53 (20)	1.82±1.08 (23)	0.09±0.09 (1)	1.10±0.20 (14)	0.40±0.03 (5)	0.09±0.06 (1)
42	3.59±0.77 (39)	0.64±0.01 (7)	1.08±0.60 (12)	0.62±0.02 (7)	1.69±0.07 (19)	1.13±0.17 (12)	0.29±0.04 (3)	0.06±0.03 (1)
49	2.46±1.00 (36)	0.69±0.38 (10)	1.85±0.90 (27)	0.71±0.13 (10)	0.46±0.27 (7)	0.58±0.18 (8)	0.09±0.03 (1)	0.01±0.01 (0)

<sup>z</sup>Mean±SD (ratio to total glucosinolate, %).

Table. II-3. Parameters determined by regression analysis for glucosinolate amounts per plant and their coefficients of determination.

Substance	Cultivar	u	a	b	R <sup>2</sup>
Total glucosinolate	Manchoo collad	760	1.13	0.83	0.94
	Jangsoo collad	770	11.4	0.78	0.94
Glucoiberin	Manchoo collad	290	11.7	0.80	0.95
	Jangsoo collad	270	416	0.73	0.90
Sinigrin	Manchoo collad	190	2.32	0.85	0.81
	Jangsoo collad	200	15.6	0.82	0.79
Glucobrassicin	Manchoo collad	80	0.71	0.87	0.73
	Jangsoo collad	60	1.47	0.86	0.81

Table. II-4. Parameters determined by regression analysis for numbers of plant per yearly and their coefficients of determination.

Cultivar	a	b	R <sup>2</sup>
Manchoo collad	1.41	61	0.93
Jangsoo collad	1.27	64	0.97

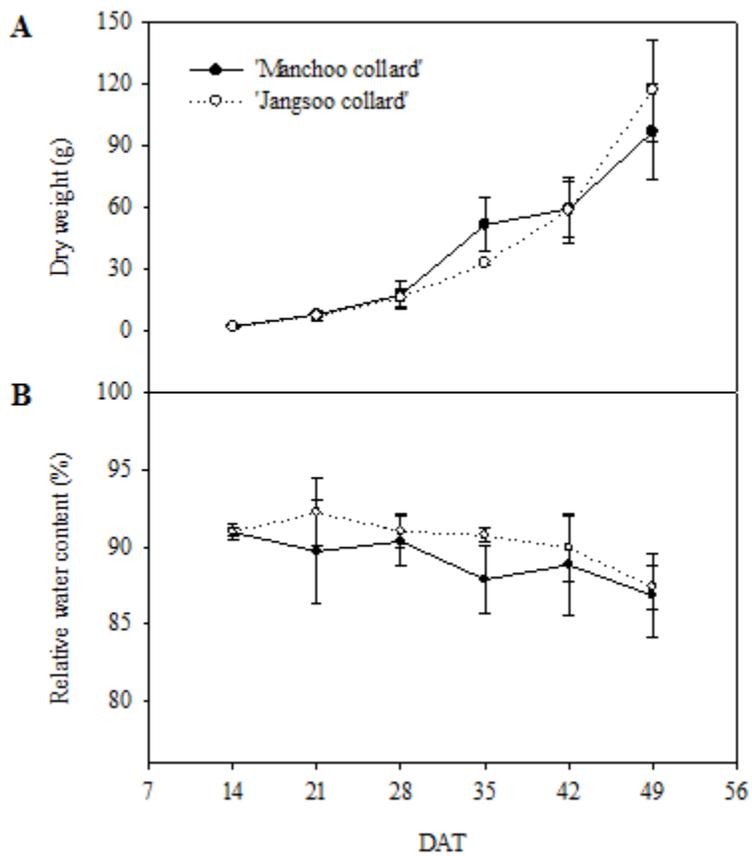


Fig. II-1. Exponential increases in dry weights (A) and their relative water contents (B) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

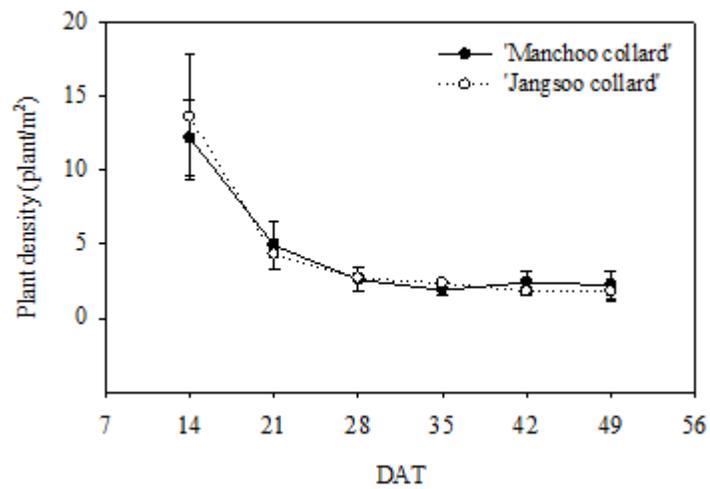


Fig. II-2. Decrease in optimum planting density to avoid mutual shadings of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting (DAT).

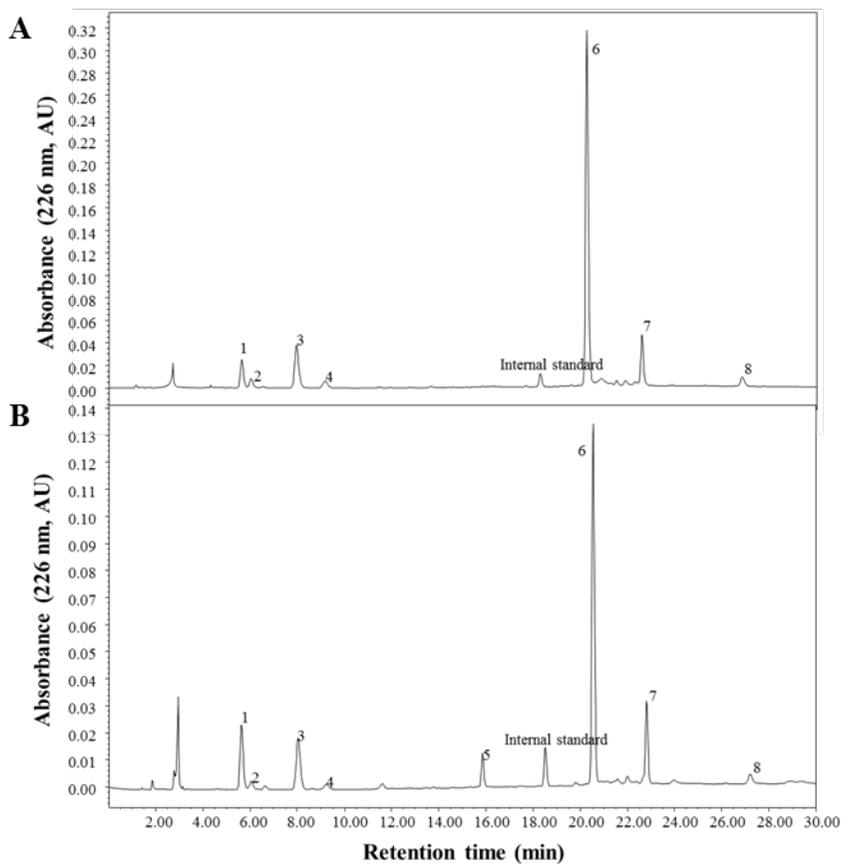


Fig. II-3. Chromatograms of ‘Manchoo collard’ (A) and ‘Jangsoo collard’ (B). Each number indicates that 1. glucoiberin, 2. progoitrin, 3. sinigrin, 4. glucoraphanin, 5. 4-hydroxyl glucobrassicin, 6. glucobrassicin, 7. 4-methoxy glucobrassicin, and 8. neo glucobrassicin

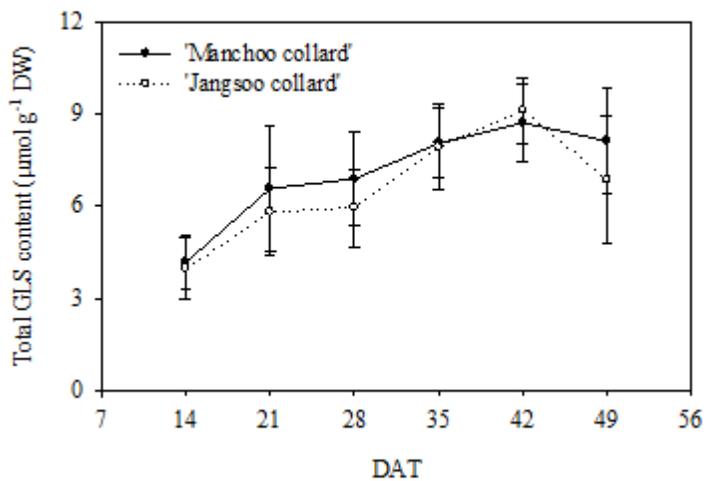


Fig. II-4. Changes in total glucosinolate content (GLS) of 'Manchoo collard' and 'Jangsoo collard' with days after transplanting.

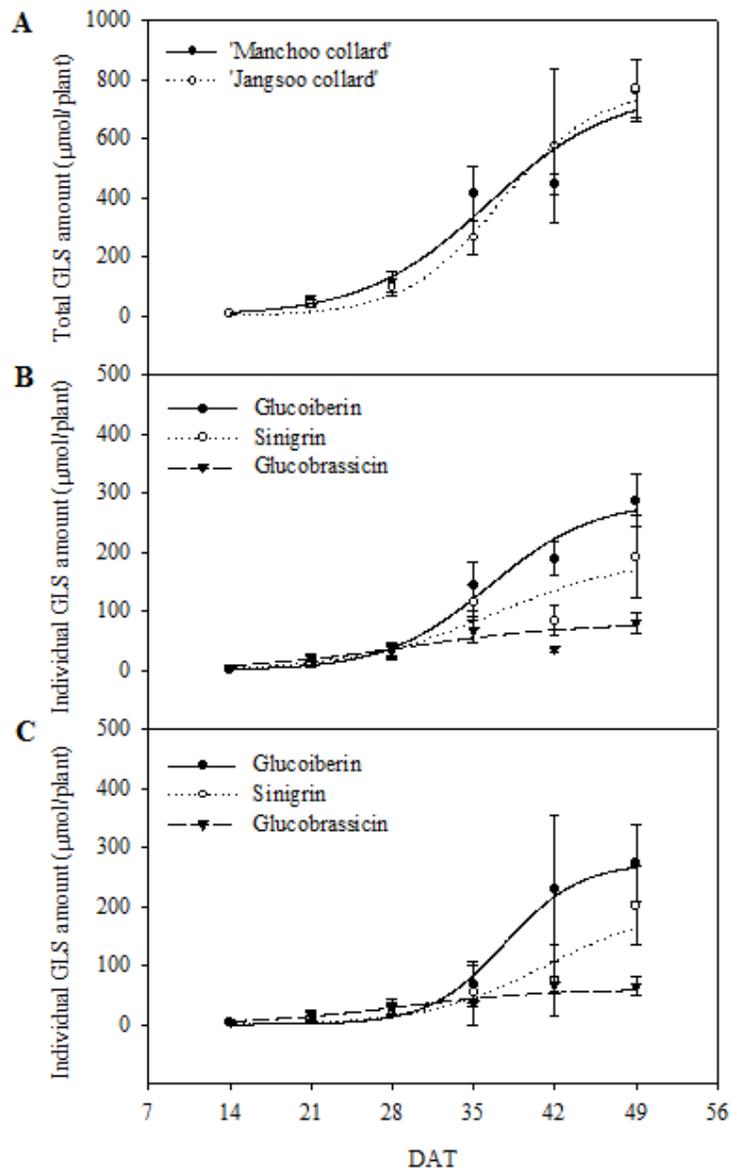


Fig. II-5. Changes in total glucosinolate (GLS) amounts per plant of both 'Manchoo collard' and 'Jangsoo collard' (A), three major GLS amounts per plant of 'Manchoo collard' (B), and 'Jangsoo collard' (C) with days after transplanting (DAT).

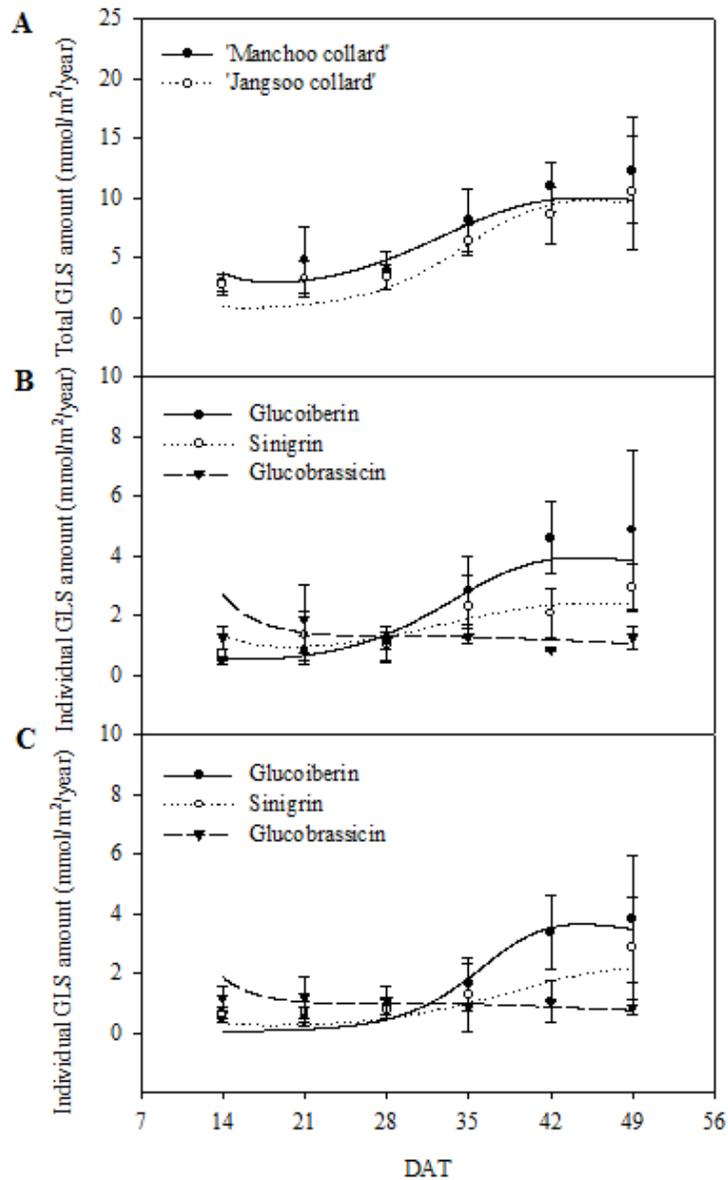


Fig. II-6. Changes in yearly amount of total glucosinolate (GLS) of 'Manchoo collard' and 'Jangsoo collard' (A), yearly amount of three major GLS of 'Manchoo collard' (B), and 'Jangsoo collard' (C) by the harvest time expressed as days after transplanting (DAT).

## CONCLUSIONS

The phases of the concentration or amount of phytochemicals in kale were investigated and adequate cultivation periods for maximum yearly production were determined. The TPC and TFC concentrations for both cultivars decreased and the antioxidant capacity concentration was not significantly changed during cultivation. The total glucosinolate concentrations for both cultivars increased with time. Glucoiberin, sinigrin, and glucobrassicin were found to be major glucosinolates in both cultivars. The increase in plant weight increased the amount of each antioxidant and glucosinolate with logistic patterns. Considering planting density and cultivation cycles per year, the longer cultivation period did not always bring the more yearly-production. The adequate cultivation periods were DAT 35 to 42 for TPC in 'Manchoo collard', DAT 42 to 49 for the other antioxidants in both cultivars, and DAT 42 to 49 for total glucosinolate in both cultivars in plant factories. In overall, 42 days of kale cultivation in plant factory should be proper for its antioxidants and glucosinolates amount. Although in this study, amount of the phytochemicals were considered to determine the adequate harvest time, other viewpoints, such as extraction efficiency or economical cost, need to be considered in further study.

## ABSTRACT IN KOREAN

본 연구에서는 식물공장에서 케일 재배 시, 이차대사산물의 연간생산량을 최대로 하는 수확시기를 결정하고자 하였다. ‘만추콜라드’와 ‘장수콜라드’ 품종을 식물 공장 모듈에서 재배하였다. 정식 후 2 주에서 7 주까지 매주 샘플링하며 생육조사와 총 페놀화합물, 총 플라보노이드, 글루코시놀레이트 농도와 항산화능을 분석하였다. 건물중, 기능성 물질 농도, 재식 간격, 연간 재배 가능 횟수를 이용하여 기능 성분의 연간 생산량을 추정하였고, 재배기간에 따른 생산량 변화를 모델화 하였다. 만추콜라드의 총 페놀화합물 생산량은 1 작기 37 일, 그 외의 물질은 42 일에서 최대값을 나타냈다. 장수콜라드는 전체적으로 45 일에서 최대값을 나타냈다. 본 결과는 식물공장의 연간생산량을 최대로 하는 경제적인 수확시기 결정에 활용될 수 있으리라 기대된다.

주요어: 페놀성 화합물, 플라보노이드, 생육 단계, 만추콜라드, 장수콜라드, 글루코브라시신, 글루코이베린, 시니그린

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