台灣農業研究 (J. Taiwan Agric. Res.) 58(2):114–124 (2009)

Seasonal Changes of Growth and Leaf Perillaldehyde in *Perilla frutescens* (L.) Britton [1]

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Abstract

Lee, Y. J. and C. M. Yang. 2009. Seasonal Changes of Growth and Leaf Perillaldehyde in *Perilla frutescens* (L.) Britton. J. Taiwan Agric. Res. 58:114–124.

Perilla frutescens (L.) Britton, a member of Lamiaceae and a native dicot in Asia, is now distributed worldwide including Taiwan. To further explore its uses as a commercial crop, a comprehensive study regarding the growth behavior and chemical components of this plant species is needed. In the present research, changes in growth traits, including plant height, leaf area index and weights of aerial parts, and leaf perillaldehyde (PA) concentration and content were investigated for plants grown in different growing seasons in the experimental period from 2004 to 2006. The five-leaf stage seedlings were transplanted in March (Season I), April (Season II), May (Season III) June (Season IV) and July (Season V), respectively. Concentration of leaf PA was determined by High Performance Liquid Chromatography (HPLC). Results showed that plant height was taller and leaf area index and fresh weights of aerial parts were larger for plants grown in cooler Season I relative to those plants grown in other warmer seasons (Seasons II-V), implying that warming conditions during growing periods was not in favor to plant growth. Seeds harvested at maturity were found varied in different growing seasons and seed produced in Season V was the lowest. In contrast, the highest value of 500-seed weight also obtained from seeds produced in Seasons V. The PA concentration in the primary leaves on the main stem from position 10 to 15 was higher than others and the quadratic pattern was similar in different growing seasons in 2004–2006. The distribution pattern of leaf PA content was in accordance with leaf PA concentration during the growing periods. In considering the proportions of PA distributed in leaves emerged on the main stem and the lateral branches, the proper time period to harvest the highest quantity of leaf PA for a single plant was from 110 to 120 days after transplanting.

Key words: Growth trait, Leaf perillaldehyde, Seed production, *Perilla frutescens* (L.), Growing season.

Introduction

Perilla frutescens (L.) Britton is an ornamental herb of the mint family (Labiatae) native to eastern Asia (Shu 1994), and is widely used as a condiment for foods in China, India, Thailand, and other Asian countries. There are green-leafed and red-leafed varieties which are generally recognized as separate

1. Contribution No.2357 from Taiwan Agricultural Research Institute (TARI), Council of Agriculture. Accepted: June 29, 2009.

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species by botanists. The green leaf type of perilla, called aojiso in Japan, is a popular potherb in the Orient. The red (or purple) type of perilla, called akajiso in Japan, is wealthy in anthocyanin and a number of chemical compounds and often used as garnish, flavoring and colorant (Suyama *et al.* 1983; Koezuka *et al.* 1985b; Chung *et al.* 1986). Recently the species has been cultivated in many regions of the world for medicinal and culinary uses (Li 1969; Richardson 1972; Perry & Metzger 1980; Kurita & Koike 1981; Koezuka *et al*. 1985b; Ragazinskiene *et al*. 2004).

Perilla can easily adapt to open sunny fields with humid climate. The prefer environment for cultivation is a well-drained rich soil with light to medium moist and full sun (Park *et al.* 1991). Generally the cold hardened seeds are sown in pots filled with sifted compost consisting of loam, leaf mold and sand and covered lightly with soil. In warm and humid weather, plants grow quickly and the mature plants may grow about one meter high and are bushy and self-branching. The leaves are fuzzy, dark purple or green and flowers are self-pollinated without insect visits (Brenner 1993; Preston 1998). To maintain a neat appearance, their tops are usually pinched off when the fourteenth primary appears on the main stem (Lee & Yang 2006). As a short-day summer annual, the flowering of perilla is sensitive to change in day length. It blooms in October and killed by frost in winter locally (Lee & Yang 2006). However, it may become a weed when escapes from the garden (Haragan 1991; Brenner 1993).

Perilla is also used in Oriental medicine, especially China (Chen 1997). The entire plant is very nutritious, packed with vitamins, minerals, and a variety of chemical components (Kurita & Koike 1981; Fujita & Nakayama 1997; Ueda & Yamazaki 1997; Yamazaki & Ueda 1997; Ragazinskiene *et al*. 2004). It has been found that seed oil is rich in omega-3 fatty acid (alpha-linolenic acid) which has some benefit in the treatment of allergy (Choi *et al*. 1980; Yu *et al.* 1997; Baser *et al.* 2003). The volatile oil extracted from leaves is used as a flavoring agent, in which perilla aldehyde (perillaldehyde, PA) is the most abundant that responsible for the aroma and taste of perilla (Arctander 1960; Nago *et al.* 1975; Tada *et al*. 1996; Makino *et al.* 2003). In fact, studies of perilla volatile oil have revealed that distinct chemotypes of perilla have different dramatically biological effects (Koezuka *et al*. 1985a; Koezuka *et al.* 1986; Nishizawa *et al.* 1989, 1990; Tabata 1997; Wilson *et al*. 1977). The PA chemotype is the source of Japanese "ao-shiso" and used as a medicine with an agreeable fragrance (Nitta & Ohnishe 1999). Additionally, perilla alcohol (PAL), a semio-chemical prepared from PA, can be used in fragrances and obtains pharmacological action in inhibiting or preventing the proliferation of neoplasms (Opdyke 1981). Perilla ketone (PK), a terpenoid component present in the leaves and seeds that consists of a furan ring with a six-carbon side chain, is toxic to some animals (Wilson *et al.* 1977; Kerr *et al.* 1986; Phillips & Von Tungein 1986). When cattle and horses consume perilla to a certain amount when grazing in fields, the PK may cause pulmonary edema leading to a condition called perilla mint toxicosis (Banno *et al.* 2004).

In this study, field experiments were conducted to study the differences in growth traits of red perilla grown in different seasons and years. Changes in PA concentration and content extracted from primary leaves on the main stem and leaves emerged from the lateral branches were investigated and their differences between seasons were also compared.

Materials and Methods

Field experiments were done in the experimental farm of Taiwan Agricultural Research Institute (Wufeng, Taiwan) from 2004 to 2006. The important dates of cultivation practices and growth characteristics for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons and years were listed in Table 1. Seeds were sown into sifted compost (a mixture of loam and leaf mold) within containers, one seed per container, covered lightly with soil, and then placed under a polyethylene plastic (PEP) structure nursery. The containers were sprayed daily in excess with fertilized water (4.2 g urea resolved in 4 L of tape water) until the five-leaf pair stage, in which the fifth primary leaf

pair emerged from the main stem. The excess of irrigation water was drained and flushed away the surplus fertilizer from the bottom of the containers. The uniformly seedlings of five-leaf pair stage were transplanted from the nursery to the experimental fields at spacings of 0.5 m × 0.5 m on March (Season I; in 2004–2006), April (Season II; in 2005 and 2006), May (Season III; in 2004 and 2005), June (Season IV; in 2005), or July (Season V; in 2004). On each transplanting, seedlings were transplanted into 3 plots in the field with each plot 8 m in length and 1.2 m in width and plowed into 2 lines.

The composite fertilizer Taifei-1 (granule, N : P_2O_5 : K_2O = 20% : 5% : 10%, Taiwan Fertilizer Company, Kaohsiung, Taiwan) was applied at a rate of 50 kg ha^{-1} two days after transplanting as basal dose, and urea (granule, 46% N, TFC) was sprayed every 30 days after the transplanting until the flowering of inflorescences. Pesticide methomyl (24% S, 1000×, 0.24 L a.i. ha^{-1}) was sprayed to control aphid, lambda-cyhalothrin (2.8% EC, 1000×, 0.028 L a.i. ha^{-1}) was used to control spider mite, and carbofuran (3% G, 3 kg a.i. ha^{-1}) was applied to control nematode. Mancozeb (33% WP, 500 C, 1 L a.i. ha^{-1}) was used to protect from infection by rust disease.

Leaf-removal and bud-pinching were practiced during the growing periods (Table 1). When the seventh primary leaf pair emerged from the main stem, the first to the fourth leaf pair was removed by hands with the purpose to promote a healthy plant

Table 1. The important dates of cultivation practices and growth characteristics during different growing seasons of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in years from 2004 to 2006

	Season				
Cultivation practice	I	II	III	IV	V
2004					
Seed sowing in nursery	01/28		04/01		06/14
Seedling transplanting to the field	03/15		05/03		07/30
Lower leaves removal on the main stem [z]	04/08		06/16		09/03
Bud pinching (main stem and branches)	05/07		07/08		10/01
Flowering of inflorescences	10/01		10/02		10/02
Seed harvest	11/18		11/22		11/25
Days from transplanting to seed harvest	248		203		118
2005					
Seed sowing in nursery	01/14	03/01	04/06	05/02	
Seedling transplanting to the field	03/14	04/22	05/12	06/16	
Lower leaves removal on the main stem	04/01	05/02	06/08	07/08	
Bud pinching (main stem and branches)	05/10	06/17	07/26	08/15	
Flowering of inflorescences	09/21	09/22	9/22	09/23	
Seed harvest	11/23	11/24	12/02	12/06	
Days from transplanting to seed harvest	254	216	204	173	
2006					
Seed sowing in nursery	02/07	03/01			
Seedling transplanting to the field	03/23	04/11			
Lower leaves removal on the main stem	04/18	05/04			
Bud pinching (main stem and branches)	05/10	05/29			
Flowering of inflorescences	10/02	10/03			
Seed harvest	11/18	11/18			
Days from transplanting to seed harvest	240	221			

[z] The first 4 leaf pairs of primary leaves on the main stem were removed from all plants when the seventh primary leaf pair emerged on the main stem for the purpose to stimulate a healthy plant growth and a neat appearance.

growth. When the fourteenth primary leaf pair emerged, treatment of bud-pinching was carried out in order to maintain a neat appearance and an easy-to-access plant height. Buds on the top of main stem and branches were pinched off by human labor. Plant samplings were made periodically to measure growth traits in 2004 and 2005, including plant height (PH), leaf area (LA), and fresh and dry weights of leaves (LFW and LDW), stems (SFW and SDW) and aboveground (AFW and ADW). Leaf area index (LAI) was calculated as the accumulated apparent leaf area of sampled plants divided by unit ground area (m^2 m^{-2}). At maturity in 2004–2006, seeds produced from all growing seasons were harvested and weighted and the 500-seed weight was calculated.

When the paired leaves emerged from the main stem and the lateral branches were fully expanded with leaf width greater than 8 cm during plant growth, their areas and fresh weights were measured and the concentrations of PA were determined by the method of High Performance Liquid Chromatography (HPLC) (Lee & Yang 2006). Leaf blade of 0.10–0.18 g was obtained from each of sampled leaves, cut to pieces with a scissors, and extracted by 5 mL 90% methanol using a pestle and mortar. The collected homogenate was stored in a test tube and placed standstill for at least 10 min. The clean supernatant was collected and filtered through a funnel packed with 0.2 g charcoal activated powder (Sigma-Aldrich Laborchemikalien GmbH, Germany). The filtrate was further filtered through a 13-mm disposable syringe filter (Xpertex®, P. J. Cobert Associates, Inc., St. Louis, MO, USA) with 0.45 μm pore size. A fraction of 10 μL from the filtrate was used for HPLC analysis. As indicated in the chromatogram (Lee & Yang 2006), the retention times were 7.47 min and 7.49 min for PA standard and extract of the leaves.

The HPLC grade S-(-)-perillaldehyde was purchased from Aldrich (Milwaukee, WI, USA) to build the calibration curve. Methanol was obtained from Tedia (Tedia Company, Inc. USA) and mixed with NANO-pure water (NANOpure Diamond™ system, Barnstead Inc., USA) for a mobile phase (methanol : water = 75 : 25, v/v). The PA standard and samples were solved in 90% methanol and detected by a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan), which consists of a LC-10AT VP pump, a FCV-10AL VP GASTORR 102, and a SPD-10A VP UV-VIS detector with detection wavelength set at 220 nm. Separation was carried out with an Inertsil C_8 column (150 mm × 4.6 mm I. D., 5 μm particle) (GL Sciences Inc., Japan) and isocratic mobile phase consisted of methanol-water (75 : 25, v/v) mixture and flowed at a rate of 0.5 mL min^{-1} under room temperature.

To determine PA content of all primary leaves collected from the main stem or all leaves emerged from the lateral branches, the PA concentrations of each pair of primary leaves or leaves from the sampled branches were measured and multiplied with their respective fresh weights. The PA content per plant was obtained from the summation of PA contents of both types of leaves. Data from this study were analyzed and graphed using the software packages Statistical Analysis System version 8.1 (SAS Institute 1998) and Sigmaplot 2001 (SPSS ASC BV, The Netherlands), respectively.

Results and Discussion

The important dates of cultivation practices and growth characteristics during different growing seasons of red perilla were recorded in Table 1. It showed that the growth duration from seedling transplanting to seed harvest varied in different growing seasons and years. In a descending order, seedlings transplanted in cooler Season I (seedlings transplanted in March) took a longer growing period to reach seed maturity relative to those transplanted in a warmer seasons (Seasons II, III, IV, and V). It also took a longer time to get to the designated developmental stages in Season I. Results suggest that differences in climatic conditions may affect the growth and development of this species and hence, the time periods required for the specified cultivation practices. However, the flowering of inflorescences occurred in the late September to early October irrespective to transplanting dates, revealing the characteristic of short-day nature of perilla. As pointed out by Kosuna *et al*. (1997) and

Zeevaat (1969, 1985), plants of perilla become photosensitive as early as at the fourth primary leaf pair stage and are good source for plant physiologists to investigate floral induction and photoperiodism (Brenner 1993). The results of this study confirmed such a phenomenon that long nights induced flowering of inflorescences. Moreover, plants grown in different growing seasons might have differential critical night lengths requirements but would bloom within the similar time period.

As shown in Fig. 1, plants of perilla elongated in a curvilinear fashion. Generally plants grown in Season I were taller and had higher values of LAI during plant growth; whereas, plants grown in warmer seasons (Seasons II-V) were shorter with less leaf area, similar to the report in Lee & Yang (2006). In addition, both leaf and stem fresh weights also increased curvilinearly during plant growth (Fig. 2). Plants grown in Season I generally had the highest weight values along plant development than those plants grown in other growing seasons. Results imply that warming conditions during growing periods was unfavorable to plant growth. In case of seed production, results indicated that seeds harvested at maturity varied in different growing seasons and seed produced in Season V was the lowest (Fig. 3). In contrast, the highest value of 500-seed weight also obtained from seeds produced in Seasons V. Apparently, plants grown in hot summer period (July to September) of Season V produced less seeds with a bigger seed size.

Perilla leaves contain about 0.2% of an essential oil which varies widely in composition and has been found obtaining good inhibitory activity against *Aspergillus niger*, *Candida albicans*, *Bacillur subtilis* and *Enchericchia coli* that attributed to the high contents of PA and caryophyllene oxide (Omer *et al*. 1998). The compound PA showed moderate and broad-spectra activity against many Gram-positive and Gram-negative microbes and fungi (Kang *et al.* 1992), and was also demonstrated having a sedative activity and antimicrobial properties (Terao *et al.* 1991; Duke & Fulton 2002).

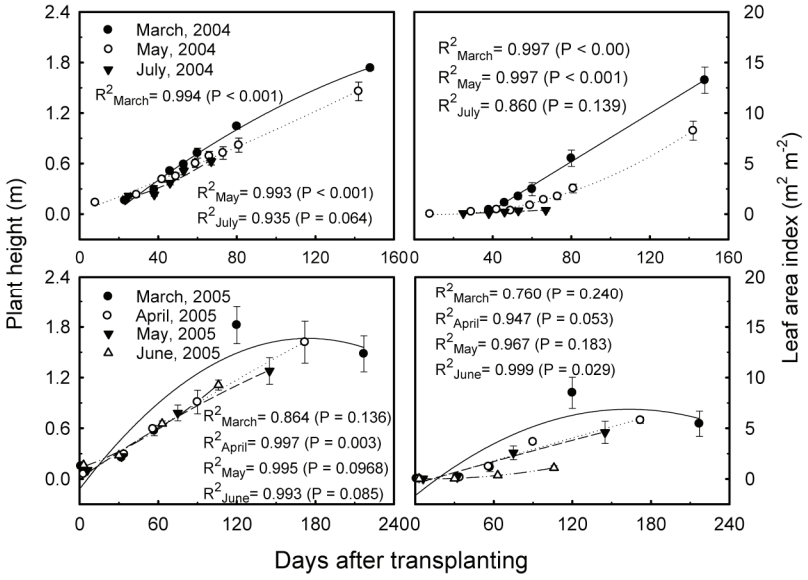

Fig. 1. Changes in plant height and leaf area index (LAI) after transplanting for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in 2004 and 2005. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April; Season III: seedlings transplanted in May; Season IV: seedlings transplanted in June; and Season V: seedlings transplanted in July.

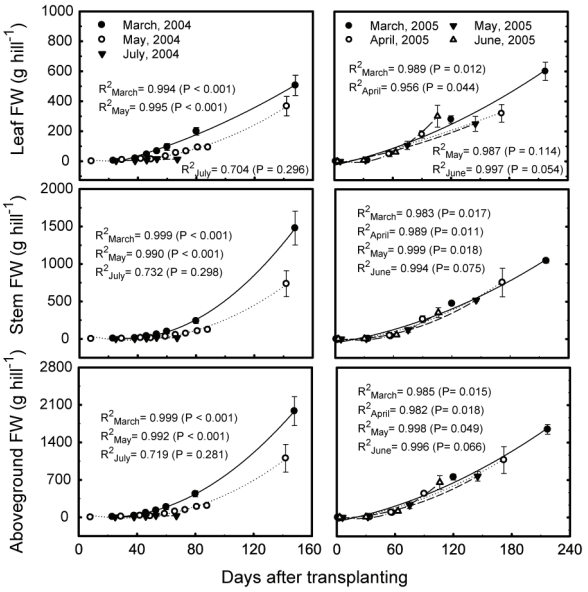

Fig. 2. Changes in fresh weights of leaves, stems, and aboveground after transplanting for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in 2004 and 2005. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April; Season III: seedlings transplanted in May; Season IV: seedlings transplanted in June; and Season V: seedlings transplanted in July.

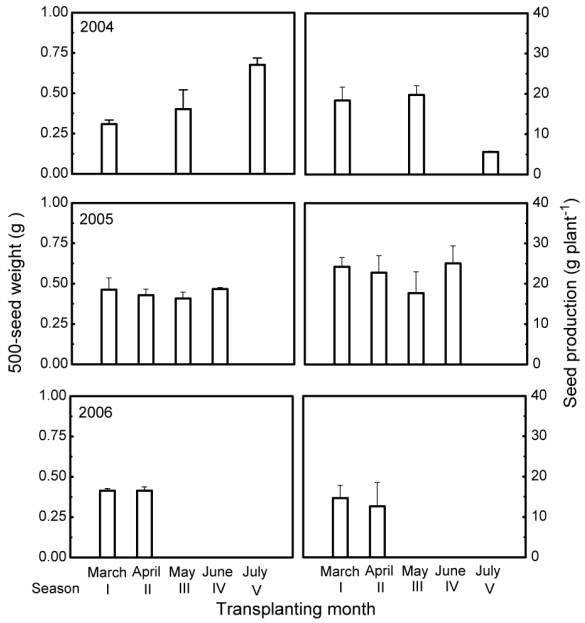

Fig. 3. Comparisons of the values of 500-seed weight and seed production measured at maturity for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in years from 2004 to 2006. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April; Season III: seedlings transplanted in May; Season IV: seedlings transplanted in June; and Season V: seedlings transplanted in July.

We examined the concentration of PA of fresh fully expanded primary leaves along the main stem upwards. With a quadratic pattern, leaf PA concentration increased with the upscaling of leaf position and then decreased after reaching the plateau in the range from leaf position 10 to 15 (Fig. 4). As PA concentration of the same leaf position varies among years of the same growing season, results indicate that the environment of growth habitat plays a role in the formation of this phytochemical. The concentration of leaf PA measured in this study was comparable to that reported by Kang *et al*. (1992), yet was a little bit higher than that reported by Omer *et al*. (1998). By further multiplying the concentration with fresh weight, PA content of each of primary leaves was obtained (Fig. 5). A similar quadratic distribution was acquired in the examined growing seasons and the plateau value was also located in the range of 10–15.

To further compare the differences in PA content in leaves of different plant parts between growing seasons, the temporal changes of PA content extracted from either primary leaves collected from

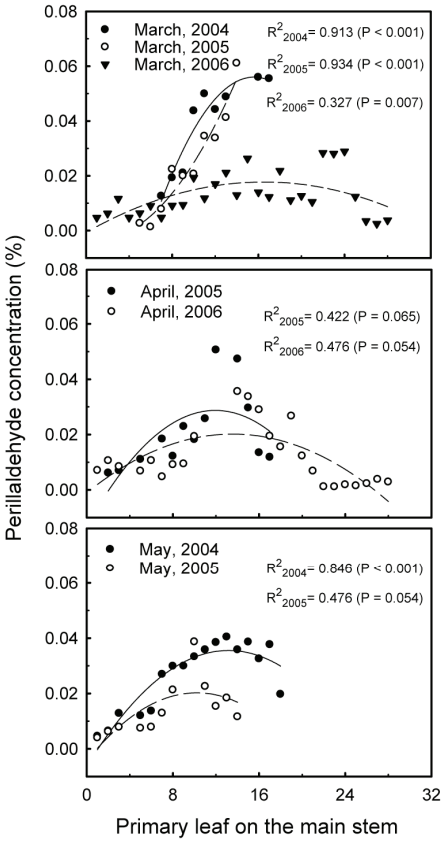

Fig. 4. Comparisons of the perillaldehyde (PA) concentration in different primary leaves emerged from the main stem for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in years from 2004 to 2006. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April; and Season III: seedlings transplanted in May.

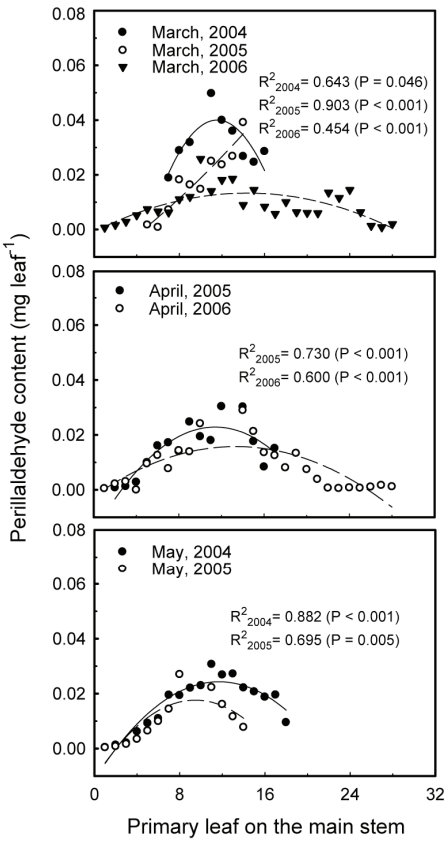

Fig. 5. Comparisons of the perillaldehyde (PA) content per leaf in different primary leaves emerged from the main stem for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in years from 2004 to 2006. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April; and Season III: seedlings transplanted in May.

the main stem or leaves collected from the lateral branches were monitored (Fig. 6). Results showed that, with limited leaf numbers, PA content of all primary leaves collected per plant changed in a quadratic function. However, as the amount of leaves collected from the lateral branches increased after transplanting until the end of PA extraction in July, their PA content built up progressively with plant development. Thus, the optimal time period for the highest quantity of leaf PA should be referred to the potential amount of leaves collected from all parts of whole plant during growing seasons. In perilla, both leaves from the main stem and the lateral branches should be considered.

Since perilla has long being used as a folk medicine, vegetable, garnish and flavoring, this plant species may be considered as a valuable herb and is worthy of studying in depth. To further expand its uses for the benefits of human health and living, changes in the interested compounds during plant development should be uncovered. Its growth performance under different growing seasons and years should also be extensively investigated, especially when the production of its plant parts and valued components are the major targets.

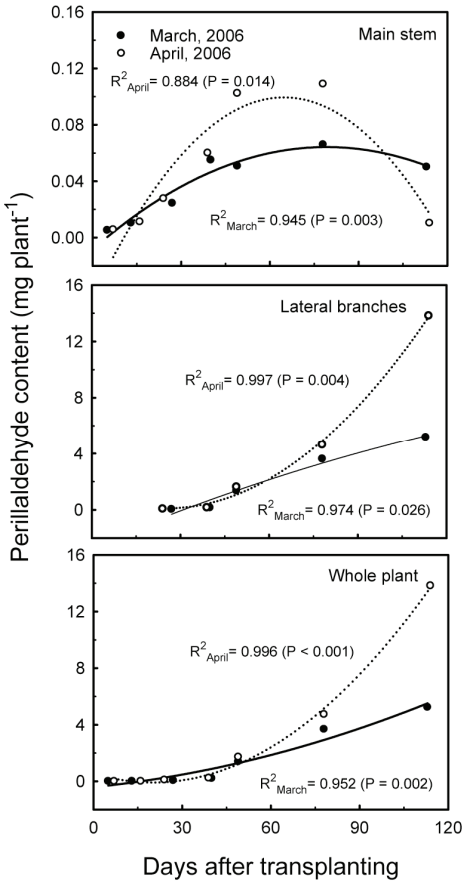

Fig. 6. Changes in perillaldehyde (PA) content per plant after transplanting in primary leaves collected from the main stem, leaves collected from the lateral branches, and leaves of whole plant for plants of red perilla, *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type), grown at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in Seasons I and II of 2006. Season I: seedlings transplanted in March; Season II: seedlings transplanted in April.

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紅葉紫蘇植株生長及葉片紫蘇醛之季節變化[1]

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摘 要

李裕娟、楊純明。2009。紅葉紫蘇植株生長及葉片紫蘇醛之季節變化。台灣農業研究 58:114–124。

紅葉紫蘇 [*Perilla frutescens* (L.) Britton] 係唇形科 (Lamiaceae) 原產亞洲之雙子葉植物，如今已廣泛分佈於包括臺灣在內之世界各地。欲將植物提升為農作物，殊有必要針對該植物生長行為及化學組成進行深入探討，以提供栽培管理與多元利用之參考。本研究調查紫蘇植株在 2004 年至 2006 年期間不同生同季節之生長性狀及葉片紫蘇醛濃度與含量季節變化，其中生長性狀以株高、葉面積指數 (leaf area index, LAI) 及植體地上部位 (葉片、莖桿) 鮮重為主。以種子育苗至五葉齡期，分別於 3 月 (Season I)、4 月 (Season II)、5 月 (Season III)、6 月 (Season IV) 及 7 月 (Season V) 進行移植工作，將苗株定植於試驗田間。葉片紫蘇醛濃度則以高效液體層析儀 (High Performance Liquid Chromatography, HPLC) 予以分析。根據試驗結果，發現生長於相對較為冷涼之 Season I 植株之株高、LAI 及植體地上部位鮮重均高於相對較為溫暖之其他栽培季節 (Seasons II-V)，似乎溫暖環境相對較不利於植株生長。植株成熟時採收之種子生產量因栽培季節而異，惟以 Season V 收刈之種子量最低，然而其 500 粒種子重量最高。無論年份或季節，主桿節位伸出葉片之紫蘇醛濃度，以第 10 至第 15 節位之間為高原期，高於其他節位葉片之紫蘇醛濃度，呈現凸形曲線分佈。其等葉片之紫蘇醛含量，在各栽培季節亦呈現類似凸形曲線變化。又紫蘇醛含量於生育期間及不同栽培季節在主桿葉片及分支葉片會有不等之變化，因此考量兩者加總量後，建議以移植後 110–120 日期間為獲得葉片紫蘇醛最大收取量之最佳時期。

關鍵詞：生長性狀、葉片紫蘇醛、種子生產量、紫蘇 [*Perilla frutescens* (L.)]、栽培季節。

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1. 行政院農業委員會農業試驗所研究報告第 2357 號。接受日期：98 年 6 月 29 日。
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