Growth Behavior and Perillaldehyde Concentration of Primary Leaves of *Perilla frutescens* (L.) Britton Grown in Different Seasons

Yuh-Jyuan Lee and Chwen-Ming Yang *
Agronomy Division, Taiwan Agricultural Research Institute, Wufeng, Taichung Hsien 41301, Taiwan ROC

ABSTRACT

Perilla frutescens (L.) Britton, a member of Lamiaceae and a native dicot in Asia, is now widely distributed in many regions of the world. It is regarded as a common annual weed in some areas and is generally ignored by grazing livestock because of the toxic compound perillaketone presented in the plant. However, it is also considered a commercial crop used as a condiment for foods in many Asian countries such as Japan, Korea and Taiwan. In the present study, changes in growth traits and perillaldehyde concentration of primary leaves along the main stem were investigated for plants grown at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) in different growing seasons of 2004. The growth traits, including plant height, leaf area and weights of aerial parts, were measured regularly during the growing periods for plants transplanted on March 15 (Season 1), May 3 (Season 2) and July 30 (Season 3), respectively. The concentration of perillaldehyde was determined by High Performance Liquid Chromatography (HPLC). Results show that plant height was shorter and leaf area and plant weights were smaller for plants grown in Season 3 relative to those plants grown in Seasons 2 and 1. Plants grown in Season 3 had shorter growth duration (118 days) and days to flowering (64 days), compared to 203 days and 152 days for Season 2 and 248days and 200 days for Season 1, respectively. However, plants grown in these three seasons all had the similar flowering date, October 1 or 2. Area and weight of primary leaves increased with the increasing of leaf position upwards until leaf position 10 and then decreased thereafter, irrespective of growing seasons. On the same leaf position, primary leaves from Season 3 were smaller in area and lighter in weight than those leaves from Season 2 and Season 1. The concentration of perillaldehyde of fully expanded primary leaves increased with the increasing of leaf position upwards, and was higher in leaves from Season 1, followed by Season 2 and Season 3, after leaf position 10. Results suggest that growth behavior and perillaldehyde concentration were influenced by planting/growing seasons, and primary leaves in the upper positions have a higher concentration of perillaldehyde than those in the lower positions.

Key words: Growth behavior, Perillaldehyde concentration, Primary leaf, *Perilla frutescens* (L.) Britton, Growing season.

不同栽培季節紫蘇(*Perilla frutescens* (L.) Britton)之生長行為及主桿不同節位葉片紫蘇醛濃度變化

李裕娟、楊純明*

行政院農業委員會農業試驗所農藝組

摘要

紫蘇(*Perilla frutescens* (L.) Britton)屬於Lamiaceae科，係原產於亞洲的雙子葉植物，

* 通信作者，cmyang@wufeng.tari.gov.tw
投稿日期：2005 年 4 月 7 日
接受日期：2006 年 2 月 12 日
作物、環境與生物資訊 3:135-146 (2006)
Crop, Environment & Bioinformatics 3:135-146 (2006)
189 Chung-Cheng Rd., Wufeng, Taichung Hsien 41301, Taiwan ROC

目前已散見於世界各地。紫蘇在許多地區被視為常見的一年生雜草，由於植體內含有毒害性物質紫蘇酮(perilla ketone)，因此多不為放牧之牲畜所覓食。在許多亞洲國家如日本、韓國及臺灣，紫蘇亦被視為作物栽培，供作食物之添加物等多種用途。本文研究旨在探討紫蘇於2004年不同栽培季節之生長行為及主桿不同節位葉片紫蘇醛(perillaldehyde)濃度，田間試驗係於位於臺中縣霧峰鄉之行政院農委會農業試驗所農場進行，幼苗分別於3月15日(第1季)、5月3日(第2季)及7月30日(第3季)定植於田區，生育期間調查之生長性狀包括株高、葉面積與地上部植體重量(葉重、桿重及葉加桿重)。主桿(莖)之不同節位葉片紫蘇醛濃度以高效能液態層析儀(HPLC，High Performance Liquid Chromatography)予以分析。根據試驗結果，與生長於第2季及第1季植株相比較，第3季生長之植株高度較矮且葉面積與植體重量較小。在生育天數與開花期方面，第3季、第2季及第1季生長之植株分別需要118天與64天、203天與152天及248天與200天。然而無論栽培季節，植株長出之花苔概於10月1日或2日開花。結果亦顯示，各栽培季節植株主桿完全展開葉片之面積與鮮重皆隨著節位上升而增加，直至第10節位，其後節位葉片則呈現下降趨勢。在相同主桿節位之完全展開葉片，以第1季生長之植株具有最大之葉面積與葉鮮重，其次為第2季生長之植株，再次為第3季生長之植株。在主桿第10節位以後之完全展開葉片紫蘇醛濃度，隨著節位上升而濃度增加，且第1季高於第2季及第3季者。綜合試驗結果，顯示栽培季節將影響紫蘇植株之生長行為及葉片紫蘇醛濃度，一般言較高節位葉片之紫蘇醛濃度大於較低節位葉片者。

關鍵詞： 生長行為、紫蘇醛濃度、主桿節位葉片、紫蘇、生長季節。

INTRODUCTION

Perilla frutescens (L.) Britton, known by varied common names in different places, is a C_3 species in Mint Family of Lamiaceae. It is a native dicotyledonous plant in eastern Asia (Shu 1994) and is found wild in India and China. This short-day annual herbaceous plant is also listed as a weed in the United States and Canada (Brenner 1993, Haragan 1991, Southern Weed Science Society 1998). However, since ancient times, perilla has been known as an herbal medicine, vegetable, garnish, flavoring, and natural colorant in Asian countries. Recently the species has also been cultivated in many European and North American countries for medicinal and culinary uses (Koezuka *et al*. 1985b, Kurita and Koike 1981, Li 1969, Perry and Metzger 1980, Ragazinskiene *et al*. 2004, Richardson 1972). In addition to be used as a condiment for foods, it is used as an antidote for sea food allergy. It is believed that some components of the leaf can prevent or neutralize the so-called 'poisoning' existing in sea food (Chen 1997). In Korea, perilla is a traditional oil seed crop and the seed oil is used for cooking and various industrial applications (Choi *et al*. 1980). In the United States, perilla food products are available in Korean ethnic markets, and red-leafed cultivated plants are used in landscaping (Brenner 1993).

Although perilla is ordinarily avoided by cattle (Kerr *et al*., 1986; Phillips and Von Tungein, 1986), traditionally it is regarded as a medicinal plant and is often prescribed in traditional Chinese medicine relieving allergic symptoms and symptoms of the common cold, cough and others (Duke and Fulton 2002, Yu *et al*. 1997). The anti-allergic effect using mice ear-passive cutaneous anaphylaxis (PCA)-reaction indicated that perilla extract significantly suppressed the PCA-reaction as that found with the treatment of rosmarinic acid (Makino *et al*. 2003). Banno *et al*. (2004) identified 8 triterpene acids from *P. frutescens* showing inhibitory effects on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 μg ear^{-1}) in mice, and among which five compounds had a potent inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) induction. In Lithuania, this species is considered as a new perspective annual medicinal deadnettle family (Lamiaceae Lindl.) plant. The extracts produced from medicinal raw material

with perilla are characterized by the variety of pharmacological effects: desensitizing, anti-microbial, antitumorous, and antioxidative (Ragazinskiene *et al*. 2004). There is also scientific research indicating that perilla extract has the ability to inhibit TNF-a (Tumor Necrosis Factor) over-production associated with auto-immune, acute and chronic inflammation and allergies (Ueda and Yamazaki 1997, Yamazaki and Ueda 1997).

Perilla is rich in anthocyanin and contains a number of chemical compounds in the extracts of plant parts. Fujita and Nakayama (1997) described in detail the chemical structures and the biological activities of more than 60 active constituents existed in the perilla plant. The pharmacological effects of perilla extract may vary with differences in varieties. There are six distinct chemotypes classified on the basis of the main components in the essential oils extracted from leaves, include perillaldehyde (PA), elsholtziaketone (EK), perillaketone (PK), citral (C), specific phenyl-propanoids (PP) and perillene (PL) (Tabata 1997). The primary toxic compound found in the plant is PK which causes serious lung edema in cattle grazing plants of wild perilla in the pasture (Koezuka *et al*. 1985a, Wilson *et al*. 1977), but the toxic dose of PK to humans is still unknown. The PA, with the synonym of laevo-isopropenyl-1-cyclohexene-1-arboxaldehyde, is the major component, up to 50%, found in the essential oil of plant extracts (Tada *et al*. 1996). This cyclic monoterpenoid, soluble in alcohol and insoluble in water, occupies a large part of the essential oil, and is a natural occurrence of grapefruit oil, orange oil, lime peel oil, peppermint, blackberry, etc. Omer *et al*. (1998) showed that the volatile oil extracted from leaves, stems (stalks) and flowers, using hydrodistillation method, reached 0.17%, 0.01% and 0.16% of fresh weight of the respective aerial parts. The hydrodistillates mainly consist of PA, caryophyllene oxide, limonene and caryophyllene, with PA as the main constituent (greater than 30%). Baser *et al*. (2003) further identified 15 compounds from the water-distilled essential oil from the aerial parts of *P. frutescens*, representing about 88% of the oil with PA (35.6%) and isoegomaketone (35.1%) as the main constituents. All these constituents extracted from perilla have a tendency to be easily oxidized in air when used in food products.

Plants of perilla are locally naturalized in many regions such as Asia, North America, and Europe. Its single upright axis bearing opposite leaves and branches, plant height can reach more than 0.9 m, and flowers are irregular in shape and purple or white in color. The indeterminate inflorescences produce from all active meristems along the stem and branches. With the simple vascular architecture, leaves and branches emerge on the same side of the square stem share vascular bundles, leaves and branches on adjacent sides share half of their bundles, while leaves and branches on the opposite sides have no bundles in common (Preston 1998). In Japan cultivation, there are two distinct variants of this species, variety crispa (know as shiso) for leafy vegetable and variety frutescens (known as egoma) for oil extraction from seeds, and each formed a cluster in the phenogram (Nitta and Ohnishe 1999). Of var. crispa, the plant is grown for aromatic leaves, which may be red (aka-shiso type) or green (ao-shiso type) but are always used fresh or frozen. In the Flora China (Shu 1994), *P. frutescens* is divided into three varieties, i.e., var. frutescens, var. purpurescens (Hayata) H.W.Li, and var. crispa (Benth.) W. Deane ex C. Bailey. The species, wild Perilla and *P. frutescens* (L.) Britton var. japonica (Hassk.) Hara, that are not purple colored and do not have an aroma having no use as medicine (Yu *et al*. 1997).

In this study, red perilla was used to compare the differences in growth behavior of this plant species when transplanted in different months and grown in different growing seasons, and in concentration of PA extracted from primary leaves of different positions along the main stem.

MATERIALS AND METHODS

Field experiments were conducted in the experimental farm of Taiwan Agricultural Research Institute (Wufeng, Taiwan) during three growing seasons from January to November of 2004. The dates of seed sowing, seedling transplanting, leaf removal, bud pinching and seed harvest of three growing seasons are listed in Table 1. Seeds of *Perilla frutescens* (L.) Britton var. crispa (aka-shiso type) were sown into sifted compost (a mixture of loam and leaf mold) within

containers, 1 seed per container, covered lightly with soil, and then placed under a polyethylene plastic (PEP) structure nursery. The containers were irrigated daily and sprayed with fertilized water (4.2 g urea resolved in 4 L of tape water) every other day until the 5-leaf pair stage, the stage when the fifth 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 5-leaf pair stage were selected and transplanted from the nursery to the experimental field at spacings of 0.5 × 0.5 m on March 15 (Season 1), May 3 (Season 2) and July 30 (Season 3) of 2004. 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. On each transplanting, seedlings were transplanted into 3 subplots with each subplot 8 m in length and 0.9 m in width and plowed into 2 lines.

Leaf-removal and bud-pinching were practiced during the growing periods (Table 1). When the seventh leaf pair were emerged from the main stem, the first to the fourth leaf pairs were removed by hands with the purpose to promote a healthy plant growth. The treatment of bud-pinching was carried out when plants grown to the fourteenth leaf pair stage. Buds on the top of main stem and branches were pinched off in order to maintain a neat appearance and an easy-to-access plant height. Samplings were in progress by 1- or 2-week intervals to measure growth traits, plant height (PH), leaf area (LA) and plant weights, including leaf fresh (LFW) and dry weights (LDW), stem fresh (SFW) and dry weights (SDW), and aboveground fresh (AFW) and dry weights (ADW).

When the primary leaves on main stem were fully expanded 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). Leaf disc of 0.16-0.18 g was punched off from each of sampled leaves, cut to pieces with a scissors, and homogenized in 5 mL 90% methanol using a pestle and mortar. The homogenate was placed standstill for at least 10 min and the clean supernatant was collected and filtered through a funnel packed with 0.2 g charcoal activated powder (Sigma-Aldrich Laborchemikalien GmbH, Germany), with a recovery rate of 20.725%. 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 then used for HPLC analysis.

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

Table 1. The important dates recorded during the cultivation of *Perilla frutescens* (L.) Britton in different growing seasons at Taiwan Agricultural Research Institute Experimental Farm (Wufeng, Taiwan) from January to November of 2004.

Important date of cultivation	Season I[x]	Season II	Season III
Seed sowing in nursery	01/28/2004	04/01/2004	06/14/2004
Seedling transplanting to the field	03/15/2004	05/03/2004	07/30/2004
Lower leaves removal on the main stem[y]	04/08/2004	06/16/2004	09/03/2004
Bud pinching (main stem and branches)	05/07/2004	07/08/2004	10/01/2004
First flowering (main stem and branches)	08/23/2004	none	none
Flowering of inflorescences	10/01/2004	10/02/2004	10/02/2004
Seed harvest	11/18/2004	11/22/2004	11/25/2004

[x] Growth duration from transplanting to harvest was 248 , 203 and 118 days for seasons 1, 2 and 3, respectively.

[y] The first 4 leaf pairs of primary leaves on the main stem were removed from all plants for the purpose of enhancing a healthy plant growth.

Diamond™ system, Barnstead Inc., USA) for a mobile phase. The standard PA 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. Separation was carried out with an Inertsil C_8 column (150 x 4.6 mm I.D., 5 µm particle) (GL Sciences Inc., Japan) and isocratic mobile phase consisted of methanol-water (75:25, v/v) and flowed at a rate of 0.5 ml min^{-1} under room temperature. The detection wavelength was set at 220 nm for PA. The chromatogram of PA from standard and extract of the primary leaf is plotted in Fig. 1, in which the retention times were 7.47 and 7.49 min for standard and extract, respectively.

RESULTS AND DISCUSSION

The weather conditions during the growing seasons of 2004 for *Perilla frutescens* (L.) Britton grown in the experimental farm of Taiwan Agricultural Research Institute were graphed in Fig. 2. Generally daily mean air temperature (DMAT), daily irradiance (DI) and daily sunshine hour (DSH) were higher in summer months (June to September) and lower in winter (December to February). Variation in daily precipitation (DP) was significant, a wet season from April to September and a dry season after September.

Daily evaporation (DE) was higher in the warmer and brighter seasons and lower in the cooling and gloomy months. Plants of Season 1 were transplanted in the early spring (March 15) when DMAT starting to increase and DI and DSH bounding back from the yearly lower values in winter time. Those plants transplanted in Season 2 (May 3) had a warming and shinny weather, while the weather was hot and humid with plenty of solar radiation for plants transplanted in Season 3 (July 30). Consequently, growth of *P. frutescens* can be expected to vary in these three growing seasons since values and patterns of weather variables altered within and between the growing durations.

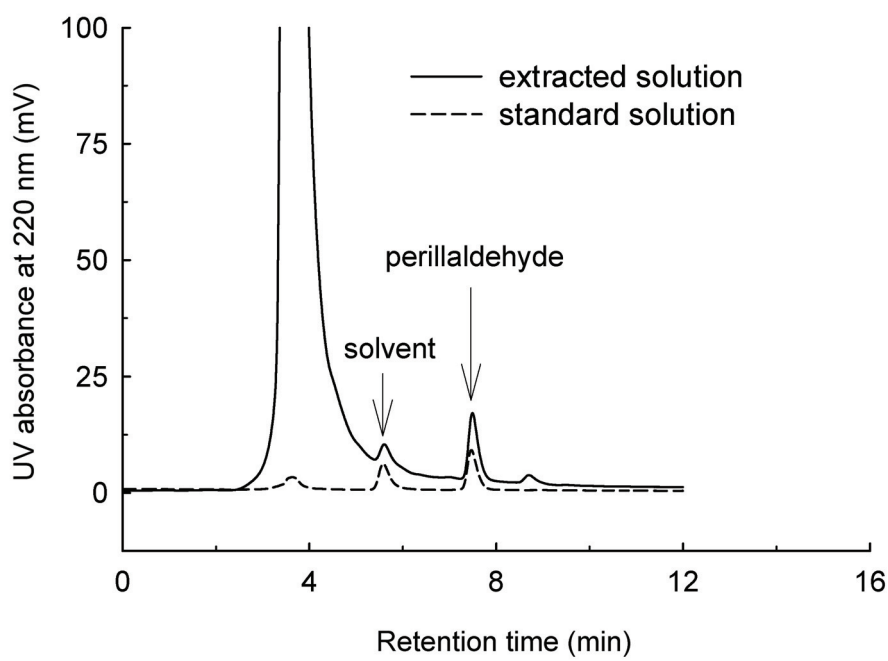

Fig. 1. Chromatogram of perillaldehyde extracted with 90% methanol from fully expanded primary leaf of *Perilla frutescens* (L.) Britton. HPLC conditions: Inertsil C_8 column (150×4.6 mm I.D., 5 µm particle); mobile phase consisting of methanol-water (75:25, v/v); flow-rate of 0.5 ml min^{-1}; detection wavelength of 220 nm.

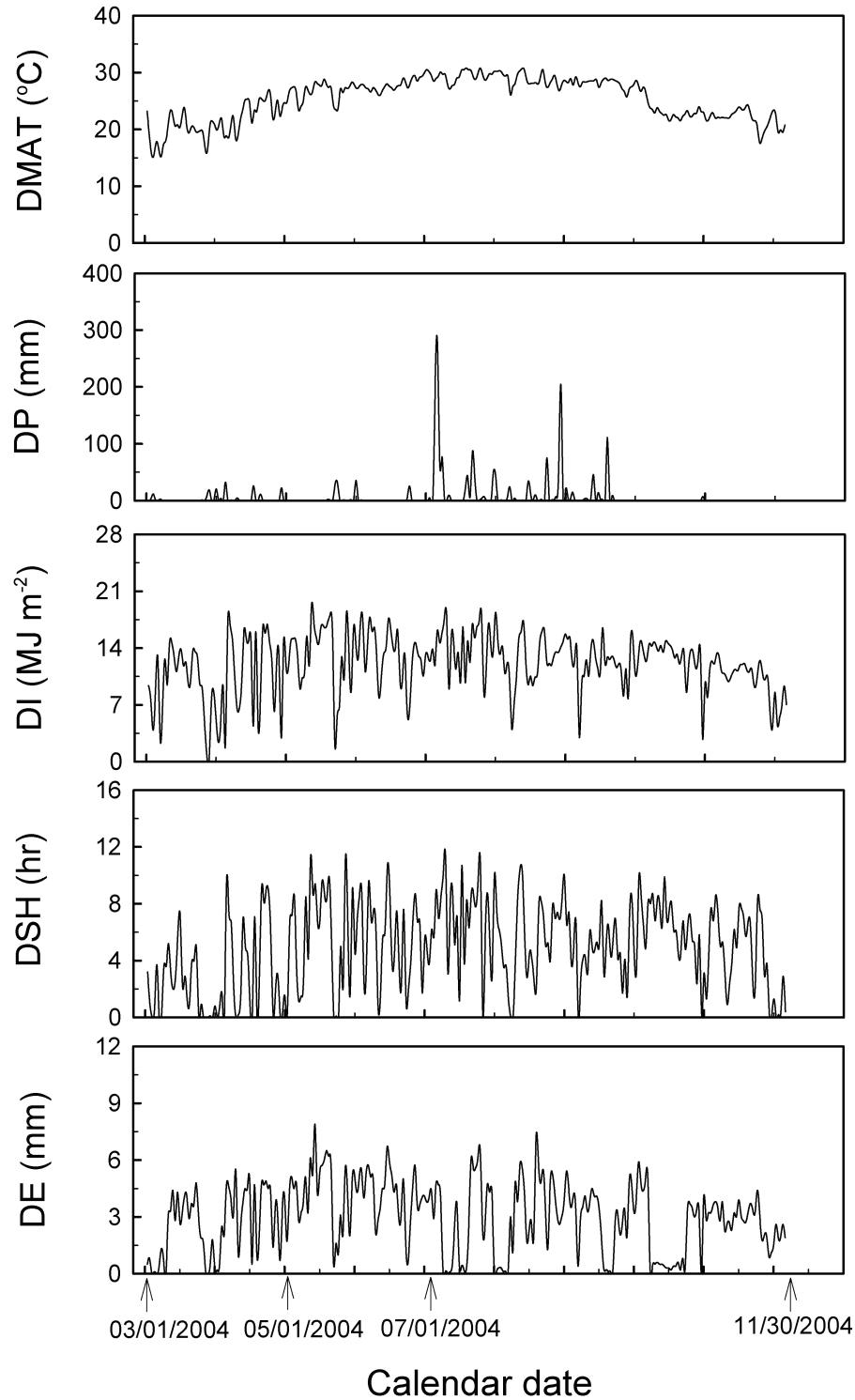

Fig. 2. Changes in weather variables for plants of *Perilla frutescens* (L.) Britton during the growing seasons of 2004.

The important dates recorded during the cultivation of this red perilla in different growing seasons are listed in Table 1. The growth duration from seedling transplanting to seed harvest was 248 days, 203 days and 118 days for Seasons 1, 2 and 3, respectively. Although plants were grown in different growing seasons with varied growth durations and days to flowering, the flowering of inflorescences on main stem and branches was almost occurred on the same date (October 1 or 2) and the seeds matured in the similar period of time, from November 18 to November 25. Apparently this short-day annual species adapts to the long night length environment of the experimental field, no matter transplanted in the early spring, late spring or mid summer. However, for plants transplanted on the March 15, there were some flowers emerged from the active meristems along main stem and branches on August 23. This is not surprising, as indicated by Kosuna *et al*. (1997) and Zeevaat (1969, 1985), that plants of perilla become photosensitive at the fourth leaf pair stage. Since the transplanted seedlings had the fifth pair of primary leaves, they might have sensed to short-day environment of the early spring (sunshine hours < 7 hr day^{-1}, Fig. 1). Similar results were found in plants of *Perilla ocymoides* cv. Yuepsildlggae (Park *et al*., 1991). Zeevaart (1969, 1985) reported that long nights induce flowering of perilla and flowering may start in 18 to 20 days after the beginning of long nights. Thus, as long as these plants grown in the same field and sensed the equivalent amounts of long nights, they should be able to flowering at the right time even though transplanted in different months.

It is also worthy to note that seeds sown in the mid winter (Season 1) and the early summer (Season 3) tend to inhibit seedling growth, when compared with those seeds sown in the mid spring (Season 2) (Table 1). Thus, days to reach the fifth leaf pair stage for seedling transplanting were 47 days, 32 days and 46 days for Season 1, Season 2 and Season 3, respectively. It implies that weather conditions of the sowing season play a key role in determining seedling growth and the time period required to reach the transplanting stage. To reduce the seedling preparation duration, therefore, a controlled environment with the appropriate temperature and radiation control is suggested.

The plant height and plant total leaf area of *P. frutescens* were progressively increased with plant development, and weights of the aerial parts (i.e., fresh and dry weights of leaves, stems and aboveground) followed the similar patterns (Fig. 3). However, plants grown in different seasons had various growth performances. Based on the measured growth traits, generally plants grown in Season 1 grew better than those plants gown in Seasons 2 and 3 after the early lag phase. That is, the later the transplanting time the shorter the plant height and the smaller the leaf area and plant weights. As these growth traits are positively correlated, plants with higher plant height would be expected to obtain more leaf area and plant biomass. In combination with the results shown in Table 1, spring season seems a better timing for transplanting of *P. frutescens* if production of the aerial parts is the main purpose in the weather conditions such as Wufeng, Taiwan.

The relations of leaf fresh weight and leaf area to leaf position were plotted in Fig. 4. As shown in the figure, fresh weight of primary leaf increased with the upward direction of leaf position along the main stem, reached the maximum, and then decreased hereafter. Similar pattern was found in the changes of leaf area of the fully-expanded primary leaves. On the same leaf position, generally area and fresh weight of the primary leaves from Season 1 were larger than those leaves from Seasons 2 and 3. There was a trend that primary leaves of later transplanting were smaller in area and lighter in weight than those of early transplanting counterparts. Whereas, from the 11^{th} primary leaf pair and above, no significant differences in area and fresh weight were found between leaves from Season 1 and Season 2. Again, results imply that plants transplanted in the mid summer grow in an inferior environment and thus perform less satisfactory in vegetative growth than the other seasons. The Season 1 is the premier season of choice to obtain better plant growth as well as larger primary leaves.

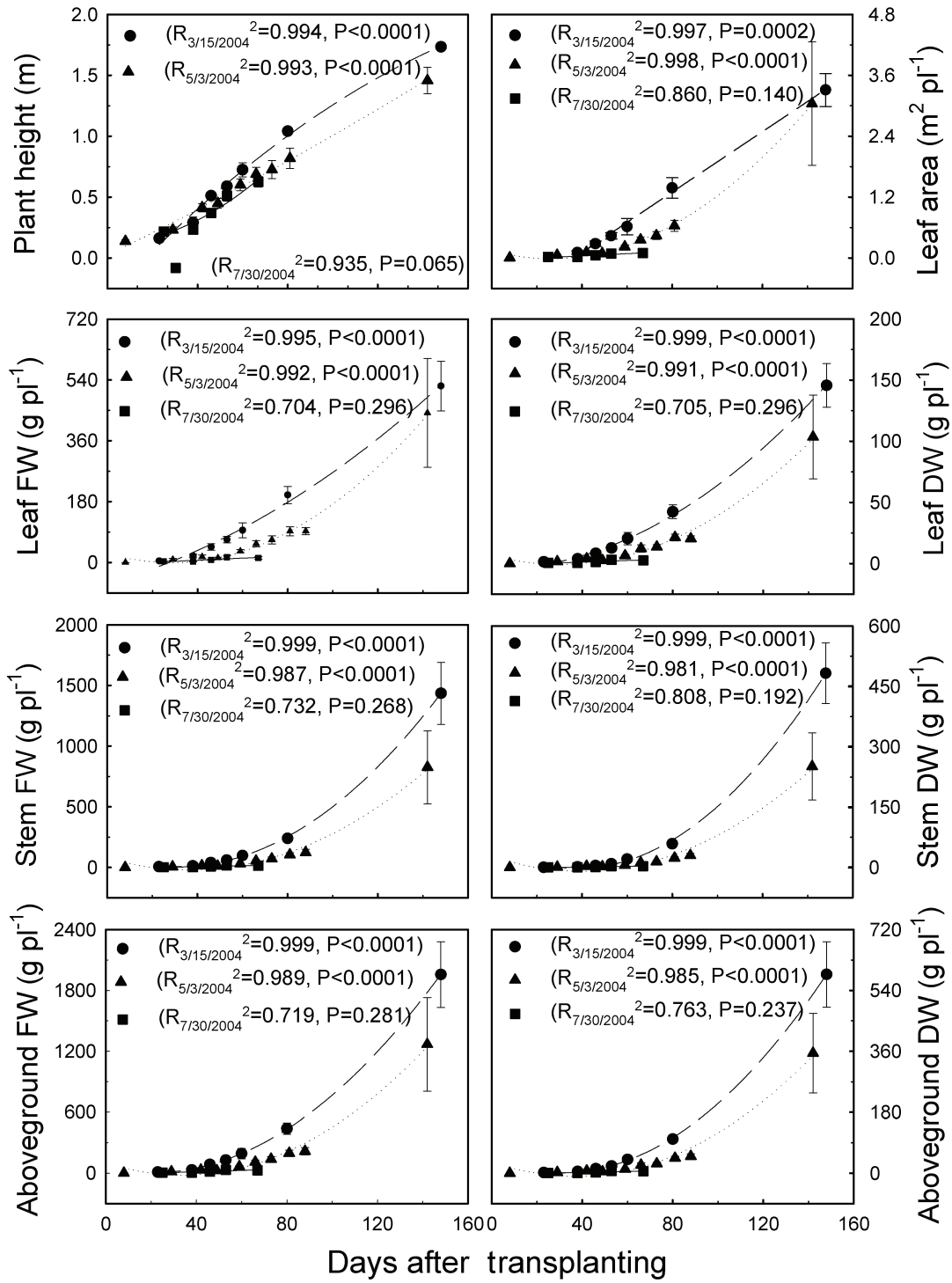

Fig. 3. Changes in growth traits of *Perilla frutescens* (L.) Britton after transplanting grown in different growing seasons of 2004.

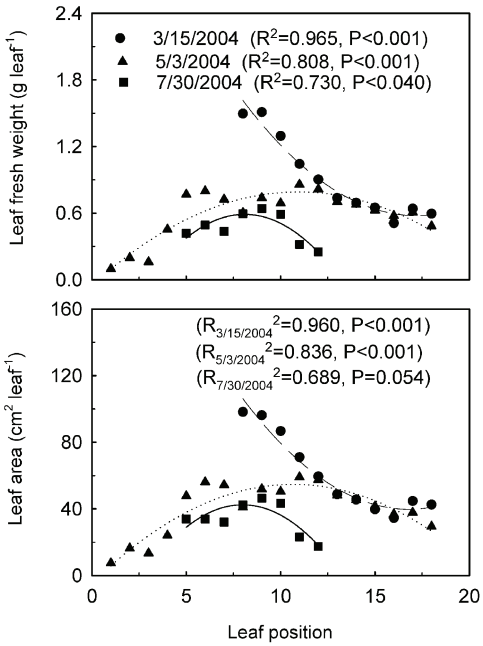

Fig. 4. Changes in leaf fresh weight and leaf area in primary leaves along the upward direction of main stem of *Perilla frutescens* (L.) Britton grown in different growing seasons of 2004.

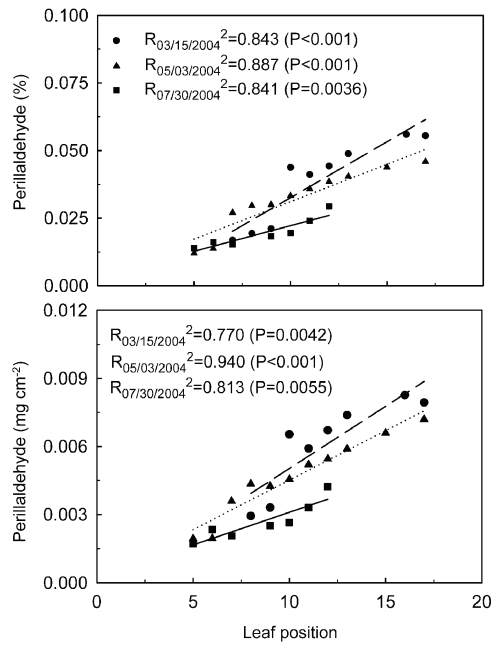

Fig. 5. Changes in concentration of perillaldehyde in primary leaves along the main stem of *Perilla frutescens* (L.) Britton grown in three different growing seasons of 2004.

Oil of perilla is used as a flavoring agent, in which PA is the desirable flavoring compound (Guenther 1949, Arctander 1960). The volatile oil can be distilled from the foliage of this species (Guenther 1949, Koezuka *et al*. 1986, Nago *et al*. 1975, Nishizawa *et al*. 1989, 1990). Omer *et al*. (1998) showed that yield of volatile oil extracted by hydrodistillation reached 0.17%, 0.01% and 0.16% of fresh weight from leaves, stem and flowers of *P. frutescens*. There were 23 components identified from volatile oil of this species cultivated in Egypt. This essential oil showed good inhibitory activity against *Aspergillus niger*, *Candida albicans*, *Bacillur subtilis* and *Encherichia coli*, and was attributed to the high contents of PA and caryophyllene oxide (Omer *et al*. 1998). Kang *et al.* (1992) pointed out that PA showed moderate and broad-spectra activity against many Gram-positive and Gram-negative microbes and fungi. Others also demonstrated that PA has a sedative activity and antimicrobial properties (Duke and Fulton 2002, Terao *et al.* 1991).

From the fully expanded primary leaves, this study found that concentration of PA linearly increased with the increase of the upward leaf position, and was higher in leaves of the early transplanted plants than those of the later transplanted plants after leaf position 10, either on per weight or on per area basis (Fig. 5). Results suggest that planting time/season not only has a strong influence on plant growth but also has a profound effect on PA concentration presented in the primary leaves, and perhaps the other leaves. As primary leaves in the upper positions having a greater concentration of PA than those in the lower positions, collecting the same amount of leaves from the upper plant parts would be expected to produce better yield of this valuable compound. The concentration of PA measured in this study is similar to that reported in plants cultivated in Japan and in California (Kang *et al.* 1992), but is a little bit higher than that cultivated in Egypt as reported in Omer *et al*. (1998). The PA measured in this study is extracted from the fully expanded primary leaves on main stem rather than mixed leaves of diversified size from the whole plant as that used in Omer *et al*. (1998).

In study of carbohydrate translocation in

perilla, Preston (1998) found that plants of *P. frutescens* were both highly integrated and markedly sectorial for carbohydrates. Both integration and sectoriality changed throughout development and that each labeled leaf played a particular role in supporting plant growth. Similar case may also exist in PA, its concentration may vary in leaves of different ages and developmental stages as indicated in different leaf positions. This is reasonable because plant parts are produced throughout the life of a plant with each subunit arises from another, and each leaf may be a unique portion not functioning uniformly throughout it lifespan. A single leaf may support its own physiological subunit as well as other parts of different physiological subunits.

Based on knowledge of its long traditional use as a folk medicine, vegetable, garnish and flavoring in association with modern scientific research findings, *P. frutescens* may be considered as a valuable herb containing a lot of beneficial constituents for human health and living. To expand the uses of this species, changes in its ingredients during plant development should be further clarified. Its growth behavior under different growing seasons needs also to be extensively explored if the production of its plant parts is the major benefit of concern.

ACKNOWLEDGMENTS

This work was supported by the research grant (94AS-1.3.1-CI-C7) from Council of Agriculture, Executive Yuan, Taiwan ROC.

REFERENCES

Arctander S (1960) Perfume and Flavor Materials of Natural Origin. Allured Pub Corp., Elizabeth, NJ.

Banno N, T Akishisa, H Tokuda, K Yasukawa, H Higashihara, M Ukida, K Watanabe, Y Kimura, J Hasegawa, H Nishino (2004) Triterpene acids from the leaves of *Perilla frutescens* and their inflammatory and antitumor-promoting effects. **Biosci. Biotechnol. Biochem.** 68(1):85-90.

Baser KHC, B Demirci, AA Donmez (2003) Composition of the essential oil of Perilla frutescens (L.) Britton from Turkey. **Flav. Fragr. J.** 18(2):122-123.

Brenner DM (1993) Perilla: Botany, uses and genetic resources. p.322-328. *In:* New Crops. J Janick, JE Simon (eds.) Wiley, New York.

Chen YP (1997) Applications and prescriptions of Perilla in traditional Chinese medicine. p.37-45. *In:* Perilla: the Genus Perilla. K Kosuna, M Haga, HC Yu (eds.) Harwood Academic Publishers, Amsterdam.

Choi IS, SY Son, OH Kwon (1980) Effect of seedling age and planting density on the yield and its component of perilla (*Ocymcides* var. Typica MAKINO) intercropped with tobacco or aftercropped. (in Korean) **Korean Soc. Hort. Sci.** 25:68-75.

Duke A, M Fulton (2002) Handbook of Medicinal Herbs. 2nd ed. CRC Press, Boca Raton, Fl. 896pp.

Fujita T, M Nakayama (1997) Chemical studies on the constituents of *Perilla frutescens*. p.109-128. *In:* Perilla: the Genus Perilla. K Kosuna, M Haga, HC Yu (eds.) Harwood Academic Publishers, Amsterdam.

Guenther E (1949) The Essential Oils. Vol 3. D. Van Nostrand, New York.

Haragan PD (1991) Weeds of Kentucky and Adjacent States: A Field Guide. The Univ. Press of Kentucky, Lexington, Kentucky. 278pp.

Kang R, R Helms, MJ Stout, H Jaber, Z Chen, T Nakatsu (1992) Antimicrobial activity of the volatile constituents of *Perilla frutescens* and its synergistic effects with polygodial. **J. Agric. Food Chem.** 40:2328-2330.

Kerr LA, BJ Johnson, GE Burrows (1986) Intoxication of cattle by *Perilla frutescens* (purple mint). **Vet. Hum. Toxicol.** 28:412-416.

Koezuka Y, G Honda, M Tabata (1985a) An intestinal propulsion promoting substance from *Perilla frutescens* and its mechanism of action. **Planta Med.** 6:480-482.

Koezuka Y, G Honda, M Tabata (1985b) Genetic control of anthocyanin production in *Perilla frutescens*. **Shoyakugaku Zasshi.** 39:228-231.

Koezuka Y, G Honda, M Tabata (1986) Genetic

control of phenylpropanoides in *Perilla frutescens*. **Phytochemistry** 25:2085-2087.

Kosuna K, M Haga, HC Yu (eds.) 1997. Perilla: the Genus Perilla. Harwood Academic Publishers, Dallas. 187pp.

Kurita N, S Koike (1981) Synergistic antimicrobial effect of perilla and NaCl. (in Japanese) **Nippon Nogeikagaku Kaishi** 55:43-46.

Li HL (1969) The vegetables of ancient China. **Econ. Bot.** 23:253-260.

Makino T, Y Furuta, H Wakushima, H Fujii, K Saito, Y Kano (2003) Anti-allergic effect of *Perilla frutescens* and its active constituents. **Phytotherapy Res.** 17(3):240-243.

Nago Y, S Fujioka, T Takahashi, T Matsuoka (1975) Studies on the quality of the Chinese drug "soyo" and the cultivation of the original plant. (in Japanese) **Takeda Res. Lab.** 34:33-42.

Nishizawa A, G Honda, M Tabata (1989) Determination of final steps in biosyntheses of essential oil components in *Perilla frutescens*. **Planta Med.** 55:251-253.

Nishizawz A, G Honda, M Tabata (1990) Genetic control of perillene accumulation in *Perilla frutescens*. **Phytochemistry** 29:2873-2875.

Nitta M, O Ohnishi (1999) Genetic relationships among two Perilla crops, shiso and egoma, and the weedy type revealed by RAPD markers. **Gene Genetic Syst.** 74(2):43-48.

Omer EA, ME Khattab, ME Ibrahim (1998) First cultivation trial of *Perilla frutescens* L. in Egypt. **Flavour Fragr. J.** 13:221-225.

Park HC, DG Choi, SK Kim, SK Chin, KH Park, BJ Choi (1991) Studies on suitable sowing times, seedling growing periods and planting distances for late-planted Perilla ocymoides cultivar Yuepsildlggae. (in Korean) **Res. Rep. Rural Devel. Administ.** (Upland & Indus. Crops) 33(3):47-53.

Perry LM, J Metzger (1980) Medicinal Plants of East and Southeast Asia. Massachusetts Inst. of Technol., Cambridge, MS, USA. 620 pp.

Phillips WA, D Von Tungein (1986) Acute pulmonary edema and emphysema in steer fed old-world bluestem hay. **Mod. Vet. Pract.** 67:252-253.

Preston K (1998) The effects of developmental stage and source leaf position on integration and sectorial patterns of carbohydrate movement in an annual plant, *Perilla frutescens* (Lamiaceae). **Amer. J. Bot.** 85(12):1695-1703.

Ragazinskiene O, V Gailys, K Jankauskiene, G Simoniene, V Jurkstiene (2004) Common perilla (*Perilla frutescens* (L.) Britton) as a perspective immuno- modulator. **Medicina** 40(3):220-224.

Richardson IBK (1972) Perilla L. p.186-187. *In:* Flora Europaea. 3rd ed. TG Tutin, VH Heywood, NA Burges, DH Valentine, SM Walters, DA Webb (eds.) Cambridge University Press, Cambridge, UK.

Shu ZS (1994) Perilla L. *In:* Flora of China. (English version) I. Al-Shehbaz *et al*. (eds.) Science Press and Missouri Botanical Garden Press, Beijing and St. Louis.

Southern Weed Science Society (1998) Weeds of the United States and Canada. CD-ROM. Southern Weed Science Society. Champaign, Illinois.

Tabata M (1997) Chemotypes and pharmacological activities of Perilla. p.129-142. *In:* Perilla: the Genus Perilla. K Kosuna, M Haga, HC Yu (eds.) Harwood Academic Publishers, Amsterdam.

Tada M, R Matsumoto, K Chiba (1996) An oxidative metabolite of perillaldehyde from *Perilla frutescens*. **Phytochemistry** 43(4):803-804.

Terao J, A Nagao, H Suzuki, M Yamazaki (1991) Effect of dietary fats (Perilla oil, lard, rapeseed oil) on peroxidizability of mouse brain lipids. **J. Agric. Food Chem.** 39:1477-1481.

Ueda H, M Yamazaki (1997) Inhibition of TNF-alpha production by orally administering a Perilla leaf extract. **Biosci. Biotech. Biochem.** 61:1292-1295.

Wilson BJ, JE Garst, RD Linnabary, RB Channell (1977) A potent lung toxin from the mint plant, *Perilla frutescens* Britton. **Science** 197:573-574.

Yamazaki M, H Ueda (1997) Anti-inflammatory and antiallergic activities of Perilla extract. p.47-54. *In:* Perilla: the Genus Perilla. K Kosuna,

M Haga, HC Yu (eds.) Harwood Academic Publishers, Amsterdam.

Yu HC, A Niskanen, J Paananen (1997) Perilla and the treatment of allergy-A review. p.55-70. *In:* Perilla: the Genus Perilla. K Kosuna, M Haga, HC Yu (eds.) Harwood Academic Publishers, Amsterdam.

Zeevaart JAD (1969) Perilla. p.116-155. *In:* The Induction of Flowering: Some Case Histories. LT Evans (ed.) Cornell Univ. Press., Ithaca, NY.

Zeevaart JAD (1985) Perilla. p.239-252. *In:* CRC Handbook of Flowering. AH Halevy (ed.) CRC Press, Inc., Boca Raton, FL.

－編輯：謝兆樞