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

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**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 248 days 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

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摘要

紫蘇(*Perilla frutescens* (L.) Britton)屬於Lamiaceae科，係原產於亞洲的雙子葉植物，
Perilla frutescens (L.) Britton, known by varied common names in different places, is a C₃ 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

INTRODUCTION
with perilla are characterized by the variety of pharmacological effects: desensitizing, antimicrobial, 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 phenylpropanoids (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:P₂O₅:K₂O= 20%:5%:10%, Taiwan Fertilizer Company, Kaoshiung, 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.

<table>
<thead>
<tr>
<th>Important date of cultivation</th>
<th>Season I</th>
<th>Season II</th>
<th>Season III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed sowing in nursery</td>
<td>01/28/2004</td>
<td>04/01/2004</td>
<td>06/14/2004</td>
</tr>
<tr>
<td>Seedling transplanting to the field</td>
<td>03/15/2004</td>
<td>05/05/2004</td>
<td>07/30/2004</td>
</tr>
<tr>
<td>Lower leaves removal on the main stem</td>
<td>04/08/2004</td>
<td>06/16/2004</td>
<td>09/03/2004</td>
</tr>
<tr>
<td>Bud pinching (main stem and branches)</td>
<td>05/07/2004</td>
<td>07/08/2004</td>
<td>10/01/2004</td>
</tr>
<tr>
<td>First flowering (main stem and branches)</td>
<td>08/23/2004</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Flowering of inflorescences</td>
<td>10/01/2004</td>
<td>10/02/2004</td>
<td>10/02/2004</td>
</tr>
</tbody>
</table>

* Growth duration from transplanting to harvest was 248, 203 and 118 days for seasons 1, 2 and 3, respectively.
* 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 C8 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. 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). Zeevaat (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 grown 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 11th 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.
Growth Behavior and Perillaldehyde Concentration of Primary Leaves of *Perilla frutescens*

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*, *Bacillus subtilis* and *Escherichia 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 arising from another, and each leaf may be a unique portion not functioning uniformly throughout its 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.

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