

Variation in Growth and Development, and Essential Oil Yield between Two *Ocimum* Species (*O. tenuiflorum* and *O. gratissimum*) Grown in Georgia

Noelle J. Fuller¹

Department of Horticulture, University of Georgia, 1111 Miller Plant Sciences Building, Athens, GA 30602

Ronald B. Pegg

Department of Food Science and Technology, University of Georgia, 100 Cedar Street, Athens, GA 30602

James Affolter

State Botanical Garden of Georgia, 450 South Milledge, Athens, GA 30605

David Berle

Department of Horticulture, University of Georgia, 1111 Miller Plant Sciences Building, Athens, GA 30602

Additional index words. *Ocimum sanctum*, holy basil, tulsi, hydrodistillation, eugenol

Abstract. The use of medicinal plants in the United States is increasing. Holy basil (*Ocimum tenuiflorum* L. and *Ocimum gratissimum* L.), a medicinal herb native to India, has become increasingly popular for its therapeutic benefits. Traditionally, holy basil has been used to promote longevity by reducing stress and restoring balance to the body. Because it is easy to grow and adapts to a wide range of growing conditions, there is great potential for holy basil production in the southeastern United States. The purpose of this study was to evaluate holy basil varieties for harvestable weight and essential oil content. Fourteen varieties of holy basil were grown during the 2015 and 2016 seasons and compared. The main active compound in the holy basil essential oil fraction, eugenol, was quantitated and compared for each variety, because it is believed to be responsible for many of the health-promoting effects. Overall, there were significant differences in harvestable weights and essential oil yields among the varieties, and a significant effect of growing season. The eugenol content was highly variable among the varieties examined, with higher eugenol contents in 2016 than in 2015. The variety that had the overall highest yield, essential oil content, and eugenol concentration was PI 288779, a USDA accession, suggesting its use in future breeding research.

Medicinal herbs represent an emerging market with many opportunities for innovative research because they are generally safe, effective, economical, and easily accessible by consumers looking for health-promoting products (Prakash and Gupta, 2005). *Ocimum tenuiflorum* L. syn. *Ocimum sanctum*, and *O. gratissimum* L., known as tulsi or holy basil (Winston and Maimes, 2007), is a medicinal herb native to India with a wide distribution over the subcontinent. It is best known for its stress-reducing adaptogenic properties, i.e., it has the capability to help the body adapt to stress, normalize physiological function, and restore balance regardless of the origin of the stressor (Kuhn and Winston, 2000; Pattanayak et al., 2010; Singh et al., 2012).

Holy basil has been broadly researched and widely cultivated in India (Aggarwal and Mali, 2015; Raina et al., 2013; Sharma et al., 2011). With a growing demand for natural alternatives for managing stress (Esch et al., 2002), it is likely that the popularity and demand for holy basil will continue to increase in the coming years (Pattanayak et al., 2010). According to Roy Upton, Executive Director of the American Herbal Pharmacopoeia, with the implementation of good manufacturing practices, fewer overseas suppliers can meet the requirements for quality and product verification (Smith, 2011). With the promising therapeutic potential of holy basil, it shows capacity as a high-value cash crop that can be grown in the United States, using production methods that meet quality and traceability standards. According to a recent publication by the American Botanical Council, tea sales for the United States exceeded 15 billion USD in 2013, and tea drinking is predicted to increase in future years (Keating et al., 2014).

To increase cultivation of holy basil in the southeastern United States, the first step is to evaluate available holy basil varieties to determine which are most suited for commercial production. At present, growers typically select varieties based on seed availability, market demand, and harvestable weight, and not necessarily on the presence or concentration of biologically active compounds (Zhang et al., 2012). With medicinal herbs, an important consideration is the measurable difference in therapeutic constituents, such as essential oils, that are indicators of quality and efficacy. For example, a notable phenolic compound found in holy basil essential oil is eugenol. It is a versatile molecule with application in many industries (Kamatou et al., 2012). It has a spicy clove-like scent and has been shown to be therapeutically effective for neurological, inflammatory, allergic, and immunological disorders (Bakkali et al., 2008; Kamatou et al., 2012; Sen, 1993). Eugenol is largely extracted from natural sources, most commonly clove essential oil (*Eugenia caryophyllata*), which has a gross market value of US\$30–70 million annually for use in food and cosmetics (Bohnert et al., 2008). However, clove production is labor-intensive and not easily grown in the United States. Holy basil has lower production costs, can be grown easily in the southeast, and is a less-expensive option for extracting eugenol commercially (Saran et al., 2017).

For this study, varieties of holy basil consisted of five commercially available cultivars and nine USDA germplasm accessions. Presently, there is considerable confusion regarding labeling: many U.S. seed companies list only the genus and species name, with no mention of a cultivar. Thus, it is difficult to differentiate between available seed stock or know which ones should be grown to achieve maximum yield and quality. Furthermore, details concerning yield, essential oil content, and eugenol concentration are lacking for many of the USDA accessions. This investigation is unique in that it provides a comparison of a large selection of holy basil varieties grown in the southeastern United States and offers important information for growers seeking diversification and alternative crop options. Moreover, such details provide important baseline information to be used in future studies evaluating the effect of specific horticulture practices on yield and essential oils and can potentially help identify future breeding goals.

Materials and Methods

Plant and growth conditions. The experiments were conducted during the 2015 and 2016 growing seasons at the University of Georgia UGArden farm in Athens, GA (lat. 33°53'55.5"N; long. 83°22'09.2"W), using a randomized complete block design with three replications. Nine holy basil (*O. tenuiflorum* L.) accessions (PI 288779, PI 652059, PI 652057, PI 652056, PI 414201, PI 414202, PI 414203, PI 414204, and PI 414205) were

Received for publication 19 Apr. 2018. Accepted for publication 3 July 2018.

Funding was provided by the NIFA-USDA National Needs Fellows Grant Award #: 2013-38420-20497, and is greatly appreciated.

¹Corresponding author. E-mail: njfuller@uga.edu.

acquired from the USDA-ARS National Plant Germplasm System (Ames, IA), and four named cultivars (Kapoor, Rama, Krishna, and Amrita) were obtained commercially from Strictly Medicinal Seeds (Williams, OR). The cultivar Vana was obtained from Strictly Medicinal Seeds. It is a different species (*O. gratissimum*) but was included in this study because it is also commonly called tulsi in India and has similar therapeutic applications (Winston and Maimes, 2007). In both years, seeds were planted in June into 50-cell plastic trays filled with Sunshine Natural and Organic Professional Growing Mix (SunGro, Agawam, MA). Young transplants were watered once a day in the greenhouse. Thirty-eight-day-old seedlings were transplanted into the field in raised beds during the third week of July in both years.

A walk-behind tractor [BCS, Abbiatograsso (MI), Italy] was used to shape the raised beds (1 m wide, 0.3 m high, and 9 m long). Two lines of drip irrigation tubing (Toro 16-mm Blue Stripe with 3.8 L·h⁻¹ pressure-compensating emitters every 30.5 cm) were placed on the raised beds and covered with pine straw mulch. The total plot size was 9 m².

Soil was a Cecil sandy clay loam. Soil samples were taken at the beginning of the growing season in both years and analyzed by the UGA Agricultural and Environmental Laboratory in Athens, GA, using the Mehlich-1 extraction method to determine pH, lime buffer capacity, and available soil nutrients. The levels of P and K in the soil from both years were described as high to very high, with no P or K supplementation recommended (Kissel and Sonon, 2008). Beds were fertilized with nitrogen using hydrolyzed poultry feathers (13–0–0) (Mason City Byproducts, Inc., Mason City, IA) at a rate of 120 kg N/ha following the recommendation of Zheljzkov et al. (2008) for holy basil grown in Mississippi. Furthermore, in 2016, pulverized dolomitic lime (Austinville Limestone Co., Austinville, VA) was added at the rate of 2800 kg·ha⁻¹ to raise the pH from 5.69 to 6.0, based on the soil test recommendations from the UGA Agriculture and Environmental Laboratory.

Each holy basil variety was planted in an experimental unit of six plants. Transplants were spaced 30 cm apart in two rows, with 60 cm of space between the experimental units. After planting, each seedling was watered with 0.18 L dilution of Chilean nitrate of soda (16–0–0) (Allganic, Eugene, OR) at a concentration of 4.8 g·L⁻¹. Twelve days after planting, holy basil plants were pruned back to the third node to remove the shoot apical meristem to encourage growth of lateral branches (Eve et al., 2016). Plants were irrigated with ≈38 mm of water per week in weeks it did not rain. The University of Georgia Weather Station at the Plant Sciences Research Farm in Watkinsville, GA, recorded average monthly maximum and minimum temperatures and total monthly rainfall for the area over both years.

Table 1. Average daytime and nighttime air temperatures, and total monthly rainfall for 2015 and 2016 growing seasons.

Month	Avg. max temp (°C)		Avg. minimum temp (°C)		Rainfall (mm)	
	2015	2016	2015	2016	2015	2016
July	33.3	33.4	21.8	21.6	106	41.7
August	31.8	32.3	20.7	21.9	188	99.8
September	27.8	31.4	17.7	18.8	133	31.5
October	23.3	26.6	11.4	12.3	183	0
November	19.5	20.5	9.1	6.6	250	69.9

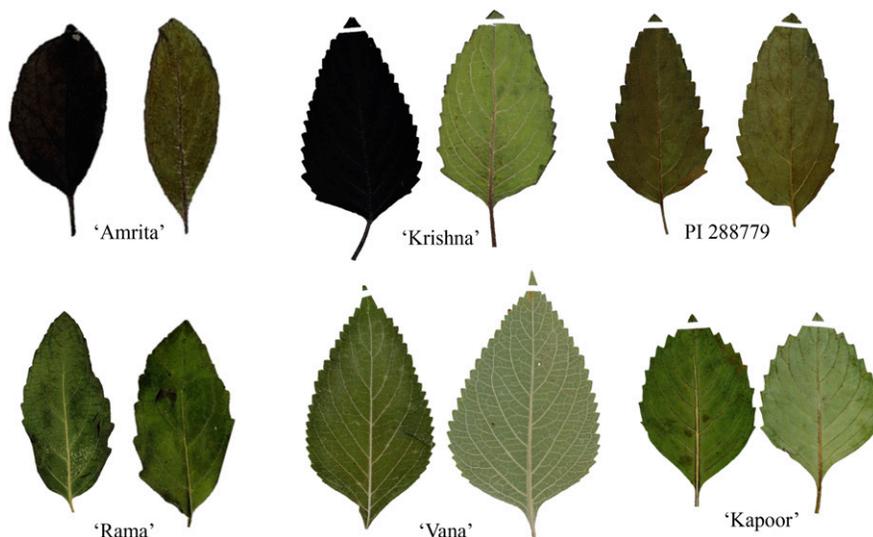


Fig. 1. Representative differences in leaf shape and color among holy basil varieties. PI 288779 similar to PI 652057. 'Kapoor' similar to PI 414201, PI 414202, PI 414203, PI 414204, PI 414205, PI 652056, and PI 652059.

Holy basil was harvested ≈6 weeks after planting, by hand cutting all stems above 15 cm. This height permitted secondary branches to remain intact and enabled regrowth to allow for a second harvest (Zheljzkov et al., 2008). During the 2015 trial, the second and final harvests were completed roughly 6 weeks later by harvesting the plants at a lower height, 10 cm above the ground, so plants would not regrow. In 2015, all of the plants were collected at the same time for consistency across varieties. However, following this protocol, not all varieties were flowering at the time of harvest. Investigators were made aware that collection during full flower has been shown to result in the highest quantity of essential oil and the greatest concentration of desirable essential oil compounds such as eugenol (Abdel-Hamid et al., 2005). As a result, in the 2016 trial, each variety was harvested twice as per the previous season, but the harvest time was determined based on the flowering stage and not a predetermined time period. Plants were considered to be at the flower stage when inflorescence occurred on 80% of the shoots and blooms were opening halfway up the flower stalk.

For each harvest, in both seasons, plants were weighed and bundled individually and air-dried at 24 °C in an herb room with a dehumidifier (Model DH50W; Honeywell,

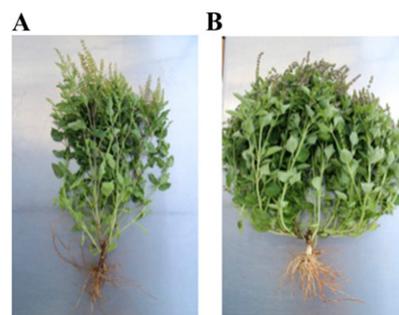


Fig. 2. (A) An example of clumping growth (PI 652057) and (B) an example of sprawling growth (PI 414205).

Mahwah, NJ). Plants were considered fully dried when there was a consistent relative humidity of 15% in the dry room and a test leaf was easily powdered. Dried weights were recorded for each plant because holy basil is typically shipped and marketed in the dry state. Six plants of each variety were combined and the leaves and flowers stripped from the stems. This was performed for three replications of each variety for each harvest. The combined mass of the dried leaves and flowers of six plants was recorded for each replication and then packed into 16 × 24-cm aluminum foil mylar zip-lock food-grade pouches followed by storage at –18 °C.

For each harvest, four samples were collected from different areas of the drying room to perform a moisture analysis. Each moisture analysis was performed in triplicate by preparing a representative mixture from the samples collected. The mixture was milled in a blade coffee grinder (Model F2037051; Krups, Millville, NJ). Roughly 2 g of the ground composite were transferred to a tared, aluminum weight pan (VWR International, Suwanee, GA), weighed, and then placed in a preheated Isotemp oven (Model 650G; Fisher Scientific Company, Dubuque, IA) set at 103 °C. The samples were checked intermittently until a constant mass was achieved (≈ 4 h). They were then removed from the oven and placed in a stainless steel desiccator cabinet. Once the samples had cooled, masses were recorded, the mass of the aluminum pans were subtracted from the initial and final values, and the moisture content was calculated using the following equation:

$$\begin{aligned} \% \text{ Moisture content} \\ &= (\text{initial mass} - \text{dry mass}) \\ &\quad / \text{initial mass} \times 100. \end{aligned}$$

In addition, 14 voucher specimens were prepared, one for each variety in the study, and verified by Dr. James Affolter, Director of Research at the State Botanical Garden of Georgia, Athens, GA. They are being stored in the University of Georgia Herbarium, Athens, GA (276066, 276068, 276069, 276070, 276072, 276074, 276075, 276076, 276077, 276079, 276080, 276081, 276083, and 276085).

Hydrodistillation. To extract the essential oils, a Clevenger trap (Wilmad-LabGlass, Kingsport, TN) for oils lighter than water was used (Clevenger, 1928). A representative sample of dried plant material (leaf and flower, 50 g) was taken from each replication for each variety. The plant material was finely ground in the blade coffee grinder to increase surface area (Milojević et al., 2013) and transferred to a 2-L round-bottom flask with deionized water at a material-to-solvent ratio of 1:13 (w/v) (Charles and Simon, 1990) and placed in a heating mantle connected to a rheostat. The mixture was brought to a boil, and then the heat was reduced to maintain a gentle simmer during collection. Distillation was carried out for 180 min per sample and was determined by waiting 30 min after the last increase in volume of essential oil in the collecting arm of the Clevenger trap. To facilitate condensation, water passing through the condenser was first chilled to 5 °C and then reprocessed through a water cooling bath (Model 4100 R20; Fisher Scientific Company). Essential oils were filtered through a glass pipette containing glass wool and 600 mg of anhydrous sodium sulfate (Avantor Performance Materials, Inc., Center Valley, PA) to remove water residue. The mass was recorded, and the collected essential oil fractions were stored in 1/4 dram (0.92 mL) amber glass vials (Premium Vials, Bristol, PA) at -18 °C until analyzed.

The recovery rate of essential oils was determined by taking a preweighed mass of essential oil and placing it in the collecting

Table 2. Varietal comparison by leaf color, growth habit, and average number of weeks for holy basil plants to reach full bloom stage for both harvests.^z

Variety	Phenotypic differences between varieties		
	Leaf color	Growth habit ^y	No. weeks to flower
Vana	Green	Clumping	9
Krishna	Purple	Clumping	9
Rama	Green	Clumping	5
Amrita	Purple	Clumping	7
PI 288779	Green	Clumping	8
PI 652057	Green	Clumping	6
Kapoor	Green	Sprawling	6
PI 652059	Green	Sprawling	6
PI 652056	Green	Sprawling	6
PI 414205	Green	Sprawling	6
PI 414201	Green	Sprawling	6
PI 414202	Green	Sprawling	6
PI 414203	Green	Sprawling	6
PI 414204	Green	Sprawling	6

^zWeeks to bloom stage determined from date of transplanting in the field.

^yClumping = upright growth, fewer branches, and stems were more tough and woody; sprawling = stems would bend down to the ground and then grow vertically, branches were more numerous, and stems were tender and more herbaceous.

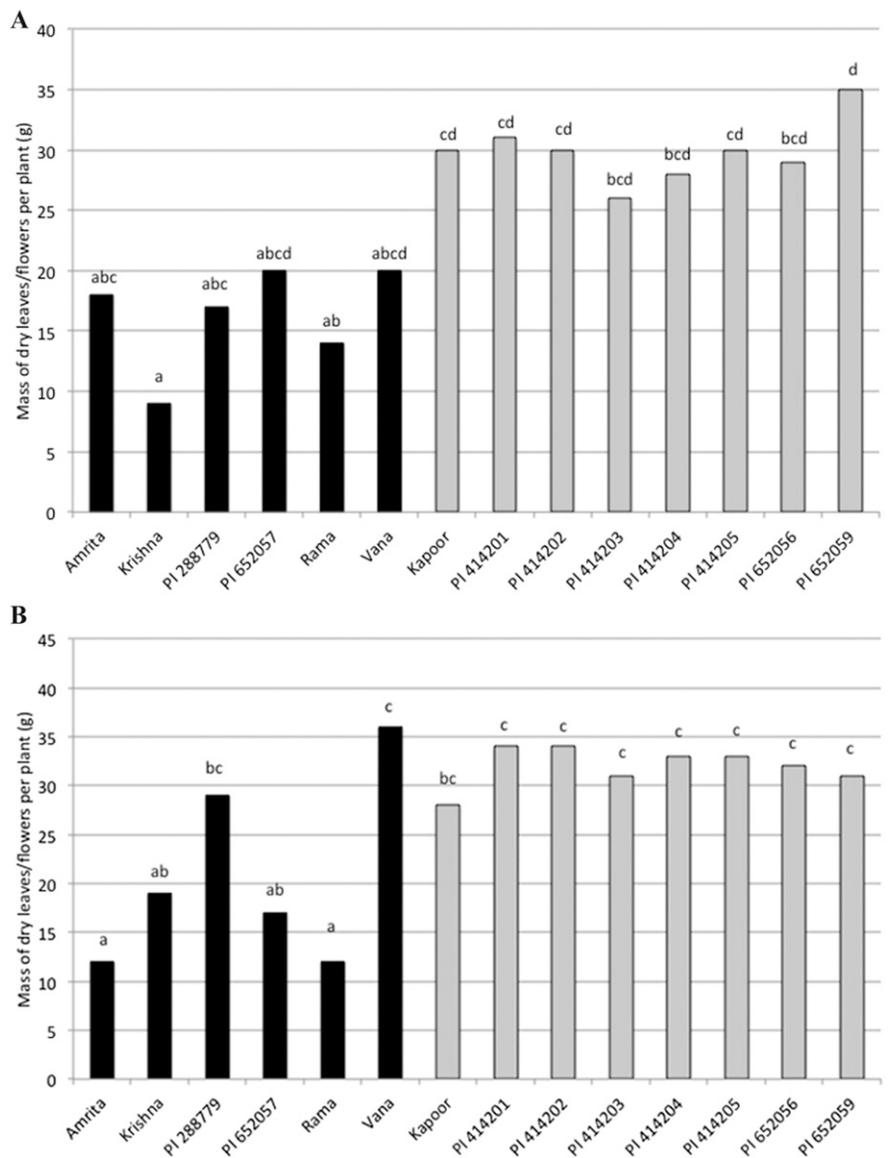


Fig. 3. Mean mass of dried leaf/flower of per plant for each variety. (A) The 2015 growing season and (B) the 2016 growing season. Black bars indicate clumping growth varieties and gray bars indicate sprawling growth type varieties. Similar letters above bars indicate no significant difference ($P > 0.05$) between varieties.

arm of the Clevenger trap to simulate the endpoint of distillation. It was then removed from the trap, filtered through sodium sulfate, and the mass was recorded. This was performed in triplicate and the percent recovery was calculated using the following equation:

$$\% \text{ Recovery} = \frac{\text{recovered mass of essential oil}}{\text{initial mass}} \times 100.$$

The percentage of essential oil in each sample was calculated using the following equation:

$$\text{Essential oil \%} = \frac{\text{mass essential oil}}{\text{mass of dried sample}} \times 100.$$

Finally, the essential oil yield (g/plant) was calculated as follows:

$$\text{Essential oil yield (mg)} = \text{concentration of essential oil (g/g)} \times \text{average mass (g) dried leaf and flower of single holy basil plant} \times 1,000.$$

Essential oil analysis. Essential oil samples were dissolved in high-performance liquid chromatography (HPLC)-grade acetonitrile (Sigma-Aldrich Chemical Company, St. Louis, MO) in a 5-mL volumetric flask, giving a final concentration of $\approx 20 \text{ mg}\cdot\text{mL}^{-1}$ of the oil. A 1- μL aliquot was injected into a single taper split liner (Agilent Technologies, Santa Clara, CA) at a split ratio of 80:1. All injections were performed in duplicate.

The analysis was carried out with a 6890N gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID) and auto-injector (Model 7683B). The data were recorded with ChemStation software [v. E.02.02]. A non-polar HP-5-fused silica capillary column (J&W Scientific, Folsom, CA) with 5% phenyl-methylpolysiloxane, 30 m, 0.32 mm i.d., 0.25 μm film thickness was used for analysis. An initial oven temperature of 50 $^{\circ}\text{C}$ was held for 5 min, then programmed to increase 3 $^{\circ}\text{C}\cdot\text{min}^{-1}$ to 120 $^{\circ}\text{C}$, then at 5 $^{\circ}\text{C}\cdot\text{min}^{-1}$ to 250 $^{\circ}\text{C}$ and finally at 15 $^{\circ}\text{C}\cdot\text{min}^{-1}$ to 300 $^{\circ}\text{C}$, followed by a 5-min hold for a total run time of 62.67 min. For the FID, airflow was set at 450 $\text{mL}\cdot\text{min}^{-1}$, Ultra High Purity (UHP)-grade hydrogen flow at 40 $\text{mL}\cdot\text{min}^{-1}$, and makeup gas (He) at 25 $\text{mL}\cdot\text{min}^{-1}$. The carrier gas was UHP-grade helium at a flow rate of 30 $\text{mL}\cdot\text{min}^{-1}$. The inlet pressure was 18.07 psi. The analysis was performed in constant flow mode, and the injector and detector temperatures were set at 250 and 300 $^{\circ}\text{C}$, respectively. Identification and quantitation of eugenol in the holy basil essential oil samples was carried out by comparison of retention time indices, employment of a commercial standard, and development of a calibration curve.

Quantitative analysis. A solution of 20.29 $\text{mg}\cdot\text{mL}^{-1}$ of eugenol (TCI America, Portland,

OR) in HPLC-grade acetonitrile was prepared and injected onto the GC-FID at seven different concentrations in a range expected for eugenol in the samples, established by literature values. A calibration curve was created ($y = 132.96x - 4.6827$, $r^2 = 0.99962$) with a concentration range of 1.01–20.29 $\text{mg}\cdot\text{mL}^{-1}$. The standard curve allowed for quantitation of eugenol in each essential oil sample. The eugenol content was calculated by the following equation:

$$\text{Eugenol content} = \frac{\text{mass eugenol}}{\text{mass essential oil}} \times 100.$$

Results are expressed as g eugenol/100 g essential oil.

Statistical analysis. The data were analyzed statistically using one-way analysis of variance by R 3.2.2 (The R Foundation for

Statistical Computing). Significance was determined using Tukey's Studentized range test. Averages are represented as the mean \pm SE.

Results

Weather. The average monthly minimum and maximum temperatures were similar over both years; however, the total rainfall for the area presented greater contrast with a total of 860 mm of rainfall in 2015 and 243 mm in 2016 between the months of July–November (Table 1).

Differences in phenotype and physiology. There were several phenotypic and physiological differences observed among the holy basil varieties evaluated. Differences were seen in leaf color and shape (Fig. 1), growth habits, and average number of weeks to flowering time. Most of the varieties displayed green leaves, with two exceptions,

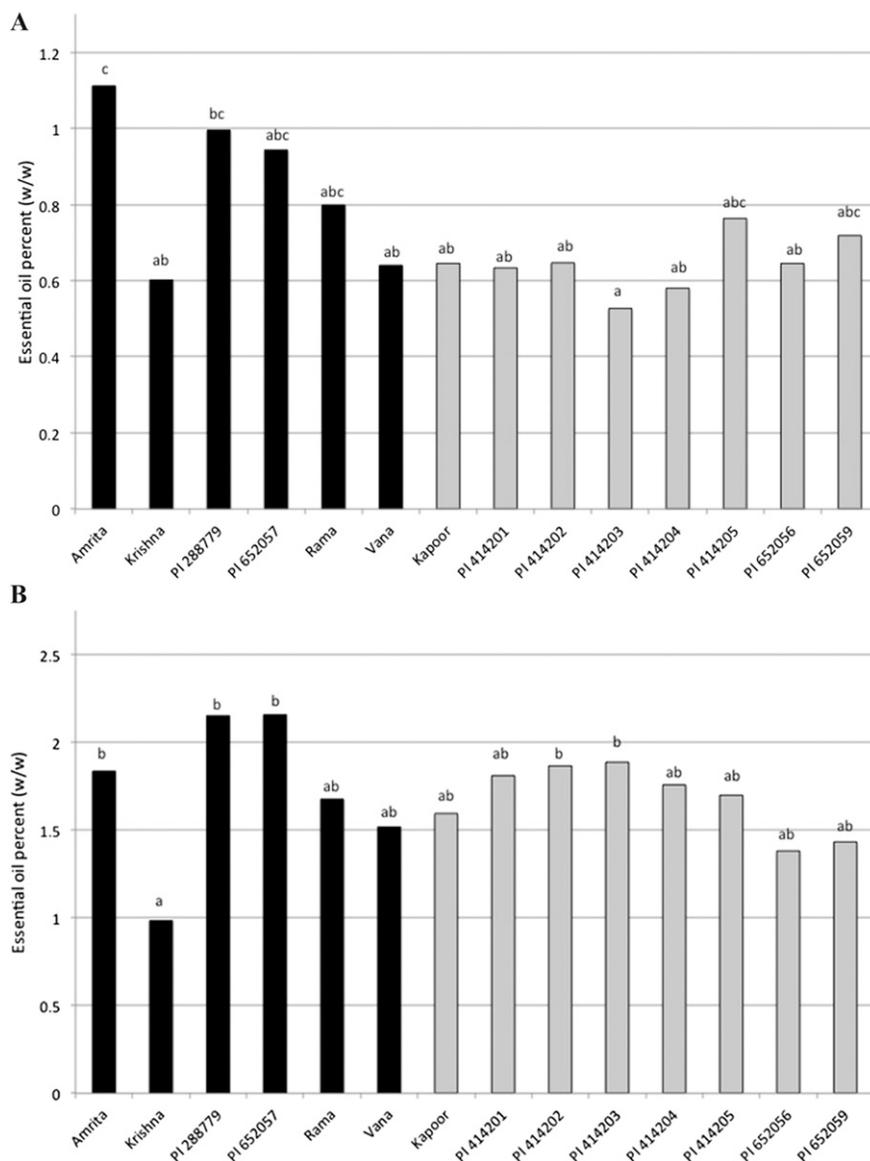


Fig. 4. Mean percentage of essential oil per plant for each variety. (A) The 2015 growing season and (B) the 2016 growing season. Black bars indicate clumping growth varieties and gray bars indicate sprawling growth type varieties. Similar letters above bars indicate no significant difference ($P > 0.05$) between varieties.

'Krishna' and 'Amrita', which had dark purple leaves. Six varieties exhibited a more clumping upright growth pattern with fewer stems that were more rigid and woody ('Amrita', 'Krishna', PI 288779, PI 652057, 'Rama', and 'Vana') (Fig. 2A) and eight varieties exhibited similar leaf shape and color, with a more sprawling growth habit and greater number of stems which were more herbaceous ('Kapoor', PI 414201, PI 414202, PI 414203, PI 414204, PI 414205, PI 652056, and PI 652059) (Fig. 2B). In 2016, the time to flower was recorded when plants were allowed to reach maturity before harvest. The 14 varieties differed in the amount of time required to achieve the flowering stage: most flowered at ≈ 6 weeks; however, notable exceptions were 'Vana' and 'Krishna' which took an average of nine, whereas 'Rama' took only 5 weeks (Table 2).

Variety evaluation. Statistical analysis revealed a significant effect of growing year on yield ($P < 0.005$) and essential oils ($P < 0.0001$); therefore, aggregate yield and essential oil data could not be statistically compared. The average moisture of the dry leaf and flower was $10.2 \pm 0.2\%$ in 2015 and $9.9 \pm 0.1\%$ in 2016. The average recovery rate of essential oils was $90.9 \pm 0.6\%$.

Effect of growing season on mass and essential oil yield. There were significant differences between the 2015 and 2016 growing seasons for yield of dry weight, essential oil percent, essential oil yield, and eugenol content. Statistically, the average dry weight yield per plant was higher in 2016 than in 2015: 27 ± 3.4 g and 24 ± 4.6 g, respectively ($P < 0.05$). However, 'Vana' was the only variety that possessed a significantly higher dried mass in 2016 when individual varieties were compared between years. In 2015, the six clumping varieties all displayed lower dry weight yields compared with the sprawling varieties (Fig. 3A). In 2016, 'Vana' had the highest dry weight yield of all the varieties and PI 288779 possessed a higher dry weight than 'Kapoor'. Otherwise, the trend was the same as the previous year with the clumping varieties producing lower yields than the sprawling varieties (Fig. 3B).

Essential oil percent between growing seasons was significantly different, with mean essential oil contents in 2015 and 2016 of $0.73 \pm 0.13\%$ and $1.70 \pm 0.25\%$, respectively ($P < 0.001$) (Fig. 4). In 2015, 'Amrita' contained the highest essential oil content at 1.1% and the accession PI 414203 displayed the lowest at 0.5% (Fig. 4A). In 2016, all of the varieties showed an increase in essential oil percent in 2016, with 'Krishna' containing the lowest at 0.9% and the accessions PI 288779 and PI 652057 possessing the highest at 2.2% (Fig. 4B).

Similarly, there was a significant difference in essential oil yield between years. In 2015, the mean essential oil yield was 168 ± 37 mg per plant, where it was 466 ± 95 mg per plant in 2016 ($P < 0.001$) (Fig. 5). 'Krishna' was the lowest in essential oil yield in both growing seasons yielding an average of

61 mg and 181 mg per plant in 2015 and 2016, respectively. Conversely, accession PI 652059 gave the highest essential oil yield in 2015 (Fig. 5A), whereas PI 288779 yielded the greatest essential oil yield in 2016 (Fig. 5B). PI 288779 and PI 414202 showed the greatest difference between years: PI 288779, a clumping type variety, had an average essential oil yield of 189 mg per plant in 2015 and 649 mg in 2016. Similarly, PI 414202 showed a remarkable increase from 183 mg of essential oil per plant in 2015 to 649 mg in 2016 and was a sprawling type variety. 'Amrita' was the only variety that exhibited a slight decrease in essential oil yield in 2016.

Effect of growing season on eugenol. There was a significant difference in mean eugenol contents between years ($P < 0.0001$), with 21.5 ± 6.8 g/100 g of essential oil in

2015 and 37.0 ± 4.1 g/100 g in 2016 (Fig. 6). In both growing seasons, the lowest and highest eugenol-containing varieties were the same. 'Amrita' displayed the lowest eugenol content, with only trace amounts detected, whereas the cultivar Vana clearly displayed the greatest concentrations at ≈ 66 g/100 g of essential oil consistently for both seasons. Except for 'Vana', all of the other varieties increased in eugenol in 2016 compared with 2015. With the exception of the clumping type cultivar Amrita that showed only trace amounts of eugenol, there was a clear trend in both years, demonstrating that the sprawling type varieties (PI 414205, 'Kapoor', PI 652056, PI 414201, PI 652059, PI 414202, PI 414204, and PI 414203) had lesser eugenol relative to the clumping type varieties (PI 652057, 'Rama', 'Krishna', PI 288779, and 'Vana'). Moreover, the lower

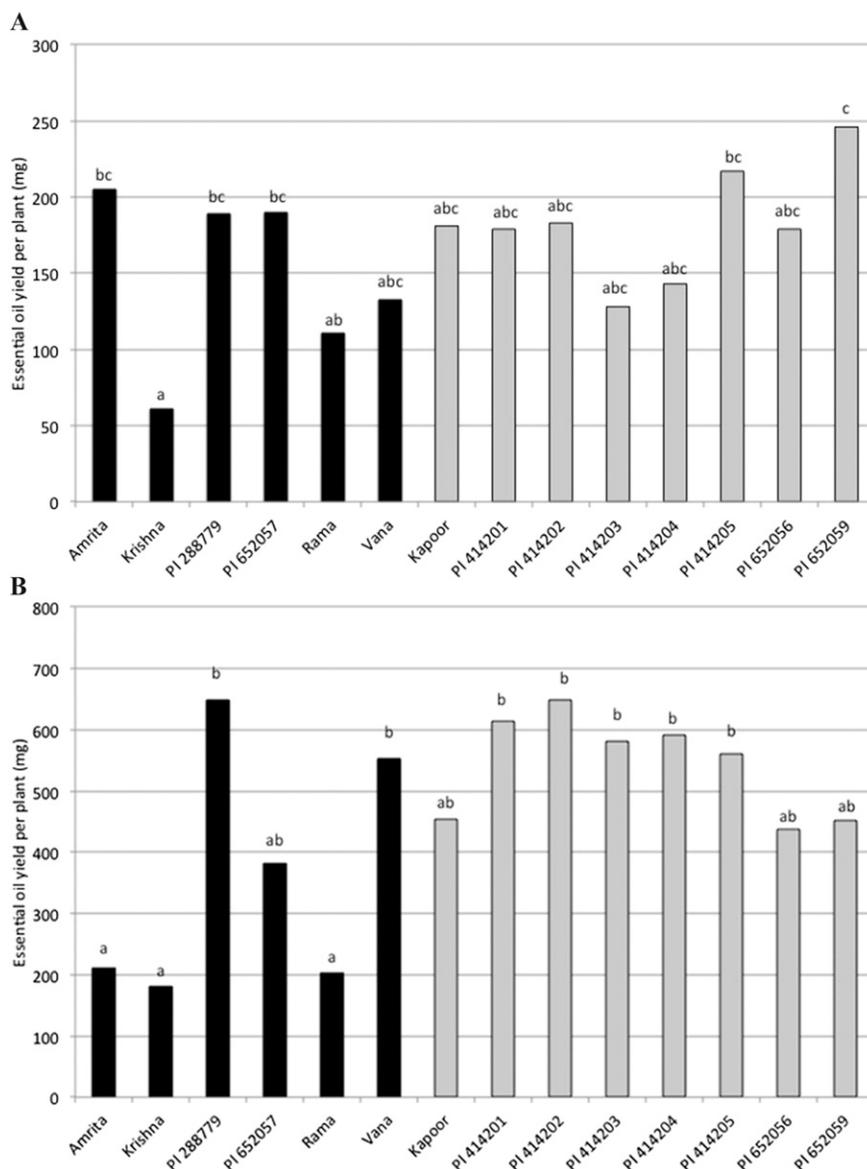


Fig. 5. Mean yield of essential oil per plant for each variety, calculated as concentration of essential oil (g/g), multiplied by the average mass of dried leaf and flower per plant (g) times 1000. (A) The 2015 growing season and (B) the 2016 growing season. Black bars indicate clumping growth varieties and gray bars indicate sprawling growth type varieties. Similar letters above bars indicate no significant difference ($P > 0.05$) between varieties.

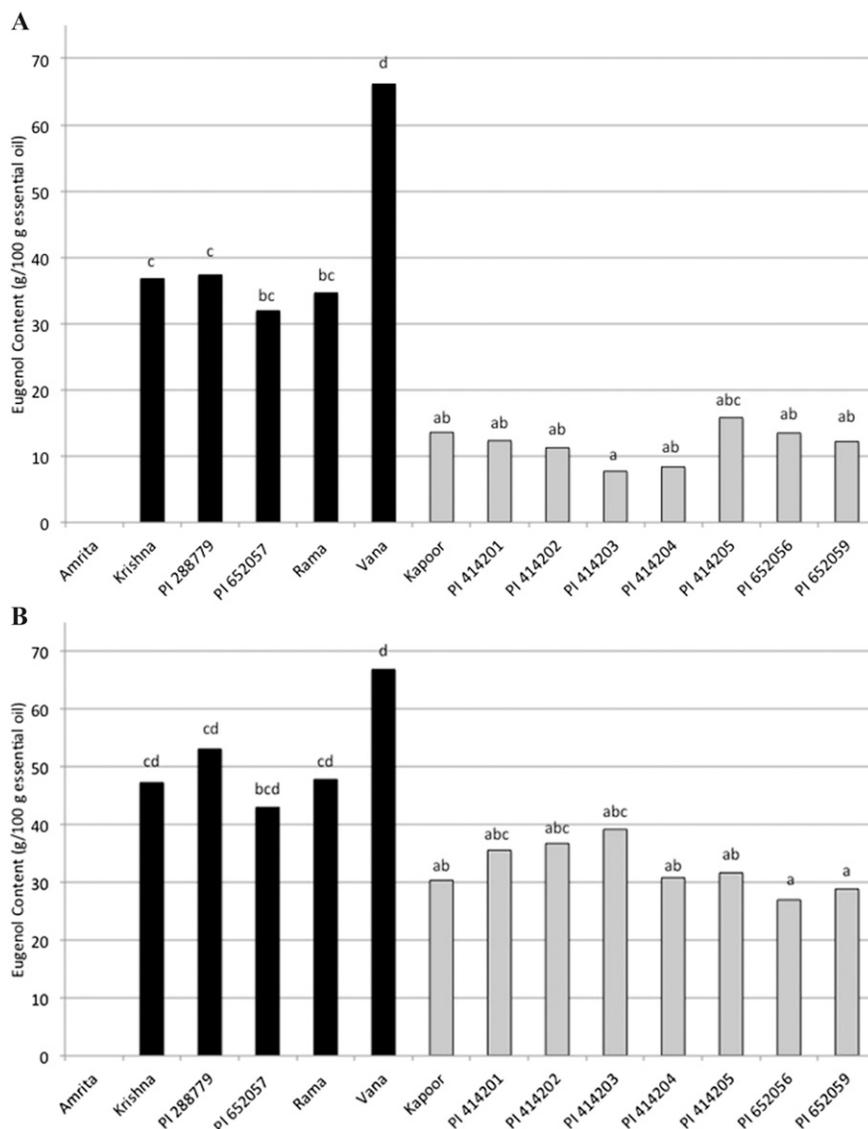


Fig. 6. Mean eugenol content in holy basil essential oils for each variety. (A) The 2015 growing season and (B) the 2016 growing season. Black bars indicate clumping growth varieties and gray bars indicate sprawling growth type varieties. Similar letters above bars indicate no significant difference ($P > 0.05$) between varieties.

eugenol varieties, relating to the same grouping by sprawling growth habits, also had the highest change in eugenol between years with accession PI 414203, exhibiting the greatest difference with an increase in eugenol from 7 to 39 g/100 g essential oil in 2016.

Flowering time. Three holy basil varieties, PI 288779, 'Vana', and 'Krishna' had not begun flowering at the time of harvest in 2015 and were allowed to mature to the full bloom stage before harvesting in 2016. The mass of dried leaves and flowers, essential oil percent, essential oil yield, and eugenol content were compared for these three varieties to evaluate effects of different harvest times between growing seasons (Fig. 7). The dry weight yield was higher for all three varieties in 2016; however, 'Vana' was the only one that was significantly greater (Fig. 7A). In terms of essential oil percent (Fig. 7B) and essential oil yield (Fig. 7C), the varieties PI 288779 and 'Vana' were signif-

icantly greater in 2016, whereas 'Krishna' was only slightly higher. Finally, although PI 288779 and 'Krishna' were slightly greater in eugenol level in 2016 as compared with 2015, there was no significant difference ($P > 0.05$) in eugenol content for any of the three varieties between growing seasons (Fig. 7D).

Varietal evaluation. Of the nine USDA accessions, seven (PI 652059, PI 652056, PI 414205, PI 414201, PI 414202, PI 414203, and PI 414204) were very similar to the commercial cultivar Kapoor. They displayed similar characteristics in phenology, had the same sprawling type growth habit, and exhibited correlative trends of higher yields and lower essential oil percent. In addition, these seven varieties displayed lower eugenol content matching 'Kapoor', and overall had a more pronounced increase in eugenol content in 2016, indicating that these sprawling 'Kapoor'-like varieties are possibly more heavily influenced by environment than the

more clumping type varieties. Unfortunately, there is considerable confusion regarding the origin of the Kapoor cultivar, and it is difficult to identify botanically. Because of the presence of eugenol, it is, nevertheless, considered as a suitable alternative to other varieties, and it is popular with many domestic growers because it grows well in temperate climates and is very prolific. For these reasons, the Kapoor cultivar it is the most common type of holy basil sold by seed suppliers in the United States.

Two other USDA accessions, PI 288779 and PI 652057, were similar to the Rama and Krishna cultivars. They all displayed the clumping type growth habit and similar eugenol contents. The cultivars Rama and Krishna are traditionally grown in India. Unfortunately, 'Rama' had the smallest harvestable weight and 'Krishna' had the lowest essential oil yield. Meanwhile, PI 288779 had a reasonable harvestable weight, rendered the maximum essential oil yield, and was the second highest in terms of eugenol content. 'Vana' possessed the greatest eugenol content and superior yields, but its essential oil yield was low compared with many of the other varieties examined. Finally, 'Amrita' was the only variety with no detectable eugenol content and had inferior yields, suggesting it is a less desirable option for growers or buyers wanting a holy basil product with marked amounts of eugenol.

Discussion

It is clear that varietal selection and environment both play a significant role in the yield and quality of holy basil, and individuals or groups of varieties have strengths and weaknesses, depending on the variable measured. For example, there appeared to be higher yielding sprawling varieties and lower yielding clumping varieties that remained consistent over both years. Ultimately, the preferences of the buyer and end use application will determine the variety(ies) to be grown.

The 2016 growing season was challenging for local growers, with very hot, dry weather. Yet, these conditions resulted in a higher percentage of essential oil, increased essential oil yield, and greater eugenol content among most varieties. Research on the effect of environmental factors on holy basil is lacking, but there have been many environmental studies on culinary basil (*Ocimum basilicum*). Eugenol content in culinary basil has been reported to increase under conditions of water stress, higher temperatures, and higher daily light integrals and decrease with heavy shading (Chang et al., 2005, 2008; Ekren et al., 2012). It is clear that further research is needed to evaluate these influences on holy basil to better understand their effect and to provide guidance for future production practices.

Growth habits also affected the ease of harvest. Varieties with a more sprawling growth habit had problems with branches that broke away from the main stem and

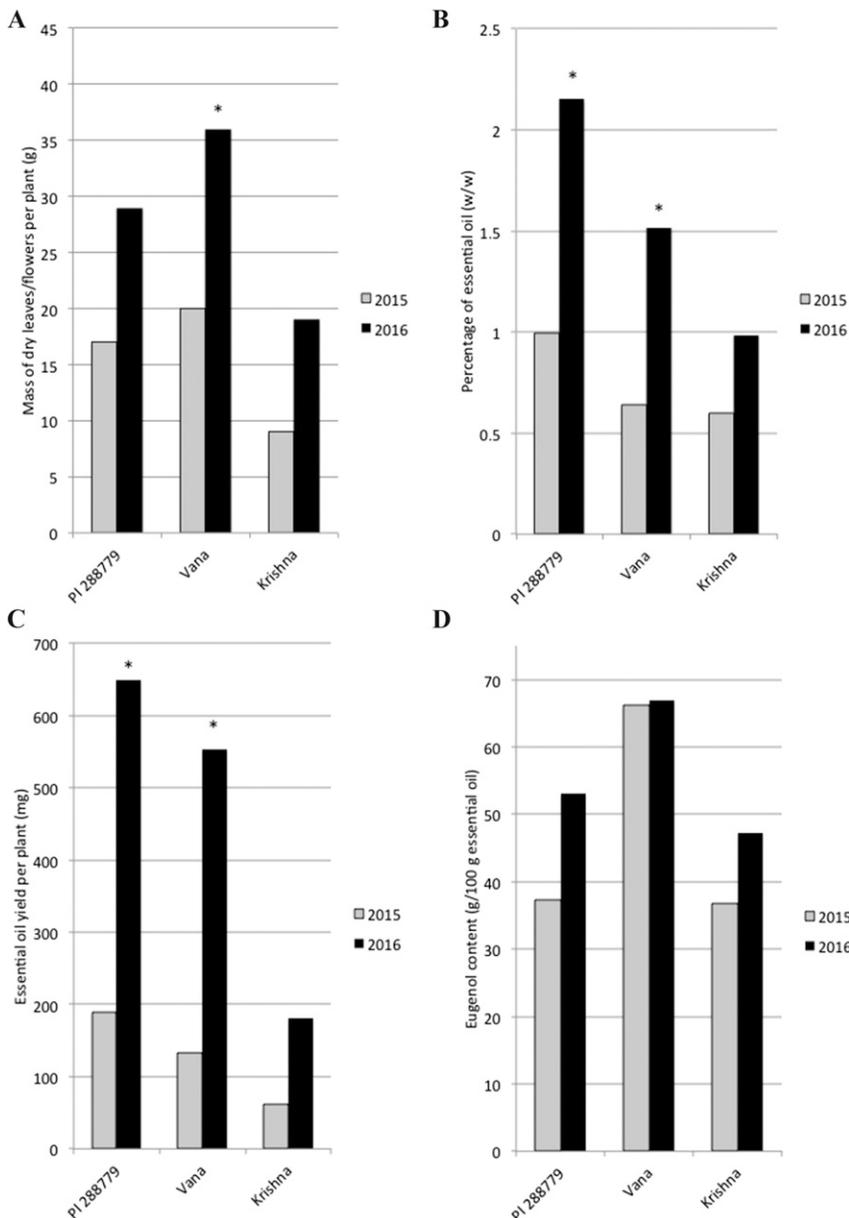


Fig. 7. (A) Dry weight yield; (B) essential oil percent; (C) essential oil yield; and (D) eugenol content of varieties harvested before flowering in 2015 and during flowering in 2016. An asterisk (*) denotes the varieties that are significantly higher ($P \leq 0.05$) in 2016 as compared with 2015.

reduced harvestable yield. The sprawling growth habit also made postharvest handling more difficult because the lower leaves had a tendency to be splashed with dirt and required additional rinsing. On the other hand, varieties with a clumping growth habit had branches that remained upright and allowed a more efficient and hygienic harvest. Another factor affecting harvest was variation in the time to flower. Certain varieties took longer to reach the flowering stage, and this affected the number of harvests that could be taken before a killing frost.

Of the nine USDA accessions, seven (PI 652059, PI 652056, PI 414205, PI 414201, PI 414202, PI 414203, and PI 414204) were very similar to the commercial cultivar Kapoor. Two other USDA accessions, PI 288779 and PI 652057, were similar to cultivars Rama and Krishna. Finally,

‘Amrita’ was the only cultivar with no detectable amounts of eugenol. Based on this study, one particular variety, USDA accession PI 288779 stood out as a target for future breeding work. It had a reasonable harvestable weight, gave the greatest essential oil yield, and was the second highest in terms of eugenol content. PI 288779 is a landrace collected from India with an upright growth habit, making it easy to harvest. A logical next step would be to work with this variety, along with other accessions with promise, over several years to prepare them for commercial production or for use in a breeding program.

Conclusion

Results of this study suggest that holy basil has potential as a value-added crop for

Georgia. All varieties of holy basil produced superior essential oil yield, with increased eugenol content in a season of minimal rain and extended heat. This suggests that holy basil may be especially suitable for areas where temperatures are increasing and precipitation falls short of plant needs, provided irrigation is an option. Moreover, there is an opportunity for breeding and development research that includes USDA accessions not currently available commercially.

Literature Cited

- Abdel-Hamid, M.F., S. El-Hady, M.E. Ibrahim, and F. Ibrahim. 2005. Effect of harvesting time on the production and chemical composition of tulsi leaves essential oil. *Ann. Agr. Sci.* 50:3–13.
- Aggarwal, A. and R.R. Mali. 2015. *Ocimum tenuiflorum*—A medicinal plant with its versatile uses. *Intl. J. Rec. Adv. Sci. Tech.* 2:1–10.
- Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar. 2008. Biological effects of essential oils—A review. *Food Chem. Toxicol.* 46:446–475.
- Bohnert, H.J., H.R. Nguyen, and N.G. Lewis. 2008. *Bioengineering and molecular biology of plant pathways*. Elsevier, San Diego, CA.
- Chang, X., P.G. Alderson, and C.J. Wright. 2005. Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum* L.). *J. Hort. Sci. Biotechnol.* 80:593–598.
- Chang, X., P.G. Alderson, and C.J. Wright. 2008. Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. *Environ. Expt. Bot.* 63:216–223.
- Charles, D.J. and J.E. Simon. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *J. Amer. Soc. Hort. Sci.* 115:458–462.
- Clevenger, J. 1928. Apparatus for the determination of volatile oil. *J. Amer. Pharm. Assn.* 17:345–349.
- Ekren, S., C. Sönmeza, E. Özcakal, Y.S. Kukul Kurttas, E. Bayram, and H. Gürgülü. 2012. The effect of different irrigation water levels on yield and quality characteristics of purple basil (*Ocimum basilicum* L.). *Agr. Water Mgt.* 109:155–161.
- Esch, T., G.B. Stefano, G.L. Fricchione, and H. Benson. 2002. The role of stress in neurodegenerative diseases and mental disorders. *Neuroendocrinol. Lett.* 23:199–208.
- Eve, B., M. Tuairira, M. Moses, and M. Thomas. 2016. The influence of pinching on the growth, flowering pattern and yield of butternuts (*Cucurbita moschata*). *Intl. J. Hort. Ornamental Plants.* 2:19–25.
- Kamatou, G.P., I. Vermaak, and A.M. Viljoen. 2012. Eugenol—From the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. *Molecules* 17:6953–6981.
- Keating, B., A. Lindstrom, M.E. Lynch, and M. Blumenthal. 2014. Sales of tea & herbal tea increase 5.9% in United States in 2013. *HerbalEGram*. 11.
- Kissel, D.E. and L.S. Sonon. 2008. *Soil test handbook for Georgia*. Georgia Coop. Ext., Athens, GA.
- Kuhn, M.A. and D. Winston. 2000. *Herbal therapy and supplements: A scientific and traditional approach*. Walters Kluwer Health, Philadelphia, PA.
- Milojević, S.Ž., D.B. Radosavljević, V.P. Pavićević, S. Pejanović, and V.B. Veljković. 2013. Modeling the kinetics of essential oil hydrodistillation from plant materials. *Hem. Ind.* 67:843–859.

- Pattanayak, P., P. Behera, D. Das, and S.K. Panda. 2010. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacogn. Rev.* 4:95–105.
- Prakash, P. and N. Gupta. 2005. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. *Indian J. Physiol. Pharmacol.* 49:125–131.
- Raina, A.P., A. Kumar, and M. Dutta. 2013. Chemical characterization of aroma compounds in essential oil isolated from “Holy Basil” (*Ocimum tenuiflorum* L.) grown in India. *Genet. Resources Crop Evol.* 60:1727–1735.
- Saran, P.L., V. Tripathy, A. Saha, K.A. Kalariya, M.K. Suthar, and J. Kumar. 2017. Selection of superior *Ocimum sanctum* L. accessions for industrial application. *Ind. Crops Prod.* 108: 700–707.
- Sen, P. 1993. Therapeutic potentials of Tulsi: From experience to facts. *Drugs News Views* 1:15–21.
- Sharma, A., A. Meena, and R.K. Meena. 2011. Biochemical estimation of primary metabolites in *Ocimum tenuiflorum*. *Bioscan* 6:463–465.
- Singh, E., S. Sharma, J. Dwivedi, and S. Sharma. 2012. Diversified potentials of *Ocimum sanctum* Linn (Tulsi): An exhaustive survey. *J. Nat. Prod. Plant Resources* 2:39–48.
- Smith, T. 2011. Nonprofit collaboration addresses adulteration of botanical ingredients. *Herbal-Gram.* 92:14.
- Winston, D. and S. Maimes. 2007. *Adaptogens: Herbs for strength, stamina, and stress relief.* Healing Arts Press, Rochester, NY.
- Zhang, J., B. Wider, H. Shang, X. Li, and E. Ernst. 2012. Quality of herbal medicines: Challenges and solutions. *Comp. Ther. Med.* 20:100–106.
- Zheljzakov, V.D., C.L. Cantrell, B. Tekwani, and S.I. Khan. 2008. Content, composition, and bioactivity of the essential oils of three basil genotypes as a function of harvesting. *J. Agr. Food Chem.* 56:380–385.