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ORIGINAL RESEARCH PAPER

Essential oil yield and yield components of basil (*Ocimum basilicum* L.) as affected by genotype and intrarow spacing at Jimma, SW Ethiopia

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Abstract

Basil is an aromatic, medicinal, culinary, and multifunctional herb which is grown in different parts of Ethiopia. Although the oil distilled from the herb, as well as its herbal yields, are a crucial input in the pharmaceutical industry and for culinary purposes, the yield obtained is below its potential due to various challenges. Genotype and plant spacing are two of the factors which contribute to the low production of the crop. A field trial was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) in 2016 and 2017 to assess the effect of genotype and plant spacing on essential oil yield and other yield-related traits of this important herb. Four promising genotypes (BO-1, BO-3, BO-4, and BO-5) and three plant spacings (20, 30, and 40 cm) were studied in a 4 × 3 factorial design arranged as randomized complete blocks (RCBD) with three replications. Data on plant height, leaf area, number of primary branches, essential oil content and oil yield were collected and analyzed. The analysis of variance revealed that there were highly significant ($p < 0.01$) interaction effect of genotype with plant spacing for all parameters tested. The maximum essential oil yield (7.88 kg ha^{-1}) was obtained from genotype BO-5 at 30 cm spacing, whilst the least (2.68 kg ha^{-1}) was recorded from BO-1 at 40 cm spacing. The maximum oil content (101 mL g^{-1}) was gained from BO-1 at 20 cm, but BO-3 at 30 cm spacing recorded the least oil content though there were no significant differences between the three treatment combinations. Further studies at different locations and seasons will be important to for future local recommendations.

Keywords

competition; essence yield; genotypes; plant density

Introduction

Basil (*Ocimum basilicum* L.) is an important species of cultivated aromatic and medicinal plants belonging to the Lamiaceae (mint family). This family Lamiaceae includes about 3,200 species of annuals and nonwoody perennials which are widely distributed almost all over the temperate and tropical regions of the world [1–3].

Basil is usually referred as the "king of the herbs", being widely utilized due to its economic, culinary, industrial, and medicinal importance. An extract of the herb is used in preventing cardiovascular diseases through improved diet and several antioxidant compounds it contains display a high antioxidant power [4]. The extracts have been shown to display important effects at the cellular level, including a platelet antiaggregant property and inhibitory activity against HIV to decrease plasma lipid content. It has also demonstrated strong hypolipidemic action in a murine model of induced hyperlipidemia, decreasing both plasma triglycerides (TG) and cholesterol in acute hyperlipidemia induced by Triton WR-1339 in rats [5].

In Ethiopia, the tender stems, leaves, and flowers are dried, ground, and added to sauces either alone or mixed with other spices to provide a fine flavour to stews. It is an important ingredient in berbere and shiro powders and the preparation of clarified (spiced) butter. The dried leaves can be used for preparing roast beef locally known as "tibs" and both dried and fresh inflorescences and leaves are used as flavoring agents in the preparation of all kinds of "wote". According to a report [6], Ethiopia has exported 68,786 kg of basil essential oil to Sudan and the USA, from which a total foreign currency of $54,991.20 and $746.00, respectively, was obtained back in 2009. The export volume accounted 19.77% between 2006 and 2009 and exhibited 0.15% and 0.14% both volume and share value of the total spice export. These figures from nearly 20 years ago indicate that the productivity of basil is much below its potential.

There are different factors which can limit production and productivity of the basil plant. Between the major factors, the effects of genotype and intrarow spacing have been reported. According to Telci et al. [7], the differences of essential oil content between 18 genotypes can range from 0.4% to 1.5%. Zheljazkov et al. [8] also evaluated the oil content, composition, and bioactive characteristics of three *Ocimum* species as affected by growth stages. Their results revealed 115, 123, and 51 kg ha^{-1} total essential oil yields were obtained from *O. basilicum* 'German', 'Mesten', and local cultivars respectively. Essential oil content and yield are also affected by intrarow spacing [9]. Daneshian et al. [10] and Gill and Randhawa 1999, as cited by [11], also stated that a narrow plant spacing can increase essential oil yield and plant height in contrast to Sullivan et al. [12] who reported essential oil content decreases as plant spacing decreases. Taking into consideration the results of previous trials, the present work was initiated with the following aims:

- to examine the variation in essential oil yield and yield-related traits in different basil genotypes at different plant spacings at Jimma, SW Ethiopia;
- to assess possible interactive effects of genotype and plant spacing on essential oil yield and yield-related traits of basil at Jimma, SW Ethiopia.

Material and methods

Description of the study site

The study was conducted at the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) horticulture research site, Jimma Zone, Oromia Regional State in the 2016/2017 cropping season. The research site is located 356 km southwest of Addis Ababa at a latitude and longitude of about 7°33′9″ N and 36°57′6″ E, respectively, and an altitude of 1,710 m a.s.l. The area receives an annual rainfall of 1,250 mm and the maximum and minimum temperatures are 26.8°C and 11.4°C, respectively. The soil of the area is characteristically a reddish-brown clay soil with a pH ranging from 5.07 to 6 (BPEDORS 2000, unpublished).

Trial treatment and design

Four promising genotypes (BO-1, BO-3, BO-4, and BO-5) were obtained from Wondo Genet Agricultural Research Center (WGARC), based on their variety trial results conducted in different parts of Ethiopia. Three plant spacings, 20 cm (eight plants m^{-2}), 30 cm (six plants m^{-2}), and 40 cm (four plants m^{-2}) were used (Tab. 1).

Tab. 1 Treatment combinations for the trial conducted in 2016/2017 at Jimma, SW Ethiopia.

No.	Treatment combination
1	BO-1 × 20 cm
2	BO-1 × 30 cm
3	BO-1 × 40 cm
4	BO-3 × 20 cm
5	BO-3 × 30 cm
6	BO-3 × 40 cm
7	BO-4 × 20 cm
8	BO-4 × 30 cm
9	BO-4 × 40 cm
10	BO-5 × 20 cm
11	BO-5 × 30 cm
12	BO-5 × 40 cm

Genotypes: BO-1, BO-3, BO-4, and BO-5; Intrarow spacings: 20, 30, and 40 cm.

The field trial was conducted in as a factorial design arranged in randomized complete blocks (RCBD), with three replications. Data (plant height, leaf area per plant and “umbrella” (basil inflorescence) number, and the number of primary branches) were collected from the middle two rows. The total plot size was 7.2 m^2 (3 × 2.4 m) with a net plot size of 1.80 m^2 (3 × 0.6 m) and the full trial area was 487.2 m^2 (width: 11.2 m and length: 43.5 m). The total number of rows per plot and number of plants per row were 4 and 15, 10 and 7, respectively, based on plant spacing. Spaces between blocks and plots were 1 m and 0.5 m, respectively.

Trial procedures

The seed bed (1 × 10 m, width and length) was prepared 1 week prior to sowing. The soil was mixed with 40 kg cow manure as recommended by Asgharipour et al. [13] to enhance soil fertility and its water-holding capacity. The seed was drilled into rows spaced 15 cm apart and at a depth of 0.25 cm. Each genotype was randomly assigned to eight rows to avoid biased allocation of genotypes, and mulched for 1 week to maintain soil moisture and inhibit weed growth. Seven days after sowing, the mulch was removed and shade was provided at a height of 1.5 m and kept in place until 10 days prior to transplanting. After 50 days, the shade screen was removed in order to harden off the seedlings. Watering seedlings was carried out early morning and late afternoon every day for 50 days to facilitate germination and growth of seedlings. However, for hardening purposes, watering was reduced to once in 2 days for 10 consecutive days prior to transplanting.

Seedlings were transplanted after 2 months of nursery establishment, and were planted late afternoon to reduce evapotranspiration. As with traditional agronomic practices, irrigation water was applied to the transplants from a watering can once a day to facilitate plant establishment, and this was continued up to the time of full plant establishment. Hand weeding was performed frequently, depending on the emergence of weeds.

Essential oil distillation procedure

The oil distillation process for the present study was undertaken at the Chemistry Laboratory of Wondo Genet Agricultural Research Center (WGARC). Leaves, umbrellas, and stems were separated manually to prepare samples for oil distillation. The composite of leaves and umbrellas (herbage yield) was mixed evenly and weighed using a sensitive balance from the Agronomy Laboratory at Jimma University College of Agriculture and Veterinary Medicine. A 300-g composite sample was packed into a polythene container and stored in an ice box to conserve the moisture in the fresh herbage. The essential oil was distilled by a hydro-distillation method by using a Clevenger-type apparatus [14]. After arrival at the WGARC Chemistry Laboratory, the sample was added to the round-bottomed flask containing 300 mL distilled water which was then heated. When the water was boiling, steam was injected into the part of the apparatus containing the plant sample. The vapor mixture of water and oil was condensed with cold water. The distillate was then directed into a separator where the oil separated from the distillate water.

Data analysis

For each measured response variable, analysis of variance (ANOVA) and a mean separation procedure were undertaken . The ANOVA model used for the analysis was:

$$Y_{ikj} = \mu + G_i + PS_j + (GPS)_{ij} + R_k + \varepsilon_{ijk}$$

where, Y_{ikj} – the mean value of the response variable of the ith genotype at the jth plant spacing in kth blocks, μ – the overall mean, G_i – effect of genotype, PS_j – the effect of plant spacing $(GPS)_{ij}$ interaction effect of genotype and plant spacing, R_k – effect of blocks, and ε_{ijk} is a random error term. After fitting an ANOVA model for significant interactions or main effects, LSDs for means were calculated at the 5% probability level. Pearson’s correlation analysis between variables was also computed to detect associations between plant traits. All the statistical analysis was carried out using SAS version 9.3 software.

Essential oil content (mL g^{-1}) (EOC) was calculated based on a formula recommended by [15] and [16]:

$$Essential\ oil\ content = \frac{Volume\ of\ essential\ oil\ recovered\ (\mathrm{mL})}{Weight\ of\ herbage\ biomass\ distilled\ (\mathrm{g})}$$

Essential oil yield (kg ha^{-1}) (EOY) was calculated by the formula as suggested by [16]:

$$Essential\ oil\ yield = Herbage\ dry\ biomass\ (\mathrm{kg\ ha^{-1}}) \times Essential\ oil\ content$$

Leaf area per plant (cm^2) (LA) was measured according to the model available in the literature [17,18], which states:

$$LA = 0.013(L^2W^2) + 4.963$$

where: LA – leaf area, L – leaf length from the tip of a leaf to the petiole, W – leaf width from the widest lamina or middle part of the leaf. Values 0.013 and 4.963 are fitted coefficients and constants.

Results

Plant height was significantly affected by genotype, plant spacing, and their interaction ($p = 0.001$). The tallest plant heights of 0.52 m and 0.48 m were recorded from genotypes BO-3 and BO-5, respectively, planted at the 20-cm spacing (Tab. 2). The shortest plants (0.32 m) and (0.35 m) were of genotype BO-1 planted at 40 cm and 30 cm, respectively. The ANOVA revealed a highly significant interaction effect in the number of primary branches ($p = 0.0019$). Genotype BO-1 planted at the 40-cm spacing gave the greatest number of primary branches, 17.2 (Tab. 2). Leaf area was also strongly influenced by the genotype and plant spacing interaction ($p = 0.0015$). Maximum leaf area per plant

Tab. 2 Mean values for herbage dry biomass, aboveground dry biomass, essential oil content, and essential oil yield as affected by genotype and plant spacing of basil genotypes at Jimma, SW Ethiopia, in 2016–2017.

Genotype	Plant spacing (cm)	Plant height (m)	Number of primary branches	EOC (mL g^{-1})	EOY (kg ha^{-1})
BO-1	20	0.39 ^{d}	12.13 ^{d}	101 ^{a}	6.15 ^{ab}
	30	0.35 ^{e}	13.73 ^{bcd}	52 ^{cbd}	2.88 ^{d}
	40	0.32 ^{e}	17.20 ^{a}	41 ^{cb}	2.68 ^{d}
BO-3	20	0.52 ^{a}	12.80 ^{bcd}	61 ^{b}	3.72 ^{cd}
	30	0.46 ^{b}	13.00 ^{bcd}	34 ^{d}	3.49 ^{cd}
	40	0.42 ^{dc}	12.53 ^{d}	45 ^{cbd}	5.52 ^{abc}
BO-4	20	0.42 ^{d}	12.93 ^{bcd}	48 ^{cbd}	2.85 ^{d}
	30	0.41 ^{d}	12.73 ^{cd}	45 ^{cbd}	3.16 ^{cd}
	40	0.39 ^{d}	13.93 ^{bcd}	52 ^{cbd}	4.35 ^{bcd}
BO-5	20	0.48 ^{ab}	14.86 ^{b}	53 ^{cbd}	3.68 ^{cd}
	30	0.45 ^{bc}	14.66 ^{bc}	57 ^{cb}	7.88 ^{a}
	40	0.46 ^{bc}	13.00 ^{bcd}	37 ^{cd}	5.13 ^{bcd}
$LSD_{0.05}$		0.038	2.09	23.00	2.46
CV(%)		5.34	9.07	26.14	22.10

Means with the same letter within a column are not significantly different at $p = 0.05$. EOC – essential oil content; EOY – essential oil yield.

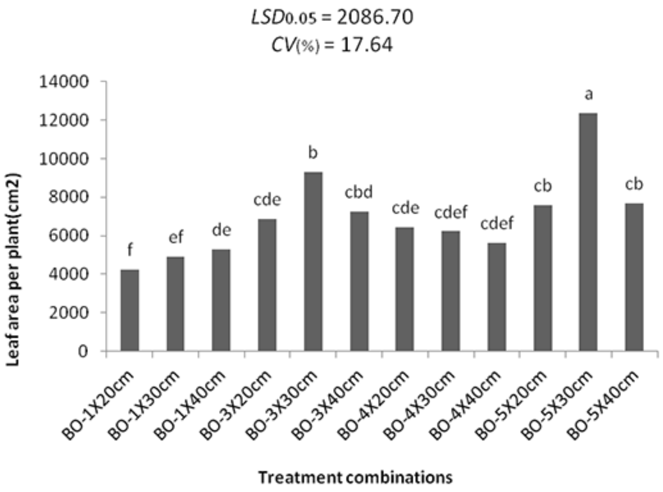

Fig. 1 Mean values of leaf area as affected by the interaction of genotypes and plant spacing of basil plants in 2016/2017 at Jimma, SW Ethiopia. Means with the same letters are not significantly different at $p = 0.05$.

(12,378 cm^2) was obtained from BO-5 planted at the 30 cm spacing (Fig. 1). The basil genotypes in this trial adopted an open growth habit allowing horizontal expansion here since competition was low compared to a densely planted growth pattern. Accordingly, leaf area per plant increased as the available space between and within plants increased.

The statistical analysis showed highly significant differences between the treatment combinations in essence content. The strong mutual influence of genotype and plant spacing on essence content was highly significant ($p = 0.0005$). Genotype BO-1 at the 20-cm spacing had the highest essence content (101 mL g^{-1}), whilst genotype BO-3 at 30 cm had the least (34 mL g^{-1}) (Tab. 2). Essential oil production and its concentration in the herb is clearly influenced by genetic, ontogenetic, and environmental effects. The interaction between genetic and environment effects causes secondary metabolites to be synthesized and essential oils are produced in response to stress. Most of these compounds are biosynthesized by metabolic pathways which are sensitive to the growing environment.

The result of the present study also revealed that the interactive effect of genotype and plant spacing on essence yield was highly significant ($p = 0.0021$). The highest essential oil yield (7.88 kg ha^{-1}) was obtained from genotype BO-5 at the 30-cm spacing. However, this was statistically similar to genotype BO-1 at 20 cm (6.15 kg ha^{-1}) and genotype BO-3 at 40 cm (5.52 kg ha^{-1}) treatment combinations. The least essential oil yield (2.68 kg ha^{-1}) was obtained from the genotype BO-1 × 40 cm treatment combination, but it was statistically on a par with BO-1 × 30 cm and other treatment combinations (Tab. 2). This could be due to the increase in dry matter yield and essence content.

Tab. 3 Pearson correlation coefficients for essential oil yield and yield-related variables.

	PH	PB	LA	EOC	EOY
PH	1	−0.43*	0.5**	−0.14	0.22
PB		1	−0.001	−0.16	−0.03
LA			1	−0.23	0.42*
EOC				1	0.66**
EOY					1

PH – plant height; PB – primary branches; LA – leaf area; EOC – essential oil content; EOY – essential oil yield. * $p < 0.05$; ** $p < 0.01$.

Correlations

Correlation analysis of parameters showed positive and negative associations between yield and yield-related traits. Essential oil yield was also strongly and positively associated with leaf area per plant ($r = 0.42$) and essential content ($r = 0.66$). This implies that the essence yield had an increasing trend with leaf area and essence content; increasing leaf area and essence content traits results in increasing essential oil yield [19]. Plant height was strongly and negatively correlated with the number of primary branches ($r = 0.43$). Plants grown at a narrow plant spacing can produce tall plants but these have a small number of primary branches. In contrast, leaf area per plant was positively and strongly associated with plant height ($r = 0.5$) (Tab. 3).

Discussion

The combined effect of genotype and plant spacing on plant height of basil could be due to competition for nutrients, photosynthetically active radiation (PAR), and available water [20]. As the bush density increases, competition for light and nutrients increases and in turn, the stem length increases. Conversely, the wider the space between the plants, the less competition for the resource and this has results in shorter plants [21].

In the case of the 20-cm plant spacing, there was little intrarow space, which might have led to high competition for light and a nutrient deficiency resulting in stem elongation. In this situation, the resources that plants have gained are sacrificed for vertical growth in order to obtain adequate sunlight [22]. Different reports also indicate the effect of variety and plant spacing on the height of basil plants in different countries. Pirkouhi et al. [22] also noted variation in height between two varieties of basil grown at different plant densities. Their report indicated that basil cultivars planted at high density produced the tallest plants as compared to the cultivars planted at low density. As cited by Lotfi et al. [23], the mutual effect of cultivars and plant density on plant height is highly significant.

The interactive effect of genotype and plant spacing on primary branching of the basil plant could be due to adequate space for absorption of light, nutrients and water [23]. Decreasing planting density brings about an increasing light absorption capacity in the plant population and more space is available for plant development [24]. Therefore, genotype BO-1 had enough space (40 cm) as a result of being less affected by neighboring plants, and more small and large plant branches can begin to grow and develop [25]. The current investigation is in line with those of Pirkouhi et al. [22] who reported a significant interaction of bush density and variety on the primary branching of basil plants.

Lotfi et al. [23] reported a similar interactive effect of genotype and plant spacing on leaf area of the basil plant. They found a decrease in leaf area as plant density increased, which they postulated was due to the rise in temperature within the plant and low availability of nitrogen. Bekhradi et al. [24] also reported that leaf area can be affected by light and water availability. Sharma and Kanjilal [26] noted significant variation in the leaf area between 14 accessions of basil, which implies that basil genotypes differ in their leaf morphology. This observation is also in agreement with the report of Bazaz et al. [17] who found that the leaf area of two basil cultivars was strongly affected by the interaction of plant density and cultivar. However, Pirkouhi et al. [22] reported a nonsignificant interaction between plant density and genotype on the leaf area of basil cultivars that might be due to genotypes being genetically similar for leaf area.

The variation of essential oil content between genotypes could be due to both environmental and genetic effects. As plants face environmental stresses (e.g., lack of nitrogen and potassium), these secondary metabolites are synthesized in response (Reymond et al. 2000, Hermsmeier et al. 2001 as cited by Lee and Ding [27]). Our findings on the variation in essence content between genotypes could be due to the variation in genetic constitution and planting density. Cowling and Field [28] also reported the effect of cultivars and planting density on essence content, stating that genotypes had different capacities to accumulate the essence. Lee and Ding [27] further found highly significant differences in essence content between three basil genotypes that ranged from 29 to 33 mL/g. Daneshian et al. [10] also obtained a similar result that the effects of genotype and plant spacing of purple basil with 40 plants m^{-2} had the maximum essence content (47 mL/g), whereas when planted at 80 plants m^{-2}, the essence content was only 25%. In our trial, the variability in essential oil yield between genotypes was due to optimum plant spacing that minimized competition. However, many other intrinsic and environmental considerations such as plant ontogeny, photosynthetic activity, light quality, photoperiodic responses, seasonal and climatic variations, nutritional relationships, plant growth regulators, oil production, plant density, moisture, salinity, and temperature have been shown to have a direct effect on the production of essential oil yield [28]. Our current findings are consistent with those of other workers (e.g., [27]) in that the interaction effect of cultivars and plant density highly affects the essential oil yield of basil. However, the present results did not agree with the findings of Khorshidi et al. [29] that the interaction of cultivars of *Foeniculum vulgare* and plant density was not significant; this could be due to genetic and environment variations. Optimizing plant densities and therefore plant spacing could be the key to having high essence yield. This could be because plant density affects plant growth by subsequently influencing the absorption of nutrients and exposure of the plant to the light which has a direct effect on photosynthesis and the production of essential oil [30].

Conclusion

The mutual effect of genotype and plant spacing showed highly significant variation in different essence yield and yield components. Oil yield and content were affected by the interaction of genotype and plant spacing. The maximum oil yield (7.88 kg ha^{-1}) was obtained from genotype BO-5 planted with the 30-cm plant spacing, and the highest oil content (101 mL g^{-1}) was obtained from BO-1 planted at the 20-cm spacing. Genotypes differ in their essential oil-bearing capacity and gross oil yield, which was affected by plant spacing in our trial. Genotypes planted with narrow plant spacing can synthesize more essential oil than those planted with a wide plant spacing. Identification of the correlations between yields and growth parameters was part of the present study; a positive correlation was found between oil content, oil yield, and leaf area per plant.

It is suggested that producers around the study area cultivate genotype BO-5 with a plant spacing of 30 cm and a row spacing of 60 cm for maximum essential oil yield. The yield of oil can also be affected by temperature, day length, soil conditions, moisture, and the growth stage and genetic make-up of the plant. Further study is needed on genotypes and planting densities under various environmental and/or climatic conditions to establish the ideal combinations at different harvesting times.

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Wpływ genotypu i typu uprawy na produkcję olejków eterycznych oraz wskaźniki plonowania bazylii (*Ocimum basilicum* L.) uprawianej w Jimma (południowo-zachodnia Etiopia)

Streszczenie

Bazylia jest aromatyczną rośliną szeroko wykorzystywaną w lecznictwie i gastronomii, powszechnie uprawianą w różnych rejonach Etiopii. Produkcja ziela bazylii oraz destylacja olejków eterycznych są bardzo istotne ze względu na szerokie zainteresowanie przemysłu farmaceutycznego oraz zastosowań w gastronomii. Potencjał tej rośliny jest wciąż nie w pełni wykorzystany ze względu na ograniczenia w uzyskiwaniu wysokich plonów.

Celem badań była ocena wpływu genotypu oraz zagęszczenia roślin w uprawie polowej na produkcję olejków eterycznych oraz wybrane wskaźniki plonowania bazylii (*Ocimum basilicum* L.). Doświadczenie prowadzono w latach 2016–2017, wykorzystując cztery genotypy bazylii (BO-1, BO-3, BO-4 i BO-5) oraz trzy warianty odległości między rzędami (20, 30 i 40 cm). Eksperyment prowadzono metodą bloków losowych w trzech powtórzeniach. Analizowano wysokość roślin, powierzchnię liści, liczbę rozgałęzień, a także zawartość i plon olejków eterycznych. Wykazano istotne interakcje pomiędzy genotypami i typem uprawy (odległość między rzędami) dla wszystkich testowanych parametrów. Najwyższy plon olejków eterycznych (7,88 kg/ha) uzyskano w wariancie BO-5 × 30 cm, podczas gdy najniższy (2,68 kg/ha) w układzie BO-1 × 40 cm. Najwyższą zawartość olejków eterycznych (101 mL/g) otrzymano w układzie BO-1 × 20 cm, a najniższą przy BO-3 × 30 cm, chociaż nie stwierdzono istotnych statystycznie różnic dla tego genotypu w trzech wariantach odległości między rzędami. W celu uzyskania najwyższego plonu olejków eterycznych, sugeruje się farmerom uprawianie genotypu BO-5 w odległości 30 cm między rzędami. Należy przeprowadzić dodatkowe badania w celu ustalenia zależności między lokalizacją i sezonową zmiennością upraw bazylii.