Journal of Agricultural Research Advances
ISSN: 2582-7227

Research Article
Open Access

Emergence of stable genotypes by parallel selection under the influence of genotype-environment interactions in basil

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Received on: 19/09/2025 Accepted on: 21/12/2025 Published on: 25/12/2025

ABSTRACT

Aim: The aim of the study was to evaluate the stability, reliability and suggestions for making essential oils from commercial basil genotypes.

Materials and Methods: To examine the stability of genotypes, field experiments were conducted in three years and twelve distinct basil cultivars were examined in order to identify those that consistently produced high levels of linalool oil under various settings and seasons.. The RBD two factor design and Principal component analysis (PCA) were performed to explore the interrelationships among traits under different environments.

Results: Results revealed that the genotypes for essential oil yield and O-2, O-6, and O-10 for linalool content (%) had the most adaptability and were the most stable variety due to their capacity to endure a broad range of environmental conditions over time. Given this, it can be said that the genotypes/varieties O-6, OC-8, and OC-10 were the most stable and had good yield performance for linalool content (%) and essential oil production. PCA revealed significant genetic variation in this basil sample as well, with PC1 through PC12 exhibiting high variable data values for each PC. The PCA also revealed the considerable genetic variety in this set of basil materials. For example, PC1 to PC11 showed extremely varying data values for each PC. Among the twelve characteristics of the basil, the corresponding greatest eigenvalues were 5.121 and the percentage of variances was 42.671.

Conclusion: It was concluded that genotypes O-6, OC-8, and OC-10 and variability can be efficiently used for additional genetic progress through heterosis breeding.

Keywords: Basil; environments; essential oil; genotypes; linalool; stability.

How to cite this article: Lal RK and Chanotiya CS (2025). Emergence of stable genotypes by parallel selection under the influence of genotype-environment interactions in basil. J. Agri. Res. Adv., 07(04): 17-32.

Introduction

The genus *Ocimum*, which is a member of the Lamiaceae family, consists of numerous species of herbs and shrubs known as basils. There are reportedly between 50 and 60 species in the genus, and because of their inherent ability to generate essential oils, these species are valued economically (Lal, et al., 2020; 2022; Kumar and Jnanesha, 2019). Steam distilling the herb yields a bright yellow, aromatic essential oil (Lal et al., 2000; Sastry et al., 2015; Kumar et al., 2016). The food, cosmetics, and pharmaceutical industries use the aromatic compounds found in essential oils—such as eugenol, methyl eugenol, citral, methyl chavicol, linalool, geraniol and camphor—as raw materials.

The antibacterial and insecticidal qualities of basil essential oils are widely recognized (Lal et al., 2021a,b; Singh and Lal, 2021; Lal et al., 2023a,b,c,d). Because of its pleasant scent and antibacterial properties, basil essential oil is a significant aroma chemical with applications in a number of industries, including food, pharmaceutical, cosmetic, and aromatherapy (Akcura, et al., 2005; Jnanesha et al., 2019; Srivastava et al., 2021; 2022).

A variety of antioxidants found in basil may promote longevity and well health (Kumar et al., 2024; 2025a,b). There is a lot of variation among the species that make up basil, including physical features, and chemical components of essential oil. Basil is frequently grown as a pot herb for use in cooking, and Israel alone exports 4 million US dollars' worth of basil to the fresh herb market per year (Yalçın et al., 2023). It also plays a significant role in the constantly expanding herbal sector in India. Basil species are grown commercially in India's hot, humid regions so that farmers and growers can produce leaves. *O. gratissimum* and *O. basilicum* are primarily planted over an area of around 3000 ha in Assam, West Bengal, Bihar, U.P., Haryana, Maharashtra, Punjab, M.P. and Jammu in India (Lal et al., 2021b). These basil crops produce 250–300 t of essential oils annually (Lal et al., 2021b). Methyl chavicol and linalool are the primary components of the 250 tonnes of basil oil that are estimated to be produced in India each year. Kusumohak, Vikarsudha, CIM-Saumya, CIM-Sharada, CIM-Surabhi, CIM-Ayu, CIM-Angana, CIM-Kanchan, CIM-Jyoti, and a hybrid of *O. basilicum* × *O. kilimandscharicum* CIM Suvaas and CIM Shishir are just a few of the basil varieties that CSIR-CIMAP, Lucknow has successfully developed through research and development (Kumar et al., 2021; Kumar and Lal., 2022; Kumar et al., 2022).

This study will assess the basil cultivars for consistency in critical yield over time in order to identify the optimal cultivar for India's agroclimate. Studies on the stability and variety of basil crops through environmental interactions (GE) are quite rare. For the basil genotypes, however, no stability or reliability studies have been conducted or assessed concurrently in a multi-environment essential oil yield trial. The current study's objective was to evaluate the stability, reliability, and suggestions for making essential oils from commercial genotypes.

Materials and Methods

All basil cultivars/varieties' plants and seeds were procured from the CSIR-CIMAP in Lucknow, India. There were 12 different genotypes (O1 to O12) of basil used in this study (Table 1). These basil cultivars were grown in the research farm CSIR-CIMAP, Lucknow (India), using only standard agronomical methods.

Experimental site: To examine the stability of genotypes, field experiments were conducted the study in the two factors RBD in the three replications with plot size = 4.0 m^2 for three years from 2022, 2023, and 2024 at the CSIR- CIMAP, Lucknow (India).

Experiments: Three replications of the experiments were conducted in the three factor RBD over three years (2022, 2023, and 2024). Every multicultural operation was conducted as needed. In all tests, all basil varieties and genotypes were transplanted with a 50×50 cm spacing. At intervals of 15 days, two split doses of 50 kg ha^{-1} nitrogen were applied. Weeds were manually eliminated 25 and 45 days following seedling transplantation. Before planting, each plot got 1.5 t ha of vermicompost, 40 kg/ ha of DAP, and 40 kg/ ha of K_2O.

Table 1. Basil genotype details with places of collections

S.No.	Genotypes	Origin/ palces of collection
1.	O1	CIM Soumya (CSIR-CIMAP, Lucknow)
2.	O2	CIM Surabhi (CSIR-CIMAP, Lucknow)
3.	O3	Tamilnadu
4.	O4	Rameshwaram
5.	O5	Trissur
6.	O6	Tamilnadu
7.	O7	Trivandrum
8.	O8	Krishna nagar, West Bengal
9.	O9	Madurai
10.	O10	Bareilly, Uttrakhand
11.	O11	Chennai, Tamil Nadu
12.	O12	Jodhpur, Rajasthan

Observations: The data were recorded on the 12 traits namely, X1 = plant height (cm); X2 = branches/plant; X3 = leaves/plant; X4 = leaf area (cm^2); X5 =herb yield (tonnes/ha); X6 = essential oil content (%); X7 = essential oil yield (kg/ha); X8 = methyl chavicol (%); X9 = linalool (%); X10 = eugenol (%); X11 = methyl content (%).; X12 = camphor content (%).

Distillation: Clevenger's apparatus was used to hydro-distill harvested crops for three hours using 500 g of each sample (Clevenger, 1928). The oil was collected and kept in sealed glass vials with a 5 ml capacity at 4^{o}C for testing and analysis after being dried over anhydrous sodium sulphates.

GC and GC-MS analysis: The GC and GC-MS tests were carried out using the established methodology (Pragadheesh et al., 2013; Chanotiya et al., 2024). Helium (He) was employed as the mobile phase (carrier) at a constant gas flow rate of 1 mL/min. The interface (transfer line) temperatures were set at 290°C, the electron impact ionization (EI) mode was used to ionize the gaseous ions, and the mass scan time was 0.39 sec with an inter-scan delay of 0.01 sec. The intended mass range of 40 to 450 amu was used to record the mass spectra of each component. Relative retention index (RRI) was used to characterize the constituents using a standardized series of saturated alkanes (C7-C30, CRM, 49451-U, Sigma-Aldrich), and 9th Edition a mass spectral data reference book (Adams, 2007).

Statistical analysis: Each variety was observed for each of the 12 traits. The RBD two factor design and Principal component analysis (PCA) was performed to explore the interrelationships among traits under different environments. PCA was carried out using PAST version 4.17. The resultant data from these programs includes the ANOVA, PCA analysis, genotype, and genotype × environment interactions. Years were treated as environments in the study.

Results and Discussion

The treatments and genotypes were highly significant for each of the twelve traits in the combined ANOVA of the mean of the twelve traits of the basil. The G × E is also highly significant for the all traits except X2, X3, and X9 traits which were only significant at only P<0.05 % only. Except for the X2 trait, the E was likewise highly significant for the most of traits (Table 2). It showed how the genotypes and genotypes of basil have such wide variations. GEI continues to be a difficult problem for basil breeders who conduct field trials of genotypes performance over environments or in various locations when choosing basil genotypes for the essential oil yield and stability. The effectiveness of basil breeders' progress towards the choice of well-accepted and stable cultivars in the basil can be increased by using the AMMI model for GEI (Shukla, 1972; Kang and Pham, 1991; Gupta et al., 2021a,b; Jaiswal et al., 2021). In three consecutive years, the mean yield of the twelve basil types ranged from 112.91 to 148.38 kg/ha (Tables 3-6).

Descriptive statistics revealed a wide range of variation among the studied basil genotypes for all measured traits. The genotypes showed considerable variation in essential oil content, essential oil yield, and its chemical constituents. The essential oil content of the genotypes ranged from 0.40 to 0.60%, essential oil yield: 112.91 to 148.38 kg/ha, methyl chavicol content (%): 0.39 to 29.93, linalool content (%): 0.58 to 25.45, eugenol content (%): 0.39 to 21.89, methyl eugenol content (%): 0.00 to 25.58, and camphor content (%): 0.02 to 12.60 %. The studied genotypes also showed range of variability for the plant height (57.54-97.50cm), branches per plant (16.61 – 21.24), leaves per plant (114.91 - 225.19), leaf area (cm²) (7.91 -9.14), herb yield (tonnes/ha) (18.89 - 33.10), essential oil content (%) (0.40- 0.60) (Table 6). The average means of these traits were for the essential oil content (0.51), essential oil yield: 134.13, methyl chavicol content (%):20.42, linalool content (%): 14.15, eugenol content (%): 14.15, methyl eugenol content (%): 8.74, and camphor content (%): 12.19, respectively. Similarly, other traits also demonstrated the same patterns (Table 6; figures 1a-d to 6a-d). Among the tested genotypes, the highest plant height showed by O7 (97.50) followed by O11 (97.48), and O3 (97.44) cm, while the genotype O1 was minimum (57.54) cm.

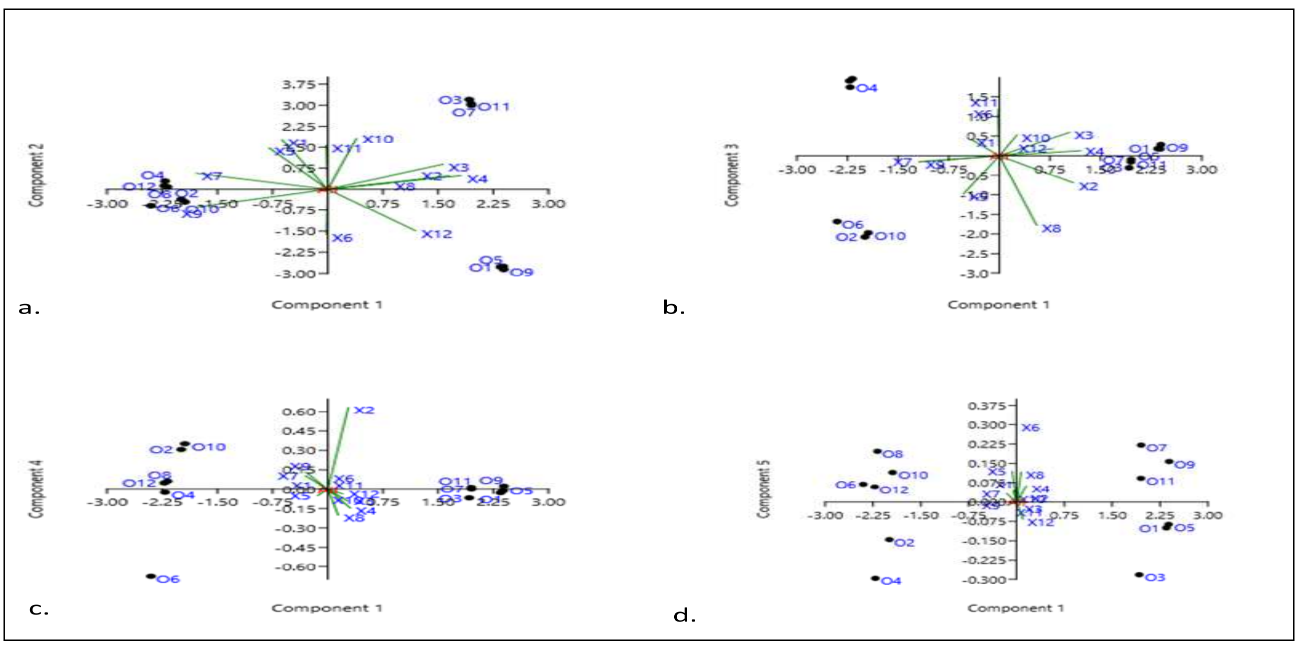


Fig. 1.a-d. Components (1 and 2 to 5) analysis in the twelve genotypes of basil

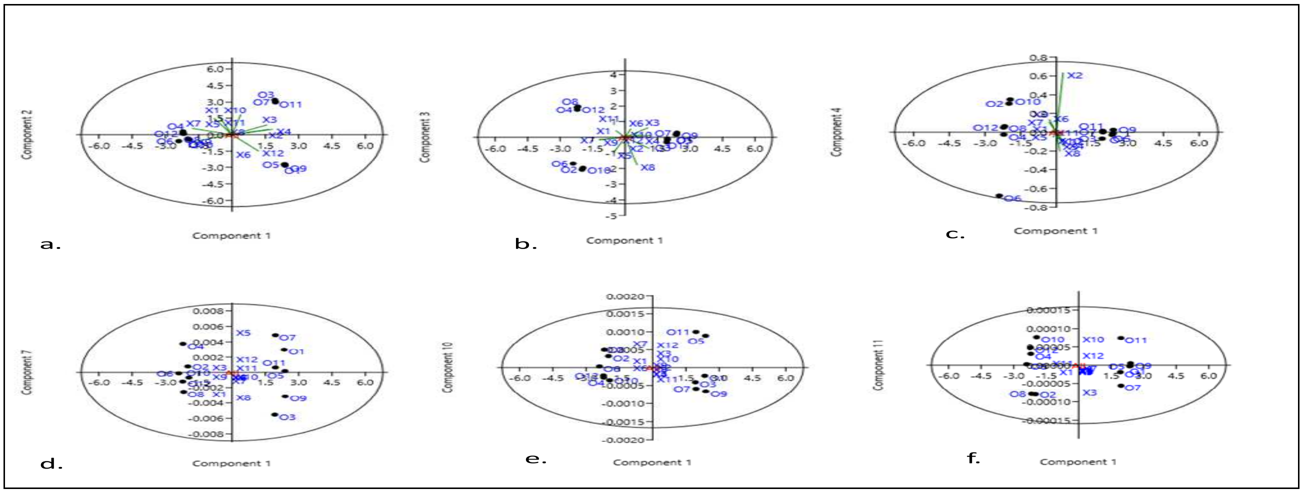


Fig. 2a-f. Components (1 and 2-4 , and 7,10,11) analysis in the twelve genotypes of basil

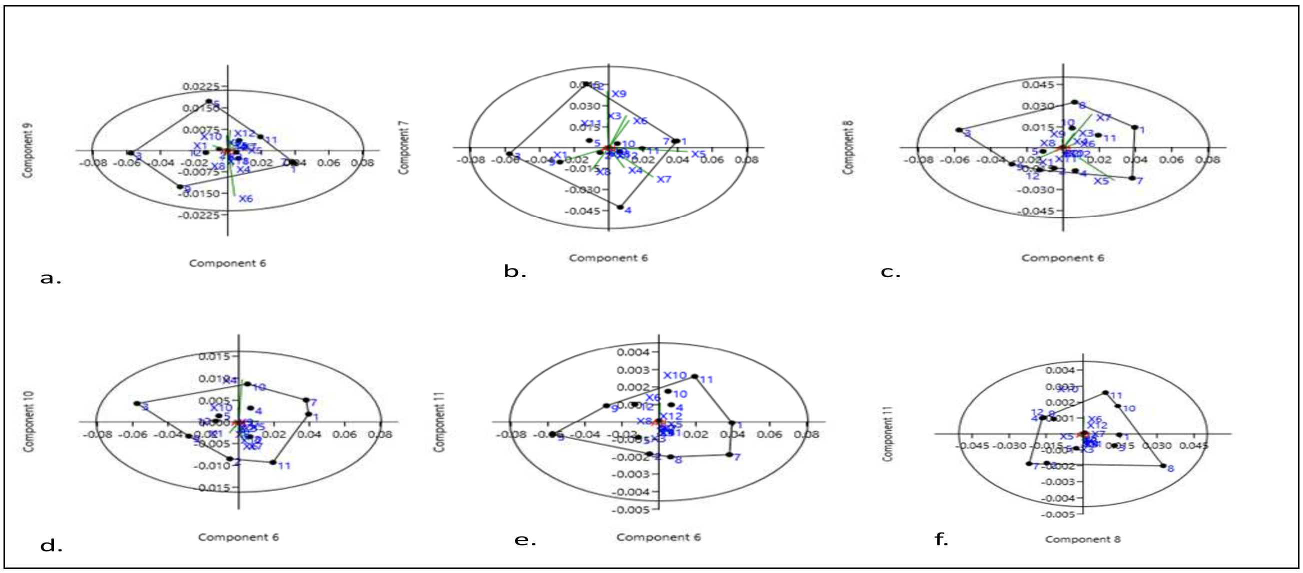


Fig. 3a-f. Components (6 and 7 to 9) analysis in the twelve genotypes of basil

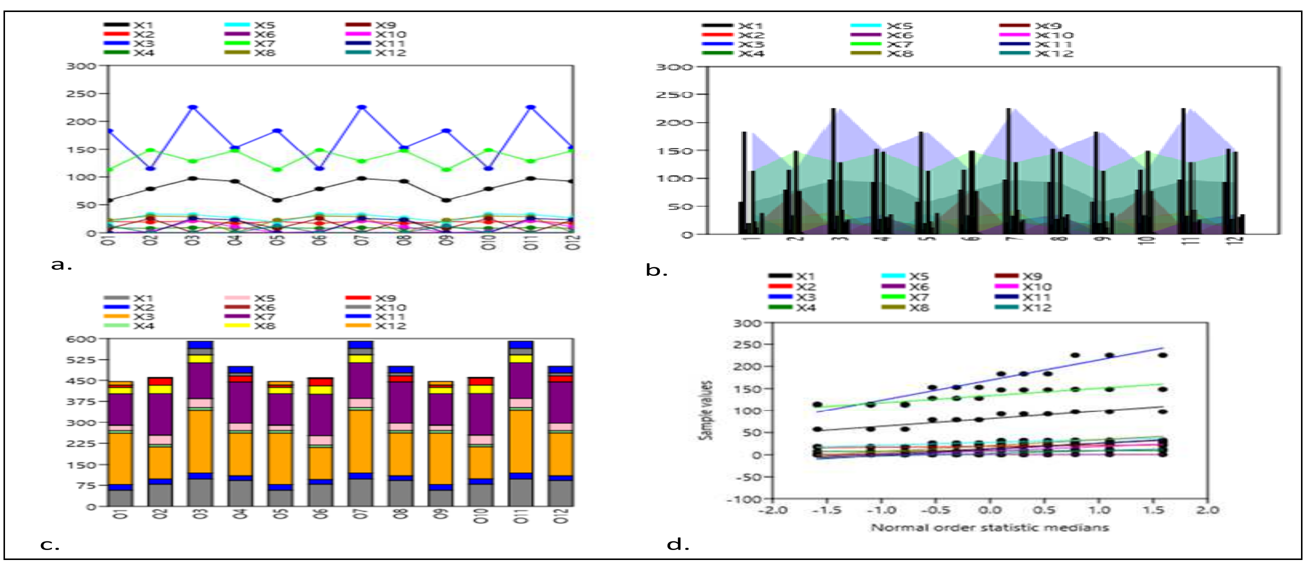


Fig. 4a-d. Mean performance and normal order statistic medians for the 12 traits of basil

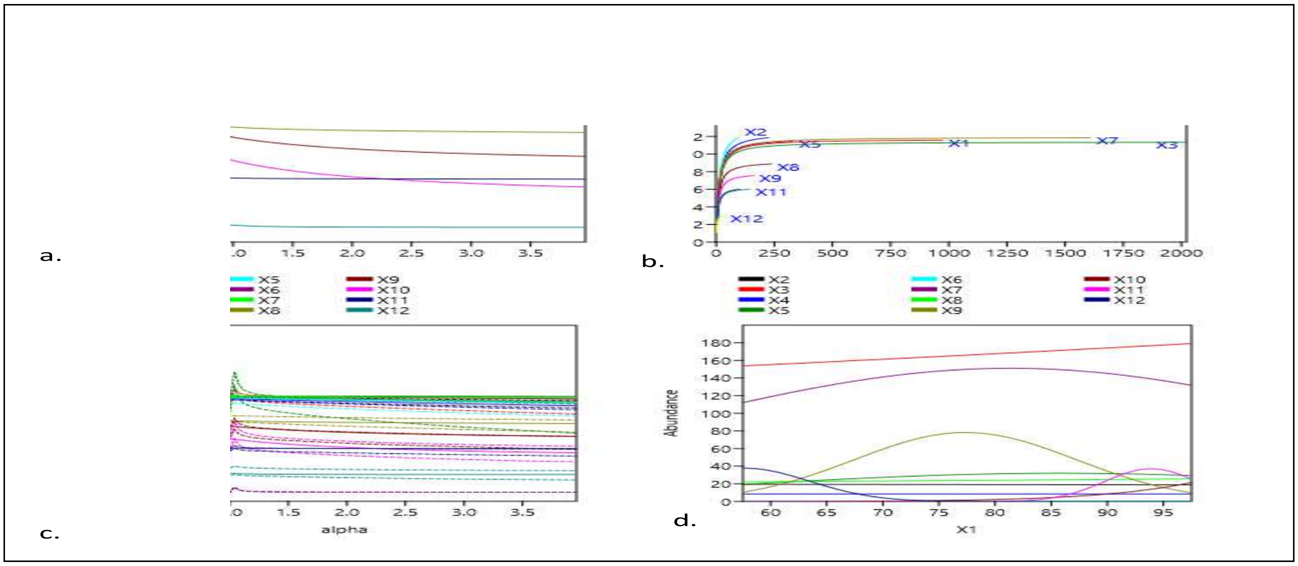


Fig. 5 a-d. Mean performance and normal order statistic medians for the 12 traits of basil

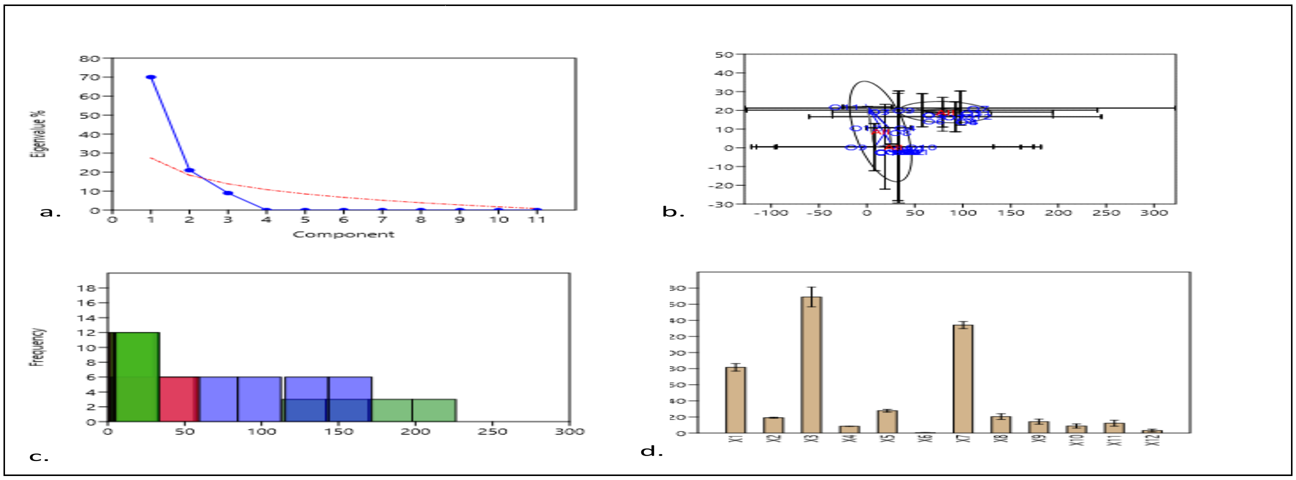
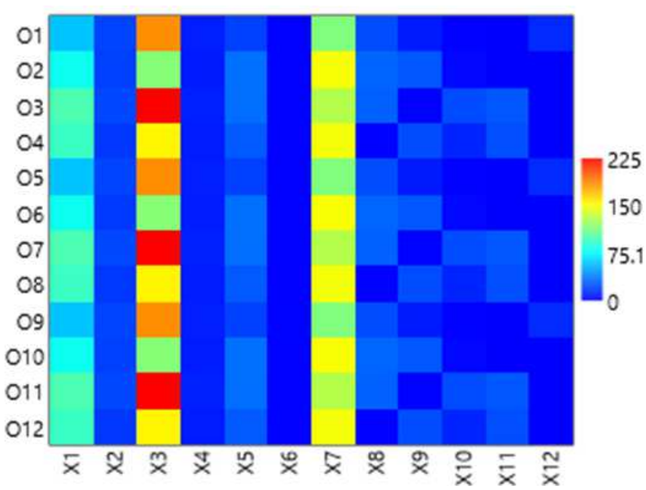


Fig. 6a-d. Mean performance, scree plot with Eigen value % for the 12 traits of basil



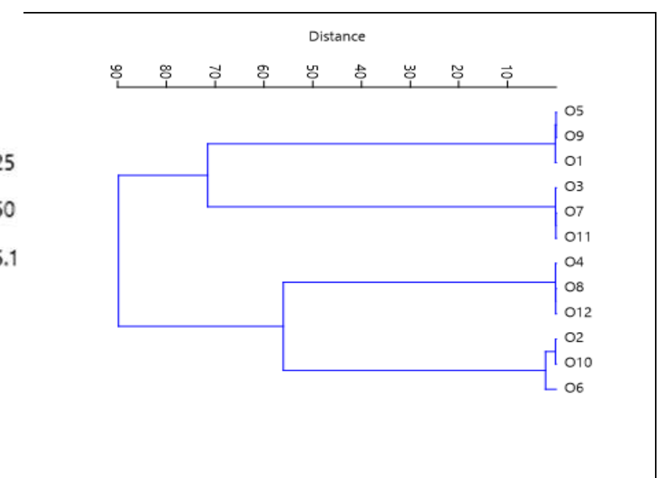


Fig. 7. Heat map for the correlations among the 12 traits of basil, Fig. 8. Dendogram for the 12 genotypes for diversity estimation in basil genotypes

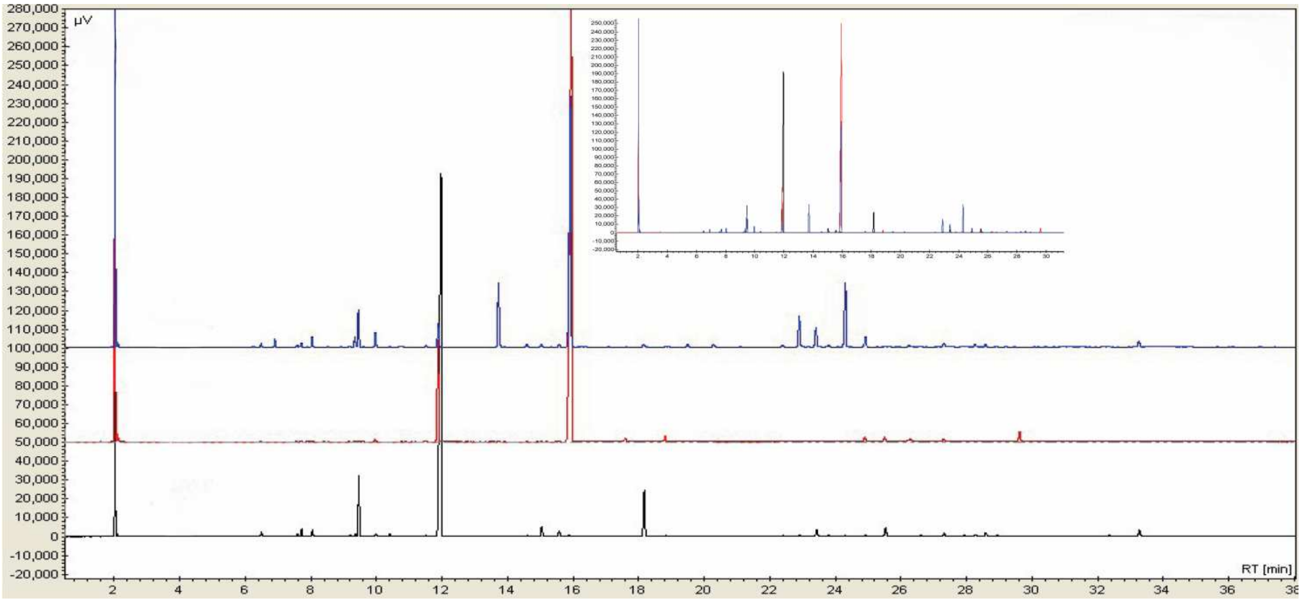


Fig. 9. GC/MS choreography of basil lines O1, O2, and O3 essential oils showing separations of aroma compounds.

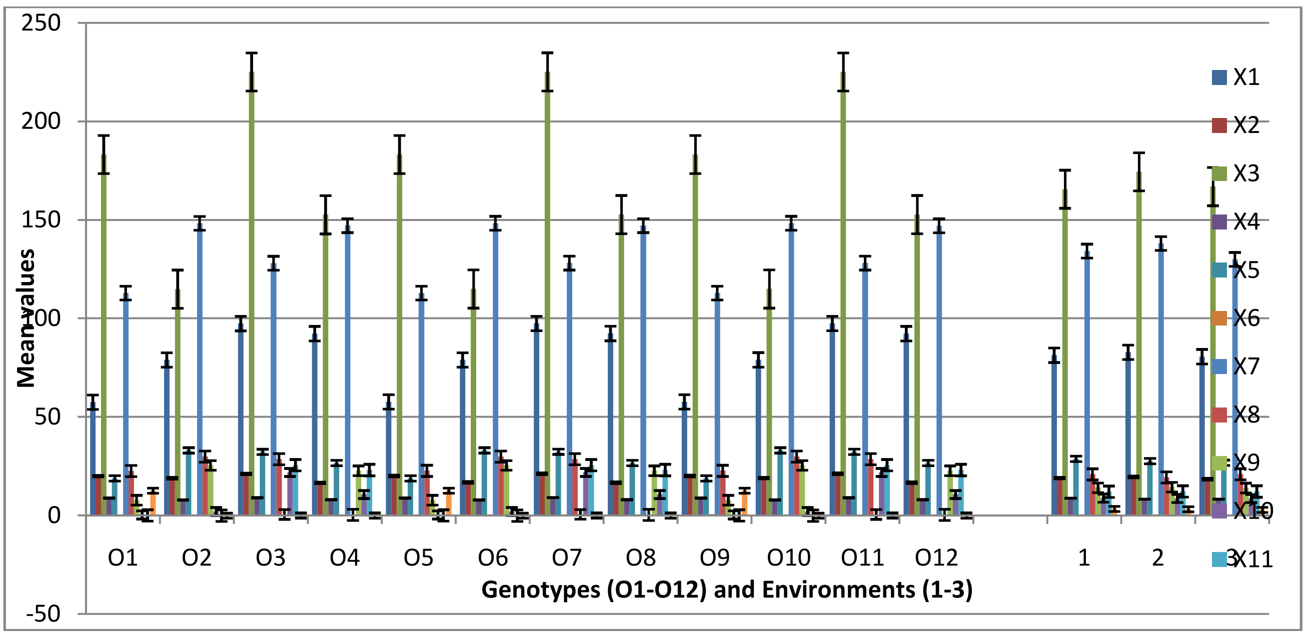


Fig 10. Mean performance of the 12 genotypes over environments in the basil

Similarly, the highest branches per plant was in the genotype O11 (21.24) followed by O7 (21.19), and O3 (21.16), while the genotype O4 expressed low branches/plant. For the leaves per plant, the highest leaves per plant demonstrated by genotype O7 (225.19) followed by genotype O11 (225.17), and O3 (225.13), while genotype O2 having 114.91 leaves. Like, methyl chavicol content (%), the highest methyl chavicol content (%) was in the genotypes O6, and O10 (29.93) followed by O2 (29.91), and O7, and o11 (28.67) %, while the genotype O4 was least (0.39) %. Among the tested basil genotypes, the highest essential oil yield was O6 (148.38) followed by O10 (138.36), and O2 (138.34) kg/ha, while the genotype O9 was least yielder (112.91) kg/ha. Similarly, qualitative traits like, methyl chavicol content (%), the highest methyl chavicol content (%) was in the genotypes O6, and O10 (29.93) followed by O2 (29.91), and O7, and o11 (28.67) %, while the genotype O4 was least (0.39) %. Similarly, other traits also demonstrated the same patterns (Table 6-7). Knowing the essential oil yield and its chemical constituents are among the most important attributes that need to be considered in selecting genotypes for the variety recommendation. Overall, the results demonstrated significant genetic variability among the tested genotypes for all traits including the essential oil yield, and quality yield-related traits, highlighting the potential for selection and genetic improvement of stable basil genotypes. The rankings of the types in the different locations and environments varied, indicating the existence of GEI (Tables 8).

A critical examination of the results reveals some significant findings, such as PC1 to PC11 showed highly variable data values for each PCs (Table 8-9, Figures 1-5a-d), the Principal Component Analysis (PCA) also demonstrated the high genetic diversity in this set of basil materials. Corresponding highest eigenvalues was 5.121 and percentage of variances 42.671 amongs twelve traits of the basil (Table 8-11).

Table 2. Combined analysis of variance (ANOVA) for the twelve traits in basil genotypes

Source of variation	d.f.	Mean Sum of Squares											
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
Treatment	35	737.91**	15.15**	5313.82**	1.76**	105.24**	0.014**	788.56**	711.91**	2587.89**	216.26**	727.45**	827.681**
Genotype (G)	11	2327.76**	32.27**	16058.69**	2.53**	318.25**	0.042**	2104.41**	2253.00**	8176.44**	685.89**	2314.26**	2620.865**
Environment (E)	2	45.64**	10.14	829.92*	2.81**	37.53**	0.009**	588.56**	234.43*	123.76**	30.73**	30.53*	16.957**
G × E	22	15.92**	7.05*	349.01*	1.27**	24.90**	0.001**	148.82**	222.96**	127.63*	41.03**	40.13**	4.791**
IPC1	12	7.18	12.57**	566.71**	2.06**	8.23	0.001**	265.25**	4.47	32.16	1.53	0.19	8.781**
Residual	10	4.41	0.43	87.77	0.32	0.90	0.0002	9.12	1.15	0.19	0.42	0.06	0.003
Error	72	6.26	4.16	170.18	0.33	6.66	0.0003	28.55	70.04	74.84	4.72	8.10	0.013
Total	107												

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%).

Table 3. Mean performance for the genotype (G) and environment (E) over the three years in the basil.

Genotype (G)	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
O1	57.54	20.04	183.23	8.84	18.89	0.58	112.92	22.66	7.80	0.31	0.068	12.5989
O2	78.96	18.98	114.91	7.91	33.09	0.46	148.34	29.91	25.45	2.04	0.003	0.0167
O3	97.44	21.16	225.13	9.10	32.31	0.40	128.09	28.66	0.58	21.88	25.581	0.0589
O4	92.35	16.61	152.65	8.04	26.67	0.53	147.13	0.39	22.75	10.68	23.120	0.0189
O5	57.69	20.10	183.26	8.87	18.90	0.58	112.92	22.69	7.82	0.33	0.068	12.5989
O6	78.99	16.82	114.99	7.95	33.10	0.48	148.38	29.93	25.45	2.05	0.003	0.0167
O7	97.50	21.19	225.19	9.14	32.43	0.44	128.12	28.67	0.60	21.89	25.581	0.0589
O8	92.41	16.67	152.75	8.07	26.66	0.57	147.15	0.40	22.77	10.69	23.120	0.0189
O9	57.71	20.11	183.24	8.88	18.89	0.60	112.91	22.69	7.81	0.32	0.068	12.5989
O10	79.02	19.04	114.98	7.96	33.10	0.48	148.36	29.93	25.45	2.05	0.003	0.0167
O11	97.48	21.24	225.17	9.12	32.39	0.43	128.14	28.67	0.59	21.89	25.581	0.0589
O12	92.35	16.64	152.75	8.02	26.66	0.56	147.07	0.40	22.77	10.69	23.120	0.0189
Environment (E)												
1	81.35	19.04	165.61	8.82	28.83	0.50	134.24	20.93	14.09	8.86	12.09	3.3758
2	82.86	19.58	174.51	8.33	27.64	0.53	138.11	19.36	14.41	8.71	12.25	3.2192
3	80.65	18.52	166.95	8.34	26.80	0.51	130.03	20.96	13.97	8.63	12.24	2.9250

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%).

The summary statistics from Principal Component Analysis for the 12 basil traits are shown in the Tables 6-9. Significant variations in in the all traits were found for qualitative and qualitative features, supporting previous research by Lahiri et al. (2018) showing that these traits were strongly genotype-dependent. It's interesting to note that several genotypes with high production had unusually high linalool and methyl chavicol content, suggesting the possibility of creating specialist cultivars aimed at the essential oil, perfumery, and pharmaceutical industries, which needs particular perfumery compounds. Divergent paternal lines might be identified to the successful selection of genotypes based on trait performance made possible by G × E analysis over the years. To maximize heterosis in subsequent crosses, such G × E is essential (Shah et al., 2023; Lal et al., 2023a,b,c,d; Maurya et al., 2022; Rai et al., 2023; Kumar et al., 2024; Singh et al., 2024).

Table 4. Mean performance for the genotype × environment (G ×E) over the three years in the Ocimum

Treatments	G×E	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
1	1×1	59.03	19.82	162.83	9.70	19.08	0.57	113.59	22.66	6.85	0.23	0.073	13.4200
2	2×1	79.16	19.87	111.38	7.38	34.38	0.43	146.42	32.01	25.38	1.85	0.003	0.0067
3	3×1	95.78	20.31	233.52	9.83	34.53	0.38	130.20	28.62	0.51	22.07	25.403	0.0700
4	4×1	91.12	16.00	154.35	8.25	27.32	0.52	146.73	0.38	23.58	11.27	22.863	0.0067
5	5×1	59.27	19.90	162.96	9.73	19.06	0.58	113.55	22.68	6.87	0.24	0.073	13.4200
6	6×1	79.20	19.90	111.49	7.40	34.40	0.46	146.47	32.03	25.38	1.87	0.003	0.0067
7	7×1	95.90	20.34	233.60	9.88	34.55	0.43	130.23	28.64	0.51	22.07	25.403	0.0700
8	8×1	91.15	16.07	154.53	8.29	27.32	0.56	146.75	0.39	23.59	11.29	22.863	0.0067
9	9×1	59.25	19.85	162.96	9.75	19.03	0.60	113.54	22.70	6.87	0.25	0.073	13.4200
10	10×1	79.30	19.90	111.57	7.45	34.42	0.45	146.44	32.05	25.39	1.87	0.003	0.0067
11	11×1	95.90	20.45	233.62	9.85	34.51	0.42	130.22	28.64	0.51	22.06	25.403	0.0700
12	12×1	91.17	16.07	154.45	8.28	27.34	0.55	146.72	0.39	23.60	11.29	22.863	0.0067
13	1×2	57.45	20.22	193.99	8.36	18.90	0.60	114.56	21.28	8.71	0.34	0.070	12.7833
14	2×2	81.18	19.34	120.65	7.54	33.33	0.47	155.31	27.51	25.67	2.69	0.003	0.0167
15	3×2	99.49	21.63	232.19	9.13	32.87	0.43	140.23	28.28	0.81	21.26	25.520	0.0433
16	4×2	93.15	16.99	151.15	8.18	25.29	0.55	142.31	0.34	22.39	10.53	23.413	0.0333
17	5×2	57.62	20.29	193.89	8.41	18.92	0.59	114.58	21.32	8.73	0.36	0.070	12.7833
18	6×2	81.22	19.43	120.72	7.57	33.35	0.49	155.34	27.53	25.68	2.69	0.003	0.0167
19	7×2	99.52	21.68	232.25	9.18	33.18	0.47	140.31	28.29	0.83	21.28	25.520	0.0433
20	8×2	93.25	17.05	151.20	8.21	25.25	0.59	142.32	0.35	22.41	10.52	23.413	0.0333
21	9×2	57.69	20.35	193.82	8.41	18.93	0.61	114.57	21.31	8.72	0.35	0.070	12.7833
22	10×2	81.20	19.33	120.70	7.58	33.33	0.49	155.32	27.52	25.68	2.68	0.003	0.0167
23	11×2	99.53	21.66	232.32	9.16	33.11	0.46	140.37	28.29	0.83	21.28	25.520	0.0433
24	12×2	92.99	17.00	151.26	8.20	25.23	0.59	142.14	0.36	22.41	10.53	23.413	0.0333
25	1×3	56.15	20.07	192.88	8.45	18.68	0.58	110.61	24.05	7.83	0.37	0.060	11.5933
26	2×3	76.53	17.75	112.70	8.82	31.55	0.47	143.29	30.22	25.29	1.57	0.003	0.0267
27	3×3	97.04	21.52	209.68	8.34	29.53	0.39	113.82	29.09	0.43	22.32	25.820	0.0633
28	4×3	92.79	16.82	152.45	7.70	27.40	0.52	152.36	0.45	22.27	10.23	23.083	0.0167
29	5×3	56.20	20.10	192.93	8.47	18.71	0.57	110.62	24.07	7.85	0.38	0.060	11.5933
30	6×3	76.57	11.12	112.75	8.89	31.54	0.51	143.33	30.24	25.30	1.58	0.003	0.0267
31	7×3	97.08	21.55	209.70	8.36	29.56	0.42	113.83	29.09	0.44	22.32	25.820	0.0633
32	8×3	92.83	16.88	152.52	7.72	27.40	0.56	152.38	0.46	22.30	10.25	23.083	0.0167
33	9×3	56.20	20.11	192.95	8.48	18.70	0.58	110.61	24.07	7.83	0.38	0.060	11.5933
34	10×3	76.56	17.88	112.68	8.86	31.56	0.50	143.32	30.22	25.29	1.59	0.003	0.0267
35	11×3	97.02	21.59	209.56	8.35	29.54	0.42	113.82	29.08	0.44	22.32	25.820	0.0633
36	12×3	92.87	16.85	152.55	7.59	27.40	0.54	152.35	0.46	22.31	10.25	23.083	0.0167

Table 5. Genotype (G), environment (E), and G × E related SE, SED, CD and GM over the three years in the basi.

Genotype (G)	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
SE	8.29	3.19	43.38	0.23	3.11	0.03	16.68	9.72	9.26	5.30	9.41	3.21
SED	11.73	4.51	61.35	0.33	4.40	0.04	23.59	13.74	13.10	7.49	13.31	4.53
CD 5%	23.23	8.93	121.46	0.65	8.71	0.08	46.70	27.21	25.94	14.84	26.35	8.98
CD 1%	30.85	11.86	161.34	0.87	11.57	0.10	62.03	36.14	34.46	19.71	35.00	11.92
Environment (E)												
SE	4.15	1.60	21.69	0.47	1.56	0.01	8.34	4.86	4.63	2.65	4.71	1.60
SED	5.87	2.26	30.67	0.66	2.20	0.02	11.79	6.87	6.55	3.75	6.65	2.27
CD 5%	11.61	4.47	60.73	1.30	4.36	0.04	23.35	13.60	12.97	7.42	13.18	4.49
CD 1%	15.43	5.93	80.67	1.73	5.78	0.05	31.02	18.07	17.23	9.85	17.50	5.96
G × E												
SE	14.37	5.53	75.13	0.81	5.39	0.05	28.89	16.83	16.05	9.18	16.30	5.55
SED	20.32	7.81	106.25	1.14	7.62	0.07	40.85	23.80	22.69	12.98	23.05	7.85
CD 5%	40.23	15.47	210.38	2.26	15.09	0.13	80.89	47.12	44.93	25.70	45.64	15.55
CD 1%	53.43	20.55	279.45	3.00	20.04	0.17	107.45	62.59	59.69	34.14	60.63	20.65
GM	81.62	19.05	169.02	8.49	27.76	0.51	134.13	20.42	14.15	8.73	12.19	3.17

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%); GM = grand mean, SE = Slandered error; SED = Slandered error difference; CD = Critical difference.

Table 6. Summary statistics Univariate fir the twelve traits of basil

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
Min	57.54	16.61	114.91	7.91	18.89	0.40	112.91	0.39	0.58	0.31	0.00	0.02
Max	97.50	21.24	225.19	9.14	33.10	0.60	148.38	29.93	25.45	21.89	25.58	12.60
Sum	979.44	228.60	2028.25	101.90	333.09	6.11	1609.53	245.00	169.84	104.82	146.31	38.10
Mean	81.62	19.05	169.02	8.49	27.76	0.51	134.13	20.42	14.15	8.74	12.19	3.18
Std. error	4.64	0.55	12.19	0.15	1.72	0.02	4.41	3.58	3.11	2.58	3.68	1.64
Variance	258.69	3.59	1784.36	0.28	35.35	0.00	233.81	153.92	116.17	79.72	162.07	32.30
Stand. dev	16.08	1.89	42.24	0.53	5.95	0.07	15.29	12.41	10.78	8.93	12.73	5.68
Median	85.69	19.54	167.99	8.46	29.49	0.51	137.61	25.68	15.29	6.37	11.60	0.04
25 prcntil	63.02	16.71	124.41	7.98	20.84	0.45	116.71	5.97	2.40	0.76	0.02	0.02
75 prcntil	96.18	20.90	214.66	9.05	32.93	0.58	148.04	29.60	24.78	19.08	24.97	9.47
Skewness	-0.67	-0.32	0.08	0.09	-0.70	-0.16	-0.43	-1.13	-0.18	0.64	0.02	1.33
Kurtosis	-1.16	-1.66	-1.27	-2.21	-1.22	-1.57	-1.67	-0.57	-2.03	-1.29	-2.41	-0.33
Geom. mean	80.02	18.96	164.06	8.48	27.11	0.50	133.30	9.37	7.19	3.52	0.00	0.13
Coeff. var	19.71	9.94	24.99	6.27	21.42	13.52	11.40	60.77	76.15	102.22	104.41	179.01
N	12	12	12	12	12	12	12	12	12	12	12	12

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm^2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%)

Table 7. Top three ranks and their means of from the 12 genotypes for the 12 traits in the Ocimum

Treats	Genotype (G) ranks			Genotype	Environment (E) ranks		
Mean	I	II	III	Lowest rank	I	II	III
X1	O7	O11	O3	O1	2	1	3
Mean	97.50	97.48	97.44	57.54	82.86	81.35	80.65
X2	O11	O7	O3	O4	2	1	3
Mean	21.24	21.19	21.16	16.61	19.58	19.04	18.52
X3	O7	O11	O3	O2	2	3	1
Mean	225.19	225.17	225.13	114.91	174.51	166.95	165.61
X4	O7	O11	O3	O2	1	3	2
Mean	9.14	9.12	9.10	7.91	8.82	8.34	8.33
X5	O6, O10	O2	O7	O1, O9	1	2	3
Mean	33.10	33.09	32.43	18.89	28.83	27.64	26.80
X6	O9	O1, O5	O8	O4	2	3	1
Mean	0.60	0.58	0.57	0.40	0.53	0.51	0.50
X7	O6	O10	O2	O9	2	1	3
Mean	148.38	148.36	148.34	112.91	138.11	134.24	130.03
X8	O6, O10	O2	O7, O11	O4	3	1	2
Mean	29.93	29.91	28.67	0.39	20.96	20.93	19.36
X9	O2, O6, O10	O8, O12	O4	O3	2	1	3
Mean	25.45	22.77	22.75	0.58	14.41	14.09	13.97
X10	O7, O11	O3	O8, O12	X10	1	2	3
Mean	21.89	21.88	10.69	0.31	8.86	8.71	8.63
X11	O3, O7, O11	O4, O8, O12	O1, O5, O9	O2, O6, O10	2	3	1
Mean	25.581	23.120	0.068	0.003	12.25	12.24	12.09
X12	O1, O5, O9	O3, O7, O11	O4, O8, O12	O2, O6, O10	1	2	3
Mean	12.5989	0.0589	0.0189	0.0167	3.3758	3.2192	2.9250

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm^2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%).

Table 8. Principal component analysis data, Eigenvalue and % variance for the 12 genotypes related to 12 traits of basil

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11
O1	2.35	-2.76	0.18	-0.03	-0.10	-0.03	0.003	-0.00309	0.00026	-0.00023	0.00000
O2	-1.99	-0.36	-2.07	0.31	-0.15	-0.02	0.001	0.00089	-0.00054	0.00032	-0.00008
O3	1.92	3.20	-0.31	-0.07	-0.28	0.003	-0.006	-0.00041	0.00035	-0.00041	-0.00002
O4	-2.21	0.29	1.76	-0.02	-0.30	0.030	0.004	0.00027	-0.00056	-0.00028	0.00003
O5	2.38	-2.75	0.17	-0.01	-0.09	0.021	0.0002	0.00205	0.00081	0.00089	0.00000
O6	-2.40	-0.59	-1.68	-0.68	0.07	-0.0004	-0.0001	0.00002	-0.00001	0.00003	0.00000
O7	1.95	3.00	-0.09	0.00	0.22	0.007	0.005	0.00105	0.00036	-0.00059	-0.00006
O8	-2.18	0.08	1.97	0.06	0.20	0.018	-0.003	-0.00185	0.00006	0.00050	-0.00008
O9	2.39	-2.86	0.28	0.02	0.16	0.007	-0.003	0.00104	-0.00107	-0.00066	0.00001
O10	-1.94	-0.45	-1.97	0.35	0.12	0.025	-0.001	-0.00091	0.00055	-0.00035	0.00008
O11	1.95	3.05	-0.16	0.01	0.09	-0.010	0.001	-0.00065	-0.00071	0.00100	0.00007
O12	-2.22	0.12	1.92	0.05	0.06	-0.048	-0.001	0.00158	0.00050	-0.00022	0.00005
X1	-0.147	0.420	0.147	0.047	0.123	0.040	-0.394	0.635	-0.380	0.161	-0.161
X2	0.388	0.114	-0.246	0.880	0.036	0.008	0.000	-0.002	0.000	0.001	0.000
X3	0.369	0.210	0.211	-0.129	-0.006	-0.251	0.092	-0.072	0.362	0.391	-0.625
X4	0.426	0.115	0.045	-0.205	0.172	0.854	0.015	-0.024	0.029	-0.030	0.007
X5	-0.188	0.348	-0.345	-0.071	0.329	-0.026	0.755	0.197	-0.029	-0.018	-0.027
X6	-0.004	-0.381	0.375	0.122	0.830	-0.104	-0.007	-0.004	-0.003	-0.001	0.002
X7	-0.421	0.135	-0.055	0.145	0.088	0.226	-0.060	-0.562	-0.249	0.578	-0.076
X8	0.195	0.052	-0.627	-0.280	0.322	-0.197	-0.414	-0.047	0.073	0.088	0.041
X9	-0.416	-0.148	-0.053	0.187	-0.035	0.275	-0.074	0.338	0.696	0.176	-0.032
X10	0.092	0.430	0.187	-0.047	0.100	-0.155	-0.082	-0.075	0.337	0.216	0.698
X11	-0.005	0.369	0.417	0.073	-0.037	-0.058	0.076	-0.134	-0.021	-0.223	0.037
X12	0.281	-0.350	0.064	-0.052	-0.183	-0.042	0.271	0.311	-0.241	0.593	0.292
Eigenvalue	5.121	4.792	1.993	0.063	0.032	0.001	0.00001	0.000002	0.0000003	0.0000003	0.000000001
% variance	42.671	39.932	16.606	0.522	0.264	0.005	0.00007	0.00002	0.000003	0.000003	0.00000002

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm^2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%).

Table 9. Similarity and distance indices for the 12 genotypes related to 12 traits of basil

Parameters	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12
O1	0.00	84.32	71.56	70.05	0.17	84.34	71.66	70.04	0.19	84.29	71.63	69.98
O2	84.32	0.00	120.73	56.03	84.30	2.16	120.78	56.11	84.29	0.13	120.76	56.10
O3	71.56	120.73	0.00	84.37	71.45	120.71	0.16	84.28	71.45	120.65	0.14	84.27
O4	70.05	56.03	84.37	0.00	69.99	55.93	84.42	0.14	69.98	55.99	84.40	0.13
O5	0.17	84.30	71.45	69.99	0.00	84.32	71.55	69.98	0.04	84.27	71.52	69.92
O6	84.34	2.16	120.71	55.93	84.32	0.00	120.77	56.01	84.31	2.22	120.75	56.00
O7	71.66	120.78	0.16	84.42	71.55	120.77	0.00	84.34	71.55	120.71	0.08	84.32
O8	70.04	56.11	84.28	0.14	69.98	56.01	84.34	0.00	69.98	56.06	84.32	0.12
O9	0.19	84.29	71.45	69.98	0.04	84.31	71.55	69.98	0.00	84.26	71.53	69.91
O10	84.29	0.13	120.65	55.99	84.27	2.22	120.71	56.06	84.26	0.00	120.69	56.05
O11	71.63	120.76	0.14	84.40	71.52	120.75	0.08	84.32	71.53	120.69	0.00	84.30
O12	69.98	56.10	84.27	0.13	69.92	56.00	84.32	0.12	69.91	56.05	84.30	0.00

Table 10. Correlations (Pearsion) between different characters in the basil genotypes

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
X1	1.000	0.685*	0.518	0.820**	0.006	0.021	0.052	0.485	0.990**	0.000	0.000	0.000
X2	-0.131	1.000	0.006	0.000	0.957**	0.209	0.007	0.010	0.000	0.307	0.980**	0.292
X3	0.207	0.737**	1.000	0.000	0.643*	0.463	0.014	0.619*	0.000	0.014	0.071	0.521
X4	-0.074	0.874**	0.942**	1.000	0.442	0.571*	0.000	0.194	0.000	0.136	0.471	0.170
X5	0.741**	-0.017	-0.149	-0.245	1.000	0.000	0.018	0.287	0.556	0.097	0.291	0.000
X6	-0.653*	-0.391	-0.234	-0.182	-0.881**	1.000	0.386	0.058	0.456	0.023	0.245	0.016
X7	0.572*	-0.726**	-0.684*	-0.849**	0.666*	-0.276	1.000	0.311	0.001	0.855**	0.526	0.001
X8	-0.224	0.708**	0.160	0.403	0.335	-0.561	-0.320	1.000	0.210	0.920**	0.158	0.730**
X9	0.000	-0.870**	-0.960**	-0.997**	0.189	0.238	0.809**	-0.390	1.000	0.083	0.352	0.254
X10	0.852**	0.322	0.686*	0.456	0.501	-0.646*	0.059	-0.033	-0.521	1.000	0.000	0.055
X11	0.869**	-0.008	0.538	0.231	0.332	-0.364	0.203	-0.435	-0.295	0.914**	1.000	0.052
X12	-0.898**	0.332	0.206	0.424	-0.898**	0.677*	-0.838**	0.111	-0.358	-0.566	-0.573	1.000

X1 = Plant height (cm); X2 = Branches per plant; X3 = Leaves per plant; X4 = leaf area (cm2); X5 =herb yield (tonnes/ha); X6 = Essential oil content (%); X7 = Essential oil yield (kg/ha); X8 = Methyl chavicol content (%); X9 = Linalool content (%); X10 = Eugenol content (%); X11 = Methyl eugenol content (%).; X12= Camphor (%).

Table 11. Compositional variability in oil constituents of most promising breeding lines of *Ocimum x basilicum*

Physicochemical Parameters	**ISO 11043:1998**	**RI$_a$**	**RI$_b$**	**O1 (CIM-Saumya)**	**O2 (CIM Surbhi)**	**O 3**	**O4**	**O5**	**O6**	**O7**	**O8**	**O9**
Refractive index @20°C(nD)	1.5100-1.5200			1.5128	1.4634	1.5109	1.5303	1.4746	1.4673	1.4988	1.4766	1.4682
Specific Gravity@20°C(t/t)	0.948-0.970			0.9553	0.8874	0.9697	1.0019	0.9018	0.8892	0.9581	0.9044	0.8880
Flash Point(T_F)	(+) 75°C			77.5°C	74.5°C	72.5°C	77.5°C	70.5°C	72.5°C	63.5°C	72.5°C	71.5°C
Constituents												
α-Pinene	-	932	937	t	0.5	0.4	0.3	0.3	0.3	1.3	0.3	0.4
Camphene	-	946	952	t	t	0.9	0.5	0.7	0.7	1.9	0.8	0.9
Sabinene		974	977	t	0.3	0.2	t	0.3	0.3	t	0.2	0.3
β-Pinene	-	976	981	t	0.9	0.6	0.7	0.6	0.5	2.6	0.6	0.7
β-Myrcene	-	988	993	t	0.7	1.2	0.7	0.8	0.7	2.4	0.8	0.9
Limonene	-	1024	1031	t	0.4	1.3	t	1.1	1.0	t	1.2	1.3
1,8-cineole	1.0-3.5 %	1026	1034	t	7.5	4.7	4.4	6.4	6.4	17.1	5.2	5.3
(Z)-β-ocimene		1032	1038	t	t	0.1	t	t	t	t	t	t
(E)-β-ocimene	0.9-2.8 %	1044	1049	0.2	0.3	1.9	1.2	0.6	0.4	3.5	0.6	0.4
γ-Terpinene	-	1054	1061	t	0.3	0.1	t	0.1	0.1	0.2	0.1	0.1
Terpinolene	-	1086	1092	t	0.1	0.2	0.1	0.2	0.2	t	0.2	0.2
Linalool	**0.5-3.0%**	**1095**	**1102**	**10.9**	**71.1**	**3.4**	**2.3**	**59.8**	**71.0**	**5.3**	**56.0**	**66.2**
Camphor	**0.15-0.8 %**	**1141**	**1148**	**t**	**0.1**	**10.0**	**6.0**	**8.0**	**9.2**	**14.1**	**8.4**	**8.9**
Terpinen-4-ol	0.2-0.6%	1174	1180	t	0.2	0.4	0.3	0.5	0.6	0.7	0.6	0.6
α-Terpineol	-	1186	1194	0.1	1.4	0.4	t	t	0.4	t	0.4	0.4
Methyl chavicol	**75.0-87.0 %**	**1195**	1202	**81.3**	**0.8**	**44.9**	**34.7**	**7.8**	**2.6**	**6.1**	**6.6**	**3.1**
Geraniol	-	1249	1257	0.1	7.0	0.4	0.5	0.3	0.2	0.3	0.6	0.3
Bornyl acetate	-	1254	1259	t	t	t	t	t	t	0.9	0.2	0.2
cis-Methyl cinnamate		1305	1308	t	t	0.4	4.7	0.4	0.2	0.5	0.8	0.1
Eugenol	-	1356	1360	0.1	0.1	0.3	0.2	0.2	0.2	0.7	0.2	0.1
Chavibetol	-	**1370**	1373	t	0.2	5.3	1.5	0.7	0.1	11.7	0.4	0.1
***trans*-Methyl cinnamate**		**1384**	1385	0.1	1.0	3.5	31.7	3.0	1.3	4.1	5.5	1.0
β-Elemene	-	1389	1395	t	t	0.4	t	0.3	0.1	0.8	0.4	0.4
Methyl eugenol	0.3-2.5%	1403	1408	t	0.2	10.5	3.9	0.9	0.1	15.4	0.6	0.2
β-Caryophyllene	-	1417	1424	0.6	0.2	1.5	1.0	1.3	0.5	t	1.9	1.7
α-trans bergamotene		1435	1442	0.5	1.4	0.2	0.2	t	t	t	0.2	0.1
α-humulene		1457	1458	0.2	t	0.3	0.3	0.2	0.1	0.6	0.3	0.3
Germacrene D	-	1484	1485	0.4	0.5	0.7	0.5	0.9	0.3	1.3	1.3	1.2
Bicyclogermacrene	-	1500	1501	t	0.1	0.2	T	0.3	0.1	0.7	0.4	0.3

Germacrene A	-	1508	1510	0.1	0.2	0.5	0.5	0.3	0.1	1.5	0.5	0.4
γ-Cadinene	-	1514	1518	t	0.5	0.4	0.3	0.3	0.1	t	0.3	0.3
δ-Cadinene	-	1522	1527	t	0.1	0.1	0.1	0.1	t	0.1	0.1	0.1
Caryophyllene oxide		1582	1582	0.1	t	0.1	0.4	0.1	t	0.4	0.1	0.1
1,10-di-epi-cubenol	-	1618	1619	t	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Epi-α-cadinol	-	1638	1645	t	1.0	1.0	0.8	0.6	0.5	1.2	0.6	0.5
α-cadinol		1654	1659	t	0.1	0.1	t	t	t	0.3	t	t
				94.7	97.3	96.7	97.9	97.2	98.7	95.9	96.5	97.2

RI_a : Reported retention index by Dr. RP Adams, 2017 edition 4.1, RI_b : Calculated Retention Index in ELITE-5 capillary columns using a homologous series of n-alkanes (C_7-C_{30} hydrocarbons). tr; trace < 0.1%, nd; not detected.

Fig. 11(a-g). a-d: Morphological variability of lines O1, O2, O3, and O4, and in the e-f: inflorescences, and g. flowers of basil

The correlations between the characters also have a significant impact on the type, stability, and level of genetic variability. The trait X1 is highly significant and positively correlated with X4 and X9. Similarly, the trait X2 is highly significant and positively correlated with X5 and X11. Likewise the traits X7 with X10, and X8 with X10 and X12 were positive and highly significantly correlated with each other. On the other hand, the weak the positive and negative associations were also recorded between traits (Table 10, Figures 7-10, and Figure 11 a-g). In other traits the weak positively or negatively correlations were noted. Therefore, correlations should also be considered when selecting different genotypes for hybridization programs. An extensive assessment of the genetic diversity in the basil accessions with respect to both quantitative yield-contributing variables and qualitative traits features is given in this investigation (Table 11). Significant variation among the examined genotypes is revealed by the results, suggesting the existence of a wide genetic foundation that can be successfully utilized in breeding initiatives.

Nevertheless, it is feasible to simultaneously improve both quantitative and qualitative features, which is a difficulty frequently mentioned in the basil breeding, according to the occurrence of variations with both high essential oil yield and essential oil profiles. According to earlier studies, the essential oil production and quality can be modulated by variables like temperature, light intensity, and soil nutrient availability. In essence, this thorough evaluation of genetic diversity offers a strategic basis for creating high-yielding, compound-specific, and locally adapted basil genotypes. Finding genetically varied and agronomical superior accessions has potential applications as breeding parents for the development of enhanced cultivars with specific chemical profiles, as well as direct cultivation under controlled conditions. Consider the stability of performance to be a crucial component of yield testing throughout years and situations. Stability and yield must be taken into account concurrently to be useful in a breeding program and to increase the accuracy and dependability of the varieties chosen. The aforementioned trends were found in the basil characteristic that has been examined the most. These findings concur with those of Lal, 2012, Lal et al., 2020; 2022; Sastry et al., 2015, Jakovljević et al., 2023; Kumari et al., 2023; Kumar et al., 2024. The various basil varieties can be categorized as stable variants based on stability data. The statistical analysis revealed that the genotypes/varieties O-6 and O-10 for essential oil yield and O2, O-6 and O-10 for linalool content (%) were the most adaptable and stable varieties due to their capacity to withstand a broad range of environmental conditions over time. It follows that the genotypes/varieties O-6, O-10, and O-2 for methyl chavicol content (%) and essential oil production are not only the most stable but also have good yield performance. Therefore, it is suggested that these genotypes and cultivars be grown economically.

Conclusions

It was concluded that the genotypes/varieties O-6, OC-8, and OC-10 were the most stable and had good yield performance for linalool content (%) and essential oil production. These genotypes and variability can be efficiently used for additional genetic progress through heterosis breeding.

Acknowledgement

Authors are grateful to Director, CSIR-CIMAP, for providing the necessary facilities. The work was supported by the Council of Scientific and Industrial Research, India.

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