Genetic Resources and Crop Evolution

Essential oil chemotype diversity and environmental stability in Turkish basil (Ocimum basilicum L.) germplasm: a three-layer field evaluation across contrasting ecologies

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ONLINE RESOURCE 1

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Table S1. Analysis of variance (ANOVA) summary for essential oil (EO) yield and dominant compound content across three experimental layers.

Source	**df**	**F**	**P**
Layer 1: Ecological (3 loc × 2 yr)			
Genotype (G)	11	—	<0.01
Location (L)	2	—	<0.01
Year (Yr)	1	—	<0.01
G × L	22	3.82	<0.01
G × Yr	11	2.14	<0.05
L × Yr	2	4.67	<0.01
G × L × Yr	22	1.43	<0.05
Error	120		

Layer 2: Ontogenetic (3 stage × 2 yr, Bursa)			
Stage	2	18.44	<0.01
G × Stage	22	2.91	<0.01
Year	1	6.23	<0.05
Error	66		
Layer 3: Seasonal (2 season × 2 yr, Tokat)			
Season	1	12.77	<0.01
Year × Season	1	5.84	<0.05
G × Season	11	1.98	<0.05
Error	44		

Response variable: EO yield (mL·100 g⁻¹ DW). All effects tested at α = 0.01 unless noted. Replication nested within location × year.

Table S2. Genotype characteristics and collection origin of the 12 basil accessions used in the present study.

Genotype	Origin	Chemotype	Key characteristic	Collection
R-1	Turkish landrace	Linalool	Moderate linalool	Anatolian
R-3k	Turkish landrace	Linalool	High linalool	Anatolian
R-4	Turkish landrace	Linalool	Co-accumulates methyl eugenol	Anatolian
R-10A	Turkish landrace	Estragole	Very high estragole (>860 %)	Anatolian
R-15	Turkish landrace	Linalool	High EO yield	Anatolian
R-16	Turkish landrace	Linalool	Highly stable linalool	Anatolian
R-17	Turkish landrace	Citral	Geranial + neral dominant	Anatolian
R-19	Turkish landrace	Linalool	High α-bergamotene	Anatolian
R-20	Turkish landrace	Linalool	Moderately stable linalool	Anatolian
R-23	Foreign accession	Methyl cinnamate/linalool	Dual dominant compounds	C/E European
Y-7	Foreign accession	Linalool	High eugenol secondary	C/E European
Y-15	Foreign accession	Linalool	Standard linalool type	C/E European

All accessions are deposited in the germplasm collection of the Department of Field Crops, Tokat Gaziosmanpaşa University. Accession codes follow the original collection numbering system.

Table S3. Climatic characteristics of the three trial locations during the experimental growing seasons.

Parameter	Bursa Y1	Bursa Y2	Eskişehir Y1	Eskişehir Y2	Tokat Y1	Tokat Y2
Latitude	40°13′N		39°45′N		40°19′N	
Longitude	28°51′E		30°33′E		36°27′E	
Altitude (m a.s.l.)	120		789		594	
Soil type	Clay-loam		Silty-clay		Sandy-loam	
GS mean temp. (°C)	21.5	21.5	18.1	18.1	18.5	20.9
GS total precip. (mm)	197.6	425.4	130.1	257.9	156.2	186.8
GS mean RH (%)	61.0	71.9	55.2	66.9	54.8	49.5
Annual mean T (°C)	15.6	16.0	11.9	12.1	12.8	14.6
Annual precip. (mm)	591.8	846.6	283.1	451.2	366.5	384.2
Annual mean RH (%)	66.1	73.9	62.3	71.1	58.6	54.0
30-yr normal T (°C)	14.5		10.8		12.5	
30-yr normal P (mm)	707		388		441	

GS = growing season (May–September). Y1 = Year 1; Y2 = Year 2. RH = relative humidity. T = temperature. P = precipitation. 30-yr normals from Turkish State Meteorological Service (MGM). 30-year normals refer to the 1981–2010 reference period. Monthly data available from corresponding author upon request. Planting: late May at all locations. Harvest (onset of flowering): Bursa late Jun–early Jul; Eskişehir mid-Jul; Tokat early–mid Jul.

Table S4. Retention indices (RI) of major essential oil compounds identified by GC-MS on BPX5 column.

Compound	RI calc.	RI lit.	ID method	Chemotype(s)
α-Pinene	932	932	RI, MS	Minor (all)
β-Pinene	974	974	RI, MS	Minor (all)
Myrcene	988	988	RI, MS	Minor (all)
Limonene	1024	1024	RI, MS	Minor (all)
Eucalyptol (1,8-cineole)	1026	1026	RI, MS	Secondary (R-15)
(Z)-β-Ocimene	1032	1032	RI, MS	Minor (R-15)
γ-Terpinene	1054	1054	RI, MS	Minor (all)
Linalool	1095	1095	Std, RI, MS	Dominant (9 gen.)
Camphor	1141	1141	RI, MS	Minor (R-3k)
α-Terpineol	1186	1186	Std, RI, MS	Minor (all)
Estragole (methyl chavicol)	1195	1195	Std, RI, MS	Dominant (R-10A)
Neral (citral b)	1235	1235	RI, MS	Co-dominant (R-17)
Geraniol	1249	1249	Std, RI, MS	Minor (R-17)
Geranial (citral a)	1264	1264	RI, MS	Co-dominant (R-17)
Eugenol	1356	1356	Std, RI, MS	Secondary (Y-7)
Methyl cinnamate	1378	1376	Std, RI, MS	Dominant (R-23)
Methyl eugenol	1403	1403	Std, RI, MS	Secondary (R-4)
α-Bergamotene	1411	1411	RI, MS	Secondary (R-19)
δ-Cadinene	1522	1522	RI, MS	Secondary (R-20)

RI calc. = retention index calculated on BPX5 column (30 m × 0.25 mm i.d. × 0.25 µm) relative to C8–C20 n-alkane series. RI lit. = literature values from Adams RP (2017). ID method: Std = co-injection with authentic reference standard (Sigma-Aldrich, ≥0.980 g g−1 purity); RI = retention index matching; MS = mass spectral matching (WILEY and NIST databases). Compounds present at <0.1% relative peak area not included; this threshold corresponds to approximately 3× the mean injection-to-injection variability (RSD <3%), ensuring only reliably quantifiable peaks are reported.