



Yield and Essential Oil Accumulation of *Stachys setifera* Linked to Aerenchyma Development

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Abstract

This study examined the effects of self- and cross-pollination on agro-morphological traits, aerenchyma formation, and essential oil composition in *Stachys setifera* C.A.Mey. Ten clonally propagated genotypes and their seed progenies were evaluated over two years under greenhouse and field conditions. Significant genetic variation was detected among genotypes for biomass, seed yield, and essential oil content. Self-pollination led to contrasting effects: it enhanced traits such as hundred-seed weight and essential oil content in some genotypes, while causing inbreeding depression in wet and dry weight or seed number in others. GC–MS analysis identified 15 essential oil compounds, with geraniol, linalool, hexadecenoic acid, tetradecanoic acid, and geranyl acetate as the dominant constituents. Inbreeding differentially influenced these compounds, indicating both enhancement and reduction across genotypes. Aerenchyma traits were significantly higher in parents than progenies, and correlation and multivariate analyses revealed strong associations between root and rhizome aerenchyma with yield components and essential oil content. Genotypes with higher oil content and yield such as Rahnan, Ji Shir, Rodehen, and Dastgerd were identified for high biomass and essential oil production. Given the crucial role of aerenchyma in vegetative storage and reproduction through facilitating gas exchange with the rhizosphere, an increase in its area and number appears to directly enhance the medicinal potential of *S. setifera*.

Keywords *Stachys setifera* · Self- fertilization · Cross- hybridization · Aerenchyma · Essential oil

Abbreviations

WW	wet weight
DW	dry weight
NS	number of seeds in main stem
WS	weight of 100 seeds
GF	growth form (status)
NMS	number of main stems
EP	essential oil content
AR	total area of aerenchyma in root surface
AS	total area of aerenchyma in stem surface
ARh	total area of aerenchyma in rhizome surface
NAR	number of aerenchyma in roots
NAS	number of aerenchyma in stem
NARh	number of aerenchyma in rhizome

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Introduction

Medicinal plants hold a unique position in agriculture and medicine, serving as valuable genetic resources. Among them, the mint family (*Lamiaceae*) is one of the most diverse and economically important plant families, well recognized for its richness in medicinal species and bioactive compounds (Ulewicz- Magulska and Wesolowski 2023; Mansinhos et al. 2024).

Stachys setifera C.A.Mey., a perennial herb or small shrub of the *Lamiaceae* (Mohammadi and Kharazian 2022), is native to the Eastern Mediterranean and Western Asia (Tomou et al. 2023; Pashova et al. 2024). Owing to its distinctive morphological features, diverse phytochemical composition, and pharmacological potential, this species has attracted growing scientific and conservation interest (Slimani et al. 2023). Its vulnerable conservation status further indicates the urgency of comprehensive biological investigations (Güner 2022; Xue et al. 2023). Beyond their medicinal relevance, *Stachys* species are also ecologically significant, contributing to soil health and ecosystem stability through interactions with soil microbiota that enhance nutrient cycling and structure (Tomou et al. 2020; Benedec et al. 2023). Phytochemically, *Stachys* species are enriched in essential oils, polyphenols, tannins, flavonoids, and phenolic acids with potent antibacterial, anti-inflammatory, and antioxidant properties (Slimani et al. 2023; Slobodianiuk et al. 2021). From a breeding perspective, reproductive strategies in *Lamiaceae* are shaped by self-incompatibility, male sterility, and protandry (Naghiloo et al. 2022). While controlled selfing is used to generate pure lines or eliminate undesirable alleles, cross-pollination fosters heterozygosity and heterosis, thereby supporting both conservation

and genetic improvement (Cropano et al. 2021; Revell et al. 2025).

Although *S. setifera* is considered drought-tolerant due to its adaptation to arid habitats, anatomical adaptations such as aerenchyma specialized gas-filled tissue reported in related *Stachys* species remain poorly characterized in this taxon (Klein et al. 2020; Haghpanah et al. 2024). Aerenchyma plays critical roles in oxygen diffusion, nutrient uptake, and tolerance to waterlogging (Sou et al. 2021; Yamauchi and Nakazono 2022). However, the anatomical diversity, functional relevance, and potential breeding implications of aerenchyma in *S. setifera* remain unexplored. Moreover, no study has systematically evaluated the reproductive outcomes of self- versus cross-pollination, the performance of early inbred generations, or their impact on essential oil composition.

To address these gaps, the present study provides the first integrated anatomical, reproductive, and phytochemical characterization of *S. setifera* and its progeny. Therefore, the present study was designed to achieve the following goals: (a) investigate genotypic diversity in anatomical structures (root, rhizome, stem) and quantify the number and area of aerenchyma and their relationship with yield and seed production; (b) evaluate vegetative and reproductive traits in the progeny of the first selfed generation (S_1) versus cross-pollinated progeny. (c) compare the essential oil composition of self vs. cross-pollinated progeny to assess the impact of inbreeding on plant traits and bioactive compounds.

Materials and Methods

Plant material and Field Growth Conditions

The genetic materials investigated in this research included 10 clones (also referred to as genotypes in this study) of *Stachys setifera* naturally collected from various regions of Iran, including Isfahan (Rahnan, Jishir, Tad, and Dastgerd), Tehran (Rodehen), Hamedan (Shahrab and Arabloo), Chaharmahal va Bakhtiari (Garm Dareh and Cham Khorram), and Mazandaran (Kelardasht) provinces. Their geographic distribution is shown in Fig. 1.

The experiment was conducted over two consecutive years at the Research Farm of Isfahan University of Technology (32°N, 51°E). The site is characterized by a cold and dry climate with an average annual precipitation of 123 mm, mean annual temperature ranging from 9.1 to 23.4 °C, soil bulk density of 1.34 g cm⁻³, and a pH of 7.5.

Initially, the collected genotypes were propagated in pots (20 × 30 cm) filled with a soil mixture consisting of equal proportions (v/v) of farm soil, sand, and leaf mold. The propagation was carried out in three replicates. During



Fig. 1 Geographic distribution of ten genotypes of *Stachys setifera* used in this study

the early growth stages, irrigation was applied frequently to prevent desiccation, and the frequency was later adjusted according to plant development. The pots were maintained outdoors on the research farm until early October, after which they were transferred to a greenhouse. In the following year (April), the genotypes were transplanted to the field under a randomized complete block design (RCBD) with three replications. Each plot measured 2.5 m² (1 × 2.5 m) and consisted of two rows with 50 cm spacing between rows and five plants per row.

Growth, Morphological and Anatomical Traits of Parents

Growth Form

Growth form was quantified as the ratio of plant height in the natural position to height when gently held upright, following Rowe and Speck (2005):

$$\text{Growth status} = \frac{(\text{normal state in plant height})}{(\text{standing state in plant height})} \quad (1)$$

Plant Biomass (Wet and Dry Weight)

For each genotype and replication, three plants were randomly sampled. Aerial parts were harvested at the crown, immediately weighed for fresh biomass (g), then shade-dried to constant weight and reweighed for dry biomass (g). Genotype means were calculated across the three replications.

Number of Seeds per Main Stem and 100-Seed Weight

At the bud stage, three branches per genotype each with one node bearing six flowers were bagged. After one month, bags were removed and self-pollinated seeds were counted. Open-pollinated seed set was assessed on three unbagged branches per genotype, and seed production per node was recorded. For 100-seed weight, 100 seeds per genotype were randomly counted and weighed.

Aerenchyma Number and Area (Stem, Root, and Rhizome)

For anatomical evaluations, from each genotype, samples of rhizome, stem, and root were collected in the field and fixed in water: ethanol: glycerin at 1:1:1 (v/v/v, i.e., 33.3% each) (Mano et al. 2006). Cross-sections were prepared and stained with carmine alum and methyl green. The number and cross-sectional area of the aerenchyma tissue in each

genotype and each part of the plant were measured using an OLYMPUS microscope and Dino software.

Essential oil Content

To extract the essential oil, leaves were collected at flowering stage, air-dried at room temperature, and ground. Essential oils were extracted from 20 to 80 g of the powdered leaf material by distillation with water for 4 h using a Clevenger-type apparatus. The distillates were collected from the Clevenger column with diethyl ether. The essential oil content (%) was calculated based on the dry weight of the sample following the method described by Arabsalehi et al. (2018):

$$\text{Essential oil content (\%)} = \frac{\text{The amount of essential oil}}{\text{Dry weight of the sample}} \times 100 \quad (2)$$

Assessment of Reproductive Strategies on Agromorphological and Anatomical Traits in the Offspring

Controlled pollinations both self and cross-fertilizations were conducted. For cross-fertilization, each clone was artificially pollinated with nine other genotypes. The crossing schemes are presented in Table S1.

Self-pollination to carry out self-pollination in each genotype, five flowers were bagged at the bud stage. After 4 weeks, the bags were removed, and seed yield was recorded. Genotypes that produced no seeds were classified as obligate outcrossers (self-incompatible), while those that produced seeds were considered facultative selfers.

Cross-pollination to check cross-pollination, 45 flowers in each genotype were emasculated and bagged after 2–4 days, when stigmas were fully receptive, five flowers were manually pollinated using pollen from one of nine other genotypes. Seed set was evaluated after one month. Pairs that produced seeds were deemed cross-compatible; those failing to do so were recorded as cross-incompatible.

Apomixis Assessment

Apomixis, the possibility of producing seeds without fertilization was also investigated. For this purpose, six flowers in each genotype were first emasculated and then bagged. After one month, the bags were removed, and the seed yield of the plants was determined. None of the genotypes produced apomictic seeds suggesting that this reproduction system is likely not prevalent in this plant species.

The Evaluation of Progeny

Seeds derived from self- and cross-pollination of 10 selected genotypes were germinated in trays (one seed per entry) in May. Seedlings at the 3–4 leaf stage were transplanted to the field in a randomized complete block design with three replications. Each plot contained 10 plants with 70 cm row spacing and 40 cm intra-row spacing. At maturity, agromorphological and anatomical traits were measured, including fresh and dry leaf weight, number of stems per plant, seed number per main stem, 100-seed weight, essential oil content, and aerenchyma number and area in root, stem, and rhizome tissues.

Essential Oil Composition

Essential oils from self- and cross-derived progenies were analyzed using gas chromatography–mass spectrometry (GC–MS). Analyses were performed on an Agilent 6890 GC system equipped with an HP-5 MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was 250 °C; column thermal planning from 50 to 290 °C with a temperature increase of 5 °C/min, FID detector with a temperature of 290 °C. Helium (99.999%) served as the carrier gas at a constant flow of 1 mL/min (Agilent Technologies, Avondale, PA, USA). One microliter of essential oil from pooled replicates of each genotype was injected, and compounds were identified based on retention time and mass spectra (Ragno et al. 2020).

Statistical Analysis

Data from each experiment were analyzed using a randomized complete block design (RCBD) with three replications. Analysis of variance (ANOVA) was performed in SAS software (ver. 9.4; SAS Institute Inc., Cary, NC, USA), and treatment means were compared using the least significant difference (LSD) test at ($P < 0.05$). To evaluate inbreeding depression, the performance of self-pollinated offspring was compared with that of cross-pollinated progeny for each trait. Inbreeding depression (%) was calculated as:

$$ID = (\text{Mean}_{\text{self}} - \text{Mean}_{\text{cross}}) / \text{Mean}_{\text{cross}} \times 100 \quad (3).$$

Differences between parents and offspring, as well as among genotypes, were further analyzed using GraphPad Prism (ver. 9.3.1). Multivariate relationships among traits and genotypes were explored through principal component analysis (PCA) with biplot and heat maps prepared in Stat Graphics XVIII (<http://www.statgraphics.com>), while JMP (ver. 16) was used for hierarchical clustering of genotypes and traits.

Results

Genetic Diversity

Analysis of variance (Table 1) revealed pronounced genetic variation among genotypes and their cross-pollinated progenies for all measured traits ($p < 0.01$). Parental effects were also highly significant across nearly all traits, with the exception of one-hundred-seed weight (WS), which showed no detectable difference among parents.

In contrast, populations derived from self-pollination exhibited significant differences only for growth form (GF), number of main stems (NMS), and essential oil percentage (EP), suggesting a narrower phenotypic range in selfed lines. When cross- and self-pollinated progenies were compared, significant differences emerged for wet weight (WW), dry weight (DW), weight of 100 seeds (WS), number of seeds (NS), growth form (GF), number of main stems (NMS), and essential oil percentage (EP). The contrast between parental genotypes and their offspring was also statistically significant for all measured traits (Table 1), indicating substantial segregation and recombination effects in the progeny populations. Percentage changes associated with inbreeding depression or improvement were assessed through ANOVA followed by LSD mean separation tests; statistically significant differences are presented in Tables 2 and 4 (LSD, $p < 0.05$).

Plant Wet Weight

Wet weight was evaluated in parents as well as in self- and cross-pollinated offspring. Analysis of variance (Table 1) indicated that genotypes, self-pollinated populations, and cross-pollinated populations all exhibited significant variation for this trait.

Wet Weight in Parents (Clones)

Mean comparisons showed a wide range of parental wet weight: Rahnan displayed the highest wet biomass (1241.72 g.m⁻²) whereas Arabloo was the lowest (273.30 g.m⁻²). These parental contrasts were supported by LSD comparisons ($p < 0.05$; Table 2).

Wet Weight in Offspring

Among cross-pollinated offspring, Ji Shir recorded the highest wet weight (378.83 g.m⁻²) and Arabloo the lowest (129.58 g.m⁻²). In self-pollinated progenies, Rodehen and Shahrab were the extremes (360.75 and 187.14 g.m⁻², respectively) (Table 2). The effect of selfing varied by genotype: some genotypes gained biomass while others

Table 1 Analysis of variance (ANOVA) for growth, agronomical, biochemical, and anatomical traits in *Stachys setifera*

S. O. V	df	Mean squares												
		WW	DW	NS	WS	GF	NMS	EP	AR	AS	ARh	NAR	NAS	NARh
Replication	2	92.31 ^{ns}	4.17 ^{ns}	5509.2 ^{ns}	0.1 ^{ns}	0.002 ^{ns}	5.40 ^{ns}	0.0003 ^{ns}	2.84 × 10 ⁷ ^{ns}	7.3 × 10 ⁸ ^{ns}	4.45 × 10 ¹⁰ ^{ns}	162.300 ^{ns}	0.433 ^{ns}	1159.28 ^{ns}
Genotypes	25	1665.3 ^{**}	119.47 ^{**}	66,679 ^{**}	0.001 ^{**}	0.049 ^{**}	15.57 ^{**}	0.00047 ^{**}	1.55 × 10 ⁹ ^{**#19}	5.47 × 10 ¹⁰ ^{**#19}	5.6 × 10 ¹³ ^{**#19}	1332.8 ^{**#19}	408.7 ^{**#19}	9475.1 ^{**#19}
Parents	9	0.01 ^{**}	0.005 ^{**}	542.311 ^{**}	0.00001 ^{ns}	0.022 ^{**}	26.31 ^{**}	0.0031 ^{**}	94.4 × 10 ⁶ ^{**}	6.2 × 10 ⁴ ^{**}	38.4 × 10 ⁶ ^{**}	734.22 ^{**}	1211.21 ^{**}	3254.33 ^{**}
Cross-pollination	9	1308.8 ^{**}	1463.5 ^{**}	20,785 ^{**}	0.0007 ^{**}	0.019 ^{**}	20.2 ^{**}	0.0004 ^{**}	22.6 × 10 ⁷ ^{**}	30 × 10 ⁵ ^{**}	35 × 10 ⁶ ^{**}	452.12 ^{**}	980.01 ^{**}	1267.98 ^{**}
Self-pollination	5	402.2 ^{ns}	79.2 ^{ns}	278.4 ^{ns}	0.00001 ^{ns}	0.08 ^{**}	9.32 ^{**}	0.0004 ^{**}	-	-	-	-	-	-
Cross-P vs. Self-P	1	587.99 ^{**}	752.14 ^{**}	2075.8 ^{**}	0.008 ^{**}	0.01 ^{**}	3.32 ^{**}	0.0019 ^{**}	-	-	-	-	-	-
Parent vs. Offspring	1	1067.35 ^{**}	2765.4 ^{**}	2340.1 ^{**}	0.002 ^{**}	0.003 ^{**}	7.45 ^{**}	0.0028 ^{**}	32.4 × 10 ⁴ ^{**}	7.21 × 10 ⁶ ^{**}	22.43 × 10 ⁵ ^{**}	372.11 ^{**}	492 ^{**}	861.17 ^{**}
Error	50	114.5	2.98	5542.1	0.00002	0.001	1.77	0.000002	9.99 × 10 ² ^{#44}	2.95 × 10 ³ ^{#44}	9.18 × 10 ⁴ ^{#44}	60.89 ^{#44}	287.37 ^{#44}	79.21 ^{#44}
CV (%)		7.91	4.96	5.65	9.64	19.24	7.22	3.21	4.25	4.98	9.74	2.12	6.19	3.87

^{ns} Non-significant. * Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. #; degree of freedom of Aerenchyma traits. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth status, NMS: number of main stems, EP: essential oil content, AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome

declined. Garm Dareh exhibited the largest inbreeding-associated increase (+62.24%) and Shahrab the largest decrease (−33.12%); these changes were validated by LSD comparisons ($p < 0.05$; Table 2).

Plant Dry Weight

The analysis of variance (Table 1) showed highly significant differences in dry matter weight among the genotypes, including parents, self-pollinated, and cross-pollinated offspring.

Plant Dry Weight in Parents (Clones)

Based on mean comparisons, the highest and lowest dry weights among parents were recorded in Rahnan (441.12 g m^{−2}) and Arabloo (136.33 g m^{−2}), respectively, with differences being statistically significant (LSD, $p < 0.05$; Table 2).

Plant Dry Weight in Offspring

In cross-pollinated progeny Ji Shir and Arabloo represented the upper and lower extremes (130.31 and 53.75 g m^{−2}, respectively). In self-pollinated progeny, Rodehen and Shahrab showed the highest and lowest values (131.50 and 79.25 g m^{−2}, respectively). These differences were significant according to LSD ($P < 0.05$). Among self-pollinated progeny, Garm Dareh exhibited the largest improvement in dry weight (+79.91%), whereas Shahrab showed the greatest depression (−20.62%) (Table 2). Improvements in wet and dry weights resulting from cross-fertilization (relative to selfing) were also observed in Dastgerd, Tad, Rodehen and Garm Dareh.

Number of Seeds in the Main Stem

The results of analysis of variance showed a significant difference between the genotypes in terms of the number of seeds in the main stem (Table 1).

Number of seeds in the main stem in parents (clones)

Parental seed number on the main stem varied from 888.5 (Rahnan) to 523.4 (Cham Khorram); with differences being statistically significant (LSD, $p < 0.05$; Table 2).

Number of seeds in the main stem in offspring

In cross-pollinated progeny, the highest and lowest values were observed in Shahrab (695.55) and Kelardasht (438.6), respectively. In self-pollinated progeny, Rahnan (382.81)

Table 2 Comparison of the mean and changes due to inbreeding (ID) for morphological and anatomical traits in parents, cross-pollinated and self-pollinated populations in *Stachys setifera*

Genotypes	wet weight (g.m ⁻²)			Dry weight (g.m ⁻²)			number of seeds in the main stem (pp)						100-seed weight (g)			
	Parent	Cross	Self	% ID	Parent	Cross	Self	% ID	Parent	Cross	Self	% ID	Parent	Cross	Self	% ID
Shahrab	562.31	279.83	187.4 ^{min}	-33.12 ^{min}	252.11	99.83	79.25 ^{min}	20.06 ^{min}	730.22	695.5 ^{max}	272.4 ^{min}	60.8 ^{min}	0.008	0.134	0.162	20.89
Dastgerd	898.73	195.72	230.75	17.9	351.00	79.08	81.75	3.37	645.65	59.01	290	-50.86	0.01	0.128	0.136	5.98
Tad	778.75	216.08	270.67	25.26	264.33	78.42	95.33	21.57	761.98	661	373.11	55.43	0.008	0.124	0.131 ^{min}	4.81 ^{min}
Rahnan	1241.72 ^{max}	251.00	222.53	11.35	441.12 ^{max}	83.03	80.69	2.88	888.5 ^{max}	595	328.8 ^{max}	35.66	0.018 ^{max}	max0.138	0.157	13.23
Rodehen	707.00	253.33	360.7 ^{max}	42.54	271.09	105.33	131.57 ^{max}	24.84	743.91	619.56	311.6	49.70	0.01	0.120	0.158 ^{max}	31.3 ^{max}
Garm	565.70	207.72	337.04	62.24 ^{max}	211.33	73.12	131.33	79.91 ^{max}	538.09	497.33	353.87	-28.8 ^{min}	0.009	0.121	0.150	23.96
Dareh																
Arabloo	273.30 ^{min}	129.58 ^{min}	-	-	136.23 ^{min}	53.75 ^{min}	-	-	732.73	595.4	-	-	0.007 ^{min}	0.083 ^{min}	-	-
Ji Shir	567.01	0.83 ^{max}	378	-	210.11	130.31 ^{max}	-	-	661.32	609.22	-	-	0.01	0.128	-	-
Kelardasht	431.71	191.92	-	-	421.34	62.25	-	-	545.55	438.6 ^{min}	-	-	0.01	0.106	-	-
Cham	769.72	254.31	-	-	262.33	88.64	-	-	523.4 ^{min}	500.55	-	-	0.01	0.129	-	-
Khorram																
Total mean	679.56	235.83	268.13	17.22	256.76	85.34	99.98	17.69	47.14b	594.23	330.63	-44.91	0.0101	0.12	0.15	16.69
LSD (%)	28.48				11.84				24.14				0.008			

Cross: cross-pollinated offspring, Self: self-pollinated offspring, ID: inbreeding regression, pp: per plant. ^{max}: The maximum mean value of the trait. ^{min}: The minimum mean value of the trait. The data values represent mean $\pm$ SE

and Shahrab (274.4) represented the upper and lower extremes. Self-pollination resulted in reduced seed numbers across all populations. The largest inbreeding depression was recorded in Shahrab (-60.8%), and the smallest in Garm Dareh (-28.87%), with these differences being statistically significant (LSD, $p < 0.05$).

100- Seed Weight

Genotypes exhibited significant variation for 100-seed weight at the 1% probability level. Significant differences were also detected among families derived from both cross- and self-pollination (Table 1).

100-Seed Weight in Parents (Clones)

Rahnan had the highest 100-seed weight (0.018 g) and Arabloo the lowest (0.07 g); mean comparisons confirmed these differences (LSD, $p < 0.05$; Table 2).

100-Seed Weight in Offspring

Among cross-pollinating progeny, Rahnan again had the greatest 100-seed weight (0.138 g) and Arabloo the lowest (0.083 g); among self-pollinated progeny, Rodehen was highest (0.158 g) and Tad lowest (0.131 g) (Table 2). Self-pollination generally increased 100-seed weight. Rodehen exhibited the largest gain (+31.3%) while Tad showed the smallest (+4.81%); these differences were significant (LSD, $p < 0.05$).

Growth form

A significant difference was observed among the genotypes for the trait of growth form. In addition, the results of analysis of variance showed that the effect of self-pollination and cross-pollination was significant for this trait, and the effect of self-pollination versus cross-pollination was also significant (Table 1).

Growth form in Parents (Clones)

A significant difference was observed among genotypes for growth status (Table 2), so that the highest and lowest amount of this trait in parents was in Shahrab (0.967) and Ji Shir (0.907), respectively. These differences were statistically supported by LSD comparisons ($p < 0.05$; Table 2).

Growth form in Offspring

Substantial variation in growth status was observed among cross- and self-pollinated progeny. In cross-pollinated

Table 3 Comparison of the mean and changes due to inbreeding (ID) for morphological and anatomical traits in parents, open-pollinated and self-pollinated populations in *Stachys setifera*

Genotypes	Growth status			No. main stem (per plant)			Essential oil content (%)		
	Parent	Cross	Self	% ID	Parent	Cross	Self	Parent	% ID
Shahrab	0.967 ^{max}	0.429 ^{max}	0.402	-4.36	14.6d	14.42	10.58 ^{min}	0.017	200 ^{max}
Dastgerd	0.933	0.343	0.630	83.85 ^{max}	22.3	10.44 ^{min}	13	0.034 ^{max}	25 ^{min}
Tad	0.926	0.185 ^{min}	0.313	69	13.6 ^{min}	12.92	11.67	0.027	100
Rahnan	0.956	0.369	0.385	4.52	23.3	13.75	12.14	0.033	100
Rodehen	0.928	0.429 ^{max}	0.662 ^{max}	4.95	18.3	11.67	15.75 ^{max}	0.020	33.33
Garm Dareh	0.945	0.252	0.201 ^{min}	-20.37 ^{min}	21.3	10.56	12	0.025	50
Arabloo	0.935	0.258	-	-	19.6	8.08	-	0.013	-
Ji Shir	0.907 ^{min}	0.303	-	-	26.3 ^{max}	15.08 ^{max}	-	0.011 ^{min}	-
Kelardasht	0.928	0.382	-	-	26.3 ^{max}	13.37	-	0.033	-
Cham Khorram	0.959	0.258	-	-	16.6	12.42	-	0.022	-
Total mean	0.938	0.32	0.43	22.93	20.22	11.67	12.52	0.02	84.72
LSD (%)	0.06				2.22			0.008	

families, Rodehen (0.429) and Shahrab (0.420) had the highest values, whereas Tad showed the lowest (0.185). In self-pollinated progeny, Rodehen exhibited the greatest growth status (0.662), while Garm Dareh had the lowest (0.201). These contrasts were significant (LSD, $p < 0.05$; Table 2). Because all ratios were below 1, genotypes generally tended toward a prostrate growth form. The impact of inbreeding on growth status varied across genotypes. In Dastgerd (+83.85%), Tad, Rodehen, and Rahnan, self-pollinated progeny outperformed cross-pollinated ones, indicating that stronger plants were produced through selfing. In contrast, in Shahrab and Garm Dareh, cross-pollinated progeny had higher growth status values than selfed progeny, suggesting that inbreeding produced weaker plants (Table 2).

Number of Main Stems (NMS)

Analysis of variance revealed significant differences among genotypes for the number of main stems. The effects of self- and cross-pollination, as well as progeny vs. parents and selfing vs. crossing contrasts, were also highly significant (Table 1).

Number of Main Stems in Parents (Clones)

Mean comparisons indicated that Kelardasht and Ji Shir had the highest NMS (26.3), whereas Tad had the lowest (13.6). These differences were statistically significant (LSD, $p < 0.05$; Table 2).

Number of Main Stems in Offspring

In cross-pollinated progeny, Ji Shir exhibited the highest NMS (15.08) and Dastgerd the lowest (10.44). In self-pollinated progeny, Rodehen recorded the maximum NMS (15.75), while Shahrab showed the minimum (10.58) (Table 2). Selfing reduced NMS in Shahrab, Tad, and Rahnan, but increased it in Dastgerd, Rodehen, and Garm Dareh. Rodehen displayed the greatest inbreeding-related improvement (+35.12%), whereas Shahrab experienced the strongest depression (-26.59%). These contrasts were statistically supported (LSD, $p < 0.05$; Table 2). Interestingly, increased NMS in some selfed lines coincided with higher biomass and yield, highlighting its potential as a selection criterion for superior genotypes.

Essential Oil Percentage

Analysis of variance showed a significant difference among different genotypes in terms of essential oil content (EP). The effects of self- and cross-pollination, as well as selfing vs. crossing and progeny vs. parents were all significant,

with the latter contrast highly significant at the 1% level (Table 1).

Essential Oil Percentage in Parents (Clones)

Comparison of means showed that the highest content of essential oil (EO) in parent families belonged to the Dastgerd genotype (0.034%), and the lowest amount in parents belonged to Ji Shir (0.011%). These differences were statistically significant (LSD, $p < 0.05$; Table 2).

Essential Oil Content in Offspring

Among progeny, Dastgerd again showed the highest EP (CP: 0.043%, SP: 0.050%), while the lowest values were observed in Ji Shir under cross-pollination (0.010%) and Rahnan under self-pollination (0.020%). These differences were significant according to the LSD test ($p < 0.05$; Table 2). Self-pollination generally increased EP content across all genotypes. The greatest inbreeding-related improvement was observed in Shahrab (+200%), whereas Dastgerd showed the smallest increase (+25%) (Table 2).

Aerenchyma Formation in Root, Stem and Rhizome

Aerenchyma development was investigated in parents and their cross-pollinated progeny. Analysis of variance showed that the effects of genotype and cross-pollination on aerenchyma-related traits were highly significant ($p < 0.01$). The contrast between parents and progeny was also significant at the 1% level across all plant organs (Table 1). Mean comparisons confirmed substantial differences in the number and area of aerenchyma between parents and cross-pollinated offspring (Fig. 2).

Roots Aerenchyma in Parent and Offspring

Parents and cross-pollinated progeny differed significantly in both number and area of root aerenchyma (Table 1). Among parents, Garm Dareh had the largest aerenchyma area (114847 μm^2), while Tad had the smallest (44682 μm^2). In progeny, Ji Shir showed the maximum area (5127.2 μm^2), and Tad again the minimum (595.3 μm^2) (Table 3). Ji Shir and Rodehen recorded the highest number of root aerenchyma in parents (88.3) and progeny (73.59), respectively, whereas Garm Dareh had the lowest in both groups (29.2 and 12.27). Overall, parents outperformed progeny, with mean root aerenchyma values of 60.93 in number and 74,552 μm^2 in area (Fig. 2). Representative cross-sections of root aerenchyma of *Stachys setifera* are shown in Fig. 3.

Stem Aerenchyma in Parent and Offspring

Significant genotypic variation was also detected for stem aerenchyma traits (Table 1). Rahnan exhibited the largest aerenchyma area in both parents (35273 μm^2) and progeny (16004 μm^2), while the smallest values were found in Arabloo (161 μm^2 , parents) and Garm Dareh (844 μm^2 , progeny) (Table 3). Shahrab showed the highest number of stem aerenchyma (20.6 in parents, 17.12 in progeny), whereas Ji Shir, Cham Khorram, and Arabloo consistently had the lowest (1). Mean comparisons revealed that parents surpassed progeny, with average values of 10.21 (number) and 126,898 μm^2 (area) (Fig. 2). Stem cross-sections of *Stachys aerenchyma* are illustrated in Fig. 5.

Rhizome Aerenchyma in Parent and Offspring

The highest number and area of aerenchyma in *Stachys* plants was observed in the rhizome. Significant differences were observed among families for both traits (Table 1). Rahnan recorded the largest rhizome aerenchyma area in parents ($2.3 \times 10^7 \mu\text{m}^2$) and progeny (10,858.9 μm^2), while Arabloo (91,784 μm^2 , parents) and Rodehen (269.5 μm^2 , progeny) showed the lowest values (Table 3). Rahnan also had the highest number of rhizome aerenchyma (731.5 in parents, 247.5 in progeny), compared to Arabloo, which had the lowest (87 and 35, respectively). On average, parents exceeded progeny with 347.73 in number and $3.06 \times 10^6 \mu\text{m}^2$ in area (Fig. 2). A representative cross-section of rhizome aerenchyma is presented in Fig. 6.

Chemical Composition of Essential Oils

GC and GC-MS analyses of essential oils from self- and cross-derived populations identified 15 principal compounds, which together accounted for 51.44–79.93% of the total oil (Tables 4 and 5). The major constituents were geraniol, hexadecenoic acid, linalool, tetradecanoic acid, and geranyl acetate. Among these, geraniol was the predominant metabolite, followed by hexadecenoic acid and linalool. In cross-pollinated progenies, the highest geraniol content was recorded in Kelardasht (37.20%) and the lowest in Arabloo (1.36%). In selfed progenies, Garm Dareh and Dastgerd showed the highest and lowest values (19.05% and 7.65%, respectively) (Table 4). Inbreeding modified geraniol content differentially across genotypes: Dastgerd experienced the greatest reduction (−60.01%), while Rodehen exhibited the largest increase (+45.35%), with differences supported statistically (LSD, $p < 0.05$).

Hexadecenoic acid represented the second most abundant compound. In cross-progenies, Arabloo had the maximum (18.09%) and Dastgerd the minimum (1.35%); while

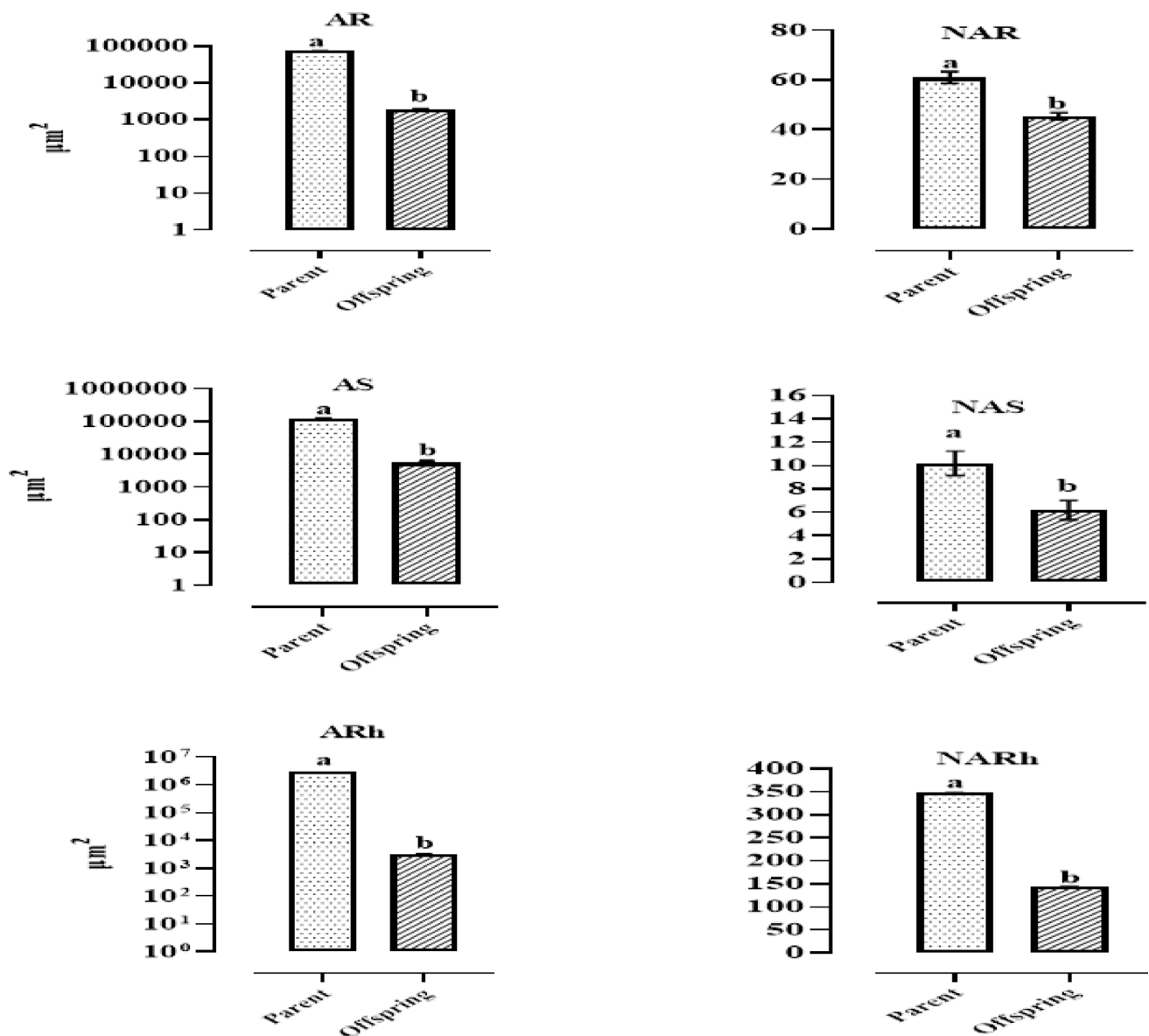


Fig. 2 Mean comparisons of the number and area of aerenchyma in parents and cross-pollinated offspring of *Stachys setifera*. Diagrams of AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface,

NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome. A same letter above the columns for each trait (diagram) indicates that the values are not statistically different ($p > 0.05$). Values are mean $\pm$ standard error

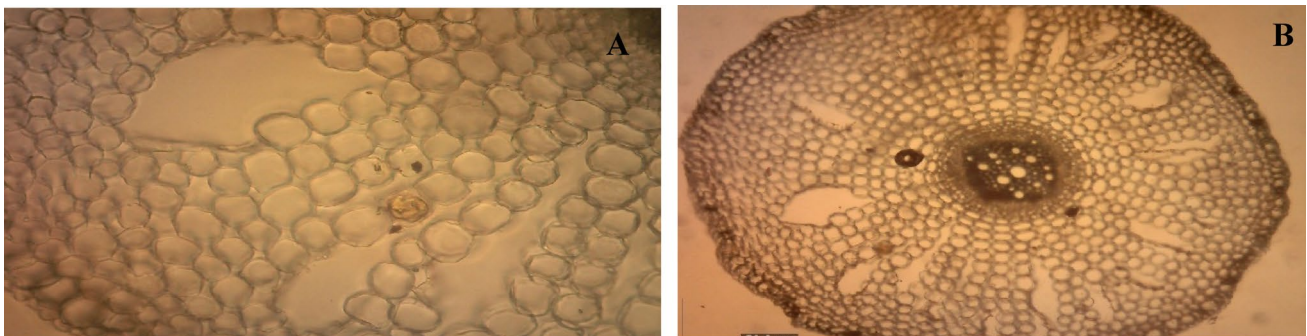


Fig. 3 A cross-section view of the aerenchyma tissue in the root under a light microscope with 40X (A) and 10X (B) magnification - Rahnan genotype

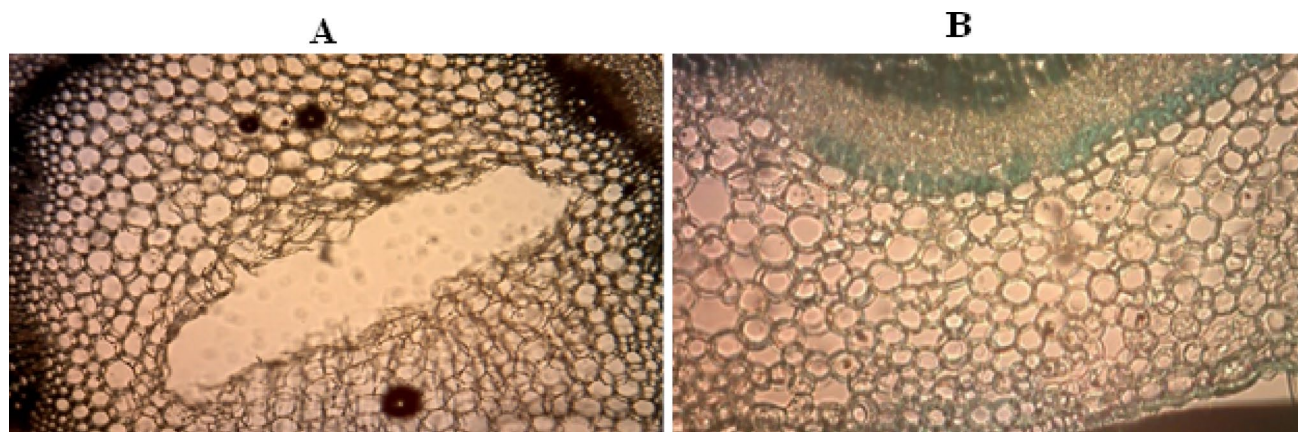


Fig. 4 A cross-section view of the aerenchyma tissue in the stem under a light microscope with 40X (A) and 10X (B) magnification - Rahnan genotype

in selfed progenies, Tad and Dastgerd represented the extremes (11.79% and 2.51%, respectively). Selfing consistently enhanced hexadecenoic acid levels across genotypes, with Dastgerd showing the highest gain (+85.92%) and Rodehen the lowest (+2.19%). Linalool ranked third, ranging from 1.90% in Arabloo to 17.77% in Dastgerd among cross-pollinated lines, and from 2.31% in Tad to 15.79% in Dastgerd among selfed lines. The effect of inbreeding on linalool was strongly genotype-dependent: Tad showed the largest reduction (−77.52%), while Rodehen displayed the greatest increase (+95.06%). Tetradecanoic acid was identified as the fourth major compound, with Cham Khorram (11.65%) and Dastgerd (1.80%) showing the highest and lowest levels in cross progenies, respectively. In selfed lines, Rodehen (8.65%) and Dastgerd (2.93%) represented the extremes. Inbreeding differentially affected this compound: levels increased in Dastgerd, Tad, and Rodehen but decreased in Shahrab, Rahnan, and Garm Dareh. Geranyl acetate was the fifth major constituent. Its levels in cross-progenies ranged from 1.09% (Arabloo) to 7.77% (Kelardasht), while in selfed lines, the highest (8.61%) and lowest (3.79%) levels were observed in Dastgerd and Tad, respectively. Inbreeding had contrasting effects, with Tad exhibiting the greatest decline (−31.34%) and Dastgerd the highest increase (+22.82%) (Table 4). Other minor compounds were also identified in both self- and cross-pollinated populations (Table 5). Overall, the inbreeding effect on essential oil composition was strongly genotype-dependent, leading to both positive and negative shifts across compounds and populations.

Relationship Among Genotypes and Agromorphological Traits

The results of principal component analysis (PCA) for parental genotypes and cross-pollinated offspring are presented in

Figs. 5 and 6. In parents, the first two principal components explained 71.62% of the total variance. PC1 (48.45%), showed positive and significant correlations with WW, WS, DW, GF, NS, AS, ARh, and NARh, while PC2 (23.17%) was positively associated with NAS, EP, NMS, and NAR, and negatively correlated with AR (Fig. 5). Most traits in PC1 were positively correlated with those in PC2. Based on the biplot, families located in the first and fourth quadrants had the highest values for PC1 traits. Rahnan was identified as the superior parent, with the highest rhizome aerenchyma traits and plant and seed yield. In addition, Rodehen, Dastgerd, Kelardasht, and Shahrab grouped together due to their high essential oil content. Interestingly, the number of root aerenchyma showed an inverse relationship with its area and was not strongly related to other traits. The largest root aerenchyma area was observed in Garm Dareh and Arabloo genotypes (Fig. 5).

In cross-pollinated progenies, the first two PCs explained more than 70% of the variance, with PC1 and PC2 accounting for 39.72% and 21.36%, respectively (Fig. 6). PC1 was positively correlated with most traits (WW, WS, DW, GF, NS, AR, EP, NMS, NAR), with no negative associations. PC2 showed positive correlations with ARh, NARh, and AS, and a negative correlation with NAS. Accordingly, PC1 can be described as a “yield index” and PC2 as an “aerenchyma index.” Genotypes such as Rodehen, Ji Shir, and Shahrab exhibited the highest PC1 values, indicating superior yield performance, while Rahnan showed the highest rhizome aerenchyma (Fig. 6).

When PCA was performed using essential oil content, its constituents, and aerenchyma traits, the first two PCs explained over 85% of total variance, with PC1 and PC2 accounting for 59.33% and 26.74%, respectively (Fig. 7). PC1 was negatively correlated with NARh, ARh, NAS, AS, and key oil components including geraniol, linalool, geranyl acetate, nonanoic acid, α -terpineol, neryl acetate, and

Table 4 Comparison of the mean for Aerenchyma traits in parent and cross-pollinated populations in *Stachys setifera*

Genotypes	AR(μm^2)			AS(μm^2)			ARh(μm^2)			NAR			NAS			NARh		
	Parent	Cross	Parent	Parent	Cross	Parent	Parent	Cross	Parent	Parent	Cross	Parent	Parent	Cross	Parent	Parent	Cross	Parent
Shahrab	65,512	3226.2	145,610	1.51 $\times 10^6$	2177	4700.47	71.6	69.45	30.6 ^{max}	17.12 ^{max}	234.67	121.22	121.22	121.22	121.22	121.22	121.22	121.22
Dastgerd	45,673	1715.63	150,724	953,255	5851	718.37	64.6	47.75	29	14	447.67	121.22	121.22	121.22	121.22	121.22	121.22	121.22
Tad	44,682 ^{min}	595.3 ^{min}	345,150	9.03 $\times 10^6$	4590	2629.27	54	38.89	3	2	467	213.11	213.11	213.11	213.11	213.11	213.11	213.11
Rahnan	76,982	1480.63	352,973 ^{max}	2.34 $\times 10^7$ ^{max}	16,004 ^{max}	10858.9 ^{max}	80.2	47.43	3.6	2.76	731.5 ^{max}	247.56 ^{max}	247.56 ^{max}	247.56 ^{max}	247.56 ^{max}	247.56 ^{max}	247.56 ^{max}	247.56 ^{max}
Rodehen	61,606	4631.93	33,397	1.43 $\times 10^6$	6202	269.5 ^{min}	88.2	73.59 ^{max}	12.3	7.34	593.5	179.23	179.23	179.23	179.23	179.23	179.23	179.23
Garm Dareh	114,847 ^{max}	4228	5832	1.36 $\times 10^7$	844 ^{min}	2306.37	29.2 ^{min}	12.27 ^{min}	13.6	5.78	692	254.44	254.44	254.44	254.44	254.44	254.44	254.44
Arabloo	100,652	678.7	161 ^{min}	91,784 ^{min}	3519.04	1897.57	65.2	44.56	1 ^{min}	7	87 ^{min}	35 ^{min}	35 ^{min}	35 ^{min}	35 ^{min}	35 ^{min}	35 ^{min}	35 ^{min}
Ji Shir	91,924	5127.2 ^{max}	40,483	209,939	1055	519.03	88.3 ^{max}	73.54	1 ^{min}	1 ^{min}	145	87	87	87	87	87	87	87
Kelardasht	72,117	397.87	2132	1.3 $\times 10^6$	3927	7947.07	37	22	7	4	236.5	123.45	123.45	123.45	123.45	123.45	123.45	123.45
Cham Khorram	71,525	1361.52	71,456	531,662	10,328	770.6	31	24.76	1 ^{min}	1 ^{min}	242.5	79.98	79.98	79.98	79.98	79.98	79.98	79.98
Total mean	74251.9	1944.29	114579.5	3.1 $\times 10^6$	5449.70	3261.715	60.93	45.421	10.21	6.2	347.73	144.22	144.22	144.22	144.22	144.22	144.22	144.22
LSD	521.5		2945	5811			7.38		3.5		102.8							

AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome. Cross: cross-pollinated offspring, ^{max}: The maximum mean value of the trait, ^{min}: The minimum mean value of the trait. The data values represent mean $\pm$ SE

E- β -ionone, while positively correlated with hexadecanol, caryophyllene oxide, spathulenol, hexadecanoic acid, and n-heneicosane. This component was therefore regarded as the “oil quality index.” In contrast, PC2 correlated positively with AR, NAR, EP, tetradecanoic acid, and dodecanoic acid, reflecting an “oil quantity and root aerenchyma index.” The analysis highlighted a positive relationship between oil content and root aerenchyma, whereas oil quality (especially geraniol and linalool) was strongly linked to stem and rhizome aerenchyma. Among cross-pollinated progenies, Dastgerd and Kelardasht were superior for oil quality, while Ji Shir, Rodehen, and Rahnan were superior for oil quantity (Fig. 7).

For grouping the genotypes based on the studied traits, heat mapping was used and graphically showed the status of each genotype concerning all the attributes. In these graphs, the dark blue color shows the lowest value of a trait, and the dark red indicates the highest (Figs. 8 and 9). In this regard, parental genotypes were divided into two groups. Rahnan genotype was placed in a separate group compared to other genotypes and had the highest value of all traits except the number of stem aerenchyma, which was consistent with the results of biplot. Also, the Garm Dareh genotype had the highest number and area of rhizome aerenchyma (Fig. 8). Similar to parental genotypes, the results of heat mapping for cross-pollination derived offspring divided the studied entries into two groups (Fig. 9). Rahnan, Shahrab, and Ji Shir genotypes were placed in a separate cluster compared to other genotypes, which had the highest values of yield traits and yield components, and had the lowest values of ARh, NARh, AS and NAS traits, and these results were consistent with the results of the biplot analysis (Figs. 5 and 6). Also, Rahnan and Tad genotypes had the highest number and area of aerenchyma.

Discussion

Genetic Variability and Ecological Responses of *Stachys setifera* Populations

The present study explored the genetic diversity among wild accessions and their progeny in *Stachys setifera* populations, providing detailed insights into various agro-morphological traits and their responses to distinct reproductive strategies. Analysis of variance (ANOVA) revealed significant effects of genotype and pollination method on all measured traits, emphasizing the considerable genetic variability within the studied populations. These findings align with previous studies highlighting the crucial role of genetic diversity in plant populations for adaptation and survival under fluctuating environmental conditions (Salgotra and Chauhan 2023;

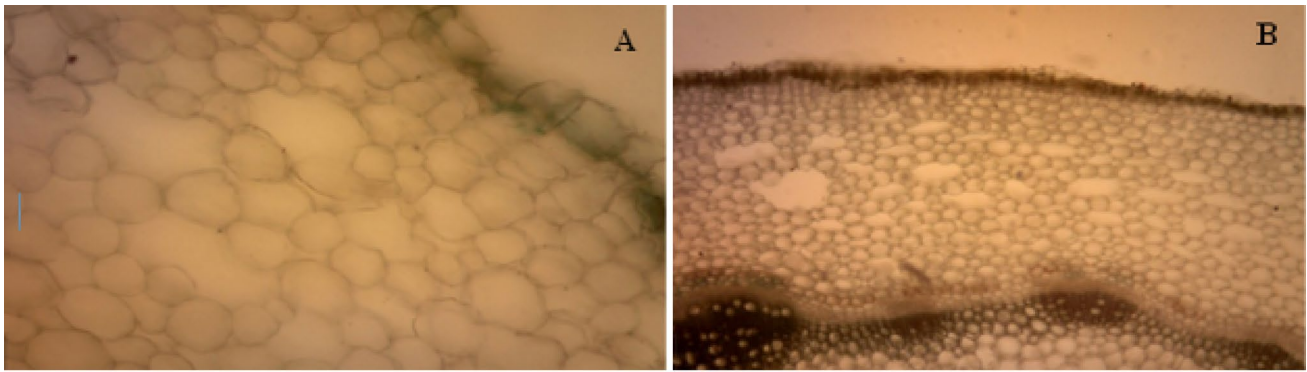


Fig. 5 Principal component analysis for the relationship among the studied traits and the distribution of parental families of *Stachys setifera* relative to these traits. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth form, NMS: number of main stems, EP: essential oil percentage, AR:

total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome

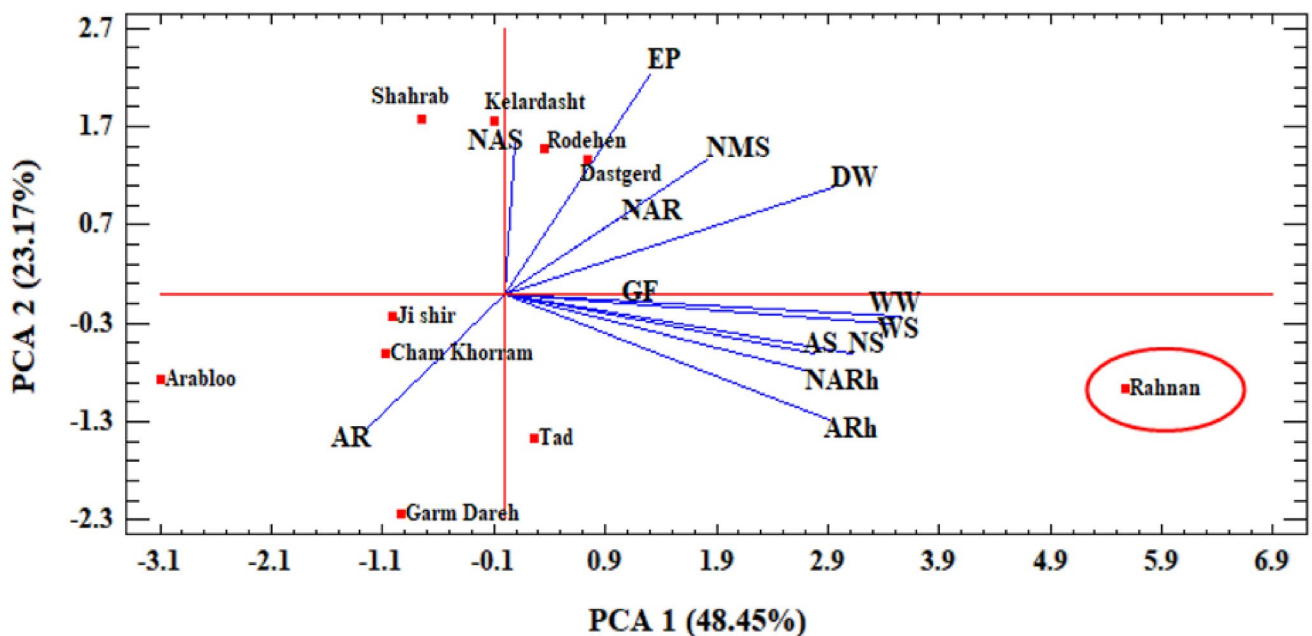


Fig. 6 Principal component analysis for the relationship among the studied traits and the distribution of parental families of *Stachys setifera* relative to these traits. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth form, NMS: number of main stems, EP: essential oil percentage, AR:

total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome

Chung et al. 2023). The observed significant parental effects on most traits indicate the contribution of parental genotypes in shaping progeny characteristics, reflecting both additive and non-additive genetic inheritance (Beavis et al. 2021). Interestingly, the weight of 100 seeds (WS) deviated from this pattern, suggests a more complex genetic architecture likely influenced by multigenic inheritance, trait correlations, and/or environmental interactions (Yuan et al. 2024).

Self-pollination induced pronounced changes in traits such as growth form (GF), number of main stems (NMS), and essential oil content (EP), highlighting the plasticity

of *S. setifera* in response to inbreeding. Similar differential effects of self-fertilization on biomass traits (DW and FW) have been reported in thyme, where some populations exhibited improved growth while others showed depression under selfing (Khanahmadi et al. 2021). Comparisons between cross- and self-pollinated progeny further illuminate the interplay between genetic diversity and reproductive strategy in shaping plant growth and biomass allocation (Parra-Tabla and Alonso 2021). From an evolutionary perspective, these contrasting outcomes indicate that while self-pollination may provide short-term reproductive

Table 5 Changes due to inbreeding in the main essential oil compounds of cross- and self-pollinated genotypes of *Stachys setifera*

Genotypes	A-terpineol			Spathulenol			n-heptacosane			1-octen-3-ol			Nonanoic acid		
	Cross	Self	% ID	Cross	Self	% ID	Cross	Self	% ID	Cross	Self	% ID	Cross	Self	% ID
Shahrab	2.23	1.49	-33.18	4.30 ^{max}	3.68 ^{max}	-14.41	2.18	2.72	24.77	-	-	2.19	1.42	-35.15	
Dastgerd	5.57 ^{max}	7.35 ^{max}	31.95	0.97	0.95 ^{min}	-20.06	min0.48	3.09	0.15 ^{max} 713	max7	max3.23	3.3	0.21 ^{max} 6	88.18 ^{max}	
Tad	3.92	0.43 ^{min} 1	0.52 ^{min} 63-	2.09	2.94	0.66 ^{max} 40	3.99	3.21	-19.54	3.68	-	2.5	1.44 ^{min}	-42.4 ^{min}	
Rahnan	2.03	2.73	34.48	3.94	3.21	-18.52	1.2	2.82	135	2.4	0.88 ^{min} 1	1.71	2.02	18.12	
Rodehen	1.82	3.39	max86.26	3.63	2.25	38.01 ^{min} -	2.4	min0.84	min65-	-	2.14	1.93	2.83	46.63	
Garm Dareh	2.43	2.23	-8.23	2.85	1.99	-30.17	3.68	0.05 ^{max} 4	10.05	1.89	2.09	1.74	1.72	-1.14	
Arabloo	1.49 ^{min}	-	-	3.14	-	-	0.30 ^{max} 8	-	-	1.11 ^{min}	-	-	-	-	
Ji Shir	1.53	-	-	2.67	-	-	0.81	-	-	1.26	-	1.37 ^{min}	-	-	
Kelardasht	3.28	-	-	1.71 ^{min}	-	-	0.84	-	-	2.34	-	3.36 ^{max}	-	-	
Cham Khorram	2.37	-	-	3.71	-	-	1.53	-	-	1.78	-	1.65	-	-	
Total mean	2.66	3.1	7.96	2.9	2.5	-10.41	2.53	2.87	133.07	2.68	2.33	2.19	2.6	12.37	
LSD	0.983			1.032			0.876			0.234		0.094			
Genotypes	Greaney-l acetate														
Tetradecanoic acid															
Shahrab	14.36	18.27	27.22	8.39	9.01	7.38	4.7	3.51	-25.31	7.15	5.17	27.69 ^{min} -	4.97	4.53	8.85-
Dastgerd	19.13	7.65 ^{min}	0.01 ^{min} -60	1.35 ^{min}	2.51 ^{min}	85.92 ^{max}	17.77 ^{max}	15.79 ^{max}	-11.14	1.80 ^{min}	2.93 ^{min}	62.77	7.01	8.61 ^{max}	0.82 ^{max} 22
Tad	14.07	10.02	-28.78	6.98	11.79 ^{max}	68.91	10.28	min2.31	-77.52 ^{min}	6.62	8.64	30.51	5.52	0.79 ^{min} 3	min-31.34
Rahnan	20.55	12.72	-38.1	7.19	9.3	25.17	5.34	5.87	9.92	8.58	8.24	-3.96	5.14	4.15	-19.26
Rodehen	10.45	15.19	max45.35	9.1	9.3	min2.19	3.65	7.12	max95.06	8.59	8.56 ^{max}	0.69	5.22	5.53	5.93
Garm Dareh	5.57	19.05 ^{max}	42.01	10.25	11.65	13.65	5.76	5.54	-3.81	5.54	5.23	-5.59	4.38	4.73	7.99
Arabloo	1.46 ^{min}	-	-	18.09 ^{max}	-	-	0.90 ^{min} 1	-	-	4.17	-	-	1.09 ^{min}	-	-
Ji Shir	21.42	-	-	7.62	-	-	3.75	-	-	8.42	-	-	5.57	-	-
Kelardasht	max37.20	-	-	3.11	-	-	8.44	-	-	3.82	-	-	7.77 ^{max}	-	-
Cham Khorram	10.82	-	-	9.2	-	-	4.55	-	-	11.65	-	-	3.08	-	-
Total mean	15.49	13.81	31.28	8.12	8.87	33.87	6.61	6.69	-2.13	6.63	6.47	9.45	4.97	5.22	-22.71
LSD	0.008			0.06			2.22			0.008			1.023		
Genotypes	E-β-ionone														
Neryl acetate															
Shahrab	2.55	2.24	0.15 ^{min} 12-	1.27	2	57.48	0.93	1.72 ^{max}	84.94	2.08	1.8	-13.46	1.54	2	29.87 ^{max}
Dastgerd	-	0.49 ^{min}	max100	0.61 ^{min}	1.06	0.77 ^{max} 73	-	0.71	100 ^{max}	3.26	3.71 ^{max}	13.8	9.21 ^{max}	5.63 ^{max}	-38.87
Tad	1.43	1.82	27.27	1.87	1.85	-1.06	0.8	1.5	87.5	2.49	0.46 ^{min} 1	-41.36	6.39	3.77	41 ^{min} -
Rahnan	1	1.77	77	1.79	1.86	5.68	1.12	1.3	16.07	2.27	1.69	25.55 ^{min} -	0.30 ^{min} 1	1.55 ^{min}	19.23
Rodehen	1.58	1.14	27.84-	2.85 ^{max}	2.01 ^{max}	0.47 ^{min} 29-	1.08	0.65 ^{min} 0	39.81 ^{min} -	1.22 ^{min}	2.39	0.90 ^{max} 95	2.4	2.04	-15
Garm Dareh	2.53	2.25 ^{max}	11.06-	0.65	0.79 ^{min}	21.53	1.56	1.27	18.58-	1.98	1.9	-4.04	1.7	1.64	3.52-
Arabloo	max2.70	-	-	-	-	-	0.33 ^{max} 2	-	-	3.29	-	-	2.27	-	-
Ji Shir	0.87	-	-	2.47	-	-	-	-	-	2.44	-	-	1.25	-	-
Kelardasht	min0.59	-	-	0.82	-	-	0.66 ^{min}	-	-	0.67 ^{max} 3	-	-	2.32	-	-
Cham Khorram	1	-	-	2.09	-	-	1.1	-	-	1.34	-	-	1.73	-	-

Table 5 (continued)

Genotypes	A-terpineol		Spathulenol		n-heneicosane		1-octen-3-ol		Nonanoic acid	
	Cross	Self	Cross	Self	Cross	Self	Cross	Self	Cross	Self

Total mean 1.58 1.61 1.59 1.59 1.22 1.19 2.4 2.15 3.01 2.77

LSD 0.08 25.53 0.032 0.021 38.35 4.21 1.043 8.21-

Cros: Cross-pollinated offspring, Self: self-pollinated offspring, ID: inbreeding regression, ^{max}: The maximum mean value of the trait, ^{min}: The minimum mean value of the trait. Values are mean ± standard error

assurance, it carries risks of reduced genetic variability, inbreeding depression, and decreased ecological resilience over generations (Cheptou 2019; Acoca-Pidolle et al. 2024). Conversely, cross-pollination enhances heterozygosity, hybrid vigor, and yield stability, thereby sustaining genetic diversity critical for adaptive potential under environmental stress (Chabert and Mallinger 2025).

Dry weight differences among parents and progeny illustrate the sophisticated interplay between genetic factors and physiological traits related to resource utilization. Cross-pollination markedly improved dry weight in specific genotypes compared to self-pollination, emphasizing the role of hybrid vigor in optimizing biomass and resource allocation. Variations in 100-seed weight further suggest that reproductive strategy influences both seed quality and overall reproductive success. The observed increase in seed weight under self-pollination likely results from reduced seed number per plant, reflecting an adaptive allocation of resources to enhance individual seed fitness (Salami et al. 2017; Dieterich Mabin et al. 2021). Such patterns highlight the potential trade-offs in reproductive strategies: selfing may increase certain traits (e.g., seed weight) while simultaneously reducing overall fitness through decreased seed number or chemical quality.

The analysis of essential oil content revealed interactions between genetics and secondary metabolite production. Self-pollination increased total essential oil content across genotypes, demonstrating that reproductive strategy can modulate biosynthesis of secondary metabolites (Shojaie-far et al. 2021). This dual response, reduced biomass (DW and FW) alongside enhanced secondary metabolite accumulation, suggests that inbreeding functions as a form of genetic stress, potentially triggering adaptive biochemical pathways. At the molecular level, stress-responsive genes are activated, regulating enzymatic pathways and protective compound synthesis, thereby facilitating adaptive responses to abiotic and biotic stresses including extreme temperatures, drought, salinity, and nutrient deficiency (Khanah-madi et al. 2021; Achrem et al. 2023). Reports of increased secretory gland numbers and essential oil percentages under self-fertilization in other medicinal plants corroborate these observations (Nazem et al. 2019).

These results collectively provide novel evidence linking reproductive strategy, genetic variation, and biochemical traits in *S. setifera*. By demonstrating genotype-specific responses in both morphological and metabolic traits, this study establishes a foundational framework for future mechanistic investigations, including transcriptomic, metabolomic, and functional analyses. Importantly, our findings offer practical implications for conservation and breeding strategies, identifying cross-pollination as a key method for maintaining genetic diversity and selecting genotypes capable of

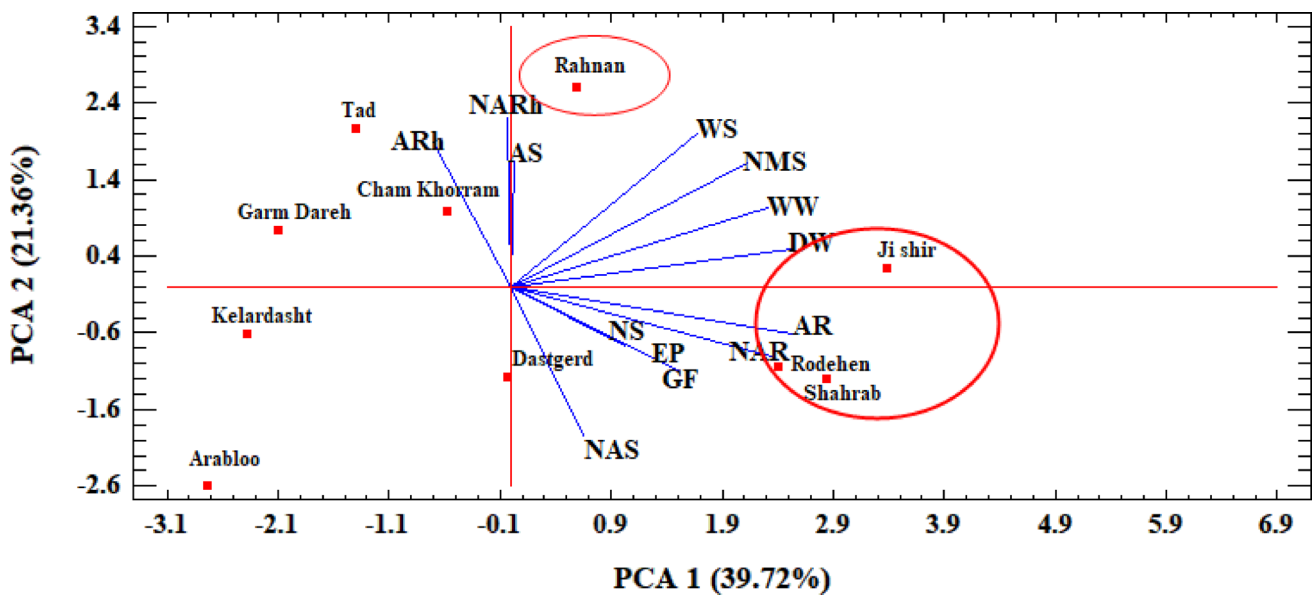


Fig. 7 Principal component analysis for the relationship among the studied traits and the distribution of offspring families (cross-pollination) of *Stachys setifera* relative to these traits. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth form, NMS: number of main stems, EP: essential oil

percentage, AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome

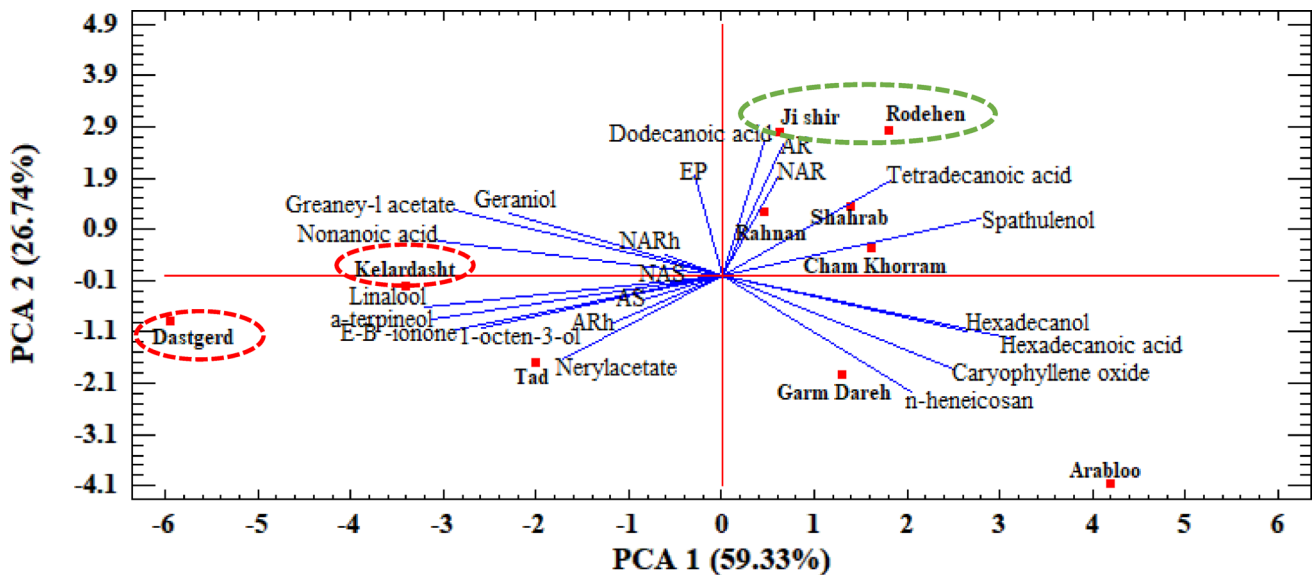
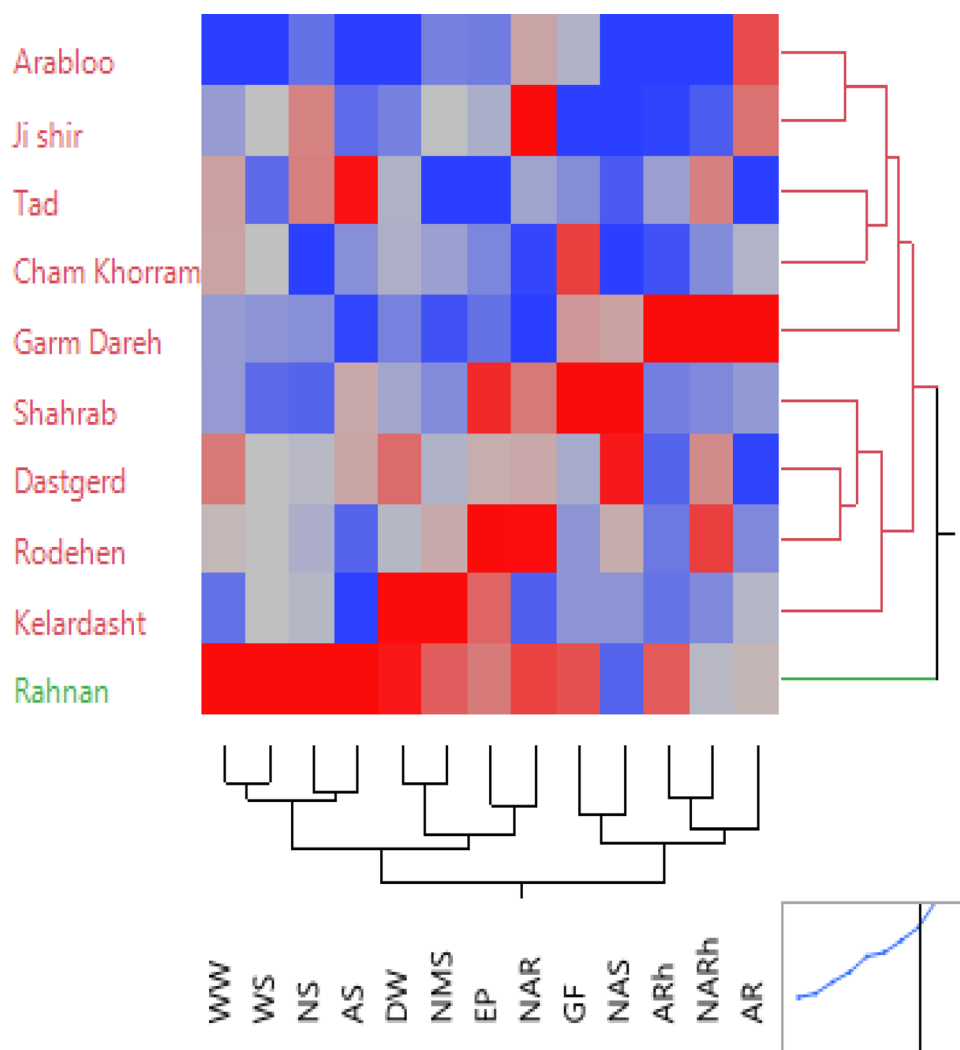


Fig. 8 Principal component analysis for the relationship among the essential oil content (EP), the main essential oil compounds and the aerenchyma traits of offspring families (cross-pollination) of *Stachys setifera*. EP: essential oil content, AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area

of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome. Red dash: the best quality of oil, Green dash: the most amount of oil

Fig. 9 Heat mapping diagram related to the studied traits of parental families of *Stachys setifera*. Heat-mapping diagram related to the studied traits of *Stachys setifera* families. The dark blue color indicates the lowest value of the characters, and the dark red color indicates the highest value. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth form, NMS: number of main stem, EP: essential oil percentage, AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome



optimizing both yield and secondary metabolite production under variable environmental conditions.

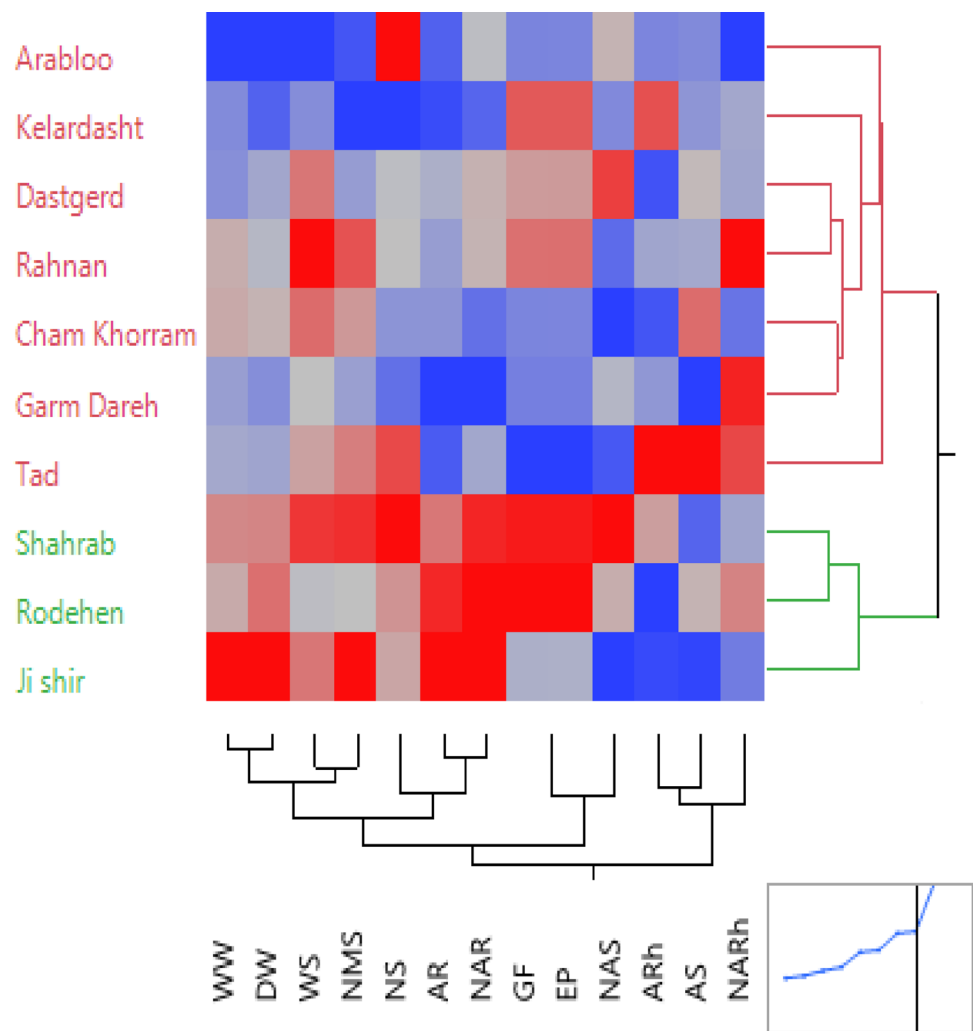
Genotypic Variation and Ecological Significance of Aerenchyma Development

Aerenchyma, the specialized tissue characterized by air spaces, plays a pivotal role in plant adaptation to waterlogged environments by facilitating oxygen transport to submerged tissues (Gao et al. 2025). In this study, we examined aerenchyma formation in roots, stems, and rhizomes of *Stachys setifera*, revealing substantial variation among parental genotypes and their cross-pollinated progeny. Variance analyses indicated significant effects of genotype and reproductive strategy on aerenchyma traits, showing the genetic basis of its development and its modulation by environmental and breeding factors. Significant differences between parents and progeny across plant organs reflect the combined influence of genetic inheritance (Schneider et al. 2023) and environmental interactions (Nomura et al.

2024) on aerenchyma morphology. Hypoxic or anoxic conditions can trigger functional and developmental responses that promote plant acclimation, including enhanced aerenchyma formation in roots and the development of impermeable barriers to radial O_2 loss (ROL) in basal root zones (Jiménez et al. 2021). Our results provide the first report of aerenchyma in *S. setifera*, a species inhabiting alternating drought and temporary waterlogging conditions. Extensive aerenchyma in rhizomes and stems likely supports oxygen supply under hypoxic stress, facilitates resource remobilization, and enhances stress tolerance, contributing to survival and reproductive success. Importantly, we observed positive correlations between aerenchyma traits and both yield- and essential oil-related traits (Fig. 7), suggesting potential mechanistic links between structural adaptations and secondary metabolism.

In roots, notable differences in aerenchyma number and area among genotypes suggest variable capacities for oxygen transport and waterlogging tolerance across generations. These differences likely arise from genetic divergence

Fig. 10 Heat mapping diagram related to the studied traits of off spring families (cross-pollination) of *Stachys setifera*. Heat-mapping diagram related to the studied traits of *Stachys setifera* families. The dark blue color indicates the lowest value of the characters, and the dark red color indicates the highest value. WW: wet weight, DW: dry weight, NS: number of seeds in main stem, WS: weight of 100 seeds, GF: growth form, NMS: number of main stem, EP: essential oil percentage, AR: total area of aerenchyma root surface, AS: total area of aerenchyma stem surface, ARh: total area of aerenchyma rhizome surface, NAR: number of aerenchyma root, NAS: number of aerenchyma stem, NARh: number of aerenchyma rhizome



in genes regulating aerenchyma formation, including those involved in programmed cell death and hormonal signaling (Schneider et al. 2023; Zhao et al. 2025). Similar genotypic variation was observed in stems, indicating that structural modifications in stem aerenchyma could affect plant stability, resource allocation, and overall fitness. The rhizome exhibited the highest aerenchyma number and area, reflecting its key role in storage and vegetative propagation. Substantial variation in rhizome aerenchyma among genotypes may influence local metabolic activity, oxygen diffusion, and adaptive responses to fluctuating hydrological conditions. These observations align with findings in *wheat*, where rhizomes showed higher aerenchyma development than roots (Gao et al. 2025). Importantly, our findings provide the first report of aerenchyma in *S. setifera*, highlighting its ecological and adaptive significance. In native habitats, these species experience alternating drought and temporary waterlogging. Extensive aerenchyma in rhizomes and stems likely supports oxygen supply under hypoxic conditions while facilitating resource remobilization and enhancing

stress tolerance, contributing to plant survival and reproductive success.

A conceptual framework integrating aerenchyma development, secondary metabolite biosynthesis, and plant fitness provides mechanistic insight into our observations. Extensive aerenchyma formation enhances internal gas exchange, which can modulate oxygen availability in roots and rhizomes, thereby influencing metabolic fluxes and carbon partitioning toward the biosynthesis of essential oils, particularly monoterpenes and other terpenoid compounds. Hormonal signaling molecules, including ethylene, auxin, and abscisic acid, as well as reactive oxygen species (ROS), are known regulators of both aerenchyma formation and secondary metabolism, and likely mediate these effects (Chen et al. 2021; Zhao et al. 2025). Consequently, genotypic differences in aerenchyma development may indirectly determine the quantity and composition of essential oils, linking structural adaptations to biochemical traits. Such integration of structural, metabolic, and signaling networks can explain how genotypes with higher aerenchyma in rhizomes and

stems achieve enhanced stress tolerance, optimized resource remobilization, and potentially improved reproductive success through modulation of ecological interactions, including herbivore deterrence and pollinator attraction. This perspective indicates that aerenchyma contributes not only to oxygen transport and tolerance to fluctuating hydrological conditions, but also to the regulation of secondary metabolism critical for the ecological and evolutionary success of *Stachys setifera* (Sarkar et al. 2023; Sherwood et al. 2025). Although our study provides correlative evidence, direct functional validation through transcriptomic, metabolomic, or hormonal analyses remains an important future direction.

Chemical Composition of Essential Oil

The chemical profile of plant essential oils is shaped by multiple factors, including genetic variability, environmental conditions, and reproductive strategies such as pollination method (Khanahmadi et al. 2021; Kanjevac et al. 2023). In the present study, GC and GC-MS analyses revealed 15 major constituents in the essential oil of cross- and self-pollinated populations of *Stachys setifera*. These compounds accounted for a substantial proportion of the oil and collectively determine both its therapeutic potential and ecological functions (Bilušić Vundać 2019). Among the identified metabolites, geraniol, hexadecenoic acid, linalool, tetradecanoic acid, and geranyl acetate were predominant. Geraniol represented the most abundant compound, followed by hexadecenoic acid and linalool; this result aligns with Gad et al. (2022) and Ivanova et al. (2023). Notably, linalool and geraniol two monoterpenes synthesized via the plastidial MEP (2-C-methyl-D-erythritol-4-phosphate) pathway by terpene synthases stood out as key constituents. These volatiles not only possess diverse pharmacological properties, including antimicrobial, antioxidant, anticancer, antidiabetic, and neuroprotective activities, but also play critical ecological roles in pollinator attraction and plant defense against herbivores and pathogens (Bahadori et al. 2020; Stegăruș et al. 2021). Geranyl acetate, a terpenoid ester, contributes to floral scent and enhances pollinator interactions. By contrast, fatty acids such as hexadecenoic and tetradecanoic acids—products of lipid metabolism serve not only as membrane components but also as precursors of oxylipins and other signaling molecules central to stress response and defense (He and Ding 2020). Overall, these findings indicate that the *S. setifera* oil profile integrates inputs from both terpenoid and fatty acid biosynthetic pathways, thereby linking its chemical composition to underlying biochemical functions and ecological roles.

Pollination strategy had a significant impact on essential oil composition. Self-fertilization altered the abundance of individual metabolites, with both increases and decreases

observed depending on genotype. For instance, hexadecenoic acid levels rose in several selfed lines, indicating potential for targeted metabolic enhancement through breeding, while geraniol and linalool showed genotype-specific reductions or gains. These contrasting trends likely reflect differences in the regulation of terpene synthases and fatty acid metabolism, as supported by recent genomic and transcriptomic studies (Ivanova et al. 2023; Rabara et al. 2023). Such compositional changes carry important medicinal and ecological implications. Reductions in geraniol and linalool may weaken antimicrobial, antioxidant, and anticancer activities, whereas elevated fatty acid levels could strengthen structural and signaling roles but contribute less to pharmacological value. Ecologically, higher monoterpene concentrations may enhance pollinator attraction, while their reduction could diminish defense against herbivores and reduce reproductive success (Bahadori et al. 2020; Stegăruș et al. 2021). Overall, these findings suggest that reproductive strategy modulates metabolic fluxes, thereby shaping both the quality and quantity of essential oils. Generally, while self-fertilization increased the total essential oil percentage (EP) across all genotypes, it often reduced the relative abundance of major bioactive compounds such as geraniol and linalool. This pattern implies a trade-off between oil yield and quality. For example, the Dastgerd genotype produced the highest EP in all three populations (parental, cross-pollinated, and self-pollinated), largely due to its elevated linalool content. Nevertheless, the overall decline in key phytochemicals under self-fertilization indicates that reproductive strategy can boost oil quantity at the expense of chemical quality (Shojaiefar et al. 2021).

Importantly, correlations between aerenchyma traits and essential oil composition indicate potential mechanistic links. Genotypes with more extensive aerenchyma in stems and rhizomes consistently exhibited higher levels of principal oil constituents, including geraniol and linalool. These results suggest that structural adaptations like aerenchyma may facilitate oxygen diffusion and metabolic efficiency, indirectly promoting secondary metabolite biosynthesis through enhanced carbon allocation and hormonal signaling (ethylene, auxin, ABA) (Hassanpouraghdam et al. 2022; Schneider et al. 2023). This integrative perspective showed that essential oil yield and quality are not solely determined by genetic makeup but are also modulated by interactions between anatomical traits, reproductive strategy, and environmental factors. Taken together, our findings provide the first comprehensive link between reproductive strategy, structural adaptation (aerenchyma), and essential oil composition in *S. setifera*, establishing a foundation for future functional genomics and breeding programs aimed at optimizing both quantity and quality of bioactive metabolites.

Understanding Genotype-trait Relationships through Principal Component Analysis

Principal component analysis (PCA) was employed to elucidate complex relationships among agro-morphological traits, aerenchyma development, and essential oil characteristics in *S. setifera* populations. The biplot results for parental and cross-pollinated populations (Figs. 5 and 6) revealed that the first principal component (PC1) was positively and significantly associated with weight-related traits (WW, WS, DW), growth form (GF), leaf traits (NS), and rhizome aerenchyma traits (ARh, NARh). The second principal component (PC2) showed a positive correlation with stem-related traits (NAS), essential oil production (EP), and leaf morphological characteristics (NMS). These patterns indicate strong integrative relationships among structural, growth, and biochemical traits, highlighting the central role of aerenchyma in linking physiological and metabolic outcomes.

Most of the variables contributing to PC1 were positively aligned with those in PC2, indicating that enhanced growth and structural vigor often coincide with improvements in oil yield and stem development. When we examined the biplot patterns of essential oil yield and composition in relation to aerenchyma traits (Fig. 7), a clear trend emerged: root aerenchyma showed the strongest link to total oil content, while rhizome and stem aerenchyma were more closely tied to oil composition especially to the five predominant compounds, geraniol, linalool, hexadecenoic acid, tetradecanoic acid, and geranyl acetate. These relationships suggest that different plant organs make distinct structural contributions to the plant's overall metabolism. Among them, stem aerenchyma appears to play a particularly influential role, shaping both total productivity and the performance of self-fertilized lines (Yamauchi and Nakazono 2022; Dzhoglova et al. 2024). Similar relationships have been observed in other crops as well. In rice, for instance, greater aerenchyma development has been linked to increases in both wet and dry biomass, improved nutrient accumulation, and stronger tolerance to environmental stress (Chakraborty et al. 2021; Vijayalakshmi and Raveendran 2023). Looking ahead, integrating more advanced multivariate approaches, such as canonical correlation analysis, partial least squares regression, or QTL–trait mapping, could help clarify how these anatomical and metabolic traits interact at the genetic level.

Comparison between progeny and parental genotypes revealed clear differences in trait distributions. Among parental genotypes, Rahnan exhibited the highest values for most traits, whereas in the progeny, Rahnan maintained the best WW and DW, with Rodehen, Ji Shir, and Shahrab demonstrating elevated values for multiple traits. The cross-pollination progeny biplot suggests that hybridization produced

superior individuals relative to their parents, consistent with heterosis effects reported in other plant systems (Thavamanikumar et al. 2020; Singh et al. 2023). Specifically, analysis of essential oil traits indicated that Dastgerd and Kelardasht genotypes exhibited the highest oil quality, while Rahnan, Ji Shir, and Rodehen had the highest oil quantity, reinforcing the importance of genotype-specific trait optimization for breeding programs.

The heat mapping graphs were consistent with the biplot diagrams, confirming that genotypes with higher aerenchyma traits in the stem exhibited greater WW, DW, and WS. Similar relationships have also been reported in other crop plants (Jia et al. 2022; Yuan et al. 2022). In contrast, these traits showed negative associations with Arh and NARh. Previous studies demonstrated that aerenchyma in the root and stem contributes more strongly to plant survival and enhanced morphological traits compared with aerenchyma in the rhizome. In plants where aerenchyma was restricted to the root, improved gas exchange and increased tolerance to stresses such as flooding were observed (Geng et al. 2023; Jin et al. 2025).

Importantly, the correlations observed between aerenchyma development and essential oil traits in *S. setifera* suggest not only descriptive variation but also potential mechanistic links. Recent studies indicate that aerenchyma formation is tightly regulated by hormonal signaling pathways (e.g., ethylene, auxin, and ABA) and programmed cell death, which also influence secondary metabolism and the allocation of carbon resources. It is therefore plausible that genotypic differences in aerenchyma development indirectly affect terpene biosynthesis through shifts in oxygen availability, carbon partitioning, and hormonal balance. This interpretation aligns with our PCA results, where aerenchyma traits clustered with both yield- and oil-related traits. By uncovering these integrative patterns for the first time in *S. setifera*, our study provides a conceptual framework for future mechanistic investigations (e.g., transcriptomic or metabolomic analyses) that can directly test how structural adaptations like aerenchyma modulate essential oil biosynthesis and quality.

Conclusion

This study provides an integrative assessment of genetic diversity, reproductive strategies, aerenchyma development, and essential oil composition in *Stachys setifera*. The results demonstrated that both genotype and pollination method significantly influenced growth, morphological, and biochemical traits. Self-pollination created trade-offs, enhancing certain metabolites (e.g., total oil content) while reducing biomass and key compounds such as geraniol and

linalool, whereas cross-pollination promoted heterozygosity, hybrid vigor, and superior performance in both yield and oil traits. Our findings revealed, for the first time, a direct association between aerenchyma development and essential oil composition, with stem and rhizome aerenchyma linked to oil quality and root aerenchyma to plant survival under stress. These results suggest that structural traits not only support ecological adaptation but also shape secondary metabolism. Multivariate analyses further confirmed that genotypes with extensive aerenchyma exhibited higher biomass and improved oil profiles, highlighting the potential of aerenchyma traits as indirect markers for breeding programs. By establishing genotype trait linkages and demonstrating the interactive effects of reproductive strategy and structural adaptation, this study advances the understanding of adaptive mechanisms in medicinal plants. The evidence highlights the broader applicability of aerenchyma-based selection for improving essential oil yield and quality while enhancing ecological resilience, providing a conceptual framework for both fundamental research and applied breeding.

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Data Availability Data will be made available on request.

Declarations

Competing Interests The authors declare no conflict of interest.

Ethical Approval Compliance with ethical standards.

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References

- Achrem M, Stępień E, Kalinka A (2023) Epigenetic changes occurring in plant inbreeding. *Int J Mol Sci* 24(6):5407. <https://doi.org/10.3390/ijms24065407>
- Acoca-Pidolle S, Gauthier P, Devresse L, Deverge Merdrignac A, Pons V, Cheptou PO (2024) Ongoing convergent evolution of a selfing syndrome threatens plant–pollinator interactions. *New Phytol* 242(2):717–726. <https://doi.org/10.1111/nph.19422>
- Arabsalehi F, Rahimmalek M, Ehtemam MH (2018) Phytochemical and morphological variation of *Stachys lavandulifolia* Vahl. populations as affected by genotype× year interaction. *Ind Crops Prod* 112:342–352. <https://doi.org/10.1016/j.indcrop.2017.12.025>
- Bahadori MB, Maggi F, Zengin G, Asghari B, Eskandani M (2020) Essential oils of hedgenettles (*Stachys inflata*, *S. lavandulifolia*, and *S. byzantina*) have antioxidant, anti-Alzheimer, antidiabetic, and anti-obesity potential: a comparative study. *Ind Crops Prod* 145:112089. <https://doi.org/10.1016/j.indcrop.2020.112089>
- Beavis W, Merrick L, Meade K, Campbell A, Muenchrath D, Fei SZ (2021) Inheritance of Quantitative Traits. *Plant Breeding E-Learning in Africa*, Ames, IW (USA), pp.1–64. <https://pbea.aron.iastate.edu>
- Benedec D, Oniga I, Hanganu D, Tiperciuc B, Nistor A, Vlase AM, Vlase L, Pușcaș C, Duma M, Login CC, Niculae M (2023) *Stachys* species: comparative evaluation of phenolic profile and antimicrobial and antioxidant potential. *Antibiotics* 12(11):1644. <https://doi.org/10.3390/antibiotics12111644>
- Bilušić Vundač V (2019) Taxonomical and phytochemical characterisation of 10 *Stachys* taxa recorded in the Balkan Peninsula flora. *Rev Plants* 8:32. <https://doi.org/10.3390/plants8020032>
- Chabert S, Mallinger RE (2025) Self-sterility, self-incompatibility and xenia: a review of the mechanisms of cross-pollination benefits in animal-pollinated crops. *Ann Bot mcaf* 047. <https://doi.org/10.1093/aob/mcaf047>
- Chakraborty K, Ray S, Vijayan J, Molla KA, Nagar R, Jena P, Mondal S, Panda BB, Shaw BP, Swain P, Chattopadhyay K (2021) Preformed aerenchyma determines the differential tolerance response under partial submergence imposed by fresh and saline water flooding in rice. *Physiol Plant* 173(4):1597–1615. <https://doi.org/10.1111/ppl.13536>
- Chen Y, Chen Y, Zhang Y, Zhang D, Li G, Wei J, Hua X, Lv B, Liu L (2021) Heterotrimeric G protein γ subunit DEP1 is involved in hydrogen peroxide signaling and promotes aerenchyma formation in rice roots. *Plant Signaling & Behavior* 16(5):1889251. <https://doi.org/10.1080/15592324.2021.1889251>
- Cheptou PO (2019) Does the evolution of self-fertilization rescue populations or increase the risk of extinction? *Ann Bot* 123(2):337–345. <https://doi.org/10.1093/aob/mcy144>
- Chung MY, Merilä J, Li J, Mao K, López-Pujol J, Tsumura Y, Chung MG (2023) Neutral and adaptive genetic diversity in plants: an overview. *Front Ecol Evol* 11:1116814. <https://doi.org/10.3389/fevo.2023.1116814>
- Cropano C, Place I, Manzanares C, Do Canto J, Lübberstedt T, Studer B, Thorogood D (2021) Characterization and practical use of self-compatibility in outcrossing grass species. *Ann Bot* 127(7):841–852. <https://doi.org/10.1093/aob/mcab043>
- Dieterich Mabin ME, Brunet J, Riday H, Lehmann L (2021) Self-fertilization, inbreeding, and yield in alfalfa seed production. *Front Plant Sci* 12:700708. <https://doi.org/10.3389/fpls.2021.700708>
- Dzhoglova V, Ivanov K, Benbassat N, Georgieva-Dimova Y, Ardashova R, Karcheva-Bahchevanska D, Ivanova S (2024) *Crithmum maritimum* L.—Study on the Histochemical Localization of Essential Oil. *Plants* 13(4):550. <https://doi.org/10.3390/plants13040550>

- Gad HA, Mukhammadiev EA, Zengen G, Musayeib NMA, Hussain H, Bin Ware I, Ashour ML, Mamadalieva NZ (2022) Chemometric analysis based on GC-MS chemical profiles of three *Stachys* species from Uzbekistan and their biological activity. *Plants* 11(9):1215. <https://doi.org/10.3390/plants11091215>
- Gao H, Chen M, Jin N, Ye L, Zhang G, Shen Q, Xu Z (2025) A comprehensive analytical method ‘Regulatome’ revealed a novel pathway for aerenchyma formation under waterlogging in wheat. *Phy Plant* 177(2):e70157. <https://doi.org/10.1111/ppl.70157>
- Geng S, Lin Z, Xie S, Xiao J, Wang H, Zhao X, Zhou Y, Duan L (2023) Ethylene enhanced waterlogging tolerance by changing root architecture and inducing aerenchyma formation in maize seedlings. *J Plant Physiol* 287:154042. <https://doi.org/10.1016/j.jplph.2023.154042>
- Güner Ö (2022) Contributions to the flora of Iraq: *Stachys bhattacharjeei* sp. nov. (Lamiaceae). *Feddes Repert* 133(4):257–267. <https://doi.org/10.1002/fedr.202100039>
- Haghighpanah M, Hashemipetroudi S, Arzani A, Araniti F (2024) Drought tolerance in plants: physiological and molecular responses. *Plants* 13(21):2962. <https://doi.org/10.3390/plants13212962>
- Hassanpouraghdam MB, Salimi Y, Morshedloo MR, Asadi M, Rasouli F, Ercisli S, Fidan H, Muresan CC, Marc RA (2022) *Stachys lavandulifolia* populations: volatile oil profile and morphological diversity. *Agronomy* 12(6):1430. <https://doi.org/10.3390/agronomy12061430>
- He M, Ding NZ (2020) Plant unsaturated fatty acids: multiple roles in stress response. *Front Plant Sci* 11:562785. <https://doi.org/10.3389/fpls.2020.562785>
- Ivanova S, Pashova S, Dyankov S, Georgieva Y, Ivanov K, Benbassat N, Koleva N, Bozhkova M, Karcheva-Bahchevanska D (2023) Chemical composition and future perspectives of essential oil obtained from a wild population of *Stachys germanica* L. distributed in the Balkan Mountains in Bulgaria. *Int J Anal Chem* 2023(1):4275213. <https://doi.org/10.1155/2023/4275213>
- Jia X, Wu G, Strock C, Li L, Dong S, Zhang J, Zhao B, Lynch JP, Liu P (2022) Root anatomical phenotypes related to growth under low nitrogen availability in maize (*Zea mays* L.) hybrids. *Plant Soil* 474(1):265–276. <https://doi.org/10.1007/s11104-022-05331-6>
- Jiménez JDLC, Pellegrini E, Pedersen O, Nakazono M (2021) Radial oxygen loss from plant roots—methods. *Plants* 10(11):2322. <https://doi.org/10.3390/plants10112322>
- Jin N, Cai Z, Ye L, Shen Q, Zhang G, Xu Z (2025) Improvement of waterlogging tolerance in wheat by the stress priming through inducing aerenchyma formation. *Plant Growth Regul* 105(1):245–255. <https://doi.org/10.1007/s10725-024-01274-3>
- Kanjevac M, Zlatić N, Bojović B, Stanković M (2023) Pharmaceutical and biological properties of *Stachys* species: A review. *Braz J Pharm Sci* 58. <https://doi.org/10.1590/s2175-97902022e20211>
- Khanahmadi F, Rahimmalek M, Sabzalain MR, Tohidi B (2021) Variation of essential oil content and composition, phenolics, and yield related traits using different pollination systems in populations of thymus species. *Chem Biodivers* 18:e2100097. <https://doi.org/10.1002/cbdv.202100097>
- Klein SP, Schneider HM, Perkins AC, Brown KM, Lynch JP (2020) Multiple integrated root phenotypes are associated with improved drought tolerance. *Plant Physiol* 183(3):1011–1025. <https://doi.org/10.1104/pp.20.00211>
- Mano Y, Omori F, Takamizo T, Kindiger B, Bird RM, Loaisiga CH (2006) Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. *Plant Soil* 281:269–279. <https://doi.org/10.1007/s11104-005-4268-y>
- Mansinhos I, Gonçalves S, Romano A (2024) How climate change-related abiotic factors affect the production of industrial valuable compounds in Lamiaceae plant species: a review. *Front Plant Sci* 15:1370810. <https://doi.org/10.3389/fpls.2024.1370810>
- Mohammadi M, Kharazian N (2022) Untargeted metabolomics study and identification of potential biomarkers in the six sections of the genus *Stachys* L. (Lamiaceae) using HPLC-MQ-API-MS/MS. *Phytochem Anal* 33(6):915–942. <https://doi.org/10.1002/pc.a.3149>
- Naghiloo S, Nikzat-Siahkoleae S, Esmaillou Z, Claßen-Bockhoff R (2022) Pollination efficiency and pollen limitation in bistaminate vs tetrastaminate Lamiaceae. *Plant Species Biol* 37(1):91–102. <https://doi.org/10.1111/1442-1984.12357>
- Nazem V, Sabzalain MR, Saeidi G, Rahimmalek M (2019) Essential oil yield and composition and secondary metabolites in self- and open-pollinated populations of mint (*Mentha* spp.). *Ind Crops Prod* 130:332–340. <https://doi.org/10.1016/j.indcrop.2018.12.018>
- Nomura Y, Arima S, Kyogoku D, Yamauchi T, Tominaga T (2024) Strong plastic responses in aerenchyma formation in F1 hybrids of *Imperata cylindrica* under different soil moisture conditions. *Plant Biol* 26(3):446–456. <https://doi.org/10.1111/plb.13618>
- Parra-Tabla V, Alonso C (2021) Autonomous pollination alleviates pollen limitation in the endemic *Cienfuegosia yucatanensis* Millsp. (Malvaceae). *Bot Sci* 99(1):80–91. <https://doi.org/10.17129/botsci.2651>
- Pashova S, Karcheva-Bahchevanska D, Ivanov K, Ivanova S (2024) Genus *Stachys*—phytochemistry, traditional medicinal uses, and future perspectives. *Molecules* 29(22):5345. <https://doi.org/10.3390/molecules29225345>
- Rabara RC, Kudithipudi C, Timko MP (2023) Identification of terpene-related biosynthetic gene clusters in tobacco through computational-based genomic, transcriptomic, and metabolic analyses. *Agronomy* 13(6):1632. <https://doi.org/10.3390/agronomy13061632>
- Ragno R, Papa R, Patsilinakos A, Vrenna G, Garzoli S, Tuccio V, Fiscarelli E, Selan L, Artini M (2020) Essential oils against bacterial isolates from cystic fibrosis patients by means of antimicrobial and unsupervised machine learning approaches. *Sci Rep* 10(1):2653. <https://doi.org/10.1038/s41598-020-59553-8>
- Revell I, Zhang P, Dong C, Salter WT, Trethowan R (2025) Heterosis in wheat: mechanisms, benefits, and challenges in hybrid development. *J Exp Bot* p eraf159. <https://doi.org/10.1093/jxb/eraf159>
- Rowe N, Speck T (2005) Plant growth forms: an ecological and evolutionary perspective. *New Phytol* 166(1):61–72. <https://doi.org/10.1111/j.1469-8137.2004.01309.x>
- Salami M, Rahimmalek M, Ehtemam MH (2017) Comprehensive research on essential oil and phenolic variation in different *Foeniculum vulgare* populations during transition from vegetative to reproductive stage. *Chem Biodivers* 14(2):e1600246. <https://doi.org/10.1002/cbdv.201600246>
- Salgotra RK, Chauhan BS (2023) Genetic diversity, conservation, and utilization of plant genetic resources. *Genes* 14(1):174. <https://doi.org/10.3390/genes14010174>
- Sarkar S, Saha S, Ghosh S, Paul SK, Dey S, Moulick D, Santra SC, Brahmachari K (2023) Abiotic Stress Sensitivity and Adaptation in Field Crops. *Climate-Resilient Agriculture, Vol 2: Agro-Biotechnological Advancement for Crop Production*. Springer International Publishing, Cham, pp 319–362. https://doi.org/10.1007/978-3-031-37428-9_15
- Schneider HM, Lor VS, Zhang X, Saengwilai P, Hanlon MT, Klein SP, Davis JL, Borkar AN, Depew CL, Bennett MJ, Kaeppler SM (2023) Transcription factor bHLH121 regulates root cortical aerenchyma formation in maize. *Proc Natl Acad Sci U S A* 120(12):e2219668120. <https://doi.org/10.1073/pnas.2219668120>
- Sherwood OL, Burke R, O'Rourke J, Whelan CV, Downey F, Ryan L, McCabe EF, Huang Z, Ng CK, McCabe PF, Kacprzyk J (2025) Transcriptional signatures associated with waterlogging stress responses and aerenchyma formation in barley root tissue. *Ann Bot* mcaf104. <https://doi.org/10.1093/aob/mcaf104>

- Shojaiefar S, Sabzalian MR, Mirlohi A, Tajdivand A (2021) Evidence for self-compatibility and variation for inbreeding depression within breeding populations of fennel (*Foeniculum vulgare* Mill.). *J Appl Res Med Aromat Plants* 22:100299. <https://doi.org/10.1016/j.jarmap.2021.100299>
- Singh A, Barb J, Singh A, Mahama AA (2023) Basic Principles of Plant Breeding. *Crop Improvement*
- Slimani W, Maioli M, Cruciani S, Zerizer S, Santaniello S, Kabouche Z, Coradduzza D, Chessa M, Fancello S, Migheli R, Serra PA (2023) Antioxidant, anti-inflammatory and anti-proliferative properties of *Stachys circinata* on HepG2 and MCF7 cells. *Plants* 12(12):2272. <https://doi.org/10.3390/plants12122272>
- Slobodianiuk L, Budniak L, Marchyshyn S, Sydor V (2021) The antibacterial activity of the extracts from the *Stachys sieboldii* MIQ. *Pharmacologyonline* 3:948–957
- Sou HD, Masumori M, Yamanoshita T, Tange T (2021) Primary and secondary aerenchyma oxygen transportation pathways of *Syzygium kunstleri* (King) Bahadur & RC Gaur adventitious roots in hypoxic conditions. *Sci Rep* 11(1):4520. <https://doi.org/10.1038/s41598-021-84183-z>
- Stegăruș DI, Lengyel E, Apostolescu GF, Botoran OR, Tanase C (2021) Phytochemical analysis and biological activity of three *Stachys* species (Lamiaceae) from Romania. *Plants* 10(12):2710. <https://doi.org/10.3390/plants10122710>
- Thavamanikumar S, Arnold RJ, Luo J, Thumma BR (2020) Genomic studies reveal substantial dominant effects and improved genomic predictions in an open-pollinated breeding population of *Eucalyptus pellita*. *G3 Genes Genomes Genet* 10(10):3751–3763. <https://doi.org/10.1534/g3.120.401601>
- Tomou EM, Barda C, Skaltsa H (2020) Genus *Stachys*: a review of traditional uses, phytochemistry and bioactivity. *Medicines* 7(10):63. <https://doi.org/10.3390/medicines7100063>
- Tomou EM, Karioti A, Tsirogiannidis G, Krigas N, Skaltsa H (2023) Metabolic characterization of four members of the genus *Stachys* L. (Lamiaceae). *Agronomy* 13(10):2624. <https://doi.org/10.3390/agronomy13102624>
- Ulewicz-Magulska B, Wesolowski M (2023) Antioxidant activity of medicinal herbs and spices from plants of the Lamiaceae, Apiaceae and Asteraceae families: chemometric interpretation of the data. *Antioxidants* 12(12):2039. <https://doi.org/10.3390/antiox12122039>
- Vijayalakshmi D, Raveendran M (2023) Introgression of Sub1 QTL alters aerenchyma-mediated gas exchange and stored carbohydrates to maintain yield under flooding stress in rice. *JCSB* 26(1):39–49. <https://doi.org/10.1007/s12892-022-00160-6>
- Xue L, Cai JH, Zhan M, Li XP, Wu L, Chen YP (2023) Molecular and morphological evidence for a new species of *Stachys* (Lamiaceae) from Hunan, China. *PhytoKeys* 236:121–134. <https://doi.org/10.3897/phytokeys.236.112741>
- Yamauchi T, Nakazono M (2022) Modeling-based age-dependent analysis reveals the net patterns of ethylene-dependent and-independent aerenchyma formation in rice and maize roots. *Plant Sci* 321:111340. <https://doi.org/10.1016/j.plantsci.2022.111340>
- Yuan Z, Ni X, Chen C, Zhang S, Chen X, Yang Z, Li C (2022) Effects of different water conditions on the biomass, root morphology and aerenchyma formation in bermudagrass (*Cynodon dactylon* (L.) Pers). *BMC Plant Biol* 22(1):266. <https://doi.org/10.1186/s12870-022-03653-2>
- Yuan X, Jiang X, Zhang M, Wang L, Jiao W, Chen H, Mao J, Ye W, Song Q (2024) Integrative omics analysis elucidates the genetic basis underlying seed weight and oil content in soybean. *Plant Cell* 36(6):2160–2175. <https://doi.org/10.1093/plcell/koae062>
- Zhao J, Wang D, Tang Y, Yu H, Zhang Y, Zhu L, Yan X, Yang J, Liu L, Chen Y (2025) The role of OsRGA1 in aerenchyma formation and adventitious root growth in rice seedlings based on the U-Gompertz model. *BMC Plant Biol* 25(1):490. <https://doi.org/10.1186/s12870-025-06526-6>

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