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Variability Assessment of New Developed Lines of Chia (*Salvia hispanica* L.)

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ABSTRACT

Three populations of chia (*Salvia hispanica*) were obtained from different sources. No meaningful differences were detected among them; therefore, a single population was selected for the present breeding assessment. Given the wide variation observed in plant height, this trait was used as a classification factor for the germplasm. Plant height is easily distinguished visually and is commonly used as an indicator of seed yield. The populations were classified into three categories based on height: tall (90–110 cm), medium (70–89 cm), and short (50–69 cm). Because chia is a self-pollinated and self-fertilized crop, it was assumed to consist of a composite of pure lines. Progeny test results confirmed the genetic purity (homozygosity) of the selected plants within the different plant height categories. Narrow-sense heritability, estimated through parent–offspring regression, ranged from 0.26 for spike length to 0.97 for plant height. Accordingly, the expected selection response values were high for most of the traits studied. All traits exhibited strong genotypic associations, with some reaching unity (1.00) in their correlation with seed weight per plant. This was clearly reflected in the parameters of correlated response and the relative efficiency of indirect selection. Selection for seed weight influenced all other traits to varying degrees through correlated responses. Following tandem multi-trait selection, twelve true-breeding chia lines were developed. Among these, two lines (1 and 7) showed particular promise for genetic improvement of chia. These lines may be directly utilized in chia production, pending stability testing, or employed as parental genetic material in cross-breeding programs.

Abbreviations Salvia hispanica (Chia), Randomized Complete-Block Design (RCBD)

Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous dicotyledonous plant native to parts of northern Guatemala and west-central Mexico. It belongs to the mint family (Lamiaceae) (Cahill 2003; Muñoz et al. 2013). Recently, chia has been introduced as a novel crop in the Egyptian agricultural system with the aim of diversifying production through the addition of new species of fragrant and medicinal plants. Within Lamiaceae, *Salvia* is the largest genus, comprising approximately 1000 species distributed across South Africa, Central America, North America, South America, and Southeast Asia (Segura-Campos et al. 2014a; Takano 2017). According to recent classifications, *Salvia* belongs to the subfamily Nepetoideae, tribe Mentheae, and subtribe Salviinae (Cahill 2003; Segura-Campos et al. 2014a). The primary center of origin of chia is located between Mexico and Guatemala (Joseph 2004).

Chia (2n = 12) is a short-day plant native to tropical and subtropical regions at elevations between 400 and 2500 meters above sea level, with an optimal temperature range of 16–26 °C (Jamboonsri et al. 2012; Bochicchios et al. 2015). It grows well not only in desert soils with adequate drainage but also in light to medium clay and sandy soils (Mohd Ali et al. 2012; Muñoz et al. 2013). Morphologically, chia has a subangular, branched stem and pubescent leaves measuring 5–8 cm in length and 3–5 cm in width. Plant height ranges from 60 to 180 cm. The flowers are small (3–5 mm), with fused floral parts and short corollas, and occur in white, blue, or purple. Self-pollination is predominant, with high seed set occurring even in the absence of pollinators (Cahill & Ehdaie 2005; Jamboonsri 2010).

From an agronomic perspective, chia cultivation has considerable potential to address the rising demand for healthy, environmentally sustainable, and locally sourced food products (Graeff-Hönninger & Khajehei 2019; Market-Growth and Trends 2020). The seeds are rich in oil, which is considered a “super nutrient” for the food, medical, and pharmaceutical sectors due to its anti-aging, anti-carcinogenic, and cardioprotective properties (Ullah Nadeem et al. 2016). Chia seeds are also a valuable source of ω-3 fatty acids, proteins, antioxidants, dietary fiber, and other nutraceuticals (Coates 2011; Segura-Campos et al. 2014b). Their functional properties have been successfully exploited in food systems: for example, chia seeds and oil have been incorporated into bakery products (Pizarro et al. 2013; da Silva et al. 2014), while the addition of chia to gluten-free flours has been shown to improve nutritional quality without adversely affecting sensory characteristics (Steffolani et al. 2014). Traditionally, hydrated seeds are consumed in beverages such as “agua fresca” or “chia fresca” in Mexico (Ahmed et al. 1994). The mucilage formed by soluble fibers acts as a prebiotic and contributes to the regulation of blood sugar and cholesterol levels (Gentry et al. 1990; Capitani et al. 2013).

Breeding programs in chia target traits such as seed yield, flowering time, maturity rate, uniformity in plant height, and disease resistance. The main drivers of genetic diversity in *S. hispanica* are its biological attributes, geographic distribution, and human selection. Among these, the breeding system has the strongest influence. The minute, homostylous flowers of chia are associated with a highly self-pollinated system (Haque & Ghoshal 1981). The species typically achieves high seed set under greenhouse or mesh-covered conditions in the absence of insect pollinators. Field studies in California reported an outcrossing rate as low as 0.24% when comparing wild and domesticated lines (Joseph 2004). Investigations into inheritance patterns have shown that seed mass responds to selection: Cahill and Ehdaie (2005) reported a 16% increase in seed mass after a single selection cycle. Because chia is largely self-pollinated and individual plants are capable of producing thousands of seeds, such selection efforts have been relatively successful (Haque & Ghoshal 1981). Nevertheless, despite the species’ economic potential, no coordinated breeding initiatives aimed at improving its agronomic performance have yet been undertaken.

Chia has not been the focus of extensive modern plant breeding efforts. The few improved cultivars or populations that exist have been developed mainly through the selection of lines from mixed germplasm sources, often landraces. Given the medicinal importance of the species and the limited information available regarding systematic breeding programs, the present study was designed with two main objectives: (i) to assess variability and statistically characterize a chia population, and (ii) to investigate selection strategies for the development of improved pure lines with enhanced seed yield and related traits.

Materials and Methods

General procedure

This study was conducted at the Ornamental Plants Farm, Faculty of Agriculture, Assiut University, Egypt, during the 2020/2021, 2021/2022, 2022/2023, and 2023/2024 growing seasons. The experimental site is located at 27°18′ latitude and 31°18′ longitude, at an elevation of 70 m above sea level. Three chia seed populations were used. The first population was imported from Mexico (Population 1, Chia 1). The second population was purchased from a commercial retailer in Assiut City, Egypt, and was originally imported from Peru (Population 2, Chia 2). The third population was obtained from Abu Auf (a roastery and nut house), and was originally imported from Argentina (Population 3, Chia 3). Laboratory tests confirmed 100% germination for all seed sources.

Each year on October 28, chia seeds were directly sown in two rows per plot (experimental unit) measuring 2 × 1 m, with 50 cm spacing between rows and five hills per row spaced 25 cm apart. After five weeks, seedlings were thinned to one plant per hill, leaving a total of 10 plants per plot. The experiment was arranged in a randomized complete block design with four replicates. The physicochemical properties of the experimental field soil were determined according to the methods described by Jackson (1958) and are presented in Table 1.

The climatic conditions (ambient temperature and relative humidity) during the growing seasons from October 2020 to May 2024 are summarized in Table 2. Standard cultural practices were applied throughout the experiment, including soil fertilization (Souza & Chaves 2017), irrigation

(Herman et al. 2016), and weeding as necessary. In total, 220 plants were cultivated in the open field from Chia 1, and 240 plants each from Chia 2 and Chia 3. Seeds were harvested at the end of each growing season.

Table 1. Physiochemical characteristics of the experimental soil.

Soluble ions meq. 100 g^{-1} soil (Extract 1:5)						
Cations				Anions		
Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	$HCO3^{-}$	Cl^{-}	$SO4^{-}$
3.10	2.48	3.77	0.007	3.19	3.65	2.59

Soil type: Clay			
Soluble K mg 100 g^{-1} soil	pH (1:2.5)	EC dS m^{-1}	Organic matter %
0.27	7.63	0.91	1.93

Table 2. Average monthly maximum and minimum temperatures and relative humidity (RH) in Assiut during chia growth in (10/2020 – 5/2021, 10/2021- 5/2022, 10/2022- 4/2023 and 10/2023-4/2024)⁽¹⁾.

Month	Temperature (°C) 2020-2021			Temperature (°C) 2021-2022			Temperature (°C) 2022-2023			Temperature (°C) 2023-2024		
	Max	Min	RH (%)	Max	Min	RH (%)	Max	Min	RH (%)	Max	Min	RH (%)
October	32.11	17.2	38.00	33.19	17.93	37.41	32.70	16.25	38.10	31.00	17.25	38.40
November	23.14	13.4	51.70	23.76	14.00	50.47	23.00	13.58	50.17	21.57	12.94	50.00
December	21.00	5.95	49.00	22.58	6.07	49.35	21.88	6.13	48.57	22.60	5.86	49.30
January	17.23	5.10	50.00	19.22	6.21	49.30	21.00	7.00	51.01	21.08	5.17	55.10
February	20.90	7.11	40.30	20.66	7.80	43.00	22.07	8.01	44.58	23.00	8.09	46.07
March	23.55	10.50	37.44	24.16	10.91	38.10	25.13	13.00	39.11	24.24	13.00	42.00
April	33.11	15.05	28.74	27.11	16.00	31.00	32.09	19.25	38.08	33.12	15.14	33.11
May	31.00	19.10	26.55	32.00	20.50	30.34	35.01	20.16	34.86	36.08	18.09	32.60

(1)Data were obtained from the Egyptian Meteorological Authority (EMA) station in Assiut University.

Specific procedure

During the 2020/2021 season, seeds from the three initial populations were planted to assess variability within and among them. The populations were grown using a randomized complete block design (RCBD) with four replications. Plants were harvested at the end of the season (April), and data were collected individually, with each plant assigned a number and a code within its respective population. The recorded traits included plant height (cm), number of shoots per plant, number of leaves per plant, stem diameter (cm), spike length (cm), number of spikes per plant, seed weight per plant (g), and days to flowering.

Considerable variability was observed in plant height, ranging from 50 to 100 cm. Based on this variation, plants were classified into three height categories: short (50–69 cm), medium (70–89 cm), and tall (90–110 cm). Analysis of variance results indicated that two populations (Chia 1 and Chia 2) were unsuitable for further study; therefore, only Chia 3 (imported from Argentina) was retained for continued evaluation. From this population, ten plants from each height category were randomly selected and designated as parents for a progeny test. Offspring of each parent were grown in 2 × 1 m plots (experimental units) under RCBD, with data collected from three to four randomly chosen plants per replication.

2021/2022: Offspring were evaluated against their parents, and ten new plants were selected from the progeny to serve as parents for a second progeny test.

2022/2023: The second progeny test was conducted using the selected offspring. The initial population was simultaneously grown to select elite lines for multiple traits using the tandem selection procedure. A second initial population was also established to perform negative tandem multi-trait mass selection against plant height categories.

2023/2024: The tandem multi-trait-derived lines were evaluated against the control for morphological, agronomic, and selected chemical traits. In addition, bulk lines derived from negative selection across the plant height categories were analyzed.

Statistical process and breeding parameters

The validity of the data was tested using graphical analyses and histograms of frequency distribution. The basic statistical parameters for populations, including the range, mean, variance, standard deviation (SD), standard error (SE), skewness, and kurtosis, were investigated. Based on the variance component of the expected mean squares (Steel and Torrie, 1960) relevant to the RCBD design (Gomez and Gomez, 1984), genotypic and phenotypic variances (Vg and Vp) were estimated. Genotypic variance $(\delta^2\mathrm{g}) = \frac{(\mathrm{MSg} - \mathrm{MSe})}{r}$ and phenotypic variance $(\delta^2\mathrm{ph}) = (\delta^2\mathrm{g} + \delta^2\mathrm{e})$, where $\delta^2 e = \frac{\mathrm{MSe}}{r}$, MSg is the means square of genotypes, MSe is the mean square of the residual (error), and r is the number of replications; $\mathrm{VP} = \mathrm{VG} + \mathrm{VE}$.

The broad sense heritability was calculated as $\mathrm{H}^2 = \frac{\mathrm{Vg}}{Vp}$ (Mather, 1949). The phenotypic coefficient of variation (PCV) was calculated as described by (Burton, 1952) $[\frac{(phenotypic\ variance)^{1/2}}{\bar{x}G}] \times 100)$ Where, $\bar{\mathrm{x}}_{\mathrm{G}}$ is the grand mean of all genotypes. Genotypic coefficient of variation (GCV) = $[\frac{(Genotypic\ variance)^{1/2}}{\bar{x}G}] \times 100$. Environmental coefficient of variation (ECV) $= \frac{[(VE)^{1/2}}{\bar{x}G}] \times 100$. Heritability values were categorized as follows: 0 – 30% = low; 31 – 60% = moderate; and > 60% = high (Robinson et al., 1949). PCV and GCV were categorized according to Sivasubramaniam and Madhava Menon (1973) , into three levels as 0 – 10% = low; 11 – 20% = moderate; and > 20% = high. The phenotypic correlation $(r_{\mathrm{p}}\,\mathrm{xy}) = \frac{Cov\ p\ XY}{\tilde{\mathrm{o}}p\ X} \times \delta\mathrm{p}\,\mathrm{Y}$, where $\mathrm{Cov\ p\ XY}$ is phenotypic covariance of traits x and y, and $\delta\mathrm{pX} \times \delta\mathrm{pY}$ is the product of the square root of phenotypic variances of traits x and y, respectively. The genetic correlation $(r_{\mathrm{p}}\,\mathrm{xy}) = \frac{\mathrm{Cov\ g\ XY}}{\tilde{\mathrm{o}}g\ X} \times \delta\mathrm{g}\,\mathrm{Y}$, where $\mathrm{Cov\ g\ XY}$ is genetic covariance between traits x and y, and $\delta\mathrm{g}\,\mathrm{X} \times \delta\mathrm{gY}$ is the product of the square root of genetic variances of traits x and y, respectively (Miller et al., 1958; Searle, 1961; Kashiani and Saleh, 2010).

Differences between parents and offspring were determined using Student's *t test*. Narrow sense heritability (h^2) was estimated by parent-offspring simple linear regression $(\mathrm{y} = \mathrm{bx} + \mathrm{a})$ method (Hasan, 1991). Expected selection response (R) was estimated (Falconer, 1996; Allard ,1999) as $\mathrm{R} = \mathrm{i} \times \mathrm{h}^2 \times \delta\mathrm{P}$, where i is standardized selection differential (tabulated for any given selected proportion for any trait that is normally distributed, so it is not specific for a trait or a population), h^2 is the heritability in narrow sense and δP is the phenotypic standard deviation.

The correlated response to selection was estimated as $\mathrm{Cry} = \mathrm{i} \times \mathrm{h}^2\mathrm{xh}^2\mathrm{y} \times \mathrm{rg} \times \delta\mathrm{Py}$, where i is the standardized selection differential for traits x, hx and hy are the square roots of narrow sense heritability of traits x and y, rg is genetic correlation between x and y and δP y is the phenotypic standard deviation for y (Falconer, 1996). Relative efficiency of indirect selection (E) is calculated (Gizaw et al., 2016) as the ratio of CRy and Rx ; $\mathrm{E} = \frac{\mathrm{CRy}}{Rx} = \mathrm{r_g} \times \frac{\mathrm{hy}}{hx}$ where CRy is the correlated response of trait y and Rx is direct response of trait x, rg is genetic correlation between the two traits, hy and hx are square roots of narrow sense heritability for the secondary trait (y) and primary trait (x), respectively. Lopes et al. (2002) suggested that the indirect selection for traits can be performed when their correlation coefficient values with the desired trait are higher than 0.50. The correlation coefficient is considered weak (less than 0.50), moderate (± 0.50 to ± 0.69) and strong (± 0.70 to ± 0.89) and very strong (± 0.90) (AlBallat and Al-Araby, 2019).For true breeding-derived lines and bulk lines. The means were separated using Duncan's multiple range test or the least significant ($\mathrm{LSD}_{0.05}$) test ($P \leq 0.05$) where appropriate.

Statistical analysis

Statistical analyses were performed using MSTAT-C software (version 2.1). Mean comparisons were conducted using Duncan's multiple range test at the 5% probability level. Graphical representations of the data were generated using Microsoft Excel (Microsoft Inc., 2007).

Results

Data validity and total variance analysis of the studied traits in the initial Chia populations

Genetic variation is a prerequisite for achieving progress through breeding. To assess this, the three initial chia populations were subjected to an F-test (Abbas et al. 2019) (data not shown). No significant differences among the populations were detected for any of the studied traits, with the exception of spike length. Based on these results, only one population (Chia 3, imported from Argentina) was selected for further evaluation.

Progeny test, parent-offspring regression and expected direct selection response.

The means of the studied traits for the selected parents and their offspring across the different plant height classes are presented in Tables 3–6. The corresponding values of the simple linear regression coefficients for the parent–offspring regression are shown in Figures 1–4. Expected selection response values were also estimated and are presented in Table 7.

Table 3. Performance of studied traits (plant height (cm) and shoot count/plant) for tall, medium and short stem parental chia plants used in progeny test.

		Plant height (cm)	
		Tall plants (cm)	
Lines	Parent	Offspring-1	Offspring- 2
Mean	91.70 ± 0.47	93.56 ± 0.78	93.03 ± 0.38
Student's t test		2.03	0.61
Significances 0.05		ns	ns
		Medium plants (cm)	
Mean	75.90 ±1.01	76.33± 0.57	76.82± 0.57
Student's t test		0.38	0.60
Significances 0.05		ns	ns
		Short plants(cm)	
Mean	56.70 ± 1.52	58.40±1.09	59.105 ± 0.99
Student's t test		0.91	0.48
Significances 0.05		ns	ns
		Shoots count / plant	
Lines		Tall plants	
	Parent	Offspring-1	Offspring- 2
Mean	19.90 ± 0.82	19.32± 0.13	19.44± 0.11
Student's t test		0.683	0.648
Significances 0.05		ns	ns
		Medium plants	
Mean	17.30 ± 0.73	17.15± 0.17	17.211± 0.18
Student's t test		0.191	0.219
Significances 0.05		ns	ns
		Short plants	
Mean	14.50 ± 0.78	13.94± 0.23	14.13± 0.12
Student's t test		0.960	0.719
Significances 0.05		ns	ns

*: significant at 5% level, ns: not significant.

Table 4. Performance of studied traits (leaf count/plant and stem diameter (cm)) for tall, medium and short parental chia plants used in the progeny test.

		Leaf count/ plant	
		Tall plants	
Lines	Parent	Offspring-1	Offspring- 2
Mean	194.3 ± 12.18	186.58 ±7.66	187.17 ± 7.56
Student's t test		0.537	0.056
Significances 0.05		ns	ns
		Medium plants	
Mean	142 ± 6.67	134.65± 3.30	135.55± 3.89
Student's t test		0.988	0.177
Significances 0.05		ns	ns
		Short plants	
Mean	106.30 ± 6.66	116.49 ±1.42	117.02 ± 1.76
Student's t test		1.497	0.235
Significances 0.05		ns	ns
		Stem diameter (cm)	
Lines		Tall plants	
	Parent	Offspring-1	Offspring- 2
Mean	0.88± 0.05	0.87± 0.22	0.90 ± 0.03
Student's t test		0.18	1.09
Significances 0.05		ns	ns

Medium plants					
Mean	0.73 ± 0.025		0.77± 0.016		0.79 ± 0.03
Student’s t test		1.48		0.636	
Significances 0.05		ns		ns	
Short plants					
Mean	0.65 ± 0.032		0.68± 0.006		0.68 ± 0.03
Student’s t test		0.93		0.234	
Significances 0.05		ns		ns	

*: significant at 5% level, ns: not significant.

Table 5. Performance of the studied traits (spike length (cm) and spike count/plant) for tall, medium and short parental chia plants used in the progeny test.

Spike length (cm)					
Tall plants (cm)					
Lines	Parent		Offspring-1		Offspring- 2
Mean	5.96 ± 0.28		5.65 ± 0.155		5.54± 0.114
Student’s t test		0.943		0.589	
Significances 0.05		ns		ns	
Medium plants (cm)					
Mean	5.23 ± 0.34		5.28 ± 0.032		5.38± 0.095
Student’s t test		0.128		0.879	
Significances 0.05		ns		ns	
Short plants(cm)					
Mean	5.42 ± 0.52		5.00 ± 0.08		5.11± 0.12
Student’s t test		0.796		0.767	
Significances 0.05		ns		ns	
Spikes count/ plant					
Lines			Tall plants		
	Parent		Offspring-1		Offspring- 2
Mean	37.5 ± 5.23		41.23±2.20		42.46± 2.16
Student’s t test		0.66		0.399	
Significances 0.05		ns		ns	
Medium plants					
Mean	23.60 ± 2.14		29.59 ± 0.53		29.88 ± 0.25
Student’s t test		2.71		0.494	
Significances 0.05		*		ns	
Short plants					
Mean	15.40 ± 1.32		20.87± 0.38		21.04± 0.58
Student’s t test		3.99		0.237	
Significances 0.05		**		ns	

*: significant at 5% level, ns: not significant.

Table 6. Performance of the studied traits (seeds weight/plant (g) and days to flowering) in tall, medium and short parental chia plants used in progeny test.

Seeds weight / plant (g)					
Tall plants					
Lines	Parent		Offspring-1		Offspring- 2
Mean	17.63 ± 3.47		16.10±1.25		18.56 ± 1.26
Student’s t test		0.42		1.399	
Significances 0.05		ns		ns	
Medium plants					
Mean	11.13 ±1.29		11.74± 0.50		12.48 ± 0.52
Student’s t test		0.44		1.03	

Significances 0.05		ns		ns	
			Short plants		
Mean	6.26 ± 0.84		8.50 ± 0.27		9.04± 0.29
Student's t test		2.55		1.37	
Significances 0.05		*		ns	
			Days to flowering		
Lines			Tall plants		
	Parent		Offspring-1		Offspring- 2
Mean	103.5 ± 2.26		100.85 ± 0.64		100.78 ± 0.45
Student's t test		1.13		0.088	
Significances 0.05		ns		ns	
			Medium plants		
Mean	104 ± 3.41		103.34 ±1.47		101.20 ± 0.89
Student's t test		0.179		1.24	
Significances 0.05		ns		ns	
			Short plants		
Mean	108.40 ± 2.97		106.38 ± 0.44		104.94 ± 0.39
Student's t test		0.673		2.44	
Significances 0.05		ns		*	

*: significant at 5% level, ns: not significant.

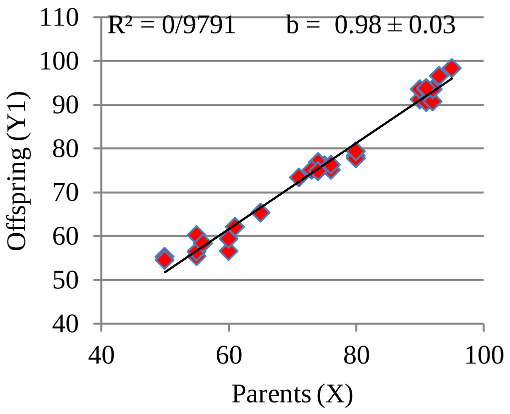
plant
s

Parent– Offspring 1

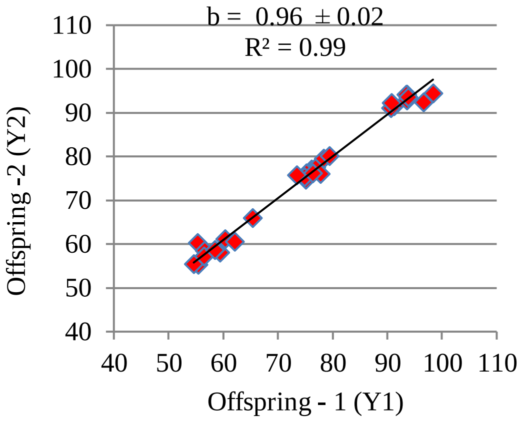
Offspring 1– Offspring 2

Plant height (cm)

Progeny

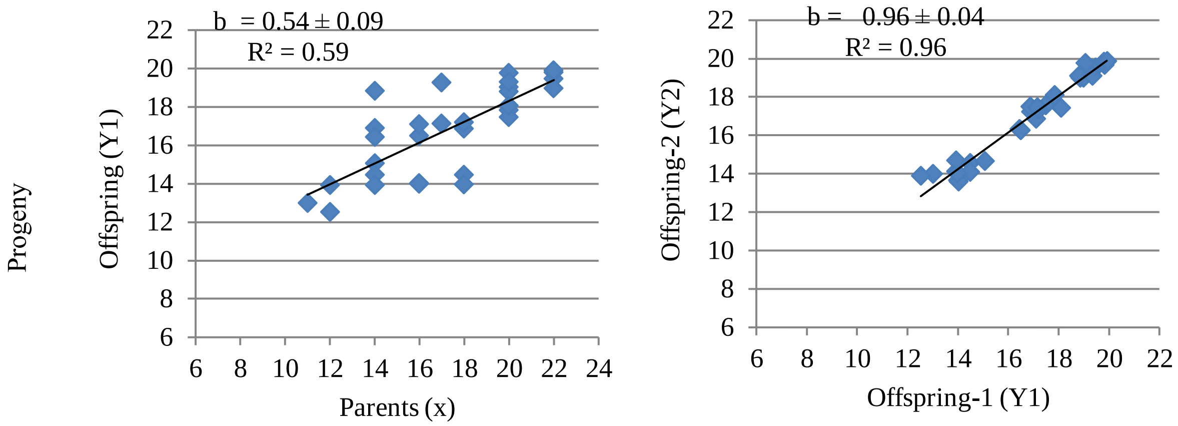


$t_{(0)}$= 32.78** $t_{(1)}$ = 0.67^{ns}



$t_{(0)}$= 48.00** $t_{(1)}$ = 2.00^{ns}

Shoot count/plant

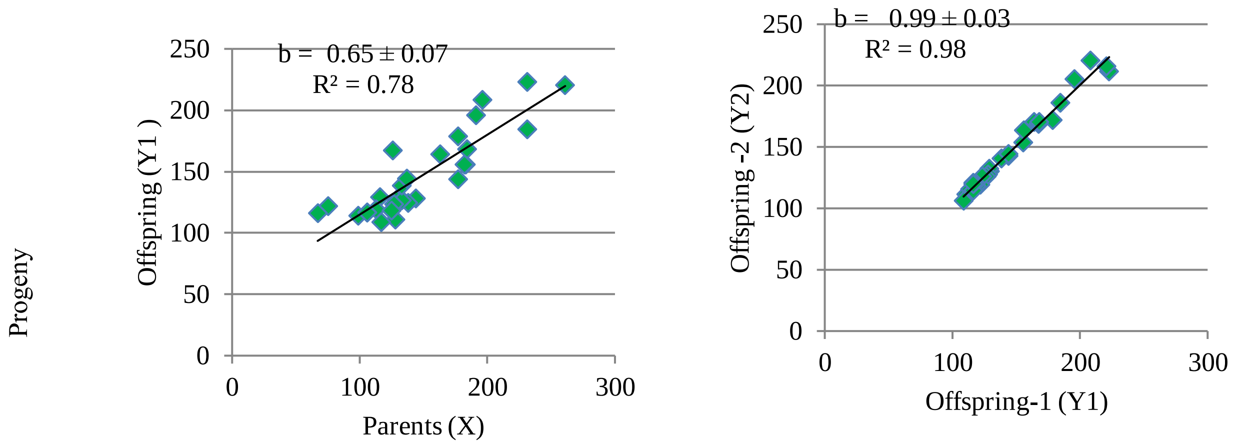


$t_{(0)}$= 6.00** $t_{(1)}$ = 5.11** $t_{(0)}$= 24.00** $t_{(1)}$ = 1.00ns

Fig. 1. Simple linear regression coefficients for the parent-offspring regression. $t_{(0)}$ and $t_{(1)}$: Significance of linear regression.

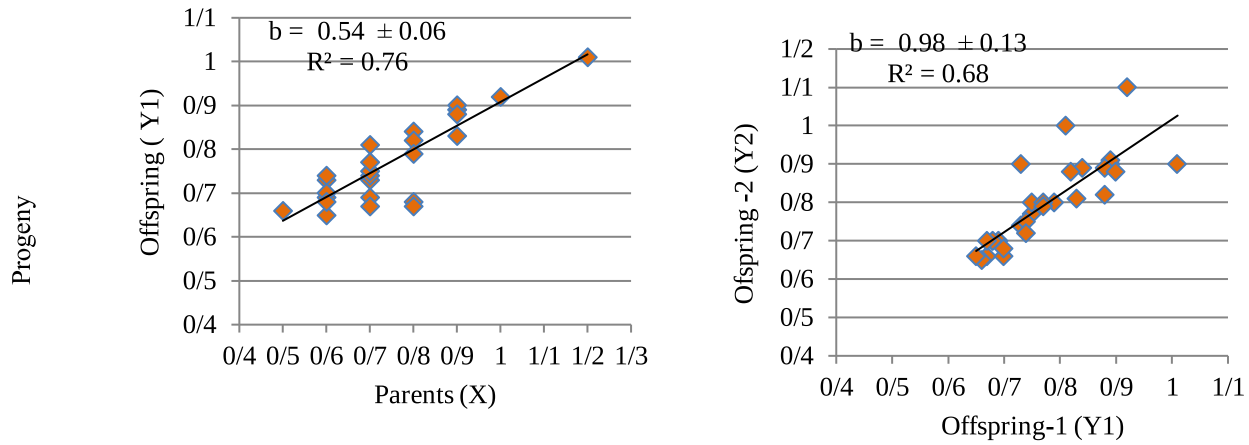
Parent– Offspring 1 Offspring 1– Offspring 2

Plants Leaf count/plant



$t_{(0)}$= 9.29** $t_{(1)}$ = 5.00** $t_{(0)}$= 33.00** $t_{(1)}$ = 0.333ns

Stem diameter (cm)

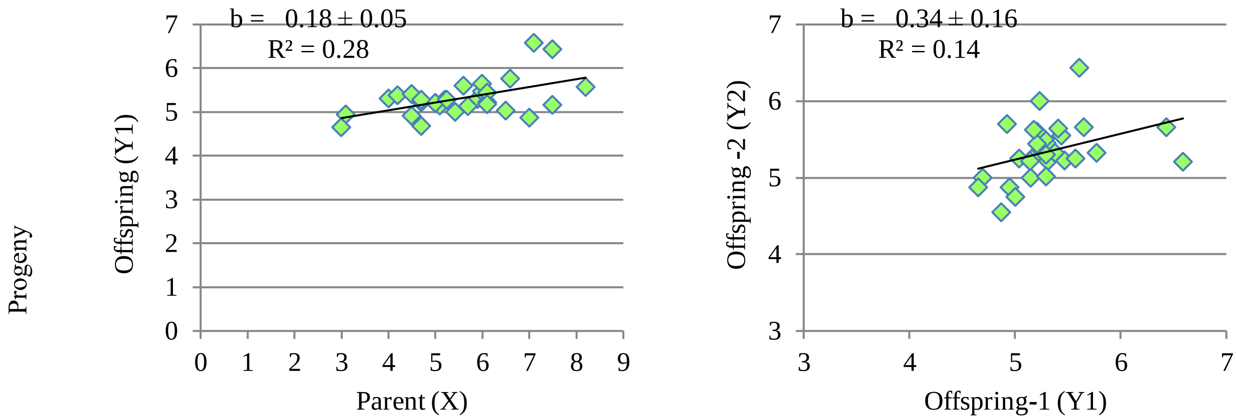


$t_{(0)}= 9.00^{**}$ $t_{(1)} = 7.67^{**}$ $t_{(0)}= 7.54^{**}$ $t_{(1)} = 0.154^{ns}$

Fig. 2. Simple linear regression coefficients for the parent-offspring regression. $t_{(0)}$ and $t_{(1)}$: Significance of linear regression.

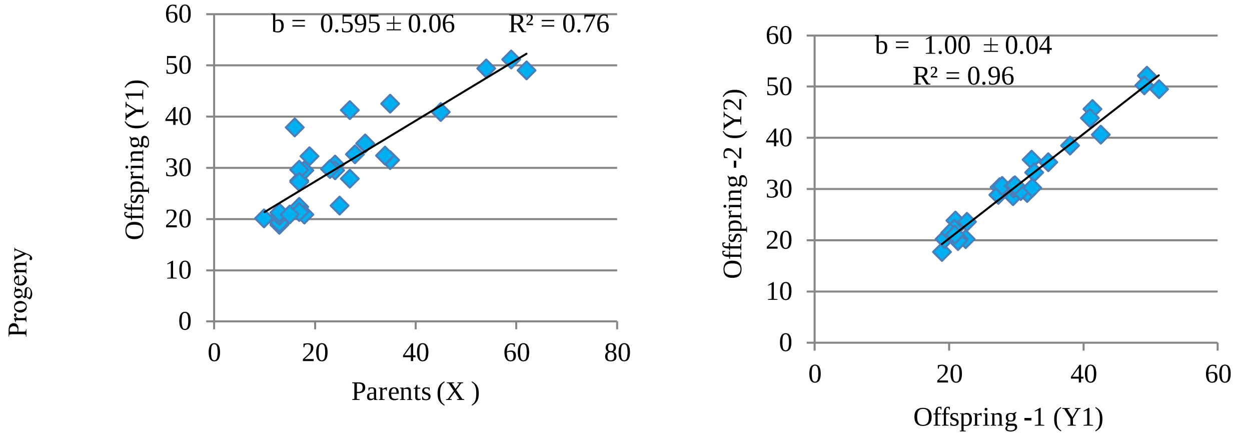
Parent– Offspring 1   Offspring 1– Offspring 2

plants   Spike length (cm)



$t_{(0)}= 3.6^{**}$ $t_{(1)} = 16.4^{**}$ $t_{(0)}= 2.13^{ns}$ $t_{(1)} = 4.13^{**}$

Spike count/plant

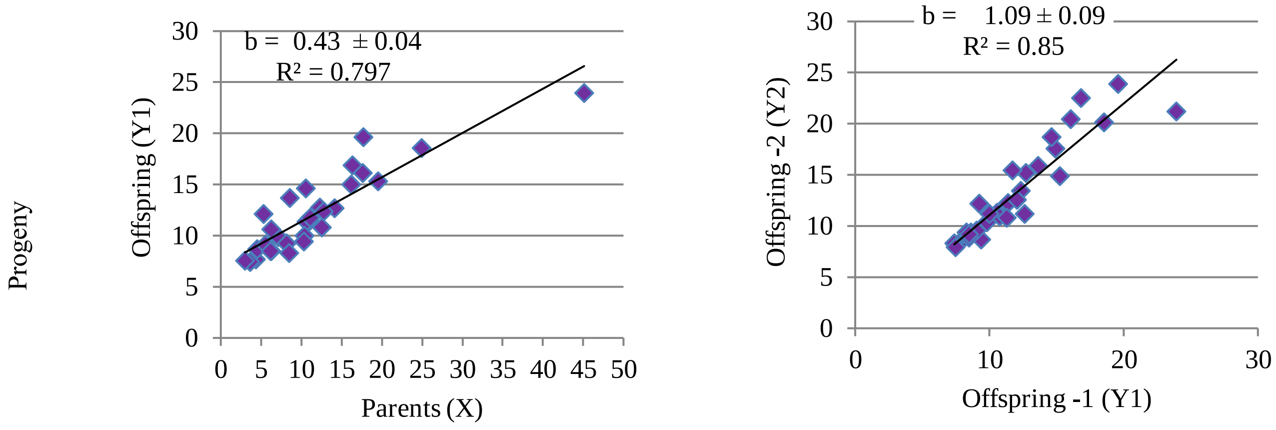


$t_{(0)}$= 9.93** $t_{(1)}$ = 6.73** $t_{(0)}$= 25.00** $t_{(1)}$ = 0.00^{ns}

Fig. 3. Simple linear regression coefficients for the parent-offspring regression. $t_{(0)}$ and $t_{(1)}$: Significance of linear regression.

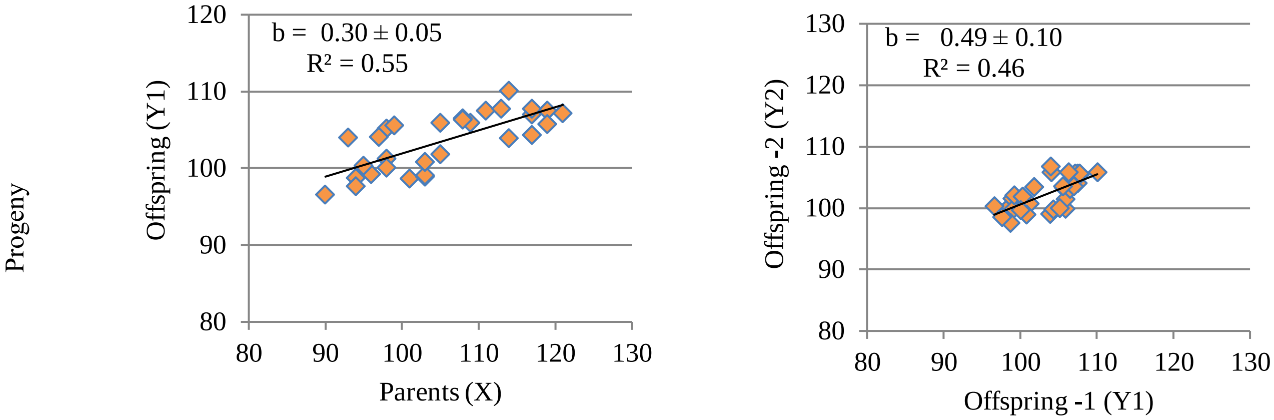
Parent– Offspring 1 Offspring 1– Offspring 2

plants Seeds weight/plant (g)



$t_{(0)}$= 10.75** $t_{(1)}$ = 14.25** $t_{(0)}$= 12.00** $t_{(1)}$ = 1.00^{ns}

Days to flowering



$t_{(0)} = 6.00^{**}$ $t_{(1)} = 14.00^{**}$ $t_{(0)} = 4.9^{**}$ $t_{(1)} = 5.10^{**}$

Fig. 4. Simple linear regression coefficients for the parent-offspring regression. $t_{(0)}$ and $t_{(1)}$: Significance of linear regression.

Table 7. Expected direct selection responses (R) following a cycle of direct mass selection in chia population.

Trait$^{(1)}$	**Selection Intensity**$^{(2)}$	
	5%	10%
Plant height (cm)	27.54	23.53
Shoot count/plant	4.30	3.67
Leaf count/plant	61.06	52.17
Stem diameter (cm)	0.26	0.223
Spike length(cm)	0.55	0.469
Spike count/plant	17.59	15.031
Seed weight/plant (g)	10.58	9.037
Days to flowering	5.38	4.595

$^{(1)}$Heritability was estimated as the mean value of regression coefficient in twice repeated simple linear parent-offspring regression analysis. $^{(2)}$Selection intensity (i) 5% = 2.06; 10% = 1.76.

Associations and correlated and indirect selection responses

Data in Table 8 present the phenotypic (r_p) and genetic correlations of the studied traits with seed weight per plant (g) in chia. In this assessment, all traits—except days to flowering—showed a significant phenotypic correlation with seed weight per plant (Table 8). The correlated response (CR) and relative efficiency (E) of indirect selection for the studied traits in the chia population are shown in Table 9.

Possible opportunities for improving chia

Assessment of novel breeding-derived lines.

The performance of the 12 tandem-selected lines is summarized in Tables 10 and 11. The tallest plants were observed in lines 1, 2, and 7. Line 7 produced the highest seed weight per plant. The greatest number of shoots per plant was recorded in lines 1, 2, 3, and 7, while the highest leaf count per plant was observed in lines 1, 3, and 7. Line 7 also exhibited the highest spike count per plant, as well as one of the greatest stem diameters, alongside lines 1 and 3. Spike length was highest in line 8, whereas the earliest flowering (lowest number of days to flower) was recorded in lines 1 and 8.

Table 8. Phenotypic (r_p) and genetic correlations of the studied traits with seed weight/plant (g) in chia.

Chia populations[1]		
Trait[1]	The phenotypic correlations (r_p)	Genetic correlations coefficient (r_g)
Plant height(cm)	0.618^{**}	1.00
Shoot count/plant	0.701^{**}	1.00
Leaf count/plant	0.688^{**}	0.998
Stem diameter (cm)	0.709^{**}	0.989
Spike length (cm)	0.145^{*}	0.781
Spike count/plant	0.862^{**}	1.00
Days to flowering	0.047^{ns}	-1.00

(1)Number of observations (n) =365.

Table 9. Correlated response (CR) and relative efficiency (E) of indirect selection in the studied chia population.

Traits	**Parameters**		
	CR(1) 5 %(2)	CR(1) 10%(2)	E(1)
		Primary trait seeds weight/plant (g)	
Plant height (cm)	24.38	20.83	1.13
Shoot count/plant	4.32	3.69	1.00
Leaf count/plant	58.72	50.16	1.04
Stem diameter (cm)	0.26	0.22	0.99
Spike length (cm)	0.73	0.62	0.46
Spike count/plant	17.18	14.68	1.02
Days to flowering	- 7.46	-6.37	- 0.72

(1)Heritability was estimated as the mean value of regression coefficient in simple linear parent-offspring regression analysis repeated twice. (2)Selection intensity (i) 5% = 2.06; 10% = 1.76.

Table 10. Performance of new pure lines derived from the initial population of chia (plant height, seeds weight/plant (g), shoots and leaf count/plant).

Pure developed lines(1)	**Plant height (cm)**	**Seeds weight/plant (g)**	**Shoot count/plant**	**Leaf count/plant**
Line -1	103.05^{a}	25.75^{ab}	20.90^{a}	183.00^{ab}
Line -2	100.50^{a}	22.00^{c}	21.05^{a}	176.6^{bc}
Line -3	90.40^{c}	21.75^{c}	20.70^{a}	178.85^{ab}
Line -4	61.48^{d}	10.49^{e}	15.90^{d}	105.50^{fg}
Line -5	59.95^{d}	7.21^{f}	16.05^{d}	116.70^{ef}
Line -6	57.100^{e}	6.14^{f}	13.45^{ef}	97.10^{g}
Line -7	101.85^{a}	26.80^{a}	20.25^{a}	192.30^{a}
Line -8	90.35^{c}	24.50^{b}	19.10^{b}	165.00^{c}
Line -9	95.00^{b}	20.25^{c}	18.40^{bc}	131.75^{d}
Line -10	60.10^{d}	10.10^{e}	13.48^{ef}	92.11^{g}
Line -11	60.00^{d}	7.29^{f}	13.95^{e}	96.25^{g}
Line -12	59.26^{de}	5.94^{f}	12.87^{f}	97.58^{g}
Control(2)	84.150^{f}	12.56^{d}	17.80^{c}	127.00^{de}

(1) Mean differences were separated using Duncan's multiple range test at 0.05 probability level. (2)Sample from the initial population.

Table 11. Performance of new pure lines derived from the initial population of chia (spike count/plant, days to flowering, stem diameter and spike length (cm)).

Developed pure lines(1)	Spike count/plant	Days to flowering	Stem diameter (cm)	Spike length (cm)
Line -1	43.90^{b}	98.40^{fg}	1.045^{a}	6.00^{b}
Line -2	35.50^{d}	99.45^{efg}	0.960^{b}	5.54^{cd}
Line -3	38.40^{cd}	100.00^{ef}	1.015^{a}	5.79^{bc}
Line -4	20.85^{g}	113.35^{ab}	0.620^{e}	4.97^{e}
Line -5	19.05^{g}	100.20^{e}	0.650^{e}	5.62^{cd}
Line -6	18.50^{g}	104.50^{c}	0.555^{f}	3.64^{g}
Line -7	47.65^{a}	106.10^{b}	1.025^{a}	5.16^{e}
Line -8	41.10^{bc}	98.05^{g}	0.795^{cd}	6.48^{a}
Line -9	36.30^{d}	104.45^{c}	0.790^{d}	4.59^{f}
Line -10	30.04^{ef}	100.45^{e}	0.605^{e}	5.10^{e}
Line -11	26.40^{f}	103.90^{cd}	0.615^{e}	5.04^{e}
Line -12	19.56^{g}	103.70^{cd}	0.662^{e}	5.47^{d}
Control(2)	30.50^{e}	102.45^{d}	0.835^{c}	5.79^{bc}

(1) Mean differences were determined using Duncan's multiple range test at 0.05 probability level. (2) Sample from the initial population.

Assessment of different bulk true breeding lines

Data in Table 12 present the mean performance of the different bulk lines derived from the initial chia population. The results indicated that bulk lines composed of tall plants consistently exhibited the highest values across all studied traits, followed by bulk lines of the medium-height category and then those combining tall and medium plants.

Table 12. Mean performance of different bulk- lines derived from the initial population of chia.

Bulk- lines(1) categories	Plant height (cm)	Seeds weight/plant (g)	Shoot count/plant	Leaf count/plant
Control (initial population)	78.002^{c}	13.248^{bc}	16.767^{b}	144.833^{bc}
Tall	94.235^{a}	18.815^{a}	18.085^{a}	168.073^{a}
Medium	74.005^{d}	11.885^{c}	16.697^{b}	130.147^{c}
Short	57.198^{e}	6.000^{d}	13.635^{c}	101.142^{d}
(tall + medium)	84.105^{b}	15.330^{b}	17.395^{ab}	149.138^{b}
	Stem diameter (cm)	Spike length (cm)	Spike count/plant	Days to flowering
Control (initial population)	0.782^{bc}	4.612^{c}	31.550^{b}	103.615^{a}
Tall	0.907^{a}	5.287^{a}	38.868^{a}	100.485^{b}
Medium	0.735^{c}	4.603^{c}	30.605^{b}	104.655^{a}
Short	0.590^{d}	4.778^{bc}	19.034^{c}	104.122^{a}
(tall + medium)	0.822^{b}	4.945^{b}	34.705^{ab}	102.590^{ab}

(1) Mean differences were determined using the least significant difference test (LSD0.05).

Discussion

Progeny test, parent-offspring regression, and expected direct selection response

Chia is a self-pollinated and self-fertilized plant. Successive selfing increases homozygosity by about 50% in each generation compared with the previous one. Under continuous self-pollination and fertilization, the population eventually becomes a composite of pure (true-breeding) lines.

In this study, a progeny t-test was employed to assess the genetic purity of plants selected within the population. Progeny testing is a common breeding method that relies on evaluating the phenotype of an individual's offspring to inform genetic selection. For traits with high heritability, simpler approaches such as selection based on an individual's own performance may be sufficient. In this investigation, with few exceptions, no significant differences were observed between parents and their progenies.

Although progeny testing is typically applied to quantitative traits, it also has an important role in selective breeding as a form of "test mating" to identify carriers of recessive alleles when a gene exhibits complete dominance. In the present study, data in Tables 3–6 show the mean values of the studied traits for selected parents versus their offspring, classified according to different plant height categories. Plant height was chosen as the principal classification factor because it is easily distinguished visually and is often correlated with seed yield (AlBallat and Al-Araby, 2019).

The results revealed high homozygosity not only for stem height but also across other growth and agronomic traits. In self-pollinated and self-fertilized plants, the regression coefficient directly reflects the narrow-sense heritability coefficient (Hasan, 1991). Heritability values are typically categorized as low, moderate, or high (Robinson, Comstock et al., 1949). In this study, nearly all traits showed moderate to high heritability coefficients.

As expected, the estimated selection response was higher at a 5% selection intensity compared with a 10% intensity. Notably, plant height, leaf count per plant, spike count per plant, and seed weight per plant all demonstrated high expected selection responses under mass selection in chia.

Associations, correlated and indirect selection responses

Seed yield is a complex trait controlled by both major and minor genes and strongly influenced by environmental variation (Ejara, Mohammed et al., 2017). Moreover, it is often difficult to measure accurately. For this reason, indirect selection through a simply inherited trait that is strongly correlated with seed yield can provide an efficient alternative for effective breeding.

Genetic correlation arises through gene linkage and/or pleiotropy. Pleiotropy is particularly valuable because a single gene can affect multiple traits simultaneously, producing a genotypic correlation between traits. This enables the possibility of selecting for several traits at once when only one is directly targeted (Falconer, 1996). Since genetic correlations are heritable, they represent a powerful tool for plant breeders.

In the present study, all traits examined, except days to flowering, showed a significant phenotypic correlation with seed weight per plant (Table 8). Interestingly, at the genotypic level, *all* traits exhibited strong to very strong associations with seed weight. These correlation results, together with estimates of heritability and the presence of genetic variability, were clearly reflected in the parameters of correlated response and the relative efficiency of indirect selection (Table 9) (AlBallat and Al-Araby, 2019).

Selection for seed weight had measurable effects on all other studied traits, with varying degrees of correlated response. Among them, plant height, shoot count per plant, leaf number per plant, stem diameter, and spike count per plant appear particularly useful for indirect selection aimed at improving seed weight (Table 9).

Possible opportunities for improving chia

Assessment of novel breeding-derived lines

As noted earlier, the initial population can be regarded as a composite of pure lines. The performance of 12 tandem-selected lines is presented in Tables 10 and 11. Among these, Lines 1 and 7 emerged as the most elite genotypes, ranking highest in 6 of the 8 evaluated traits. These lines could be directly utilized for chia production following stability testing, or alternatively, serve as valuable parental material in cross-breeding programs (Cahill and Ehdaie, 2005).

Assessment of different bulk true breeding lines

Negative selection-derived bulk lines were also evaluated (Table 12). This approach is relatively straightforward in practice and offers strong genetic buffering capacity against environmental fluctuations. The mean performance of the bulk lines indicated that tall-plant bulk lines consistently ranked highest across all traits studied, followed by bulk lines derived from the medium-and-tall category combination (Ejara et al., 2017).

Conclusion

Twelve true-breeding chia lines were developed following a tandem multi-trait selection procedure. Among these, two lines (Lines 1 and 7) appear particularly promising for the genetic improvement of chia. These elite lines may be directly utilized in chia production after their stability has been thoroughly tested, or they may serve as valuable parental genetic material in future cross-breeding programs aimed at further enhancing desirable traits. In addition to the tandem-selected lines, negative selection-derived bulk lines may also be employed. This approach is not only practically straightforward but also provides the advantage of high genetic buffering capacity, enabling the plants to better withstand fluctuations in climatic conditions. Considering the promising performance and potential applications of both the tandem-selected lines and the negative selection-derived bulk lines, further comprehensive studies on these strains are strongly recommended in order to evaluate their long-term stability, adaptability across different environments, and overall contribution to chia breeding and production.

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Author Contributions
AT conceived the study; MT performed the experiments and analyzed the data; AM and MM drafted the manuscript. All authors have read and approved the final version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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