Detection of High-Yielding and Orobanche crenata-Tolerant Faba Bean (vicia faba L.) Mutant Lines by Grain Yield, Infestation Severity and Tolerance indices

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Research Article

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Detection of High-Yielding and *Orobanche crenata*-Tolerant Faba Bean (*vicia faba* L.) Mutant Lines by Grain Yield, Infestation Severity and Tolerance indices

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Abstract
In Egypt, both the cultivation area and productivity of faba bean have declined significantly, mainly due to infestation by the parasitic broomrape (*Orobanche crenata Forssk*). Developing high-yielding, *O.crenata*-tolerant varieties represents a logical solution to this problem. This study aimed to evaluate the effectiveness of mutation breeding for enhancing both productivity and resistance to *Orobanche* across four generations (M1-M4) during the 2021-2024 growing seasons. Grain yield components, infestation severity, genetic parameters, and drought indices were used. Genotypes (mutant lines and their parents) were arranged in randomized complete blocks design with three replications. Significant differences ($p \leq 0.05$) were observed among genotypes across generations and a wide genetic variability. Several mutant lines exhibiting superior productivity and reduced infestation severity compared with their parents. High heritability associated with high genetic advance was recorded for all studied traits, except plant height, indicating the predominance of additive gene action and high efficiency of early–generation selection. Principal components analysis (PCA) showed that the first two components (PC1 and PC2) jointly explained 83.8% of the total variance, with three mutant lines (3, 8, and 10) exhibited a wide genetic divergence compared to their parents. Grain yield showed a significant and positive correlation with all studied traits except plant height. Selection parameter indices detected superior mutant lines under infestation conditions. Overall, these findings illustrated that mutagenesis was an effective tool for improving yield and enhancing tolerance to *Orobanche*. The selected mutant lines represent valuable genetic resources for faba bean breeding programs aiming for *O.crenta*-affected environments.
.**Keywords**: Faba bean; Genetic diversity; grain yield , *Orobanche* weed, Biplot Analysis, Tolerance indices

Introduction

Faba bean is an important legume crop in Egypt because it represents a main source of protein, dietary fiber and a number of essential vitamins for human nutrition [1,2]. In addition, faba bean enhances soil fertility by fixing nitrogen from the atmosphere, reducing the use of chemical nitrogen fertilizers [3,4], thereby reducing greenhouse gas emissions from agricultural practices [5]. Despite these advantages, its productivity has been constricted by biotic stresses, particularly infestation by broomrape (*Orobanche crenata Forssk* infestation, family Orobanchaceae), which causes significant grain yield losses in Egypt [6,7].The roots of the faba bean release chemical substances (strigolactones), which stimulate the germination of *Orobanche* seeds present in the soil [8]. This enables the parasite to attach to the host root and absorb water, mineral nutrients, and assimilates, causing severe reductions in plant growth and final grain yield [9,10].Most Egyptian faba bean varieties were highly susceptible to infestation with *Orobanche*. Unfortunately, this persistence of *Orobanche* seeds in the soil makes its management a big challenge, making conventional control ineffective [11]. Although many control methods, including chemical, mechanical, and biological methods, have been used to control *Orobanche* [12,13], none of these methods has achieved satisfactory control under field conditions [14]. In addition, the genetic basis of *Orobanche* resistance in faba bean still weakly understood and requires more studies [15]. Also, the faba bean has a large and complex genome (~13 GB), which has hindered the progress of genetic improvement [1]. Due to these restrictions, infestation severity (IS) is considered one of the best criteria for selecting faba bean lines resistant to *Orobanche* weed, because it reflects the number of *Orobanche* shoots per plant, which is easily detectable and observable in open fields [16,17]. Infestation severity with broomrape can cause 100% of grain yield losses [18,19].Consequently, lines that exhibited low number of *Orobanche* shoots per and superior yield performance, supporting the utility of infestation severity and yield-related traits as selection criteria. The development of high-yielding faba bean varieties with high yield resistance to *O. crenata* represented a sustainable strategy to maintain productivity in affected areas [7,20].In this context, mutation breeding offers a massive approach for generating and detecting a novel allele that may contribute to improved grain yield and high resistance to *Orobanche*. Mutation breeding is an effective tool for increasing the genetic diversity while keeping the master genetic makeup of elite varieties, accelerating the development of improved varieties with broader adaptability [21-23]. Host resistance to parasites may develop either passively or actively through mechanisms involving morphological, physiological, and biochemical characteristics that inhibit parasite attachment and development [24]. Many studies have studied aspects of host-parasite interactions, but none of them have been incompletely explained [25,26]. Biometrical parameters, including phenotypic (PCV%) and genotypic (GCV%) coefficients of variability, heritability (hb%), and genetic advance (GA%) from selection, were estimated to determine genetic control of key traits (additive or non-additive genetic effects). High values of PCV, GCV, hb and GA%, indicating that significant genetic variability was generated and dominant of additive gene action occurred, thereby making selection effective for genetic improvement [27,28]. Principal component analysis, as a multivariate tool, simplifies and analyzes relationships between many traits by reducing them to a smaller set of components, without losing a lot of the variability information. Principal PCA1 explains the majority of the total variance in populations, helping to detect the traits that contribute most to the total variance. Moreover, PCA containing cluster analysis offers an effective tool to detect promising mutant lines under stress conditions, thereby increasing breeding decisions to improve yield and stress tolerance [29-31]. In addition, correlation coefficients are common used to determine the relationships between the studied traits, helping to detection of indirect selection criteria [32]. Breeding for *Orobanche* tolerance in faba bean is inherently complex. Therefore, tolerance indices, including stress tolerance indices, stress tolerance, mean productivity, yield stability index, stress tolerance index, and drought resistance index, were used to detect high-yielding and *Orobanche*-tolerant mutant lines, thereby increasing selection efficiency [33,34]. However, limited information is available on the stability of mutant lines across successive generations under *O. crenata* infestation. Additionally, the effectiveness of early-generation selection based on stress tolerance indices for detecting *O. crenata* -tolerant faba bean varieties is still unclear. Therefore, this study aimed to evaluate the effectiveness of mutation breeding for enhancing both productivity and resistance to *Orobanche*, using yield performance, infestation severity and drought indices to detect and select promising faba bean mutant lines.

Materials & Methods

Plant materials

Three faba bean varieties were obtained from the Agricultural Research Center (ARC), Egypt. These varieties were selected based on grain yield and resistance to *O. crenata* infestation. Table 1 showed the characterizations and pedigree of faba bean varieties.

Table 1: The origin and pedigree of faba bean varieties

Variety	Origin	Pedigree	Characters
Giza-716	Egypt	461/842/83/503/453/83	Hybrid variety, early maturing, branching, resistant to lodging; sensitive to *O. crenata* and 100-seed weight 80–85 g.
Giza-843	Egypt	Giza716 461/845/83/561/2076/85	Hybrid variety; resistant to *O. crenata* and blight; medium branching and 100-seed weight: 60–65 g
Sakha-4	Egypt	81/35/2001 (Shkha 4) derived from Sakha 1 x Giza 3	Sensitive to *O. crenata* and high yield potential

Mutagen

Sodium azide ($NaN3$; SA) was applied as a chemical mutagen. The SA treatments were manufactured at the Field Crops Research Department, Biological and Agricultural Research Institute, National Research Center, Egypt. For each variety, batches of 300 dry and healthy grains (with a 10% moisture content) were presoaked in distilled water for 12 hours to increase mutagen penetration. SA concentrations were prepared in a phosphate buffer (pH of 7.0) to enhance mutagenic efficiency and decrease negative effects on seed viability [35]. Seeds were then treated with three SA concentrations (0.02, 0.03, and 0.04%) for 9 h, with intermittent shaking to ensure proper aeration. The treated seeds were carefully rinsed under running water to remove the mutagenic effect of the mutagen. 300 seeds from each treatment were immediately sown in the field to obtain the M1 generation.

Location characterizations

Experiments were conucted in Abshish village (28°59'18.2"N 31°04'43.0" E), Beni Suef Governorate, Egypt. This region was highly infested with *Orobanche*. As shown in Fig.1, the location characteristics that promoting infested with *Orobanche* [11]. Seeds of *Orobanche* remain viable in the soil for more than ten years [36].In addition, the most faba bean verities in Egypt more sensitive for *Orobanche.*

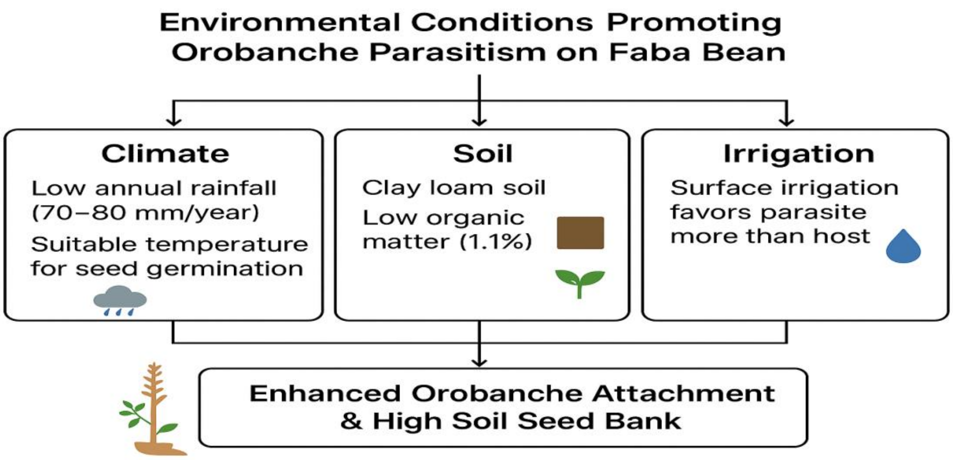

Fig.1 . Environmental conditions that enhance parasitism of *Orobanche* weed on faba bean

Agricultural practices

The soil was ploughed before cultivation, and basal fertilizers were added for sowing, consisting of 150.0 kg of superphosphate, and 125.0 kg of potassium sulphate. Sowing was done in the second half of October, using three seeds per hill. A total of 2,700 bean seeds (300 seeds of each variety with three SA concentrations) were treated and sown, along with untreated seeds, to get the M1 generation. Seeds were planted as of 70.0 cm between rows, 60 cm row width, and 25.0 cm within rows, with three rows per plot, resulting in a total plot area of 5.4 m².

Data Collection and *Orobanche* measurement

Plant height (PH) (cm), branches per plant (NB), pods per plant (PP), grain yield (t ha^{-1}) under *Orobanche* -free infested soils (GY), and grain yield under *Orobanche*-infested soils (GYS). Infestation severity (IS) per plant was recoded based on the number of shoots on the ground. At harvest, the number of *Orobanche* shoots per plant on the ground was recorded to estimate infestation severity (IS).

M1 generation (2021 season)

Mutant lines were harvested when lower pods turned from green to dark brown and the upper pods changed from green to yellow, then brown. Surviving seeds from individual plants were collected and stored to establish the M2 generation. The commercial variety, Sakha 4, which was previously classified as sensitive to *Orobanche* [37], was used as a susceptible control.

M2 generation (2022 season)

Genotypes (mutant lines and mother varieties) were evaluated under *Orobanche* -free and infested soils. Based on the studied traits, twenty-three promising mutant lines were selected, harvested separately, and stored to get the M3 generation.

M3 generation (2023season)

Genotypes were evaluated as in the previous generation. Sixteen high-yielding mutant lines exhibiting resistance to *Orobanche* were detected, harvested separately, and stored to obtain the M3 generation.

M4 generation (2024 season)

Genotypes were evaluated as in the previous generation. Ten high-yielding and resistant to *Orobanche* mutant lines were detected and selected for further study.

Experimental design

Genotypes were arranged in a randomized complete block design (RCBD) with three replicates. Data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using GenStat software.

Statistical parameters

The phenotypic (PCV) and genotypic (GCV) coefficients of variation were estimated according to Burton and Devane [38] . Broad-sense heritability (Hb%), genetic advance (GA%) as a percentage of the mean trait were calculated according to Robinson et al. [39]. Correlation coefficients between studied traits were estimated using Microsoft Excel, and the significance level was tested at (n-2) degrees of freedom, where n represented the number of genotypes**.** Principal component analysis (PCA) was computed using GENSTAT 18th Edition (VSN International,Hempstead, UK).

Stress tolerance indices

Table 2**.** Tolerance indices used for evaluation of grain yield of faba bean genotypes

Tolerance to stress (TOL)	TOL $= YP - YS$	The mutant line with low values of this index is more stable in two different conditions.	Rosielle and Hamblin [40]
Mean productivity (MP)	MP $= \frac{YS+YP}{2}$	The mutant line with high value of this index will be more. desirable	Rosielle and Hamblin [40]
Stress tolerance index (STI)	STI $= \frac{Yp * Ys}{\overline{Yp2}}$	The mutant line with high STI values will be tolerant to stress.	Bouslama and Schapaugh [41]
Yield stability index (YSI)	YSI $= \frac{Ys}{Yp}$	The mutant line with high YSI values can be regarded as stable genotypes under stress and non-stress conditions.	Fernandez [42]
Drought response index (DI)	DI $= YS \times [(YS/YP)\overline{Y}S]$	The genotypes with low values of this index 1≥ more stable.	Mousavi et al. [43]

where, Yp and Ys present the mean yield of genotype *Orobanche* -free and *Orobanche* -infested soils and the mean yield of genotype under stress, respectively. $\bar{Y}p$ and $\bar{Y}s$ were total mean of grain yield for all genotypes under *Orobanche* -free and *Orobanche* -infested soils, respectively.

Results

Increasing the genetic diversity of cultivated varieties is one of the basic pillars to face the challenges and damages resulted from climate change, such as the emergence of harmful diseases and pests. In this study, mutation breeding was applied to faba bean using sodium azide (SA) at three concentrations (0.02, 0.03 and 0.04%) to generate genetic variation. In the M1 generation, plant survival rates decreased with increasing mutagen concentrations (Fig.2).

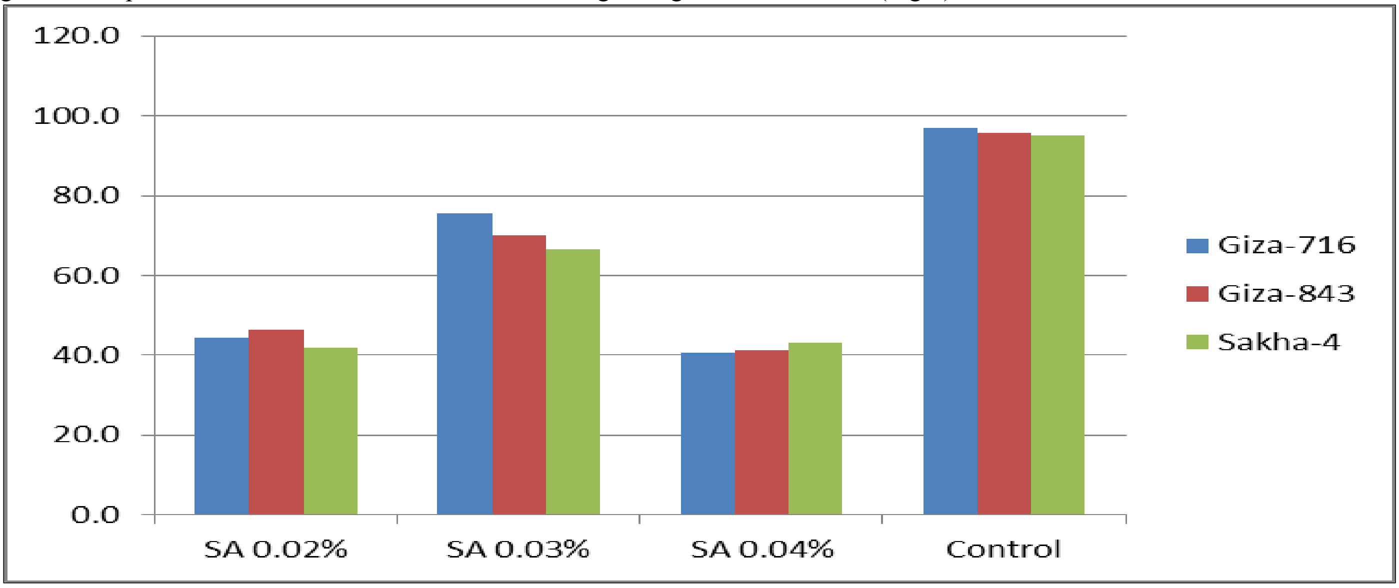

Fig.2. The survival rate % of faba bean varieties after treatment with the three concentrations of the chemical mutagen sodium azide SA

The survival rate of the control plants was 97.1%, whereas survival rates of SA-treated plants ranged from 40.5% % to 75.6%. The optimal concentration was 0.03%, which gave the highest survival rate (75.6%) in the Giza-716 variety, followed by 0.02 and 0.04%. In the M1 generation, the survival plants were cultivated and advanced to produce the M2 generation. In the first segregating generation (M2), 23 mutant lines, along with the three mother varieties, were evaluated under *Orobanche* -free and *Orobanche* -infested soils. As shown in Table 3, the combined ANOVA for grain yield of faba bean mutant lines in the M2 generation under both conditions illustrated highly significant differences ($p \leq 0.05$) among genotypes, environments, and genotypes X environments interactions.

Table 3. Combined analysis of variance for grain yield of faba mutant lines in the M2 generation

Source of variation	Degree of freedom	Sum square	Mean sum square	F -value
Replication	1	0.05	0.05	6.2
Genotypes (G)	25	12.9	0.52	63.2*
Environments (E)	1	82.3	82.3	10025.7*
G*E	25	0.75	0.03	3.6*
Residual	51	0.42	0.01	
Total	103	96.45		

Coefficient of variation (CV%) was 10.2

Table 4 showed the average performance, range, and variance of the genotypes for the studied traits and Infestation severity in the M2 generation.

Table 4. Descriptive statistics of faba bean mutant in the M2 generation for the studied traits

Genotype	PH (cm)	NB	PP	GY(t ha^{-1})	GYS (t ha^{-1})	IS
G1	132.2	18.7	129.3	4.3	2.1	2.0
G2	109.3	14.0	96.4	4.2	2.1	5.0
G3	107.0	15.6	108.0	4.1	2.0	4.0
G4	126.3	19.5	134.9	4.1	1.9	5.0
G5	95.3	17.2	118.7	4.1	2.0	6.0
G6	123.5	17.5	120.7	4.0	2.0	4.0
G7	116.1	16.4	113.1	3.9	1.9	7.0
G8	92.0	15.9	109.5	3.9	1.9	4.0
G9	129.2	11.9	82.2	3.8	1.7	7.0
G10	108.7	15.4	106.0	3.7	1.9	5.0
G11	116.9	14.9	103.0	3.8	2.1	6.0
G12	96.4	14.7	101.4	3.8	1.7	6.0
G13	96.1	12.3	84.7	3.8	1.8	5.0
G14	111.6	11.1	76.2	3.7	1.8	8.0
G15	102.1	11.4	76.1	3.7	1.8	8.0
G16	102.5	11.3	76.3	3.7	1.9	8.0

G17	84.6	11.1	76.6	3.7	2.0	6.0
G18	103.5	10.1	70.0	3.6	1.8	4.0
G19	103.2	10.3	71.0	3.6	1.8	6.0
G20	100.3	10.6	73.0	3.5	1.7	5.0
G21	120.7	11.0	77.1	3.5	1.7	7.0
G22	113.4	10.5	72.5	3.4	1.7	6.0
G23	108.7	10.4	72.0	3.4	1.7	8.0
Mean (x̄)	108.7	13.6	93.4	3.8	1.9	5.7
Range	84.6-142.4	9.7-19.5	66.9-134.9	3.1-4.3	1.7-2.11	2.0-8.0
Statistical analysis						
SL0.05	*459.9	57.0*	1580.1*	0.7*	0.4*	5.1*
CV%	11	6.5	5.9	12	5.8	8.3
LSD 0.05	20	1.5	38.5	0.7	0.55	0.8
The mothers varieties						
Sakha 4	124.5	6	82.8	2.2	1.1	4.0
G-834	129	5	76.9	2.6	1.3	9.0
G-716	140	7	78.1	2.5	1.2	10.0
Mean (x̄)	131.2	6	79.3	2.4	1.2	7.7

plant height (PH) (cm), branches per plant (NB), pods per plant (PP), grain yield (t/ha) under *Orobanche* -free infested soils (GY) , grain yield (t ha^{-1}) under *Orobanche* -infested soils (GYS), Infestation severity (IS):number of shoots of *Orobanche* per mutant line, significant level at 0.05, CV%: coefficient of variation, LSD $_{0.05}$: least significant difference

Highly significant differences (p≤0.05) were found among the mutant lines, accompanied by a wide range of variability for the studied traits. Regarding the average performance for the traits studied, mutant lines (G1-G5) consistently achieved the highest rankings for the traits studied under both under *Orobanche* -free and *Orobanche* -infested soils. As for infestation severity (IS), high significant differences (p≤0.05) were found among the faba bean mutant lines and their parents, with wide range (6.0). The mutant lines clearly outperformed the mother varieties based all studied traits. The mother varieties produced 2.2–2.6 t/ha GY and 1.1–1.3 t/ha GYS, whereas the mutant lines produced >3.5 t/ha GY and ~1.7–2.1 t/ha GYS. The top-performing mutant lines under both conditions were G1-G6, with the highest GY (4.0–4.3 t/ha) and relatively stable GYS (1.9–2.1 t/ha). The mutant line G9, G12, and G13 exhibited moderate GY but lower GYS. In this generation, six mutant lines (G1–G6) showed both tolerance to *Orobanche* infestation and high-yielding. Mutant lines recorded low mean performance for infestation severity (IS) compared to their parents. Fig.3 demonstrated that mutant lines combined high yield potential with a relatively lower yield reduction under *Orobanche* - infested soils.

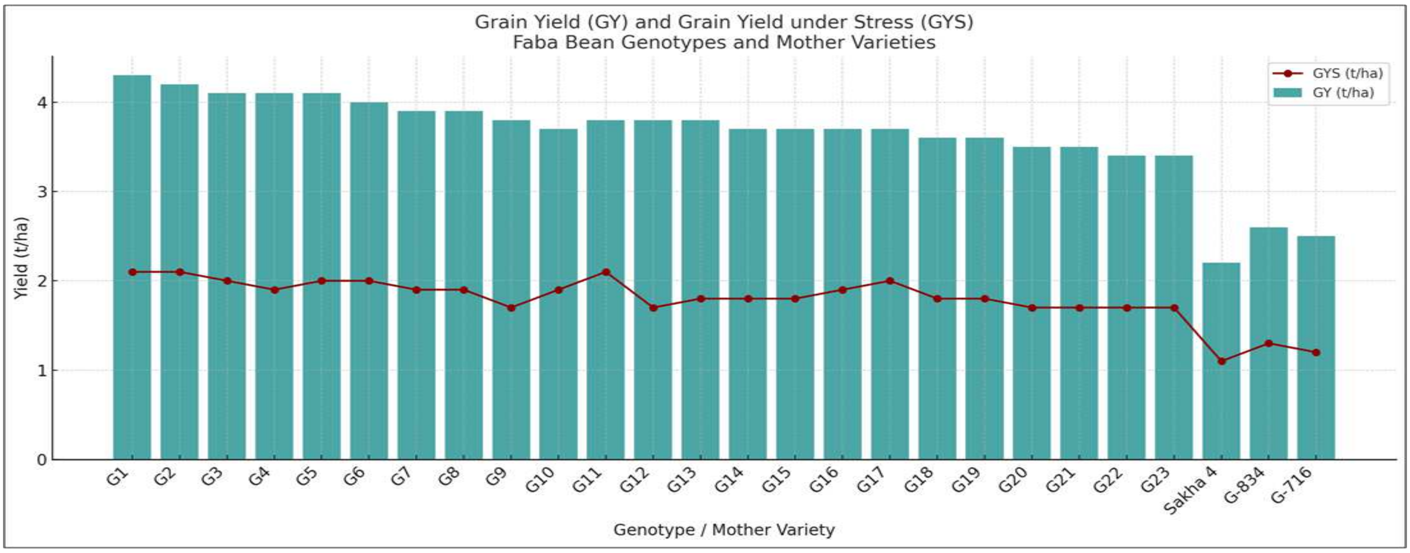

Fig.3. Comparative grain yield of the best-performing bean mutant lines vs their mother varieties under O.crenata -free (GY) and O.crenata -infested soils (GYS) in the M2 generation.

This generation, sixteen mutant lies were selected based on the studied traits and infestation severity to obtain the M3 generation.

M3 generation

The combined analysis for grain yield of the faba bean mutant lines in the M3 generation under *O.crenata*-free and *O.crenata* -infested soils showed highly significant differences among genotypes and environments (Table 5).

Table 5. Combined analysis of variance for grain yield of faba mutant lines in the M3 generation

Source of variation	Degree	Sum square	Mean sum square	F -value
Replication	1	2.26	2.26	76.45
Genotypes (G)	18	5.68	0.31	10.69*
Environments (E)	1	16.24	16.24	549.37*
G*E	18	0.11	0.00647	0.22^{ns}
Residual	37	1.09	0.02957	
Total	75	25.4		

Coefficient of variation (CV%) was 16.6

The environmental effect was the major contributor to total variation, whereas the G × E interaction was non-significant. Residual variance was moderate. The findings in Table 4 showed significant differences ($p < 0.05$) among the mutant lines for the studied traits, accompanied by a wide range of variability (Table 6).

Table 6. Descriptive statistics of faba bean mutant in the M3 generation for the studied traits

Genotype	PH (cm)	NB	PP	GY (t ha^{-1})	GYS (t ha^{-1})	IS
G1	145.2	8.3	100	3.88	2.7	1.6
G2	157.6	5.7	95	3.7	2.6	4.2
G3	148.8	7.1	93.7	3.6	2.3	3.3
G4	118.7	11.7	129.9	3.58	2.25	4.0
G5	117.5	12	131.6	3.57	2.24	5.3
G6	145.5	8.5	99.4	3.55	2.23	3.8
G7	139.7	6	89.9	3.53	2.28	6.0
G8	139.8	6.5	99.3	3.44	2.5	3.5
G9	134.1	10.8	110.8	3.41	2.2	6.2
G10	161.7	4.6	48	3.4	2.15	4.2
G11	156.5	5.2	89.2	3.26	2.11	5.0
G12	159.5	4.2	46.5	3.25	2.1	5.1
G13	153.8	4.4	45.9	3.24	2.08	4.7
G14	145	6.8	89	3.23	2.07	7.1
G15	127.1	5.2	94.3	3.1	2	7.0
G16	123.9	4.7	86.5	3	2	7.2
Mean ($\bar{x}$)	142.2	7.0	90.6	3.4	2.2	4.8
Range	117.5-161.7	4.2-12.0	54.9-131.6	3.0-3.88	2.0-2.7	1.6-7.2
Statistical analysis						
SL0.05	450.0*	66.0*	3000.0*	0.72*	0.30*	0.003*
CV%	12.3	11.0	12.0	13.2	2.7	16.6
LSD 0.05	25.0	2.1	25.0	0.50	0.30	0.9
Mother varieties						
Sakha 4	118.3	5.7	78.7	2.6	1.6	4.9
G-834	122.6	4.8	73.1	3.2	2.0	8.0
G-716	133.0	6.7	74.2	2.7	1.8	9.0
x	124.6	5.7	75.3	2.8	1.7	7.3

Plant height (PH) (cm), branches per plant (NB), pods per plant (PP), grain yield (t/ha) under *Orobanche* -free infested soils (GY), grains yield (t ha^{-1}) under *Orobanche* -infested soils (GYS), infestation severity (IS):number of shoots of *orbanche* per mutant line, SL: significant level at 0.05, CV%: coefficient of variation, LSD 0.05: least significant difference.

Regarding the average performance for the traits studied, mutant lines (G1-G10) consistently achieved the highest rankings under both *Orobanche* -free and *Orobanche* -infested soils. Mutant lines recorded low mean performance for infestation severity (IS) compared to their parents. In this generation, ten mutant lines were selected to obtain the M4 generation (Fig.4).

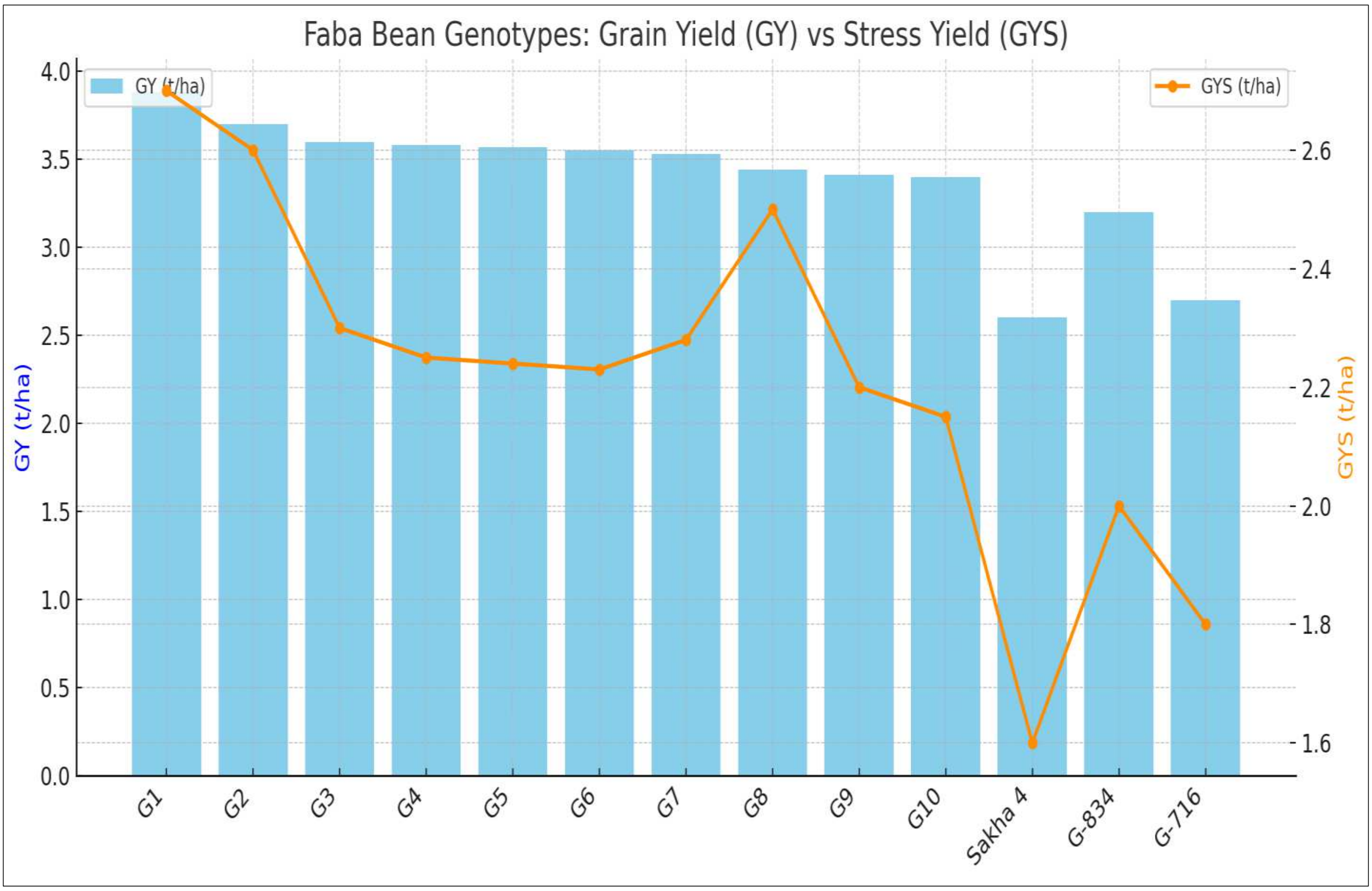

Fig.4.Comparative grains yield of the best-performing bean mutant lines vs their mother varieties under *Orobanche* -free (GY) and *Orobanche* -infested soils (GYS) in the M3 generation**.**

M4 generation

The combined analysis for grain yield of the faba bean mutant lines in the M4 generation under *Orobanche*-free and *Orobanche*-infested conditions revealed significant differences among genotypes and environments (Table 7). In contrast, the genotype × environment interaction (G x E) was non-significant. The small residual variation was found.

Table 7 Combined analysis of variance for grain yield of faba mutant lines in the M4 generation

Source of variation	Degree of freedom	Sum square	Mean sum square	F -value
Replication	1	0.3039	0.30	2.69
Genotypes (G)	12	11.8	0.98	8.73*
Environments (E)	1	7.6	7.6	67.28*
G*E	12	0.22	0.018	0.16 ^{ns}
Residual	25	2.8	0.11	
Total	51	22.7		

Coefficient of variation (CV%) was 13.0%

The findings in Table 8 showed significant differences ($p < 0.05$) among the mutant lines for the studied traits, accompanied by a low wide range of variability for studied traits.

Table 8. Descriptive statistics faba bean mutant in the M4 generation for the studied traits

Genotype	PH (cm)	NB	PP	GY (t ha^{-1})	GYS (t ha^{-1})	IS
G1	139.8	17.0	147.2	4.2	2.8	0.0
G2	130.0	15.6	141.7	4.0	2.4	2.3
G3	162.4	15.3	150.0	3.9	2.7	2.2
G4	139.5	16.1	144.4	3.7	2.6	3.1
G5	154.5	14.2	133.3	3.4	2.3	4.2
G6	125.2	13.8	130.6	3.6	2.2	3.0
G7	125.9	13.3	127.8	3.5	2.3	5.5

G8	166.0	12.8	125.0	3.3	2.3	2.8
G9	161.3	12.4	122.2	3.1	2.2	4.2
G10	152.9	11.0	113.9	3.0	2.1	3.2
Mean ($\bar{x}$)	145.8	14.2	133.6	3.6	2.4	3.1
Range	125.2-166.0	11.0-17.0	113.9-150.0	3.0-4.2	2.1-2.8	2.2-5.5
Statistical analysis						
SL $_{0.05}$	37.8**	45.3**	1950.6**	1.0*	0.45*	0.05
CV%	7.3	8.1	9.4	11	7.1	12.3
LSD $_{0.05}$	19.1	2	21.1	0.8	0.4	1.0
Mother varieties						
Sakha 4	131.0	7.1	88.7	2.8	1.4	4.3
G-834	136.0	6.1	83.0	2.7	1.3	7.8
G-716	139.0	8.0	84.1	2.6	1.3	8.8
	137.5	7.1	85.3	2.7	1.3	7.0

Plant height (PH) (cm), branches per plant (NB), pods per plant (PP), grains yield (t/ha) under *Orobanche* -free infested soil (GY) , grains yield (t/ha) under *Orobanche* -infested soil (GYS), Infestation severity (IS):number of shoots of *Orobanche* per mutant line, SL: significant level at 0.05, CV%: coefficient of variation, LSD $_{0.05}$: least significant difference

Ten mutant lines were detected as promising lines because of superiority in the studied traits compared to mother varieties under *Orobanche* -free and *Orobanche* -infested soils (Fig.5).

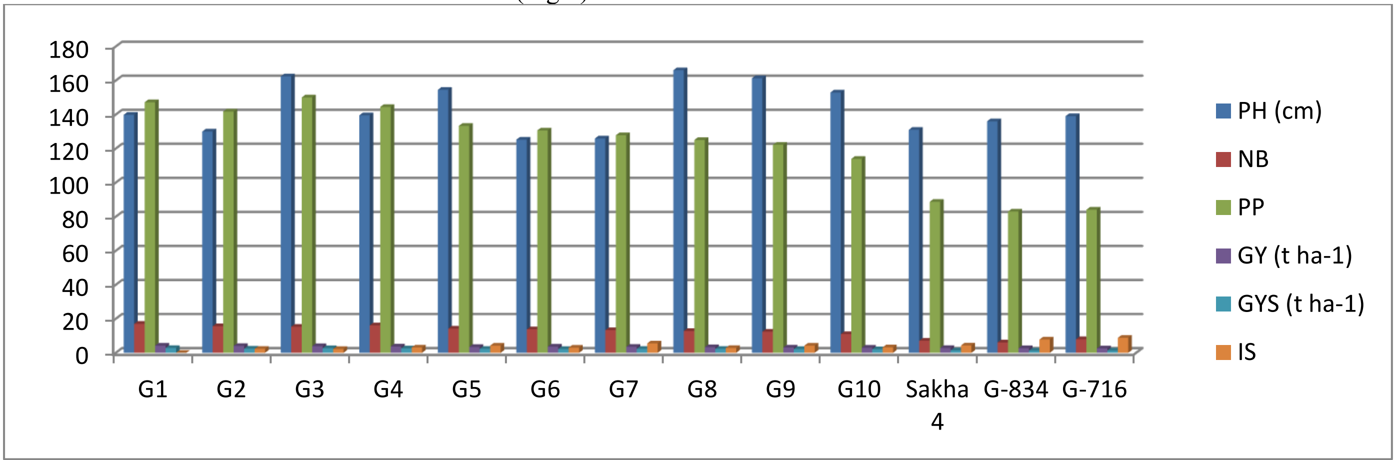

Fig. 5.Comparative studied traits of the best-performing bean mutant lines vs their mother varieties under *Orobanche* -free and *Orobanche* -infested soils in the M4 generation.

3.2. Genetic parameters

The results in Fig.6 showed that variation was presented in that study, as it showed high values for the phenotypic and genetic coefficients of variation, exceeding 20% for the studied traits, except that plant height was moderate (10-20%).

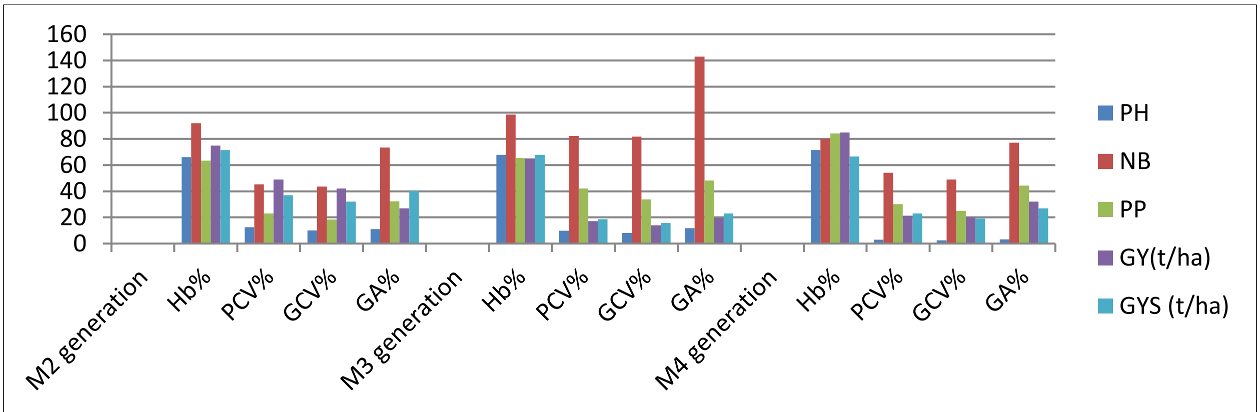

Fig.6 . Estimates of heritability (Hb%),phenotypic (PCV %) and genotypic (GCV %) coefficients of variation and genetic advance as % of the mean (GA %) for quantitative traits in the M2, M3 and M4 generations of faba bean mutant lines.PH: plant height, NB: branches per plant (NB), pods per plant (PP), GYS grains yield under *Orobanche* -free infested soils, GYS (t ha^{-1}): under *Orobanche* -infested soils

The highest values of PCV and GCV values were recorded for NB, followed by PP, GYS and GY. In the M2 and M3 generations, high heritability estimates (60% ≤) were associated with genetic advanced (20%≤) for most studied traits, whereas in the M4 generation, PH showed high heritability (60%≤) but was moderate genetic advance (10-20%). High variability (PCV ≥ 20% and GCV ≥ 20%) was observed for all the studied traits, except for PH, which showed moderate variation (10-20%).In the M3 generation, both GY and GYS showed moderate variability.

Principal component analysis (PCA)

Principal component analysis (PCA) illustrated that PC1 accounted for 61.53% and PC2 for 22.35% of the total variance, which jointly explained 83.88% of the variance (Fig.7).

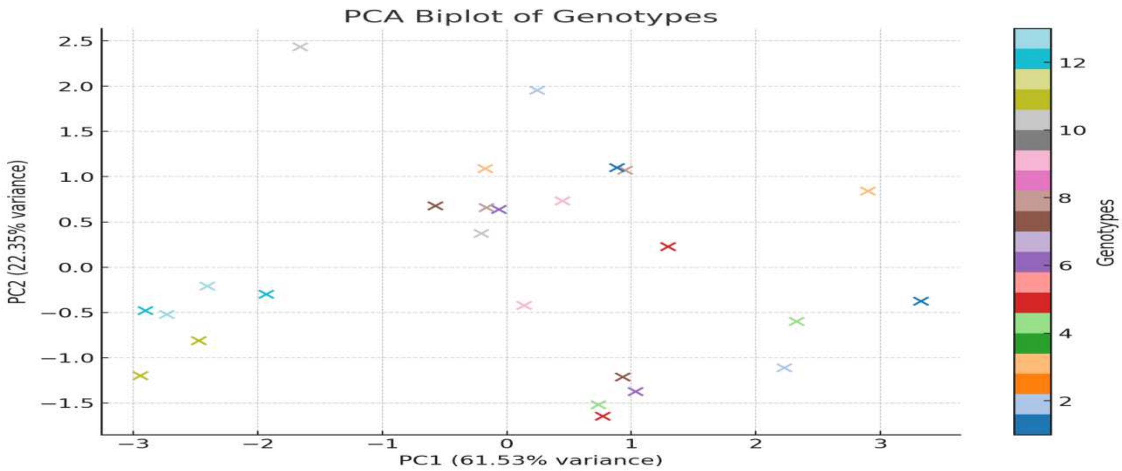

Fig 7. The scatter plot showed genotypes distributed based on similarity across the studied traits.

PC1 was dominated by branches per plant, pods per plant, and grain yield under both *Orobanche* -free and *Orobanche* - infested soils (all around 0.48–0.50). PC2 explained 22.3% of the total variance and was dominated by plant height (0.81), which contributed to the vertical separation in the PCA plot. Three mutant lines (3, 8, 10) were positioned further apart. Fig.8 showed strong relationships between studied traits.

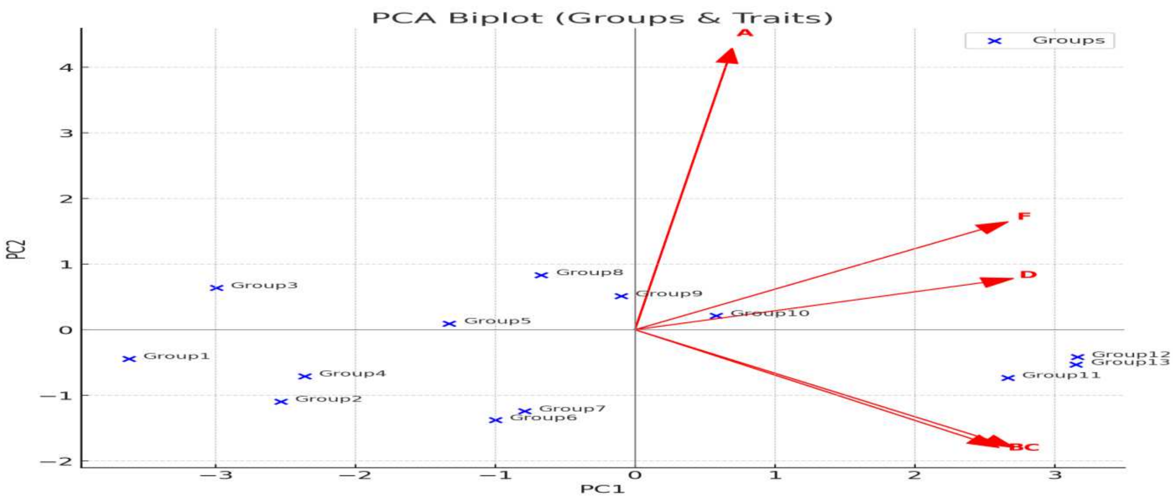

Fig. 8. Showed the correlation among studied traits and the distribution of faba bean genotypes under *O. crenata*-infested conditions

A very strong positive correlation was found between the number of branches/plant (B) and number of pods/plant (C). Grain yield under *O.crenata*-free infested soils showed a positive correlation with grain yield under *Orobanche* -infested conditions (F). Plant height (A) showed a weak or negative correlation with other traits. Under *Orobanche* -infested soils. Fig. 9 showed clear separation between the mutant lines and mother varieties.

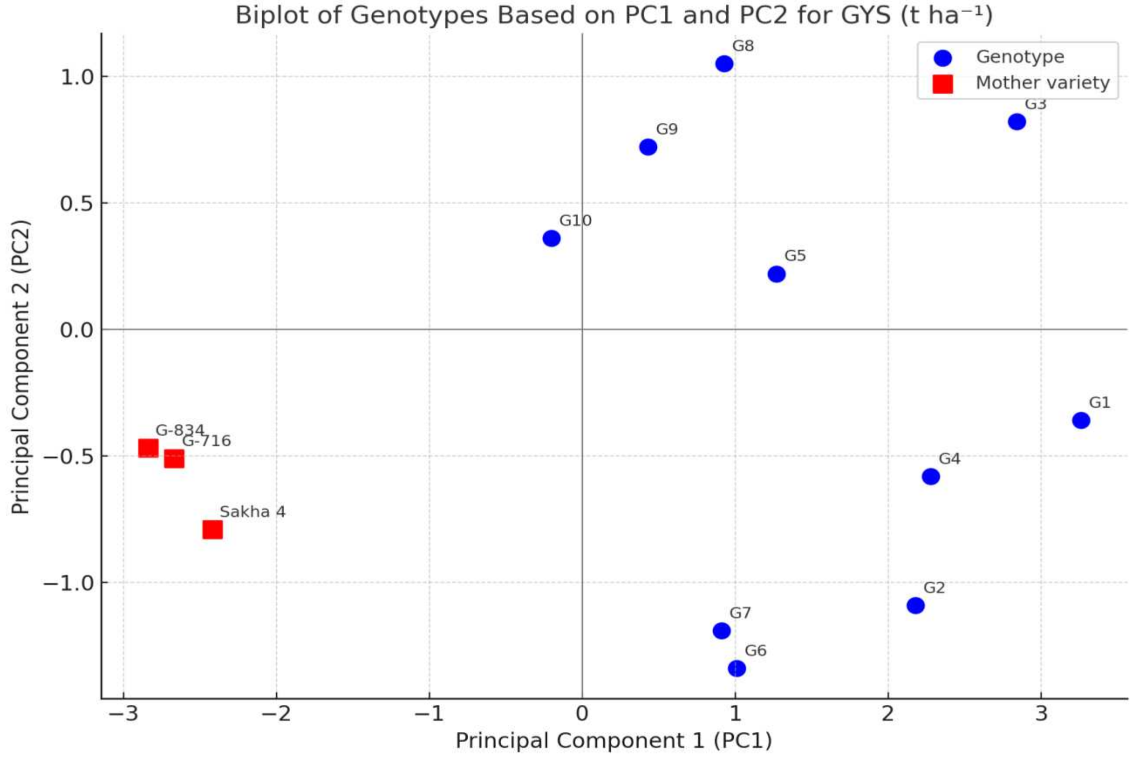

Fig.9. Showed principal component, PC1 and PC2, scores of mutant lines and their mother varieties based on GYS.

Mutant lines (G1–G4) were positioned far on the positive side of PC1. Mutant lines (G5–G9) occupied intermediate positions. In contrast, G10 and the mother varieties (Sakha 4, Giza 716, and Giza 843) showed lower yield potential.

Correlation coefficients

A highly significant and positive correlation was found between the branches per plant and the pods per plant in both M_3 and M_4 generations. Both traits also exhibited strong positive associations with grain yield under both *Orobanche* -infested and *Orobanche* -free infested soils. In contrast, plant height showed negative correlations with most traits in both generations under contrasting environments. A very strong and highly significant correlation was found between grain yield under *Orobanche* -infested soils and grain yield under *Orobanche* -free infested soils across generations (Table 9). Infestation severity showed significant negative and correlations with grain yield under both *Orobanche* -infested soils and grain yield under *Orobanche* -free infested across generation.

Table 9. Simple correlation between the studied traits in mutant lines faba bean in the M3 and M4 generations

Traits	NB	PP	GY (t ha^{-1})	GYS (t ha^{-1})	IS
			M3 generation		
PH (cm)	-0.910	-0.863**	-0.267	-0.018	-0.156
NB		0.877**	0.372	0.045	0.044
PP			0.466**	0.271	-0.022
GY (t/ha^{-1})				0.811**	-0.626**
GYS (t/ha^{-1})					-0.659**
			M4 generation		
PH (cm)	0.144	0.202	-0.037	0.278	-0.207
NB		0.985**	0.937**	0.970**	-0.788**
PP			0.938**	0.984**	-0.813**
GY (t/ha-1)				0.909**	-0.819**
GYS (t/ha-1)					-0.835**

PH: plant height, branches per plant (NB), pods per plant (PP), GY grain yield under *Orobanche* -free soils, GYS (t/ha^{-1}): grain yield under *Orobanche* -infested soils, IS:Infestationseverity, NS: non-significant,**:significant level at 0.01.

Stress tolerance indices

In the M2 generation, mutant lines, G1–G6, recorded the highest values of MP, STI, and DI (Table 10).

Table 10. Tolerance indices of mutant lines based on grain yield in the M2 generation

Genotype	TOL	MP	YSI	STI	DI	SD
G1	2.20	3.20	0.49	0.660	0.87	1.2
G2	2.10	3.15	0.50	0.644	1.87	1.1
G3	2.10	3.05	0.49	0.599	2.87	1.2
G4	2.20	3.00	0.46	0.569	3.87	1.5
G5	2.10	3.05	0.49	0.599	4.87	1.8
G6	2.00	3.00	0.50	0.584	5.87	2.2
G7	2.00	2.90	0.49	0.541	6.87	2.6
G8	2.00	2.90	0.49	0.541	7.87	3.0
G9	2.10	2.75	0.45	0.472	8.87	3.5
G10	1.80	2.80	0.51	0.514	9.87	3.9
G11	1.70	2.95	0.55	0.583	10.87	4.3
G12	2.10	2.75	0.45	0.472	11.87	4.8
G13	2.00	2.80	0.47	0.500	12.87	5.2
G14	1.90	2.75	0.49	0.486	13.87	5.7
G15	1.90	2.75	0.49	0.486	14.87	6.1
G16	1.80	2.80	0.51	0.514	15.87	6.5
G17	1.70	2.85	0.54	0.541	16.87	7.0
G18	1.80	2.70	0.50	0.473	17.87	7.4
G19	1.80	2.70	0.50	0.473	18.87	7.9
G20	1.80	2.60	0.49	0.435	19.87	8.3
G21	1.80	2.60	0.49	0.435	20.87	8.8
G22	1.70	2.55	0.50	0.422	21.87	9.2
G23	1.70	2.55	0.50	0.422	22.87	9.7
The mother varieties						
Sakha 4	1.10	1.65	0.50	0.177	23.87	10.3
G-834	1.30	1.95	0.50	0.247	24.87	10.7
G-716	1.30	1.85	0.48	0.219	25.87	11.2

TOL: Stress tolerance, MP: Mean productivity, YSI: Yield stability index, STI: Stress tolerance index, DI: Drought resistance index, and SD: standard deviation.

Mutant lines G7-G9, G12-G15, and G18-G23 recorded moderate values for these indices. Four mutant lines exhibited balanced performance under both *Orobanche*-free and *Orobanche* infested soils. In contrast, the mother varieties recoded the lowest values of both STI and DI. In the M3 generation, mutant lines G1-G8 showed high values of MP, STI, and DI with the lowest (favorable) value of TOL (Table 11).

Table 11. tolerance indices of mutant lines based on grain yield in the M3 generation under *Orobanche*-free and *Orobanche* -infested soils.

Genotype	TOL	MP	YSI	STI	DI	SD
G1	1.18	3.29	0.70	1.004	0.87	1.1
G2	1.10	3.15	0.70	0.922	1.87	1.0
G3	1.30	2.95	0.64	0.794	2.87	1.1
G4	1.33	2.92	0.63	0.772	3.87	1.4
G5	1.33	2.91	0.63	0.766	4.87	1.8
G6	1.32	2.89	0.63	0.759	5.87	2.2
G7	1.25	2.91	0.65	0.771	6.87	2.6
G8	0.94	2.97	0.73	0.824	7.87	3.1
G9	1.21	2.81	0.65	0.719	8.87	3.5
G10	1.25	2.78	0.63	0.701	9.87	3.9
G11	1.15	2.69	0.65	0.659	10.87	4.4
G12	1.15	2.68	0.65	0.654	11.87	4.8
G13	1.16	2.66	0.64	0.646	12.87	5.2
G14	1.16	2.65	0.64	0.641	13.87	5.7
G15	1.10	2.55	0.65	0.594	14.87	6.2
G16	1.00	2.50	0.67	0.575	15.87	6.6
The mother vareities						
Sakha 4	1.00	2.10	0.62	0.399	16.87	7.1
G-834	1.20	2.60	0.63	0.613	17.87	7.5
G-716	0.90	2.25	0.67	0.466	18.87	8.0

TOL: Stress tolerance, MP: Mean productivity, YSI: Yield stability index, STI: Stress tolerance index, DI: Drought resistance index, and SD: standard deviation.

In contrast, the mother varieties showed the lowest (unfavorable) STI and DI values. Mutant lines recorded the lower slandered deviation (SD) values compared to their parents. Similarly, in the M4 generation, mutant lines G1, G3 and G4 showed high MP, STI, DI values connected with low TOL (Table12).

Table 12. Stress tolerance indices of mutant lines based on grain yield in the M4 generation under *Orobanche* -free and *Orobanche* infested soils.

Genotype	TOL	MP	YSI	STI	DI	SD
G1	1.40	3.50	0.67	1.04	0.87	1.15
G2	1.60	3.20	0.60	0.85	0.67	1.09
G3	1.20	3.30	0.69	0.93	0.87	1.08
G4	1.10	3.15	0.70	0.85	0.85	1.03
G5	1.10	2.85	0.68	0.69	0.73	0.93
G6	1.40	2.90	0.61	0.70	0.16	1.07
G7	1.20	2.90	0.66	0.71	0.27	1.03
G8	1.00	2.80	0.70	0.67	0.23	1.00
G9	0.90	2.65	0.71	0.60	0.13	0.97
G10	0.90	2.55	0.70	0.56	0.12	0.93
The mother varieties						
Sakha 4	1.40	2.10	0.50	0.35	0.06	0.85
G-834	1.40	2.00	0.48	0.31	0.07	0.81
G-716	1.30	1.95	0.50	0.30	0.11	0.77

TOL: Stress tolerance, MP: Mean productivity, YSI: Yield stability index, STI: Stress tolerance index, DI: Drought resistance index, and SD: standard deviation.

In contrast, the mother varieties showed the lowest STI and DI values. The standard deviation (SD) values were almost identical for mutant lines and their parents.

Discussion

Broomrape weed represented a big challenge in *faba bean* cultivation, and effective control of this problem has not yet been solved. The development of resistance varieties is a viable strategy. The current study was intended to generate genetic variation through mutagenesis and to detect and select faba bean mutant lines under *Orobanche*-infested soils based on grain yield. Selection for yield per se in early generations under stressful conditions was inevitable, helped to reduce the number of genotypes tested in subsequent generations [42].The combined analysis of variance across the M2, M3 and M4generations illustrated notable genetic variability among the mutant lines, as clearly demonstrated by highly significant differences ($p \leq 0.05$) among the mutant lines for the studied traits and the wide range expressed over generations [7,44]. These findings showed the effectiveness of mutagen in creating genetic diversity by altering the nucleotide sequence, leading to the emergence of genes controlled in the studied traits [35,45]. The non-significant genotype × environment interaction detected in the M3 and M4 generations indicated stable performance of mutant lines under both *Orobanche*-free and *Orobanche*- infested conditions, suggesting that direct selection for grain yield, regardless of stress level, was effective in detecting and selecting high-yielding and *Orobanche*-tolerant mutant lines. These improvements may be due to accumulation and fixation of promising mutant lines, resulting in improved performance compared to the mother varieties over the generations under both conditions (Fig.3-5).This trend was shown in Fig. 10; the separation between mutant lines in the M4 generation and their parents reflected the role of mutagenesis on the studied traits.

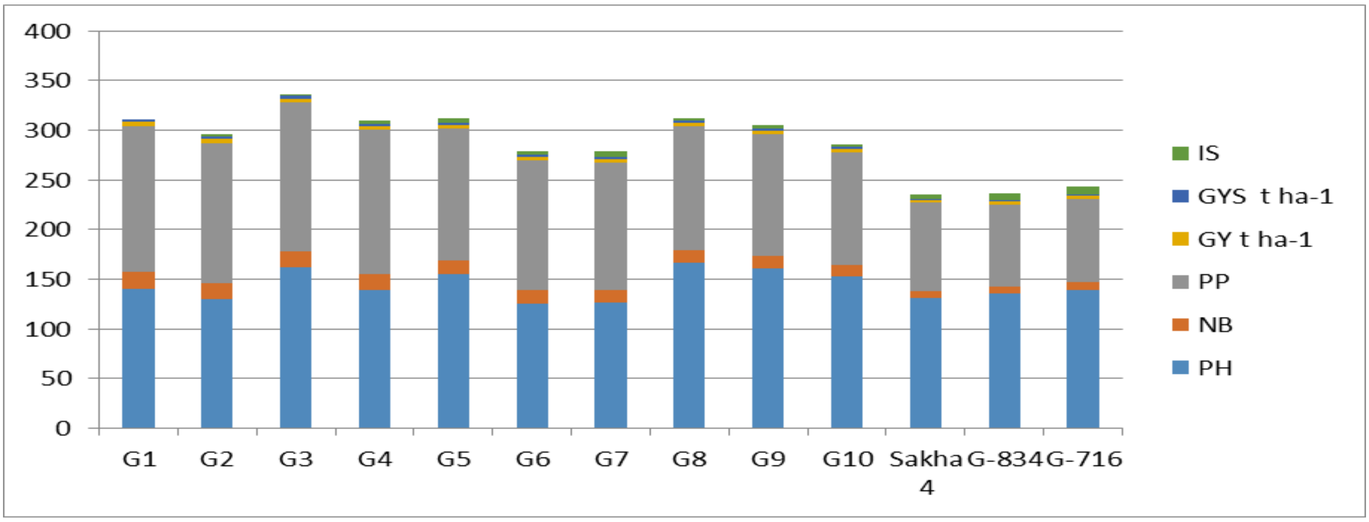

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Fig.10 showed effect of mutagen on the studied traits in mutant lines of the M4 generation compared to the mother varieties

The mutant lines and mother varieties occupied a different space in the bar area, reflecting significant differences among them under *Orobanche*-free and *Orobanche* -infested soils. According to the results shown in Tables 4,6 and 8, mutant lines (G1, G2, and G3) consistently outperformed their parents for the studied traits and showed very low *Orobanche* infestation across generations. These results confirmed the effectiveness of using infestation severity (IS) as a reliable, simple selection criterion for detecting *Orobanche*-tolerant mutant lines (Fig.11).

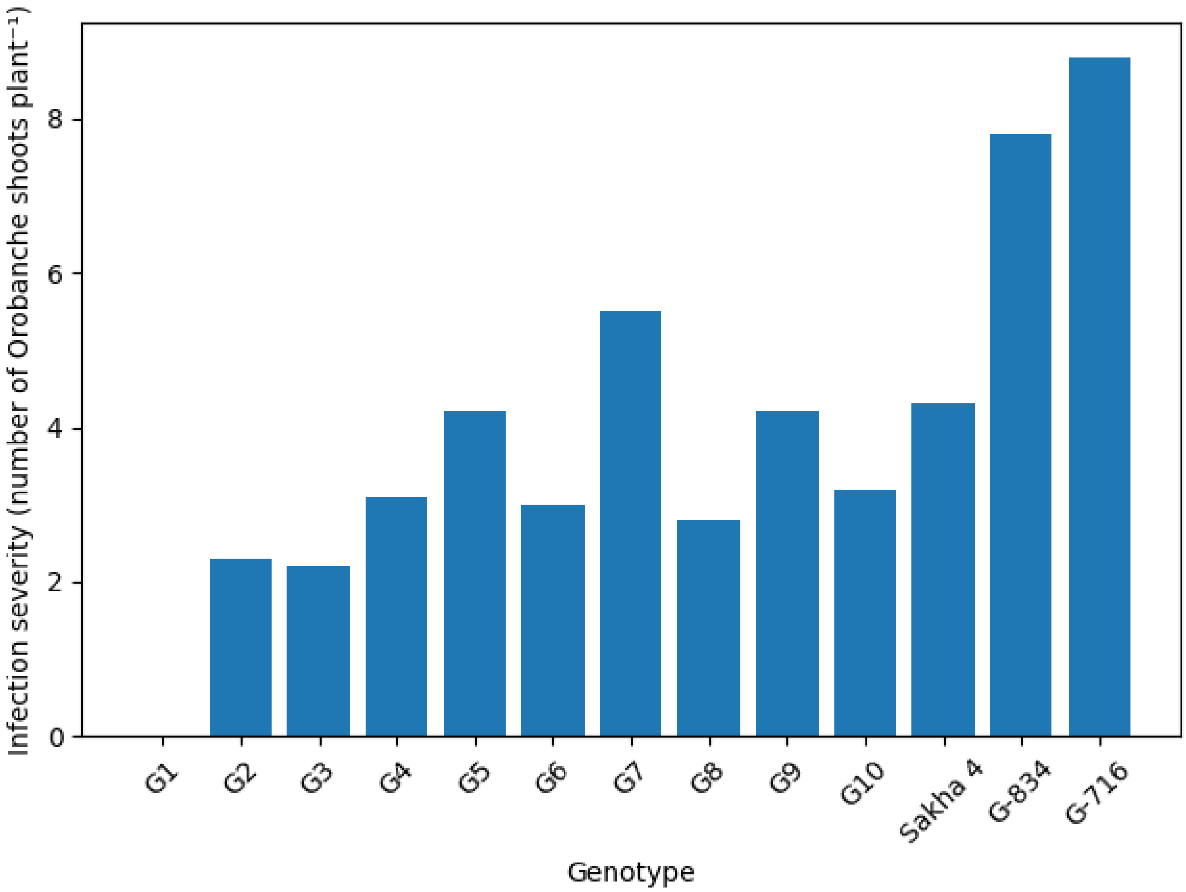

Fig.11. comparison between the best mutant lines in the M4 generation and their parents based on infestation severity

In agreement with previous results reported by Sauerborn [19] and Fernandez-Aparicio et al. [20]. As shown in Fig.12, the promising mutant line G1, positioned on the right side of the Fig.12, exhibited complete resistance to *O. crenata*, despite being grown in infested soil, whereas the parent check was infested.

Fig.12 showed the G1 mutant line, located on the right side of the Fig. as uninfested, whereas the infested control is located on the left side of the Fig. (infested).

This mutant line reflected resistance mechanisms by preventing the *O.crenata* from establishment and development. However, this resistance was connected with a reduction in the studied agronomic traits, suggesting a possible trade-off between *O. crenata* resistance and grain yield components. Nevertheless, this line represents a novel genetic source of resistance for faba bean improvement. Also, resistance of mutant lines to *O. crenata* may be attributed to morphological resistance, as they were taller and more productive, thereby reducing the ability of *O. crenata* to attach and establish. According to Niks et al. [46], host resistance to parasitic weed may develop through active mechanisms, including physiological and morphological characteristics. Previous studies have used mutation breeding in faba bean improvement [47-49].

The high heritability was connected with high genetic advance for all studied traits except plant height, indicating that these traits were controlled by genetic factors more than environmental influences. These results supported direct selection in the early generation (M2 generation) [27, 28]. The increase in heritability and genetic advance in the M3 generation reflected the fixation and accumulation of desirable mutations. In the M4 generation, heritability values were enhanced, indicating greater genetic stabilization, making this generation as a final stage for selection high-yielding and highly resistant to *Orobanche* [50].

Principal component analysis (PCA) supported previous findings, with PC1 and PC2 jointly explained 83.88% of the total variance, confirming the efficiency of PCA in determining most of the genetic variation among genotypes. Similar findings have been reported by Soliman et al.[7] and Yeken et al. [51]. The wide distribution of mutant lines (G1-G10) in the PCA biplot, compared with their parents (Figs.8 and 9), indicated that mutagenesis broadened the gene pool for breeding pipelines targeting *Orobanche* tolerance and yield improvement. Notably, there were close correlations of best-performing mutants with yield-related traits under both conditions, suggesting that they present harbor tolerance mechanisms that may reduce parasite attachment and damage [7,52].To confirm the previous findings, the simple correlation analysis revealed that direct selection for grain yield under both conditions reflected good yield stability among mutant lines. A strong significant negative correlation between infestation severity (IS) and grain yield under both conditions, confirmed that higher *Orobanche* infestation reduces grain yield [30, 53]. Therefore, direct selection for mutant lines with vigorous growth and high potential yield represents an effective approach to detect *Orobanche*–tolerant faba bean lines. Grain yield is a complex trait controlled by multiple genes located on different alleles and influenced by environmental conditions [54]. It provides complete information on the performance of mutant lines under both *Orobanche*-free and *Orobanche*- infested soils.As shown in Fig.13, tolerance indices illustrated that mutant lines showed higher mean productivity (MP) and greater yield stability across generations compared to their parent under both *Orobanche*-free and *Orobanche*-infested soils.

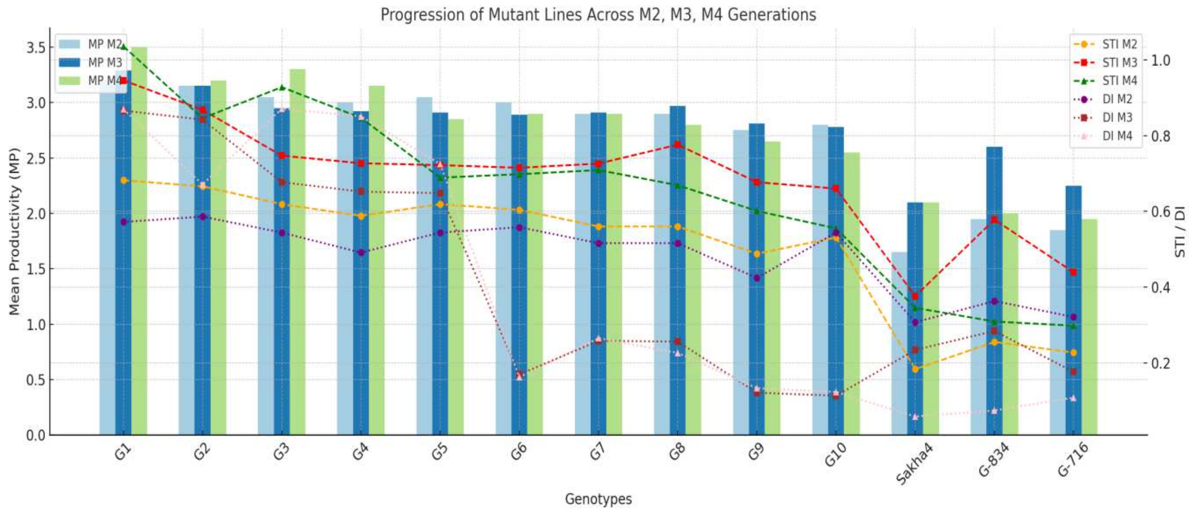

Fig.13 . Tolerance indices of faba bean mutant lines across the M2,M3 and M4 generations, along with their parents under *Orobanche*-free and *Orobanche* infested soils

The increasing of mean productivity values reflected increasing homozygosity and allelic fixation over generations. Moreover, higher stress tolerance index (STI) values and lower drought intensity (DI) values in mutant lines compared with their parents confirmed tolerance to infestation with *Orobanche*. In this respect, Sedghiyeh et al. [33] Basavara et al. [34] emphasized the efficiency of tolerance indices in detecting stress –tolerant varieties. Collectively, phenotypic selection from M2 to M4 generations enabled the detection of ten promising mutant lines of faba bean with superior grain yield and enhanced resistant to *O.crenata*. These lines as valuable genetic resources for improving grain yield and increasing resistant to *O.crenata* and will be advanced to confirmation trials. Overall, the mutagenic treatments induced genetic modifications in the studied traits, leading to an enhancement of the mutant's resistance to *O. crenata* and improving grain yield.

Conclusion

In Egypt, sustainable faba bean production was hindered by *Orobanche* infestation, and the development of new *Orobanche* -tolerant varieties is a logical solution to address this issue. This study revealed the effectiveness of sodium azide as a mutagen for enhancing genetic diversity in faba bean, leading to improvements in grain yield and resistance to *Orobanche*. High phenotypic and genetic coefficients of variation observed across generations indicated the generation of genetic variability for the studied traits. Moreover, high heritability estimates connected with high expected genetic advances suggested the predominance of additive gene action, thereby raising the effectiveness of selection. Principal components analysis (PCA) jointly explained 83.8% of the total variance. Significant positive correlations between the studied traits and grain yield under both *Orobanche*-free and *Orobanche*- infested soils, indicating the suitability of these traits as selection criteria for detecting *Orobanche*-tolerant faba bean varieties. In addition, tolerance indices proved effective in detecting *Orobanche*–tolerant faba bean mutant lines. Ten mutant lines were detected based on their yield and resistance to *Orobanche*, as valuable genetic resource. These lines will be advanced to confirmation trials across multiple locations over many seasons to estimate their yield stability, accompanied by molecular and physiological studies to explain and unravel the mechanisms of their resistance.

Data availability statements.
All data presented in the Tables and Figures in the article "Detection of High-Yielding and *Orobanche crenata*-Tolerant Faba Bean (*vicia faba* L.) Mutant Lines by Grain Yield, Infestation Severity and tolerance indices" are public available. This paper is intended to serve as a practical reference for faba bean breeders.
Author contributions
Single author
Declaration of Competing Interest
No declaration of conflict of interest
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Declaration
- Consent to participate declaration: not applicable
- Consent to publish declaration: not applicable
- Ethics declaration: not applicable

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