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First Induced Mutant Population for Drought Tolerance in *Vicia faba* L.: Yield Traits and Stress Indices Across Generations and Water Regimes

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Abstract

Drought is a critical constraint for legume production in semi-arid regions, yet breeding for drought tolerance in faba bean through induced mutagenesis remains largely unexplored. To our knowledge, this is the first EMS-derived mutant population in faba bean specifically developed for drought tolerance, comprising 45 M_2/M_3 lines derived from small-seeded cv. Zina and large-seeded cv. Aguadulce Superlonga), evaluated under two irrigation regimes—100% field capacity (well-watered control) and 40% field capacity (severe stress)—over two consecutive growing seasons in a randomized complete block design with three replications. Drought stress caused severe yield losses, reducing mean seed number per plant by 42.2% and mean seed weight per plant by 47.1%. Analysis of variance revealed highly significant effects of genotype, irrigation, and generation/year on both yield components. The non-significant genotype × irrigation interaction indicated similar proportional drought response across genotypes, while the non-significant three-way interaction suggested relatively consistent genotype rankings across generations/growing seasons. Among the ten drought tolerance indices evaluated, seed-number-based mean productivity (MPn) and stress tolerance index (STIn) were the most discriminating, whereas weight-based indices failed to differentiate genotypes due to the inherent seed-size contrast between botanical backgrounds. Dunnett's comparisons identified genotype 23 (Zina-derived) as the top performer, significantly exceeding its parent for both MPn and STIn; genotypes 22, 24, 12, 3, and 15 similarly outperformed controls. Cluster analysis broadly distinguished three groups: a tolerant cluster dominated by Zina-derived lines, a moderately tolerant cluster (Zina wild-type), and a sensitive cluster of Aguadulce Superlonga-derived lines. These findings suggest that EMS mutagenesis generated potentially heritable and exploitable variation for drought tolerance, with selected lines representing promising candidates for further multi-environment validation.

Keywords: *Vicia faba*; EMS mutagenesis; drought tolerance; stress tolerance index; mutation breeding; semi-arid environments

Academic Editors: Nasya Tomlekova and Jerzy Henryk Czembor

Received: 18 April 2026
Revised: 12 May 2026
Accepted: 25 May 2026
Published: 28 May 2026

1. Introduction

Climate change is a major threat to agriculture, particularly in dryland regions such as North Africa. Rising temperatures, prolonged droughts, shifting rainfall patterns, and

declining water resources are expected to reduce agricultural productivity and intensify food insecurity. The agricultural sector consumes a large share of the available freshwater resources and is particularly exposed to water scarcity. Across Africa, more than 250 million people are currently undernourished, which is projected to rise to over 25% by 2030, placing the continent far from achieving the "zero hunger" goal [1].

Morocco is exposed to many of these challenges. Its agricultural sector, which is highly climate-dependent, is under increasing pressure from recurrent droughts and reduced water supplies. The country has experienced more dry years than wet ones in recent decades across its main rainfed crop-producing areas. At the same time, demographic growth, industrial activity, and irrigation demand continue to strain water and environmental resources, with annual per capita water availability declining to only 700 m^3 [2–6]. According to projections by the World Bank [7] and Van Praag et al. [8], Morocco, along with Tunisia and Algeria, will likely become global hotspots of climate change by the end of the 21st century.

In this context, the resilience of Moroccan agriculture depends on transforming current cropping systems. One urgent priority is to reduce the dominance of monoculture cereals through crop diversification. Legumes, particularly faba bean (*Vicia faba* L.), offer a promising alternative. In addition to their high nutritional value and role in food and feed security, faba beans enrich soil fertility through biological nitrogen fixation, reducing the need for chemical fertilizers. They are well-suited to rotation with cereals and can enhance the sustainability of farming systems in vulnerable regions.

However, the wider adoption of legumes such as faba bean is limited by several challenges, including their susceptibility to drought stress [9]. To ensure their viability in increasingly arid environments, it is critical to develop new, drought-resilient varieties adapted to the stress conditions of Moroccan agroecosystems.

Mutation breeding has been widely adopted for crop improvement worldwide, with over 3500 mutant varieties released across 235 species in 79 countries [10]. Among these, approximately 7% (237 varieties) have been developed for abiotic stress tolerance, including more than 100 improved specifically for drought resistance [11]. Notable examples illustrate the success of this approach. In the USA, the rice cultivar *Calrose 76*, released in 1976, was the first semi-dwarf table rice variety and exhibited a 15% yield advantage over traditional tall cultivars [12]. In India, the rice mutant *Nagina 22* (*N22*) has become a key variety known for its deep root system and tolerance to both drought and heat stresses [13]. Another example is mutant rice variety *Zhefu 802*, recognized for its high yield potential, broad adaptability, strong resistance to rice blast, and cold tolerance; it was widely cultivated on over one million hectares between 1986 and 1994 [14,15]. More recently, Indonesia released three grain sorghum mutant varieties that combine drought tolerance with early maturity and semi-dwarf stature, making them suitable for dry-season cultivation in drought-prone regions [16].

Chemical mutagens such as EMS (ethyl methanesulfonate) have played a central role in mutation breeding programs, particularly for legumes. EMS has been extensively used to improve key agronomic traits, contributing to the development of 486 mutant legume varieties, 20 of which are faba bean mutant varieties. Several legume success stories demonstrate the effectiveness of this approach. In Pakistan, chickpea (*Cicer arietinum*) mutant varieties such as *CM-72*, *CM-88*, and *CM-98* showed resistance to *Ascochyta blight* and *Fusarium wilt*, significantly improving productivity. In India, the urad bean (*Vigna mungo*) mutant variety *TAU-1* became widely adopted in Maharashtra, contributing an estimated $64.7 million in additional production between 1989 and 1999 [17].

Despite this broader progress, induced mutagenesis in faba bean has remained confined to objectives of genetic diversity and morphological characterization. The historical

Jan Sjödin collection, developed between 1960 and 1970 using X-ray, neutron, gamma-ray, and EMS treatments [18], was recently re-evaluated through SNP-based genotyping and morphological phenotyping, with no abiotic stress component [19]. Likewise, gamma- and DES-induced M_2 mutant populations have been assessed for morphological diversity [20] and genetic diversity via molecular markers [21], while studies on mutagen effectiveness in faba bean focused solely on mutation frequencies [22]. Concurrently, drought tolerance research in faba bean has been conducted exclusively on registered cultivars, inbred lines, and germplasm accessions, using physiological traits [23], germplasm screening strategies [24], stress indices [25,26], and genomic approaches [27], with breeding progress explicitly acknowledged as slow due to the limited genetic material evaluated [28]. To date, no study has combined induced mutagenesis with drought tolerance as a breeding objective in *Vicia faba*, leaving this intersection entirely unexplored.

The present study addresses this gap by reporting on the first EMS-induced mutant population in *Vicia faba* developed and evaluated with drought tolerance as an explicit breeding objective. To this end, drought tolerance was assessed in selected mutant lines under controlled water regimes, lines that were previously identified from a broader EMS-derived mutant population as potentially resilient to water deficit.

2. Material and Methods

2.1. Plant Material

The plant material used in this study consisted of a mutant population derived from two faba bean varieties representing two botanical types. The first variety, *Aguadulce Superlonga*, is a faba bean *major* type characterized by its large seeds and long pods containing up to seven seeds per pod. The second variety, *Zina*, is a faba bean *minor* type known for its broad adaptability, small seeds, and short pods typically containing 3 to 4 seeds per pod.

EMS mutagenesis was performed in December 2022, prior to the evaluation period reported in this study. Seeds were pre-soaked in distilled water for 8 h and subsequently treated for 7 h at 22 °C with EMS solutions prepared in 2% DMSO (Sigma-Aldrich, St. Louis, MO, USA) (*v*/*v*). A preliminary dose–response assay was conducted using six EMS (Sigma-Aldrich, St. Louis, MO, USA) concentrations (0.03%, 0.04%, 0.05%, 0.1%, 0.2%, and 0.5% *v*/*v*), with 100 seeds per variety treated at each concentration. Following treatment, seeds were neutralized in 3% sodium thiosulfate (Sigma-Aldrich, St. Louis, MO, USA) for 16 h and rinsed three times for 15 min. Concentrations below 0.1% EMS resulted in survival rates above 60% in both varieties. Based on survival response and mutagenic efficiency [22], the concentrations selected for M_1 population development were 0.04%, 0.05%, 0.1%, and 0.5% for cv. Zina, and 0.04%, 0.05%, 0.1%, and 0.2% for cv. Aguadulce Superlonga. Mutant populations derived from the LD_{50} treatment were retained separately, and the corresponding mutant codes were prefixed with "DL50".

Mutant identification codes followed the format $C_nL_nCultivar_n$, where C_n denotes the EMS concentration level (from 1 to 4, corresponding from the lowest to the highest retained concentration), L_n refers to the M_1 planting line within each concentration, and $Cultivar_n$ indicates the position of the mutagenized seed within the corresponding line. Selection of mutant lines for the present study was based primarily on seed yield performance under the exceptionally hot and dry growing season of 2022–2023 at the Douyet experimental station of INRA (Institut National de la Recherche Agronomique, Morocco). During that season the site received a total precipitation of 215.4 mm, below the long-term average for the Saïs region, characterized by an uneven distribution. Notably, rainfall ceased from March onward, coinciding with the critical reproductive growth stage [29], thereby providing natural conditions for preliminary drought screening. Mutants derived from the highest EMS concentration (C4) generally exhibited reduced performance during the first

generation of evaluation, likely due to the combined effects of increased mutagenic load and incomplete physiological recovery following EMS treatment.

Based on this initial assessment, 45 promising mutant lines (M_2 and M_3) were selected for this evaluation in the subsequent growing seasons of 2023–2024 and 2024–2025. These included mutants 1 to 33, derived from the *Zina* variety, and mutants 34 to 45, derived from *Aguadulce Superlonga* (Table 1). The original non-mutated varieties were included as controls: genotype 46 (*Zina*) and genotype 47 (*Aguadulce Superlonga*), to allow performance comparison between the mutants and their respective parental lines.

Table 1. List of mutant lines selected with assigned identification numbers and origin.

Genotype	Genotype Code	Origin
C1L4Z11	1	Zina-derived
C1L7Z27	2	Zina-derived
C2L2Z29	3	Zina-derived
C2L3Z26	4	Zina-derived
C2L4Z14	5	Zina-derived
C2L4Z29	6	Zina-derived
C2L6Z19	7	Zina-derived
C2L8Z28	8	Zina-derived
C2L13Z7	9	Zina-derived
C2L15Z12	10	Zina-derived
C2L15Z16	11	Zina-derived
C2L17Z25	12	Zina-derived
C2L17Z27	13	Zina-derived
C2L20Z14	14	Zina-derived
C2L20Z25	15	Zina-derived
C3L5Z15	16	Zina-derived
C3L5Z18	17	Zina-derived
C3L6Z30	18	Zina-derived
C3L11Z33	19	Zina-derived
C3L14Z30	20	Zina-derived
C3L16Z27	21	Zina-derived
C3L17Z19	22	Zina-derived
DL50L1Z7	23	Zina-derived
DL50L1Z14	24	Zina-derived
DL50L1Z17	25	Zina-derived
DL50L1Z41	26	Zina-derived
DL50L1Z18	27	Zina-derived
DL50L1Z22	28	Zina-derived
DL50L1Z24	29	Zina-derived
DL50L1Z38	30	Zina-derived

Table 1. *Cont.*

Genotype	Genotype Code	Origin
DL50L2Z38	31	Zina-derived
DL50L3Z1	32	Zina-derived
DL50L5Z18	33	Zina-derived
C1L10Ag3	34	Aguadulce Superlonga-derived
C1L16Ag16	35	Aguadulce Superlonga-derived
C1L1Ag30	36	Aguadulce Superlonga-derived
C2L16Ag18	37	Aguadulce Superlonga-derived
DL50L1Ag6	38	Aguadulce Superlonga-derived
DL50L1Ag10	39	Aguadulce Superlonga-derived
DL50L1Ag17	40	Aguadulce Superlonga-derived
DL50L2Ag1	41	Aguadulce Superlonga-derived
DL50L2Ag2	42	Aguadulce Superlonga-derived
DL50L2Ag5	43	Aguadulce Superlonga-derived
DL50L2Ag14	44	Aguadulce Superlonga-derived
DL50L2Ag46	45	Aguadulce Superlonga-derived
Zina	46	INRA Morocco
Aguadulce Superlonga	47	AGRIN

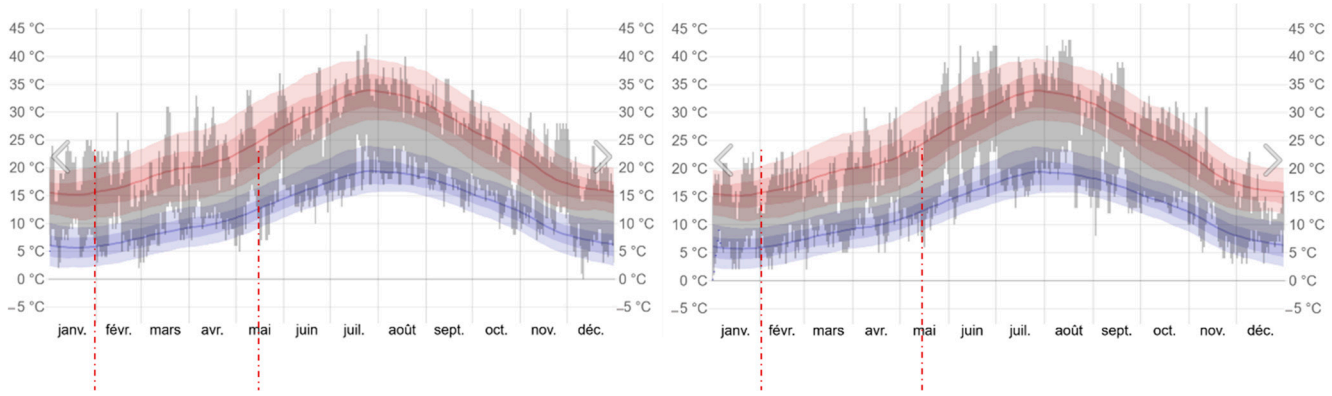
2.2. Experimental Design and Irrigation Treatments

The trial was conducted in an insect-proof net tunnel covered with a transparent plastic canopy, which protected plants from rainfall while maintaining near-natural environmental conditions. Ambient temperature was not actively controlled, allowing exposure to natural thermal variation across the two experimental years (Figure 1). Water supply was carefully managed to simulate two contrasting soil moisture levels: 100% field capacity (i.e., maximum water-holding capacity of the pot substrate; well-watered control) and 40% field capacity (severe water stress), across a total of 47 genotypes. The experimental design was a randomized complete block design (RCBD) with three replications, applied over two consecutive growing seasons (2023–2024 and 2024–2025). Each experimental unit consisted of a single plant grown in one pot. For each genotype, three replicates were used for each irrigation treatment, resulting in three pots at 100% field capacity and three pots at 40% field capacity.

Plants were grown in 12 kg pots filled with a substrate mixture consisting of 50% sand and 50% peat. Field capacity was determined gravimetrically and expressed on a dry-weight basis according to the protocol described by Cassel and Nielsen [30]. To determine field capacity, pots were fully saturated with water and then allowed to drain for 24 h while covered with black plastic to minimize evaporation. The pots were then weighed to obtain the saturated weight (W_{sat}). The substrate was subsequently oven-dried at 105 °C for 48 h, and the dry weight (W_{dry}) was recorded. Field capacity (FC) was calculated as follows:

$$FC = \frac{(W_{sat} - W_{dry})}{W_{dry}} \times 100$$

This value represents the percentage of water the substrate retains after drainage.



(**A**) (**B**)

Figure 1. Monthly temperature patterns in Meknes for 2024 (**A**) and 2025 (**B**). The red and blue shaded bands represent daily maximum and minimum temperatures, respectively; the grey central band indicates mean temperature; vertical red dashed lines indicate the onset of the reproductive phase. Month abbreviations are in French: janv. = January, févr. = February, mars = March, avr. = April, mai = May, juin = June, juil. = July, août = August, sept. = September, oct. = October, nov. = November, déc. = December.

Two irrigation treatments (IT) were applied: T1 corresponded to 100% FC (control), and T2 corresponded to 40% *FC* (water stress treatment). Sowing took place on 29 December 2023 and 20 December 2024. Water stress was imposed from the intermediate vegetative stage, corresponding to early February 2024 and late January 2025 for each respective season. It was maintained continuously through the reproductive stages (flowering and pod filling) until physiological maturity (indicated by pod blackening and stem desiccation), with irrigation applied twice weekly. The amount of water required to adjust each pot to the desired soil moisture level was managed gravimetrically following the approach described by Earl [31]:

$$\text{Target weight} = W_{dry} \times \left(1 + \frac{FC}{100} \times \theta_{Target}\right)$$

$$Water\ to\ add\ (\text{g or mL}) = Target\ weight - W_{current}$$

where W_{dry} represents the reference weight of the soil at oven-dry condition, *FC* is the gravimetrically determined field capacity (%), $\theta_{Tarrget}$ is the target soil water content expressed as a fraction of *FC* (e.g., 0.4 for 40% FC), and $W_{current}$ is the weight of the pot at the time of watering (g). Water was applied twice weekly to bring each pot to its target weight.

Initially, plant biomass was considered negligible in pot weight measurements. As growth progressed, target pot weight was adjusted for estimated plant fresh mass: the onset of flowering, the beginning of pod formation, and the completion of podding. These measurements were obtained from destructively harvested supplementary pots and were subtracted from $W_{current}$ prior to irrigation.

2.3. Trait Measurements and Drought Tolerance Indices

Drought Tolerance Indices Calculation

Five drought tolerance indices, namely stress susceptibility index (SSI) [32], mean productivity (MP) [33], tolerance index (TOL) [33], stress tolerance index (STI) [34], and yield stability index (YSI) [35], were calculated to evaluate the response of faba bean genotypes under drought stress using the following formulas:

$$\text{SSI} = (1 - (\text{Ys}/\text{Yp}))/(1 - (\bar{\text{Y}}\text{s}/\bar{\text{Y}}\text{p}))$$

$$MP = (Ys + Yp)/2$$

$$TOL = Yp - Ys$$

$$STI = (Ys \times Yp)/(\bar{Y}p)^2$$

$$YSI = Ys/Yp$$

where

Yp = yield of a genotype under well-watered conditions
Ys = yield of a genotype under drought stress
$\bar{Y}p$ = mean yield of all genotypes under well-watered conditions
$\bar{Y}s$ = mean yield of all genotypes under drought stress

In this study, yield was expressed in two complementary ways: seed yield per plant, expressed as seed weight (YPg) and total number of seeds per plant (YPn). This dual approach was used because faba bean comprises both large-seeded and small-seeded botanical types, and assessing drought tolerance solely on seed weight may not fully capture the genotypic response. To clearly distinguish between the two measures, indices calculated based on seed weight are denoted with the suffix "g" (e.g., SSIg, MPg), while those based on seed number are denoted with "n" (e.g., SSIn, MPn). This allows a comprehensive evaluation of drought tolerance considering both quantitative and numerical seed production.

2.4. Statistical Analyses

Yield components (YPn and YPg) were analyzed by three-way ANOVA with genotype (Gp), irrigation treatment (IT), and generation/year (Gr/Y) as fixed factors and blocks (replications) as a random factor, including all two-way and three-way interactions within a randomized complete block design. The factor generation/year (Gr/Y) was treated as a single combined factor because the mutagenic generations were inherently associated with the experimental years; specifically, the M_2 generation was evaluated during the 2023–2024 cropping season, whereas the M_3 generation was evaluated during the 2024–2025 cropping season. Drought tolerance indices were subjected to two-way ANOVA (Gp × Gr/Y). Pairwise comparisons of mutant lines against their respective parental controls were performed using Dunnett's two-sided *t*-test ($\alpha = 0.05$). Estimated marginal means, adjusted for generation/year and replication effects, are presented with 95% confidence intervals; due to the balanced experimental design, these are identical to arithmetic means. Hierarchical cluster analysis used Ward's linkage with squared Euclidean distance on z-score-standardized drought indices and raw yield values under both water regimes.

All statistical analyses were performed using IBM SPSS Statistics (Version 26.0).

3. Results and Discussion

3.1. Effects of Genotype, Water Regime, Generation/Year and Their Interactions

Analysis of variance showed significant main effect of genotype on both yield traits examined, namely total number of seeds per plant (YPn) and yield per plant (YPg) (Table 2). The significant genotype effect indicates that mutant lines differ from the parental controls and from each other in their seed-setting capacity and overall seed weight. Irrigation treatment (100% vs. 40% field capacity) significantly reduced both YPn and YPg, which is agronomically expected given that water deficit at reproductive stages directly compromises pollination success, pod retention, seed filling, and final seed weight [36]. Mean YPg declined from 34.77 g to 18.40 g (−47.1%) and YPn from 31.86 to 18.41 grains (−42.2%), reflecting a comparable impact of water deficit on both seed filling and seed setting. This pattern was consistent across botanical types: YPg reductions reached 42.0% in *V. faba* major and 43.5% in *V. faba* minor, while YPn declined by 46.3% and 41.1%, respectively

(Figures 2–5). Notably, the *V. faba* minor Zina exhibited higher baseline productivity than the *V. faba* major Aguadulce Superlonga for both components, suggesting that parental background contributed strongly to yield performance, though proportional losses under drought were similar across types. These reductions (41–47%) are consistent with those reported by Balko et al. [28] in faba bean, where plot yield and seed number per plant declined by 46.4% and 42.8%, respectively, under drought stress. Among the three dominant grain legumes in Morocco, the observed yield reductions align with those reported for chickpea under comparable stress conditions (40–50%) [37–40], but remain considerably lower than the 70% yield loss documented in lentil under reproductive-stage drought [41], positioning faba bean as moderately sensitive within the regional legume cropping system.

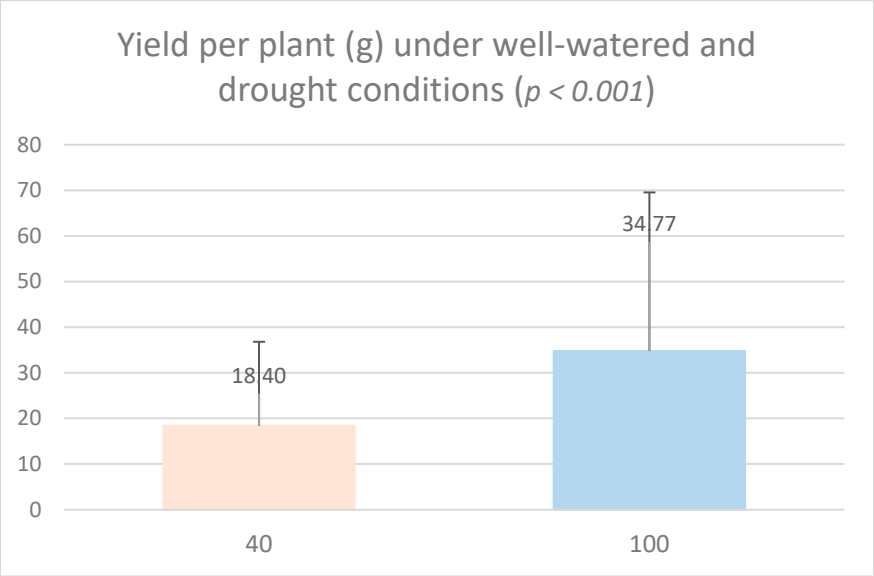


Figure 2. Overall mean yield per plant (g) of *Vicia faba* L. pooled across all genotypes under well-watered (100% field capacity) and water stress (40% field capacity) conditions. Bars represent standard deviations. Differences between water regimes were statistically significant ($p < 0.001$).

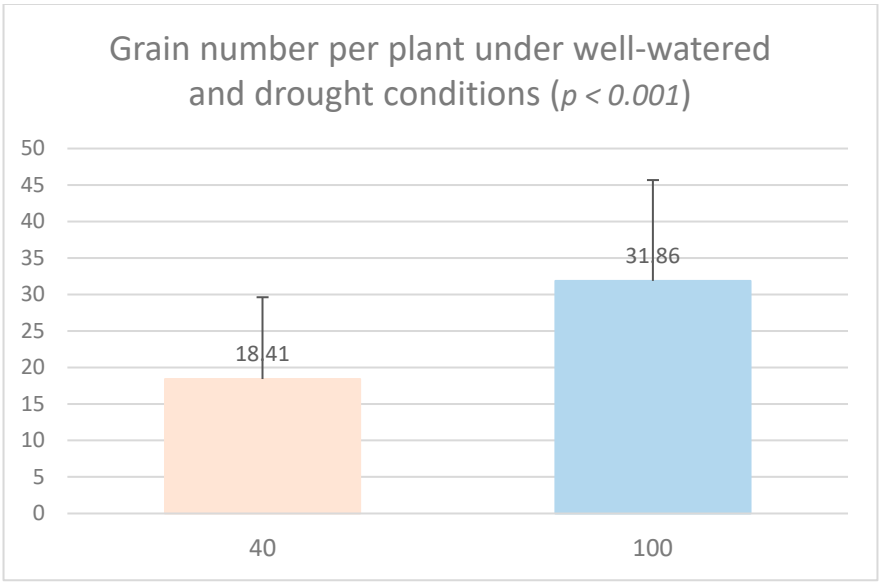


Figure 3. Overall mean of total number of seeds per plant of *Vicia faba* L. pooled across all genotypes under well-watered (100% field capacity) and water stress (40% field capacity) conditions. Bars represent standard deviations. Differences between water regimes were statistically significant ($p < 0.001$).

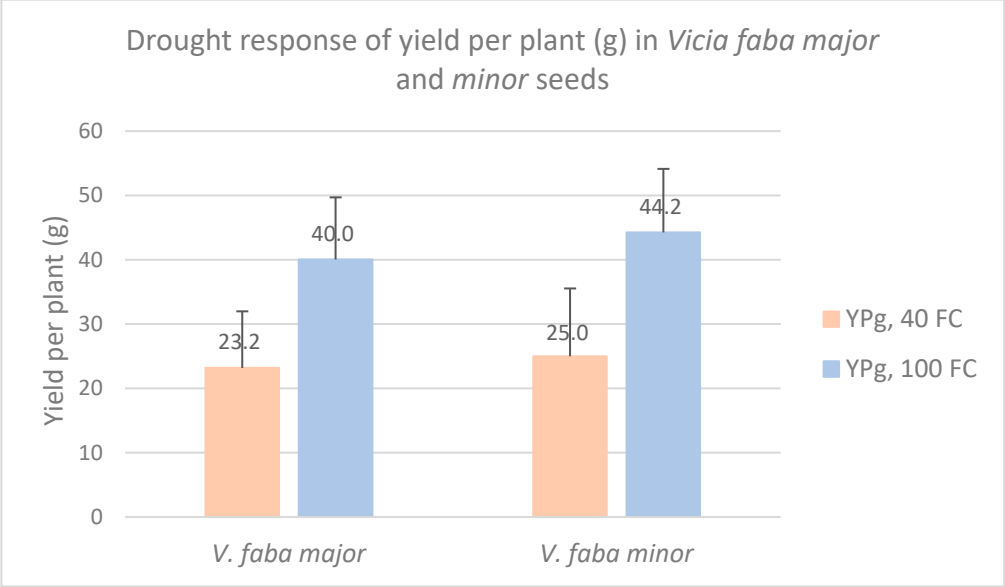


Figure 4. Mean yield per plant (g) pooled across all genotypes of *Vicia faba* var. *major* and *Vicia faba* var. *minor* under water stress (40% field capacity) and well-watered (100% field capacity) conditions. Bars represent standard deviations. Values reflect the overall response of each botanical variety to water regime regardless of genotypic differences.

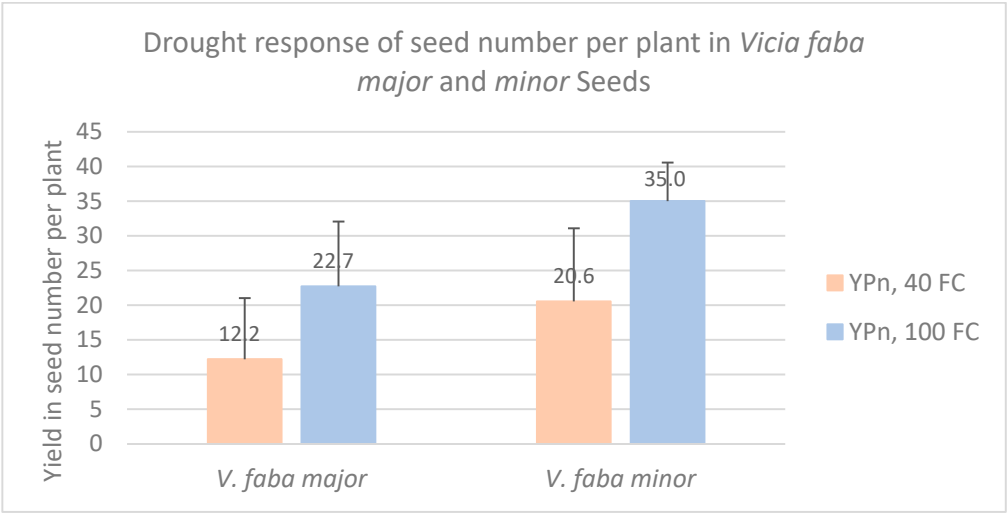


Figure 5. Mean seed number per plant pooled across all genotypes of *Vicia faba* var. *major* and *Vicia faba* var. *minor* under water stress (40% field capacity) and well-watered (100% field capacity) conditions. Bars represent standard deviations. Values reflect the overall response of each botanical variety to water regime regardless of genotypic differences.

Table 2. Analysis of variance (mean squares and significance levels) for total seed number per plant (YPn) and seed yield per plant (YPg) in faba bean mutant lines evaluated under contrasting irrigation regimes across two generations.

	Df	YPn	YPg
Genotype (Gp) *p* value	46	446.254 <0.001	107.333 0.005
Irrigation Treatment (IT) *p* value	1	25,060.913 <0.001	36,811.857 <0.001
Generation/year (Gr/Y) *p* value	1	33,675.312 <0.001	35,161.047 <0.001

Table 2. *Cont.*

	Df	YPn	YPg
Gp × IT	46	41.482	22.755
p value		0.854	1.000
Gp × Gr/Y	46	146.385	93.121
p value		<0.001	0.031
IT × Gr/Y	1	312.129	976.230
p value		0.016	<0.001
Gp × IT × Gr/Y	46	50.105	27.730
p value		0.594	1.000
Error	355	53.510	63.468

Df: degrees of freedom, YPn: total seed number per plant, YPg: seed yield per plant.

Generation/year corresponding to M_2 evaluated in 2023–2024 and M_3 evaluated in 2024–2025, also significantly affected both traits. In fact, temperature records for Meknes show that from February to mid-April, M_3 (2025) experienced more frequent and intense heat peaks than M_2 (2024), with several temperature spikes approaching 40 °C in 2025, compared with peaks generally remaining below 35 °C during the same period in 2024 (Figure 1). As this window coincides with the reproductive and grain-filling stages of faba bean, the elevated thermal stress during M_3 likely drove the reduction in YPn and YPg, consistent with the well-documented sensitivity of legume reproductive phases to transient heat episodes [29,36,42]. It cannot be excluded, however, that part of the generation effect also reflects the shift in genetic constitution between generations: M_2 plants are heterozygous and segregating, potentially inflating line means or masking recessive mutations, whereas M_3 lines are advancing toward homozygosity and expressing a relatively more fixed genetic background. The observed reduction from M_2 to M_3 may therefore reflect a combination of stage-specific thermal stress and progressive genetic fixation. The convergence of both effects toward reduced mean performance in M_3 reinforces the relevance of lines that maintained or improved their ranking across generations as candidates for stable, broad-adaptation selection. To the best of our knowledge, this study represents the first attempt to develop and evaluate a mutagenesis-derived population in faba bean with the objective of enhancing drought tolerance, making the patterns of genetic and generational variation documented here a novel contribution to legume stress breeding.

The genotype × irrigation interaction was non-significant for both YPn and YPg, suggesting that genotypes maintained broadly consistent relative rankings under both irrigation levels for these yield traits (Table 2). The significant genotype × generation/year interaction for both YPn and YPg indicated that genotypic rankings for yield shifted between the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025), reflecting the combined influence of the segregation dynamics and differential thermal exposure discussed above. Similarly, the significant irrigation × generation/year interaction for both YPn and YPg indicates that the magnitude of drought-induced yield reduction varied between M_2/2023–2024 and M_3/2024–2025 evaluations. Water deficit imposed a greater penalty on seed number and seed weight in M_3 than in M_2, consistent with a compounding effect of concurrent stage-specific heat and drought stress. Additionally, the relatively more fixed genetic background of M_3 lines may have reduced the phenotypic buffering capacity that residual heterozygosity could have provided in M_2, making M_3 plants more uniformly sensitive to the imposed stress combination.

The three-way interaction (genotype × irrigation × generation/year) was non-significant for both YPn and YPg, suggesting that genotypic responses to drought stress were likely consistent across the two generations/years (Table 2). Despite the yield penalty

recorded in M_3 relative to M_2, the relative differentiation among genotypes under deficit versus well-watered conditions did not shift materially between seasons. The absence of a three-way interaction therefore suggests that differential drought response appeared relatively consistent across both irrigation levels and generational backgrounds, though year and generation effects remain confounded. The tolerance phenotype of top-performing lines is likely heritable and relatively stable across the generations evaluated, supporting their consideration as candidates for advancement in the breeding pipeline.

The significant effects of genotype and water regime on seed yield per plant observed in our mutant population are consistent with those reported in conventional faba bean germplasm, where highly significant effects of both factors on seed yield per plant have been documented across multiple studies and seasons [25,43,44], confirming that water deficit consistently depresses seed yield regardless of the genetic background evaluated. Notably, these studies also reported a significant genotype × irrigation interaction, indicating that natural genotypes differ in their relative yield response to water deficit. In contrast, this interaction was non-significant in our mutant population for both YPn and YPg, suggesting that while mutant lines differed meaningfully in absolute yield capacity, their relative response to water deficit was broadly uniform across genotype.

Drought tolerance indices derived from YPn and YPg were calculated to integrate performance under contrasting irrigation regimes into composite metrics reflecting both yield potential and stress response. Among the ten indices evaluated, significant genotype effect was detected only for the grain-number-based mean productivity (MPn; $p \leq 0.001$) and stress tolerance index (STIn; $p \leq 0.001$), whereas the genotypes were statistically comparable for the rest of indices (Table 3). This pattern is largely attributable to population structure. The mutant lines derived from two botanically distinct backgrounds, the large-seeded 'Aguadulce Superlonga' and the small-seeded 'Zina', whose inherent contrast in seed size introduces a systematic background effect into weight-based metrics. In contrast, grain-number-based indices provide a relevant measure of reproductive performance.

Table 3. Analysis of variance (mean squares and significance levels) for drought tolerance indices derived from seed number (SSIn, MPn, TOLn, STIn, YSIn) and seed yield (SSIg, MPg, TOLg, STIg, YSIg) in faba bean mutant lines across two generations/growing seasons.

	Df	SSIg	SSIn	MPg	MPn	TOLg	TOLn	STIg	STIn	YSIg	YSIn
Genotype (Gp)	46	0.163	0.336	53.192	218.486	50.224	92.361	0.139	0.629	0.036	0.060
p value		0.961	0.579	0.103	<0.001	0.995	0.258	0.061	<0.001	0.961	0.579
Generation/year (Gr/Y)	1	8.198	12.317	19,252.596	17,984.898	1271.597	317.508	35.084	47.901	1.812	2.184
p value		<0.001	<0.001	<0.001	<0.001	<0.001	0.048	<0.001	<0.001	<0.001	<0.001
Gp × Gr/Y	46	0.195	0.316	45.533	75.610	60.594	97.570	0.124	0.366	0.043	0.056
p value		0.854	0.673	0.283	<0.001	0.969	0.186	0.149	<0.001	0.854	0.673
Error	180	0.254	0.356	40.284	35.259	96.892	80.322	0.099	0.106	0.056	0.063

Df: degrees of freedom, SSIg/SSIn: stress susceptibility index based on seed weight (g) or seed number (n), respectively; MPg/MPn: mean productivity index based on seed weight or seed number; TOLg/TOLn: tolerance index based on seed weight or seed number; STIg/STIn: stress tolerance index based on seed weight or seed number; YSIg/YSIn: yield stability index based on seed weight or seed number.

The absence of significant genotype main effect for the remaining seed-number-based indices, SSIn, TOLn, and YSIn (Table 3), is agronomically informative: unlike SSI, TOL, and YSI, which capture only the relative or absolute magnitude of yield loss under stress, MP and STI simultaneously integrate performance under both water regimes, rewarding genotypes that combine high yield potential under well-watered conditions with strong yield maintenance under drought. A genotype can achieve a favorable SSI or YSI value simply by being uniformly low-yielding, whereas a high STI or MP value necessarily reflects sustained productivity across environments. The absence of significant genotype

main effect for SSIn, TOLn, and YSIn therefore indicates that genotypic variation in this population resides not in stress sensitivity per se, but in overall reproductive capacity. This pattern is consistent with findings in chickpea, where STI and MP proved more reliable discriminators than TOL, SSI, and YSI [45], and corroborates in a large-scale evaluation of 100 faba bean genotypes where STI revealed highly significant genotypic variation under moderate water deficit [28]. The non-significance of all weight-based indices is consistent with the population structure of this study, given that the two botanical backgrounds carry an inherent seed-size contrast. STIg showed a marginal trend ($p = 0.061$) but did not reach conventional significance.

Generation/year significantly affected all ten indices ($p < 0.05$), consistent with the significant generation/year effect documented for YPn and YPg. More informative is the genotype × generation/year interaction, which was significant exclusively for MPn ($p < 0.001$) and STIn ($p < 0.001$) and non-significant for all remaining indices (Table 3). This means that although genotypes differed in grain-number-based drought resilience, their relative rankings for MPn and STIn were not stable across seasons, mirroring the genotype × generation/year interaction detected for YPn itself. The Gp × Gr/Y interaction was non-significant for all weight-based indices and for SSIn, TOLn, and YSIn ($p > 0.05$), indicating that genotypic drought responses measured through these metrics were consistent across generations/growing seasons.

3.2. Seed Yield Performance of Mutant Lines Under Well-Watered and Deficit Irrigation

The estimated marginal means plots for both YPg and YPn (Figures 6 and 7) revealed a consistent separation between the two irrigation treatments across all genotypes ($p < 0.001$), with well-watered plants (100% FC) systematically exceeding the observed grand mean, while water-stressed plants (40% FC) predominantly fell below it, confirming that drought stress significantly compromised grain filling and overall plant productivity regardless of genetic background. Nevertheless, considerable genotypic variation was detected for both YPn ($p < 0.001$) and YPg ($p < 0.01$), with certain mutant lines maintaining relatively higher means compared to others, suggesting differential drought tolerance among the mutant population.

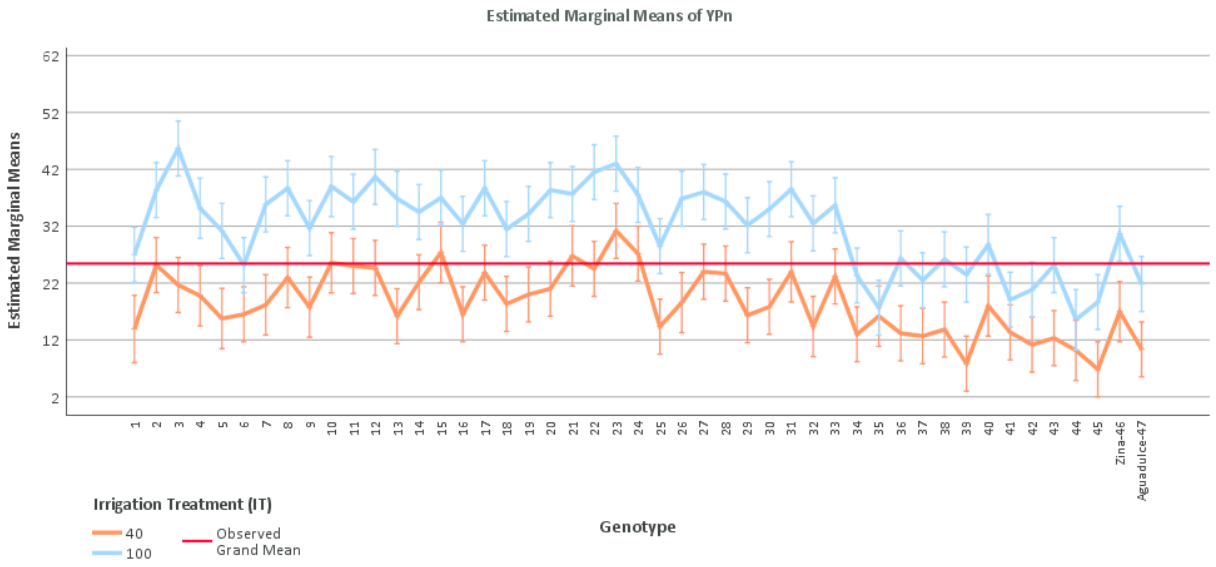


Figure 6. Estimated marginal means of total seed number per plant (YPn) for 45 faba bean mutant lines and their respective parental checks (Zina-46 and Aguadulce Superlonga-47) under well-watered (100% field capacity, blue) and deficit irrigation (40% field capacity, orange) conditions, pooled across the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025). The horizontal red line

represents the observed grand mean across all genotypes and irrigation treatments. Error bars indicate 95% confidence intervals. Mutant lines 1–33 are derived from *Vicia faba minor* cv. Zina and lines 34–45 from *Vicia faba major* cv. Aguadulce Superlonga.

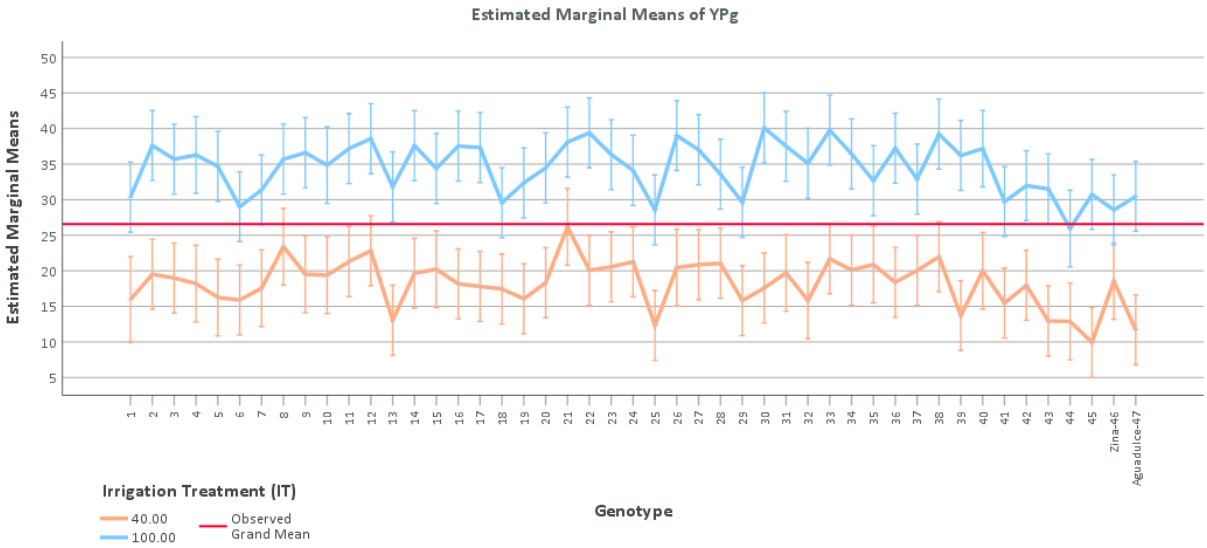


Figure 7. Estimated marginal means for seed yield per plant (YPg, g plant^{-1}) for 45 faba bean mutant lines and their respective parental checks (Zina-46 and Aguadulce Superlonga-47) under well-watered (100% field capacity, blue) and deficit irrigation (40% field capacity, orange) conditions, pooled across the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025). The horizontal red line represents the observed grand mean across all genotypes and irrigation treatments. Error bars indicate 95% confidence intervals. Mutant lines 1–33 are derived from *Vicia faba minor* cv. Zina and lines 34–45 from *Vicia faba major* cv. Aguadulce Superlonga.

Mean performance of the mutant genotypes was then evaluated relative to their respective wild-type parents, Zina and Aguadulce Superlonga, using Dunnett's two-sided *t*-test, to identify which genotypes statistically outperformed their controls. For the majority of genotypes assessed, no statistically significant differences were detected in comparison to either check variety, except for mutant line 23, which exhibited a significantly higher grain number per plant than its parent Zina (mean difference = 12.64 seeds, $p \leq 0.01$), positioning it as a valuable genetic resource for breeding programs targeting drought-tolerant, high-yielding faba bean varieties adapted to arid environments. Subsequent genotypic comparisons are based on estimated marginal means adjusted for generation/year effects within the ANOVA model.

Under deficit irrigation (40% FC), mutant 23 consistently occupied the highest position for YPn marginal mean, recording 31.0 seeds per plant, exceeding its parental check Zina by 14.2 seeds (Figure 6), confirming its statistical superiority established by Dunnett's test. Although plant height was not included as a measured parameter in the present study, mutant line 23 is characterized by a notable reduction in this trait relative to Zina, which may partly account for its superior seed number under deficit irrigation; reduced vegetative biomass potentially lowering source-sink competition between vegetative and reproductive organs, thereby sustaining pod set and seed number under water stress.

Several additional Zina-derived mutants also substantially exceeded the overall mean for YPn under 40% FC (18.4 seeds), notably lines 15 (27.2), 24 (27.0), 21 (26.6), and 10 (25.4), all surpassing Zina under stress conditions (16.8 seeds), suggesting that mutagenesis generated favorable variation for reproductive drought tolerance within the minor seed-type background (Figure 6).

Within the Aguadulce Superlonga-derived population, no mutant line reached statistical significance relative to the parental check under either irrigation regime. Nevertheless,

a general trend of improved seed-setting capacity was observable across most lines, with the exception of lines 39, 44, and 45, which failed to exceed the parental check under stress conditions (Figure 6). While these gains remain agronomically modest relative to Zina-derived lines, they suggest that mutagenesis introduced detectable variation in reproductive capacity within the large-seeded background, providing a basis for further selection within this botanical type.

Examination of YPg marginal means revealed a slightly different pattern to that observed for YPn. No Zina-derived mutant line reached statistical significance relative to the parental check for this trait under either irrigation regime. Descriptively, weight-based yield advantages within the Zina-derived group appeared more pronounced under well-watered conditions than under deficit irrigation, suggesting that seed weight expression may be more sensitive to water availability than seed number in this population (Figure 7). Mutant 23, the top performer for YPn, also recorded a moderate YPg advantage over Zina under both regimes, indicating that its superior seed-setting capacity partially translates into weight-based yield gain while conserving the small, uniform seed morphology of the *V. faba minor* background. This combination is particularly relevant in the Moroccan agricultural context, where the national ambition of reaching one million hectares under conservation agriculture [46] is constrained by a critically limited portfolio of varieties compatible with no-till mechanized seeding, for which small-seeded minor-type faba bean is inherently better suited. If validated across broader environments, its combination of enhanced seed number with an unaltered seed morphology may therefore represent a meaningful contribution to this breeding gap.

On the other hand, despite their inferior seed number performance, Aguadulce Superlonga-derived lines showed a descriptive trend of improved YPg relative to their parental check under both irrigation regimes, though none reached statistical significance (Figure 7). Percentage gains over the parental check were particularly notable under deficit irrigation, suggesting that mutagenesis may have introduced variation in individual seed weight expression or seed retention under stress within this large-seeded background. Line 38 consistently ranked first under both irrigation regimes, emerging as the most resilient Aguadulce-derived mutant for this trait (Figure 7). From a market perspective, large-seeded major-type lines with improved weight-based yield represent promising candidates for fresh and green markets, where individual seed size is a primary commercial criterion, providing a basis for further selection within this botanical type pending statistical confirmation across additional environments.

The absence of significant differentiation in weight-based yield under deficit irrigation may reflect the particular sensitivity of seed filling to severe water stress in *Vicia faba*: at 40% FC, assimilate supply during grain filling is likely uniformly limited across genotypes, constraining the expression of genetic variation in this component. Nevertheless, the degree of yield retention demonstrated by the top-performing mutant lines under 40% FC (Figure 7), compares favorably with yield reductions reported in natural faba bean germplasm under a comparable deficit level. In Alshameri et al. [43], yield among their selected best-performing genotypes declined by approximately 28–37% between full irrigation and 50% FC, with stressed yields ranging from 21.36 to 26.13 g/plant in L4, X. 735, and 989/309/95. Notably, multiple mutant lines matched or exceeded these values under the more severe 40% FC: within the Zina-derived group, lines 21 (26.4 g), 8 (23.6 g), 12 (23.0 g), 33 (21.9 g), 24 (21.5 g), 27 (21.1 g), 22 (20.3 g), and 23 (20.8 g) all fell within or above that range, as did Aguadulce-derived lines 38 (22.2 g) and 35 (21.1 g). This performance is particularly important given that water deficit in our trial was compounded by above-average temperatures during reproductive and grain-filling stages, a stress combination known to

amplify yield penalties beyond drought alone, further highlighting the resilience potential of these lines for semi-arid production environments.

3.3. Performance of Mutant Lines Across Drought Tolerance Indices

Among the drought tolerance indices evaluated in the present study, analysis of variance revealed statistically significant differences among genotypes only for the mean productivity index (MPn) ($p < 0.001$) and the stress tolerance index (STIn) ($p < 0.001$), while the remaining indices did not yield significant overall effects. Subsequent pairwise comparisons using Dunnett's two-sided test further identified specific mutant lines that differed significantly from their respective wild-type parents, enabling the identification of mutant lines whose drought tolerance outperforms significantly the wild-type reference.

Analysis of the mean productivity index (MPn) across the mutant populations revealed notable differences in drought tolerance between the two genetic backgrounds (Figure 8), with Zina-derived mutants consistently outperforming their Aguadulce Superlonga counterparts. The wild-type estimated marginal means of Zina (22.25) and Aguadulce Superlonga (15.92) already established baseline differences of approximately 6.3 units, and this differential was further amplified across their respective mutant progenies. Among the Zina-derived lines, MPn values ranged from 18.3 (genotype 1) to 36.9 (genotype 23), with approximately 60% of lines exceeding the grand mean of the experiment (24.6) (Figure 8). Dunnett's two-sided test against the Zina control identified two mutant lines as significantly superior to their wild-type parent: genotype 23, which exhibited the highest MPn value recorded across the entire trial (36.92 $\pm$ 4.58) and differed significantly from Zina by 14.67 units ($p < 0.001$), and mutant 3, which showed a mean difference (MD) of 11.25 units above the wild-type ($p < 0.05$) (Table S1). In contrast, the Aguadulce Superlonga-derived mutant population (genotypes 34–45) demonstrated a different response pattern, with MPn values ranging from 12.25 to 20.0, none of which exceeded the grand mean and none of which differed significantly from the Aguadulce Superlonga wild-type (Figure 8). The two lowest-performing genotypes across the entire experiment, genotypes 44 (12.25) and 35 (15.42), both originated from the Aguadulce Superlonga background. This pattern underscores a well-established principle in mutagenesis breeding: the genetic ceiling of the parental genotype constrains the range of variation achievable in mutant progenies, making the initial selection of an agronomically competitive parent a critical determinant of breeding success [47].

Analysis of the stress tolerance index (STIn) revealed a pattern consistent with that observed for MPn, whereby Zina-derived mutants displayed superior productivity relative to Aguadulce Superlonga and its derived mutants (Figure 9), with estimated marginal means of 0.490 for the wild-type control Zina versus 0.239 recorded for Aguadulce Superlonga. Among the Zina-derived mutant lines, STIn values ranged from 0.283 (genotype 1) to 1.365 (genotype 23), with a considerable proportion of lines exceeding the grand experimental mean (0.670). Dunnett's two-sided test identified four Zina-derived mutant lines as significantly superior to the wild-type control. Genotype 23 recorded the highest STIn value across the entire trial (STIn = 1.365; mean difference (MD) = +0.874; $p < 0.001$), followed by genotype 22 (STIn = 1.250; MD = +0.759; $p < 0.01$), genotype 24 (STIn = 1.190; MD = +0.700; $p < 0.01$), and genotype 12 (STIn = 1.110; MD = +0.616; $p < 0.05$), all differing significantly from Zina in the same direction (Table S1). These values are among the higher STI-based drought tolerance responses documented for faba bean, in line with those reported for tolerant genotypes by Mansour et al. [44] and Memari et al. [48], and within the range described by Abdelhaleim et al. [25] for Egyptian cultivars screened under severe drought. In contrast, the Aguadulce Superlonga-derived mutant population (genotypes 34–45) displayed uniformly low STIn values ranging from 0.128 to 0.398, none of which exceeded

the grand mean or differed significantly from the Aguadulce Superlonga wild-type. The lowest-performing entries across the entire experiment, genotypes 45 (0.128) and 44 (0.153), again originated from the Aguadulce Superlonga background, consistent with the pattern observed for MPn.

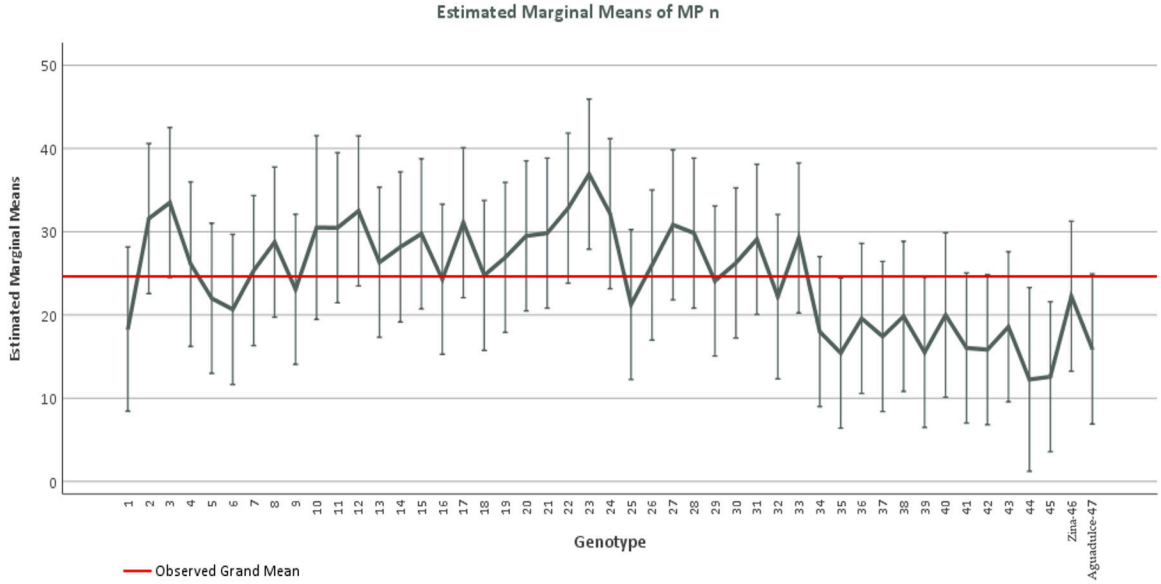


Figure 8. Estimated marginal means of the mean productivity index (MPn), calculated on the basis of seed number per plant, for 45 faba bean mutant lines and their respective parental checks (*Vicia faba minor* cv. Zina (genotype 46) and *Vicia faba major* cv. Aguadulce Superlonga (genotype 47)), pooled across the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025) under well-watered (100% field capacity) and deficit irrigation (40% field capacity) conditions. Mutant lines 1–33 are derived from cv. Zina and lines 34–45 from cv. Aguadulce Superlonga. The horizontal red line represents the observed grand mean across all genotypes and irrigation treatments. Error bars indicate 95% confidence intervals.

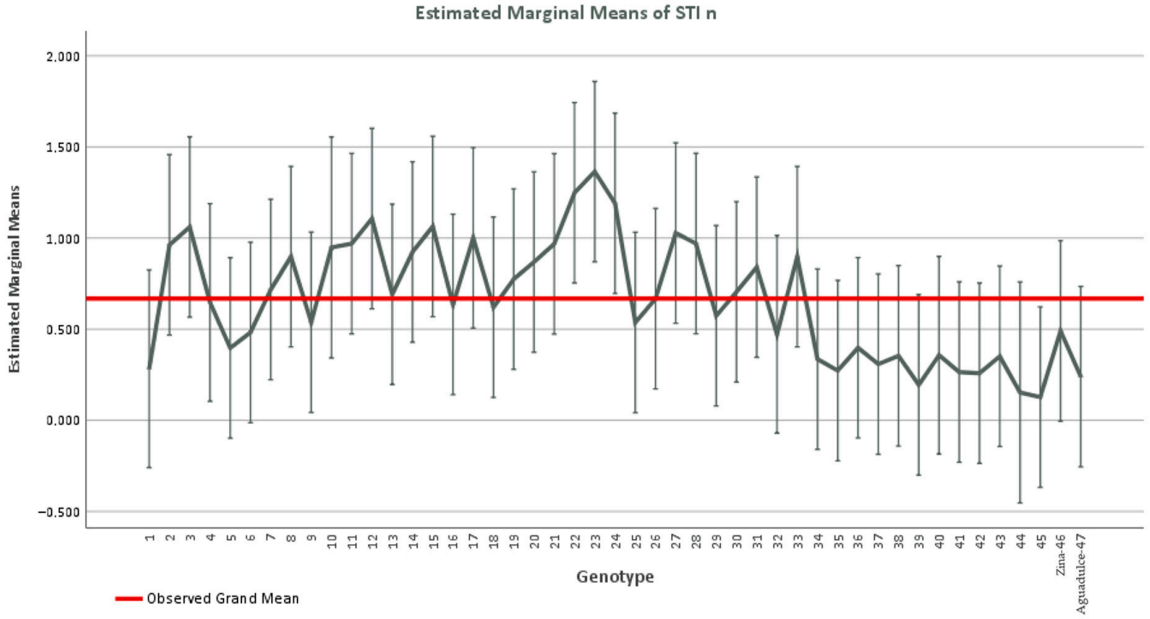


Figure 9. Estimated marginal means of the stress tolerance index (STIn), calculated on the basis of seed number per plant for 45 faba bean mutant lines and their respective parental checks (*Vicia faba minor* cv. Zina (genotype 46) and *Vicia faba major* cv. Aguadulce Superlonga (genotype 47)), pooled across the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025) under well-watered

(100% field capacity) and deficit irrigation (40% field capacity) conditions. Mutant lines 1–33 are derived from cv. Zina and lines 34–45 from cv. Aguadulce Superlonga. The horizontal red line represents the observed grand mean across all genotypes and irrigation treatments. Error bars indicate 95% confidence intervals.

Collectively, across both drought tolerance indices evaluated, six Zina-derived mutant lines emerged as noteworthy candidates: genotype 23, which achieved statistically significant superiority over the wild-type for both MPn and STIn ($p < 0.001$) and recorded the highest values across the entire trial for both indices; genotypes 22 and 24, which attained significance for STIn ($p < 0.01$) while approaching the threshold for MPn; genotype 12, which reached significance for STIn ($p < 0.05$) with a near-significant MPn response; and genotype 3, which demonstrated significant above-average performance for MPn ($p < 0.05$).

In the present study, the lack of significant genotypic differences for SSI, TOL, and YSI helped clarify which indices were truly informative as MPn and STIn appeared to provide more meaningful discrimination among the tested lines. The tolerance index (TOL = Yp − Ys) represents the absolute reduction in yield under drought. However, this index is strongly influenced by yield potential under non-stress conditions. Genotypes that naturally produce high yields under good conditions tend to show larger numerical reductions under stress even when they maintain a good proportion of their productivity. The yield stability index (YSI = Ys/Yp), on the other hand, measures proportional yield retention under stress but does not account for the absolute yield level. A genotype with consistently low yield under both conditions can produce the same YSI value as a high-performing genotype that sustains strong productivity during drought. Therefore, relying solely on YSI may not be agronomically useful for selecting superior material. Similarly, the stress susceptibility index (SSI = $(1 - Ys/Yp)/(1 - \bar{Y}s/\bar{Y}p)$) adjusts individual yield reduction according to overall stress intensity in the experiment. However, when stress conditions are relatively uniform across genotypes, variation in SSI values tends to be limited, which reduces its power to discriminate among lines. In contrast, MPn and STIn simultaneously reward performance under both water regimes. MPn reflects the average yield across stressed and non-stressed environments, while STIn emphasizes genotypes that perform well in both conditions by integrating their yields multiplicatively. In this study, these indices better captured both productivity potential and drought resilience, making them more appropriate for identifying promising mutant lines within the context of the current evaluation.

The superiority of multiplicative productivity-based indices is corroborated in natural faba bean germplasm, where STI, MP, and Geometric Mean Productivity (GMP) consistently identified the most drought-tolerant [25]. Moreover, the patterns of faba bean mutant response to drought find a notable parallel in a mutagenesis study conducted in lentil, where four varieties spanning small-seeded and bold-seeded groups were mutagenized and evaluated under stress and non-stress conditions. Drought-tolerant mutant lines were more frequently induced in the small-seeded background, mirroring the contrast between Zina-derived and Aguadulce Superlonga-derived lines in our population and GMP proved a more reliable selection criterion than SSI alone [49], consistent with the discriminating superiority of STIn and MPn observed here given their shared multiplicative structure.

3.4. Hierarchical Clustering of Genotypes Based on Drought Tolerance Indices

Hierarchical cluster analysis based on Ward's linkage method, integrating all ten drought tolerance indices (SSIg, SSIn, MPg, MPn, TOLg, TOLn, STIg, STIn, YSIg, YSIn) alongside yield performance under both irrigation regimes (YPg and YPn), partitioned the 47 entries into three statistically distinct groups reflecting a gradient of drought tolerance (Figure 10). The first cluster grouped 18 Zina-derived mutant lines, collectively classified

as drought-tolerant (genotypes 2, 3, 8, 10, 11, 12, 14, 15, 17, 20, 21, 22, 23, 24, 27, 28, 31, 33) which consistently recorded high values for multiplicative productivity indices (STIn, MPn) and low stress sensitivity, exhibiting the highest phenotypic similarity across all drought-related metrics as reflected by their tight grouping within the dendrogram. The second cluster, representing moderately tolerant genotypes, included a broader and more heterogeneous assemblage of Zina-derived mutant lines alongside the wild-type parent Zina itself (genotype 46), reflecting the intermediate performance of these entries relative to both the tolerant and sensitive groups. The positioning of Zina in the intermediate cluster, distinct from its top-performing mutant progenies, supports the possibility that the improvements observed in the first cluster reflect mutagenesis-derived gains, beyond the inherent capacity of the parental genotype. On the other hand, the third cluster, classified as drought-sensitive, was almost exclusively composed of Aguadulce Superlonga-derived mutant lines (genotypes 34–45) together with the Aguadulce Superlonga wild-type (genotype 47), which merged at a large rescaled distance from the other two clusters, indicating a fundamentally different and less favorable drought response profile.

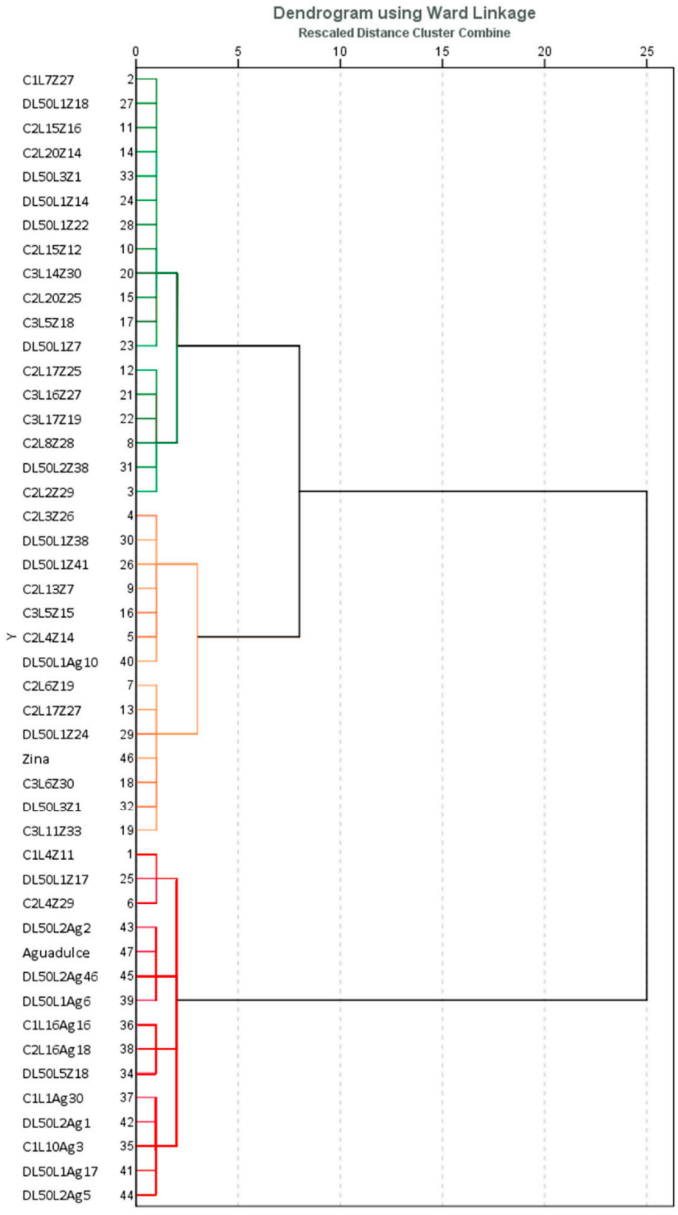


Figure 10. Hierarchical cluster analysis dendrogram (Ward's linkage method, rescaled distance) of 45 EMS-derived faba bean mutant lines and their two wild-type parents (Zina, genotype 46; Aguadulce

Superlonga, genotype 47) based on ten drought tolerance indices (SSIg, SSIn, MPg, MPn, TOLg, TOLn, STIg, STIn, YSIg, YSIn) and seed yield per plant (in mass and number of seeds) under well-watered (100% FC) and deficit irrigation (40% FC) conditions across the two generation/year combinations (M_2/2023–2024 and M_3/2024–2025). Three clusters are identified: drought-tolerant (green), moderately tolerant (orange) and drought-sensitive (red).

Collectively, the hierarchical clustering structure synthesizes and spatially validates the conclusions emerging from individual index analyses: mutagenesis-driven improvements in drought tolerance were largely confined Zina background, whereas the large-seeded Aguadulce Superlonga background, despite yielding lines with marginally superior performance relative to their wild-type parent, remained within the drought-sensitive range of the population, highlighting the critical importance of carefully selecting the starting genetic material prior to mutagenesis, as the genetic potential of the parental genotype ultimately determines the effectiveness of mutational gain in drought tolerance breeding.

The hierarchical clustering structure obtained in the present study is consistent with Mansour et al. [44] and Memari et al. [48], both of whom similarly applied Ward's linkage method to partition faba bean genotypes into tolerance classes based on drought indices, with tolerant groups consistently recording superior STI and MP values relative to sensitive counterparts.

4. Conclusions

This study is the first work designed to develop and characterize an EMS-induced mutant population in faba bean (*Vicia faba* L.) with the explicit objective of enhancing drought tolerance, addressing a critical gap in legume stress breeding research. Two-generational evaluation across contrasting water regimes, with each generation corresponding to a distinct growing season, provides preliminary evidence that induced mutagenesis can generate measurable, potentially heritable variation in reproductive drought resilience in this species.

Severe water deficit at 40% field capacity reduced seed number and yield per plant by 42% and 47%. Among ten drought indices tested, seed-number-based MPn and STIn were the most discriminating, a methodologically relevant finding when evaluating populations from divergent botanical types.

Genotype 23 (Zina-derived), emerged as the most consistent top performer, significantly exceeding its parental check for both MPn and STIn ($p < 0.001$ vs. wild-type), with genotypes 22, 24, 12, and 3 identified as secondary candidates for continued advancement.

In contrast, Aguadulce Superlonga-derived mutant lines demonstrated limited mutational gain for drought tolerance, suggesting that parental background influences the expression of useful variation under stress. Hierarchical clustering partitioned the 47 entries into three coherent drought-response classes, with 18 Zina-derived lines grouping within the tolerant cluster.

These results support advancing the identified lines to multi-location field trials under semi-arid conditions, alongside physiological and molecular characterization of the underlying tolerance mechanisms. If validated across broader environments, their integration into variety registration pipelines would represent a tangible outcome for applied semi-arid breeding. Additionally, the small seed size of genotype 23 may confer compatibility with no-till mechanized seeding systems, warranting evaluation in the context of Morocco's expanding conservation agriculture program.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy16111064/s1, Table S1. Mean ± standard deviation of drought tolerance indices for faba bean (*Vicia faba* L.) mutant lines comprising Zina-derived

lines (1–33) and Aguadulce Superlonga-derived lines (34–45), with significance of pairwise comparisons against the respective wild-type parent (Dunnett's two-sided test) indicated by asterisks.

Author Contributions: Conceptualization, O.C., L.B., E.B., and A.N.; methodology, O.C. and A.N.; software, A.D.; formal analysis, O.C. and A.D.; investigation, O.C., S.O., M.E.F., C.E.K., and K.K.M.; data curation, O.C., S.O., M.E.F., C.E.K., and K.K.M.; writing—original draft preparation, O.C.; writing—review and editing, A.D., K.A., L.B., and A.N.; visualization, O.C. and A.D.; project administration, L.B., E.B., and A.N.; supervision, L.B., E.B., and A.N.; funding acquisition, O.C., L.B., E.B., K.A., and A.N.; validation, A.N. and L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was was conducted within the framework of a project partially funded by the Moroccan-Swiss Cooperation between the Ministry of Higher Education, Scientific Research and Innovation of the Kingdom of Morocco and the State Secretariat for Education, Research and Innovation of the Swiss Confederation (Project n° 3, 2023).

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Acknowledgments: The authors gratefully acknowledge the support of the Moroccan Collaborative Grants Program (MCGP) between INRA Morocco and ICARDA for its continuous support and technical assistance throughout this work.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EMS	Ethyl methanesulfonate
FC	Field capacity
YPg	Yield per plant (in grams)
YPn	Yield per plant (in number of seeds)
SSIg/SSIn	Stress susceptibility index (seed weight/seed number)
MPg/MPn	Mean productivity index (seed weight/seed number)
TOLg/TOLn	Tolerance index (seed weight/seed number)
STIg/STIn	Stress tolerance index (seed weight/seed number)
YSIg/YSIn	Yield stability index (seed weight/seed number)
GMP	Geometric Mean Productivity
ANOVA	Analysis of variance
RCBD	Randomized complete block design
IT	Irrigation treatment
INRA	Institut National de la Recherche Agronomique
M1/M2/M3	Mutagenic generations 1, 2, and 3
IBM SPSS	IBM Statistical Package for the Social Sciences

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