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EFFECT OF ETHYL METHANESULFONATE AND SODIUM AZIDE ON THE PERFORMANCE AND YIELD COMPONENTS OF FABA BEANS

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SUMMARY

Faba bean (*Vicia faba* L.) is one of the most important legume crops worldwide because of its multiple uses and ability to grow over a wide range of climatic conditions. The presented study aimed to determine the efficiency of ethyl methanesulfonate (EMS) and sodium azide (SA) concentrations on two faba bean cultivars. Two field experiments during the crop seasons of 2019–2020 and 2020–2021 took place at Cairo University, Giza, Egypt. Genetically pure, uniform, dry, and dormant seeds of the faba bean cultivars Cairo-4 and Nubaria-1 received three different doses of EMS (0.05%, 0.10%, and 0.15%) and SA (1500, 3000, and 4500 ppm). The results indicated EMS was more effective in inducing mutation than the SA. The mutagenic effectiveness appeared to be better with higher concentrations of both mutagens (EMS and SA). The possible reason for the decrease in mutagenic effectiveness indicated an association with a decreased concentration of both mutagens, which also revealed a less damaging effect.

Keywords: Faba bean (*V. faba* L.), ethyl methanesulfonate (EMS), sodium azide (SA), mutation, performance, yield components

Key findings: The EMS with a higher concentration proved more effective in inducing mutation than the SA in the faba bean (*V. faba* L.). The optimal doses of mutagens are crucial for enhancing the frequency of desirable mutations and minimizing undesirable ones in faba bean breeding programs.

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INTRODUCTION

Faba beans (*Vicia faba* L.) are a multipurpose legume crop used both as human food and animal feed because of their supreme protein content and biomass (Musallam *et al.*, 2004; Yasmin *et al.*, 2020). They also play a vital role in crop rotation, nitrogen fixation, and improvement in soil structure (Ye *et al.*, 2005). However, the last three decades showed a meager growth rate of the faba bean's grain yield, which has been alarming because of the slow and steady progress in increasing the genetic diversity (Nedumaran *et al.*, 2015). The general use of high-yielding cultivars for selection and extensive faba bean cultivation led to an increased production worldwide. Yet, there is a narrowing of the faba bean's genetic diversity, which is the main constraint for its higher productivity (Praveen and Prashant, 2020).

Induced mutagenesis has been instrumental in developing high-yielding cultivars by improving yield-related traits while preserving the cultivars' original genetic composition (Raina *et al.*, 2018). Chemical mutagenesis is a process that uses chemical compounds to alter the DNA structure and affect the gene function. Chemical mutagenesis, with its ability to induce random variations throughout the genome, offers several advantages. It generates a wide array of mutations in target genes, and a single plant can harbor numerous distinct mutations, leading to a manageable population size (Raina *et al.*, 2018).

Mutation induced by chemical mutagens significantly enhances the genetic variability for specific traits in crop plants, facilitating more effective selection and enhancing the likelihood of obtaining desired genotypes. Mutagenesis is a promising technology for augmenting genetic variability in species with limited natural genetic diversity, and where losing the desired phenotypes has occurred due to poor competitiveness under natural conditions. According to FAO reports (2019), approximately 20 mutant cultivars have succeeded in their development, thus far. Among them, the recently released faba bean

mutant cultivar Geca-5 exhibited improved attributes such as superior quality and early maturity.

The mutagens' efficiency depends on the specific crop genotype and the type of mutagen applied. Various researchers have reported varying degrees of effectiveness and efficiency by using EMS and SA mutagens in different crops. For instance, studies conducted by Waghmare and Mehra (2001) focused on grass pea; Gaikwad and Kothekar (2004), as well as, Laskar *et al.* (2015), examined the chickpea; Laskar *et al.* (2018) investigated the lentil; Amin *et al.* (2016) studied the black cumin; Khan and Tyagi (2010) explored the soybean; and Wani *et al.* (2017) conducted research on the mung bean. Each of these studies sheds light on the differing efficiency of mutagens within their respective crop species. The following study aimed to assess the effectiveness of the mutagens ethyl methanesulfonate (EMS) and sodium azide (SA) in the M₁ and M₂ generations of the faba bean.

MATERIALS AND METHODS

Two faba bean (*V. faba* L.) cultivars (Cairo-4 and Nubaria-1), with considerable variations in seed size, were specimens used in this study. The cultivar Cairo-4, having medium seeds, is an Egyptian local selection and synthetic cultivar provided by the Cairo University, Giza, Egypt. The cultivar Nubaria-1, with a large seed size, is an individual plant selection from the Rena Blanka, obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

M₁ generation

Healthy seeds of uniform size of each faba bean cultivar incurred presoaking for six hours in distilled water before treating with EMS "CH3SO3C2H5" (0.05%, 0.10%, and 0.15%) and SA "NaN3" (1500, 3000, and 4500 ppm) with intermittent shaking at room temperature of 24 °C ± 2 °C. Later, the seeds' washing under running water took two hours before planting in the field with the control treatment

during the crop season of 2019–2020 as M₁ generation at the Cairo University, Giza, Egypt (30° N, 31°; 28 E with an altitude of 19 m). The faba bean field experiment started the planting on October 27, 2019, in a randomized complete block design (RCBD) layout with three replications. The experimental plot consisted of three ridges, and each ridge was 4 m long and 60 cm apart. Conducting the dry sowing of seeds continued on one side of each ridge, with 1 seed per hill and a 20-cm plant-to-plant distance.

M₂ generation

Twenty healthy seeds from each normal-looking M₁ plant in all treatments in both faba bean cultivars reached sowing on October 17, 2020, as the M₂ generation. The plants and rows' distance remained at 20 and 60 cm, respectively. The field trial, conducted in an RCBD layout with three replications, proceeded in each treatment. The M₂ generation experiment also transpired at Cairo University, Giza, Egypt.

Data recorded

The recording of data ensued on traits, i.e., emerged seedlings (%) (after seven days), emerged seedlings (%) (after 14 days), survived plants (%), plant height (cm), number of branches, pods per plant, seeds per plant, plant dry weight (g), seed yield per plant (g), seed yield per ridge (g), and seed index. The weight of a random sample of 100 seeds from each plant and harvest index as the percentage are calculated as (seed yield per plant/plant dry weight) × 100.

Assessment of mutation frequency, mutagenic effectiveness, and efficiency

Individual plants of each treatment and the control plot underwent separate harvesting, with desirable M₁ variants selected and raised in the M₂ generation. Later, calculating the mutation frequency and mutagenic effectiveness succeeded, as expressed in percentages (Konzak *et al.*, 1965).

Mutation frequency

On the M₂ plant basis (mutated M₂ plants percentage), mutation frequency estimation as the percentage of segregating M₁ plant progenies (Gaul, 1964) followed the formula below.

$$\text{Mutagenic frequency} = \frac{\text{Number of mutant seedlings}}{\text{Total number of M}_2 \text{ seedlings}} \times 100$$

Mutagenic effectiveness

Mutagenic effectiveness is a measure of the frequency of mutation induced by a unit dose of a mutagen. The formula suggested by Konzak *et al.* (1965) served to evaluate the mutagenic effectiveness of the mutagens.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency in M}_2 (M_p)}{\text{Concentration} \times \text{duration of treatment}}$$

Mutagenic efficiency

The ratio of factor mutation to biological damage means desirable changes are free from associated undesirable changes on mutagenesis. Mutagenic efficiency also represents the proportion of mutation in relation to biological damage. Mutagenic efficiency was calculated by the formula suggested by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency in M}_2 (M_p)}{\text{Lethality abnormality M}_1\%}$$

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency in M}_2 (M_p)}{\text{Sterility abnormality M}_1\%}$$

Where, calculating lethality was a percentage of the plant survival reduction, and sterility as a percentage of the seed fertility reduction.

RESULTS AND DISCUSSION

Analysis of variance

In the M₁ generation during 2019–2020, the faba bean's two cultivars treated with different concentrations of ethyl methanesulfonate (EMS) and sodium azide (SA) revealed significant differences for most traits (Table 1). Cultivars displayed significant ($P \leq 0.05$ and $p \leq 0.01$) variations for all the studied traits, except emerged seedlings (after seven and 14 days) and survived plants' percentages. Therefore, the genotypic means indicated considerable differences for most growth- and yield-related traits. The results proved these attributes acquired substantial effects from both faba bean cultivars. Moreover, the EMS significantly ($P \leq 0.01$) affected all studied traits, which could be due to the different EMS concentrations. Khursheed *et al.* (2016a) reported that yield-related traits and seed yield received considerable influences from different concentrations of EMS in faba beans. The interactions between cultivars and EMS showed significant differences for all studied characters, except the survived plants, seed yield per plant, and seed yield per ridge. This proved that despite the effectiveness of faba bean cultivars, such varied performances resulted from different EMS concentrations for the growth and yield traits.

The SA concentrations as a source of variation revealed significant ($P \leq 0.05$ and $P \leq 0.01$) differences for all studied traits. Haridy *et al.* (2022) reported that growth traits, seed yield, and its components obtained noteworthy effects from different concentrations of SA doses in faba beans. The interactions between faba bean cultivars and SA concentrations showed significant ($P \leq 0.05$) differences for all studied traits, except the survived plants (%), seeds per plant, seed yield per plant, seed yield per ridge, and harvest index. This proved the effectiveness of faba bean cultivars did not limit varied outcomes for various traits as a result of the different SA concentrations.

In the M₂ generation during the 2020–2021 cropping season, the two cultivars of the faba bean treated with different concentrations of EMS and SA demonstrated significant

differences for most traits (Table 2). Faba bean cultivars as a source of variation expressed significant ($P \leq 0.05$ and $P \leq 0.01$) disparities for all studied traits, except emerged seedlings (after seven days), survived plants (%), and pods per plant. Hence, establishing the validity of comparing genotypic means was successful. These findings proved that the various attributes gained considerable influence from various faba bean cultivars. Furthermore, the EMS concentrations exhibited a significant ($P \leq 0.01$) impact on all traits under study, as anticipated due to the variations in EMS concentrations. Ambreen *et al.* (2012) observed that pods per plant, seeds per pod, and seeds per plant had notable changes from different concentrations of EMS in *Linum usitatissimum*.

The interactions between the faba bean cultivars and EMS concentrations showed significant ($P \leq 0.05$) variations for all traits, except for emerged seedling percentage (after seven days), seeds per pod, and plant dry weight. This proved that even if faba bean cultivars are superior, the outcomes varied across different EMS concentrations for various traits. In contrast, SA concentrations as a source of variation exhibited significant ($P \leq 0.05$ and $P \leq 0.01$) effects on all studied traits. Haridy *et al.*'s (2022) findings disclosed that growth traits, seed yield, and yield components of faba beans incurred sizable alterations from different SA doses. The interactions between cultivars and SA concentrations exhibited significant differences for all studied traits, except plant height, seeds per pod, and seed yield per ridge. This indicates the effectiveness of faba bean cultivars had little effect, resulting in varied outcomes across different SA doses for plant height, seeds per pod, and seed yield per ridge. These results were greatly analogous to past findings in faba bean genotypes treated with chemical mutagens (Al-Shamma, 2014; Al-Nuaimi and Al-Shamma, 2015).

Mean performance

The results showed a significant increase in pods per plant, plant dry weight, and seed yield per plant in mutants of the M₁ generation compared with the control in both faba bean

Table 1. Significance of mean squares due to genotypes (G), EMS, SA, and their interactions for studied traits during the crop season of 2019–2020 in M₁ generation of faba beans.

Traits	Cultivars (C)	EMS	C x EMS	SA	C x SA
Emerged seedlings % (7 days)	37.500 ^{ns}	381.944**	126.389**	480.556**	101.389 ^{ns}
Emerged seedlings % (14 days)	176.042 ^{ns}	612.153**	103.819**	841.667**	323.639*
Survived plants %	126.021 ^{ns}	559.375**	39.931 ^{ns}	651.042**	95.486 ^{ns}
Plant height	1066.67*	543.278**	171.733*	452.556*	249.676*
Branches' number	114.415*	284.785**	98.9654**	100.614**	91.414*
Pods/plant	294.324*	3521.154**	587.441**	2601.391**	962.541**
Seeds/pod	876.235**	944.365**	236.236*	545.951**	124.329*
Seeds/plant	501.420*	529.070**	75.352*	543.268**	41.798 ^{ns}
Plant dry weight	19193.072**	1885.486**	537.029*	1883.364**	473.631**
Seed yield/plant	4137.000**	567.366**	71.874 ^{ns}	520.085**	253.249 ^{ns}
Seed yield /ridge	76017.329*	12852.552**	7271.165 ^{ns}	19582.692**	1502.683 ^{ns}
Seed index	4277.340**	1616.518**	401.406**	1066.379**	682.131*
Harvest index	380.457*	170.428**	71.118 ^{ns}	782.321**	150.235 ^{ns}

*, ** = Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively, NS = Nonsignificant.

Table 2. Significance of mean squares due to genotypes, EMS, SA, and their interactions during the crop season of 2020–2021 in M₂ generation of faba beans.

Traits	Cultivars (C)	EMS	C x EMS	SA	C x SA
Emerged seedlings % (7 days)	759.375 ^{ns}	587.153**	28.819 ^{ns}	1862.153**	228.819**
Emerged seedlings % (14 days)	234.375*	317.708**	84.375*	1304.167**	430.289*
Survived plants %	137.500 ^{ns}	502.778**	112.507*	1326.042**	470.365*
Plant height	120.167*	219.611**	207.833**	660.111**	137.000 ^{ns}
Branches' number	711.094**	445.521**	235.541*	369.184**	214.587*
Pods/plant	266.325 ^{ns}	961.254**	321.110**	248.740**	151.874*
Seeds/pod	442.321**	165.210**	74.216 ^{ns}	251.365**	99.654 ^{ns}
Seeds/plant	582.321*	798.210**	387.324*	685.829**	229.076*
Plant dry weight	7280.166**	1320.129*	520.223 ^{ns}	2673.589**	522.254*
Seed yield/plant	622.202**	313.089**	70.616*	690.998**	410.275*
Seed yield/ridge	10384.656**	17625.712**	1596.117*	20794.085**	9710.348 ^{ns}
Seed index	3802.683**	1267.325**	612.275*	1280.265**	178.057*
Harvest index	383.251*	191.234*	168.279*	80.651**	106.787*

*, ** = Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively, NS = Nonsignificant.

Table 3. Mean performance of faba bean cultivars affected by different EMS concentrations for some yield traits during the crop season of 2019–2020 in M₁ generation.

Cultivars	Concentrations	Pods plant ⁻¹		Plant dry weight (g)		Seed yield plant ⁻¹ (g)	
		Mean	Relative ¹⁾	Mean	Relative ¹⁾	Mean	Relative ¹⁾
Cairo-4	Control	16.7	100.0	99.1	100.0	33.0	100.0
	0.05%	14.4	86.1	80.8	81.7	25.1	76.3
	0.10%	19.3	115.3	131.7	132.9	44.1	134.3
	0.15%	14.0	83.7	79.8	80.5	21.7	65.5
	LSD _{0.05}	1.942	11.39	15.60	15.80	5.99	21.28
Nubaria-1	Control	15.2	100.0	150.5	100.0	59.1	100.0
	0.05%	17.2	113.2	163.1	108.6	54.6	92.6
	0.10%	18.9	124.7	168.7	112.0	69.2	117.3
	0.15%	15.8	104.0	135.3	90.2	46.1	78.1
	LSD _{0.05}	1.569	11.16	ns	ns	6.442	10.41

cultivars (Tables 3). However, the cultivar Nubaria-1 exhibited more increase in seed yield than the cultivar Cairo-4. The highest plant dry weight and seed yield per plant appeared in EMS (0.10%) (168.7 and 69.2 g, respectively) in cultivar Nubaria-1. Unlike other polygenic traits, these characters had reports of being controlled by many genes (Khan, 1990). Past studies reported that the shift of seed yield and its components depends on the favorable association between the components in response to mutagenic treatments and could be the direct selection for seed yield in the M₂ generation (Khan and Qureshi, 2006). However, the dose of EMS (0.15%) gave the lowest values of pods per plant, plant dry weight, and seed yield per plant in the cultivar Cairo-4 (14.0, 79.8, and 21.7 g, respectively). Moreover, the cultivar Nubaria-1 provided the minimum values with EMS of 0.15% for plant dry weight and seed yield per plant, thus indicating the differential genotypic response of plants toward mutagenic treatments. Shahnawaz *et al.* (2016) also stated a similar decrease for the seed yield and its components in faba beans. Many efforts have proceeded for the induction of genetic variability in crop plants using chemical mutagenesis, like growth habits, yield, and yield components (Khursheed *et al.*, 2016b).

The SA concentrations revealed significant differences for various traits, and the SA concentration of 3000 ppm showed the highest values for pods plant⁻¹, plant dry weight, and seed yield plant⁻¹ traits in cultivars

Cairo-4 and Nubaria-1 (19.8, 114.0 g, 43.8 g, 18.5, 153.9 g, and 58.5 g, respectively) (Table 4). However, the SA concentration (4500 ppm) presented the lowest values for all studied traits in both cultivars (12.8, 69.9 g, 20.8 g, 15.4, 119.0 g, and 38.1 g, respectively). These results agree with past findings of Bhat *et al.* (2007) and Haridy *et al.* (2022). The latest study took place as an innovative approach using EMS and SA treatments. Therefore, it was possible to combine the high-yielding traits with high-yielding components in faba beans (Jensen *et al.*, 2010).

Effectiveness and efficiency of mutagenesis

Based on the number of mutations per unit dose of the EMS mutagen, it calculated the mutagenic effectiveness. Mutagenic effectiveness emerged to be higher at lower doses of EMS treatments in both faba bean cultivars Cairo-4 and Nubaria-1 (81.31%, 1.35%, 65.66%, and 2.14%, respectively). The estimates of effectiveness ranged from 57.25% to 81.31% in the Cairo-4 cultivar and 54.87% to 65.66% in the cultivar Nubaria-1 with EMS treatments. The mutagenic efficiency reached calculation based on pollen sterility (%). The efficiency of EMS treatments ranged from 0.94 to 1.35 in the cultivar Cairo-4 and 1.09 to 2.14 in the cultivar Nubaria-1. However, in both cultivars, the EMS of 0.05% appeared to be more effective than its other doses. In both faba bean cultivars, the

Table 4. Mean performance of faba bean cultivars affected by different SA concentrations for some yield traits during the crop season of 2020–2021 in M₂ generation.

Cultivars	Concentrations	Pods plant ⁻¹		Plant dry weight (g)		Seed yield plant ⁻¹ (g)	
		Mean	Relative ¹⁾	Mean	Relative ¹⁾	Mean	Relative ¹⁾
Cairo-4	Control	16.7	100.0	99.1	100.0	33.0	100.0
	1500 ppm	16.7	100.3	87.9	88.8	30.9	94.5
	3000 ppm	19.8	118.2	114.0	115.0	43.8	132.5
	4500 ppm	12.8	76.4	69.9	70.6	20.8	63.4
	LSD _{0.05}	3.55	21.45	18.91	19.15	8.30	23.79
Nubaria-1	Control	15.2	100.0	150.5	100.0	59.1	100.0
	1500 ppm	16.4	107.9	168.9	112.3	57.1	96.6
	3000 ppm	18.5	122.2	153.9	102.4	58.5	99.1
	4500 ppm	15.4	101.3	119.0	79.1	38.1	64.6
	LSD _{0.05}	1.48	10.88	16.34	10.57	8.46	14.03

Table 5. Effectiveness and efficiency of EMS and SA in M₂ faba bean progenies in cultivars Cairo-4 and Nubaria-1.

Treatments		Pollen sterility (%)	Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency
EMS					
Cairo-4	0.05%	8.24	10	81.31	1.35
	0.10%	10.21	12	65.54	1.13
	0.15%	15.54	14	57.25	0.94
	0.05%	5.71	8	65.66	2.14
Nubaria-1	0.10%	7.92	10	54.87	1.43
	0.15%	13.72	14	59.46	1.09
SA					
Cairo-4	1500 ppm	6.14	8	64.37	0.81
	3000 ppm	11.74	10	71.22	1.95
	4500 ppm	13.71	16	47.65	1.07
	1500 ppm	4.47	10	31.33	2.03
Nubaria-1	3000 ppm	10.53	12	51.79	0.97
	4500 ppm	12.26	12	57.69	1.22

comparative efficacy ranking of mutagens revealed EMS doses of 0.05% to be superior to both 0.10% and 0.15% doses (Table 5). Based on pollen sterility, EMS (0.15%) was the most efficient in both cultivars among all mutagen treatments. On the basis of pollen sterility, the efficiency of mutagens was in descending order with EMS (0.15% > 0.10% > 0.05%) in both faba bean cultivars (Table 5).

Mutagenic effectiveness computation relied on the number of mutations per unit dose of the SA mutagen. Mutagenic effectiveness was evidently higher at the SA 3000 ppm treatment in the cultivar Cairo-4 (71.22); however, the SA 4500 ppm showed higher values in the cultivar Nubaria-1 (57.69). The estimates of effectiveness ranged from 47.65% to 71.22% in the cultivar Cairo-4 and

31.33% to 57.69% in the cultivar Nubaria-1 with SA treatments. Meanwhile, the mutagenic efficiency had calculations based on the pollen sterility (%). The efficiency of SA treatments ranged from 0.81 to 1.95 in the cultivar Cairo-4 and 0.97 to 2.03 in the cultivar Nubaria-1. In the Cairo-4 cultivar, SA 3000 ppm proved more effective than other concentrations. The SA 1500 ppm was more effective than other concentrations in the cultivar Nubaria-1. Based on pollen sterility, it was notable that the SA 4500 ppm treatment exhibited the highest efficiency among all the mutagen treatments in both faba bean cultivars. According to pollen sterility, the descending order of mutagenic efficiency occurred as SA (4500 ppm > 3000 ppm > 1500 ppm) in both cultivars (Table 5).

Mutagenic effectiveness and efficiency play a crucial role in mutation breeding. The mutagenic effectiveness measures the frequency of mutation induced by a specific concentration of the mutagen, while the mutagenic efficiency assesses the mutation based on their biological effects, such as sterility and lethality. In the M₂ generation, the genotypic response to increased concentrations of mutagen represents the mutagenic effectiveness, while efficiency indicates the proportion of mutation relative to undesirable biological effects. Ambreen *et al.* (2012) observed the highest effectiveness of EMS compared with individual doses of SA in *Linum usitatissimum*. Khan and Tyagi (2010) reported a higher efficiency at lower concentrations of mutagens in *Glycine max*. Wani *et al.* (2011) stated similar results in *Vigna radiata*, while Khursheed *et al.* (2016b) disclosed the same trend in *Vicia faba*. It is noteworthy that higher concentrations of mutagens can lead to increased biological damage, as indicated by factors, like pollen sterility (Konzak *et al.*, 1965).

Past studies have considered mutagenic efficiency based on criteria, i.e., seedling height and lethality, as more reliable. Consistent with this, Gaikwad and Kothekar (2004) observed the higher biological damage (pollen sterility) with the higher doses of mutagens in lentils. Girija and Dhanavel (2009) reported similar findings in *Vigna unguiculata*. Khursheed *et al.* (2015) also described the influence of higher doses of mutagens in *Vicia faba*. The optimal choice of mutagens, doses, and duration of exposure is essential for successful mutation breeding (Bhosle and Kothekar, 2010; Khursheed *et al.*, 2015). However, the lower doses of EMS have been better options for achieving a higher frequency of mutation with minimal biological damage in *Vicia faba* (Khursheed *et al.*, 2016b).

In summary, the knowledge of mutagenic effectiveness and efficiency, as reported by various authors, is vital for the successful implementation of mutation breeding. Understanding the balance between desirable and undesirable mutations and selecting the appropriate mutagen, dose, and duration of exposure are the key factors in

achieving the desired outcomes through mutation breeding.

CONCLUSIONS

The application of ethyl methanesulfonate (EMS) and sodium azide (SA) can induce genetic variability in faba bean genotypes, leading to unique variations in agronomic and morphological traits. These induced variations can be valuable for breeding and developing new, high-yielding cultivars that meet the needs of farming communities and the market.

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