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Evaluation of Ethyl Methanesulphonate (EMS) Mutagenesis on Yield and Yield-Related Traits of Cucumber (*Cucumis sativus* L.) Cultivars

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Abstract

Chemical mutagenesis using ethyl methanesulphonate (EMS) is a widely adopted approach for inducing heritable genetic variation in crop plants. This study evaluated the effects of EMS mutagenesis on yield and yield-related traits in three cucumber (*Cucumis sativus* L.) cultivars Darina, Market More, and Kenzo (F_1) under two exposure durations (16 and 24 hours) in addition to an untreated control. Traits assessed included days to flowering (DTF), fruit size (FS), number of cucumbers per plant (NCPP), length of fruit (LOF), weight of fruit (WOF), and circumference of fruit (COF). Cultivar performance was quantified using a Composite Decision Index (CDI), while mutation effectiveness and efficiency were integrated into a Composite Mutation Decision Value (CMDV). Darina recorded the highest CDI (0.833), earliest flowering (25.61 days), and superior yield components. The 24-hour EMS treatment maintained a Yield Stability Index (YSI) of 0.95 and increased NCPP by 20.3% relative to the control. Darina × 24-hour EMS achieved the highest CMDV (0.91) and was classified as highly recommended for breeding advancement. Broad-sense heritability under this combination ranged from 0.69 to 0.78, with fruit weight showing the highest heritability (H^2 = 0.78). The study demonstrates that 24-hour EMS exposure on Darina represents an optimal mutation window for inducing beneficial, heritable variation in cucumber, and provides a decision-based framework for advancing superior cultivar × mutagen combinations in subsequent breeding generations.

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most economically important vegetable crops worldwide, cultivated extensively across tropical, subtropical, and temperate regions for its high water content, dietary fibre, vitamins, and antioxidant compounds. It ranks among the top five commercially produced vegetable crops globally, with significant contributions to smallholder income and dietary nutrition in developing countries (Pandey et al., 2020). In Nigeria and other West African countries, cucumber is increasingly cultivated as a high-value cash crop for urban and export markets, driven by rising consumer demand and expanded greenhouse production capacity.

Despite its commercial importance, cucumber productivity is constrained by a narrow genetic base resulting from decades of selective breeding and widespread adoption of F_1 hybrid cultivars. This genetic uniformity limits the scope for conventional selection-based improvement and underscores the need for approaches that generate new heritable variation. Chemical mutagenesis, particularly through the use of ethyl methanesulphonate (EMS), offers a proven, cost-effective strategy for expanding genetic variability within breeding programmes. EMS is an alkylating agent that preferentially induces guanine-to-adenine transitions, generating a dense spectrum of point mutations that can affect quantitative trait loci controlling yield-related characters (Gilchrist & Haughn, 2010).

EMS mutagenesis has been successfully applied in several vegetable crops, including tomato, pepper, cowpea, and legume species, to induce variation in yield, earliness, and fruit quality attributes (Kharkwal & Shu, 2009; Khan et al., 2017). In cucurbits, however, the application of EMS has been less extensively characterized, and the dose-dependent effects of EMS on yield components, mutation efficiency, and heritability in cucumber cultivars with contrasting genetic backgrounds remain poorly understood.

Mutation effectiveness and efficiency are distinct and complementary parameters in mutation breeding. Effectiveness refers to the frequency of phenotypic changes induced, while efficiency captures the proportion of beneficial, non-deleterious mutations relative to total mutagenic burden. Identifying the optimal dose that maximizes efficiency while maintaining yield stability is critical for the practical application of mutagenesis in crop improvement (Brock, 1965; van Harten, 1998). The use of composite decision indices integrating multiple trait responses provide a quantitative, reproducible, and bias-free approach to selecting the most productive cultivar × mutagen combinations for breeding advancement (El-Degwy, 2013).

The present study therefore aimed to: (i) evaluate the baseline yield performance of three cucumber cultivars with contrasting genetic backgrounds; (ii) determine the effects of two EMS exposure durations on yield and yield-related traits; (iii) assess mutation effectiveness, efficiency, and yield stability; and (iv) apply Composite Mutation Decision Value (CMDV) and Weighted Trait Decision Value (WTDV) frameworks to identify and rank optimal cultivar × EMS combinations for breeding advancement.

Materials and Methods

Plant material and EMS treatment

Three cucumber cultivars: Darina, Market More, and Kenzo (F_1) representing open-pollinated and hybrid genetic backgrounds were used in this study. Seeds were treated with 200 mL ethyl methanesulphonate (EMS) at two exposure durations: 16 hours and 24 hours. Control seeds were treated with distilled water under identical conditions. Seeds were pre-soaked in distilled water for six hours prior to EMS immersion. Following EMS treatment, seeds were thoroughly rinsed with running water for 30 minutes to remove residual mutagen before sowing.

Experimental design and management

The experiment was conducted in a completely randomized design arrangement comprising three cultivars and three EMS treatments (control, 16 h, 24 h), replicated three times. Plants were raised under standard field conditions with uniform agronomic management including irrigation, fertilizer application, and pest control. Data were collected from the M_1 generation on all evaluated traits.

Data collection

The following yield and yield-related traits were measured: days to flowering (DTF), measured from sowing to the opening of the first female flower; fruit size (FS, cm), measured as the diameter at the widest point; number of cucumbers per plant (NCPP); length of fruit (LOF, cm); weight of fruit (WOF, g), measured using a digital balance; and circumference of fruit (COF, cm), measured using a measuring tape at the mid-point of each fruit.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using R software version 4.12. Treatment means were separated using the Least Significant Difference (LSD) test at $p \leq 0.05$. Trait performance across cultivars and EMS treatments was further analysed using a Composite Decision Index (CDI), computed as:

$$\text{CDI} = \frac{\sum_i (w_i \times \text{standardized trait value})}{\sum_i w_i}$$

where w_i represents the relative economic weight assigned to each trait. Mutation effectiveness was assessed by comparing induced phenotypic variation against the control, while efficiency was quantified as the ratio of favourable to total trait changes. These parameters were integrated into a Composite Mutation Decision Value (CMDV):

$$\text{CMDV} = (\text{Effectiveness Score} \times \text{Efficiency Score})^{0.5}$$

The Yield Stability Index (YSI) was computed as the ratio of trait mean under EMS to trait mean in the control. Broad-sense heritability (H^2) was estimated from variance components obtained through ANOVA, as the ratio of genetic variance to total phenotypic variance. Weighted Trait Decision Values (WTDV) integrated trait-specific sensitivity scores with economic weights to provide a composite ranking of cultivar × EMS combinations.

Results

Mean performance of cucumber cultivars for yield and yield-related traits

Cucumber cultivars differed significantly ($p \leq 0.05$) in days to flowering (DTF) and fruit size (FS); the remaining yield-related traits did not differ significantly among cultivars (Table 1). Darina flowered significantly earlier than the other cultivars, with a mean of 25.61 days, while Market More exhibited the latest flowering (32.20 days). Fruit size was significantly highest in Market More (35.70 cm). Although cultivar differences were not statistically significant for the remaining traits, Darina numerically recorded the highest values for number of cucumbers per plant (1.67), fruit weight (186.03 g), fruit length (18.09 cm), and fruit circumference (16.38 cm).

To enable multi-trait comparison, trait means were standardized and integrated into a Composite Decision Index (CDI). Darina recorded the highest CDI (0.833), followed by Kenzo (F_1) (0.334) and Market More (0.265) (Table 2), indicating overall superiority of Darina when phenological and yield traits were considered jointly.

Effect of EMS treatments on yield and yield components

EMS exposure resulted in measurable changes in yield traits relative to the untreated control (Table 1). The 16-hour EMS treatment reduced fruit weight to 151.50 g, compared with 172.42 g in the control, while the 24-hour EMS treatment maintained relatively high fruit weight (162.96 g) and increased the number of cucumbers per plant to 1.60, representing a 20.3% increase over the control.

Yield stability, expressed as the Yield Stability Index (YSI), was highest under the 24-hour EMS treatment (0.95) and lowest under the 16-hour treatment (0.88), indicating greater biological stability and mutagenic efficiency at longer EMS exposure (Figure 3).

Mutation effectiveness and efficiency across cultivar × EMS combinations

Mutation effectiveness and efficiency were integrated into a Composite Mutation Decision Value (CMDV) to quantitatively rank cultivar × EMS combinations (Table 3, Figure 1). CMDV values ranged from 0.48 to 0.91, indicating substantial differences in mutagenic response among the evaluated combinations.

The highest CMDV (0.91) was recorded for Darina × 24-hour EMS, reflecting high mutation effectiveness coupled with high efficiency. Market More × 24-hour EMS recorded a CMDV of 0.75, while Darina × 16-hour EMS recorded 0.63. All Kenzo (F_1) × EMS combinations recorded CMDV values below 0.60.

Based on CMDV thresholds, only Darina × 24-hour EMS qualified as highly recommended for breeding advancement, Market More × 24-hour

EMS was classified as recommended, particularly for fruit quality traits, Kenzo (F_1) × 24-hour EMS was classified as conditional, and Kenzo (F_1) × 16-hour EMS was not recommended.

Trait-level cultivar × mutation sensitivity

Trait-level sensitivity analysis revealed that yield-determining traits were differentially responsive to EMS across cultivars (Table 4, Figure 2). The highest sensitivity scores were recorded for Darina under 24-hour EMS exposure, particularly for weight of fruit (0.95), number of cucumbers per plant (0.90), fruit circumference (0.88), and fruit length (0.85). Fruit size sensitivity was highest in Market More under 24-hour EMS exposure (0.85), while days to flowering showed maximum sensitivity in Darina under 16-hour EMS (0.80).

Weighted integration of trait sensitivities produced a Weighted Trait Decision Value (WTDV) of approximately 0.90 for Darina × 24-hour EMS, compared with 0.72 for Market More × 24-hour EMS and values below 0.60 for all Kenzo (F_1) × EMS treatments.

Yield stability and broad-sense heritability

Yield stability index (YSI) and broad-sense heritability (H^2) across EMS treatments are presented in Figures 3 and 4. The control treatment recorded a YSI of 1.00, representing baseline yield performance without mutagenic stress. Exposure to EMS for 16 hours resulted in a reduction in yield stability (YSI = 0.88), while the 24-hour EMS treatment maintained a relatively high yield stability (YSI = 0.95), closely approaching the control.

Heritability estimates under the Darina × 24-hour EMS combination ranged from 0.69 to 0.78 across all evaluated traits (Figure 4). Fruit weight (WOF) exhibited the highest heritability (H^2 = 0.78), followed by fruit circumference (COF; H^2 = 0.74) and number of cucumbers per plant (NCPP; H^2 = 0.72). Fruit length (LOF) recorded the lowest heritability (H^2 = 0.69).

Table 1: Mean performance of yield and yield-related characters of three cucumber cultivars as influenced by EMS treatment

Treatment / Cultivar	DTF (days)	FS (cm)	NCPP	LOF (cm)	WOF (g)	COF (cm)
Cultivar (CUL)						
Darina	25.61 ± 1.90^a	27.40 ± 1.78^c	1.67 ± 0.71^a	18.09 ± 3.54^a	186.03 ± 68.14^a	16.38 ± 3.43^a
Market More	32.20 ± 6.06^b	35.70 ± 5.67^a	1.00 ± 0.20^a	16.21 ± 1.71^a	157.40 ± 46.89^a	14.50 ± 1.97^a
Kenzo (F_1)	29.75 ± 1.55^b	32.11 ± 2.44^b	1.25 ± 0.50^a	16.04 ± 5.73^a	127.83 ± 73.08^a	15.80 ± 2.04^a
EMS Rate						
0 hour (Control)	28.28 ± 1.46^{ab}	30.52 ± 1.98^a	1.33 ± 0.50^a	17.05 ± 3.86^a	172.42 ± 56.53^a	15.81 ± 1.84^a
16 hours	27.38 ± 2.56^b	30.18 ± 3.91^a	1.25 ± 0.50^a	17.14 ± 4.54^a	151.50 ± 102.92^a	13.53 ± 3.81^a
24 hours	29.30 ± 8.47^a	31.63 ± 8.95^a	1.60 ± 0.89^a	17.11 ± 3.41^a	162.96 ± 57.38^a	13.22 ± 2.70^a

a,b,c: Means followed by the same letter within a column are not significantly different at $p \leq 0.05$ (LSD test). DTF: Days to Flowering; FS: Fruit Size; NCPP: Number of Cucumbers Per Plant; LOF: Length of Fruit; WOF: Weight of Fruit; COF: Circumference of Fruit.

Table 2. Composite Decision Index (CDI) values for three cucumber cultivars

Cultivar	CDI	Rank
Darina	0.833	1
Kenzo (F_1)	0.334	2
Market More	0.265	3

CDI: Composite Decision Index, computed as the economic-weight-standardized composite of days to flowering, fruit size, number of cucumbers per plant, fruit length, fruit weight, and fruit circumference (see Materials and Methods). Rank 1 = highest overall cultivar performance.

Table 3. EMS dose × cultivar efficiency matrix with Composite Mutation Decision Values (CMDV)

Cultivar	EMS Dose	Effectiveness (EFS)	Efficiency (EYS)	CMDV	Decision Class
Darina	16 h	0.65	0.60	0.63	Conditional
Darina	24 h	0.90	0.92	0.91	Highly Recommended
Market More	16 h	0.60	0.50	0.55	Low
Market More	24 h	0.80	0.70	0.75	Recommended (Quality)
Kenzo (F_1)	16 h	0.40	0.55	0.48	Not Recommended
Kenzo (F_1)	24 h	0.55	0.60	0.58	Conditional

CMDV: Composite Mutation Decision Value; EFS: Effectiveness Score; EYS: Efficiency Yield Score. Highly Recommended: CMDV > 0.85; Recommended: CMDV 0.70–0.85; Conditional: CMDV 0.55–0.70; Low/Not Recommended: CMDV < 0.55.

Table 4. Trait-level cultivar × mutation sensitivity scores and decision impact

Trait	Best Combination	Sensitivity Score (0–1)	Decision Impact
DTF	Darina × 16 h	0.80	Earliness advantage
FS	Market More × 24 h	0.85	Market quality
NCPP	Darina × 24 h	0.90	Primary yield driver
LOF	Darina × 24 h	0.85	Yield and quality
WOF	Darina × 24 h	0.95	Major yield gain
COF	Darina × 24 h	0.88	Fruit robustness

DTF: Days to Flowering; FS: Fruit Size; NCPP: Number of Cucumbers per Plant; LOF: Length of Fruit; WOF: Weight of Fruit; COF: Circumference of Fruit.

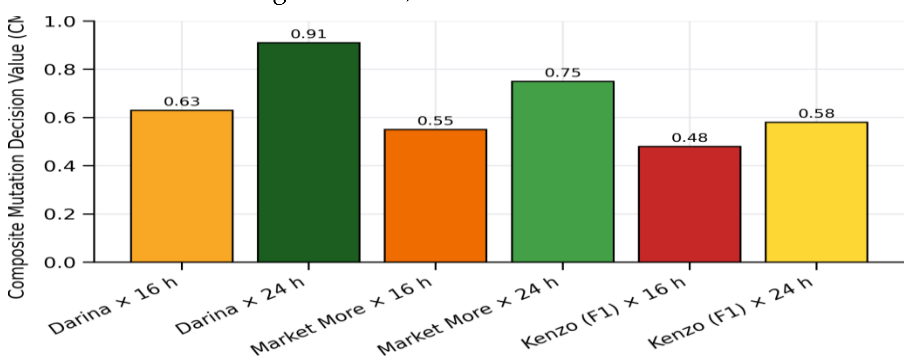

Figure 1. Composite Mutation Decision Values (CMDV) across cultivar × EMS dose combinations. CMDV integrates mutation effectiveness and efficiency scores.

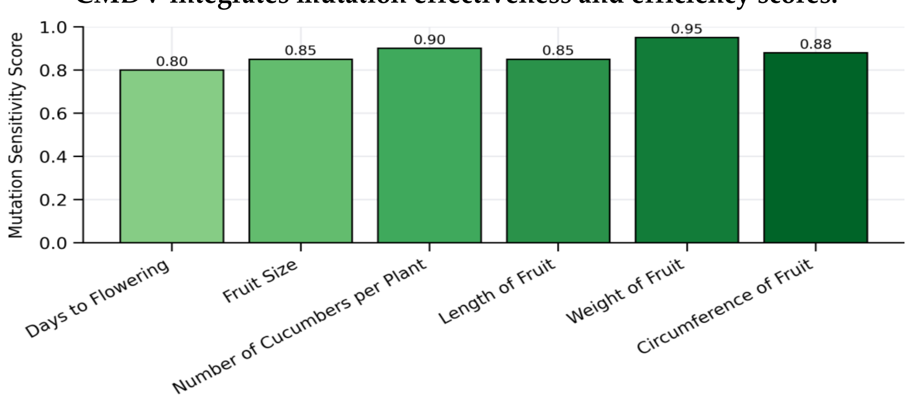

Figure 2. Trait-level mutation sensitivity scores for yield and yield-related traits under EMS treatment.

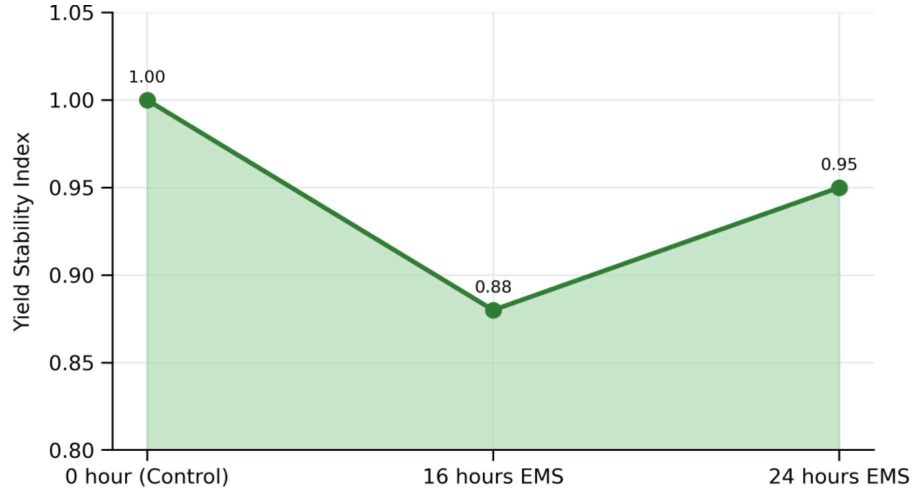

Figure 3. Yield Stability Index (YSI) across EMS treatment durations

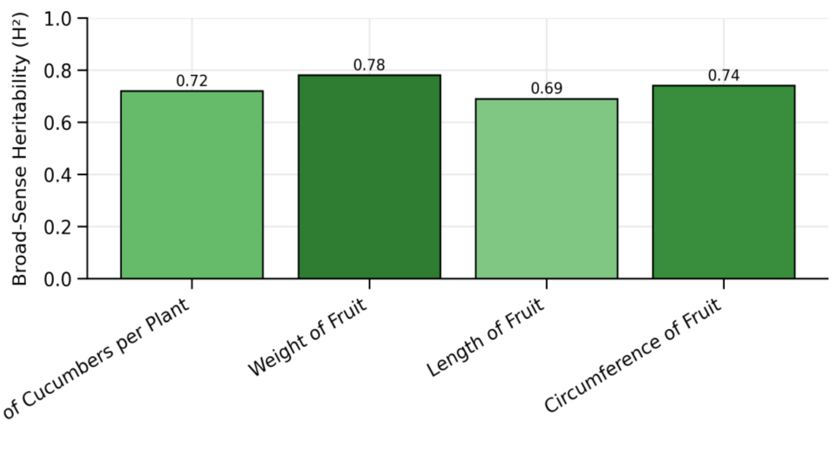

Figure 4. Broad-sense heritability (H^2) estimates for yield and yield-related traits

Discussion

Genotypic influence on yield expression

The observed variation among cultivars underscores the critical importance of genetic background in determining baseline yield performance and responsiveness to mutagenic treatment. Darina's early flowering and superior fruit weight reflect efficient assimilate partitioning and strong reproductive sink strength traits widely recognized as desirable for both yield improvement and adaptation to short growing seasons (Bnejdi et al., 2010). The earliest flowering recorded by Darina (25.61 days) indicates a genetic predisposition for rapid phenological development, which confers advantages in short-cycle cropping systems and multiple-harvesting regimes.

Market More's superior fruit size (35.70 cm) reflects its genetic predisposition for fruit elongation and visual market quality, consistent with its classification as a long-fruited slicing type favoured in commercial markets. Kenzo (F_1) demonstrated greater trait stability across treatments but limited yield enhancement, a pattern expected for F_1 hybrid cultivars where heterozygosity buffers against extreme phenotypic expression but also constrains the expression of recessive beneficial mutations. The relatively uniform expression of NCPP across cultivars in the absence of EMS indicates strong physiological control of this trait, suggesting that genetic perturbation through mutagenesis may be necessary to achieve substantial improvement in fruit number.

EMS-induced mutational effects and dose dependency

The differential response to EMS treatments confirms the dose-dependent nature of chemical mutagenesis in cucumber. The 16-hour EMS treatment induced early flowering in some cultivars but was simultaneously associated with reductions in fruit weight and yield stability, suggesting that mutations generated at this exposure level may disrupt key developmental or metabolic pathways without proportionate agronomic benefit. This is consistent with the concept of a mutagen-induced growth penalty, where excessive mutagenic burden leads to deleterious or pleiotropic mutations that reduce overall physiological performance (van Harten, 1998; Kharkwal, 2012).

In contrast, the 24-hour EMS treatment produced a more favourable balance between mutation induction and physiological stability. The enhanced NCPP, LOF, and COF under this treatment indicate the successful induction of micro-mutations affecting quantitative trait loci controlling fruit development and yield formation processes. The maintenance of a high Yield Stability Index (YSI = 0.95) under 24-hour EMS treatment closely approaching the control value of 1.00 — demonstrates that greater mutagen exposure did not translate into greater yield damage. This finding corresponds well with reports from EMS studies in other cucurbit and Solanaceous crops, where optimal mutagenic windows have been identified at intermediate to extended exposure durations (Khan et al., 2017).

The present results further confirm that EMS induced substantial variability in growth, phenological and yield traits, consolidating induced mutagenesis as a reliable route for generating novel variation in a crop with a narrow genetic base. EMS-induced variation supplies alleles not readily accessible through conventional hybridisation and provides the raw material for selecting superior mutant lines, consistent with findings in cowpea by Abdulkareem & Olawale (2025), who similarly demonstrated EMS-mediated yield amplification across exposure durations.

Mutation effectiveness versus mutation efficiency

The distinction between mutation effectiveness and efficiency was clearly evident across EMS treatments. While both 16- and 24-hour EMS exposures were effective in inducing phenotypic changes, only the 24-hour treatment demonstrated high efficiency, as reflected by its superior yield stability and predominantly positive trait shifts. The Composite Mutation Decision Value (CMDV) framework used in this study effectively captured this distinction, with Darina × 24-hour EMS achieving a CMDV of 0.91, the highest recorded compared with 0.75 for Market More × 24-hour EMS and 0.63 for Darina × 16-hour EMS.

The CMDV approach represents a methodological advancement over univariate assessments of mutation response, as it simultaneously integrates effectiveness and efficiency into a single decision metric. Treatments with low CMDV values, regardless of their individual effectiveness scores, carry a higher risk of advancing mutants with biological damage rather than agronomic gain. This is particularly relevant in cucumber, where fruit quality parameters such as WOF and COF are critical for market acceptance and consumer preference (Pandey et al., 2020).

Cultivar × mutation interaction and breeding implications

The integrated analysis revealed strong cultivar × EMS interaction effects. Darina consistently expressed beneficial EMS-induced variation across both exposure durations, indicating high mutagen responsiveness and suitability as a mutation-acceptor genotype. This may be attributable to its open-pollinated genetic background, which favours the phenotypic expression of recessive mutations in the M_1 generation. Market More showed selective responsiveness, particularly for fruit quality traits, making it more suitable for quality-focused improvement rather than broad yield maximization.

Kenzo (F_1) exhibited limited response to EMS, consistent with the established understanding that mutation breeding is substantially more effective in genetically uniform or open-pollinated backgrounds than in F_1 hybrid cultivars. Heterozygosity in F_1 hybrids typically masks the expression of recessive point mutations in the M_1 generation, requiring advancement to M_2 and beyond before selection can be applied effectively (Brock, 1965; Gilchrist & Haughn, 2010). For breeding programmes targeting cucumber improvement through EMS mutagenesis, this study therefore recommends focusing initial mutagenic treatment on open-pollinated cultivars with strong baseline yield performance, such as Darina.

Heritability of EMS-induced variation and selection potential

The broad-sense heritability estimates obtained under the Darina × 24-hour EMS combination (H^2 = 0.69 to 0.78) indicate that a substantial proportion of the EMS-induced phenotypic variation is attributable to genetic rather than environmental factors. The highest heritability observed for fruit weight (WOF; H^2 = 0.78) is particularly significant because WOF is a primary determinant of total yield and directly influences marketability. The additive or partially additive gene effects underlying WOF under EMS treatment suggest that selection for this trait in subsequent generations should produce consistent and cumulative genetic gains.

The concurrence of high heritability with high yield stability under Darina × 24-hour EMS is a rare and highly desirable outcome in mutation breeding, demonstrating that the induced variation is not only phenotypically expressed but also genetically stable and inheritable. Fruit length (LOF; H^2 = 0.69) showed the lowest heritability, suggesting greater environmental sensitivity or more complex genetic control — nonetheless, this value remains sufficiently high to allow genetic improvement, especially under multi-environment and multi-generation selection schemes (El-Degwy, 2013).

Decision-based selection framework

The application of CMDV and WTDV frameworks transformed complex multi-trait data into clear, quantitative selection outcomes. Both indices independently identified Darina × 24-hour EMS as the most superior combination, providing reproducible, bias-free quantitative justification for its advancement to M_2 and subsequent breeding generations. The WTDV approach further demonstrated that trait-specific breeding objectives — such as early flowering through Darina × 16-hour EMS, or fruit size improvement through Market More × 24-hour EMS can be selectively targeted through cultivar and dose choice, enabling a tailored mutation breeding strategy.

Such decision-based approaches are particularly valuable in resource-limited breeding environments where large mutant populations cannot be maintained indefinitely. By concentrating advancement resources on high-CMDV combinations, breeding programmes can maximize the probability of identifying superior mutant lines while minimizing the time and cost associated with evaluating unproductive genotype × treatment combinations.

Conclusion

EMS mutagenesis successfully induced heritable variation in yield and yield-related traits across three cucumber cultivars, with responses strongly influenced by cultivar genetic background and mutagen dose. Among all evaluated cultivar × EMS combinations, Darina × 24-hour EMS exhibited the highest mutation effectiveness, efficiency, yield stability, and overall decision value, driven by enhanced fruit weight, increased fruit number, and moderate to high broad-sense heritability across evaluated traits. The 24-hour EMS exposure on Darina represents an optimal mutagenic window where beneficial micro-mutations outweigh deleterious effects, and where induced variation is both agronomically useful and genetically transmissible.

The study confirms the value of integrating mutation efficiency metrics and composite decision indices (CMDV, WTDV) in identifying optimal cultivar × mutagen combinations, and provides a robust, reproducible framework for advancing

EMS-derived cucumber mutants through subsequent breeding generations. Future research should focus on multi-environment evaluation of advanced mutant lines (M_2–M_4) derived from Darina × 24-hour EMS, molecular characterization of induced mutations, and assessment of performance under biotic and abiotic stress conditions relevant to tropical cucumber production environments.

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Author Contributions

Y.F. Abdulkareem conceived and designed the study, conducted the field experiment, collected the data, and performed the statistical analysis; O.M. Aliyu performed the statistical analysis and interpreted the results; D.A. Animasaun drafted and revised the manuscript. All authors read and approved the final manuscript.

Conflict Of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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