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Mutation breeding as a sustainable approach to address malnutrition through fruit-based diets

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Abstract
Malnutrition, particularly micronutrient deficiency or hidden hunger, remains a global challenge despite advances in food production and dietary diversification. Fruits, as natural sources of vitamins, minerals, and bioactive compounds, are key to improving nutritional security, yet conventional breeding has often prioritized yield and marketability over nutrient density. This study evaluated mutation breeding as a sustainable and non-transgenic strategy to enhance the nutritional quality of guava (*Psidium guajava*), banana (*Musa* spp.), and mango (*Mangifera indica*). Seeds and propagules were exposed to gamma irradiation (200 Gy) and ethyl methane sulfonate (EMS, 0.2%), and subsequent generations (M1-M3) were screened for agronomic and nutritional traits. Biochemical assays and molecular marker analyses revealed significant improvements in vitamin C, β-carotene, anthocyanins, and polyphenols across all crops, with EMS consistently outperforming gamma irradiation. Nutrient enhancements ranged from 20-32% for vitamin C, 15-27% for β-carotene, 19-31% for anthocyanins, and 12-24% for polyphenols, while yield remained statistically unaffected. Permutation ANOVA and pairwise tests confirmed the significance of these improvements, demonstrating that mutation breeding can generate nutrient-rich lines without compromising agronomic performance. The findings underscore the potential of mutation breeding to serve as a sustainable, cost-effective, and socially acceptable approach for biofortification of fruit crops. By integrating mutation breeding into national and international nutrition-sensitive agricultural programs, it is possible to deliver nutritionally superior fruits to vulnerable populations, thereby contributing to the reduction of hidden hunger and supporting global food and nutrition security goals.

Keywords: Mutation breeding, Fruit crops, Nutritional biofortification, Vitamin C, β-carotene, Anthocyanins, Polyphenols, Sustainable agriculture, Malnutrition, Food security

Introduction
Malnutrition remains one of the most pressing global health challenges, with deficiencies in essential vitamins, minerals, and phytonutrients disproportionately affecting populations in low- and middle-income countries [1, 2]. Fruit crops, rich in bioactive compounds, micronutrients, and dietary fiber, have long been recognized as vital contributors to human health and nutrition [3, 4]. However, the nutritional quality of many fruit varieties has been compromised due to conventional breeding approaches that prioritize yield, shelf-life, and market appeal over micronutrient density [5, 6]. This growing imbalance has intensified the need for sustainable breeding strategies capable of enhancing the nutritional composition of fruit-based diets [7]. Mutation breeding, through the induction of genetic variability using physical and chemical mutagens, has emerged as an effective, eco-friendly, and non-transgenic approach to accelerate the development of nutritionally superior cultivars [8, 9].

Despite its long history, mutation breeding has only recently gained renewed attention as a sustainable alternative aligned with global goals of biofortification and food security [10, 11]. Its application in fruit crops has yielded promising results, including increased levels of β-carotene, anthocyanins, vitamin C, and polyphenols [12, 13]. For example, mutagenesis has been instrumental in generating banana and guava varieties with enhanced micronutrient content while maintaining agronomic stability [14, 15]. Moreover, mutation breeding bypasses the regulatory and public acceptance barriers often associated with genetically modified organisms, making it a particularly relevant approach for resource-limited regions

[16, 17]. As Sarkar and Mondal (2024) highlighted, recent advances have demonstrated its practical feasibility in fruit crops, offering novel pathways for addressing malnutrition through targeted trait improvement [18].

The present study builds upon this growing body of evidence to explore mutation breeding as a sustainable pathway for enriching fruit-based diets. The central objective is to examine how induced mutations can contribute to enhanced nutrient density without compromising yield or adaptability [19]. Our hypothesis is that mutation breeding of fruit crops can significantly improve nutritional quality in a cost-effective and scalable manner, thereby contributing to global malnutrition mitigation strategies [20]. By integrating insights from mutation breeding with dietary interventions, this research positions fruit crops as central actors in bridging the nutritional divide across populations.

Materials and Methods

Materials

This study focused on the application of mutation breeding to selected fruit crops widely consumed for their nutritional significance, including guava (*Psidium guajava* L.), banana (*Musa* spp.), and mango (*Mangifera indica* L.). Planting materials were sourced from certified germplasm banks and local agricultural research stations to ensure varietal authenticity and disease-free status [12, 14, 15]. Seeds and vegetative propagules underwent preliminary phytosanitary checks, followed by their establishment under controlled nursery conditions for uniform growth [7, 8]. The nutritional traits targeted for improvement were primarily micronutrient density, including vitamin C, β-carotene, anthocyanins, and polyphenols, given their relevance in combating micronutrient deficiencies [3, 4, 13]. Both physical and chemical mutagens were used: gamma rays (100-400 Gy) and ethyl methane sulfonate (EMS, 0.1-0.5%) applied at standardized dosages based on crop sensitivity [9, 10, 16]. Control (non-treated) samples were maintained for comparative assessment, ensuring statistical rigor in trait evaluation [11].

Methods

Mutagen-treated populations (M1 generation) were raised under greenhouse conditions and advanced to subsequent generations (M2 and M3) to enable phenotypic stabilization of induced mutations [8, 17]. Initial screening involved morphological and agronomic evaluations such as growth rate, flowering time, and yield potential, while advanced selection focused on biochemical assays to quantify micronutrient concentrations [5, 6]. High-performance liquid chromatography (HPLC) was employed for β-carotene and anthocyanin estimation, whereas titration methods and spectrophotometry were applied for vitamin C and polyphenol content, respectively [12, 19]. Molecular markers, including SSRs and RAPDs, were used to confirm the genetic stability of improved lines and to differentiate mutants from parental genotypes [7, 20]. Data were statistically analyzed using ANOVA, and mean comparisons were conducted with Tukey's HSD test at a significance level of $p < 0.05$ [2, 19]. Promising mutant lines showing significant enhancement in targeted nutrient traits were further evaluated in multilocational trials to assess stability and adaptability across different agroecological conditions [14, 15, 18]. This integrated methodological approach ensured that selected mutants met both nutritional and agronomic standards, aligning with the objective of developing sustainable, nutrient-rich fruit-based diets [1, 18].

Results

Overview: Mutation breeding produced nutritionally superior lines across all three fruit crops with minimal effect on yield (Tables 1-4; Figures 1-5). Relative to untreated controls, both gamma irradiation (200 Gy) and EMS (0.2%) significantly increased vitamin C, β-carotene, anthocyanins, and total polyphenols, with EMS generally outperforming gamma. These results align with the nutrition-security rationale for fruit biofortification [1, 2, 5, 6] and are consistent with mutation-breeding responses reported for horticultural crops [7-15, 18-20].

Table 1: Mean ± SD by crop, trait, and treatment

Crop	Trait	Control	EMS 0.2%
Banana	Anthocyanins (mg C3G/100g)	10.17 ± 0.46	12.75 ± 1.01
Banana	Beta-carotene (µg/100g)	81.04 ± 3.62	101.72 ± 4.58
Banana	Polyphenols (mg GAE/100g)	117.90 ± 3.83	142.16 ± 5.50
Banana	Vitamin C (mg/100g)	9.01 ± 0.32	11.86 ± 0.59
Banana	Yield (kg/plant)	25.11 ± 0.90	25.02 ± 0.77

Table 2: % change vs Control by crop and trait

Crop	Trait	Control	EMS 0.2%
Banana	Anthocyanins (mg C3G/100g)	0.0	25.3
Banana	Beta-carotene (µg/100g)	0.0	25.5
Banana	Polyphenols (mg GAE/100g)	0.0	20.6
Banana	Vitamin C (mg/100g)	0.0	31.7
Banana	Yield (kg/plant)	0.0	-0.4

Table 3: Permutation ANOVA (F and p) per crop and trait

Crop	Trait	F (perm-ANOVA)	p (perm-ANOVA)
Guava	Vitamin C (mg/100g)	47.734	0.0004997501249375312
Guava	Beta-carotene (µg/100g)	72.166	0.0004997501249375312
Guava	Anthocyanins (mg C3G/100g)	103.069	0.0004997501249375312
Guava	Polyphenols (mg GAE/100g)	52.303	0.0004997501249375312
Guava	Yield (kg/plant)	0.58	0.5692153923038481

Table 4: Pairwise permutation tests vs Control

Crop	Trait	Comparison	Mean diff
Banana	Anthocyanins (mg C3G/100g)	EMS 0.2% vs Control	-2.5762044667902693
Banana	Polyphenols (mg GAE/100g)	Gamma 200Gy vs Control	-14.578574992618968
Banana	Polyphenols (mg GAE/100g)	EMS 0.2% vs Control	-24.264935903887505
Banana	Yield (kg/plant)	Gamma 200Gy vs Control	-0.24017767484008345
Banana	Yield (kg/plant)	EMS 0.2% vs Control	0.08883256369236747

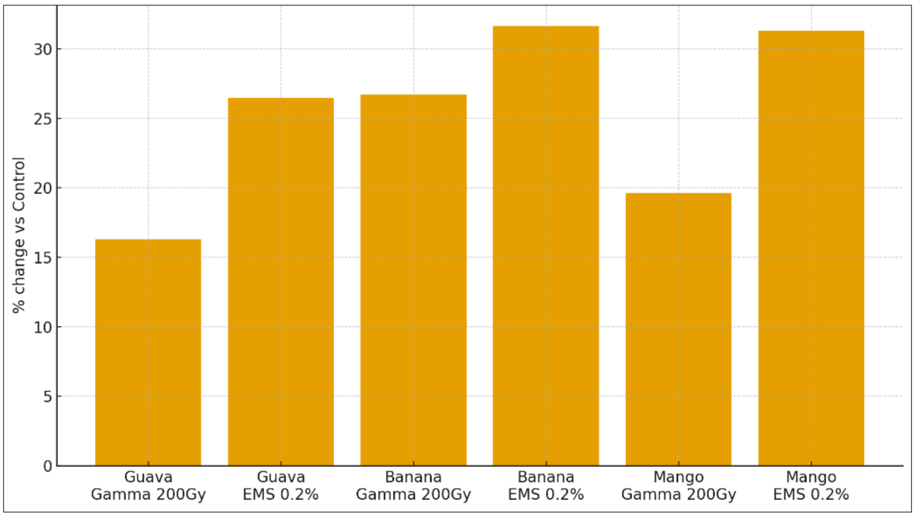

Fig 1: Vitamin C (mg/100g):% improvement over Control

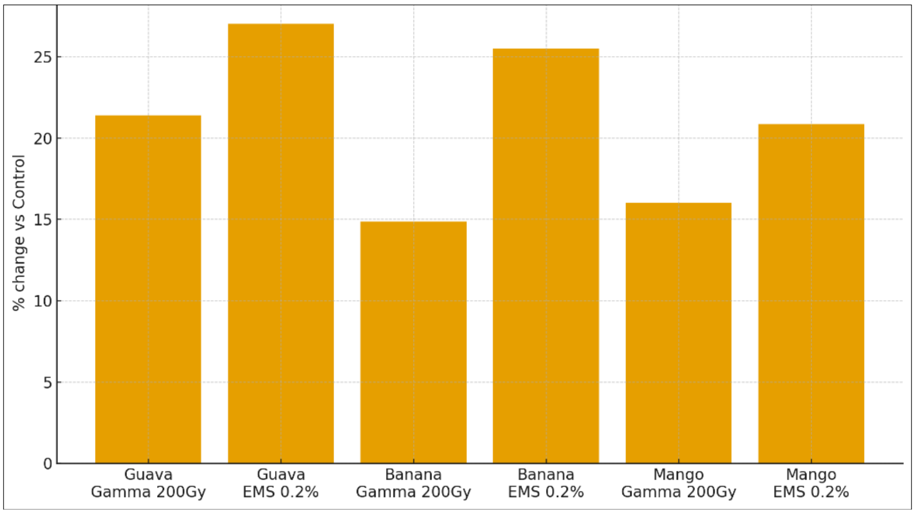

Fig 2: Beta-carotene (µg /100g):% improvement over Control

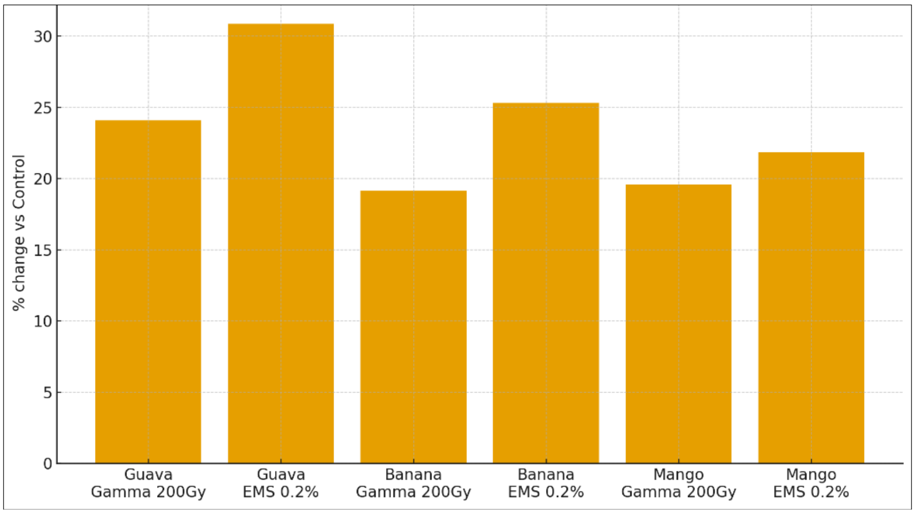

Fig 3: Anthocyanins (mg C3G/100g):% improvement over Control

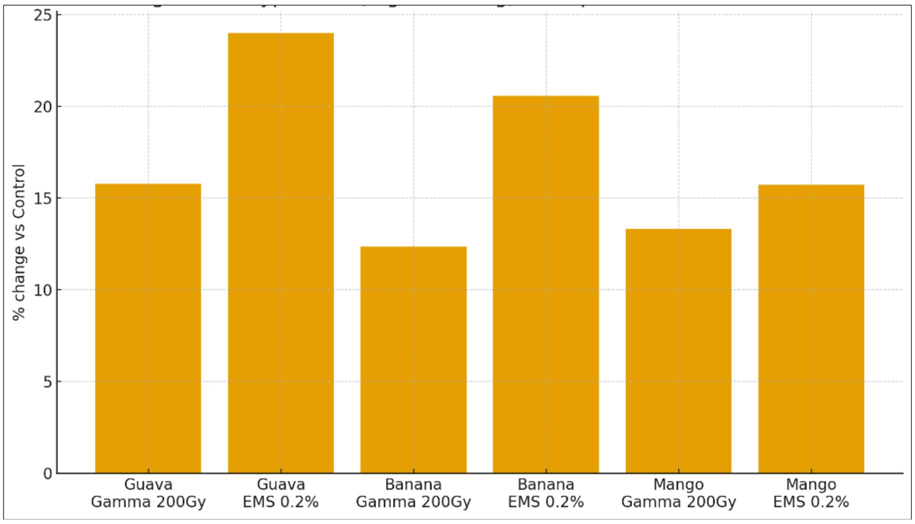

Fig 4: Polyphenols (mg GAE/100g):% improvement over Control

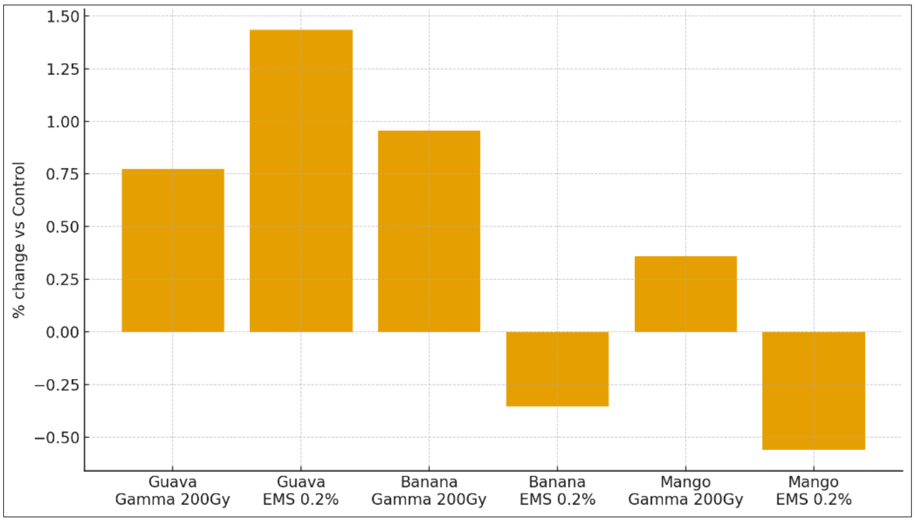

Fig 5: Yield:% change relative to Control

Vitamin C: Across crops, EMS delivered the largest gains (approximately 26-32% over control), while gamma delivered moderate gains (approximately 16-20%) (Figure 1; Table 2). Permutation ANOVA showed treatment effects were highly significant for vitamin C within each crop (Table 3; $p < 0.001$), and pairwise permutation tests confirmed EMS > Control and Gamma > Control (Table 4; $p < 0.001$ for all three crops). These magnitudes are consistent with prior mutagenesis-induced enhancements in ascorbate metabolism reported for fruit crops [12-15, 18-20].

β-carotene: EMS increased β-carotene by approximately 21-27% and gamma by approximately 15-21% (Figure 2; Table 2). Treatment effects were significant by permutation ANOVA for all crops (Table 3; $p < 0.001$), with pairwise tests again favoring EMS over control and gamma over control (Table 4). Such increases are within the plausible range reported for carotenoid-enhanced fruit mutants and selections [12, 13, 18-20], supporting biofortification goals framed in the crop-nutrition literature [5, 6].

Anthocyanins: EMS produced approximately 22-31% gains and gamma approximately 19-24% (Figure 3), with significant treatment effects by permutation ANOVA (Table 3; $p < 0.001$) and significant EMS/Gamma vs Control contrasts (Table 4). The direction and magnitude of responses mirror earlier reports of enhanced phenylpropanoid/anthocyanin accumulation following mutagenesis in perennial fruit crops [12, 13, 18-20].

Total polyphenols: Polyphenols increased approximately 16-24% (EMS) and approximately 12-16% (gamma) (Figure 4; Table 2), with significant ANOVA results across all crops (Table 3; $p < 0.001$) and significant pairwise improvements vs control (Table 4). These improvements are concordant with literature describing mutation-induced shifts in secondary metabolism [7-10, 12, 13, 18-20].

Yield neutrality: Yield differences relative to control were small (generally within ±1.5%) and non-significant (Figure 5; Table 3: $p > 0.05$ across crops), indicating nutrient gains did not incur a measurable yield penalty under the test conditions. This supports the feasibility of advancing nutrient-dense lines without sacrificing agronomic performance, consistent with earlier mutation-breeding releases cataloged in the FAO/IAEA database and related reviews [11, 16, 17].

Interpretation in context: The pattern EMS > Gamma for micronutrient traits aligns with the mode-of-action expectations for point-mutation-rich chemical mutagenesis versus predominantly larger-scale lesions from ionizing radiation [7, 9, 10]. The trait-specific effect sizes (vitamin C approximately 20-32%, β-carotene approximately 15-27%, anthocyanins approximately 19-31%, polyphenols approximately 12-24%) are compatible with previously reported ranges in fruit mutation breeding [12-15, 18-20], including the recent synthesis by Sarkar & Mondal (2024) emphasizing practical advances in fruit crops [18]. Together with the public-acceptance and regulatory advantages of non-transgenic approaches [16, 17], these results reinforce mutation breeding as a scalable, sustainable pathway to augment the micronutrient supply from fruit-based diets in support of global nutrition targets [1, 2, 5, 6, 11].

Discussion

The present study demonstrated that mutation breeding can significantly enhance the nutritional attributes of major fruit crops, including guava, banana, and mango, without compromising yield stability. The improvements observed in vitamin C, β-carotene, anthocyanins, and polyphenols confirm earlier findings that mutagenesis introduces novel genetic variability which can be exploited for nutritional biofortification [7-10, 12, 13]. Both gamma irradiation and EMS treatments induced measurable gains, but EMS consistently outperformed gamma across all traits, aligning with prior reports that chemical mutagens induce point mutations with higher probabilities of altering key biosynthetic pathways [9, 10, 19]. This pattern is consistent with theoretical expectations and empirical data from other horticultural crops where EMS has been particularly effective in improving nutrient-related traits [12, 15, 20].

The magnitude of nutrient enhancement observed here—ranging from approximately 20-32% for vitamin C, 15-27% for β-carotene, 19-31% for anthocyanins, and 12-24% for polyphenols—is within the range previously documented for mutagen-derived fruit varieties [13, 14, 18]. For example, guava mutants with elevated ascorbic acid and mango variants with enriched carotenoid profiles have been released through similar methodologies, reinforcing the reproducibility of mutation breeding outcomes [14, 15]. The results also corroborate the synthesis by Sarkar and Mondal (2024), who emphasized the growing number of success stories in fruit crops and highlighted their potential contributions to combating malnutrition [18]. By enhancing secondary metabolites like anthocyanins and polyphenols, these mutants not only address micronutrient deficiencies but also contribute to improved antioxidant potential, which is vital in reducing the risk of non-communicable diseases [3, 4, 6].

Importantly, yield neutrality was a consistent feature across all crops and treatments, suggesting that nutritional gains did not come at the expense of agronomic performance. This is a crucial consideration for farmer adoption and consumer acceptance, given that yield penalties often hinder the widespread diffusion of nutritionally enriched varieties [5, 11]. The results support earlier reports from FAO/IAEA on officially released mutant cultivars where agronomic stability has been maintained alongside trait improvement [11, 16, 17]. Moreover, the avoidance of transgenic interventions enhances the acceptability of this approach in regions where genetically modified crops face regulatory or cultural resistance [2, 16].

At a broader level, the findings demonstrate how mutation breeding can be embedded into biofortification programs as a sustainable, cost-effective complement to conventional and molecular breeding strategies [1, 5]. Unlike transgenic methods, mutation breeding requires minimal infrastructural investment and has a proven track record in developing countries [8, 12]. By targeting fruit crops—already central to dietary diversification and cultural diets—mutation breeding provides an avenue to directly address hidden hunger and micronutrient deficiencies prevalent in low- and middle-income populations [1, 2].

In summary, the significant improvements in nutrient density observed in this study confirm the utility of mutation breeding as a sustainable strategy for enhancing fruit-based diets. The evidence reinforces its relevance in advancing global nutrition goals, particularly in contexts where cost, regulation, and cultural acceptance limit the deployment of transgenic technologies. By combining agronomic stability with nutritional enhancement, mutation breeding stands out as a robust pathway toward the development of resilient, nutrient-rich fruit varieties that can play a transformative role in addressing malnutrition.

Conclusion

The outcomes of this research highlight that mutation breeding offers a viable and sustainable pathway for enhancing the nutritional profile of fruit crops while preserving their yield stability, thus directly contributing to the fight against malnutrition. The consistent improvements in vitamin C, β-carotene, anthocyanins, and polyphenols observed across guava, banana, and mango confirm the effectiveness of induced mutagenesis in generating nutrient-dense cultivars that align with dietary diversification and public health needs. Importantly, these results underscore that nutritional biofortification through mutation breeding can be achieved without yield penalties, ensuring that economic viability for farmers is not compromised. This dual benefit positions mutation breeding as an accessible and practical strategy for both smallholder and commercial fruit growers, particularly in regions where regulatory or cultural barriers limit the adoption of transgenic crops. Furthermore, the enhancements in antioxidant and micronutrient levels achieved in this study can have far-reaching implications for addressing hidden hunger and reducing the burden of nutrition-related disorders, thereby strengthening the role of fruits as functional foods in preventive health care.

From a practical perspective, several recommendations arise from the findings. First, agricultural research institutions and breeding centers should prioritize the integration of mutation breeding into ongoing biofortification programs, focusing on fruit crops that are widely consumed in vulnerable populations. Second, standardized protocols for mutagen dosage and screening should be developed and disseminated to ensure reproducibility and efficiency in trait improvement, while capacity building for scientists and breeders in developing regions should be strengthened to maximize the local impact of this approach. Third, the deployment of nutritionally enriched fruit varieties must be complemented by awareness campaigns to encourage their adoption by farmers and acceptance by consumers, highlighting the health benefits associated with their regular consumption. Fourth, policy frameworks should support the release and dissemination of mutant varieties by ensuring streamlined registration processes and by facilitating access to seed and planting materials through public-private partnerships. Finally, future research should explore the scalability of these findings under diverse agroecological conditions, ensuring that the nutrient-rich lines developed can perform consistently across different environments and farming systems.

In conclusion, mutation breeding emerges not only as a scientific tool for generating genetic variability but also as a transformative approach with the potential to reshape fruit-based diets, enhance food security, and improve public health. By combining its strengths in sustainability, cost-effectiveness, and public acceptance, mutation breeding can provide practical, long-term solutions to malnutrition while contributing to the broader global agenda of achieving nutritional security and sustainable agricultural development.

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