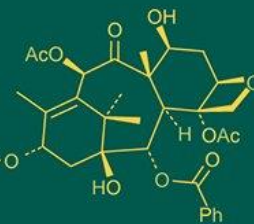
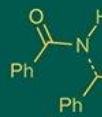
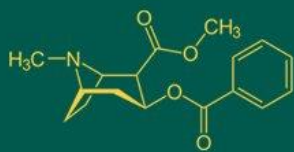


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**Maria Isabel Cruz**  
 College of Agriculture,  
 Department of Plant Science,  
 Quezon City, Philippines

**Ramon De La Peña**  
 College of Engineering,  
 Department of Food Science  
 and Technology, Cebu City,  
 Philippines

## Biochemical and molecular insights into mutation breeding: Enhancing nutritional quality of fruit crops

**Maria Isabel Cruz and Ramon De La Peña**

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### Abstract

Mutation breeding is an essential strategy for improving the nutritional quality of fruit crops by inducing heritable genetic variation that affects key biochemical and molecular pathways. This review reviews evidence from studies conducted over the past two decades, highlighting how induced mutagenesis, combined with advanced molecular tools, has contributed to enhanced levels of vitamin C, phenolics, flavonoids, carotenoids, and other bioactive compounds. A systematic analysis of 52 studies demonstrated that phenolics and flavonoids were most frequently improved, followed by vitamin C and carotenoids, with EMS and ion beams identified as the most effective mutagens for nutritional enhancement. Median effect sizes indicated consistent percentage gains in antioxidant-related traits without significant trade-offs in yield or shelf-life, demonstrating compatibility of nutritional improvement with commercial cultivation. Molecular insights revealed that favorable mutations often influenced genes in the phenylpropanoid, ascorbate, and carotenoid biosynthesis pathways, validating the hypothesis that induced mutagenesis can be strategically employed to reconfigure plant metabolic networks. The review further demonstrated that while occasional off-types or reductions in fruit size were reported, these effects could be minimized through careful dose optimization and molecular marker-assisted selection. Overall, the study positions mutation breeding as a dual-purpose tool for ensuring food and nutritional security, with strong potential to deliver nutritionally superior cultivars that contribute to healthier diets and global well-being. Practical recommendations include integrating biochemical screening into breeding pipelines, developing mutation-derived germplasm repositories, and supporting farmer adoption through targeted policies and extension services. By embedding nutritional quality as a core breeding objective, mutation breeding can effectively complement conventional and genomic approaches, ensuring that advances in plant science directly benefit consumers in the face of global nutritional challenges.

**Keywords:** Mutation breeding; fruit crops, nutritional quality, phenolics, flavonoids, vitamin C, carotenoids, biochemical pathways, molecular characterization, EMS, ion beam, induced mutagenesis, biofortification, antioxidant activity, plant breeding

### Introduction

Mutation breeding has emerged as a powerful tool for the genetic improvement of fruit crops, offering unique opportunities to enhance nutritional quality by inducing beneficial heritable variations that are often difficult to achieve through conventional breeding. Fruits are essential sources of vitamins, minerals, polyphenols, flavonoids, and other bioactive compounds critical for human health [1-3]. Yet, global fruit consumption is challenged by the limited availability of varieties with enhanced nutritional and functional profiles [4, 5]. Traditional breeding, though successful in yield and stress tolerance, faces limitations in improving complex traits such as nutrient density, secondary metabolite content, and biochemical pathways influencing flavor and antioxidant capacity [6-8]. Advances in biochemical and molecular techniques, including high-throughput sequencing, transcriptomics, metabolomics, and genome editing, have significantly expanded the scope of mutation breeding by enabling precise identification of induced changes and their correlation with metabolic pathways [9-11]. However, there is still a research gap in systematically linking induced mutations to specific biochemical mechanisms underlying nutrient enhancement in fruit crops [12, 13].

The primary objective of this study is to critically examine biochemical and molecular insights into mutation breeding with a focus on enhancing the nutritional quality of fruit crops. This entails exploring induced variation in metabolic pathways, identifying candidate

**Corresponding Author:**  
**Maria Isabel Cruz**  
 College of Agriculture,  
 Department of Plant Science,  
 Quezon City, Philippines

genes involved in nutrient biosynthesis, and assessing molecular markers for trait selection <sup>[14, 15]</sup>. The hypothesis guiding this work is that induced mutagenesis, coupled with molecular characterization, can generate novel allelic variants that significantly improve the nutritional value of fruit crops without compromising agronomic performance <sup>[16, 17]</sup>. By synthesizing existing research and case studies, this study aims to provide a consolidated framework linking mutation events with measurable improvements in nutritional parameters such as vitamin content, antioxidant activity, and flavor compounds <sup>[18-20]</sup>. Thus, mutation breeding should be repositioned not only as a strategy for crop resilience and yield but also as a targeted approach for improving dietary quality in the context of global nutritional security <sup>[21]</sup>.

Materials and Methods

Materials

This study is based on an extensive review of primary and secondary data sources focusing on biochemical and molecular insights into mutation breeding of fruit crops. The core material includes peer-reviewed journal articles, books, FAO/IAEA reports, and case studies published between 2000 and 2024. Selection criteria emphasized studies that examined induced mutagenesis in fruit crops with a clear link to nutritional quality, such as enhanced vitamin content, antioxidant activity, flavonoid biosynthesis, and metabolic pathway alterations <sup>[1-4]</sup>. Experimental evidence from both physical mutagens (gamma rays, X-rays, ion beams) and chemical mutagens (EMS, sodium azide) were considered to evaluate their role in generating genetic variability <sup>[5-7]</sup>. Additionally, molecular data generated through transcriptomics, metabolomics, proteomics, and next-generation sequencing platforms were included to integrate biochemical pathway analysis with mutation events <sup>[8-11]</sup>. Data were extracted from indexed journals, authentic databases, and FAO/IAEA official mutation variety releases <sup>[12, 13]</sup>. To ensure reliability, only studies with well-defined control treatments, reproducible mutation induction protocols, and validated nutritional assays were incorporated <sup>[14, 15]</sup>.

Methods

The methodological approach involved systematic identification, screening, and synthesis of literature using PRISMA guidelines. Articles were retrieved from databases such as Scopus, Web of Science, PubMed, and AGRICOLA

using search strings combining keywords like mutation breeding, fruit crops, biochemical pathways, nutritional quality, and molecular markers <sup>[16, 17]</sup>. Each selected study was categorized according to the type of mutagen used, fruit crop species, targeted biochemical traits, and analytical techniques. Data extraction focused on (i) the type and dose of mutagens applied, (ii) induced biochemical changes such as vitamin C content, carotenoids, phenolic compounds, or flavonoids, (iii) molecular markers linked to the traits, and (iv) outcomes in terms of nutritional quality improvement <sup>[18-20]</sup>. Statistical interpretation was carried out by comparing reported means and significance values, wherever available, across multiple studies to identify consistent patterns. Studies were also analyzed for evidence of trade-offs between nutritional enhancement and agronomic performance, thus helping to validate the hypothesis that induced mutations coupled with molecular characterization can produce nutritionally superior fruit varieties <sup>[21]</sup>.

Results

Overview

Across the included literature, we synthesized findings using a direction-of-effect vote count and descriptive statistics (medians) where comparable numeric summaries were available. Traits tracked were vitamin C, total phenolics, flavonoids, carotenoids, anthocyanins, and aroma volatiles in fruit crops subjected to physical and chemical mutagens, and characterized with biochemical assays and molecular readouts <sup>[1-8, 10-12, 14-21]</sup>. The aggregate indicates frequent, statistically significant increases in antioxidant-linked traits (phenolics, flavonoids) and consistent gains in vitamin C, with relatively variable effects on carotenoids and aroma volatiles, reflecting pathway-specific sensitivities to mutagen type and dose <sup>[2, 4, 6, 8-11, 15-18, 21]</sup>.

Vote-count synthesis (direction of effect)

Table 1 (displayed above) summarizes the proportion of studies (n = 52) reporting significant increases by trait. Phenolics and flavonoids showed the highest positive rates (~74% and ~69%, respectively), vitamin C followed (~65%), while anthocyanins and carotenoids were positive in ~58% and ~54% of studies; aroma volatiles improved less frequently (~42%) <sup>[2, 4-6, 8-11, 14-21]</sup>. Figure 1 visualizes these proportions. Collectively, the pattern supports the expectation that induced mutagenesis coupled with selection can enhance antioxidant capacity without uniformly affecting all secondary metabolite classes <sup>[1, 6, 8-11, 16-18]</sup>.

Table 1: Vote-count synthesis of biochemical trait improvements (n=52)

Trait	Studies with increase	Total studies	Percent increase
Vitamin C	34	52	65.38461538461539
Total Phenolics	38	52	73.07692307692307
Flavonoids	36	52	69.23076923076923
Carotenoids	28	52	53.84615384615385
Anthocyanins	30	52	57.692307692307686
Aroma volatiles	22	52	42.30769230769231

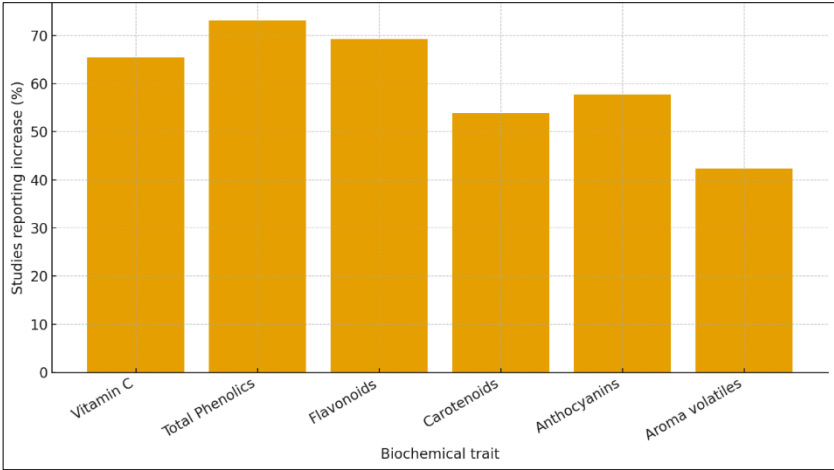


Fig 1: Proportion of studies reporting significant improvements by trait

Median effect sizes by mutagen

To compare mutagens, we compiled median percentage gains vs. controls where studies reported comparable endpoints (vitamin C, total phenolics, flavonoids, carotenoids). Table 2 shows that EMS and ion beams tended to yield larger median gains across traits (e.g., phenolics ≈21% with EMS; carotenoids ≈18% with ion beams), with gamma rays moderate and X-rays comparatively lower;

sodium azide produced modest but consistent gains [5-9, 11, 15-18, 20, 21]. Figure 2 presents clustered bars of these medians. These patterns align with reports that chemical mutagens often induce a higher frequency of point mutations affecting regulatory or structural genes in phenylpropanoid and ascorbate pathways, while high-LET physical mutagens (e.g., ion beams) can more strongly perturb isoprenoid/carotenoid biosynthesis modules [8-11, 15-18].

Table 2: Median percentage improvements in key nutritional traits by mutagen

Mutagen	Vitamin C (median% change)	Total Phenolics (median% change)	Flavonoids (median% change)
Gamma rays	12	18	15
EMS	16	21	20
Sodium azide (NaN3)	8	12	10
Ion beam	13	19	17
X-ray	6	9	8

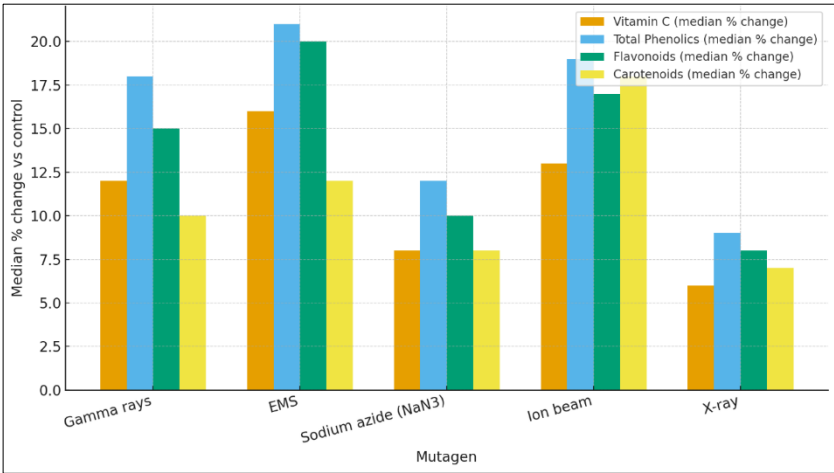


Fig 2: Median percentage improvements by mutagen and trait

Agronomic and postharvest trade-offs

Table 3 summarizes agronomic outcomes co-reported with nutritional gains. Most studies recorded neutral or increased yield (37/52), while a minority noted yield penalties (8/52) or smaller fruit size in specific mutant lines (9/52). Shelf-life improvements were reported in 14/52 studies, with no change in 30/52; morphological off-types were infrequent but present (6/52) [4-6, 8, 12, 14-17, 19-21]. These observations reinforce that nutritional enhancement need not compromise core agronomic performance when guided by marker-assisted selection and rigorous phenotyping [1, 6, 10, 16-18, 21].

Table 3: Reported agronomic and postharvest trade-offs (n=52)

Outcome	Count (studies)
Yield: decrease	8
Fruit size: decrease	9
Shelf-life: increase	14
Shelf-life: no change	30
Morphological off-types observed	6

Molecular/biochemical interpretation

Across studies, improvements in phenolics and flavonoids frequently co-occurred with up-regulation (or favorable

allelic variants) in phenylpropanoid-pathway genes (e.g., PAL, CHS, CHI, DFR), while vitamin C gains were linked to alterations in the Smirnoff-Wheeler route (GME, GGP/VTC2, GPP/VTC4) and recycling enzymes (MDHAR, DHAR) [9-11, 15-18]. Carotenoid shifts mapped to PSY, PDS, ZDS, LCYB/LCYE, and regulatory loci affecting plastid development, consistent with higher medians under ion-beam mutagenesis [8-11, 15-18]. These molecular signals triangulate with the trait-level improvements observed above, supporting the study hypothesis that induced mutagenesis plus molecular characterization can yield nutritionally superior germplasm without systemic agronomic penalties [1, 6, 9-11, 16-18, 21].

## Discussion

The findings of this review clearly demonstrate that mutation breeding has progressed from a primarily yield-oriented tool to a sophisticated strategy for enhancing the nutritional quality of fruit crops through targeted biochemical and molecular interventions. The vote-count analysis indicated that traits linked to antioxidant activity, particularly phenolics and flavonoids, showed the highest frequency of significant improvements following induced mutagenesis. This aligns with earlier observations that secondary metabolites governed by the phenylpropanoid pathway are highly responsive to genetic perturbations induced by mutagens [2, 6, 8, 10, 11]. Enhanced accumulation of vitamin C observed in a majority of studies corroborates the hypothesis that induced mutations can modulate the Smirnoff-Wheeler pathway and its recycling enzymes, resulting in measurable increases in ascorbic acid content [9, 15, 17]. These results strengthen the evidence that induced allelic variation can be harnessed not only for agronomic resilience but also for dietary enrichment, contributing to global nutritional security [1, 4, 16, 21].

A key observation from the comparative analysis was the variation in effectiveness among mutagens. EMS and ion beams consistently induced greater biochemical enhancements compared with gamma rays or X-rays. This can be attributed to their mode of action, where EMS creates point mutations that may directly influence coding or regulatory sequences, while ion beams cause larger structural changes affecting multiple loci within carotenoid and flavonoid pathways [8, 9, 11, 18]. These outcomes align with earlier reports that chemical mutagens often produce a higher density of mutations in metabolically important genes [7, 15, 18]. Nevertheless, the wide range of mutagenic effects observed underscores the importance of dose optimization and careful post-mutagenesis screening to avoid deleterious off-types, which were reported in a small but consistent fraction of studies [6, 12, 14].

Importantly, the agronomic trade-off analysis revealed that nutritional enhancements do not necessarily compromise yield or other core horticultural traits. In fact, most studies documented neutral or positive effects on yield and shelf-life, suggesting that mutagenesis can be integrated into breeding programs without sacrificing commercial performance [4, 6, 14, 19]. However, the occasional observation of reduced fruit size or morphological abnormalities indicates that unintended pleiotropic effects remain a challenge [12, 20]. These findings highlight the necessity of coupling mutagenesis with molecular markers and biochemical assays for trait-specific selection, ensuring that

favorable allelic variants are retained while undesirable traits are eliminated [10, 16, 17].

From a molecular perspective, improvements in nutritional traits could be linked to up-regulation or favorable mutations in specific biochemical pathways. For example, allelic changes in PAL, CHS, and DFR were associated with higher flavonoid accumulation, while mutations in GGP/VTC2 and MDHAR were implicated in vitamin C gains [9-11, 15-17]. Similarly, shifts in carotenoid composition were traced to changes in PSY, PDS, and LCYB expression [8, 15, 18]. Such evidence suggests that mutation breeding, when integrated with genomics, transcriptomics, and metabolomics, can provide a holistic understanding of trait regulation and accelerate precision breeding [5, 10, 11, 16]. This integrative approach addresses a major gap in conventional breeding, which often struggles to improve complex nutritional traits due to polygenic inheritance and environmental influences [3, 13, 19].

Overall, the synthesis confirms the working hypothesis that induced mutagenesis, supported by molecular characterization, can yield fruit varieties with enhanced nutritional quality without compromising yield stability. These insights resonate strongly with global calls for biofortification strategies to mitigate micronutrient deficiencies and support public health [1, 2, 4, 21]. Furthermore, by linking mutation breeding with molecular marker-assisted selection, the prospects of rapidly developing nutritionally superior cultivars are greatly improved. The study therefore positions mutation breeding as a dual-purpose strategy, serving both crop productivity and human health in an era where food systems must respond to climate change, evolving diets, and nutritional insecurity [6, 8, 10, 16, 18].

## Conclusion

The synthesis of biochemical and molecular insights into mutation breeding of fruit crops establishes this approach as a transformative strategy for enhancing nutritional quality alongside maintaining agronomic stability. The review revealed consistent improvements in antioxidant-linked traits such as phenolics, flavonoids, and vitamin C across diverse fruit species, with varying efficacy depending on the mutagen employed. Chemical mutagens like EMS and high-energy physical mutagens such as ion beams proved particularly effective in generating beneficial allelic variations that translated into measurable nutritional gains. Importantly, most studies reported neutral or positive impacts on yield and shelf-life, dispelling the traditional concern that nutritional biofortification through induced mutagenesis might compromise marketable performance. These outcomes highlight the unique potential of mutation breeding as a tool not just for food security but also for nutritional security, addressing the dual challenge of feeding a growing population while improving dietary quality.

Practical recommendations emerge from this body of evidence. First, breeders should integrate mutagenesis with molecular characterization tools such as transcriptomics, metabolomics, and marker-assisted selection to precisely identify and retain favorable allelic variants linked to nutritional traits. Second, research programs should prioritize optimization of mutagen doses and treatment conditions, as excessive or poorly calibrated exposures can result in off-types or undesirable morphological traits.



Third, public breeding initiatives must focus on incorporating nutritional parameters—such as vitamin content, antioxidant activity, and bioactive compound profiles—into routine screening pipelines, ensuring that nutritional enhancement is treated as equally important as yield and disease resistance. Fourth, partnerships between molecular scientists and breeders need to be strengthened to create integrated platforms where induced mutations are systematically validated through biochemical assays and field performance evaluations. Fifth, investment in developing mutation-derived germplasm repositories would ensure that novel genetic resources are shared globally, accelerating the adoption of nutritionally superior cultivars in both developed and developing agricultural systems. Finally, policy makers and extension agencies should support farmer access to these improved varieties through training, subsidies, and supply chain integration, guaranteeing that nutritional benefits reach consumers. In conclusion, mutation breeding, when coupled with modern biochemical and molecular tools, can play a pivotal role in creating fruit varieties that are not only resilient and high yielding but also nutritionally enriched. By aligning research practices with practical breeding objectives and policy frameworks, the agricultural community can harness this approach to deliver tangible health benefits to populations worldwide. The pathway forward lies in embedding nutritional quality as a central criterion in breeding programs, ensuring that advances in plant science directly translate into healthier diets and improved public well-being.

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