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Effects of Colchicine and ^{60}Co-γ Radiation Treatments on the Leaf Size and Fruit Quality of Kiwifruit 'Donghong'

Limei Wang [1,2], Qing Liu [1,2,3], Xishi Li [1,2], Guanglian Liao [1,2] and Xiaobiao Xu [1,2,*]

[1] College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, China; limeiwang0520@163.com (L.W.); liuqingwx89@163.com (Q.L.); lxs0329@163.com (X.L.); liaoguanglian@jxau.edu.cn (G.L.)
[2] Kiwifruit Institute, Jiangxi Agricultural University, Nanchang 330045, China
[3] Guizhou Institute of Pomological Sciences, Guizhou Academy of Agricultural Science, Guiyang 550006, China
* Correspondence: xbxu@jxau.edu.cn

Abstract: Colchicine and ^{60}Co-γ radiation are commonly used breeding techniques for kiwifruit, offering advantages such as low cost, rapid execution, and high efficiency. The buds of red-fleshed kiwifruit (*Actinidia chinensis*) cv. 'Donghong' were used as experimental material and subjected to different concentrations of colchicine and different doses of ^{60}Co-γ radiation, respectively. Then, the buds were grafted on rootstock, and the ploidy, leaf size, and fruit quality of mutant fruit were evaluated, and principal component analysis (PCA) and simple sequence repeat markers were used to comprehensively assess and detect genetic variations, respectively. The results indicated that a total of 19 buds successfully germinated, with 13 branches successfully bearing fruit. Significant changes were observed in both leaf and fruit morphology following the mutation treatments. Most of the mutant materials showed significant increases in fruit weight, flesh firmness, and soluble sugar content, while titratable acidity and ascorbic acid content significantly decreased. Notably, the 25Gy ^{60}Co-γ radiation (25d) treatment demonstrated outstanding results, with fruit weight increasing by 256.10%, soluble sugar content rising by 88.29%, titratable acidity decreasing by 29.86%, and ascorbic acid content increasing by 35.60%. PCA results showed that the 25d mutant had the best comprehensive traits. And, except for the 0.4c mutant, all other mutant materials exhibited significant genetic changes at the DNA level.

Keywords: ^{60}Co-γ radiation; colchicine; fruit quality; kiwifruit; simple sequence repeat

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1. Introduction

Kiwifruit (*Actinidia*) is a perennial vine fruit tree in the Actinidiaceae family [1], renowned for its rich content of ascorbic acid (AsA), proteins, sugars, amino acids, and other organic compounds, making it highly favored by consumers [2,3]. Based on flesh color, kiwifruit can be classified into yellow-fleshed, red-fleshed, and green-fleshed cultivars. Among these, the 'Donghong' cultivar is a popular red-fleshed kiwifruit variety favored by both growers and consumers. It possesses strong resistance to *Pseudomonas syringae* pv. *actinidiae* and retains anthocyanins well under high temperatures [4]. However, its relatively small single fruit weight has become a major limitation in the promotion and development of this cultivar [5].

Kiwifruit breeding still primarily relies on traditional methods, such as wild selection, seedling selection, and hybridization [6]. These methods typically involve long breeding cycles and require extensive manual intervention, resource input, and significant financial support. Therefore, exploring more efficient and controllable breeding approaches has become increasingly important in modern horticulture [7]. With the continuous development of biotechnology, mutation breeding has gradually emerged as a promising alternative. Mutation breeding not only shortens the breeding cycle but also offers higher breeding efficiency and lower costs, making it a favored approach among breeders. Mutation breeding is mainly achieved through two methods: physical mutation and chemical mutation [8]. Physical mutation typically involves radiation to induce gene mutations, with commonly used radiation sources including high-energy rays, such as ^{60}Co-γ radiation. These rays can break the DNA structure of plant cells, triggering mutations [9]. Chemical mutation, on the other hand, uses specific chemical agents to induce genetic variations. Among these, colchicine is a commonly used mutagen that effectively inhibits cell division, leading to the production of polyploid plants [10], which often exhibit stronger growth vigor and higher yields. Since radiation itself does not leave residues and the concentration of chemical treatments is low, coupled with the long growth cycle of fruit crops, the mutagenic materials no longer pose any toxic effects to humans. These techniques have been extensively studied in fruit crops, such as the successful induction of polyploid *A. chinensis* using colchicine [10,11]. However, there is still limited research on the application of these mutagenic technologies in the superior red-flesh kiwifruit cultivar 'Donghong'.

This study used the red-flesh cultivar 'Donghong' of *A. chinensis* as the material, applying different concentrations of colchicine and varying doses of ^{60}Co-γ radiation for mutagenic treatments. The mutagenized materials were evaluated for ploidy, leaf morphological traits, and both internal and external fruit quality. Finally, principal component analysis (PCA) was employed for comprehensive assessment, and simple sequence repeat (SSR) molecular markers were used to detect DNA-level variations. Our study aimed to enrich the theoretical foundation of kiwifruit mutation breeding and provide a theoretical basis for cultivar structure optimization and the industrial development of kiwifruit.

2. Materials and Methods

2.1. Materials and Treatment

One-year-old branches of *A. chinensis* cv. 'Donghong', sourced from the kiwifruit germplasm nursery at the Agricultural Bureau of Fengxin County, Jiangxi Province, were used in this study. For the colchicine treatment, cotton pads were wrapped around the buds and then saturated with colchicine solutions of varying concentrations (0%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%) using a disposable syringe. The buds were thoroughly soaked and treated for 8 d. For the ^{60}Co-γ radiation treatment, branches were irradiated with different doses (0, 25, 75, and 100 Gy) of ^{60}Co-γ rays at the Hunan Irradiation Center. The radiation cabinet operates at an efficiency of 25 Gy/min, so the exposure times required for each dose were 0, 1, 3, and 4 min, respectively (Figure 1). All treated branches were grafted onto five-year-old *A. chinensis* cv. 'Wuzhi 1'. For each treatment, 20 buds were used per biological replicate, and 3 biological replicates were set up for each treatment.

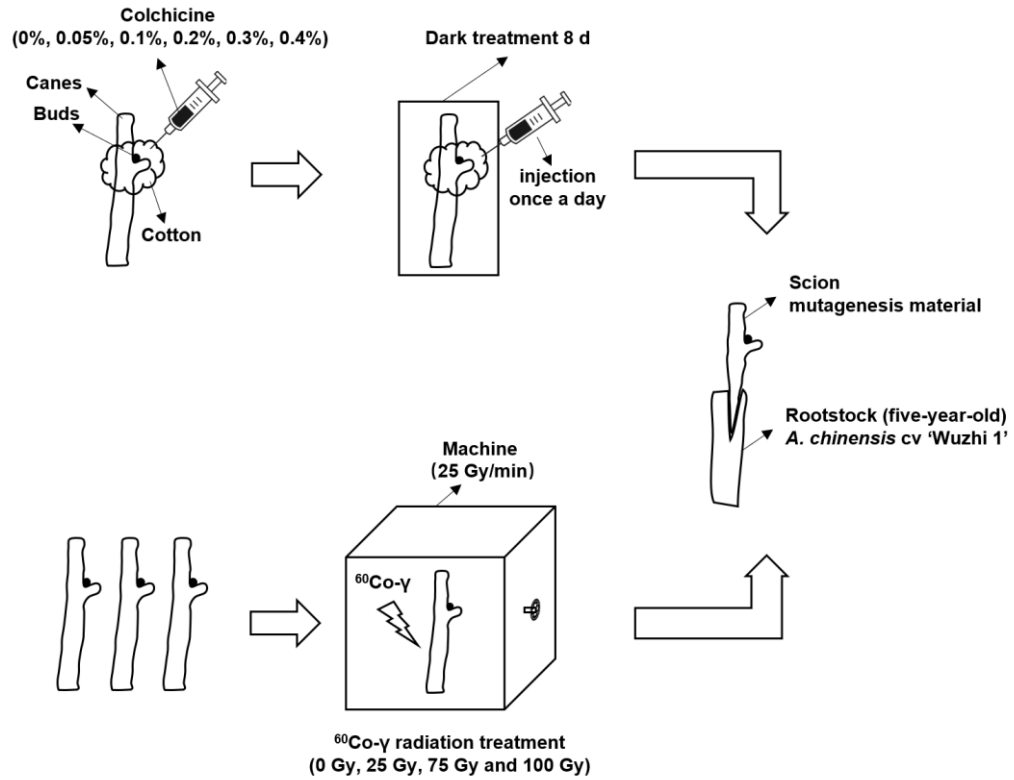

Figure 1. Infographic of kiwifruit (*Actinidia chinensis*) cv. 'Donghong' buds treated with colchicine and ^{60}Co-γ radiation. Gy, gray.

2.2. Ploidy Determination

The ploidy of the treated materials was determined using flow cytometry. Young leaves were sampled and prepared following standard protocols to analyze their nuclear DNA content [12]. Comparisons were made against the control samples to confirm changes in ploidy levels. The control sample was a known diploid *A. chinensis* cv. 'Donghong'. Ploidy analysis was conducted using a CyFlow Ploidy Analyzer flow cytometer (Sysmex, Norderstedt, Germany).

2.3. Traits of Leaf and Fruit Determination

Leaf length, leaf width, petiole length, petiole diameter, fruit transverse diameter, longitudinal diameter, and lateral diameter were measured using a vernier caliper with an accuracy of 0.01 mm. The leaf shape index (leaf shape index = leaf length/leaf width) and fruit shape index (fruit shape index = longitudinal diameter/transverse diameter) were calculated accordingly. The weight of individual fruits was measured using an electronic balance with an accuracy of 0.001 g. Flesh firmness was assessed using a texture analyzer (model: TA-XT plus, Stable Micro System, London, UK). Fruit color was measured using a colorimeter (model: CHROMA METER CR-400, Konica Minolta, Tokyo, Japan).

The soluble solids content (SSC) was measured using a digital refractometer (model: 0–50% 'Pocket' PAL-1, Atago, Tokyo, Japan). Dry matter (DM) content was determined by slicing 1–2 g thin sections from the transverse cross-section of fruits, drying them at 65 °C in a constant-temperature drying oven for 24 h, and calculating the dry matter content (%) as: DM content (%) = (dry weight of fruit (g)/fresh weight of fruit (g)) × 100 [13]. The soluble sugar content was determined using the anthrone colorimetric method [14]. The AsA content was measured using the 2,6-dichlorophenolindophenol titration method [14,15]. The titratable acid content was determined by acid-base titration [14].

2.4. Simple Sequence Repetition (SSR)

DNA was extracted using the CTAB method, and the quality-verified DNA was diluted to a concentration of 150–200 ng/μL for subsequent use [16]. SSR primers were sourced from the laboratory's collection, specifically selected for kiwifruit germplasm indention [17,18]. The SSR amplification reaction was performed in a 10 μL system, comprising: 5 μL of 2× Taq PCR Mix (Tiangen, Tianjin, China), 0.8 μL of forward primer, 0.8 μL of reverse primer, 1 μL of template DNA, and 2.4 μL of ddH_2O. The PCR amplification conditions were as follows: initial denaturation at 94 °C for 3 min, 28–34 cycles of 94 °C for 30 s, annealing at 57–64 °C for 30 s (annealing temperatures set according to Table 1), and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The amplified products were separated using 8% polyacrylamide gel electrophoresis, detected by rapid silver staining, and photographed for record-keeping. Bands were manually scored, with the presence of a band at the same position recorded as "1" and the absence of a band as "0" [19].

Table 1. Information of eight pairs of primers for simple sequence repetition (SSR) analysis.

Primer	Sequence 5′-3′	Annealing Temperature (°C)	Allele Size Range (bp)
UDK96-019	F: ATACACTTGAAGCGCCGC R: AAGCAGCCATGTCGATACG	57	100–200
UDK96-035	F: AAGAGCCATAGCTTATTCACCG R: AAGTAAAGCCATTGTCATTGCA	60	100–150
UDK96-039	F: GGTTTGATCGGTCTTCGAAA R: ATAAATGTGTGCCAGTGCGA	57	150–200
UDK96-040	F: TCGAGTTACCTAGCTACTCCGC R: CAAGGGAAGAAAATGTTGAACC	62	100–200
UDK96-053	F: GTAAGGTCATTTTTGCGAAAGG R: TTTGTTGGGAGTAACGTGAGG	64	50–100
For13	F: ACTAACAGACAAAAACTGGGGG R: ATGGAAGGAGATGGCGATG	58	200–250
ST-Acd04	F: CCCTTCCCCTCTCTCTCTC R: CGGAAGATCTGGCCATAGG	57	200–400
EST-Ad42	F: GTTAATTTGATCGGGATGG R: GAGGAGCTTGAGCTGCTAT	62	250–400

2.5. Data Analysis

Data were analyzed and bar charts were plotted using GraphPad Prism v8. Statistical significance and PCA analysis were conducted using IBM SPSS Statistics v25. Clustering analysis of the SSR band data was performed using NTsys v2.1 software. The polymorphic variation coefficient was calculated as the ratio of the sum of the number of increased and decreased polymorphic loci to the total number of polymorphic loci in the control.

3. Results

3.1. Ploidy Identification of Mutagenic Materials

The scions treated with colchicine and ^{60}Co-γ radiation were grafted onto trees (age > 5 a), resulting in a total of 19 buds from the scions that germinated normally (Table 2). Among them, 13 were from colchicine-induced branches and 6 were from ^{60}Co-γ radiation-induced branches. And only branches treated with a colchicine concentration of 0.05% (0.05a), 0.1% (0.1a, 0.1d), 0.3% (0.3b), 0.4% (0.4c), and ^{60}Co-γ radiation doses of 75Gy (75a) did not bear fruit. In addition, flow cytometry analysis revealed that all surviving branches were diploid.

Table 2. Ploidy statistics of surviving branches. Gy, gray. Young leaves were used for flow cytometry analysis to determine ploidy.

Sample	Sample ID	Fruit	Ploidy
Wild type	CK	yes	diploid
Colchicine concentration (0.05%)	0.05a	no	diploid
	0.05b	yes	diploid
Colchicine concentration (0.1%)	0.1a	no	diploid
	0.1b	yes	diploid
	0.1c	yes	diploid
	0.1d	no	diploid
Colchicine concentration (0.2%)	0.2a	yes	diploid
	0.2b	yes	diploid
Colchicine concentration (0.3%)	0.3a	yes	diploid
	0.3b	no	diploid
Colchicine concentration (0.4%)	0.4a	yes	diploid
	0.4b	yes	diploid
	0.4c	no	diploid
^{60}Co-γ radiation dose (25Gy)	25a	yes	diploid
	25b	yes	diploid
	25c	yes	diploid
	25d	yes	diploid
^{60}Co-γ radiation dose (75Gy)	75a	no	diploid
^{60}Co-γ radiation dose (100Gy)	100a	yes	diploid

3.2. Measurement of Leaf Morphological Traits

Compared to the control, the leaf shape and size of the mutagenized materials showed significant changes (Figure 2A). Specifically, the leaf length and width of the colchicine-treated materials were significantly smaller than those of the control, while the ^{60}Co-γ radiation-treated materials exhibited significantly larger leaves than the control (Figure 2B,C). Regarding leaf shape index, both colchicine-induced and ^{60}Co-γ radiation-treated materials had significantly lower leaf shape indices compared to the control group. Among them, the leaf shape index of 0.1b was the smallest at 0.85, with a decrease of 24.03% compared to the control (Figure 2D). The petioles of colchicine-treated materials were significantly shorter than the control, with the petiole length of 0.05b being 55.54 mm, which was 58.17% shorter than the control (Figure 2E). In contrast, both colchicine-induced and ^{60}Co-γ radiation-treated materials had larger petiole diameters than the control group, with the petiole diameter of 25a being the largest, increasing by 54.24% compared to the control (Figure 2F).

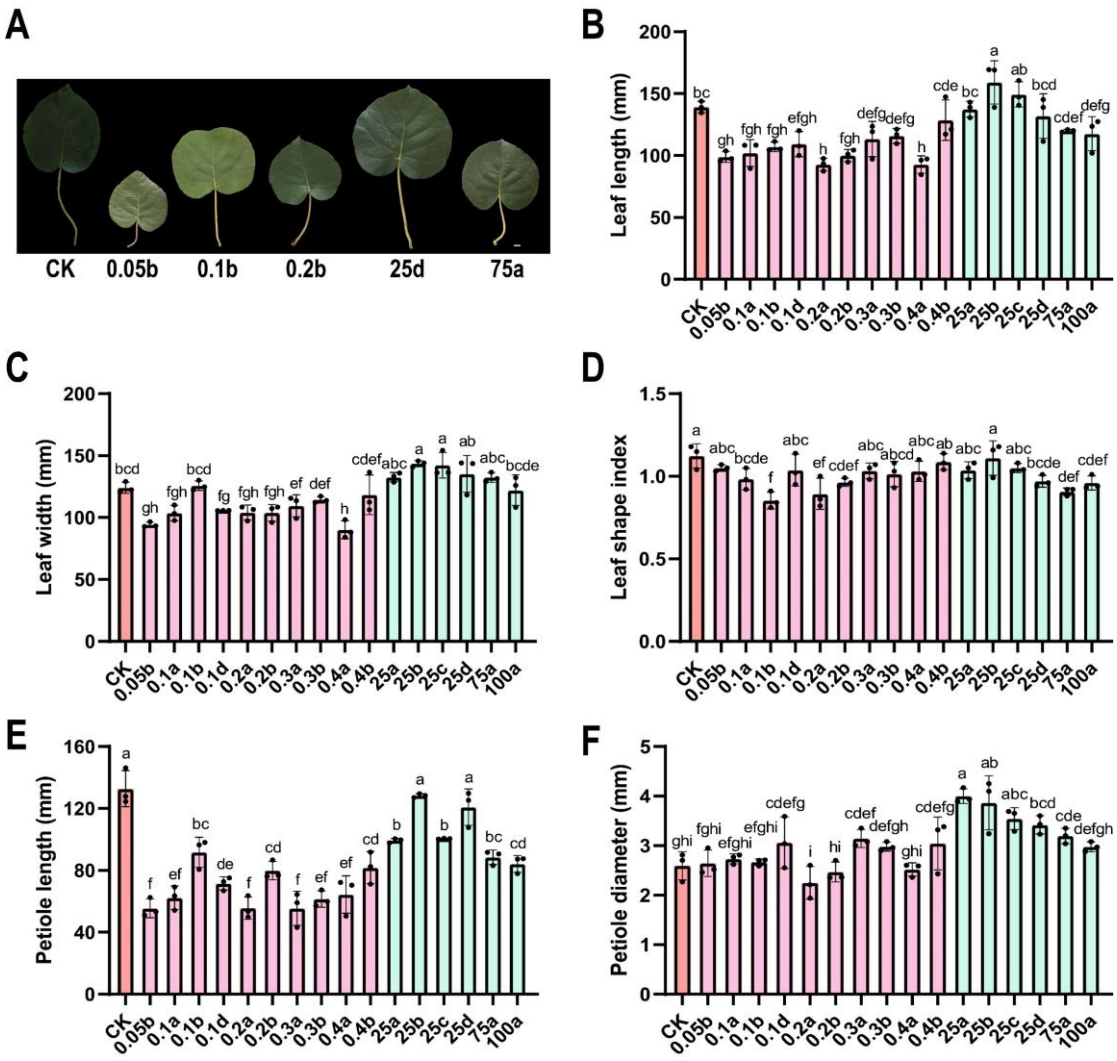

Figure 2. The effect of colchicine-induced mutation and ^{60}Co-γ radiation on the leaf morphology. (**A**), leaf appearance, the bar value represents 1 cm; (**B**), leaf length; (**C**), leaf width; (**D**), leaf shape index; (**E**), petiole length; (**F**), petiole diameter. If two groups share the same lowercase letter, it indicates no significant difference between the two groups at the 0.05 level.

3.3. Fruit Appearance Quality Determined

Compared to the control, the appearance of the fruit in the materials treated with colchicine-induced mutation and ^{60}Co-γ radiation showed significant changes (Figure 3A). Overall, the fruit length, width, side diameter, single fruit weight, and flesh firmness of the mutagenized materials exhibited a significant increase. Among them, the 25d mutant showed the most significant changes, with the transverse diameter, longitudinal diameter, lateral diameter, and fruit weight increasing by 53.97% (Figure 3B), 51.38% (Figure 3C), 39.21% (Figure 3D), and 256.10% (Figure 3E) compared to the control, respectively. However, the fruit shape index did not show a significant difference from the control (Figure 3F). For flesh firmness, the 25c mutant exhibited the most significant increase, with an increase of 198.36% (Figure 3G).

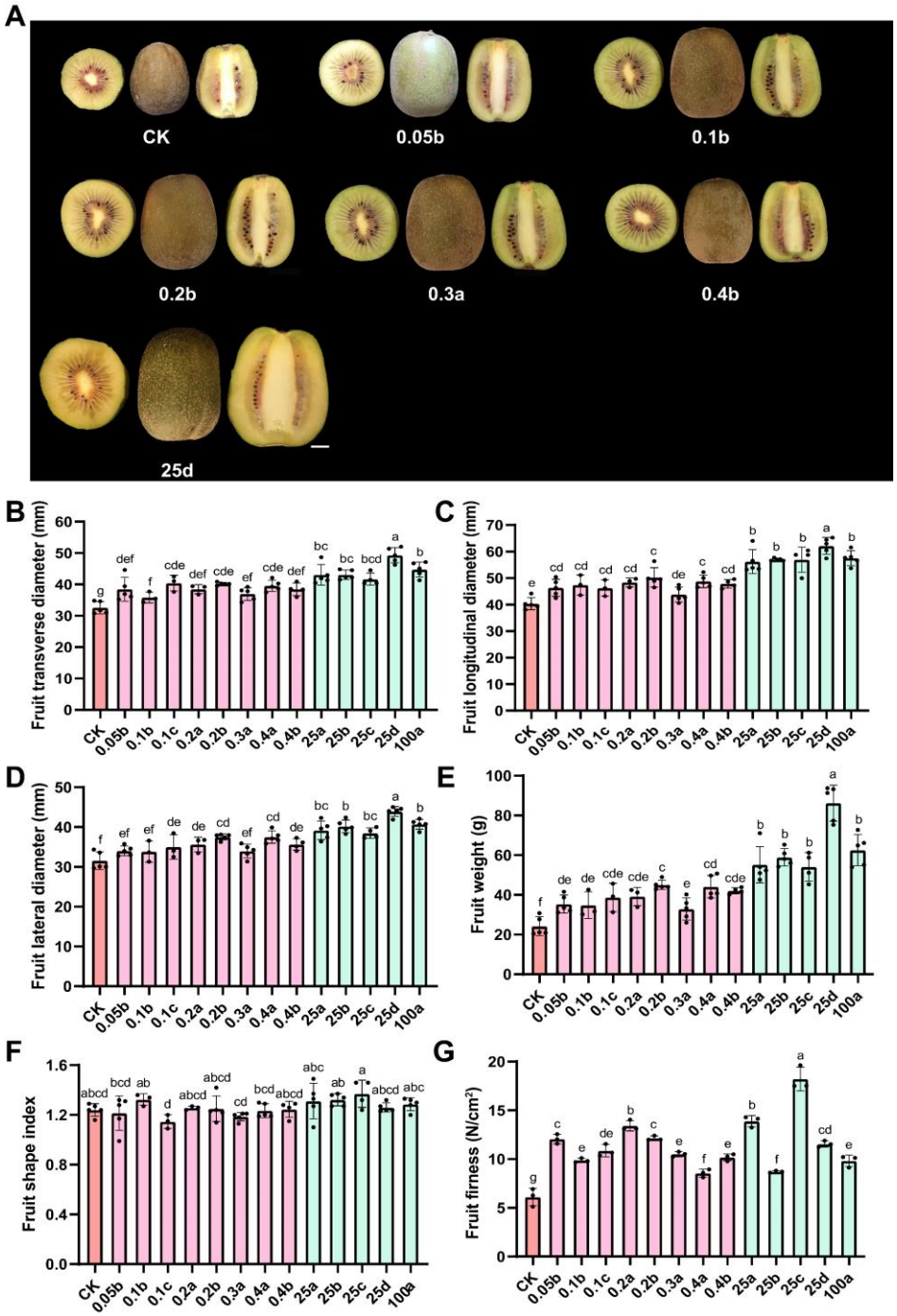

Figure 3. Effects of colchicine-induced mutation and ^{60}Co-γ radiation on fruit appearance quality. (**A**), fruit appearance, bar value represents 1 cm; (**B**), transverse diameter; (**C**), longitudinal diameter; (**D**), lateral diameter; (**E**), fruit weight; (**F**), fruit shape index; (**G**), flesh firmness. If two groups share the same lowercase letter, it indicates no significant difference between the two groups at the 0.05 level.

For the mesocarp color, the L* value of the materials treated with colchicine and ^{60}Co-γ radiation showed an increasing trend, with the highest L* value of 58.59 observed in 0.4b (Figure 4A). The a* values of the mesocarp in 0.1c, 0.2a, 0.2b, 25c, and 25d were significantly higher than in the control (Figure 4B). Most of the mutagenized materials showed no significant difference in the b* values of the mesocarp compared to the control, except for 0.05a, 25a, and 25c, which were significantly lower than the control (Figure 4C). The h* values of the mesocarp in 0.1c, 0.2a, 0.2b, and 25d were significantly lower than the control

(Figure 4D). For the endocarp color, the L* values of the endocarp in 0.2b and 25c were significantly higher than the control (Figure 4E). Most of the mutagenized materials showed significantly lower a* and b* values in the endocarp compared to the control. Among them, the a* value of 0.1b decreased the most, by 63.48%, and the b* value of 0.4a decreased the most, by 36.59% (Figure 4F,G). The h* values of the endocarp in 0.1b, 0.1c, 0.3a, 25b, and 100a were significantly higher than the control group (Figure 4H).

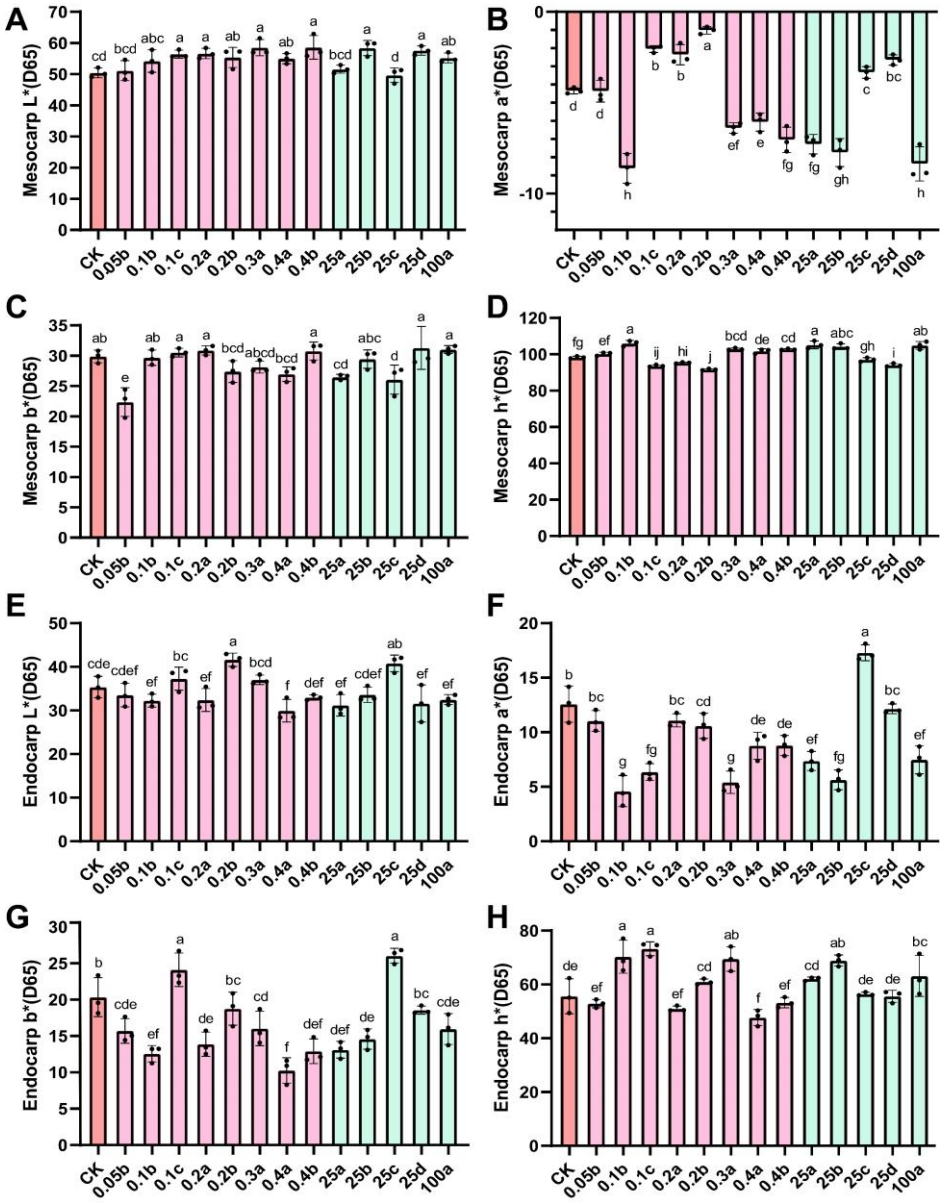

Figure 4. Effects of colchicine-induced mutation and ^{60}Co-γ radiation on fruit color. (**A**–**D**), L* value, a* value, b* value, and h* value of the mesocarp. (**E**–**H**), L* value, a* value, b* value, and h* value of the endocarp. The L* value represents brightness, with higher values indicating brighter color; the negative a* value indicates green, and the positive value indicates red; the negative b* value indicates blue, and the positive value indicates yellow; the h* value represents the color angle, with values closer to 0° indicating a color closer to red, 60° indicating yellow, 120° indicating green, 180° indicating cyan, and 240° indicating blue. If two groups share the same lowercase letter, it indicates no significant difference between the two groups at the 0.05 level.

3.4. Fruit Intrinsic Quality Determined

After colchicine-induced mutation and ^{60}Co-γ radiation, the fruit dry matter content, soluble solids content, and soluble sugar content of the materials all showed an increasing

trend. Among them, 25d exhibited significant increases in soluble solids and soluble sugar content, which increased by 23.35% and 88.29%, respectively (Figure 5A and Figure 5C). The dry matter content of 0.05b showed the most significant increase, rising by 20.18% (Figure 5B). The titratable acid content of 0.2b was significantly higher than the control, increasing by 42.30% (Figure 5D). The AsA content in 25d fruit was significantly upregulated, increasing by 35.60% (Figure 5E).

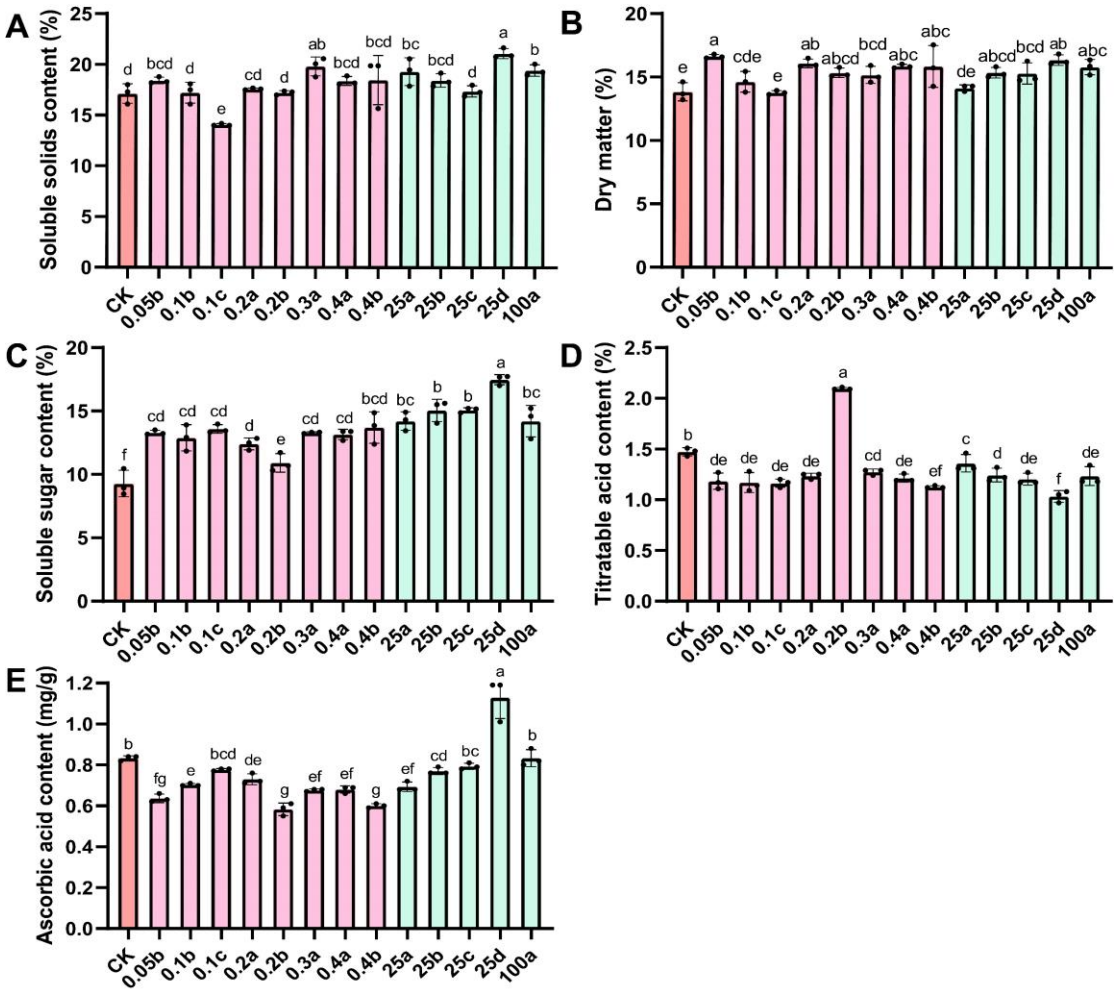

Figure 5. Effects of colchicine mutagenesis and ^{60}co-gamma radiation on fruit intrinsic quality. (**A**), soluble solid content; (**B**), dry matter content; (**C**), soluble sugar content; (**D**), titrable acid content; (**E**), ascorbic acid (AsA) content. If two groups share the same lowercase letter, it indicates no significant difference between the two groups at the 0.05 level.

3.5. Principal Component Analysis

The principal component analysis was conducted on nice fruit quality indicators, including fruit weight, flesh firmness, mesocarp h* value, endocarp h* value, dry matter content, soluble sugar content, titratable acid content, soluble solids content, and AsA content. Five principal components were selected, with a cumulative contribution rate of 92.47%, indicating that the five principal components could reflect most of the information about fruit quality. Among them, the first principal component had a contribution rate of 37.26%, mainly integrating seven major positive indicators and two negative indicators. The second principal component had a contribution rate of 18.40% (Tables 3 and 4).

Table 3. Variance contribution of principal components of fruit quality. Extraction method: principal component analysis.

Principal Components	Eigenvalue	Variance Contribution %	Cumulative Contribution Rate %
1	3.35	37.26	37.26
2	1.66	18.40	55.66
3	1.48	16.46	72.12
4	1.00	11.14	83.26
5	0.83	9.20	92.47
6	0.39	4.29	96.75
7	0.20	2.23	98.98
8	0.08	0.93	99.91
9	0.01	0.09	100.00

Table 4. Fruit quality composition load matrix. Extraction method: principal component analysis.

Quality Index	Principal Components				
	1	2	3	4	5
Fruit weight	0.87	−0.01	0.28	−0.09	0.30
Flesh firmness	0.30	−0.44	0.37	0.71	0.02
Mesocarp h* value	0.08	0.68	−0.63	0.27	0.13
Endocarp h* value	−0.24	0.71	0.45	0.19	0.31
Dry matter content	0.62	−0.49	−0.45	0.05	−0.03
Soluble solids content	0.72	0.02	−0.46	−0.13	0.40
Soluble sugar content	0.90	0.21	0.21	0.26	−0.01
Titratable acid content	−0.57	−0.44	0.12	−0.13	0.66
Ascorbic acid content	0.65	0.16	0.45	−0.53	−0.13

The comprehensive score F_{total} was calculated based on the principal components Y_1, Y_2, Y_3, Y_4, and Y_5.

$$F_{total} = 0.3726 \times Y_1 + 0.1840 \times Y_2 + 0.1646 \times Y_3 + 0.1114 \times Y_4 + 0.0920 \times Y_5$$

Compared with CK, the fruit quality was improved after colchicine mutagenesis and ^{60}Co-γ radiation. The 25d has the highest comprehensive score of 1.70, indicating that it has the best comprehensive quality (Table 5).

Table 5. Fruit quality principal component score calculate. $F_{total} = 0.3726 \times Y_1 + 0.1840 \times Y_2 + 0.1646 \times Y_3 + 0.1114 \times Y_4 + 0.0920 \times Y_5$.

Sample ID	Y_1	Y_2	Y_3	Y_4	Y_5	F_{total}	Ranking
CK	−2.77	0.23	−0.14	−2.28	−0.49	−1.31	14
0.05b	0.18	−1.07	−1.38	0.65	−0.68	−0.35	10
0.1b	−1.13	1.88	−0.22	0.53	−0.15	−0.07	7
0.1c	−1.81	1.06	2.73	0.16	−1.21	−0.12	8
0.2a	−0.07	−1.74	−0.15	0.18	−0.97	−0.44	12
0.2b	−2.35	−2.43	0.84	−0.22	2.03	−1.02	13
0.3a	−0.43	1.06	−0.70	0.42	0.68	0.03	6
0.4a	0.10	−0.55	−1.60	−0.55	−0.64	−0.45	11
0.4b	0.11	−0.16	−1.52	0.47	−0.54	−0.24	9
25a	0.24	0.78	0.09	1.08	1.06	0.47	5

Table 5. *Cont.*

Sample ID	Y_1	Y_2	Y_3	Y_4	Y_5	F_{total}	Ranking
25b	0.75	1.55	−0.05	−0.11	0.67	0.60	3
25c	1.12	−1.13	1.40	1.59	−0.53	0.57	4
25d	4.68	−0.39	1.22	−1.57	0.09	1.70	1
100a	1.39	0.92	−0.53	−0.36	0.69	0.62	2

3.6. Simple Sequence Repetition (SSR) Analysis

Overall, 8 pairs of SSR primers were used for SSR detection, resulting in the identification of 36 loci, of which 31 were polymorphic, yielding a polymorphism rate of 86.11%. The number of polymorphic loci per primer pair ranged from three to seven. Among them, the primers EST-Ad42 and UDK96-040 had the highest number of polymorphic loci, with seven and six loci, respectively, while UDK96-053 and For13 had the lowest, with three loci each (Figure 6A). Compared to the control, the variation coefficients of 0.3a, 0.4a, and 0.4b were the highest, at 47.22%, each showing changes in 17 polymorphic loci. The 0.4c showed an increase and decrease of one polymorphic locus each, with the lowest variation coefficient of 5.56% (Table 6). Based on the analysis of SSR polymorphic loci, a phylogenetic tree was constructed. All treatment groups were relatively distant from the control, with 100a and the control being the most distant, having a similarity coefficient of around 0.7, while 0.4c and the control were closest, with a similarity coefficient of 0.92 (Figure 6B).

Table 6. Fingerprint profiles and polymorphic locus variation analysis of 20 samples. Banding was manually read, with "1" indicating the presence of a band at the same position and "0" indicating the absence of a band.

Sample	EST-Ad42	ST-Acd04	UDK96-019	UDK96-040	UDK96-035	UDK96-053	UDK96-039	For13	Number of Sites Increased	Number of Sites Decreased	Coefficient of Variation (%)
CK	0110100	1111	1111	000011	1111	111	11111	101	0	0	0
0.05a	0110100	0001	1111	111110	1111	111	11111	011	5	5	27.78
0.05b	1110100	1111	1111	111110	1111	111	11111	111	6	1	19.44
0.1a	1110100	1111	1111	111110	1111	111	11111	111	6	1	19.44
0.1b	1110100	1111	1111	111110	1011	111	11111	111	6	2	22.22
0.1c	1110100	1101	1111	111110	1111	111	11111	101	5	2	19.44
0.1d	0110100	0011	0010	111110	1111	010	11111	111	5	8	36.11
0.2a	1110100	1111	1111	011110	1111	111	11111	101	4	1	13.89
0.2b	1111111	1111	1111	111110	1111	111	11111	101	8	1	25.00
0.3a	0110100	0111	1100	111110	1011	000	00010	111	5	12	47.22
0.3b	1110100	1111	1111	011110	1111	111	11111	111	5	1	16.67
0.4a	0000100	0110	1000	111110	1111	010	00111	111	5	12	47.22
0.4b	0000000	0011	1010	111110	1111	000	00111	101	4	13	47.22
0.4c	1110100	1111	1111	000010	1111	111	11111	101	1	1	5.56
25a	0000100	0111	1110	111110	1111	000	11111	111	5	8	36.11
25b	0111100	0111	1110	011010	1111	111	11111	101	3	3	16.67
25c	0110100	0111	1111	111110	1111	011	11111	101	4	3	19.44
25d	0111111	1111	1111	111110	1111	111	11111	101	7	1	22.22
75a	1111101	1111	1111	111110	1111	111	11111	101	7	1	22.22
100a	0010100	1111	0011	111110	1001	000	11111	001	4	10	38.89

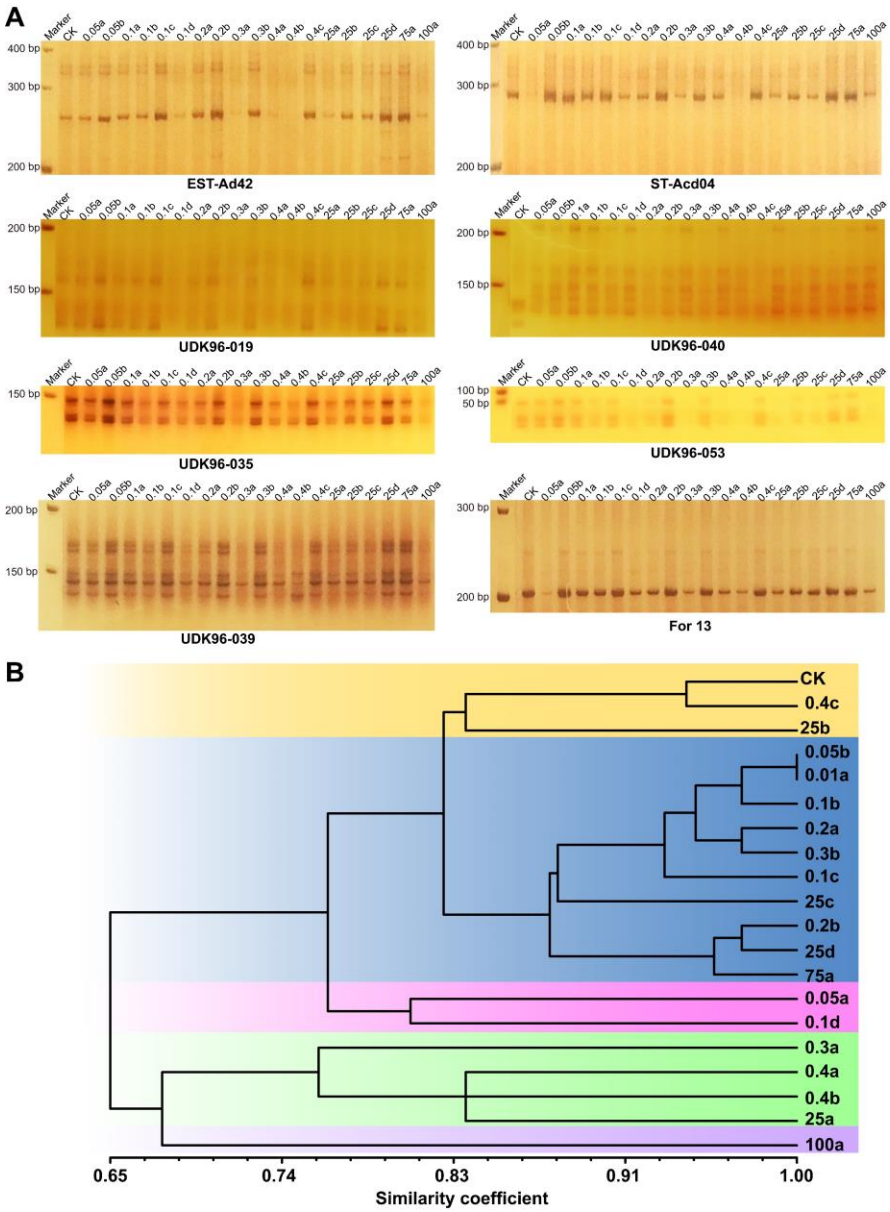

Figure 6. The simple sequence repetition (SSR) analysis of colchicine mutagenesis and ^{60}co-gamma radiation materials. (**A**), SSR amplification map of 8 primers; (**B**), evolutionary analysis tree based on SSR.

4. Discussion

The diversity of consumer choices has driven the breeding of kiwifruit towards diversification, with new breeding goals focusing on sweet flavor, large fruit size, and high nutritional metabolites. Among these goals, many studies have been conducted to increase fruit weight, such as the use of plant growth regulators and chemical sprays [20], trunk and cane girdling [21], and fruit thinning [22]. Although these methods have achieved good results, they cannot be maintained in the long term and have even led to the excessive use of plant regulators, resulting in a series of issues, such as food safety [23]. The best solution is to breed varieties that naturally meet the needs of consumers under normal growth conditions.

Fruit trees are perennial plants with long breeding cycles and slow breeding progress. Therefore, methods that can rapidly induce mutations in popular varieties to create new breeds are highly favored. For example, the use of chemical reagents or physical methods

for induction not only shortens the breeding cycle but also facilitates the rapid innovation of germplasm, enriching breeding materials. Among all chemical mutagens, colchicine is the most commonly used with a high induction rate, and it has been widely applied in fruit tree breeding, particularly in polyploid breeding [24]. Colchicine has been successfully used to induce polyploid varieties in fruit trees such as jujube [25], mulberry [26], fig tree [27], and kiwifruit [11]. Commonly, these polyploid varieties generally exhibit heavier fruit and enhanced photosynthetic characteristics, and the results of our study are consistent with previous studies [11]. The number and size of fruit cells after treatment significantly increased [28,29], which led to a significant increase in the transverse diameter and longitudinal diameter of fruit, thereby resulting in a higher fruit weight. Although no chromosome doubling events occurred in the materials mutated in our study, a significant increase in fruit weight after colchicine treatment was observed, which suggests that the increase in fruit weight in polyploids may not only be related to chromosome doubling but also closely associated with colchicine. Colchicine has toxic effects, and while inducing chromosome doubling, it can also cause toxicity to plants [30]. Therefore, future research should focus more on detecting colchicine residues in mutagenized materials.

In addition, our study revealed an overlooked phenomenon, where colchicine and ^{60}Co-γ treatment led to a significant decrease in the a* value and AsA content; the a* value decrease suggested that the redness of the endocarp was a significant decrease. The red color of kiwifruit flesh is determined by the anthocyanin content. Both colchicine and radiation mutagenesis are non-biological stresses for plants. As anthocyanins and AsA are important antioxidant compounds [2,31], it is inferred that these treatments induced mutations in some metabolism-related genes to help the plant resist the non-biological stress, and these mutations have been retained over time. Further identification of the expression levels of related genes using transcriptome technology will be the focus of the later stages of our research.

Radiation treatment is one of the commonly used methods in physical mutagenesis breeding, characterized by inducing a small number of mutations while having minimal impact on the favorable traits of the original varieties [32]. The most commonly used radiation source is ^{60}Co-γ, which can cause cell death or mutation in plants after irradiation, primarily manifesting as abnormalities in the germination rate, the survival rate, and morphology [33,34]. ^{60}Co-γ has been extensively studied in horticultural crops. For instance, the irradiation of watermelon seeds with ^{60}Co-γ at 350 Gy for 3 h established a mutagenesis library of over 4000 seeds, serving as an important resource for discovering novel phenotypes and providing a basis for genetic breeding and functional gene exploration in watermelon [35]. However, studies using ^{60}Co-γ radiation to create mutants in kiwifruit are rare. In this study, a material labeled 25d with a comprehensive evaluation significantly higher than its maternal parent was developed using ^{60}Co-γ at 25 Gy. This material exhibited a significantly higher individual fruit weight and AsA content compared to other treatments and the maternal parent, addressing a critical limitation in the industrial development of the kiwifruit cultivar *A. chinensis* cv. 'Donghong.' The increase in fruit AsA content aligns with previous findings where radiation mutagenesis enhanced antioxidant enzyme activity in tea plants [36]. The discovery of this new germplasm also provides excellent research material for studying the regulatory mechanisms of AsA in kiwifruit.

5. Conclusions

After treatment with colchicine and ^{60}Co-γ radiation, no changes in ploidy were observed in any of the materials. However, significant changes in the shape of the leaves and fruits were noted. The petioles of most mutant materials were shortened, while the single fruit weight, flesh firmness, and soluble sugar content increased significantly, and

both titratable acid and AsA content decreased significantly. Among these, 25d showed the most notable improvements, with a 256.10% increase in single fruit weight, an 88.29% increase in soluble sugar content, a 29.86% decrease in titratable acid content, and a 35.60% increase in AsA content. PCA analysis indicated that 25d had the best overall traits. Phylogenetic tree analysis based on SSR results showed that, except for the 0.4c mutant, all other mutant materials exhibited significant changes at the DNA level compared to the parental material.

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