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Original Article

Analysis of Morphological Features and Flavonoid Compositions of Crabapple Exposed to ^{60}Coγ Radiation Treatments

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Abstract

Flavonoids are important secondary metabolites, whose content and composition such as anthocyanin and flavonol have been associated with fruit quality and affect fruit coloration. Crabapple (*Malus* spp.), with rich color and nutritive value, belongs to the *Malus* genus, Rosaceae family, and it is widely used in landscape for its rich flavonoid compositions. Radiation mutagenesis breeding may increase the gene mutation frequency and enhance diversity of species, so it is a predominant approach for plant germplasm innovation. However, the changes of crabapple morphological characteristics and flavonoid compositions by radiation are not clear. In this study, we employed ^{60}Coγ radiation to *Malus* cv. 'Royalty' crabapple, and then surveyed the changes of leaves and flowers in the growth and morphology traits, color parameters, flavonoid composition, and the genetic diversity. The result found that ^{60}Coγ radiation decreased plant height, stem diameter and leaf and flower areas. And it promoted the flavonoids accumulation in leaves, but inhibited that in flowers. Additionally, ^{60}Coγ radiation improved DNA diversity. W60-7, W40-2 and W40-5 plants showed clear phenotypic variation by AFLP analysis. These results provide evidence that mutagenesis breeding may change the morphological features and flavonoid compositions, and expand crabapple germplasm resources and improve its application value.

Keywords: crabapple, flavonoids, genetic diversity, morphology, radiation

Introduction

Flavonoid compounds, such as flavonols and anthocyanins, originate from several branches of the phenylala-nine metabolism pathway in plants (Jaakola, 2013). The flavonoid compositions in fruit directly affect fruit coloration phenotype. Flavonoid not only protects the fruit from stress but also provides diverse benefits for human health, including strong antioxidant activities, anti-cancer activities andanti-atherosclerotic effects (Czank *et al*., 2013). Crabapple (*Malus* spp.) is used in ornamental, medicinal and processing industries for its abundant color, phenotype and the compositions of flavonoids in the leaves, flowers and fruits. Increasing attention has been paid to the rapid breeding and development of apple germplasm resources, which motivated by radiation mutagenesis breeding.

Radiation mutagenesis is a crucial plant breeding method that can result in better plant characteristics than conventional breeding. This approach accelerated plant breeding and improved its choice efficiency (Sen and Alikamanoglu, 2014; Takouridis *et al*., 2015). In recent years, isotope radiation as an important modern breeding method has an increasingly obvious effect on the induction of mutations in plant tissues, organs and even whole plants. Over the past thirty years, ^{60}Coγ has often been used for radiation breeding, production stimulation, pest control and food preservation, among other applications. The action of ^{60}Coγ radiation in plants always results in an improved mutation rate that expands the spectrum of mutations and induces new characteristics and rare types or mutations that do not exist in nature; this approach overcomes the hybrid incompatibility of distant source (Zou *et al*., 2015). One study showed that the seed germination of groundnut (*Arachis hypogaea* L.) was increased by 10-25% at lower doses of ^{60}Coγ radiation, and the improvement in plant vigor in the same dose range was much higher (22-84%) than that in the control without radiation (Ahuja *et al*., 2014). After the *Gossypium hirsutum* cv. 'Liaomian No. 9' were mutagenized by ^{60}Co gamma ray, the mutant line not only developed good traits, but it also had a higher lint outturn and lint yield than that of the control. Furthermore, some investigators discovered that

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^{60}Coγ radiation inhibited the growth and proliferation of hawthorn plantlets, resulting in a variety of abnormal leaf shape variations (Kim *et al*., 2013), and ^{60}Coγ irradiation of *Pyrus communis* branches resulted in offspring with rich variations, especially the fruit quality traits (Svetlana *et al*., 2013; Cassata, 2014; Du, 2014; Yang *et al*., 2014). Although mutagenesis breeding may result in some negative effects, it can be beneficial to breeding targets that inducing dwarfing mutagenesis, changing maturity, improving quality and disease resistance in crop and horticulture production (Liu *et al*., 2015).

Morphological markers are important references in germplasm research. Researchers have demonstrated that plant height can be used to investigate *Fusarium* head blight and *Septoria tritici* blotch resistance for breeding purposes in winter wheat populations (Mirdita *et al*., 2015). Moreover, in attempts to improve seed yield, some studies screen out superior populations of faba beans (*Vicia faba* L.) in terms of plant height, number of flowers, fresh weight, dry weight, root length, nodule number, root fresh weight and root dry weight (Backouchi *et al*., 2015). Some investigators irradiated the seeds of the 'Luhua 11' cultivar with a mixed high-energy particle field at different doses, and then they developed new ways to breed peanuts by observing the vigor, fertility, plant height, branch number, and pod size and shape of M2-generation plants (Wang *et al*., 2015). Thus, for a variety of perennial horticultural plants, the external shape and internal structure of the plant are widely used during resource identification and selection of breeding materials as the primary morphological markers that indicate mutations caused by radiation.

In comparison with other genetic markers, DNA markers have advantages of being used as different organizational markers that do not affect the expression of the target traits (Hamid *et al*., 2015). With the development of molecular biology techniques, DNA molecular markers now have dozens of broad uses in genetic breeding, genome mapping, gene mapping, genetic relatedness and species identification studies, gene library construction, cloning and other applications (Wang *et al*., 2013). Among them, amplified fragment length polymorphism (AFLP) is used to detect DNA polymorphisms at high resolution, with good stability and high efficiency, which combines the products of RFLP (restriction fragment length polymorphism) and PCR (Sun *et al*., 2013). In the present mutagenesis of horticultural crops, AFLP has been widely used to study cabbage (*Brassica oleracea* L. var. *capitata* Linn.)], rapeseed (*Brassica napus* Linn), zucchini (*Cucurbita pepo*), maize (*Zea mays* L.) and other crops (Colli *et al*., 2014). The new genetic structure in these plants was revealed by AFLP marker analysis, and the variation of specific loci between different plants was assessed (Cui *et al*., 2013). However, little research has identified the relationship between DNA polymorphisms and their morphological features of crabapple after isotope-based radiation treatments.

In this study, we chose *Malus* cv. 'Royalty' crabapple varieties, and employed ^{60}Coγ radiation of different strength (Gy) to explore changes in plant height, leaf and shoot traits, leaf and flower coloration, especially flavonoid accumulation and genetic diversity by using AFLP analysis. The results demonstrated that ^{60}Coγ radiation significantly decreased plant height, stem diameter, and area of leaves and flowers. Radiation induced red coloration in leaves but decreased it in flowers; red color is associated with the accumulation of anthocyanins. In addition, 40-Gy and 60-Gy irradiation promoted the accumulation of flavonoids (except for catechin) in the leaves but inhibited their accumulation in flowers. ^{60}Coγ radiation increased DNA diversity according to AFLP analysis, which provided evidence that radiation mutation breeding maybe an effective mean to rich crabapple germplasm resources and improving its agricultural application value by changing their morphological features and flavonoid compositions.

Materials and Methods

Experimental material and experimental design

The annual *Malus* cv. 'Royalty' branches with relative consistent growth potential came from 9-11-year-old plants at the Crabapple Germplasm Resources Nursery at the Beijing University of Agriculture (BUA) from March 2009-2011. We selected 0 (CK), 40, 60, 80, 100, and 120-Gy doses for the ^{60}Coγ radiation treatments (1 Gy / min), which were conducted at the Institute of Low-Energy Nuclear Physics, Beijing Normal University (Beijing Radiation Center). Three groups were irradiated per treatment, and each group contained 15 branches. After irradiation, the branches were immediately cut into 3 sections (each containing 2-3 plump buds), which were preserved with wax seals and then grafted onto 2-year-old rootstocks of *M. micromalus* plants and labeled. The grafted plants were managed by conventional methods. We retained only one shoot with strong growth potential from each scion to measure during the growth stage.

Measurements of the growth and morphology traits

After budding of the grafted plants, we observed and recorded the survival numbers of all the grafted plants, and then we calculated the survival rate by using the following formula: survival rate (%) = (number of surviving trees / total grafted number of trees) × 100%. For each dose treatment, we selected 20 representative plants and then measured their plant height, stem diameter at 1 cm from the ground and shoot growth with a caliper. We calculated the rate of shoot growth with the following equation: shoot growth = the value for shoot growth / days. When we had recorded the total number of internodes per plant, we calculated the average length between the internodes with the following formula: average internode length = height / total number of internodes.

We selected 10 flowers from all the flowering plants that were treated with radiation to measure the petal lengths and widths with a Vernier caliper. We selected 5 fully grown functional leaves from 20 representative plants per treatment and measured the leaf lengths and widths with a Vernier caliper.

For the CK plants, all of the above measurements were taken at random from the 20 plants.

Measurement of leaf and flower color parameters

The leaf and flower color parameters were analyzed with a colorimeter (CR400, Konica Minolta, Japan) on 10 leaves and 10 flowers that were randomly selected from 20 representative plants per treatment. The color parameters included the L *, a * and b *, which represent the brightness, red-green color axis and yellow-blue color axis, respectively.

Measurement of the flavonoid contents in leaves and flowers

To investigate the effects of radiation on the flavonoid accumulation level in leaves and flowers, we analyzed the flavonoid contents with HPLC (high-performance liquid chromatography) (Lu *et al*., 2016). Each 0.8 g sample was ground in liquid nitrogen, and the flavonoids were extracted from the ground plant material in 5 ml of extraction buffer (methanol:water:formic acid:TFA = 70:27:2:1) for 72 h at 4 °C on a rotator in the dark. The liquid was separated from the solids by filtration through sheets of qualitative (or quantitative) filter paper, and the filtrate was then passed through 0.22 μm reinforced nylon membrane filters. The filtrate was evaporated at 30 °C, and the residue was dissolved in 5 ml of water and purified by solid-phase extraction (500 mg, 3 ml) on a C18 Supelclean ENVI-18 cartridge (Agilent Technologies, Santa Clara, CA, USA). The cartridge was successively rinsed with water and methanol. Detection was performed at 520 nm for anthocyanins and at 350 nm for flavonols. All the samples were analyzed in triplicate.

AFLP analysis

To explore the genetic diversity of the crabapple, we randomly selected 19 samples, including the radiation treatment group and CK, and we preserved them at -80 °C for AFLP analysis. We extracted genomic DNA using the CTAB method, and estimated the DNA concentration with a fluorometer (DyNA Quant 200, Höfer-Pharmacia) according to the manufacturer's instructions. AFLP analysis was then performed as described by Vos *et al*. (1995). In brief, 0.8 to 1.0 μg of each DNA sample was subjected to restriction digestion with EcoRI/MseI (5 U each), and the fragments were then ligated to their respective adapters. After incubation for 16 h at 37 °C, the samples were diluted (1:10) in pure water. Polymerase chain reaction (PCR) amplifications were performed with pre-selective primers, which were complementary to the adapters. For selective amplification, an initial screening was performed on 10 individuals with 64 primer combinations. Nine primer combinations were chosen for the selective PCR. The selective amplification products were resolved by electrophoresis in polyacrylamide gels (7% acrylamide:bis-acrylamide 29:1) for 3 h at 200 V and stained with 20% silver nitrate. A 70-500 bp molecular ladder (Ludwig Biotecnologia, Ltda.) was used to determine the molecular weights of the fragments. AFLP data were collected and aligned with the internal size standards using ABI Prism GeneScan analysis software 3.7.1 (Applied Biosystems). The data were then analyzed by GENESCAN software. Only bands with molecular sizes between 70 and 500 bp that could be scored for their presence or absence across all individuals were considered for a primitive matrix analysis consisting of '0' and '1', which was used for analysis by POPGENE. The Jaccard similarity coefficient was analyzed with NTSYS-pc 2.10e, and the Jaccard cluster was analyzed using UPGMA.

Statistics and analysis

The statistical significance of differences between treatments was determined with the SPSS 17.0 software package (SPSS, Chicago, IL, USA). The data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to identify differences between different treatments at $P \leq 0.05$ and $P \leq 0.01$. The data are presented as the mean ± SD.

Results

The growth and morphology traits after ^{60}Coγ radiation treatment

To investigate plant responses to different doses of ^{60}Coγ radiation, we measured the survival of grafted ornamental crabapple plants after radiation treatment. The result showed that the survival rates of plants that were treated with 0 Gy, 40 Gy and 60 Gy of radiation were 93.18%, 37.84%, and 27.27%, respectively, while the survival rates of plants treated with 80 Gy, 100 Gy and 120 Gy of radiation were less than 10% (Fig. 1A). Thus, we selected the data from the 0-Gy, 40-Gy and 60-Gy treatments for further analysis.

In the present study, we also analyzed the influence of different ^{60}Coγ radiation doses on plant height, stem diameter and internode length to identify the effects of radiation on dwarfing characteristics. With ^{60}Coγ radiation treatment, the growth rates of the new shoots from surviving plants were half of plants in the 0-Gy group. Compared with the CK, plant height and stem diameter were decreased significantly, without a clear difference between the 40-Gy and 60-Gy groups, and ^{60}Coγ-induced changes in the shoot internode length were not obvious (Fig. 1B-1E). Radiation may have inhibited plant growth, and it promoted the appearance of dwarf characteristics.

Radiation also affected the morphology of the leaves and the flowers in the study plants. With ^{60}Coγ radiation, the leaves appeared to crack and the numbers of petals increased relative to the control (Fig. 2A). The leaf length and width after ^{60}Coγ treatment were both smaller than the corresponding CK values (Fig. 2B, D), as were the petal length and width (Fig. 2C, E). These findings indicated that the morphology traits of leaves and flowers changed after ^{60}Coγ radiation treatment.

The color parameters and flavonoid composition after ^{60}Coγ radiation treatment

Analysis of the phenotypic variance in leaves and flowers showed that increasing the ^{60}Coγ radiation dose caused the leaves to gradually be less green, and the red color of the flowers also faded (Fig. 2A). Compared with the control, a* color parameters increased gradually and the L* color parameter showed little increase in irradiated leaves. The b* color parameter declined, showing that the red characteristic of the leaves became increasingly obvious, while the green characteristic was reduced gradually and the lightness increased slightly (Fig. 3A, C, E). Because plants

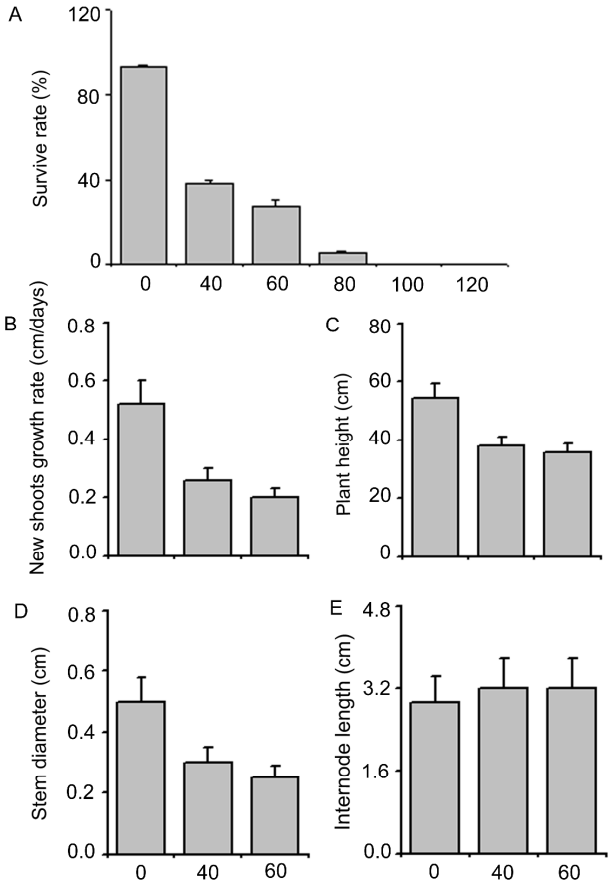

Fig. 1. The growth and dwarf characteristics of 'Royalty'. (A) The calculated survival rates of 'Royalty' plants treated with 0 Gy (CK), 40 Gy, 60 Gy, 80 Gy, 100 Gy, and 120 Gy. (B) The calculated new-shoot growth rate of 'Royalty' treated with 0 Gy (CK), 40 Gy, and 60 Gy. (C) The measured plant height of 'Royalty' under 0-Gy (CK), 40-Gy, and 60-Gy treatment conditions. (D) The measured stem diameter of 'Royalty' after 0-Gy (CK), 40-Gy, and 60-Gy treatments. (E) Measurements of the internode lengths of 'Royalty' when treated with 0 Gy (CK), 40 Gy, and 60 Gy

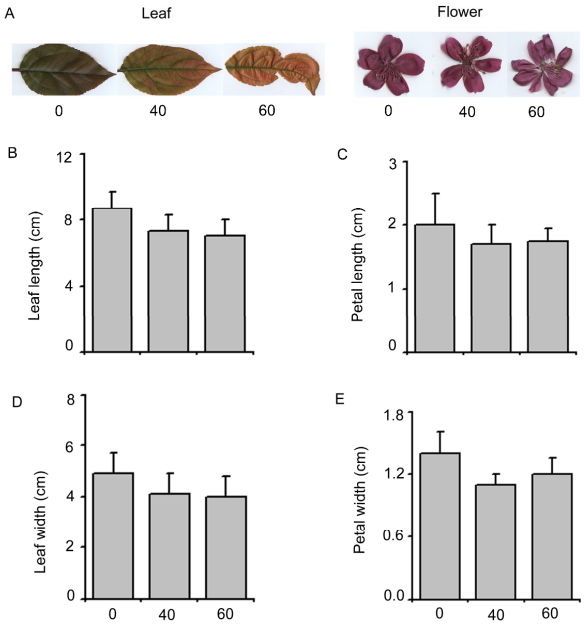

Fig. 2. The morphology of 'Royalty' leaves and flowers. (A) The observed phenotypes of 'Royalty' after 0-Gy (CK), 40-Gy, and 60-Gy treatments. (B, D) The measured leaf lengths and widths of 'Royalty' after 0-Gy (CK), 40-Gy, and 60-Gy treatments. (C, E) The measured petal lengths and widths of 'Royalty' after 0-Gy (CK), 40-Gy, and 60-Gy treatments

treated with 60 Gy of radiation could not produce flowers, we only measured the color parameters of flowers that were treated with 0 Gy and 40 Gy of radiation. As shown in Figs. 3B, 3D and 3F, there is no obvious change compared with the control. This finding illustrated that different ^{60}Coγ radiation levels affected the color of leaves and flowers to varying degrees.

To explore the physiological mechanism of color transformation, we analyzed the flavonoid composition in leaves and flowers that were treated with ^{60}Coγ radiation. Among the flavonoids in 0-Gy, 40-Gy and 60-Gy irradiated leaves, the anthocyanin contents were 70, 80 and 125 (mg / g), respectively (Fig. 4A). With increasing radiation dosage, the anthocyanin content of the leaves gradually increased. Although ^{60}Coγ radiation also increased the rutin, apigenin and quercetin contents, the apigenin content at 40 Gy declined (Fig. 4B, C, D). The catechin content decreased after ^{60}Coγ treatment (Fig. 4E). These results indicated that ^{60}Coγ radiation generally increased the flavonoid content of the leaves, with the exception of catechins, which exhibited the opposite trend. In flowers, the anthocyanin contents after 0-Gy, 40-Gy and 60-Gy treatments were 2,500, 1,700 and 1,500 (mg / g), thus showing a decreasing trend (Fig. 5A). Other flavonoids, such as rutin, apigenin, quercetin and catechin, also showed a decreasing concentration trend, and the degree of rutin and apigenin decline at 40 Gy was smaller than that at 60 Gy (Fig. 5B-5E). Together, these results indicated that ^{60}Coγ radiation influenced the flavonoid contents and the different radiation doses led to different effects.

AFLP analysis of genetic diversity following ^{60}Coγ radiation

In AFLP experiments, the amplification efficiencies of different primer combinations are different. The test experiment employed 19 groups of material and 9 pairs of primers (E-AAC/M-CAG, E-AAC/M-CTG, E-ACT/M-CAA, E-ACC/M-CAG, E-ACC/M-CAT, E-ACG/M-CTA, E-ACG/M-CTC, E-AGC/M-CAA, E-AGG/M-CAA) identified from 64 pairs of primers by 6% polyacrylamide gel electrophoresis for formal amplification.

Different plants exhibited rich polymorphisms. Among the AFLP amplification results for 'Royalty', the E-AAC / M-CTG primer combination produced the largest number of bands, at 134, and the percentage of polymorphic loci was also the highest, at 97.76%. And its observed number of alleles(Na) was 1.6204, effective number of alleles(Ne) was 1.3012, Nei's gene diversity(H) was 0.181, Shannon's Information Index (I) was 0.2796, which were analyzed by POPGENE. The E-ACG / M-CTC primer combination produced at least 73 bands, and the percentage of polymorphic loci was 89.04%; the E-AAC / M-CAG primer combination produced the lowest percentage of

polymorphic bands, at 78.13% (Table 1). Nine pairs of primers were used to amplify 916 bands, of which 820 were polymorphic loci, and the percentage of polymorphic loci reached 89.52%. The levels of 'Royalty' DNA exhibited a wide range of differences among the individual irradiated plants.

To explore the effect of $^{60}Co\gamma$ radiation on the genetic variability of the plants, we analyzed the average Jaccard similarity coefficients between the CK and the radiation groups by UPGMA. It showed that $^{60}Co\gamma$ radiation increased the genetic variability of the plants, as shown by their decrease of Jaccard similarity coefficients (Fig. 6A). The Jaccard similarity coefficients between CK and CK was 1, which was acted as control, and the average of Jaccard similarity coefficients between 40Gy group and CK was 0.74, the average of Jaccard similarity coefficients between 60Gy group and CK was 0.76. When we set the similarity coefficient at 0.75, the experimental plants were divided into 6 clusters. The first cluster included 5 plants that were treated with 40 Gy of radiation (W40-1, 3, 4, 6, and 9), 4 plants that were treated with 60 Gy (W60-1, 2, 8, 9), and the CK. The second cluster consisted of the 40 Gy-7 plant and the 60 Gy-10 plant. All the plants in the third cluster were 40 Gy-irradiated plants (W40-8, 10, 11, and 12). The other 3 clusters were represented by the 40 Gy-2, 40 Gy-5 and 60 Gy-7 plants, which displayed large genetic distances from the CK (Fig. 6B). Our study provides a reliable basis for species identification or genetic mutation breeding in crabapple.

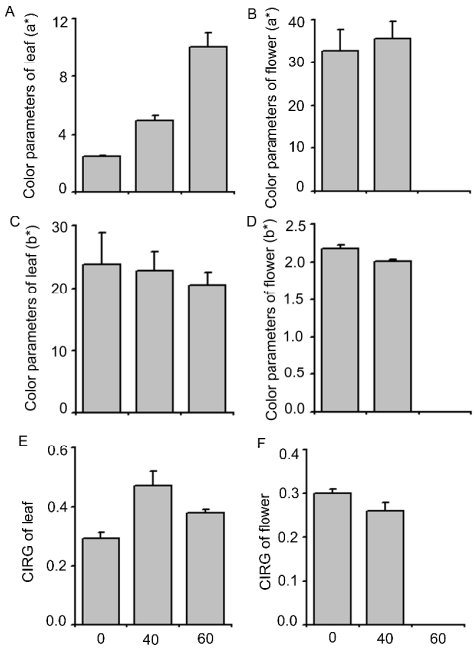

Fig. 3. The color parameters of 'Royalty' leaves and flowers. (A, B) The measured a* color parameters for 'Royalty' leaves and flowers after 0-Gy (CK), 40-Gy, and 60-Gy treatments. (C, D) The measured b* color parameters for 'Royalty' leaves and flowers after 0-Gy (CK), 40-Gy, and 60-Gy treatments. (E, F) The measured L* color parameters of 'Royalty' leaves and flowers after 0-Gy (CK), 40-Gy, and 60-Gy treatments

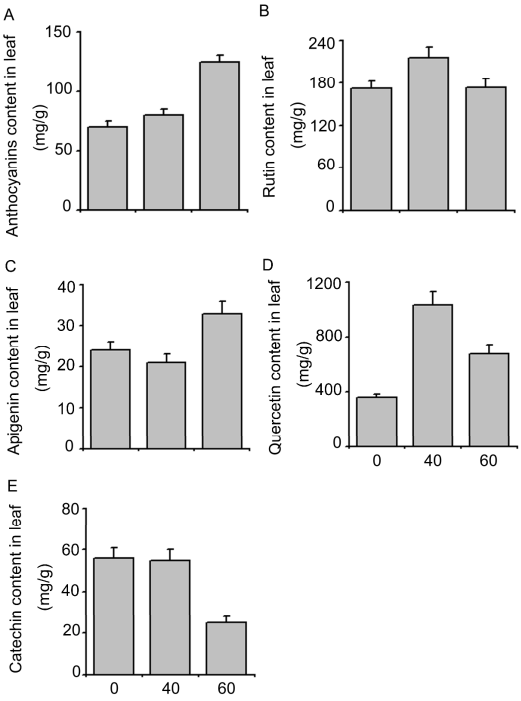

Fig. 4. The flavonoid contents in 'Royalty' leaves. The flavonoid contents of anthocyanin, rutin, apigenin, kaempferol, quercetin and catechin (A-E, respectively) in irradiated 'Royalty' leaves after 0-Gy (CK), 40-Gy, and 60-Gy treatments analyzed by HPLC. The data represent the mean ± SE of three biological replicates

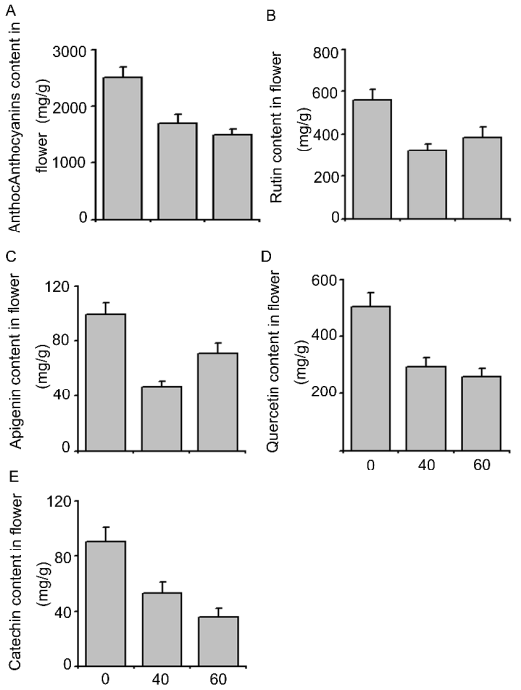

Fig. 5. The flavonoid contents in 'Royalty' flowers. The flavonoid contents of anthocyanin, rutin, apigenin, kaempferol, quercetin and catechin (A-E, respectively) in irradiated 'Royalty' flowers after 0-Gy (CK), 40-Gy, and 60-Gy treatments analyzed by HPLC. The data represent the mean ± SE of three biological replicates

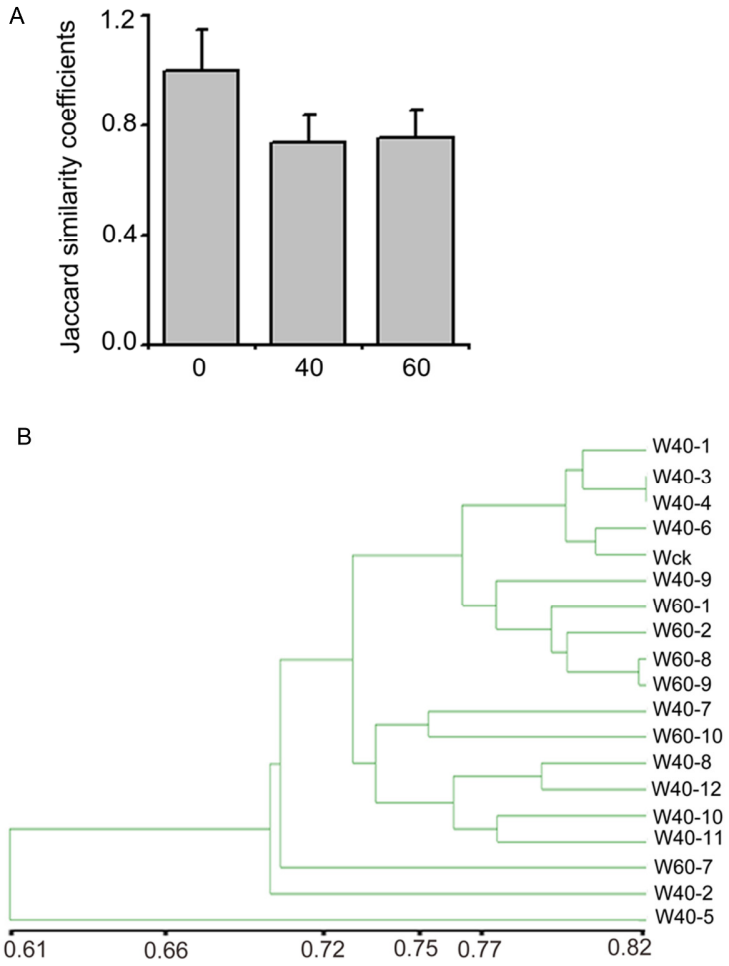

Fig. 6. Analysis of Jaccard similarity coefficients and Jaccard cluster based on AFLP markers in 'Royalty'. (A) The mean calculated values of Jaccard similarity coefficients between the control plants and the irradiated plants (B) The Jaccard cluster of 'Royalty' analysed by NTSYSpc2.1.

Table 1. AFLP amplified results of 'Royalty'

Primer combinations	Total number of AFLP bands	Polymorphic bands	Percent of polymorphic loci	Observed number of alleles (Na)	Effective number of alleles (Ne)	Nei's gene diversity (H)	Shannon's Information index (I)
E-AAC/M- CAG	96	75	78.13%	1.3472	1.1659	0.0996	0.1528
E-AAC/M- CTG	134	131	97.76%	1.6204	1.3012	0.181	0.2796
E-ACT/M- CAA	105	94	89.52%	1.4352	1.1969	0.1202	0.1879
E-ACC/M- CAG	114	110	96.49%	1.5139	1.2298	0.1436	0.2255
E-ACC/M- CAT	113	106	93.81%	1.4861	1.1814	0.1164	0.188
E-ACG/M- CTA	98	86	87.76%	1.3935	1.1959	0.1196	0.1839
E-ACG/M- CTC	73	65	89.04%	1.3009	1.1448	0.0896	0.1392
E-AGC/M- CAA	91	78	85.71%	1.3657	1.1952	0.1145	0.174
E-AGG/M- CAA	92	75	81.52%	1.3565	1.1467	0.095	0.151
Total	916	820	89.52%	12.8194	10.7578	1.0795	1.6819

Discussion

Crabapple has abundant color, phenotype and the contents and compositions of flavonoids in the leaves, flowers and fruits. So it is a promising variety for ornamental, medicinal and processing values. With the rapid development of fruit production, more emphasis has being putted on the development and utilization of ornamental crabapple.

Radiation mutagenesis is an important technique for plant breeding. The identification of the morphological characteristics, physiological changes and molecular biological mechanisms induced by radiation is a basic task in early selection and identifying new plant germplasm (Yoshihara *et al*., 2013). Some researchers have selected irradiated citrus materials to investigate their morphological and physiological characteristics for cultivating fine varieties.

They found that the leaves appeared to be cracked and grew larger, the branch angles opened and the leaf shape index became smaller. Ha *et al*. (2013) engaged in a great radiation mutagenesis effort to develop drought-tolerant soybean cultivars by assessing plant water loss and growth rates under drought stress. Herrero *et al*. (2007) used a new laser-induced fluorescence detection method to identify and quantify the formation of new maize varieties by analyzing chiral amino acids. In the present study, we found that ^{60}Coγ radiation significantly decreased the growth rates of new shoots, the plant height and stem diameter of surviving plants (Fig. 1B-1E). At the same time, the leaves that were treated with radiation appeared crack, and the numbers of petals increased relative to the control (Fig. 2A). The length and width of both leaves and flowers after ^{60}Coγ treatment were reduced relative to the CK (Fig. 2B-2E). Radiation improved the growth characteristics and promoted the appearance of dwarf characteristics, which may be an important focus for breeding dwarf apple stock.

The apparent plant characteristics are the basic criteria for judging plant health and vitality. The luminosity and coloration of plants are closely related to their function. Researchers believe that coloration can protect plants from abiotic and biotic environmental stress damage and promote antioxidant, anti-cancer and anti-cardiovascular activity (Telias *et al*., 2011). Our study indicated that ^{60}Coγ radiation promoted the a* and L* color parameters but decreased the b* color parameters in leaves, meaning that the red characteristic of the leaves became more and more obvious, while the green characteristic of the leaves diminished gradually and the lightness of the leaves increased slightly, but there is no obvious change compared with the control of flowers (Fig. 3). Moreover, Researchers have shown that UV radiation can induce plant stress-resistance in pineapple by promoting the biosynthesis of bioactive compounds, including vitamins (C and E) and carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, neoxanthin, violaxanthin and zeaxanthin) (Freitas *et al*., 2015). Yao *et al*. (2014) indicated that the appropriate ultraviolet (UV)-B radiation intensity could regulate PAL activity and increase the active ingredient content of a chrysanthemum variety, which provided a basis for breeding new varieties. In our study, according to the results in Figure 4 and 5, we found that ^{60}Coγ radiation significantly increased the anthocyanin, rutin, apigenin, and quercetin (but not catechin) contents in the leaves; however, in the flowers, ^{60}Coγ radiation decreased the anthocyanin, rutin, apigenin, quercetin and catechin contents, with a lower decrement of rutin and apigenin at 40 Gy than that at 60 Gy. This finding indicates that ^{60}Coγ radiation influences flavonoid content and that different radiation doses induce different effects. Flavonoids are involved in UV filtration, pathogen infection, aluminum poisoning, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors to regulate plant growth, development, blossoming, fruit bearing and antimicrobial activities. According to the above principles, we speculate that ^{60}Coγ radiation may rich plant coloration and affect plant activities by changing the accumulation of flavonoids. Our findings may bring new hope for the improvement of economical crops.

In recent years, AFLP has been applied to germplasm identification, genetic diversity and genetic relationship analysis, genetic linkage map construction, gene localization and cloning, gene expression and regulation, and marker-assisted selective breeding for crops, vegetables, trees, fruit trees and other plants. In this study, we selected 19 types of typical plants and investigated the differences among irradiated plants by using AFLP analysis. We found that the E-AAC/M-CTG primer combination produced the largest number of bands, at 134, and the highest percentage of polymorphic loci, at 97.76%. These results indicated that ^{60}Coγ radiation boosted the genetic variability of ornamental crabapples at the DNA level. We also analyzed the Jaccard similarity coefficients of plants by UPGMA. It showed that ^{60}Coγ radiation increased the genetic variability of the plants, as reflected by the decreasing Jaccard similarity coefficients (Fig. 6A). By setting the similarity coefficient at 0.75, the experimental plants clustered into 6 groups. Three of them were 40 Gy-2, 40 Gy-5 and 60 Gy-7 plants, respectively, displaying great genetic distances. And we can further study them with respect to their phenotypes and genetic characteristics. Our results provide a reliable basis for species identification or genetic mutation breeding in crabapple.

Conclusions

^{60}Coγ radiation improved growth characteristics and promoted the appearance of dwarf characteristics of crabapple, at the same time, the coloration of leaves and flowers also changed for the flavonoids contents altered. These all boosted crabapple ornamental value and application value. And it is needed to further study the hereditary character of the plants after ^{60}Coγ radiation to promote genetic breeding in crabapple.

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