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Full Length Research Paper

Mutation breeding of sweet potato by gamma-ray radiation

Ji-Min Shin[1], Bong-Kyu Kim[1], Sang-Gyu Seo[1], Seo Bum Jeon[1], Ji-Seong Kim[1], Byung-ki Jun[1], Si-Yong Kang[2], Joon-Seol Lee[3], Mi-Nam Chung[3] and Sun-Hyung Kim[1]*

[1]Department of Environmental Horticulture, The University of Seoul, Jeonnong-dong 90, Dongdaemun-gu, Seoul 130-734, South Korea.
[2]Advance Radiation Technology Institute, KAERI, Jeongeup, Jeonbuk 580-185, South Korea.
[3]National Institute of Crop Science, RDA, Muan, Jeonnam 293-5, South Korea.

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This gamma irradiation study was conducted to produce sweet potato mutants having high yield and high starch content. Regenerated plants from gamma-irradiated stems were grown in the field. Twenty-nine plant lines were selected by estimating storage root size, shape and yield. Genetic variation and phylogenetic relationship among the lines were investigated assuming the specific traits. Also, the flowering of sweet potato should be artificially induced by grafting and short-day treatment in north-east regions of Asia, such as Korea and Japan (Ahn et al., 2002). Furthermore, the self- and cross-incompatibility of this crop limits the use of genetic resources, and it is very difficult to breed new varieties and improve varieties through cross breeding (Ahn et al., 2002; Martin, 1965) . Mutation breeding can be used as a major approach in improving sweet potato varieties, since it is a clonally propagated crop. Irradiation-induced mutation breeding is effective in improving sweet potato characters such as yield, starch and soluble sugar content, carotenoids content of storage roots and disease resistance (Kukimura, 1986; Wang et al., 2007). Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003; Yoon et al., 1990). Sweet potato is a typical starch crop that is used as a foodstuff and, importantly, for industrial applications. In the present study, gamma irradiation-mediated mutation breeding was applied to sweet potato to produce useful varieties having a high yield and high starch content. Irradiation-induced mutants were grown in the field and assessed for their starch and sugar characteristics.

Key words: Sweet potato (*Ipomoea batatas* (L.) Lam.), gamma-ray, mutation breeding, RAPD.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is one of the most important food crops in the world, especially in developing and undeveloped countries, where the sweet potato yields are the greatest. Sweet potato is enriched in vitamins, proteins and carbohydrates, and so represents good nutritional source. Appropriately, it is used widely a as a food, feed, source of starch and as a dietary supplement (Woolfe, 1992). The current focus on energy production from biomass has led to the recognition of the potential of sweet potato as a biomass species (Liu and Cantiliffe, 1984; Ziska et al., 2009); Sweet potato has greater potential as an ethanol source than the current choice, corn, and so could productively replace or offset corn as a biofuel source (Ziska et al., 2009).

Improvement of sweet potato variety by conventional breeding methods has some limitations. Conventional methods are able to generate many mutations at once because sweet potato exhibits polyploidy and has many chromosomes. However, it is hard to determine the

*Corresponding author. E-mail: mongdal@uos.ac.kr. Tel: +82-2-2210-5728.

Abbreviations: PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; UPGMA, unweighted pair-group method with arithmetic average.

genetic phenomenon of specific traits. Also, the flowering of sweet potato should be artificially induced by grafting and short-day treatment in north-east regions of Asia, such as Korea and Japan (Ahn et al., 2002). Furthermore, the self- and cross-incompatibility of this crop limits the use of genetic resources, and it is very difficult to breed new varieties and improve varieties through cross breeding (Ahn et al., 2002; Martin, 1965).

Mutation breeding can be used as a major approach in improving sweet potato varieties, since it is a clonally propagated crop. Irradiation-induced mutation breeding is effective in improving sweet potato characters such as yield, starch and soluble sugar content, carotenoids content of storage roots and disease resistance (Kukimura, 1986; Wang et al., 2007). Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003; Yoon et al., 1990). Sweet potato is a typical starch crop that is used as a foodstuff and, importantly, for industrial applications. In the present study, gamma irradiation-mediated mutation breeding was applied to sweet potato to produce useful varieties having a high yield and high starch content. Irradiation- induced mutants were grown in the field and assessed for their starch and sugar characteristics.

MATERIALS AND METHODS

Plant material and gamma irradiation

Sweet potato (*I. batatas* L.) cultivar, Yulmi, a major recommended cultivar in Korea, was used in this study. Stems with axillary buds (50 to 100 cm) cut from the sweet potato grown in a greenhouse, under natural day length were used as experimental material after removing leaves and exposure to 30, 50 and 70 gray (Gy) doses of a 60Co source, in the radiation facility at the Advance Radiation

Technology Institute, Jeongeup, Korea. The gamma-irradiated stems with axillary buds were excised from two or three nodes, and immediately planted in garden soil (Evergreen, Seoul- bio, Korea). The planted stems were covered and grown with a transparent polyvinyl sheet to retain moisture. The axillary buds surviving the gamma irradiation were used in regeneration through shoot apex culture and/or re-irradiation with 80, 100 and 120 Gy doses of 60Co.

In vitro regeneration and field trial

Regeneration of gamma-irradiated sweet potato was carried out via *in vitro* regeneration and direct cut-planting in soil. Nodal stems including axillary buds for *in vitro* culture were excised and surface-sterilized by immersion in 70% (v/v) ethanol for 1 min and 2% (v/v) sodium hypochlorite for 20 min. The sterilized materials were washed five times with distilled water. Axillary buds isolated from the sterilized materials (approximately 0.5 to 1 mm) were transferred to MS medium supplemented with 30 g L^{-1} of sucrose and 3 g L^{-1} of gelite (Murashig and Skoog, 1962) and cultured in a growth room operating at a temperature of 24 ± 2°C and using light/dark periods of 16 h/8 h. After incubation for 4 to 6 weeks, the regenerated plantlet -induced shoots from meristem were transferred to a plant culture vessel to develop into whole plants. The regenerated plants were transferred to a pot containing garden soil and used in the field trial after acclimatization. The field trial was conducted in Yangpyeong-gun, Gyeonggi-do, Korea. The regenerated plants were planted at a distance of 30 cm and mulched with black polyethylene films to maintain the soil temperature in the early stage of planting and to minimize weed growth. After 123 days of culture in the field, storage roots of the developed sweet potato were harvested and selected by size, shape and the number of storage roots.

Random amplification of polymorphic DNA (RAPD) assay

Genomic DNAs were extracted from the leaves of sweetpotato as described by Kim and Hamada (2005). Thirty decamer RAPD primers (Bioneer, Korea) and an AccuPower polymerase chain reaction (PCR) pre-mix kit (Bioneer) were used for PCR amplification. For RAPD assay, PCR amplification was carried out by adding 30 ng of genomic DNAs and 5 pmole of primer to PCR pre-mixture (Bioneer) containing buffer, dNTP and Taq DNA polymerase. PCR reaction was performed by pre-denaturation for 5 min at 94°C; 45 amplification cycles each made up of 1 min denaturation at 94°C, 1 min annealing at 36°C and 2 min extension at 72°C; and stabilization at 72°C for 10 min, with storage thereafter at 4°C. The amplified products were loaded onto a 1.5% (w/v) agarose gel containing ethidium bromide and were separated by electrophoresis in 0.5 x TAE buffer for 50 min. RAPD polymorphic bands were visualized and photographed using ultraviolet illumination, and were scored as present (1) or absent (0). A dendrogram was generated by unweighted pair-group method with arithmetic average (UPGMA) in the SHAN program.

Assay of starch and soluble sugar

For the assay of the contents of starch and amylose/amylopectin, sweet potato storage roots were dried at 80°C for 48 h, finely ground and passed through a 0.1 mm sieve prior to analysis. Total starch and amylose analyses were performed using an amylose/amylopectin assay kit (Megazyme, Ireland) according to the manufacturer's instructions. Soluble sugar contents in storage roots of sweet potato were analyzed accordingly, as previously described (Antonio et al., 2007) with slight modifications. Briefly, fresh sample of sweet potato root (0.2 g) was ground to a fine power in liquid nitrogen using a mortar and pestle. 50 mg of frozen ground material was transferred to a microfuge tube (2 ml) to which was added 800 µl of 16% (w/v) trichloroacetic acid (TCA) in diethylether. The sample mixture was mixed by vortexing and stored on ice for 30 min. The mixture was added to 1000 µl of 16% TCA/water (w/v) containing 5 mM ethylene glycol-bis (2-aminoethylether) -N,N,N',N'-tetraacetic acid (EGTA), mixed well, and stored on ice for 2 h.

After centrifugation at 17900 × g at 4°C for 10 min, the upper phase was discarded. The remaining water phase was transferred to a new microfuge tube and 1000 µL ice-cold diethylether was added to the tube. After centrifugation as earlier stated, the separated upper phase was discarded and the ether washing step detailed earlier was repeated twice. The extract was evaporated to completely remove the ether under a flow of helium gas and was neutralized with 2.5 M KOH aliquots (pH 5 to 6). The neutralized extract was stored at -80°C prior to analysis. All extracts were centrifuged as previously stated and filtered through 0.45 µm filters before analysis. Soluble sugar contents were determined using a model ICS3000 Bio liquid chromatography system (Dionex, Sunnyvale, CA) with a CarvonPAC1 (20 cm x 4 mm; Dionex) at a flow rate of 0.7 ml min^{-1} at 30°C. As a solvent system, distilled water

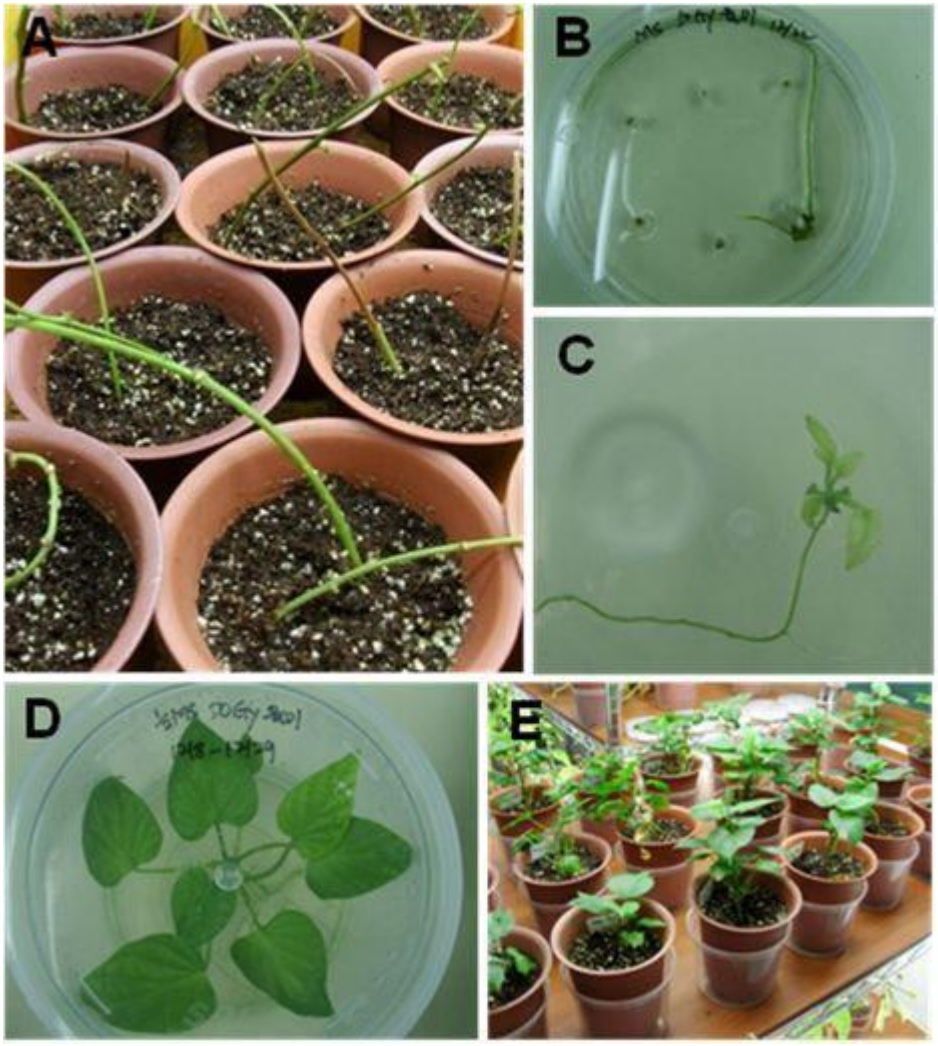

Figure 1. Plant regeneration from gamma-irradiated auxillary buds. (A) Plant regeneration using direct cut-planting and culture in soil. (B–D) *In vitro* regeneration from gamma-irradiated auxillary buds. (E) Putative mutant plants from *in vitro* culture after accumulation.

and 1 M NaOH (80:20, v/v) was used for the elution of neutral sugars. Detection was realized using a pulsed-amperometric detector.

RESULTS AND DISCUSSION

Regeneration of plant irradiated by gamma-ray

After stems of gamma- irradiated sweet potato were cut-planted in garden soil, the surviving axillary buds were regenerated to whole plants by *in vitro* culturing (Figure 1). Culture of axillary buds including a partial nodal stem on MS medium resulted in the direct induction of shoots and roots (Figure 1B, 1C) . As plant regeneration arose in the absence of the callus phase and involved direct induction of explants into shoots and roots, plants were easily and quickly regenerated from the irradiated axillary buds. There was a difference in the time of formation of shoot, according to explant size. Early formation of shoot and root were observed in the larger explants, indicating that explant size affects plant regeneration, perhaps because the larger explants contain more nutrient reserves and plant growth regulators to sustain plant regeneration. Plant development was affected by plant growth regulators, consistent with the suggestion that explants could have a different endogenous hormone level and nutrient contents (Ivanova et al., 1994; Centeno et al., 1997) . The difference in internal tissue factors could result in different timing of shoot and root development.

The method of direct cut-planting and culture in soil without *in vitro* culture offered a simpler way to obtain whole plants; although, it produced a lower survival rate until mutants were acquired from the irradiated axillary buds. For the irradiation-mediated production of mutants, the present approach offer time and cost benefits. The

Figure 2. Twenty nine selected mutant lines based on the yield potential, seed color and other characteristics. These putative mutant lines were selected from 600 lines.

combination of chronic gamma irradiation and tissue culture is a well-established means of inducing mutants (Nagatomi et al., 1996, 2000; Wang et al., 2007), and enhances mutation frequency, which increases the types of mutations that arise (Nagatomi et al., 2000).

However, for the direct use of mutants as new cultivars in practical breeding, mutants should not have any unwanted mutations or abnormalities. Mutation frequency is not particularly important in this case. Two methods were used for obtaining regenerated whole plant from axillary buds with irradiation in the present study: regeneration that directly induced shoot and root from explants using *in vitro* culture and soil culture. The combination of acute irradiation and direct regeneration simplified the processes of mutagen treatment and plant regeneration, allowing quicker generation of mutations. Although problems remain, such as chimera formation and a low-mutation rate, the present method offers promise in practical mutation breeding as a simple method of mutant generation.

Field trial and RAPD analysis

Over 600 regenerated plants from irradiated axillary buds were obtained via *in vitro* culture or soil culture. These were used for the field trial and sweet potato were harvested 4 months after field planting. Five root flesh color variants and many abnormalities were found in the harvested plants (data not shown). From the putative mutants obtained, 28 lines were selected by size, shape and the number of storage roots (Figure 2). In these selected mutants, genetic variations were detected RAPD analysis, an approach that detects genetic differences (Lynch and Milligan, 1994; Williams et al., 1990). The analysis was performed for the mutant lines and the donor cultivar, Yulmi, using 30 primers; 234 bands were produced from 25 primers. Gamma irradiation produced

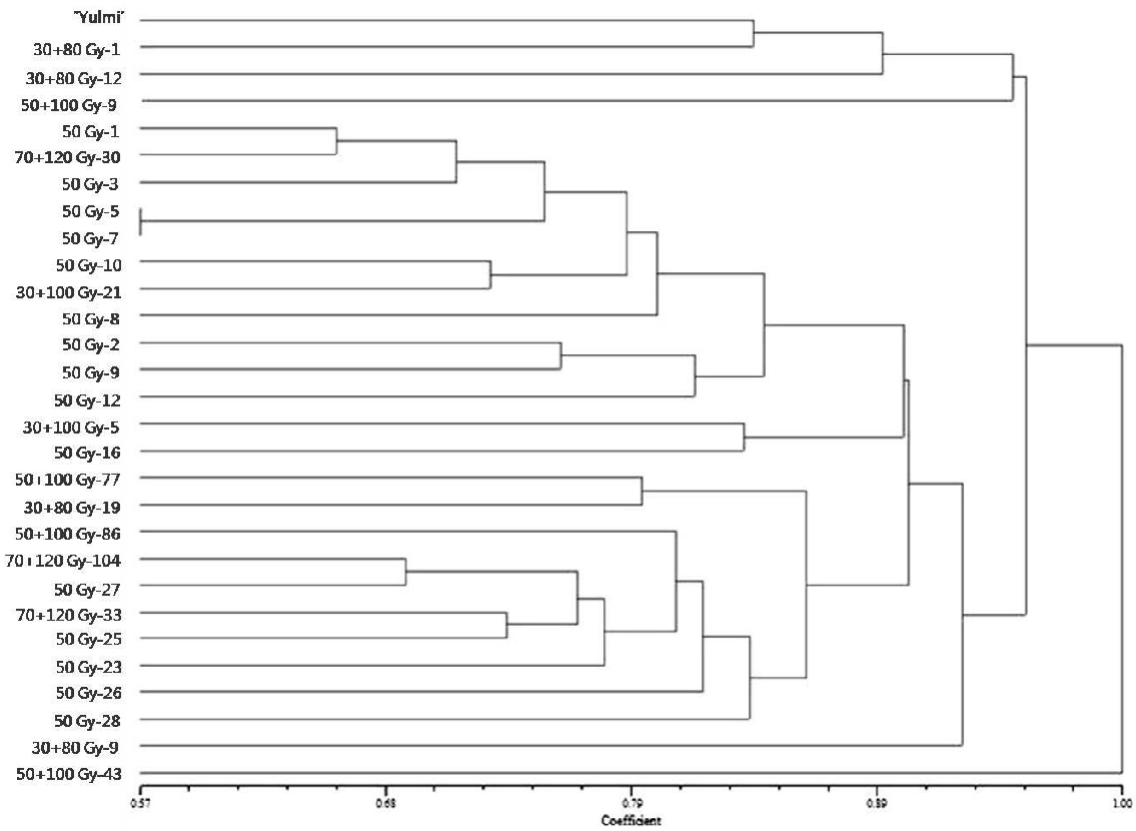

Figure 3. Dendrogram showing the genetic relationship between the wild type plant 'Yulmi' cv. and the selected putative mutant lines.

genetic variations in the 28 selected lines as compared with the Yulmi cv. (Figure 3).

Starch assay of mutant lines

Starch is the most abundant material in the storage roots of sweet potato, which is beneficial as a foodstuff, a source of energy, and for industrial applications. Starch consists of two glucose polymers, amylose and amylopectin. The ratio of these components is influential in granularity and utilization (Jobling, 2004) . The amylose and total starch contents of storage roots were investigated in the 28 selected lines (Table 1). Amylose contents were generally low in the selected lines (12 to 17%) and were not appreciably different from parental plants, which had an amylose content of 13.3%. The lines 50Gy- 10 and 50Gy-8 displayed relatively high amylose levels (17.2 and 15.8%, respectively) (Table 1). The selected mutant lines displayed wide variation in total starch content. Lines 70+120Gy-33 and 50Gy-27 displayed low starch levels compared with 'Yulmi' cv., while some lines, including 50Gy-23, 50Gy-26 and 50Gy-28, displayed very elevated levels of starch (>30% on a fresh weight basis) (Table 1). Sweet potato is a typical starch crop, that contains a high proportion of starch, which makes it valuable as a food and an industrial raw material. Both glucose polymer of starch, amylase and amylopectin, have a linear and branched structure, and their ratio affects physical and physicochemical properties, such as gelatinization and recrystallization (Karlsson et al., 2007). The amylose content in typical starch sources like a corn, wheat, rice and potato ranges from 15 to 25%, with the amylase and amylopectin ratio in wheat and corn being approximately 1:3 (Zobel and Stehen, 2006). In a previous study, the amylose contents from 51 sweet potatoes ranged from 14 to 24% (mean 19.7%), which was slightly lower than wheat (mean 24%) (Noda et al., 1998). In comparison, amylose contents in the present study were lower and displayed a narrower range (12 to 17%) in both the mutant lines and donor cultivar. A previous study of the 'Yulmi' cv. reported a low amylose content with an amylose/amylopectin ratio of 12.8:87.2% (Ahn et al., 2010).

Alteration in starch structure was presently attempted using gamma irradiation, but was unsuccessful.

Contrarily, significant differences in starch content were evident among the selected mutant lines ranging from 60 to 80% on a dry weight basis and from 15 to 35% on a fresh weight basis (Table 1). Most of the selected mutant lines displayed increased starch content compared to 'Yulmi' cv. In particular, 50Gy-10, 50Gy-16, 50Gy-23,

Table 1. Amylose and starch contents of storage roots of the selected mutant lines and the donor cultivar.

Plant line	Amylose content (%)	Starch content (% based on dry weight)	Starch content (% based on fresh weight)
Yulmi cv.z	13.25 a-ey	68.47 b-g	19.49 bcd
50 Gy-1	13.96 a-f	70.85 b-j	26.32 ghi
50 Gy-2	14.78 c-f	63.51 ab	17.74 abc
50 Gy-3	14.53 c-f	64.61 abc	20.05 cde
50 Gy-5	14.10 a-f	74.16 e-j	27.15 hij
50 Gy-7	14.07 a-f	77.37 g-j	27.55 hij
50 Gy-8	15.84 fg	74.07 d-j	26.26 ghi
50 Gy-9	15.41 efg	68.28 b-f	27.99 hij
50 Gy-10	17.21 g	76.73 f-j	32.34 lm
50 Gy-12	14.31 b-f	69.57 b-h	25.87 gh
50 Gy-16	12.75 abc	77.00 f-j	31.36 kl
50 Gy-23	14.10 a-f	76.54 f-j	34.83 m
50 Gy-25	14.21 b-f	74.62 e-j	27.32 hij
50 Gy-26	14.48 c-f	79.57 j	34.44 m
50 Gy-27	13.02 a-d	64.52 abc	16.86 ab
50 Gy-28	13.34 a-e	78.56 ij	34.21 m
30+80 Gy-1	13.39 a-e	65.44 a-d	20.85 de
30+80 Gy-9	14.39 c-f	78.38 hij	27.22 hij
30+80 Gy-12	15.28 d-g	70.58 b-i	23.81 fg
30+80 Gy-19	13.28 a-e	69.75 b-i	22.95 ef
30+100 Gy-5	14.34 b-f	64.06 abc	19.40 bcd
30+100 Gy-21	14.67 c-f	74.80 e-j	27.25 hij
50+100 Gy-9	12.09 ab	72.50 c-j	24.93 fgh
50+100 Gy-43	11.89 a	76.54 f-j	29.08 ijk
50+100 Gy-77	13.23 a-e	69.02 b-g	22.16 def
50+100 Gy-86	12.57 abc	77.09 f-j	29.68 jkl
70+120 Gy-30	13.94 a-f	72.14 b-j	22.26 def
70+120 Gy-33	14.52 c-f	58.65 a	15.07 a
70+120 Gy-104	14.60 c-f	69.29 b-g	24.98 fgh

zOriginal plant which was not exposed to gamma-ray; yMean separation within columns by Tukey's test at p = 0.05.

50Gy-26 and 50Gy-28 had a starch content exceeding 30%, implicating them as potentially valuable as processing raw materials. Starch has been widely used as a raw material in the food and beverage industries, as well as in the paper, plastics, pharmaceutical and cosmetic industries (Ellis et al., 1998; Röper, 2002). These uses are influenced by the amylose: amylopectin ratio. As well, starch quantity attributes, such as content and yield are important economic considerations. It needs to be determined whether the selected mutant lines possess these desirable stable traits and sufficient yield. However, it seems clear from the present results that mutation breeding using gamma irradiation can improve the starch quantity of sweet potato storage roots.

Soluble sugar contents of mutant lines Water-soluble sugar contents are closely related with sweetness, which is one of the important characteristics of sweet potato in food processing and cooking (Kohyama and Nishinari, 1991). The contents of soluble sugars for the 28 selected mutant lines were examined. Sucrose was the main soluble sugar, while glucose and fructose were minor components in sweet potato roots. Sucrose content differed appreciably only in a few lines, while glucose and fructose contents differed significantly among the selected lines (Table 2). The lines 50Gy-2, 50Gy-27, 30+80Gy-12 and 70+120Gy-33 were significantly increase in the glucose content compared to Yulmi' cv., but the lines 50Gy-5, 50Gy-28 and 30+80Gy-1 possessed very low levels (Table 2). Fructose content was obviously increased in line 50Gy- 27, while most of the selected mutant lines showed a low or similar fructose level to 'Yulmi' cv. (Table 2). Glucose and fructose content of line 50Gy- 27 was highest at 15.56 and 9.21 mg g^{-1} fresh weight, over 2.5 and 1.5-fold higher, respectively, than that of 'Yulmi' cv., which was 5.81 and 5.95 mg g^{-1}

Table 2. Comparison of glucose, fructose and sucrose contents of storage roots of the selected mutant lines and the donor cultivar.

Plant line	Glucose content (mg/g FW)	Fructose content (mg/g FW)	Sucrose content (mg/g FW)
Yulmi cv.z	5.81 f-iy	5.95 hij	20.63 abc
50 Gy-1	3.01 a-f	2.02 a-d	20.57 abc
50 Gy-2	12.14 l	6.32 ij	20.16 abc
50 Gy-3	8.81 jk	4.15 fg	25.92 c
50 Gy-5	1.02 ab	0.90 ab	22.81 abc
50 Gy-7	4.05 c-h	2.99 c-f	22.04 abc
50 Gy-8	7.54 ij	3.71 efg	23.10 bc
50 Gy-9	3.73 b-g	1.50 abc	24.95 bc
50 Gy-10	5.37 e-i	1.87 a-d	24.55 bc
50 Gy-12	2.53 a-e	1.70 a-d	20.27 abc
50 Gy-16	4.41 c-h	1.63 a-d	20.44 abc
50 Gy-23	4.26 c-h	1.12 ab	16.20 ab
50 Gy-25	9.44 jk	4.18 fg	23.83 bc
50 Gy-26	2.92 a-f	0.90 ab	20.84 abc
50 Gy-27	15.56 m	9.21 k	22.11 abc
50 Gy-28	0.74 a	0.47 a	26.94 c
30+80 Gy-1	1.48 abc	0.99 ab	13.76 a
30+80 Gy-9	3.38 a-g	2.13 bcd	21.66 abc
30+80 Gy-12	10.79 kl	5.12 ghi	19.64 abc
30+80 Gy-19	6.76 hij	4.52 fgh	25.60 bc
30+100 Gy-5	7.74 ij	5.03 ghi	22.99 bc
30+100 Gy-21	5.88 f-i	2.42 b-e	20.68 abc
50+100 Gy-9	2.97 a-f	2.14 bcd	21.53 abc
50+100 Gy-43	2.46 a-e	1.36 ab	21.80 abc
50+100 Gy-77	4.01 c-h	3.20 def	24.86 bc
50+100 Gy-86	6.02 ghi	3.08 c-f	27.97 c
70+120 Gy-30	5.14 d-i	2.98 c-f	25.00 bc
70+120 Gy-33	11.29 kl	7.13 j	23.07 bc
70+120 Gy-104	4.00 c-h	1.85 a-d	22.00 abc

zOriginal plant which was not exposed to gamma-ray. yMean separation.

fresh weight, respectively (Table 2). As well, the lines 50Gy-2 and 70+120Gy-33 displayed low starch content, but had high soluble sugars content, in contrast to the lines 50Gy-26 and 50Gy-28 with high starch and low sugar content (Tables 1 and 2). There was a negative correlation between content of soluble sugars and starch content in sweet potato storage root of the selected mutant lines (data not shown). It is known that starch can be converted to soluble sugars in times of plant need for use as an energy source. Presently, the correlation between soluble sugars and starch content was apparently related to the conversion of starch to soluble sugars in the sweet potato roots. Increased sugar levels can be accompanied by decreased starch content (Besma and Mounir, 2010). However, the regulation of starch synthesis and starch degradation is a complex process involving many endogenous factors (Alison et al., 2005; Beck and Ziegler, 1989), making it difficult to invoke this as the explanation of the present observations with the selected mutant lines. Thus, endogenous enzymes and metabolites associated with starch synthesis and degradation during sweet potato root development and root storage in the selected mutant lines should be examined in further detail.

In the present study, mutation breeding using gamma-ray radiation was used to produce useful sweet potato mutants. Two approaches for obtaining regenerated whole plant from axillary buds with irradiation were followed. They simplified regeneration, possibly through direct induction of shoot and root from explants without a callus phase. Significant variation of the levels of starch and soluble sugars was evident in the selected mutant lines. Starch and sugars contents are important characteristics of sweet potato that influence the plant's uses. Mutants with high starch content and sugars content could be beneficial in the industrial use of sweet potato. Furthermore, the altered properties of starch and sugars in mutant plants could be exploited to study the mechanism of starch synthesis and degradation in sweet potato.

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