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Effect of Proton Beam Radiation on Bulbil Yield and Gibberellins of Chinese Yam (*Dioscorea opposita* Thunb.)

Sang-Kuk Kim*, Hong-Jib Choi*, Kye-Ryung Kim**, In-Jung Lee***, and Hak-Yoon Kim****†

**Division of Crop Sciences, Gyeongsangbuk-do Agricultural Research & Extension Services, Daegu 702-708, Republic of Korea*

***Proton Engineering Frontier Project, Korea Atomic Energy Research Institute, Daejeon 305-353, Republic of Korea*

****Division of Plant Biosciences, Kyungpook National University, Daegu, 702-701, Republic of Korea*

***** Department of Global Environment, Keimyung University, Daegu, 704-701, Republic of Korea*

ABSTRACT The study was carried out to evaluate the effect of proton beam radiation on production of bulbil and tuber including change of endogenous gibberellins, of *Dioscorea opposita* Thunb. The yield of bulbils and tubers from non- and irradiated *D. opposita* Thunb at doses of 5, 10, 15 and 20 Gy were determined. Endogenous gibberellins were also quantified by GC/MS analysis. *D. opposita* tubers irradiated at 15 Gy produced higher bulbil production than non-irradiated plants. Enlarged bulbil (above size diameter 4 mm) was significantly increased at 15 Gy. Bioactive endogenous GA_4 was dominant in bulbils and tubers irradiated with proton beam rather than GA_1. Major gibberellins biosynthetic pathways in bulbils and tubers of *D. opposita* plants were non C-13 hydroxylation route. From the results of this study, 15 Gy proton beam radiation was suggested as an optimal dose that can produce high amounts of bulbil for mass production of *D. opposita* plant.

Keywords : proton beam irradiation, chinese yam, bulbil, gibberellins

Chinese yam (*Dioscorea opposita* Thunb; Tsukuneimo in Japanese; Deunggunma in Korean) has been cultivated as a major medicinal crop and is also grown widely as a raw healthy food in Korea, China and Japan. In our country, chinese yam is specially the most popular medicinal crop after ginseng. Mass production of bulbils and tubers is reproductively essential for reducing the propagation cost for yam farmers. Of these Chinese yams variety Nagaimo, Ichoimo and Tsukuneimo, only two varieties, Nagaimo and Ichoimo, are able to produce high amounts of bulbils and tubers under natural conditions. Otherwise, the variety 'Tsukuneimo' has been shown to have a lowest bulbil production among the yam varieties (Okagami and Tanno, 1977). For this reason, we founded new cultural technologies that foliar spray of mepiquat chloride at high concentration induces the enlargement of bulbils under field conditions (Kim *et al.*, 2005). It is one of the efficient methods for induction of bulbil formation promoting bulbil enlargement in agriculture practices. However, these technologies need two years for stable production of bulbils. Furthermore bulbil production is occasionally dependent on plant growth stage and application times in agricultural aspects.

Radiation tool of food crops is one of the most common techniques for induction of crop mutations. These mutants are useful for developing new crop cultivars as well as for improving starch properties, especially in starchy crops such as rice, wheat, barley, potatoes, and chinese yam. The type of mutagenic treatment is an important factor to obtain successful results in mutation breeding. Physical mutagens, such as gamma rays and X-rays, have mainly been used to induce mutations, and many varieties have been released (Yamaguchi *et al.*, 2009). Recently, ion beams have attracted attention as mutagens. A characteristic feature of ion beams is their ability to deposit high energy on a target, densely and locally, as opposed to low linear energy transfer (LET) radiation such as gamma rays and X-rays (Yang and Tobias, 1979; Tanaka, 1999). Mutation induction with ion beams, using various plants, has been attempted since the 2000s in Korea, the 1990s in Japan.

†Corresponding author: (Phone) +82-53-580-5918 (E-mail) hykim@kmu.ac.kr
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Until now, no attempts have been made by researchers to enhance the tuber yield and to produce stable aerial tuber production from chinese yams, particularly, *Dioscorea opposita* cv. Deunggunma.

Therefore, the objective of this study was to evaluate effect of proton beam on the growth characteristics and changes of endogenous gibberellins in *D. opposita* in relation to tuber productivity.

MATERIAL AND METHODS

Chinese yam (*Dioscorea opposita* Thunb. cv. Deunggunma) which bred from Institute for Bioresources Research, Andong, Republic of Korea, was used in this study. The samples were packed in plastic (polyethylene) bags and irradiated at room temperature in 45-MeV proton beams of the MC 50 cyclotron at doses of 5, 10, 15, and 20 Gy. This treatment was done at the Korean Institute of Radiological Sciences, Korea Atomic Energy Research Institute, in Republic of Korea. Irradiated tubers were stored in growth cabinets at 5℃ keeping relative humidity of 85% for 25 days. Each tuber was divided into 5 or 6 segments. Tuber pieces (about 40 g fresh weight) were planted in a depth of 5 cm. The planting date was 13 June. Cultural practices were those commonly used for the cultivation of Chinese yam plant as described previously (Kim *et al.*, 2005).

A randomized block design with four replications was used for the experiment. Prior to planting, 43-28-32 kg/10a of N-P_2O_5-K_2O was applied and incorporated as basal and topdressing (7:3, w/w) to the experimental field. Bulbils and tubers were harvested on 25 October. The bulbils and tubers harvested were immediately frozen in liquid nitrogen and stored at -80℃. When all the required materials for gibberellins analysis had been collected, these samples were lyophilized for 48 h. The extraction of endogenous gibberellins was followed as described by Lee *et al.* (1998). Three major ions of the supplemented [$^2H_2$] GA internal standards (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia) and the endogenous GA were monitored simultaneously. Retention time was determined by using the hydrocarbon standards to calculate the KRI value (Kim *et al.*, 2003a).

Quantification of gibberellin was based on the peak area ratios of endogenous (non-deuterated, sample) to deuterated GAs, after correcting for any contribution from the deuterated standard to non-deuterated GAs. The endogenous contents of GA_{53}, GA_{12}, GA_{15}, GA_{44}, GA_{24}, GA_{19}, GA_9, GA_{20}, GA_{36}, GA_4, and GA_1 were calculated from the peak area ratios of 450/448, 302/300, 241/239, 432/434, 316/314, 436/434, 300/298, 420/418, 286/284, and 508/506, respectively. The collected data for endogenous gibberellins and tuber yield were analyzed by using SAS package for Duncan's multiple range tests.

RESULTS AND DISCUSSION

The effect of irradiation on viability and sprouting rate in *Dioscorea opposita* Thunb was estimated. Viability degree in *D. opposita* tubers irradiated with proton beam ranged 81.4 to 95.5% as compared to the control. When dose intensity was increased, viability in *D. opposita* tubers was slightly decreased. However, sprouting rate was also estimated after planting each irradiated tuber (Table 1). Sprouting rate among irradiated tubers at 20 DAP was increased in 5 and 10 Gy. Sprouting rate was also decreased in irradiated tubers compared with the control. Highest sprouting rate at 40 DAP was 15 Gy, and 5 Gy at 60 DAP, respectively. *D. opposita* tuber has long dormancy periods over 5 or 6 months. When tubers are planted into soils, sprouting periods are also delayed because of endogenous gibberellins sand some phenolic compounds such as batatasins (Okagami and Nagao, 1971; Okagami and Nagao, 1973; Kim *et al.*, 2002). Early sprouting in Chinese yam was accelerated by proton beam irradiation. Beam utilization to shorten dormancy period is considered to be one of the powerful tools. Growth and tuber characteristics of *D. opposita* irradiated with proton beam were evaluated. Vine length in *D. opposita* irradiated with proton beam ranged 2.2 to 2.8 m. Vine length was decreased with increased proton beam irradiation. Aerial tuber was significantly produced in *D. opposita* plants irradiated with proton beam. At 5, 10, 15, and 20 Gy, number of bulbil per plant resulted in 20, 77, 435, and 196, respectively (Table 2). Highest aerial tuber yield was observed in 15 Gy as 19.2 kg/10a. Otherwise, underground tuber yield did not change by proton beam irradiation as compared to the control.

Table 1. Viability and sprouting rate of *Dioscorea opposita* Thunb at different radiation doses.

Dose (Gy)	Viability (% of control)	Sprouting rate (%)		
		[b]15 DAP	25 DAP	35 DAP
0	[a]100.0 a	25.5 a	58.3 c	100.0 a
5	95.5 b	21.2 b	67.5 b	96.5 b
10	93.8 bc	22.8 b	64.1 b	95.2 b
15	89.5 c	18.6 c	69.7 a	93.4 b
20	81.4 d	19.4 c	65.4 b	94.6 b

All data are means of three replications.
[a] The same letters in each column are not significantly different at 1% level by DMRT.
[b] DAP means days after planting tuber pieces.

Table 2. Yield of bulbil and tuber of *Dioscorea opposita* Thunb at different radiation doses.

Dose (Gy)	Vine length (m)	Bulbil (no./plant)	Bulbil yield (kg/10a)	Tuber yield (kg/10a)
0	3.5	[a] 4 e	0.5 e	1,596 a
5	2.8	20 d	1.4 d	1,553 a
10	2.7	77 c	3.2 c	1,589 a
15	2.4	435 a	19.2 a	1,574 a
20	2.2	196 b	10.5 b	1,563 a

All data are means of three replications.
[a] The same letters in each column are not significantly different at 5% level by DMRT.

Table 3. Bulbil size distribution of *Dioscorea opposita* Thunb at different radiation doses.

Dose (Gy)	Bulbil size distribution (%)		
	≤ 2 mm	2 - 4 mm	4 mm ≤
0	[a] 100.0 a	0.0 d	0.0 e
5	23.5 b	75.7 a	0.8 d
10	19.6 b	65.5 b	14.9 c
15	11.4 c	60.2 c	28.4 a
20	14.5 c	66.3 b	19.2 b

All data are means of three replications.
[a] The same letters in each column are not significantly different at 1% level by DMRT.

Changes of distribution rate of bulbil in *D. opposita* plant irradiated with proton beam showed in Table 3. Exposure to 5 to 20 Gy of proton beams increased an enlarged bulbil which ranges 2 - 4 mm and above 4 mm. Only enlarged bulbil (2 - 4 mm and above 4 mm, diameter) is considered to available values in farmers cultivating *D. opposita* plant in Korea.

Bulbil size distribution rate showing 2 - 4 mm in *D. opposita* plant exposed with proton beam was 60.2 to 75.7%. Distribution rate of bulbil size ranging 2 - 4 mm was 75.7% at 5 Gy. The greatest bulbil size (above 4 mm) was induced in exposure of 15 Gy. In our previous studies (Kim *et al.*, 2003a; Kim et al, 2003b), trinexapac-ethyl and mepiquat chloride increased the yield of bulbils in *D. opposita* plant. Bioactive gibberellins in both GA_1 and GA_4 were first quantified from the bulbils of *D. opposita* irradiated with proton beam (Table 4). Endogenous GA_1 content was decreased gradually by increased proton beam. Otherwise endogenous GA_4 content was significantly increased. Total gibberellin contents in *D. opposita* tubers were highly

Table 4. Bioactive and total gibberellins in bulbils and tubers of *Dioscorea opposita* Thunb at different radiation doses.

Organs	Dose (Gy)	Endogenous GA contents (ng/g dry weight)		
		GA_1	GA_4	Total GAs
Bulbils	0	[a] 1.37±0.12 c	10.42±0.32 e	64.68±1.73 e
	5	1.25±0.11 d	11.69±0.17 d	70.85±2.21 d
	10	1.34±0.15 c	15.30±0.20 c	90.63±2.05 c
	15	0.29±0.05 a	27.12±0.12 a	128.21±2.33 a
	20	0.25±0.06 b	22.10±0.14 b	102.05±0.83 b
Tubers	0	0.65±0.13 a	6.37±0.26 e	45.20±1.73 e
	5	0.44±0.12 b	9.53±0.13 d	47.32±1.11 d
	10	0.20±0.03 e	11.37±0.11 c	65.37±1.29 c
	15	0.24±0.04 d	13.42±0.01 b	78.49±2.01 b
	20	0.27±0.05 c	14.19±0.06 a	84.00±1.32 a

The data presented in gibberellins are the means of 3 replications±SE.
[a] The same letters in each column are not significantly different at 1% level by DMRT.

increased at the 15 Gy. Its increase was 2-fold higher than that of the control. Bioactive gibberellins in both GA_1 and GA_4 were quantified from the tubers of *D. opposita* plant irradiated with proton beam (Table 4). Endogenous GA_1 content was significantly decreased by increased proton beam exposure. However endogenous GA_4 content was significantly increased by increased proton beam irradiation. Total gibberellins content including eleven endogenous gibberellins GA_{53}, GA_{12}, GA_{15}, GA_{44}, GA_{24}, GA_{19}, GA_9, GA_{20}, GA_{36}, GA_4 and GA_1 (data not shown) in tubers irradiated with proton beam ranged from 47.32 to 84.00 ng/g dry weight. Total gibberellin contents were highly increased at the 20 Gy. It was 1.86-fold higher than that of the control. In conclusion, the proton beam radiation showed it has a direct influence on enhanced bulbil production yield, increasing enlarged bulbil (size diameter above 4 mm) in *Dioscorea opposita* Thunb. In particular, enlarged bulbil was significantly increased at 15 Gy. The proton beam radiation at 15 Gy might be an optimal dose that can produce the high yielding bulbil for mass production of *D. opposita* plant.

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