RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
51 (1) 1-11, 2019

DETERMINATION OF LETHAL DOSE AND EFFECT OF GAMMA RAYS ON GROWTH AND TUBER YIELD OF JERUSALEM ARTICHOKE MUTANT

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SUMMARY

Mutation induced by radiation may result in increased variation in Jerusalem artichoke. The objective of this study was to determine the LD 50 and effect of gamma rays on growth and tuber yield of two Jerusalem artichoke genotypes HEL 65 and CN 52867. The experimental design was a completely randomized design (CRD) with 4 replications. The results demonstrated that LD_{50} for the genotypes HEL 65 and CN 52867 were 22 and 27 Grays (Gy), respectively. The radiation treatments did not change flower characters in either genotype. Germination percentage and plant height in both genotypes were decreased by increasing the radiation dose. In contrast, the number of branches was increased with higher gamma radiation doses (20 and 25 Gy).Gamma ray radiation at 5 Gy increased tuber yield in both Jerusalem artichoke genotypes. The present study indicated that gamma radiation might be useful for improvement of important traits such as yield in Jerusalem artichoke.

Key words: *Helianthus tuberosus* L., irradiation, plant height, fresh tuber yield

Key findings: The LD_{50} for Jerusalem artichoke genotypes HEL 65 and CN 52867 were 22 and 27 Gy, respectively. Plant height and germination percentage decreased with increasing doses of gamma rays. Low dose at 5 Gy could be used to increase tuber yield of these two genotypes of Jerusalem artichoke.

Manuscript received: July 15, 2018; Decision on manuscript: November 10, 2018; Accepted: December 3, 2018.

Communicating Editor: Dr. Ramakrishnan M. Nair

INTRODUCTION

Jerusalem artichoke *(Helianthus tuberosus* L.) can be grown successfully in the Semi-Arid Tropics including Thailand (Puangbut *et al*., 2015a). Jerusalem artichoke is a tuber crop containing inulin at about 39-77% of dry weight, and consisting of fructose chain (Puangbut *et al*., 2017; Puttha *et al*., 2012). Inulin is considered as a prebiotic substance with beneficial affects to human health and may reduce the risk of many diseases (Roberfroid, 2007).The Jerusalem artichoke is considered as a functional food crop in Thailand. Therefore, a breeding program is being carried out to improve Jerusalem artichoke genotype with high inulin content and tuber yield.

The Jerusalem artichoke is rather wild with minimal breeding efforts compared to other well-utilized crops, and genetic information about important Jerusalem artichoke traits is limited. Breeding programs for improving Jerusalem artichoke as a functional food crop in the tropics have been carried out at Khon Kaen University, Thailand. Many studies on genetic diversity using morphological traits, agronomic traits and inulin content have been reported (Puttha *et al*., 2012; 2013).

The Jerusalem artichoke is an obligate out crosser and produces seeds only when cross-pollinated. Seed production varies with clone (Konvalinková, 2003) and usually wild clones produce more seed per flower than cultivated clones (Kays and Nottingham, 2007). Several factors contribute to this low seed production including late flowering, cool temperatures, and self-incompatibility during seed development. Self-incompatibility has been demonstrated to reduce population size and leads to a reduction of genetic diversity and may be increase the expression of inbreeding depression (Busch and Schoen, 2008; Silva *et al*., 2016). Therefore, self-incompatibility is a genetic barrier for improvement of Jerusalem artichoke. In addition it may reduce the genetic diversity of desirable traits such as high inulin and large tuber size.

Induced mutations by gamma ray irradiation can increase genetic variation of economically important traits in germplasm. It can enhance conventional breeding by directly improving genotypes for specific traits or providing valuable material for further breeding work. Radiation mutations have been used in the improvement of tuber crops such as in potato (Cheng *et al*., 2010), yam (Yalindua *et al*., 2014) and sweet potato (Shin *et al*., 2011).

Gamma radiation has been widely used for the improvement of various traits of many plant species (Cheng *et al*., 2010; Songsri *et al*., 2011; Shin *et al*., 2011; Aney, 2013; Roslim *et al*., 2015). Gamma radiation is a tool which can be used to increase genetic variation for different plant characters such as plant height, flower shape and flower color in horticulture plants (Aslam *et al*., 2016). The success of mutation breeding using gamma rays depends on the level of irradiation and the genotype of the plant (Roslim *et al*., 2015). In addition, mutation rate depends on phase of plant growth, and size and the thickness of the material (Yalindua *et al*., 2014).

Determination of LD_{50} is very important for mutation breeding. Previous studies demonstrated that LD_{50} was different between plant species and varieties in the same species (Roslim *et al*., 2015; Aslam *et al*., 2016). Irradiation dose is a key factor in the radiation treatment of plant materials. Several researches have determined LD_{50} values in many species such as 50 Gy for Yulmi sweet potato variety (Shin *et al*., 2011), 25 Gy for rodent tuber (Sianipar *et al*., 2013), 620 Gy for Kampar mungbean variety and 540 Gy for K-851 mungbean variety (Roslim *et al*., 2015; Tah, 2006), 30 Gy for yam (Yalindua *et al*., 2014) and 27.5 Gy for cassava (Kangarashu *et al*., 2014).

Limited information is available on the determination of lethal dose and effects of gamma ray irradiation on growth and yield of Jerusalem artichoke. The objectives of this study were to determine the LD_{50} of Jerusalem artichoke and measure effects of gamma rays on growth and tuber yield.

MATERIALS AND METHODS

Experimental design and plant preparation

A pot experiment was carried out under a rainout shelter in the post rainy season during September to December 2016 at the Field Crop Research Station of Khon Kaen University. The experimental design was a 2 × 7 factorial in a completely randomized design (CRD) with four replications with two Jerusalem artichoke genotypes as factor A and seven gamma radiation doses including control (0 Gy) as factor B. Two commercial genotypes of Jerusalem artichoke grown in Thailand were used in this study. CN 52867 was donated by the Plant Gene Resource of Canada (PGRC) and HEL 65 was donated from the Leibniz Institute of Plant Genetics and Crop Plant Research of Germany (IPK). Tubers with axillary buds for the two genotypes were treated with gamma radiation at the doses of 0, 5, 10, 15, 20, 25 and 30 Grays (Gy) at the department of Applied Radiation and Isotop, Kasetsart University, Bangkok. The next day irradiated tubers were cut into small pieces each of which had one bud. The tuber pieces were incubated in coconut peat media under ambient conditions for 7 days or until a sprout developed from the bud on the tuber (VE stage) (Puangbut *et al*., 2015b).

Crop management

Sprouted seed tubers of two Jerusalem artichoke were grown in plastics pots with 32 cm in diameter and 28 cm in height. The healthy seedlings were selected and transplanted to pots as one seedling per pot. Fertilizer of formula 15-15-15 was applied at 30 days after transplanting (DAT) at a rate of 156 kg per ha^{-1} (2 g per pot) (Ruttanaprasert *et al*., 2013).

Data collection

Determination of Lethal Dose 50 (LD_{50})

The LD_{50} was determined using Microsoft Excel 2010 based on the number of survival plants at the different doses of gamma radiation. A regression equation was used to determine the LD_{50}. Data were recorded for a percentage of germination at 10 day after incubation (DAI). Germination was confirmed when a sprout developed from a bud on a tuber and percentage of emergence was computed. After that they were transferred to plug plastic trays.

After 7 days of growth in plug plastic trays, sprouted seed tubers which had two leaf-sprouted seedlings (V2) were transplanted to pots. Number of surviving plants was recorded at 7, 14 and 21 days after transplanting and then percentage of survival plant was calculated.

Effect of gamma radiation on number of branches, plant height and fresh tuber yield

Number of branches was recorded at 8 weeks after radiation. The observation of plant height was recorded at 2, 4, 6 and 8 weeks after radiation. Tubers were harvested at maturity depending on genotypes. The plants were cut at the soil surface and separated into shoots and tubers. Tubers were washed in tap water to remove the soil and then tuber fresh weight was determined.

Statistical analysis

Individual analysis of variance was performed for each character following a factorial in a completely randomized design (CRD). Least Significant Difference (LSD) test was used to compare means using a STATISTIX8 software program. Regression analysis was used to estimate the lethal dose (LD_{50}) of Jerusalem artichoke.

RESULTS AND DISCUSSION

Determination of LD_{50} of Jerusalem artichoke genotypes

The results indicated that plantlet survival decreased with increasing dose of gamma radiation (Figure 1). The LD_{50} of Jerusalem artichoke genotypes was 22 and 27 Gy for HEL 65 and CN 52867, respectively. HEL 65 was large tuber size (dry weight of individual tuber) than CN 52867 (Pimsaen *et al*., 2010). The results revealed that the LD_{50} of Jerusalem artichoke was depends on varieties and tuber size. This supports previous findings that LD_{50} was different between varieties of the same species (Aslam *et al*., 2016; Roslim *et al*., 2015). In addition, the genotype with smaller tubers was more resistant to gamma irradiation than the genotype with larger tubers. The portion of the area irradiated is an important exposure parameter because the larger area exposed to radiation lead to the greater damage of plant tissue. Likewise, Bado *et al*. (2016) demonstrated that micro-tuber sprouting ability was most resistant to gamma irradiation in potato.

There were no deaths in the control treatment (0 Gy), while treatments with irradiation levels higher than the LD_{50} value caused a high number of plantlet deaths. An irradiation dose above 30 Gy caused complete death for both genotypes. Likewise, previous study demonstrated that higher dose of irradiation given caused a decreased of plant growth in rodent tuber (Sianipar *et al*., 2013). This could be due to high dose of gamma rays leads to DNA damage in the cell which are responsible for retarding the growth and development of plants.

Radiation effect on emergence ability

The percentage of emergence of Jerusalem artichoke after irradiation decreased with the increased irradiation dose (Table 1). The highest

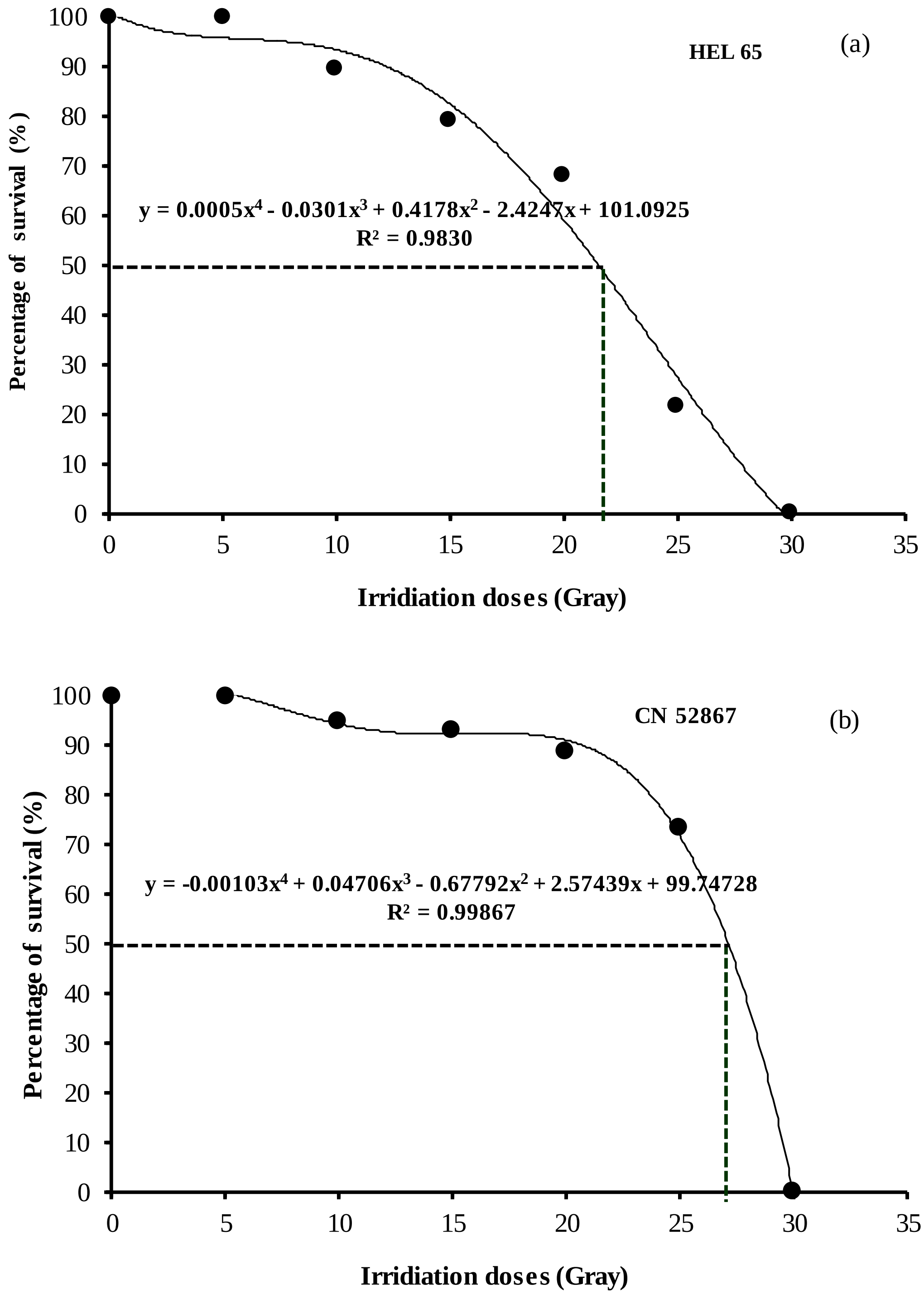

Figure 1. Lethal dose 50 (LD50) of Jerusalem artichoke genotype HEL 65 (a) and CN 52867 (b).

Table 1. Effect of gamma irradiation doses on percent emergence in Jerusalem artichoke genotypes at 10 days after incubation.

Irradiation doses	Percentage of emergence (%)	
	HEL 65	CN 52867
0 Gy	100.0 a	100.0 a
10 Gy	84.2 b	84.5 b
20 Gy	63.9 c	78.9 b
30 Gy	0.0 d	0.0 c
Mean	62.03	65.84

Means in the same column with the same letters are not significantly different by LSD ($P \leq 0.05$).

percentage of emergence was observed in the control treatment (100%) followed by 10 Gy and 20 Gy treatments in both genotypes. No plants emerged for the 30 Gy treatments for both genotypes. This could be due to chromosome aberrations, physiological damage, and cell damage (Cheng *et al*., 2010). Marcu *et al*. (2013) also reported that a high dose of gamma (>30 Gy) inhibited emergence ability of lettuce. High gamma irradiation caused damage to proteins along with failure of RNA and leads to inhibition of tissue culture growth (Kiong *et al*., 2008).

Effect of radiation on morphological traits

Figure 2 showed the observed plant abnormalities caused by gamma irradiation to Jerusalem artichoke at 4 weeks after irradiation. Plant height decreased with increasing doses of gamma irradiation while higher doses induced greater branching of stems. Plant mutants from the 20 and 25 Gy treatments had a higher stem branching than the other gamma ray doses (Table 2, Figure 3) and usually plant mutants produce more branches (6) than normal plants (1 or 2). These findings were similar to other tuber crops such potato and sweet potato (Cheng *et al*., 2010; Wang *et al*., 2007). In contrast, Sianipar *et al*. (2013) reported a higher irradiation dose had decreased number of shoots in rodent tuber. The differences in the observations could be due to difference in plant species. This suggested that the higher gamma dose had a positive effect on the number of shoots in Jerusalem artichoke. However, the gamma ray could not change the flower color of Jerusalem artichoke (data not presented).The yellow color in Jerusalem artichoke is apparently a very stable color.

Plant height differed significantly among gamma irradiation doses at 4, 6 and 8 weeks after irradiation (Table 3). The results indicated irradiation at 25 Gy result in reduced plant height compared to lower gamma irradiation doses in both HEL 65 and CN 52867 genotypes. This was similar to previous studies where higher gamma ray doses had a negative effect on the morphological characteristics in tomato, rodent tuber and potato (Norfadzrin *et al*., 2007; Sianipar *et al*., 2013; Cheng *et al*., 2010). Radiation of plants with a high dose of gamma rays disturbs the hormone balance and enzyme activity which inhibit the rate of cell elongation and result in a reduction in plant growth such as leaf area and plant height (Stoeva, 2002; Kiong *et al*.,

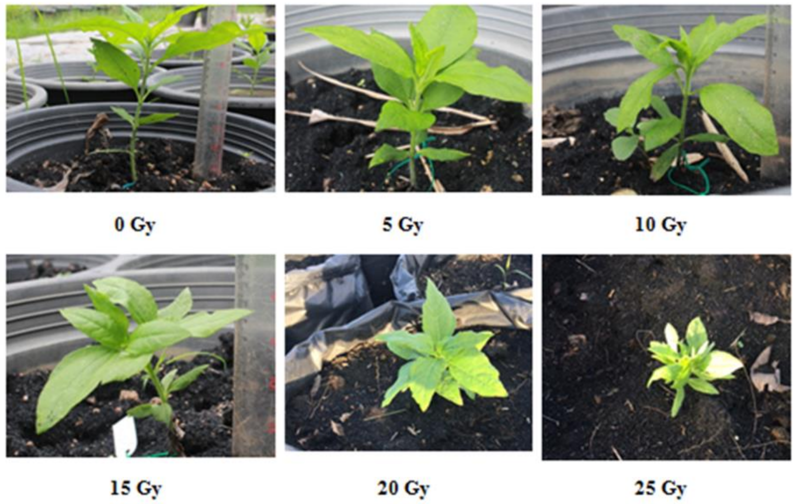

Figure 2. Morphology appearance of HEL 65genotype at 4 weeks after irradiation at different irradiation doses.

Table 2. Number of stem branching of HEL 65 and CN 52867 genotypes at 4 weeks after irradiation at different irradiation doses.

Irradiation doses	Number of stem branching	
	HEL 65	CN 52867
0 Gy	2.0 b	1.0c
10 Gy	2.2 b	1.8 b
15 Gy	3.0 b	2.0 b
20 Gy	6.0 a	3.8 a
25 Gy	6.0 a	4.0 a
Mean	3.8	2.6

Means in the same column with the same letters are not significantly different by LSD ($P \leq 0.05$).

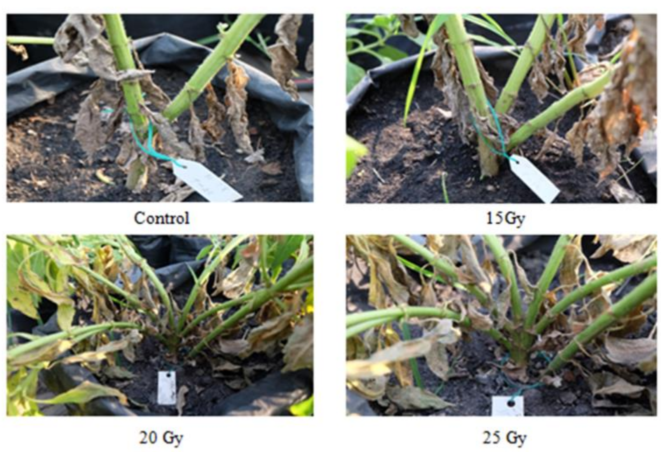

Figure 3.Effect of gamma irradiation on branching on the main stemof Jerusalem artichoke genotypes.

Table 3. Effect of gamma irradiation on plant height of Jerusalem artichoke genotypes at 2, 4, 6 and 8 weeks after irradiation.

Irradiation doses	2 weeks		4 weeks		6 weeks		8 weeks	
	HEL 65	CN 52867	HEL 65	CN 52867	HEL 65	CN 52867	HEL 65	CN 52867
0 Gy	8.1 a	9.9 a	43.0 a	63.0 a	90.8 a	90.4 a	96.7 a	92.8 a
5 Gy	8.3 a	9.0 a	36.4 a	49.8 b	95.6 a	76.8 d	96.0 a	79.6 c
10 Gy	7.4 b	7.3 b	30.4 b	35.4 c	88.1 ab	76.4 d	94.7 a	77.6 c
15 Gy	7.1 b	6.1 c	24.6 c	32.7 c	84.0 b	73.2 d	95.7 a	74.0 c
20 Gy	6.7 b	7.4 b	20.9 c	41.0 bc	78.3 b	79.5 b	90.2 b	81.4 b
25 Gy	5.4 c	5.9 c	15.4 d	18.1 d	58.6 c	65.7 d	73.5 c	70.7 d
Mean	7.2	7.6	28.5	40.0	82.6	77.0	91.1	79.4

Means in the same column with the same letters are not significantly different by LSD ($P \leq 0.05$).

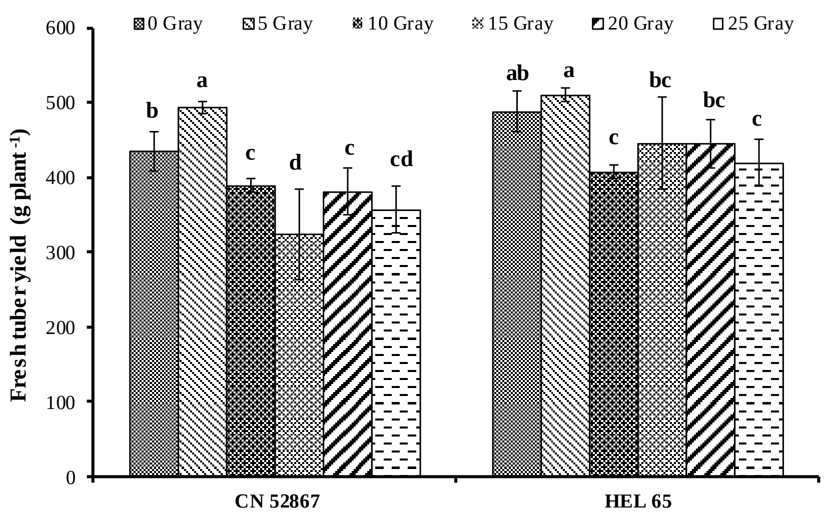

Figure 4. Effect of gamma irradiation on fresh tuber yield of Jerusalem artichoke genotypes at harvest. Means of three replications ± SD.

2008). However, low dose of gamma ray may increase the enzymatic activation which stimulates the rate of cell division and result in promotion of plant height.

These results indicated that the irradiation dose given to the plant significantly affected the number of shoot and plant height. The type of morphological variations also showed a regular change with radiation dose. For both Jerusalem artichoke genotypes, the increase of branches with an increase in the radiation dose but inhibited plant height. Similar results was found in rice exposure to radiation resulted in increased tiller numbers (Kataria and Guruprasad, 2012; Efendi *et al*., 2017).Similar results regarding reductions in plant height were also visible in various plant species such as rice (Efendi *et al*., 2017), mungbean (Roslim *et al*., 2015), wheat (El- Degwy and Hathout, 2014) and potato (Cheng *et al*., 2010).

Effect of radiation on fresh tuber yield

The difference in gamma ray doses had a significant effect on fresh tuber yield in both HEL 65 and CN 52867 genotypes (Figure 4).The results revealed that irradiation at 5 Gy promoted fresh tuber yield compared to the control and other gamma irradiation doses in both HEL 65 and CN 52867 genotypes. Irradiation at 5 Gy increased fresh tuber yield an average of 10% over the control. In potato, tuber yield was increased by 70% of control (Cheng *et al*., 2010), while the root yield of sweet potato was increased (up to 3-fold) than that normal plant (Wang *et al*., 2007). In contrast, tuber yield of yam mutant plants were reduced compared to the control treatment (Yalindua *et al*., 2014). The different observations on yield may be due to different plant species and irradiation doses.

With an increase in the radiation dose at 10, 15, 20 and 25 Gy, the fresh tuber yield was decreased an average of 13%, 17%, 11% and 15% over the control. The results indicated that 15 and 10 Gy of gamma dose had higher reduced tuber yield for CN 52867 and HEL 65, respectively. The results demonstrated that high dose of radiation was decreased tuber yield of Jerusalem artichoke. Similar to previous researches reported that tuber yield was decreased with increase of radiation dose in potato and yam (Cheng *et al*., 2010; Yalindua *et al*., 2014).

The present study revealed that tuber yield increased with low gamma dose at 5 Gy and this could be due to low dose had increased enzyme activation which stimulates the rate of cell division along with plant growth such as leaf area and plant height and ultimately increase tuber yield (Ali *et al*., 2016). The present study revealed that lower doses of irradiation given could be promoted tuber yield of Jerusalem artichoke.

CONCLUSION

The LD_{50} of Jerusalem artichoke was determined based on percentage of surviving plants. The LD_{50} of HEL 65 and CN 52867 genotypes were 22 and 27 Gy, indicating that Jerusalem artichoke had low radio-sensitivity to gamma ray. Irradiation dose of 10 and 20Gy promoted emergence ability of Jerusalem artichoke. Gamma irradiation at 20 and 25 Gy increased number of shoots compared to the control treatment, whereas decreased plant height. The present study revealed that tuber yield increased with irradiation dose of 5 Gy for both genotypes. Low dose can cause changes in morphological characteristic and increased tuber yield of Jerusalem artichoke.

ACKNOWLEDGEMENT

This work was supported by the Peanut and Jerusalem Artichoke Improvement for Functional Food Research Group, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University and the research funding support of Khon Kaen University. The acknowledgement is extended to the Thailand Research Fund for providing partial financial support through the Senior Research Scholar Project of Prof. Dr. Sanun Jogloy (Project no. RTA 6180002).We also thank the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany and the Plant Gene Resource of Canada for the contribution of Jerusalem artichoke germplasm.

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