



RESEARCH ARTICLE

Impact of gamma irradiation doses on early growth of Philippine garlic (*Allium sativum* L.) varieties

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Abstract

This study aimed to determine the effect of different gamma irradiation doses on the early growth characteristics of the 5 National Seed Industry Council (NSIC)-registered garlic varieties. Garlic cloves of the varieties, Ilocos Pink, Ilocos White, Ilocos Tan Bolters, Mexican and MMSU Gem, were exposed to different doses (1, 2, 4, 6, 8, 10 and 12 Gy) of gamma irradiation. Non-irradiated garlic served as a control. Both irradiated and non-irradiated garlic cloves were planted in plastic cups under screenhouse conditions. The treatments were arranged in a factorial completely randomized design with 3 replications. Generally, gamma irradiation was observed to have an inhibitory effect on plant height, wherein, as irradiation doses increase, plant height decreases. Regarding leaf length, a decreasing trend was observed as gamma irradiation increased from 1–6 Gy. Higher doses of irradiation (4–8 Gy) had an inhibitory effect on root length, while lower doses were comparable to untreated. Root count increased with gamma irradiation, with the effective dose varying depending on the garlic variety. The results indicate that the optimal gamma irradiation dose is variety-dependent. These results are vital for successful crop improvement works on garlic, as specific doses of irradiation showed phenotypic changes that signify alteration of the genetic make-up of the crop, which needs further study.

Keywords: bulb crop; inhibitory effect; NSIC-garlic variety; radiosensitivity

Introduction

Garlic (*Allium sativum* L.) is a popular bulb crop due to its wide use in culinary and medicine. This crop is commonly propagated through vegetative propagation, but displays differences in bulb size, shape and colour; number and size of cloves; maturity date; flavour and pungency; bolting capacity; numbers and sizes of top sets; and flowers in the inflorescence (1–3). However, genetic diversity in garlic field collections has been revealed by morphological, developmental, biochemical and molecular differences (3). To date, more than 16 cultivars have been locally collected. These are being maintained at the Mariano Marcos State University and most have been morphologically characterised using the International Plant Genetic Resources Institute' (IPGRI) Descriptor's list for Alliums. Five registered garlic varieties are part of the study as they are proven to be adaptable to the existing conditions in the Philippines and they have good agronomic characteristics. The phenotypic characteristics of these garlic varieties may be improved by gamma irradiation technique.

Gamma irradiation is a powerful tool being used in the agriculture sector, which enhances the crop quality and production, wherein it can cause mutations with the improvement of the characteristics of the crops (4). Gamma irradiation is one of the most used mutagens in mutation breeding due to its ease of application

and controllability (5). The genetic characteristics are improved through gamma irradiation, making this an appealing method. However, the study will concentrate on the phenotypic characteristics as influenced by gamma irradiation doses. Various studies reported that growth traits of crops were improved by gamma irradiation, but this depends on the crop species. At 5 Gy, plant height and fresh weight of artichoke were improved as compared to lower and higher doses (6). In another study, plant height in garlic (cv. Lumbu Kuning) was improved with a lower gamma irradiation dose of 4 Gy than a higher dose (7). Furthermore, they have reported that in another garlic variety (Tawangmangu Baru), the plant height declined as the doses increased from 2 to 8 Gy. It indicates that the impact of the gamma irradiation is species and variety dependent. Moreover, an increasing trend in the plant stature of the garlic Doulu cultivars was reported from 2–10 Gy and using the same irradiation doses, leaf count per plant was also enhanced, particularly 8 and 10 Gy (8). They have reported that gamma rays disrupt the chromosomal structure in the seed and affect the auxin synthesis that accelerates cell division, hence affect seed growth processes. Whereas high doses of gamma irradiation resulted in an inhibitory impact on cell division, resulting in cell death, it then decreases the growth capacity of the plant (8). In a previous study, a 20 Gy gamma irradiation dose increased 18–32 % root count in wheat (9). Gamma irradiation may stimulate the

hormonal balance modulation and induce signaling pathways. The generation of reactive oxygen and upregulation of auxin-related pathways are involved in the process (10). A gamma irradiation dose of more than 200 Gy may have a harmful impact on plant cells by inducing DNA mutations that disrupt cell differentiation and impede growth responses (11). In a separate study, high doses (100–400 Gy) of gamma irradiation reduced the root and shoot lengths in Fescue plants with lower concentrations of enzymatic antioxidants (catalase and superoxide dismutase) and an increase of hydrogen peroxide and malondialdehyde (MDA) (12).

Based on the previous studies, it is essential to identify the suitable gamma irradiation dose for every crop species due to the varying impact on the growth characteristics. Hence, this study was conducted to determine the effect of different gamma irradiation doses on the early growth characteristics of the selected National Seed Industry Council (NSIC)-registered garlic varieties (Ilocos Pink, Ilocos White, Ilocos Tan Bolters, Mexican and MMSU Gem). The results will provide baseline information to enhance the growth traits of the garlic varieties.

Materials and Methods

Source and preparation of planting materials and irradiation of cloves

Healthy, disease-free cloves of the 5 garlic varieties (Ilocos Pink, Ilocos White, Ilocos Tan Bolters, Mexican and MMSU Gem) were acquired from the Mariano Marcos State University. These cloves were submitted to the Philippine Nuclear Research Institute for irradiation with different doses (1, 2, 4, 6, 8, 10 and 12 Gy). Non-irradiated cloves served as the control. They were exposed to the same dose rate of 1.126 kGy/hr in an Obseirn Sanguir self-shielded gamma irradiator, which uses a Cobalt-60 source to emit gamma rays. After irradiation, the samples were immediately shipped back to MMSU and the cloves were sown upon arrival (4 days before their arrival at MMSU). The gamma irradiation doses were adopted from the previous study on garlic (13).

Growing conditions

The temperature during the growing period of garlic was $28 \pm 2^\circ\text{C}$.

Planting of garlic cloves

The irradiated garlic cloves were planted in plastic cups, each measuring 11 cm in height, 5.5 cm in top diameter and 9.5 cm in bottom diameter. Thoroughly sterilised garden soil was used as the potting media; the soil was sterilised by heating it over a fire. Planting was accomplished by using a stick to create a hole, into which two-thirds of the clove's length was inserted vertically into the soil. The plants were watered using a sprinkler at least 2 times a week. A total of 396 cloves were planted per treatment.

Plant height and stem diameter

At 30 days after planting (DAP), the plant height of the garlic plants was measured using a standard ruler from the surface of the growing media to the tip of the tallest leaf. Whereas the stem diameter was measured 2 cm from the growing media's surface using a Vernier caliper.

Leaf count, length and width

At 30 DAP, leaf count, length and width were measured. Leaf count was done by counting the leaves of every garlic variety. Leaf length, however, was measured using a ruler from the base of the longest

leaf to its tip. Whereas a Vernier caliper was used to measure the leaf width from the widest leaf part.

Root length and count per plant

Roots were measured by using a standard ruler from root attachment to the root tip for the root length and counting the number of roots manually.

Statistical analysis

The data obtained were analysed using a factorial completely randomized design with 3 replications. The Statistical Tool for Agricultural Research (STAR) program was used to analyse the data. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$.

Results and Discussion

Plant height and stem diameter

Plant height was significantly affected by gamma irradiation dose and varietal variation in plant height was observed. Moreover, an interaction effect was observed on the plant height (Fig. 1A). Generally, irradiation significantly reduced the plant height of garlic varieties (Fig. 1A). For Ilocos Pink, higher doses (4, 6 and 8 Gy) of irradiation reduced its height as compared to untreated plants, but they had comparable plant height with the other irradiation doses. For Tan Bolters, Ilocos White and MMSU Gem, the higher the dose, the shorter the plant's stature. There was a declining trend of plant height as the radiation dose increased. The non-irradiated MMSU Gem was observed to be the tallest across all irradiation treatments and varieties. Under 1 Gy, Ilocos Tan Bolters garlic variety was the tallest among the varieties, except in Mexican and MMSU Gem, which had similar plant stature. Likewise, MMSU Gem showed a gradual decrease in plant height as the gamma irradiation dose increased. High doses (100–400 Gy) of gamma irradiation reduced the root and shoot length in fescue plant with lower concentrations of enzymatic antioxidants (catalase and superoxide dismutase) and the increase of hydrogen peroxide and MDA (12). The irradiated garlic varieties may produce a high concentration of these reactive oxygen species with low concentrations of enzymatic antioxidants, which resulted in the decrease of the plant stature.

On the other hand, it was reported that low doses of gamma irradiation promote faster cell division in meristematic tissues, thereby enhancing and stimulating vegetative growth (14). It was reported that stimulated cell division at low doses may be due to the enhanced nucleic acid synthesis and enhanced antioxidants (15, 16). A further study is recommended to identify the nucleic acid synthesis and antioxidant levels of the garlic varieties and gamma irradiation doses used in the study.

The stem diameter, however, was not affected irrespective of the gamma irradiation doses across garlic varieties (data not shown). The range of the stem diameter of garlic varieties was 1.79–2.00 mm, while across irradiation doses was 1.69–2.08 mm. Although there was a decreasing trend in the stem diameter with an increasing dose of gamma irradiation, results indicate that gamma irradiation showed a significant impact on the plant height, but no significant effect on the stem diameter.

Leaf length, count and width

A decreasing trend of the leaf length as the gamma irradiation

dose increased was observed from 1–6 Gy. At 1, 2, 4 and 6 Gy, the 5 garlic varieties generally exhibited similar leaf lengths (Fig. 1B). Specifically at 4 Gy, MMSU Gem had longer leaves than Ilocos Pink, though MMSU Gem's leaf length was similar to the remaining garlic varieties. At 8 Gy, Ilocos White displayed longer leaves than MMSU Gem, but they were similar to other varieties. At 10 Gy, Mexican Tan Bolters produced longer leaves than the other varieties.

On the other hand, there was no interaction effect between garlic varieties and gamma irradiation doses on the leaf count and width (Table 1). Leaf count was higher in Ilocos Pink than in the other garlic varieties. MMSU Gem had the least leaf count. Leaf count decreased as gamma irradiation doses increased. Signifying

that the impact of gamma irradiation on the leaf count is inhibitory. This trend was also observed in the leaf width. However, non-irradiated garlic showed similarity in leaf width with the higher doses, particularly from 1–4 Gy. This may be due to the inhibitory impact on cell division, resulting in cell death, which in turn decreases the plant's growth capacity (8). Irrespective of the gamma irradiation dose, the leaf count per plant in *Philodendron scandens* K. Koch & Sello was decreased (17). The gamma irradiation (100–300 Gy) exposure may be due to the decrease in photosynthetic efficiency and a high production of MDA (a key indicator of lipid peroxidation) that destroys the cellular components, hence, impeding growth response (12, 18).

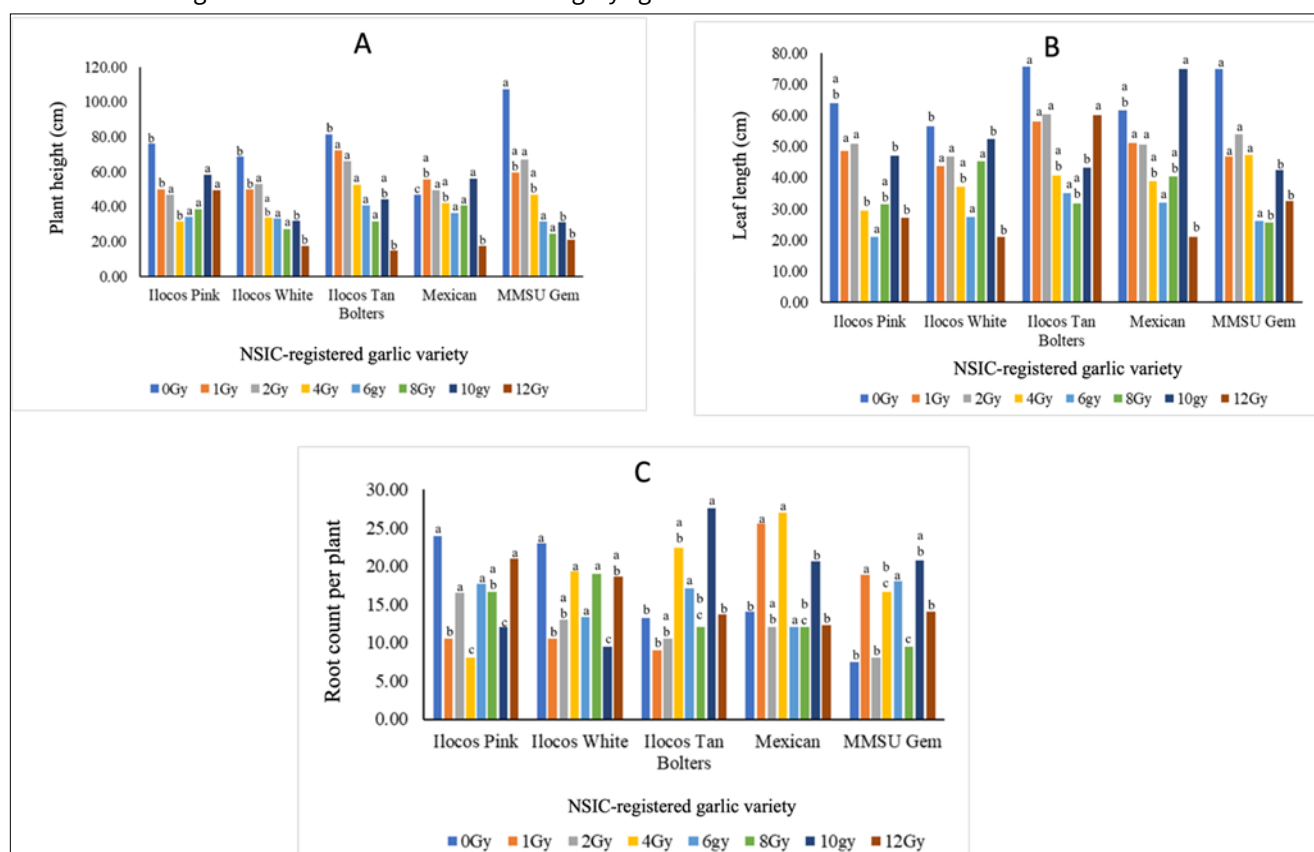


Fig. 1. Effect of gamma irradiation doses on growth parameters of garlic varieties. A: Varying plant height between garlic varieties and irradiation doses; B: Comparison on the leaf length between garlic varieties and gamma irradiation doses; C: Varying root count between garlic varieties and irradiation doses. Bars with different letters indicate a significant difference among garlic varieties and gamma irradiation doses using Tukey's Honest Significant Difference test at $p \leq 0.05$ level.

Table 1. Leaf count and width and root length per plant of NSIC-registered garlic varieties as influenced by gamma irradiation doses

Treatment	Leaf count	Leaf width (cm)	Root length (cm)
Garlic variety (a)	*	*	*
Ilocos Pink	2.93 a	0.3179 b	8.41 b
Ilocos White	2.19 b	0.3354 b	10.76 a
Ilocos Tan Bolters	2.13 b	0.4025 a	11.31 a
Mexican	2.10 b	0.3438 b	11.06 a
MMSU Gem	1.38 c	0.4021 a	12.02 a
Gamma Irradiation Dose (Gy) (b)	*	*	*
0	2.93 a	0.4567 a	14.12 a
1	2.19 b	0.4407 ab	12.78 a
2	2.13 b	0.4227 abc	11.64 ab
4	2.10 b	0.3767 bcd	9.82 bc
6	1.38 c	0.3527 cd	8.26 c
8	1.07 c	0.3067 d	8.26 c
10	1.29 c	0.3133 d	11.31 ab
12	1.33 c	0.2133 e	9.52 bc
Variety x Dose (c)	ns	ns	ns
CVa (%)	30.56	19.48	23.57
CVb (%)	30.56	19.48	23.57
CVC (%)	30.56	19.48	23.57

Columns with different letters indicate a significant difference among garlic varieties and gamma irradiation doses using Tukey's Honest Significant Difference test at $p \leq 0.05$ level.

Root count and length per plant

The root counts of irradiated garlic varieties varied from one to another. It was observed that Ilocos Tan Bolters showed an evident increase in the root count after gamma irradiation of 4, 6 and 10 Gy. Increased root count was also observed in MMSU Gem (1, 6 and 10 Gy) and Mexican (1 and 4 Gy) (Fig. 1C). These results indicate that these varieties are responsive to the stimulatory effect of gamma irradiation at these particular doses. Other garlic varieties, such as Ilocos Pink and Iloco White, showed similar root count even at higher irradiation doses. A previous study reported that irradiation improved the root count in fig (19). Previous works reported that irradiation may activate auxin-responsive genes (10). These garlic varieties can be exposed to higher gamma irradiation doses to determine their impact on their root count. The increase may be due to the auxin synthesis, which is responsible for the increase in root count in plants. In a previous study, a 20 Gy gamma irradiation dose increased 18–32 % root count in wheat (9). The difference between the current and previous studies was the crop species variation, which may have a varying impact. But it showed that a low dose of gamma irradiation is needed to obtain a high root count. Gamma irradiation may stimulate the hormonal balance modulation and induce signaling pathways. The generation of reactive oxygen and upregulation of auxin-related pathways are involved in the process (10). A gamma irradiation dose of more than 200 Gy may have a harmful impact on plant cells by inducing DNA mutations that disrupt cell differentiation and impede growth responses (11). A further study is needed at the molecular level to confirm this impact of garlic.

On the other hand, all the garlic varieties had similar root length, except for Ilocos Pink, which had the shortest. A decreased root length was observed as the gamma irradiation doses increased. However, non-irradiated and garlic exposed to 1 and 2 Gy gamma irradiation had similar root length. There was no interaction effect was observed between irradiation doses and garlic varieties. It was previously reported that at 30 Gy, gamma irradiation induced longer roots in strawberry (20). It should be noted that the response from gamma irradiation doses was crop species-dependent, which may have a similar or opposite effect. In another study, a similar observation as the study was reported in garlic (Balady cv.) after gamma irradiation of 100 Gy as compared to 50 Gy (21). Despite a similar inhibitory effect, the doses and garlic varieties used were varied. Varietal variation effect and dose-dependent effect must be considered. A previous study reported that high doses (100–400 Gy) of gamma irradiation reduced the root length in fescue plant and along with shorter roots, it exhibited lower concentrations of enzymatic antioxidants (catalase and superoxide dismutase) and an increase of hydrogen peroxide and MDA (12). Enzymatic antioxidants are essential to neutralise the free radicals and reactive oxygen species to minimise the impact on the seedling growth.

Conclusion

Gamma irradiation generally negatively influenced the plant height of all National Seed Industry Council (NSIC)-registered garlic varieties, leading to a decreasing trend as irradiation doses increased. The leaf length of the five garlic varieties was reduced after gamma irradiation exposure, even how low dose. However, at 1 and 2 Gy, all 5 garlic varieties were comparable to each other in

terms of leaf length. MMSU Gem showed longer leaves than Ilocos Pink, but they exhibited similarity with the other garlic varieties after 4 Gy gamma irradiation. Mexican garlic variety displayed longer leaves among the garlic varieties at 10 Gy. In contrast, root count per plant was enhanced by gamma irradiation in Ilocos Tan Bolter, Mexican and MMSU Gem with the response varying depending on the garlic variety and irradiation dose. The variety-specific response of the garlic may contribute to the mutation breeding for further study at the molecular level. Further studies are recommended to evaluate additional growth and yield characteristics of these garlic varieties using similar gamma irradiation doses.

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Authors' contributions

JQL, NBL, DSB and RJGR carried out the conceptualisation, methodology, investigation, data analysis, writing draft and final manuscript. COP and MBSGB participated the methodology, data analysis and writing and editing draft and final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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