Biological Forum – An International Journal **15(12): 91-95(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Gamma Irradiation on Induction of Mutations in *Zamioculcas zamiifolia* Engl. for Novel Plant Architecture

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(Received: 12 September 2023; Revised: 08 October 2023; Accepted: 25 November 2023; Published: 15 December 2023)
(Published by Research Trend)

ABSTRACT: *Zamioculcas zamiifolia* Engl. (ZZ) is an ornamental foliage plant, recently introduced to the world of interior plantscape. The present study on inducing variability in ZZ plants using mutagens was carried out during the year 2021-2022 in shade net at the 'D' Block, UAS, GKVK, Bengaluru. Mature leaflets with rhizome initiated were irradiated with 10 Gy, 12 Gy, 14 Gy, 16 Gy, 18 Gy and 20 grays of gamma irradiation. In gamma irradiated treatments, LD_{50} was observed at 18 Gy. In untreated control mature leaflets, early rhizome formation at 16.50 days after rhizome initiation (DAI), root initiation at 4.80 days DAI, highest number of roots (4.90) at 120 DAI, new shoot bud initiation at 62.50 DAI, maximum rhizome diameter of 1.62 cm at 90 DAI was recorded. Formation of cotyledonary like leaves at 93.60 DAI, maximum leaflet number (3.10), leaflet area (1.45 cm^2) and plant height (3.35 cm) were recorded in control mature leaflet at 120 DAI. Variations could not be recorded in any of the gamma irradiated treatments with respect to leaf colour, leaflet variegation and leaflet shape due to detrimental effect of mutagen which was lethal and resulted in mortality of all the treated mature leaflets.

Keywords: *Zamioculcas zamiifolia*, Mature leaflet, Gamma irradiation, Mutation, EMS.

INTRODUCTION

Zamioculcas zamiifolia Engl., also referred to as ZZ, is a member of the Araceae family and is commonly known as African coontie, Aroid palm, Arum fern, Cardboard palm and Emerald frond. ZZ is native to tropical east and subtropical southeast Africa, with chromosome number 2n =34. It grows in dry grassland and often stony ground and has several fleshy stalks bearing alternate pinnate leaflets. The leaflets can sprout and produce new plants and form tiny rhizomes at their base. ZZ is monocotyledonous stemless (Feng *et al*., 2006) sub-erect, evergreen perennial plant. It produces succulent rhizomes at the base of attractive dark green and glossy foliage. ZZ needs 80 to 90 per cent shade or 1,250 to 2,500-foot-candles of light for its production with the temperature of 21°C to 32°C and relative humidity 50 to 95 percent (Chen *et al*., 2003).

ZZ is mainly propagated by rhizome division or leaf and petiole cuttings. There are no variants found under natural conditions. Very few varieties *viz.*, Raven ZZ, Zenzi ZZ, Lucky classic etc., are available commercially. There is a requisite to create variability as variants have high value in plant scapes. Among the conventional plant breeding techniques, mutation breeding can be very well employed for this purpose. This study was carried out to cognize the effect of gamma irradiation on mature leaflets with a rhizome initiated with the objective of developing variants in ZZ.

MATERIAL AND METHODS

The present study was conducted during the year 2021-2022 in Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru. Well-developed dark green mature leaflets were collected from the middle five pairs from the stock plants. The leaflets were placed in potting media {vermiculite: coir pith (1:1, v/v)} for rhizome initiation after dipping in NAA (3000 ppm) for 30 sec. Rhizome initiation was noticed in the planted mature leaflets after 30 days of planting. Such mature leaflets with rhizome initiated and its size measuring 0.5-0.6 cm in width were used for the present study. This plant material was treated with various doses of gamma rays *viz*., 0 Gy, 10 Gy, 12 Gy, 14 Gy, 16 Gy, 18 Gy and 20 Gy with 10 replications. The irradiation was carried out at Gamma Chamber 5000, Indian Institute of Horticultural Sciences, Bengaluru. The data was analyzed using completely randomized design (CRD). Mortality percentage was recorded and the dose at which 50 per cent mortality was observed is given as LD_{50}.

RESULT AND DISCUSSION

LD_{50} for gamma irradiation: In mature leaflets LD_{50} was calculated at 90 days after rhizome initiation

(DAI). All the untreated leaflets showed 100 per survivability, while survivability decreased with increase in dosage of gamma ray with 50 per cent survivability being observed in 18 Gy as shown in table 1 (Fig. 1). Vijaykumar (2020) irradiated mature ZZ leaflets with 15 Gy, 25 Gy, 35 Gy and 45 Gy, where 50 per cent survivability noticed between 15 Gy and 25 Gy gamma irradiated ZZ plants. The cause of reduction in survival per cent might be due to the influence of mutagen on meristematic cell or due to acute chromosomal damage, delay in onset of mitosis, chromosomal aberration induced enzyme activity such as catalase and lipase production (Tiwari *et al*., 2010). The survival rate of *Curcuma alismatifolia* plants irradiated with 0 Gy, 10 Gy, 20 Gy, 25 Gy 35 Gy, 40 Gy, 60 Gy and 100 gray were recorded as 100 %, 100 %, 68.8 %, 41.3 %, 22.5 %, 8.8 %, 1.3 % and 0 %, respectively by Taheri *et al*., (2014). The lethal dose of 50 per cent survival rate (LD_{50}) was noticed at 21 gray in Chiang mai red variety. The death of plants was attributed to the interaction of radiation with other molecules in the cell, particularly water, to produce free radicals (H, OH). The free radicals could combine to form toxic substances, such as hydrogen peroxide (H_2O_2), which contribute to the destruction of cells. In a study conducted by Kumari and Kumar (2020) on inducing mutations in gladiolous using gamma rays concluded that there was 100 per cent survival in control (untreated plants) of all 8 varieties. Survival percentage was 98.62 per cent at 25 Gy dose, which was at par with survival percentage in untreated plants and it was significantly reduced with further increase in dose.

Days taken for rhizome formation

Early rhizome formation was noticed in untreated mature leaflets compared to gamma irradiated leaflets. Among the irradiated-mature leaflets, early rhizome formation was recorded in 10 Gy dose. Delayed rhizome formation (Fig. 1) was noticed in 20 Gy as shown in table 1. Time taken for rhizome formation increased with increase in irradiation dosage.

Days taken for root initiation. Root initiation at the base of leaflet was noticed early in untreated mature leaflets (Table 1). There was no root initiated from irradiated mature leaflets. Similar results were reported by Vijaykumar (2020) in all gamma irradiated mature leaflets with high doses of irradiation. Auxins are a group of naturally occurring and artificially synthesized plant hormones. The toxicity of the gamma dosage may be the cause for affecting the auxin synthesis which is responsible for induction of rhizome and roots and has resulted in ceasing of root initiation.

Number of roots. Highest number of roots (4.90) was noticed in untreated control mature leaflets at 120 days after initiation of rhizomes. However, no roots formed in gamma irradiated leaflets due to toxicity of gamma irradiation that inhibited root initiation. Hence, further root development has not taken place.

Days taken for new shoot bud initiation. In ZZ plants, the synergistic interaction of cytokinin and auxin results in the formation of high-quality new shoots. New shoot bud initiation was observed in untreated mature leaflets, at 62.50 days after the rhizome initiation, while this process of shoot bud initiation was not perceived in any of the irradiated mature leaflet. The inhibitory effect of gamma irradiation leading to blocking the initiation of new shoot buds might be due to disturbances in physiological and biochemical processes related to the action of gibberellic acid. In the cells of growing shoot, mitotic and meiotic abnormalities arise during mutation which may cause growth-inhibition. Shukla *et al*. (2018) reported that the lower doses of mutagenic treatments had a remarkable stimulative effect on parameters like sprouting percentage and days taken to sprouting in gladiolus (*Gladiolus grandiflora*s L.). Whereas, most of the morphological parameters *i.e.*, survival rate, plant height and number of leaves, showed a decrease from desired levels.

Days taken for the formation of cotyledonary - like leaves

Formation of cotyledonary like leaves is a unique phenomenon in ZZ plants and is noticed in any of the plant part used as propagule. This was noticed only in untreated mature leaflets in which rhizomes had formed at 93.60 days after rhizome initiation. Mortality was observed in irradiated mature leaflets due to selected dose of gamma irradiation for which they are exposed and was lethal to the plant tissue affecting the normal growth. Gamma irradiated mature leaflets started shrinking and drying over period of time with no response. Such responses were also noticed by Vijaykumar (2020) when mature leaf lets of ZZ were irradiate with a dosage of 15 Gy, 25 Gy, 35 Gy and 45 Gy gamma rays, which indicates that the dosage of gamma ray was much higher than that was employed in the present study.

Number of leaves. ZZ plants show very slow growth. Fleshy stalks or the true leaves bearing alternate pinnate leaflets will be produced from the underground rhizome that forms at the base of any part of the plant used as propagule. Also, very few leaves develop on the plant. Hence, on an average number of leaves produced was only on in control untreated mature leaflets. Gamma irradiation of mature leaflets inhibited the formation of rhizomes which prevented the further growth and formation of new shoots from mature leaflets. As observed by Ritonga (2017) in *Aglonema* varieties, the induction of gamma ray irradiation could decrease the per cent of viable plants, number of leaves, leaf length and leaf width. The dosage of gamma rays in the present study appears to have reduced the ability of the mature leaflets cells to develop into rhizome thus hindering the formation of new plants. The same results were obtained by Hajizadeh *et al.* (2022) in Lilium bulbs cv. Tresor, they observed a significant reduction in leaf number of plants with increasing in levels of gamma rays. Untreated plants had average number of leaves (86.33) whereas irradiated plants with 50 Gy gamma ray had the least (41.17) number of leaves. High levels of γ-rays doses had the negative effect on the number of leaves per ornamental. It seems that alteration in plant growth regulators status inside the plant especially kinetin, can be responsible for the

variation in leaf number. Similar results were recorded by Rawat *et al.* (2021) in gladiolus. Among all the gamma dose, the maximum number of leaves were found in control and minimum was at 6.5 Kr (8.43) irrespective of varieties.

Number of leaflets. As untreated mature leaflets alone showed rhizome and new leaf formation, leaflet formation was also seen these control plants at 120 DAI.

Area of leaflets. Emergence of new leaf from shoot bud was observed only in control leaflets which were not irradiated and leaflet area measured (1.45 cm^2) at 120 DAI. Similar results were reported by Vanzie-Canton (2006) in their studies on ZZ plant where leaflets irradiated with x rays showing 100 per cent mortality at 40 Gy. There was no initiation of new shoot in irradiated leaflets. New shoot initiation was observed only in control leaflet, leaf area was 1.53 cm^2 at 180 days (Vijaykumar, 2020). Similar results were observed by Hajizadeh *et al.* (2022), in Lilium bulbs cv. Tresor. Maximum leaf area was observed in the untreated plants and was seen decreasing with increase in dosage.

Plant height. Plants regenerated from untreated mature leaflets recorded a height of 3.35 cm at 120 DAI. In irradiated bougainvillea, with increase in gamma ray dosage, a reduced pant height was noticed with minimum plant height of 67 cm being recorded at 62.75 days (Swaroop *et al*., 2015). Difference in all the growth and floral characters was observed plants as compared to the control when cuttings treated with gamma rays.

Diameter of the rhizome. Development of rhizomes from the cut end as a response to wound healing is a very slow process in ZZ plants. Rhizomes of untreated control showed maximum diameter of 1.62 cm at 120 days after rhizome initiation. Among the irradiated leaflets, 10 Gy showed highest rhizome diameter of 1.11 cm. while the rhizome diameter decreased with increase in irradiation dosage, and the size of the rhizome reduced to 0.83 cm in 20 Gy as shown in Table 1 (Fig. 1). The size of the rhizome may possibly have been negatively impacted by higher doses due to growth inhibition brought about by the decrease in auxin synthesis and inability of cells to use available resources. Dhaduk (1992) reported that reduction in size is a result of negative impact of radiation on cell division and multiplication as it disrupted the physiological system of treated gladiolus corms.

Chlorophyll content

Chlorophyll content was estimated in the mature leaves that were used for the study as well as in the newly newly formed leaflets. The freshly planted mature leaflet had a chlorophyll content of 58.90 SPAD while in the newly formed leaflet it was 49.10 SPAD. The chlorophyll is an important photosynthetic pigment to the plant, which largely determines the photosynthetic capacity and plant growth. The new young leaflets were less greenish in colour and turned dark green as the growth continued.

Variations could not be recorded in any of the irradiated treatments with respect to leaf colour, leaflet variegation and leaflet shape due to detrimental effect of gamma rays which was lethal and resulted in mortality of all the irradiated mature leaflets. In a similar study by Hajizadeh *et al.* (2022), gamma irradiation of Lilium bulbs cv. Tresor with increasing beam intensity at a dose of 50 Gy, the mortality rate increased and beyond this dose led to lack of plant growth.

Table 1: Effect of gamma irradiation on Mortality (%), days taken for rhizome formation (DAI), diameter of rhizome in mature leaflets with rhizome initiated.

Treatments	Mortality (%)	Days taken for rhizome formation (DAI)	Diameter of rhizome 120 days DAI (cm)
Control	0.00	16.50	1.62
10 Gy	26.00	20.80	1.11
12 Gy	32.00	24.70	1.00
14 Gy	38.50	27.30	0.99
16 Gy	48.00	31.30	0.97
18 Gy	49.90	34.10	0.88
20 Gy	52.50	37.40	0.83
S.Em. ±	0.30	0.66	0.02
C.D.@ 5 %	0.84	1.86	0.07
F	*	*	*

DAI- Days after rhizome initiation

Mean values are showing significant difference among control untreated and irradiated mature leaflets with rhizome initiated.

Gy – Gray S.Em. ± - Standard error of mean

C.D.- Critical difference * Significant at 5 %

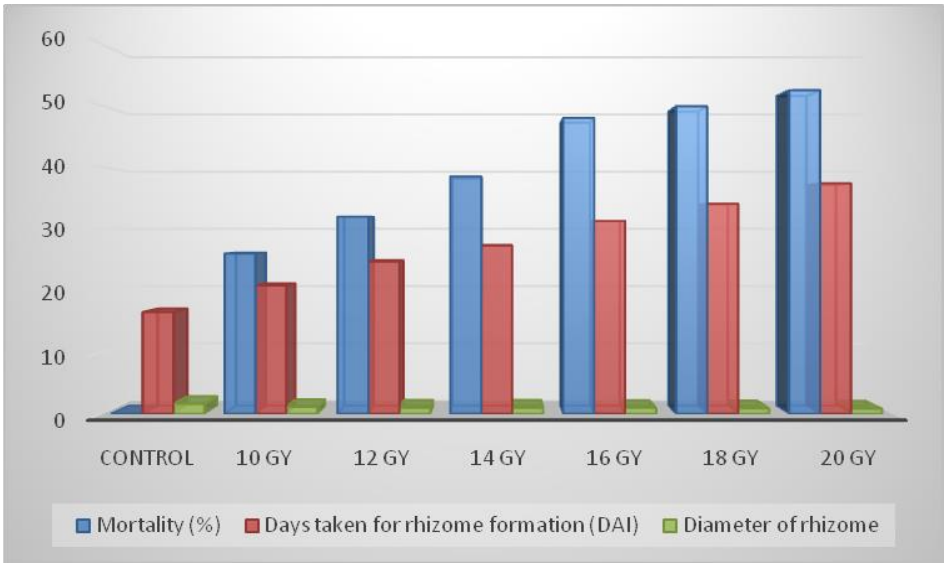

Fig. 1. Effect of gamma irradiation on mortality (%), days taken for rhizome formation and rhizome diameter at 120 DAI.

CONCLUSIONS

Mutation induction using gamma ray is an exploration method for creating new variation in *Zamioculcas zamiifolia* Engl.. In the gamma irradiated mature leaflets with rhizome initiated, increase in the irradiation dosage had an adverse effect on the growth of rhizomes. The regular cycle and sequence of events in the formation of new plantlets from mature leaflets was hindered. Formation of roots, shoot bud, cotyledonary like leaves and development of new leaf was completely inhibited in irradiated mature leaflets with rhizome initiated. LD_{50} was found to be 18 Gy where 50 per cent of the rhizomes was survived.

FUTURE SCOPE

As ZZ is considered as major indoor plant, there is a huge scope in bringing up leaf variegation in order to make it more beautiful than the present ones. Variations can be induced in ZZ by using other physical mutagens like ion bean radiation and chemical mutagens like Diethyl sulphate- DES, dimethyl nitrosamine -DMN.

Conflict of interest. None

Author Contribution. Basana Gouda, S. carried out research work, recorded observations and wrote the article.
Vijaykumar, carried out research and provided basement information to carry out the research work.
Dr. Nirmala, K. S., facilitated necessary inputs for research and guidance during period of study.
Dr. R. Vasantha Kumari, supervision and guidance during period of study.

Acknowledgement. With sincerity and immense pleasure, I feel great pride and privilege to express a profound sense of gratitude and heartiest indebtedness and obligations to the chairperson of my advisory committee Dr. Nirmala, K. S. Professor, Department of Horticulture, UAS, GKVK, Bengaluru and member Dr. R. Vasantha Kumari Professor, Department of Horticulture, UAS, GKVK, Bengaluru. I feel obliged and grateful to Mr. Vijaykumar for his valuable contribution for this research.

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How to cite this article: Basana Gouda S., Vijaykumar, Nirmala K. S. and R. Vasantha Kumari (2023). Effect of Gamma Irradiation on Induction of Mutations in *Zamioculcas zamiifolia* Engl. for Novel Plant Architectur. *Biological Forum – An International Journal, 15*(12): 91-95.