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RESEARCH ARTICLE

Induced Variability of Tomato Seeds *(Lycopersicon esculentum)* by Gamma Irradiation and Early Detection of Tomato Variants Resistance to Anthracnose

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A B S T R A C T

Gamma irradiation (GI) is an effective tool used in mutation breeding programs for improving various characteristics in horticultural plants, including obtaining tomato cultivars resistant to diseases. The study aims to improve various characteristics in tomato plants, particularly in enhancing resistance to anthracnose disease, through physical mutagenesis using GI. Tomato seed cultivar Timothy F1 was exposed to different doses of GI at 50 to 120 Gy, with un-irradiated seed used as a control. The effect of GI on tomato seeds involves aspects of viability, vigor testing, and the resulting phenotypic changes. Viability and Vigor testing of gamma irradiated seeds showed that germination potential ranged from 46-84%, maximum growth potential ranged from 78-94%, germination vigor index ranged from 40-80%, and seed emergence uniformity ranged from 62-82%. Analysis of the lethal dose for tomato seeds was determined to be 132.49 Gy. GI at 60-120 Gy significantly affects plant characteristics, including lower plant height, reduced number of leaves, narrower leaf width, and phenotypically longer leaves compared to the control plants. Most irradiated seeds showed susceptibility to *Colletotrichum* sp., with disease incidence ranging from 90-100% and disease severity index (DSI) ranging from 32.5-70.0%. However, the 70 Gy-irradiated tomato plant exhibited enhanced resistance to anthracnose disease, with the lowest DSI among the treatments. Identified tomato variants showing potential resistance to anthracnose will be further evaluated in a greenhouse to confirm their resistance.

Keywords: *Colletotrichum* sp., cultivar 'Timothy' F1, gamma irradiation, lethal doses.

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* Mill.) are widely popular in Indonesia and essential in various raw materials, cooked or processed foods. According to Statistics Indonesia (2023), the production of tomatoes in Indonesia has increased from 2021 (1.114.399,00 tons) to 2022 (1.168.744,00 tons). Despite the increase in production, the quality of tomato fruit's resistance to diseases has not improved significantly. Plant diseases, including those caused by *Colletotrichum* sp., are particularly harmful. Diseases interfere with photosynthesis, hamper nutrient absorption, and disrupt the plant life cycle (Nazarov *et al.,* 2020). This interference leads to a decrease in the quality and yield of tomatoes, resulting in economic and time losses. *Colletotrichum* sp. is identified as a common biotic factor contributing to low-quality tomato fruit. The impact of this pathogen on tomato plants may result in compromised growth, development, and overall fruit quality.

Resistance to diseases is crucial for maintaining the quality and yield of tomato crops. Strategies to enhance disease resistance, such as mutation breeding programs, can be vital in addressing these challenges. *Colletothricum* is a genus of a mold-type pathogen causing anthracnose disease in many plants and its non-host-specific characteristics (Pardo-De la Hoz *et al.,* 2016; Cabrera *et al.,* 2018). Most of the agronomically important crop and horticultural plants reported are susceptible to this filamentous fungus, such as maize (Vargas *et al.,* 2012), chili (Herwidyarti *et al.,* 2013), tomato (Diao *et al.,* 2014; Pardo-De la Hoz *et al.,* 2016), and eggplant (Saha *et al.,* 2010). Anthracnose disease caused by *Colletotrichum* sp. is also recorded to be found in fruit plants such as bananas (Huang *et al.,* 2020), strawberries (Istifadah *et al.,* 2017), and mango (Alkan *et al.,* 2014; Li *et al.,* 2019).

Colletotrichum sp. exhibit the ability to attack a wide range of host plants. The challenges posed by the broad host range of *Colletotrichum* pp. have caused researchers to explore solutions for obtaining anthracnose-resistant tomato lines. Previous studies have investigated various approaches, focusing on controlling anthracnose disease through fungicides, pesticides, and plant-based ingredients to suppress the growth of *Colletotrichum* sp. (Istifadah *et al.,* 2017). Another method involved the application of chitosan as a protective layer for harvested tomatoes (Suryadi *et al.,* 2016). Despite these efforts, the provided information suggests that additional research is needed to explore further and refine the methods used to control anthracnose disease.

Increasing genetic variability to provide a tomato variant resistant to anthracnose disease by conventional breeding is considered more time and cost-effective. Hence, mutation induction, specifically using gamma irradiation, is proposed as an effective technique for rapidly generating genetic variation in plants, including tomato plants (Sikder *et al.,* 2013; Lamo *et al.,* 2017; Chaudhary *et al.,* 2019). The effectiveness of mutation induction varies with the type of mutagen and its dosage (Jankowicz-Cieslak *et al.,*2017). Induce mutation with the desire for specific character changes has been a critical material for functional genomics and breeding in tomatoes and other plants (Sikder *et al.,* 2013; Chaudhary *et al.,* 2019; Atiq *et al.,* 2022). The irradiated tomato seeds were predicted to find a positive mutant tomato seed. Once sufficient genetic variation is induced, the next step involves selecting plant materials with the desired altered traits, including resistance to anthracnose (Jankowicz-Cieslak *et al.,*2017).

MATERIALS AND METHODS

Seeds treatment and Irradiation of tomatoes seeds: The experiment involved subjecting tomato seeds to various radiation treatments and assessing the viability and vigor of the seeds. Tomato cultivar 'Tymothy' F1 seeds were gamma irradiated treatment with a dose 0, 50, 60, 70, 80, 90, 100, 110, and 120 Gy. The viability test was conducted by planting tomato cultivar Tymothy F1 seeds on a Petri dish lined with wet filter paper for each treatment. The viability test was conducted by planting tomato cultivar Tymothy F1 seeds on a Petri- dish lined with wet filter paper for each treatment. The vigor test was conducted by growing seeds on rolled rice paper for each treatment. The seeds are then stored in a dark room for 14 days. The number of seeds for viability and vigor testing per treatment is 100.

The viability of seeds was determined by calculating their Maximum Growth Potential (MGP), which measured the maximum growth potential of the seeds. Germination Potential (GP) assesses seeds' potential to germinate. Vigor testing was determined by the Germination Vigor Index (GVI), which determined the vigor of germination. This index may be involved in assessing seed germination's speed and uniformity and Seed Emergence Uniformity (SEU), which measures the uniformity of seed emergence. This parameter could provide insights into how evenly the seeds sprout, a crucial aspect for research and agriculture (Sajad, 1994).

Determination of Lethal Dose: This study aimed to determine the lethal dose of irradiated seeds. Tomatoes dry seeds were irradiated with 50, 60, 70, 80, 90, 100, 110, and 120 Gy of gamma rays. Gamma irradiation was conducted using Cobalt (^{60}Co). Un-irradiated seed was used as a control, and the number of seeds per treatment was 50 for each treatment. The tomato seed was grown on rock wool media to support germination. Seeds were observed for germination four weeks after sowing to determine the LD_{50} using the software CurveExpert 1.4. The growth of surviving plants was evaluated under controlled hydroponic conditions at room temperature 26 ± 2 ^{o}C and LED light with 3100 lumens/5m for four weeks. Nutrient solution containd KNO_3, Fe EDTA, K_2SO_4, $(NH_4)2SO_4$, $MgSO_4$, $MnSO_4$, and H_3BO_3.Statistical analysis for plant growth was

conducted to determine the significance of the observed effects using ANOVA and DMRT to determine each treatment's effect on the plant.

Isolation and Disease Resistance Evaluation: Chilli fruit showing anthracnose disease symptoms was used as the source of the fungal isolate. The surface of the fruit was sterilized using 2% sodium hypochlorite and rinsed with distilled water. The isolated fungus was propagated for two weeks using the blotter method (Momtaz *et al.,* 2022). After incubation, the growing fungi were identified using a stereomicroscope based on the characteristics feature of the genus *Colletotrichum*. The identified fungi were isolated on Potato Dextrose Agar (PDA) at 25 ◦ C in a dark room for two weeks. The environment is conducive to the growth and sporulation of isolates. The purification process was carried out in stages, ensuring the isolation of a single, pure strain of the fungus *Colletotrichum.* sp**.** The purification process likely involves separating and culturing individual colonies to ensure the isolation of a single, pure strain. The microscopic features used for characterization include unicellular conidia, cylindrical and hyaline spores, septate and branched mycelium.

The resistance of tomato plants to anthracnose disease was identified by infecting plant stems with a suspension of conidia *Colletotrichum* sp. The conidial suspension was prepared by taking 1-month-old colonies (0.5 in diameter) on solid media and then diluting them in 200 ml of sterile distilled water (Indrayanti and Sudarsono, 2011). The density of the conidia suspension was calculated under a microscope with a 5-sphere Haemacytometer. The standard density value of conidia suspensions used for infection is approximately 10^{-6} conidia.ml^{-1} (Alkan *et al.,* 2014). Tomato plants, both irradiated and un-irradiated at six weeks old, were selected for this experiment. Infection was induced following the method of Saini *et al.* (2017) by injecting 1 ml of conidia suspension of *Colletothricum* sp. into the plant stem. Plant resistance is assessed using a scoring system (Herdwiyarti *et al.,*2013; Cui *et al.,* 2023). Scoring occurs 15 days after infection (Nurbailis *et al.,* 2017). Disease incidence (DI) and severity index (DSI) were calculated to evaluate the plant response to the infection (Chiang *et al.,* 2017). Score scale of anthracnose disease intensity was no visible symptom (score 0), 0-25% of plant surface was infected (score 1), >25-50% of plant surface was infected (3), >50-75% of plant surface was infected (3), >75-100% of plant surface was infected (score 4) (Herdwiyarti *et al.,*2013).

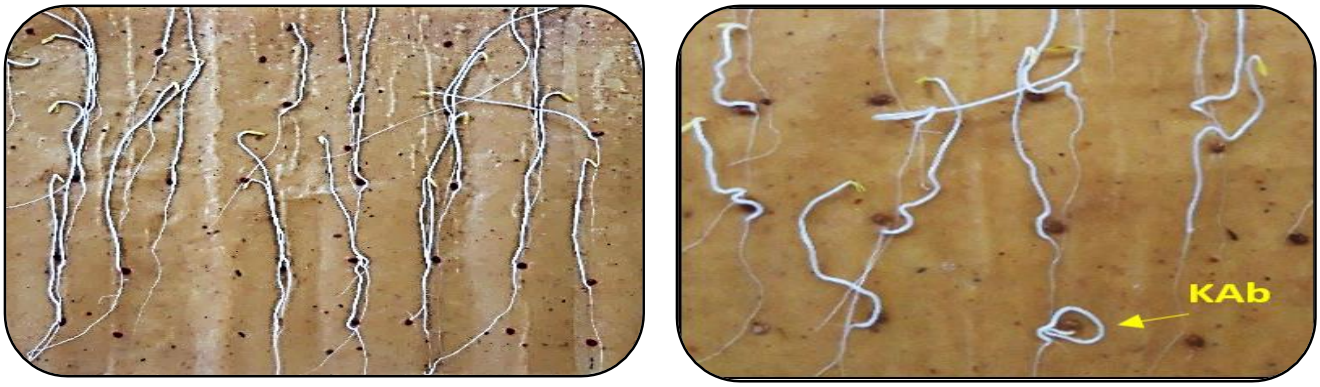
RESULTS

Seeds treatment and Irradiation of tomatoes seeds: Germination was described as a process that initiates water uptake by the quiescent dry seed and terminates with the elongation of the embryonic axis, which was completed by radicle emergence (Bewley, 1997). The study indicated variability in the viability and vigor of un-irradiated and irradiated tomato seeds (Table 1). The Germination Potential (GP) was reported to range from 46 to 92%, and the Maximum Growth Potential (MGP) ranged from 78 to 94%. It was found that tomato seed cv. 'Tymothy'F1 radiated from 70 Gy showed the highest viability. The MGP for these seeds was 94%, and the GP was 84%.

Seedling vigor was an index of how well a seed will establish seedlings Finch-Savage and Bassel (2016). The seed vigor of irradiated tomato seeds was generally lower than the control seeds. The Germination Vigor Index (GVI) was reported to range from 40 to 80%, and the Seed Emergence Uniformity (SEU) ranged from 62 to 82% (Table 2). The unirradiated seeds (control) exhibited the highest vigor, and no abnormal sprouts were found in the control seeds. Abnormal growth was observed at doses of 60 Gy. These abnormal sprouts were characterized by features such as the curve of the hypocotyl, wilt, and rot (Figure 1).

Table1. Viability and vigor testing of unirradiated and irradiated gamma seeds of tomato cv. 'Timothy' F1 seeds at 14 days after germination.

Percentage (%) of Viability and	Seed germination from each treatment (Gy)								
Vigor of Seeds	0	50	60	70	80	90	100	110	120
Germination Potential (GP)	92	80	46	84	78	76	76	70	60
Maximum Growth Potential (MGP)	92	90	88	94	90	92	84	82	78
Germination VigorIndex (GVI)	80	74	40	74	74	70	70	66	66
Seed Emergence Uniformity (SEU)	82	80	62	80	78	78	76	80	76



A B (Abnormal sprouts)

Figure 1. A normal seedling of un-irradiated seeds (0 Gy) (a) and an abnormal seedling of 60 Gy tomato seeds cv. 'Timothy' F1 (b).

Determination of Lethal Dose: The survival seeds germinating after the irradiation were used to calculate the LD_{50} Four-week-old tomato plants were used as data to determine the LD_{50} of tomato cv. 'Timothy' F1. Tomato seeds resulting from irradiation in the first generative cycle were labelled M1G1 seedlings, while seeds before irradiation were labelled M_0G_0 (Table 2). The data on growth were standardized with control plants to establish the relationship between irradiation doses (x) and plant growth responses (y). Using Curve Expert 1.4, the relationship was expressed as a quadratic equation: $y = a + bx + cx^2$ with coefficient a = 9.97; b = -4.36, and c = -2.80; hence the calculated data showed that $y = = 9.97 – 4.36x – 2.80x^2$ (Figure 2). The analysis of the lethal dose showed a 50% reduction in plant growth of tomato cv. 'Timothy' F1 was obtained at 132.49 Gy. This study also showed that increasing the irradiation dose led to a 91% inhibition of seed germination (r = 0.91) (Figure 2).

Table 2. The number of tomato seed before irradiated (M_0G_0) and the number of tomato seedling at 4 week afer germination (M_1G_1).

The number of seeds at	Gamma Irradiation doses (Gy)								
	0	50	60	70	80	90	100	110	120
M_0G_0	50	50	50	50	50	50	50	50	50
M_1G_1	46	42	38	44	40	32	30	32	27
Growth standardization (%)	100	91	83	96	87	70	65	70	59

Note. Standardization of seeds growth (%) at each irradiation dose was obtained using the formula: Σ irradiated seedling on M_1G_1 / Σ control seed (0 Gy) on M_1G_1 x 100%.

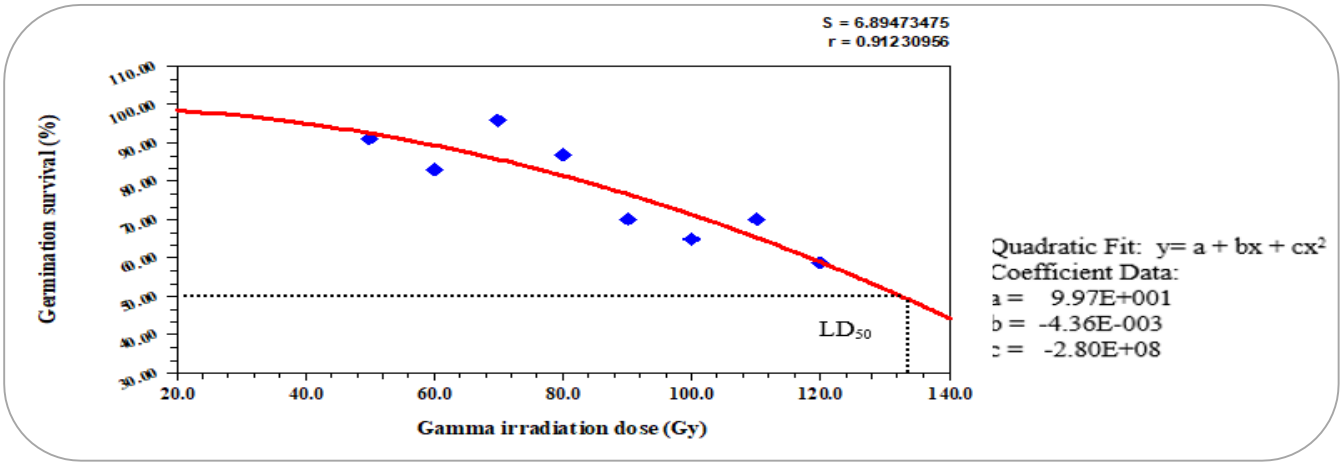


Figure 2. Lethal dose of tomato seed after gamma irradiation

Evaluation of gamma irradiation effect on the plant height and the number of leaves: In plant research, mutation frequencies were generally determined based on observable phenotypes, such as development changes or leaf or flower colour (Jo *et al.,* 2019). In this study, variability of phenotypes was observed at 2 – 8 weeks after germination. Different doses of gamma radiation affected several growth traits of tomato genotypes. Evaluation of phenotype characteristics showed that the highest plant height (15.15 cm) grew from un-irradiated gamma (0 Gy) seeds (Figure 3). There was no significant difference in plant height between un-irradiated (0 Gy), 50 Gy, and 100 Gy seeds (Table 3).

The number of tomatoes leaves 2 (two) weeks after germinating showed no significant difference among all treatments (Table 3). However, 6- 8 weeks after germination, it was shown that the highest average number of leaves was recorded from unirradiated seeds (7.25). It significantly differs from other treatments, except for seed irradiated with 50 Gy (6.25). Irradiation doses above 50 Gy were predicted to inhibit the shoot's meristem cell growth. Zafar *et al.,* (2022) reported that the 150 Gy treatment showed the least damaging impact and induced maximum genetic variability. This research was similar to Tomato's cv. Mawar that irradiated gamma at 50 Gy and 100 Gy produces optimum plant growth (Sutapa and Kurniawan (2016). Otherwise, Naheed (2014) reported that tomato seeds cv. Rio Grande, with a dose of 100 Gy, had an average plant height lower than the control plants.

Table 3. Effect of gamma irradiation on the variability of the plant height (cm) and the number of leaves of tomato cv. 'Timothy' F1.

Gamma irradiation Dose (Gy)	The average plant height (cm) after 2-8 weeks of irradiation				The average number of leaves after 2-8 weeks of irradiation			
	2	4	6	8	2	4	6	8
0	8.75^{d}	11.00^{e}	13.25^{c}	15.15^{d}	2.50^{a}	4.25^{c}	6.25^{d}	7.25^{d}
50	8.25^{cd}	10.45^{de}	11.75^{bc}	13.15^{d}	2.25^{a}	3.25^{b}	4.50^{bc}	6.25^{cd}
60	4.10^{a}	6.10^{a}	9.75^{a}	10.85^{ab}	2.00^{a}	2.25^{a}	3.25^{a}	4.50^{a}
70	6.05^{abc}	7.10^{abc}	9.25^{a}	10.25^{a}	2.25^{a}	3.00^{ab}	4.25^{bc}	5.50^{abc}
80	5.75^{abc}	8.25^{abcd}	9.25^{a}	10.25^{a}	2.25^{a}	3.25^{b}	4.75^{c}	5.25^{abc}
90	5.75^{abc}	8.60^{bcd}	10.75^{abc}	11.75^{c}	2.00^{a}	2.75^{ab}	3.75^{ab}	4.75^{ab}
100	5.90^{abc}	8.25^{abcd}	10.75^{abc}	13.55^{d}	2.00^{a}	3.50^{bc}	4.25^{bc}	5.50^{abc}
110	7.30^{bcd}	9.10^{cde}	10.25^{ab}	11.25^{bc}	2.25^{a}	3.25^{b}	4.50^{bc}	5.75^{bc}
120	5.40^{ab}	6.60^{ab}	8.85^{a}	10.75^{ab}	2.00^{a}	3.00^{ab}	3.75^{ab}	5.25^{abc}

Note: Mean with a different letter within each treatment(cultivars) are significantly different at the 5% level according to the Duncan Multiple Range Test (DMRT)

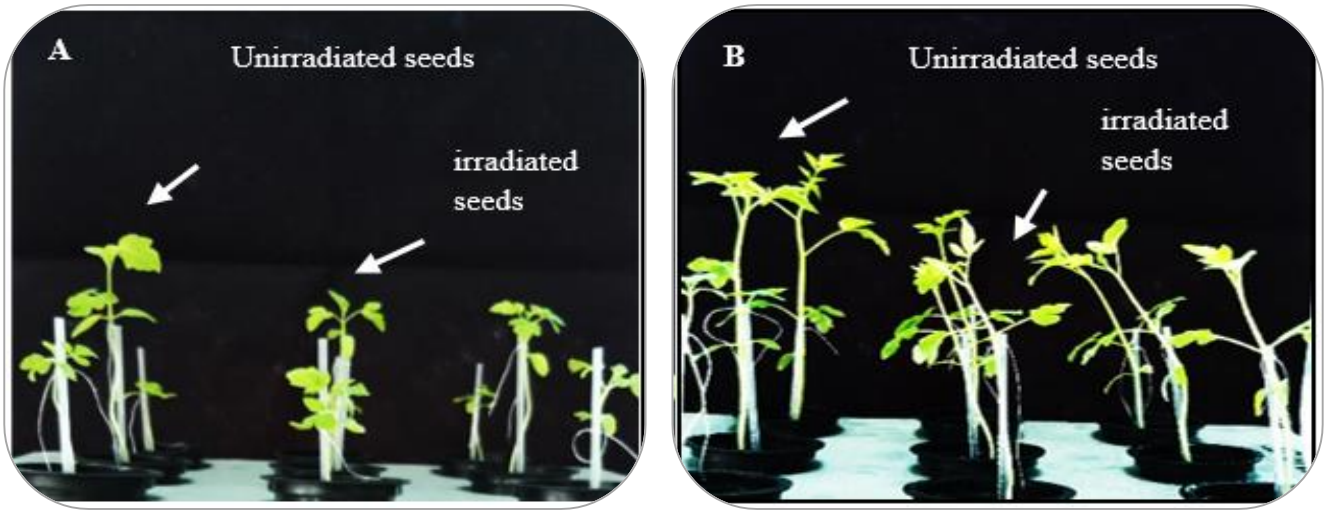


Figure 3. Growth of plant height from unirradiated seeds and irradiated tomato seeds (70 Gy) in hydroponics system at (a) 6 weeks old; (b) 8 weeks after germination.

Evaluation of gamma irradiation effect on variability of tomato leaf: Gamma irradiation significantly differed the average leaf length and width at eight weeks after germination. The un-irradiated seeds significantly produced the highest leaf width (4.61 cm) and smaller leaf length and width ratio (1.29). A smaller ratio indicates a wider leaf shape. Irradiated Seeds (50–120 Gy) produced hhigher ratios (1.40–1.69), suggesting a phenotypically longer leaf shape (Table 4). Sikder *et al.,* (2014) reported similar findings in tomato seeds cv. 'Rio Grande,' where unirradiated seeds had smaller leaf sizes than plants irradiated with 50 and 100 Gy.

The morphological changes were also observed in plant leaves as a result of gamma irradiation, and the potential causes of these changes were found, including the influence of epigenetic variations and random mutations in genetic material. The morphological observations of tomato leaves showed differences in leaf shape. The young plants exhibited fused compound leaves; as the plant matures, the leaves undergo morphological change, resembling an adult plant (Figure 4). In this study, the morphology of leaf type and leaf shape were identified based on the descriptor for tomato from sources IPGRI (1996) and BI (2013). The leaf type of tomato was identified as potato leaf type, and compound leaves were marked as the predominant type. The morphology of the compound leaves showed variability in leaf blade shape, leaf apex shape, and leaf margin (Figure 5).

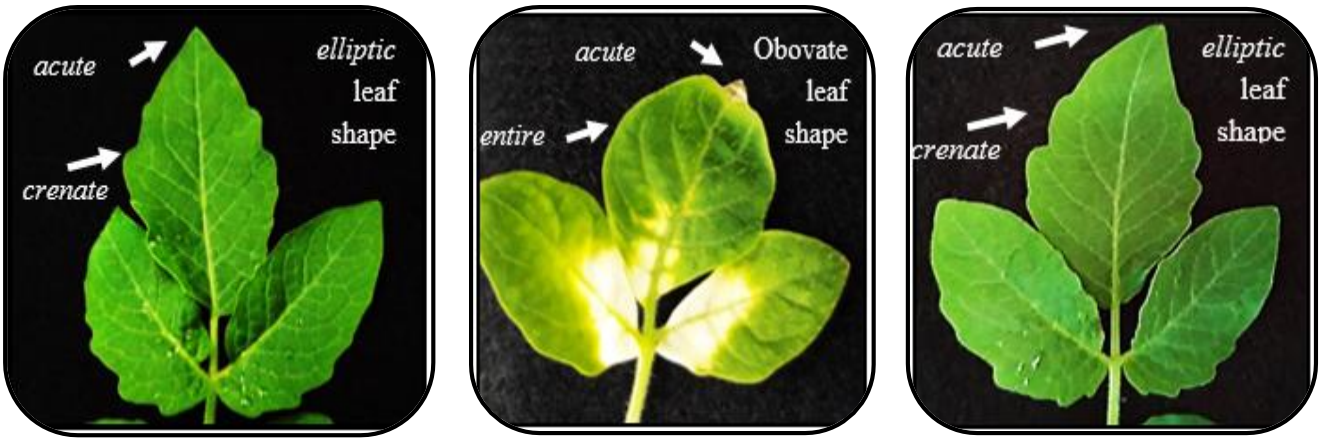
Table4. Effect of gamma irradiation on the variability of tomato leaf length and width (cm), ratio of leaf length and width (L:W) of tomato cv. 'Timothy' F1

Gamma irradiation Dose (Gy)	The average leaf length (cm) after 2-8 weeks of irradiation				The average leaf width (cm) after 2-8 weeks of irradiation				Leaves ratio
	2	4	6	8	2	4	6	8	(L:W)
0	2.99^{a}	3.75^{b}	4.75^{b}	6.00^{c}	1.75^{a}	2.75^{c}	3.75^{c}	4.61^{c}	1.29^{a}
50	2.91^{a}	3.40^{ab}	4.25^{ab}	5.08^{ab}	1.75^{a}	2.40^{bc}	2.75^{ab}	3.15^{ab}	1.56^{bc}
60	2.77^{a}	3.25^{ab}	3.65^{ab}	4.25^{a}	1.25^{a}	1.88^{ab}	2.60^{ab}	2.85^{ab}	1.44^{bc}
70	2.83^{a}	3.50^{ab}	4.21^{ab}	5.22^{abc}	1.60^{a}	2.00^{ab}	2.65^{ab}	2.75^{a}	1.69^{bc}
80	2.56^{a}	3.10^{ab}	3.95^{ab}	4.96^{ab}	1.88^{a}	2.21^{abc}	2.75^{ab}	3.15^{ab}	1.50^{bc}
90	2.52^{a}	3.25^{ab}	4.25^{ab}	5.33^{bc}	1.75^{a}	2.06^{ab}	2.39^{ab}	3.00^{ab}	1.65^{bc}
100	2.75^{a}	3.15^{ab}	4.26^{ab}	5.25^{abc}	1.71^{a}	2.75^{c}	3.15^{bc}	3.60^{b}	1.40^{b}
110	2.65^{a}	3.35^{ab}	4.42^{ab}	5.21^{abc}	1.25^{a}	2.16^{abc}	2.75^{ab}	3.06^{ab}	1.65^{bc}
120	2.48^{a}	3.00^{a}	4.50^{a}	5.23^{abc}	1.25^{a}	1.75^{a}	2.10^{a}	2.75^{a}	1.60^{bc}

Note: Mean with a different letter within each treatment(cultivars) are significantly different at the 5% level according to the Duncan Multiple Range Test (DMRT)

A B C

Figure 4. Representative figure of leave growth from irradiated seeds of 50 Gy (a), at 4 6 (b) and 8 (c) weeks after gamma irradiation.



A B C

Figure 5. Variability of leaf shape of tomato cv. 'Tymotyi' F1 after gamma irradiation: (a) elliptic leaf blade shapes and crenate leaf margin of seed irradiated of 50 Gy, (b) obovate leaf blade and entire leaf margin of 100 Gy irradiated plants, and (c) elliptic leaf blade shape of control leaves (0 Gy) at 8 weeks after planting

The leaf blade shapes observed from 0 Gy and 50 Gy some are elliptic (a single vast middle portion tapers as it approaches the apex or base), and leaf margins are crenate (having rounded teeth). The leaf blade shapes observed from 100 Gy are obovate (the widest portion of leaves is near the tip), and the entire leaf margin (has a smooth edge). All leaf apexes were acute (forming an angle less than 90º) (Figure 5). In other treatments, leaf morphology was the same as control leaves. Different doses of gamma irradiation seemed to have distinct effects on leaf morphology. The control leaves (unirradiated) had a wider shape than the irradiated leaves. The irradiated leaves at 0 Gy and 50 Gy exhibited elliptic shapes with crenate margins, while those at 100 Gy show obviate shapes with smooth margins. Additionally, all leaves, including controls, had acute leaf apexes.

Isolation and Disease Resistance Evaluation: *Colletotrichum* was isolated from a chili (*Capsicum* sp.). A characteristic of *Colletotricum* isolate is the black acervuli, which were structures containing spores or conidia (Figure 6). Colony morphology features on PDA were uneven circular colonies with white color and compact with abundant aerial mycelium. Unicellular conidia shape, cylindrical spores, and septate mycelium determined microscopic features. Macroscopic characteristic colonies were similar to those described by Manova *et al.,* (2022). In mango fruit, the colony changed color to dark gray, white, and orange (Li *et al.,* 2019).

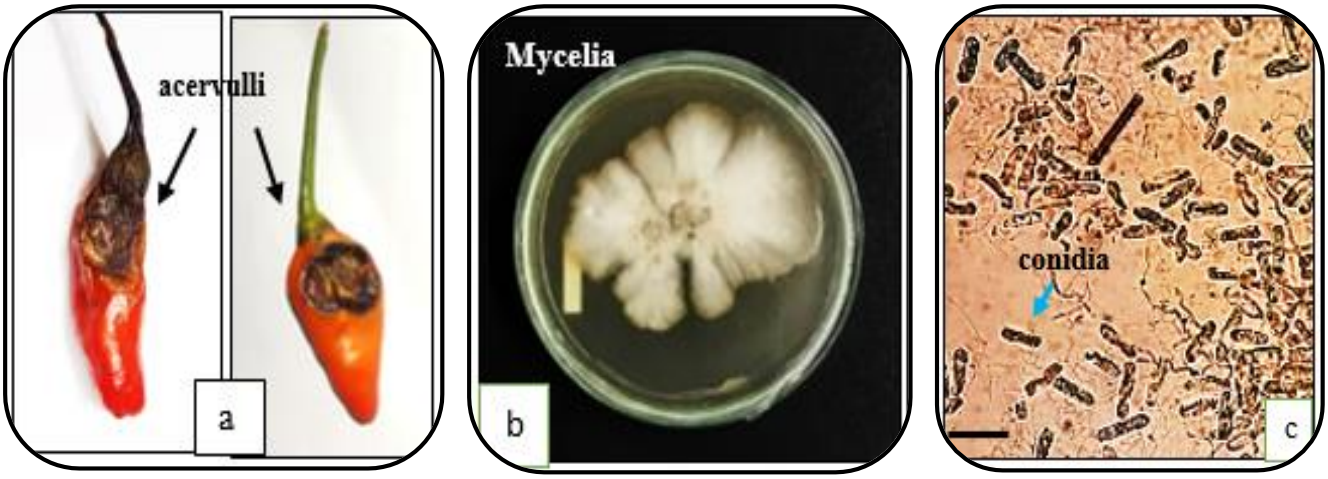


Figure 6. Representative figure of (a) detached chili fruit showing black *acervuli*; (b) mycelium colony of *Colletotrichum* sp colonies two weeks after inoculation on PDA media; (c) *Colletotrichum* spp. conidia under a microscope 400x. Bars: 10 µm.

The pathogenic properties of *Colletotrichum* isolates were conducted on tomato variants to identify tomato resistance to anthracnose disease. Six-week-old tomato stem tissue was injected with 1 ml of *Colletotrichum* suspension into the epidermis layer of the plant stem, where the xylem and phloem transport tissue was located. Conidial density was 1.08 x 10^{-6} conidia per ml. Infection was conducted on the stem. In this study, symptoms in plants due to infection appeared two days after infection. The white fungal colonies grow on the infected part and change in stem color to pale. Observations 4 days after the disease showed the leaves becoming chlorosis and the colonies on the stems turning black and necrotic.

Leaf death occurred on the sample plants eight days after infection; some unirradiated and irradiated plants showed symptoms of plant death ten days after infection (Figure 7). According to Cannon *et al.,* (2012), anthracnose symptoms included limited necrotic lesions on leaves, stems, flowers, fruit, and crown and stem rot. The results of the study's results for assessing disease incidence and severity in tomato variants following infection are presented in Table 5. The number of plants showing symptoms and death after 14 days of infection was calculated for the percentage of disease incidence and severity (Table 5). Tomato variants regenerated from gamma irradiation at 60, 70, 90, 100, and 120 Gy exhibited 100% disease incidence (DI). Tomato variants irradiated at 50, 80, and 110 Gy showed a disease incidence of 90%. The disease severity index (DSI) of the unirradiated and irradiated tomato variants ranged from 32.5 - 70.0%, showing that plants were susceptible to anthracnose disease. Plants from gamma irradiation 70 Gy showed the lowest DSI and fewer pathogenic lesions in stems.

Figure 7. Representative figure of tomato plants symptoms after infection of *Colletotrichum* sp: (a) Plants that do not show any symptoms of disease on tomato leaf, (b) Appearance of white colonies on the tomato stem after irradiation at a dose of 80 Gy, (c) Necrosis leaf. (d) Plant death as a result of irradiation at 60 Gy, observed 14 days after infection.

Table 5. Percentage of Disease Incidence (DI) and Disease Severity Index (DSI) of tomato variants regenerated from gamma irradiation after 14 days of *Colletotrichum* infection.

Detection of tomato resistance	Tomato seed from irradiated gamma (Gy) from								
	0	50	60	70	80	90	100	110	120
DI (%)	100.0	90.0	100.0	100.0	90.0	100.0	100.0	90.0	100.0
DSI (%)	47.5	50.0	70.0	32.5	55.0	65.0	60.0	50.0	55.0

Noted: Percentage of DI $= \frac{A}{A+B}$ x 100%. A: the number of infected plants B: the number of non-infected plants. $DSI = \frac{\Sigma (n \times v)}{Z \times N} \times 100\%$ n: the number of infected plants in every score; v = the score of disease intensity; Z: the highest score; N: the number of plant infections.

DISCUSSION

Seed viability is the ability of seeds to germinate in suitable conditions (Bradbeer 1988), and it is expressed as the percentage of seeds that grow in the standard germination test. Seed vigor is the activity and performance of seed lots to germinate under various field conditions (Milosevic *et al.,* 2010; ISTA, 2015). The viability and vigor seed testing provides an overview of the germination characteristics of un-irradiated and irradiated tomato seeds. The study suggests that irradiation at a specific dose (70 Gy) resulted in the highest viability, as measured by both MGP and GP,

exceeding the 90% threshold recommended by International Standards for Genebanks (Rao *et al.,* 2006). The control seeds, which were not irradiated, showed higher vigor with no abnormal sprouts. Seed irradiation at a dose of 60 Gy negatively impacts seed vigor, leading to abnormal sprouts characterized by specific growth abnormalities. Seedling vigor is highlighted as an important factor influencing the uniformity of seedling emergence. Seedlings from high-vigor seed lots are expected to emerge more uniformly than those from low-vigor lots (Finch-Savage and Bassel 2016; Egli and Rucker 2012).

Gamma irradiation on seeds could affect a few critical germination processes, such as (1) a decrease in cell respiration due to cytochrome oxidase inhibition, (2) disturbing the cell division phase, and (3) disturbing the synthesis of enzymes used in the germination process (Sikder *et al.,* 2013; Usman *et al.,* 2024). The high energy of gamma rays can damage the DNA structure and cause abnormal growth of germs due to changes in the encoded properties (Ahmad *et al.,* 2024; Majeed *et al.,* 2017). According to Hase *et al.* (2018), the irradiation of dry seeds gave a higher mutation frequency than the irradiation of seedlings. This difference is mainly due to the increased frequency of insertions and deletions upon dry seed irradiation. This study found lower viability and vigor on seed irradiated from gamma irradiation 100 – 120 Gy. This experiment enables observing the impact of different gamma irradiation doses on the viability (ability to germinate) and vigor (subsequent growth and development) of the tomato seeds.

The lethal dose (LD) is considered the main factor for assessing the level of radio sensitivity in plants (Kumar *et al.,* 2013). LD_{50} is a specific dose in which 50 percent of irradiated seeds cannot germinate due to several biochemical and molecular changes (Zafar *et al.,* 2022). This study suggests a strong correlation between irradiation dose and the inhibition of seed germination ($r = 0.91$). Other research using different gamma irradiation sources (^{137}Cs) found that LD_{50} of 300 Gy caused a 50% germination inhibition in various tomato genotypes from Pakistan. However, maximum genetic variability and the least damaging effect were found in the 150 Gy treatment (Zafar *et al.,* 2022).

The frequency of mutations is influenced by various factors, including radiation type, linear energy transfer, radiation dose, and plant tissue type and condition (Tahir *et al.,* 2023; Jo and Kim 2019). LD_{50} is essential in irradiation studies to obtain a population with a high mutation frequency (Albokari *et al.,* 2012). Plants survive better under low-dose irradiation, which may result in a low-frequency mutation (Aisyah *et al.,* 2009). Therefore, a high-frequency mutation is needed to increase the chances of finding tomato cultivars resistant to anthracnose disease, suggesting a balance between survival and mutation frequency. This study emphasized the importance of LD_{50} in assessing radiosensitivity, and the need for a balance between survival and mutation frequency for successful mutation breeding to develop disease-resistant tomato cultivars.

Evaluation of plant growth for eight weeks indicates that gamma radiation doses impact the growth traits of tomato genotypes, specific to plant height and the number of leaves, with significant differences observed at 6-8 weeks. The highest tomato plant height and the number of leaves are recorded in un-irradiated seeds (Table 3 and 4). Leaf observation shows the passage details the temporal effects of gamma irradiation on tomato leaf characteristics. While there was no significant difference at two weeks, un-irradiated seeds exhibit wider leaves by eight weeks, while irradiated seeds show a phenotypically longer leaf shape. These findings align with a reference study by Sikder *et al.,* (2014) on a different tomato cultivar. Effect gamma irradiation on a tomato leaf significantly differs in the average leaf length and width at eight weeks after germination. According to Kovacs and Keresztes (2002), gamma irradiation causes plant cells to shrink and produce morphological differences in plants. The growth in leaf area and its relationship to biomass accumulation depend on how carbon is distributed in all parts of plant and respiration processes (Weraduwage *et al.,* 2015).

The morphology changes of plant leave in this research return to separate compound leaves from resembling the nature of epigenetic variations. Epigenetic variation involves modification of gene expression. These modifications can be inherited but are reversible. Epigenetic variants have a higher rate than genetic variants, and eenvironmental factors can influence epigenetic variation (Atiq *et al.,* 2023; Hirsh *et al.,* 2023). Random mutations in genetic material are predicted to influence the emergence of varied phenotypes, including differences in leaf shape. It suggests a connection between morphological changes in plant leaves,

particularly the separation into compound leaves, and the influence of epigenetic variations. Additionally, the role of random mutations in genetic material is acknowledged as a factor predicting differences in morphology character and the emergence of diverse phenotypes (Iqbal *et al.,* 2022). The reference to Zafar *et al.,* (2022) adds a comparative perspective on the impact of radiation doses, emphasizing the random nature of physical mutations in the observed plant phenotypes.

Colletotrichum is well-known as a pathogen that attacks many plants and affects many host plants, including the Solanaceae family (Cannon *et al.,* 2012; Manova *et al.,* 2022). The typical symptom is black sunken necrotic tissue with a ring of water-soaked black *acervuli* (asexual fruiting bodies) or conidial masses, microsclerotia (Manova *et al.,* 2022; Cui *et al.,* 2023). The *Colletotrichum* isolates from chilli exhibit specific characteristics regarding acervuli, colony morphology, and conidia. The macroscopic features of PDA resemble those found in a previous study, and there are variations in colony colour, such as mango fruit, in different host environments. Multiplication of isolate sources was carried out using the blotter test method (filter paper test method) for two weeks. This method utilizes the moist conditions of wet filter paper to stimulate mold growth. Detection of tomato variants' resistance to anthracnose indicates that all tomato variants, both control (unirradiated) and irradiated, are susceptible to anthracnose disease. The disease incidence is high across all irradiation levels, with 100% in several cases and 90% in others (Table 5). However, the disease severity index is comparatively lower for tomato variants regenerated from seeds irradiated at 70 Gy, suggesting a potentially lower anthracnose disease severity in these particular variants than others.

The success of plant breeding for anthracnose resistance is greatly influenced by the method used for inoculation and evaluation (Cui *et al.,* 2023). The inoculation method is crucial, and various techniques have been employed in plant breeding studies. Inoculation can be carried out on fruit or leaves using detached fruit/leaf assay or whole plants through wounding or without wounding before inoculation (Souza *et al.,* 2020; De Silva *et al.,* 2017). Tissue injury methods cause damage to plant tissue to facilitate the entry of spores. It can be carried out through pinprick methods using pins or microinjections (Cui *et al.,* 2023; Giacomin *et al.,* 2020; Kumari *et al.,* 2017) or root dip inoculation (Singh *et al.,* 2019; Indrayanti *et al.,* 2012). In this particular study, inoculation of conidia *Colletotrichum* was conducted through injection. The advantages of wounding methods, as highlighted by Auyong *et al.,* (2015), include a more direct entry of spores into plant tissue, bypassing the cuticle and epidermis layers that serve as primary defence mechanisms.

The defence mechanisms of plants against pathogens mainly focus on the interaction between plants and *Colletotrichum*. Susceptible plants are predicted to lack genes for resistance to pathogens infection or may express their defense mechanism late (Shad *et al.,* 2024; Vargas *et al.,* 2012). Hence, the plant and the *Colletotrichum* conidia interaction leads to disease development. After infection, the growing colonies enter the biotrophic growth phase, initially in the infected tissue, without external symptoms. The biotrophic phase may last a short or longer time. Following the biotrophic phase, there is a switch to necrotrophic growth characterized by the production of secondary hyphae, causing extensive colonization of plant tissue, host cell death, and pathogenic lesions appear in infected plants (Cannon *et al.,* 2012; Vargas *et al.,* 2012). In this study, most tomato variant plants died 10 – 14 days after infection (Figure 7). This research provides a comprehensive understanding of the effects of gamma irradiation on tomato seeds, encompassing viability, vigor testing, and resulting phenotypic changes. The identification of the LD_{50} and the observation of anthracnose resistance in the 70 Gy-irradiated tomato plant are essential, suggesting potential applications in mutation breeding programs for developing tomato cultivars with enhanced disease resistance. However, the resistance plant candidates obtained in this research are limited. Further, the number of variants to be selected should be increased, and evaluation in the greenhouse will provide additional insights into the resistance of surviving plants to anthracnose. This research contributes valuable insights to mutation breeding programs and offers potential solutions for developing tomato cultivars with enhanced resistance to anthracnose disease.

CONCLUSIONS

The gamma irradiation on tomato seed induces variability in viability and vigor testing. Seed viability is assessed through Maximum Growth Potential and Germination

Potential. Seed vigor is evaluated using the Germination Vigor Index and Seed Emergence uniformity. LD_{50} for tomato seeds was obtained at 132.48 Gy. Gamma irradiation at 60-120 Gy significantly caused lower plant height, reduced number of leaves, narrower leaf width, and phenotypically longer leaves than the control plant. Early detection of anthracnose resistance in the Timothy F1 tomato plant cultivar reveals that the plant irradiated at 70 Gy exhibits signs of potential resistance to *Colletotrichum*, as evidenced by the lowest Disease Severity Index and fewer pathogenic lesions. This research contributes to mutation breeding programs and offers potential solutions for developing tomato cultivars with enhanced resistance to anthracnose disease.

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