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Morpho-Physiological, ISSR-Based Molecular Characterization of Gamma-Irradiated Soybean Under Waterlogging Stress

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Abstract. Soybeans (*Glycine max*) are Indonesia's third-most important food crop after rice and maize. However, because of its susceptibility to waterlogging, its productivity decreases. Gamma-ray irradiation has been used to create soybean varieties that are resistant to waterlogging. This study investigates the genetic variation and morpho-physiological traits of irradiated Grobogan soybean varieties under waterlogging stress. Soybean seeds were exposed to gamma-ray doses of 0 Gy, 25 Gy, 50 Gy, 75 Gy, and 100 Gy, then the soybean plants were submerged in water at concentrations of 0%, 100%, 150%, 200%, and 250%. The study's morpho-physiological parameters include seed weight, pod count, plant height, chlorophyll content, and seed viability. The results imply that varying waterlogging levels and radiation dosages show varied responses. All irradiation doses showed optimal growth in the control treatment, whereas 75 Gy and 100 Gy under 250% waterlogging conditions showed the most severe growth inhibition. To evaluate genetic variation among the irradiated soybean variants, five ISSR markers were employed. Because of its high PIC value of 0.391, marker ISSR-1 was found to be the most effective in detecting polymorphisms. ISSR-1 is a promising marker for assessing genetic diversity in irradiated soybean plants, according to the results. These results are valuable for the development of soybean breeding strategies targeting improved tolerance to waterlogging stress.

Keywords: Morpho-physiological, *Glycine max*, ISSR, Waterlogging, Irradiation

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INTRODUCTION

Soybeans (*Glycine max*) are Indonesia's third most important food crop, after rice and maize (Harsono et al., 2021). According to Tewari et al. (2023), soybeans are a valuable potential food source due to their well-known nutritional composition, which includes 20% oil, 30% to 50% protein, and a superior amino acid profile. However, soybean production has faced significant obstacles, including declining national yields over time. This deficiency is primarily caused by environmental factors, particularly Indonesia's heavy rainfall, as soybeans are susceptible to waterlogging stress (Oh et al., 2014).

Waterlogging occurs when soil pores are saturated with water exceeding 20% of the soil’s capacity (Kim et al., 2015), causing the soil to undergo oxygen deficiency, which limits plant growth and productivity (Khan et al., 2022). Addressing this challenge, it requires the development of superior soybean varieties that have stable traits under adverse environmental conditions. Such varieties must be able to resist waterlogging. Improving soybean tolerance to various stresses is crucial. For example, anatomical traits have been shown to contribute to disease resistance, as demonstrated in soybean cultivars selected for resistance to *Cercospora* leaf blight (Husen et al., 2022). Anatomical and physiological traits have also been shown to support tolerance to abiotic stresses. For instance, the Mahameru soybean cultivar exhibits high salinity tolerance through increased epidermis thickness, stomatal density, and chlorophyll stability under NaCl stress (Juwarno et al., 2018). However, currently no natural sources of waterlogging-resistant genes have been identified in soybeans. Consequently, genetic variation must be artificially induced, with gamma irradiation being a widely used method. Gamma irradiation is one of the approaches used to enhance genetic diversity in plants (Hanafiah, 2010). Gamma irradiation introduces random genetic mutations, creating opportunities to select desirable traits such as waterlogging tolerance. This method has also been successfully applied to microalgae, such as Nannochloropsis sp., to modify biochemical traits including lipid and fatty acid composition (Ermavitalini et al., 2017).

Moreover, irradiation has been proven to increase soybean production (Mudibu, 2011; and Aminah, 2015). Past studies have shown that giving soybeans a low dose of gamma rays (25–50 Gy) can boost their growth traits and create useful genetic differences when the plants face waterlogging stress (Saputro et al., 2018; Saputro et al., 2019). This means that combining gamma irradiation with waterlogging could be a practical way to find soybean varieties that are more tolerant.

The Grobogan soybean variety, a high-performing cultivar recognized for its early maturity and productivity exceeding 2.5 tons per hectare, was selected for this study (Mustikawati et al., 2018). Since the genetic changes induced by irradiation occur randomly, systematic selection is necessary to obtain variants with desirable traits, particularly improved tolerance to waterlogging stress. The identification and confirmation of such tolerance can be strengthened by employing molecular marker techniques, including Inter Simple Sequence Repeats (ISSR).

Recent studies on soybean cultivars resistant to waterlogging emphasize the urgent need to integrate morphological, molecular, and proteomic data (Adegoye et al., 2023). Despite the availability of numerous molecular markers, ISSR remains underexplored in waterlogging-related soybean research. Therefore, to better characterize irradiated soybean variants under waterlogging stress, this study employs ISSR in addition to morpho-physiological responses. In this context, the ISSR technique is particularly valuable for understanding both the physiological traits of irradiated soybean plants and the genetic alterations induced by gamma irradiation. Scientifically, this research advances the application of ISSR markers for assessing gamma-induced genetic variation in soybean under abiotic stress, an area that remains insufficiently explored. Practically, the findings provide a basis for breeding programs to identify and develop waterlogging-tolerant soybean cultivars, thereby supporting sustainable agriculture and strengthening national food security.

Therefore, this study aims to evaluate the morpho-physiological responses of gamma-irradiated Grobogan soybean under waterlogging stress, and assess the genetic variation using ISSR markers to identify potential mutant lines with improved waterlogging tolerance.

METHODS

Waterlogging Measurement

To apply waterlogging treatments, field capacity measurements were conducted to determine the appropriate watering volume as a reference. The procedure involved saturating the planting media in polybags by watering until excess water began to drip out. The media were then left to sit for three days until no more water dripped from the polybag hole. Afterward, the planting media were weighed to record their fresh weight. This fresh weight was taken after ensuring no further dripping from the polybag. Subsequently, the dry weight was measured after the planting media were oven-dried at 105°C until a constant weight was obtained. Water requirements based on field capacity are calculated using the following formula:

$$\text{FC (\%)} = \frac{FWS - DWS}{DWS} \times 100\%$$

where: FC : Field Capacity; FWS : Fresh Weight of Soil; DWS : Dry Weight of Soil (Kondhia et al., 2015).

Seed Irradiation and Germination

Grobogan soybean seeds were obtained from the Legumes and Tubers Research Center (BALITKABI) in Kendal, Payak-Malang. A total of 500 seeds were sterilized by soaking in 70% ethanol for 15 minutes and then treated with a 1% NaOCl solution for 2 minutes. The sterilized seeds were then put in Petri dishes and sealed to avoid contamination. Gamma irradiation to the seeds was conducted at the National Atomic Energy Agency of Indonesia (BATAN) at doses of 0 Gy, 25 Gy, 50 Gy, 75 Gy, and 100 Gy (Saputro, et al., 2018; Saputro, et al., 2019).

After irradiation, the treated seeds were planted in prepared germination media. Before planting, the seeds were soaked in distilled water (aquades) for two hours and then drained. The seeds were then planted in seedling trays filled with planting media, and the seeds were left to germinate until they developed two true leaves.

Seed Viability and Vigor Assessment

The germination rate is used to evaluate seed viability, reflecting the potential of seeds to grow normally under optimal field and environmental conditions. Germination rate (GR), speed of germination (SG), and vigor (Vg) were the viability parameters that were observed in this study. The germination rate, also known as the percentage of seedlings observed seven days after planting (DAP), is determined using the formula below:

$$\text{GR} = \frac{Number\ of\ seedlings}{Total\ Number\ of\ Seed\ Planted} \times 100\%$$

(Saibari et al., 2023)

The speed of germination (SG) is determined by calculating the number of days required for the emergence of radicles or plumules over a specified period (7 days). The speed of germination is calculated using the formula below (Noe & Zedler, 2000):

$$\text{SG} = \frac{N_1T_1 + N_2T_2 + \ldots\ldots + N_xT_x}{\text{Total Number of Germinated seeds}}$$

SG : Speed of germination
N : Number of seeds that germinated at a specific time interval
T : Time interval between the start of the test and the end of a specific observation interval

Seed vigor refers to the seeds' ability to germinate and thrive under a range of environmental conditions, even under suboptimal field conditions and varying environmental factors. Robust seeds can establish growth under a range of field conditions (Cécel & Barbedo, 2023). In this study, seeds' vigor was assessed based on the synchrony of seed germination (*Vg*). The vigor percentage was determined by calculating the proportion of normal, vigorous seedlings on the fourth day using the following formula:

$$\text{Vg} = \frac{N}{TS} \times 100\%$$

(Noe & Zedler, 2000)

Vg : Vigority of seed germination
N : Number of viable seedlings
TS : Total number of seeds analyzed

Acclimatization and waterlogging stress

The planting medium was prepared by combining garden soil and organic fertilizer in a ratio of 3:1, with 3 kgs of soil to 1 kg of compost, resulting in a total of 4 kgs per polybag. After weighing, the mixture was thoroughly blended and placed into polybags, which were then arranged on greenhouse racks according to the experimental layout. Each polybag was labeled to indicate its respective treatment. The acclimatization period for soybean plants (*Glycine max*) lasted ten days, during which they were maintained with regular watering every morning and afternoon as needed. Waterlogging stress treatments were conducted in the greenhouse of the Department of Biology, ITS. After determining field capacity, 14-day-old acclimatized soybean plants were exposed to waterlogging stress for 14 days under varying treatment conditions. Waterlogging involved putting water into each polybag until it reached a certain level based on calculations of field capacity. To keep the plants stable in saturated soil, bamboo supports were installed in the polybags to stabilize the plants. The water level was monitored daily using a ruler, and adjustments were made as necessary to maintain the required water levels (Kim et al., 2018).

Measurement of plant growth

Soybean plants were harvested after 14 days of treatment with waterlogging stress. After the harvest, the plants were washed with water to remove soil residues, then dried and stored in labelled ziplock bags. After that, the samples were put in a freezer for further analysis. Before and after the waterlogging stress treatment, the plants' height was measured from the base of the stem to the growth point or tip. The number of pods was determined after the 14-day waterlogging stress period by counting the total pods produced per plant. The weight of seeds was assessed by shelling pods and weighing groups of five seeds using an analytical balance. The experiment employs a Completely Randomised Design (CRD) with two factors and three replications. The factors include waterlogging, with four treatment levels: G0 is the control with no waterlogging; G1 represents 100% waterlogging; G2 represents 150% waterlogging; G3 represents 200% waterlogging; and G4 represents 250% waterlogging. A two-way analysis of variance (ANOVA) will be used to examine plant growth and assess how treatments impact the measured parameters. The significance level (α) will be 0.05%. We will use the Duncan Multiple Range Test (DMRT) at a 5% significance level, along with IBM SPSS Statistics Version 23, to compare the best waterlogging treatments with one another.

ISSR markers to identify the differentiation among variants

DNA isolation was performed using a total of 80 mg of leaves were placed in a mortar containing liquid nitrogen and ground until completely crushed. The samples were extracted using the Genomic DNA Mini Kit (Plant) and following the protocol provided by the manufacturer. DNA pellets were added with 40 µl of sterile aquabidest and homogenized. Subsequently, all the samples were stored at -20°C (Akhtar et al., 2021).

Data Analysis

The Effective Multiplex Ratio (EMR) was calculated using the formula α × β, where α represents the average number of amplified fragments per marker, and β was determined as the ratio of polymorphic loci (PB) to the total number of loci (PB + MB), where MB denotes monomorphic loci. The Marker Index (MI), which indicates the discriminatory power of each primer in detecting polymorphic loci, was calculated as MI = EMR × PIC. The Polymorphic Information Content (PIC) for each primer was estimated according to Saputro et al. (2019) using the formula:

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

PIC_i : Polymorphic Information Content for the i^{th} marker

P_{ij} : frequency of the j^{th} allele for the i^{th} marker

n : total number of alleles detected for the i^{th} marker

$\sum_{j=1}^{n} P_{ij}^2$: the sum of squared frequencies of all alleles for the i^{th} marker

RESULTS AND DISCUSSIONS

Enhancing productivity begins at the seed level, mainly through improving seed viability and vigor. The quality of the seeds can be determined by seed viability and seed vigor, which include both physiological and physical characteristics. The Gamma irradiation technique is a seed treatment method that can be used to improve the seed quality, increasing seeds' viability and vigor (Manjusha et al., 2024). To evaluate the effects of irradiation dosage on seed viability and vigor, measurements of these parameters were conducted after the seeds were sown.

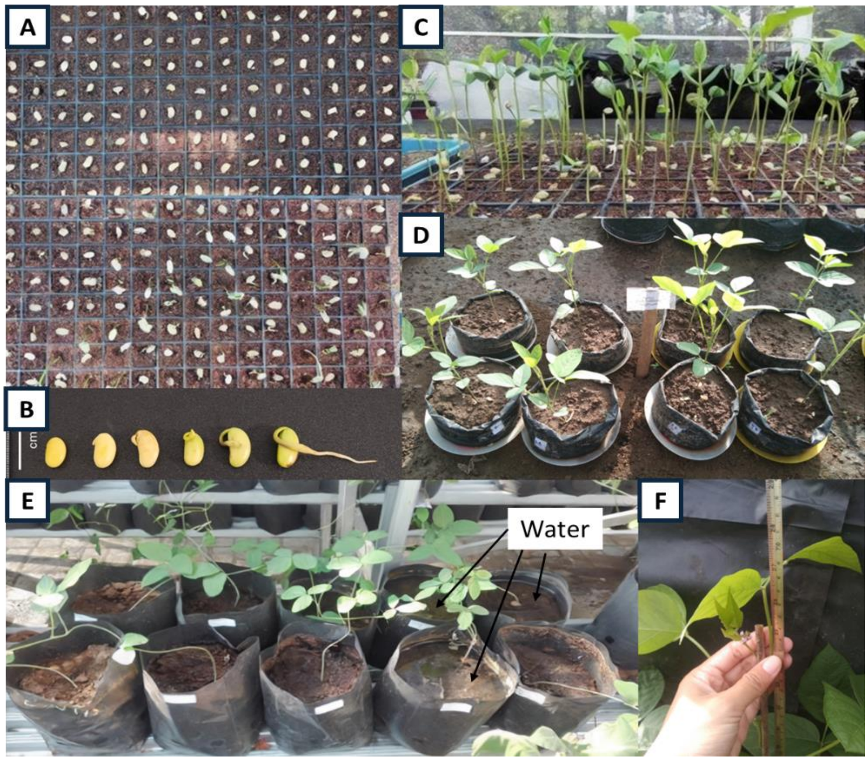

Figure 1. A. Seeds were arranged in germination trays at the beginning of the experiment. B. Stages of seed germination from swelling to the emergence of the root (The bar = 1 cm). C. Young seedlings with two true leaves that are ready to transfer to polybags. D. Plants prior to treatment, after transplantation, during the initial vegetative phase. E. Plants during the treatment, displaying growth in the later vegetative stage. F. Plant height measurement.

Seed Viability and Vigority of Grobogan Soybean Seeds

Enhancing productivity begins at the seed level, mainly through improving seed viability and vigority. Seed viability and vigority determine the quality of seeds used, encompassing both physical and physiological aspects of seed quality. One method to enhance seed quality is through gamma irradiation techniques (Manjusha et al., 2024), which can increase seed viability and vigority (Ikram et al., 2010). The measurement of seed viability and vigority is often conducted after sowing to evaluate the effects of irradiation dosage

The average viability of Grobogan soybean seeds, assessed through germination rate (GR, Figure 2A), was highest at 98,33%, with the fastest speed of germination (SG, Figure 2B) occurring in 3 days and 7 hours at irradiation doses of 25 Gy and 0 Gy. In contrast, seeds exposed to 100 Gy showed the lowest germination rate (76,66%) and the slowest germination speed (4 days and 4 hours). The average of seed vigority peaked at 80% at 25 Gy. whereas the lowest growth uniformity or seed vigority was observed at 100 Gy, with a value of 45%. Seed vigority is a multifaceted characteristic, not a singular property, encompassing aspects like germination rate, uniformity, and growth potential. Vigority tests are designed to evaluate the performance of seeds even after storage and their ability to maintain germination capacity (Reed et al., 2022).

The optimal irradiation dose for seed germination parameters GR and SG was determined to be 25 Gy. This is because low-dose irradiation generally has a stimulatory effect on germination by increasing enzyme activity, improving cellular respiration, and enhancing the production of reproductive structures (Saibari et al., 2023). Seeds with high germination uniformity exhibit high vigority. Seeds with good/high vigority germinate quickly and uniformly, as rapid and uniform germination indicates that the seeds can adapt to environmental conditions. The higher of germination uniformity indicates the high storage potential of the seeds. Germination non-uniformity can be caused by genetic differences or by non-homogeneous environmental conditions (Noe & Zedler, 2000). This phenomenon is consistent with radiation hormesis, where low doses enhance biological performance by activating antioxidant defenses, DNA repair, and growth-regulating signals (Volkova et al., 2022).

An irradiation dose of 100 Gy resulted in the lowest GR, SG, and Vg responses. This is because high-dose gamma irradiation generally inhibits germination, leading to decreased auxin levels or chromosomal damage (Saibari et al., 2023). Additionally, there is a deterministic effect, which is an effect caused by cell death due to irradiation exposure. The deterministic effect occurs when the dose received by the plant is above the threshold dose and generally occurs shortly after irradiation (Rafiuddin et al, 2015). The inability of seeds to germinate at higher gamma-ray doses has been attributed to several reasons: (1) Numerous histological and cytological changes; (2) Disruption and disorganization of the seed coat or testa in direct proportion to the intensity of gamma-ray exposure; (3) Disruption of mitosis or virtual elimination of cell division in the meristematic zone during germination. The inhibition of seed germination and seedling growth induced by irradiation is often attributed to the formation of free radicals in irradiated seeds (Jan et al., 2012).

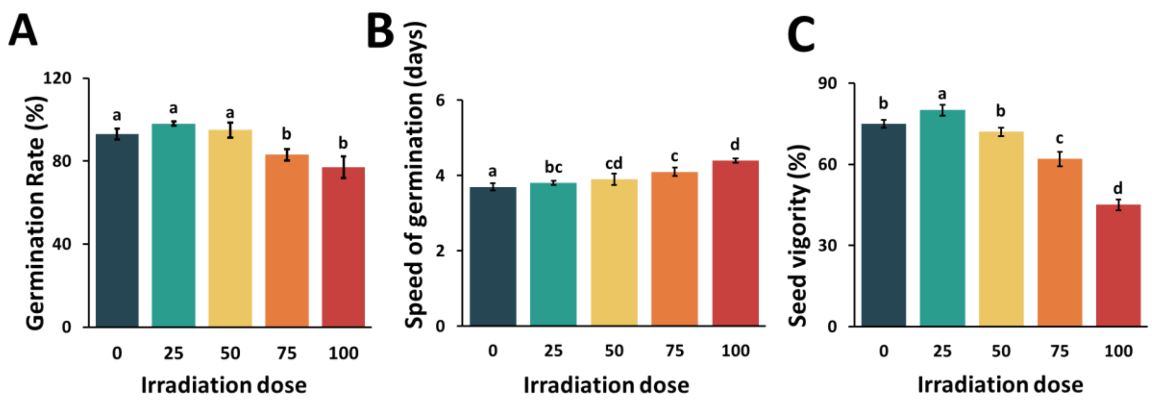

Figure 2. The germination profile of Grobogan Soybean Seeds. A. Germination rate; B. Speed of germination; and C. Seed vigority.

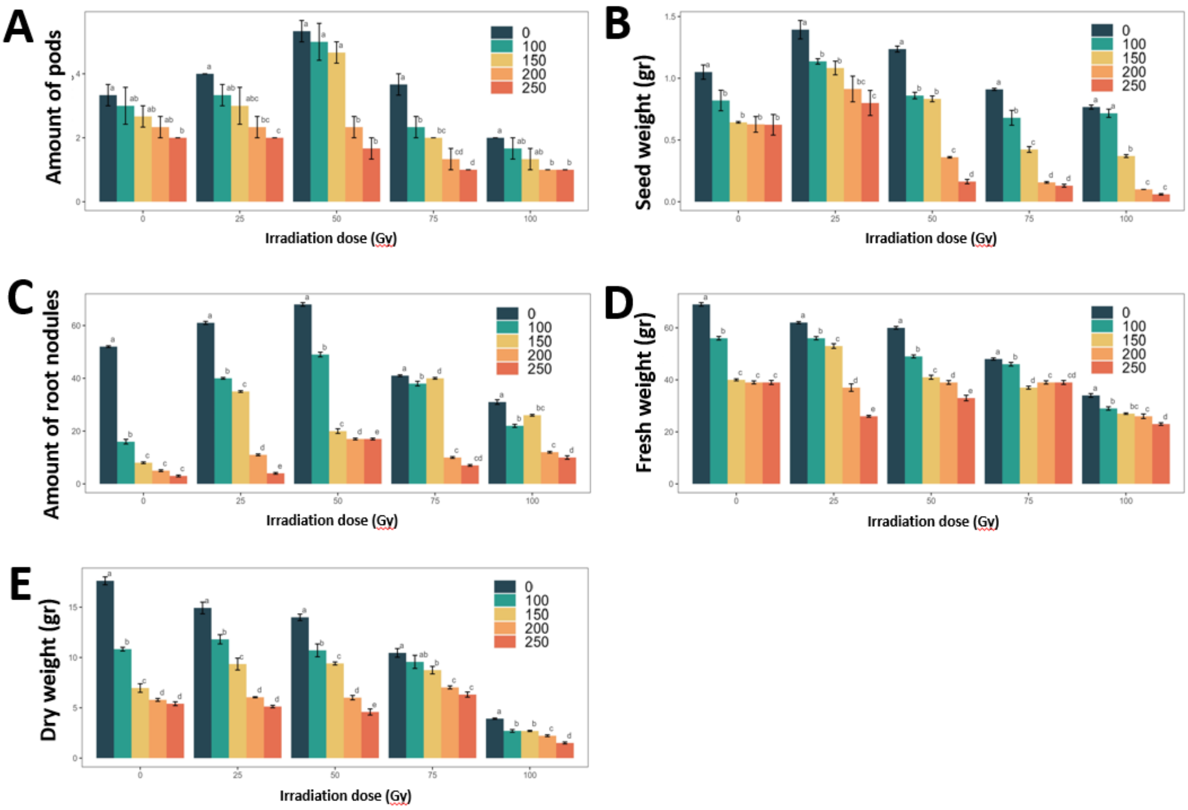

Figure 3. Effect of irradiation dose and waterlogging stress on plant growth parameters of Grobogan soybean variety. A. Number of pods. B. Seed weight; C. Amount of root nodules; D. Fresh weight; E. Dry weight.

Growth and Yield-Related Morphological Traits of Soybean as Affected by Irradiation and Waterlogging

The morphological performance of Grobogan soybean plants was markedly influenced by irradiation dose and waterlogging stress, as reflected in pod production, seed weight, root nodules, and biomass accumulation (Figure 3A–E). Similar reductions in growth parameters under waterlogging stress have also been documented in other crops. In tobacco, waterlogging significantly decreased plant height, root length, dry biomass, and induced adventitious root and aerenchyma formation (Nurhidayati et al., 2021). These observations support the general pattern that waterlogging disrupts morphological performance across plant species. Pod number was highest in plants exposed to 50 Gy under control conditions, averaging five pods per plant (Figure 3A). In contrast, plants subjected to 100 Gy irradiation combined with 250% waterlogging stress produced only a single pod, indicating that excessive irradiation and severe waterlogging synergistically reduce reproductive capacity. Similar observations have been reported in legumes where waterlogging disrupts flower initiation and pod retention, while high irradiation levels impair reproductive development through oxidative stress and DNA damage (Gowdra et al., 2025; Caplin et al., 2018). The importance of pod number in legumes cannot be overstated, as it directly reflects the plant's ability to translate vegetative growth into seed yield. A higher pod count is generally associated with increased productivity, serving as a key trait for breeders and farmers alike (Antra et al., 2024). This suggests that while moderate irradiation may stimulate pod formation, higher doses in combination with flooding stress impair flower and pod development.

Seed weight exhibited a comparable dose-dependent pattern (Figure 3B). In the absence of stress, unirradiated plants generally produced heavier seeds compared to irradiated plants. However, a notable exception was observed at 25 Gy, where seed weight significantly increased under both non-stressed and waterlogged conditions, supporting the concept of radiation-induced hormesis in which low doses stimulate plant metabolism and seed filling (Saibari et al., 2023). At 50 Gy, seed weight remained higher than the control under mild waterlogging (100 and

150%) but declined sharply under severe waterlogging (200 and 250%). Conversely, at 75 and 100 Gy, seed weight consistently decreased across all stress conditions, with the lowest value recorded in plants exposed to 100 Gy and 250% waterlogging. These findings are consistent with previous studies reporting that excessive gamma irradiation reduces assimilate partitioning toward seeds due to impaired photosynthesis and accelerated senescence (Kim et al., 2004; Mohammadi et al., 2005).

Root nodule formation was particularly sensitive to both irradiation and waterlogging stress (Figure 3C). The highest nodule numbers occurred in control plants without irradiation, whereas plants subjected to 100 Gy under 250% waterlogging produced the fewest nodules. The sharp decline in nodulation at high doses suggests impaired rhizobial colonization and disruption of root physiology, possibly due to radiation-induced oxidative damage that interferes with nodulation signaling pathways. Waterlogging itself is known to reduce nodulation and nitrogen fixation in soybean by limiting oxygen diffusion to root tissues (Ploschuk et al., 2022) and the addition of high-dose irradiation appears to intensify this inhibition. Reduced nodulation may have contributed to the overall decline in biomass and seed yield under combined stress conditions (Sathi et al., 2022).

Biomass accumulation, measured as fresh and dry weight, followed a similar trend (Figures 3D-E). Unirradiated plants under non-stressed conditions had the highest fresh and dry weights, reflecting optimal growth in the absence of stress. In contrast, plants treated with 100 Gy under severe waterlogging had the lowest biomass, confirming the detrimental impact of high-dose irradiation in combination with flooding stress. Interestingly, at moderate waterlogging stress (150%), plants exposed to 25 Gy exhibited higher fresh weight than the control, suggesting that low-dose irradiation may enhance stress resilience and support biomass production under suboptimal conditions. Previous reports have linked this stimulatory effect to enhanced antioxidant activity, repair of DNA damage, and improved energy metabolism induced by low radiation doses (Bansal et al., 2022; Yang et al., 2024).

Overall, Grobogan soybeans exhibited a hormetic response to irradiation. Low doses (25-50 Gy) enhanced pod production, seed weight, and biomass under mild to moderate waterlogging, likely by stimulating physiological processes that improve stress tolerance. In contrast, high doses (75-100 Gy) consistently reduced all morphological traits, particularly under severe waterlogging, due to cellular damage, impaired nodulation, and limited assimilate allocation. These findings highlight the dual role of irradiation, with beneficial effects at low doses and detrimental impacts at higher exposures.

ISSR Analysis of Irradiated Plants in the Condition of Waterlogging

The CTAB method was used to extract DNA from soybean leaves. This method is suitable for plants that contain a high concentration of polysaccharides and phenols (Turaki et al., 2017). The modified CTAB method consistently yields DNA of sufficient quality and quantity for PCR-based molecular analysis (Nunes et al., 2011; Dubey et al., 2017). A PCR-based molecular marker, such as ISSR (Inter-Simple Sequence Repeats), is widely used for evaluating genetic variation among plant species due to its high reproducibility and consistency compared to RAPD (Reddy et al., 2002; Nghia et al., 2007). In soybeans, ISSR has been effectively used to detect genetic polymorphisms in gamma-irradiated populations exposed to waterlogging stress (Saputro et al., 2019). In that study, primers such as (AC)8G yielded the highest polymorphism and PIC values, supporting their reliability in identifying tolerant variants. This research employed ten ISSR primers to evaluate the genetic diversity of soybean under waterlogging stress.

Among the primers tested, ISSR-1 showed the most polymorphisms, with 9 loci (4 polymorphic), 45 bands, and a PIC value of 0.391. Polymorphisms were observed at 75 Gy and 100 Gy. ISSR-2 formed 6 loci (1 polymorphic), 30 bands, and a PIC value of 0.64, with polymorphism observed at 25 Gy. ISSR-3 formed 8 loci (1 polymorphic), 40 bands, and a PIC value of 0.32 with polymorphisms observed at 0 Gy, 25 Gy, and 100 Gy. ISSR-4 formed 7 loci (1 polymorphic), 35 bands, and a PIC of 0.055. Polymorphisms observed at 75 Gy. ISSR-5 formed 6 loci (2 polymorphic), 30 bands, and a PIC of 0.357, with polymorphisms showing up at 75 Gy. These results indicate that ISSR-1 and ISSR-2 are the most effective primers for detecting genetic variations between irradiated soybean genotypes.

Table 1. Polymorphism characterization of irradiated soybean genotypes using ISSR primers

Primer Name	Sequence	Tm (°C)	Total Loci	Total Polymorphic Loci	Percentage of Polymorphic Loci	Total Bands Formed	Total Polymorphic Bands	PIC Value
ISSR-1	(AC)8G	53.0	9	4	44.4	45	12	0.391
ISSR-2	(TCC)5GC	58.8	9	1	16.7	30	1	0.064
ISSR-3	(GA)8T	45.4	8	4	50	40	8	0.32
ISSR-4	(GT)8TC	51.6	7	1	13	35	1	0.055
ISSR-5	(AG)8T	47.0	6	2	33	30	7	0.357
Total			36	12	147.1	190	29	1.187
Average			7.2	2.4	29.42	38	5.8	0.24

The primers ISSR-1 to ISSR-5 amplified a total of 36 loci, with an average of 7.2 loci per primer and 12 polymorphic loci (2.4 on average). ISSR-1 had produced the highest number of bands (45) and the highest PIC value (0.391), while the lowest PIC (0.055) was observed in ISSR-4. The number and intensity of DNA bands were primer-dependent, indicating the primers' capacity to anneal with complementary sequences. According to the PIC classification by Botstein et al. (1980), ISSR-1, ISSR-3, and ISSR-5 are moderately informative ($0.25 < PIC < 0.50$), while ISSR-2 and ISSR-4 are less informative ($PIC < 0.25$). These results demonstrate that gamma radiation induces genetic variation in Grobogan soybeans, with ISSR-1 serving as the most effective primer for identifying these alterations.

This finding also underlines the novelty of the study, as gamma irradiation successfully generated new soybean variants with previously unknown genetic characteristics that require further selection to evaluate their tolerance to waterlogging stress. Beyond methodological insights, the study contributes scientifically by expanding the application of ISSR markers to mutation-derived diversity under abiotic stress. From a practical perspective, the results provide a basis for identifying promising soybean variants that could support breeding programs aimed at developing waterlogging-tolerant cultivars, thereby contributing to sustainable agriculture and national food security.

CONCLUSION

This study investigated the genetic variation and morpho-physiological traits of irradiated Grobogan soybean under waterlogging stress. Low doses of gamma irradiation (25–50 Gy) enhanced seed vigority, biomass, and yield-related traits, while higher doses (≥75 Gy) reduced performance due to physiological and genetic damage. ISSR marker analysis confirmed genetic polymorphisms, with ISSR-1 identified as the most informative primer. These findings highlight that gamma irradiation at optimal doses can generate soybean lines with improved tolerance to waterlogging, offering a potential strategy for sustaining production in flood-prone areas. Future research should validate the stability of selected lines across generations and field conditions, while integrating molecular and omics approaches to better elucidate mechanisms of tolerance

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AUTHOR CONTRIBUTION STATEMENT

A.S. wrote the original manuscript, performed data and formal analysis, and processed the data. D.I. contributed to data and formal analysis as well as data processing. B.A.M. was involved in data and formal analysis and data processing. A.S.F. contributed to data and formal analysis and data processing. W.M. was responsible for material and data collection in addition to formal analysis. T.N. supervised data analysis and data processing. R.C. contributed to manuscript writing and data analysis. T.B.S. conceptualized and designed the study, provided funding, supervised the project, and finalized the manuscript. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the research.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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