Radiosensitivity of post-gamma irradiated *Indigofera zollingeriana*

P I Khaerani\(^1\), Y Musa\(^2\), R Sjahri\(^2\) and M Nadir\(^3\)

\(^1\)Postgraduate Program, Agriculture Science, Universitas Hasanuddin Makassar, 90245 Indonesia
\(^2\)Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin Makassar, 90245 Indonesia
\(^3\)Faculty of Animal Science, Universitas Hasanuddin Makassar, 90245 Indonesia

E-mail: yunusmusa@yahoo.com

**Abstract.** Gamma Irradiation by employing gamma-ray enables genetic transformation to produce the target gene activation functioning as a productivity determinant. A number of studies had found that the employment of gamma-ray is capable in improving the plant’s tolerance against drought treatment of irradiation. The purpose of this study was to determine the Lethal Dose 50 (LD\(_{50}\)) and Reduction Dose 50 (RD\(_{50}\)) of *Indigofera zollingeriana* post gamma irradiated. The irradiation dosage consisted of 0Gy (control, 50 Gy, 100 Gy, 150 Gy, and 200 Gy. Each dose consisted of 250 seeds were sown in the tray for 2 weeks after germination to determine the values of LD\(_{50}\) and RD\(_{50}\). Each LD\(_{50}\) and RD\(_{50}\) value of the Indigofera seeds was determined by processing the data on the survival rate of the radiation dose treatment survival rate using the Curve Expert 1.3 program. The observed parameter encompassed the germination percentage, seedling length, and seedling vigor index. This research shows the percentage of germination showed a value of 

\[ 45.125 + 0.11 X -0.0006 x^2 \]  
with LD\(_{50}\) = 320.282 Gy; seedling length = 4.156 - 0.00472 X, with RD\(_{50}\) = 406.356 Gy; and Seedling vigor index = 153.916 - 0.31244 X with RD\(_{50}\) = 246.435 Gy.

1. **Introduction**

The availability of good quality forages is one key component in achieving successful livestock maintenance and enterprise. A sufficient and sustainable amount of feed should also be taken into account. There are a number of methods to improve forage productivity, including agricultural intensification, plant breeding, plant protection against pest and disease, and the employment of high-quality plant variety. However, these methods are commonly restricted by the availability of productive land. Therefore, one effort to sustain forage availability, especially during the dry season and low groundwater availability, is to utilize tolerant alternative forages against stresses and marginal land. To ensure the availability of tolerant forages, plant breeding was performed.

Legume is one of perennial forages that plays an important role in sustaining forages efficiently with good nutritional content and digestibility [1]. One type of legume forages with good nutritional content and digestibility is *Indigofera zollingeriana*. This legume also possesses excellent adaptability. A number of studies had indicated the tolerance capability of Indigofera against stresses and drought [2-4].
Plant genetic modification through plant breeding could produce better plant characteristics than the original species [5]. Plant breeding programs can be performed using physical mutagens, for example, gamma rays [6, 7]. Increased genetic variability can be achieved by mutation treatment (irradiation). Gamma-ray irradiation is a treatment of (artificial) mutation induction using gamma rays that produce resistant plants [8]. Gamma rays also contribute to physiological damages such as growth inhibition, sterility, and plant death at M1 generation and therefore, it was majorly applied to increase genetic variability. Physiological symptoms in plants exposed to different Gamma rays have been investigated in a number of studies [9-14]. The symptoms frequently observed in plants irradiated both at low and high dosage are the stimulating or inhibitory germination, seed growth, and other biological responses [5, 12].

Some benefits from gamma rays are a more accurate dosage, highly penetrating irradiation to a homogenous cell encouraging newly modified genetic combinations with high mutation frequency [15]. Gamma rays are electromagnetic short wavelengths with high energy interacting with atoms and molecules, producing free radicals in cells. These free radicals will induce mutation in plants because they produce cell damages or contribute important effects on the plant's cell components [16, 17].

A dosage determination was performed to identify the dosage of gamma-ray radioactive irradiation on a plant variety because each organism or material has a different radiosensitivity [18]. The initial experiments for investigating the optimal irradiation dosage are performed to simulate genetic variability by identifying the LD$_{50}$ and RD$_{50}$ values. Plant breeding could promote quantity and the quality improvement of tolerant plants against abiotic stress such as drought and salinity through the plant's modified characters [5]. Therefore, this study focused on forage genetic improvement, especially for Indigofera legume, by employing physical mutagens of gamma rays.

2. Material and methods
The early experiment was performed to identify optimal irradiation dosage that may stimulate genetic variability through LD$_{50}$ and RD$_{50}$ value identification. In identifying the values of LD$_{50}$, Indigofera zollingeriana was irradiated at a dosage of 50, 100, 150, and 200 Gray excluding the 0 Gray treatment as control. Irradiation was performed at PAIR-BATAN (National Nuclear Energy Agency of Indonesia) with 300 seeds for each irradiation treatment. The post-irradiated seeds were planted in trays and their germination percentage, germination length, seedling length, seedling vigor index 4 weeks after planting to identify LD$_{50}$ and RD$_{50}$ values. Each value of LD$_{50}$ and RD$_{50}$ from Indigofera seeds was identified by processing the survival rate data using Curve Expert 1.3.

3. Results and discussion
The effect of irradiation in improving the variability was determined by the radiosensitivity of irradiated plants. Variability induction can be revealed through LD$_{50}$ calculation results. Radiosensitivity curve for Indigofera was presented according to the Curve Expert 1.3. analysis calculation. The analysis results, including germination percentage, seedling length, and seedling vigor index, are presented in tables 1 and 2 and figure 1-3.

Table 1. The LD$_{50}$ of gamma-ray post-irradiated Indigofera seed at 32 days after germination.

| Parameter                  | Model     | Equation                        | R       | LD$_{50}$ |
|---------------------------|-----------|---------------------------------|---------|-----------|
| Germination Percentage (%)| Quadratic | $45.125 + 0.11X -0.0006X^2$     | 0.2146  | 320. 282  |

The curve of the radiosensitivity test is presented in figure 1. which indicated a curve shape with different patterns. S value is the curve intersection value. R value is the accuracy value (almost or more accurate).
Figure 1. Radiosensitivity curve of the gamma irradiation effect on germination percentage.

The result of the radiosensitivity test curve is presented in figure 1. The plot indicated a curve shape with different patterns. S value is the curve intersection value. R value is the accuracy value (almost or more accurate). Indigofera has a quadratic curve pattern where the irradiation treatment at the dosage of 50 Gy could increase a higher survival rate compared to the control treatment (0 Gy). However, a decrease was identified in other irradiation dosages.

The gamma irradiation treatment at low dosage on wheat in the study performed by Singh & Datta [19] could be used as an example for stimulating a plant's vigor. This phenomenon was known as hormesis, a stimulating process that occurred in plant's seeds such as acceleration in germination, leaf, and root growth as a consequence of low dosage irradiation. Gamma-ray is an electromagnetic wave triggering ionizing radiation resulting in biological effects such as inhibition, stimulation, mutation, and cell death. Low dosage gamma-ray irradiation could promote the germination percentage and seed growth [6], [20-22].

Based on the study performed by Sri [23] and Yamaguchi [24] the range of dosages in achieving optimal genetic variability was in LD20 - LD50. The radiosensitivity of a plant has variability depending on genetic makeup, irradiation level, the amount of DNA, moisture content, and genotype growth stage [25, 26].

One possible method to identify the radiosensitivity level of a plant to gamma-ray irradiation is to find out its reduction dose (RD50) [27, 28]. One trend that can be observed from the seedling length and seedling vigor index was the higher the irradiation dosage, the more it affects the growth response of a plant. The reduction dose (RD50) equation on both parameters is presented in table 2.

Table 2. RD50 of post-irradiated Indigofera seed after 32 days of germination.

| Parameter               | Model | Equation               | R    | RD50   |
|-------------------------|-------|------------------------|------|--------|
| Seedling length (cm)    | Linear| 4.156 - 0.00472 X     | 0.611| 406,356|
| Seedling vigor index    | Linear| 153.916 – 0.31244 X   | 0.951| 246.435|
The lower the RD$_{50}$ of a plant, the higher its radiosensitivity. As it was known that RD$_{50}$ is the irradiation dosage where 50% of plants experience growth inhibition (abnormal). The result of the radiosensitivity test curve is presented in Figure 2 and Figure 3. The plot indicated a curve shape with linear pattern. S value is the curve intersection value. R value is the accuracy value (almost or more accurate). Indigofera has a linear curve pattern where the irradiation dosage treatment decreases the seedling length and seedling vigor index. It was presumed that gamma rays damaged the plant's chromosome arrangement and therefore had an effect on the plant's growth. This is in line with the study performed by Kim et al. [14] and Surya et al. [29] the higher the radiation dosage, the lower the plant's growth. The decrease in plant growth occurred due to the damages to the plant's chromosome caused by irradiation.

Some studies concerning the effect of irradiation on germination. Arvind-Kumar and Mishra [30] found that the gamma rays at 10 and 20 Kr combined with the other mutagens increase seedling vigor index. Rao and Suvatha [31] found that gamma rays at around 30 Kr resulted in the highest germination percentage in tomato seeds. Ahmadi et al. [32] confirmed that soaking seeds for 12 hours could increase growth acceleration, vigor index, and seedling dry weight of various wheat cultivars.
Findings from a study performed by Nobre et al. [33] confirmed that seed vigor was affected by irradiation dosage and storage duration. The index of the seeds' vigor tended to decrease as the gamma irradiation dosage and storage duration increased. The dosage between 50 and 100 Gy indicated low or no effect on seed viability and vigor compared to control seeds (0 Gy). Gamma irradiation at a lower dosage than 25 and 50 Gy could significantly promote germination index and resistance compared to no irradiation.

4. Conclusion
The effect of irradiation in improving the variability was determined by the radiosensitivity of irradiated plants. Variability induction could be identified by the calculation of LD$_{50}$ and RD$_{50}$ values. The LD$_{50}$ value of Indigofera was around 320 Gy and RD$_{50}$ ranges between 246-406 Gy. Although radiosensitivity curve demonstrating a quadratic and linear shape does not have a significant effect on the observed parameters of irradiation percentage, seedling length, and seedling vigor index, however, it can be seen that the higher the irradiation dose applied, the lower the germination percentage, seedling length, and seedling vigor index. This study requires a more in-depth research in terms of accurate irradiation dosage on Indigofera seed.

References
[1] Radovic L R 2009 Active Sites in graphene and the mechanism of CO2 formation in carbon oxidation J. Am. Chem. Soc. 131 (47) 17166–17175
[2] Abdullah and Suwarlinn 2010 Herbage yield and quality of two vegetative parts of indigofera at differen Lt times of first regrowth defoliation Media Peternak 33 (1) 44–49
[3] Nadir M Ansyar I Khaerani P I and Syamsuddin 2019 Effect of various polyethylene glycol concentrations on the growth of seedlings of Indigofera zollingeriana IOP Conf Ser Earth Environ Sci 343 012040
[4] Nadir M Anugrah M J and Khaerani P I 2018 Salt Salinity Tolerance on Nursery of Indigofera zollingeriana IOP Conf Ser Earth Environ Sci 156 (1) 012027
[5] Shu Q Y Forster B P and Nakagawa H 2012 Plant Mutation Breeding and Biotechnology (Wallungford: CABI 2012)
[6] Beyaz R and Yildiz M 2017 The Use of Gamma Irradiation in Plant Mutation Breeding (Plant Breeding) Plant Engineering Chapter 3 Snježana Jurić, IntechOpen, DOI: 10.5772/intechopen.69974. Available from: https://www.intechopen.com/books/plant-engineering/the-use-of-gamma-irradiation-in-plant-mutation-breeding
[7] Soedjono S 2003 Application of induced mutation and somaclonal variation in plant breeding J Litbang Pertan 22 (2) 70–78
[8] Ahloowalia B S Maluszynski M and Nichterlein K 2004 Global impact of mutation-derived varieties Euphytica 187–204
[9] Yamaguchi H 2018 Mutation breeding of ornamental plants using ion beams Breed Sci. 68 (1) 71–78
[10] Cheema A A and Atta B M 2003 Radiosensitivity studies in basmati rice Pakistan J. Bot. 35 (2) 197–207
[11] Vandenhove H Vanhoudt H Cuypers A Van Hees M Wannijn J and Horemans N Life-cycle chronic gamma exposure of Arabidopsis thaliana induces growth effects but no discernable effects on oxidative stress pathways Plant Physiol. Biochem. 2010
[12] Shahabi S Farhood N Majdabadi A… Fatem S M 2014 Effect of gamma irradiation on structural and biological properties of a PLGA-PEG-hydroxyapatite composite Sci World J 14 1-8
[13] Akyuz S Akyuz T Celik O and Atak C 2013 FTIR and EDXRF investigations of salt tolerant soybean mutants J Mol Struct 1044 67–71
[14] Kim S Y Sung S Y Jo Y D Lee H J and Kim S H 2016 Effects of gamma ray dose rate and sucrose treatment on mutation induction in chrysanthemum Eur J Hortic Sci 81 (4) 212–218
[15] FAO/IAEA Mutation Breeding Newsletter Report from the FAO/IAEA Plant Breeding and
Genetics Section FAO/IAEA 1987
[16] Kovács E and Keresztes 2002 Effect of gamma and UV-B/C radiation on plant cells Micron. 3 199–210
[17] Shaban M 2013 Review on physiological aspects of seed deterioration Int. J. Agric. Crop. Sci. 11 (6) 627–631
[18] National Nuclear Energy Agency of Indonesia (BATAN) 2012 New Varieties of Soybean from Radiation Mutation Breeding (Jakarta: Atoms Media Informasi Ilmu Pengetahuan Teknologi Nuklir)
[19] Singh B and Datta P S Effect 2010 of low dose gamma irradiation on plant and grain nutrition of wheat Radiat. Phys. Chem. 79 (8) 819–825
[20] Hakeem K R 2015 Crop Production and Global Environmental Issues Springer
[21] Songsri P Surinam B Sanitchon J Srisawangwong S and Kemsala T 2011 Effects of gamma radiation on germination and growth characteristics of physic nut (Jatropha curcas L) J. Biol. Sci. 11 (3) 268–274
[22] Mokobia C E and Anomohanran O 2005 The effect of gamma radiation on the germination and growth of certain Nigerian agricultural crops J. Radiol. Prot. 25 (2) 181
[23] Suhesti S Khumaida N Wattimena G H Syukur M Husni A and Hadipoenyanti E 2015 Gamma irradiation and in vitro selection could increase drought tolerance in sugarcane Int. J. Sc.i Basic Appl. Res. 23 (2) 370–380
[24] Yamaguchi Y Shimizu A Degi K and Morishita T 2008 Effects of dose and dose rate of gamma ray irradiation on mutation induction and nuclear DNA content in chrysanthemum Breed. Sci. 58 (3) 331–335
[25] Ambavane A R, Sawardekar S V Sawantdesai S A and Gokhale N B 2015 Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (Eleusine coracana L Gaertn) J. Radiat. Res. Appl. Sci. 120–125
[26] Nurmansyah M Alghamdi S S Migdadi H M and Farooq M 2018 Morphological and chromosomal abnormalities in gamma radiation-induced mutagenized faba bean genotypes Int J Radiat Biol 174–185
[27] Herison S I A Rustikawati C Sujono H S 2008 Mutation induction through gamma rays on seeds to increase the diversity of the basic population of maize (Zea mays L) Akta Agrosia 11 (1) 57–62
[28] Lelang M A Setiadi A and Fitria 2016 Effect of gamma irradiation on the performance of the plant seed from the chicken’s comb (Celosia cristata L) Savana Cendana 1 (1) 47–50
[29] Surya M and Soeranto 2006 Effect of Gamma Irradiation on the Growth of Sweet Sorghum (Sorghum bicolor L) (Pus Apl Teknol Isot dan Irradiasi BATAN)
[30] Arvind-Kumar M M N 2004 Effect of gamma-rays EMS and NMU on germination seedling vigour pollen viability and plant survival in M1 and M2 generations of okra (Abelmoschus esculentus L Moench) Adv. Plant. Sci. 17 (1) 295–297
[31] Rao P K and Suvartha C 2006 Effect of gamma rays on in vivo and in vitro seed germination seedling height and survival percentage of Lycopersicon esculentum cv Pusa Ruby Adv. Plant. Sci. 19 (2) 335–339
[32] Ahmadi A Sio-Se A Mardeh Poustini K and Esmailpour M Jahromi 2007 Influence of osmo and hydopriming on seed germination and seedling growth in wheat (Triticum aestivum L) cultivars under different moisture and temperature conditions Pakistan J. Biol. Sci. 10 (22) 4043–4049 2007
[33] Nobre D A C Sediyama C S Arthur V Piovesan N D and Da Silva A S H 2016 High gamma irradiation doses and long storage times reduce soybean seed quality Semin. Agrar.