
| RESEARCH ARTICLE

Testing the Quality of Chilli Seeds Resulting from Gamma-Ray Irradiation and Genetic Diversity

Zulkifli Maulana¹ ✉ Muhammad Arief Nasution², Andi Muhibuddin³, Rachmawaty⁴ and Haeruiddin Saleh⁵

^{1,2,3}Department of Agrotechnology, Faculty of Agriculture, Bosowa University, Makassar, 90245, Indonesia.

⁴Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Makassar, Indonesia.

⁵Department of Economics, Faculty of Economics and Business, Bosowa University, Makassar, Indonesia.

Corresponding Author: Zulkifli Maulana, **E-mail:** zulkifli.maulana@universitasbosowa.ac.id

| ABSTRACT

Gamma-ray irradiation is used to improve seed germination and plant seedling growth and is a way to increase plant genetic diversity. This research aims to (1) analyze the response of seed irradiation to germination and (2) identify genetic diversity. This study used a Randomized Group Design (RAK), which consisted of 6 treatment levels used, namely R0 = 0 Gy (control), R1 = 100 Gy, R2 = 200 Gy, R3 = 300 Gy, R4 = 400 Gy and R5 = 500 Gy. Each treatment had 20 replications, so there were 100 experimental units. Based on the results of this research show that the reticulum emergence test can be used as a basis for determining the germination and vigor of mutant and non-mutant chili seeds, while the use of the special molecular marker RAPD can be used to determine the level of diversity for mutant chili plants.

| KEYWORDS

Chili seeds, irradiation, germination and genetic diversity.

| ARTICLE INFORMATION

ACCEPTED: 01 June 2024

PUBLISHED: 05 June 2024

DOI: 10.32996/bjbs.2024.4.1.3

1. Introduction

Chili is a horticultural plant and is used in traditional medicine (Karma et al., 2019). The production is used domestically and exported in dry products. Chili is used as a stimulant, carminative, and tonic, and also to treat asthma, impotence, symptoms of fever, colds, influenza, cholera, anthelmintic, antifatulent, expectorant, antitussive, antifungal, and cholesterol medication.

Indonesia has experienced an increase in chili production from year to year. The Central Statistics Agency (BPS) noted that national chili production reached 2.77 million tons in 2020. This figure increased by 183.96 thousand tons or 7.11% compared to 2019. Throughout 2020, the highest chili production occurred in August, reaching 280.78 thousand tons with a harvest area of 73.77 thousand hectares. East Java Province is the largest chili producer in Indonesia, producing 784.05 thousand tons or 28.28% of the national chili production (Rizaty, 2021).

Even though there is an increase in production every year, the stability of chili production throughout the year cannot be maintained; in fact, in various regions, there is still an imbalance between production and demand (Pankrasius et al., 2022). If production decreases and demand is high, it will cause inflation. This phenomenon occurred in March 2022, which resulted in inflation increasing by 0.66%; the main contributor to this inflation, among others, came from red chili commodities (Silaban, 2022; Andi & Nailah, 2023; Hesti et al., 2024) Chili production decreased Due to significant climate change, superior varieties are needed, which are resistant to biotic stress factors and abiotic stress factors. (Surni et al., 2023)

Meanwhile, if chili production is high, followed by less demand, then there will be overproduction, which will result in drastically lower chili prices. If this happens, it will be very unprofitable for farmers. Sometimes, farmers' production costs exceed the price of chilies that farmers get. This phenomenon occurred on September 3, 2021, when the price of large red chilies fell 3.38 percent to IDR 28,600 per kilogram. Meanwhile, red bird's eye chilies fell 1.83 percent, and curly red chilies fell 2.77 percent per Ha (National Food Price Strategy Information Center (PIHPS), 2021).

Given the two problems mentioned above, superior varieties that have high adaptability to climate change are needed, in addition to high production and metabolic compounds. Superior varieties with broad adaptability are expected to be more tolerant to both biotic and abiotic stresses. With this character, it is hoped that it can adapt to extreme environments, so it is hoped that it will still have high productivity.

Under certain conditions, chili production is sometimes abundant, so chili farmers sometimes throw away their harvest. This action by farmers would not need to be taken if farmers had a good understanding of Good Handling Processing (GHP) (Lukas, 2023; Anggra & Medria, n.d.). If previously farmers only understood selling in the form of chilies (unprocessed), with an understanding of GHP, all parts of the chili plant, starting from stems, leaves, and fruit, could become an alternative and opportunity for the red chili industry. Apart from that, chilies have natural compounds that can provide benefits to humans. One of the most important compounds in chilies is capsaicin, which is a secondary metabolite. In other research, chemical compounds related to secondary metabolites were alkaloids, terpenoids, steroids, and saponins. Chili has a spicy taste and distinctive smell mediated by capsaicinoid compounds. Capsaicinoids include ordihydrocapsaicin, capsaicin, dihydrocapsaicin, norcapsaicin, homodihydrocapsaicin, homocapsaicin, and nonivamide. In addition, chilies have high nutritional value and are an excellent source of vitamins C and A, along with minerals such as folate, potassium, thiamine, molybdenum, and manganese. The antioxidant properties in chilies are caused by -carotenoids and vitamins A and C (Kumar, T. H., 2020); Amit et al., 2022; Monika et al., 2023; Esther et al., 2023)

With various forms of potential processed chili products and secondary metabolite products that are very beneficial for health and can provide added value for farmers, it is necessary to develop superior varieties that have the characteristics of being resistant to environmental stress (biotic and abiotic), high productivity and containing secondary metabolism tall. According to (Ruiying et al., 2023), to develop superior varieties, it is necessary to expand genetic diversity, one of which can be done by radiation mutation.

Through radiation mutations, new genetic diversity will be obtained, thus providing more opportunities for selection. According to Suprasanna (2013), developing the potential of local plants needs to be done by improving the characteristics of existing local varieties so that productivity increases, is resistant to biotic and abiotic stress, and has high secondary metabolic characteristics, one of which is by mutation breeding. The advantage of mutations in plants is that changes occur in the genotypic structure so that species variability increases and can support plant adaptability to various selection pressures (Salas, R. A et al., 2013; Brain et al., n.d.) These mutations can be induced by physical mutagenic agents such as ionizing radiation (X-rays and gamma rays), non-ionizing radiation (ultraviolet), and cellular radiation (protons, neutrons, alpha, and beta particles) (Ludovici, et. al., 2022; Gian et al., 2022; Hara, 2023).

One of the local chili genotypes that has the potential to be developed is Katokkon chili. Katokkon chilies are one of the leading local commodities in TanahToraja Regency, South Sulawesi Province, which needs serious attention in development efforts (Marano et al., 2017; Peter & Tatiana, 2017; Budi & Reisky, 2024). Katokkon chilies have good potential to be developed because apart from their spicy taste, they also have a distinctive aroma with a specific spicy taste; they have a unique shape like small peppers and have been registered with the Center for Plant Variety Protection and Agricultural Licensing.

Efforts to increase the genetic diversity of the katokkon chili population have never been carried out either through conventional or non-conventional techniques. For this reason, a structured and targeted plant breeding program is needed to obtain superior varieties from the genetic mutations of this chili. The specific aim of this research is to expand the genetic diversity of katokkon chilies through gamma-ray induction so that selection can be carried out to obtain new superior varieties that suit the objectives of this breeding program. In the end, the Regional Government of Tanah Toraja Regency, in particular, and the Regional Government of South Sulawesi Province, in general, received new superior varieties which have different characteristics from those of other chilies. The urgency of this research is to overcome fluctuations in chili production that occur every year as a result of inconsistent chili productivity caused by climate change, pest and disease attacks, and the implementation of crop management that is not yet optimal. Various efforts have been made to overcome problems in the field of plant breeding science. Related to the above and especially considering that Indonesia is a country with high biodiversity, one strategy that has great potential to increase the productivity, quality, and competitiveness of plant commodities is through a plant breeding approach. Through breeding activities, it is hoped that a variety of new superior cultivars can be produced, which, apart from having high productivity, also have several other characteristics that support efforts to improve quality and competitiveness. Plant breeding itself is defined

as a series of research activities and genetic development of plants (modification of genes or chromosomes) to produce superior cultivars/varieties that are useful for human life (Priyadarshini et al., 2024)

2. Research Methods

This research starts from September to November 2022. Gamma-ray irradiation treatment was carried out at the Central Irradiation and Instrumentation Laboratory of the National Atomic Energy Agency (BATAN) South Jakarta, Jakarta, and seed experiments were carried out at the Science Laboratory of the Faculty of Agriculture, Bosowa University, while field experiments were carried out at Bosowa University Faculty of Agriculture Education Garden in Gowa Regency.

The activities carried out are as follows:

1. **Gamma-ray radiation treatment** of katokkon chili seeds and seed quality testing. This research used a Randomized Group Design (RAK) consisting of 6 treatment levels, namely R0 = 0 Gy (control), R1 = 100 Gy, R2 = 200 Gy, R3 = 300 Gy, R4 = 400 Gy and R5 = 500 Gy. Each treatment had 20 replications, so there were 100 experimental units.

The chili seeds that will be irradiated are first selected and selected according to the desired seeds, namely normal, good, and healthy seeds. After getting good seeds, they are put in plastic packaging, and the seeds are sent to the lab. Radiation Process Technology- Laboratory. Irradiation, Directorate of Laboratory Management, research facilities, and science and technology areas. National Research and Innovation Agency (BRIN) Jakarta – Lebak Bulus to carry out gamma ray radiation according to the predetermined dose. The chili seeds that have been irradiated are then sown to test the quality of the seeds at the Seed Laboratory, Faculty of Agriculture, Bosowa University.

Chili seeds that have been irradiated with gamma rays before sowing are first soaked in lukewarm water (temperature 43 oC) for \pm 24 hours. This aims to speed up seed germination, in addition to separating submerged seeds from floating seeds. Floating seeds are discarded because floating seeds are not good. The chili seeds sown in each treatment consisted of 100 seeds. Because there are 6 treatments, the seeds needed for the test are $6 \times 3 \times 30 = 540$ chili seeds. The chili seeds were sown as many as 30 seeds per petri dish. After that, observations were carried out every day for 14 days.

Observation parameters include:

(1) Germination Power (DB): The germination test was carried out by testing on paper (top paper) using filter paper; in an electric germinator, the temperature changed by 20-30°C. Observation and counting of normal sprouts was carried out on the 7th and 14th days (27).

(2) Vigor Index (IV), using the following formula:

$$\text{Vigor Index} = \frac{\sum \text{normal sprouts on the 4th day}}{\sum \text{germinated seeds}}$$

Observation of the vigor index was carried out by calculating the percentage of normal sprouts on the 7th day. The growth speed value was carried out by calculating the percentage of normal sprouts for each etmal (24 hours) starting from day 1 to day 14. (Deandrasari et al., 2023; Nura & Halimursyadah,

(3) Growth Speed (K_{cr}), using the following formula:

$$\sum_{k=0}^n \frac{n}{t}$$

Information:

K_{cr} = Growth speed (%KN/etmal); N = Percentage of normal sprouts at each observation time; t = Observation time; tn = End of observation time

(4). Radicle Emergence Test, using the following formula:

$$\text{Radicle Emergence Test} = \frac{\sum \text{The radicle appears}}{\sum \text{seeds germinate}} \times 100\%$$

Observations of the average germination time were carried out from 24 to 168 hours. The criterion for seeds to germinate is the appearance of a minimum radicle of 2 mm. The average germination time is calculated every 24 hours (Ellis, RM & Roberts, E 1980; Ullah, 2023). The results of test observations in the seed laboratory used a randomized block design with one factor, namely the dose of gamma-ray irradiation. After that, an analysis of variance (ANOVA) was carried out, and the Duncan Multiple Range Test (DMRT) was continued at the 5% level. Correlation coefficient calculations were carried out to calculate the close relationship between the radicle emergence test values and other test benchmarks.

2.1 Molecular Marker Analysis

Research on genotypic diversity was carried out at the Molecular Laboratory of the Research Center for Biotechnology and Agricultural Genetic Resources (BB-Biogen) Agricultural Research and Development, Ministry of Agriculture of the Republic of Indonesia. Genotypic observations were carried out using DNA band pattern analysis based on the RAPD technique. Carrying out RAPD analysis includes two main activities, namely a) DNA isolation (template preparation) and b) RAPD analysis.

2.2 Isolation and purification of chili plant DNA

DNA isolation was carried out by extracting DNA from 30 young leaves of mutant chilies, which came from gamma-ray irradiation. DNA isolation was carried out following the Doyle and Doyle CTAB method (Doyle JJ., JL. Doyle. 1987; Bignya et al., 2023). The material analyzed was 0.5 g of young leaves and cut into small pieces. The leaf pieces are put into a mortar, then PVPP and liquid nitrogen are added, and then crushed until smooth. The scoured results were put into an Eppendorf tube containing 600 µL of CTAB extract buffer solution (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM ethylene diamine extra acetic acid (EDTA) pH 8.0, 2% (m/v) cetyltrimethylammonium bromide (CTAB), and 0.2% β-mercapto-ethanol), the mixture was shaken and heated in a water bath for 30 minutes at 65oC (every 10 minutes the mixture was turned). The mixture was allowed to reach room temperature, and then 600 µL of chloroform-isoamyl alcohol solution (24:1) was added and shaken. Centrifuge the mixture at room temperature at 12000 rpm for 15 minutes to form two liquid phases. The upper liquid phase (supernatant) was poured into a new tube, and then the same volume of cold isopropanol was added and stored in the freezer overnight. The mixture was thawed at room temperature and centrifuged at 12,000 rpm for 15 minutes. The liquid was carefully discarded, and the pellet was added with 100 µL of cold 70% ethanol, then centrifuged at 12,000 rpm for 5 minutes. The liquid was discarded, and the pellet was dried by inverting the tube. The dried pellets were added to 100 µL of ion-free water and shaken until dissolved.

DNA purification using the method of Sambrook et al. (1989). The DNA solution was added with 1 µL of RNAase and left at room temperature for 2 hours. Next, add 100 µL of cold isoamyl alcohol phenol-chloroform and centrifuge at 12,000 rpm for 15 minutes. The supernatant was pipetted into a new tube, and an equal volume of isoamylalcohol chloroform was added. Then, it was centrifuged at 12,000 rpm for 15 minutes. The supernatant was pipetted into a new tube, and 1/10 volume of 3 M sodium acetate pH 5.2 and 2.5 volume of cold isopropanol were added. The solution was shaken until homogeneous and stored in the freezer overnight. The solution was centrifuged at a speed of 12,000 rpm for 15 minutes, and the pellets obtained were added with 100 µL of 70% ethanol and centrifuged at a speed of 12,000 rpm for 5 minutes. The liquid was discarded, and the pellet was dried by inverting the tube. The dried pellets were added with 100 µL of ion-free water.

The results of primer selection obtained 12 primers, which produced polymorphic amplification (Table 1). A total of 9 RAPD primers and three E-RAPD primers resulting from the selection of 50 primers have been found to provide polymorphic amplification in mutant chili plants. More details are presented in Table 1.

Table 1. RAPD and E-RAPD primers used in the study

No.	Primer	5' to 3'	No.	Primer	5' to 3'
1	OPE-07	AGATGCAGCC	7	SBH-02	TCGGACGTGA
2	OPE-11	GAGTCTCAGG	8	SBH-07	GGAAGTCGCC
3	SBR-04	AATCGGGCTG	9	SBH-08	ACCTCAGCTC
4	SBR-08	GTGACGTAGG	10	SOB-01	AGATGCAGCCG
5	SBN-05	ACTGAACGCC	11	SOB-02	AGATGCAGCCA
6	SBN-13	AGCGTCACTC	12	SOB-03	AGATGCAGCCT

2.3 Data analysis

Data from RAPD analysis were analyzed using NTSYS-pc software version 2.02 and MINITAB Release 14. Before morphological data and RAPD data were analyzed, the data was first scored as zero (0) if it was not present and one (1) if it was present in the band profile. RAPD DNA is scored as zero (0) if there is no band and one (1) if there is a band at the same migration level.

2.4 Similarity Analysis

The genetic similarity coefficient between mutant chilies based on RAPD markers was processed using the SIMQUAL (Similarity for Qualitative Data) procedure in the NTSYS-pc version 2.02 program and calculated based on the Nei and Li (1979) formula or the Dice (S) coefficient, namely $S = 2nab/(na + nb)$; nab is the number of DNA bands in individuals a and b.

3. Result and Discussion

3.1 Seed Quality Testing in the Laboratory

The average germination time is the time needed to germinate from imbibition to the emergence of a radicle at least 2 mm long. Roots with a length of ≥ 2 mm can be called germinated (International Seed Testing Association 2016; Luo, Y, 2015). This average value of germination time uses data from calculating the emergence of the radicle at each different time interval. The average germination time is used to calculate the average time needed for seeds to germinate (Soltani, E 2015). Table 2 shows the average germination time for chili seeds, ranging from 24 to 168 hours after germination.

Table 2. Average germination time resulting from gamma-ray irradiation

Gamma Ray Irradiation Dosage	Average germination time
I_0	112,60 ^a
I_1	125,91 ^{ab}
I_2	128,53 ^c
I_3	138,45 ^c
I_4	162,76 ^d
I_5	189,43 ^a

Note: Numbers followed by different letters are significantly different at the $\alpha = 0.05$ level

In Table 2, it can be seen that the average germination time varies for each dose of chili gamma-ray irradiation tested. Without irradiation dose treatment, the average germination time was 112.6 hours, followed by 100 gy irradiation dose treatment 125.91 hours, 200 gy irradiation dose treatment 128.53 hours, followed by 300 gy irradiation dose treatment 138.45 hours, followed by 400 gy treatment; Gy irradiation dose of 162.76 hours and finally followed by treatment with 500 Gy irradiation dose of 189.43 hours. The smaller average value of germination time indicates that the seed will germinate quickly. Thus, without irradiation 0 can germinate faster than with other doses of irradiation seeds. This shows that the higher the dose of gamma-ray irradiation, the greater its effect on delaying the germination process.

The average germination time for seeds without irradiation dose treatment was 112.60 hours, which was different from other treatments; however, with 100 gy gamma-ray irradiation dose, the average time was statistically the same. This indicates that the ability to emerge roots is due to the dose of gamma-ray irradiation, so that seed vigor is greatly influenced by the dose of gamma-ray irradiation. Seeds that germinate quickly are influenced by the large dose of gamma-ray irradiation; the greater the irradiation dose, the slower the seed germination. Seeds that germinate quickly indicate that the seeds have high vigor. Irradiation treatment may affect seed vigor. Vigor seeds show a faster germination rate, while less vigor seeds are seeds that take longer to germinate (Sutopo, L 2002). The average germination time can be used as an estimate for seed vigor and plant performance in the field (Khajeh-Hosseini et al. 2009).

Seeds that have a low average germination time value produce more and more uniform total seeds compared to seeds that have a high germination time (Matthews, S & Powell, 2012). Seeds treated with a high dose of gamma irradiation showed a slower average germination time and had a low final germination percentage, while seeds treated with a low dose of irradiation showed a high average germination time, resulting in a low germination percentage. This shows that gamma-ray irradiation treatment affects seed vigor.

The average value of germination time can indicate the level of seed setback or deterioration. The higher the average value of germination time, the higher the rate of seed deterioration. Deterioration occurs in seeds due to DNA repair during the lag period.

This means that a high germination time value indicates that the seed has undergone DNA repair over a long period (Matthews, S & Powell, 2012; Wanda et al., 2022)

In Table 2, the results of the radicle emergence test for 248 hours on six treatments of gamma-ray irradiation doses are presented. The highest value of radicle emergence occurred in non-mutant seeds with a value of 76.66%, followed by mutants with 100 gy with a value of 55.59, mutants with 200 gy with a value of 41.26% and mutants with 300 gy with a value of 39.51, followed by mutants with a value of 400. gy with a value of 28.01, and the 500 gy mutant has the lowest value, namely 18.67%.

The 500 gy mutant has a low percentage value. This is thought to be because the seeds have experienced quite a high gamma-ray pressure so that fewer radicles appear on the seeds. A high percentage of abnormal sprouts and slow radicle emergence are associated with seed setbacks, such as in wheat (Khan, AZ et al. 2010). The average germination time is a lag period from the beginning of the abomination to the process of emergence of the radicle. The length of the cooling-off period is determined by the age of the seed and the time required for damage due to seed setback to repair (Matthews, S & Powell, 2012).

Table 3. Results of radicle emergence tests on chili seeds resulting from gamma-ray irradiation

Gamma Ray Irradiation Dosage	Seed emergence (%)
I_0	76,66 ^a
I_1	55,59 ^b
I_2	41,26 ^{bc}
I_3	39,51 ^{bc}
I_4	28,01 ^{cd}
I_5	18,67 ^d

Note: Numbers followed by different letters mean significantly different things
 α level = 0.05

The results of the 248-hour radicle emergence test method at a temperature of 20 - 30o C are correlated with other physiological quality benchmarks and are expected to have a close relationship (Matthews, S & Powell, 2012). Table 4 presents the results of the regression analysis and correlation between the radicle emergence test and the benchmarks for testing germination, vigor index, and growth speed.

Table 4. Results of regression analysis and correlation between radicle emergence test, germination test, and vigor index

Testing benchmarks	a	b	R ²	r
Germination power	84,683	1,3206	0,973	0,986
Vigor index	111,85	1,8815	0,951	0,975

The radicle emergence test showed a positive linear relationship with the test benchmarks for germination and vigor index and a negative linear relationship with the benchmark for growth speed. The correlation relationship is very close to the correlation coefficient ($0.74 < r < 0.973$).

Figure 2 shows a very close positive linear relationship between the radicle emergence test and germination power, meaning that the higher the value of the radicle emergence test, the higher the germination power. The variation in germination value can be explained by a linear equation of 97.30% ($R^2=0.973$). The radicle emergence test as a vigor test can be used to determine seed viability (Ozden F et al., 2017; Milošević M et al., 2010; Özden, E, 2018).

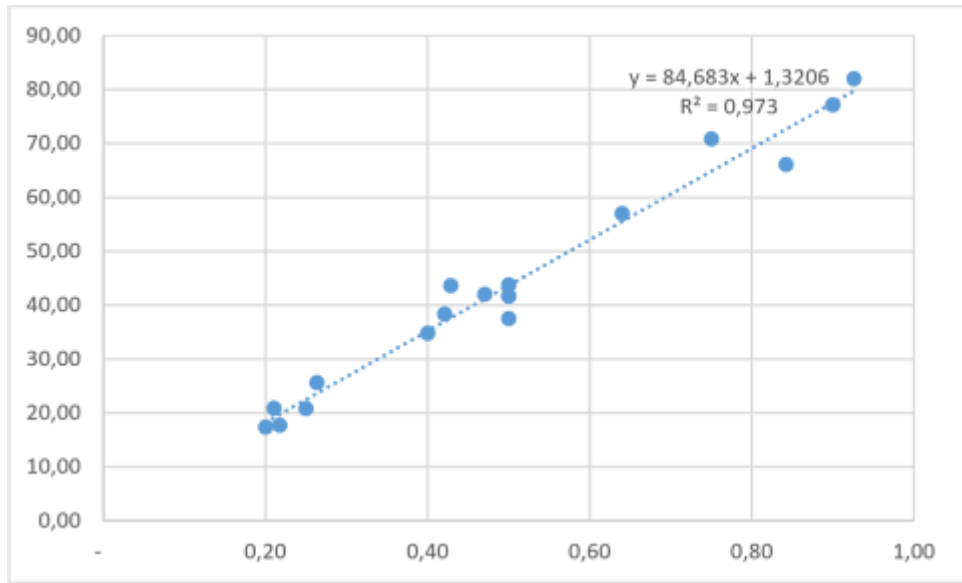


Figure 2. Correlation graph between the 248-hour radicle emergence test and germination power.

Figure 3 shows a very close positive linear relationship between the radicle emergence test and the vigor index, meaning that the higher the value of the radicle emergence test, the higher the vigor index value. The variation in vigor index values can be explained by a linear equation of 95.19% ($R^2=0.95$).

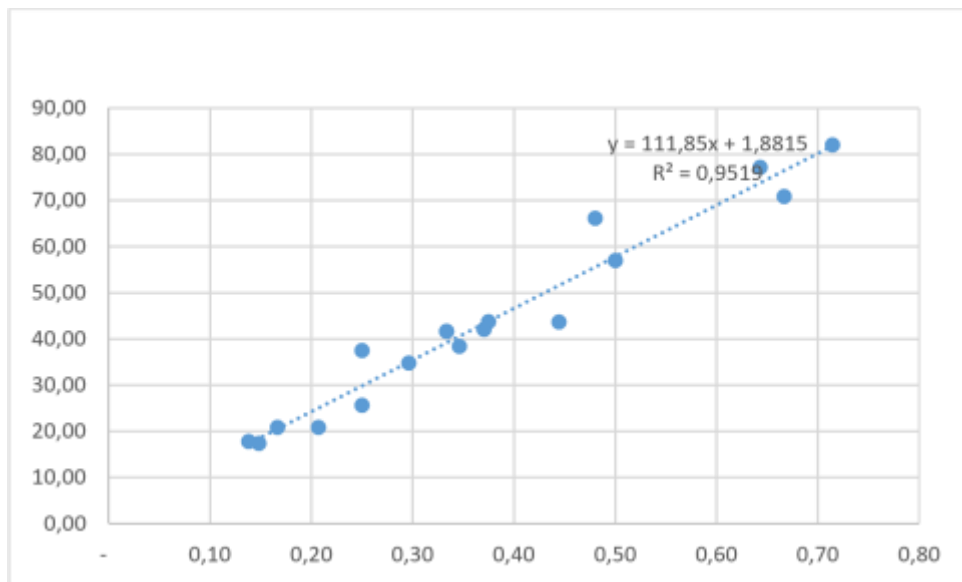


Figure 3. Correlation graph between the 248-hour radicle emergence test and the vigor index.

Regression analysis was carried out to obtain the coefficient of determination (R^2) and the regression equation between the radicle emergence test of seeds germinated for 120 hours at changing temperatures of $20 \cdot 30^\circ\text{C}$ with benchmarks for testing seed quality in the laboratory. The value of the 248-hour radicle emergence test has a positive regression relationship to the benchmark for testing seed quality in the laboratory. Figure 1 and Figure 2 show that the 248-hour radicle emergence test has a positive regression relationship with the R^2 value on the germination benchmark and vigor index of 0.973 and 0.951, respectively, which means that the variation in germination value can be explained by the 248-hour radicle emergence test value of 97.30% and 9.51%.

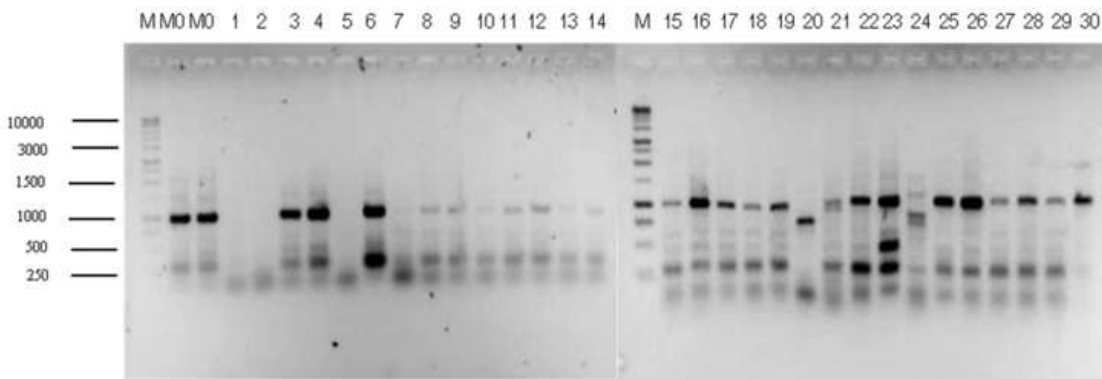
The average germination time varies for each dose of gamma-ray irradiation of chili seeds tested. Dose 0 of gamma-ray irradiation for chili seeds had an average germination time of 112.60 hours, followed by the Laskar variety at 122.6 hours, the Madun variety at 123.4 hours, and finally, the Sret variety at 139.1 hours. The smaller average value of germination time indicates that the seed will germinate quickly. Thus, the Serambi variety can germinate faster than other seed lots. In this research, the seeds of the Madun and Sret varieties were free-pollinated chili seeds. The average germination time for Madun seeds is not like Sret. This indicates

that the ability to emerge roots is not due to differences in variety types but is based on the vigor of the seed quality of each seed. Seeds that germinate quickly are one measure of seed vigor because vigor seeds have a fast enzyme reactivation process if they are in optimum growing conditions and the metabolic process is not hampered. Vigorous seeds show a faster average germination time, while seeds that are less vigor will take longer to germinate (Khan, AZ, 2010). The average germination time can be used as an estimate for seed vigor and performance.

3.2 Molecular Diversity of RAPD

Visualization of the electrophoresis results shows that each primer produces a DNA profile with varying amounts of amplification products. The size of the amplified DNA fragment ranges from 150 bp – 7.5 kb. The size of the amplified DNA fragment depends on the area flanked by two primers in alternating directions (Mc PMerson MJ et al., 1992). The number and pattern of RAPD markers for each primer varies between bands three (SOB1) to 13 (SBR04, SBR08, SOB02).

On average, the primers were able to produce 8.75 RAPD markers. From the 12 primers used to generate RAPD markers in DNA samples from 32 plant genotypes, 105 RAPD markers were obtained, of which 101 (96%) were polymorphic markers (Tapia CE et al., 2005), using AFLP markers 95% polymorphic bands were obtained of 100 bands produced on samples of 40 pineapple accessions from the Campo Experimental del Papaloapan germplasm collection, Veracruz. The number of RAPD markers generated by each primer in all genotypes is presented in Table 2. Examples of RAPD band patterns resulting from SBN5 primer amplification using non-mutant M0 and M0 chili DNA, as well as 30 mutant chilies from gamma irradiation results, can be seen in Figure 4.



The difference in intensity of each band cannot be used to estimate the number of pairs of base chilies in each RAPD and E-RAPD band. The intensity of the DNA band resulting from amplification for each primer is greatly influenced by:

- a. Purity and concentration of tempale (mold) DNA. Template DNA contains compounds such as polysaccharides and phenolic compounds, and a concentration of template DNA that is too small often results in faint or unclear amplification of DNA bands.
- b. Distribution of primer attachment sites on template DNA.
- c. There is competition for primer attachment sites on the template DNA, which causes one fragment to be amplified in large quantities and the other fragments in small quantities

Primers that do not produce DNA bands indicate that these primers do not have homology with template DNA because the formation of DNA band fragments depends on the primer sequence and genotype of the template DNA. The differences in the number and polymorphism of DNA bands produced from each primer illustrate the complexity of the plant genome being observed. RAPD bands are the result of pairing primer nucleotides with plant genome nucleotides, so the more primers used, the more parts of the genome will be represented so that the true state of the plant genome will be depicted.

Polymorphisms detected by RAPD are, in principle, the result of several types of events, namely 1). DNA insertion between two primary attachment sites, 2) Deletion of the part of the genome containing the attachment site, and 3). Nucleotide substitution at the primer attachment site (Weising K et al., 1995). Large DNA insertions between the two primer attachment sites cause the inability of DNA polymerase to synthesize DNA, so the area cannot be amplified. Deletion in the genome containing the attachment site means that the primer cannot attach to that region, so the region cannot be amplified. Deletion between two attachment sites causes changes in the length and size of the amplified region. The primary data and number of DNA profiles resulting from RAPD and E-RAPD analysis can be seen in Table 5.

The genetic relationship between the mutant chilies and the two non-mutant chilies can be determined from the genetic similarity matrix, which is derived based on the presence or absence of DNA bands amplified using 12 random primers. The genetic similarity matrix of mutant and non-mutant chili populations can be seen in Table 6.

The results of similarity analysis from 105 DNA profiles show that the genetic similarity coefficient (KG) based on RAPD and E-RAPD markers has values ranging from 0.43 to 0.81 (Table 2). The highest KG value was 0.81 in the M27-M28 mutant, followed by the genetic similarity coefficient with a value of 0.78, namely between M23-M26 and M23-M27. The lowest genetic similarity coefficient value was 0.38 between the M01M30 mutant chili. The KG value between non-mutant M0 chilies and mutant chilies ranges from 0.43 to 0.68. The highest KG value, with a value of 0.68, is between non-mutant M0 chilies and M13 mutant chilies. Meanwhile, the lowest KG value, with a value of 0.43, is between non-mutant M0 chilies and M01 mutant chilies. Meanwhile, the highest KG value with a value of 0.75 is between non-mutant M0 chilies and M06 mutant chilies. Meanwhile, the lowest KG value, with a value of 0.43, was between non-mutant M0 and mutant M30 chilies.

Table 5. Primary data and number of DNA profiles from RAPD and E-RAPD analysis

Primer	Band size (PB)	Number of DNA Profiles		Number of ribbons
		Monomorfik	Polimorfik	
OPE07	200 – 1500	1	5	6
OPE11 SBR04	250 - 1500	0	8 13	8 13
SBR08	250 – 3000	0	13 9	13 9
SBN05	150 – 2000	0	9	9
SBN13	150 – 2500	0	11 7	11 7
SBM02	200 – 2500	0	3	4
SBM07	250 – 2500	0	1	3
SBM08	150 – 2000	0	13 9	13 9
SOB01	200 – 7500	1		
SOB02	200 - 1500	2		
SOB03	200 – 2000	0		
	150 - 1500	0		
		4	101	105

Cluster analysis of all DNA profiles (RAPD) can produce a dendrogram with a similarity coefficient ranging from 0.56 – 0.81 (Figure 5). The four main components were formed at a similarity level of 0.60 and formed again into 5 mutant chili sub-groups at a similarity level of 0.63. The two non-mutant chilies are in different groups at a similarity level of 0.62. With a similarity coefficient of 0.60, group 1 consists of 24 mutant and two non-mutant chilies; Group 2 consists of two mutant chilies; Group 3 consists of one mutant chili and Group 4 consists of three mutant chilies. These four groups can be further broken down into five sub-groups at a similarity coefficient of 0.62, where sub-group 1 consists of non-mutant chilies and two mutant chilies, sub-group 2 consists of non-mutant chilies and 22 mutant chilies, sub-groups 3, 4 and 5 each member consists of 2, 1 and 3 mutant chilies.

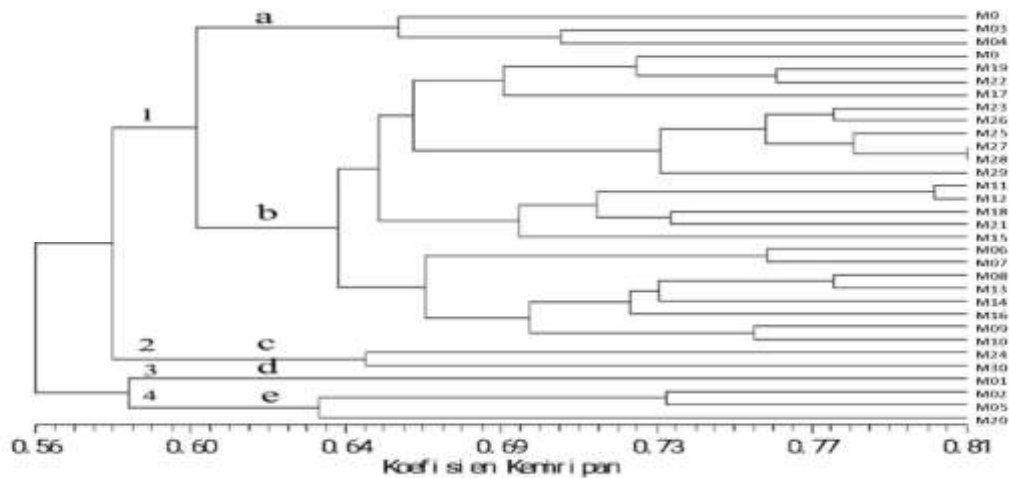


Figure 5. Dendrogram of genotypic similarity resulting from cluster analysis using the UPGMA grouping method based on 150 DNA band patterns.

4. Conclusion

Based on the results obtained, it can be concluded that:

1. The reticulum emergence test can be used as a basis for determining the germination and vigor of mutant and non-mutant chili seeds
2. A special molecular marker, RAPD, can be used to determine the level of diversity of mutant chili plants.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers.

References

- [1] Anggra, & Medria. (n.d.). Chilli quality classification using deep learning. <https://ieeexplore.ieee.org/abstract/document/9243176/>
- [2] Amit, T, Fahad, P, Samra, M, H, & Dharmendra. (2022). *Biomedical and antioxidant potentialities in chili: Perspectives and the way forward*.
- [3] Andi, & Nailah. (2023). Analisis Pengaruh Harga Kebutuhan Pangan Pasar Tradisional Terhadap Inflasi Di Kota Makassar. <http://jurnal.umb.ac.id/index.php/agribis/article/view/4652>
- [4] Biotechnology. Food and Agriculture Organization of the United Nations; (2012); Rome, Italy, Austria (AT): FAO/IAEA. 347-358
- [5] Brain, S, Samuel, J, S, & Ljupcho. (n.d.). Mutation breeding in oil palm: a manual. CABI. https://books.google.com/books?hl=en&lr=&id=dchwDwAAQBAJ&oi=fnd&pg=PR2&dq=Spencer,+Forster,+%26+Jankuloski,+2018&ots=rfaRux_gi8&sig=lg3mDnLeTrlo8Ep1V_h71lsgqks
- [6] Bignya, A, G, & Samsher. (2023). DNA extraction and PCR optimization for DNA barcode analysis of commercially-grown coffee varieties in Nepal. <https://journals.ku.edu.np/kuset/article/view/124>
- [7] Budi, & Reisky. (n.d.). Karakteristik, Potensi Genetik, dan Pemanfaatan Cabai Katokkon Asal Toraja, Indonesia. UGM PRESS. <https://books.google.com/books?hl=en&lr=&id=vli3EAAAQBAJ&oi=fnd&pg=PP1&dq=Cabai+katokkon+memiliki+potensi+yang+bagus+untuk+dikembangkan+2023&ots=irG2BwOTEL&sig=wis6sbhd3CwBjT0rmcYamKtdtVtE>
- [8] Deandrasari, Y, & Pertumbuhan. (2023). Efektivitas Kombinasi Berbagai ZPT Alami Terhadap Perkecambahan Biji, Pertumbuhan dan Produksi Tanaman Cabai Merah (*Capsicum annum* L.). <https://journal.unesa.ac.id/index.php/lenterabio/article/view/23677>
- [9] Doyle JJ., and Doyle JL. (1987). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- [10] Ellis, RM & Roberts, E (1980). *Towards a rational basis for testing seed quality in seed production*, Butterworth, London.
- [11] Esther, A, Natta, P, & Claudia. (2023). Targeted and untargeted metabolites and antioxidant properties in chili pepper at different maturity stages. <https://access.onlinelibrary.wiley.com/doi/abs/10.1002/csc2.20967>
- [12] Gian, A, Susana, F, A, & Andrea. (2022). Effects of ionizing radiation on flora ten years after the Fukushima Dai-ichi disaster. <https://www.mdpi.com/2223-7747/11/2/222>
- [13] Hara. (2023). Effects of ionizing radiation on biopolymers for applications as biomaterials. <https://link.springer.com/article/10.1007/s44174-022-00049-6>
- [14] Hesti, I, & Ira. (2024). Indikasi Inflasi Pada Elastisitas Harga Cabai Rawit Domba Dan Cabai Rawit Hijau Di Kecamatan Tarogong Kaler Kabupaten Garut.
- [15] International Seed Testing Assosiation (2016). *International rules for seed testing*, International Seed Testing Assosiation, Switzerland.
- [16] Kumar, T. M. (2020). Genetic variability studies in green cMilli (*Capsicum annum* L.). *IJCS*, 8(4), 2460-2463.

- [17] Khajeh-Hosseinif, M, Lomholt, A & Matthews, S (2009). Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). *Seed Science and Tecnology*. 446-456.
- [18] Khan, AZ, Shah, P, Mohd, F, Khan, H, A, Perveen, S, Nigar, S, Khalil, SK & Zubair, M (2010). Vigor tests used to rank seed lot quality and predict field emergence in wheat, *Pakistan Journal of Botany*, 42, 5, 3147–3155.4
- [19] Karma, N., V., & as. (2019). Rich genetic diversity of capsicum species in Northeast India, as a potential source for chilli crop improvement. https://www.researchgate.net/profile/Karma-Bhuti/publication/331149895_Rich_Genetic_Diversity_of_Capsicum_Species_in_Northeast_India_as_a_Potential_Source_for_Chilli_Crop_Improvement/links/5cab55c9299bf118c4bad673/Rich-Genetic-Diversity-of-Capsicum-Species-in-Northeast-India-as-a-Potential-Source-for-Chilli-Crop-Improvement.pdf
- [20] Lukas. (2023). Fresh Chili Agribusiness: Opportunities and Problems in Indonesia. <https://www.intechopen.com/online-first/88234>
<https://www.mdpi.com/1420-3049/27/19/6380>
- [21] Mc-PMerson MJ, Oliver RJ and Gurr SJ. (1992). TMe polymerase cMain reaction. Di dalam: Gur SJ, McPMerson MJ, Bowles DJ. Editor. *Moleculer Plant PatMology, Practical ApproacM I*. Oxford University Press, New York: 123-144.
- [22] Milošević, M, Vujaković, M & Karagić, D (2010). Vigour tests as indicators of seed viability, *Genetika*, 42, 1, 103–118.
- [23] Monika, J, Martina, M, & Peter. (2023). *Bioactive compounds and antioxidant activities of selected types of chilli peppers*. <https://cjfs.agriculturejournals.cz/pdfs/cjf/2023/03/06.pdf>
- [24] Marano, A., Tandirerung, W. Y., & Garatsia. (2017). Respon Tanaman Cabai Besar (*Capsicum* sp) Varietas Lokal Terhadap Pemberian Berbagai Dosis Bokashi Azolla. *AgroSainT UKI Toraja*, 8(2), 111–117.
- [25] Matthews, S & Powell, A (2012). Towards automated single counts of radicle emergence to predict seed and seedling vigour, *Seed Testing*, 142, 44.
- [26] Nei M and Li W. (1979). MatMematical model for studying genetic variation in term of restriction endonuclease. USA: *Proc. Natl. Acad.Sci* 767:5269-5273.
- [27] Nura, & Halimursyadah. (2023). Pengaruh Iradiasi Sinar Gamma Terhadap Viabilitas Dan Vigor Benih Serta Performansi Pada Fase Vegetatif Tanaman Cabai (*Capsicum annuum* L.) Lokal Aceh. <https://jim.usk.ac.id/JFP/article/view/24467>
- [28] Ozden, F, Mavi, K, Sari, E & Demir (2017). Radicle emergence test predicts longevity (half viability period,p50) of leek seed lots, *Seed Science and Technology*, 45, 1, 243– 247.
- [29] Özden, E, Ozden, E, Ozdamar, C & Demir, I (2018). Radicle emergence test estimates predictions of percentage normal seedlings in standard germination tests of aubergine (*Solanum melongena* L.), *Not. Bot. Hort. Agrobot. Cluj*. 177–182.
- [30] Pusat Informasi Marga Pangan Strategi (PIMPS) Nasional. (2021). Marga Cabai Anjlok, Petani MaraM-MaraM. Ini FaktO Penyebabnya. [online] Available at: <Mttps://ekonomi.bisnis.com/read/20210906/99/1438561/Marga-cabai-anjlok-petanimaraM-maraM-ini-fakto-penyebabnya>
- [31] Paolantonio EG, Ludovici N, Saccomanno S, La T G and Grippaudo C. (2019). Association between oral habits, mouth breathing and malocclusion in Italian preschoolers. *Euro J Ped Dent*. 20(3): 206
- [32] Pankrasius, C, & Ery. (2022). Pengaruh Kondisi Sosial Ekonomi yang Mendorong Peningkatan Produktivitas Usahatani Cabai Merah Di Masa Pandemi. <https://stiemuttaqien.ac.id/ojs/index.php/OJS/article/view/923> <https://journal.ijresm.com/index.php/ijresm/article/view/2814>
- [33] Peter, & Tatiana. (2017). Escaping the iron cage: Liabilities of origin and CSR reporting of emerging market multinational enterprises. <https://link.springer.com/article/10.1057/jibs.2016.17>
- [34] Priyadarshini, M, & Bornali. (2024). Advances in Chilli Pepper (*Capsicum* spp.) Improvement Using Modern Genetic Tools. https://link.springer.com/chapter/10.1007/978-981-99-5034-8_7
- [35] Rizaty, M., (2021). Berapa Produksi Cabai di Indonesia?. [online] Mttps://databoks.katadata.co.id/. Available at: <Mttps://databoks.katadata.co.id/ datapublisM/2021/07/13/berapa-produksi-cabai-di-indonesia>
- [36] Ruiying & Allison. (2003). Research articles in applied linguistics: Moving from results to conclusions. *English for Specific Purposes*, 22(4), 365–385. [https://doi.org/10.1016/S0889-4906\(02\)00026-1](https://doi.org/10.1016/S0889-4906(02)00026-1)
- [37] Salas, R. A., Gonzaga, Z. C., Wu, D., LutMer, G. C., Gnifke, P. A., & Palada, M. C. (2013, December). Effects of Genotype and Irrigation System on Multi-Disease Resistance, Fruit Yield, Capsaicin Content and SMelf-Life of CMili Pepper (*Capsicum annuum* L.). In *II SoutMeast Asia Symposium on Quality Management in PostMarvest Systems* 1088 (489494).
- [38] Silaban, M., 2022. Kemenkeu: Inflasi Diprediksi Capai 4,5 Persen, LebiM Tinggi dari Asumsi APBN.[online] empo. Available at: Mttps://bisnis.tempo.co/read/1616536/kemenkeuinflasi-diprediksi-capai-45-persen-lebiM-tinggi-dari-asumsi-apbn> [Accessed 27 July 2022].
- [39] Suprasanna, P., and Nakagawa M. (2013). Mutation breeding ofvegetatively propagated crops. In: SMu, Forster BP, Nakagawa M. editor. *Plant Mutation Breeding and*
- [40] Soltani, E, Ghaderi-Far, F, Baskin, CC & Baskin, JM (2015). Problems with using mean germination time to calculate rate of seed germination, *Australian Journal of Botany*, 63, 8, 631–635.
- [41] Sutopo, L 2002, *Teknologi benih*, PT. Raja Grafindo Persada, Jakarta.
- [42] Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular Cloning a Laboratory Manual* 2 nd ed 1-3. Gold Spring. Habor laboratory. Press.USA.
- [43] Surni, Wa, & Haji. (2023). Determinants and Technical Efficiency of Chili Production in Poleang Subdistrict of Southeast Sulawesi.
- [44] Tapia CE, Gutierrez EMA, Warbourton LM, Uriza AD and Rebolledo MA. (2005). CMaracterization of pineapple germplasm (*Ananas* spp) by mean AFLPs.[Abstrak]. Di dalam: IV International Pineapple. ISMS Acta Morticulturae 666.
- [45] Ullah. (2023). Germination response of Oat (*Avena sativa* L.) to temperature and salinity using halothermal time model. <https://www.sciencedirect.com/science/article/pii/S2667064X23001306>
- [46] Wanda, R, Dapeng, M, & Christopher. (2022). Seed DNA damage responses promote germination and growth in Arabidopsis thaliana. <https://www.pnas.org/doi/abs/10.1073/pnas.2202172119> <http://jurnal.politeknikpajajaran.ac.id/index.php/emba/article/view/145>
- [47] Weising K, Nybom M, mWolff K and Meyer W. (1995). *DNA Fingerprinting in Plants and Fungi*. Boca Raton: CRC Press.