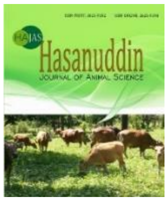

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Productivity of *Indigofera zollingeriana* Mutant 2 Result Gamma Irradiation planted in Coastal Areas

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ABSTRACT

Coastal areas have great potential to be developed. One of the good sectors developed in coastal areas is livestock, but the obstacle in the development of livestock in coastal areas is saline land conditions that can affect the process of plant growth. *Indigofera zollingeriana* is a plant that is tolerant to various types of environmental stress, one of which is salinity stress. Induced mutation is currently the most effective breeding method for plant genetic improvement. One example of induced mutation treatment is gamma irradiation. This study used a complete randomized design method with 10 treatments and 6 replicates. The treatments consisted of P0 (Control/Without irradiation); P1 (50 Gy gamma irradiation); P2 (100 Gy gamma irradiation); P3 (150 Gy gamma irradiation); P4 (200 Gy gamma irradiation); P5 (Irradiation was given salinity stress); P6 (50 Gy gamma irradiation and salinity stress); P7 (100 Gy gamma irradiation and salinity stress); P8 (150 Gy gamma irradiation and salinity stress); and P9 (200 Gy gamma irradiation and salinity stress). The results of the analysis of variance showed that the treatment of gamma irradiation dose and salinity stress had a significant effect ($P<0.05$) on the total weight, leaf weight and leaf production of *Indigofera* but no significant effect ($P>0.05$) on stem weight and production.
Keywords: Salinity stress, *Indigofera zollingeriana*, gamma irradiation

INTRODUCTION

Coastal areas are known as aquatic ecosystems with enormous potential for marine resources [1]. In addition to marine potential, several sectors in coastal areas also have potential that could be more promising. Animal husbandry is a leading sector with good potential for development in coastal areas. Still, the obstacle is the availability of feed crops because, generally, soil conditions in coastal areas have high salinity levels, which can affect plant growth. Nisak and Supriadi [2] explain that the obstacle in using saline soil for plant cultivation is the high dissolved salt level, especially NaCl. The presence of high Na^+ and Cl ions causes an increase in the osmotic pressure of the soil solution, so plants have difficulty absorbing water. The absorption of Na^+ and Cl ions is more than other ions, so there is an imbalance of electrolyte solutions in the plant body, and Na+ and Cl- poisoning occurs, which has an impact on reducing plant growth and production [3].

One of the efforts to utilize saline land is through tolerant genotypes [4]. Ali *et al*. [5] said that *Indigofera zollingeriana* is one type of forage fodder from a group of leguminous shrubs tolerant to various environmental stresses, one of which is salinity stress. Still, the results of research by Nadir et al. [6] show that the adaptation of Indigofera growth in NaCl media shows that this plant is tolerant to salinity up to a salinity level of 9.99 ds/S, so high salinity stress has a stressful impact on plants and affects their growth. Therefore, it is necessary to improve the quality of Indigofera even though it is planted on land with high salinity stress. *Indigofera zollingeriana* mutant 2 is a second-generation gamma-irradiated *Indigofera zollingeriana* plant that has gone through seed selection. According to Hutasoit *et al*. [7], there are superior morphological characters in *Indigofera zollingeriana* second mutant (M2) when tested for salinity stress adaptation.

Mutation induction is currently the most effective breeding method for plant genetic improvement. Physical mutation induction through gamma rays is one of the possible efforts to expand the genetic diversity of plants [8]. One example of induced mutation treatment is gamma irradiation [9]. Several studies have found that gamma rays on plants can increase germination potential [10], growth and productivity [11], and resistance to environmental stress [12]. Therefore, gamma-ray mutation induction technology is expected to increase the genetic variability of *Indigofera* to maximize growth even though it is planted in an environment with salinity stress.

MATERIALS AND METHODS

The materials used in this study were *Indigofera zollingeriana* seedlings of Gamma irradiation with different doses that were six months old. *Indigofera zollingeriana* seedlings of the second mutant of gamma irradiation or M2 were obtained from the selection of seeds produced by Indigofera M1, which has been through the process of gamma irradiation at the Badan Tenaga Atom Nasional (BATAN). The cultivation process was carried out in the Faculty of Animal Husbandry Nursery, Hasanuddin University. *Indigofera zollingeriana* was given salinity stress during seedling using a 50 mM NaCl solution. The design used was a completely randomized design (CRD) consisting of 10 treatments. The treatments were P0 (Control/Without irradiation); P1 (50 Gy gamma irradiation); P2 (100 Gy gamma irradiation); P3 (150 Gy gamma irradiation); P4 (200 Gy gamma irradiation); P5 (Irradiation was given salinity stress); P6 (50 Gy gamma irradiation and salinity stress); P7 (100 Gy gamma irradiation and salinity stress); P8 (150 Gy gamma irradiation and salinity stress); and P9 (200 Gy gamma irradiation and salinity stress).

Research Procedure

Indigofera zollingeriana seedlings, the second mutant, were planted in Untia Fishery Harbour, Makassar City, South Sulawesi Province, Indonesia. Because this research was conducted on the coast, soil salinity testing was performed, and 0.67 dS/m was obtained. The site's climatic conditions were tropical, with a rainy season.

All *Indigofera zollingeriana* seeds were planted in a plastic cell–tray before transplanting into the experimental area up to 4–5 weeks old plants. The transplanted legumes were grown at 75 × 150 cm spacing (inter–and intra–spacing, respectively). Individual plots consisted of 2 rows 4 m in length and 1.5 m apart, and each row contained 25 plants for a total of 50 plants per plot. Plant cultivation was carried out by paying attention to water availability, providing manure compost fertilizer of as much as 3 kg per planting hole, and controlling weeds that grow around the plants.

Observed Parameters

The parameters observed in this study were total fresh weight, leaf fresh weight, leaf production, stem fresh weight, and stem production. The fresh weights were measured from each plant with leaves and stems cropped to 100 cm above the ground. The total biomass was weighed as fresh matter (FM) in the field, and a representative sub-sample of 400–500 g FM was taken to separate by hand into leaf and stem components. The leaf stem ratio was also calculated. Data collection was carried out 45 days after planting.

Data Analysis

Data were analyzed using variance analysis based on a completely randomized design. Each treatment was repeated six times, resulting in 60 treatment combinations. Statistical analysis was carried out with SPSS Software (Version 16.0). If the treatment's effect was significantly different in the variance analysis, then a further test was continued with Duncan's test.

The materials used in this study were *Indigofera zollingeriana* seedlings of Gamma irradiation with different doses that were six months old. *Indigofera zollingeriana* seedlings of the second mutant of gamma irradiation or M2 were obtained from the selection of seeds produced by Indigofera M1, which has been through the process of gamma irradiation at the Badan Tenaga Atom Nasional (BATAN). The cultivation process was carried out in the Nursery of the Faculty of Animal Husbandry, Hasanuddin University. Indigofera zollingeriana was given salinity stress during the seedling process using a 50 mM NaCl solution. The design used was a completely randomized design (CRD) consisting of 10 treatments. The treatments were P0 (Control/Without irradiation); P1 (50 Gy gamma irradiation); P2 (100 Gy gamma irradiation); P3 (150 Gy gamma irradiation); P4 (200 Gy gamma irradiation); P5 (Irradiation was given salinity stress); P6 (50 Gy gamma irradiation and salinity stress); P7 (100 Gy gamma irradiation and salinity stress); P8 (150 Gy gamma irradiation and salinity stress); and P9 (200 Gy gamma irradiation and salinity stress).

RESULTS AND DISCUSSIONS

Indigofera productivity can be expressed through fresh-weight leaves and stems, dry-weight production of leaves and stems, and leaf/stem ratio [13]. As shown in Table 1, the treatment of salinity stress and gamma irradiation with different doses significantly affected total fresh weight, fresh weight of leaves, and leaf production.

Table 1. Total Fresh Weight, Fresh Weight of Leaves, and Leaf Production of *Indigofera zollingeriana* with doses of gamma irradiation and salinity stress

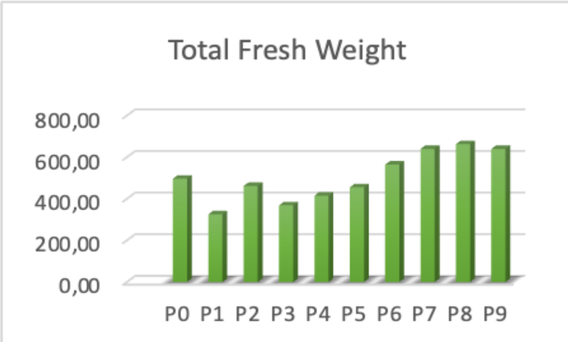
Treatment	Total Fresh Weight (g/plant)	Leaf Fresh Weight (g/plant)	Leaf Production (g/plant)
P0	496.67±79.43^{bc}	302.18±62.73^{abc}	70.86±16.82^{a}
P1	325.00±45.00^{a}	189.73±39.76^{a}	48.13±10.11^{a}
P2	461.67±112.51^{abc}	270.48±51.60^{abc}	68.89±14.37^{a}
P3	368.33±20.82^{ab}	218.73±9.87^{a}	55.60±2.48^{a}
P4	415.00±52.92^{abc}	238.16±29.39^{ab}	56.53±8.14^{a}
P5	455.00±17.32^{abc}	319.28±38.38^{abcd}	89.83±6.85^{ab}
P6	564.33±152.07^{cde}	392.600±171.38^{bcde}	115.46±52.28^{bc}
P7	640.00±37.75^{de}	464.17±51.45^{de}	143.49±15.55^{c}
P8	662.67±22.50^{e}	494.20±57.00^{e}	142.35±15.73^{c}
P9	640.00±140.00^{cd}	425.20±162.68^{cde}	115.27±35.12^{bc}

Description: P0 (Control/Without irradiation); P1 (50 Gy gamma irradiation); P2 (100 Gy gamma irradiation); P3 (150 Gy gamma irradiation); P4 (200 Gy gamma irradiation); P5 (Irradiation was given salinity stress); P6 (50 Gy gamma irradiation and salinity stress); P7 (100 Gy gamma irradiation and salinity stress); P8 (150 Gy gamma irradiation and salinity stress); and P9 (200 Gy gamma irradiation and salinity stress). Different superscripts in the same line show significantly different (P<0.05)

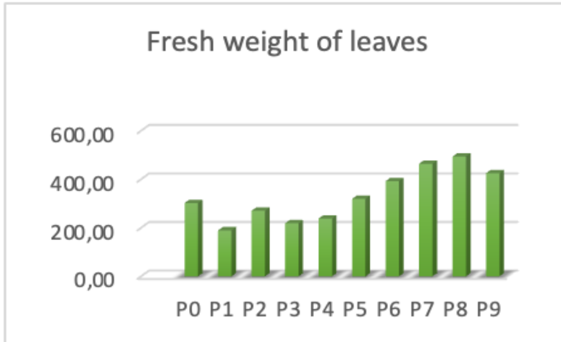
Based on the results of the analysis of variance showed that the treatment had a significant effect (P<0.05) on the total fresh weight, fresh weight of leaves, and leaf production. The highest data value was obtained in the treatment P8 (150 Gy gamma irradiation and salinity stress) with the respective values for total fresh weight of 662.67 g, fresh weight of leaves 494.20 g, and leaf production 142.35 g. This was because gamma irradiation can cause changes in all parts of the plant, including the leaves. This aligns with the research results by Harianja *et al*. [4], which has a higher fresh weight than the control.

Table 1 shows that the treatment with an irradiation dose of 100 Gy - 200 Gy (P2,3,4,7,8,9) has the highest leaf production value. Jonaidi *et al*. [15] said that the higher the radiation intensity, the higher the chance of mutation, and it can have a positive impact at specific doses. This study's results align with research by Maulana *et al*. [16], which concluded that the irradiation level of 200 Gy gave the best results on the test of germination and growth of chili katokkon. Increasing the irradiation dose will cause more physiological damage due to mutations [17]. Doses in the range of 400 Gy to 1000 Gy caused many deaths of chili plants [18].

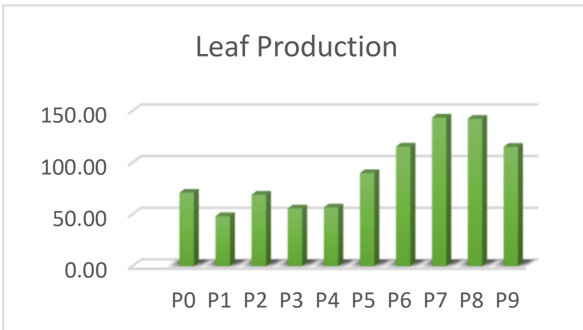
Salinity also affects leaf development; the higher the salinity, the smaller the leaf area. This is to the statement by Samanhudi *et al*. [19], which states that high concentrations of NaCl suppress plant growth by inhibiting cell enlargement and division because plants find it difficult to absorb water, resulting in smaller leaf size [20], which says that the influence of NaCl will reduce leaf area which will ultimately reduce the production of crown dry matter. Leaf size is closely related to leaf production. The more leaves there are, the better the quality of the legume is because the leaves are part of the plant tissue with the highest nutrient content compared to the stem/branch. Huang *et al*. [20] state that when the dry mass of leaves increases, the increase in leaf area decreases. This indicates plants that have small leaf size have more production. In addition, high salinity stress also causes thickening of the leaves, thus affecting the weight and production of leaves. Yao *et al*. [21] believe that high salt stress encourages leaf thickening, increases leaf thickness, reduces water loss, and increases leaf water retention capacity.



Picture 1. Total Fresh Weight (g/Plant)



Picture 2. Fresh Weight of Leaves (g/Plant)



Picture 3. Graph of Leaf Production (g/Plant)

Table 2. Stem fresh weight and stem production of *Indigofera zollingeriana* treated with doses of gamma irradiation and salinity stress

Treatment	Stem Fresh Weight (g/plant)	Stem Production (g/plant)
P0	194.49±97.45	43.79±21.55
P1	135.27±5.34	42.47±2.99
P2	191.19±61.09	62.27±25.07
P3	149.60±17.05	46.99±9.89
P4	176.84±34.77	51.79±9.48
P5	135.72±27.60	43.39±9.85
P6	171.73±23.00	40.91±2.94
P7	175.83±60.09	61.24±23.06
P8	168.54±63.29	58.42±21.13
P9	214.80±23.24	68.56±7.65

Description: P0 (Control/Without irradiation); P1 (50 Gy gamma irradiation); P2 (100 Gy gamma irradiation); P3 (150 Gy gamma irradiation); P4 (200 Gy gamma irradiation); P5 (Irradiation was given salinity stress); P6 (50 Gy gamma irradiation and salinity stress); P7 (100 Gy gamma irradiation and salinity stress); P8 (150 Gy gamma irradiation and salinity stress); and P9 (200 Gy gamma irradiation and salinity stress). Different superscripts in the same line show significantly different (P<0.05)

The results of variance analysis showed that the treatment had no significant effect (P>0.05) on the fresh weight of the stem and stem production. This is thought to be because *Indigofera* is one type of plant included in the Fabaceae family, in which Fabaceae plants have a relatively high level of tolerance to osmotic stress, including salinity stress. This is supported by Sunandi *et al*. [22], who state that plants from the Fabaceae family have a mechanism for reducing osmotic stress by synthesizing peroxidase compounds. Peroxidase compounds will break down Na+ compounds by adding hydrogen (H) from donor molecules in the reduction-oxidation (redox) reaction. Peroxide is reduced to form water and other oxidized molecules. Relatively more minor plant production is thought to be due to adding nutrients to the soil and plants that need to be balanced [23]. Competition for water and nutrient absorption results in plant development being disrupted so that plant production decreases [24].

Gamma irradiation doses create plant varieties and alter the amino acid composition of plants [25]. Gamma radiation affects the growth and productivity of *Indigofera* sp. plants [26]. Due et al. [27] stated that irradiation doses that are too high will inhibit cell division and cell death, significantly affecting plant growth, decreased growth power, and plant morphology. However, this study showed that the irradiation dose level did not affect Indigofera's fresh weight and stem production. In this study, it can be seen that the production of leaves is higher than that of stems. In accordance, Witariadi and Kusumawati [28] state that the higher value of the ratio of leaves to stems indicates higher carbohydrate and protein content. Keraf *et al*. [29] state that the lack of water content in plants significantly affects the increase in cell wall thickness in plant stems. Plant biomass production highly depends on plants' variety and ability to absorb more water and nutrients [30].

CONCLUSIONS

The results showed that treatment with gamma irradiation doses and administration of salinity stress significantly affected total weight, leaf weight, and Indigofera leaf production but had no significant effect on stem weight and output. The best results obtained from this study were 100 and 150 Gy gamma irradiation and salinity stress.

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