The tolerance improvement of local soybean in waterlogging condition through the combination of irradiation and in vivo selection

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Abstract. Soybean (Glycine max L.) is wellknown as a plant that posses an extra nutrition and many proteins and has a major role in improving people's nutrition status. The environmental factors are the main obstacle in soybean production. High rainfall frequency can lead the waterlogging phenomenon which potentially decrease the production rate of soybean. Crop improvement are the best solution to solve the problems. The purpose of this research was to determine the tolerance level and physiological response of irradiated soybean, Grobogan and Anjasmoro varieties in waterlogging condition. 60CGamma were used to irradiate the seeds with 25Gy, 50Gy, and 100Gy dosages. This research were conducted in vegetative stadia with 100%, 150%, 200% concentration of water that dispersed into medium and control as comparison. Based on the data, the irradiation has a complex responses in plant morphology. The irradiated plant height was not significantly different among all treatments. While the leaf’s width, root length, nitrogen in leaf, root nodules, chlorophyll content, fresh weight and dry weight are decline as the waterlogging concentration increased.

1. Introduction

Soybean (Glycine max L.) become the main food commodities that has long been cultivated in Indonesia after rice and corn. Soybean (Glycine max L.) currently not only positioned as a raw material for food industry, but also placed as raw material for non-food industry. Soybean (Glycine max L.) is one of the most important edible oil producers legumes plants because of its high nutritional value [1]. Soybean containing protein, oil, carbohydrates insoluble, carbohydrates soluble, the water level and various functional a material such as anthocyanin, isoflavon, saponin, and fibers food [1], [2] and [3].

The increased of demand were not followed by an increase in production rate. The average of soybean yearly needs as much as 2.2 million tons/year and will be increased every year. However, until 2016, soybean production in Indonesia were estimated to be difficult to improved from the range of 800-900 thousand tons. One of the main factors in decrement of soybean production is climate change including the prolonged periods of rainy season and distribution of precipitation that carries broad impact on various sectors of human life. The amount of water that exceeds the absorption average capacity of land could potentially causing waterlogging. The occurrence of waterlogging...
stress is defined when all the soils pores saturated with water and over capacity at least 20\% of soils capacity [4].

Standing upon the conditions of water, the excessive water can be classified into two, namely: (1) water-saturated condition (waterlogging) where only root crops is flooded, and (2) the condition of the whole part of the plant is flooded (complete submergence) [5]. The main problems in waterlogging stress is the lack of $O_2$ on the submerged part of plants. This is a major factor that causes damage to the soybean crop, both physiological and physical.

The control in environmental condition of cultivation area can be simply conducted since has a complex factors and also cost consume [6; 7], so the tolerance characteristic of soybean against waterlogging stress need to be improved. Recently, research on food crops especially on soybean tolerance to waterlogging stress is still limited. Furthermore, there is no source of tolerance genes to waterlogging stress in the soybean plant. Based on the background, we need to induce the variation by using a irradiation technique. Gamma irradiation has positive effect in improving growth of *Abelmoschus esculentus* L. Monech plant [8]. Irradiation will produce a random mutation, all the variants will be selected by using in vivo selection. The selected variant or surviving variants will be compared with its initial lines or original varieties. This information is important in development of resistant varieties of soybean to waterlogging stress.

2. **Materials and Methods**

This research were conducted in Laboratory of Plant Bioscience and Technology, Biology Department, Sepuluh Nopember Institute of Technology (ITS) Surabaya. Gamma rays was used and performed in BATAN.

**Measurement of Field Capacity**

Measurement of field capacity was aimed to determine the water volume as a linemark of the waterlogging treatment. Furthermore, planting medium weighed water saturated weight and dry weight. Water saturated weight was measured after no water dripped from polybags. Dry weight was measured after planting medium was dried in oven at 105$^\circ$ C until a constant weight was obtained.

Water requirement based on the field capacity is calculated by the following formula:

$$ FC (\%) = \frac{T_b - T_k}{T_k} \times 100\% $$

Where:

- $T_b$: Wet weight of soil
- $T_k$: Dry weight of soil

**Preparation, irradiation and seeding of Seeds**

The seeds of Grobogan and Anjasmoro varieties were obtained from Center of Bean and Tuber Crops Research Institute (BALITKABI), in Kendal Payak, Malang. Those two varieties were soaked for 6 hours using aquadest and then drained. The soybean seeds were then sterilized on the surface with 70\% ethanol for 2 minutes and 1\% NaOCl for a minute. Furthermore, seeds were placed on petridish and wrapped. The exposure of Gamma rays was performed at several levels of 0 Gy, 25 Gy, 50 Gy, and 75 Gy and 100 Gy at the Center of National Atomic Technology Agency (BATAN). Soybean seeds that have been exposed to Gamma rays are grown on prepared media, sowing until 2 leaves appear and then acclimatized for 7 days.

**Waterlogging Selection**

The soybean plants that have been acclimatized for 7 days are given the treatment of waterlogging stress according to the calculation obtained. The waterlogging was carried out for 14 days at all treatment levels by giving water into each polybag as much as the concentration determined based on the preliminary field capacity study results. Plants in each treatment were taken and cleaned from the rest of the soil using water and air drained. Furthermore, plants are inserted into a ziplock plastics and labeled. Several growth parameters were observed during and after the treatment.

**Plant height (cm)**

Measurement on plant height were carried out every 2 days for 14 days of waterlogging treatment. Soybean plants are measured from the base of the stem to the tip of the stem or the growth point.
**Leaf area**

Measurement of the leaf area was done after 14 days of waterlogging by gravimetric method, that is by drawing the leaf to the paper. The leaf area is calculated based on the weight ratio of the leaf replica to the total paper. The leaf samples used were taken from the same plant. The formula calculation is as follows:

\[ LA = \frac{W_r (g)}{W_t (g)} \times PA \]

LA: leaf area; Wr: weight of paper replica (g); Wt: weight of total paper (g); PA: paper area [10].

**Root parameters**

Root length measurement were made after 14 days waterlogging from the base of the root until the tip of the longest root using a ruler. While, the observation of the root nodule was done after 14 days waterlogging stress by counting the number of root nodules on the root of each levels of waterlogging. The observation of adventitious root was performed after 14 days of waterlogging by counting the adventitious root that formed at the root of each level of waterlogging.

**Wet weight and dry plant**

The procedure of measuring the wet weight of the plant is cutting the plant based on each organ including leaves, stems, and roots, then weighted using analytical scales. The wet weight of the plants is the accumulation of the total number of all plant organs. While dry weight is done by drying the plants using the oven first until the constant weight and then weighed using an analytical scale. The dry weight obtained is the productivity of the plant.

**Chlorophyll content**

The measurement of chlorophyll content was conducted at each treatment of the waterlogging concentration. Soy leaves are weighed as much as 0,1 grams with analytical balance. Chlorophyll of soybean leaf extracted using 96% ethanol 10 ml and then macerated for 24 hours. The extract was filtered using whatman 42 filter paper and taken into cuvette and measured with UV-Vis spectrophotometre in 649 nm and 665 nm wavelength. The calculation formula as follow:

Total Chlorophyll Content = \[20,0 \times A_{649} + 6,10 A_{665}\]

3. Result and Discussion

Viability test needed after seeds completely irradiated in term to find the influence of irradiation of gamma rays to the ability of seed to germinate. The ability of seed germination is an important parameter related their resistance level to the environment condition. Seed viability is an ability of seeds to germinate demonstrated by the high rate of metabolic activity in germination and growth of seedling. Viability can be observed based on two categories i.e seedling ability and seedling rate. The results of testing the rate of germination can be seen in Table 1.

Table 1. Germination rate of soybean after irradiated with Gamma rays

| Varieties | Irradiation Dosage (Gy) | Seeding percentage (%) | Seedling rate (day) |
|-----------|-------------------------|------------------------|---------------------|
| Anjasmo   | Kontrol                 | 87.50                  | 1.57                |
|           | 25 Gy                   | 87.50                  | 1.33                |
|           | 50 Gy                   | 87.50                  | 1.29                |
|           | 75 Gy                   | 87.50                  | 1.95                |
|           | 100 Gy                  | 83.33                  | 2.50                |
| Grobegan  | Kontrol                 | 92.67                  | 2.65                |
|           | 25 Gy                   | 91.87                  | 2.34                |
|           | 50 Gy                   | 79.17                  | 2.16                |
|           | 75 Gy                   | 79.17                  | 2.16                |
|           | 100 Gy                  | 75.00                  | 2.22                |
Seeds that have irradiated with gamma rays in 100 Gy doses showed the decrement in both Anjasmoro and Grobogan varieties. Anjasmoro were more stable in seed viability parameters eventhough indicate the decrease in the highest dosage. Seeds with treatment 100 doses Gy took more time to grow its radicle. The sensibility plant in the dose of radiation depending on plants type and even varieties in the same species. The maximum irradiated dose that can be given in soybean plant is not yet known. Hameed et al., 2008 reported that the germination percentage will be declined significantly on irradiated high doses ranged from 400 - 500 Gy and in 500 doses the germination percentage were declined significantly in chickpea plant [11]. High doses of irradiation will impede the entire germination by decreasing the seedling rate. Khawar et al., 2010 reported that the increase in irradiation dose of gamma rays on the several plant seed will negatively affect the germination [12]. But in low doses, irradiation will stimulate germination through a process for activation of RNA for protein synthesis [13].

Waterlogging stress can affect the plant growth including plant height, root length, root nodule, amount of adventif root, and chlorophyll. The elongation of plant stems caused by interactions between Giberellic acid (GA) and Ethylene. Rapid elongation process in internodal produced by Ethylene that induce the specific ratio between Giberelic acid (GA) and Absicic acid (ABA). Furthermore, in waterlogging there has been a decrease in the ABA indigenous and increase of GA [14]. The result show that in 25 and 50Gy plant able to elongate higher than the other dosages. Detail data about plant height can be shown in Figure 1.

![Figure 1. Plant height of soybeans in waterlogging stress A. Anjasmoro variety; B. Grobogan variety](image)

Irradiation can increase the sensitivity of plants. According to Mudibu, et al., 2012, irradiation can improve the sensitivity of plant with reducing the number of Phytohormones, especially Cytokinins [15]. Irradiation can degrades mitochondria and the nucleus because the cell membrane and nucleus membrane is sensitive to the irradiation exposure, but Cytokinins that been accumulated in granules will not be degraded. The influence of water exceeds for vegetative stage were signed by the smaller leaves that can reduce the absorption of light, reduce chlorophyll and reduce the activity of some enzymes (e.g. nitrate reductase), the decline in plant productivity (biomass) due to the decline of primary metabolism, depreciation leaves wide and the photosynthesis.furthermore, waterlogging can affect the uptake of macro and several micronutrients N, P, K, Ca, Mg, S, Fe [16] . In addition, water also plays an important role in regulating cell turgidity that necessary for growth and cell enlargement. Those roles make a waterlogging stress causing decrement in metabolic processes directly or indirectly. The response of plants to waterlogging can be seen in metabolic activity, morphology, physiology and the growth level. The effect of waterlogging in fresh and dry weight of plant can be shown in Figure 2.
Figure 2. The fresh and dry weight of soybean in waterlogging stress 14 DAP. A. Anjasmoro variety; B. Grobogan variety; note : blue bars : fresh weight; red bars : dry weight.

Generally there are many physiological mechanism in plant that can improve its ability to stand or adapt with water exceeds condition. The concentration of several hormones also were decrease in responding the water exceeds condition [16]. In the otherhand, the ethylene production were increase. Ethylene can be the main mechanism in plant response to stress. Furthermore, ethylene can induce the formation of aerenchyma and also formation of adventif roots [17; 18]. Aeration stress can induce plant to held the plasticity mechanism. One of the main plasticity mode of plant can be shown in basal stem area, reforming that part to become unusual root (adventif root). The measurement of adventif root can be shown in Figure 3.

Figure 3. The adventif roots of plant in waterlogging conditions A. Adventif roots; B. Anjasmoro variety; C. Grobogan variety.

Waterlogging stress also affect the ratio of stomata that opens and closes and decrease of root physiological metabolism (roots permeability, root dry weight, the number of and effectiveness of roots nodules). In addition, the condition hypoxia or anoxia also affect in fixation and distribution of nitrogen and other minerals due to the dead roots and physiological drought that causes inhibition in root growth and nodulation. The Nitrogen content in soybean plant related to the formation of root nodes. Root nodules is a symbiotic mutualism between root cells to bacteria especially genus Rhizobium. This symbiotic relationship will produce nodules structure in roots that are mostly in plants legum.
Figure 4. The amount of root nodules in waterlogging conditions A. Root nodules; B. Anjasmoro variety; C. Grobogan variety.

The result shows that the chlorophyll content were significantly decline along with the increase of water concentration in medium. Nitrogen serves in forming green pigmen substance in leaves. Hence, general visual symptoms of chlorophyll deficient is pale green or even pale yellow leaves. Nitrogen and Magnesium plays a vital role in determine the amount of chlorophyll in leaves. Figure 5 explain

Figure 5. The chlorophyll content in waterlogging conditions A. Anjasmoro variety; B. Grobogan variety. Note : Red bars is chlorophyll A; Blue bars is Chlorophyll B.

4. Conclusion
Controlled experiments on vegetative stage of soybean plants conducted under greenhouse conditions confirmed results that the viability of seeds after irradiation were decrease, the significant decrease shown in 100 Gy dosage. The plant height and chlorophyl content shows that the optimum height were obtained in 25 and 50 Gy, while the amount of root nodules were decreased inline with the increasing of water concentration. The highest amount of adventif roots were obtained in 200% of water exceeds. All in all, Anjasmoro variety more tolerant compared to Grobogan variety in waterlogging. Further functional genomic in protein levels and transcriptomic analysis will be needed to evaluate the role of several genes involved in plant dynamic response to waterlogging stress.

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References

[1] Waqas M, Khan A L, Kang S M, Kim Y H and Lee I J 2014 Phytohormone-producing fungal endophytes and hardwood-derived biochar interact to ameliorate heavy metal stress in soybeans *Biol. Fertil. Soils* 50 1155–67

[2] Thomas J M G, Boote K J, Allen L H, Gallo-Meagher M and Davis J M 2003 Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance *Crop Sci.* 43 1548–57

[3] Bellaloui N, Hu Y, Mengistu A, Kassem M A and Abel C A 2013 Effects of foliar boron application on seed composition, cell wall boron, and seed δ15N and δ13C isotopes in water-stressed soybean plants *Front. Plant. Sci.* 4 270

[4] Alam I, Lee D G, Kim K H, Park C H, Sharmin S A and Lee H 2010 Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage *J. Biosci.* 35 49–62

[5] Van Toai T T, Martin S K St, Chase K, Boru G, Schnipke V, Schmitthenner A F and Lark K G 2001 Identification of a QTL associated with tolerance of soybean to soil waterlogging *Crop Sci.* 41 1247–52

[6] Chen X P, Cui Z L, Vitousek P M, Cassman K G, Matson P A and Bai J S 2011 Integrated soil–crop management system for food security *Proc. Natl. Acad. Sci. U.S.A.* 108 6399–6404

[7] Ahmed F, Rafii M Y, Ismail M R, Juraimi A S, Rahim H A and Asfaliza R 2013 Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects *Biomed. Res. Int.* 2013 963525

[8] Hegazi A Z and Hamideldin N 2010 The effect of gamma irradiation on enhancement of growth and seed yield of okra (Abelmoschus esculentus L. Monch) and associated molecular changes *Journal of Horticulture and Forestry* 2 38-51

[9] Foth H D 1984 *Fundamentals of Soil Science, Sixth Edition* ed C E Millar and L M Turk (Canada: Jhon Willey and Sons, Inc.)

[10] Sitompul S M 1995 *Analisis Pertumbuhan Tanaman* ed Guritno B (Yogyakarta: Gadjah Mada University Press) 409-412

[11] Hameed A, Mahmud T S, Atta B M, Haq M A and Sayed H 2008 Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea *Pak. J. Bot.* 40 1033–41

[12] Khawar A, Bhatti I A, Khan Q M, Bhatti H N and Sheikh M A 2010 A germination test: an easy approach to know the irradiation history of seeds *Pak. J. Agri. Sci.* 47 279-285

[13] Borzouei A, Kafi M, Khazaei H, Naseriyan B and Majdabadi A 2010 Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings *Pakistan J. Bot.* 42 2281-90

[14] Kende H 1998 Deepwater rice: a model plant to study stem elongation *Plant Physiol.* 118 1105–10

[15] Mudibu J, Nkongolo K K C, Kalonji-Mbuyi A and Roger V R 2012 Effect of gamma irradiation on morphoagronomic characteristics of soybean (*Glycine max* L.) *Am. J. Plant Sci.* 3 331-337

[16] Akhtar I, Nazir N 2013 Effect of Waterlogging and Drought Stress in Plants *International Journal of Water Resources and Environmental Sciences* 2(2): 34-40

[17] Rohmah E A and Saputro T B 2016 Analisis Pertumbuhan Tanaman Kedelai (*Glycine max* L.) Varietas Grobogan Pada Kondisi Cekaman Genangan *Jurnal Sains Dan Seni ITS* 5(2) 2337-3520

[18] Fatimah V S, Saputro T B 2016 Respon Karakter Fisiologis Kedelai (*Glycine max* L.) Verietas Grobogan terhadap Cekaman Genangan *Jurnal Sains Dan Seni ITS* 5(2) 2337-3520