Germination Pattern of Four Soybean Varieties on Media Containing Al, Fe, and Mn

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Abstract

Acid soils in Lampung contain a lot of Al, Fe, and Mn, which inhibit soybean growth and production. The study of seed germination is important since plant growth begins with seeds. The study aimed to determine (1) the viability of various local soybean seeds: Anjasmoro, Grobogan, Dena-1, and Devon-1 on Al, Fe, and Mn heavy metal media and (2) the highest effect of dissolved metals among Al, Fe, and Mn on the viability of soybean seeds. The research was conducted at the Laboratory of Seed and Plant Breeding, Faculty of Agriculture, the University of Lampung from November 2019 to March 2020. The research applied a randomized complete block design (RCBD) with three replications and the data were averaged and presented as bar charts and standard deviation. The experiments were evaluated based on germination variables including germination capacity, average seed germination time, primary root length of normal germination, and dry weight of normal seedlings.The results showed that Grobogan and Anjasmoro varieties had high resistance to dissolved metals Al, Fe, and Mn, followed by Dena-1 varieties while Devon-1 was the most susceptible. The fourth day of germination showed that Fe caused the highest damage to the seeds, followed by Mn and finally Al. Interestingly, on the fifth day of germination, dissolved metal Mn had the highest toxicity effect, followed by Fe and Al.

Keywords: acid soil, seeds, soybean, germination, and viability

1. Introduction

Soybean (Glycine max L. Merr) is widely grown in Indonesia because it is one of the essential food crops in Indonesia. The need for soybeans is increasing as the population increases. In 2020, the average soybean consumption level was around 11–12 kg/per capita/year while during the period 2000 to 2019, domestic production only contributed 30–35% to the total need, which meant that 65–70% of soybean need was imported. Indonesian Ministry of agriculture projected that in the year 2022 soybean domestic production only able to cover less than 10 percent of the total national needs since the production was only 200,315 tons while the need was 2,983,511 tons (BPS, 2020).

In Indonesia, one of the efforts to increase soybean yield is by expanding soybean cultivation outside Java island, one of which is in Lampung Province. For high production, soybean farming is best suited to fertile, well-drained loam soils with a pH range of 6.0 to 7.5 (Taufiq and Sundari, 2012). However, most of the soil in Lampung is acidic characterized by a pH of less than 5.5 and soil fertility is low. (Riwandi et al., 2017).

Soil acidity affects nutrient and microbial activity as well as plant growth. Chihacek et al. (2021) explained that plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), and molybdenum (Mo) have low availability at strongly acidic pH values. Other nutrients such as manganese (Mn), copper (Cu), and zinc (Zn) tend to be more available until the soil gets very acidic (less than pH 5). Iron (Fe) and aluminum (Al) availability increases as soil acidity increases and Al become toxic to plants at pH values less than 5. On crops, soil pH below 5 caused poor nodules formation and function because of the toxicity effect of Fe and Al. Acidic soil also reduced organic matter breakdown, nutrition cycling by the microorganism, nutrient uptake by root, and inhibits root growth (Bakari, et al., 2020). The soybean did not grow well in the soil with pH below 5.2 and above 6.5 therefore the yield is low. Soybean also requires high nutrients, with P and K being most crucial for optimal production, and acidic soils that have a high concentration of Al and Fe cause P unavailable for soybean.

It was found that low soybean yields in Africa are mostly also due to nutrient deficiency such as P, Ca, Mg, Mo, and K which can be associated with soil acidity. A decrease in soil pH increases the concentration of Fe and Al ions, and these ions cause P absorption through their reaction with phosphate ions to form insoluble compounds (Keino, 2015). Kuswantoro (2017) explained that the response of several soybean genotypes to Mn toxicity in the germination phase until the sixth day found that high Mn concentrations affected the roots and shorten the hypocotyl became, which contributed to low dry weight. With those backgrounds, this research aimed to investigate the effects of heavy metal solutions Al, Fe, and Mn which exist in acidic soil on soybean germination through the viability of four soybean seeds.

Seed viability studies are important to carry out as a form of testing of seeds' qualities. Viability is reflected by the ability of seeds to grow and develop into normal plants at optimum conditions. Seeds with high viability will produce a high-quality crop. The presence of heavy metals will inhibit the absorption of water by the seeds. Heavy metals will have concurred in the macrophiles so that the water necessary for the activation of the hormone gibberellin that will synthesize DNA, RNA, and proteins for the formation of enzymes becomes slow, therefore the overhaul of food reserves and cell division is also hampered. From the four local soybean varieties, there should be some variety that showed the highest viability that could be recommended to be planted in Lampung Province.

This research aimed to study whether the low growth and production of soybeans in Lampung is related to planting the soybean on acidic soil that generally contained Al, Fe, and Mn. This research was conducted using heavy metal media Al, Fe, and Mn in the laboratory as a simulation of acidic field conditions to determine their effect on the viability of four soybean seeds. The hypothesis was heavy metal media Al, Fe, and Mn affect the viability of seeds of 4 local soybean varieties (Anjasmoro, Grobogan, Dena 1, and Devon 1).

2. Materials and Method

The research was conducted at the Laboratory of Seed and Plant Breeding, Faculty of Agriculture, University of Lampung, Indonesia from November 2019 to March 2020.

The materials used in the study were four local soybeans varieties - Anjasmoro, Grobogan, Dena-1, and Devon-1. AB mix solution (macro + micro nutrients); $AlCl_3.6H_2O$ solution 0.01M; $FeCl_3.6H_2O$ solution 0.01M; and $MnCl_2.4H_2O$ solution 0.01M. Each treatment used 100 seeds with a total of 1,600 seeds for one group. The seeds were planted on plastic trays size 25x35x5 cm filled with 2 kg gravel.

The experimental design was a completely randomized block design with a 4x4 combination and three replications. The combined 4x4 treatments were constructed from the four varieties of soybeans and four media contained metal which was control, Al, Fe, and Mn. Results data were then presented with bar charts and standard deviations.

Al 1 M solution was made by weighed $AlCl_3.6H_2O$ 241.5 g and then put into Erlenmeyer and added aqua distillation 100 ml, stirred until homogeneous and stamped to the limit of 1,000 ml. Al 1 M 100 ml was homogenized and stamped up to 1,000 ml to make Al 0.1 M; then to make a solution of Al 0.01 M, a 100 ml solution of Al 0.1 M was taken homogenized and glued to 1,000 ml. Similar steps were taken to make Fe 0.1 M using 270.5 g $FeCl_3.6H_2O$ and to make Mn 1 M using 198 g of $MnCl_2.4H_2O$.

On each plastic tray introduced control solution (AB mix), 350 ml Al solution, Fe solution, and Mn solution following each treatment. Then the trays were placed on a shelf and arranged based on the experimental layout that has been randomized according to the layout of the perfectly randomized group design.

The test was carried out by the hydroponic method. Previously the seeds to be germinated were whispered on moist paper for 12 hours. In each of the experimental media planted 100 soybean seeds arranged with a row spacing of 1x1 cm. Observations were made on the fifth day. Good seed viability is indicated by germination capacity, germination rate (time), primary root length of normal seedlings, hypocotyl length, and average normal seedlings dry weight. The methodologies for that variables is described as follow:

1.Germination capacity (%) = total normal seedling/total seeds x 100% (1)

2. Average germination rate (%/day)= $\sum_{t=0}^{t=5}\frac{NS}{t}$, NS is the number of normal seedlings (2)

3. The primary root length of a normal seedling; root length was measured on the fifth day from root base to root tip (cm).

4. The hypocotyl length of a normal seedling; hypocotyl is the upper part of the seedlings; hypocotyl length was measured on the fifth day from the base to the top of the upper part of the seedlings.

5. The dry weight of a normal seedling was measured on the fifth day from normal seedlings without the cotyledons. The seedlings were dried at oven temperature 800C until the dry weight is constant. The total dry weight is then divided by the number of seedlings to get the average dry weight of normal seedlings.

3. Results

Germination Capacity

The germination capacity of each four soybean varieties in different media was presented in Figure 1. It showed that Anjasmoro, Grobogan, Dena-1, and Devon-1 varieties maintained high seed viability (germination above 70%) in control media or without metals Al, Fe, and Mn solutions. Al, Fe, and Mn gave the same effect which is slightly less germination percentage on the soybean seeds of Grobogan varieties, Al then Mn caused lesser seed viability on Anjasmoro; on Dena-1 Al decrease the germination percentage while FE and Mn gave similar percentage. In contrast, all metal solutions gave the worst effect on Devan-1. Metal solutions Al, Fe, and Mn gave different effects on different varieties' germination percentages.

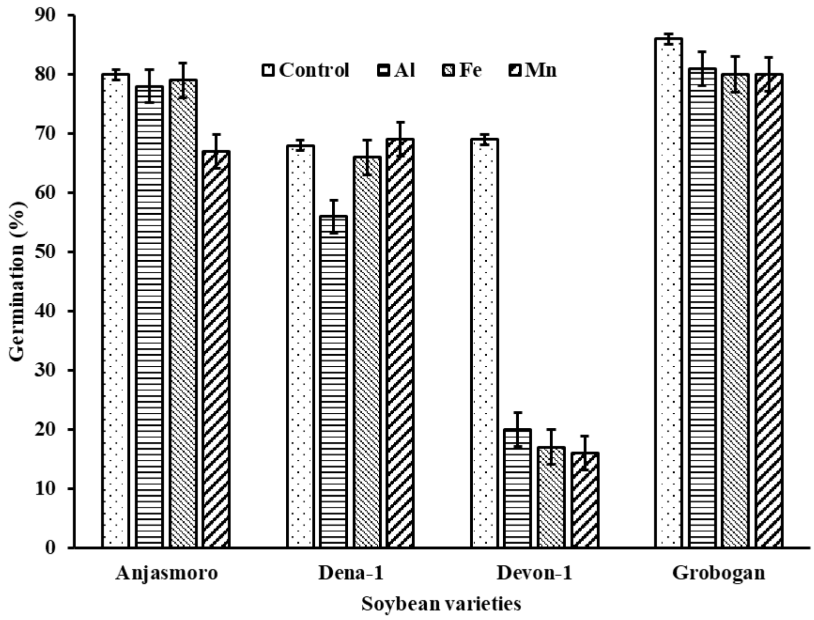


Figure 1. The germination capacity of four soybean varieties on the fifth day comparing control to media with Al, Fe, and Mn. Anjasmoro and Grobogan maintained high germination capacity while devon was the lowest

To observe which metal solutions caused a lesser germination percentage, the average germination capacity of the four soybean varieties simultaneously tested under conditions of Al, Fe, and Mn media is (Figure 2. The media containing Fe gave the worst effect on the first day to the fourth day of germination compared to Mn and Al. However, on the fifth day of germination, the effect of heavy metal Fe is equal to that of Mn or Al. On the other hand, to observe which soybean variety was affected most by the metal solutions media, the average germination percentage on each variety was counted (Figure 3)

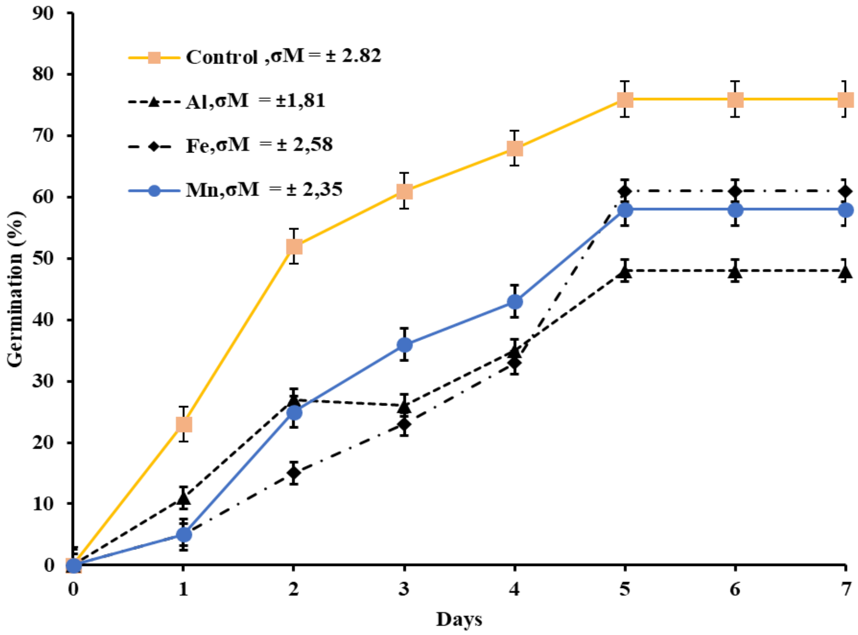


Figure 2. The germination capacity as affected by heavy metals of Al, Fe, and Mn. Fe caused the lowest germination capacity up until the fourth day but on the fifth day seeds has adapted to Fe. σM=standard error of the mean

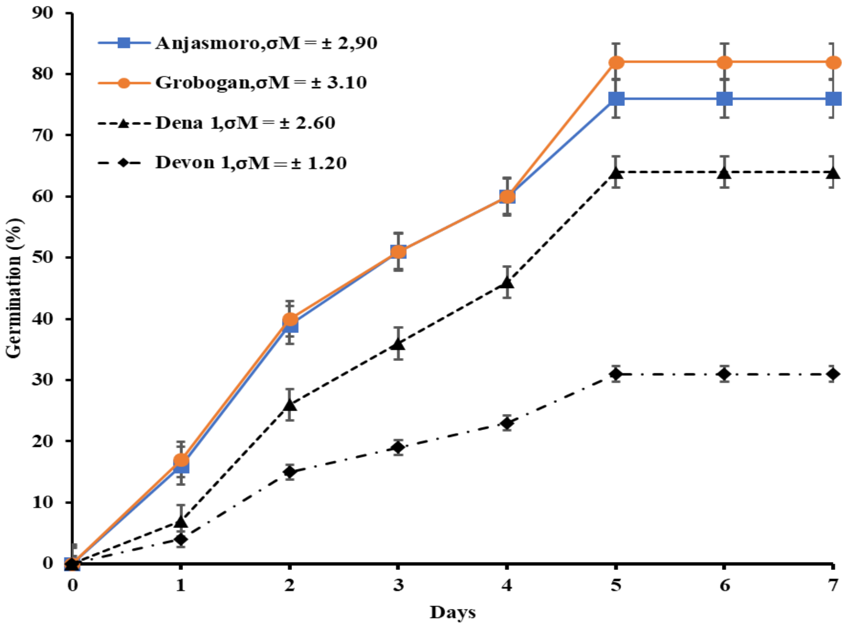
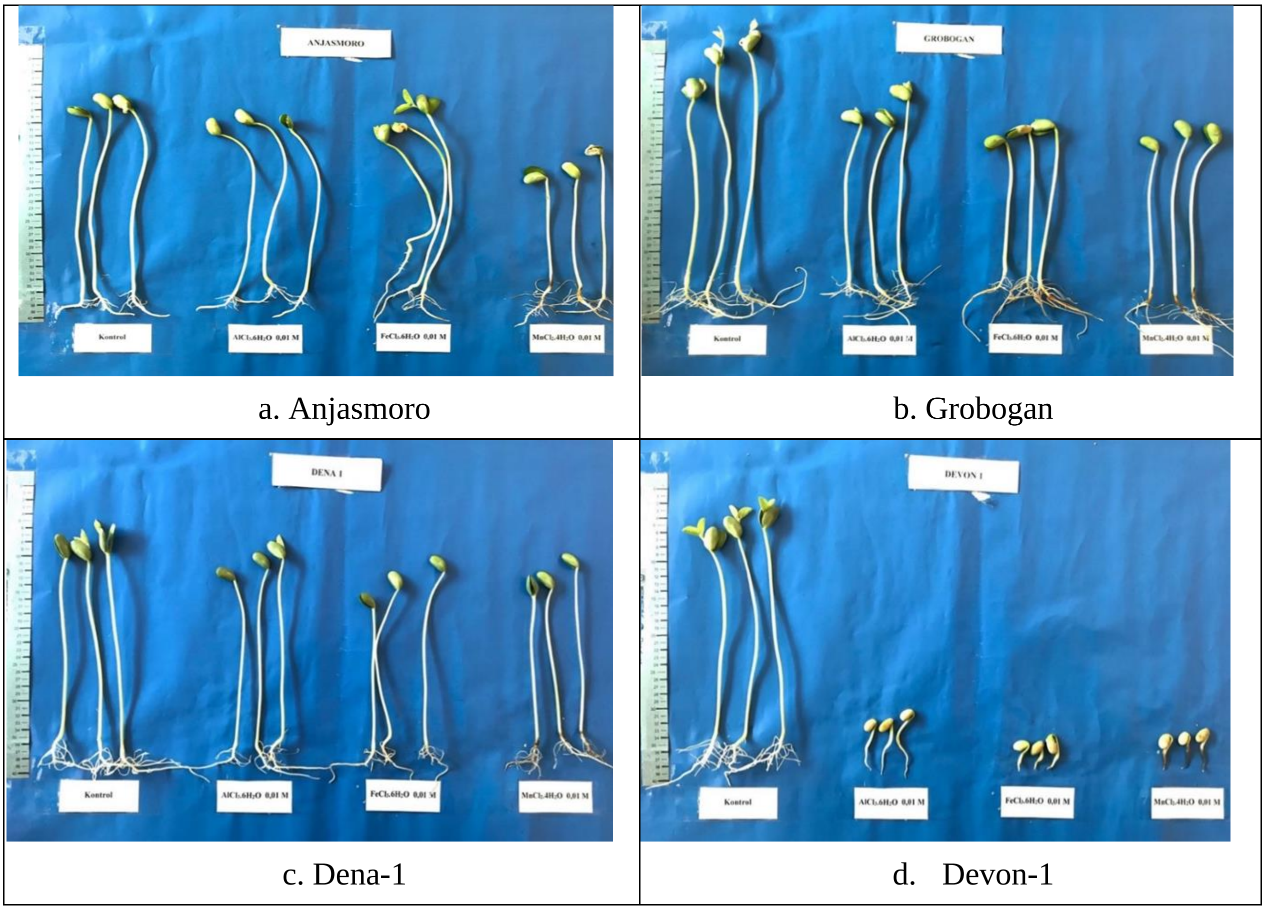


Figure 3. The average germination rate of four soybean varieties as affected by Al, Fe, and Mn from the first to the fifth day. Grobogan and Anjasmoro showed high germination rate while Devon was much the lowest., σM= standard error of the mean

Figure 3 showed the average of germination rate of the soybean varieties Grobogan and Anjasmoro, from day one to day five, had consistent germination capacity, being higher than Dena-1 and Devon-1, while the Devon-1 variety had the lowest germinability.

The growth of soybean variety seedlings up to the fifth day when grown on control media, Al, Fe, and Mn, were shown in Figure 4. Besides the germination ability, Mn media made soybean seedlings colored blackish brown roots, Fe medium made the soybean seedlings have roots with a rust-like color, while heavy metal Al, caused seedlings to have less root hair.



a. Anjasmoro | b. Grobogan | c. Dena-1 | d. Devon-1

Figure 4. Seedling performance of soybean, compared between control and those affected by the Al, Fe and Mn.

As described before, up to the fifth day, the heavy metal media Al, Fe, and Mn gave the worst effect on Devon-1 germination. Those heavy metals caused the seedlings of Devon-1 to be unable to elongate the roost and the crown, so the soybean seedlings were significantly stunted

Average germination time

Figure 5 showed that the four soybean varieties need time to germinate, which tends to be the same. In the control media, when ordered from the fastest to slowest, there were Grobogan, Anjasmoro, Dena-1, and Devon-1 varieties respectively. The germination speed on the heavy metals media Al, Fe, and Mn showed that germination on Fe medium for the four soybean varieties required more time, resulting slowest germination process when compared to Mn or Al (the sequence from the fastest was Al <Mn < Fe).

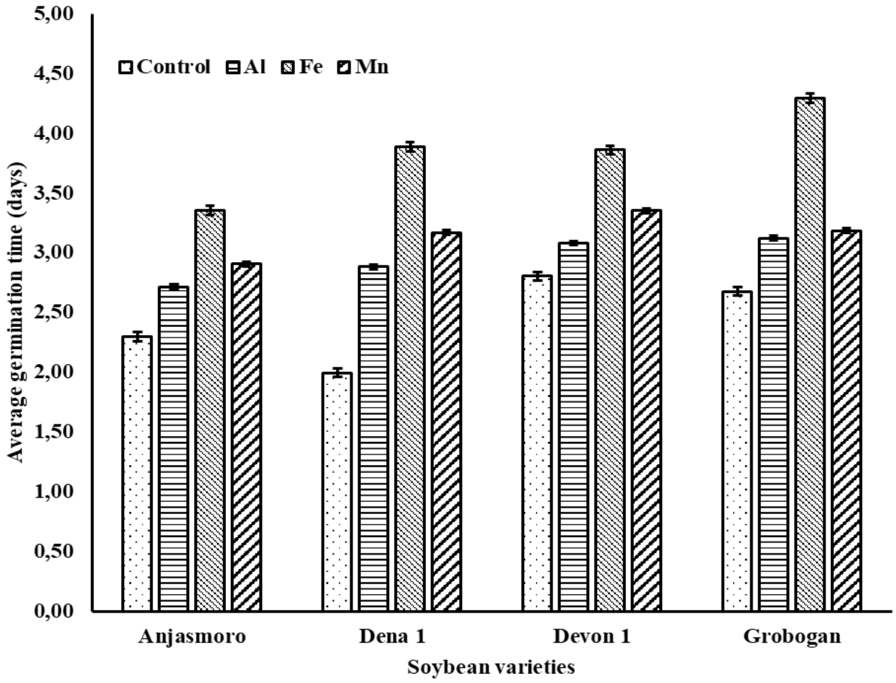


Figure 5. The average germination time of the soybean varieties

On the fifth day, the primary root length of normal seedlings compared between control to heavy metal media Al, Fe, and Mn was 6.65 cm, 5.10 cm; 5.42 cm; and 4.04 cm respectively (Figure 6). Similarly that the highest hypocotyl length was the control media at 14.71 cm, followed by Fe at 12.07 cm; Al at 11.82 cm; and Mn at 10.10 cm (Figure 7). The average hypocotyl root ratio was presented in Table 1.

Table 1. Ratio hypocotyl to root compared between the medium and the four soybean varieties

Medium	Ratio hypocotyl to root	Varieties	Ratio hypocotyl to root
Control	2,21	Anjasmoro	3,23
Al	2,31	Grobogan	1,85
Fe	2,21	Dena 1	2,26
Mn	2,67	Devon 1	1,79

Interestingly to observe, the Fe medium had inhibited germination (0-4 days) see Figure 3, but on the fifth day, soybean seeds had adapted to the effect of heavy metal Fe. This also could be observed on normal seedling primary root length, normal hypocotyl length, and normal germination dry weight observed on the fifth day; seedlings on Fe medium had reached the highest length.

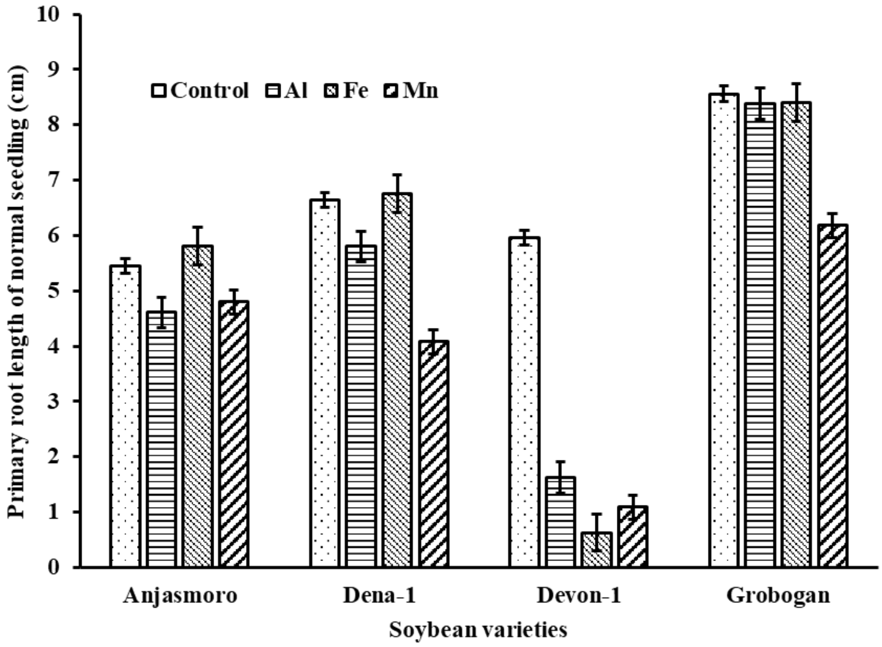


Figure 6. The primary root length of a normal seedling on the fifth day

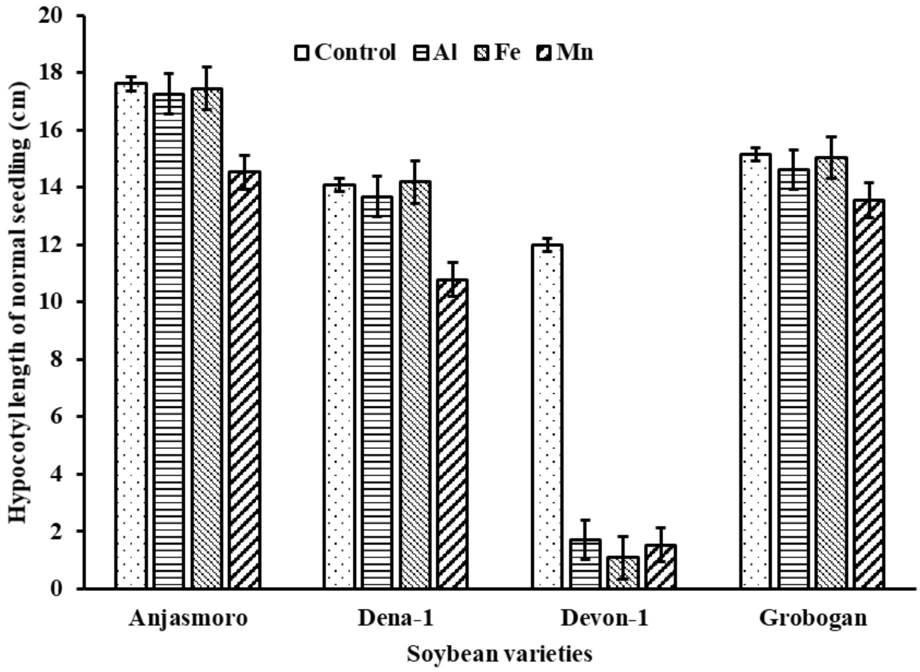


Figure 7. The hypocotyl length of a normal seedling on the fifth day

Root and crown length determined the light germination weight. The dry weight from the control, Al, Fe, and Mn was 0.76 g, respectively; 0.61 g; 0.57 g; and 0.40 g (Figure 8).

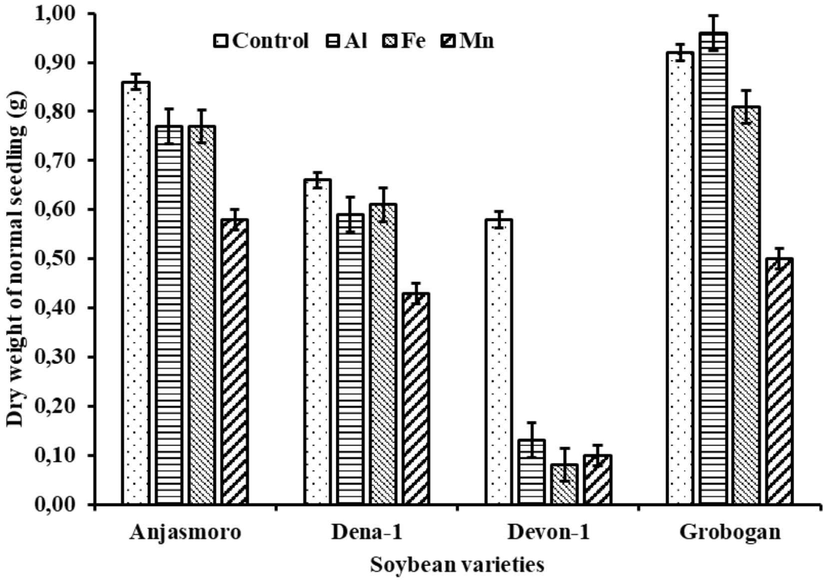


Figure 8. The dry weight of a normal seedling on the fifth day

4. Discussion

Seed viability results in this study were discussed from two sides.

First, based on the chemical content of seeds. The tested soybean varieties on heavy metals Al, Fe, and Mn media showed that Grobogan had the highest viability, followed by Anjasmoro, Dena-1, and the lowest Devon-1 variety. Soybeans contain a lot of protein rather than carbohydrates and fats. for chemical content, especially protein, the Grobogan variety has the highest protein content (43.9%), followed by Anjasmoro (41.8-42.1%), Devon-1 (37.97%), and Dena-1 (36.7%) (Balitkabi, 2016). Protein character is easy to absorb water or hydrophilic. The protein, carbohydrates, fats, and minerals in seeds functioned as raw materials and energy for embryos during germination. (Artika et al., 2017). Large soybean seeds contain more food reserves and have many more enormous sources than tiny seeds. The content of food reserves will be disported by enzymes after the water enters the seeds due to the imbibition process. The water that enters the seeds will activate enzymes. Protein sources are converted to amino acids by protease, carbohydrates such as starch and hemicellulose into sugar, and lipase converts fat into fatty acids and glycerin (Bahri et al., 2012).

Second, in terms of seed size, Grobogan is the largest seed size, followed by Anjasmoro, Dena-1, and Devo-1. Large seeds benefit from seedling survival and produce more vigorous seedlings, which support seedling establishment in more stable habitats (Arellano and Peco, 2012; Souza and Fagundes, 2014). In this study, different soybean varieties have different tendencies to imbibition. Grobogan, Anjasmoro, and Dena-1 seeds were more tolerant to media containing heavy metals than Devon-1, but heavy metals of Al, Fe, and Mn tended to affect soybean seed imbibition in the same pattern. In the media containing dissolved metals, it turned out that the Devon 1 variety (Figures 1,2,3, and 4-7) was not resistant to the stress of dissolved metals containing Al, Fe, and Mn. The Devon 1 variety is rich in isoflavones. The Devon 1 variety is an extension of the promising line KXIAC 100-997-1035 and has a total isoflavone content of 2219.74 g/g. Isoflavones in soybean seeds can be in the form of aglycone and glucoside compounds. The main aglycone compounds which consist of genistein, daidzein, and genistein are the main parts of glucoside compounds. Genistein is the primary isoflavone in soybeans, reaching 75% of the total isoflavones (Balitkabi, 2016).

The presence of isoflavone compounds allows metallic bonds with flavones and forms a complex of flavones and metals that affect the germination of Devon-1 varieties. Generally, flavone compounds are present in the seed coat and affect dormancy and seed germination (Shirley, 1998; Debeaujon et al., 2007). Several studies on the effect of dissolved metals on seed germination have been reported (Athar and Masood, 2002; Li et al., 2005; Datta et al., 2011). In addition, the impact of metals on seed germination decreases water uptake and transport (Bewley and Black, 1994; Becerril et al., 1989). The difference in water entering the seeds originated from zero until the second day of germination; the heavy metals Fe and Mn tended to enter less water than the control and Al. According to Vezza et al. (2018), Al, Fe, and Mn in germination media affect the ability of seeds to absorb water. Water distribution into the seeds is slow because the metals Al, Fe, and Mn have larger molecules than water so the metals will cover the seed micropyle. Water will be difficult to enter the seeds because the potential for water in the seeds is higher than in heavy metal media. It will slow down the water entering the seeds.

Water supply in the seed cells is very important for the continued germination of seeds. Lack of water during germination will disrupt the cell division process, inhibiting the production of the hormone gibberellin for the formation of enzymes that break down food reserves. Finally, radicle formation slows as the translocation of food back to the embryo occurs gradually. Heavy metals were found to alter the selection permeability properties of the cell membrane, accelerate the breakdown of stocked nutrients in the endosperm, decrease hydrolysis of starch by -amylase, and disrupt cellular homeostasis in some experiments (Shafiq et al., 2008, Mittal et al., 2015) and some stress-related proteins (Ahsan et al., 2007). This elucidation is following the results of the study because the presence of heavy metals Al, Fe, and Mn affected the germination process of soybean seeds from the first to the fourth day; it appears that the media with heavy metal Fe had the worst effect on soybean seeds, followed by Mn and Al. The form of heavy metals of Al, Fe, and Mn that will inhibit germination were Al3+, Fe2+, and Mn2+ (Aggarwal et al., 2015; Rout and Sohoo, 2015; Seran, 2017).

Rout and Soho (2015) stated that Fe3+ would be reduced to Fe2+, which can poison germination because Fe2+ plays an active role in photosynthesis and respiration. Based on the presence of Fe(II) more than 100 mg per liter has been reported in some fields, with serious Fe toxicity for rice (Sahrawat 2004). Research by Ling Lu et al. (2013) using x-ray fluorescence (XRF) showed that Fe2+ in rice germination after 24 hours mostly deposited on the radicle; this allowed the inhibition of radicular growth through cell division. During the 0-4th day of germination, the stress by heavy metal Al was less than Mn and then less than Fe (Figure 2), but on the fifth day, the soybean seeds had adapted to the heavy metal Fe, so that in the Mn media condition, the effect was more visible on the yield of seedlings on the fifth day, the pattern became Al less than Fe and Fe less than Mn.

Heavy metals of Al, Fe, and Mn after seedlings roots formatting will accumulate in the roots so that the root tips are blackish brown, see Figures 7-10. Also, the ratio of hypocotyl to root (Tables 1 and 2) showed that the media contained dissolved metals caused inhibition of the water into the seeds and caused poisoning. The possibility of dissolved metals and forming chelates binds to free auxins in the meristematic part of the roots, thereby inhibiting cell division. Perrot-Rechenmann (2010) noted that the elongation of plant cells requires water absorption and Auxin is one of the primary stimuli that affect the mechanism of cell elongation.

According to Budiyanto (2016), at the molecular level, heavy metals are related to DNA, which affected phytochemical properties and biological functions such as cell division in meristems, cell addition, and DNA and RNA synthesis. This results in a lighter dry weight because the food reserves in the cotyledons only accelerate cell elongation and inhibit new cell division. As the results of overhauling the food reserves in the seeds are more prioritized for the elongation of the hypocotyl, the hypocotyl grows higher than the root. Even though plant defense techniques exist to manage metal harmfulness through diminished take-up into the cell, sequestration into vacuoles by the arrangement of complexes, authoritative by phytochelatins, an amalgamation of osmolytes, enactment of different agents to combat ROS, changed expression of enzymes, that overexpression of gene exist (Sharma et al. 2011, Mustafiz et al.,2011, and Wang et al. 2011), the mechanism of germinating seeds combat overwhelming metal stress remains to a great extent obscure.

From these experiments, it can be concluded that local soybean varieties Grobogan and Anjasmoro showed higher resistance to Al, Fe, and Mn stress with average germination of 80% followed by the Dena-1 with average germination of 63% and dropped to the most vulnerable variety Devon-1 with average germination 18%. Also, in the germination process, Fe gave the highest heavy metals impacts on soybean seedlings, followed by Mn and Al (Al <Mn <Fe); however, on the fifth day of seedlings yield, heavy metal Mn showed the highest effect, followed by Fe and Al (Al <Fe <Mn). This research should be continued by planting the seeds on the field contained with Al, Fe, and Mn to confirm the results gained from a laboratory experiment. Soybean varieties such as Grobogan and Anjasmoro should be available widely to increase soybean production in Indonesia. However, since acid soil with the potential presence of heavy metal compounds is common in the tropical area, seeds breeding to develop seeds that resistance to heavy metal toxicities is a high priority.

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