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Effect of Iron (Fe) heavy metal content at different pH on the germination of seven soybean varieties in Indonesia

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ABSTRACT

Greater use of acid soil has expanded the area under cultivation for soybeans; however, acid soil is associated with heavy mineral toxicity, including Iron (Fe). This investigation looked at how well soybean seeds germinated in media containing heavy metal Fe and how the pH of the media affected the viability of soybean seeds. This research was conducted at the Seed and Plant Breeding Laboratory at the University of Lampung, Indonesia. The experimental design was a randomized block design. The first factor was seven soybean local varieties – Grobogan, Anjasmoro, Derap 1, Detap 1, Dena 1, Deja 1, and Dega 1 – and the second factor was heavy metal Fe solution pH of 6–7 and 4.5 and without heavy metal Fe (control). Seed viability in heavy metal Fe medium was assessed using radicle emergence, germination capacity or percentage, germination speed, number of normal seedlings, normal seedling hypocotyl length, main root length of regular seedlings, and normal shoot dry weight. This research found that heavy metal Fe affected soybeans' seeds' viability in pH 6–7 and 4.5. The observed data showed that all seeds' viability variables in media with heavy metal Fe, both in pH 6–7 and in pH 4.5, differ from the control media. The seed viability in media heavy metal Fe pH 6–7 was not significantly different from the control media, but in pH 4.5, the difference is significant. Soybean varieties' characteristics also influence how heavy metal Fe in different pH affects seed viability. Overall, Anjasmoro, Deja, Grobogan, and Dega were the types that consistently demonstrated resistance or adaptation to heavy metal Fe existence, while Dena, Derap, and Detap are susceptible to heavy metal Fe existence. Seed viability in Iron medium is not always related to seed physical performance; therefore, before planting soybean in acid soil, it is recommended to conduct a seed viability test.

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1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a substantial source of protein for humans and a high-quality animal feed (Karges et al., 2022) since soybeans contain necessary food supplements. In the future, as the human population and awareness of the importance of healthy living are steadily increasing, a global soybean demand is unavoidable. Regretfully, soybean and other agricultural items have yet to keep pace with expected demand. For example, the Indonesian Statistics Bureau (BPS, 2020) stated that the soybean Indonesian national product was only 632,300 tons, while the average need is 3,200,000 tons per year; the gap should be covered by importing soybeans, which in 2020 reached about 2,475,286 tons. Considering the effect of climate change, researchers projected that Indonesia's soybean production is lower than that stated by the BPS. It is 574,600 tons in 2020 with the current climate conditions and 571,400 tons by 2050 with modest climate change mitigation efforts (RCP 4.5) and 528,330 tons if there is no significant mitigation efforts (RCP 8.5) (Table 1) (Fischer et al., 2021). RCP stands for representative concentration pathways, which is the term for the quantitative future greenhouse gas concentration and additional energy taken up by the Earth system. RCP 2.6 means radiation intensity of 2.6 Wm^{-2} which is the lowest scenario level, RCP 4.5 is the middle level, and RCP 8.5 is the maximum level since there is no mitigation effort (Nazarenko et al., 2015). Moreover, the area suitable for soybean cultivation has remained the same (Nazarenko et al., 2015). Since soybean is among the 16 major crops grown worldwide, soybean research to boost their production is still desperately needed (Pagano & Miransari, 2016). In Indonesia, besides soybean being an important food, the primary cause of the demand's striking growth is the move toward more industries employing those commodities as raw materials (Rachmat & Erwidodo, 1996).

Due to the limited reserves of productive agricultural land to meet national food needs, the Indonesian Agriculture Ministry must utilize suboptimal land, particularly acid soil. Of Indonesia's 189.2 million ha landmass, about 108.8 million ha includes acidic dry land, with the widest spread in Sumatra, Kalimantan, and Papua (Mulyani & Sarwani, 2013). The large acid soil area could be related to heavy precipitation that caused intense washing of alkaline soil, resulting in 70% of the land being acidic. Suboptimal land naturally has low productivity due to internal and external factors. Acid soil (pH < 5.5) has been used to increase soybean production; however, acid soil is a global problem to plant production mainly because soil pH influences the availability of nutrients and heavy metal level of toxicity makes acid soils often to lack essential nutrients (Bian et al., 2013; Sintorini et al., 2021).

The Agricultural Research and Development Agency of the Indonesian Agriculture Ministry has issued some superior soybean varieties with the hope that the types could withstand suboptimal land. Some of those superior varieties with high protein and lipid content are Grobogan, Anjasmoro, Deja-1, Dega-1, Detap, Dena, and Derap. The Anjasmoro variety is a large seed category of soybean that is adaptive to all land agroecosystems, more adaptive than all other soybeans. This is a new superior variety for tidal marshlands, a good reason for possible resistance to heavy metal, with similar characteristics to the Grobogan variety, which grows well in shallow marshland. Detap 1 seed variety displayed an outstanding growth rate of 91.67 % at 7 DAP (days after planting) (Aisah et al., 2020; Endrizal & Jumakir, 2015; Haitami et al., 2021; William & Saleh, 2016).

The problem in cultivating soybeans in acid soil is that optimum soybean yields require a pH of at least 6.3. Low pH will restrict soybean nodulation production and function due to the toxicity effects of Al and Fe ions (Bakari et al., 2020). At pH 5.5, soybeans can still produce with severe growth inhibition, but the obtained results are better at pH 6–6.8.

Overall, the occupancy of fast-growing soybean significantly increased at alkaline pH (Cruz et al., 2019). For extending the soybean plantation area, finding a suitable variety to overcome soil acid problems is necessary. National soybean productivity, in general, was 1.44 $tons.ha^{-1}$; in contrast, the adapted variety to acid soil had higher performance than the superior national variety. Research in the acid soil of Central Lampung resulted in the highest grain yield shown by soybean line SC5P2P3.5.4.1-5 with 2.51 $tons.ha^{-1}$ (Kuswantoro, 2014). A seed viability test is necessary to select those superior varieties to soil pH and heavy metal Fe resistance, since it is proven that if the adapted variety could be found, the soybean production is high.

Certain heavy metal elements such as Cu, Zn, and Fe at low quantities are necessary for plant growth but become hazardous in higher amounts. Deficiency or toxicity is caused by any imbalance of heavy metal solubility plant uptake from the soil (Ghori et al., 2019; Sethy & Ghosh, 2013; Vácha, 2021). Heavy metals have also hampered seed germination (El Rasafi et al., 2016). High levels of Fe oxides often characterize subtropical and tropical acid soils. Fe is required for photosynthesis and chlorophyll production. It has been hypothesized that Fe may play a key role in regulating NO_3^- immobilization in acid forest soils (Jiang et al., 2015). Similarly, nitrification inhibition by large concentrations of iron from pyrite (FeS_2) can also occur. On the other hand, Fe is highly reactive and poisonous due to the Fenton reaction. Fe toxicity occurs in soils with high active Fe, hydrogen sulfide, acidity, and aluminum. Furthermore, Fe toxicity occurs in the presence of aluminum, regardless of the existence of organic materials (Haque et al., 2022).

Before planting soybeans in acidic soil, it is essential to test soybean seeds' viability and vigor in media containing heavy metal Fe in various pH levels. Crop production starts with good seeds since seeds contain the embryo of the plant; thus, verifying seed viability is crucial (Finch-Savage & Bassel, 2015). The use of high-vigor planting seeds is required for all crops; poorer yield could be indirectly related to reduced seed vigor. Seeds of good strength will prevent replanting and the consequent delay in maturity or decreased productivity. Seed germination is crucial in determining crop yields, particularly in dry and semiarid regions, and guarantees crop sustainability in changing environment (Bezini et al., 2019; Reed et al., 2022).

This research aimed to investigate soybeans' ability to grow in soil containing heavy metal Fe. The study suggests an assessment of pH and heavy metal Fe pollution effects on the germination of seven Indonesian local soybean cultivars.

2. MATERIAL AND METHODS

2.1 Research site and soybean varieties

This research was conducted at the Seed and Plant Breeding Laboratory and the Integrated Laboratory and Centre of Technology and Innovation at the University of Lampung, Indonesia, from May to July 2021. Seven varieties of local soybean were used in this study, including Anjasmoro, Dena 1, Detap 1, Deja 1, Grobogan, Dega 1, and Derap 1.

2.2 Experimental design

The experimental design was group randomized design (7 × 3) with three replications. The first factor was seven varieties of local soybeans, and the second factor was comprised of the pH 6–7 heavy metal Fe solution, pH 4.5 heavy metal Fe solution, and a pH 7 or neutral solution without heavy metal Fe solution. Each variety used 50 seeds, 350 seeds for the 7 varieties, and 1050 seeds for the 3 replications. To conduct the experiment, Fe solution was first created using $FeCl_36H_2O$ at 0.01 M. The pH of the obtained solution was adjusted using NaOH (0.01 M) solution to reach 6–7 and 4.5 pH values.

Table 1. Soybean area and production in Indonesia as predicted with recent climate condition (RCP 2.6), modest climate change mitigation effort (RCP 4.5), and if there is no significant mitigation effort (RCP 8.5)

Climate scenarios	Water supply	Area (km^2)			Production x 1000 tons		Max potential yield (kg ha^{-1})
		Total	VS	S	VS	S	
RCP 2.6 year 2020	Irrigated	655.73	484.82	155.37	226.73	60.22	5368
	rainfed	719.88	553.84	153.90	235.71	51.94	4887
RCP 4.5 year 2050	Irrigated	655.73	474.79	151.03	217.87	58.16	5264
	rainfed	719.88	591.02	116.87	256.34	39.03	4985
RCP 8.5 year 2050	Irrigated	655.73	393.46	224.41	178.31	89.95	5174
	rainfed	719.88	507.80	189.11	199.03	61.04	4477

Remarks: VS = very suitable land, S = suitable land
Source: FAO Gaez v4 Data Portal (Fischer et al., 2021)

2.3 Preparation for heavy metal Fe solution

The $FeCl_3 6H_2O$, 0.01 M solution, was produced by weighing 0.275 g Fe and adding aqua distillation up to 1000 ml and then stirred until homogenous in the Erlenmeyer flask. The NaOH 0.01 M solution was developed by weighing 0.275 g of NaOH and adding aqua distillation up to 686 ml and then stirred until homogenous in the Erlenmeyer tube. The NaOH solution was added to the heavy metal Fe solution until it reached pH 6–7 and 4.5 (500 ml and 250 ml, respectively).

2.4. Preparation for seed observation

The planting medium used for this experiment was perforated plastic trays (25 × 35 × 5 cm) which were put over the non-perforated tray containing the heavy metal Fe solution, and then the trays were put on racks arranged as the block randomized experimental design. The experiment used hydroponic techniques; seeds that would be germinated were first disinfected with sodium hypochlorite 0.1% for 3 minutes and then drained and imbibed for 2 hours on running water, and after that, the seeds were planted on scribbling paper at 1 × 1 cm. The paper was soaked using $FeCl_3 6H_2O$ pH 6–7, pH 4.5, and control (aqua distillation). The paper was rolled, put on a seed germination appliance, and observed for 1 day, and then the seedlings were transferred to trays containing the treatment solutions for 5 days.

2.5. Seed viability parameters

Radicle emergence, germination capacity or percentage, germination speed, number of normal seedlings, normal seedling hypocotyl length, main root length of regular seedlings, and normal shoot dry weight were variables used to evaluate the viability of seeds in a heavy metal Fe medium.

2.5.1. Radicle Emergence (%)

Radicle emergence is defined as the appearance of a radicle after breaking through the seed coat (imbibition). Observations are made from the first to the fifth day and are expressed in percentage (Eq. 1).

$$Radicle\ emergence\ (\%) = \left(\frac{\Sigma\ radicle\ (days\ 0)}{total\ seeds} + .. + \frac{\Sigma\ radicle\ (days\ 5)}{total\ seeds}\right) x100 \quad .... [1]$$

2.5.2. Germination Percentage (GP)

A germination test determines the percentage of seeds alive in any seed lot. Germination was determined by counting the number of seeds germinating normally or normal seedlings at days 3 and 5 divided by the total seeds sprouted (Eq. 2).

$$Germination\ percentage\ (\%) = \frac{\Sigma\ normal\ seedlings\ (days\ 3 + days\ 5)}{total\ seeds} x100 \quad .... [2]$$

2.5.3. Germination Speed (%/etmal)

Germination speed provides a measure of the time course of seed germination divided to total germinated seeds expressed in percent (Eq. 3).

$$Germination\ speed = \frac{n3}{d3} + \frac{n4}{d_4} + \frac{n5}{d5} \quad .... [3]$$

where n = number of germinated seeds and d= number of days (from days 3 to 5).

Note: etmal stands for estimation time of arrival; essentially, this is a 24 hours' time unit which is employed frequently in seed science.

2.5.4. Vigor Index (VI)

Vigor testing not only measures the percentage of viable seeds in a sample; it also indicates the capacity of those seeds to provide healthy seedlings under less than ideal or unfavorable growing environments similar to those in the field. In this research, vigor index was obtained from some normal seedlings on the first observation (Eq. 4) (Milivojević et al., 2018).

$$\text{Vigor index} = \frac{\Sigma\ normal\ seedling\ on\ first\ count}{N} x100 \quad ....(4)$$

2.5.5. Abnormal Seedlings

Abnormal seedlings are identified based on traits such seedlings with damaged cotyledon; seedlings with constriction, splits, and cracks; seedlings with damaged primary leaves and apical bud; and seedlings with weak and unbalanced development of the essential structures, e.g., injurious hypocotyls and epicotyls and inferior root system.

The abnormal seedling percentage is the percentage of the observed seeds with abnormal appearance, including seeds that germinate but then rot at the end of the observation day. Abnormal seedlings were tallied on the last day of observation (the fifth day).

Normal seedlings were considered after 5 days of planting for growth parameter determination. Indeed, hypocotyl and root length (cm) were measured at the conclusion of the study. For dry weight determination (mg), hypocotyls and the roots from the normal seedlings of each experimental unit were separated from the cotyledons. Then hypocotyls and roots were wrapped and individually dried in a Memmert-type oven at 80°C for 3 x 24 hours or until they reached a constant dry weight. The hypocotyl length of normal seedling was measured 5 days after planting. The crown length was measured from the cotyledon root's base to the growing point's tip. Seedling root length was measured 5 days after planting. Root length was measured from the root's base to the root's tip.

The hypocotyl and the roots from the normal seedlings of each experimental unit were separated from the cotyledons. Then the seedlings were wrapped and dried in a Memmert-type oven at 80°C for 3 x 24 hours or until their dry weight remained constant. The weighing was done with an Ohaus-type analytical or precision balance.

The separated roots of normal seedlings were weighed, placed in an envelope, and dried in a Memmert-type oven at 80°C for 3 x 24 hours or until the dry weight remained consistent. The weighing was done with an Ohaus-type analytical or precision balance.

2.6. Metal content analysis for the soy seed sample

The seed sample was weighed 0.5–1 gram and put in a deconstruction flask and then filled with 5 ml HNO_3 1:1, 5 ml HCl 1:1, and 5 ml H_2O_2 30 %. The flask is heated using heavy metal digester with a temperature of 95 °C for 30 minutes, and the sample is cooled; then the sample is filtered into a 50 ml measuring flask using Whatman no.41 filter paper and diluted with ultrapure to the mark of volume measurement limit. Finally, the metal content of the sample is ready to be measured using MPAES (microwave plasma atomic emission spectrometer) Agilent 4210.

Table 2. Recapitulation of analysis of variance of seven soybean variety seeds as impacted by the pH level of heavy metal Fe media

Variables	Treatments		
	pH	V	PxV
Radicle emergence (%)	**	**	ns
Germination (%)	**	**	ns
Vigor index (%)	**	**	ns
Germination rate (%)	*	**	ns
Hypocotyl length (cm)	**	**	ns
Primary root length (cm)	**	**	ns
Hypocotyl dry weight (mg)	**	**	*
Root dry weight (mg)	*	*	ns
Abnormal seedlings (%)	*	**	ns

Remarks: P, pH; V, soybean varieties; ns, not significant at 5%; *, significant at α 5 %; **, significant at α 1 %

2.7. Statistical analysis

The statistical analysis was done using R program (https://cran.r-project.org/bin/windows/base/) and GerminaQuant program (https://cran.r-project.org/web/packages/GerminaR/vignettes/GerminaQuant.html). When the treatment effect was found significant, the mean difference was tested using the LSD test at P ≤ 0.05.

3. RESULTS

The recapitulation of the analysis of variance for all variables is presented in Table 2. The table demonstrated that pH level of the heavy metal Fe affected all variables; likewise, seeds variety characteristics. However, there was no interaction between pH and seed variety in most of the variables; interaction between pH and soybean varieties only existed on seeds' hypocotyl dry weight variable.

3.1. Effect of pH of Fe media on seed viability variables

Table 3 presented the effect of heavy metal Fe pH level on seed viability variables including radicle emergence, seed germination, germination speed, vigor index, hypocotyl length, root length, and root dry weight of normal seedling and percentage of abnormal seedlings.

Radicle emergence evaluation showed that in control media and media with heavy metal Fe at pH of 6–7, no discernable difference was observed (78% and 76.19%). While in media with heavy metal Fe at pH of 4.5, the radicle emergency was significantly reduced (63.42%).

Heavy metal Fe affected the germination. Germination percentage was lower in heavy metal Fe media compared to control media without heavy metal Fe. The rate of germination in heavy metal Fe with a pH of 6–7 was, however, much lower than in control media (80.96% and 82.57%, respectively). The lowest percentage of germination was caused by the heavy metal Fe at pH 4.5 (72.28%). In comparison with the control media without heavy metal Fe, germination speed was shorter in the heavy metal Fe media. Comparing heavy metal Fe medium with pH 6–7 to control media (24.35% and 25.09%, respectively), no significant difference was seen. The shortest germination speed, however, was seen in heavy metal Fe media with pH 4.5 (22.07%) (Table 3).

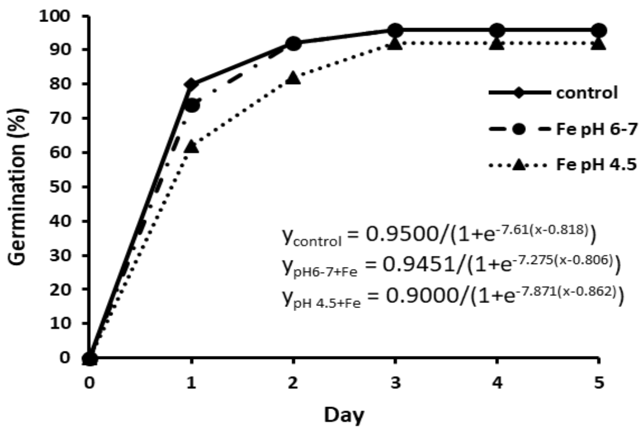

Figure 1. Nonlinear germination model of seven varieties of soybeans on a medium without heavy metal Fe (control) and contains heavy metal Fe with a pH of 6–7 and 4–5. Based on germination data (%) (Table 4)

Table 3. Effect of heavy metal Fe media pH on seed viability variables

pH media	Radicle emergence (%)	Germination (%)	Germination speed (%.etmal^{-1})	Vigor index (%)	Hypocotyl length (cm)	Root length (cm)	Root dry weight (mg)	Abnormal seedlings (%)
Control	78.00^{a}	82.57^{a}	25.09^{a}	62.19^{a}	13.64^{a}	8.51^{a}	5.08^{a}	3.11^{b}
Fe pH 6–7	76.19^{a}	80.95^{a}	24.35^{a}	58.66^{a}	13.62^{a}	6.94^{b}	5.10^{a}	3.63^{b}
Fe pH 4.5	63.42^{b}	72.28^{b}	22.07^{b}	46.19^{b}	12.43^{b}	5.84^{c}	4.66^{b}	4.42^{a}
LSD 0.05	7.50	5.26	2.06	7.32	0.54	0.76	0.35	0.64

Remarks: Two mean values followed by the same letter do not differ based on the 5% LSD test

Table 4. Effect of variety characteristics on seed viability variables

Varieties	Radicle emergence (%)	Germination (%)	Germination speed (%.etmal^{-1})	Vigor index (%)	Hypocotyl length (cm)	Root length (cm)	Root dry weight (mg)	Abnormal seedlings (%)
Grobogan	84.22^{a}	83.77^{a}	26.07^{a}	65.33^{a}	13.23^{bcd}	7.12^{ab}	4.81^{bc}	3.59^{abc}
Anjasmoro	70.22^{bc}	85.11^{a}	26.12^{a}	62.88^{a}	13.88^{ab}	7.06^{ab}	5.34^{ab}	2.86^{c}
Derap 1	61.77^{c}	70.88^{bc}	20.94^{c}	47.33^{c}	12.41^{de}	6.46^{bc}	4.76^{c}	4.04^{ab}
Detap 1	63.33^{c}	69.11^{c}	20.13^{c}	43.33^{c}	13.58^{bc}	5.85^{c}	4.60^{c}	4.36^{a}
Dena 1	67.33^{bc}	77.77^{ab}	22.87^{bc}	49.33^{bc}	12.94^{cd}	7.33^{ab}	4.80^{c}	4.28^{a}
Deja 1	82.44^{b}	80.66^{a}	25.45^{ab}	63.33^{a}	14.66^{a}	8.09^{a}	4.90^{abc}	3.29^{bc}
Dega 1	78.44^{ab}	82.88^{a}	25.27^{ab}	58.22^{ab}	11.92^{e}	7.76^{a}	5.40^{a}	3.61^{abc}
LSD 0.05		8.04	3.15	11.19	0.83	1.16	0.54	0.97

Remarks: Two mean values followed by the same letter do not differ based on the 5% LSD test

In addition, the average seed germination rate for seven soybean types is also described in Figure 1. The control medium had the highest rate of germination on the first day, but from the second to the fifth day, the germination rate of the heavy metal media treatment was greater. While heavy metal Fe pH 4.5 indicated the lowest germination rate, heavy metal Fe pH 6–7 tended to match the control. On the first through third days, media influence is discernable. On the fourth and fifth days, however, germination rates were fixed, indicating that media influence had ended.

Vigor index showed significant decrease at heavy metal Fe media with pH 4.5 (46.19 %), instead of heavy metal Fe media with pH 6–7 where the vigor index did not significantly change compared to control media (62.19 % and 58.66 %, respectively).

Hypocotyl length was shorter in heavy metal Fe media compared to control media without heavy metal Fe. Hypocotyl length in heavy metal Fe with pH 6–7 did not show significant difference from control media (13.64 cm and 13.62 cm, respectively). However, heavy metal Fe with pH 4.5 resulted in the shortest hypocotyl length (12.43 %).

Root length was noticeably shorter in heavy metal Fe media compared to control media lacking heavy metal Fe (Table 3). Root length in heavy metal Fe with pH 6–7 was significantly shorter compared to the control media (8.51 cm and 6.94 cm, respectively) and root length in heavy metal Fe with pH 4.5 significantly shorter than in heavy metal Fe with pH 6–7 (5.84 cm).

Evidently, heavy metal Fe impacted root dry weight of the typical seedlings. Root dry weight in control media and heavy metal Fe with pH 6–7 were not significantly different (5.08 mg and 5.10 mg, respectively). Root dry weight (4.66 mg) was considerably decreased by the presence of the heavy metal Fe at pH 4.5.

Heavy metal Fe also affected percentage of abnormal seedlings. Abnormal seedling percentage was greater in heavy metal Fe media compared to control media without heavy metal Fe. However, abnormal seedling percentage was significantly higher in heavy metal Fe with pH 4.5 (4.42%).

3.2. Effect of variety characteristics on seed viability variables

Conversely, Table 4 displayed the impact of various seed variety attributes on seed viability variable as previously mentioned.

The effects of several soybean varieties on the appearance of radicles were displayed for control media devoid of heavy metals Fe and for media containing heavy metals Fe with pH ranges of 6–7 and 4.5. The cultivars Grobogan, Deja 1, Dega 1, and Anjasmoro demonstrated improved radicle emergence, with respective radicle emergence rates of 84.22%, 82.44%, 78.44%, and 70.22%. The radicle percentages of the variants Dena 1, Detap 1, and Derap 1 are lower, at 67.33%, 63.33%, and 61.77%, respectively.

The seed germination rates for the cultivars Anjasmoro, Grobogan, Dega 1, and Deja 1 were better and not noticeably different, at 85.11%, 83.77%, 82.88%, and 80.66%, respectively. The radicle percentages of the variants Dena 1, Derap 1, and Detap 1 are lower, at 77.77%, 70.88%, and 69.11%, respectively.

For the germination speed**,** Anjasmoro (26.12%), Grobogan (26.07%), Deja 1 (25.45%), Dega 1 (25.27%), and Dena 1 (22.87%) are some kinds that showed comparable germination speeds; Derap 1 and Detap 1 had slower germination speeds, at 20.94 and 20.13%, respectively.

Vigor index was also impacted by numerous factors. The Grobogan, Deja 1, and Anjasmoro vigor indices, which were 65.33, 63.33, and 62.88%, respectively, did not substantially

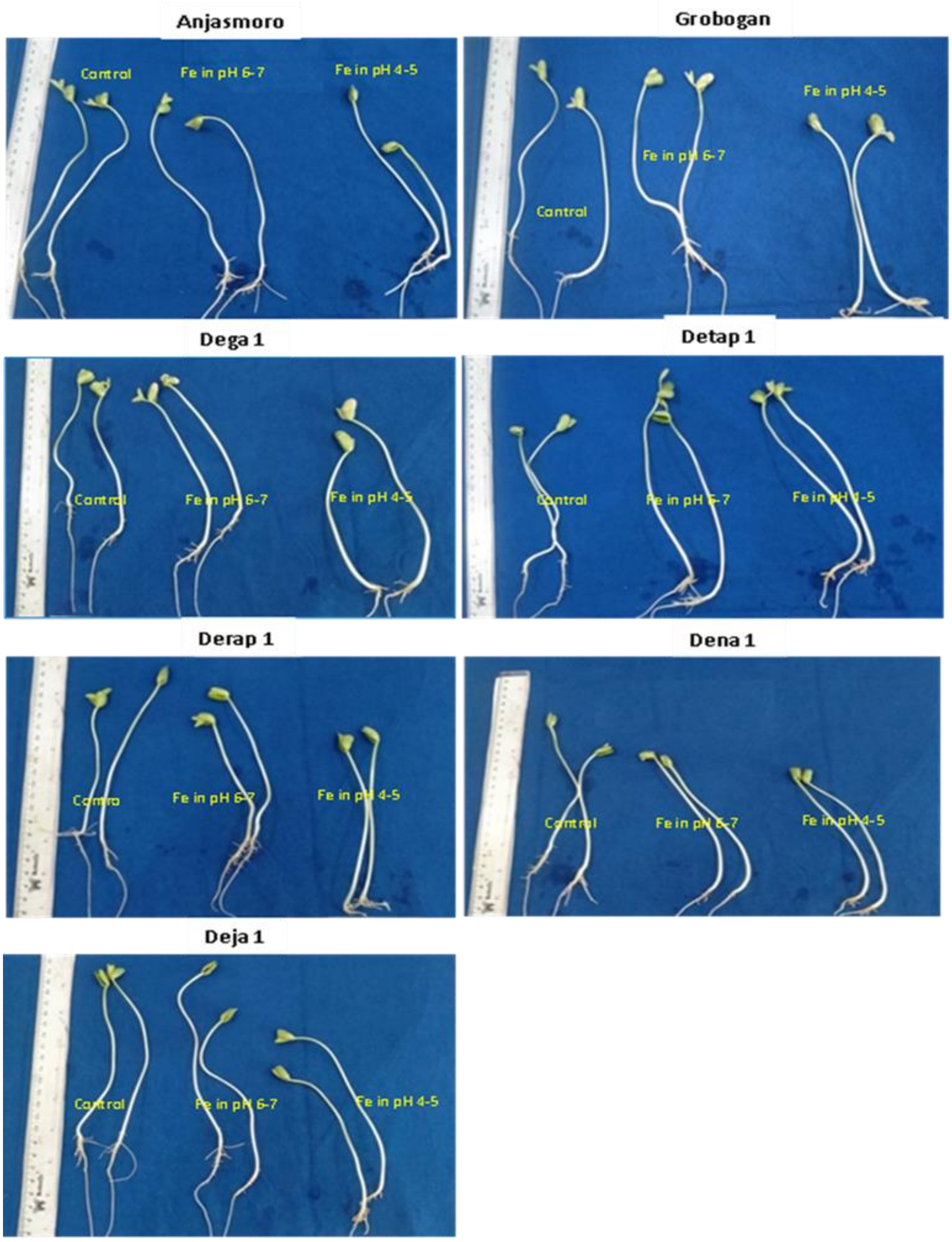

Figure 2. Visualization of roots and hypocotyl of seven soybean varieties affected by media without heavy metal Fe and with heavy metal Fe media with pH 4.5 and 6–7

differ from one another. Dega 1, Dena 1, Derap 1, and Detap 1 were varieties with low vigor indices, with corresponding values of 58.22%, 49.33%, and 43.33%.

The hypocotyl length of normal seedlings was considerably influenced by a variety of features, as shown in Table 4. Detap 1, Grobogan, and Dena 1 came in second (13.58, 13.23, and 12.94 cm respectively), followed by Derap 1 and Dega 1 with shorter hypocotyl lengths (12.41 and 11.92 cm) and variants Deja 1 and Anjasmoro with longer hypocotyls compared to other kinds (14.66 cm and 13.88 cm).

The root length of a typical seedling was determined by a variety of characteristics. Detap 1 and Derap 1 had the smallest roots, measuring 6.46 cm and 5.85 cm, respectively. Deja 1 variety had the largest root (8.09 cm), followed by Dega 1, Dena 1, Grobogan, and Anjasmoro.

Furthermore, Figure 2 illustrated the effect of Fe on the hypocotyl and root length of each seed type during germination. The hypocotyl and root length performances of the seeds varied. According to Table 4, Deja (located at the bottom at the picture) had the best seed germination outcomes since it had the longest hypocotyl and roots. The Grobogan is the next plant, and it is the fourth in terms of hypocotyl and root length (top right).

Root dry weight of normal seedlings was greatly impacted by several factors. The kinds with the best root dry weights are Dega 1, Anjasmoro, and Deja 1 (5.40, 5.34, and 4.90 mg, respectively). These are followed by Grobogan, Dena 1, Derap 1, and Detap 1 (4.81, 4.80, 4.76, and 4.60 mg, respectively).

The features of the variety influenced the percentage of abnormal seedlings. The percentage of aberrant seedlings in the varieties Detap 1 and Dena 1 was highest (4.36 and 4.28%), followed by Derap 1, Dega 1, and Grobogan (4.04, 3.61, and 3.59%, respectively). The least number of abnormal seedlings was found in Anjasmoro and Deja, at 2.86% and 3.29%, respectively.

3.3 Interaction of media pH and soybean varieties

The hypocotyl dry weight variable showed a pH and variety interaction. Deja 1 and Dega 1 generated the best hypocotyl dry weights at neutral pH (control) and on heavy metal Fe medium with pH 6–7 variations, with respective hypocotyl dry weights of 23.77 and 22 g (control media) and 20.05 and 21.54 g (heavy metal Fe media with pH 6–7). The weight of the hypocotyl did, however, significantly decrease in both types at pH 4.5. Both types lacked low pH resistance. The Grobogan, Anjasmoro, Derap 1, Detap 1, and Dena 1 cultivars, with dry weights of 17.21, 18.97, 18.45, 19.34, and 18.39 g, were more robust at pH 4.5. The weights that were observed did not differ significantly from the weights at control media and heavy metal Fe media at pH 6–7. Apiece variety reacted differently to controls without Fe metal or neutral pH; the Deja 1 variety had the largest hypocotyl weight, followed by the Dega 1, Anjasmoro, Dena 1, Grobogan, and Detap 1 variations, with hypocotyl weights of 23.77, 22, 20.82, 19.27, 18.55, and 17.86 g apiece. The Dega 1 variety had the highest hypocotyl dry weight in the Fe metal concentration with a pH of 6–7, followed by the Detap 1, Anjasmoro, Deja 1, Grobogan, Derap 1, and Dena 1 variations, with corresponding hypocotyl dry weights of 21.54, 20.83, 20.72, 20.05, 18.96, 18.20, and 16.48 g. In Fe heavy metal media with pH of 6–7, all varieties except Deja 1 and Dega1 produced the same response, i.e., decreased, but the effect was not significant. Conversely, the Deja 1 and Dega 1 varieties declined notably.

An additional experiment had been done in this research to support the assertion that heavy metals are capable of inhibiting soybean germination. This was conducted by examining the heavy metal Fe content in the soybean seeds with pH 6–7 and 4.5. The results showed that for seeds imbibed on control media, the Fe concentration is 149.62 ppm; on heavy metal Fe solution pH 6–7, the Fe concentration rose to 316.75 ppm, and the maximun was on heavy metal Fe pH 4.5, 483.15 ppm. Average seed increasing weight after imbibition processes showed weight among the treatments: control, heavy metal Fe pH 6–7, and heavy metal Fe pH 4.5 are not much different – 0.2 $g.seed^{-1}$, 0.19 $g.seed^{-1}$, 0.18 $g.seed^{-1}$, respectively.

4. DISCUSSION

This study found that heavy metal Fe impacted soybeans' seeds' viability particularly at pH 4.5. The observed data showed that all seed viability variables (seed germination, germination speed, vigor index, hypocotyl length of normal seedling, root dry weight, and abnormal seedlings) in media with heavy metal Fe at pH 4.5 showed significant differences. Soybean seeds demonstrated high viability without heavy metal Fe solution; indeed, soybean seeds showed significantly low viability in media Fe with pH 4.5.

From the heavy metal content study, it was clear that heavy metal Fe toxicities were stronger in lower pH solutions. Some research concluded that extractable Fe is mainly influenced by soil pH. High soil pH limits the amount of Fe that access plants; as pH rises by 1 unit, the amount of Fe^{3+} considerably decreases because insoluble Fe^{3+} hydroxide forms (Fageria & Nascente, 2014; Mari et al., 2020; Xu et al., 2022). The impact of Fe on germination processes has appeared since the beginning of germination, the radicle emergence or radicle protrusion from seed (Ali & Elozeiri, 2017). Water is a fundamental necessity for seed germination, which is referred to as imbibition. Seventy-eight percent of the recorded seeds germinated in the control media, which is water without Fe. Since Fe entered the seed when the pH was 6–7 and more when the pH was 4.5, the heavy metal Fe caused the radicle emergence to decrease to 76.19% and to 63.42%, respectively. As stated by Fageria and Nascente (2014), minimum Fe solubility occurs between pH 7.5 and 8.5, which is the pH range of most calcareous soils. It can be implied that Fe solution with pH 6–7 intakes by the seeds is the same as water in control state since Fe solubility is minimum. Therefore, both solutions (control and heavy metal Fe pH 6–7) entered the seeds with the same rate as Louf et al. (2018) found that initial imbibition in soy seeds is driven primarily by capillary forces and since soy seed is a single pore scale porous material, the imbibition process in soy seeds is homogenous.

The above assertion was supported by Figure 1, which showed the radicle emergence. On the first day, the control medium showed the highest germination rate; however, on the second day, germination rate of heavy metal media Fe pH 6–7 matched the control, while heavy metal Fe pH 4.5 still indicated the lowest germination rate. From the third day onward, the radicle germination rate remained constant and became plateau for both the control and heavy metal Fe with pH 6–7; this showed that seeds in heavy metal Fe pH 6–7 had absorbed water the same amount with the control and then the radicle emergence. The general nonlinear equation is expressed as $Y = a/(1+ e^{-b(x-c)})$, and this fitted to the event time model (Ritz et al., 2013). Using data of germination in Table 4, the equations for each pH treatment were presented in Figure 1. According to the formula, in average radicle emergence fast on the first day are 76% for both control and the heavy metal Fe media with pH 6–7 and 54% for media with pH 4.5. On the second day, all treatments reached the maximum (95% for both control and the Fe media with pH 6–7 and 87% for media with pH 4.5) and then became a flat line until the fifth day. Aung and Masuda (2020) stated that the form of Fe that is hazardous and absorbed by plants is Fe^{2+}; and Fe^{2+} form increases at a pH of about 4.5–6 and decreases at pH of >6 (de Mello Gabriel et al., 2021; Pennisi & Thomas, 2015). These are reflected in this study; germination on the first 3 days on soil containing rich heavy metal Fe with pH 4.5 showed that the number of radicles and germination percentage decreased. However, at pH 6–7, Fe^{2+} decreased resulting in the amount of radicle emergence, and the percentage of germination was not different from the control media (Table 3). In the growth rate variable and abnormal seedlings, heavy metal Fe pH 4.5 treatment showed the highest percentage of abnormal shoots compared to the control treatment and heavy metal Fe pH 6–7 (Table 3). Again, Fe elements will be hazardous if the pH value is low; Iron content in a solution with a pH of 4.5–6.0 will increase Fe levels in tissues and show symptoms of poisoning in plants (Asadi-Kavan et al., 2020; Das et al., 2017; Gülser et al., 2019; Rengel, 2015; Rodrigues Filho et al., 2020).

Since toxicity of Fe affects the radicle germination, then the following seed viability will also be impacted: seed germinating speed, the proportion of germination, decrease in root growth, and increase in the quantity of abnormal seedlings (El Rasafi et al., 2016). Excessive Fe even affects almost all cellular functions, including embryos in seeds, protein damage, DNA synthesis, and cell division; still, this study is restricted to seed viability factors. Another feature of Fe heavy metal poisoning is the thickening of the root base and the hypocotyls (Figure 2) since Fe accumulated in the roots. On the medium heavy metal Fe pH 4.5, the growth of the roots of shoots was shorter, and the color resembled rust. The root hypocotyl ratio decreased due to heavy metals. Heavy metal causes structural and morphological alterations in roots, including non-formation of root hairs, stunted growth, and browning of the meristematic portion of the roots (Dey et al., 2019; Nikolic & Pavlovic, 2018). Reviews related to the effect of heavy metal inhibition had been done by Lapaz et al. (2022); and Baruah et al. (2019) demonstrated that Cd and Fe have the greatest effects on wheat germination. However, the germination of red bean seeds is only reduced in the presence of Cd, while these seeds seem to be more tolerant of Fe even at high metal concentrations.

The roots are part of the plant that gets contaminated initially; therefore, they are more sensitive to metal toxicity than shoots (Chai et al., 2022; Kuswantoro, 2014; Li et al., 2016; Rizvi et al., 2020; Shahid et al., 2017; Sultana et al., 2022). Figure 2 showed that soybean seed germination in an iron-rich solution resulted in seedlings that were thinner, smaller, and of a different color from those that germinated in an iron-free treatment (control). Additionally, to the increased level of the heavy metal Fe, the germination process will be severely hindered (Zielińska-Dawidziak et al., 2014). As shown in this study, the Fe pH 4.5 treatment factors of hypocotyl length, root length, and dry weight of the roots showed the lowest values compared to the control treatment and the heavy metal Fe pH 6–7 (Table 3). The deadly effect of Fe^{3+} toxicity is the nonexistence of germination or suppression of germination, and the damage occurs mainly to the root area. Mittal et al. (2015) found that contamination of the metal Fe leads to inhibition of germination and the ratio of root length to hypocotyledons by 70 ± 11.55% and 1.15 ± 0.90 cm, respectively. The hypocotyl dry weight variables showed the interaction between heavy metal Fe and soybean varieties. The influence of the heavy metal Fe on hypocotyl elongation and, ultimately, its weight was depending on pH and soybean varieties. Grobogan, Derap 1, and Detap 1 on pH 6–7 had heavier hypocotyl dry weight compared to control, while Derap 1 and Detap 1 had heavier hypocotyl dry weight on pH 4.5 compared to control.

In terms of hypocotyl dry weight, Derap 1 and Detap 1 are cultivars that were more tolerant to heavy metal Fe both at pH 6–7 and at pH 4.5 (Table 5). This interaction also indicated that the prominent soybean varieties' characteristics also influenced how heavy metal Fe in different pH affected seed viability. From this study, soybean cultivars independently gave different responses in all observation variables.

Table 5. Interaction of media pH and soybean varieties on hypocotyl dry weight

Varieties	pH			LSD 5%
	Control	pH 6–7	pH 4.5	
Grobogan	18.55^{Acd}	18.96^{Abc}	17.21^{Aa}	
Anjasmoro	20.82^{Abc}	20.72^{Aa}	18.97^{Aa}	
Derap 1	17.86^{Ad}	18.20^{Acd}	18.45^{Aa}	
Detap 1	18.49^{Ad}	20.83^{Aab}	19.34^{Aa}	2.18
Dena 1	19.27^{Acd}	16.48^{Ad}	18.39^{Aa}	
Deja 1	23.77^{Aa}	20.05^{Aabc}	19.40^{Ba}	
Dega 1	22.00^{Aab}	21.54^{Aab}	18.80^{Ba}	

Remarks: Two mean values followed by the same letter do not differ based on the 5% LSD test. Upper case refers to horizontal rows; lower case refers to vertical columns

Table 6. Soybean variety seed characteristics as released by the Indonesia Ministry of Agriculture

Varieties	Seed size		Weight	Hypocotyl	Crude protein	Oil
	EI (mm)[a]	FI (mm)[b]	100 seeds (g)	Color	(g.100 g^{-1})	(g.100 g^{-1})
Anjasmoro	1.22± 0.08	0.35±0.02	14.81±0.45	Purple	42.27±0.15	18.27±0.67
Dega 1	1.214±0.04	0.39±0.04	19.80±0.45	Purple	37.13±0.60	17.03±0.55
Deja 1	1.27±0.06	0.224±0.04	11.20±0.45	Purple	39.63±0.15	17.17±0.32
Dena 1	1.30±0.06	0.30±0.06	16.00±0.00	Purple	37.00±0.98	18.33±0.90
Derap 1	1.22t±0.10	0.29±0.04	15.60±0.89	Purple	38.82±0.39	17.77±0.85
Detap 1	1.22±0.07	0.26±0.07	14.80±0.45	Purple	40.34±0.25	15.55±1.45
Grobogan	1.25±0.07	0.29±0.06	18.00±0.71	Brown	43.80±0.36	18.47±0.31

Remarks: a, eccentricity index; b, flatness index
Source: (Arifin et al., 2023)

Different varieties exhibited distinct properties even within the exact origin means that Indonesian soybean varieties also have variations in agronomical and morphological characteristics (Arifin et al., 2023; Zhen et al., 2021). Overall, Anjasmoro, Deja, Grobogan, and Dega were kinds that consistently showed resistance or adapted to Fe existence, while Dena, Derap, and Detap were sensitive to Fe existence. The seed characteristics of the observed seeds (Table 6) showed that the main characteristics of those varieties are relatively similar. Therefore, the seed viability test is essential to ascertain seeds' vigor when the seeds experience heavy metal stress.

5. CONCLUSION

This research finds that heavy metal Fe affected soybean seeds' viability both in pH 6–7 and 4.5. All seed viability characteristics in media with heavy metal Fe, both in pH 6–7 and in pH 4.5, differ from the control media (without the heavy metal Fe). Soybean can grow in soil containing heavy metal Fe if the pH is 6–7. Soybean varieties influence the seeds' resistance to heavy metal Fe existence. Anjasmoro, Deja, Grobogan, and Dega were the varieties that consistently showed resistance or adapted to heavy metal Fe existence. However, the differences were not shown in the seeds' physical or chemical properties. The viability test is vital in investigating seeds' vigor against heavy metal stress.

Declaration of Competing Interest

The authors declare that no competing financial or personal interests that may appear and influence the work reported in this paper.

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