Growth response of three forest seedlings to iron exposure

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Abstract. Fe is one of the microessential plants needed in low concentrations. The purpose of this study was to examine the growth response of three forest seedlings to iron exposure in water culture media. This study used a completely randomized design (CRD) with Fe dose treatment and consisted of 9 levels, namely 0, 0.5, 0.1, 1.5, and 2, with 3 replications each and each replication consisting of 3 plant units. The plants used consisted of Ochroma bicolor, Shorea macrophylla, and Senna siamea. The results of this study showed that Fe exposure treatment had a significant effect on the three seedlings on all growth parameters (height, root length, root dry weight, shoots, and total dry weight). Increasing the concentration of Fe can reduce all growth parameters. The three seedlings have different tolerance levels. Fe exposure treatment had a significant effect on the SPAD index only on O. bicolor and S. siamea seedlings. S. macrophylla seedlings had the highest tolerance index value compared to the other two seedlings. This indicated that S. macrophylla seedlings had higher resistance than the other two seedlings to Fe exposure.

1. Introduction
Iron (Fe) is one of the microelements needed by plants in low concentrations [1,2,3,4]. Works for plant development and growth processes, especially plant physiologic processes, DNA synthesis, photosynthesis, respiration, and becomes a cofactor for various enzymes [3,5,6,7]. However, the presence of Fe in high concentrations can be toxic to plants [8] [9]. Toxicity Fe is present in the form of Fe2+ [10,11], and is widely available in acid soils [12]. The high availability of Fe2+ can cause an imbalance of nutrients which will have an impact on inhibiting plant growth and causing plant productivity [13].

Revegetation is one of the important aspects in reforesting marginal lands, especially acid lands that have high Fe content. The selection of forestry plant species in revegetating land with high Fe content is very important, to support the success of revegetation. This shows that the selection of plant species that are resistant to Fe exposure is very important. So far, there have not been many studies on the types of forestry plants that are resistant to Fe exposure. Therefore, this study aimed to examine the growth of forestry plant species against Fe exposure.
2. Method

2.1 Seed germination and media preparation
Seed dormancy was broken on *Ochroma bicolor* and *Senna siamea* seeds, while *S. macrophylla* seeds were directly sown using zeolite media. Breaking dormancy refers to the method of Algofari *et al.* [14] with a modification, where the seeds were soaked in 80 °C hot water (80 °C) for 15 minutes and then soaked in water. After that, the seeds were sown in a media bath that was filled with zeolite media. Seeds are maintained until they grow and are ready for weaning, watering is carried out 2 times a day taking into account the humidity of the media. Weeding and seed acclimatization is done when the seeds have started to grow 2-3 leaves.

The medium used in this research is water culture (aquadest). The nutrient solution used included 1.5 mM Ca(NO$_3$)$_2$.4H$_2$O, 1.0 mM NH$_4$NO$_3$, 1.0 mM KCl, 0.4 mM MgSO$_4$.7H$_2$O, 1.0 mM KH$_2$PO$_4$, 0.50 ppm MnSO$_4$.H$_2$O, 0.02 ppm CuSO$_4$.5H$_2$O, 0.05 ppm ZnSO$_4$.7H$_2$O, 0.50 ppm H$_3$BO$_3$, 0.01 ppm (NH$_4$)$_6$Mo$_7$O$_{24}$.4H$_2$O [15], while for Fe exposure using FeSO$_4$.7H$_2$O.

2.2 Seedling adaptation test and Fe exposure
Seedlings were weaned or transferred to containers that already contained media and maintained for ±14 days (acclimatization). Seedlings are placed on a serofom that has been perforated and wrapped with cotton so that it is able to stand upright. After the adaptation test, the seedlings were transferred to a new container that already contained the media and had been added with Fe in accordance with the predetermined concentration (0, 0.5, 1, 1.5, and 2 mM). During the adaptation test and experimental treatment of Fe exposure, the condition of the media was maintained at pH 4. The pH adjustment was carried out by adding 1 N HCl and NaOH. The addition of media is done when the media volume has started to decrease. The media was changed after 14 days to keep the seedlings growing optimally. Seedlings are maintained for up to 30 days.

2.3 Parameter evaluation and crop harvesting
Parameters measured included: plant height, root length, root and shoot dry weight, total seedling dry weight, chlorophyll index, and tolerance index. Plant height was measured once a week for 4 weeks using a ruler. Root length was measured at week 4 and measured using a ruler. The chlorophyll level was measured using a SPAD chlorophyll meter. The plants were harvested after 30 days, the roots and shoots of the seedlings were separated and oven-baked for 2 days at 80 °C and then weighed to obtain the dry weight.

2.4 Research design and data analysis
This study used a one-factor completely randomized design (CRD) with 5 levels of Fe dose (0, 0.5, 1, 1.5, and 2 mM). Each treatment was repeated 3 times and each replication consisted of 3 plant units. Data analysis using the Anova test was then continued with the Duncan Multiple's Ranget Test (DMRT) at a 95% confidence level (α = 5).

3. Result and Discussion

3.1 Result

3.1.1. Plant height
Fe treatment was able to give a significant effect on the height growth of the three seedlings (Table 1). The increase in Fe concentration was able to reduce the height growth of the three seedlings. Several concentrations of Fe were able to give significant differences in the height growth of the three seedlings, especially the control, only the type of *S. macrophylla* treated with Fe 0 mM did not show a significant difference with Fe concentration of 0.5 mM. In addition, the treatment of Fe 1-2 mM on the types of *O.*
bicolor and S. macrophylla did not show a significant difference. These results indicate that each type of plant has a different height growth response to Fe exposure.

### Table 1. The height of the three seedlings on Fe exposure after 4 weeks

| Species         | Concentrations (mM) | P-value |
|-----------------|---------------------|---------|
|                 | 0       | 0.5    | 1      | 1.5    | 2       |
| O. bicolor      | 16.03 ± 1.53 a      | 8.83 ± 2.17 b | 1.18 ± 0.73 c | 0.70 ± 0.34 c | 0.10 ± 0.13 c | **       |
| S. macropylla   | 2.47 ± 1.72 a       | 2.23 ± 0.59 a | 1.23 ± 0.13 b | 0.43 ± 0.26 b | 0.12 ± 0.48 b | **       |
| S. seamea       | 3.62 ± 0.52 a       | 2.43 ± 0.44 b | 1.45 ± 0.26 c | 0.60 ± 0.17 d | 0.47 ± 0.47 d | **       |

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level.

** significant effect at the 1% level.

#### 3.1.2 Root length

Fe exposure treatment was able to give a significant effect on the root length of the three seedlings (Table 2). The increase in Fe concentration was able to reduce the root length of the three seedlings. The decrease in length of O. bicolor and S. siamea was higher than that of S. macrophylla seedlings. In addition, at a concentration of 2 mM in the species S. macrophylla produced a higher root length than the Fe concentration of 0.5-15 mM.

### Table 2. Root length of the third seedling to Fe exposure after 4 weeks

| Species         | Concentrations (mM) | P-value |
|-----------------|---------------------|---------|
|                 | 0       | 0.5    | 1      | 1.5    | 2       |
| O. bicolor      | 33.13 ± 15.77 a     | 12.07 ± 1.56 bc | 8.75 ± 1.50 c | 8.50 ± 5.12 c | 8.83 ± 1.84 c | **       |
| S. macropylla   | 12.28 ± 1.85 a      | 6.32 ± 1.10 c | 5.96 ± 0.23 c | 7.56 ± 1.16 c | 7.38 ± 1.27 bc | **       |
| S. seamea       | 22.16 ± 2.99 a      | 8.86 ± 1.93 d | 13.56 ± 3.31 b | 10.30 ± 3.40 bcd | 8.80 ± 2.05 d | **       |

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level.

** significant effect at the 1% level.

#### 3.1.3 Seedling Dry Weight

Fe treatment had a significant effect on the dry weight of the three seedlings (Table 3). The increase in Fe concentration was able to reduce the dry weight of the three seedlings. Type O. bicolor experienced a very significant reduction in dry weight compared to other species. Seedling dry weight is also strongly influenced by shoot and root growth of each type of seedling. When the shoot and root growth of the seedlings is high, the dry weight of the seedlings will also increase, and vice versa.

### Table 3. Dry weight of the third seedling to Fe exposure after 4 weeks

| Species         | Concentrations (mM) | P-value |
|-----------------|---------------------|---------|
|                 | 0       | 0.5    | 1      | 1.5    | 2       |
| O. bicolor      | 3.58 ± 1.46 a      | 1.30 ± 0.19 c | 0.14 ± 0.06 d | 0.09 ± 0.02 d | 0.07 ± 0.03 d | **       |
| S. macropylla   | 0.84 ± 0.07 a      | 0.59 ± 0.02 a | 0.57 ± 0.13 b | 0.43 ± 0.05 b | 0.38 ± 0.04 b | **       |
| S. seamea       | 0.19 ± 0.04 a      | 0.13 ± 0.07 bc | 0.20 ± 0.03 a | 0.08 ± 0.02 cd | 0.05 ± 0.01 d | **       |

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level.

** significant effect at the 1% level.
3.1.4 SPAD chlorophyll index
Fe exposure treatment had a significant effect on the SPAD chlorophyll index in O. bicolor and S. seamea species, while in S. macropylla, Fe exposure did not show a significant effect. (Table 4). The 0.5 mM Fe concentration was able to increase the chlorophyll content of the type O. bicolor, and decreased with increasing Fe concentration. In the type of S. seamea the decrease in SPAD chlorophyll index was highest when the Fe concentration was 2 mM.

Table 4. SPAD chlorophyll index values of the three types of seedlings on Fe exposure

| Species          | Concentrations (mM) | P-value |
|------------------|---------------------|---------|
|                  | 0           | 0.5      | 1          | 1.5         | 2           |
| O. bicolor       | 21.56 ± 1.63 ab    | 24.27 ± 2.21 a | 19.37 ± 3.96 bc | 17.32 ± 0.47 bc | 14.76 ± 2.63 c | **         |
| S. macropylla    | 32.47 ± 9.29 a     | 34.65 ± 1.43 a | 34.25 ± 3.10 a  | 29.13 ± 3.65 a  | 32.99 ± 3.15 a | ns         |
| S. seamea        | 30.62 ± 3.66 a     | 30.24 ± 1.27 a | 29.12 ± 5.38 a  | 28.66 ± 1.16 a  | 18.50 ± 4.19 b | **         |

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level.
** significant effect at the 1% level.

3.1.5 Seedling tolerance index
The tolerance index shows the response of the seedling resistance or tolerance to heavy metal exposure, one of which is Fe. Fe exposure showed a significant difference compared to the control (Figure 1). The increase in Fe concentration was able to decrease the tolerance index of O. bicolor and S. macropylla seedlings, while in S. siamea seedlings the 1 mM Fe concentration was able to increase the tolerance index, but decreased again with increasing Fe concentration.

Figure 1. Tolerance index of three types of plants to Fe exposure. The different letters above the bar chart show significant differences based on the DMRT test (α=5)

3.2 Discussion
Fe is one of the essential metals needed for plant growth and development [3,16,17]. Fe exposure had a significant effect on the growth parameters (height, root length, and dry weight) of the three seedlings (Tables 1, 2, and 3). The increase in Fe concentration was able to reduce the growth of the three seedlings. The results of several studies also reported that increasing the concentration of Fe can reduce plant growth, such as pineapple [18], Vaccinium macrocarpon [19], soybean [20,21], and Discorea spp. [22]. Growth inhibition due to Fe toxicity is thought to be caused by an increase in Fe content in plants which interferes with plant metabolic processes and damages plant cell structures [23].
and an increase in Fe concentration is able to inhibit nutrient absorption, so that plants experience nutrient deficiency (deficiency) nutrients. Snowden and Wheeler [24] reported that P and N concentrations in shoots decreased due to increased Fe concentrations. In addition, the formation of Fe plaques in the roots prevents the absorption of Fe into plants, prevents the absorption of other nutrients, and damages the root surface epidermis tissue [25].

Fe toxicity is able to induce cellular oxidative damage that causes changes in morpho-physiological properties which will reduce plant productivity [11,26]. Plants experiencing Fe toxicity are characterized by stunted growth and poor root systems [17]. The toxicity of Fe causes the growth of plant roots to be inhibited, the roots become few, short and dark brown in color [27]. The increase in Fe concentration inhibited the growth of P. australis roots [28]. This can be seen from the results of the study which showed that increasing the concentration of Fe was able to reduce the root length of O. bicolor and S. seamea seedlings (Table 2).

The decrease in height and root length of the seedlings resulted in a decrease in the dry weight of the three seedlings. The results also showed that increasing the concentration of Fe was able to reduce the dry weight of the seedlings (Table 3). However, at a concentration of 1 mM the dry weight of S. siamea seedlings was able to increase, even higher than the control. This shows that at a certain concentration of Fe is able to stimulate plant growth. Chakralhoseini et al. [19] reported that the Fe concentration of 2.5 mg kg\(^{-1}\) was able to increase the dry weight of soybean.

Fe plays a role in chlorophyll activity and is an important element in the processes of photosynthesis and respiration [29,30]. Fe plays a role in the formation of the chloroplast ultrastructure [31]. The results showed that Fe treatment was able to reduce the SDPA chlorophyll index of O. bicolor and S. seamea seedlings, while in S. macrophylla seedlings, Fe exposure did not show a significant effect. Fageria et al. [32] reported that Fe toxicity in rice was also able to reduce chlorophyll content. The toxicity of Fe also causes oxidative stress, so that it can reduce the chlorophyll content [33]. Low Fe supply is also able to reduce the chlorophyll content and will have an impact on decreasing the photosynthesis process, thereby reducing plant growth [34]. The results of several studies show that increasing the concentration of Fe can increase the SPAD value of plants [35,36]. The decrease in chlorophyll content was also due to the function of S-aminolevulinate acid in chlorophyll biosynthesis and the effectiveness of Fe during protochlorophyll formation [37].

Each plant is able to respond to Fe exposure differently, and each type has an optimum Fe concentration limit for growth. This has an impact on different plant tolerances. The results showed that Fe exposure was able to reduce the tolerance index of seedlings (Figure 1), except for S. siamea seedlings which increased when the Fe concentration was 1 mM. Each plant is able to develop different defense systems against Fe exposure. This can be seen from the difference in the tolerance index of seedlings at various Fe concentrations (Figure 1). S. macrophylla has the highest tolerance level compared to other seedlings. This can be a recommendation that S. macrophylla species can be used to revegetate lands that have high Fe concentrations.

4. Conclusion

Fe exposure had a significant effect on all growth parameters of the three seedlings. The increase in Fe concentration was able to reduce the growth parameters of the three seedlings. Fe exposure treatment also significantly affected the SPAD chlorophyll index of O. bicolor and S. siamea seedlings. Seedling tolerance index to Fe exposure decreased with increasing Fe concentration, especially in O. bicolor and S. macrophylla seedlings. S. macrophylla seedlings had the highest tolerance to Fe exposure compared to other seedlings. This shows that the type of S. macrophylla is very suitable to be planted on land that has a fairly high concentration of Fe.

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