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Interactive Effects of Aluminum and Iron on Several Soybean Genotypes Grown in Nutrient Solution

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Abstract: The effects of combined stress of aluminum (Al) and iron (Fe) on plant root were studied using four soybean genotypes differing in Al+Fe tolerance in nutrient solutions. Those genotypes were selected based on their root length sensitivity index. The results showed that the root growth inhibition of Anjasmoro and Yellow Biloxi (Al-Fe tolerant genotypes) was 14.04%, less than Tanggamus and Lawit (Al-Fe sensitive genotypes) which was 60.38%. Anjasmoro and Yellow Biloxi accumulated less Al and Fe in root and shoot than Tanggamus and Lawit.

Key words: Aluminum, iron, soybean

INTRODUCTION

Aluminum is the 3rd most abundant element in the earth's crust (Kochian, 1995) which is about 7% (Zhan *et al.*, 2013). This element belongs to the beneficial element (Marschner, 1995) but often the limiting factor in acid soils (Li *et al.*, 2012). The main effect of Al stress is, inhibits root growth (Sopandie *et al.*, 2003; De Macedo *et al.*, 2009; Posmyk *et al.*, 2008; Yu *et al.*, 2011; Miyasaka *et al.*, 2007; Nagy *et al.*, 2004; Delhaize *et al.*, 2012).

Iron (Fe) is the most important metal and one of the major constituents of the lithosphere (Kabata-Pendias, 2011). Its average content of the earth's crust is about 5%. Iron is an essential element in plant nutrition, involved in important processes such as photosynthesis, respiration and nitrogen assimilation (Stein *et al.*, 2009). However, excessive amounts can cause toxicity such as inhibiting the extension of rice roots (Fu *et al.*, 2012) and protein synthesis (Wei *et al.*, 2010), hampering photosynthesis (Adamsky *et al.*, 2011; Olaleye *et al.*, 2001), changing the chromatin structure and enzyme activity (Connolly and Guerinot, 2002).

Acid Sulfate Soil (ASS) utilization is one of the options in increasing soybean production due to limited arable land in Indonesia. Our country has 6.71 million hectares ASS distributes in 3 big islands (Sumatra, Papua

and Kalimantan). Aluminum and iron are together contained in the ASS. Generally, the concentration of these elements increases as the oxidation of pyrite increases (Dent and Pons, 1995; Ritsema and Groenenberg, 1993).

This study aims to investigate the differences resistance between 4 soybean genotypes selected from 10 genotypes in various combinations of Al and Fe stress. For this purpose, we were used root length as variable to find out the combination of threshold concentration. The reference of this concentration then used to explore the distribution of Al and Fe in the roots and their accumulation in plant roots and shoot.

MATERIALS AND METHODS

Plant materials and seedling growth: Ten soybean genotypes were used to investigate their tolerance to the combination of aluminum and iron toxicity. Four genotypes were Al-tolerant, consisted of Sindoro and Wilis, Yellow Biloxi and Slamet (Sopandie *et al.*, 2003), based on their description, Lawit and Menyapa were used as check genotypes for ASS, whereas Tanggamus and Anjasmoro showed highest productivity on ASS (Ghulamahdi *et al.*, 2009). Two other genotypes were Tidar and Kaba.

Based on their root length sensitivity index at combination of Al and Fe stress, two genotypes were selected as Al and Fe-tolerant and two for Al and Fe-sensitive. The inhibition percent of root growth of each genotype was used to select the threshold concentration. This concentration then used to observe Al and Fe distribution in root, their concentration in root and shoot compared to control and the highest combination of Al and Fe stress.

Soybean seeds were germinated in sand for 5 days. The criteria to be used for transplanting was based on the uniformity of root length. Seedlings were transplanted carefully, rinsed twice with distilled water, clamped with foam and placed on perforated styrofoam. Each pot contained 2 L of nutrient solution consisted of 5 plants. Each pot was connected with other through 1/4 inch size PVC pipe. The air pump was used to create oxidizing conditions because soybean cannot be growth in water-logged condition.

Plants were transferred into nutrient solution at pH 4 for 2 days prior to stress treatment of Al and Fe. The pH was maintained at pH 4 using 1 N NaOH and 1 N HCl while the volume adjusted to 2 L through the addition of distilled water. This activity was carried out every two days upto 14 days after transplanting (harvest). The root length was measured and weigh for dry root. These samples then used to analyze the Al and Fe concentration of root and shoot.

The experiment was arranged in completely randomized design with 3 replications, consisted of 3 factors. The first factor was the concentration of Al, consisted of: Control (A0), 0.5 mM Al (A1), 0.7 mM Al (A2) and 0.9 mM Al (A3). The second factor was the concentration of Fe, consisted of: Control (B0); 0.1 mM Fe (B1), 0.2 mM Fe (B2); 0.3 mM Fe (B3) and 0.4 mM Fe (B4). The third factor was 10 soybean genotypes.

The composition of nutrient solution was based on Sopandie (1990), consisted of: 1.5 mM Ca (NO₃)₂.4H₂O, 1.0 mM NH₄NO₃, 1.0 mM KCl, 0.4 mM MgSO₄.7H₂O, 1.0 mM KH₂PO₄, 0:50 ppm MnSO₄.4H₂O, 0:02 ppm CuSO₄.5H₂O, 0:05 ppm ZnSO₄.7H₂O, 0:50 ppm H₃BO₃, 0:01 ppm (NH₄)₂MO₇O₂₄.4H₂O and 0.068 mM FeSO₄.7H₂O. Aluminum and iron were given in the form of AlCl₃.6H₂O and FeSO₄.7H₂O, respectively. The collected data was analyzed by Minitab 16 program.

Staining: Root staining was used to investigate the distribution of Al and Fe of four selected genotypes. After 5 days germinated in sand, the uniform root length plants were transferred into nutrient solution containing control, threshold combination and the highest concentration of Al and Fe for 24 h, respectively.

Hematoxylin staining for Al: After stress period, then soybean rinsed with distilled water and soaked in hematoxylin solution for 15 min (hematoxylin 2%; NaIO₃ 0.2%) (Polle *et al.*, 1978) and rinsed again. The purple root tip (2-3 mm) was examined under the microscope to obtain images of Al distribution.

Pear's Prussian blue staining for Fe: The observations were made by soaking fresh root tissue in a solution containing 2% potassium ferrosianida and 2% HCl (1:1) for 10 min, transferred into 2% HCl solution for 10 min, washed quickly with water and stained with 0.5% eosin. Roots were washed quickly with water and dehydrated with 95% absolute alcohol as much as 2 times. Samples were clarified by xylen, examined under the microscope to obtain images of Fe distribution.

RESULTS

Root growth: After 14 days grown in nutrient solution containing 20 combinations of Al and Fe stress, the Sensitivity Index (SI) showed that root length of Anjasmoro and Yellow Biloxi is 0.42 and 0.36 (tolerant) at the treatment of 0.5 mM Al+0.2 mM Fe, whereas Menyapa, Slamet and Wilis are moderate with the SI of 0.84, 0.86 and 0.91, respectively. Tidar, Sindoro, Tanggamus, Lawit and Kaba are sensitive genotypes with SI value more than 1.0. There are no tolerant genotypes at the higher combination of 0.5 mM Al+0.2 mM Fe. From these results, Anjasmoro and Yellow Biloxi were selected as tolerant Al and Fe genotypes, contrary to Tanggamus and Lawit.

Tanggamus and Lawit are 2 genotypes that have the longest roots at control but began to show root inhibition more than 50% at 0.5 mM Al+0.1 mM Fe. The higher inhibition (more than 50%) has been seen with the higher concentrations of Al and Fe stress. Based on the response of root length of all genotypes (Fig. 1), it was obvious that the concentration of the stress concentration of 0.5 mM Al+0.2 mM Fe was required to assess differential tolerance of the Anjasmoro, Yellow Biloxi, Tanggamus and Lawit genotypes (Fig. 2).

Aluminum and iron accumulation in root and shoot: Data in Table 1 shows that Al accumulation root and shoot of all genotypes increased on a higher stress combination of Al and Fe. Tolerant genotypes (Anjasmoro and Yellow Biloxi) had lower accumulation than sensitive genotypes (Tanggamus and Lawit). All genotypes had the lowest root-Fe accumulation at control, then increase at 0.5 mM Al+0.2 mM and decrease at 0.9 mM Fe Al+0.4 mM Fe (Table 2).

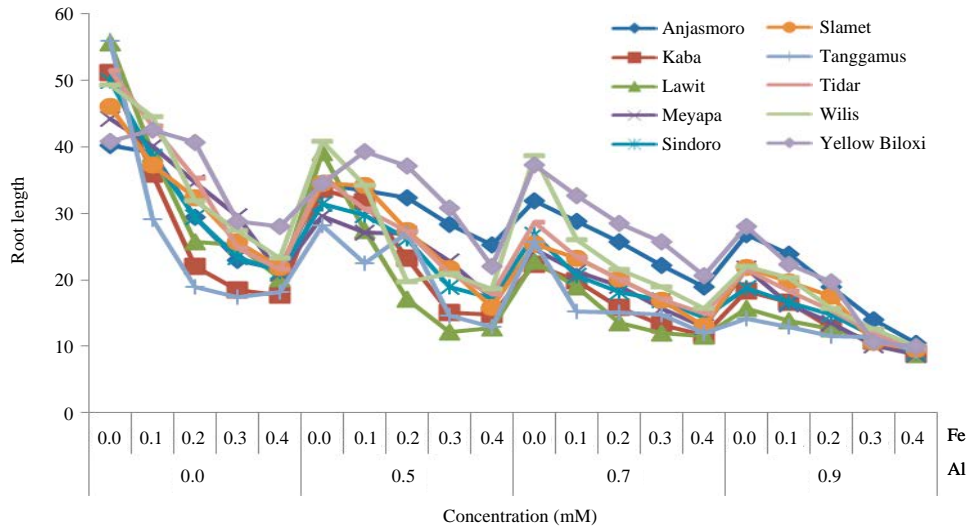


Fig. 1: Root length of ten soybean genotypes at twenty combination of Al and Fe stress

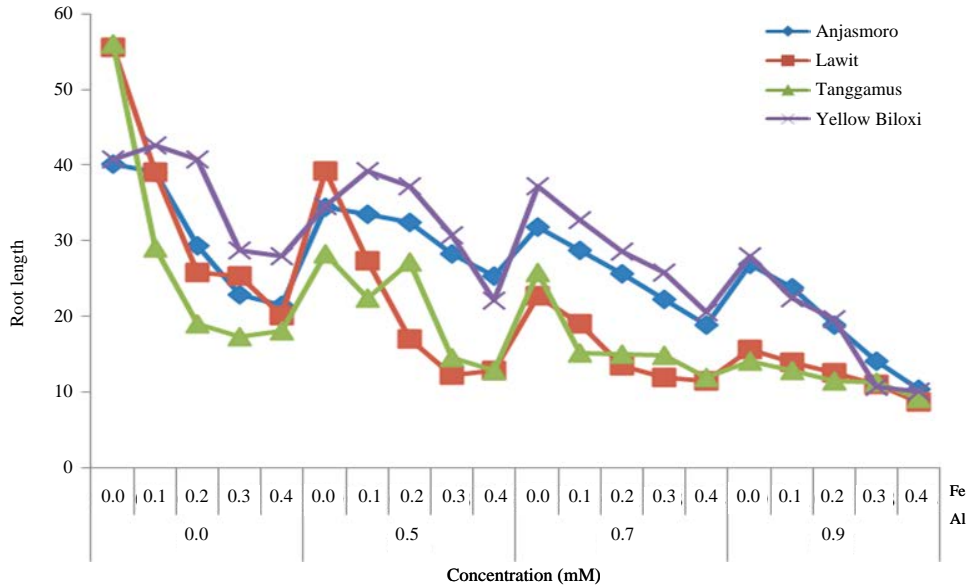


Fig. 2: Root length of tolerant and sensitive soybean genotypes

Table 1: Aluminum accumulation in root and shoot (ppm) of four soybean genotypes at various concentrations of Al and Fe

Genotype	Concentration of Al and Fe (mM)					
	0.0+0.0		0.5+0.2		0.9+0.4	
	Root	Shoot	Root	Shoot	Root	Shoot
Anjasmoro	0	0	604.0 ^c	51.3 ^d	946.7 ^c	172.0 ^b
Yellow Biloxi	0	0	781.3 ^c	91.3 ^c	1218.7 ^b	184.7 ^{ab}
Tanggamus	0	0	1022.7 ^b	127.3 ^b	1420.0 ^a	185.3 ^{ab}
Lawit	0	0	1215.3 ^a	165.3 ^a	1425.3 ^a	191.3 ^a

Distribution of aluminum and iron in soybean roots: Root cross-sectional images (Fig. 3) showed that no blue color

on control treatment, indicates the absence of aluminum. At 0.5 mM Al+0.2 mM Fe stress, Anjasmoro and Yellow

Table 2: Iron accumulation in root and shoot (ppm) of four soybean genotypes at various concentrations of Al and Fe

Genotype	Konsentrasi Al and Fe (mM)					
	0.0+0.0		0.5+0.2		0.9+0.4	
	Root	Shoot	Root	Shoot	Root	Shoot
Anjasmoro	516.2 ^b	219.6 ^b	2039.0 ^b	366.5 ^c	1709.8 ^b	628.3 ^b
Yellow Biloxi	466.1 ^b	204.4 ^b	2290.2 ^b	554.8 ^{bc}	1310.3 ^{bc}	831.7 ^a
Tanggamus	1647.6 ^a	358.8 ^a	2653.5 ^a	823.6 ^a	2388.0 ^a	919.0 ^a
Lawit	723.5 ^b	276.2 ^b	2137.9 ^b	712.3 ^{ab}	1084.1 ^c	843.2 ^a

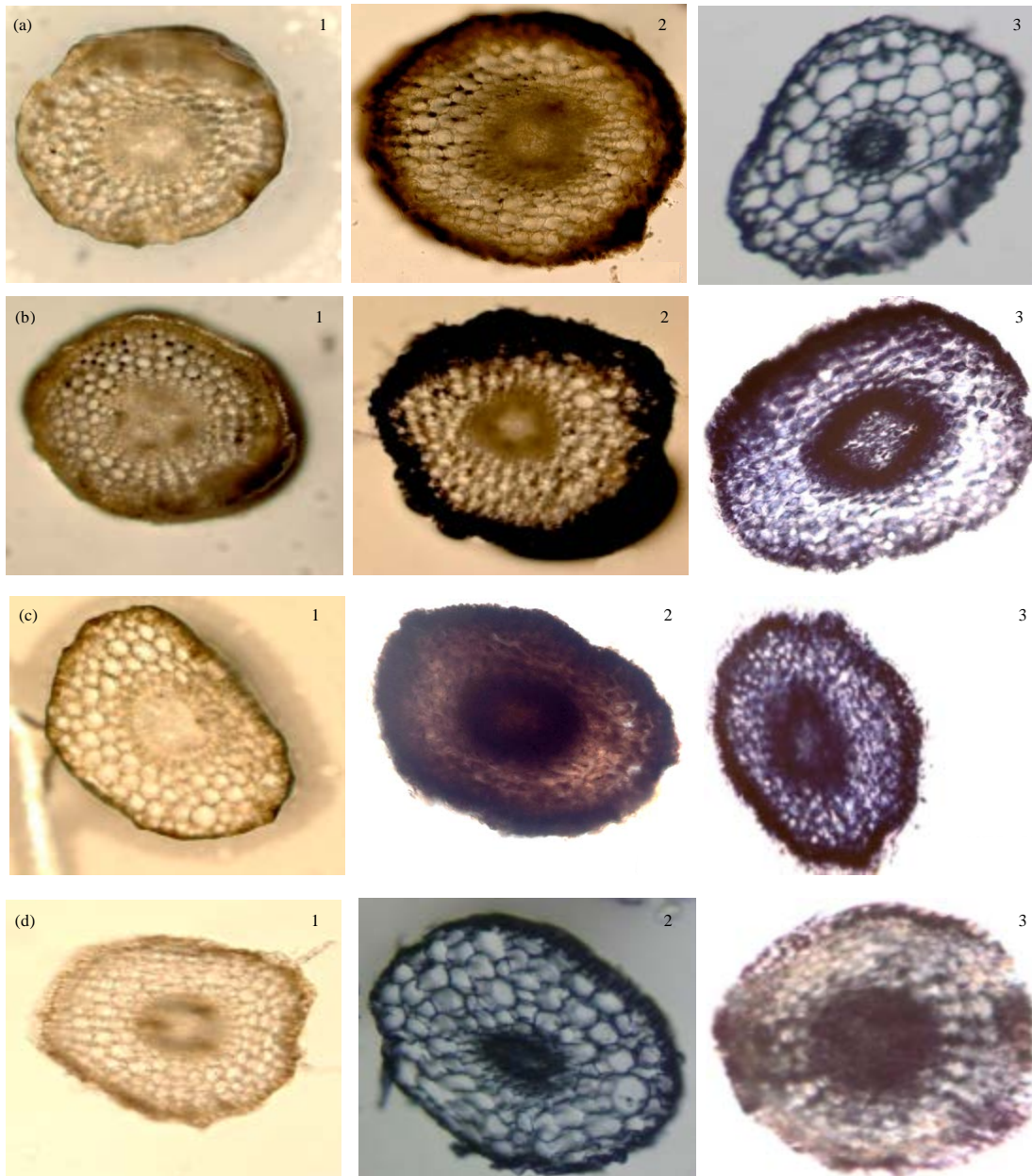


Fig. 3(a-d): Distribution of aluminum in the roots of four soybean genotypes (a) Anjasmoro, (b) Yellow Biloxi, (c) Tanggamus and (d) Lawit. 1: Control, 2: 0.5 mM Al+0.2 mM Fe and 3: 0.9 mM Al+0.4 mM Fe

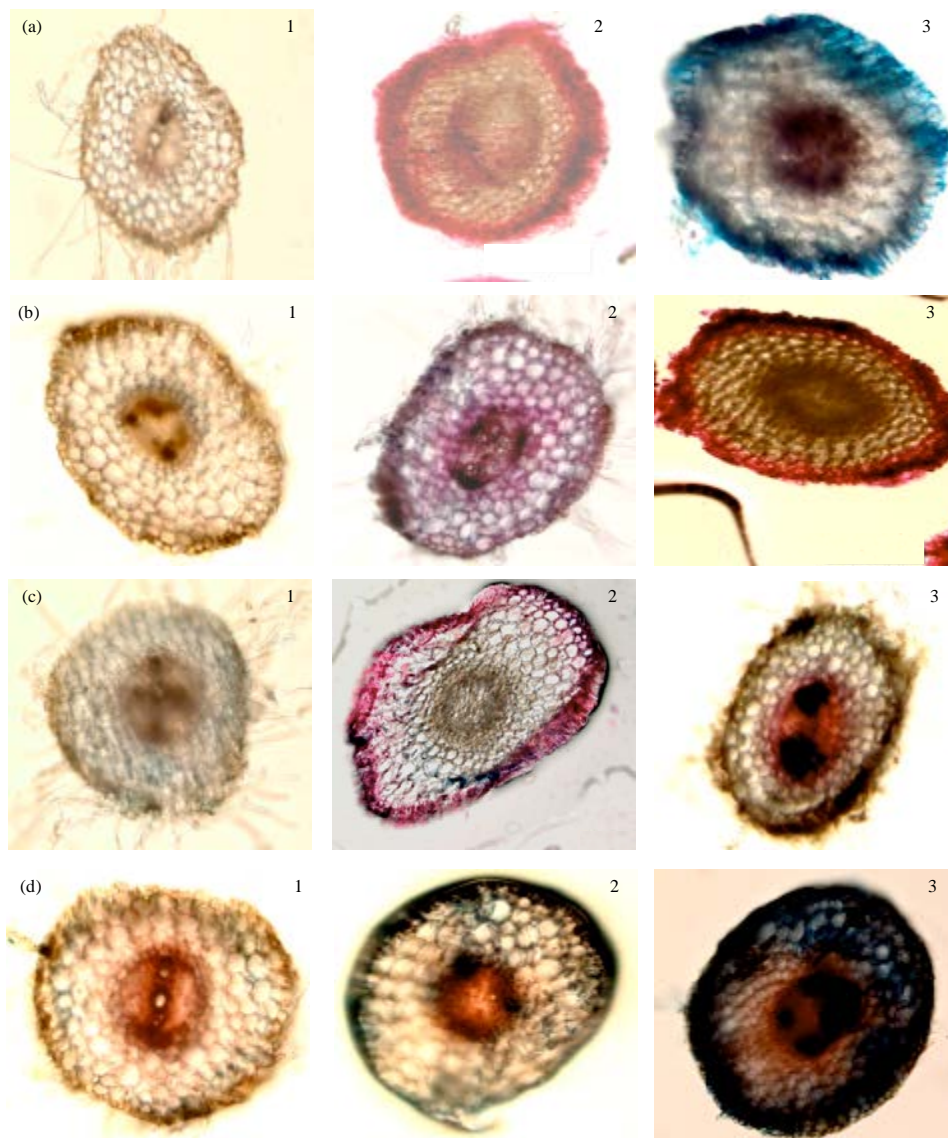


Fig. 4(a-d): Distribution of iron in the roots of four soybean genotypes (a) Anjasmoro, (b) Yellow Biloxi, (c) Tanggamus and (d) Lawit. 1: Control, 2: 0.5 mM Al+0.2 mM Fe and 3: 0.9 mM Al+0.4 mM Fe

Biloxi hold Al in the root epidermis (dark blue) and some small reach the cortex but the xylem is still bright. On the contrary, Lawit and Tanggamus showed a dark blue vascular tissue indicates more Al translocated to shoot. At the higher stress combination, xylem and phloem of all genotypes were dark blue, show more Al reach xylem.

Root cross-sectional images (Fig. 4) showed that more Fe (dark and blue) distributed into the inner root with higher stress combination. This was indicated that more Fe transported to shoot.

DISCUSSION

The results study of Sopandie *et al.* (2003) showed that Yellow Biloxi genotype was tolerant to the concentration of 0.7 mM Al. Stress combination of Al and Fe causes a greater negative effect that Biloxi Yellow tolerance down to 0.5+0.2 mM Al. The root growth of Anjasmoro and Yellow Biloxi genotypes (tolerant) on 0.5 mM Al+0.2 mM Fe stress were inhibited 19.28 and 8.81% (average 5.14%), whereas Tanggamus and

Table 3: Root length (cm) of soybean genotypes treated by Al and Fe stress

Soybean genotypes	Control	0.5 mM Al+0.2 mM Fe	Inhibition (%)
Tolerant			
Anjasmoro	40.15556 ^a	32.41389 ^{am}	19.279190
Yellow Biloxi	40.75555 ^a	37.16389 ^{ab}	8.812704
Average	40.45556	34.78889	14.045840
Sensitive			
Tanggamus	55.94667 ^b	27.13333 ^b	51.501430
Lawit	55.61333 ^b	17.09833 ^c	69.254970
Average	55.78000	22.11583	60.378200

Lawit were 51.50 and 69.25%, respectively (average of 60.38%), (Table 3). Further root inhibition (more than 50%) of Anjasmoro and Yellow Biloxi were at 0.7 mM Al+0.4 mM Fe.

Shamsi *et al.* (2008) reported that combination of Al and Cd give additive effects to inhibition of soybean. Ali *et al.* (2011) concluded that Al and Cr stress resulting a greater oxidative stress than single stress at pH 4. It was characterized by a decrease in dehydrogenase activity and increased MDA and H₂O₂. The results (Ikegawa *et al.*, 2000) showed the presence of Al accumulation in tobacco cells treated with a single Al for 24 h but had no significant effect on cell death. The addition of Fe²⁺ to the cells for 12 h increased lipid peroxidation and cell death. Fang and Kao (2000) concluded that Fe²⁺ ion have a higher peroxidase activity than SO₄²⁻, Ni²⁺, Co²⁺ and Zn²⁺ but to Cu²⁺.

Matsumoto and Motoda (2012) and Bian *et al.* (2013) stated that inhibition of root extension is the first symptoms to the Al toxicity in plant and this response differed among genotypes. Sopandie *et al.* (2003) reported that root extension of Yellow Biloxi (Al tolerant genotype) was inhibited 10.4% at concentration of 0.7 mM Al after 4 days of stress. Yu *et al.* (2011) reported that at higher concentration of Al in the nutrient solution was decreased the root of Zhechun 2 genotype (Al tolerant soybean) and Zhechun 3 (Al sensitive) compared to control. Zhechun 3 genotype root length was reduced to 20.8, 45.5, 69.6 and 81.3%, respectively at concentrations of 10, 30, 60 and 90 mg L⁻¹ Al while the reduced of Zhechun 2 was 15.1, 39.4, 81.5 and 86.2%.

Plant responses to Al stress was reported by Miftahudin *et al.* (2007). The growth of the seminal roots of rice plants was stunted and adventitious root initiation was blocked at a concentration of 15 ppm. The results De Macedo *et al.* (2009) showed that root meristem of all rice cultivars are very sensitive to Al, though have the lowest concentration. Yan *et al.* (2012) suggested the relative root length of centipedegrass accessions E006 (Al sensitive) and E041 (Al tolerant) was reduced more than 50% at 0.5 and 1.5 mM Al, respectively. According to Posmyk *et al.* (2008), the inhibition of mitotic cells activity are involve to the destruction of the basic processes during the cell cycle phase and this is the main cause of growth retardation (Maksymiec, 2007).

This study showed that Anjasmoro and Yellow Biloxi genotypes (tolerant of Al and Fe) had accumulated lower Al and Fe roots and shoot than Tanggamus and Lawit (sensitive Al and Fe). The results are consistent with reports of Guo *et al.* (2007) that Al-sensitive barley genotypes had a higher levels of root-Al and shoot than tolerant genotype (aluminum and cadmium stress), as well as Al-sensitive wheat genotype (Dong *et al.*, 2002).

Table 1 and 2 also showed that soybean root accumulated higher Al and Fe than shoot, similarly to the report of Blair and Taylor (1997) that wheat accumulated higher Al in root than shoot at combination of Al and Mn stress. This indicates, relative response, although Fe²⁺ ions have a higher peroxidase activity than Mn²⁺ (Fang and Kao, 2000).

Accumulation of Al-root each genotypes significantly different in treatment of 0.5 mM Al+0.2 mM Fe and 0.9 mM Al+0.4 mM Fe. Al and Fe-tolerant genotypes have lower accumulation of Al-root and Al-shoot than sensitive genotypes. Anjasmoro genotype has the lower root-Al, followed by Yellow Biloxi, Tanggamus and Lawit. At the stress of 0.5 mM Al+0.2 mM Fe, Anjasmoro accumulated the smallest root-Al (604.0 ppm) which is not different to Yellow Biloxi (781.3 ppm), whereas to Tanggamus (1022.7 ppm) and Lawit (1215.3), respectively.

Increase stress concentration upto 0.9 mM Al+0.4 mM Fe enhance 56.74% accumulation of Anjasmoro's Al-root to 946.7 ppm, significantly different to Yellow Biloxi, Tanggamus and Lawit. The accumulation root-Al of these genotypes increased 55.98, 38.85 and 17.28% from 0.5 mM Al+0.2 mM Fe stress. At this stress concentration, Anjasmoro has the lowest accumulation of shoot-Al (51.3 ppm), statistically different to Yellow Biloxi, Tanggamus and Lawit which have 91.3, 127.3 and 165.3 ppm, respectively. Accumulation shoot-Al of Anjasmoro increase 2.35 fold (235.28%) to 172 ppm in the 0.9 mM and Al+0.4 mM Fe stress, whereas Yellow Biloxi, Tanggamus and Lawit increase 102.3, 45.56 and 15.73%.

The increase of root and shoot accumulated Al is supported by cross-sectional image showing the root of Anjasmoro and Yellow Biloxi genotypes able to block Al in the root epidermis, in contrast to Tanggamus and Lawit. Smith *et al.* (2011) stated that the difference of poplar plant resistance to Al stress was associated to the differences in binding and compartmentation Al in roots. Furthermore, poplar resistant-Al accumulated apoplast-Al, whereas the symplastic-Al for sensitive poplar. According to Ma (2007), accumulated Al in shoot shows that Al was transported through the plasma membrane to symplastic.

All genotypes have the lowest root-Fe at control, increase at 0.5 mM Al+0.2 mM, then decrease at 0.9 mM Fe and Al+0.4 mM Fe while the shoot-Fe tend to

increase at the higher stress combination level. Tanggamus has the highest root-Fe (1647.6 ppm) in all treatments compared to other genotypes. The accumulation were 1647.6, 2653.5 and 2388.0 ppm at control, 0.5 mM Al+0.2 mM and 0.9 mM Fe and Al+0.4 mM Fe, respectively. The shoot-Fe of Anjasmoro, Yellow Biloxi and Lawit genotypes were not statistically different in each treatment. The accumulation shoot-Fe of these genotypes increased to 2039.0, 2290.2 and 2137.9 ppm at 0.5 mM Al+0.2 mM Fe and then decreased to 1709.8, 1310.3 and 1084.1 ppm at 0.5 mM Al+0.2 mM, then decreased at 0.9 mM Fe and Al+0.4 mM Fe.

Nagajyoti *et al.* (2010) stated that Fe toxicity in plants is close related to the high absorption of Fe²⁺ by roots which is transport to leaf through transpiration. Some organelles of Fe storage in plant include of vacuole (root and shoot), mitochondria, vesicles and plastids (Thomine and Lanquar, 2011) and chloroplasts (Shingles *et al.*, 2002). Iron in chloroplast is in the form of Fe-S (Shingles *et al.*, 2002) and approximately 90% of leaf-Fe is in this organelle (Abdel-Ghany *et al.*, 2005). Excess of Fe²⁺ absorption lead to produce free radicals that damage the cell structure and membrane, DNA and protein (De Dorlodot *et al.*, 2005).

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