Differential response of roots growth of soybean germplasm under low pH and manganese toxicity

HERU KUSWANTORO1,*

1Indonesian Legume and Tuber Crops Research Institute, Indonesian Agency for Agricultural Research and Development. Jl. Raya Kendalpayak Km. 8 Malang - Indonesia 65101. Tel./Fax. +62-341-801468/801496, *email: herukusw@gmail.com.

Abstract. Kuswantoro H. 2016. Differential response of roots growth of soybean germplasm under low pH and manganese toxicity. Biodiversitas 18: 257-262. Most of Indonesia dry land are covered by acid soil. The growth and development of the plant in this acid soil cope with low pH and micronutrients toxicity. The objective of this research was to study the response of root growth of soybean germplams to low pH and manganese toxicity. Nine soybean germplasm were treated in aquadest with pH 7 as control, aquadest with pH 4, and 75 ppm Mn with pH 4 in Seed Laboratory of Indonesian Legume and Tuber Crops Research Institute, Malang, Indonesia. Results showed that root length, number of lateral roots, root dry weight were lower in low pH and Mn toxicity than in the control. Shoot dry weight, hypocotyl length and epicotyl length were not influenced by low pH and Mn toxicity. Some genotypes showed increasing root length in low pH, and increasing number of lateral roots and root dry weight in Mn toxicity. Based on the acid soil adaptation index (ASAI) on root length, MLGG 0493 and MLGG 0496 showed the highest value index in three comparative conditions. The genotype of MLGG 0496 also showed the highest ASAI value on numbers of lateral root in three comparative conditions. MLGG 0494 achieved the highest ASAI value of root dry weight. However, the use of root dry weight as a criterion in soybean adaptation in low pH and Mn toxicity should be studied further because the tolerance is not just in increasing root dry weight as a result of root thickening.

Keywords: ASAI, germplasm, low pH, Mn toxicity, root growth, soybean

INTRODUCTION

Soil acidity is one of the main problems in Indonesia which covers about 69% of the dry land. Soil acidity is also the main problem in the wet land due to the materials of wet land derived from the decomposition of organic materials. In this acid soil, plant growth and development face low pH and micronutrients toxicity. H+ and Al3+ have main role toxicity in low pH condition. Usually, the influence of H+ is correlated with Al3+. However, some authors demonstrate that as H+ has different role than Al3+ in acid soil (Kidd and Proctor 2001; Kinraide 2003; Shavrukov and Hirai 2016). Excess of H+ ion is an important factor in soil acidity because it can affect plant growth and development. Excess of H+ ion, also called as H+ toxicity, can decrease root elongation (Kidd and Proctor 2001).

The low pH leads micronutrients toxicity, such as manganese toxicity. Manganese (Mn) toxicity involves physiological and biochemical processes (Millaleo et al. 2010), such as induce oxidative stress (Ribera et al. 2013). Blamey et al. (2015) proposed two mechanisms that tolerance to high Mn is, in the root environment, due to the prevention of Mn accumulation in the cytoplasm and apoplast. Mn tolerance also involves other nutrient, such as Si in detoxifying Mn through some mechanism from physiological responses (Che, et al. 2016; Li et al. 2015), gene expression (Li et al. 2015) and Se in alleviating Mn-induced oxidative stress (Saidi et al. 2014). In soil, Mn toxicity also has a relationship with Sr-nitrate extractable metals, while liming decreases this compound (Beyer et al. 2015).

Low pH and Mn toxicity have similar effect on plant growth and development. Interrelationship of these factors and the plant genotypes is expressed in plant growth and development resulting in variation response in crops. Soybean genotypes have different response to low pH (Kuswantoro 2015a) and Mn toxicity (Kuswantoro 2015b). The genetics of low pH response is complicated, involving many genes expression (Shavrukov and Hirai 2016). Genes constitution that developing a genotype plays the main role in these response. In Mn toxicity, additive gene action is found at seedling stage (Moroni et al. 2013). In physiological point of view, expression of the genes appears in changes of enzyme activities (Zhang et al. 2015). In natural ecosystems, genetic variation of tolerance to Mn toxicity may drive changes composition of the community (Fernando and Lynch 2015).

MATERIALS AND METHODS

Experimental design

The experiment was conducted in Seed Laboratory of Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang-Indonesia. Factorial design of randomized complete block design (RCBD) with two factors was applied in this experiment with three replications. The first factor was pH level consisting three
treatments i.e. aquadest with pH 7 as control, aquadest with pH 4, and aquadest with 75 ppm of Mn and pH 4. The form of manganese used was MnCl₂·4H₂O. The second factor was soybean germplasm consisting of nine accessions of soybean germplasm originating from ILETRI’s collection. Four genotypes originated from Gunung Kidul District, Yogyakarta Province, Indonesia. Two genotypes originated from Wonogiri District, Central Java Province, Indonesia. The district and province of the three genotypes are unknown (Table 1). Therefore, there were 27 treatments combination.

Seed preparation
Before soybean seeds were applied for the experiment, they were germinated in a petri dish. Each petri dish contained 25 sterilized soybean seeds. To ensure the germinated seeds remain standing straight up, gauze was put inside the petri dish. Seed respiration is important in germination to keep the seed live. Therefore, the solution was poured up to half of seed size in the petri dish. The volume of the solution was monitored everyday and solution was added up to the specified limit when solution decreased due to the absorption by the seeds. Room temperature for germination was maintained at 25ºC.

Data collection
The observation was conducted at 6 days after germinating on root length, number of lateral roots, root dry weight, hypocotyl length, epicotyl length, shoot dry weight and seedling dry weight. Root dry weight was assessed from whole root system including primary root and lateral roots. The tolerance of soybean genotypes was calculated by using acid soil adaptation index (ASAI) according to Howeler (1991).

Table 1. Source of plant materials

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLGG 0491</td>
<td>Gunung Kidul District, Yogyakarta, Indonesia</td>
</tr>
<tr>
<td>MLGG 0492</td>
<td>Gunung Kidul District, Yogyakarta, Indonesia</td>
</tr>
<tr>
<td>MLGG 0493</td>
<td>Gunung Kidul District, Yogyakarta, Indonesia</td>
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<tr>
<td>MLGG 0494</td>
<td>Gunung Kidul District, Yogyakarta, Indonesia</td>
</tr>
<tr>
<td>MLGG 0496</td>
<td>Wonogiri District, Central Java, Indonesia</td>
</tr>
<tr>
<td>MLGG 0507</td>
<td>Wonogiri District, Central Java, Indonesia</td>
</tr>
<tr>
<td>MLGG 0527</td>
<td>District and province are unknown, Indonesia</td>
</tr>
<tr>
<td>MLGG 0529</td>
<td>District and province are unknown, Indonesia</td>
</tr>
<tr>
<td>MLGG 0532</td>
<td>District and province are unknown, Indonesia</td>
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</tbody>
</table>

Data analysis
Data were analyzed using Statistical Tool for Agricultural Research (STAR) from International Rice Research Institute (IRRI). Analysis of variance was performed for all data. The significant data based on the analysis of variance were analyzed again using least significant different for pH level and honestly significant different for genotype. If the interaction between genotype × stress conditions was significant, the advanced analysis would be conducted on genotype × stress condition interaction. If there was no interaction, the analysis would be conducted on the significant genotype and the significant stress condition.

RESULTS AND DISCUSSION
Low pH and Mn toxicity affect plant growth and development that were indicated by the response of its characters. The response of the observed characters in low pH and Mn toxicity varied. Root length, number of lateral roots, root dry weight and hypocotyl length in low pH (pH) and Mn toxicity with low pH (Mn) were lower than in control or same with that in control. However, these four characters in Mn toxicity were not always lower than in low pH condition. Even, root dry weight and hypocotyl length in Mn toxicity were higher than in low pH (Table 2).

Alteration of root length was in line with the stresses (Table 2). In this study, Mn toxicity was assumed to be severer than low pH because Mn toxicity was in low pH condition as well. The alteration of root length includes decreasing and increasing of the root length. Decrease percentage of root length in different stress conditions varied among the tested genotypes. There was no negative value in Control-Mn and pH-Mn, but negative values existed in Control-pH (Table 3). Negative value suggests an increasing variable from the optimal or relatively optimal condition to the severer condition. There were six genotypes showing an increase in root length in low pH condition. In Control-pH, the highest increasing was shown by MLGG 0527, while the highest decreasing was shown by MLGG 0507. In Control-Mn and pH-Mn, the lowest decreasing was shown by MLGG 0493. Compared to the control, other studies reported the lower root length was in Mn toxicity (Kuswantoro 2015b) and inconsistent response in low pH depended on the genotypes (Kuswantoro 2015a).
The alteration of root length is influenced by environment and genetic factors. Different treatment performed different root length (Table 2). Control condition showed the longest root length than in low pH and in Mn toxicity, and in Mn toxicity, it showed shorter root length than in low pH. This alteration was due to the low pH inhibits root growth (Liang et al. 2013) as well as the Mn toxicity (Abou et al. 2002). However, root length is also affected by the genotypes. In this study, there was no interaction between genotype × stress condition suggested that these two factors had an equal role. Root length of the tested genotypes was shown in Fig. 1. There were four genotypes with the longest root length, i.e. MLGG 0496, MLGG 0493, MLGG 0507 and MLGG 0491. Different performance in root length is expressed by the genetic constitution of the tested genotypes.

Similar to root length, number of lateral root was also in line with the stresses. In identifying of the tolerant genotype, a sensitive organ is needed. Number of lateral roots is very important, because its alteration can identify the tolerant genotype. Lateral root is also more important than primary root, because lateral root is more sensitive (Haling et al. 2011). However, a difference was found between root length and number of lateral roots. Statistically, number of lateral roots was not different between low pH and Mn toxicity.

Decrease percentages on number of lateral roots based on Control-pH, Control-Mn and pH-Mn varied among nine genotypes (Table 3). There was no negative value in Control-pH, but some negative values were found in Control-Mn and many negative values were found in pH-Mn. No negative value on Control-pH indicates the decreasing number of lateral roots. Irreversible damage occurring in low pH stress (Koyama et al. 2001) causes decreasing number of lateral roots. Negative values in Control-Mn suggests that some genotypes increase in number of lateral roots in Mn toxicity, negative values in pH-Mn suggests that a number of lateral roots of most genotypes grew better in Mn toxicity than in low pH. Presumably, some external factors exist in the increasing number of lateral roots, such as Ca concentration (Murata et al. 2003) and P concentration (Hissinger et al. 2003) in low pH or Si (Liang et al. 2007) and compounds of H₂O₂ and NO (Kováčik et al. 2014) in Mn toxicity. Stimulating lateral roots under high concentration of Mn may be also due to the function of Mn as an essential element. Mn plays many roles in plant’s metabolic processes such as activation and cofactor of enzymes, amino acids synthesis and hormone activation (Burnell 1988). This metabolic process can increase plant organs growth including number of lateral roots. The lowest decreasing number of lateral roots in Control-pH was found by MLGG 0532 followed by MLGG 0493. These two genotypes had different characteristics in Control-Mn and pH-Mn, where MLGG 0532 had positive values and MLGG 0493 had negative values. It means that MLGG 0532 could not maintain the number of lateral roots, while MLGG 0493 could maintain/increase number of lateral roots.

Some components constituting root dry weight are root length and number of roots. Alteration in root length and number of roots causes an alteration in root dry weight. Alteration also occurs on root hair length and root hair density (Haling et al., 2011) that cause an alteration in root dry weight. In this study, root dry weight was not in line with root length and number of lateral roots, where root dry weight increased in Mn toxicity but equal to low pH (Table 2). It may be due to the thickening of the roots. Root thickening has been reported by some authors in response to metal toxicity, such as aluminum (Alvarez et al. 2012), iron (Fu et al 2012), silicon (Fu et al 2012), and cadmium (Felici et al. 2014), and copper (Kang et al. 2015).

Decrease percentage based on three stress conditions varied among the tested genotypes. Only one genotype, MLGG 0532, that showed negative values on those three stress conditions consistently. Most of the tested genotypes and all of the genotypes showed negative value in Control-Mn and pH-Mn respectively (Table 3). It indicates increasing root dry weight in Mn toxicity. This study is also in line with previous study (Kuswantoro 2015b) that reported some genotypes have higher root dry weight in Mn toxicity than in control, but contrast to studies of Izaguirre-Mayoral and Sinclair (2005), Hadjiboland et al. (2008) and Khabaz-Saberi et al. (2009) that reported decreasing root dry weight was in high Mn concentration. Increasing root diameter (Junior et al. 2009) and/or root thickening may be the cause in increasing of root dry weight.

Hypocotyl and epicotyl lengths were not significantly different among the three solution treatments. These results are not similar to Abou et al. (2002) that stated high Mn concentration was involved in shortening of the shoot. However, previous studies reported inconsistent results, where hypocotyl length in low pH is not significantly different (Kuswantoro 2015a), while in Mn toxicity hypocotyl length is lower than in control, and epicotyl length is higher in Mn toxicity than in control (Kuswantoro 2015b). Low pH adversely influences more on the root growth than on the shoot growth (Ritchey and Carter 1993).

Shoot dry weight was not affected by the low pH and Mn toxicity treatments. Genetic factor played main role in this character than environmental factor. The highest shoot dry weight was shown by MLGG 0496, while the lowest dry weight was shown by MLGG 0492 and MLGG 0532 (Fig. 4). Lin et al. (2012) reported similar result that shoot dry weight in pH 6.8 and pH 4 is not significantly different. Different result was reported by Murata et al. (2003) that decreasing solution pH and Ca concentration decreased the shoot dry weight. Hypocotyl and epicotyl lengths (Fig 2-3) seemed no contribution on shoot dry weight. The pattern of these two characters differed to shoot dry weight. These results were not similar to the previous studies (Kuswantoro 2015a; Kuswantoro 2015b). Interestingly, the lowest shoot dry weight was resulted by genotype of MLGG 0492 with the highest epicotyl length. It indicated no correlation between shoot dry weight and epicotyl length.

Acid Soil Adaptation Index (ASAI), proposed by Howeler in 1991, is an index for discovering a genotype that adapts to the low pH condition. Based on the root
length, MLGG 0493 and MLGG 0496 achieved the highest ASAI in three comparative conditions. MLGG 0496 was also supported by ASAI on number of lateral roots in three comparative conditions. A different result was shown in ASAI of root dry weight, where MLGG 0494 achieved the highest ASAI (Table 4). The genotype with the highest ASAI is assumed as the adaptive genotype. However, the use of root dry weight as a criterion in soybean adaptation in low pH and Mn toxicity should be studied further. This is because the mechanisms in tolerating low pH and Mn toxicity was by accumulating Mn in roots, which supposed to allow the increasing of root elongation and the number of lateral roots. Kuswantoro (2015a) reported the highest ASAI on seven characters in one genotype.

**Figure 1.** The average of root length of some soybean germplasm under control, low pH, and Mn toxicity conditions.

**Figure 2.** The average of hypocotyl length of some soybean germplasm under control, low pH, and Mn toxicity conditions.

**Figure 3.** The average of epypocotyl length of some soybean germplasm under control, low pH, and Mn toxicity conditions

**Figure 4.** The average of shoot dry weight of some soybean germplasm under control, low pH, and Mn toxicity conditions.

**Table 3.** Decrease percentage of root length, number of roots, and root dry weight of some soybean germplasm under low pH and Mn toxicity conditions.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Root length</th>
<th>Number of lateral roots</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control-pH</td>
<td>Control-Mn</td>
<td>pH-Mn</td>
</tr>
<tr>
<td>MLGG 0491</td>
<td>24.54</td>
<td>51.85</td>
<td>36.20</td>
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<tr>
<td>MLGG 0492</td>
<td>-9.77</td>
<td>32.18</td>
<td>38.22</td>
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<tr>
<td>MLGG 0494</td>
<td>-9.88</td>
<td>41.86</td>
<td>47.09</td>
</tr>
<tr>
<td>MLGG 0496</td>
<td>5.32</td>
<td>25.37</td>
<td>21.18</td>
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<td>MLGG 0507</td>
<td>32.20</td>
<td>42.93</td>
<td>15.83</td>
</tr>
<tr>
<td>MLGG 0527</td>
<td>-16.67</td>
<td>45.16</td>
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</tr>
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<td>MLGG 0529</td>
<td>-1.89</td>
<td>14.47</td>
<td>16.05</td>
</tr>
<tr>
<td>MLGG 0532</td>
<td>-10.00</td>
<td>55.63</td>
<td>59.66</td>
</tr>
</tbody>
</table>
For summary, the observed characters showed a different response in low pH and Mn toxicity. Root length, number of lateral roots, and root dry weight in low pH and Mn toxicity were lower than in control condition. However, these three characters did not always decrease in low pH and Mn toxicity. Increasing root length in low pH, number of lateral roots and root dry weight in Mn toxicity were found in some genotypes. On the other hand, low pH and Mn toxicity did not influence shoot dry weight, hypocotyl length and epicotyl length. These three characters cannot be used as the criterion of soybean tolerance in low pH and Mn toxicity. Tolerance criteria may be laid on root length, number of lateral roots, and root dry weight. Genotypes of MLGG 0493, MLGG 0496 and MLGG 0494 were the tolerant genotypes based on the ASAI on root length and number of lateral roots, and root dry weight respectively. The increasing root dry weight may due to the result of root thickening. Therefore, the use of root dry weight as a criterion in soybean tolerance in low pH and Mn toxicity should be studied further to ensure the increasing root elongation, number of lateral roots and nutrients uptake to support its tolerance.

## REFERENCES


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