Acid soil tolerance of 28 soybean varieties in hydroponics and soil-based evaluation

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Abstract. Utilization of marginal soil plays important role in improving soybean production in Indonesia. Soybean breeding program targeting high yielding varieties and tolerant to acid soils is estimated to be more cost-effective and environmentally friendly approach in improving plant productivity on acid soil. The aim of this research was to study plant response under acid soil stress and to identify tolerant and sensitive genotype which might be useful for further breeding program. The experiment was conducted using 28 soybean varieties as genetic material. The experiment was carried out in two parts, including seedling evaluation in hydroponics and plant growth evaluation in polybag using acid soil. The first experiment was arranged in factorial randomized block design, consisting of genotype, pH, and AlCl$_3$ treatment as the factors with 5 replications. The second experiment was conducted using randomized complete block design with 5 replications to identify the plant growth in different genotype and acid soil conditions. The results showed that the low pH and high Al inhibits seedlings root growth of soybean genotypes. The estimation of STI from root and shoot dry weight suggests Detam 1 as the most tolerant genotype followed by other varieties with lower level of tolerance. Plant response was different each genotype to both seedling evaluation in hydroponics and plant growth evaluation using acid soil.

1. Introduction
Soybean is one of the staple commodities for food and industry in Indonesia. As the main source of plant-based protein, soybean has the highest priority after rice and corn in terms of food security. In the last decade, national soybean production was relatively low. According to BPS data [5], in 2017 the national soybean production was 986,000 tons from a harvested area of 785,500 ha and in 2018 it was 995,000 tons with a harvested area of 790,000 ha. This figure is still far below from the national demand which is estimated to reach 2.8 million tons per year [1], which lead to an inevitable import of soybeans.

Improving soybean production in Indonesia can be conducted through increasing crop productivity and expand the planting area. Land extensification is possible but the option now left to the marginal land that has relatively low fertility. Marginal land in Indonesia consists of dry land, acid land, saline land, peat land and others that has physical and chemical conditions that are unfavorable for plants. To overcome the drawbacks from marginal land, several
strategies can be selected, such as improving soil conditions or by using superior varieties adaptive to these stressed soil conditions.

The dry land in Indonesia reaches 148 million ha, of which 69.46% of the dry land is acid dry land [18]. Acid dry land is characterized by low pH and soil fertility. The acidity of acid dry land is below 5.5 which can dissolve some toxic minerals for plants, such as Al and Mn. Al solubility in soil is one of the main problems in crop production. Al stress causes stunted root growth thereby reducing the efficiency of water and nutrient absorption [12]. Al-stressed plants are usually accompanied by symptoms of P, Ca, Mg, B, Zn, Mo, Cu, K and Na deficiency [21], [15].

Soybean has relatively low tolerance to high soil acidity (pH < 4.5) and Al saturation > 20% [6]. Under these conditions, the plants show some symptoms like dwarfing, non-developing roots, and leaf chlorosis. The high content of Al mainly affects the division and elongation of meristematic cells in the roots, thereby inhibiting root growth [19]. Roots grow weak and dark, hence reducing the absorption of water and nutrients, especially Ca, Mg and P [17]. The level of soil acidity is very influential on the growth and yield of soybeans [21]. Soybean root growth was severely stunted at pH 3.5, i.e. the root length was only 5.1 cm, the plant height was only 1 cm and seeds could not be formed. Increasing soil pH to 4, can increase soybean root length to 16.1 cm, plant height to 19.8 cm, but seeds still do not form. Soybean seeds formed at pH 4.5 with a yield of 0.96 g/plant.

The approach that can be taken to overcome acid soil is through soil amelioration like liming. Lime application could overcome Al toxicity and improve the pH [24]. However, this approach is not cost efficient and cannot overcome acidity in deeper soil layers. Plant breeding program to obtain high yielding varieties but tolerant to acid soils is estimated to be more cost-effective in improving plant productivity on acid soils and also environmentally friendly because no chemical is released to the environment [20]. However, soybean genetic diversity adaptive to acid soil is still very limited. Tanggamus and Demas-1 are soybean varieties tolerant to acid soil released by Indonesian Legumes and Tuber Crops Research Institute (ILETRI), but their yield potential is still low at 2.5 tons/ha with average yields of 1.2 tons/ha to 1.7 tons/ha, Therefore, the breeding of adaptive soybean varieties in acid soils still needs to be pursued.

Screening methods for acid soil tolerance are very important in practical breeding. Previous studies used several methods with various advantages and disadvantages, such as screening methods with hydroponics, screening on sand cultures, and soil-based screening [22]. Screening with hydroponics can be conducted quickly and involves many genotypes, but is usually limited to the seedling stage. Screening with sand culture is generally similar to hydroponics, but can lead to poor aeration and accumulation of nutrient salts. Acid soil based screening is highly dependent on the type of soil used, therefore it is region specific and cannot be used widely.

This research was performed to study plant response under acid soil stress and to identify tolerant and sensitive genotype which might be useful for further breeding program.

2. Materials and Methods
A total of 28 soybean varieties were used in the study, from the collection of CIRA (Center for Isotopes and Radiation Application), ICABIOGRAD (Indonesian Centre for Agriculture Biotechnology and Genetic Resources and Development) and ILETRI (Table 1). The experiment was carried from October 2020 to May 2021 at the CIRA. The study was carried
out in two phases, seedling evaluation of soybean varieties in hydroponics using the treatment of pH and Aluminum (Al) and plant growth evaluation of soybean varieties by using latosols and ultisols soil in a polybag.

### Table 1. List of 28 varieties in the study and the origin

| No | Variety   | Source      | No | Variety   | Source      |
|----|-----------|-------------|----|-----------|-------------|
| 1  | Anjasmoro | ILETRI      | 15 | Detam 4   | ILETRI      |
| 2  | Argomulyo | ILETRI      | 16 | Detap 1   | ILETRI      |
| 3  | Biosoy 1  | ICABIOGRAD  | 17 | Devon 1   | ILETRI      |
| 4  | Biosoy 2  | ICABIOGRAD  | 18 | Devon 2   | ILETRI      |
| 5  | Burangrang| ILETRI      | 19 | Gamasug 2 | CIRA        |
| 6  | Dega 1    | ILETRI      | 20 | Gepak Kuning | ILETRI   |
| 7  | Deja 1    | ILETRI      | 21 | Grobogan  | ILETRI      |
| 8  | Deja 2    | ILETRI      | 22 | Kemuning 1| CIRA        |
| 9  | Demas 1   | ILETRI      | 23 | Meratus   | CIRA        |
| 10 | Dena 1    | ILETRI      | 24 | Mitani    | CIRA        |
| 11 | Dena 2    | ILETRI      | 25 | Muria     | CIRA        |
| 12 | Derap 1   | ILETRI      | 26 | Panderman | ILETRI      |
| 13 | Dering 1  | ILETRI      | 27 | Rajabasa  | CIRA        |
| 14 | Detam 3   | ILETRI      | 28 | Tengger   | CIRA        |

#### 2.1. Seedlings evaluation in hydroponics media

Soybean genotypes were grown in hydroponics by using a modified method of Bianchi-Hall [3]. Soybean seeds germinated on petri dish which were coated with wet cotton containing a 800 mM CaSO₄ for 72 hours. Afterwards, the sprouts were measured and transferred to the tank containing a solution of hydroponics with a capacity of 10 L. The growth media for soybean seeds was a hydroponic nutrient inserted into the PVC hydroponic tank with 70 holes each tank. The experiment was arranged in a randomized complete design with three factorial factors. The first factor was soybean genotype, consisting 28 soybean varieties. The second factor was the acidity of the hydroponic media solution which consisted of two pH levels, namely pH 4 and pH 6 (the acidity level of the solution was adjusted using 0.025 M H₂SO₄). The last factor was the exposure to aluminum (AlCl₃) at concentration of 0 and 24 ppm. All treatments were repeated with 5 replications so there were 560 experimental units in 8 hydroponic tanks. Plants were maintained with the media solution hydroponics at a stable temperature (27°C) by adjusting the pH of the solution every day. The parameters observed were the root length. Data were analyzed with R to calculate the F test. Factors with significant effect were compared using Tukey’s honest significant difference (HSD) test at the level of 5%.

#### 2.2. Plant growth evaluation by using latosol and ultisols soil in polybag

Two soil types were used in the experiment, latosol soil from Pasar Jumat (6°17'43.3"S 106°46'28.8"E) and ultisols soil from Jasinga (6 ° 27'38.0 "S106 ° 26'59.0" E). The characteristics for the two soil types are described in Table 2.
Table 2. The soil profile from Pasar Jumat and Jasinga

| Soil Parameter | Pasar Jumat | Notes   | Jasinga | Notes   |
|---------------|-------------|---------|---------|---------|
| pH            | 5.4         | Acidic  | 4.0     | Very acidic  |
| C-organic     | 1.51%       | Low     | 1.73%   | Low     |
| N total       | 0.19%       | Low     | 0.02%   | Very low |
| P total       | 78.14 ppm   | Very high | 111.11 | Low     |
| Al            | 1.22 cmol kg\(^{-1}\) | High     | 11.52 cmol kg\(^{-1}\) | Very high |
| Texture       | Clay        | -       | -       | -       |

The soil was taken at a depth of 0-20 cm. The soil was air dried, sieved using 10 mesh filter and thoroughly mixed. Each experimental unit was consisted of 5 kg soil in 30 x 30 cm polybag. The experiment was arranged in a randomized complete block with two factors, which were soybean genotype and soil type. Each combination treatment was repeated five times so that there were 280 experimental units.

The N fertilizer, with urea (46.72% N), were applied twice, on 7 and 35 days after planting about 10 ppm per polybag. The P and K fertilizers were applied by using 50 ppm SP-36 (33.59% P\(_2\)O\(_5\)) and 37.5 ppm KCl (55.06% K\(_2\)O) at planting time. Several parameters were observed, including plant height, number of branch, number of node, seed dry weight, shoot dry weight and root dry weight. Data were analyzed with R to calculate the F test. Factors with significant effect were further tested with Tukey’s honest significant difference (HSD) test at the level of 5%. The estimation of tolerant genotypes was calculated using the following formula [9]:

\[
\text{Stress tolerance index (STI)} = \frac{Y_p \cdot Y_s}{\bar{Y}^2}
\]

In this experiment, \(Y_p\) and \(Y_s\) are the parameter of a genotype in pH 5.4 and pH 4.0 respectively. Meanwhile, \(\bar{Y}^2\) was the average yield in pH 5.4 treatment.

3. Results and Discussions

3.1. Root growth of seedlings in hydroponics

The effect of pH and Al on soybean genotype was studied at pH 4 and 6, while Al at concentrations of 0 and 24 ppm. According to Balittanah [2], Al stress is classified as high in soil at concentrations above 21 ppm.

Analysis of variance showed that genotype, pH and Al gave significant differences in root length, respectively (Table 3). The interaction between genotype-pH and pH-Al also gave a significant difference to root length, while the genotype-Al interaction did not show a significant difference. This might be due to the similarity of root length response of each genotype in Al treatment.
The genotypes showed a decrease in root length in Al treatment, especially at pH 4 (Table 4). The combination of pH 4 and Al 24 treatment gave the lowest root growth compared to other treatments. In general, the growth of primary and secondary roots seemed to be inhibited on pH 4-Al 24 ppm media hence the pH 6-Al 0 ppm treated seedlings grew longer roots (Fig. 1). Low pH has been found as a main factor inhibiting root nodulation in soybean [8]. The inhibition of root nodule development starts early as the root is exposed to low pH media. This effect would remain although the plant is transferred to optimum pH soil [14].

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### Table 3. Analysis of variance for seedling root length on different soybean genotype, pH and Al concentration

|               | Df | Sum Sq | Mean Sq | F value |
|---------------|----|--------|---------|---------|
| Replicate     | 4  | 48.97  | 12.24   | 4.3945  |
| Genotype      | 27 | 651.26 | 24.12   | 8.6577  |
| pH            | 1  | 370.34 | 370.34  | 132.9244*** |
| Al            | 1  | 98.62  | 98.62   | 35.396  |
| Genotype:pH   | 27 | 140.41 | 5.2     | 1.8665  |
| Genotype:Al   | 27 | 69.64  | 2.58    | 0.9258  |
| pH:Al         | 1  | 215.51 | 215.51  | 77.3532*** |
| Genotype:pH:Al| 27 | 87.53  | 3.24    | 1.1635  |
| Residuals     | 444| 1237.02| 2.79    |         |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The roots of seedlings from 30 genotypes treated with low pH and AlCl3

| Genotype       | pH 4      | pH 6      | STI based on pH 4 Al 24 and pH 6 Al 0 |
|----------------|-----------|-----------|-------------------------------------|
|                | Al 0 ppm  | Al 24 ppm | Al 0 ppm  | Al 24 ppm |                      |
| Anjasmoro      | 4.90      | 2.28      | a-n      | 5.38      | 6.78                  | a-m          | 0.36          |
| Argomulyo      | 5.42      | 4.08      | b-n      | 6.84      | a-l                  | 6.48         | a-m          | 0.83          |
| Biosoy 1       | 7.40      | 4.14      | b-n      | 6.08      | a-n                  | 4.92         | a-n          | 0.75          |
| Biosoy 2       | 7.96      | 4.24      | b-n      | 8.12      | a-d                  | 8.38         | ab           | 1.02          |
| Burangrang     | 5.86      | 2.80      | h-n      | 6.06      | a-n                  | 7.88         | a-f          | 0.50          |
| Dega 1         | 3.42      | 2.38      | k-n      | 4.30      | b-n                  | 4.48         | b-n          | 0.30          |
| Deja 1         | 7.28      | 4.08      | b-n      | 6.46      | a-m                  | 7.16         | a-j          | 0.78          |
| Deja 2         | 5.66      | 4.46      | b-n      | 7.42      | a-h                  | 6.48         | a-m          | 0.98          |
| Demas 1        | 3.64      | 2.96      | h-n      | 3.80      | b-n                  | 5.36         | a-n          | 0.33          |
| Denam 1        | 5.38      | 4.76      | a-n      | 7.70      | a-g                  | 8.08         | a-d          | 0.68          |
| Dena 2         | 6.32      | 3.16      | g-n      | 7.24      | a-i                  | 8.20         | abc          | 1.09          |
| Derap 1        | 4.98      | 2.20      | mn       | 5.36      | a-n                  | 6.02         | a-n          | 0.35          |
| Dering         | 3.32      | 2.28      | lmn      | 2.70      | i-n                  | 4.02         | b-n          | 0.18          |
| Detam 3        | 4.56      | 2.46      | k-n      | 2.26      | lmn                  | 2.88         | h-n          | 0.17          |
| Detam 4        | 4.20      | 3.32      | g-n      | 4.32      | b-n                  | 6.30         | a-n          | 0.41          |
| Detap 1        | 4.90      | 1.78      | n        | 4.24      | b-n                  | 5.42         | a-n          | 0.22          |
| Devon 1        | 6.50      | 2.38      | k-n      | 5.56      | a-n                  | 7.16         | a-j          | 0.39          |
Devon 2 5.84  a-n  1.80  n  4.78  a-n  5.48  a-n  0.26
Gamasugen 6.64  a-m  3.76  b-n  6.62  a-m  6.56  a-m  0.74
Gepak Kuning 7.16  a-j  6.16  a-n  6.98  a-k  6.86  a-l  1.28
Grobogan 3.52  d-n  3.10  g-n  4.76  a-n  4.18  b-n  0.44
Kemuning 5.02  a-n  3.20  g-n  4.80  a-n  5.60  a-n  0.46
Meratus 6.10  a-n  5.06  a-n  6.08  a-n  6.46  a-m  0.91
Mitani 5.60  a-n  2.76  i-n  5.84  a-n  6.36  a-n  0.48
Muria 4.38  b-n  2.56  j-n  6.98  a-k  5.50  a-n  0.53
Panderman 5.62  a-n  5.24  a-n  6.92  a-k  7.14  a-j  1.08
Rajabasa 5.48  a-n  2.88  h-n  9.20  a  7.12  a-j  0.79
Tengger 4.74  a-n  3.38  e-n  5.80  a-n  6.58  a-m  0.58

Mean 5.42  b  3.34  c  5.80  ab  6.20  a

Note: The lane number followed by the same letter is not significantly different based on the HSD test at the 5% level.

It can be seen that acidic medium suppress root growth while the addition of Al put more constraint to the root development. In acid environment with low pH (below 4.5-5.0), plant roots absorb more Al and caused serious disturbance in root elongation and root cell damage [10], [16]. The suppressing effect of Al was absent in pH 6. Instead, the average root length was higher in Al treatment than without treatment eventhough not significant (Table 4). Aluminum was found to have multiple effects in plant growth, either toxic or beneficial [4]. In wheat, the supporting effect from Al is related to prevention of H+ toxicity [11]. However, the exact mechanism of Al during stress environment remains elusive.

There were four genotypes that have the highest STI values, above 1.0, namely Gepak Kuning, Dena 1, Panderman and Biosoy 2. The high value of STI is an indication of a better level of tolerance to stress.

Figure 1. The seedlings performance of Rajabasa, Grobogan, and Demas 1 in pH 4 Al 24 ppm and pH 6 Al 0 ppm in hydroponic media

3.2. Plant growth evaluation in polybag
All genotypes experienced visible decline in vegetative growth under pH 4.0 treatment. The figure confirms the effect of soil pH towards plant height [23]. Low pH contributes to low
availability of macronutrients like P and N and also to poor survival of beneficial microorganisms in soil that can help nutrient uptake.

The plant height ranged from 24.8 to 46.2 cm on pH 4.0 while the range almost doubled on pH 5.4 at 36.5-66.9 cm. The pH 4 treatment shows an obvious difference with an average of 33.99 cm compared to pH 6 treatment at 52.33 cm. Burangrang and Dega 1 stood at the highest plant growth at almost 67 cm in pH 5.4.

Stressed plant conditions can also be seen from the low number of branches and number of nodes per plant under low pH soil. For pH 5.4, the average number of branches was 5.04 while pH 4.0 the figure decreased significantly at 1.14. The number of node showed a relatively small decrease from 13.44 for pH 5.4 to 9.75 for pH 4.0.

![Plant height](image1)

![Number of branches](image2)

![Number of nodes](image3)

**Figure 2.** Plant height (a), number of branch (b) and number of node (c) of soybean genotypes under soil of Pasar jumat (pH 5.4) and soil of Jasinga (pH 4.0)

Shoot and root dry weight were used to estimate the stress tolerance index in this study. Yield component was ignored due to severe pest attack from Riptortus linearis (Hemiptera : Coreidae) which made difficulty in the analysis to distinguish the plant response to acid soil or pest.

Demas 1 was the most tolerant genotype based on the STI of shoot dry weight at 0.54, then followed by varieties of Kemuning 1, Rajabasa, and Detam 4 (Table 5). These genotypes also showed the highest performance of shoot dry weight in pH 4.0 at over 5 g for Demas 1 and
Detam 3. However, these figures were sharp decrease when compared to their performance in pH 5.4 ranging from 13.64 g for Detam 1 to 19.90 g for Kemuning.

The STI of root dry weight showed a similar trend from shoot dry weight. Demas 1 also showed the highest STI for this trait at 1.43 which was the only genotype with STI figure more than 1. The outstanding root dry weight of Demas 1 in pH 5.4 was much higher than the average weight of the all genotypes, eventhough significantly decreased at pH 4.0 which also performed by other genotypes.

**Table 5.** Shoot and root dry weight of 28 soybean varieties genotypes in polybag using soil of pH 4 and pH 6

| Genotype       | Shoot dry weight | Root dry weight |
|----------------|------------------|-----------------|
|                | pH 4.0 | pH 5.4 | STI | pH 4.0 | pH 5.4 | STI |
| Agromulyo      | 1.70    | 10.05  | d-k | 0.09   | 0.27   | f    | 2.21  | def 0.07 |
| Anjasmoro      | 3.27    | 15.03  | a-e | 0.26   | 0.44   | ef   | 3.11  | c-f 0.15 |
| Biosoy 1       | 2.31    | 8.31   | e-l | 0.10   | 0.40   | ef   | 2.81  | c-f 0.13 |
| Biosoy 2       | 1.55    | 12.06  | b-h | 0.10   | 0.33   | ef   | 2.26  | def 0.08 |
| Burangrang     | 1.35    | 14.98  | a-e | 0.11   | 0.22   | f    | 2.40  | c-f 0.06 |
| Dega 1         | 2.44    | 12.78  | b-g | 0.17   | 0.49   | ef   | 1.08  | def 0.06 |
| Dena 1         | 2.14    | 16.18  | a-d | 0.19   | 0.39   | ef   | 3.59  | b 0.16  |
| Dena 2         | 4.04    | 16.60  | a-d | 0.36   | 0.87   | def  | 2.45  | ed 0.24 |
| Derap 1        | 2.98    | 12.25  | b-h | 0.20   | 0.37   | f    | 1.54  | c-f 0.06 |
| Demas 1        | 5.84    | 17.34  | abc | 0.54   | 1.66   | def  | 7.69  | def 1.43 |
| Dena 2         | 3.24    | 13.70  | a-e | 0.24   | 0.34   | f    | 0.96  | ef 0.04 |
| Detam 3        | 4.43    | 14.13  | a-e | 0.34   | 0.83   | ef   | 3.46  | c-f 0.32 |
| Detam 4        | 5.76    | 13.64  | a-e | 0.42   | 1.25   | def  | 4.16  | c-f 0.58 |
| Detap 1        | 2.19    | 14.19  | a-e | 0.17   | 0.38   | ef   | 2.75  | cde 0.12 |
| Devon 1        | 5.81    | 11.31  | c-i | 0.35   | 0.63   | ef   | 2.31  | def 0.16 |
| Devon 2        | 2.50    | 12.90  | b-f | 0.17   | 0.53   | ef   | 3.50  | a 0.21  |
| Gamasugen 2    | 2.47    | 10.27  | d-j | 0.14   | 0.36   | ef   | 2.96  | def 0.12 |
| Gepak          | 1.08    | 11.39  |     | 0.19   | 1.64   |     |       |       |
| Kuning         |         |        |     |        |        |     |       |       |
| Grobogan       | 2.34    | 11.23  | c-i | 0.14   | 0.35   | f    | 1.51  | c-f 0.06 |
| Kemuning 1     | 4.13    | 19.90  | a   | 0.44   | 0.52   | ef   | 4.06  | bc 0.24 |
| Meratus        | 1.95    | 12.93  | b-f | 0.14   | 0.22   | f    | 1.25  | def 0.03 |
| Mitani         | 3.86    | 15.87  | a-d | 0.33   | 0.57   | ef   | 3.50  | cde 0.22 |
| Muria          | 2.21    | 14.27  | a-e | 0.17   | 0.65   | ef   | 3.51  | cde 0.26 |
| Panderman      | 2.62    | 12.61  | b-h | 0.18   | 0.45   | ef   | 1.98  | def 0.10 |
| Rajabasa       | 4.43    | 18.43  | ab  | 0.44   | 0.53   | ef   | 3.87  | cde 0.23 |
| Tengger        | 2.53    | 13.09  | a-f | 0.18   | 0.39   | ef   | 1.69  | ef 0.07 |

**Mean** 3.14 b 13.58 a 0.53 b 2.04 a
The results of the experiment of seedling stage in hydroponics and plant growth in polybags showed different results for each genotype tested. The Gepak Kuning genotype showed inconsistent results for both experiments. In hydroponic experiments, Gepak Kuning showed a slight reduction in root length and high STI so it was classified as a tolerant genotype, but in the polybag experiment, Gepak Kuning indicated as the most sensitive genotype because it had the smallest STI on shoot and root dry weight. Polybag experiments showed Demas 1 as a tolerant genotype, eventhough in hydroponic experiments Demas 1 didn’t show better tolerance with STI of 0.33. Demas 1 was developed and released as acid soil tolerant varieties, so the polybag experiment result strengthen Demas 1 as tolerant genotype.

The impact of pH towards root development can be seen from the direct root measurement at seedling stage and also root dry weight at mature stage. Both data in this experiment showed that acid environment affect root growth which disturb nutrient intake needed for plant growth and seed formation. However, the root growth might not suggest an increase in plant shoot formation. An increase in root dry weight could be influenced by root thickening. Hence, it is suggested to not select for root dry weight as a criterion for acid tolerant soybean breeding program [13].

4. Conclusion
The low pH and high Al inhibits seedlings root growth of soybean genotypes. The estimation of STI from root and shoot dry weight suggests Detam 1 as the most tolerant genotype followed by other variety with lower level of tolerance. The plant response of each genotype was different to the hydroponic seedling evaluation and plant growth evaluation using acid soil, so it is not recommended to use root parameters as selection criteria.

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