Seedling-stage screening method for tolerance of upland rice genotypes to low pH stress

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Abstract. One of the causes of generally low upland rice productivity in Indonesia is low fertility and soil pH. The use of genotypes with high yielding capacity and tolerant of acid soils can increase rice productivity. This study aimed to develop an early and efficient selection method, to determine rice genotypes tolerant to low pH. This research was conducted using a seed germination technique on rice paper medium with varied acidity level (pH 6.5, 5.5 and 4.5). Research data on the germination test showed that germination variables: growth uniformity, relative growth rate, vigour index, T50, and root length can be used as early indicators for determining rice genotypes tolerant to low pH. Research data showed that genotype Pae Huko is relatively more tolerant to low pH than the other three tested genotypes. Pae Huko had the highest values for relative growth rate, growth uniformity, vigour index, and T50 of 92.12%, 87%, 73.3, and 1.6 days, respectively. It had also the highest root length of 14.27 cm. The results give an indication that the method can be used as an early screening for determining tolerant rice genotypes to low pH. Further test (glasshouse experiment) is required to confirm the current results.

1. Introduction
The improvement of upland rice productivity can be achieved with the use of improved varieties including high yielding and adaptive varieties planted in a specific environment. Southeast Sulawesi has many local upland rice germplasms considerable potential, which have traditionally been cultivated by local farmers. Some local upland rice genotypes of Southeast Sulawesi have been reported to have a relatively good yield, and quite resistant to blast disease [1,2]. Meanwhile, the local upland rice genotypes Wagamba and Wangkoito are two examples of local upland rice in Southeast Sulawesi which have good grain quality [3].

In order to get more comprehensive data, it is necessary to evaluate the potential of local upland rice genotypes to biotic and abiotic stress factors, including to low pH (acidic soil) stress, which is one of the characteristics and constraints of rice production in dry land Ultisol in Southeast Sulawesi. At low pH, which limits the growth of plants are, such as, the level of toxicity or deficiency to certain minerals, and the solubility of aluminum (Al) are high, because the solubility of Al will increase with decreasing pH. Common symptoms encountered are poorly developed root system (short and thick) because the process of cell elongation is too late and the damage of plasmalema root cells [4]. Excess Al causes
inhibition of elongation and cell division, resulting in root stunt accompanied by a reduced absorption of water and nutrients.

Currently, the average productivity of upland rice in Southeast Sulawesi is still below 2 tons per hectare, far below the productivity of paddy rice, which reaches > 4 tons per hectare, and productivity of upland rice nationwide and productivity of upland rice in several regions in Indonesia, which reach approximately 3.5 - 5.0 t/ha (Department of Agriculture and Horticulture in South Sulawesi 2009). Increased productivity of upland rice can be done through the intensification program, including through the assembly and use of improved and adaptive varieties.

Development of early selection method is quite important and urgent considering the number of germplasm available to be tested / selected. Relying only on field testing will be quite time and cost consuming necessary to carry out testing the potential of the upland rice genotypes. Hence, the urgency of this research was to develop faster (early) and more efficient selection methods that can be used to define upland rice genotypes tolerant to low pH stress.

Biotic and abiotic factors could provide a major threat to agriculture. Therefore, efforts to develop stress-resistant crops are very important to improve crop productivity. The use of rice cultivars tolerant to Al is an effective and environmentally friendly approach. In general, the method of selection against Al, which generally related to soil pH, can be grouped on two selection approaches, in the laboratory and on the field [5].

Selection in the field is a method for screening genotypes that have tolerance to Al. Acid soil testing method is an effective way for selecting plants tolerant to Al, but the method is costly and sometimes difficult to apply because of the concentration of Al is not uniform, as well as the influence of the environment [6].

Selection in the laboratory can be done by selecting using specific selection agent, using a nutrient culture, and use the land in a greenhouse with a low pH (high Al). Test on artificial medium or using a nutrient solution is one of the alternative methods that can be used to test genotypes prior to the tests in the field.

Plants selected in the laboratory, should further be tested for the tolerance traits in a greenhouse or in the field. Testing in the greenhouse is useful for selecting plants with unstable tolerances as well as to determine the correlation between tolerance in the laboratory and in the greenhouse. In this research, a glasshouse testing on soil medium will later be conducted to confirm the results of laboratory testing.

The specific objective of this research was to develop methods of faster and more efficient early selection, in providing information about suspected upland rice genotypes that are more tolerant of low pH stress. This method is expected to be used to improve efficiency (faster and cheaper) in testing / evaluation and selection of potential rice plants to stress low pH.

2. Materials and methods

2.1. Research procedure

Rice genotypes for the testing materials were some local upland rice genotypes originating from various regions in Southeast Sulawesi [1-3,7,8]. The genotypes were Waburi-buri (G1), Pae Parigi (G2), Warumbia (G3), Tinangge (G4), and Pae Huko (G5). Seeds used were as uniform as possible, especially in terms of harvesting age. Treatment used for germination test was pH level of rice paper medium: pH 4.5, 5.5, and 6.5.

2.2. Procedure on germination testing on rice paper in laboratory

For the germination test on paper, after imbibition process, the seed was grown on two layers of sterile rice paper in petridish, about 25 seeds germinated per petridish. Rice paper was moistened with sterile water pH 4.5, 5.5 or 6.5 (according to treatments). Petridish was then closed and placed in a germination chamber, at a temperature of about 30° C with 12 hours photoperiod. Data were collected for seed germination for the period of 14 days.
2.3. Observation variables
Observations were done every week, on routine germination variables: root number, and root length, on at least 3 samples of sprouts. Observation of root length was used to calculate the Relative Root Length (RRL), according to previous method [3]. RRL > 0.5 = tolerant and RRL < 0.5 = sensitive. Research data were tabulated descriptively and analyzed using analysis of variance, followed by HSD test at the level of 95%.

3. Results and discussion
This research was done by using seed germination method on rice paper medium (Fig. 1) with different acidity levels: pH 6.5 (P0); pH 5.5 (P1) and pH 4.5 (P2). Local upland rice genotypes of Southeast Sulawesi used in this study were Waburi-buri (G1), Pae Parigi (G2), Warumbia (G3), Tinangge (G4), and Pae Huko (G5).

![Figure 1](image)

**Figure 1.** Germination testing of upland rice seeds on paper medium to varied acidity level treatments: (A) rice seeds on germination paper medium, (B) and (C) germinated rice seeds on paper medium.

Data showed that most of the germination variables such as growth uniformity, relative growth rate, vigor index, T50, and root length could be used as early indicators for determining rice genotypes tolerant of low pH, because their responses were generally differed on different conditions of medium acidity. Similarly, there were also variations of the response, although not all them were significant, on each local upland rice genotype tested on different medium pH conditions (Table 1).

| Treatment       | RGR    | GR     | GU     | VI     | MGP    | T50   |
|-----------------|--------|--------|--------|--------|--------|-------|
| G5 (Huko)       | 92.12* | 97.33* | 87.11* | 73.33* | 98.67* | 1.60* |
| G4 (Tinangge)   | 81.12 b| 87.11 b| 79.11 b| 56.89 b| 96.00 b| 1.82 b|
| G2 (Parigi)     | 88.91 ab| 96.89 a| 84.00 ab| 58.22 b| 99.11 b| 2.52 c|
| G1 (Waburi-buri)| 78.30 b| 90.67 b| 68.89 c| 32.00 c| 96.89 b| 2.96 d|

Notes: RGR: relative growth rate, GR: germination rate, GU: growth uniformity, VI: vigor index, MGP: maximum growth potential, T50: time (day) to 50% of the seeds grew.

The research data on Table 1 and Table 2 shows that the genotype Pae Huko (G5) is relatively more tolerant to low pH than the other three genotypes tested. Pae Huko had the highest values for the relative growth rate, growth uniformity, vigor index, and T50 of 92.12%, 87%, 73.3, and 1.6 days, respectively. Similarly, it also had the highest length of root seedlings at the age of 7 days of 14.27 cm. Aluminum can cause harmful effects on plant growth both directly and indirectly [3]. The most direct and obvious
Effect of Al toxicity on plant growth is the inhibition of root elongation (Figure 2). Therefore, relative root elongation is considered to be the parameter most directly related to Al tolerance in plants [9,10]. Effect of Al stresses is not the same in all plants, even within the same species. The roots of the plants that is most sensitive to Al-toxicity [6]. The initial symptoms are seen in plants that Al toxicity, i.e. the development of the root system as a result of inhibition of cell elongation. This is due to the incorporation of Al in the cell wall and cell division inhibition, thus inhibiting the absorption of water and nutrients.

Table 2. Responses of root length (cm) and root number on various local upland rice genotypes of Southeast Sulawesi.

| Treatment       | Root Length    | Root Number |
|-----------------|----------------|-------------|
|                 | 7 days | 14 days | 7 days | 14 days |
| G5 (Huko)       | 14.27  | 18.54    | 3.55   | 4.76    |
| G4 (Tinangge)   | 12.73  | 17.59    | 3.42   | 4.56    |
| G2 (Parigi)     | 13.89  | 18.51    | 3.89   | 4.87    |
| G1 (Waburi-buri)| 11.67  | 20.64    | 3.33   | 5.42    |

Table 3. Responses of various seed germination variables of local upland rice genotypes to different medium pH conditions.

| Treatment | RGR    | GR    | GU    | VI    | MGP   | T50   |
|-----------|--------|-------|-------|-------|-------|-------|
| P0 (6.5)  | 83.60  | 93.78 | 76.44 | 45.11 | 98.00 | 2.74  |
| P1 (5.5)  | 80.95  | 92.22 | 72.67 | 38.56 | 97.44 | 2.85  |
| P2 (4.5)  | 82.28  | 93.00 | 74.56 | 41.83 | 97.72 | 2.80  |

Notes: RGR: relative growth rate, GR: germination rate, GU: growth uniformity, VI: vigor index, MGP: maximum growth potential, T50: time (day) to 50% of the seeds grew.

Table 4. Responses of root length (cm) and root number to various germination medium pH.

| Treatment | Root Length    | Root Number |
|-----------|----------------|-------------|
|           | 7 days | 14 days | 7 days | 14 days |
| P0 (6.5)  | 12.78  | 19.57   | 3.61   | 5.14   |
| P1 (5.5)  | 12.23  | 20.10   | 3.47   | 5.28   |
| P2 (4.5)  | 12.50  | 19.84   | 3.54   | 5.21   |

The data in Table 3 and Table 4 also shows that there are variations in seed germination variables of various local upland rice genotypes on different medium acidity levels. This indicates that different genotypes have different levels of tolerance to low pH condition. Tolerant rice seedlings seen to have long branchy roots, fresh and bright in color, otherwise less tolerant seedlings have short, brownish and less branchy roots (Figure 2). In peas (Pisum sativum) and similar legumes, prolonged treatment with aluminum caused the formation of cracks in root surfaces which allowed the metal to reach inner root tissues, leading to loss of plasma membrane integrity [4,11,12]. In the present research, different genotype responses differently under different medium pH conditions, and this indicates that the testing method could be used as an initial screening method for determining rice genotypes tolerant of low pH. Further testing (using other approaches such as in vitro testing and rice growing in a greenhouse) is to be conducted, to confirm the current research results.
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Figure 2. Appearance of seedling roots of some local upland rice genotypes at different medium pH treatments.

4. Conclusions
Results of the study indicated that the testing method applied could be used as an initial screening method for determining rice genotypes tolerant of low pH. The research data showed that genotype Pae Huko relatively more tolerant to low pH than the other tested genotypes. This can be seen that Pae Huko has the highest values for the relative growth rate, growth uniformity, vigor index, and T50 of 92.12%, 87%, 73.3, and 1.6 days, respectively. Pae Huko had also the highest root length of 14.27 cm.

References
[1] Taufik M, Wijayanto T, Wahab A 2012 The characterization and evaluation of local upland rice cultivars to blast disease (Pyricularia oryzae) resistance in Southeast Sulawesi in Indonesia International Conference on Sustainable Agriculture and Food Security: Challenges and Opportunities 2011 (Bandung, Indonesia) Accepted
[2] Hasfiah, Taufik M, Wijayanto T 2012 Uji daya hasil dan ketahanan padi gogo lokal terhadap penyakit blas (Pyricularia oryzae) pada berbagai dosis pemupukan [Yield test and resistance of upland rice to blast disease Pyricularia oryzae with different dosages of fertilizer] Berkala Penelitian Agronomi 1 pp 26-36
[3] Sadimantara G R, Muhidin, Cahyono E 2014 Genetic analysis on some agro-morphological characters off hybrid progenies from cultivated paddy rice and local upland rice Adv Studies in Biol 6(1) pp 7-18
[4] Yamamoto Y, Kobayashi Y, Matsumoto H 2001 Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation in pea roots Plant Physiol 125 pp 199-208
[5] Mariska I, Lestari E G 2010 Pemanfaatan bioteknologi untuk mengatasi cekaman abiotik pada tanaman [The use of biotechnology to overcome abiotic stresses on plants] Seminar Nasional Balai Besar Penelitian dan Pengembangan Bioteknologi dan Sumberdaya Genetik Pertanian 2010 [National Proceeding Institute for Research and Development of Biotechnology and Agriculture Genetic Resources 2010] (Bogor, Indonesia) Accepted
[6] Anas, Yoshida T 2000 Screening of al-tolerant sorghum by hematoxylin staining and growth response Plant Prod Sci 3(3) pp 246-53
[7] Suliartini N W S, Sadimantara G R, Wijayanto T, Muhidin 2011 Pengujian kadar antosianin padi gogo beras merah hasil koleksi plasma nutfah Sulawesi Tenggara [Examination of anthocyanin contents in red upland rice obtained from germ plasm collection in Southeast Sulawesi] Crop Agro 4(2) pp 43-48
[8] Khaeruni A, Syair, Wijayanto T 2013 The utilization of indigenous rhizobacteria as inducer for rice plant resistance against bacterial leaf blight disease (Xanthomonas oryzae pv. Oryzae) International
Conference CRISU-CUPT Innovation and Collaboration Towards ASEAN Community 2015 (Sulawesi, Indonesia) Accepted

[9] Khatiwada S P, Senadhira D, Carpena A L, Z eigler R S, Fernandez P G 1996 Variability and genetics of tolerance for aluminum toxicity in rice (Oryza sativa L) Theor Applied Gen 93 pp 738-44

[10] You J F, He Y F, Yang J L, Zheng S J 2005 A comparison of aluminum resistance among Polygonum species originating on strongly acidic and neutral soils Plant and Soil 276 pp 143-51

[11] Nguyen A H, Wijayanto T, Erskine W, Barker S J 2016 Using green fluorescent protein shed light on Lupinus angustifolius L. transgenic shoot development Plant Cell, Tissue Organ Cult 127 665-74

[12] Suliartini N W S, Wijayanto T, Madiki A, Boer D, Muhidin, Tufaila M 2018 Yield potential improvement of upland red rice using gamma irradiation on local upland rice from Southeast Sulawesi Indonesia Bios Res 15(3) pp 1673-78

Acknowledgements
The authors gratefully acknowledge the financial support from Directorate General of Higher Education, Ministry of Education and Culture, Indonesia, under Fundamental Research Program 2016 and Higher Education Excellence Applied Research Program 2019-2020.