Tolerance limits of Indonesian rice varieties to drought and salinity in germination phase using PEG and NaCl as selection agents

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Abstract. This research aims to study tolerance limit of various Indonesian rice varieties to drought and salinity in germination phase using PEG and NaCl as selection agents and to obtain a resistance benchmarks of the rice varieties on drought and or salinity. Two sets of experiments were conducted, both set using Split Plot design, to analyse the plant tolerance to drought and salinity stress, respectively. Four levels of salinity i.e. Control, EC 4, 6 and 8 mmhos/cm, respectively were used in the first experiment and four levels of drought condition in the second experiment i.e. control, -0.33, -0.67 and -1.0 MPa, respectively. 25 varieties used in both experiments were germinated using wet papers previously applied with PEG and NaCl. High genetic diversity was shown by the rice varieties with the highest heritability index (H>0.7) on the PEG and NaCl with concentration of -0.67 MPa and 6 mmhos/cm, respectively. The tolerance limit of the rice varieties to salinity was on the NaCl concentration of 6 g.L\(^{-1}\), while to drought was on the PEG concentration of 100 g.L\(^{-1}\) (-0.67 MPa). Parameters that can be used as benchmarks for tolerance to drought and salinity in the germination phase were vigor index, radicle and plumula length.

1. Introduction
Rice is a strategic commodity that plays an important role in the economy and national food security so that it becomes the main base in the revitalization of agricultural development in the future. Efforts in developing the agricultural sector are always faced with a dynamic domestic and international environment. As a country located in the tropics, Indonesian agriculture has a comparative advantage. Such comparative advantage is the basis for the building of national and international competitive advantage.

Indonesian Agricultural Research Agency has been working to produce innovations to increase rice production. Through the implementation of intensive research and innovation of Integrated Crop Management and Resources (ICMR), adoption of suitable technology for local conditions that can improve production, yield quality, and preserve the environment can be conducted by farmers to increase farmers' income. However, the approach taken is still partial for each region [1].

Based on data from Indonesian Agricultural Research and Development Agency [2] the national rice productivity for the last 5 years only ranges from 4.98 to 5.34 t ha\(^{-1}\), while the yield potential of the released varieties ranges from 8 to 12 t ha\(^{-1}\) (table 1). This shows that the yield potential of the developed varieties was far from the expected target. Thus, the use of locally specific varieties is necessary to be implemented, especially on marginal land with appropriate package of cultivation technology.
Table 1. Land area, production and productivity of Indonesian national rice in 2011-2015.

| Indicator       | Unit | 2011          | 2012          | 2013          | 2014          | 2015          |
|-----------------|------|---------------|---------------|---------------|---------------|---------------|
| Harvest Area    | Ha   | 13,203,643    | 13,445,524    | 13,835,252    | 13,793,640    | 14,117,000    |
| Production      | T    | 65,756,904    | 69,056,126    | 71,279,709    | 70,846,465    | 75,398,000    |
| Productivity    | t ha\(^{-1}\) | 4.98          | 5.14          | 5.15          | 5.14          | 5.34          |

In order to meet Indonesian needs for food, rice cultivation can not only be planted on fertile land and available water, but also needs to be taken further in marginal lands. To increase productivity, utilization of marginal lands that suffer from drought stress, salinity, acidity, and its combination is necessary to be considered. Data of 2012, show that Indonesia has dry land of about 148 million ha (78%) and wetland area of 40.20 million ha (22%) of 188.20 million ha of total land area [3]. Swamp land in Indonesia reaches 33,393,570 million ha which spread in some areas of Sumatra, Kalimantan, Sulawesi, and Papua. In Indonesia, dry land is quite large with estimated about 60.7 million ha or 88.6% of the land area, while the rice field area is only 7.8 million ha or 11.4%. However, the expansion of cultivation in the area of new openings often faces ecological limiting factors such as drought and salinity or both. Drought and salinity stress reduce rice yield. Reduction of the rice yield caused by drought can be ranged from 20 to 100% in rainfed areas while yield reduction from salinity stress can range from 20 to 50% [4]. Salinity interferes with rice growth and development [5] include in the earlier stages such as germination phase [6]. However, the response of plant to salinity were varied between varieties and level of salinity [7].

2. Methodology
The research was carried out in the Laboratory of Agronomy Department, Faculty of Agriculture Hasanuddin University. Two sets of trial were set both in a Split Plot design with main plot for each cultivation in the area of new openings often faces ecological limiting factors such as drought and salinity or both.

A test on paper method was employed in the trial to germinate the 25 varieties of rice on various concentration of NaCl and PEG. Parameters measured were percentage of germination, germination rate, radicle and plumule length, plant fresh weight and vigor. Heritability was calculated for each of parameter. Concentration of NaCl and PEG that result in highest heritability will be used as concentration to test the varieties at growth and production stages in a further test hydroponically in screen house.

3. Results and Discussion
Table 2 shows that the germination percentage, germination rate, plumule length and vigor on the test on paper decreased with increasing Polyethylene glycol (PEG) concentration. The highest percentage of reduction in growth compared to normal condition (P0) was on seedling vigor with reduction reached 25% when water potential dropped to -0.67 MPa. Further reduction in vigor to more than 50% was shown in PEG concentration that resulted in water potential of -1.0 MPa. This justifies the method used in establishing the drought condition. The more PEG absorbed by the seed the less amount of water imbibed into the seed, because the PEG compound has polar properties, which is a characteristic that can bind water and priming seeds to control imbibition. This is in line with Hardegree and Emmerich [8] that Polyethylene glycol (PEG) is a compound used in priming where PEG has the properties of...
controlling the imbibition and hydration of the seed. Water is an indispensable factor in germination. The presence of water is essential for enzyme activity and decomposition of food reserves, nutrient translocation, metabolism/ biosynthesis, cell division, growth and other physiological processes [9]. Decrease in water potential increased the root length of the rice seedlings. This might be caused by the response of the plant as one of the mechanisms of resistance to drought stress.

In the test on paper, the higher the concentration of NaCl as selection agent for salinity tolerance from EC of 4 to 8 mmhos cm$^{-1}$ resulted in reduction of seedlings growth that range from 30 to 70%.

Table 2. Growth of various Indonesian rice varieties on different concentration of Polyethylene glycol (PEG).

| Parameter          | P0  | P1 (-0.33 MPa) | P2 (-0.67 MPa) | P3 (-1.0 MPa) |
|--------------------|-----|---------------|----------------|---------------|
| Percentage of Germination (%) | 91.47 | 78.93 | 13.71 | 73.51 | 19.63 | 66.22 | 27.60 |
| Germination Rate    | 39.08 | 35.16 | 10.02 | 30.02 | 23.17 | 23.60 | 39.61 |
| Root Length (cm)    | 5.13  | 5.90  | 13.05 | 6.67  | 30.02 | 7.08  | 38.01 |
| Plumula Length (cm) | 6.33  | 5.72  | 9.64  | 5.19  | 18.01 | 3.46  | 45.34 |
| Plant Fresh Weight (g) | 0.06  | 0.07  | 12.86 | 0.08  | 26.23 | 0.07  | 16.39 |
| Vigor               | 11.50 | 11.00 | 4.35  | 8.65  | 24.78 | 5.51  | 52.09 |
| Average             | 25.59 | 63.62 | 23.64 | 36.51 |

Table 3. Growth of various Indonesian rice varieties on different concentration of Sodium Chloride (NaCl).

| Parameter          | P0  | P1 (EC 4 mmhos cm$^{-1}$) | P2 (EC 6 mmhos cm$^{-1}$) | P3 (EC 8 mmhos cm$^{-1}$) |
|--------------------|-----|--------------------------|--------------------------|--------------------------|
| Percentage of Germination (%) | 91.47 | 77.96 | 14.77 | 72.98 | 20.21 | 66.22 | 27.60 |
| Germination Rate    | 39.17 | 35.16 | 10.24 | 30.37 | 22.47 | 39.37 |
| Root length (cm)    | 2.50  | 4.98  | 99.20 | 7.43  | 197.20 | 6.15  | 146.00 |
| Plumula Length (cm) | 6.80  | 5.44  | 20.00 | 3.90  | 42.65 | 1.03  | 84.85 |
| Plant Fresh Weight (g) | 0.04  | 0.05  | 31.71 | 0.06  | 65.85 | 0.06  | 51.22 |
| Vigor               | 12.51 | 11.13 | 11.03 | 8.57  | 31.49 | 6.68  | 46.60 |
| Average             | 25.42 | 31.16 | 63.31 | 65.93 |

Table 3 shows that the percentage of germination, germination rate, plumula length and vigor parameters of the rice varieties decreased with increased in concentration of Sodium Chloride (NaCl) in the test on paper. The higher the concentration of NaCl as selection agent for salinity tolerance from EC of 4 to 8 mmhos cm$^{-1}$ resulted in reduction of seedlings growth that range from 30 to 70%.

Salinity or salt stress can cause poisoning in plants so that the germination phase and growth of the seedlings become inhibited. In the germination phase, the growth of sprouts will be hampered due to the amount of Cl$^{-}$ content dissolved with water has been seeded by sprouts [12]. Some anions such as Cl$^{-}$ can cause severe cell membrane damage in excessive amounts and cause leakage of cell membranes. High salt levels inhibit seed germination, plant height, yield quality, production and damage to plant tissue [13]. As for the parameters of root length and wet weight of the plant, it increases with increasing...
concentration of NaCl. Root growth exposed to the saline environment is usually less affected [14]. This is thought to be due to improved balance by maintaining water-absorbing ability.

Heritability analysis is a quantitative benchmark to determine whether the phenotypic differences of a character are caused by genetic or environmental factors, so it can give an idea whether the observed character is more influenced by genetic or environmental factors. The highest heritability value as plant response to the concentration of PEG shown by the plant vigor character (95.28), while response to NaCl concentration was shown by germination rate character (90.24) as shown in table 4. Characters with high heritability will increase the effectiveness of selection in tolerance test because the observed character is a reflection of the influence of genetic factors compared with environmental influences [15]. Quantitative characters that have high heritability will produce a selection progress for desirable traits, whereas if low heritability is less effective to be used as selection material.

Table 4. Heritability values (%) of parameters as response of various Indonesian rice varieties to different concentration of Sodium chloride (NaCl) and Polyethylene glycol (PEG).

| Parameter                  | NaCl Heritability (%) | PEG Heritability (%) | Criteria |
|----------------------------|-----------------------|----------------------|----------|
| Percentage of Germination  | 79.31 High            | 69.45 High           | High     |
| Germination rate           | 90.24 High            | 91.01 High           | High     |
| Root Length                | 59.42 High            | 54.13 High           | High     |
| Plumula Length             | 61.94 High            | 56.41 High           | High     |
| Plant Fresh Weight         | 67.94 High            | 72.64 High           | High     |
| Vigor                      | 77.64 High            | 95.28 High           | High     |

Notes: $0 < h^2 \leq 20$ (low), $21 < h^2 \leq 50$ (medium), $50 < h^2 \leq 100$ (high)

4. Conclusion
The tolerance limit of rice resistance to drought was at PEG concentration of 100 g.L$^{-1}$ (-0.67 MPa) and to salinity was at NaCl concentration of 6 g.L$^{-1}$ (EC 6 mmhos.cm$^{-1}$). There is a high genetic diversity of Indonesian rice varieties to drought and salinity with high heritability ($H> 0.7$) at water potential of -0.67 MPa and salinity of EC 6 mmhos.cm$^{-1}$.

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