Breeding of abiotic tolerant varieties with high grain quality to meet the consumer preference at rice swampy land

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Abstract. Acid sulfate is common constraint in swamp rice land, however, salinity and submergence become a problem as well especially in the coastal part. Hight yielding and tolerant varieties were one of cheaper technology could be adopted by farmers to cope with the problems. Screening were the starting point in the steps of breeding programs. There were 41 lines identified tolerant to acid sulfate, while under salinity there were 37 lines had tolerant to salinity stress up to 12 dSm⁻¹. Among the lines, 6 promising lines were adaptive to both stresses, such as B13981E-KA-34, B14316E-KA-48, B13134-4-MR-1-KA-1, B14354E-KA-2, B14339E-KA-27, and B14357E-KA-48. Besides the yield and tolerance, grain quality was also important to be evaluated to meet consumer preference. There was variation for amylose content among the lines because Indonesian farmers had a wide range of texture preference. The selected lines have moderate amylose content, varied from 20.72 to 24.92 %. Most of the lines were slender with medium shape, and no chalkiness. There were two lines which had small shapes, such as B13983E-KA-12-2 and B13578E-KA-5-B. It seems meets the preference of Kalimantan farmers who prefer small and long-grain, as same as Siam type.

1. Introduction
One of target of Sustainable Development Goals (SDGs) is decrease hunger prevalence at 2030. Indonesia people was predicted will increase from 238.5 M (2010) to 305.6 M people at 2035. It is mean that Indonesia have to increase the food availability to feed more population [1]. Rice is a staple food, it has a role to fulfill 45% of food intake or 80% of the main carbon hydrate sources in the consumption patterns of the Indonesian people [2]. Leverage analysis results [2] explain that there are several variables related to land resources aspects which very sensitive on national rice production, such as the availability of irrigated land, conversion of paddy fields, land compatibility, land clearing and land productivity.

Optimalization of sub-optimum lands such as wetland at the swampy area, upland and rain-fed lowland used as one strategy to increase rice productivity. Swampland is one of the ecosystems with huge potency for agricultural development, including rice production. In Indonesia, it has been agreed that the wetland of the swampy area is divided into two types, namely tidal swamp, and lebak (freshwater) swamp or basin area. Tidal land is a wetland whose water regime is affected by tidal or river water, while a lebak or basin is a land whose water regime is affected by rain came from the site or upstream area. Most of the swamp area farmers consider rice as the main commodity because it is relatively easy to cultivate in swamp environment, especially on tidal swampland types A and B.
The area of tidal swamps suitable for rice reaches 6.10 million ha, consist of 3.42 million ha located on tidal swamps and 2.67 million ha on peatlands [3]. The complex constraint was happen in the area. Swampland is generally characterized by unpredictable water fluctuations because the macro and micro drainage systems cannot be controlled yet. Then, low soil fertility, soil acidity, iron (Fe) toxicity, Aluminium (Al) toxicity [4], pest and disease become constraints as well.

Traditional rice cultivars are still commonly cultivated by wetland farmers, which is a long duration and photoperiod sensitive, less respond to fertilizer and low yield. The effort to shifting the local varieties into tolerant and high yield improved varieties increased the rice production up to 32.4% [5, 15]. It means the use of adaptive and high yielding varieties has an important role in a rice production increase in tidal swampland. In general, farmers in swamp areas are subsistence farmers with minimal input use in their rice cultivation. The use of high yielding varieties in the area is still very low [6], so the yield was not optimal because they are susceptible to iron toxicity and disease. On the other hand, the introduction of new high yielding varieties is limited because sometime it doesnt match the farmer’s preference and local wisdom. Therefore, breeding for abiotic tolerant, high yield and good grain quality are very important to meet the farmer preference and accelerate the adoption process.

2. Materials and Methods

2.1. Saline screening

A total of 100 swampy rice lines will be evaluated for tolerance to salinity stress in saline soluble, along with susceptible check variety (IR29) and resistant check variety (Pokkali). The plant material that we used is a derivative of saline tolerant parents. Screening the tolerance properties of swamp rice lines to salinity stress was carried out on seedlings stages in the greenhouse of ICRR, west java in DS 2015.

The experiment was carried out using a strip check design with 4 replications. Each selected accession was tested at a saline stress level of 12 dS/m and carried out following [7] method. The solution is renewed every eight days and the pH is maintained at 5.0. Scoring was done at 21 days after salinization to have the worst stresses. Observation of salinity stress was carried out in accordance with SES [8].

2.2. Acidic sulphate screening

Acid sulfate tolerance testing of swamp rice lines was carried out in acid sulphate fields in Belandean, Barito Koala, South Kalimantan in WS 2015. Three weeks old of seedlings were planted in plots of 2 x 10 hills using a spacing of 25 m x 25 cm, and field design were Augmented. Inpara 7, Inpara 9 and IR42 were used as check varieties. Visual symptoms observed after the susceptibility check begin to show withering or at ± 25-30 days after transplanting.

2.3. Observational yield trial under iron toxicity stress (tidal swamp land)

100 selected swamp rice lines were transplanted in the observation yield trial in WS 2015, at Karang Agung, Banyuasin, using an augmented design with 4 blocks. Each line was planted in a 1m x 5m plot with a spacing of 25cm x 25cm. Twenty days old of seedling were transplanted with 1 seedling per hill. Inpara 3, Inpara 4, Inpara 7, Inpara 9, IR42 used as check varieties and transplanted at each block. Fertilizer was applied three times with doses: 100 kg Urea + 100kg TSP + 100kg KCl/ha applied at 7 days after transplanting (DAT), 50 kg/ha of Urea was applied at 28-30 DAT and 49-50 DAT. Observation were done for plant height, productive tiller number, flowering date and grain yield.

Data were statistically analyzed using the SAS statistical software (9.1). Analysis of Variance (ANOVA) was performed for all traits. Mean separation was done using the Least Significant Difference (LSD) at P=0.05.
2.4. Grain quality evaluation
Evaluation of grain quality was carried out at Rice Quality Laboratory, ICRR, West Java for amylose content (AC), length and shape of grain, and chalkiness portion.

Size and shape of the grain. The size and shape of rice were measured from 10 whole grains of rice using a measuring instrument "Dial Caliper". The shape of rice was the ratio between length and width of grain. The length and shape of rice classification follows the international guidelines [8]. The classification of each line is measured based on the average percentage of observation and it will classify by follows the guidelines that have been standardized [8].

Chalkiness: Chalkiness of rice was measured based on the average percentage of the chalkiness portion of each line. Classification of the chalkiness portion follows the standardized guidelines [8].

Amylose content (AC): Analysis of amylose content was carried out by the colorimetric iodide method which was used as a standard method for the analysis of rice amylose content. Amylose levels of rice samples were classified as sticky rice (0-2%), very low (3-9%), low (10-19%), moderate (20-25%) and high (> 25%) [9].

3. Results and Discussion

3.1. Saline and acidic sulphate tolerance
Acidic-sulphate soil is a complex type of soil, whereas plants which exposed to the soil will face iron toxicity, sulphate toxicity and soil acidity [10]. It will inhibit root growth and rice root will appear black and shorter [11]. The use of tolerant varieties treated by good management strategies has been found to give a great production increase. Liming and some other mineral additional which may play an important role to reduce the effect of iron toxicity and increasing the soil pH were given a positive effect on the expression of tolerant varieties [12].

![Figure 1. frequency of saline and acidic sulphated tolerance among the swampy rice lines](image)

Salt stress is abiotic stress limiting rice productivity, particularly in coastal areas. In most Asian countries, salinity was happened iatthe same time with drought, especially during the dry season, where there is no enough irrigation to be used reducing soil salt in rice paddy fields. Reduced freshwater in irrigation canal induced secondary salinization and the effect of salinity will get worse [13]. It will affect directly to rice growth and yield punishment will happen. Therefore, selected the tolerant improved lines to be used in the salt soil will very important.

The selection of adaptive improve rice lines under acidic sulphate soil and saline solution is one of the first step on the breeding of tolerant rice varieties. In this study, we found a good result in the screening of both characters. About 20.9% of tested lines were categorized adaptive to the acid
sulphate soil whereas 17.2% were moderately tolerant and 3.7% were tolerant with almost no symptom appear in the leaf and it has normal growth (Figure 1). It’s means that there was variation among improved rice lines for both characters and we have the opportunity to select the best lines of them.

The susceptible symptom of IR29 was appear in 7-14 days after treatment, while Pokkali remained tolerant even though it has been tested for 21 days. Among tested varieties, Inpara 7 gave a consistent reaction to salt stress, i.e. moderately tolerant (score 5) at 12 dSm\(^{-1}\) of stress. The IR42 and other variety has a susceptible response (score 7-9). There were 31 lines that had a moderately resistant response (score 5). There were several lines that were good vigor up to 21 days after treatment, and its remaining give a good performance in the field after transferring from the salt solution. There were 20 improved lines that showed a good vigor with scores 3 – 5 (data not shown).

### Table 1. Improved rice lines adaptive to saline and acidic sulphate stresses

| Lines/Check Varieties | Tolerance Score to Saline (12 dS/m) | Tolerance to Acid-sulphate soil Score | PH (cm) | PT |
|-----------------------|------------------------------------|--------------------------------------|--------|----|
| B13981E-KA-34         | 5                                  | 1                                    | 103.6  | 20 |
| B14316E-KA-48         | 5                                  | 3                                    | 105.4  | 18 |
| B13134-4-MR-1-KA-1    | 5                                  | 3                                    | 92.6   | 15 |
| B14354E-KA-2          | 5                                  | 3                                    | 109.6  | 18 |
| B14339E-KA-27         | 5                                  | 3                                    | 101.0  | 18 |
| B14357E-KA-48         | 5                                  | 3                                    | 107.4  | 14 |
| Inpara 7              | 5                                  | 3                                    | 92.2   | 22 |
| Inpara 9              | 7                                  | 5                                    | 105.0  | 12 |
| IR42                  | 9                                  | 5                                    | 104.3  | 12 |
| IR29                  | 9                                  | 3                                    | 92.2   | 22 |
| Pokkali               | 1-3                                |                                      |        |    |

Note. Score of acid sulphate: 1 tolerant, 3 moderately tolerant, 5 moderately susceptible, 7 susceptible, 9 very susceptible; score of saline stress : PH plant height, PT productive tillers

Table 1 showed the tolerant improved lines which have the tolerance to acid sulphate and saline stress. The six lines were moderately tolerant to 12 dSm\(^{-1}\) of saline stress and it was also moderately tolerant (MT) to tolerant (T) to acid sulfate condition. B13981E-KA-34 was tolerant to acid soil (score 1) and it performed well under acid sulphate soil. The leaves were remaining green, with no leaf bronzing with normal growth such as plant height and tillers number. Another 5 lines were moderately tolerant with score 3. Some of the improved lines showed growth disorders such as chlorosis, yellowish, and inner venial spots on the leaves blade due to pyrite toxicity and soil acidity. High concentration of iron-induced nutrient imbalance such as K and Zn. It will affect plants’ ability to reduce uptake of iron in the shoots through a physiological mechanism by roots such as iron oxidation, iron exclusion, and iron retention [14].

### 3.2. Observational yield trial under iron toxicity stress

The average plant height was varied among improved rice lines in tidal swampland (Table 2). It was varied from 93.62 to 116.81 cm. In general, the plant height was suitable for the wetland to avoid lodging, except B13134-4-MR-1-KA-3-4 which taller than others. The tall plant ideotype should be supporting by the strong stem to anticipating lodging [16]. Lodging induced by stem breaking or pulling out of the root from the soil. It can results in inhibiting transport of assimilates, water and other minerals, and leading the yield reduction [17].

Productive tillers number has been reported that has a good correlation with rice yield [18, 19]. The number of tillers was related to the phyllochron formation period. Phyllochron is the time interval
when the first cell of stem, leaf and root appeared in the stem bud and germination. It was correlated with the old of seedling which had transplanted to the field. Transplanting using younger seedlings will produce more tillers number due to the richer of phyllochron [20].

Flowering date of the tolerant-high yield lines was categorized as early maturity, earlier that IR42 and Inpara 4, both were popular varieties in the wetland. B14315E-KA-59 was the best line in terms of yield. It produced 9.63 t/ha and significantly higher than the best check named Inpara 9 which produced 5.63 t/ha. Varieties which high yielding and adaptive to the ecosystem have important role to increase rice production in the wetland, especially with complex constraints such as acid sulphate ecosystem.

| No | Lines/check varieties     | PH (cm) | PT | FD (DAS) | Yield (t/ha) |
|----|---------------------------|---------|----|----------|-------------|
| 1  | B14315E-KA-59             | 99.31   | 20 | 85       | 9.63*       |
| 2  | B13983E-KA-12-2           | 108.58  | 16 | 87       | 8.65        |
| 3  | B13136-6-MR-2-KA-2-1-7    | 110.62  | 21 | 84       | 7.02        |
| 4  | B13589E-KA-14-B           | 94.62   | 15 | 89       | 6.98        |
| 5  | B14308E-KA-7              | 110.22  | 13 | 86       | 6.98        |
| 6  | TDK 1-Sub 1-MR-1          | 111.22  | 22 | 84       | 6.82        |
| 7  | B14308E-KA-34             | 112.22  | 15 | 84       | 6.74        |
| 8  | B13578E-KA-5-B            | 110.22  | 16 | 89       | 6.69        |
| 9  | B13100-2-MR-3-KY-2        | 93.62   | 16 | 84       | 6.69        |
| 10 | B13134-4-MR-1-KA-3-4      | 116.81  | 7  | 87       | 6.54        |

IR42 88.07 17 104 4.30
Inpara 3 95.63 14 84 4.69
Inpara 4 86.63 16 111 3.47
Inpara 7 91.93 18 83 3.82
Inpara 9 108.82 13 91 5.63

LSD 19.96 7.26 4.22 3.26
CV (%) 8.23 21.76 2.09 28.18
Rata-rata 105.15 14.48 87.40 5.02

Note. PH plant height, PT productive tillers no., FD flowering date, DAS days after sowing

Preference of people to rice quality was very various in Indonesia, but in general, they prefer soft to moderate rice. To meet their preference, our study was also considering to develop various grain quality attributes, such as amylose content, shape, and chalkiness. Table 3 showed the grain quality of selected improved lines that have the tolerance to saline stress and adaptive to acid sulphate soil. Grain size and shape are not only related to the rice quality, but it was important components of grain yield too [21]. It can be understandable that grain size and shape will affect grain weight. The eight of improved lines have moderate amylose content, it was varied from 20.72 to 24.92 % (Table 3). Most of the lines were slender with medium shape, and no chalkiness. There were two lines which had small shapes, such as B13983E-KA-12-2 and B13578E-KA-5-B. It seems meet the preference of Kalimantan farmers who prefer small and long grain [22], as same as Siam type.
Table 3. Grain quality of best rice improved lines

| Best Lines of OYT | AC (%) | Grain Quality |
|------------------|-------|---------------|
|                  |       | Length | Shape | Chalkiness |
| B14315E-KA-59    | 23.45 | L      | M     | N          |
| B13983E-KA-12-2  | 22.75 | L      | S     | N          |
| B13589E-KA-14-B  | 20.72 | L      | M     | N          |
| B14308E-KA-7     | 21.00 | L      | M     | N          |
| B14308E-KA-34    | 24.92 | L      | M     | N          |
| B13578E-KA-5-B   | 23.31 | L      | S     | N          |
| B13100-2-MR-3-KY-2 | 22.40 | M      | M     | N          |
| B13134-4-MR-1-KA-3-4 | 23.31 | L      | M     | N          |

Note: L slender (long), M medium, S small, N very small portion or none chalkiness

4. Conclusion

There were 6 improved rice lines which tolerant to both saline and acidic sulphate stresses. It were B13981E-KA-34, B14316E-KA-48, B13134-4-MR-1-KA-1, B14354E-KA-2, B14339E-KA-27, and B14357E-KA-48. They were well performed under saline and acid sulphate soil with score 5 and 3, respectively. B14315E-KA-59 was the best line and produced 9.63 t/ha, significantly higher than the best check named Inpara 9 (5.63 t/ha). The improved lines have moderate amylose content, varied from 20.72 to 24.92 %. Most of the lines were slender with medium shape, and no chalkiness. There were two lines which had small shapes, such as B13983E-KA-12-2 and B13578E-KA-5-B. It was meet the preference of Kalimantan farmers who prefer small and long-grain, as same as Siam type.

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