

LEAF BRONZING SYMPTOM SCORE AS RELATED TO GRAIN YIELD OF RICE UNDER IRON TOXICITY OF ACID-SULFATE SOIL AREA

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ABSTRACT

Excessive Fe²⁺ uptake of rice plant can cause iron toxicity in most of acid wet land environment. Deleterious effect of iron may interfere metabolic processes and cause damage to rice plants, which is marked by red-brownish leaf discoloration or leaf bronzing symptoms (LBS). In some extent of level scoring of LBS may relevant to reducing of grain yield and can be use as criterion selection. Since, high yielding is main goal of rice breeding for acid-sulfate soil environment, it is therefore important to understand the correlation between yield and leaf bronzing symptoms. Here, we study the yield performance of 22 breeding lines under iron toxicity and determine correlation between yield and leaf bronzing symptom of iron toxicity. A randomized complete block design with three-four replications was used in the two acid-sulfate soil area, Karang Agung (South Sumatera) and Belandean (South Kalimantan) in the dry season 2016. Response of rice genotypes to iron toxicity was observed on leaf bronzing symptom, yield, and yield components. The research resulted there were 6 lines have consistency high grain yield and LBS score in two locations, that were (1) B13586E-KA-9-B; (3) B13988E-KA-20, (8) B13983E-KA-13-3, (10) B13983E-KA-13-1, (14) B13925E-KA-46, (17) B13926E-KA-1 with LBS score 3 - 5. Two lines have high yield, but are not consistent with LBS score, that were (21) B14039E-KA-1 and (22) B14009E-KA-39. Leaf bronzing symptoms has significant and negative correlation with grain yield ($r=-0.537$). Leaf bronzing symptoms is effective as one of criterion selection to get high yielding under iron toxicity in hotspot area.

Keywords: criterion selection, iron toxicity, leaf bronzing symptom

INTRODUCTION

Iron is a micro nutrient needed by plants on very small quantities. Concentration of iron in plant tissue is normal on range of 100-200 ppm. If concentration of iron in soil more than 300 ppm, this condition is toxic for rice plant (Tanaka & Yoshida, 1972).

Iron toxicity in rice can be observed on bronzing and dark symptoms, plant root systems is not developed, inhibited flowering, asimilat synthesis process is stopped, plant is dwarf, roots is thick, brown, rough, and retracted, stems and leaves is decay (Yamauchi & Peng, 1995; Dobermann & Fairhurst, 2000). Bronzing symptoms are fully visible on the leaves that play role as a source of photosynthesis, beginning with the presence of a small brown stain spread from

leaf tip to the base of the leaf. More symptoms are leaves become yellowing and dry up, followed by a very high rate of respiration which in turn all leaves become yellowish and brown (rust), or the leaves will be purple, stiff, and hard. This condition indicates a very severe level of iron toxicity (Yamanouchi & Yoshida, 1981).

Iron toxicity in rice plants can decrease grain yield. Grain yields decreased by 52% (Ismunadji *et al.*, 1973), 70% in susceptible varieties and 30% in tolerant varieties (Virmani 1977), even 90% in severe-level of iron in the fields (Suhartini, 1992). Growth abnormalities due to iron stress decrease rice yields by 15 - 30%, even harvest failures in severe iron poisoning (Audebert & Sahrawat, 2000). Hotspot for Fe toxicity is an area with a relatively high Fe levels, which can seriously affect rice production. Fe levels vary from 10 to 2000 ppm (Diatta *et al.*, 1998).

In plant genetic improvement, selection strategy is a key to progress. While breeding for complex traits such as abiotic stress tolerance, two types of selection are distinguished: direct selection based on yield and indirect selection using secondary traits such as leaf rolling, leaf bronzing score, plant height, root and shoot biomass, tissue Fe concentration, chlorophyll content or agronomic traits other than yield. Rice breeding programs aiming at improving the tolerance to Fe toxicity have mainly used yield for direct selection and leaf bronzing score as a secondary trait under Fe toxicity (Abifarin, 1989; Gridley *et al.*, 2006).

Audebert and Fofana (2009) reported that for every increase in LBS by one grain, the yield is reduced by 500 kg ha⁻¹. There are a few other reports of a negative correlation between bronzing score and yield under Fe toxicity stress (Sahrawat *et al.*, 1996; Nozoe *et al.*, 2008; Onaga *et al.*, 2013). The relation between LBS and grain yield could depend on the type and number of entries used, and experimental conditions.

The key secondary trait should have sufficient genetic variability, it should be easier and less expensive to measure than grain yield itself, it should have high heritability, and it should have positive genetic correlation with yield (Laffite *et al.*, 2003). The leaf bronzing symptom (LBS) is a key secondary trait. Leaf symptoms are commonly rated visually using LBS (IRRI, 2002). The score is graded on a scale of 0 – 9 where 0 means normal growth and 9 indicates that almost all plants are dead or dying. As it is only a visual score it can be measured rapidly and it is also relatively easy to measure. However, several contradictory reports exist regarding the relationship between yield and LBS. It is possible to develop good varieties combining high yield with low LBS (Gridley *et al.*, 2006).

Here, we study the yield performance of 22 breeding lines under iron toxicity and determine correlation between yield and leaf bronzing symptom of iron toxicity.

MATERIALS AND METHODS

Experiment was conduct in acid-sulfate area, which were Karang Agung, Banyuasin, South Sumatera and Belandean, Barito Kuala, South Kalimantan on dry season of 2016. The materials used were 22 breeding lines for swampy area and 4 check varieties.

Experimental design used in each location was Randomized Complete Block Design with four replication in Karang Agung, and three replication in Belandean. Response of plant to iron toxicity was observed on leaf bronzing symptom (LBS)

score (Shimizu *et al.*, 2005), plant height, time 50% flowering, yield, and yield components.

Data were analysis using analysis of variance, combine analysis of variance, and correlation analysis. Person correlation was calculated to identify correlation between quantitative traits. Spearman's rank correlation was calculated to determine the relationship between yield, yield component, plant height, time of 50% flowering and LBS. All analysis was perform using STAR software.

RESULTS AND DISCUSSION

Performance of Genotypes

Grain yield of genotype tested in Karang Agung were range from 3.11 to 4.18 ton ha⁻¹. INPARA varieties were not significantly different with susceptible variety IR 42. Average of INPARA 9 grain yield was relatively higher so it was used as check in this analysis.

Table 1. Grain yield and leaf bronzing score of breeding line in acid-sulfate area

| No | Genotype | Grain yield (ton ha ⁻¹) | | | LBS | |
|----|-----------------|-------------------------------------|------|------|-----|-----|
| | | KA | BLD | Mean | KA | BLD |
| 1 | B13586E-KA-9-B | 4.18 | 3.50 | 3.95 | 3 | 4 |
| 2 | B14299E-KA-46 | 3.13 | 2.84 | 2.96 | 3 | 3 |
| 3 | B13988E-KA-20 | 3.81 | 3.15 | 3.59 | 3 | 4 |
| 4 | B14308E-KA-34 | 3.45 | 3.48 | 3.40 | 3 | 4 |
| 5 | B14360E-KA-17 | 3.35 | 2.78 | 3.10 | 7 | 5 |
| 6 | B13983E-KA-6-3 | 3.58 | 3.07 | 3.39 | 3 | 5 |
| 7 | B13971E-KA-23-1 | 3.60 | 3.16 | 3.35 | 3 | 5 |
| 8 | B13983E-KA-12-3 | 4.06 | 3.39 | 3.75 | 3 | 4 |
| 9 | B13984E-KA-8-2 | 3.24 | 2.44 | 2.86 | 3 | 4 |
| 10 | B13983E-KA-13-1 | 3.60 | 3.46 | 3.55 | 3 | 5 |
| 11 | B13983E-KA-7-3 | 3.67 | 2.87 | 3.25 | 5 | 5 |
| 12 | B13970E-KA-23-3 | 3.65 | 3.11 | 3.38 | 3 | 4 |
| 13 | B13925E-KA-7 | 3.07 | 2.86 | 2.97 | 3 | 5 |
| 14 | B13925E-KA-46 | 4.14 | 3.25 | 3.71 | 3 | 5 |
| 15 | B13926E-KA-23 | 4.12 | 2.96 | 3.55 | 3 | 5 |
| 16 | B13925E-KA-42 | 3.26 | 2.63 | 2.94 | 3 | 4 |
| 17 | B13926E-KA-1 | 4.14 | 3.15 | 3.65 | 3 | 5 |
| 18 | B14010E-KA-20 | 3.72 | 3.18 | 3.51 | 3 | 5 |
| 19 | B14039E-KA-15 | 3.11 | 2.94 | 3.01 | 3 | 5 |
| 20 | B13996E-KA-1 | 3.19 | 2.35 | 2.81 | 3 | 5 |
| 21 | B14039E-KA-1 | 3.66 | 3.15 | 3.51 | 1 | 3 |
| 22 | B14009E-KA-39 | 3.55 | 3.77 | 3.75 | 1 | 4 |
| 23 | IR42 | 3.45 | 3.25 | 3.31 | 1 | 2 |
| 24 | INPARA 3 | 3.88 | 3.47 | 3.63 | 1 | 3 |
| 25 | INPARA 8 | 3.96 | 2.76 | 3.36 | 1 | 5 |
| 26 | INPARA 9 | 3.60 | 4.28 | 4.09 | 1 | 3 |
| | LSD (5%) | 0.51 | 0.75 | - | | |
| | CV (%) | 10.0 | 14.6 | 12.1 | | |

LBS = leaf bronzing score, KA = Karang agung, grain yield was calculated from four replication, BLD = Belandean, grain yield was calculated from three replications; Mean = overall mean, was calculated from three replication of each location.

Four genotype have grain yield higher than INPARA 9 in Karang Agung, that were (15) B13926E-KA-23, (14) B13925E-KA-46, (17) B13926E-KA-1, and (1)

B13586E-KA-9-B. Other genotypes were not significantly different with INPARA 9. Leaf bronzing symptom (LBS) score ranged from 1 to 7. LBS score of four genotypes with higher grain yield was 3. B14039E-KA-1 (21) and B14009E-KA-39 (22) have low LBS, even the grain yield were not significantly different with INPARA 9 (Table 1). Six genotype could be considered to evaluate in next trials.

Grain yield in Belandean was ranged from 2.76 to 4.28 t/ha. INPARA 8 has low grain yield (2.76 ton ha⁻¹). INPARA 9 has highest grain yield (4.28 ton ha⁻¹). Only B14009E-KA-39 (22) that has an equivalent yield with INPARA 9. Other genotypes have lower yield than INPARA 9. Leaf Bronzing Symptom (LBS) score of Belandean showed higher than Karang Agung, ranged from 2 to 5. B14009E-KA -39 (22) was 4 and INPARA 9 was 3.

Table 2. Plant height, number of productive tillers, and time of 50% flowering of breeding line in acid-sulfate soil area

| No | Genotype | Plant height (cm) | | | No. of tillers | | | 50 % Flowering (HSS) | | |
|----|-----------------|-------------------|-----|------|----------------|-----|------|----------------------|-----|------|
| | | KA | BLD | Mean | KA | BLD | Mean | KA | BLD | Mean |
| 1 | B13586E-KA-9-B | 103 | 107 | 105 | 11 | 20 | 15 | 92 | 89 | 91 |
| 2 | B14299E-KA-46 | 95 | 105 | 102 | 14 | 18 | 16 | 91 | 79 | 85 |
| 3 | B13988E-KA-20 | 94 | 104 | 100 | 23 | 17 | 22 | 96 | 90 | 92 |
| 4 | B14308E-KA-34 | 101 | 117 | 108 | 11 | 18 | 15 | 91 | 77 | 83 |
| 5 | B14360E-KA-17 | 93 | 108 | 101 | 12 | 18 | 16 | 92 | 79 | 86 |
| 6 | B13983E-KA-6-3 | 101 | 108 | 106 | 14 | 18 | 16 | 93 | 82 | 86 |
| 7 | B13971E-KA-23-1 | 105 | 124 | 116 | 13 | 16 | 15 | 93 | 81 | 88 |
| 8 | B13983E-KA-12-3 | 104 | 110 | 109 | 14 | 16 | 14 | 92 | 80 | 85 |
| 9 | B13984E-KA-8-2 | 108 | 121 | 116 | 13 | 18 | 16 | 94 | 80 | 87 |
| 10 | B13983E-KA-13-1 | 103 | 116 | 111 | 13 | 18 | 16 | 93 | 86 | 89 |
| 11 | B13983E-KA-7-3 | 102 | 109 | 106 | 13 | 17 | 15 | 96 | 86 | 90 |
| 12 | B13970E-KA-23-3 | 113 | 127 | 121 | 16 | 18 | 17 | 92 | 80 | 85 |
| 13 | B13925E-KA-7 | 92 | 107 | 101 | 15 | 15 | 16 | 92 | 77 | 84 |
| 14 | B13925E-KA-46 | 99 | 122 | 112 | 14 | 16 | 16 | 92 | 80 | 85 |
| 15 | B13926E-KA-23 | 103 | 109 | 107 | 13 | 17 | 15 | 92 | 81 | 86 |
| 16 | B13925E-KA-42 | 94 | 105 | 101 | 15 | 17 | 17 | 93 | 78 | 85 |
| 17 | B13926E-KA-1 | 94 | 107 | 99 | 14 | 17 | 15 | 91 | 77 | 85 |
| 18 | B14010E-KA-20 | 110 | 111 | 111 | 13 | 16 | 15 | 89 | 82 | 85 |
| 19 | B14039E-KA-15 | 96 | 113 | 104 | 9 | 18 | 13 | 96 | 81 | 89 |
| 20 | B13996E-KA-1 | 107 | 116 | 113 | 13 | 16 | 15 | 91 | 76 | 83 |
| 21 | B14039E-KA-1 | 96 | 110 | 105 | 12 | 19 | 15 | 95 | 86 | 90 |
| 22 | B14009E-KA-39 | 99 | 105 | 102 | 15 | 18 | 17 | 89 | 86 | 88 |
| 23 | IR42 | 89 | 103 | 96 | 19 | 19 | 21 | 105 | 90 | 98 |
| 24 | INPARA 3 | 93 | 109 | 103 | 13 | 16 | 15 | 92 | 83 | 87 |
| 25 | INPARA 8 | 106 | 108 | 108 | 16 | 16 | 16 | 92 | 87 | 89 |
| 26 | INPARA 9 | 105 | 109 | 108 | 10 | 17 | 14 | 93 | 79 | 85 |
| | LSD (5%) | 8.9 | 5.1 | 8.3 | 7 | 2.2 | - | 5 | 4.3 | 4.8 |
| | CV (%) | 6.3 | 2.8 | 4.8 | 35 | 7.6 | 24.9 | 3.7 | 3.2 | 3.4 |

KA = Karang agung, was calculated from four replication, BLD = Belandean, was calculated from three replications; Mean = overall mean, was calculated from three replication of each location.

Combined analysis of variance showed genotype and genotype × environmental interaction was not significantly different. The interaction was not significant may because the narrowness of the genetic diversity of the tested lines (sister line). Field evaluation of large population in Fe-toxic soil conditions with high precision and repeatability would be the ideal situation since varieties

would be tested in the target environment or in very similar conditions. However high variability in the distribution of Fe, even in the same field, resulting in large experimental errors and large $G \times E$ interaction on grain yield greatly impairs varietal selection and breeding efficiency for tolerance to Fe toxicity (Sikirou *et al.*, 2015; Cherif *et al.*, 2009; Drame *et al.*, 2010).

Average of grain yield was ranged from 2.81 - 4.09 ton ha⁻¹. INPARA 9 has high yield (4.09 ton ha⁻¹). Based on consistency of grain yield and LBS score in two locations, there were 6 lines have consistency high grain yield and LBS score in two locations. The six lines were (1) B13586E-KA-9-B; (3) B13988E-KA-20, (8) B13983E-KA-13-3, (10) B13983E-KA-13-1, (14) B13925E-KA-46, (17) B13926E-KA-1 with LBS score 3 - 5. Two lines have high yield, but are not consistent with LBS score, that were (21) B14039E-KA-1 and (22) B14009E-KA-39.

Plant height in Karang Agung was ranged from 89 to 113 cm (Table 2), was lower than Belandean which ranging from 104 to 124 cm (Table 2). Mean of plant height was ranged from 99 to 121 cm, the range is ideal for swampy rice.

Table 3. Number of filled and unfilled grains tillers, and weight of 1000 grains of breeding line in acid-sulfate soil area

| No | Genotype | No. of filled grains | | | No. of unfilled grains | | | W1000 (g) | | |
|----|-----------------|----------------------|------|------|------------------------|------|------|-----------|------|------|
| | | KA | BLD | Mean | KA | BLD | Mean | KA | BLD | Mean |
| 1 | B13586E-KA-9-B | 133 | 111 | 126 | 27 | 27 | 29 | 23.6 | 21.9 | 22.7 |
| 2 | B14299E-KA-46 | 111 | 116 | 120 | 22 | 29 | 26 | 24.3 | 23.1 | 23.6 |
| 3 | B13988E-KA-20 | 128 | 149 | 139 | 18 | 27 | 22 | 23.2 | 22.6 | 22.9 |
| 4 | B14308E-KA-34 | 126 | 113 | 121 | 12 | 24 | 18 | 26.0 | 26.1 | 26.1 |
| 5 | B14360E-KA-17 | 145 | 137 | 140 | 18 | 27 | 21 | 23.1 | 22.3 | 23.0 |
| 6 | B13983E-KA-6-3 | 130 | 122 | 127 | 13 | 24 | 17 | 24.5 | 21.3 | 22.8 |
| 7 | B13971E-KA-23-1 | 152 | 133 | 144 | 27 | 30 | 30 | 23.5 | 23.1 | 23.1 |
| 8 | B13983E-KA-12-3 | 140 | 155 | 150 | 23 | 29 | 27 | 23.5 | 22.3 | 22.9 |
| 9 | B13984E-KA-8-2 | 106 | 123 | 114 | 39 | 40 | 42 | 25.4 | 23.8 | 24.5 |
| 10 | B13983E-KA-13-1 | 145 | 137 | 145 | 25 | 63 | 44 | 23.2 | 21.0 | 22.1 |
| 11 | B13983E-KA-7-3 | 138 | 122 | 128 | 20 | 31 | 26 | 23.2 | 21.8 | 22.5 |
| 12 | B13970E-KA-23-3 | 155 | 164 | 160 | 17 | 31 | 26 | 25.3 | 23.8 | 24.6 |
| 13 | B13925E-KA-7 | 109 | 139 | 126 | 12 | 14 | 14 | 23.9 | 21.7 | 22.9 |
| 14 | B13925E-KA-46 | 156 | 152 | 160 | 22 | 23 | 23 | 23.3 | 21.3 | 22.2 |
| 15 | B13926E-KA-23 | 166 | 159 | 165 | 26 | 39 | 33 | 22.6 | 20.2 | 21.4 |
| 16 | B13925E-KA-42 | 119 | 149 | 139 | 8 | 22 | 15 | 24.0 | 19.9 | 21.9 |
| 17 | B13926E-KA-1 | 147 | 131 | 139 | 12 | 29 | 21 | 21.4 | 18.4 | 19.9 |
| 18 | B14010E-KA-20 | 199 | 156 | 175 | 17 | 42 | 29 | 23.4 | 21.8 | 22.6 |
| 19 | B14039E-KA-15 | 153 | 137 | 144 | 37 | 53 | 45 | 24.7 | 23.8 | 24.3 |
| 20 | B13996E-KA-1 | 123 | 107 | 120 | 18 | 28 | 22 | 25.5 | 23.1 | 24.4 |
| 21 | B14039E-KA-1 | 143 | 131 | 144 | 29 | 45 | 38 | 24.5 | 23.5 | 24.2 |
| 22 | B14009E-KA-39 | 156 | 137 | 149 | 15 | 19 | 17 | 23.5 | 21.2 | 22.2 |
| 23 | IR42 | 115 | 114 | 121 | 24 | 23 | 21 | 19.6 | 18.5 | 19.0 |
| 24 | INPARA 3 | 147 | 150 | 151 | 29 | 33 | 32 | 24.9 | 23.6 | 24.3 |
| 25 | INPARA 8 | 137 | 122 | 136 | 15 | 40 | 25 | 25.4 | 23.6 | 24.7 |
| 26 | INPARA 9 | 131 | 154 | 150 | 20 | 29 | 25 | 23.5 | 23.3 | 23.6 |
| | LSD (5%) | 29 | 36 | - | 11 | 18.7 | - | 1.2 | 1.3 | 1.3 |
| | CV (%) | 15.0 | 16.2 | 15.2 | 36.3 | 36.1 | 36.8 | 3.5 | 3.6 | 3.5 |

KA = Karang agung, was calculated from four replication, BLD = Belandean, was calculated from three replications; Mean = overall mean, was calculated from three replication of each location.

Number of productive tillers in Karang Agung was ranged from 9 to 23, while in Belandean ranged from 15 to 20. Average number of tillers ranged from 14 to 22. Number of tiller of INPARA 9 was relatively low, despite having high grain yield. Otherwise, number of tillers of IR 42 was higher but the grain yield was low. It may be compensation of assimilate partition from number of tillers to other yielding components, which affects grain yield.

Number of filled grain in Karang Agung and Belandean is quite large. Average number of filled grain was ranged from 114 to 175 grains per panicle (Table 3). Average number of unfilled grains was low. In general, the size of weight of 1000 grains of breeding lines tested (swampy rice) was smaller than irrigated varieties (<26 grams). Based on these traits, in general the lines tested adapt well in tidal swamp land.

Relationship between Traits

The rank correlation between LBS, and grain yield and yield components, plant height, and time of 50% flowering was shown in Table 4. The correlation between grain yield and leaf bronzing symptom was negative and significant with $r = -0.537$ (Table 4). The coefficient of correlation was medium. There is a strong correlation between LBS and grain yield as seen in this study at 60 DAS and several others (Audebert & Fofana, 2009; Audebert & Sahrawat, 2000; Elec *et al.*, 2013; Onaga, Edema, & Asea, 2012). However, in some other studies no significant correlation between LBS and grain yield was observed (Nozoe *et al.*, 2008; Sahrawat *et al.*, 1996) as seen at 40 and 80 DAS. Level of correlation between the two traits is likely to depend on stress intensity, testing condition, and type of varieties used. When the stress level is moderate, there is no strong relationship between LBS and yield (Audebert & Fofana, 2009). Stress level was rather high with regards to the LBS of the susceptible variety (IR64) which reached the score of 6 (Sikirou *et al.*, 2016). On average, in Fe-toxic soils one unit increase in LBS was related to a yield decrease of about 20%.

Table 4. Correlation of LBS and grain yield, yield component, plant height, and time of 50% flowering

| Traits | No. of tiller | Flower | Filled grain | Unfilled grain | W1000 | Yield | LBS |
|----------------|---------------|----------|--------------|----------------|----------|----------|----------|
| Plant Height | 0.313* | -0.701** | 0.126 | 0.453** | -0.017 | -0.351** | 0.529** |
| No. of tiller | | -0.402** | -0.248 | 0.245 | -0.434** | -0.287* | 0.308* |
| Flower | | | -0.049 | -0.303* | 0.253 | 0.510** | -0.588** |
| Filled grain | | | | 0.011 | -0.046 | 0.365** | 0.000 |
| Unfilled grain | | | | | -0.137 | -0.305* | 0.329* |
| W1000 | | | | | | 0.185 | -0.445** |
| Yield | | | | | | | -0.537** |

Correlation between plant height, number of productive tillers, and unfilled grain and LBS was positive and significant with low-medium of coefficient of correlation. Time of 50% flowering and weight of 1000 grains have significant and negative correlation with LBS.

CONCLUSION

There is six lines have consistency high grain yield and LBS score in two locations, that are (1) B13586E-KA-9-B; (3) B13988E-KA-20, (8) B13983E-KA-13-3, (10) B13983E-KA-13-1, (14) B13925E-KA-46, (17) B13926E-KA-1 with LBS score 3 - 5. Two lines have high yield, but are not consistent with LBS score, that were (21) B14039E-KA-1 and (22) B14009E-KA-39.

There is sufficient evidence to show that the grain yield is negatively correlated to LBS. This relation should be exploited in breeding. LBS is a key secondary trait as it fulfills all the requirements necessary to use it in breeding programs.

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