Rice tolerance variation to long-term stagnant flooding and germination ability under an-aerobic environment

I A Rumanti1,2, T Sitaresmi1 and Y Nugraha1

1 Indonesian Center for Rice Research (ICRR), Indonesian Agency of Agriculture Research and Development, Jl. Raya Sukamandi No 9, Subang, Jawa Barat, 41256, Indonesia
2 Corresponding author: indrastuti.apri@gmail.com

Abstract. Erratic and unpredicted weather due to climate change cause flood in agriculture production area especially when it has poor drainage system. Flood during early season of crop establishment will induce anaerobic germination (AG) in the area where farmers practiced a direct seeding method, and the flood can be prolonged as a stagnant flooding (SF) stress if the water could not recede. AG and SF stress not only will affect the crop establishment but also reduce grain yield. The study on selection of tolerant lines to AG and SF based on phenotypic performance was conducted. We identified a total of 22 rice lines had ability to germinate under low oxygen after initial screening under 10 cm of flooding for 2 weeks. The survival rate was 82.5 – 100% with seedling growth rate about 0.9 – 1.5 cm per day. Eight lines had better performance and out yield the best check of SF. SF was increased the plant height, but reduce the panicle length and unfilled grain per panicle. Aerenchyma areas of tolerant variety were bigger than a sensitive variety. The results of study will increase the chance to developing of flood tolerant rice varieties to anticipate the climate change.

1. Introduction

Agriculture faces increasing problems with extreme weather due to climate change and it leads the significantly yield losses. Unpredicted weather due to climate change cause flood in poor drainage system agriculture areas. There are two types of flooding stresses in the rice lowland, such as transient complete submergence and long-term water stagnation (stagnant flooding and deep water) [1].

In Indonesia, mostly farmers did trans-planting (TPR), rather than direct seeded (DSR). But, recently due to limited labour and natural resources such as water availability, then farmers are encouraged to shift from TPR to DSR. DSR is expected to reduce water use by 30%, particularly in seedling nursery, puddling and rice growth maintaining [2]. In the area where DSR is practiced, flooding resulted absence of oxygen that important for seed germination, called as anaerobic germination (AG) condition. However, flooded condition during seed establishment is also needed to inhibit weeds growth.

As consequent of limited oxygen, flood results poor crop establishment, as seeds are sensitive to low oxygen during germination and early seedling growth. Therefore, farmer might earn extra seed and labour cost to reseed another crop establishment. Anaerobic conditions during germination (AG) is inhibits coleoptile emergence [3]. Flooding inhibited O2 movement in the soils, and roots experiences to hypoxia (low oxygen) and anoxia (absence of oxygen). Oxygen is the terminal electron acceptor of mitochondrial electron transport, then anoxia inhibits respiration and causing energy deficit. In addition, blocking of gas exchange during waterlogging leads to root hypoxia or anoxia, abundance of CO2 in the...
root zone, and phytotoxins in reduced soils. All gives consequences for root metabolism, nutrient acquisition, and roots and shoots growth [4].

Rice is unique instead its growth is exhibited during germination under waterlogged, however it capable to growing well after developed leaves and stem because of its well-developed aerenchyma system that facilitates aeration of the roots and the rhizosphere [5]. However, this escape mechanism is insufficient for longer durations of floods such as a few weeks of submergence and several months of stagnant flood [6]. Complete submergence by flash floods could be occurs for up to 2 weeks. Tolerant genotypes (+Sub1) remaining dormant under water and it will recovery once water receded. In other types of flooding, namely stagnant flooding (SF or medium deep water), tolerant varieties will partially elongate in response to rising water and trying to keep the leaves to contact with the air [7].

Climate change significantly affect to the rice production. Unpredicted weather increased pest-disease population and erratic rain which imposed floods or drought in rice lowland. Developing rice varieties that can struggle under soil flooding, such as germinate under flooded or and have capability to partially elongate are important to be widely adopted by farmers in flood-prone areas. In term to developing climate ready rice in flood-prone area, screening of improving rice lines under both stresses are needed, with the final aim to select the high yielding lines under stresses.

2. Materials and Methods

2.1. An-aerobic germination screening
An-aerobic germination screening of 109 improved rice lines were conducted during dry season (DS) of 2018. IR64 and Khao Hlan On (KHO) were used as a sensitive and tolerant check varieties, respectively. Dry direct seeding was done in plastic trays containing a shallow layer of soil. Forty dry seeds of each lines were sown at about 5 mm soil depth in a single row, with 10 lines per try including check varieties. Seeds were immediately submerged just after sowing. The water depth was maintained on 10 cm for 2 weeks. Percentage germination and survival rate were determined based on the number of seedlings which reached the water surface. Seedling growth were observed at 5, 10, and 15 days after submerged.

2.2. Observational yield trial under SF stress
During wet season of 2018, 159 improved rice lines and 7 check varieties (INPARA 4, INPARA 8, INPARA 9, INPARI 30, IR 42, and IRRI 119) were screened under SF. Flooded started at 30 days after transplanting (DAT) with 20 cm of water depth. The water depth gradually increased weekly by 5 cm up to 60-70 cm and it maintained up to maturity. None of the entries were completely submerged [8]. The experiment was done at submergence ponds, NPK fertilizers (40:40: 40 N–P–K kg ha⁻¹) were used. A molluscicide was applied 5-7 days before transplanting to control golden apple snail. 21 days old of seedlings were transplanted with one seedling per hill. Four blocks of augmented design were used as a field lay-out with 25 x 25 cm of spacing. Each check varieties were grown replicated in every block. All tested lines and varieties were transplanted at 1 x 5 cm of plot. Observation were done for plant height and tiller number at 30 DAT, 60 DAT and before harvest, yield components and grain yield per plant. Aerenchyma of tolerant and sensitive check were observed by dissect of second internode of main culm and root tip, then observed the number and size of aerenchyma using light microscope.

Data were statistically analysed using SAS statistical software (9.1). Analysis of Variance (ANOVA) was conducted for all traits and mean separation was done using Least Significant Difference (LSD) at P=0.05. Pearson correlation analysis were conducted using STAR software at P=0.05.

3. Results and discussion

3.1. An-aerobic germination tolerance
Climate change are induced unpredicted weather such as temperature increase and uncertain rain, it created stresses to rice plants. Uncertain rainy after seeding induced partial or complete flooding. It causing crop failure especially in the direct seeded rice as rice is highly sensitive to flooding during
Breeding rice varieties which have capability to germinate under standing water stress are important to help the farmers. There was variation in term of germination ability under anaerobic condition (Fig. 1). Twenty-two lines have good survival rate range from 81 – 100% germinated, while 37 improved lines were moderate tolerant.

![Figure 1. Distribution of survival rate](image1)

**Figure 1.** Distribution of survival rate

![Figure 2. Correlation of plant survival with elongation rate under an aerobic germination](image2)

**Figure 2.** Correlation of plant survival with elongation rate under an aerobic germination

BP17830e-1-6-1 has a fastest seedling growth (2.5 cm per day) among all tested lines (Fig 2). The three most tolerant lines with 100% of survival rate, such as IR11T210, B14366E-KY-3 and B14299E-KY-46 had faster seedling growth compare to the check varieties and general mean. It grouped with other 12 lines inside the circle (Fig. 2). Rice seed need oxygen induced functioning of the enzymes which needed for the breakdown and mobilization of the carbohydrates which stored in the endosperm, and generate energy to supported the embryos growth \[7\]. The adaptive mechanism of rice when grown in hypoxia or anoxia condition is the coleoptile ability to grow faster to facilitate contact with the air and provide the good aeration. The same mechanism showed by tolerant improved lines in this study. The ability to break down starch in the germinating rice seeds was genetically controlled. Tolerant genotypes showed greater ability to mobilize stored carbohydrates for seedling growth. Total amylase activity reported had strong correlation with both of survival and elongation rate of the coleoptile \[9\].

3.2. **Phenotyping of stagnant tolerant rice lines**

There was variation of stem elongation under SF and it have a significant different among lines (Table 1). Internode elongation is important to maintain the leaf tip above of the water surface. Stem elongation known as an important mechanism to escape anaerobic respiration when submerged condition was occurred. The elongate genotypes would be able to perform photosynthesis and produce energy to continuing the growth. The previous study founded that IRRI 119 had the longest stem but with low
elongation increment (7%) under SF when it compared to normal condition. These suggested that the tolerance mechanism was not only reflected from the elongated stem, but also on plant stature at normal conditions [10]. Stagnant flooding tolerance varieties should have a relatively higher stature than lowland varieties even in normal conditions.

Table 1. Plant height and tillers number at 30, 60 day after transplanting (dat) and harvest time

| No | Genotypes           | PH30 (cm) | PH60 (cm) | PHh* (cm) | Till30 | Till60 | Tillh |
|----|---------------------|-----------|-----------|-----------|--------|--------|-------|
| 78 | FeY-4-42-KA-3-1-1   | 65        | 68        | 123       | 12     | 17     | 8     |
| 132| B14894E-SKI-8-4-1   | 77        | 96        | 124       | 15     | 24     | 11    |
| 136| BP30201E-SKI-1-1    | 68        | 89        | 115       | 16     | 31     | 11    |
| 137| BP30158E-SKI-15-2   | 76        | 91        | 114       | 16     | 25     | 13    |
| 140| BP30155E-SKI-10-3   | 67        | 91        | 125       | 13     | 17     | 13    |
| 141| B15084E-SKI-5-3     | 79        | 98        | 123       | 15     | 18     | 10    |
| 143| B15084E-SKI-13-1    | 70        | 84        | 134       | 15     | 4      | 13    |
| 146| BP30201E-SKI-2-3    | 66        | 93        | 124       | 16     | 28     | 10    |
| 153| B14354E-KA-5        | 58        | 63        | 124       | 15     | 11     | 10    |
| 157| BP29578E-SKI-13-6-1 | 63        | 76        | 119       | 18     | 24     | 10    |
| 158| B14357E-KA-48       | 63        | 92        | 131       | 18     | 20     | 11    |
| 159| B14377E-KY-30-KA-2  | 68        | 96        | 129       | 15     | 24     | 13    |
| 152| Khao Hlan On (AG-tol) | 62       | 80        | 159       | 15     | 10     | 14    |
| A  | IRRI 119 (SF tolerant) | 74      | 94        | 120       | 16     | 15     | 8     |
| B  | IR 42 (SF sensitive) | 67       | 103       | 105       | 17     | 18     | 11    |
| V1 | Inpara 4            | 69        | 89        | 118       | 15     | 18     | 7     |
| V2 | Inpara 8 (SUB1)     | 69        | 93        | 132       | 15     | 19     | 8     |
| V3 | Inpara 9            | 66        | 97        | 135       | 15     | 19     | 11    |
| V4 | Inpari 30 (SUB1)    | 67        | 102       | 116       | 16     | 18     | 9     |
| V5 | IR 64              | 71        | 87        | 110       | 14     | 15     | 12    |
| CV (%) |                      | 12        | 24        | 5         | 21     | 58     | 29    |
| LSD |                      |           |           |           |        |        | 14    |

Note. PH30: plant height at 30 dat, PH60: plant height at 60 dat, PHh: plant height at harvest time, Till30: tillers number at 30 dat, Till60: tillers number at 60 dat, Tillh: tillers number at harvest time.

Figure 3. Yield of single plant relativity with plant increment rate under stagnant flooding.
Plant increment rate under SF were also varied. Genotypes with increment rate under 1 cm/day during vegetative and a bit faster during generative stage had higher survival and grain yield (Fig. 3). All the best improved lines which have higher yield compare to IRRI 119 (tolerant check) were also have moderate stem elongation with the same pattern. Under floods, a sensitive check, IR42 have faster growth during vegetative, but it exhausted when generative stage due to limited photosynthates production.

**Table 2.** Yield and yield attributes of several tolerant improved lines and varieties.

| No  | Genotypes               | FG  | UG* | PL  | B1000 (g) | YR (g) |
|-----|-------------------------|-----|-----|-----|-----------|--------|
| 78  | FeY-4-42-KA-3-1-1       | 106 | 20  | 26.5| 26.0      | 35.2   |
| 132 | B14894E-SKI-8-4-1       | 60  | 18  | 29.1| 25.4      | 39.7   |
| 136 | BP30201E-SKI-1-1        | 103 | 31  | 42.9| 27.6      | 36.7   |
| 137 | BP30158E-SKI-15-2       | 67  | 22  | 17.6| 26.6      | 35.1   |
| 140 | B150515E-SKI-10-3       | 129 | 35  | 25.1| 24.3      | 38.4   |
| 141 | B15084E-SKI-5-3         | 107 | 38  | 22.0| 28.4      | 37.1   |
| 143 | B15084E-SKI-13-1        | 89  | 15  | 27.8| 21.3      | 35.6   |
| 146 | BP30201E-SKI-2-3        | 104 | 31  | 21.7| 23.0      | 34.6   |
| 153 | B14354E-KA-5            | 80  | 24  | 26.1| 25.7      | 34.4   |
| 157 | BP29578E-SKI-13-6-1     | 91  | 35  | 17.3| 24.7      | 34.2   |
| 158 | B14357E-KA-48           | 114 | 25  | 28.2| 26.0      | 38.1   |
| 159 | B14377E-KY-30-KA-2      | 105 | 27  | 27.7| 27.1      | 46.1   |
| 152 | Khao Hlan On (AG-tol)   | 35  | 58  | 14.1| 23.7      | 11.4   |
| A   | IRRI 119                | 105 | 17  | 31.8| 27.6      | 29.9   |
| B   | IR 42                   | 91  | 18  | 22.7| 15.8      | 21.3   |
| Cek-1| Inpara 4                | 98  | 25  | 23.8| 22.5      | 23.4   |
| Cek-2| Inpara 8                | 106 | 16  | 35.6| 17.9      | 24.5   |
| Cek-3| Inpara 9                | 115 | 16  | 31.2| 25.5      | 26.7   |
| Cek-4| Inpara 30               | 92  | 15  | 22.1| 20.6      | 18.7   |
| Cek-5| IR 64                   | 101 | 25  | 24.9| 25.6      | 26.1   |
|     | KK (%)                  | 35  | 25  | 46.3| 30.4      | 45.1   |
|     | LSD                     | 15  |     |     |           |        |

Note. FG: filled grain per panicle, UG: unfilled grain per panicle, PL: panicle length, B1000: 1000 grain weight, YR: yield per hill

Stagnant flooding had negative effect to sensitive varieties such as IR42 and Khao Hlan On, an AG tolerant variety (Table 2). Panicle length became shorter and total grain number per panicle were reduced. Thousand grain weight of IR42 were lower than tolerant lines. The genotypes with moderate stem elongation had higher survival and yield under SF [10]. A moderate elongation rate could anticipate plant lodging, therefore the line could produce good yield under SF [11]. On the opposite, slower elongation inhibits plant growth then plant parts may remain under the water and hamper the photosynthesis.

Plant height, tillers number on 60 dat and harvest time, filled grain per panicle, panicle length and aboveground dry matter had positive association with yield of single plant (Table 3). In the beginning of SF stress, plants utilized photosynthates more for culm formation and biomass development. Plant were grown faster and produce enough tillers number in order to produce yield. Genotypes with SUB1 which had intermediate plant height with faster shoot elongation under rising water, resulting in higher canopy expansion and yield when comparing with the moderate submersion tolerant genotypes [1]. Plant increment and biomass were not significantly associate with panicle weight [11]. It indicates there were differentate among lines in order to cope stagnant flooding condition. There is no significant interaction among genotypes x SF condition x duration of SF stress, but two ways interactions were highly significant. It showed that even within one species, the different accessions were showed different response to SF [11].

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**Table 3.** Yields of several tolerant improved lines and varieties under stagnant flooding.

| Genotypes               | FG  | UG* | PL  | B1000 (g) | YR (g) |
|-------------------------|-----|-----|-----|-----------|--------|
| FeY-4-42-KA-3-1-1       | 106 | 20  | 26.5| 26.0      | 35.2   |
| B14894E-SKI-8-4-1       | 60  | 18  | 29.1| 25.4      | 39.7   |
| BP30201E-SKI-1-1        | 103 | 31  | 42.9| 27.6      | 36.7   |
| BP30158E-SKI-15-2       | 67  | 22  | 17.6| 26.6      | 35.1   |
| B150515E-SKI-10-3       | 129 | 35  | 25.1| 24.3      | 38.4   |
| B15084E-SKI-5-3         | 107 | 38  | 22.0| 28.4      | 37.1   |
| B15084E-SKI-13-1        | 89  | 15  | 27.8| 21.3      | 35.6   |
| BP30201E-SKI-2-3        | 104 | 31  | 21.7| 23.0      | 34.6   |
| B14354E-KA-5            | 80  | 24  | 26.1| 25.7      | 34.4   |
| BP29578E-SKI-13-6-1     | 91  | 35  | 17.3| 24.7      | 34.2   |
| B14357E-KA-48           | 114 | 25  | 28.2| 26.0      | 38.1   |
| B14377E-KY-30-KA-2      | 105 | 27  | 27.7| 27.1      | 46.1   |
| Khao Hlan On (AG-tol)   | 35  | 58  | 14.1| 23.7      | 11.4   |
| IRRI 119                | 105 | 17  | 31.8| 27.6      | 29.9   |
| IR 42                   | 91  | 18  | 22.7| 15.8      | 21.3   |
| Inpara 4                | 98  | 25  | 23.8| 22.5      | 23.4   |
| Inpara 8                | 106 | 16  | 35.6| 17.9      | 24.5   |
| Inpara 9                | 115 | 16  | 31.2| 25.5      | 26.7   |
| Inpara 30               | 92  | 15  | 22.1| 20.6      | 18.7   |
| IR 64                   | 101 | 25  | 24.9| 25.6      | 26.1   |
| KK (%)                  | 35  | 25  | 46.3| 30.4      | 45.1   |
| LSD                     | 15  |     |     |           |        |
Table 3. Pearson correlation among traits

|          | PH30 | PH60 | PHh | Till30 | Till60 | Tillh | FG    | UG    | PL    | B1000 | YR    | biomass |
|----------|------|------|-----|--------|--------|-------|-------|-------|-------|-------|-------|---------|
| PH30     |      | 1    | 0.026 | -0.263 | -0.003 | 0.033 | -0.071 | 0.127 | **-0.241** | 0.107 | 0.027 | -0.006 | -0.017 |
|          |      |      | 0.743 |        |        |       |        |       |       |       |       |         |
| PH60     |      | 1    | -0.118 | -0.042 | 0.059 | 0.167 | -0.211 | 0.118 | **-0.324** | -0.102 | -0.128 | 0.138   |
|          |      |      | 0.129 | 0.588 | 0.453 | 0.031 | 0.006 | 0.130 | **0.000** | 0.192 | 0.101 | 0.077   |
| PHh      |      |      |       | 1      | **-0.160** | -0.008 | 0.010 | 0.137 | 0.088 | 0.146 | 0.026 | **0.286** | 0.196 |
|          |      |      |      | 0.039 | 0.918 | 0.896 | 0.078 | 0.261 | 0.061 | 0.738 | **0.000** | 0.011   |
| Till30   |      |      |       |        |        | 1     | -0.054 | 0.011 | -0.074 | 0.017 | 0.024 | -0.010 | 0.046 |
|          |      |      |      | 0.041 | 0.492 | 0.886 | 0.345 | 0.833 | 0.763 | 0.895 | 0.558 |         |
| Till60   |      |      |       |        |        | 1     | 0.049 | 0.098 | -0.134 | 0.061 | **-0.151** | **0.153** | **0.171** |
|          |      |      |      | 0.535 | 0.207 | 0.086 | 0.437 | 0.053 | 0.049 | 0.028 |         |          |
| Tillh    |      |      |       |        |        | 1     | -0.110 | -0.075 | **-0.314** | **-0.273** | 0.175 | 0.446   |
|          |      |      |      | 0.158 | 0.338 | **0.000** | **0.000** | 0.024 | 0.000 | 0.028 |          |          |
| FG       |      |      |       |        |        | 1     | -0.269 | **0.162** | 0.029 | 0.415 | 0.178 |         |
|          |      |      |      | 0.001 | **0.037** | **0.000** | 0.022 |         |       |       |         |
| UG       |      |      |       |        |        | 1     | -1.012 | 0.078 | -0.006 | -0.104 |         |          |
|          |      |      |      | 0.189 | 0.318 | 0.941 | 0.182 |         |       |       |         |
| PL       |      |      |       |        |        | 1     | 0.144 | **0.222** | -0.136 |         |          |
|          |      |      |      | 0.065 | 0.004 | 0.081 |         |       |       |         |
| B1000    |      |      |       |        |        | 1     | 0.044 | **-0.264** |         | 0.577 | 0.001   |
|          |      |      |      | 0.577 | 0.001 |         |          |       |       |         |
| YR       |      |      |       |        |        | 1     | 0.497 |         |         |         | **0.000** |          |

Note. PH30: plant height at 30 dat, PH60: plant height at 60 dat, PHharvest: plant height at harvest time, Till30: tillers number at 30 dat, Till60: tillers number at 60 dat, Till harvest: tillers number at harvest time, FG: filled grain per panicle, UG: unfilled grain per panicle, PL: panicle length, B1000: 1000 grain weight, YR: yield per hill

Figure 4. Aerenchyma gas space in internode of sensitive genotype (IR42, left) and tolerant genotype (IRRI 119, right)

In internodes, aerenchyma formation which occurs simultaneously with the elongation of internode was induced by ethylene [12]. Fig 4 showed that aerenchyma formation occurs event in sensitive genotypes under stagnant flooding. But clearly seen that aerenchyma of tolerant genotype were bigger compared to sensitive genotype. It allowed plant to have an effective internal aeration to support photosynthesis process [13]. Aerenchyma observation in root tip showed a different result, sensitive genotype had wider space compared to tolerant genotype, however the tolerant genotype was produced small numerous aerenchyma (Fig 5). The formation of aerenchyma increase the oxygen availability of roots, but diffusion gradient along the root caused limited oxygen [14]. The severe hypoxia or anoxia will occur in the stelar tissues of roots and the condition can adversely impact ion transport. Therefore, a narrow stele diameter will useful to increase the waterlogging tolerance, then diffusion path into the
The ratio of cortex to stele were greater as well. Cortex is known as an oxygen source, while stele is an oxygen sink tissue. Therefore, narrow stele would greater avoid anoxia compared to the roots with wider stele, because it could take advantage of limited oxygen [14].

**Figure 5.** Aerenchyma in root tip of sensitive genotype (IR42, right) and tolerant genotype (IRRI 119, left)

4. Conclusion

Based on our research, a total of 22 rice lines have an ability to germinate under low oxygen after initial screening. There were 8 lines have a good in term of phenotypic and produce high yield under stagnant flooding stress. Aerenchym could be used as a selection trait in the vegetative phase for stagnant flooding. The information and selected promising lines were increase the opportunity to developing stress tolerant varieties and anticipate floods caused by the erratic rainy and unpredictable weather as a climate change effect.

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