Drought resistance of various rice genetic background based on raised bed screening system

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Abstract. Drought is one of the major constraints of rice production in Indonesia. Climate change increases the risk of drought incidences. The evaluation of drought resisting rice genotypes available in Indonesia would hopefully find and enhance such resistance which could be utilized to be further tested in releasing a variety or donor parent within the drought-resistant breeding program. Raised bed system (RBS) is a relatively reliable method to screen drought resistance up to generative growth phase. This research aimed to screen 69 rice genotypes including 26 IRRI accession included in 3000 rice genome sequencing projects, 29 elite lines, 6 old released variety, 6 released variety, and each of susceptible and resistant drought check variety raised bed screening system. The trial was conducted in RBS facility in ICRR from January to May 2017 following a randomized complete block design of two replications. The materials were directly planted in a 10 hill row with planting space of 20 cm x 20 cm. The result showed that HHZ5-SKI-9-3-0Kr-JK-IND and IR64 Dro1 had good leaf drying and rolling and a number of field grain/panicle. IR84636-13-12-2-6-3-3-2-2-B, Inpari 18, and Huanghuazhan had the highest total grains/plant. Some drought-resistant mechanism might be found within the tested genotypes.

1. Introduction
Drought is one of the major obstacles for rice production in Indonesia. There are roughly 50,000 to 350,000 ha of areas that are affected by drought every year[1]. Besides, around 1 million out of 5.14 million ha of lowland areas in Indonesia are prone to drought [2]. Due to climate change conditions, drought risk is increasing more and more [3]. Global warming is also predicted to reach a 1.5°C increase in air surface temperature over land in 2018 compared to the pre-industrial period [4]. It causes hot extremes in most inhabited regions, heavy precipitation in several regions, drought and precipitation deficits in some regions [5]. Growing rice in the rainy season, especially in the upland area, faces many drought problems due to erratic rainfall in the area. Some areas have a longer dry season under global climate change, such as Java Island, West Sumatera, East and South Kalimantan, Nusatenggara, and South Sulawesi [6]. These provinces are rice production centers in Indonesia so maintaining rice production in this area is important.

Drought stress on the field possibly happened either during vegetative or generative growth phase. Planting rice plant at early rainy season facing vegetative growth phase drought stress, while planting at the end of rainy season has more risk on generative growth phase drought stress. Erratic and unpredictable rainfall patterns under global climate change increases drought stress and the plant growth phase. Therefore, drought resistance screening should be conducted during vegetative and generative growth phases. It is reported that drought stress during vegetative growth phase is affecting yield [7]. Nevertheless, other study
showed that vegetative growth phase drought tolerant genotypes showed poor drought tolerance to generative growth drought stress [8]. Genotype having consistent tolerance to drought stress during vegetative and generative growth phase is also identified [9]. Final decision for drought resistance in rice should consider yield [10]. It suggested that drought resistance screening should be conducted until generative growth phase.

Maximizing biomass and rice yield under drought stress can be obtained by maximizing plant ability to extract available soil moisture from the soil [11]. Root architecture is a very important trait in developing drought-resistant rice varieties. One of the important root traits is deep root development. A study on deep root architecture in rice is initiated to find some rice variation [12]. A major Quantitative Trait Loci (QTL) related to deep vertical root development is then identified called Dro1 [13]. This QTL confers deeper root architecture and during generative stage, it enhances Nutrient uptake and cytokinin fluxes from root to shoot, resulting in increasing grain filling and yield [14]. Deep roots are supposed to improve drought resistance in rice due to their contribution in uptaking water from deeper soil layers, especially during drought incidence [15]. Various traits are actually correlated with drought resistance such as root traits, leaf traits, osmotic adjustment capabilities, water potential, ABA content, and the stability of the cell membrane [16], but root traits are more massively studied.

A methodology to study drought-resistant rice during generative growth phase based on presumption on plant deep rooting ability has been developed. It is called a Raised Bed System [17]. This research aimed to study the response and screen the drought resistance of 69 rice genotypes from various genetic backgrounds to drought stress during the generative growth phase using a raised bed system screening method.

2. Methodology
The experiment was conducted from January till May 2017 at the Raised Bed System (RBS) facility of ICRR in Sukamandi. RBS was developed by digging up topsoil of around 25 cm depth and around 11 m x 22 m square area, followed by sand for 2 cm and gravel for 5 cm. Two sets of RBS were developed side by side. The topsoil was put over the gravel for 25 cm. Plastic fence surrounding and roof over the RBS were established. It is useful to avoid rainfall spills, especially during rainy season, to keep the treatment uniform.

This experiment's materials were 69 rice genotypes, consisting of 26 rice accessions originating from the IRRI (International Rice Research Institute), which had genome sequence information, 29 lines, 12 released varieties, and two checks. Each genotype was planted in a row with 10 hills and 20 cm x 20 cm between and within the rows. Around 5 seeds were put at each hill. The layout was followed by a randomized complete block design with two replications. Watering was conducted for four weeks just after planting. Leaf rolling and drying were scored two times during generative phase with the interval of a week following the Standard Evaluation System release by IRRI [18]. Plant height, tiller number, and yield components were also measured. Variance and correlation were analyzed.

3. Results and discussion
The results showed that the drought treatment caused severe drought to the plants. Out of 69 tested materials, only 55 genotypes survived until harvesting and provided grains for harvesting. Among the tested genotypes, leaf rolling scores ranged from 3 to 6, while leaf drying ranged from 3 to 7. Leaf was strongly affected by drought stress, which was shown by altering the leaf phenotypic on chlorophyll fluorescence parameters and chlorophyll index. Resistant variety has less significant differences than the susceptible ones [19].

It was identified that 10 genotypes had the best leaf drying response (score 3) (Figure 1). Three uppmost leaves drying is reported to be strongly correlated with drought tolerance in rice [20]. Severe drought in this experiment was supposed to be representing the resistance of the plant to drought stress. Raised bed system was supposed to have stronger drought screening ability than other methods such as concrete box methods, which was screen drought response during the vegetative growth phase. This study confirmed drought resistance from previous studies using concrete box method, such HHZ5-SKI-9-3-0Kr-JK-IND, and Lipigo 4 [21].
LR1 = score of leaf rolling at first data collection; LR2 = score of leaf rolling at second observation; LD2 = score of leaf drying at the second observation

**Figure 1.** Leaf rolling characters of selected rice genotypes under drought stress by Raised Bed System, Sukamandi, first cropping season, 2017

FGP = number of total filled grain/panicle; FG = number of filled grain/panicle; SS = seed set (%)

**Figure 2.** Leaf rolling characters of selected rice genotypes under drought stress by Raised Bed System, Sukamandi, first cropping season, 2017

Final resistance to drought would be determined by its capability to give yield [10]. Due to severe had no single grain. Those survived and had yield genotypes were suspected to be resistant to drought stress. Drought resistance is defined as the crop survival ability and production capacity under drought conditions [22]. The number of filled grain/panicle was one of the traits representing the plant’s ability to produce grains under severe drought conditions. Based on the number of filled grain/panicle, 10 best genotypes were identified, namely IR64 Dro1, HHZ5-SKI-9-3-0Kr-JK-IND, and B14288F-MR-1. These genotypes had good score of leaf drying (score 3), and IR64 Dro1 and HHZ5-SKI-9-3-0Kr-JK-IND also had good score of leaf rolling (score 3). These two lines also among the ten best genotypes based on seed set, namely 63% and 66%, respectively (Figure 2). KATUMANI::IRGC 69695-1 and MARHARORA::IRGC 63511-1 include in 3000 rice genome project [23]. Molecular and genomic study using these genotypes would become easier.

Plant yield in this experiment could also be identified based on the total of filled grain produced. Based on this trait, the best ten genotypes were IR84636-13-12-2-6-3-3-2-2-B (394.36 grains), Inpari18 (321.85 grains), Huanghuazhan (260.79 grains), BP17298M-53D-SKI-1-4-2 (259.73 grains), IR 83383-B-B-11-4 (251.82 grains), HZ5-SKI-9-3-0Kr-JK-IND (231.79 grains), IR64 Dro1 (229.12 grains), KATUMANI::IRGC 69695-1 (225.24 grain), BP17280M-46D-IND (220.25 grains), and BP14352e-2-
2-0Kr (207.30 grains). Seven of them had a high number of filled grain/panicle with a total filled grain/plant (Figure 2). HHZ5-SKI-9-3-0Kr-JK-IND and IR64 Dro1 consistently showed good performance on leaf drying, number of filled grain/panicle and number of filled grain/plant simultaneously. It indicated these two genotypes had resistant to drought. It was assumed that 1000 grain weight was comparable among genotypes, with total filled grain/panicle would be the final selection. In this case, IR84636-13-12-2-6-3-3-2-2-B, Inpari 18, and Huanghuazhan would have the best performance under severe drought conditions. It indicated that the genotypes had tolerance ability against drought stress.

IR64 Dro1 is a line having Dro1 gene, which controls the deep vertical root system[13][24]. It indicated that deep vertical root system was effective in catching moisture from deeper soil areas. Dro1 gene has been reported to enhance rice yield in paddy field [14]. HHZ5-SKI-9-3-0Kr-JK-IND is a GSR (Green Super Rice) line as an improvement of Huanghuazhan which is a cross with OM1723 [25]. Huanghuazhan was released in Indonesia as a new variety in 2016 namely Inpari 42 Agritan GSR [26]. On the other hand, Inpari 18 is also reported to have a relatively high number of roots and proportion of vertical root architecture [27]. IR84636-13-12-2-6-3-3-2-2-B is related to the study about panicle improvement to increase yield [28]. Various genetic backgrounds showed various responses to drought stress. Some identified resistant genotypes in this study are advanced lines or released varieties. It indicated that obtaining drought-resistant rice variety with best agronomic performance and yield is feasible.

Root traits such as root length and weight are reported to be strongly correlated with yield [29]. Raised bed system is designed to select rice genotypes with deep vertical root system. This root architecture increases the plant’s ability to catch moisture from below the gravel layer, which is deeper than the usual rice rooting system area [17]. It indicated that the outstanding genotypes identified in this study also had deep root system. Genetic materials tested in this study had various genetic backgrounds. Some genotypes also showed good performance in one or more observed traits. It indicated that deep vertical roots might effectively support the plant's drought resistance level, while other mechanisms may also exist.

Raised bed system can screen genotypes under severe drought conditions at generative growth phase. The soil’s water potential at 30 cm depth reached at least -60kpa at generative growth phase until harvesting. Drought resistant plant might be obtained by using this screening method. Previous study reported that severe condition could be developed [30]. Field screening can not maximally control drought treatment and it can not be conducted during dry season.

**Table 1.** Correlation among 7 traits of 55 rice genotypes under drought stress using Raised Bed System, in Sukamandi, first cropping season, 2017

|      | FG   | UFG  | SS         | PH          | FGP         | TN           | LR2         |
|------|------|------|------------|-------------|-------------|---------------|-------------|
| UFG  | -0.469*** |      |            |             |             |               |             |
| SS   | 0.784**   | -0.767*** |            |             |             |               |             |
| PH   | -0.097   | 0.304*   | -0.28*     |             |             |               |             |
| FGP  | 0.861**   | -0.508*** | -0.126     | 0.769**     |             |               |             |
| TN   | 0.159     | -0.518*** | 0.399**    | -0.603**    | -0.327*     |               |             |
| LR2  | -0.011    | -0.068   | -0.039     | -0.16       | 0.263       | 0.263*        |             |
| LD   | 0.109     | 0.009    | 0.056      | -0.216      | 0.202       | 0.202         | 0.027       |

FG = number of filled grain/panicle; UFG = number of un-filled grain/panicle; SS = seed set (%); PH = plant height (cm); FGP = total of filled grain per plant; TN=productive tiller number/plant; LR2 = leaf rolling at second observation; LD = leaf drying; *** significant at α=1%; * significant at α=5%.

On the other hand, managing the uniformity of the experimental field was difficult. In contradiction with field screening, a raised bed system screening method gives advantage of flexibility time to do drought screening in dry season and during rainy season. The uniformity condition of the raised bed is
easier to be managed. Raised bed system screening method will hopefully strengthen the screening capability and capacity and increase the chance to obtain drought-tolerant rice varieties.

Under drought stress conditions created by using raised bed system screening method, the correlation between key traits correlated to drought resistance such as leaf drying and yield components is considerably weak (Table 1). On the other hand, correlation among yield components such as filled grain/panicle, empty grain/panicle, and seed set were detected. Plant height is positively correlated with filled grain/panicle and negatively correlated with tiller number. Tiller number is weakly correlated in positive way with leaf rolling score. Previous study showed that some traits supporting high yield with drought tolerance are panicle length, grains per panicle, harvest index, biomass yield, root/shoot ratio and root length [31].

4. Conclusion
Among the 69 rice genotypes tested using RBS method, drought-resistant rice genotypes were identified based on some suspected traits correlated with drought resistance. HHZ5-SKI-9-3-0Kr-JK-IND and IR64 Dro1 ha-based on some traits supporting high yield with drought tolerance are panicle length, grains per panicle, harvest index, biomass yield, root/shoot ratio and root length [31].

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