Drought tolerant selection of rice genotypes using raised bed system

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Abstract. Drought is one of major constrain in rice production in Indonesia. Developing of rice tolerant to drought would hopefully overcome the problem. Raised bed system is a modified selection method to screen drought tolerant in rice up to generative growth phase. This research aimed to evaluate 60 various genetic background of rice genotypes using raised bed method. The trial was managed according to randomized complete block design with two replications. The materials we directly planted with planting space of 20 cm x 20 cm in row plot consisted of 10 hills each. The trial was conducted in raised bed system screening facility in ICRR experimental station in Sukamandi during August – November 2016. There were five genotypes considered as tolerant based on leaf rolling and leaf drying score. There were 15 genotypes produced filled grains, ranged from 2 to 121 grains/plant or 0.12 to 4.48 grains/panicle. The seed set was ranged from 2 to 40%. Among the producing grain genotypes, BP16146M-1D-SKI-2-13-1-3, BP14352e-2-3-3Op-JK-0, and BP17280M-46D-IND considered to have better drought tolerance based on number of filled grain produced and seed set rate. Yield trial of the lines would confirm the possibility for further testing for releasing variety.

1. Introduction
Drought is one of major problems in producing rice in Indonesia. Every year, around 50,000 to 300,000 ha areas evenly over tehe country were affected by drought [1]. Drought risk is also exist in lowland area. It was reported that 1 million ha out of 5.14 million ha lowland areas in Indonesia are drought prone area [2]. On the other hand, global climate change caused longer dry season in rice production centers in Indonesia, such as java, west part of Sumatera, East and South part of Kalimantan, Nusatenggara, and South Sulawesi. There was also reduction on rainfall during June – August [3]. Drought is also happen in upland area, due to unpredictable erratic rainfall in the area.

Maintaining yield relying on increasing world water supply is not a viable option. Therefore, genetic management strategies for drought focus on maximum extraction of available soil moisture and its efficient use in crop establishment and growth to maximize biomass and yield is become crucial [4]. Genetic variation on drought tolerance is required in developing the variety [5]. Drought tolerance itself is controlled through various mechanism. Plants have evolved a series of mechanisms at the morphological, physiological, biochemical, cellular, and molecular levels to overcome water deficit or drought stress conditions. The drought resistance of plants can be divided into four basic types-drought avoidance, drought tolerance, drought escape, and drought recovery. Various drought-related traits, including root traits, leaf traits, osmotic adjustment capabilities, water potential, ABA content, and stability of the cell membrane, have been used as indicators to evaluate the drought resistance of plants [6].
Out the various traits, root architecture is very important in drought tolerant controlling in rice plant. Deep root development is important for the drought resistance in rice (*Oryza sativa* L.) [7]. Deep roots are a key trait for improving drought resistance in rice in upland environments, it is due to its contribution for water uptake from deeper soil layers during drought [8]. A major QTL controlling deep vertical root development had been identified, called as *Dro1* [9]. *Dro1* is a gene controlling root growth in more downrad direction. It resulting the line to avoid drought by increasing deep rooting which maintain high yield under drought condition. Introducing *Dro1* gene into shallow rooting rice had been reported increased avoidance from drought stress [10].

A methodology to study drought tolerance (avoidance) of rice to drought during generative growth phase had been developed, called as Raised Bed System [11]. This research is aimed to study tolerance of 60 rice genotypes to drought stress during generative growth phase by using raised bed system.

2. Methodology
The experiment was conducted during August – November 2016. The testing was conducted in Raised Bed System (RBS) facility in the field of ICRR experimental station in Sukamandi. RBS facility was developed by digging the top soil of upland (not a lowland area) field for around 25 cm and put 5 cm layer of gravel and cover back with top soil for around 25 cm. The gaver layer was dedicated to percolate water into deeper layer and avoid the humidity in the upper layer. This method would select rice genotypes with deep vertical root to get water below the gravel layer. Plastic roof and fence were established to avoid rainfall seeps into the trial.

As many as 60 genotypes were tested during this study. The experiment was managed following randomized complete block design of two replications. The material were directly planted on the RBS one genotype one row of 10 hills with plant spacing of 20 cm x 20 cm, aroung 5 seeds in each hill. The plants were watered until 4 weeks after planting. Observation was conducted into leaf rolling and drying of the plants during generative growth phase (flowering time) for two times with interval of one week between the scoring time. Yield especially number of filled grain/panicle was observed into survive plants. Scoring for leaf rolling and leaf drying was taken following Standard Evaluation System release by IRRI [12] applied into generative growth stage as listed in Table 1 and Table 2.

| Scale | Symptom                                |
|-------|----------------------------------------|
| 0     | Leaves healthy                         |
| 1     | Leaves start to fold (shallow)         |
| 3     | Leaves folding (deep V-shape)          |
| 5     | Leaves fully cupped (U-shape)          |
| 7     | Leaf margins touching (0-shape)        |
| 9     | Leaves tightly rolled                  |

Table 2. Scale of leaf drying of rice plant as response to drought stress

| Scale | Symptoms                                  |
|-------|-------------------------------------------|
| 1     | Slight tip drying                         |
| 3     | Tip drying extended up to 1/4             |
| 5     | One-fourth to 1/2 of all leaves dried     |
| 7     | More than 2/3 of all leaves fully dried   |
| 9     | All plants apparently dead. Length in most leaves fully dried |

3. Results and discussion
The results showed that the plants were suffered from drought severely. Nine of them were not survive. The scoring for leaf drying was ranged from 3 to 7, while for leaf rolling was from 4 to 9. The data was average scoring of two replications. It indicated that leaf rolling is more responsible to
drought compared to leaf drying. It could be seen that plant firstly respond to drought stress by rolling
the leaves and in such a point, the leaf become drying as a process of plant dying.

Based on leaf rolling and drying at the threshold score of 4, there were 5 genotypes were
considered to be relatively tolerant to drought stress, i.e. BP14352e-2-3-3Op-JK-0, CT 18510-23-4-4-
1-MP-JK-0, BP17282M-41D-1-SKI, BP14352e-2-2-0Kr, and HHZ5-SKI-9-3-0Kr-JK-IND (Table 3).
Drought stress affected rice leaf strongly, which is altered the leaf phenotypic traits based on
chlorophyll fluorescence parameters and chlorophyll index, with more significant differences in
susceptible variety than in traditional landraces (resistant varieties) [13]. Leaf trait, especially drying
of three upmost leaves strongly correlated with drought tolerant and reported as new method for
drought tolerant screening [14].

Table 3. Score of leaf rolling and drying of 60 rice genotypes on Raised Bed system
screening facility, ICRR, WS 2016

| No | Genotype                   | Leaf Rolling | Leaf Drying |
|----|---------------------------|--------------|-------------|
| 1  | Dro1 IR64 NIL             | 5            | 3           |
| 2  | Gajah Mungkur             | 6            | 4           |
| 3  | Lipigo 4                  | 8            | 6           |
| 4  | Inpari 10                 | 9            | 6           |
| 5  | Inpari 18                 | 8            | 6           |
| 6  | Towuti                    | 8            | 5           |
| 7  | HHZ12-SKI-1-2-0Kr-JK-IND  | 7            | 5           |
| 8  | TIL3 (IR84636-13-12-2-6-3-2-2-B) | 7 | 5 |
| 9  | Huanghuazhan              | 5            | 5           |
| 10 | Kelimutu                  | 7            | 5           |
| 11 | Batang Lembang (6661)     | 7            | 5           |
| 12 | Dular                     | 7            | 6           |
| 13 | **HHZ5-SKI-9-3-0Kr-JK-IND** | 4 | 4 |
| 15 | Limboto                   | 6            | 4           |
| 16 | IR20                      | 8            | 6           |
| 17 | IR64IRRI                  | 7            | 6           |
| 18 | BP17552-1c-SBY-0-CRB-0    | 8            | 6           |
| 19 | Inpari 21                 | 8            | 7           |
| 20 | Bahbutong (1182)          | 7            | 6           |
| 21 | HHZ5-SKI-7-1-0Kr-JK-IND   | 6            | 5           |
| 22 | IR83142-B-20              | 5            | 4           |
| 23 | TIL2                      | 6            | 5           |
| 24 | TIL24                     | 5            | 5           |
| 25 | BP16734e-3                | 6            | 5           |
| 26 | BP14262e-1-1              | 7            | 5           |
| 27 | IR83383-B-B-11-4          | 6            | 4           |
| 28 | PR40781b-3-4-SBY-0-CRB-2-SKI-0-5 | 6 | 4 |
| 29 | BP17300M-54D-SKI-8-2-2    | 6            | 4           |
| 30 | BP17298M-53D-SKI-1-4-2    | 5            | 4           |
| 31 | BP15994M-4D-SKI-18-1-3    | 7            | 5           |
| 32 | BP16146M-1D-SKI-2-13-1-3  | 5            | 5           |
| 33 | BP16178M-4D-SKI-16-6-2-2  | 7            | 5           |
| 34 | BP16178M-1D-SKI-13-8-1    | 7            | 5           |
| 35 | BP16188M-2D-SKI-19-2-1    | 6            | 4           |
| 36 | GSR IR1- 11-D3-S3         | 6            | 4           |
| 37 | GSR IR1- 11-D1-Y1         | 5            | 4           |
| 38 | GSR IR1-11-D3-S2          | 6            | 5           |
| 39 | **BP17282M-41D-1-SKI**    | 4            | 3           |
| 40 | BP17280M-46D-IND          | 5            | 4           |

Continued
The plants were suffering from drought severely, especially during generative growth phase. There were only 15 genotypes were able to produce filled grain, ranged from 2 to 121 grains/plant or 0.12 to 4.48 grains/panicle and seed set ranged from 2 to 40%. (Table 4). Among the producing grain genotypes, BP16146M-1D-SKI-2-13-1-3, BP14352e-2-3-3Op-JK-0, and BP17280M-46D-IND considered to have better drought tolerance based on number of filled grain produced and seed set rate. Four out of the five genotypes having good scoring on leaf rolling and leaf drying above, produces filled grains. It indicated that the leaf rolling and leaf drying is reliable to screen drought tolerance of rice plant.

Table 4. Grains produced by survive lines under severe drought stress of Raised Bed System Treatment, Sukamandi, DS 2016

| No | Genotype | Leaf Rolling | Leaf Drying |
|----|----------|--------------|-------------|
| 41 | IR83142-B-20 | 6 | 4 |
| 42 | IR61336-4B-14-3-2 (PSBRC94) | 7 | 4 |
| 43 | BP14262e-2-8 | 6 | 4 |
| 44 | BP14262e-1-1 | 7 | 5 |
| 46 | **BP14352e-2-2-0Kr** | 4 | 3 |
| 48 | **BP14352e-2-3-3Op-JK-0** | 4 | 3 |
| 49 | BP17280M-1 | 5 | 4 |
| 50 | BP17280M-66-1 | 6 | 4 |
| 52 | BP14342f-7 | 6 | 5 |
| 53 | BP14352e-2-3-1Op-JK-0 | 5 | 4 |
| 57 | BP14352e-1-2-2Kr-JK-0 | 6 | 4 |
| 59 | **CT18510-23-4-4-1-MP-JK-0** | 4 | 3 |

Raised bed system is designed to select drought tolerant rice genotypes having deep vertical root system, due to its ability to catch moisture below the gravel layer under commonly root area of the plant [11]. This research result is also indicating that the genotypes producing filled grain had deep root system.
Yielding ability of plant under stress is finally become the key to screen drought tolerance in rice plant [15]. Variation had been identified on the quantity of the produced filled grains in this study indicating the variation of yielding ability of the plant under severe drought condition. Nevertheless, yield testing under mild drought and optimum condition is needed to identify genotypes having yield ability under various drought level had been conducted [16] and [17]. Study on interaction between genetic of drought related traits including yield and environmental factors has also been conducted [18].

For further field application on various field condition, it need to consider that dvarious environmental stresses, such as high irradiance, high temperatures, nutrient deficiencies, and toxicities, may challenge crops simultaneously. Interdisciplinary deep study into metabolic pathways and moleculars underlying drought tolerant in rice crop is needed based on the physiological / morphological and molecular mechanisms found in resistant parent lines, a strategy is suggested to select a particular environment and adapt suitable germplasm to that environment [19]. Genetic mapping to rice traits related to drought tolerance either through drought tolerance (DT) and drought avoidance (DA) had identified QTLs for drought indicating that DT and DA had distinct genetic bases [20]. Combining of both is possible. Study of drought using recent transgene technology such as bombardment of marker free ascorbate peroxidase-coding gene OsAPX2 had also been conducted [21]. Drought study is still challenging due to its complexity and comprehensive effort to further develop drought tolerant rice variety should be continuously taken anyway. Developing new method and approach such as raised bed system would be very worthy for the such efforts.

4. Conclusions
Raised bed system is effective for drought screening during generative growth phase for rice plant. Within this study, BP16146M-1D-SKI-2-13-1-3, BP14352e-2-3-3Op-JK-0, and BP17280M-46D-IND was selected as tolerant to drought stress at generavi growth stage based on leaf rolling, leaf drying, number of filled grain produced and seed set rate. The gentypes would hopefully be useful as source of severe drought tolerant gene donor. Yield trial of the lines would confirm the possibility for further testing for releasing variety. Continuous comprehensive study and developing new approach to drought study is still needed for further answer drought challange in producing more rice in the world.

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