Selecting Traits for Drought Tolerance Screening in Rice

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Abstract. Drought is one of major constrains for rice production in Indonesia. Development of drought tolerant rice variety is believed as an efficient and effective strategy to face the challenge. Nevertheless, drought is a complex trait thus many approach had been implemented in the selection of drought tolerant rice genotypes. This study aimed to search traits correlated with drought tolerance in rice plant. Two screening methods, i.e. seedling box and mini pot methods was conducted into 60 rice genotypes. The experiments were conducted during dry season 2016. The results showed that based on % recovery, TIL24 had the highest (82.5 %) while number of green leaves had strong positive correlation with root length, root number in zone 1 (horizontal), 2 (medium), and 3 (vertical), total root number, leaf rolling, and plant recovery. Nevertheless, proportion of root in zone 3 negatively correlated with shoot length, green leaf number, root number in zone 1 and 2, total root number, and plant recovery. It seem that proportion of vertical root compensate with other traits in developing drought tolerance, while number of green leaf under stress and root number in zone 2 correlated more with traits supporting drought tolerant in rice.

1. Introduction
Drought is one of major constrains for rice production globally and include Indonesia. Drought stress mostly happen in unbunded upland, bunded upland, shalow rainfed, mid-lowland rainfed and in water-short irrigate. Drought affects around 23 million ha of rainfed rice accross the world [1]. The condition is predicted to be worse in teh future, due to global climate change. It would also interact with other abiotic and biotic stresses [2]. In Indonesia, it is reported that around 50.000 to 300.000 ha areas are affected by drough every year [3]. It is predicted that due to climate change, there will be also reduction on rainfall during June – August [4].

Development of drought tolerant rice variety is believed as an efficient and effective strategy to face the challenge. Nevertheless, drought is a complex trait thus many approach had been implemented in the selection of drought tolerant rice genotypes [5]. This study aimed to search traits correlated with drought tolerance in rice plant at the early vegetative growth.

2. Methodology
The materials of this research is 60 rice genotypes which is simultaneously selected using two screening methods, i.e. (1) minipot for root angle pattern and (2) Seedling box for seedling dry. Both methods are used for early vegetative growth phase drought tolerance selection. The experiment was conducted in ICRR green house in Sukamandi during October to December 2016.

The seedling box experiment was followed randomized complete block design with two replications. The seeds of the 60 rice genotypes we sown in petri dished with weath paper inside for around two days to let seed for initiating germination. The well initially germinated seeds were transfered into the
seedling box made of wooden materials, each row contained 20 seeds with the distance of around 1 cm within row and 5 cm between row. IR20 as susceptible check and Salumpikit as tolerant check were planted in every 20 tested entries. IR20 was also planted surrounding the tested genotypes. Watering was conducted for seven days then wait until IR20 died or severely dried. Scoring was done into leaf rolling (Table 1) and drying (Table 2) \cite{6}, and plant recovery (percentage of survive plants). After both scoring, watering was given again to the plants for three days. Plant recovery was counted one week after the watering. Soil moisture content/soil osmotic was monitored using Irrometer moisture meter in 15 cm depth. Observation was executed into leaf rolling score, leaf drying score, and recovery (percentage of recover plants after stopping the drought treatment).

| Score | 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          |

| Score            | Symptom                                      |
|------------------|----------------------------------------------|
| 0                | No symptoms                                 |
| 1                | Slight tip drying                           |
| 3                | Tip drying extended up to ¼ length in most leaves |
| 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                  |

The minipot experiment was managed according to randomized complete block design of three replications. A single plant in a pot is representing one unit data. Media was prepared as mixed of soil and sand in an equal ratio. Minpots were filled up with the prepared media with sieve at the top of the media in each pot. The pots were made of paralon pipe with the diameter of 2 inch with 20 cm depth. Sieve was made in sunken dimension with the dept of 5 cm. Sieve diameter was manged to be fitted with the pot diameter. The sieve was marked by red colored string weaving into the sieve in two lines horizontally separating the sieve proportionally into three zone, i.e. representing vertical, medium, and horizontal root growth direction. The seeds were placed on the media on the sieve on the pot. The pots were placed in box with around 3 cm standing water to keep the humidty of the media. The plant was establish for 42 days, so that the final obsavasion was done at the end of vegetative plant growth phase. Observation was executed into shoot length, tiller number, root length, and root number at zone 1 (horizontally growth), zone 2 (medium), and zone 3 (vertically growth). Observations were conducted to Shoot length (cm), Tiller Number, Root length (cm) Zone 1, Root Number Zone 2, Root Number Zone 3, Total Root Number, and proportion of Zone 3 Root Number to Total Root Number. Correlation coefficient and T value of the coefficient were analyzed among the observes traits in both experiments. The Minitab Release 14 Software Analysis is used for the analysis.

3. Results and Discussion
The results showed that leaf rolling of the genotypes ranged from 5 to 9 (average of two replications). It showed that the stress level is very high (Figure 1). Based in leaf rolling, Kelimutu (5), Bahbutong (5), Huanghuazhan (6), and HHZ5-SKI-9-3-0Kr-JK-IND (6) had the best performance, while IR 20 had
score of 8 and 19 genotypes had score of 9 (Table 3). Leaf rolling is one of plant response to drought that might be correlated with reducing leaf expansion, lower stomatal conductance, and transpiration rate [7]. It showed that leaf rolling may correlate with drought tolerance of the plant.

Based on leaf drying, Inpari21, TIL24, IR64IRRI, and BP16188M-2D-SKI-19-2-1 had the best performance (score 1). IR 20 along with other 37 genotypes had relatively good score (2), including Kelimutu, Huanghuazhan, Gajang Mungkur, and Bahbutong (Table 3). IR 20 in some cases showing relatively good performance comparable with the tolerant check. Nevertheless, there are some lines having lower performance compare to IR 20. It indicate that the selection was very thight. Leaf rolling and drying are significantly associated with rapid ability to recover after drought stress [8]. This trait is still widely used to screen drought tolerant mateials of rice.

![Image of rice plants under drought stress treatment](image)

**Figure 1.** Performance of 60 rice genotypes under drought stress treatment using seedling box method, Sukamandi, 2016

**Table 3.** Leaf rolling, leaf drying, and plant recovery of 60 rice genotypes under drought screening at seedling stage using seedling box method, Sukamandi, 2016

| No | Genotype                      | Leaf Rolling | Leaf Drying | Plant Recover (%) |
|----|-------------------------------|--------------|-------------|-------------------|
| 1  | Dro1                          | 9            | 3           | 30.00             |
| 2  | GajahMungkur                  | 9            | 2           | 15.00             |
| 3  | Lipigo4                       | 7            | 2           | 40.00             |
| 4  | Inpari10                      | 8            | 2           | 60.00             |
| 5  | Inpari18                       | 9            | 3           | 55.00             |
| 6  | Towuti                        | 7            | 3           | 67.50             |
| 7  | HHZ12-SKI-1-2-0Kr-JK-IND       | 8            | 2           | 30.00             |
| 8  | TIL3(IR84636-13-12-2-6-3-3-2-2-B)| 7            | 3           | 37.50             |
| 9  | Huanghuazhan                  | 6            | 2           | 7.50              |
| 10 | Kelimutu                      | 5            | 2           | 2.50              |
| 11 | BatangLembang(6661)           | 8            | 3           | 22.50             |
| 12 | Dular                         | 8            | 4           | 15.00             |
| 13 | HHZ5-SKI-9-3-0Kr-JK-IND       | 6            | 3           | 5.00              |
| 14 | BP14342f-7                    | 7            | 3           | 52.50             |
| 15 | Limboto                       | 7            | 2           | 10.00             |
| 16 | IR20                          | 7            | 2           | 17.50             |
| 17 | IR64IRRI                     | 9            | 1           | 2.50              |
| 18 | BP17552-1c-SBY-0-CRB-0        | 7            | 3           | 57.50             |
| 19 | Inpari21                      | 7            | 1           | 12.50             |
| No | Genotype | Leaf Rolling | Leaf Drying | Plant Recover (%) |
|----|----------|--------------|-------------|-------------------|
| 20 | Bahbutong(1182) | 5 | 2 | 20.00 |
| 21 | HHZ5-SKI-7-1-0Kr-JK-IND | 9 | 2 | 7.50 |
| 22 | IR83142-B-20 | 8 | 2 | 65.00 |
| 23 | TIL2 | 9 | 2 | 25.00 |
| 24 | TIL24 | 7 | 1 | 82.50 |
| 25 | BP16734e-3 | 8 | 4 | 45.00 |
| 26 | BP14262e-1-1 | 8 | 2 | 55.00 |
| 27 | IR83383-B-B-11-4 | 8 | 2 | 65.00 |
| 28 | PR40781b-3-4-SBY-0-CRB-2-SKI-0-5 | 8 | 3 | 65.00 |
| 29 | BP17300M-54D-SKI-8-2-2 | 9 | 3 | 12.50 |
| 30 | BP17298M-53D-SKI-1-4-2 | 7 | 2 | 65.00 |
| 31 | BP15994M-4D-SKI-18-1-3 | 9 | 3 | 12.50 |
| 32 | BP16146M-1D-SKI-2-13-1-3 | 8 | 3 | 50.00 |
| 33 | BP16178M-4D-SKI-16-6-2-2 | 7 | 2 | 42.50 |
| 34 | BP16178M-1D-SKI-13-8-1 | 8 | 2 | 35.00 |
| 35 | BP16188M-2D-SKI-19-2-1 | 9 | 1 | 50.00 |
| 36 | GSRIR1-11-D3-S3 | 9 | 3 | 45.00 |
| 37 | GSRIR1-11-D1-Y1 | 9 | 2 | 45.00 |
| 38 | GSRIR1-11-D3-S2 | 9 | 4 | 65.00 |
| 39 | BP17282M-41D-1-SKI | 9 | 4 | 52.50 |
| 40 | BP17280M-46D-IND | 9 | 4 | 75.00 |
| 41 | IR83142-B-20 | 8 | 3 | 57.50 |
| 42 | IR61336-4B-14-3-2(PSBRC94) | 9 | 2 | 30.00 |
| 43 | BP14262e-2-8 | 8 | 2 | 42.50 |
| 44 | BP14262e-1-1 | 8 | 2 | 32.50 |
| 45 | BP14352e-1-1-2Op-JK-0 | 7 | 2 | 32.50 |
| 46 | BP14352e-2-2-0Kr | 7 | 2 | 75.00 |
| 47 | HHZ5-SKI-7-3-0Kr-JK-0 | 8 | 3 | 42.50 |
| 48 | BP14352e-2-3-3Op-JK-0 | 9 | 2 | 57.50 |
| 49 | BP17280M-1 | 9 | 2 | 52.50 |
| 50 | BP17280M-66-1 | 8 | 3 | 32.50 |
| 51 | BP17292M-22 | 8 | 2 | 47.50 |
| 52 | BP14342f-7 | 8 | 2 | 47.50 |
| 53 | BP14352e-2-3-1Op-JK-0 | 9 | 4 | 55.00 |
| 54 | HHZ9-SKI-19-8-0Kr-JK-0 | 8 | 5 | 60.00 |
| 55 | BP14476e-3-4-2Kr-JK-0 | 7 | 4 | 10.00 |
| 56 | BP14476e-3-9-1Kr-JK-0 | 8 | 3 | 65.00 |
| 57 | BP14352e-1-2-2Kr-JK-0 | 8 | 3 | 22.50 |
| 58 | HHZ18-SKI-2-1-0Kr-JK-0 | 8 | 3 | 20.00 |
| 59 | CT18510-23-4-4-1-MP-JK-0 | 9 | 4 | 7.50 |
| 60 | WAB99-2-1-JK-0 | 8 | 2 | 5.00 |
| 61 | | 2.58 | 3.04 | 60.74 |

5%LSD

CV (%) | 16.30 | 62.00 | 78.70
Based on % recovery, TIL24 had the highest value (82.5 %). It is an IR64 isolines improved on root characteristics. Analysis variance of recovery of the genotypes showing no significance among the genotypes (Table 2). It frequently happen for drought research. Nevertheless, the value seem to be reasonable showing the variation among the genotypes. We could disect the difference among the genotypes at the confidential level of 44%. It migh due to the complexity of the complexity of drought tolerance traits.

The frequency distribution of the genotypes are scattered in the upper midle of the population (Figure 2). It indicate that the genotypes had relatively good tolerance to drought through percentage of plant recovery mechanism. Other genotypes following TIL 24, such as BP14352e-2-2-0Kr (75.00 %), BP17280M-46D-IND (75.00 %), Towuti (67.50 %), BP17298M-53D-SKI-1-4-2 (65.00 %), IR83142-B-20 (65.00 %), IR83383-B-B-11-4 (65.00 %), PR40781b-3-4-SBY-0-CRB-2-SKI-0-5 (65.00 %), BP14476e-3-9-1Kr-JK-0 (65.00 %), GSRIR1-11-D3-S2 (65.00 %), and Inpari10 (60.00 %). Inpari 10 had been reported to have drought tolerance and water use efficiency. IR83142-B-20 and GSRIR1-11-D3-S2 (65.00 %) are GSR lines, which were designed to be tolerance to multiple stress [9; 10]. IR83383-B-B-11-4 is also dedicated for drought tolerance in rainfed lowland area. This results indicating that the drought tolerant systems which developed by many groups are working.

Table 4. Analysis of variance of plant recovery of 60 rice genotypes under drought Screening at Seedling Stage using Seedling Box Method, Sukamandi, 2016

| No | Source of Variance | Degree of Freedom | Sum of Square | Mean Square | F Value | Probability |
|----|--------------------|-------------------|---------------|-------------|--------|-------------|
| 1  | Replication        | 1                 | 213.334       | 213.334     | 5.79   | 0.018       |
| 2  | Genotype           | 59                | 2264.37       | 38.3791     | 1.04   | 0.439       |
|    | Residual           | 59                | 2174.67       | 36.8588     |        |             |
|    | Total              | 119               | 4652.37       | 39.0955     |        |             |

Figure 2. Frequency distribution of recovery of 60 rice genotypes under drought Screening at Seedling Stage using Seedling Box Method, Sukamandi, 2016

The second experimen Inpari21, BP16178M-1D-SKI-13-8-1, HHZ5-SKI-7-1-0Kr-JK-IND, Inpari18, Towuti, and Bahbutong (1182) had either high number of vertical root growth (more than 10 roots) as well as high proportion of the vertical root growth (more than 40%). The genotypes were suspected to have good drought tolerant due to the root architecture [11]. The data had been discussed in the publication.
Correlation analysis among the traits of experiment 1 and 2 showed that the number of green leaves had strong positive correlation with root length, root number in zone 1 (horizontal), 2 (medium), and 3 (vertical), total root number, leaf rolling, and plant recovery (Table 3). It indicated that number of green leaves under drought condition might be used as indirect selection criteria for drought tolerance screening material. This finding is relevant with the finding [12] which defining Drought Tolerant Degree (DTD) as ratio of green leaf length to total leaf length of the top three leaves in every seedling after drought treatment. DTD is significantly correlated with drought tolerant considerable traits such as water potential, survival rate, panicles per plant, spikelets per panicle, seed setting rate, yield per plant, and contents of proline, chlorophyll, and malondialdehyde (MDA). This study concluding that DTD is a simple method to directly select drought tolerant material. The number of green leaves after drought stress treatment found in this study correlated with some drought tolerant related traits also indicating that the trait could be used as new criteria in selecting drought tolerant materials. This trait is relatively simple and it is possible to do high-throughput selection using this trait. On the other hand, the ability of plant to keep the leaves green might be correlated with the vigoring ability of the plant at early plant growth. This early vigor is also positively correlated with yielding ability either under optimum or drought stress condition [13].

Number of zone 3 root (grow vertically to the basal) in this study is positively correlated with shoot length, tiller number, and green leaf number, total root number, proportion of zone 3 root number from the total root number. It is also relevant with the study [14] which conclude that deep root architecture is reported to increase plant capability to uptake water from the deeper soil zone. Under drought stress, it would benefit to plant in collecting the deep. Dro1 gene has been proven for this mechanism. Among the root zones, number of root in zone 2 had higher correlation coefficient with the number of green leaf (0.625) followed by Zone 3 (0.415) and zone 1 (0.400). It indicated that the existence of root in zone 2 is also important for the plant to survive. Nevertheless, proportion of root in zone 3 negatively correlated with shoot length, green leaf number, root number in zone 1 and 2, total root number, and plant recovery. It seems that proportion of vertical root compensate with other traits in developing drought tolerance. It indicated that developing cultivars having deep root would helpful for drought, but should manage the proportion from the total roots. Finally, it could be seen that root trait strongly correlated with drought tolerance [15]. Thus the study is continuously being conducted by many scientists. Selection for drought tolerant during vegetative growth phase is still widely being used with new modifications, and it shows promising results [16; 17]. This method is relatively simple, quickly done, and could suitable for high-throughput running.

Single trait selection might not be enough to find robust durable drought tolerance variety. The rice plants response to drought stress in many traits, including morphological, physiological, biochemical, and molecular [18]. It makes the traits very complicated and a single approach may not be enough to overcome the stress. More over, drought stress may come with other stresses all together on field. Final selection on yield under drought condition might work better in selecting drought tolerant lines [19]. Nevertheless, on field selection under natural condition would be very difficult to control. The selection might be succeefull during dry season only (once a year), still the risk of unpredictable rainfall occurrence. On the other hand, a quick request to cope with drought incidence is almost every year to come.

Screening of breeding materials and finding a new donors while studying physiological mechanism of drought tolerant should be conducted continuously. Molecular tools, in such stage would also be very helpful [20; 21]. Variety combining drought tolerance with drought avoidance like very early maturing traits might be feasible for this immediate requirement recently. Agronomical effort such as developing ponds and modifying irrigation system would help more to overcome the drought problem.

4. Conclusion
Number of green leaf under stress and root number in zone 2 correlated more with traits supporting drought tolerant in rice. Both traits are supposed to be useful for drought stress tolerance selection of rice plants. Between both of the traits, number of green leaves seem to be simples and more practive to be applied. Further confirmation is needed and considering drought is a complex trait, simultaneous selection using some methods would be more powerful.
5. Appendix

Tabel 5. Correlation among seedling characteristics, root pattern, and seedling stage response to drought of 60 rice genotypes simultaneously tested using mini pot and Seedling box methods into 60 rice genotypes.

| Tiller Number | Shoot length | Tiller Number | Grean Leaf Number | Dry Leaf Number | Root Length | Root Number Zone 1 | Root Number Zone 2 | Root Number Zone 3 | Total Root Number | Zone 3 Root Number Proportion | Leaf Rolling Score | Leaf Drying Score |
|---------------|--------------|---------------|-------------------|-----------------|-------------|-------------------|-------------------|-------------------|-----------------|-----------------------------|------------------|------------------|
|               |              |               |                   |                 |             |                   |                   |                   |                 |                             |                  |                  |
| Tiller Number | 0.524        | 0             |                   |                 |             |                   |                   |                   |                 |                             |                  |                  |
| Grean Leaf Number | 0.599  | 0.872        |                   |                 |             |                   |                   |                   |                 |                             |                  |                  |
| Dry Leaf Number | -0.378 | -0.033  | -0.072            |                 |             |                   |                   |                   |                 |                             |                  |                  |
| Root Length    | 0.341        | 0.284        | 0.311             | -0.344          |             |                   |                   |                   |                 |                             |                  |                  |
| Root Number Zone 1 | 0.582 | 0.336  | 0.406             | 0.033           | 0.133       |                   |                   |                   |                 |                             |                  |                  |
| Root Number Zone 2 | 0.531 | 0.489  | 0.625             | -0.126          | 0.274       | 0.507             |                   |                   |                 |                             |                  |                  |
| Root Number Zone 3 | 0.393 | 0.458  | 0.415             | -0.193          | 0.106       | 0.177             | 0.198             |                   |                 |                             |                  |                  |
| Total Root Number | 0.69    | 0.61   | 0.686             | -0.158          | 0.248       | 0.701             | 0.813             | 0.649             |                 |                             |                  |                  |
| Proportion of Zone 3 | -0.297 | -0.251 | -0.335            | -0.029          | -0.133      | -0.518            | -0.601            | 0.423             | -0.304          |                             |                  |                  |
| Leaf Rolling Score | 0.237  | 0.167  | 0.299             | 0.064           | 0.127       | 0.217             | 0.035             | 0.181             | 0.191           | -0.006          |                             |                  |                  |
| Leaf Drying Score | -0.068 | 0.005  | -0.044            | 0.015           | 0.099       | -0.277            | -0.209            | -0.029            | -0.232          | 0.14            | 0.231                   |                  |                  |
| Plant Recovery | 0.232        | 0.184        | 0.266             | -0.095          | 0.193       | 0.198             | 0.188             | -0.094            | 0.11            | -0.276         | 0.15          | 0.111                   |
|                | 0.075        | 0.159        | 0.471             | 0.14            | 0.129       | 0.15              | 0.476             | 0.403             | 0.033           | 0.253          | 0.397                   |                  |                  |
6. References

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