Study of rice root distribution patterns for breeding of drought tolerant varieties

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Abstract. Root growth and development is one of the morphological characters which related to drought tolerant traits. This study aims to evaluate the root distribution pattern of 30 rice genotypes to support the breeding of drought tolerant varieties. The research was conducted in ICRR greenhouse in Sukamandi, Subang, Indonesia from October to December 2015. Thirty rice genotypes, consisting of cultivars, promising lines (prior to be released as new varieties), and check varieties, were arranged using randomized complete block design with three replications. Seed were planted in mini pots containing a mixture of sand and soil media with a concave sieve at the top. The filter is divided into three zones, namely upper (1), middle (2), and lower (3). The pot was placed in a plastic box filled with water to maintain the humidity. The results showed that Mekongga had the number of tillers, the number of fresh leaves, the number of roots in zone 1, and the total number of roots significantly higher than the best check variety, Salumpikit. In this study, it was found that the amount of metaxylem between genotypes was different. Salumpikit has the most metaxylem among other genotypes. Further research is expected to be carried out both in drought and optimum condition as a control to see the correlation between root architecture with drought tolerance in the field.

1. Introduction

Drought is the main environmental factor that can inhibit plant growth and reduce production depending on the level of stress and the phase of plant growth when under drought stress. Low water availability in the early stages of plant growth can lead to drought stress [1].

The physiological impact of drought is decreasing intracellular CO₂ concentrations, over-reduction of the photosynthetic electron chain, which results the formation of ROS compounds that cause imbalance in metabolic processes in plants [2, 3, 4]. Plants respond by synthesizing antioxidant compounds (ascorbic acid), and accumulation of proline compounds [5, 6].

Root growth and development is one of the morphological characters reported to tolerance to drought stress [7, 8]. The root system plays an important role for plants in obtaining water and is an indicator of plant tolerance to water shortages. Root elongation is related to the osmotic potential which affects water absorption [9]. The ability of root penetration to reach deeper zones in some millet species is a mechanism to avoid drought stress to increase water absorption ability. The xylem anatomy in the roots is also affected by drought stress.
Screening for tolerance to drought stress using concrete box method is routinely carried out at ICRR which had identified several genotypes supposed to be tolerant to drought stress. This study aims to determine the root distribution pattern of rice genotypes supposed to be tolerant to drought stress based on concrete box screening method.

2. Methodology
This research was carried out in the greenhouse of the Indonesian Center for Rice Research in Sukamandi, Subang, West Java from October to December 2015. The materials were 30 genotypes consisting of superior varieties, promising lines and check varieties (Table 1). In this study, the check varieties were IR64 Dro1 and Salumpikit. Salumpikit is a genotype which commonly used in drought screening as tolerant check because it has very good vigor even under drought condition. Dro1 is a major QTL related to tolerance to drought stress through the mechanism of deep root growth in an upright downward direction [10]. NIL IR64 Dro1, which is the IR64 genotype that has been enriched with the Dro1 gene, was obtained from Prof. Dr. Yusaku Uga from NIAS (National Institute of Agricultural Sciences) through Dr. Tsutomu Ishimaru from IRRI (International Rice Research Institute).

Table 1. Rice genotypes we used in study of root distribution patterns, BB Padi, 2015

| No. | Genotype                                      | Year of released | Maturity age | Yield Potential | Agroecosystem |
|-----|-----------------------------------------------|------------------|--------------|-----------------|---------------|
| 1   | Inpago 4                                      | 2010             | 124          | 6.1             | Upland        |
| 2   | Inpago 8                                      | 2011             | 119          | 8.1             | Upland        |
| 3   | Huanghuazhan (Inpari 42 Agritan GSR)          | 2016             | 112          | 10.58           | Upland        |
| 4   | Inpago 6                                      | 2010             | 113          | 5.8             | Upland        |
| 5   | Inpago 7                                      | 2011             | 111          | 7.4             | Upland        |
| 6   | Gajah Mungkur                                 | 1994             | 95           | 2.5             | Upland        |
| 7   | IR64 (from NIAS)                              |                  |              |                 |               |
| 8   | Ciherang                                      | 2000             | 116-125      | 7.0             | Irrigated     |
| 9   | Inpari 30                                     | 2012             | 111          | 9.6             | Irrigated     |
| 10  | Zhongzu 14 (Inpari 43 Agritan GSR)            | 2016             | 111          | 9.02            | Irrigated     |
| 11  | Inpago 5                                      | 2010             | 118          | 6.2             | Upland        |
| 12  | Inpago 9                                      | 2012             | 109          | 8.4             | Upland        |
| 13  | Inpari 10                                     | 2009             | 112          | 7.0             | Irrigated     |
| 14  | Inpari 13                                     | 2010             | 99           | 8.0             | Irrigated     |
| 15  | Inpara 4                                      | 2010             | 135          | 7.6             | Swampy        |
| 16  | Situbagendit                                  | 2003             | 110          | 5.0             | Irrigated     |
| 17  | Mekongga                                     | 2004             | 116          | 6.0             | Irrigated     |
| 18  | IR20                                         |                  |              |                 |               |
| 19  | Lipigo 1                                      |                  | 110          | 8.18            | Upland        |
| 20  | Lipigo 2                                      |                  | 113          | 8.15            | Upland        |
| 21  | Lipigo 4                                      | 2014             | 113          | 7.1             | Upland        |
| 22  | Obs 8412 (UDH OPT)                            |                  |              |                 |               |
The experiment was conducted following a Randomized Complete Block Design with three replications. Seeds were planted in pots in the form of 2.5-inch round paralon of 20 cm depth with a small mess (1 mm x 1 mm hole) metal coneve sieve. The filter is divided into 3 zones proportionally, namely zone 1 (the top third to the soil surface: angle of about 45 – 90°), zone 2 (the middle third (45 – 30°), and zone 3 (the lower third to perpendicular to the bottom (30 – 0°). The planting media is a mixture of soil: sand as much as 1: 1. The mini pots are then placed in a plastic box which is always filled with water to maintain the humidity (Figure 1). Seedlings are planted as much as 5 seeds per pot, planted right in the middle of the sieve. At the age of about ten days, the plants are spaced out to grow one best plant in each pot. When the plants were 42 days old, they were removed from the pots and the number of roots and length of each zone were observed. Measurement of root length from each zone was measured from the outside of the sieve to the tip of the root in that zone, while the total root length was measured from the root base to the tip of the root after the plant was removed from the sieve. The distribution of roots from each zone shows the angle of the plant roots to the main axis (Figure 2). Observations were also made on plant height, number of tillers, and number of leaves. Analysis of variance and differences between treatments was carried out using the CropStat 7.2 program [11].

![Figure 1. Plant performance on the medium](image-url)
3. **Result and discussion**

According to [12] and [13], the ability of root growth under drought stress conditions is genetically controlled, so [14] suggested that root morphological characters could be used to select of maize genotype tolerance to drought stress directly or indirectly.

The deep rooting character indicates that the roots of the rice plant are mostly located at the bottom, not sideways, which in this study was shown in zone 3 (Figure 3). Such roots support plant tolerance to drought. This is related to the efforts of plants to reach deeper soil layers, where in general the deep layer has higher humidity than the top layer [15].

![Figure 2](image)

**Figure 2.** Root distribution pattern on zona 1 (top), zona 2 (middle), dan zona 3 (above)

![Figure 3](image)

**Figure 3.** Root distribution pattern performance

Dro 1  Salumpikit  Mekongga
Most of the tested genotypes had more roots in zone 1 than in other zones, except Inpari 30 Ciherang Sub1 and Salumpikit which had more roots in zones 2 and 3 (Figure 3). Based on the total number of roots, Mekongga was the genotype that had the highest total number of roots. So far, Mekongga is known as a variety that is quite adaptive in rainfed and irrigated rice fields with lack of water supply. According to [16], the strategy of plants in dealing with drought stress starts from the germination phase and vegetative growth by forming deep root formations and many root branches. This is an important character of plant tolerance to drought stress.

**Table 2.** Number of roots in each zone and total numbers of roots of 30 rice genotype

| No | Genotype                        | Number of roots in Zone 1 | Number of roots in Zone 2 | Number of roots in Zone 3 | Number of total roots |
|----|--------------------------------|---------------------------|---------------------------|---------------------------|-----------------------|
| 1  | Inpago 4                        | 9                         | 14                        | 8                         | 31                    |
| 2  | Inpago 8                        | 8                         | 9                         | 3                         | 20                    |
| 3  | Huanghuazhan                    | 14                        | 7                         | 4                         | 25                    |
| 4  | Inpago 6                        | 9                         | 10                        | 2                         | 21                    |
| 5  | Inpago 7                        | 15                        | 11                        | 2                         | 28                    |
| 6  | Gajah Mungkur                   | 10                        | 11                        | 6                         | 27                    |
| 7  | IR64 from NIAS                   |                           |                           |                           |                       |
| 8  | Ciherang                        | 12                        | 11                        | 6                         | 29                    |
| 9  | Inpari 30 Ciherang Sub1          | 2                         | 9                         | 5                         | 17                    |
| 10 | Zhongzu 14                      | 10                        | 7                         | 3                         | 20                    |
| 11 | Inpago 5                        | 11                        | 10                        | 7                         | 28                    |
| 12 | Inpago 9                        | 11                        | 9                         | 3                         | 23                    |
| 13 | Inpari 10                       | 11                        | 11                        | 6                         | 28                    |
| 14 | Inpari 13                       | 11                        | 10                        | 4                         | 26                    |
| 15 | Inpara 4                        | 13                        | 9                         | 4                         | 26                    |
| 16 | Situbagendit                    | 17                        | 11                        | 4                         | 32                    |
| 17 | Mekongga                        | 18                        | 12                        | 8                         | 38                    |
| 18 | IR20                            | 12                        | 8                         | 6                         | 27                    |
| 19 | Lipigo 1                        | 14                        | 11                        | 3                         | 29                    |
| 20 | Lipigo 2                        | 16                        | 6                         | 2                         | 25                    |
| 21 | Lipigo 4                        | 12                        | 9                         | 3                         | 24                    |
| 22 | Obs 8412 (UDH OPT)              | 13                        | 8                         | 5                         | 26                    |
| 23 | BP17280M-26D-IND                | 14                        | 10                        | 8                         | 32                    |
| 24 | BP17280M-48D-0-SKI              | 15                        | 10                        | 8                         | 33                    |
| 25 | BP17282M-41D-1-SKI              | 21                        | 8                         | 1                         | 31                    |
| 26 | BP17280M-66C-2-IND              | 21                        | 7                         | 3                         | 30                    |
| 27 | BP17280M-50D-IND                | 14                        | 10                        | 6                         | 31                    |
| 28 | BP14352e-2-3-3Op-JK-0           | 10                        | 7                         | 4                         | 21                    |
IR64, Situbagendit, Mekongga, Lipigo 2, BP17282M-41D-1-SKI, and BP17280M-66C-2-IND had a significantly higher number of roots in zone 1 than IR64Dro1 (Table 2). Roots in zone 1 will increase the plant's ability to expand the root system. The main approaches to see the tolerance to drought stress are (1) the ability to absorb water optimally by expanding and deepening the root system, and (2) the ability of plants to maintain turgor through decreasing osmotic potential [17].

The average root length in zone 2 was identified as the longest (22.93 cm) followed by zone 1 (20.18 cm) and zone 3 (19.74 cm) (Table 3). Roots in zone 2 grew longer with a narrower angle than roots in zone 1. It was estimated that they have the ability to expand the root zone to obtain water supplies in the soil. Salumpikit (tolerant check) has longer roots than IR64 Dro1. Salumpikit has 20.18 cm, 22.93 cm, and 19.74 cm root length in zone 1, zone 2, and zone 3, respectively. The longest roots in zone 1 were obtained in Inpago 8 (26.2 cm), in zone 2 in Lipigo 2 (36.2 cm), and in zone 3 in Ciherang (28.9 cm) (Table 3). Genotypes with longer roots in each zone indicated the ability of plants to increase root area under drought stress conditions. Increasing root length downward and laterally can extend root reach into deeper soil layers. In this layer the soil moisture is higher than the top layer, so the roots have the opportunity to absorb water. The results of [18] showed that maize genotypes tolerant to drought stress conditions had deeper root growth than vulnerable genotypes.

### Table 3. Root length in each zone of 30 rice genotypes, ICRR, 2015

| No | Genotype               | Length of roots | Total length of root |
|----|------------------------|-----------------|----------------------|
|    |                        | Zona 1          | Zona 2          | Zona 3          |                      |
| 1  | Inpago 4               | 18              | 20.1            | 26.8            | 33.9                |
| 2  | Inpago 8               | 26.2            | 29.8            | 19.1            | 30.1                |
| 3  | Huanghuazhan           | 14.6            | 24.7            | 28.7            | 31.1                |
| 4  | Inpago 6               | 21.9            | 23.8            | 10.7            | 29.4                |
| 5  | Inpago 7               | 17.4            | 18.2            | 11.7            | 20.7                |
| 6  | Gajah Mungkur          | 25.6            | 23.6            | 27.6            | 30.3                |
| 7  | IR64 from NIAS         | 23.3            | 17.1            | 5.4             | 23.4                |
| 8  | Ciherang               | 14.7            | 22.2            | 28.9            | 24.4                |
| 9  | Inpari 30 Ciherang Sub1| 21.5            | 26.3            | 22.1            | 32.5                |
| 10 | Zhongzu 14             | 21.9            | 23.7            | 12.1            | 25.5                |
| 11 | Inpago 5               | 27.2            | 18              | 17.5            | 27.5                |
| 12 | Inpago 9               | 19.7            | 21.7            | 19.1            | 23.6                |
| 13 | Inpari 10              | 16.7            | 24.1            | 26.4            | 31.1                |
| No | Genotype             | Length of roots          | Total length of root |
|----|----------------------|--------------------------|----------------------|
|    |                      | Zona 1 | Zona 2 | Zona 3 |                  |
| 14 | Inpari 13            | 19.2   | 18.5   | 15.7   | 21.4              |
| 15 | Inpara 4             | 22     | 26.6   | 18     | 29.7              |
| 16 | Situbagendit         | 16.7   | 19.4   | 19.1   | 20.9              |
| 17 | Mekongga             | 18     | 16.3   | 23.3   | 23.2              |
| 18 | IR20                 | 19.4   | 27     | 19.5   | 28.3              |
| 19 | Lipigo 1             | 16.4   | 36.2   | 21.6   | 37.2              |
| 20 | Lipigo 2             | 18.6   | 21.1   | 17.1   | 23.9              |
| 21 | Lipigo 4             | 21.6   | 28.7   | 21.7   | 35.6              |
| 22 | Obs 8412 (UDH OPT)   | 21.8   | 27.3   | 24.7   | 28.2              |
| 23 | BP17280M-26D-IND     | 16.9   | 16     | 17.6   | 23.8              |
| 24 | BP17280M-48D-0-SKI   | 19.2   | 26.1   | 23.2   | 27.2              |
| 25 | BP17282M-41D-1-SKI   | 25.9   | 18.6   | 12.1   | 26.1              |
| 26 | BP17280M-66C-2-IND   | 22.1   | 20.3   | 18.8   | 23                |
| 27 | BP17280M-50D-IND     | 25.8   | 20.3   | 16.9   | 28.8              |
| 28 | BP14352e-2-3-3Op-JK-0| 18.3   | 22.6   | 20.5   | 24.3              |
| cek| IR64 Dro 1           | 13.8   | 17.6   | 20.8   | 21.3              |
| cek| Salumpikit           | 20.9   | 32     | 25.5   | 33.7              |
|    | CV                   | 23.2   | 19.5   | 23.7   | 12.8              |
|    | LSD                  | 7.7    | 7.3    | 1.7*   | 5.7               |
|    | Average              | 20.4   | 22.8   | 19.5   | 27.3              |

The genotype with the tallest posture was Lipigo 2 (65.6 cm), not significant to Salumpikit (61.7 cm), but significant taller than IR64 Dro1 (53 cm). There were three genotypes that had more tillers than IR64 Dro1 (2 stems), namely Inpari 10 (3 stems), Mekongga (4 stems) and IR20 (3 stems). There were 3 genotypes that had more leaves than IR64 Dro1 (7 leaves), namely Inpari 10 (10 leaves), Inpara 4 (10 leaves), and Mekongga (11 leaves) (Table 4). The ability of plants to maintain root growth is very important in maintaining water and nutrient absorption in drought stress conditions. [19] also reported that drought stress tends to increase plant root length and root shoot ratio. The same thing was conveyed by Breseghello et al. (2008) that changes in root architecture due to drought depend on the genotype. Several genotypes showed the same root density between control and drought treatment. While the other genotypes showed different changes in root architecture, for example expressing deep roots when drought stress occurred.

Ajithkumar and Panneerselvam reported that Setaria italica decreased stem length with increasing drought [19]. The decrease occurred due to the plant's response to reduce cell elongation due to reduced water and decreased turgor pressure in cells. Cell volume also decreased because the amount of water in the xylem was reduced. Translocation of food materials in the phloem was disrupted.
**Figure 4.** Root performance of several genotype

**Table 4.** Plant height, tiller number, and leaf numbers of 30 rice genotype, ICRR, 2015

| No. | Genotype        | Plant height | Tiller number | Leaf numbers |
|-----|-----------------|--------------|---------------|--------------|
| 1   | Inpago 4        | 58.6         | 1             | 6            |
| 2   | Inpago 8        | 59.7         | 1             | 5            |
| 3   | Huanghuazhan    | 56.2         | 2             | 6            |
| 4   | Inpago 6        | 63.3         | 2             | 7            |
| 5   | Inpago 7        | 59.5         | 2.7           | 9            |
| 6   | Gajah Mungkur   | 56.8         | 2.3           | 8            |
| 7   | IR64 asal NIAS  | 50.2         | 2.7           | 9            |
| 8   | Ciherang        | 58.5         | 2.3           | 9            |
| 9   | Inpari 30       | 60           | 1             | 4            |
| 10  | Zhongzu 14      | 51.4         | 1.7           | 7            |
| 11  | Inpago 5        | 56.2         | 2.7           | 8            |
| 12  | Inpago 9        | 48           | 1.7           | 6            |
| 13  | Inpari 10       | 52.6         | 3             | 10           |
| 14  | Inpari 13       | 56           | 2.7           | 8            |
| 15  | Inpara 4        | 51.3         | 2.7           | 10           |
| 16  | Situbagendit    | 53.3         | 2.3           | 9            |
| 17  | Mekongga        | 55.7         | 4             | 11           |
| 18  | IR20            | 56.6         | 3             | 8            |
| No. | Genotype             | Plant height | Tiller number | Leaf numbers |
|-----|----------------------|--------------|---------------|--------------|
| 19  | Lipigo 1             | 61.1         | 2             | 7            |
| 20  | Lipigo 2             | 65.6         | 2             | 6            |
| 21  | Lipigo 4             | 59           | 2             | 6            |
| 22  | Obs 8412 (UDH OPT)   | 56.8         | 2.3           | 9            |
| 23  | BP17280M-26D-IND     | 57.2         | 2             | 7            |
| 24  | BP17280M-48D-0-SKI   | 57.8         | 2             | 8            |
| 25  | BP17282M-41D-1-SKI   | 57.5         | 2             | 7            |
| 26  | BP17280M-66C-2-IND   | 57           | 2.3           | 8            |
| 27  | BP17280M-50D-IND     | 60           | 2.3           | 9            |
| 28  | BP14352e-2-3-3Op-JK-0| 49.5         | 2             | 7            |
| cek | IR64 Dro 1           | 53           | 2             | 7            |
| cek | Salumpikit           | 61.7         | 1.3           | 6            |
|     | CV                   | 5.7          | 23.6          | 20           |
|     | LSD                  | 5.3          | 0.8           | 3            |
|     | Rata-rata            | 56.6         | 2.2           | 8            |

Anatomical changes, especially in the roots, have been reported to occur under conditions of lack of water to protect and adapt. This adaptation is because the presence of lignin or suberin found in the exodermis, endodermis and cell layer of the medullary cortex [20] which protects against desiccation and death of cortical cells [21]. Research conducted by [22] reported that tolerant rice root anatomy produces thick cell walls from the endodermis, xylem vessels, mendulla and sclerenchyma cell layers. In addition, the proportion of aerenchyma decreased when rice plants were under water shortage conditions. In this study, it was found the variation amount of root metaxylem among the genotypes. Salumpikit has the most metaxylem among other genotypes (Figure 4).

This research showed the variation of plant characteristics which is supposed to be correlated with drought tolerance of the plant. Salumpikit as common check for drought showed the most robust in metaxylem, highest number of root in zone 3, longest root in zone 2, fifth longest root in zone 3, and second tallest seedling. Those traits may correlated to drought tolerance. Root length and number in zone 3 and zone 2 is strongly indicating the tolerance to drought. The genotypes tested in this study were prescreened for drought tolerance, so that actually the genotypes are relatively tolerant to drought. They may have variation in the tolerance to drought, but variation in the observed traits in this study indicating that those genotypes may have various mechanism to withstand under drought stress.

The materials consisted of for irrigated, upland, and swampy agroecosystem considering that drought stress is happened in the such agroecosystems. The materials consisted of new released varieties, promising lines, and checks. Introduces promising lines, i.e. Huanghuazhan was then released as Inpari 42 Agritan GSR and Zhongzu 14 as Inpari 43 Agritan GSR. Both varieties were designed for efficient in external inputs such as water and fertilizers, while efficient in pesticides due to its wide spectrum of resistance to pests and diseases. It relevant with this study which showed that both variety having at least more than the average of the population in each observed traits.
4. Conclusion
The tested genotypes showed variation in the suspected traits correlated with drought tolerance, indicating each the genotypes bearing specific mechanism in dealing with drought condition. Number of root in zone 3, and length of root in zone 2 and 3 were confirmed with metaxylem traits in drought tolerant genotype (Salumpikit). Root architecture indicated to be strongly affecting the drought tolerance of the plant, eventhought it is not as the only one major traits. The further research should be carried out on both drought and optimum condition to see the correlation of root architecture and drought tolerance under field condition. Selection of drought tolerance into breeding materials increase the chance to obtain at least medium drought tolerant varieties such as Inpari 42 Agritan GSR and Inpari 43 Agritan GSR.

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