Effect of PEG-8000 imposed drought stress on rice varieties germination

V Violita*, S Azhari
Departemen of Biology, Faculty of Mathematics and Science (FMIPA), Universitas Negeri Padang, Indonesia

*violita@fmipa.unp.ac.id

Abstract. Drought is the major abiotic stress factor that plays crucial role in reduction in plant production of agrycutural fields in the world. In order to evaluate drought stress on rice varieties germination, total 9 rice varieties were collected from varios place in West Sumatera. PEG-8000 is often used to determine tolerance and susceptible in germination level. Three different concentrations of PEG-8000 0%, 10 %, and 20% were used in the experiment. Germination percentage, shoot length, root length, root dry weight and shoot dry weight were determined as parameters studied. The result showed that rice varieties germination was significantly reduced with the increased of PEG concentration level in all parameters studied. Base on Drought Sensitively Index (DSI) we can classified the rice varieties as tolerant (Harum and Baroto), moderate (Situbagendit and Randam Kaus) and sensitive one (Keriting, Batang Palo, Kuning rendah, Indragiri, and Rosna putih).

1. Introduction
Rice is the main staple food in some countries in Asia [1]. At least 40% more of rice production needs to feed the population of the world by 2025 [2]. Unfortunately rice is not resistant to stress conditions one of which is drought. Drought plays a crucial role in reduction in plant production of agricultural fields in the world. Drougt stress is a result of limited water from the environment. Germination is one of the most important states of plant development. The success in yield and productions of plant also depending on germination states growth. Seedling growth is limited by lack of moisture in dry areas in the region and establishment of seedling greetly speed processing time and performances [3].

Polyethylene glycol (PEG) can simulated drought stress in early stage of growth. PEG being a high molecular weight osmotic substance has been used frequently as artificial abiotic stress inducer in many studies. PEG was used because it has a high molecular weight, it cannot pass through the cell wall, can induce uniform water stress without causing direct physiological damage [4] and therefore it is used to regulate water potential in germination tests [5].

Previous research has been carried out on 6 rice varieties found in West Sumatra and obtained 2 tolerant varieties, 2 moderate varieties and 2 sensitive varieties [6]. However, this study used 9 rice varieties from various regions in West Sumatra. The identification of several rice varieties is required for selection of drought-resistant rice in the germination phase.

Due to the importance germination and early plant establishment, objective of this study is to examine the effect of germination response to different level of water stress (PEG) as the rice varieties and to identify rice resistant to drought stress.
2. Material and Methods

The study explored the germination parameters of 9 rice varieties (Table 1) from West Sumatera against drought stress induced by polyethylene glycol (PEG). Root length, shoot length, root dry weight, shoot dry weight, and seed vigour were evaluated against different concentrations (0 (P0), 10% (P1), 20% (P2)) of PEG-8000 at germination stage.

| Varieties          | Origin                                      |
|-------------------|---------------------------------------------|
| Situbagandit      | Pariaman                                    |
| Keriting          | Banja Laweh, Kab. 50 Kota                   |
| Batang Palo       | Sijunjuang                                   |
| Harum             | Padang Air Dingin, Kab. Solok Selatan       |
| Kuning Rendah     | Padang Air Dingin, Kab. Solok Selatan       |
| Indragiri         | Pasaman                                     |
| Rosna Putih       | Banja Laweh, Kab. 50 Kota                   |
| Baroto            | Sicincin                                    |
| Randam Kaus       | Batusangkar                                 |

The experiment was carried out as factorial in the form of randomized complete design with 2 factor and three replications. The first factor contained 9 rice varieties (Table 1). The second factor included four levels of drought stress created by adding polyethylene glycol-8000 (PEG-8000) at 3 concentrations level: 0, 10, 20 %. PEG was used because it has a high molecular weight, it cannot pass through the cell wall and therefore it is used to regulate water potential in germination tests. Polyethylene glycol 8000 was used to evaluate resistance to drought at germination stage and to create different levels of water potential.

The seeds were immersed in the solution of sodium hypochlorite 1% for 5 min and were disinfected, then, washed by distilled water three times. Twenty seeds of each variety were transferred into each container in which the stencil papers were placed. 100 ml of the solution related to each treatment was add to the stencil paper. The germinated were counted until full germination. The seeds whose root length is 2 mm or more are considered as the germinated ones. In 7th day, the germinated seeds were taken out of the petri dishes and the shoot and root were separated to assess the parameters.

Germination test of the seeds each using between paper method in container. The germination percentage was calculated based on the normal seedling evaluated on 7th day after treatment [7].

2.1. Parameters

Germination percentages (%) were calculated as total number of germinated seeds by total number of seed used into 100. The Seedling vigor index (SVI) was calculated as shoot and root length into germination percentage divided by 100. Root and shoot length, root and shoot fresh weight, root and shoot dry weight was evaluated. Drought sensitivity Index (DSI) was used to characterize the relative stress tolerance of all rice varieties.

The index was calculated from genotype means using a generalized formula [8] in which DSI = [(1-YD/YP)] D, where; YD = Yield (KRWT) in stress environment; YP = Yield (KRWT) in non stress environment = Potential Yield (KRWT); D (environmental stress intensity) = 1-(Mean YD of all genotypes/Mean YP of all genotypes). Yield potential (YP) of each genotype was defined as the maximum mean response of each genotype averaged over the two years in the well-watered condition [9].

Vigour index: Vigour index value was computed using the following formula suggested by [10] and expressed as whole number.

\[ Vigour \ index = Germination \ (%) \times Total \ seedling \ length \ (cm) \] (1)
3. Result

3.1. Germination percentage (GP)
The percentage of rice seed germination 7th days after treatment are above 90% (0% PEG). While in the PEG-8000 10% treatment, the germination percentage ranged from 80%-90% but none of them reached 100%. Whereas in the PEG 8000 20% treatment, the germination percentage rate below 60% (Table 2).

Table 2. Germinate Percentage of Rice Seed on the 7th day after treatment

| Varieties       | PEG 8000 (drought treatment) |
|-----------------|------------------------------|
|                 | P0 (0%) | P1 (10%) | P2 (20%)       |
| Situbagandit    | 98a     | 97a       | 61b             |
| Keriting        | 100a    | 99a       | 66b             |
| Batang Palo     | 96a     | 81a       | 31b             |
| Harum           | 90a     | 86a       | 79a             |
| Kuning Rendah   | 89a     | 74b       | 37c             |
| Indragiri       | 90a     | 82a       | 22b             |
| Rosna Putih     | 99a     | 96a       | 3b              |
| Baroto          | 99a     | 97a       | 92b             |
| Randam Kaus     | 95a     | 94a       | 63b             |

3.2. Root length (RL)
The root length of rice seed was decreased with the increased of the PEG concentration. Meanwhile Baroto seminal root length was not significantly different between the control and PEG-8000 treatment (Table 3).

Table 3. Seminal Root Length of Rice Seeds (cm) on the 7th day after treatment

| Varieties       | PEG 8000 (drought treatment) |
|-----------------|------------------------------|
|                 | P0 (0%) | P1 (10%) | P2 (20%) |
| Situbagandit    | 8,39a   | 5,10b    | 3,74b    |
| Keriting        | 4,89a   | 2,60b    | 1,41c    |
| Batang Palo     | 8,95a   | 7,00ab   | 6,36b    |
| Harum           | 8,09a   | 6,19b    | 4,61b    |
| Kuning Rendah   | 7,41a   | 4,66b    | 4,63b    |
| Indragiri       | 6,54a   | 4,08b    | 3,64b    |
| Rosna Putih     | 6,54a   | 3,64b    | 2,74b    |
| Baroto          | 7,53a   | 6,25a    | 5,43a    |
| Randam Kaus     | 7,45a   | 6,47ab   | 5,03b    |

3.3. Shoot length (SL)
The shoot length in the control treatment ranged from 7-10 cm. The shoot length in 10% PEG treatment ranged from 6-9 cm. While the shoot length in the 20% PEG-8000 treatment ranged from 3-7 cm. It can be seen that the shoot length was decreased with the increased of PEG concentration (Table 4).
Table 4. Length of Rice Seed (cm) on the 7th day after treatment

| Varieties     | PEG 8000 (drought treatment) |
|---------------|------------------------------|
|               | P0 (0%) | P1 (10%) | P2 (20%) |
| Situbagandit  | 8.25a   | 7.51a   | 6.21b   |
| Keriting      | 9.04a   | 7.28b   | 4.08c   |
| Batang Palo   | 9.54a   | 8.67ab  | 7.45b   |
| Harum         | 9.54a   | 7.69ab  | 7.39b   |
| Kuning Rendah | 8.54a   | 7.15a   | 4.91c   |
| Indragiri     | 9.21a   | 6.82b   | 4.15c   |
| Rosna Putih   | 7.38a   | 6.18b   | 4.00c   |
| Baroto        | 7.16a   | 6.23ab  | 4.83b   |
| Randam Kaus   | 7.80a   | 7.01ab  | 5.86b   |

3.4. Seedling Vigor Index (SVI)

Seed vigor decreased with increasing of PEG-8000 concentration. Among all rice varieties, Harum and Baroto had the highest seed vigor in P2 (20% PEG) (948.69 and 942.16). While the lowest seed vigor was in Rosna Putih (16.76). A high vigor value in P2 indicates that these varieties are more capable of growing into normal plants than those with lower vigor (Table 5).

Table 5. Seedling Vigor Index (SVI) of rice on the 7th day after treatment

| Varieties     | PEG 8000 (drought treatment) |
|---------------|------------------------------|
|               | P0 (0%) | P1 (10%) | P2 (20%) |
| Situbagandit  | 1634.28a| 1226.54b | 608.43c |
| Keriting      | 1392.00a| 978.12b  | 361.77c |
| Batang Palo   | 1775.49a| 1269.75b | 433.24a |
| Harum         | 1586.31a| 1204.08ab| 948.69b |
| Kuning Rendah | 1406.90a| 868.41b  | 355.87c |
| Indragiri     | 1424.62a| 900.28b  | 177.05c |
| Rosna Putih   | 1374.83a| 937.26b  | 16.76c  |
| Baroto        | 1450.26a| 1205.69ab| 942.16b |
| Randam Kaus   | 1454.43a| 1271.84a | 683.96b |

3.5. Seminal Root Dry Weight (RDW)

Seminal root dry weight decreased with increasing of the PEG-8000 concentration, but, there were 5 varieties were not significantly different from the control treatment; Situbagandit, Harum, Kuning rendah, Rosna putih, and Randam Kaus (Table 6).

Table 6. Seminal root dry weight of seed rice on the 7th day after treatment

| Varieties     | PEG 8000 (drought treatment) |
|---------------|------------------------------|
|               | P0 (0%) | P1 (10%) | P2 (20%) |
| Situbagandit  | 4.37a   | 4.15c   | 3.60a   |
| Keriting      | 3.20a   | 2.19ab  | 1.42b   |
| Batang Palo   | 5.35a   | 4.63ab  | 3.99b   |
| Harum         | 4.54a   | 4.39a   | 3.98a   |
| Kuning Rendah | 4.28a   | 4.16a   | 3.71a   |
3.6. **Shoot Dry Weight (SDW)**

Shoot dry weight decreased as the PEG-8000 concentration increased. However, there were 4 varieties that were not significantly different between the control and treatment of PEG-8000; Situbagendit, Keriting, Kuning rendah, and Randam kaus (Table 7).

### Table 7. Rice seed shoot weight (mg) on the 7th day after treatment

| Varietas          | PEG 8000 (drought treatment) |       |       |       |       |       |       |       |
|-------------------|-------------------------------|-------|-------|-------|-------|-------|-------|-------|
|                   | P0 (0%)                        | P1 (10%) | P2 (20%) |       |       |       |       |       |
| Situbagendit      | 4.73a                          | 4.61a  | 4.03a  |       |       |       |       |       |
| Keriting          | 4.90a                          | 4.65a  | 2.32a  |       |       |       |       |       |
| Batang Palo       | 6.62a                          | 5.03b  | 4.82b  |       |       |       |       |       |
| Harum             | 6.79a                          | 6.38a  | 4.10b  |       |       |       |       |       |
| Kuning Rendah     | 5.05a                          | 4.89a  | 4.08a  |       |       |       |       |       |
| Indragiri         | 5.91a                          | 4.87ab | 3.50b  |       |       |       |       |       |
| Rosna Putih       | 5.90a                          | 4.87a  | 3.20b  |       |       |       |       |       |
| Baroto            | 4.45a                          | 4.39a  | 2.65b  |       |       |       |       |       |
| Randam Kaus       | 5.29a                          | 5.22a  | 4.56b  |       |       |       |       |       |

3.7. **Drought Sensitivity Index (DSI)**

Based on the values of the DSI for some of these parameters, we can classify rice into tolerant (Harum and Baroto), moderate (Situbagendit and Randam Kaus), and sensitive ones (Keriting, Batang Palo, Kuning rendah, Indragiri, and Rosna putih) (Table 8).

### Table 8. Drought Sensitivity Index (DSI)

| Varietas          | T | GP  | RL  | SL  | SVI | RDW | SDW | SDI  | X SDI | Description |
|-------------------|---|-----|-----|-----|-----|-----|-----|------|------|-------------|
| Situbagandit      | P1 | 0.17| 1.44| 0.78| 1.05| 1.27| -0.45| 0.71 | 0.83 | Moderate   |
|                   | P2 | 1   | 1.35| 0.73| 1.05| 0.96| 0.58| 0.95 |      |            |
| Keriting          | P1 | 0.17| 1.72| 1.69| 1.25| 8.22| -0.92| 2.02 | 1.89 | Sensitive  |
|                   | P2 | 0.9 | 1.74| 1.62| 1.34| 3.03| 2.05| 1.76 |      |            |
| Batang Palo       | P1 | 2.62| 0.8 | 0.79| 1.20| -4.06| 0.63| 1.33 | 1.08 | Sensitive  |
|                   | P2 | 1.8 | 0.71| 0.64| 1.27| 0.76| -0.17| 0.84 |      |            |
| Harum             | P1 | 0.62| 0.86| 1.69| 1.01| -3.65| 1.15| 0.28 | 0.44 | Tolerant   |
|                   | P2 | 0.33| 1.05| 0.66| 0.67| -0.56| 1.39| 0.59 |      |            |
| Kuning Rendah     | P1 | 2.89| 1.36| 1.42| 1.61| 0.75| 0.57| 1.43 | 1.24 | Sensitive  |
|                   | P2 | 1.55| 0.92| 1.25| 1.25| 0.72| 0.64| 1.06 |      |            |
| Indragiri         | P1 | 1.55| 1.38| 2.25| 1.55| 7.4 | -3.14| 1.83 | 1.74 | Sensitive  |
|                   | P2 | 2.01| 1.08| 1.62| 1.47| 2.06| 1.59| 1.64 |      |            |
| Rosna Putih       | P1 | 0.51| 1.63| 1.41| 1.34| 2.2 | -3.09| 0.67 | 1.12 | Sensitive  |
|                   | P2 | 2.59| 1.42| 1.35| 1.66| 0.63| 1.78| 1.57 |      |            |
| Baroto            | P1 | 0.34| 0.63| 1.3  | 0.71| -8.66| 0.22| -1.34| -0.26| Tolerant   |
|                   | P2 | 0.19| 0.68| 0.66| 0.59| 1.28| 1.54| 0.82 |      |            |
| Randam Kaus       | P1 | 0.12| 0.48| 0.88| 0.53| 1.33| -0.25| 0.52 | 0.55 | Moderate   |
|                   | P2 | 0.19| 0.79| 0.73| 0.89| -0.29| 0.54| 0.59 |      |            |

Note: T (PEG 10 %), T (PEG20%), T (treatment), GP (Germination Percentage), RL (Root length), SL (Shoot length), SVI (Seedling Vigor Index), RDW (Root Dry Weight), SDW (Shoot Dry Weight), DSI (Drought Sensitivity Index), X DSI (Mean of Drought Sensitivity Index).
4. Discussion
Generally seed germination determined by the seed quality and environmental condition \[11\]. In our study, germination percentage of rice seed in the control treatment (without considering drought stress) was higher than that of the PEG 8000 treatment (drought stress treatment), which was above 90%. The high percentage of rice germination in the control treatment showed that germination was going well because water requirement fulfilled. Germination percentage decreased with the increased of PEG concentrations in all rice varieties. According to \[5\], the germination percentage value was inversely related to the PEG concentration level. When the PEG concentration is increased, the osmotic potential value around the seeds becomes increasingly negative, so that it is difficult for the seeds to absorb water \[12\].

The germination process occurs when there is metabolic activity from the seeds and water to be an important think to stimulate that process. If there is a lack of water, the metabolic process in the seeds becomes disrupted and even stops \[13\]. Apart from being influenced by water, the variety and shelf life of seeds also greatly influence the germination percentage. Different seed varieties also have different metabolisms and different germination times.

In rice seeds treated with PEG 8000, it is much shorter than P0 because the water potential is also decreasing so that the water that can be absorbed by the roots also decreases and affects the root growth of the sprouts. According to \[14\], seed water uptake showed a decreased trend as stress intensity increased. This conditions coused distraction of seed metabolism to growth. The elongation of plant roots in an effort to find water is one indicator of rice being tolerant of water shortages. Seminal roots are roots that develop from the radicles when germinating and are only temporary. Seminal root growth will slow down after the plumules appear to the soil surface and seminal root growth will stop 10-18 days after germinating \[15\].

Water potensial decreased with the increased of PEG-8000, which makes it is difficult for water to be absorbed by plants. Lack of water during the vegetative stage can reduce the rate of widening and elongation of leaves, inhibiting shoot growth as indicated by decreased shoot height increase. PEG treatment inhibits shoot length increase \[12\] because drought stress will affect aspects of growth morphology \[16\], anatomy \[17\] and physiology \[12\]. Root dry weight indicates the ability of a plant to absorb water, because plants with high root dry weight have larger roots and a higher level of tolerance to drought compared to plants with low root dry weight \[15\].

Water is related to the process of plant growth as a raw material in the photosynthesis process. Water is an essential part of the protoplasm and forms 80-90% of the fresh weight of active growing tissue, water is a solvent, in which there are various kinds of salts and gases and other solutes, which move in and out of cells, from organ to organ in the process of transpiration. In addition, water is a reagent in photosynthesis and the hydrolysis process and maintains turgidity, including in cell enlargement and opening of stomata \[12\]. It will also affect cell size and plant cell biomass.

PEG factors and varieties caused significant differences in seed vigor between the control treatment and the 10% and 20% PEG treatment. The average seed vigor in the control treatment was 1453 and decreased as the PEG 8000 concentration increased. In the 10% PEG 8000 treatment it was 1107 and in the PEG 8000 20% treatment it was 586. It can conclude that seed vigor decreased as PEG concentration increased. High seed vigor causes tolerant seeds to grow and develop in sub-optimum land conditions in the form of an environment that is not suitable for seed growth and germination. In general, seeds with low vigor value use less energy than seeds with high vigor values \[11\].

Shoot dry weight showed that the treatment without PEG 8000 (control), resulted in weight differences with PEG 8000 treatment at a concentration of 10% and 20%. This can occur due to drought caused by reduced water potential. Drought can cause the leaf cuticle to be less permeable to water so that it will slow down metabolism, reduce protein synthesis, slow down ion transport and inhibit division in leaf cells \[12\]. In addition, drought also causes less turgor pressure on leaf cells. If the turgor pressure of small leaf cells and cell division is inhibited, it will cause a decrease in leaf biomass. Drought in plants can cause stomata closure, thereby reducing CO\(_2\)uptake and reducing dry weight \[18\].
Base on DSI we can classified rice varieties into 3 level of drought resistance are: tolerant (Harum and Baroto), moderate (Situbagendit and Randam Kaus), and sensitive ones (Keriting, Batang Palo, Kuning rendah, Indragiri, and Rosna putih). The highest DSI value is owned by Keriting (1.89), followed by Batang Palo, Kuning rendah, Indragiri and Rosna putih, which indicates that those variety is the most sensitive to drought stress. There are two varieties classified into the moderate (Situbagendit and Randam Kaus). The best varieties Harum and Baroto varieties are tolerant varieties, with DSI values of 0.44 and -0.26. Harum and Baroto have the potential to be used as national superior rice varieties. With the low DSI value of the two varieties, it can be said that the morphology of the two is not too affected by drought stress due to the lack of water availability that can be absorbed by the roots. In addition to the factor of water availability in the planting medium, genetic factors also have a big influence on the germination response.[12]

References
[1] Bandumula N 2017 Proc. Natl. Acad. Sci. India Sect. B Biol. Sci 88
[2] FAO 2002 The International Rice Commission “Issues and Challenges in Rice Technological Development for Sustainable Food Security Bangkok Thailand
[3] Shitole SM and Dhumal KN 2012 International J. Pharmaceutical Sci. Res 3 528-31
[4] Almaghrabi OA 2012 Life Sci. J. 9 590-8
[5] Wang C, Zhou L, Zhang G, Xu Y, Gao X, Jiang N, Zhng L, and Shao M 2018 Agri. Sci. 9 991-1006
[6] Azhari S and Violita V 2019 Bio Sains 4 21-8.
[7] ISTA 1999 Seed Sci. Techno 27 27-32
[8] Fischer RA and Maurer R 1978 Ausr. J. Agric. Res. 29 897-907
[9] Bruckner PL and Frohberg RC 1987 Crop Sci. 27 31-6
[10] Abdul-Baki AA and Anderson JD 1973 Crop Sci. 13 630-33
[11] Khodarahmpour Z 2011 African J. Biotechnology 10 18222-7
[12] Basal O, Szabó A and Veres S 2020 Current Plant Bio. 22 100-35
[13] Taiz L and Zeiger E 2010 Plant Physiology (Sunderland: Sinauer Associates Inc) p 782
[14] Pavli OI, Foti C, Skoufogianni G, Karastergiou G, Panagou A, M Khah E 2020 Agri. Res & Tech 23 556250
[15] Bowes BG and James DM 2008 Plant Structure (London: Manson Publishing) p 450
[16] Mardita S and Violita V 2018 Biosciences 3 60-8
[17] Zagoto ADP and Violita V 2019 Eksakta 20 42-52
[18] Lawlor DW. 1993. Photosynthesis Molecular, Physiological and Environmental Processes (England: Longman Scientific and Technical) p 318