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| Pakistan Journal of Botany  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **EFFECT OF DROUGHT STRESS ON ROOT ANATOMY AND LIPID PEROXIDATION OF TOLERANCE, MODERATE AND SENSITIVE RICE VARIETIES**    **VIOLITA VIOLITA1\*, APNITA ZULMA PUTRI2, NURMA DWI SAFITRI2, YUDI AGUSTIRA RAHMATULLAH2, DES M1, ZULYUSRI1, YUSNI ATIFAH1, AFIFATUL ACHYAR1**    1Biology Department, Universitas Negeri Padang, Indonesia  2Graduated student of Biology Department, Universitas Negeri Padang, Indonesia  \*Corresponding author’s email: [violita@fmipa.unp.ac.id](mailto:violita@fmipa.unp.ac.id) and [violitavioviolita@gmail.com](mailto:violitavioviolita@gmail.com)    **Abstract:** Rice is an important crop in the world, especially in Asia. Rice productivity is influenced by biotic and abiotic factors. The main abiotic factor that limits rice production is drought caused by water deficits. In reality, each variety of rice has a different response to drought. We have identified 3 groups of rice varieties based on their resistance to drought stress. However, the mechanism of the rice variety to drought are not yet known. This research aim to describe the mechanism of rice to drought in anatomy and physiological effect. Three rice varieties (Harum, Situbagendit, and Rosna) were treatment in 20% PEG 8000 solution as a simulation of drought stress for three days. The anatomical and peroxidation lipid were observed as a responses to drought stress. The result showed that the highest lipid peroxidation occurs in Rosna varieties followed by Situbagendit and Harum. Changes in anatomical structure of root rice to drought occurred in all anatomical parameter (stele diameter, cortex thickness, and xylem diameter).  **Key words: Anatomy, drought, lipid peroxidation, rice, root**  **Introduction:** Rice (Oryza sativa L.) is a staple food for the people in the world, with about 761.5 million tonnes produced in 2018 (FAO, 2022). After all, rice crops do not tolerate to drought. Drought known as one of the main limiting factor of plant growth. Long period of water deficit can greatly impact the yield stability and productivity of plant (Richard *et al.,* 2015) include rice (Panda *et al.,* 2022). The fluctuation of temperature and rainfall influence of soil moisture and soil water content. This condition affect root as the main organ to respond to drought. The root becomes deeper as the long drought period (Boyer, 1996).  The characteristics anatomy and damage level of root system is known as one of the main factors affected the plant’s responses to drought. According to Fenta *et al.,* (2014), drought can result in anatomical and physiological changes in plants, especially in roots. Furthermore, plants develop root systems in responses to water shortage conditions (Lynch & Brown, 2012).  How changes in the anatomical level of the roots that occur during drought are an indication of drought in plants. One of the indications of root damage due to drought is the formation of lipid peroxidation (Selote & Kanna-Copra, 2010; Hasanuzzaman *et al.,* 2020). According to Hossain *et al* (2015). The extent of root damage due to drought can be seen from how much the degree of lipid peroxidation including changes in anatomical structure (Lexa *et al.,* 2019).  In the early stages of drought results in the formation of oxidative stress. Oxidative stress is related to the presence of free radical compounds that can damage plants or commonly known as Reactive Oxigen Species (ROS) (Huang *et al.*, 2019). The increase in ROS is also accompanied by an increase in malondyaldehyde and antioxidants (Hamim *et al*., 2017). This ROS increasing can cause lipid peroxidation in the roots including changes in root anatomy.  The level of plant damage and survival due to drought stress is different for each species, even for each variety within a species (Laxa *et.al.,*2019). Tolerant plant varieties have a lower level of damage than sensitive varieties. Meanwhile the defense ability is higher than sensitive one. We have identified 3 groups of rice varieties based on their resistance to drought stress (Violita and Azhari 2021). The first variety is Harum known as tolerant to drought, the second is Situbagendit known as moderate to drought, and the last one is Rosna the sensitive one. However, the mechanism of the rice variety to drought are not yet known especially in the root system.  Environmental simulation in drought can be done with the PEG (Polyethylene glycol) treatment. PEG dissolved in water and can be used to mimic the magnitude of the water potential (Michel, 1983). PEG is the best material to control water potential and it does not cause poisoning in plants (Verslues *et al*., 2006). In this study, PEG 8000 was used which has a molecular weight of approximately 8000 Dalton with the form of crystalline solids. According to Violita and Azhari (2021) the use of PEG 8000 can cause a decrease in water potential in the planting medium so as to reduce sprout growth.  This research aim to describe the mechanism of root rice to drought in anatomy and physiological effect for each varieties based on the resistance level.  **Materials and Methods**  The research was conducted in July until September 2022 at the research laboratory of Biology Departement FMIPA, Universitas Negeri Padang using a complete random design arranged factorially with 2 factors. The first factor is rice varieties, namely Harum (tolerant), Situbagendit (Moderate), and Rosna (sensitive) (Violita and Azhari, 2021). The second factor is the concentration of PEG 8,000 which is 0% (aquades; without solution PEG = control) and 20% (200 g L-1) which are equivalent to water potentials of 0 Mpa and -0.3 Mpa (respectively) (Mexal et al., 1975). The two factors were combined so that 6 treatments were obtained and repeated three times so that there were 18 experimental units. Observations of histochemical lipid peroxide, the combination of treatments are not repeated so that one experimental unit consists of one container containing three plants for each treatment.  We have selected 10 varieties of rice in West Sumatra Indonesia which are seen based on the initial stage of germination (Violita and Azhari 2021) (Table 1). It was obtained that there are 3 levels of drought resistance represented by several varieties of rice. The level of resistance is: tolerant (Harum and Baroto varieties), moderate (Situbagendit and Randam kaus varieties), sensitive (Keriting, Batang Palo, Kuning Rendah, Indragiri and Rosna) (table 1).  **Table 1**. 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** | **1,24** | **3,03** | **2,05** | **1,76** |  |  | | **Batang Palo** | **P1** | **2,62** | **0,8** | **0,79** | **1,20** | **-4,06** | **6,63** | **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** | **P1** | **2,89** | **1,36** | **1,42** | **1,61** | **0,75** | **0,57** | **1,43** | **1,24** | **Sensitive** | | **Rendah** | **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** | **0,9** | **0,79** | **0,73** | **0,89** | **-0,29** | **0,54** | **0,59** |  |  |   Note: P1 (PEG 10 %), P1 (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).  In this study, we used 3 varieties that represent each level of drought resistance, the Harum variety (tolerant), the Situbagendit variety (moderate), and the Rosna variety (sensitive) as representatives for each level of drought resistance.  *Anatomical character.* Cross-section of root anatomy was observed by using the paraffin embedding method with safranin and fast green dyes. Measurement of the cortex and stele using a trinocular electric microscope with a digital microscope camera (DP25 program) on a scale of 100 μm (100x magnification) while xylem measurements on a scale of 50 μm (200x magnification).  *Lipid Peroxidation.* Histochemical root lipid peroxidation observed base on Kubis *et, al* (2004) method. The primary root tip is cut by one-third of the root length, then soaked for 15 minutes in Schiff's reagent. Furthermore, the roots are rinsed with a solution of sulfite (K2S2O5 0.5%) in 0.05 M HCl then the roots are cut again approximately 1 cm long from the root tip, then the roots are observed under a stereo microscope that has been connected to a computer. The red root is then measured by using an application that is already on the computer.  **Result and Discussion:**  ***Lipid peroxidation***  PEG 20% as a drought simulation causes lipid peroxidation in all roots of rice varieties. Harum varieties get the lowest lipid peroxidation, followed by Situbagendit and Rosna (Figure 1 and Table 1).    Figure 1. The appearance of the roots of rice varieties with PEG treatment of 0% and 20%. The arrow indicates the area of accumulation of lipid peroxides. Observations using stereo microscopes (40x magnification), samples photographed using Sony's 14.1 MP digital camera, staining with Schiff's reagent  According to Violita and Azhari (2021) and Mardhita and Violita (2019), Harum is among the drought-tolerant varieties. In this study, it was seen that the lowest lipid peroxidation occurred in Harum varieties. Drought checking causes oxidative congestion, increasing lipid peroxidation.  Table 1. Percentage of root peroxidation lipid   |  |  |  | | --- | --- | --- | | Varietas Padi | PEG concentration | | | 0% | 20% | | Harum (Tolerant) | 0a | 20,15a | | Situbagandit (moderate) | 0a | 43,53ab | | Rosna (Sensitive) | 0a | 80,89c |   Description: Numbers followed by the same letter on the same line of each variety did not differ markedly on the 5% Duncan test.  Drought-stricken treatment with PEG causes a clearer and more pronounced and even discoloration of Rosna varieties than Situbangandit and is also Harum. This is indicated by the appearance of redder roots with a wider area after being colored with Schiff's reagent.  Schiff's reagent is a product formed in several formulation reactions of color substances such as the reaction between sodium bisulfite and fukhsin. This product is used to check for the presence of aldehydes. The presence of the red color indicates the presence of Malondialdehyde content. Malondialdehyde (MDA) is the final product of lipid peroxides and its presence can indicate the level of oxidative congestion that occurs in plants.  According to Pandey *et al* (2010), The high level of MDA content, indicates more lipid peroxidation and more membrane permeability and are comparatively more susceptible for water stress such species have better capability for moisture stress tolerance. The peroxidation of lipids in biological membranes is the most obvious symptom of stress in plants, included drought stress. During drought stress, the lipids that constitute cellular membranes become damaged via peroxidation. Fundamentally, the process of lipid peroxidation comprises three distinct stages: initiation, propagation, and termination. The initiation phase of lipid peroxidation is the step by which a fatty acid radical is produced. The hydroxyl radicals or superoxides can react with methylene groups, consequently generates hydroperoxides, lipid peroxy radicals and conjugated dienes (Smirnoff 1995).  In our experiment, lipid peroxidation of root rice did not occur in the control treatment. Lipid peroxidation increased in drought treatment. The highest lipid peroxidation occurred in Rosna varieties, followed by Situbagendit and Harum varieties. Those Rosna varieties known as a sensitive one. The highest lipid peroxidation in Rosna varieties indicated that the plants are damaged and have not been able to overcome the damage. Whereas in Situbagendit and Rosna, moderat and tolerant varieties respectively, had the lowest lipid peroxidation. This condition indicated that the plant can survive and more less damage occurred in drought stress situation.  According to Pitzsche *et el,* (2006) those damage caused by the induction of reactive oxygen species (ROS). The general nature of ROS can cause oxidative damage to proteins, DNA, and lipids including lipid peroxides. Plant has some mechanism to resolve ROS, it is related to the efficient antioxidant system and also antioxidant compounds and antioxidant enzymes. Those component has an important rules in resistant of plant to drought. Plant that tolerant to drought has the lowest level of lipid peroxidation related to the activity of the antioxidant compounds and antioxidants enzyme (Jia *et al.,* 2022).  ***Root anatomy***  Changes in root anatomical parameters under water deficit are shown in Figure 2. Drought stress affects anatomical changes in the roots of rice plants. Resizing occurs in the diameter of stele, the thickness of the cortex and the diameter of the xylem. This change occurs in all rice varieties (Figure 2).    Situbagendit  Harum      Rosna  Figure 2. The anatomical of rice roots in PEG 0% (left) and PEG 20% (right); TK = thick cortex; DS = stele diameter; X = xylem  The highest diameter of the rice plant root stele is at a concentration of 0% PEG, with an average value of 33.64. In fragrant varieties the average value of 35.6 differs markedly from the varieties Situbagendit and Rosna. A decrease in the size of the diameter of the stele occurs in all varieties of rice. The highest decline occurred in the Rosna variety followed by the Situbagendit and Rosna varieties (Figure 4). Rosna is a drought-sensitive variety (Violita and Azhari 2021). A decrease in the size of the stele diameter in Rosna proves the occurrence of damage to the diameter of the stele compared to other varieties that are more resistant to drought.  Knowledge of anatomical root modifications is essential for the explication of plants growth under drought stress and therefore to understand the mechanisms used to confront drought conditions is important one. One of the parameter of anatomical characteristic is stele diameter of root. The stele has an important functions in the transport of water, nutrients, and photosynthates, and related to plant growth development.  Figure 3. Stele diameter of rice root in drought (PEG). Description: error bar indicated of standard error  Prolonged water deficit (3 days PEG 20% treatment) caused decreases in the cortex thickness. As compared with the control, root total thickness decreased from 18.32 to 17.25% in Harum varieties (tolerant) and the highest decreased of cortex thickness occurred in Rosna varieties (sensitive one) from 22.20 to 15.04 % (Figure 3).  Drought leads to a decrease in the thickness of the cortex, this occurs due to a decrease in the number of parenchymal cells (Pena valdivia, 2010). Drought control treatment is a plant tolerance mechanism to shorten the distance of water transport into the stele and xylem so that the roots more effectively transport water and increase the thickness of the cortex. It is a plant mechanism to multiply cells in the cortex in an attempt to store more water. The same is true of drought-sensitive soybean genotypes (Makbul *et al*., 2011) and corn (Fraser *et al*., 1990). The thickness of the cortex corresponds to the storage capacity of water at the root. An increase in the number of cells present in the cortex increases plant tolerance to drought stress.  Figure 3. Thickness cortex of rice root in drought (PEG). Description: error bar indicated of standard error  Drought effected changes of root xylem diameter (Figure 6). In tolerance (Harum varieties) has a significantly different between drought and control. However in moderate (Situbagendit varieties) and sensitive (Rosna varieties) has no significantly different between drought and control (Figure 6).  Figure 5. Xylem diameter of root rice in drought (PEG). Error bar indicated of standard error.  A decrease in xylem root diameter in response to drought is a mechanism to avoid the influence of embolism on the xylem (Comas *et al*., 2013). Embolism is the blocking of a xylem vessel or tracheid by an air bubble or cavity. In our experiment, xylem diameter of the tolerance (Harum varieties), significantly decreased compared with the control. Whereas in the moderate and sensitive one had not significantly different between control and drought treathment. This is indicated that the decrease of xylem root diameter is one of the mechanism of plant to survive in drought condition.  Embolism always remains low in leaf veins, even when plants exhibit clear water-stress symptoms (Cardoso *et al.,* 2019; Cochard 2002). Futhermore Cochard (2002) said that embolism xylem and drought induced stomatal closure. Stomatal closure during drought contains xylem embolism to a minimum value.  **Conclusion**  Drought stress causes lipid peroxidation of root rice. Sensitive rice varieties (Rosna) has the highest level of damage due to lipid peroxidation, followed by rice moderate varieties (situbagendit) and rice tolerant varieties (Harum).  Drought stress causes changes in the anatomical dimensions of the roots, which include the stele diameter, cortex and xylem. This change in root anatomy can be used as an estimation of drought-tolerant plants.  **Acknowledgement**  The present study was mostly financed by PNBP Research Grants of Universitas Negeri Padang under contract No. 947/UN35.15/LT/2022.  **References**  Boyer, J.S. 1996. Advances in drought tolerance in plants*. Adv. Agron,* 56:187-218.  Cochard, H. 2002. Xylem embolism and drought-induced stomatal closure in maize. *Planta.,* 215(3):466-71  Fenta, B.A., S.E, Beebe., K.J, Kunert., J.D, Burridge., K.M, Barlow., J.P, Lynch., C.H, Foyer. 2014. Field phenotyping of soybean roots for drought stress tolerance. *J. Agro*., 4:418-435.  FAO 2022. (<http://www.fao.org/faostat/en/#data/QC>)  Hamim, H., V, Violita., T, Triadiati., M, Miftahudin. 2017. Oxidative stress and photosynthesis reduction of cultivated (*Glycine max*L.) and wild soybean (*G. tomentella*L.) exposed to drought and paraquat. *Asian J. Plant Sci.,* 16(2): 65-77.  Hasanuzzaman, M., M.H.M. 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