JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 7 NO. 3 September 2025

DOI :https://doi.org/10.36378/juatika.v7i3.3811
eissn 2656-1727
pissn 2684-785X
pages : 694 – 700

RESEARCH ARTICLE Open Access

Drought Resistance Of Several Local Upland Rice Genotypes (*Oryza sativa* L.) From West Sumatra Province

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Abstract

Rice is a major staple food for the Indonesian population, therefore, its productivity must be improved to accomplish the domestic consumption. Efforts to increase rice production in a sustainable manner in the future not only rely exclusively on lowland rice production but also from other genotypes including upland rice. West Sumatra is one of the province in Indonesia that preserves various local upland rice germplasm. The characterization of local upland rice genotypes to drought stress are required in order to support the increase of national rice production. This study aims was to obtain the West Sumatra local upland rice genotypes that are resistant to drought. The drought resistance screening was carried out in three ways, namely: 1) Testing of seed resistance with Polyethylene Glycol (PEG), 2) Testing of root penetration using a wax coating, and 3) Testing of proline content. From the study, two genotypes, namely: Susun Porti and Ladungan which have good resistance to drought.

Keywords: Drought, PEG, Prolin, Resistance, Upland Rice

1. Introduction

Almost 95% of Indonesians are consumed rice as a staple food (Rokhmah *et al*., 2022). Indonesia is the third largest rice consumer in the world with a rice consumption reaching of 35.8 million tons in 2020 (Darmawan *et al*., 2021). In the same periods, Indonesia rice dry grain production was decreased from 54.65 million tons in 2020 to 54.42 million tons in 2021 (BPS, 2021). The decay of rice production is caused by the reduction of the harvested area. The rice harvested area was decreased by 2.3 percent from 10.66 million/ha in 2020 to 10.41 million/ha, in 2021 (BPS, 2021). The other serious problems is the reducing of productive land for paddy rice planting, such as the conversion of irrigated rice area to non-agricultural uses, and climate change effect which causes an increase in dry area in Indonesia. These obstructions are becoming a big challenges in order to provide sufficient rice demand for Indonesia populations. Therefore, to accomplish the large amount of national rice demand is achieved by imported from other countries. During January - December 2021, Indonesian had importeds of 407.74 thousand tons of rice, increased by 14.44% compared to the same period of the previous year (BPS, 2021).

One of the potential lands that can be used for increasing of national rice production is acidic dry land which occupy of 74.3% of the total suboptimal dry land area of 107.36 million hectares (Directorate General of Food Crops, 2022). This dry land can be optimally used for planting of dryland rice known as upland rice. The local upland rice has some superior characters including high adaptability, good taste, high-stress tolerance, and high yield potential. While, the other important characters are need to improve including longer life span and taller plants, makes them more susceptible to lodging. The dry land can be utilized for planting of drought-tolerant rice genotypes. One of the object-ive of rice plant breeding is to produce new varieties that have superior properties for the specific area (Ali & Wani, 2021).

West Sumatra is one province in Indonesia which diverse local upland rice germplasm. The local rice genotype is a beneficial resource for rice breeding prog-ram. The centers of upland rice production in West Sumatra are East and West Pasa-man, South Solok, and Dharmasraya Regency. In 2017, the upland rice harvested

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in East Pasaman, West Pasaman, South Solok and Dharmasraya was 1,270 ha, 2,760 ha, 717 ha and 117 ha, respectively with a production of 2,120 tons, 9,326 tons, 2,333 tons and 248 tons, respectively (BPS, 2018).

The development of drought-tolerant upland rice varieties is crucial for facing of water deficit phenomena in rice cultivation in dry land areas. Drought can significantly reduce crop yields. Therefore the identifi-cation and the development of new geno-types resistant to drought is necessity. Several techniques can be applied to exam-ine of plant resistance to drought, including growth environment modificati-on for minimizing the stress and improving of plant resistance to drought stress (Soemar-tono, 1995). To achieve this objectives, various approaches was employed, includ-ing drought simulations with Polyethylene Glycol (PEG), root penetration tests, and proline content analysis.

PEG enables researchers to mimicry drought environment which controlable and reproducible, avoiding environmental variability usually found in the field testing. This method is highly effective for testing of plant responses to drought stress during the germination and early seedling stages, which are the most vulnerable phase. Drought stress applied in seeds germination stages is disrupt of seed metabolism system, leading to drought tolerant seeds only are be able to germinate (Firdausya *et al*., 2016)

Drought stress is stimulates plants to modify of root architecture including root length and number in order to maximize of water and mineral absorption. The root penetration test directly evaluates the roots physical ability to penetrate the media. This is a crucial trait for accessing water in dry or hard-layered soils. A strong and efficient root system is important traits for plant's surviving in drought stress; This test is measures the root's physical capacity to penetrate a specific density of media. The ability of roots to penetrate hard soil layers is an effective way to characterize drought-tolerant plants (Hanson *et al*., 1990).

Proline is an amino acid that significantly accumulated in plant tissues when they are facing various abiotic stresses, including drought. This accumu-lation is plant important physiological res-ponse and adaptation mechanism. Proline plays an important role in maintaining cell turgor and root growth in low osmotic potential conditions (Ali *et al.*, 2014). Overall, the combination of these three testing methods, including PEG, root penetration, and proline content provides a highly effective approach for identifying of upland rice genotypes resistant to drought stress. The main objective of the research was to obtain local upland rice genotype from West Sumatera that are resistant to drought.

2. Material and Methods

This research was conducted at the Agronomy and Plant Physiology Laboratory, Faculty of Agriculture, Andalas University, Padang (Latitude: -0.9121171, Longitude: 100.4597830), at an altitude of approximately 255 meters above sea level. The materials used in this study included 12 local upland rice genotypes from West Sumatra, 20% of polyethylene glycol (PEG) 6000 which equivalent to an osmotic tension of 6.7 bar, stencil paper, 60% paraffin, 40% vaseline, 3% sulfosalicylic acid, ninhydrin, glacial acetate, toluene, soil, sand, and distilled water. The tools used included 90x90 mm Petri dishes, 240 ml plastic cups, digital scales, mortars, measuring cups, test tubes, aluminum foil, dropper pipettes, wooden racks, tweezers, label paper, centrifuges, spectrophotometers, water baths, ice baths, ovens, stationery, and photography equipment.

PEG study was conducted using a factorial Completely Randomized Design (CRD) with two directions and repeated three times. The first factor was 12 local rice genotypes from West Sumatra consisting of Siputri, Silampung, Sigubal, Sigoyang, Sigudang, Simarus, Sigotik, Kulambom, Kukubalam, Kapundung Darmasraya, Susun Porti, and Ladungan. The second factor was the concentration of PEG 6000 solution consisting of: P0 (aquades without PEG) and P1 (PEG 6000 solution with a concentration of 20%). The good quality seeds provided by Prof. Dr. Ir. Irfan Suliansyah, MS with a storage time less than 1 year at the Agronomy Laboratory, Faculty of Agriculture, Andalas University was used. Selected seeds were washed with running water, then washed with distilled water, and followed by washing in 70% ethanol for 30 seconds. The seeds were then washed with 2% NaOCl for 15 minutes. The sterilized seeds were immediately washed 4 times with distilled water to remove all remaining sterilant and the seeds were allowed to air dried at room temperature. The dry sterile seeds were then placed in a 90 mm diameter Petri dish which previously been lined with 2 sheets of stencil paper. Each Petri dish was filled with 10 seeds, for (P1) 2 ml of PEG 6000 solution (20%) was added, and for the control (P0) only 2 ml of distilled water was added. Then, the Petri dish was closed and the germination rate was observed for 7 days at room temperature. Data collection was includes the maximum growth potential of germination ability.

The next experiment was examined the strength of the upland rice roots on the wax layer media. Wax layer was prepared using Vaseline and Paraffin (40%: 60%), a 40 g of Vaseline and 60 g of paraffin were mixed and diluted by heating at 70 ^{0}C for approximately 20 minutes. The mixture then put into a perforated 240 ml plastic cup with a diameter of 3 cm, then 3 mm of layer wax was added which provides a hardness level approximately of 12 bar (Suardi *et al*., 2001). In total, two units of 240 ml plastic cups were used. The first was used for the planting medium and the second plastic cup was used for the nutrient solution. After the wax dried, then the first plastic cup was filled with soil and sand (1:1), then five rice seedlings was planted. Then,

the first plastic cup was placed on the top of the second plastic cup containing the nutrient solution. Data was collected 28 days after planting which included maximum growth potential, drought sensitivity index, number of roots penetrating the wax layer, length of the roots penetrating the wax layer, and proline content.

Proline levels was measured using the Bates, Waldren and Teare method (1973). In brief, 0.1 g of leaves was extracted with 3 ml of 3% sulfosalicylic acid in a mortar. The leaf extract then centrifuged at 6000 rpm for 15 minutes. The clear supernatant was carefully poured into a glass bottle, then, the remaining residue was more added with 2 ml of sulfosalicylic acid and centrifuged in the same conditions. The second supernatant was poured into the first supernatant and mixed thoroughly, called the final supernatant. Then, 2 ml of the final supernatant was mixed with 2 ml of 3% ninhydrin acid and 2 ml of glacial acetic acid. The mixture was then heated at 100 °C for 1 hour in a water bath, and cooled in an ice bath. Four ml of toluene was added to the mixture and stirred for 15 seconds to form of chromoform. The absorbance of the formed chromoform was measured using a spectrophotometer at a wavelength of 520 nm.

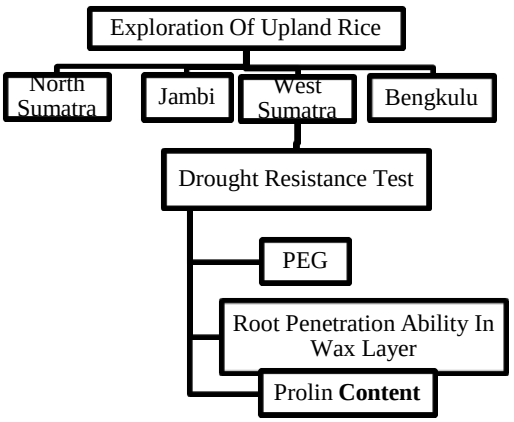

Figure 1. Research flow diagram

2.1. Data analysis

Maximum growth potential was calculated by the formula: PTM = ∑ (number of seeds grown)/(number of seeds planted) X 100 %

Germination capacity (DB) was calculated based on the number of normal sprouts (KN) on the first and second observations, namely on day 5 and day 7 (ISTA 2021).

DB % =∑ (KN count 1+count 2)/ (seeds planted) x 100%

The resistant or susceptible index was calculated using the formula Fernandes (1993) cit chaniago *et al*., 2021)

Resistant Index = Yd/yn x Yd/Hyd

Information: Yd: plants under drought conditions, Yn: plants under normal conditions, Hyd: the result of the highest stress genotype. Criteria: Ti > 0.5 = Resistant and Ti < 0.5 = Susceptible

To determine proline levels, data was obtained from a regression equation with proline levels (x) and absorbance (y). The proline levels obtained were still in the form of µM, and were converted into µ grams/ml by multiplying the BMP (Proline Molecular Weight) 115.13 grams/mol. The proline content of the leaves was expressed in µM/g fresh leaf weight with the formula (Bates, 1973).

$$\mu M/g = \frac{\text{"Concentration x ml Toluene"}}{\frac{\text{MW Proline/5}}{\text{Fresh Weight}}}$$

3. Results and Discussion

3.1. Maximum Growth Potential (%)

The results showed that the interaction of PEG and local West Sumatra upland rice genotype, as well as single treatment both for rice genotype and PEG was significantly affected the maximum growth potential (Table 1). Maximum growth potential of all rice genotypes was significantly different between 0% and 20% PEG treatment. PEG 20% was generally reduced the maximum growth potential of all local West Sumatra upland rice genotypes. The potential maximum growth capability of these genotypes is indicated that these genotypes are resistant to drought.

Table 1. Maximum growth potential of 12 local West Sumatra upland rice genotypes in 20% PEG

Genotypes	PEG	
	0%	20%
Siputri	100,00 aA	57,60 eB
Silampung	100,00 aA	28,80 fB
Sigubal	98,30 aA	79,20 cdB
Sigoyang	96,60 aA	83,53 bcB
Sigudang	96,60 aA	81,26 cdB
Simarus	100,00 aA	88,96 abA
Sigotik	94,90 aA	57,43 eB
Kulambom	98.300 aA	91,23 abA
Kukubalam	100,00 aA	70,70 deB
Kapundung Dharmasraya	98,30 aA	77,30 cdB
Susun Porti	100,00 aA	96,46 aA
Ladungan	100,00 aA	85,43 abA
CV 12.15 %		

The numbers in rows followed by the same capital letter and in columns followed by the same lower case letter are not significantly different according to the DNMRT test at the 5% level.

Rice seeds are hygroscopic. The rice seeds water content is highly dependent on the humidity and

temperature of the storage room. Environmental and genetic factors are influenced the ability of seeds to maintain their quality during storage periods. If the maximum growth potential more than 80% is categorized as good seed quality standard (Taghfir *et al*., 2018). Based on this parameter, all seeds used in this study were good quality seeds, because they maximum growth potential bigger than 80%. Meanwhile, in the 20% PEG, the maximum growth potential of some seeds was declined, it might be caused by the different abilities of each genotype to 20% PEG. Water absorption inhibition was observed in Mustakmal rice seeds, a superior rice genotype treated with 10% and 25% of PEG 6000 (Nazirah, 2024).

PEG is affected water absorption process, leading to plant tissue experienced water stressed. Water deficit is reduced of turgor pressure on the cell walls. PEG 6000 20% is inhibit the seed imbibition process. The amount of water and oxygen absorbed by the seeds are restricted by PEG molecules located outside of the cell membranes. PEG is causing of low seed environmental potential and decrease of seed water absorption rates (Fata, *et al*., 2020). Wider surface seeds have a greater absorption or imbibition capacity and require higher concentrations of osmo-conditioning solutions (Aisyah, Kendarini & Ashari, 2018).

3.2. Germination Ability (%)

The analysis showed that the local West Sumatra upland rice germination ability was affected by the interaction of PEG treatment and the genotype. Similarly, the single treatment, both genotype and PEG treatment, also significantly affected of the seed germination ability (Table 2).

Table 2. Germination rate of 12 Local West Sumatra Upland Rice Genotypes in PEG treatment

Genotypes	PEG	
	0 %	20 %
Siputri	100.00 aA	33.33 fB
Silampung	100.00 aA	13.33 gB
Sigubal	96.66 aA	80.00 bcB
Sigoyang	93.33 aA	70.00 cdB
Sigudang	99,50 aA	66.66 deB
Simarus	100.00 aA	80.00 bcB
Sigotik	90.00 aA	36.66 fB
Kulambom	100.00 aA	83.33 abB
Kukubalam	100.00 aA	53.33 deB
Kapundung Dharmasraya	96.66 aA	60.00 deB
Susun Porti	100.00 aA	93.33 aA
Ladungan	100.00 aA	73,33 cdB
CV 11.94 %		

The numbers in rows followed by the same capital letter and in columns followed by the same lower case letter are not significantly different according to the DNMRT test at the 5% level.

PEG 20% treatment generally reduces the germination rate of local West Sumatra upland rice. The genotypes with high germination rate in PEG 20% treatment was Susun Porti and Kulambom with germination abilit of 93.33% and 83.33%, respectively. The decrease of seed germination rate is caused by PEG can reduce the water potential in the media, thereby inhibiting rice germination (Maemunah *et al*., 2023).

3.3. Drought Sensitivity Index (DSI)

Drought sensitivity index (DSI) is one of the important indicators used for screening of a genotype resistant to drought stress. The classification later can be used as a reference index to predict genotype based on the DSI. Genotype tested under sub-optimal conditions show a significant decrease is categorized as susceptible genotypes (DSI>0,5). Table 3 shows that the resistance index of the tested West Sumatra upland rice genotypes. Eight (66.67%) of them was categorized as resistant and 4 genotypes were susceptible. Kulambom and Susun Porti were having the highest drought sensitivity index of 0.80. Simulation using PEG solution is open possibility to detect and differentiate of plant responses to drought stress. PEG is inhibiting water absorption which causes plants lack of water, thereby slowing seed germination process and plant growth. Water absorption by seeds treated with high concentration of PEG is inhibited, thereby slower the germination process (Maemunah *et al*., 2023). PEG was used as an alternative strategy in genotype selection for drought stress at the germination phase. Seeds of three chili genotypes was reported able to grow at PEG 15%, while the seeds was failed to germinate at 20% of PEG treatment (Budiyanti et al., 2023).

3.4. Number of Translucent Roots in the Wax Layer

The results of the analysis of variance showed that the number of roots penetrating the wax layer was not significantly affected by the genotype of local upland rice from West Sumatra. Data from the results of further analysis of DNMRT 5% on the variable number of roots

penetrating the wax layer of several genotypes of local upland rice from West Sumatra can be seen in Table 4.

Table 3. Drought sensitivity index of 12 West Sumatera local upland rice genotypes

Genotipes	ISK Maximum Growth Potential	Criteria	ISK Germination Ability	Criteria
Siputri	0.3	Susceptible	0.28	Susceptible
Silampung	0.1	Susceptible	0.09	Susceptible
Sigubal	0.52	Resistant	0.51	Resistant
Sigoyang	0.64	Resistant	0.61	Resistant
Sigudang	0.53	Resistant	0.53	Resistant
Simarus	0.6	Resistant	0.6	Resistant
Sigotik	0.11	Susceptible	0.11	Susceptible
Kulambom	0.82	Resistant	0.8	Resistant
Kukubalam	0.2	Susceptible	0.2	Susceptible
Kapundung Dharmasraya	0.52	Resistant	0.51	Resistant
Susun Porti	0.8	Resistant	0.8	Resistant
Ladungan	0.61	Resistant	0.6	Resistant

Tabel 4. Number of penetrating roots of several local West Sumatra upland rice genotypes in drought resistance tests using wax coatings

Genotypes	Number of penetrating roots (quantity)
Siputri	1.16
Silampung	0.84
Sigubal	1.12
Sigoyang	0.95
Sigudang	1.09
Simarus	1.42
Sigotik	1.10
Kulambom	1.11
Kukubalam	1.02
Kapundung Dharmasraya	0.84
Susun Porti	1.00
Ladungan	1.50
:%	11 %

However, the highest root penetrating wax layer was found in Sigudang, Ladungan, and Simarus genotype. Thus, these genotypes are promising for drought resistant genetic materials. It is assumed that increasing the number and length of roots is a rice defense mechanisms tolerant to drought stress.

Drought resistant rice genotype could maintained their growth and development in a stress environment and keep high grain production. One of the mechanisms is by the extending their roots to find water sources. The ability to utilize deep soil water is determined by the penetration power and root length. The number of roots penetrated the wax layer is influenced by the thickness of the wax layer media (Prasetyo *et al*., 2020).

Drought resistant plants can minimize the impact of drought and damage with adaptation mechanisms (Oktavianti *et al*., 2019). Plants can achieve their higher yield potentials optimaum nutrient *(*Afmerta *et al.*, 2019)

3.5. Length of Translucent Roots in the Wax Layer

The analysis of variance indicated that the length of roots penetrating the wax layer was not significantly influenced by the genotype of local upland rice from West Sumatra. Results of the 5% DNMRT post hoc test for this variable across several local upland rice genotypes from West Sumatra are presented in Table 5.

Table 5 showed that several genotypes have long penetrating roots including Ladungan at 8.75 and Simarus 8.32 cm. It is suspected that genotypes with longer penetrating roots are genetically more responsive to drought stress, through extending their roots mechanism.

Rice plants that experienced drought stress tend to extend their roots. Root plants are more difficult to absorp the water in long drought stress periods (Laila Nazirah, 2024). Better root penetration was observed in several rice mutants resistant to drought stress (Ma'sumah *et al*., 2016). Long root that be able to penetrate of hard soil layer is important characteristics for adaptation to drought stress. Therefore, plants can be easily absorb of water in deep soil layers (Lynch, 2013).

Root length is related to the root penetration. Roots are difficult to penetrate of more dense soils. Plants grown in stress environment are trying to penetrate the wax layer in order to get more nutrients needed for their growth and development. Plants that are resistant to drought are extended of their roots in order to get more water and nutrients needed for their growth (Ilyani, *et al*., 2017).

Table 5. The length of the roots penetrating of 12 local upland rice genotpes from West Sumatra in the drought resistance test using a wax layer

Genotype	Length of penetrating roots (cm)
Siputri	6.93
Silampung	4.83
Sigubal	6.59
Sigoyang	5.57
Sigudang	6.48
Simarus	8.32
Sigotik	6.48
Kulambom	6.56
Kukubalam	5.94
Kapundung Dharmasraya	4.83
Susun Porti	5.80
Ladungan	8.75
KK	

3.6. Proline Content (µmol/g)

The analysis of variance demonstrated that proline content was significantly affected by the genotype of local upland rice from West Sumatra. The results of the 5% DNMRT test on the proline content of various local upland rice genotypes from West Sumatra are presented in Table 6.

Table 6 shows that the proline content of each local upland rice genotype from West Sumatra was different. The highest proline content was observed in Kulambom genotype, namely 0.37 µmol, followed by Susun Porti and Ladungan genotype, namely 0.34 µmol and 0.33 µmol, respectively. It is believed that the high amount of proline of these 3 genotypes is due to the high resistance of them to drought stress.

Proline is the most rapidly accumulated compound by plants for adaptation to the stress. Proline has high solubility and compatibility with the cell environment. Proline plays as an osmoregulator for maintainng the cells osmotic balance. Proline is a free amino acid formed and accumulated in higher amount of plant leaves exposed to drought stress. Proline accumulation is one of plants mechanismrespons to drought stress (Larasani, 2021). Plants are prevention of dehydration by increasing of contration of cell solute and with maintaining of high water levels.

Table 6. Proline content of several genotypes of local upland rice from West Sumatra drought resistance test using wax layers

Genotypes	Proline Content (µmol/g)
Siputri	0.20 c
Silampung	0,16 c
Sigubal	0.18 c
Sigoyang	0.21 c
Sigudang	0.17 c
Simarus	0.29 b
Sigotik	0.09 d
Kulambom	0.37 a
Kukubalam	0.11 d
Kapundung Dharmasraya	0.22 c
Susun Porti	0.33 ab
Ladungan	0.34 ab
KK%	17.64 %

The numbers in columns followed by the same lower case letter are not significantly different according to the DNMRT test at the 5% level.

Proline accumulation in rice treated with salinity stress is genotype dependents. Tolerant genotype is accumulated more proline than susceptible genotypes. In addition, proline accumulation in roots was higher than in the stems or leaves (Zhang, *et al.*, 2014). Proline is accumulated in plant tissue, and plays an important role in regulating osmotic pressure to protect and reduce cell damage. The proline accumulation in plant tissues are used to evaluate the resistance of genotype to the stress (Bates *et al*, 1973).

4. Conclusion

Based on the results from the serial selection stages that have been carried out on local West Sumatra upland rice genotypes, there are two resistant genotypes, namely the Susun porti genotype and the Ladungan genotype.

Acknowledgments

The research was funded by the Directorate of Research, Technology, and Community Service, Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and

Technology through Master's Thesis Research Scheme with Research Contract Number: 086/E5/PG.02.00PT/2022, Fiscal Year 2022. We would like to express our gratitude for the opportunity to conduct the research. All other parties for any kind of assistance are very much appreciated.

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