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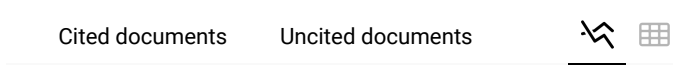
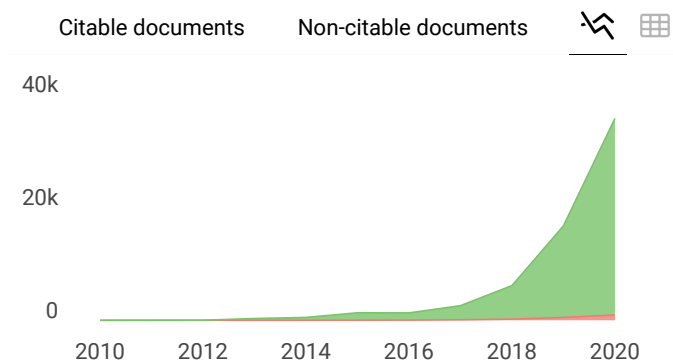
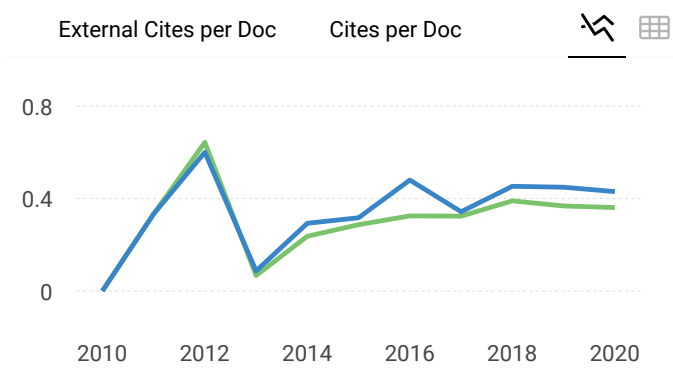
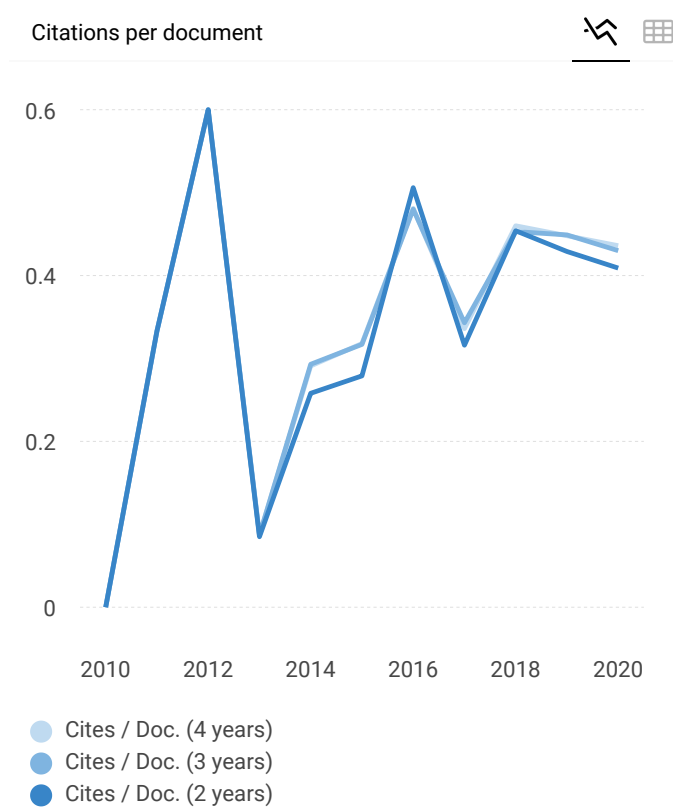
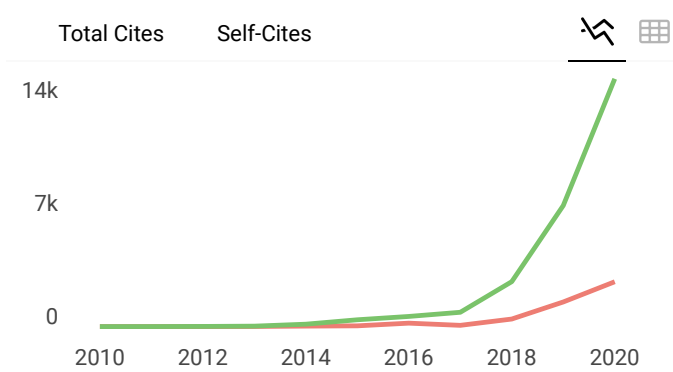
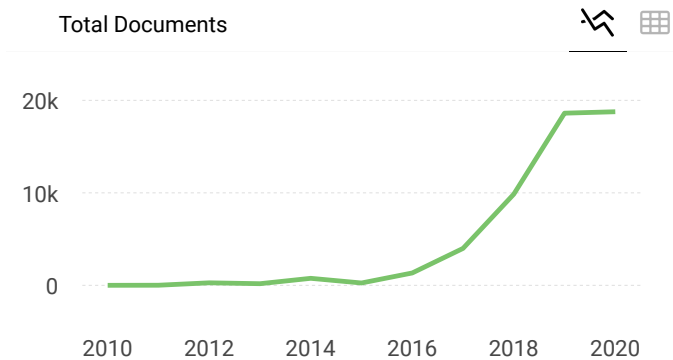
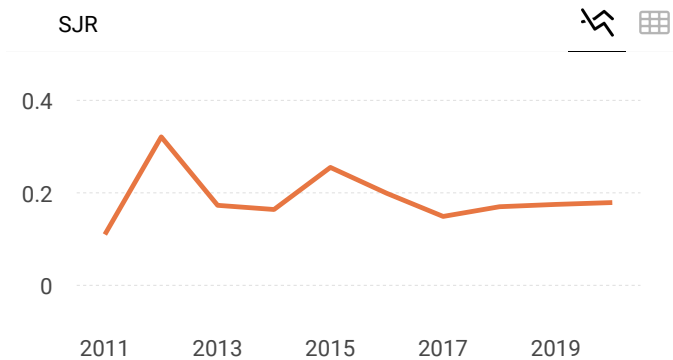
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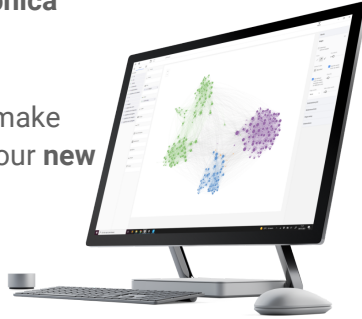
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The performance of three local rice (*Oryza sativa* L.) cultivar from East Kalimantan - Indonesia under drought stress at early seedling stage

I B M Artadana*, I T Dewi and J Sukweenadhi

Faculty of Biotechnology, University of Surabaya Jalan Raya Kalirungkut, Surabaya 60292, East Java, Indonesia

*Corresponding author: arta@staff.ubaya.ac.id

Abstract. Climate change made rain fall unpredictable leading to sudden drought stress during crop cultivation period. Drought stress affects the crop growth and development, especially during the seedling period. This research aims to evaluate the performance of three local rice seedling under drought stress induced by PEG 6000. Three local rice cultivars were germinated and then divided into five groups. In the 3 d after germination, each group were treated *in vitro* with either 0 %, 10 %, 15 %, 20 % or 25 % PEG 6000 solution for 10 d *in vitro*. Drought stress was continued for 14 d *ex vitro* in rice treated with 0 % and 25 % PEG. Osmotic stress induced by PEG significantly reduced plant growth and total chlorophyll content in the leaf. *In vitro* study showed rice seedling growth reduction was increased when the PEG 6000 increased. *Ex vitro* study with application of 25 % PEG showed cv. Telaseh more tolerant to drought stress at the seedling stage compare to cv. Mayas Kuning and cv. Mayas Putih. Additionally, drought stress induced increasing of soluble sugar in the leaf of all rice cultivars. This might indicate that soluble sugar plays a role as osmoprotectant under drought stress in those three cultivars.

Keywords: Cultivar Telaseh, rice seedling, soluble sugar

1. Introduction

According to the Indonesia statistic department (BPS) [1], Indonesia population are increasing and will be reaching 3×10^6 people in 2035. Increasing in population is a coincidence with increasing demand for food. Rice is the main source for carbohydrate in Indonesia. To balance the future rice demand and consummation, rice production must be increased every year. Several approaches have been done to balance between rice demand and production; one of them is optimizing rice production in rain feed plantation.

Around 25.02×10^6 ha area In Indonesia is dry land which is potentially used to increase rice production in rain feed system [2]. Rain feed system completely depends on water from the rain for rice cultivation [3]. Recently, global warming induces climate change which affected rain patter around the globe. Low water precipitation from the rain in crop season will induce drought stress leading to low productivity. Rice is sensitive to drought stress, especially at the early seedling stage [3]. Drought stress at early seedling stage inhibits rice germination [4], growth [4–6], and reduced chlorophyll contents in the leaf [7, 8]. The plant usually accumulates compatible solute when grown under drought stress. Compatible



solute maintains water balance inside the cell under drought stress. Elevated concentration of compatible solute correlate with enhanced tolerance to drought stress [7]. Soluble sugar, glicyne betanin, and proline are an example of compatible solute found in rice [9].

Indonesia is rich with rice germ plasm. Exploring local rice cultivar which tolerant to drought stress is a challenge to increase rice production in the rain feed system. *Oryza sativa* (L.) cv. Mayas Putih, cv. Mayas Kuning and cv. Telaseh are local rice cultivar cultivated in rain feed system at East Kalimantan. The aim of this study is to evaluated the effect of drought stress on germination and growth of those cultivars at early vegetative stage. Additionally, the relation between drought stress and sugar accumulation also evaluated.

2. Material and method

2.1. Plant material

All seed was directly collected from a farmer at East Kalimantan. The viable seed was selected by soaking seed in water. All floating seed were removed, and the remaining seed was germinated in petridis containing tissue wet tissue paper. The days of germination (DAG) was defined as 2 mm radicle emerged from the seed.

2.2. Drought tolerance evaluation

All germinated seeds were divided into five groups and culture inside petridis containing wet tissue paper in dark condition for 3 d. After 3 d in dark condition or seed was at 4 DAG, germinated seeds were cultured in *in vitro* under light illumination and treated with drought stress for 10 d. Drought stress was applied by pouring either 0 %, 10 %, 15 %, 20 % or 25 % Polyethylene glycol (PEG 6000) into petridis. At the end of treatment, shoot and root length were measured, and all rice seedling were transferred into the pot containing soil. After seven days growing in the soil or 21 DAG, rice seedlings which previously treated with 0 % and 25 % PEG were treated again with 0 % and 25 % PEG, respectively, for 14 d. At the end of treatment, shoot length was measured, and leaf was harvested for chlorophyll and sugar measurement.

2.3. The measurement of chlorophyll content and soluble sugar

Rice leaf (8 mg for chlorophyll extraction and 100 mg for sugar extraction) were grinded to a powder using mortal with additional liquid nitrogen. Chlorophyll was extracted by dissolving leaf powder with 85.5 % acetone followed by incubation in 4 °C for 48 h. The concentration of chlorophyll a (Chl a) and chlorophyll b (Chl b) in solution were measured using spectrophotometer at 662 nm and 644 nm wavelength. The amount of chlorophyll in the leaf tissue was calculated according to the following question [10]:

$$[\text{Chl a}] = 9.784 D_{662} - 0.99 D_{644} \quad (1)$$

$$[\text{Chl b}] = 21.42 D_{644} - 4.65 D_{662} \quad (2)$$

$$\text{Total chlorophyll (TC)} = [\text{Chl a}] + [\text{Chl b}] \quad (3)$$

Reducing sugar was determine using Somogy [11]. Leaf powder was dissolved in 10 mL of water, and the 1 mL of it was mixed with 1 mL basic Cu solution. The solution was heated at a boiling temperature for 20 min and the 1 mL Phosphomolybdate was added when the solution is already cooling. The absorbance of the solution was measured using spectrophotometer at 540 nm wavelength. The amount of reducing sugar in the solution was determined according to the formulation from the standard curve.

Total sugar was determined using Phenol-Sulfuric Acid method [12]. An amount of 1 mL of leaf powder was mixed 1 mL of 5 % phenol solution followed by addition of 5 mL concentric sulfuric acid. The solution the incubated in water bath at 30 °C for 20 min. The absorbance of the solution was

measured using spectrophotometer at 488 wavelengths. The amount of reducing sugar in the solution was determined according to the formulation from the standard curve. The amount of total sugar then used to measure the amount of non-reducing sugar. The amount of non-reducing sugar was calculated according to the following question:

$$\text{Non-reducing sugar} = \text{total soluble sugar} - \text{reducing sugar.} \quad (4)$$

2.4. Statistical analysis

The effect of stress was analysis by calculating the percentage of change in morphology and physiology characters. The calculation was done according to the following formula:

$$\% \text{ of change under drought stress} = (\text{control value} - \text{treated value}) / \text{control value} \times 100 \% \quad (5)$$

The experiment was conducted as a completely randomized design with three replications. All data were analysis using Kruskal-Wallis with $P < 0.05$ to compare the percentage of change between cultivar under drought stress and were analyzed using SPSS software.

3. Result

3.1. Rice shoot and root growth

Drought stress applied *in vitro* at 4 DAG for 10 d inhibit shoot and root growth in all rice cultivars (figure 1). Shoot and root length reduction were increasing when the concentration of PEG was increasing. At highest PEG concentration, shoot length reduction in Mayas Putih, Mayas Kuning and Telaseh was 70.67 %, 64.00 % and 57.67 % compared to control, respectively. Shoot length reduction of Mayas putih significantly lower than Talaseh, and no significant different in shoot length reduction were observed between Mayas Putih and Mayas Kuning or between Mayas Kuning and Telaseh. In contrast, the shoot length reduction of Telaseh was significantly lower than Mayas Putih and Mayas Kuning when treated with 25 % PEG. Root length reduction of Mayas Putih, Mayas Kuning and Telaseh, were 65.33 %, 66.67 % and 80.33 % compared to control, respectively (table 1). Re-treated rice seedling *ex vitro* with 25 % PEG at 21 DAG for 14 d showed Mayas Kuning had the highest shoot reduction (table 2). The shoot length reduction of Mayas Putih, Mayas Kuning and Telaseh were 41.67 %, 70.00 % and 46.67 % compared to control, respectively.

3.2. Chlorophyll and soluble sugar in the leaf

Drought stress reduced chlorophyll content in the leaf of all three rice cultivars (table 2). Among the three rice cultivars, Telaseh showed the lowest chlorophyll reduction which is significantly lower than Mayas Putih and Mayas Kuning. The total chlorophyll reduction of Mayas Putih, Mayas Kuning and telaseh were 78.00 %, 82.33 % and 60.00 % compared to control, respectively.

In contrast with chlorophyll content in the leaf, the concentration of both reducing and non-reducing sugar were increased in all rice cultivars when treated with drought (table 2). Increasing in reducing and non-reducing sugar induced by drought stress, were not significant between all rice cultivars. Reducing sugar of Mayas Putih, Mayas Kuning and Telaseh increased by 490.33 %, 452.67 % and 398.67 % when treated with 25 % PEG. For non-reducing sugar, drought stress reduced non-reducing sugar of Mayas Putih, Mayas Kuning and Telaseh by 276.00 %, 221.67 % and 330.67 % compared to control, respectively.

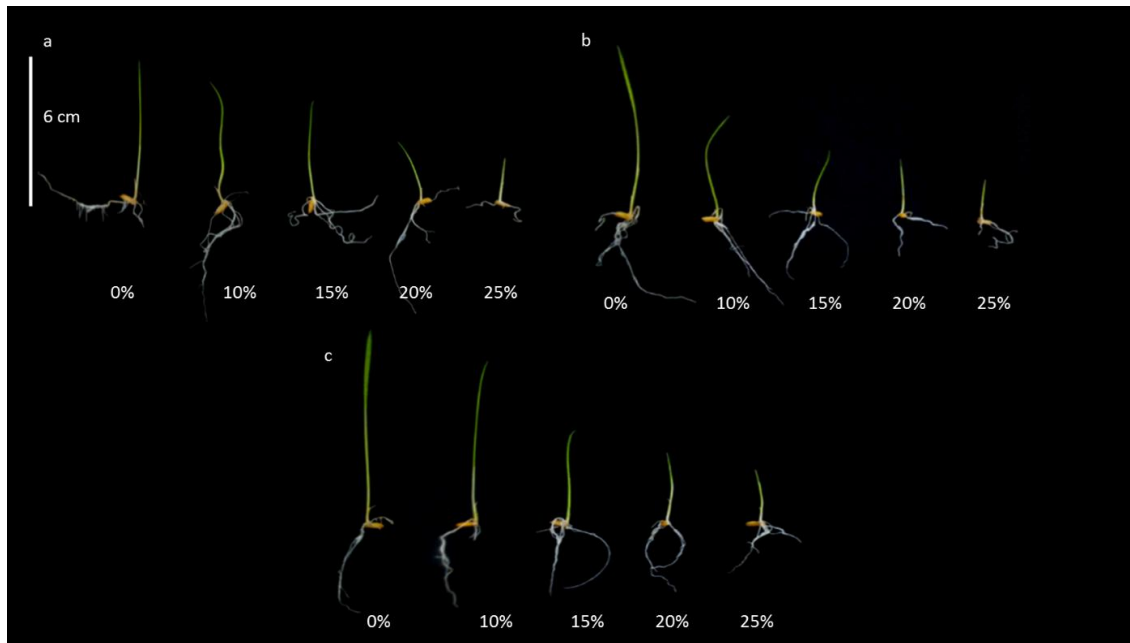


Figure 1. Morphology of rice seedling at 10 d after cultivated with various concentration of PEG 6000. a. Mayas putih; b Mayas kuning; c Telaseh.

Table 1. The reduction of shoot and root length of rice seedling after 10 d cultivated with various concentration of PEG 6000

Variety	[PEG 6000]	Percentage of reduction	
Rice	(%)	Shoot length (%) *	Root length (%) *
Mayas Putih	10	23.00 ± 2.00 ^h	34.00 ± 2.65 ^f
	15	35.33 ± 4.73 ^{fg}	56.33 ± 3.51 ^c
	20	49.00 ± 4.36 ^{cde}	59.00 ± 2.65 ^c
	25	70.67 ± 5.13 ^a	65.33 ± 1.53 ^b
Mayas Kuning	10	11.00 ± 1.00 ⁱ	41.00 ± 3.61 ^e
	15	32.67 ± 4.62 ^{gh}	46.33 ± 2.08 ^d
	20	44.00 ± 3.61 ^{def}	60.00 ± 3.00 ^c
	25	64.00 ± 5.57 ^{ab}	66.67 ± 1.53 ^b
Telaseh	10	11.00 ± 2.65 ⁱ	33.00 ± 4.00 ^f
	15	40.67 ± 3.51 ^{efg}	47.00 ± 1.00 ^d
	20	52.33 ± 5.13 ^{cd}	77.33 ± 0.58 ^a
	25	57.67 ± 4.16 ^{bc}	80.33 ± 1.53 ^a

The difference letter indicated significant different at $P < 0.05$ analyzed by Kruskal-Wallis

Table 2. The reduction of the shoot, root length, chlorophyll content, and increase in reducing dan non-reducing sugar of rice seedling after re-treated with PEG 6000 from 21 DAG to 35 DAG

Variety	Percentage of reduction (%)		Percentage of increase (%)	
	Shoot length	Total chlorophyll	Reducing sugar in the leaf	Non-reducing sugar in the leaf
Mayas Putih	4.67 ± 21.96 ^b	78.00 ± 5.20 ^a	490.33 ± 29.48	276.00 ± 225.58
Mayas Kuning	70.00 ± 3.46 ^a	82.33 ± 10.60 ^a	452.67 ± 149.35	221.67 ± 62.43
Telaseh	46.67 ± 18.56 ^b	60.00 ± 7.55 ^b	398.67 ± 287.34	330.67 ± 97.50

The difference letter indicated significant different at $P < 0.05$ analyzed by Kruskal-Wallis.

4. Discussion

Rice germination and early growth depend on the ability to absorb water from the surrounding environment. Water imbibition into the seed activated cell metabolism which produces energy to support seedling growth and development [13]. Moreover, water also important for cell elongation which needed for the shoot and root elongation in plant [14, 15]. PEG 6000 is a non-ionic molecule which commonly used to induced drought stress in the plant. The previous report showed drought stress induced by PEG 6000 inhibit shoot and root development in rice seedling [8, 16]. Similarly, the shoot and root growth of Mayas Putih, Mayas Kuning and Telaseh seedling were inhibited by drought stress produced by the application of PEG 6000 (figure 1 and table 1). The inhibition effect of PEG 6000 was increasing when its concentration was increase which means less water available leading to less growth. Moreover, a seedling of Mayas Kuning showed the highest shoot reduction and seedling of Telaseh showed the highest root reduction when treated with 25 % PEG. The variation responses of the shoot and root growth under drought stress in these three cultivars might cause by genetic variation between them.

At the early stage of seedling, rice growth predominantly using the nutrition available in seed. When the nutrition in the seed is already run out, rice grows to depend on the ability to absorb nutrient from the soil and harvesting light energy through photosynthesis. Rice has already in vegetative stage at 21 DAG. In this stage, rice completely dependent on their photosynthetic capability to produced energy for growth and development [17]. This reserach found that Mayas Kuning re-treated by 25 % PEG 600 for 14 d at 21 DAG showed significantly higher shoot growth reduction compare to Mayas Putih and Telaseh (table 2). Chlorophyll was a pigment responsible for light harvesting during photosynthesis. The previous report showed the reduction of chlorophyll content in the leaf induced by abiotic stress, such as drought [18, 19] stress, related to a reduction in plant growth. Among the three rice cultivars, Mayas Kuning showed the highest chlorophyll reduction under drought stress which is significantly lower than Telaseh (table 2). It might indicate that Mayas Kuning has the lowest ability to harvest light energy due to high chlorophyll degradation under drought stress leading to highest growth reduction.

In order to survive and still growth under drought stress, the plant must able to absorb water from the soil. The plant can continue to absorb water only when potential water in the plant lower than soil. Drought stress reduced water potential in the soil making it harder for the plant to absorb water from the soil. In order to keep water balance inside the cell, plant accumulates compatible solute to reduced water potential inside the cell [14]. Proline [20], glycine betanin [21], and soluble sugar [22] are compatible solute found in the plant. Some report showed the accumulation of soluble sugars was enhanced plant tolerance to drought stess [23, 24]. In this research was found that the concentration of reducing sugar and non-reducing sugar were increasing in the leaf of Mayas Putih, Mayas Kuning and Telaseh under drought stress (table 2). However, no significant different was found in increasing of reducing and non-reducing sugar between Mayas Putih, Mayas Kuning and Telaseh. This result indicated that Mayas putih, Mayas Kuning and Telaseh tried to protect their osmotic balance by accumulating osmotic solute such as

reducing and non-reducing sugar inside the cell. Telaseh has better growth and lowers chlorophyll reduction than Mayas Kuning, but the increasing of both reducing and non-reducing sugar were not significantly different between the two cultivars. It seems reducing, and non-reducing sugar was not the only mechanism to protect Mayas Putih, Mayas Kuning and Telaseh from drought stress.

5. Conclusion

Compare to *Oryza sativa* (L.) cv Mayas Putih and cv. Mayas Kuning, cv. Telaseh was more tolerant to drought stress. cv. Mayas Putih, cv. Mayas Kuning and cv. Telaseh used reducing and non-reducing sugar keep water balance under drought stress. The accumulation of reducing and non-reducing sugar is not the only mechanism to protect cv. Mayas Putih, cv. Mayas Kuning and cv. Telaseh from drought stress.

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