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Morphological Response of Local Cowpea Varieties of Southwest Maluku to Drought Stress with PEG 6000 Induction in the Germination Phase

Merlys Tri Novembry Tanasale[1], Ritha Lusian Karuwal[1*], Kristin Sangur[1]

Study Programme of Biology Education, Faculty of Teacher Training and Education, Pattimura University, Ambon, Indonesia
*email: rithakaruwal8@gmail.com

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Abstract. Cowpea (*Vigna unguiculata* L. Walp), commonly known as kacang merah, is a type of legume native to southwest Maluku. This area is one of the areas with limited water availability, which causes stress to the plants. Drought stress can inhibit plant morphological growth starting from germination phase to reproductive phase. Simulation of drought stress environment can use PEG 6000. This study aims to analyze the morphological response of drought stress treatment with PEG 6000 induction in the germination phase of local cowpea varieties in Southwest Maluku. The study used a completely randomized design with PEG 6000 concentration treatment on different local varieties. The research stages consisted of preparation of PEG 6000 solution according to treatment concentration, seed germination for 7 days, and measurement of germination parameters (number of seventh day sprouts, final germination percentage, root and shoot length of sprouts, wet and dry weight of sprouts). The data obtained were analyzed using ANOVA and then Duncan's test. The results showed that PEG 6000 induction affected the morphological response of each local cowpea variety in Southwest Maluku at the germination stage. Variety KM6 showed better morphological response than other varieties in all PEG 6000 concentration treatments.

Keywords: Cowpea; Drought stress; Germination phase; PEG 6000; Morphological responses

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a minor legume with high varietal diversity. This plant is found in the southwestern region of Maluku. Cowpea in this area is cultivated by the community and used as a carbohydrate supplementary food (Karuwal et al., 2021). This region has a rainfall distribution that consists of a dry season that is longer than the rainy season. Low water availability can lead to drought conditions or drought stress (Susanto & Sirappa, 2005). Drought is a condition that describes the lack of water availability for plant needs, so that plants cannot perform the process of growth, development and production optimally, both in terms of yield and plant quality (Dietz et al., 2021; Bhattacharya 2021). Drought stress can reduce yield by 30-90% under field condition (Hussain et al., 2019). The level of plant resistance to drought stress is influenced by its growth phase, starting from the germination phase to the reproductive phase (Zahra et al., 2021; Mahpara et al., 2022). Germination is the emergence and development of the radicle and hairs from the seed (Kaya and Rehatta, 2013). The germination stage is the initial stage of a plant's growth and is also a critical stage for the plant (El-Maarouf-Bouteau, 2022). For a plant to germinate, the seed must absorb approximately 50% of its weight in water to germinate. If water is not available in sufficient quantities, germination can be delayed by up to three weeks (Adi, 2011).

To determine plant resistance to drought stress can be done chemically with polyethylene glycol (PEG) (D'souza & Shegokar, 2016). According to Verslues et al. (2006), PEG is the best material to control water potential and cannot be absorbed by plants (Siaga et al., 2016). Jiang and Lafitte (2007) stated that PEG is an

inert and non-toxic chemical with a high molecular weight. At certain concentrations, PEG 6000 can induce water deficit conditions in dry soils through the activity of ethylene oxide subunit matrices capable of binding water molecules with hydrogen bonds. The use of PEG 6000 to identify drought tolerance has been widely used in legumes such as lentil, cowpea, chickpea (Singh et al. 2016; Carvalho et al., 2019; Himaja et al., 2023).

Previous research by Faidah (2013) on the effect of PEG 6000 on the viability of mung bean seeds. From the results of the study, it was reported that the effective concentration of PEG 6000 was 2.5%, the concentration was able to produce a germination percentage of 95.78%, a concurrent growth percentage of 90.44%, a sprout length of 30 cm and a dry weight of 1.3267 g. However, no research has been conducted on the drought stress resistance of local cowpea varieties with PEG 6000 induction at the germination stage. On the other hand, the Southwest Maluku community is breeding local cowpea varieties without knowing which varieties are drought tolerant. The results of this study are expected to select drought tolerant varieties in an effort to develop the cultivation of superior varieties. This study was conducted to analyze the morphological response of local cowpea varieties in Southwest Maluku to drought stress with PEG 6000 induction at the germination stage.

MATERIALS AND METHODS

This research was conducted from March to June 2023 at the Laboratory of Biology Education FKIP Pattimura University. The research design used was a complete randomized design with treatment of local cowpea varieties (KM1, KM3, KM4, KM6, KM7) and PEG 6000 concentration P0 = 0%, P1 = 3%, P2 = 5% and P3 = 7% and repeated three times. The stages in this study include:

a. Seed preparation,

In the preparation stage, cowpea seeds collected from Southwest Maluku were sterilized by soaking in 20% NaClO solution for 20 minutes, after which the seeds were rinsed three times with distilled water. In addition, the seeds were immersed in alcohol for 30 seconds and rinsed with sterile distilled water.

b. Preparation of PEG-6000 solution,

Prepare a stock solution of PEG 6000 by taking 7 g of the required 7% PEG 6000 and dissolving it in 100 ml of distilled water. This solution will be diluted to different concentrations as follows:

Table 1. Concentration of solution PEG 6000

Volume 2 (mL)	Molaritas 2 (%)	Volume 1 (mL)	Molaritas 1 (%)	Add water (mL)
100	0	0	7	100
100	3	60	7	71.42
100	5	71.42	7	60
100	7	100	7	0

c. Germination with PEG 6000 Induction.

After soaking, cowpea seeds were germinated in petri dishes covered with filter paper and treated with PEG 6000 solution at a concentration of 0%, 3%, 5%, 7%. Each petri dish was then labeled according to the treatment. The petri dishes were then placed in a closed room for 7 days.

The parameters measured were number of sprouts, final germination percentage, root length, shoot length, wet weight of sprouts, dry weight of sprouts. The data obtained were analyzed using two-way analysis of variance (ANOVA) and continued with Duncan's test at 95% significance using SPSS software version 22.

RESULTS AND DISCUSSION

The ANOVA test results showed that the treatment of PEG 6000 concentration in the germination stage of cowpea had a very significant effect on the number of sprouts, final germination percentage, root length, wet weight of sprouts, dry weight of sprouts observed. While the interaction of PEG 6000 and variation had no significant effect on shoot length. In Figures 1 and Figure 2, the number of sprouts and percentage of final germination after treatment with PEG 6000 concentration in each variety can be seen.

Compared to the control, KM1, KM6 and KM7 varieties were able to germinate by showing better germination percentage compared to other varieties. At 7% concentration, KM6 variety was also able to germinate well. Treatment with PEG 6000 in the media can reduce the germination ability of the varieties used.

The application of PEG 6000 at a concentration of 5% already shows a sufficient limit of drought tolerance, on the other hand, the treatment with PEG 6000 at a concentration of 7% experiences a drought stress that exceeds the limit of drought tolerance, so that the percentage of germination is significantly different from the control. The results of this study are in agreement with Basal et al., (2020) who found that increasing the concentration of PEG 6000 caused a decrease in the percentage of germination in soybean. This is also supported by Verslues et al., (2006) who stated that the higher the concentration of PEG, the more ethylene subunits that bind water. This makes it more difficult for the shoots to absorb water and eventually the plants experience drought stress. Ali & Eloseiri (2017) states that if there is a lack of water during the germination process, the metabolism of the seed will be disrupted, thus affecting germination.

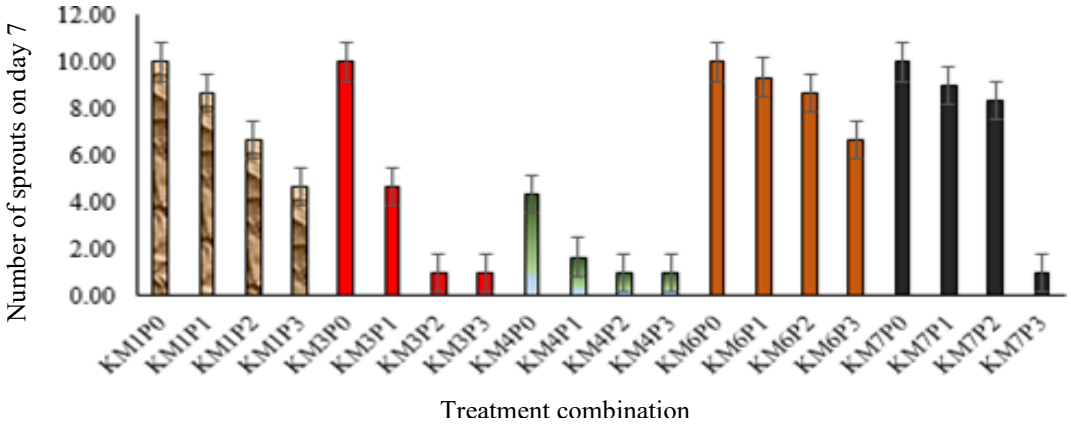

Figure 1. Average number of sprouts due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

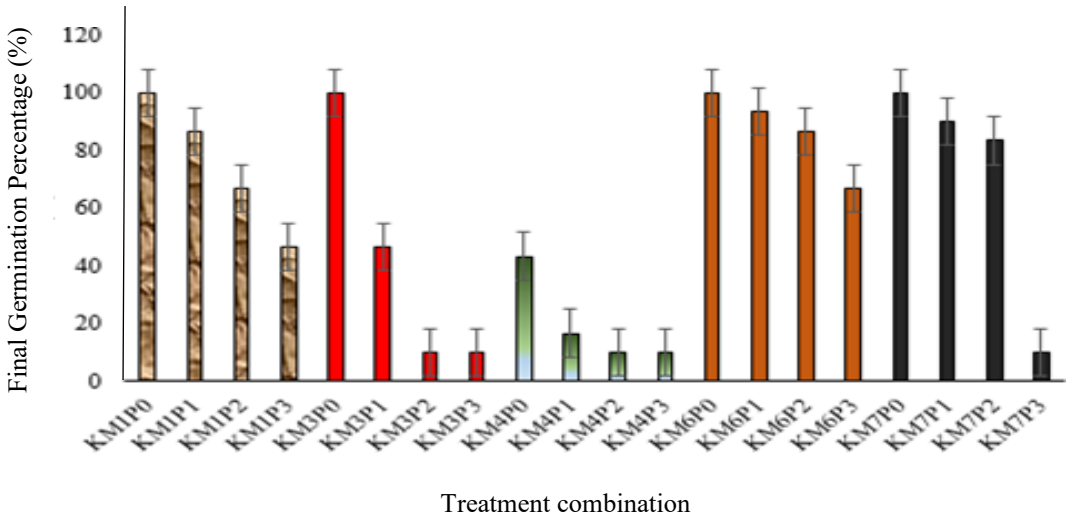

Figure 2. Average final germination percentage due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

Figure 3 shows the average root length due to drought stress with PEG 6000 induction in local cowpea varieties from Southwest Maluku. In this study, it can be seen that the higher the concentration of PEG used, shows a decrease in root length in each variety. Furthermore, it was also shown that KM1 and KM6 variety with 3%, 5% and 7% concentration treatment had more root length than other varieties. Root length is one of the criteria used to measure the morphological response of germination to drought stress (Carvalho et al., 2019). Purcell et al., (2014) explained that the root is the first structure to appear in the germination process because it functions as a nutrient absorber, water supplier, and maintains an adequate water balance in the shoots. This is in agreement with Nio et al., (2010) who reported that there was an increase in root length in rice plants under drought stress. This was also reported by Efendi (2009) who found that root length growth decreased with

increasing PEG stress. Furthermore, Verslues et al., (2006) stated that root and shoot growth decreased due to treatment with PEG 6000, which can bind water so that it is not available to plants experiencing drought stress.

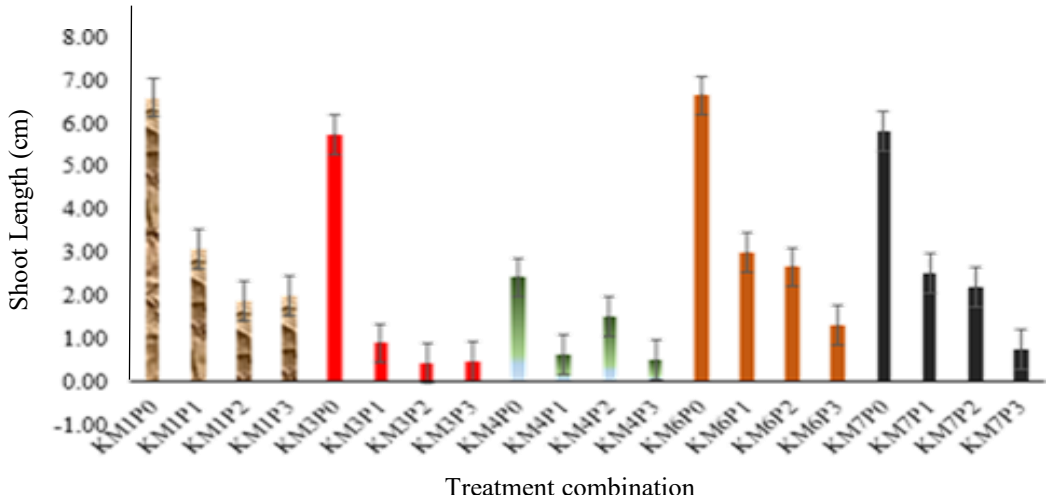

Figure 3. Average root length due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

Figure 4 shows the average shoot length due to drought stress with PEG 6000 induction in local cowpea varieties from Southwest Maluku. Similar to the root length, the shoot length also decreased with increasing concentration of PEG 6000 used. The results showed that KM6 variety had the longest shoots in all PEG 6000 concentration treatments. The results of this study are consistent with previous research conducted by Rosawanti (2015) on soybean plants, which showed that the PEG treatment had an effect on shoot length and a decrease in shoot length compared to the control. According to Indraswati (2015), when there is a lack of water, root system growth generally increases while shoot growth decreases. In this case, root growth is more dominant than shoot growth, considering the function of the root as a water absorber.

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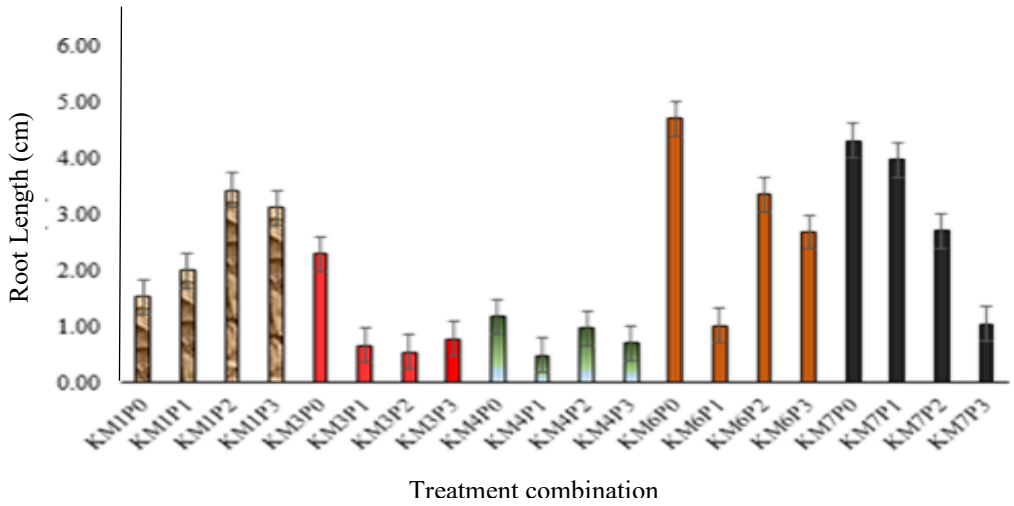

Figure 4. Average shoot length due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

Figure 5 shows the average wet weight of sprouts due to drought stress with PEG 6000 induction on local cowpea varieties in Southwest Maluku. In this study, the application of PEG 6000 to variety KM1 showed a decrease in the wet weight of the sprouts compared to the control. Seedling weight can also be used as an indicator of morphological response to drought stress. In a previous study conducted by Indraswati (2015) on

upland rice plants, both upland rice varieties studied showed a decrease in the wet weight of the sprouts. Indraswati (2015) also reported that the decrease in plant wet weight was influenced by the accumulation of water content in the sprouts, which decreased during stress. The results of other studies also showed a decrease in the wet weight of sprouts in ginger plants (Nio, 2010).

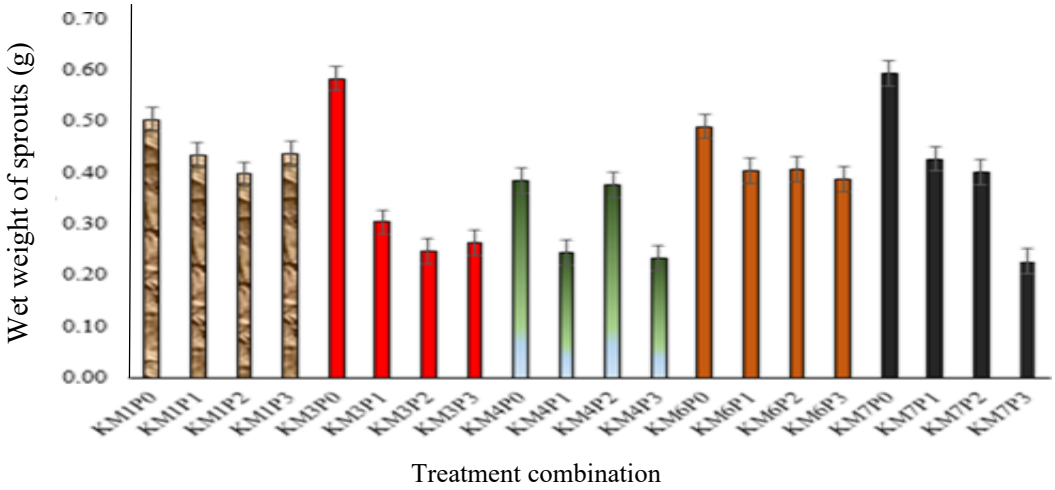

Figure 5. Average wet weight of sprouts due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

Furthermore, for the parameter of dry weight of sprouts show that in Figure 6. The study conducted by feeding PEG 6000 to KM1 and KM6 varieties showed a decrease in dry weight of sprouts compared to the control. The results of this study are in agreement with Indraswati (2015), who found a decrease in dry weight of sprouts in upland rice. The same was also reported by Effendi (2009) who found that increasing drought stress also resulted in a decrease in number of tillers, plant dry weight, and relative growth rate.

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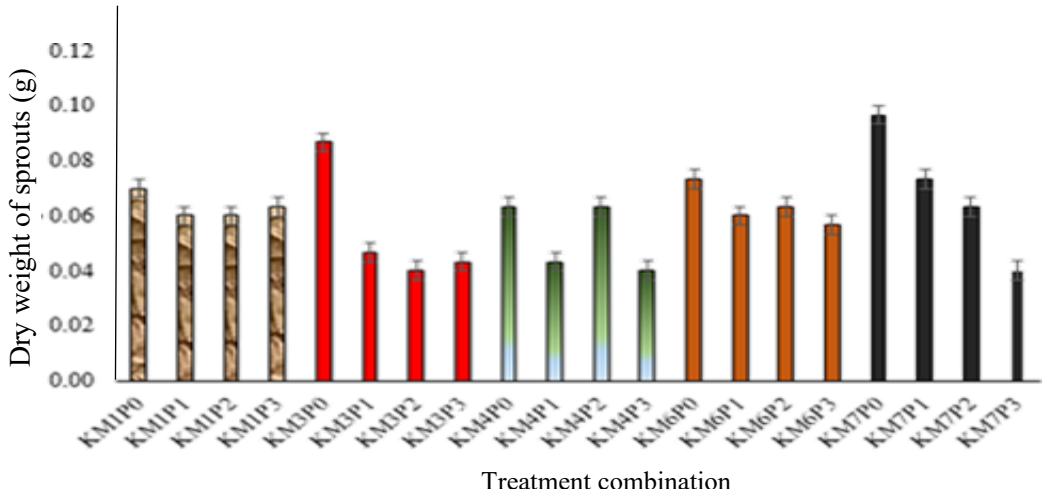

Figure 6. Average dry weight of sprouts due to drought stress with PEG 6000 induction in local cowpea varieties in Southwest Maluku

Based on all parameters measured, KM6 showed a better morphological response than other varieties in all PEG 6000 concentration treatments. This is because the morphological response to drought stress is influenced by internal and external factors. According to Peijic et al., (2013), drought stress tolerance is influenced by genotype, period and intensity of stress, and stage of plant development. Each plant variety will show different responses because it is controlled by genetic factors.

CONCLUSION

Based on the research results, it can be concluded that:

1. PEG 6000 affects the morphological response of local cowpea varieties in Southwest Maluku at the germination stage. The morphological responses differed among varieties and PEG 6000 concentrations.
2. The higher the PEG concentration applied, the more the morphological response decreases for each variety.
3. Variety KM6 showed a better morphological response compared to other varieties in all PEG 6000 concentration treatments.

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