Germination performance of selected local soybean (*Glycine max* (L.) Merrills) cultivars during drought stress induced by Polyethylene Glycol (PEG)

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Abstract. Drought stress is one of the factors that can decreased growth and production, so that required a variety that has the ability to sustain cellular metabolism, and growth during the stress. This research was aimed to investigated the involvement of germination performance *in vitro* of five local soybean cultivars, Grobogan, Kaba, Anjasmoro, Argomulyo, and Dering to drought stress induced by polyethylene glycol (PEG) 6000 (0%, 2%, 4%, and 6%). The measurable seedling traits as the day appearance of shoots and roots, total of leaves, shoot length, root length, fresh plant weight, dry plant weight, fresh root weight, and dry root weight under control as well as water stress condition were recorded. The experiment units were arranged in factorial completely randomized design with four replications. The result showed that the value for most parameters was recorded highest for Argomulyo cultivar compared with Dering cultivar which is known to be tolerant to drought. In terms of roots performance, Grobogan and Argomulyo cultivars produced the longest and heaviest of roots, while Grobogan cultivar had no significant different for root length compared with control. In conclusion, the root length and fresh weight root parameters can be used as quick criteria for drought tolerance.

1. Introduction

Drought or any other abiotic stresses constraining plant growth and comprehensive crop production seriously, also current global climate change has made this condition more serious [1]. Drought is a complex physical-chemical process, which in many biological macromolecules and small molecules are implicated, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements. Drought is connected with almost all aspects of biology needed of plants to increase the uptake of water, which is usually more available deep down in the soil [2]. At this time, drought study has been one of the main commands in global plant biology and biological breeding related to the fertile root system, ability to maintain stomatal opening and growth within their capacity at low levels of water potential.

Most of the agricultural plants, including soybean, rice, maize, barley, oats, sorghum, and wheat, tolerate even the most transient water stress. Stronger seed germination and seedling growth under drought conditions for requires above-normal tolerance of the low-level water conditions for the improvement of direct seeding cultivation. In rain fed agricultural conditions, low-level water conditions may prove to be a critical constraint to crop growth in the soil detrimental. The amount of
water used by a crop is closely associated with the photosynthetic activity, dry matter production and yield in many species [3].

The use of polyethylene glycol (PEG) as a screening agent has been widely used for plantsdrought screening [4], such as Wheat [5], Sorghum [6], Maize [7], Tomato [8], and Sunflower [9]. PEG is a chemical drought-induced molecules, can be used to reduce the osmotic potential of water and nutrient solution culture without causing physiological damage and maintain the condition during the experiment periods. According to Sirait [10], in vitro screening genotypes for abiotic stress (drought) resistance properties has several advantages, such as shorter selection times, requires less space, ease of handling, less laborious, and is not limited by seasons. Furthermore selection for drought tolerance at early stage of seedling using PEG traits is a potentially cheaper method than conventional planting. Seedling period is often considered to be less consistent when facing the low-level water effect in the dry soil areas during the dry season, also for the selected genotypes to study in detail on water stress on plant germination indices [11].

The present investigation was carried out to find out appropriate criteria for screen out of soybean cultivars that have higher drought resistant and to study the morpho-physiological changes of soybean seed germination in vitro under osmotic stress.

2. Materials and methods
The study was conducted using five selected soybean genotypes on the basis of their tolerance of drought. All those genotypes were provided by Indonesian Agricultural Research Centre, Malang, Indonesia. Drought stress was simulated by polyethylene glycol (PEG) with the molecular weight (MW) 6000 (Merck Schuchardt OHG, Germany). Mature Seeds were dehusked and surface washed with 70% (v/v) ethanol for 2 min followed by 40% (v/v) sodium hypochlorite for another 20 min shaking. After rinsing five times with sterile distilled water, the sterilized seeds were placed and allowed to germinate on in vitro solidified basal Murashige and Skoog (MS) medium [12]. Osmotic stress in vitro was simulated by adding PEG to MS medium in 2% w/v, 4% w/v, and 6% w/v concentrations. The pH of the media was set to 5.8 prior to autoclaving at 121°C for 25 min. The cultures were incubated at 25°C ± 2°C under artificial lightening to have photoperiod of 16 h.

About 7 days seed germinating, the day appearance of shoot and root were recorded. Seeds were considered as germinated if they exhibited radicle extension by more than 3 mm. The experiment was terminated by harvesting seedlings after 4 weeks and amount of leaves (AL), shoot length (SL), root length (RL), fresh weight shoot (FWS), fresh weight root (FWR), dry weight shoot (DWS), and dry weight root (DWR) of five soybean cultivars in four replication were recorded experimentally. Fresh shoot or root weight was measured on digital analytical balance (Sartorius, Germany), while dry shoot or root weight was obtained after drying the sample by putting shoots and roots in paper bags separately for 72h in the oven at 70°C for constant dry weight.

3. Results and discussion
Seedling development under laboratory conditions has been accepted as suitable growth stages for testing drought tolerance in soybean. The effect of osmotic stress induced by polyethylene glycol (PEG) on the day appearance root and shoot of germinated seeds of five selected soybean cultivars is presented in Fig. 1A and B. Most of the cultivars had longer time progressively for their appearance root or shoot compared to control condition, except for Grobogan, Kaba and Argomulyo cultivars which showed no significant different for the appearance shoot of their germinated seeds (Fig. 1B). Usually, the drought tolerance genotype has the highest germination rate and better survival. In this experiment, Grobogan cultivar seemed to have the fastest germination rate. The faster germination rate (in term of the day of appearance root and shoot) may be due to the capability of the seed to the increased diffusivity of seed coat to water even under osmotic stress condition. Turk et al. [13] reported that drought stress at germination stage delayed or hinder the emergence of the radicle and further germination completely. However, once the grain attains a critical level of hydration it
will lead to full germination. The earlier seed forms the roots/shoot, so it can be provided energy from photosynthesis for growth and root formation at later stages [14].

![Day Appearance Root (A)](image1)

![Day Appearance Shoot (B)](image2)

**Figure 1.** The day appearance root (A), the day appearance shoot (B) of soybean cultivars at different osmotic conditions on MS medium. Values are mean ± standard deviations based on four replications

Total of leaves was also measured, which considered as the fully open leaves. A number of leaves during osmotic stress showed different profiles depending on cultivars (Fig. 2). Cultivar grobogan at osmotic condition maintained the higher amount of leaves until 6% PEG when compared with control condition (0% PEG). A marked increased in total of leaves was observed in Argomulyo cultivar in 2% PEG, and Anjasmoro cultivar in 4% PEG compared to control (Fig. 2). Reduction in leaves perhaps due to less water absorption and decreased in external osmotic potential by PEG [15]. Grobogan cultivar showed maintain higher total of leaves that maybe it could preserve their higher leaf water-potential in the stressed environments.

![Total of Leaves](image3)

**Figure 2.** Total of leaves of soybean cultivars at different osmotic conditions on MS medium. Values are mean ± standard deviations based on four replications

The highest percentage reduction on shoot length (SL) was noted at Grobogan cultivar (32.5%), followed by Anjasmoro (31.7%), Kaba (27.0%), Dering (26.5%), and Argomulyo (5.7%) cultivars.
due to drought stress (2% PEG) in vitro MS medium (Fig 3.). The results suggested that Argomulyo cultivar was least affected by drought stress compared with other cultivars, and showed no significant different between control and osmotic stress in 2% PEG, and after that, the SL was decreased in 4% and 6% PEG.

Root length (RL) also decreased with increasing of PEG concentration, except for Grobogan cultivar (Fig. 5). At 2%, 4%, and 6% of PEG, the RL in Grobogan cultivar was slightly similar with the control (0% PEG). RL was decreased at 2% PEG, and after that the RL was increased at 4% PEG for Anjasmoro and Argomulyo cultivars.

**Figure 3.** Shoot length of soybean cultivars at different osmotic conditions on MS medium. Values are mean ± standard deviations based on four replications

**Figure 4.** Root length of soybean cultivars at different osmotic conditions on MS medium. Values are mean ± standard deviations based on four replications

All fresh shoot weight (FSW) of the soybean cultivars were decreased with the increasing concentration of PEG. Osmotic stress caused significant reduction in FSW for all cultivars, with the highest decreased at Dering cultivar (56.3%), followed by Kaba cultivar (38.2%), Argomulyo cultivar (37.9%), Anjasmoro cultivar (32.6%), and Grobogan cultivar (14.3%) (Fig. 5A).

There was no significant decrease in dry shoot weight (DSW) detected for Grobogan, Kaba, and Argomulyo cultivars compared to control at 2-6% of PEG treatment (Fig. 5B). However, Dering cultivar which is known as one of tolerant for drought stress, had significant different in DSW at 2% PEG. Osmotic stress negatively affects photosynthesis and carbohydrate production, resulting in growth disturbances [16]. The decrease of dry matter was noted in many plant species under drought conditions [17]. Some authors believe that dry matter increases in plants during water stress may
indicate tolerance to drought. In the presented experiments, DSW of Grobogan, Kaba, and Argomulyo cultivars were no significant different with control (Fig. 5B). Our results revealed that those of cultivars improved to persistent and use the limitation of their surroundings such as water, nutrients supply, to be alive under stress.

Fresh root weight (FRW) in osmotic condition had shown significant differences decreased for all soybean cultivars, except for Grobogan cultivar relative to non osmotic condition control (Fig. 6A). Kaba, Argomulyo, and Anjasmoro cultivars had decreased of their FRW at 2% PEG by 83.4%, 78.7%, and 75.9%, respectively, relative to control.

There were significantly decreased in dry root weight (DRW) for all cultivar in osmotic conditions (Fig. 6B). Among soybean cultivars, Argomulyo has shown low reduction in DRW. Fresh and dry root weight were decreased due to osmotic stress in soybean cultivars, except for Grobogan cultivar. Similar results were reported by Savitri [18]. Water uptake by the root is a complex parameter that depends on root anatomy and the pattern by which different parts of the root contribute to overall water transport [19].

The cultivar which is showing better performance can be considered as drought tolerant. The current study showed that cultivars grobogan and Argomulyo found better growth than others, such as root length, fresh weight root and dry weight root (Fig. 7). In conclusion, these three characteristics of *in vitro* development are possible candidates for criteria in developing models and quick criteria for preliminary screening of drought tolerance. In addition to other physiological components involved in the seed germination process under drought stress conditions, may be considered for breeding purposes.
Figure 6. Fresh root weight (A), dry root weight (B) of soybean cultivars at different osmotic conditions on MS medium. Values are mean ± standard deviations based on four replications.

Figure 7. Root performance of Grobogan (A) and Argomulyo (B) soybean cultivars in vitro condition on MS medium subjected to 0, 2%, 4%, and 6% PEG.

4. Conclusions
Root length, fresh weight root and dry weight root are possible candidates for criteria in developing models and quick criteria for preliminary screening of drought tolerance. In addition to other physiological components involved in the seed germination process under drought stress conditions, may be considered for breeding purposes.

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