

Induction of drought resistance in bell pepper (*Capsicum annuum* var. *grossum*) with osmopriming Polyethylene Glycol (PEG) 4000

ARSITA PRELLIA, SOLICCHATUN*, ARI PITOYO

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, *email: solichatun@staff.uns.ac.id

Manuscript received: 1 December 2022. Revision accepted: 11 February 2023.

Abstract. Prellia A, Solichatun, Pitoyo A. 2023. Induction of drought resistance in bell pepper (*Capsicum annuum* var. *grossum*) with osmopriming Polyethylene Glycol (PEG) 4000. *Asian J Agric* 7: 34-46. The bell pepper (*C. annuum* var. *grossum*) is an economically valuable chili cultivar. However, in Indonesia, the cultivation of bell peppers is hampered by drought stress. Osmopriming is an alternative method to improve seed quality. That method was used by soaking the seeds in osmotic solutions, with Polyethylene Glycol (PEG) as the solution. This research examines the effect of osmopriming with PEG 4000 on the germination and growth of bell pepper seedlings under various drought stressors. In 2020, seeds were collected from ripe bell peppers grown by farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, Indonesia. This study employed a Completely Randomized Design (CRD) with two parameters: PEG concentration and water capacity variations. The concentrations of PEG 4000 utilized were 0, 50, 100, and 150 g/L. The drought stress test is conducted by cultivating primed bell pepper seedlings in planting media with varying water capacities of 100%, 75%, and 50% Space Capacity (SC). Included in the drought group with a moderate stress level is a water capacity of 50% SC. Each treatment was replicated three times. Under drought stress, osmopriming with PEG 4000 accelerated the germination rate and affected seedling growth regarding height, number of leaves, leaf area, wet seedling weight, and shoot-root ratio. However, under drought stress, osmopriming PEG 4000 had no significant effect on the rise in proline and chlorophyll content nor on the decrease in carotenoids and nitrate reductase activity. PEG 150 g/L with 50% SC was ideal for seedling height, leaf number, leaf area, and wet weight, while PEG 100 g/L with 100% SC was optimal for the shoot-root ratio.

Keywords: *Capsicum annuum* var. *grossum*, drought, osmopriming, polyethylene glycol

INTRODUCTION

Among Indonesia's many valuable agricultural products is bell pepper (*Capsicum annuum* L.). Badan Pusat Statistik (2018) reports that after reaching a low point in 2017 with a production of 7.4 thousand tons, bell pepper output increased to 18.15 thousand tons in 2018. Overall output rose by 10.75 kilotons from 2017 to 2018. It demonstrates that bell pepper has a significant demand amongst the general populace of Indonesia. Bell pepper is a valuable commodity because to the fact that it can be utilized both fresh and dry, making it ideal for a wide variety of culinary applications. Warsi (2013) claims that bell peppers are a valuable commodity due to their high vitamin content, antioxidant properties, and versatility in food processing. These numerous advantages bolstered bell pepper's already significant value as a strategic item in the national economy. Bell pepper has enormous potential for growth in Indonesia as there is now a gap between supply and demand, despite its widespread cultivation in subtropical and tropical regions (Arifianto and Kartika 2018).

Several problems, such as plant disease disturbances and environmental stress conditions, continue to limit the success of bell pepper farming in Indonesia. Chili yields are low because of the long dry season, which also contributes to dryness on farmland (Yusniwati et al. 2008). The pepper plant, related to chilies, is very drought- and heat-sensitive and sensitive to light (Aminifard et al. 2010;

Tulung and Demmassabu 2011). The rate of growth, maturity and biomass accumulation of farmed plants can all be altered by drought stress.

Seed priming is an alternate method for enhancing seed quality that involves preparing the seeds before planting. Seed priming can prepare seeds to germinate concurrently, grow better, adapt to adverse environmental conditions such as drought, and be more resistant to disease attacks (Lutts et al. 2016). The widespread use of seed priming in horticulture crops is due to its ability to boost crop yields. For example, applying seed priming to bell pepper seeds is anticipated to optimize bell pepper plant growth and induce drought tolerance.

There are various methods for priming seeds, osmopriming being one of them. The seeds are soaked in an osmotic solution, such as sugar, glycerol, sorbitol, mannitol, or Polyethylene Glycol (PEG), for osmopriming (Lutts et al. 2016). It is known that using PEG for seed priming has increased simultaneous germination and induced tolerance to environmental stress. Sorghum seeds (*Sorghum bicolor* L. Moench), soybean seeds (*Glycine max*), wheat seeds (*Triticum aestivum* L.), and rice seeds (*Oryza sativa* L.) have all been successfully induced to develop drought stress resistance (Eivaz 2012; Syaiful et al. 2014; Salah et al. 2015; Zhang et al. 2015).

Plant morphological, anatomical, and physiological responses to drought can be considered (Kasi et al. 2017). Plants respond physiologically to drought stress by

accumulating proline molecules, which operate as osmoregulatory and osmoprotectant chemicals for cell membranes. Compared to optimal environmental conditions, the proline content of plant leaves increased when plants were exposed to drought (Yusniwati et al. 2008). Due to a loss in chlorophyll and an increase in secondary metabolites, drought stress also disturbs the metabolic activity of plants (carotenoids). Drought can also reduce Nitrate Reductase Activity (NRA) by interfering with the absorption of nitrogen fertilizers. Nitrate reductase enzymes contribute to the assimilation of nitrate, which influences plant development and yield. When plants were subjected to drought conditions, nitrate reductase activity decreased relative to optimal environmental circumstances (Fitriana et al. 2011). The nitrate reductase activity (NRA) can be employed as a plant selection measure since it correlates positively with production, dry weight, total nitrogen, and plant yield.

The application of osmopriming to bell pepper seeds to generate drought tolerance has not been performed. Therefore, it has not been as widespread as it has been with other varieties of chilies. The prior studies employed PEG with molecular weights of 6000 and 8000; however, PEG 4000 has not been utilized extensively (Syaiful et al. 2014; Yuanasari et al. 2015; Zhang et al. 2015). Furthermore, the application of PEG 4000 on seeds, followed by their cultivation under varying drought stress circumstances, is also new knowledge uncovered by researchers. Therefore, reviewing growth characteristics is necessary to identify the response of bell pepper plants to drought stress following osmopriming with PEG 4000, given this context.

Given the above context, the problem is stated as follows: (i) Does osmopriming with PEG affect the germination rate of bell pepper (*C. annuum*) seeds? (ii) Does osmopriming with PEG influence bell pepper (*C. annuum*) seedlings' growth under drought stress? (iii) Does osmopriming with PEG affect the levels of chlorophyll, carotenoids, proline, and Nitrate Reductase Activity (NRA) in bell pepper seedlings (*C. annuum*) under drought stress? (iv) What is the appropriate concentration of osmopriming and PEG treatment for bell pepper (*C. annuum*) seedling growth under drought stress? Therefore, based on the definition of the problem above, the goal of this study is as follows: (i) Determine the effect of osmopriming with PEG 4000 on bell pepper germination rate (*C. annuum*). (ii) Determine the effect of osmopriming with PEG on the growth of bell pepper (*C. annuum*) seedlings under conditions of drought stress. Determine the effect of osmopriming with PEG on the levels of chlorophyll, carotenoids, leaf proline, and activity of the enzyme nitrate reductase in bell pepper seedlings (*C. annuum*) subjected to drought stress. (iii) Determine the appropriate concentration of osmopriming and PEG treatment on the growth of bell pepper (*C. annuum*) seedlings under drought stress.

MATERIALS AND METHODS

Time and place

This research was carried out in October 2020-April 2021 in the greenhouse of the Integrated Laboratory Technical Implementation Unit, the Biology Laboratory, and the Universitas Sebelas Maret (UNS) Integrated Laboratory of the UNS, Surakarta, Central Java, Indonesia.

Materials and tools

Seeds of bell pepper (*C. annuum* var. *grossum*) were employed in this investigation. The seeds came from farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, Indonesia, in the harvest year 2020. Red pepper seeds were chosen for regeneration because they were mature and ready to be used. The chemicals used included PEG 4000 solution with concentrations of 0, 50, 100, and 150 g/L, 1% tetrazolium, 80% acetone, distilled water, Merck's standard proline compound, 3% sulfosalicylic acid, 0.14 M ninhydrin acid, glacial acetic acid, toluene 99.5%, phosphate buffer with pH 7.2, sodium nitrate (NaNO₃) 5 M, N-[1-naphthyl]-ethylenediamine (NED) 0.02%, 1% sulfanilamide acid, hydrochloric acid (HCl) 3 N. The planting medium was a mixture of alluvial soil and manure (2:1).

Germination cups (pot trays), petri dishes, measuring scales, scissors, razor blades, plastic clips, measuring cups, beakers, stir sticks, pH paper, micropipette, tweezers, mortar, pestle, glass funnel, test tube, Whatman filter paper, water bath, stereo microscope, analytical balance, cuvette, UV-VIS spectrophotometer (Perkin Elmer Lambda 25), and vortex mixer are utilized.

Research design

This research is experimental in the laboratory and greenhouse. The research design used was a Completely Randomized Design (CRD) with two factors, namely variations in PEG 4000 osmopriming (0, 50, 100, and 150 g/L) and variations in water availability (100, 75, and 50% space capacity). Based on these two factors, 12 treatment combinations were obtained. Each treatment was carried out in 3 repetitions. The following is the treatment in this study. The combination of treatment of the two factors is presented in Table 1.

Table 1. Combinations of PEG osmopriming variations and space capacity percentages

Space Capacity Variation	PEG 4000 concentration variations			
	P0	P1	P2	P3
C0	P0C0	P1C0	P2C0	P3C0
C1	P0C1	P1C1	P2C1	P3C1
C2	P0C2	P1C2	P2C2	P3C2

Note: P0 = PEG 0 g/L, P1 = PEG 50 g/L, P2 = PEG 100 g/L, P3 = PEG 150 g/L; C0 = 100% Space capacity, C1 = 75% Space capacity, C2 = 50% Space capacity

Procedure

Preparation of bell pepper seeds

Pepper seeds were obtained from farmers in Surjo Hamlet, Sukabumi Village, Cepogo Sub-district, Boyolali District, Central Java, in 2020. The seeds come from ripe bell peppers and are ready for regeneration. Peppers are split, so the seeds are visible and then dried in indirect sunlight. The dried seeds are threshed from their sockets and then selected as reasonable and uniform (color and seed size are uniform).

Osmopriming treatment

PEG 4000 solution was made in four concentration variations, namely 0, 50, 100, and 150 g/L. Bell pepper seeds are soaked in various solutions for 24 hours. Afterward, the seeds are drained in a container with a paper towel and left to air dry for 24 hours (Latifa and Rachmawati 2020). The dried seeds were then stored for the germination test.

Tetrazolium test

A preliminary test is to determine the initial viability of seeds using the tetrazolium test. The preparation of tetrazolium solution begins with a phosphate buffer solution of 9.078 grams of potassium dihydrogen phosphate (KH_2PO_4) dissolved in 1000 mL of distilled water (solution I). Then 11.876 grams of disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) dissolved in 1000 mL of distilled water (solution II). Next, 400 mL of solution I and 600 mL of solution II (2:3)(v/v) were mixed homogeneously. Finally, ten grams of tetrazolium salt (2,3,5 triphenyl tetrazolium chloride) was added to 1000 mL of buffer solution to make a 1% tetrazolium solution (Zanzibar and Herdiana 2010).

The tetrazolium test was carried out by soaking ten bell pepper seeds in distilled water for one day and one night (24 hours). After 24 hours, the seeds were split into two parts. One part was soaked in a 1% tetrazolium solution of approximately 20 mL for 24 hours at room temperature ($\pm 28^\circ\text{C}$), and the other was in the dark. Seeds whose embryonic parts and cotyledons are pink to dark red are counted as viable seeds (Varela and Albornoz, 2013). The tetrazolium test was carried out in 3 repetitions. Seeds treated with osmopriming were tested for viability again with the tetrazolium test. The percentage of seed viability is calculated using the following formula (Subantoro and Prabowo 2013).

$$\text{seed viability} = \frac{\text{number of viable seeds}}{\text{total number of seeds}} \times 100\%$$

Germination test

Seeds that had been treated with osmopriming were germinated on filter paper media. A total of 30 seeds were imbibed in distilled water for 12 hours. The seeds were then germinated in a germination cup that had been lined with filter paper. Each cup contains ten seeds, so there are three replications. The filter paper was moistened with as much as 15 mL of distilled water. Germination incubation

was carried out at room temperature for two weeks. Watering with distilled water and the same volume is done every day until the end of germination. Observations were made every day on the time the sprouts appeared, and then the germination percentage and rate were calculated. At the end of germination, the seeds were weighed to determine the wet weight of the sprouts. Seeds are said to have germinated when radicles appear at least 2 mm long (Pramana et al. 2019). The formula for calculating germination percentage and rate is as follows (Sutopo 2002; Lesilolo et al. 2013).

$$\text{germination (\%)} = \frac{\text{number of germinated seeds}}{\text{number of seeds germinated}} \times 100\%$$

$$\text{germination rate (days)} = \frac{N1T1 + N2T2 + \dots + NxTx}{\text{number of germinated seeds}}$$

$$\text{germination speed index} = \frac{N1}{T1} + \frac{N2}{T2} + \dots + \frac{Nx}{Tx}$$

Where:

N = number of seeds that germinate in a particular time unit

T = The time between the test's start and the end at a specific observation interval.

Treatment of drought stress

Drought stress treatment with variations of water stress 100%, 75%, and 50% Space Capacity (SC) lasted for 13 weeks. Space capacity was determined using the gravimetric method. The planting medium is a mixture of ground and manure with a ratio of 2:1.

Seeds treated with the priming variation were sown in the media with 100% watering at Space Capacity (SC) for 14 days beforehand. Seedlings with uniform size were then transferred to polybag media with soil moisture content according to treatment. Seedling uniformity is based on the height and number of seedling leaves. Next, the polybags were placed in greenhouse and watered every two days with the volume according to the planned treatment. After 13 weeks, growth was observed in morphological and physiological parameters.

Determination of space capacity used the appropriate gravimetric method from Swibawa and Oktarino (2010). This method is carried out by weighing the initial weight of the media (W_o) and then pouring the media with water until it is saturated (all the pore space of the media is filled with water) and left for 24 hours or until the water stops dripping from the polybag. After the water has stopped dripping, the media is weighed again to determine the final weight (W_a). The water content for 100% space capacity (W_t) is determined by subtracting the final weight of the media from the initial weight of the media. Space capacities of 75% and 50% are determined by:

$$\text{Water content to be added} = \% \text{ SC required} \times W_t$$

Observation of pepper plant growth

Seedling growth parameters. The growth of pepper seedlings was observed at the end of the drought stress treatment when the plants were 13 weeks old. Growth parameters observed included plant height, fresh weight of seedlings, root crown ratio, leaf area, and the number of leaves.

Measurement of plant height. Plant height is measured from the base of the stem above the soil surface to the apical growing point. (ii) Root canopy ratio is the ratio between the wet weight of the upper part of the plant (leaves, stems) and the lower part of the plant (roots). The formula for measuring the crown root ratio is as follows (Effendi 2008):

$$\text{root crown ratio (gram)} = \frac{\text{crown wet weight}}{\text{root wet weight}}$$

Measurement of leaves. Leaves measured in width are leaves that have opened completely. Leaf area measurement uses the gravimetric method and is calculated using the following formula (Irwan and Wicaksono 2017).

$$\text{leaf area (cm}^2\text{)} = \frac{\text{leaf replica paper weight}}{\text{total paper weight}} \times \text{total paper area}$$

Physiological parameters. The measured physiological parameters were chlorophyll, carotenoids, leaf proline levels, and the enzyme nitrate reductase.

Measurement of leaf proline content. Analysis of total leaf proline content was carried out using Bates et al. (1973). Fresh leaves (2nd to 3rd leaves from shoots) as much as 0.1 gram, cleaned, then mashed and homogenized with 5 mL of sulfosalicylic acid (3% w/v). After centrifuging at 5000xg for 15 minutes using a centrifuge, 2 mL of the supernatant obtained was taken and reacted with 2 mL of 0.14 M ninhydrin acid (C₉H₆O₄) solution (1.25-gram ninhydrin composition) and added with 2 mL of Glacial Acetic Acid (GAA) then heated using a water bath at 100°C for 60 minutes.

The test tube containing the supernatant that has been heated is put into a beaker filled with ice cubes for 5 minutes. The sample is mixed with 4 mL of toluene (99.5%) and vortexed for 15-20 seconds until two layers of liquid with different colors are formed. The red toluene-containing proline was taken, and the absorbance value was read with a UV-VIS spectrophotometer at 520 nm. The blank solution is toluene. L-proline (Sigma) dissolved in sulfosalicylic acid (3% w/v) was used as standard.

Measurement of total chlorophyll and carotenoid content of bell pepper leaves. Measurement of leaf chlorophyll and carotenoid content was carried out at the end of the observation. The content of chlorophyll and carotenoids was measured by cleaning fresh leaf samples and weighing 0.1 grams. The sample is mashed with a mortar and pestle. Once smooth, the sample is extracted with 10 mL of 80% acetone, then stirred until the color is released from the tissue. The leaf extract is filtered with filter paper. The filtrate obtained was put into a cuvette of 3 ml. The content of chlorophyll and carotenoids was

analyzed using a UV-VIS spectrophotometer at wavelengths of 480 nm, 645 nm, and 663 nm with a blank of 3 mL of 80% acetone (Kurniawan et al. 2010). This method was carried out for each treatment sample. The chlorophyll content was calculated using the equation from Hendry and Grime (1993), as follows (Anggarwulan and Solichatun 2007):

$$\text{Chlorophyll a (mg/g F.W)} = (12.7 \times A663) - (2.69 \times A645) \times 10^{-1}$$

$$\text{Chlorophyll b (mg/g F.W)} = (22.9 \times A645) - (4.68 \times A663) \times 10^{-1}$$

$$\text{Total chlorophyll (mg/g F.W)} = (8.02 \times A663) + (20.2 \times A645) \times 10^{-1}$$

The calculation of carotenoid content using the equation according to Gayathri et al. (2016) is as follows:

$$\text{Carotenoids (mg/g F.W)} = A480 + (0.114 \times A663) + (0.638 \times A645)$$

Where:

A663 = Absorbance value at a wavelength of 663 nm

A645 = Absorbance value at a wavelength of 645 nm

A480 = Absorbance value at a wavelength of 480 nm

FW = Fresh weight (wet weight of leaves)

The nitrate-reductase activity measurement. Nitrate reductase activity was measured using the first to third leaf samples from shoots of 0.25 g. The leaves were cut into small pieces and put into a dark bottle containing 5 mL phosphate buffer solution (pH 7.2-7.5) for 24 hours. After 24 hours of incubation, the phosphate buffer solution was replaced with 5 mL of new phosphate buffer solution, and 0.1 mL of 5M NaNO₃ was added as a substrate, then incubated for 2 hours (after this, the sample was called an aliquot). Finally, 0.1 mL aliquots were put into a test tube containing the coloring reagent in the form of 0.2 mL of 1% sulfanilamide acid in 3N HCl and 0.2 mL of 0.02% N-Naphtylethylen diamine (NED). Then, wait for the reaction until a pink color change occurs (10-15 minutes) as a sign of nitrate reduction to nitrite by the enzyme nitrate reductase. After a color change, 5 mL of distilled water was added and homogenized. Sample absorbance was measured using a UV-VIS spectrophotometer at 540 nm. The blank solution is a mixture of coloring reagents with a ratio of 1:1. Nitrate reductase activity (NRA) is calculated using the following formula (Latifa and Anggarwulan 2009; Putra et al. 2020).

$$\text{NRA } (\mu\text{mol NO}_2^-/\text{g/h}) = \frac{\text{sample absorbance}}{\text{absorbance standard}} \times \frac{100}{\text{WW}} \times \frac{1}{\text{IT}} \times \frac{50}{100}$$

Where:

Absorbance standard = 0.0142

WW = wet weight of the sample (grams)

IT = incubation time (hours)

Data analysis

Quantitative data were analyzed by two-way ANOVA (Analysis of Variance), followed by one-way unstacked

ANOVA, and then tested by Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Tetrazolium test

Seed viability testing was carried out using the tetrazolium test method on the treated seeds. This method is called the quick test because the seeds tested can be analyzed quickly and do not need to be germinated. Good viability (live seeds) seeds are marked with a red pattern embryo and cotyledons after soaking for 24 hours with 1% tetrazolium solution Figure 1). The tetrazolium test was carried out on seeds that had not been treated (the control) and Polyethylene Glycol (PEG) 4000-treated seeds with concentrations of 50, 100, and 150 g/L, with three replications each. The results of the tetrazolium test yielded a seed viability percentage value of 100% for all control and treated seeds. These results indicate that all seeds are still viable (alive) and can germinate and grow. Therefore, giving PEG 4000 treatment cannot affect seed viability.

The test uses tetrazolium salt (2,3,5 triphenyl tetrazolium chloride) as an indicator to detect the presence of living cells. Every living organism or tissue performs respiration and produces hydroxide gas (H_2) with the help of dehydrogenase enzymes. The dehydrogenase enzyme activity will release H^+ ions and react with tetrazolium, which was initially colorless and reduced to a red precipitate called formazan. Therefore, viable seeds absorb tetrazolium and appear red (Copeland and McDonald 2001; Warid and Palupi 2009). The nature of PEG only lowers the osmotic potential of the water in the media and the compound because it has a large molecular weight and does not get absorbed into the tissues or seed embryos, so it does not poison the seeds. Based on this description, applying PEG is safe for seeds and does not affect seed viability.

Germination test

Pepper seeds that had been soaked in PEG 4000 solution according to the treatment were germinated for 14 days. Seeds are germinated when radicles appear at least 2 mm long (Pramana et al. 2019). The seed germination process starts from the process of water absorption by the seed (imbibition). Imbibition occurs in three phases. Phase I begins with rapid water absorption due to the potential difference between the water and the seeds. In phase II, imbibition is slow because there is a potential water balance in the seed with its environment, and seed metabolism is actively taking place. Finally, in phase III, the rate of imbibition increases again because the growth and development of the sprouts are ongoing, which begins with the appearance of the radicle (Yuanasari et al. 2015; Ernita and Mairizki 2019). Table 2 shows the average number of sprouts that appeared each day. It was found that the PEG 100 and 150 g/L treatments on the first day had germinated seeds, while the control treatment sprouts

started appearing on the 4th day. The highest number of sprouts in the control treatment appeared on the 7th day. In the PEG 50 and 100 g/L treatments, the highest number of sprouts appeared on 4th day, and in the 150 g/L PEG treatment, the highest number of sprouts appeared on 5th.

In Table 3, it was found that the PEG 4000 control, 50, 100, and 150 g/L treatments produced a 100% germination percentage of bell pepper seeds with a different wet weight for each sprout. The ANOVA results showed that the PEG 4000 treatment significantly affected the fresh weight of each sprout. The 50 g/L PEG treatment produced the heaviest sprouts of 0.0575 g, which was not significantly different from the control and 100 g/L PEG treatments but significantly different from 150 g/L PEG, the lowest germination weight of 0.0414 g. These results could imply that the PEG 50 g/L and 100 g/L treatments increased the wet weight of the sprouts compared to that without the PEG 4000 osmopriming treatment. Therefore, the sprouts' wet weight reflects the sprouts' structure growing normally and optimally.

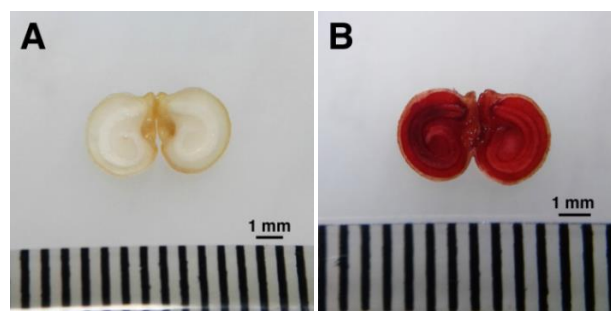


Figure 1. Morphology of cross-sectional peppercorn seeds through a stereo microscope at 1x magnification. Before soaking in 1% tetrazolium solution (A). After soaking in 1% tetrazolium solution (B)

Table 2. Daily germination of pepper seeds

Day	The number of sprouts that appear			
	PEG 0 g/L	PEG 50 g/L	PEG 100 g/L	PEG 150 g/L
1	-	-	0.33	0.67
2	-	0.33	1	0.33
3	-	0.67	1.67	0.33
4	0.33	3.33	2.67	2.33
5	2.67	3	1	3.33
6	2.33	1.33	2.33	1.67
7	3	0.67	0.67	1
8	1	0.67	-	0.33
9	0.33	-	-	-
10	-	-	0.33	-
11	0.33	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-

Table 3. Percentage of germination and average fresh weight of each bell pepper sprout (*C. annuum* var. *grossum*) after osmopriming treatment with PEG 4000

PEG 4000 variation	Germination percentage (%)	Average wet weight of sprouts (g)
0 g/L	100	0.0497 ^{ab}
50 g/L	100	0.0575 ^a
100 g/L	100	0.0528 ^a
150 g/L	100	0.0414 ^b

Note: Numbers accompanied by the same letter show no significant difference ($P>0.05$) according to the 5% DMRT test

Germination rate is an important aspect of plant vigor. The germination rate is determined by the number of days it takes for the radicle or plumule to emerge over a certain period (Sutopo 2002). The results of ANOVA showed that the PEG 4000 treatment significantly affected the germination rate and germination rate index of bell pepper seeds. Figure 2A shows that the control treatment produced the highest germination rate of 6.43 days, while the 100 g/L PEG treatment produced the lowest germination rate of 4.50 days. However, it was not significantly different from the 50 g/L and 150 g/L PEG treatments. The germination rate results indicate the seeds' ability to germinate quickly in that time range. In addition, the Germination Speed Index (GSI) value also supports seeds' ability to germinate quickly.

The average germination rate index value in Figure 2B is in line with the germination rate value. The 100 g/L PEG treatment produced the highest germination rate index value of 2.89 and was significantly different from the control treatment, which produced the lowest germination rate index value of 1.63. According to Lesilolo et al. (2013), the higher the germination rate value indicates that, the more days needed for a germination process, the lower the germination rate index value. Ernita and Mairizki (2019) reported that the soaking treatment of 2.5% PEG 6000 for 6 hours significantly affected the vigor index (germination speed) of soybean seeds, with the highest value of 1.16. Based on the results of this study, PEG 4000 treatment as an osmopriming agent at a concentration of 100 g/L increased the ability of seeds to germinate and accelerate germination rates with better vigor.

In general, the pattern of water absorption in seeds treated with osmopriming was not different from those without treatment. The osmopriming treatment only affects the water absorption rate so that it is slowed down and controlled (Varier et al. 2010). Yuanasari et al. (2015) added that Osmo conditioning causes the potential of the seed environment to be lower so that the initial rate of imbibition (phase I) can be slowed down. Then entering phase II, the seed can complete the pre-germination metabolic process optimally before planting so that the seed is ready for the emergence of radicles (prospective roots) (phase III). Based on this description, giving PEG 100 g/L in this study was thought to slow and control the rate of imbibition in phase I so that the seeds could improve and complete their metabolic processes during phase II before entering phase III. When seeds are planted

or germinated, they are ready to germinate (phase III). The faster germination rate parameter and the high germination speed index value indicate it.

Growth of pepper seedlings

Seedling height

In general, seedlings treated with drought stress and PEG 4000 produced higher seedling height than the control. The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affects the height of the bell pepper seedlings (Figure 3).

Table 4 shows that water availability (space capacity) significantly affected seedling height growth in the control treatment without PEG 4000 treatment. PEG 4000 treatment at 100% SC water availability caused the average seedling height to increase and decrease, although not significantly. At 75% SC water availability, PEG 50, 100, and 150 g/L treatment decreased the seedlings' average height, which was not too significant. Meanwhile, the availability of water at 50% SC of seeds primed with PEG 4000 increased the average height of the seedlings significantly. However, PEG concentrations of 100 and 150 g/L produced a height that was not significantly different. Based on these results, water availability at 75% SC without PEG treatment was the optimum condition for the seedlings, and did not consider it to be in a stressed environment. It was evident in the 75% SC control, which had a better height than the 100% SC control. The PEG treatment of 150 g/L was the optimum concentration for pepper seedlings because it produced the highest seedling height compared to the control, even in low water availability (50% SC).

According to Rahayu et al. (2005), polyethylene glycol (PEG) can mimic drought stress conditions because this compound lowers the osmotic potential of the solution through the activity of the ethylene oxide subunit matrix, which can bind water molecules with hydrogen bonds. In this study, the PEG 4000 treatment increased the height of bell pepper seedlings when there was water stress. Presumably, the seedlings selected with PEG had been simulated to be in a stressed environment so that when grown on planting media, they were able to adapt under unfavorable conditions (low water availability). In addition, the process of cell division and expansion in the apical meristem was not hampered, resulting in taller pepper seedlings.

Table 4. The average height of bell pepper (*C. annuum* var. *Grossum*) seedlings after treatment with drought stress and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	13.90 ^e	22.67 ^b	21.23 ^{bc}	21.00 ^{bc}
75% SC	21.07 ^{bc}	18.67 ^{cd}	17.40 ^d	17.40 ^d
50% SC	10.57 ^f	20.20 ^{bed}	28.27 ^a	31.20 ^a

Note: Numbers accompanied by the same letter indicate no significant difference ($p>0.05$) according to the 5% DMRT test

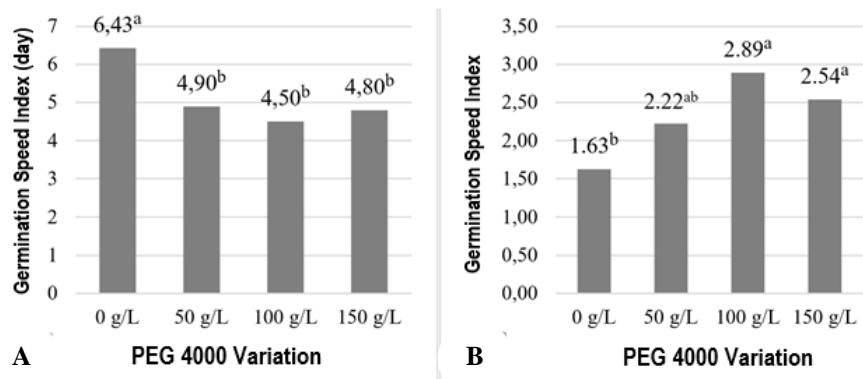


Figure 2. Average germination rate (A) and index seed germination rate (B) after osmopriming with PEG 4000. Notes: Numbers accompanied by the same letter indicate no significant difference ($P > 0.05$) according to the 5% DMRT test



Figure 3. Compilation of photos of pepper seedlings aged 13 weeks after treatment

Drought stress treatment can cause a significant reduction in plant height (Armita et al. 2017). According to Yusniwati et al. (2008), drought stress negatively impacts chili growth. Reducing the provision of water can cause vegetative growth such as plant height, root length, plant dry weight, and generative growth of chili to decrease compared to plants at optimum conditions. Subantoro (2014) added that a lack of water conditions could cause a decrease in cell turgor, affecting cell division and expansion, thereby inhibiting vegetative growth and decreasing physiological processes. Research from Nurjannati (2017) reported that the PEG 6000 treatment with a concentration of 250 g/L with 600 mL of water (equivalent to 100% SC) had an effect on the height of the chili plants with the highest average value (38.17 cm) compared to giving PEG 225 g/L and the control. The combination of 150 g/L PEG treatment with 50% SC water capacity resulted in the highest average seedling height of 31.20 cm. This treatment combination is thought to have the ability to adapt to drought stress so that the reduction in water content does not significantly hinder the growth of seedling height. The PEG 0 g/L combination shows the lowest value with a water capacity of 50% SC, which is 10.57 cm.

Number and area of leaves

The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affected the number of leaves and leaf area of the bell pepper seedlings. Leaf area will decrease if the intensity of drought stress is high. The average number and leaf area of bell pepper seedlings (*C. annuum* var. *grossum*) after drought stress and PEG 4000 treatment are presented in Table 5.

Table 5 shows that the number of leaves at 100% SC water availability with PEG 4000 treatment can increase the number of leaves. However, the number of leaves decreases as the PEG concentration increases, although not significantly different. At 75% SC water availability, PEG 4000 can reduce the number of leaves with values that are not significantly different. The highest average number of leaves was obtained in the 150 g/L PEG treatment with 50% KL water availability, namely 21 leaves, while the lowest average number of leaves was obtained in the 0 g/L PEG treatment with 50% KL water availability, namely eight strands. Generally, pepper seedlings with a water availability of 100%-50% SC primed with PEG 4000 could still produce good leaves. It is suspected that the plants did not experience growth inhibition due to drought stress so that they could grow more leaves. According to Samanhudi et al. (2021), a decrease in the number of leaves is a form of plant adaptation to water shortage conditions. If the number of leaves decreases, then the transpiration rate also decreases.

In the leaf area parameter, treatment without PEG 4000 (the control) can reduce leaf area at 50% SC water availability. In comparison, at 75% SC water availability, there is a significant increase in leaf area. It is suspected that the availability of 75% SC water is the optimal limit for plants to grow, so it does not show a significant difference with the treatment of 100% SC water

availability. In general, osmopriming with PEG 4000 increased leaf size. The treatment of 100% and 75% SC water availability did not show a significant difference after administration of PEG 4000 at concentrations of 50, 100, or 150 g/L. In contrast, at 50% SC water availability, it produced significant differences after osmopriming PEG 4000, even though the concentration was 100 g/L and 150 g/L were not significantly different. The PEG concentration of 150 g/L at 50% SC water availability resulted in the highest average leaf area of 14.72 cm². The lowest average leaf area was obtained in the PEG treatment with 0 g/L and water availability of 50% SC which was 1.80 cm².

One of the plant responses to drought stress is to reduce leaf area. The decrease in leaf area is related to transpiration which will decrease in rate so that the plants do not lose too much water (Hendrati et al. 2016). According to Hidayati et al. (2017), plants that lack water will inhibit the growth and development of young leaves, cell shrinkage occurs, and the leaf aging process is followed by the dropping of old leaves, resulting in a reduction in the photosynthetic area. Based on this study, osmopriming with PEG 4000 can increase the number and expansion of leaves. Therefore, it is suspected that pepper seedlings can adapt to drought stress conditions. Their transpiration process is at a tolerance limit so that excess water loss does not occur and growth can still run normally.

Seedling wet weight

Plants under drought stress generally experience a decrease in plant wet and dry weight. The results of ANOVA show that the treatment of variations in water availability and PEG 4000 independently or in combination significantly affected the fresh weight of each bell pepper seedling. The average wet weight of the seedlings in Table 6 shows that water availability in the 75% SC control treatment had a significantly different average wet weight per seedling with the 100% and 50% SC controls. This result is in line with the average height of the 75% SC control seedlings, which was assumed to be the optimum condition for seedlings without osmopriming treatment.

Table 5. Average number and leaf area of bell pepper seedlings (*C. annuum* var. *grossum*) after drought stress treatment and PEG 4000

PEG 4000 concentration variations	Variation of space capacity (% SC)	Number of leaves	Leaf area
0 g/L	100	10.67 ^{de}	3.18 ^{de}
	75	15.00 ^{bcd}	5.87 ^{bcd}
	50	8.00 ^e	1.80 ^e
50 g/L	100	16.33 ^b	8.19 ^b
	75	12.33 ^{bcd}	4.62 ^{cde}
	50	14.00 ^{bcd}	5.36 ^{bcd}
100 g/L	100	15.33 ^{bc}	7.58 ^{bc}
	75	14.33 ^{bcd}	6.45 ^{bc}
	50	20.67 ^a	13.53 ^a
150 g/L	100	12.00 ^{bcd}	6.23 ^{bc}
	75	11.33 ^{cde}	6.34 ^{bc}
	50	21.00 ^a	14.72 ^a

Note: Numbers accompanied by the same letter indicate no significant difference ($P > 0.05$) according to the 5% DMRT test

Table 6. Average fresh weight of bell pepper (*C. annuum* var. *Grossum*) seedlings after drought stress treatment and PEG 4000

Space capacity variation	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	1.11 ^{de}	4.83 ^{bc}	5.25 ^b	3.65 ^{bcd}
75% SC	3.90 ^{bc}	2.52 ^{cde}	3.70 ^{bcd}	2.85 ^{bcd}
50% SC	0.61 ^e	3.27 ^{bcd}	13.60 ^a	14.10 ^a

Note: Numbers accompanied by the same letter show no significant difference ($P>0.05$) according to the 5% DMRT test

Table 7. The average shoot-root ratio of bell pepper (*C. annuum* var. *grossum*) seedlings after drought stress treatment and PEG 4000

Space capacity Variation	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	4.88 ^{bcd}	6.17 ^{abc}	8.91 ^a	4.70 ^{cd}
75% SC	5.81 ^{bcd}	7.89 ^{ab}	4.66 ^{cd}	5.67 ^{bcd}
50% SC	2.90 ^d	6.98 ^{abc}	7.35 ^{abc}	5.74 ^{bcd}

Note: Numbers accompanied by the same letter indicate no significant difference ($P>0.05$) according to the 5% DMRT test

The PEG 4000 osmopriming treatment increased and decreased the fresh weight of bell pepper seedlings, which were not significantly different at 100%-75% SC water availability conditions. Whereas at 50% SC water availability conditions showed a significant increase in seedling's wet weight after being treated with PEG 4000. Concentration 150 g/L PEG produced the highest average wet weight of each bell pepper seedling at 15.10 grams, while the lowest wet weight of seedlings was found in the PEG 0 g/L treatment with 50% SC water availability, which was 0.61 grams. This study resulted in the size of the seedlings' wet weight in line with the height of seedlings and number of leaves. The higher the seedling height and the number of leaves, the greater the wet weight of the seedling.

Root header ratio

Drought stress can suppress plant growth, shoots, and roots, causing a decrease in total plant dry weight. In addition, drought stress suppresses shoot development to a greater extent than root development. It is due to the efforts of plants to adapt by reducing water loss through leaves so that plants reduce the crown size and maintain root development to meet sufficient water and nutrient needs (Jamilah 2012). The results of ANOVA showed that the treatment of variations in water availability independently had no significant effect on the shoot-root ratio of the bell pepper seedlings. In contrast, the PEG 4000 treatment and the interaction between the two significantly affected the shoot-root ratio of the bell pepper seedlings.

Table 7 shows that the treatment without the addition of PEG 4000 (control) experienced a decrease in the average crown-root ratio at 50% SC water availability, while at 100% and 75% SC water availability, there was an average increase in the crown-root ratio which was not significant. Therefore, the PEG 4000 treatment was generally not

significantly different from the average crown-root ratio under drought-stress conditions. Giving PEG 100 g/L at 100% SC water availability produced the highest average crown-root ratio at 8.91. The lowest shoot-root ratio was obtained in the PEG treatment of 0 g/L available water 50% SC which was 2.90. These results are in accordance with the research of Nurjannati (2017), who reported that the treatment of PEG administration by giving the volume of water to chili plants did not significantly affect the wet weight of the shoot-root ratio. The highest shoot-root ratio wet weight was obtained at 0.69 in the PEG treatment 0 g/L (control) with a volume of 600 mL water (equivalent to 100% SC). In comparison, the PEG treatment was 225 g/L and 250 g/L with a volume of 300 mL water (equivalent to 50% SC), equal to 0.65 and 0.59, with results that are not significantly different.

The crown-root ratio value indicates biomass allocation between the crown and roots during growth. According to Effendi (2008), a decrease in the crown-root ratio illustrates that the assimilate partitions in plant growth under drought conditions will be more distributed to form roots. Water stress can change the distribution or partition of assimilate between organs. The growth of the crown will decrease more than the roots because there is a large water deficit in the crown. A decrease in the crown-root ratio indicates that the plant is under drought stress and, in response, increases the volume and root elongation to absorb water. Based on the results of this study, the application of PEG 4000 generally increased the value of the crown-root ratio. It is suspected that plants allocate assimilate more aimed at the crown, so that shoot growth is good. Pepper seedlings treated with PEG 4000 could grow normally even under stress, so it can be said that these seedlings were tolerant to drought stress.

Leaf proline content

The mechanism of plant tolerance to drought stress is to maintain turgor pressure manifested by a decrease in osmotic potential and the ability to accumulate dissolved compounds in the form of sugars and amino acids, especially proline (Novenda and Nugroho 2016). This study shows obtained proline levels from the regression equation as measured by the proline standard curve $y = 0.3194x - 0.032$ with $R^2 = 0.9063$. The results of ANOVA showed that the treatment of drought stress and PEG 4000 independently or the interaction between the two had no significant effect on the average proline of bell pepper seedling leaves.

Table 8 shows the average response of the proline content of bell pepper leaves to the administration of PEG 4000, and water availability produces values that are not significantly different. Proline levels in the treatment without PEG 4000 with water availability of 75% SC increased and decreased at 50% SC. The highest proline content was obtained in the PEG 50 g/L treatment combination with 100% SC water, which was 1.64 M. The lowest proline content was in the 150 g/L PEG treatment with 75% SC water, which was 1.10 M. Moreover, treatment without administration of PEG 4000 at 75% SC water availability increased proline levels, meaning that

under these conditions, the plants were in a stressed environment, so they responded by accumulating proline. However, at 50% SC water availability, the proline levels decreased. It could be because the bell pepper plants in this study grew optimally at a water availability of 75% SC. When faced with a water availability of 50% SC, the plants assumed they were in a stressed condition, so their growth was disturbed (seen from the low plant height and wet weight), and the plants could not develop. Even if they adapt well, then the measured proline levels are low.

Yusniwati et al. (2008) reported that leaf proline content in plants experiencing drought stress was higher than in the optimum environment. One of the mechanisms of plant adaptation to drought stress is by regulating the osmotic potential of cells, namely through increasing leaf proline concentrations. Proline organic compounds can reduce cell osmotic potential without inhibiting enzyme function and not reducing cell turgor.

The treatment of PEG 4000 with a concentration of 50 g/L resulted in a decrease in proline levels along with low water availability (100-50% SC). The addition of PEG 100 g/L at 100%-75% SC water availability increased proline levels, and at 50% SC decreased leaf proline levels. Adding PEG 150 g/L at 100%-75% water availability decreased proline levels, and at 50% SC water availability increased leaf proline levels. According to Juswardi and Tanzerina (2003), giving PEG can lower the water potential, causing reduced cell turgor pressure. To maintain turgor pressure, plants accumulate dissolved compounds, one of which is proline. PEG-selected plants face unfavorable conditions, so they respond by changing their metabolism affecting plant growth and development. As a result, the growth of selected plants is better than non-selected plants. Effendi (2008) added that plants that are tolerant to drought stress would have higher proline levels.

The average proline content of bell pepper leaves with the control treatment and PEG 4000 administration on water availability produced values that were not significantly different. Therefore, it indicated that there was no interaction between the drought stress treatment and the PEG 4000 given on the proline content of the bell pepper seedling leaves. Therefore, based on the results of this study, a PEG concentration of 50 g/L can induce the resistance of bell pepper seedlings to water stress because there is no increase in proline levels.

Total chlorophyll and carotenoid content

Plants that experience drought stress can disrupt their metabolic activity. It is characterized by a decrease in chlorophyll content and an increase in secondary metabolites, one of which is carotenoid (Armita et al. 2017). The results of the ANOVA of this study showed that the treatment of variations in water availability and PEG 4000 independently or the interaction between the two did not significantly affect the average total chlorophyll in the bell pepper seedling leaves. Furthermore, the results of the ANOVA showed that the PEG 4000 treatment independently affected the carotenoid average of bell pepper seedling leaves, while the independent variation of water availability and the interaction between the two treatments did not affect carotenoid averages.

Table 8. Average proline content of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	1.14 ^{ab}	1.64 ^a	1.16 ^{ab}	1.41 ^{ab}
75% SC	1.46 ^{ab}	1.19 ^{ab}	1.58 ^{ab}	1.10 ^b
50% SC	1.12 ^{ab}	1.06 ^b	1.22 ^{ab}	1.46 ^{ab}

Note: Numbers accompanied by the same letter show no significant difference ($P > 0.05$) according to the 5% DMRT test

Table 9 shows the average total chlorophyll response of bell pepper seedlings at 75% and 50% SC water availability which was lower than normal conditions (100% SC), although not significantly different. Giving the PEG 4000 at 100% SC water availability increased the total chlorophyll content of the leaves. At 75%-50% SC available water and 50 g/L PEG concentration, there was an increase in the total chlorophyll content of the leaves, but the total chlorophyll content decreased at 100 g/L and 150 g/L PEG concentrations. The highest leaf chlorophyll content was obtained in the treatment of 50 g/L PEG concentration and 100% SC water availability at 1.55 mg/g wet weight. The lowest chlorophyll content was obtained in the treatment with a PEG concentration of 150 g/L with a water availability of 75% SC at 1.19 mg/g wet weight. Following Zhang et al. (2015), priming PEG 8000 with a concentration of 20% (w/v) on sorghum seeds (*S. bicolor*) can increase total chlorophyll content (chlorophyll a and b) in conditions of poor soil moisture (dryness) with a content of 1.68 mg/g wet weight compared to seeds that were not primed had a total chlorophyll content of 1.39 mg/g wet weight. Syaiful et al. (2014) reported that seeds primed with PEG 300 g/L produced soybean plants that were tolerant to drought stress (50% SC), with the highest chlorophyll, protein, and dry weight content.

According to Hendriyani and Setiari (2009), the low water content in the growing media can inhibit chlorophyll synthesis in the leaves. In addition, plants will experience an increasing temperature and transpiration, causing the disintegration of chlorophyll and affecting the decrease in the rate of photosynthesis. Finally, it will result in decreased synthesis of chlorophyll. In general, applying PEG 4000 to different water availability had no significant effect on the average total chlorophyll of bell pepper seedling leaves. These results suggest that applying PEG 4000 did not make bell pepper seedlings under drought stress conditions (plants already can adapt), so the bell pepper seedlings responded by causing no significant decrease in total chlorophyll content.

Carotenoids are one of photosynthetic pigments that plants need in small amounts. Plants experiencing drought stress have a decrease in chlorophyll content. As a result, more carotenoids are needed to continue the photosynthesis process (Armita et al. 2017). The results of the ANOVA in this study showed that the treatment of variations in water availability and PEG 4000 independently or the interaction between the two did not significantly affect the carotenoid average of bell pepper seedling leaves.

Table 9 shows that variations in water availability had no significant effect on carotenoid content, while giving PEG 4000 concentrations of 100 g/L and 150 g/L gave significant results on carotenoid content compared to the control and PEG 50 g/L. The highest average carotenoid content in bell pepper seedling leaves was obtained in the 50 g/L PEG treatment with 50% SC water availability of 1.09 mg/g wet weight, while the lowest carotenoid content was obtained in the 100 g/L PEG treatment with 75% water availability and 50% SC which is equal to 0.60 mg/g wet weight. Based on the results of this study, PEG 4000 was thought not to make pepper seedlings under drought stress conditions, so the seedlings responded with no significant increase in carotenoid levels.

Nitrate reductase enzyme activity

Nitrate reductase is an enzyme that reduces nitrate ions to nitrite ions. Putra et al. (2020) reported that increasing drought stress would reduce the activity of the nitrate reductase enzyme due to an increase in the water potential gradient between the environment and plant tissues. The results of the ANOVA in this study showed that the PEG 4000 variation treatment independently affected the average nitrate reductase activity of the bell pepper seedlings. In contrast, the independent variation of water availability and the interaction between the two treatments had no significant effect on the average of the bell pepper seedling nitrate reductase activity.

Table 9. Average total chlorophyll and carotenoid content of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

PEG 4000 concentration variations	Variation of space capacity (% SC)	Total chlorophyll (mg/g wet weight)	Carotenoids (mg/g wet weight)
0 g/L	100	1.39 ^{ab}	1.04 ^a
	75	1.29 ^{ab}	0.97 ^a
	50	1.34 ^{ab}	0.99 ^a
50 g/L	100	1.55 ^a	1.05 ^a
	75	1.41 ^{ab}	1.05 ^a
	50	1.51 ^{ab}	1.09 ^a
100 g/L	100	1.36 ^{ab}	0.67 ^{bc}
	75	1.22 ^{ab}	0.60 ^b
	50	1.27 ^{ab}	0.60 ^b
150 g/L	100	1.47 ^{ab}	0.89 ^{ab}
	75	1.19 ^b	0.68 ^{bc}
	50	1.37 ^{ab}	0.71 ^{bc}

Note: Numbers accompanied by the same letter indicate no significant difference ($P>0.05$) according to the 5% DMRT test

Table 10. Average levels of the enzyme nitrate reductase ($\mu\text{mol NO}_2^-/\text{g}/\text{hour}$) of bell pepper (*C. annuum* var. *grossum*) seedling leaves after drought stress treatment and PEG 4000

Variation of space capacity	PEG 4000 concentration variations			
	0 g/L	50 g/L	100 g/L	150 g/L
100% SC	148.27 ^{abc}	131.44 ^{bcde}	133.28 ^{bcde}	103.90 ^f
75% SC	156.79 ^a	128.26 ^{bcde}	113.87 ^{def}	100.60 ^f
50% SC	149.87 ^{ab}	134.38 ^{bcd}	127.30 ^{cde}	112.24 ^{ef}

Note: Numbers accompanied by the same letter show no significant difference ($P>0.05$) according to the 5% DMRT test

Table 10 shows the average response of the activity of the nitrate reductase (NRA) enzyme in bell pepper seedling leaves to the availability of water without PEG 4000 treatment; there were increases and decreases with values that were not significantly different. At the availability of water 100%, 75%, and 50% SC, PEG 4000 administration causes a decrease in the average activity of the enzyme nitrate reductase. The highest average NRA was obtained in the 0 g/L PEG treatment with 75% SC water availability, which was 156.79 $\mu\text{mol NO}_2^-/\text{g}/\text{hour}$, while the lowest NRA average was obtained in the 150 g/L PEG treatment with 75% water availability. % SC is 100.60 $\mu\text{mol NO}_2^-/\text{g}/\text{hour}$. The treatment of giving PEG 4000 at concentrations of 50 g/L and 150 g/L with 50% SC water availability showed an increased NRA value. A PEG concentration of 50 g/L produced a higher NRA value than a PEG concentration of 150 g/L; this was suspected to be an optimum PEG administration, and 50 g/L can still support nitrate reductase activity. The treatment of giving PEG 100 g/L with 50% SC water availability showed a decrease in NRA values.

An increase in the NRA value is suspected that plants can defend themselves against drought stress. A high nitrate reductase value indicates that water in the environment is available, which acts as a provider of protons and electrons for its activities. Water available in the soil can facilitate the transport of nitrogen from the soil into the plant. Fitriana et al. (2011) reported that soil with 100% - 90% SC water availability resulted in higher nitrate reductase activity than that with 70%, 50%, 30%, and 10% SC water availability in *Burangrang* cultivar soybean. These results were suspected because a lack of water can cause stomata to close, thereby cutting off the supply of CO_2 to the mesophyll cells and reducing the photosynthesis rate. Low photosynthetic efficiency affects the amount of NADPH formed in the light reaction (Salisbury and Ross 1992). Suppose there is not an adequate supply of NADPH₂ in the cytosol. In that case, there will be a decrease in the nitrate reductase enzyme activity because NADPH₂ plays an important role as a proton and electron donor, which can stimulate the movement of electrons in the cytosol (Fitriana et al. 2011).

Based on this research, it can be said that giving PEG 4000 to drought stress can reduce nitrate reductase activity, which is indicated by a decrease in its levels. For example, pepper seedlings treated with 150 g/L PEG priming had low nitrate reductase activity. However, the seedlings showed good growth in seedling height, leaf number and area, and the seedling's wet weight.

The conclusions of this study are (i) Osmopriming with PEG 4000 can accelerate the germination rate of bell pepper (*C. annuum* var. *grossum*) seeds. Giving PEG 100 g/L resulted in the fastest germination rate of 4.50 days with a better vigor value. (ii) Osmopriming with PEG 4000 affected the growth of bell pepper (*C. annuum* var. *grossum*) seedlings, namely increasing seedling height, number of leaves, leaf area, wet weight of seedlings, and crown-root ratio values under drought stress conditions. (iii) Osmopriming with PEG 4000 had no significant effect on increasing total proline and chlorophyll levels but

affected reducing carotenoid levels and activity of the enzyme nitrate reductase of bell pepper seedlings (*C. annuum* var. *grossum*) under drought stress conditions. The highest levels of proline and chlorophyll were obtained in the treatment combination of PEG 50 g/L with 100% SC water availability, while the highest levels of carotenoids were obtained in the treatment combination of PEG 50 g/L with 50% SC water availability. The highest nitrate reductase activity was obtained with the treatment combination of PEG 0 g/L with 75% SC water availability. (iv) The combination of PEG 4000 osmopriming treatment under drought stress conditions optimal for growth of seedling height, number of leaves, leaf area, and wet weight of pepper seedlings was PEG 150 g/L with 50% SC water availability. In contrast, the optimal combination on the canopy ratio parameter-root is PEG 100 g/L with 100% SC water availability.

REFERENCES

- Aminifard MH, Aroiee H, Karimpour S, Nemati H. 2010. Growth and yield characteristics of bell pepper (*Capsicum annuum* L.) in response to plant density. *Asian J Plant Sci* 9: 276-280. DOI: 10.3923/ajps.2010.276.280.
- Anggarwulan E, Solichatun. 2007. Kajian klorofil dan karotenoid *Plantago major* L. dan *Phaseolus vulgaris* L. sebagai bioindikator kualitas udara. *Biodiversitas* 8 (4): 279-282. DOI: 10.13057/biodiv/d080407. [Indonesian]
- Arifianto M, Kartika JG. 2018. Proses pemanenan bell pepper (*Capsicum annuum* var. *tribeli*) di Greenhouse, De Lier, Belanda Selatan, Belanda. *Bul Agrohorti* 6 (3): 372-381. DOI: 10.29244/agrob.v6i3.21104. [Indonesian]
- Armita D, Arumningtyas EL, Mastuti R. 2017. Tolerance level of three genotypes of cayenne pepper (*Capsicum frutescens* L.) toward drought stress of vegetative phase based on morphological and physiological responses. *Intl J Chem Tech Res* 10 (2): 183-192.
- Badan Pusat Statistik. 2018. Statistik Tanaman Sayuran dan Buah-Buahan Semusim Indonesia. Indonesia: BPS-Statistics Indonesia. [Indonesian]
- Bates IS, Waldren RP, Tare ID. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* 39: 205-207. DOI: 10.1007/BF00018060.
- Copeland LO, McDonald MB. 2001. Principles of Seed Science and Technology 4th Edition. Kluwer Academic Publishers, London. DOI: 10.1007/978-1-4615-1619-4.
- Effendi Y. 2008. Kajian Resistensi Beberapa Varietas Padi Gogo (*Oryza sativa* L.) terhadap Cekaman Kekeringan. [Tesis]. Program Pascasarjana, Program Studi Agronomi, Universitas Sebelas Maret Surakarta. [Indonesian]
- Eivaz A. 2012. Induction of drought tolerance with seed priming in wheat cultivars (*Triticum aestivum* L.). *Acta Agriculturae Slovenica* 99: 21-29. DOI: 10.2478/v10014-012-0003-6.
- Ernita E, Mairizki F. 2019. Penggunaan polietilen glikol sebagai teknik invigorasi untuk memperbaiki viabilitas, vigor, dan produksi benih kedelai. *Jurnal Ilmiah Pertanian* 16 (1): 8-18. DOI: 10.31849/jip.v16i1.2140. [Indonesian]
- Fitriana J, Pukan KK, Herlina L. 2011. Aktivitas enzim nitrat reduktase kedelai kultivar burangrang akibat variasi kadar air tanah pada awal pengisian polong. *Biosaintifika J Biol Biol Educ* 1 (1): 1-8.
- Gayathri N, Gopalakrishnan M, Sekar T. 2016. Phytochemical screening and antimicrobial activity of *Capsicum chinense* Jacq. *Intl J Adv Pharmac* 5: 12-20.
- Hendriani IS, Rachmawati D, Pamuji AC. 2016. Respon kekeringan terhadap pertumbuhan, kadar prolin dan anatomi akar *Acacia auriculiformis* Cunn., *Tectona grandis* L., *Alstonia spectabilis* Br., dan *Cedrela odorata* L. *Jurnal Penelitian Kehutanan Wallacea* 5 (2): 123-133. DOI: 10.18330/jwallacea.2016.vol5iss2pp123-133. [Indonesian]
- Hendriyani IS, Setiari N. 2009. Kandungan klorofil dan pertumbuhan kacang panjang (*Vigna sinensis*) pada tingkat penyediaan air yang berbeda. *Jurnal Sains dan Matematika* 17 (3): 145-150. [Indonesian]
- Hidayati N, Hendrati RL, Triani A, Sudjino. 2017. Pengaruh kekeringan terhadap pertumbuhan dan perkembangan tanaman nyamplung (*Callophylum inophyllum* L.) dan johar (*Cassia florida* Vahl.) dari provenan yang berbeda. *Jurnal Pemuliaan Tanaman Hutan* 11 (2): 99-111. DOI: 10.20886/jpth.2017.11.2.99-111. [Indonesian]
- Irwan AW, Wicaksono FY. 2017. Perbandingan pengukuran luas daun kedelai dengan metode gravimetri, regresi dan scanner. *Jurnal Kultivasi* 16 (3): 425-429. DOI: 10.24198/kultivasi.v16i3.14448. [Indonesian]
- Jamilah N. 2012. Pengujian Karakter Morfologi untuk Evaluasi Ketahanan Kekeringan Beberapa Varietas Kedelai (*Glycine max* (L.) Merrill). [Skripsi]. Jurusan Biologi, Fakultas Sains dan Teknologi, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang. [Indonesian]
- Juswardi, Tanzerina N. 2003. Akumulasi prolin dan indeks toleransi kalus padi yang diseleksi Polietilen Glikol (PEG) secara in vitro sebagai marka toleransi kekeringan. *Jurnal Penelitian Sains* 14: 92-100. DOI: 10.56064/jps.v0i14.294. [Indonesian]
- Kasi PD, Cambaba S, Illing I. 2017. Pemanfaatan mulsa serbuk gergaji untuk mengatasi pengaruh cekaman kekeringan pada bibit tanaman cabai (*Capsicum annuum* L.). *Jurnal Dinamika* 8 (1): 30-40. [Indonesian]
- Kurniawan M, Izzati M, Nurchayati Y. 2010. Chlorophyll, carotenoid and vitamin C in some species of aquatic plants. *Bul Anat Physiol* 18: 28-40.
- Latifa A, Rachmawati D. 2020. Pengaruh osmopriming benih terhadap pertumbuhan dan morfofisiologi tanaman kangkung darat (*Ipomoea reptans* Poir) pada cekaman kekeringan. *Jurnal Agronomi Indonesia* 48 (2): 165-172. DOI: 10.24831/jai.v48i2.31448. [Indonesian]
- Latifa IC, Anggarwulan E. 2009. Nitrogen content, nitrate reductase activity, and biomass of kimpul (*Xanthosoma sagittifolium*) on shade and nitrogen fertilizer variation. *Nusantara Biosci* 1 (2): 65-71. DOI: 10.13057/nusbiosci/n010203.
- Lesilolo MK, Riry J, Matatula EA. 2013. Pengujian viabilitas dan vigor benih beberapa jenis tanaman yang beredar di pasaran Kota Ambon. *Agrologia* 2 (1): 1-9. DOI: 10.30598/a.v2i1.272. [Indonesian]
- Lutts S, Benincasa P, Wojtyla L, Kubala SS, Pace R, Lechowska K, Quinet M, Garnczarska M. 2016. Seed Priming: New comprehensive approaches for an old empirical technique. In: Araujo S and Alma B (Ed) *New Challenges in Seed Biology, Basic and Translational Research Driving Seed Technology*. <https://www.intechopen.com/books/new-challenges-in-seed-biology-basic-and-translational-research-driving-seed-technology/seed-priming-new-comprehensive-approaches-for-an-old-empirical-technique> [7 Juli 2020]. DOI: 10.5772/64420.
- Novenda IL, Nugroho SA. 2016. Analisis kandungan prolin tanaman kangkung (*Ipomoea reptans* Poir), bayam (*Amaranthus spinosus*) dan ketimun (*Cucumis sativus* L.). *Pancaran* 5 (4): 223-234. [Indonesian]
- Nurjannati K. 2017. Efek Perlakuan Priming terhadap Performa Tanaman Cabai (*Capsicum annuum* L.) pada kondisi stres air. [Skripsi]. Program Studi Biologi, Jurusan Pendidikan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Yogyakarta, Yogyakarta. [Indonesian]
- Pramana SA, Pujiasmanto B, Sakyia AT. 2019. Perbandingan uji tetrazolium dan radicle emergence dalam menduga viabilitas benih kopi arabika (*Coffea arabica* L.). *Jurnal Litri* 25 (1): 1-10. DOI: 10.21082/litri.v25n1.2019.1-10. [Indonesian]
- Putra VP, Solichatun, Sugiyarto, Sutarno. 2020. Nitrate reductase activity of black rice (*Oryza sativa* L.) cempo ireng cultivar strain 13 and 46 as the result of plant breeding using ⁶⁰Co gamma ray on drought stress variaton. *J Phys: Conf Ser* 1436 (2020): 1-8. DOI: 10.1088/1742-6596/1436/1/012114.
- Rahayu ES, Edi G, Satriyas I, Sudarsono. 2005. Polyethylene Glycol (PEG) dalam media in vitro menyebabkan kondisi cekaman yang menghambat tunas kacang tanah (*Arachis hypogaea* L.). *Berkala Penelitian Hayati* 11 (2005): 39-48. DOI: 10.23869/bphjbr.11.1.20057. [Indonesian]
- Salah S, Yajing G, Dongdong C, Jie L, AAmir N, Qijuan H, Weimin H, Mingyu N, Jin H. 2015. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. *Sci Rep* 5 (14278): 1-14. DOI: 10.1038/srep14278.

- Salisbury FB, Ross CW. 1992. Fisiologi Tumbuhan Jilid 2. Terjemahan Diah R. Lukman & Sumaryono: 1995. Edisi Keempat. ITB-Press, Bandung. [Indonesian]
- Samanhudi, Rahayu M, Sakya AT, Susanti YD. 2021. Seleksi ketahanan bebrapa varietas sorgum manis (*Sorghum bicolor* L.) pada berbagai konsentrasi salinitas. *Jurnal Pertanian Presisi* 5 (1): 40-56. DOI: 10.35760/jpp.2021.v5i1.3740. [Indonesian]
- Subantoro R, Prabowo R. 2013. Pengkajian viabilitas benih dengan tetrazolium test pada jagung dan kedelai. *Mediagro* 9 (2): 1-8. [Indonesian]
- Subantoro R. 2014. Pengaruh cekaman kekeringan terhadap respon fisiologis perkecambahan benih kacang tanah (*Arachis hypogaea* L.). *Mediagro* 10 (2): 32-44. [Indonesian]
- Sutopo L. 2002. Teknologi Benih (edisi revisi). Fakultas Pertanian Universitas Brawijaya. PT. Raja Grafindo Persada, Jakarta. [Indonesian]
- Swibawa IG, Oktarino H. 2010. Pengaruh kadar air tanah terkontrol terhadap kelimpahan nematoda parasit tumbuhan. *Prosiding Seminar Nasional Sains dan Teknologi* 3: 213-219. [Indonesian]
- Syaiful SA, Dunga NE, Riadi M, Ridwan I. 2014. Seed priming with PEG 8000 for improving drought stress tolerance of soybean (*Glycine max*). *Intl J Agric Syst* 2 (1): 19-26.
- Tulung SMT, Demmassabu S. 2011. Pertumbuhan dan hasil bell pepper (*Capsicum annuum* var. *grossum*) pada beberapa jenis naungan. *Eugenia* 17 (2): 156-162. DOI: 10.35791/eug.17.2.2011.3538. [Indonesian]
- Varela RO, Albornoz PL. 2013. Morpho-anatomy, imbibition, viability and germination of the seed of *Anadenanthera colubrina* var. *cebil* (Fabaceae). *Intl J Trop Biol* 61 (3): 1109-1118. DOI: 10.15517/rbt.v61i3.11907.
- Varier A, Vari AK, Dadlani M. 2010. The subcellular basis of seed priming. *Curr Sci* 99 (4): 450-456. DOI: 10.12928/pharmaciana.v3i1.415.
- Warid, Palupi ER. 2009. Korelasi Metode Pengecambahan In Vitro dan Pewarnaan dalam Pengujian Viabilitas Polen. Makalah Seminar. Departemen Agronomi dan Hortikultura, IPB. [Indonesian]
- Warsi AG. 2013. Aktivitas Antioksidan ekstrak metanol buah bell pepper hijau (*Capsicum annuum* L.). *Jurnal Ilmiah Kefarmasian* 3 (1): 9-19. [Indonesian]
- Yuanasari BS, Kendarini N, Saptadi D. 2015. Peningkatan viabilitas benih kedelai hitam (*Glycine max* L. Merr) melalui invigorasi osmoconditioning. *Jurnal Produksi Tanaman* 3 (6): 518-527. [Indonesian]
- Yusniwati, Sudarsono, Aswidinnoor H, Hendrastuti S, Santoso D. 2008. Pengaruh cekaman kekeringan terhadap pertumbuhan, hasil dan kandungan prolin daun cabai. *Jurnal Agrista* 12 (1): 19-27. [Indonesian]
- Zanzibar M, Herdiana N. 2010. Akurasi metode uji cepat dalam menduga mutu fisiologis benih damar. *Jurnal Penelitian Hutan* 7 (4): 181-189. DOI: 10.20886/jpht.2010.7.4.181-189. [Indonesian]
- Zhang F, Yu J, Johnston CR, Wang Y, Zhu K, Lu F, Zhang Z, Zou J. 2015. Seed priming with polyethylene glycol induces physiological changes in sorghum (*Sorghum bicolor* L. Moench) seedlings under suboptimal soil moisture environments. *PLoS ONE* 10 (10): 1-15. DOI: 10.1371/journal.pone.0140620.