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Research Article
In vitro Selection of Cayenne Pepper (*Capsicum frutescens* L.) Varieties Against Drought Stress Mediated Through Polyethylene Glycol

Luthfi Aziz Mahmud Siregar, Lollie Agustina P. Putrie and Yeni Winarseh

Department Agro-Technology, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan, 20155, Indonesia

Abstract

Background and Objective: Planting chilli cultivars that are tolerant of drought stress and which have high yields offers hope for developing cayenne pepper cultivation in the dry land. An initial study aimed at selecting cayenne pepper plant varieties that are tolerant of drought stress conditions *in vitro* has been carried out using polyethylene glycol (PEG) to simulate drought stress in the form of a solution. **Materials and Methods:** This study used a completely randomized design with 2 factors, namely, cayenne pepper varieties, consisting of Tetra Hijau, Pedas, Cakra Hijau, Sigantung, Wijaya, Sapade, Sret, Bara, Genie, Hanna 08, Batari, Pelita 8 F1 and Bimantara 158 varieties) and PEG concentration factors, consisting of 0.5, 10, 15 and 20%. This study used 3 (three) replications, with the number of sample units in each bottle namely one plant, so that the total sample size was 195 plants. Parameters observed were the percentage of living plantlets, plant height, number of leaves, number of roots, root length and drought sensitivity index. **Results:** The results showed that the differences in varieties had a significant effect on the percentage of abnormal plantlets, the percentage of normal plantlets, plant height, number of leaves, number of roots and root length, but had no significant effect on the percentage of plantlets not growing. PEG concentration had a significant effect on all observed parameters, but the interaction between varieties and PEG concentrations had a significant effect on plant height, the number of leaves and root length. **Conclusion:** Varieties of Wijaya, Sapade, Genie and Hanna 08, Batari and Pelita F1 08 were included in the moderate group, while Tetra Hijau, Pedas, Cakra Hijau, Sigantung, Sret, Bara and Bimantara 158 including sensitive varieties based on drought simulations using PEG 20% *in vitro*.

Key words: Cayenne pepper varieties, drought stress, polyethylene glycol, *in vitro*

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Corresponding Author: Luthfi Aziz Mahmud Siregar, Department Agro-Technology, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan, 20155, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The need for cayenne pepper (*Capsicum frutescens* L.) continues to increase every year in line with the increasing population and the development of industries that require chilli raw materials. The increase in demand for chilli has not been followed by an increase in real results. The problem is land suitable for the development of chilli cultivation is still limited in optimal land, so it is necessary to make efforts to use marginal land such as dry land. Indonesia has the potential for dry land which includes gardens, fields and land that are temporarily not being cultivated with an area of 11,846,954; 5,172,502 and 11,945,726 ha, respectively. An area of 11,905,140 ha is untreated non-paddy dry land outside Java Island and 40,586 ha is on Java Island[1].

The dry-land has the most prominent constraints is the limited availability of water due to low rainfall and a long dry season resulting in evaporation that is greater than rainfall. So that it can cause alkalinity and salinity and disturbed nutrient balance. Drought stress is one of the widest stresses affecting growth and production in agricultural areas. It can be seen from several abiotic stress factors that the percentage of drought stress is the widest stress factor which is about 26%, followed by 20% mineral stress, 15% low-temperature stress, while the rest is biotic stress which is 39%[2].

Planting cayenne pepper seeds that are tolerant of drought stress with high yielding yields offers hope for development in a dry land. Tolerance to drought stress can occur if plants can withstand the conditions that occur or have mechanisms that allow them to avoid these stressful situations. Plants have different tolerances to drought stress due to differences in morphological, physiological, biochemical and molecular mechanisms[3].

The selection of plant genotypes for resistance to abiotic stress such as drought, Al poisoning, low soil pH and salinity can be simulated by *in vitro* culture and used to obtain plant variants that can be developed. The resulting plants tend to have phenotypes that are tolerant of the selection conditions. *In vitro* selection is more efficient because the selection conditions can be made homogeneous, the space required is relatively small and the effectiveness of selection is high. *In vitro* technology is an alternative selection method that is effective in producing individuals with characters who can adapt to certain stress conditions[4]. Therefore, it becomes an effective step in the development of drought-tolerant cayenne pepper plants by selecting *in vitro* drought-resistant cultivars of cayenne pepper. The selection agent used to find drought-tolerant cultivars is an osmotic compound. Osmoticum compounds that are widely used to simulate drought stress recently are PEG (Polyethylene Glycol) compounds[5].

Based on the description above, it is necessary to conduct a study related to the initial selection of several varieties of cayenne pepper (*C. frutescens* L.) *in vitro* which are tolerant to drought stress conditions by using PEG selection agents. This study is the initial stage of plant breeding activities to produce superior varieties of drought-resistant cayenne pepper.

MATERIALS AND METHODS

Material and experimental design: The study was conducted from April-October, 2018 at the Tissue Culture Laboratory, Faculty of Agriculture, University of North Sumatra, Medan. Plant materials used in this study were 13 explants of cayenne pepper seeds from several different varieties.

This experiment used a Completely Randomized Design (CRD) with 2 factors. The first factor is chili varieties, consisting of V1: Tetra Hijau, V2: Spicy, V3: Cakra Hijau, V4: Sigantung, V5: Wijaya, V6: Sapade, V7: Sret, V8: Bara, V9: Genie, V10: Hanna 08, V11: Batari, V12: Pelita F1 08, V13: Bimantara 158. The second factor is the provision of PEG 6000 with 5 levels of concentration, namely, P0: 0%, P1: 5%, P2: 10%, P3: 15%, P4: 20%. The treatment combinations were as many as 65 combinations, this study was repeated for 3 replications. The number of samples in each bottle is one plant. The total number of samples was 195 plants. The data obtained were analyzed using a two-way analysis of variance. If the treatment factor has a significant effect, then proceed with the mean difference test using the DMRT (Duncan Multiple Range Test) tests at the test level $\alpha = 5\%$.

Sterilization of equipment, media and explants: All bottles, synthetic foam and culture equipment were put into the autoclave for sterilization at a temperature of 121°C, the pressure of 17.5 psi for 60 min. Then the tools are stored in the oven before use. Synthetic foam (2×2×1 cm) was used as a seat for the sprouts in the treatment medium.

The media used in this study were Murashige and Skoog (MS) media which consisted of macronutrients, micronutrients, vitamins, myo-inositol 0.1 g L^{-1}, sucrose 30 g L^{-1} and the addition of PEG 6000 with the concentration according to treatment. The acidity of the media was determined with a digital pH meter at a pH of 5.8 using 0.1 N NaOH and 0.1 N HCl. Then the media solution was poured into a culture bottle with a height of 120 cm and a diameter of 4.5 cm. Each culture bottle is filled with media as much as 30 mL/bottle and sterilized foam. The media was sterilized by using an autoclave at a pressure of 17.5 psi at 121°C for 15 min.

The seed explants were washed using a detergent for 15 min then rinsed with water. The explants were sterilized with 3 drops of Benlate 2 g L^{-1}+Tween 20 solution and shaken for 15 min then rinsed with sterile distilled water three times. Furthermore, the seed explants were sterilized using Clorox 15%+Tween 20 as much as 3 drops then shaken for 15 min then rinsed with distilled water three times. The final sterilization was done by placing the seed explants in 10% betadine solution and shaking them for 5 min and then rinsing them with sterile distilled water three times. Then the seeds are germinated *in vitro* for 1 week in a sterile petri dish until the roots (radicles) appear ± 2 mm (https://www.scitepress.org/Papers/2018/100868/100868.pdf).

Explant planting: Sterile sprout explants were planted in a Laminar Air Flow Cabinet (LAFC) which had been sterilized with 70% alcohol. The one-week-old sprout explants were placed on synthetic foam in culture media bottles according to the treatment. Each bottle of treatment media was placed with one explant. After that, the *in vitro* germination culture was placed in the culture room at a temperature of 24±2°C.

Observation parameters: The observation parameters consisted of the percentage of live plantlets, plant height, number of leaves, number of roots, root length, Drought Sensitivity index (S). Observations were made at the end of the study 4 weeks after culture. The tolerance of chillies to drought stress was assessed by the stress sensitivity index (S)[6] with the formula:

$$S = \frac{1 - YD / YP}{D}$$

Where:

YD: Average observed value for one particular variety under PEG stress conditions

YP: The average observed value for one particular variety under control conditions:

$$D: \text{Drought intensity} = 1 - \frac{\text{Mean of observations of all varieties under PEG stress condition}}{\text{Mean of observations of all varieties under control condition}}$$

Chilli plants are said to be tolerant of drought stress if they have a value of S<0.5; Moderate if 0.5<S<1 and sensitive if S>1.

RESULTS

Percentage of live and normal plantlets: The average percentage of live plantlets in the treatment with PEG and variety can be seen in Table 1. PEG treatment had a significant effect on the percentage of live plantlets, while the variety and their interactions had no significant effect on the percentage of live plantlets. Table 1 shows that PEG treatment at a concentration of 0% (P0) is not significantly different from treatment 5% (P1) and is significantly different from 10% (P2), 15% (P3) and 20% (P4).

Table 1: Percentage of plantlets live on cayenne pepper varieties with PEG treatment *in vitro*

	(%)*					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	100.00	100.00	100.00	100.00	67.00	93.40
V2 (Pedas)	100.00	100.00	100.00	100.00	67.00	93.40
V3 (Cakra Hijau)	100.00	100.00	100.00	67.00	33.00	80.00
V4 (Sigantung)	100.00	100.00	67.00	67.00	100.00	86.80
V5 (Wijaya)	100.00	100.00	100.00	100.00	67.00	93.40
V6 (Sapade)	100.00	100.00	100.00	33.00	67.00	80.00
V7 (Sret)	100.00	100.00	100.00	100.00	67.00	93.40
V8 (Bara)	100.00	100.00	100.00	67.00	67.00	86.80
V9 (Genie)	100.00	100.00	100.00	100.00	100.00	100.00
V10 (Hanna 08)	100.00	100.00	100.00	100.00	100.00	100.00
V11 (Batari)	100.00	100.00	33.00	33.00	100.00	73.20
V12 (Pelita F1 08)	100.00	100.00	67.00	100.00	100.00	93.40
V13 (Bimantara 158)	100.00	67.00	33.00	67.00	67.00	66.80
Mean	100.00^{a}	97.46^{ab}	84.62^{bc}	79.54^{c}	77.08^{c}	87.74

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

Table 2: Percentage of normal plantlets on cayenne pepper varieties with PEG treatment *in vitro*

	(%)*					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	100.00	100.00	100.00	100.00	0.00	80.0^{ab}
V2 (Pedas)	100.00	100.00	100.00	100.00	0.00	80.0^{ab}
V3 (Cakra Hijau)	100.00	100.00	100.00	67.00	33.00	80.0^{ab}
V4 (Sigantung)	100.00	100.00	33.00	0.00	0.00	46.60^{cd}
V5 (Wijaya)	100.00	100.00	100.00	100.00	67.00	93.40^{a}
V6 (Sapade)	100.00	100.00	100.00	33.00	0.00	66.60^{bc}
V7 (Sret)	100.00	100.00	0.00	0.00	0.00	40.00^{d}
V8 (Bara)	100.00	100.00	100.00	67.00	0.00	73.40^{ab}
V9 (Genie)	100.00	100.00	100.00	67.00	67.00	86.80^{a}
V10 (Hanna 08)	100.00	100.00	100.00	0.00	0.00	60.00^{bc}
V11 (Batari)	100.00	100.00	0.00	0.00	0.00	40.00^{d}
V12 (Pelita F1 08)	100.00	67.00	67.00	0.00	0.00	46.80^{c}
V13 (Bimantara 158)	100.00	67.00	0.00	0.00	0.00	33.40^{d}
Mean	100^{a}	94.90^{a}	69.20^{b}	41.10^{bc}	12.90^{c}	63.62

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

Table 3: Plant height in cayenne pepper varieties treated with PEG *in vitro*

	(cm*)					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	4.70^{a-c}	4.63^{a-c}	1.67^{g-o}	1.37^{i-o}	0.23^{o}	2.52^{c-e}
V2 (Pedas)	4.70^{a-c}	5.27^{ab}	3.77^{a-f}	2.80^{b-i}	0.43^{m-o}	3.39^{a}
V3 (Cakra Hijau)	3.70^{b-f}	4.37^{a-d}	2.37^{c-k}	2.03^{e-n}	0.50^{m-o}	2.59^{a-d}
V4 (Sigantung)	4.07^{a-e}	3.77^{a-f}	1.93^{f-o}	0.80^{j-o}	0.73^{j-o}	2.26^{c-e}
V5 (Wijaya)	2.33^{c-j}	3.27^{b-h}	2.13^{d-l}	1.00^{i-o}	0.50^{l-o}	1.85^{de}
V6 (Sapade)	4.33^{a-d}	6.67^{a}	3.93^{a-e}	1.13^{j-o}	0.30^{m-o}	3.27^{a-c}
V7 (Sret)	2.43^{c-j}	3.33^{b-h}	0.87^{j-o}	1.00^{i-o}	0.33^{m-o}	1.59^{ef}
V8 (Bara)	3.53^{b-g}	3.20^{b-h}	2.27^{c-k}	1.53^{h-o}	0.37^{m-o}	2.18^{c-e}
V9 (Genie)	5.03^{ab}	4.27^{a-d}	4.50^{a-d}	1.43^{h-o}	1.07^{i-o}	3.26^{ab}
V10 (Hanna 08)	3.77^{a-f}	3.63^{b-f}	3.73^{a-f}	0.40^{m-o}	0.70^{j-o}	2.45^{b-e}
V11 (Batari)	1.83^{e-m}	1.07^{i-o}	0.30^{no}	0.50^{m-o}	0.87^{j-o}	0.91^{fg}
V12 (Pelita F1 08)	1.20^{i-o}	1.20^{i-o}	1.13^{i-o}	0.53^{k-o}	0.57^{k-o}	0.93^{fg}
V13 (Bimantara 158)	0.90^{i-o}	0.53^{k-o}	0.40^{m-o}	0.37^{m-o}	0.23^{o}	0.49^{g}
Mean	3.27^{a}	3.47^{a}	2.23^{b}	1.15^{c}	0.52^{d}	2.13

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

The average percentage of normal plantlets in the treatment of varieties and PEG concentrations can be seen in Table 2, which shows that the treatment of varieties and PEG concentrations had a significant effect, but the interaction did not significantly affect the percentage of normal plantlets. PEG at a concentration of 0% (P0) was not significantly different from treatment 5% (P1) and significantly different from PEG treatment of 10% (P2), 15% (P3) and 20% (P4). Wijaya (V5) and Genie (V9) varieties showed a high average percentage of normal plantlets (93.4 and 86.8%, respectively) but were not significantly different from the Tetra Hijau (V1: 80.00%), Pedas varieties. (V2: 80.00%), Chakra Hijau (V3: 80.00%) and Bara (V8: 73.40%). Wijaya and Genie varieties were significantly different from Sigantung (V4: 40.60%), Sapade (V5: 66.60%), Sret (V6: 40.00%), Hanna 08 (V10: 60.00%), Batari (V11: 40.00%), Pelita F1 08 (V12: 46.80%) and Bimantara 158 (V13: 33.40%) varieties. Sret (V7), Batari (V11) and Bimantara 158 (V13) varieties showed the lowest percentage of sprouts normality compared to other varieties.

Plant height: Based on the analysis of variance, it can be seen that differences in varieties, PEG concentrations and their interactions have a significant effect on plant height in Table 3. Table 3 shows that the variety of Pedas (V2) was not

Table 4: Number of leaves in cayenne pepper varieties treated with PEG *in vitro*

	Leaves*					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	4.00^{a-d}	4.00^{a-d}	2.33^{c-i}	1.33^{g-j}	0.00^{j}	2.33^{bc}
V2 (Pedas)	4.67^{a-c}	5.00^{ab}	4.67^{a-c}	2.67^{b-h}	0.00^{j}	3.40^{a}
V3 (Cakra Hijau)	4.00^{a-d}	4.00^{a-d}	2.00^{d-i}	1.67^{e-j}	0.67^{h-j}	2.47^{a-c}
V4 (Sigantung)	4.00^{a-e}	4.00^{a-d}	1.67^{f-j}	0.00^{j}	0.00^{j}	1.93^{cd}
V5 (Wijaya)	3.33^{a-f}	4.67^{a-c}	2.33^{b-h}	0.67^{h-j}	0.67^{h-j}	2.33^{a-c}
V6 (Sapade)	4.00^{a-d}	5.67^{a}	4.00^{a-d}	1.33^{g-j}	0.00^{j}	3.00^{ab}
V7 (Sret)	2.67^{b-g}	4.00^{a-e}	0.00^{j}	0.00^{j}	0.00^{j}	1.33^{de}
V8 (Bara)	3.67^{a-e}	4.00^{a-d}	3.33^{a-f}	1.33^{f-j}	0.00^{j}	2.47^{a-c}
V9 (Genie)	4.67^{a-c}	4.33^{a-c}	4.33^{a-c}	0.33^{ij}	0.67^{h-j}	2.87^{ab}
V10 (Hanna 08)	4.33^{a-c}	4.67^{a-c}	4.33^{a-c}	0.00^{j}	0.00^{j}	2.67^{a-c}
V11 (Batari)	3.00^{a-g}	1.33^{f-j}	0.00^{j}	0.00^{j}	0.00^{j}	0.87^{e}
V12 (Pelita F1 08)	3.00^{a-g}	2.00^{c-i}	0.67^{h-j}	0.00^{j}	0.00^{j}	1.13^{de}
V13 (Bimantara 158)	2.00^{c-i}	0.67^{h-j}	0.00^{j}	0.00^{j}	0.00^{j}	0.53^{e}
Mean	3.64^{a}	3.72^{a}	2.28^{b}	0.72^{c}	0.15^{d}	2.10

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

significantly different from the Cakra Hijau (V3), Sapade (V6), Genie (V9) varieties but was significantly different from other varieties. PEG treatment 0% (P0) was not significantly different from treatment 5% (P1) and significantly different from treatment 10% (P2), 15% (P3) and 20% (P4) on plant height.

The interaction of the two factors shows the existence of a combination of treatment phenomena that has a significant effect on plant height. For the combination with the highest average was obtained in the Sapade variety in PEG 5% (V6P1) treatment of 6.67 cm which showed a significant difference with PEG of 15% (V6P3) and 20% (V6P4) and not significantly different from PEG of 0% (V6P0) and 10% (V6P2). The combination of Sapade varieties with 5% PEG (V6P1) showed significant differences with the treatment of all varieties in the medium containing 15% (P3) and 20% (P4) PEG. The combination of the Sapade variety with 5% PEG (V6P1) showed no significant differences with the Tetra Hijau variety (V1) in the 0% and 5% PEG, the Pedas variety (V2) in the 0, 5 and 10% PEG, the Cakra Hijau variety (V3) at 5% PEG, Sigantung variety (V4) at 0 and 5% PEG, Genie variety (V9) at 0, 5 and 10%, Hanna 08 (V10) variety at 0 and 10% PEG, but showed significant differences outside the treatment combination. This phenomenon shows that the treatment of 15 and 20% PEG in MS medium can be used as a selective medium for drought stress because in this treatment all cayenne pepper varieties including those that can withstand 10% PEG conditions (not significantly different from the V6P1 combination) show high plants that decreased and were significantly different from V6P1.

Number of leaves: Table 4 shows that the Pedas variety (V2) was significantly different from the Sigantung (V4) and Sret (V7) varieties, but not significantly different from the other tested varieties. In the treatment, the PEG concentration difference showed that at 0% (P0) it was not significantly different from the 5% (P1) treatment, but was significantly different from the 10% (P2), 15% (P3) and 20% (P4) treatment of the number of plant leaves.

The interaction of the two factors shows the existence of a combination of treatment phenomena that has a significant effect on the number of leaves. For the combination with the highest average was obtained in the Sapade variety in PEG 5% (V6P1) treatment of 5.67 leaves which showed a significant difference with PEG of 15% (V6P3) and 20% (V6P4) and not significantly different from PEG of 0% (V6P0) and 10% (V6P2). The combination of Sapade varieties with 5% PEG (V6P1) showed significant differences with the treatment of all varieties in the medium containing 15% (P3) and 20% (P4) PEG. The combination of the Sapade variety with 5% PEG (V6P1) showed no significant differences with: the Tetra Hijau variety (V1), Cakra Hijau variety (V3), Sigantung variety (V4), Wijaya variety (V5) at 0% and 5% PEG; Pedas variety (V2), Bara variety (V8), Genie variety (V9), Hanna 08 variety (V10) at 0, 5 and 10% PEG and Sret variety (V7), Batari variety (V11) and Pelita F1 08 (V12) at 5% PEG.

Number of roots: Based on the analysis of variance, the treatment of varieties and PEG concentrations had a significant effect on the number of plant roots, but their interaction did not significantly affect the number of plant

Table 5: Number of roots in cayenne pepper varieties treated with PEG *in vitro*

	Roots*					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	7.33	9.00	4.00	3.00	1.00	4.87^{d}
V2 (Pedas)	12.67	13.67	11.00	6.33	2.33	9.20^{a}
V3 (Cakra Hijau)	13.33	10.33	3.67	4.00	1.33	6.53^{b-d}
V4 (Sigantung)	16.00	11.67	8.00	2.33	1.67	7.93^{a-d}
V5 (Wijaya)	8.33	13.00	4.67	1.67	0.67	5.67^{cd}
V6 (Sapade)	12.33	10.00	6.67	3.33	1.67	6.80^{a-d}
V7 (Sret)	9.00	10.33	2.67	4.67	1.33	5.60^{cd}
V8 (Bara)	10.67	9.67	8.00	4.33	1.00	6.73^{a-d}
V9 (Genie)	11.67	12.33	11.67	5.00	2.67	8.67^{ab}
V10 (Hanna 08)	11.33	12.00	10.00	1.67	3.67	7.73^{a-c}
V11 (Batari)	7.33	6.00	0.33	0.67	1.00	3.07^{e}
V12 (Pelita F1 08)	8.00	9.33	3.67	1.00	1.67	4.37^{de}
V13 (Bimantara 158)	2.67	1.33	0.33	0.67	0.67	1.13^{f}
Mean	10.05^{a}	9.90^{a}	5.74^{b}	2.97^{c}	1.59^{d}	6.05

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

Table 6: Root lengths in cayenne pepper varieties treated with PEG *in vitro*

	(cm)*					
	PEG (%)					
Varieties	0 (P0)	5 (P1)	10 (P2)	15 (P3)	20 (P4)	Mean
V1 (Tetra Hijau)	1.00^{e-r}	1.53^{c-p}	0.37^{l-r}	0.82^{g-r}	0.10^{r}	0.76^{cd}
V2 (Pedas)	1.77^{c-l}	2.37^{a-g}	2.43^{a-g}	0.90^{e-r}	0.53^{j-r}	1.60^{ab}
V3 (Cakra Hijau)	0.93^{e-r}	1.80^{c-l}	1.47^{c-p}	0.70^{h-r}	0.13^{qr}	1.01^{b-d}
V4 (Sigantung)	1.97^{c-j}	2.60^{a-f}	1.50^{c-r}	0.13^{qr}	0.77^{g-r}	1.39^{bc}
V5 (Wijaya)	1.33^{c-r}	1.73^{c-m}	1.10^{e-r}	1.03^{e-r}	0.53^{j-r}	1.15^{bc}
V6 (Sapade)	0.67^{h-r}	3.43^{a-c}	1.57^{c-o}	0.30^{n-r}	0.43^{k-r}	1.28^{bc}
V7 (Sret)	2.27^{b-h}	2.77^{a-e}	0.43^{j-r}	0.80^{e-r}	0.53^{j-r}	1.36^{bc}
V8 (Bara)	1.17^{d-r}	0.97^{e-r}	1.13^{e-r}	0.57^{h-r}	0.20^{o-r}	0.81^{cd}
V9 (Genie)	1.93^{c-k}	1.63^{c-n}	3.17^{a-d}	0.87^{e-r}	0.67^{h-r}	1.65^{ab}
V10 (Hanna 08)	2.03^{c-i}	4.43^{ab}	4.67^{a}	0.30^{m-r}	0.73^{f-r}	2.43^{a}
V11 (Batari)	1.40^{c-r}	1.47^{c-q}	0.10^{r}	0.57^{j-r}	0.90^{e-r}	0.89^{cd}
V12 (Pelita F1 08)	1.77^{c-m}	1.03^{e-r}	0.33^{l-r}	0.77^{f-r}	0.73^{g-r}	0.93^{bcd}
V13 (Bimantara 158)	1.37^{c-r}	0.37^{m-r}	0.27^{n-r}	0.20^{p-r}	0.27^{n-r}	0.49^{d}
Mean	1.51^{ab}	2.01^{a}	1.43^{b}	0.61^{c}	0.50^{c}	1.21

*Numbers followed by the same letter on the same line are not significantly different according to the DMRT at the 5% level. Data analysis was transformed using √(x+0.5)

roots in Table 5. Variety difference factors indicated that the Pedas variety (V2) was significantly different from the varieties of Tetra Hijau (V1), Cakra Hijau (V3), Wijaya (V5) and Sret (V7) but was not significantly different from other varieties on the number of roots. The highest number of plant roots was in the Spicy Variety (V2) treatment, namely 9.20 roots and the lowest was in the Bimantara 158 (V13) variety treatment, namely 1.13 roots. The treatment of differences in PEG concentrations showed that the treatment without giving PEG (P0) was not significantly different from the 5% (P1) treatment and was significantly different from the treatment of 10% (P2), 15% (P3) and 20% (P4) on the number of roots. The highest number of plant roots was found in treatment 0% (P0) with an average of 10.05 roots and the lowest was in treatment 20% (P4) with an average of 1.59 roots.

Root length: Based on the analysis of variance, differences in varieties, PEG concentrations and their interactions significantly affected the root length of cayenne pepper *in vitro* in Table 6. Hanna 08 (V10) was not significantly different from Pedas (V2) and Genie (V9) varieties but was significantly different from other varieties in terms of root length. The highest root length was obtained in the Hanna 08 (V10) variety, namely 2.43 cm and the shortest in the

Table 7: Stress sensitivity index (S) based on five observation variables on cayenne pepper varieties with PEG treatment *in vitro*

Varieties	Sensitivity index (S)					Mean	Tolerance criteria
	PNP	PH	NL	NR	LR		
V1 (Tetra Hijau)	1.15	1.11	1.04	1.02	1.34	1.13	Sensitive
V2 (Pedas)	1.15	1.06	1.04	0.97	1.04	1.05	Sensitive
V3 (Cakra Hijau)	0.77	1.01	0.86	1.07	1.28	1.00	Sensitive
V4 (Sigantung)	1.15	0.96	1.04	1.07	0.91	1.03	Sensitive
V5 (Wijaya)	0.38	0.92	0.83	1.09	0.89	0.82	Moderate
V6 (Sapade)	1.15	1.08	1.04	1.03	0.53	0.97	Moderate
V7 (Sret)	1.15	1.01	1.04	1.01	1.14	1.07	Sensitive
V8 (Bara)	1.15	1.05	1.04	1.08	1.23	1.11	Sensitive
V9 (Genie)	0.38	0.92	0.90	0.91	0.96	0.81	Moderate
V10 (Hanna 08)	1.15	0.95	1.04	0.80	0.96	0.98	Moderate
V11 (Batari)	1.15	0.63	1.04	1.02	0.53	0.87	Moderate
V12 (Pelita F1 08)	1.15	0.61	1.04	0.94	0.88	0.92	Moderate
V13 (Bimantara 158)	1.15	0.90	1.04	0.89	1.19	1.03	Sensitive

PNP: Percentage of normal plantlet, PH: Plant height, NL: Number of leaves, NR: Number of roots, LR: Length of roots. Tolerance criteria: Tolerant (S<0.5), moderate (0.5<S<1), sensitive (S>1)

Bimantara 158 (V13) variety, 0.49 cm. In PEG treatment, without giving PEG (P0) in culture medium significantly different from PEG treatment at 10% (P2), 15% (P3) and 20% (P4) but not significantly different at 5% (P1) to root length. The highest root length was found in 5% PEG (P1) treatment with an average of 2.01 cm and the lowest at 20% PEG (P4) with an average of 0.50 cm.

Whereas the interaction of the two factors had a significant effect on the highest average root length values for the Hanna 08 variety and 10% PEG (V10P2) of 4.67 cm, which was significantly different from the combination of all varieties in the treatment 0% (P0), 15% (P3) and 20% (P4). Several varieties, such as the varieties of Tetra Hijau, Pedas, Cakra Hijau, Sigantung, Wijaya, Sapade, Sret, Hanna 08 and Batari showed a tendency to increase root length at 5% PEG compared to 0% PEG. At concentrations of 15 and 20% PEG, all tested varieties showed a decrease in root length when compared to the PEG treatment which produced the highest root length for each variety.

Sensitivity index: The sensitivity index is used to measure the tolerance level of plant varieties to 20% PEG stress based on five observation variables, namely the percentage of normal plantlets, plant height, number of leaves, number of roots and root length. From Table 7 it can be seen that there are no tolerant varieties based on the sensitivity index value (S<0.5). Wijaya (V5), Sapade (V6), Genie (V9) and Hanna 08 (V10), Batari (V11) and Pelita F1 08 (V12) varieties are classified as moderate varieties (0.5<S<1). Meanwhile, the varieties of Tetra Hijau (V1), Pedas (V2), Cakra Hijau (V3), Sigantung (V4), Sret (V7), Bara (V8) and Bimantara are among the sensitive varieties (S>1).

DISCUSSION

Drought is a multifaceted stress condition that causes serious crops to yield limitations depending on plant growth stage, stress duration and severity. Germination is the most critical and sensitive stage in the life cycles of plants and the seeds exposed to unfavourable environmental conditions such as drought may compromise the subsequent seedling establishment[7].

Statistical analysis showed that differences in cayenne pepper varieties had a significant effect on the percentage of normal plantlets, plant height, number of leaves, number of roots and root length, while the percentage of plantlets growing had no significant effect. This is related to the existence of the genetic expression of each variety in its genetically controlled growing environment. The varieties of chilli pepper showed a significant effect on the shoot and root dry weight, shoot-root ratio, relative water content and water use efficiency[8]. The varieties play an important role in the development of cayenne pepper cultivation because each variety has a different yield potential. Wijaya and Genie varieties are varieties that have the highest average percentage of normal plantlets and this can be seen at a concentration of 20% PEG that still shows 67% of plantlets appear normal in growth. Based on the parameters of plant height, number of leaves, number of roots and root length, it still shows the existence of normal growth and is not different from the varieties that have the highest average. Through a similar approach, it was reported that the evidence for genotypic evidence showed that the lentil cultivars were the most tolerant and the most intolerant under severe drought stress[9]. Research also provides evidence that testing for

drought-tolerant germplasm can be carried out by assessing seed germination and early growth potential under water-deficient conditions. The results showed that the treatment with various concentrations of PEG had a significant effect on all observed parameters, namely the percentage of plantlets that did not grow, normal plantlets, plant height, number of leaves, number of roots and root length. Giving PEG 5% (P1) to cayenne pepper plants was not significantly different from the 0% control treatment (P0) for all parameters. This is because the PEG concentration of 5% (P1) has the potential of available water and sufficient to meet the needs of plants for the growth and development of cayenne pepper plants. It is generally known that the higher the PEG 6000 concentration, the lower the growth and development of plants. The growth and development of the worst cayenne pepper at the highest concentration, namely treatment at 20% (P4), is due to the very low water potential of the treatment so that the plant experiences stress (drought stress) which can cause plant growth and development to be very stunted, even causing sprouts to become abnormal and die. The addition of polyethylene glycol as an *in vitro* selection medium could increase seed mortality, besides that the 20% PEG concentration could be used as a sub-lethal concentration and could be used for the selection of dryness in mutant soybean seeds[10]. Giving PEG 6000 at a concentration of 20% will reduce the potential for water in the media so that the plants are unable to absorb water in the media. One form of drought-tolerant plant mechanism will reduce water potential in plants by producing proline or polyamine compounds[11,12]. Giving PEG to the culture media does not directly affect the explants, but the growth of plantlets is hampered due to the lower water potential because the water content in the media has been bound by PEG. Plant damage or death due to PEG is considered a drought effect, not a direct effect of PEG compounds because PEG is not absorbed by plants. The drought stress substantially affects parameters associated with germination and growth, with its effect being analogous to the stress level applied[9].

The initial selection of seed germination and growth of cayenne pepper against drought stress using 20% PEG shows the concentration that can be used in liquid media *in vitro*. This is shown based on the existence of cayenne pepper plants that can still grow and develop at this concentration even though there is a decreased performance in the parameters of the percentage of live plantlets, the percentage of normal plantlets, plant height, number of leaves, number of roots and root length. The drought stress simulated by PEG (15%) harmed the vegetative growth of all soybean genotypes tested, stunted phenotype, reduced the number of trifoliar leaves and nodes, reduced root length, root dry weight, shoot dry weight and plant biomass[13].

In this study, five growth indicators, percentage of plantlets normal, plant height, the number of leaves, the number of roots and root height decreased with increasing PEG concentrations. The treatment of 15 and 20% PEG in MS medium can be used as a selective medium for drought stress because in this treatment all cayenne pepper varieties including those that can withstand 10% PEG conditions (not significantly different from the V6P1 combination) show high plants that decreased and were significantly different from V6P1.

The same phenomenon was also reported for soybean, where drought stress caused severe wilting and discolouration of leaves in a sensitive genotype but relatively less damage in a resistant genotype[14]. Plants have evolved a wide range of morphological, physiological, biochemical and molecular changes to adapt to water deficit or drought stress conditions[15]. Drought stress progressively reduces the rate of CO_2 assimilation due to reduced stomatal conductance, reduced leaf size, stem extension and root proliferation, disturb plant water-relations and reduce water use efficiency[16]; decreased tuber dry weight and total biomass in Jerusalem artichoke[17] and reduced pod yield and total biomass of peanut[18].

Genetic variability within a species offers a valuable tool for studying drought tolerance mechanisms. Current results highlighted significant differences between varieties exposed to drought stress with severely decreased germination percentage, normal plantlets, plant height, number of leaves, number of roots and root length in all varieties tested. Based on the study, it was found that the interaction between varieties and PEG on plant height with the highest average value was in the Variety of Sapade at 5% PEG (V6P1) treatment of 6.67 cm, which was significantly different from other treatments and the lowest was in the Tetra Hijau and Bimantara variety at 20% PEG (V1P4 and V13P4) treatments of 0.23 cm. While the root length with the highest average value in a variety of Tetra Hijau at 10% PEG (V10P2) treatment of 4.67 cm was significantly different from other treatments and the lowest in variety of Tetra Hijau at 20% PEG (V1P4) and variety of Batari at 10% PEG (V11P2) treatment was 0.10 cm. The decrease in plant height and root length was not only caused by PEG but genetically influenced varieties, therefore the growth of each variety was different. The tolerance

mechanism of plants to drought stress varies depending on their genetic ability[19]. Four genotypes of lentil germination showed different physiological responses to drought simulations with PEG, namely differences in the increase in proline content, total soluble sugars, the activities of α-amylase and α-glucosidase[20].

The drought sensitivity index (S) is an index that can be used to assess the decrease in yield caused by a suboptimal environment compared to the optimum environment[6]. A low drought sensitivity index indicates that the genotype tested under suboptimal conditions did not show a large decrease, so it can be said that the genotype was tolerant. The characters used to calculate the drought sensitivity index were the percentage of normal plantlets, plant height, number of leaves, number of roots and root length. The results of the calculation of the average drought sensitivity index of each character are used as a reference for determining drought tolerance. Wijaya and Genie varieties have a moderate drought sensitivity index, namely 0.82 and 0.81, but also these two varieties show the lowest sensitivity index value (0.38), especially in the percentage parameter of normal plantlets and are included as the tolerance group. These two varieties are highly recommended as a source of genotypes that can be further developed in the study of drought-tolerant cayenne pepper through conventional and unconventional breeding approaches followed by various further tests both *ex vitro* and in the field. Through the same approach, the Hashim-8 variety has a low-stress tolerance index and determined to be the best candidate variety that has selection criteria for use in further wheat plant breeding programs for drought resistance[19].

CONCLUSION

PEG concentrations of up to 20% can be used as a selective medium for *in vitro* drought stress in cayenne pepper as indicated by a decrease in the percentage of normal plantlets, plant height, number of leaves, number of roots and root length. Each variety of cayenne pepper tested *in vitro* had a different response to seedling growth and development. The interaction between varieties and giving PEG had a significant effect on three parameters, namely plant height, number of leaves and root length. Varieties of Wijaya (V5), Sapade (V6), Genie (V9), Hanna 08 (V10), Batari (V11) and Pelita F1 08 (V12) were included in the moderate group, while Tetra Hijau (V1), Pedas (V2), CakraHijau (V3), Sigantung (V4), Sret (V7), Bara (V8) and Bimantara (V13) including sensitive varieties based on drought simulations using PEG 20% *in vitro*.

SIGNIFICANCE STATEMENTS

This study discovered that PEG concentrations of up to 20% can be used as a selective medium for *in vitro* drought stress in cayenne pepper. There were two genotype groups of thirteen cayenne pepper varieties tested, namely moderate genotypes and sensitive genotypes on drought stress. This study can be used by plant breeders to develop genotypes of cayenne pepper that are resistant to drought stress and by agronomists, this information can be used to develop technical cultures following the characteristics of each genotype against drought stress.

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