Screening of Varieties of Cayenne Pepper (*Capsicum frutescens* L.) on Salinity Resistance through *in vitro* Culture Technique

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Abstract: An in vitro study to select from a number of cayenne varieties possessing resistance to salinity stress derived from NaCl. The research used Randomized Complete Random (RAL) Factorial with two factors tested, namely varieties and NaCl salt concentration factor. Varieties of cayenne peppers tested were Tetra Hijau, Pedas, Cakra Hijau, Sigantung, Wijaya, Sapade, Sret, Bara, Genie and Hanna 08, while the NaCl salt concentration used on MS medium was 0, 2500, 5000, 7500 and 10,000 ppm. The study was conducted with four replications. The results showed that the varieties of cayenne pepper, NaCl concentration and interaction both significantly affected the percentage of normal planlet, plantlet height, root length and leaf number. In the abnormal percentage of planlet, the treatment of varieties and concentration had a significant effect, but the interaction of both had no significant effect. Three selected varieties can be used as populations that can be used in subsequent trials in the field.

1 INTRODUCTION

Chilli is a strategic vegetable commodity, especially red chilli and cayenne pepper. In the season of celebration or religious holidays, the need for chilli usually increases about 10-20% of normal needs. In certain seasons, chilli price increases are significant enough to affect the inflation rate (Indarti, 2016). From data obtained from the year 2013-2015, the price fluctuation of cayenne pepper is higher than the large red pepper and red curly pepper, in December 2014 the three chilli have a very high fluctuation spike (Nauly, 2016).

Cayenne pepper (*Capsicum frutescens* L.) contains nutrients that are quite complete i.e. protein, fat, carbohydrates, minerals (calcium, phosphorus, iron), vitamins, and other medicinal substances such as oleoresin, capsaicin, bioflavonoids, essential oils, carotenoids (capxhantin, capsorubin, carotene, and lutein). Compared with the large chili types (including paprika), the content of capsaicin and hydrocapsaicin in cayenne pepper is high enough that the cayenne pepper has a more spicy taste than other types of chilli (Cahyono, 2003).

The cayenne pepper plant has a shallow root system, rather diffuse, beginning with a primary roots and then growing root hairs to the side (lateral root/secondary roots). The length of the primary root is about 35-50 cm and the lateral root is about 35-45 cm. The lateral roots rapidly develop in the soil and spread at a depth of 10-15 cm (Undang, 2014). Such root morphology causes cayenne peppers to be sensitive to various stress conditions in growing media, including to salinity stress. Salt stress is first perceived by the root system and impairs plant growth both in the short term, by inducing osmotic stress the caused by reduced water availability, and in the long term, by salt-induced ion toxicity due to nutrient imbalance in the cytosol (Munns, 2005). Therefore, the two play the threats imposed by salinity are induced by osmotic stress and ionic toxicity associated with excessive Cl and Na+ uptake, leading to Ca^{2+} and K^{+} deficiency and to other nutrient imbalances (Marschner, 1995). In addition, salt stress is also manifested as oxidative stress mediated by ROS. All these responses to salinity contribute to the deleterious effects on plants (Hernández *et al*, 1993). The anatomy of the root system (length, root diameter) determines the root performance, enabling plants to acquire water and nutrients and thereby increase of the replacement rate of plant water lost (Passioura, 1988). Optimum root systems can support shoot growth and yield plant

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yields, since roots serve as an interface between plants and the soil (Vamerali, 2003).

At the present time, good land for the agricultural sector is decreasing. Agricultural extensification leads to the use of problematic land, such as land that has a high salinity which is widely found in Indonesia in the form of coastal areas, estuaries and land that have tidal influences.

Plant breeding methods in vitro culture techniques can be used as an alternative in helping the plant breeding purposes get salinity resistant stress plants. Selection in vitro can be done by determination of salt density lethal dose, salinity stability tolerance screening, and regeneration and evaluation of salinity-resistant plant yield. The study of plant responses to salinity is important in effective crop screening efforts. Screening in vitro can be used as a preliminary screening of a number of genotypes to see the effect of acidity on physiological, morphological and plant growth processes. Early screening with this tissue culture technique can be followed by field screening for more precise screening of varieties.

NaCl is the main salt in saline resistance, which makes NaCl as the salt used in the treatment of salinity stress. Inducing salinity stress in aquaculture plants through the use of NaCl salts as selective agents allows the selection or screening of plants tolerant to salt stress. The higher the induced NaCl, the surviving plants, the more tolerant the salts stress (Efendi *et al*, 2012).

Seeds are carriers of declining properties, including the resilience of saltiness. In addition, germination is the initial process of plant growth. Therefore, the less resistant or resistant properties of plants to saltiness can be seen since seed germination. Based on the description above, the purpose of this study was to screen various types of cayenne pepper for salinity stress in vitro by using agents in NaCl in the planting medium. This study is the initial stage of the plant breeding project activities to produce superior varieties of cayenne pepper which are resistant to salinity.

2 MATERIALS AND METHODS

2.1 Place, Materials and Tools

This research was conducted at Plant Culture Tissue Laboratory, Agro-technology Study Program Faculty of Agriculture, University of North Sumatra, Medan. The materials used in this study include the seeds of cayenne pepper used by many farmers in North Sumatra, namely the seeds of Tetra Hijau, MS medium: macronutrient stock solution; micronutrient stock solution, vitamin stock solution, iron stock solution, myoinositol, 3% sucrose and 0.8% agar. Materials for sterilizing seeds are alcohol, sterile aquadest, detergent, dithane, benlate, chlorox, iodine and tween 20. Buffer material pH: NaOH 0,1 N and HCl 0.1 N. label paper, pH indicator paper, aluminum foil, paper tissue and filter paper.

This research uses a Factorial Randomized Complete Random (RAL) with two treatments, namely NaCl concentration with 5 (five) levels (G0: 0, G1: 2500, G3: 5000, G4: 7500 and G5: 10000 ppm) NaCl, and various types of cayenne varieties (V1: Tetra Hijau, V2: Pedas, V3: Cakra Hijau, V4: Sigantung, V5: Wijaya, V6: Sapade, V7: Sret, V8: Bara, V9: Genie, and V10: Hanna).

2.2 Preparation Medium Culture

The media used in this study were MS solid medium, 3% sucrose, 0.8% agar with pH 5.8 before autoclave papa sterilization. The saline treatment conditions were carried out with the addition of NaCl in accordance with the prescribed treatment concentration. The treatment medium is placed in a 100 ml volume bottle of 20 ml each bottle. The culture medium was sterilized using an autoclave with a pressure of 17.5 psi, 121ºC for 15 minutes.

2.3 Aseptic Culture Technique

The chilli seeds were shaken in a 30 g/L detergent solution of distilled water for 30 minutes, then rinsed with distilled water three times. Further sterilization of chili seeds in Laminar Air Flow Cabinet aseptic. The seeds were sterilized in a benlate solution of 2 g/L for 15 minutes, and afterwards rinsed with aquadest three times. The seeds were sterilized with 20% clorox solution for 10 minutes, and soaked in 10% betadine solution for 5 minutes, then rinsed with aquadest three times. The sterilized seed is added in a sterile petridish which has four sheets of filtered paper and is moistened with sterile aquadest and then covered with petridish and wrapped with plastic wrapping to prevent contaminants from entering the petridish when the seeds are added. Two days after the seeds germinate, the sprouts are transferred into the MS medium that has been given treatment. Subsequently culture was placed on a culture rack inside the culture room with a temperature of 24 ± 2 º C.

The parameters used in this study were plant height (cm), root length (cm), number of leaves,

percentage of normal and abnormal planlet, and sensitivity index.

3 RESULTS AND DISCUSSION

In Table 1 of ten varieties tested, Sigantung varieties showed normal (100%) planlets compared to other varieties. Treatment of G2V8 shows the lowest normal planlet (0%). Variety of Pedas, Cakra Hijau and Sigantung can grow normally until the NaCl treatment is 10,000 ppm. The V7G4 treatment showed that the plantlets did not grow the highest (75%) compared to other treatments. V6, V7 and V8 showed symptoms recorded at 10,000 ppm NaCl, indicated by the highest abnormal planlets compared to other treatments. The observed percentage of abnormal planlet on V4 (0%) is different from other varieties. Giving of NaCl concentration from control G0 to 10.000 ppm concentration did not significantly affect the planlet's abnormality.

Based on analysis of variety of combination of varieties, the concentration of NaCl and interaction both significantly affect the parameters of germination height, root length, and number of leaves. Based on the analysis of various varieties known varieties, the concentration of NaCl and interaction both have a significant effect on leaf number parameters. Each genotype has different adaptability capabilities in each selection environment, knowing that genotype and environmental interactions provide opportunities for selecting and assembling lines that can adapt to specific environments (Suwignyo, 2007).

Table 2 shows an increase in NaCl concentration tends to result in a shorter planlet height in all tested varieties. The combination of G0V4 treatment (9.70 cm) yielded a higher planlet than the other treatments. While the planlet G4V1 (0.48 cm) shorter than other treatments. Treatment of G1V4 (7.28 cm) yielded the highest significantly higher plantlet height with other treatments on salinity 2500 ppm NaCl. The treatment of G2V4 (4.73 cm) yielding the highest planlet height was not significantly different from the G2V6 treatment on the salinity of 5,000 ppm NaCl. The treatment of G3V4 (3.20 cm) yielded the highest significantly higher plantlet height with G3V7 and G3V8 treatment on salinity stresses of 7.500 ppm NaCl. The treatment of G4V4 (1.93 cm) yielding the highest seedling in vitro was not significantly different from other treatments on salinity of 10,000 ppm NaCl.

Table 3 shows an increase in salt concentration causing the length of the roots to be shorter. However, G1V4 treatment yielded longer roots than G0V4 treatment. The combination of G1V4 treatment (11.58 cm) yielded longer roots, while the G4V1 treatment (0.81 cm) produced a shorter root than the other treatments. The G0V4 treatment (6.65 cm) yielded the longest root length significantly different from that of the other treatments at a salinity of 0 ppm NaCl. The G2V4 treatment (8.18 cm) yielded the longest root length significantly different from that of the other treatments on the salinity of 5,000 ppm NaCl. The treatment of G3V4 (3.05 cm) yielded the longest root length was not significantly different from the treatment of G3V3 and G3V9 on salinity of 7.500 ppm NaCl. Treatment of G4V4 (1.93 cm) yielded the longest root length significantly different from the treatment of G4V1 and G4V8 on salinity of 10,000 ppm NaCl.

Table 4 shows the increase in salt concentration causing less number of leaves. Treatment G0V4 (8 sheets) produces more leaves than other treatments. while the G2V8, G2V9, G3V5, G3V9, G4V1, G4V3, G4V7 and G4V8 treatment resulted in leafless plantlets (0 sheet). Treatment of G1V4 (7.00 sheets) yielded the highest number of leaves significantly different from the other treatments at 2500 ppm salinity of NaCl. Treatment of G2V4 (5.75 sheets) yielded the highest number of leaves significantly different from the other treatments at 5,000 ppm NaCl salinity. The treatment of G3V4 (3.25 sheets) yielded the highest number of leaves was not significantly different from the treatment of G3V2 and G3V3 at salinity of 7.500 ppm NaCl. Treatment of G4V4 (1.50 sheets) yielded the highest number of leaves was not significantly different from the other treatments on salinity of 10,000 ppm NaCl. The initial growth gap will be a potential capital to produce later growth gaps, such as plants that have more leaves at the beginning of growth will grow faster because of the ability to produce higher photosynthesis than plants with lower leaf area (Sitompul and Guritno, 1995).

Seedlings used to determine the ISC are only 3 (three) varieties, namely Spicy varieties, Cakra Hijau and Sigantung). The normal sprouts criteria in question is the sprouts that have good root system development, especially the primary roots and for plants that normally produce the roots of the seminal then this root should be no less than two, the development of a good hypocotyls without any damage to the tissue, the growth of a perfect plumula with leaves green and grows well, inside or emerging from a coleoptile or a perfect epicotile growth with normal buds and having two cotyledons for dicotyl sprouts.

Table 1: Percentage of normal, not-growing and abnormal plantlets of cayenne pepper varieties at different concentration of NaCl.

Parameter	Varieties	Salinity (ppm NaCl)					Mean
		G_1	G_2	G_3	G_4	G_5	
Normal Planlet		……%[1]..……					
	V_1 (Tetra Hijau)	100_a	100_a	50_{ab}	75_{ab}	0_b	65
	V_2 (Pedas)	100_a	100_a	75ab	75ab	25ab	75
	V_3 (Cakra Hijau)	100_a	100_a	75_{ab}	50_{ab}	25_{ab}	70
	V_4 (Sigantung)	100_a	100_a	100_a	100_a	100_a	100
	V_5 (Wijaya)	100_a	100_a	75ab	0b	0b	55
	V_6 (Sapade)	100_a	100_a	100_{ab}	25ab	0b	65
	V_7 (Sret)	100_{ab}	100_a	50_{ab}	0_b	0_b	50
	V_8 (Bara)	100_a	100_a	0_b	25_{ab}	0_b	45
	V_9 (Genie)	50_{ab}	50_{ab}	50_{ab}	0_b	0_b	30
	V_{10} (Hanna 08)	100_a	50_{ab}	75_{ab}	25_{ab}	0_b	50
Mean		95	90	65	37.5	15	60.5
Planlet Not Growing	V_1 (Tetra Hijau)	0_b	0_b	0_b	0_b	50_{ab}	10
	V_2 (Pedas)	0_b	0_b	0_b	0_b	0_b	0
	V_3 (Cakra Hijau)	0_b	0_b	0_b	0_b	0_b	0
	V_4 (Sigantung)	0_b	0_b	0_b	0_b	0_b	0
	V_5 (Wijaya)	0_b	0_b	0_b	25_{ab}	0_b	5
	V_6 (Sapade)	0_b	0_b	0_b	25_{ab}	50_{ab}	15
	V_7 (Sret)	0_b	0_b	25_{ab}	50_{ab}	75_a	30
	V_8 (Bara)	0_b	0_b	50_{ab}	0_b	25_{ab}	15
	V_9 (Genie)	0_b	0_b	0_b	0_b	0_b	0
	V_{10} (Hanna 08)	0_b	0_b	0_b	0_b	25_{ab}	5
Mean		0	0	7.5	10	22.5	8
Abnormal Planlet	V_1 (Tetra Hijau)	0	0	50	25	50	25_a
	V_2 (Pedas)	0	0	25	25	75	25_a
	V_3 (Cakra Hijau)	0	0	25	50	75	30_a
	V_4 (Sigantung)	0	0	0	0	0	0_b
	V_5 (Wijaya)	0	0	25	75	100	40_a
	V_6 (Sapade)	0	0	0	50	50	20_a
	V_7 (Sret)	0	0	25	50	25	20_a
	V_8 (Bara)	0	0	50	75	75	40_a
	V_9 (Genie)	50	50	50	100	100	70_a
	V_{10} (Hanna 08)	0	50	25	75	75	45_a
Mean		5_a	10_a	27.5_a	52.5_a	62.5_a	31.5

[1] The numbers followed by different letter notation on the same columns and rows are significantly different based on Duncan Multiple Range Test at 5% level.

Table 2: Planlet height with treatment of cayenne pepper varieties and NaCl concentration *in vitro*.

Varieties	Salinity (ppm NaCl)					Mean
	G_0 (0)	G_1 (2.500)	G_2 (5.000)	G_3 (7.500)	G_4 (10.000)	
	……cm[1]……					
V_1 (Tetra Hijau)	2.98_{c-l}	3.03_{c-l}	1.88_{f-p}	1.33_{h-p}	0.48_{p}	1.94
V_2 (Pedas)	4.03_{c-f}	4.63_{cd}	2.15_{e-p}	1.18_{i-p}	1.28_{i-p}	2.65
V_3 (Cakra Hijau)	3.28_{c-j}	4.25_{c-e}	2.45_{d-p}	1.48_{g-p}	1.08_{j-p}	2.51
V_4 (Sigantung)	9.70_{a}	7.28_{b}	4.73_{c}	3.20_{c-j}	1.93_{f-p}	5.37
V_5 (Wijaya)	2.20_{e-p}	1.98_{f-p}	2.30_{e-p}	1.30_{h-p}	1.58_{g-p}	1.87
V_6 (Sapade)	4.00_{c-f}	3.50_{c-h}	3.60_{c-g}	1.75_{g-p}	0.98_{k-p}	2.77
V_7 (Sret)	3.13_{c-k}	1.60_{g-p}	0.88_{l-p}	0.73_{m-p}	0.53_{op}	1.37
V_8 (Bara)	2.93_{c-m}	3.38_{c-i}	0.70_{op}	0.93_{k-p}	0.95_{k-p}	1.78
V_9 (Genie)	1.95_{f-p}	2.70_{c-o}	1.43_{g-p}	1.18_{i-p}	1.55_{g-p}	1.76
V_{10} (Hanna 08)	2.83_{c-n}	2.30_{e-p}	2.00_{f-p}	1.75_{g-p}	1.10_{j-p}	2.00
Mean	3.70	3.46	2.21	1.48	1.14	2.40

[1] The numbers followed by different letter notation on the same columns and rows are significantly different based on Duncan Multiple Range Test at 5% level.

Table 3: The length of plantlet roots by treatment of cayenne pepper varieties and the different concentration of NaCl *in vitro*.

Varieties	Salinity (ppm NaCl)					Mean
	G_0 (0)	G_1 (2.500)	G_2 (5.000)	G_3 (7.500)	G_4 (10.000)	
	.....……cm[1]…..…….					
V_1 (Tetra Hijau)	1.63_{c-l}	0.95_{d-l}	0.98_{d-l}	0.50_{g-l}	0.81_{l}	0.85
V_2 (Pedas)	2.43_{c-f}	2.70_{c-e}	2.38_{c-g}	0.88_{d-l}	1.28_{c-l}	1.93
V_3 (Cakra Hijau)	1.35_{c-l}	2.13_{c-g}	1.38_{c-l}	2.00_{c-j}	1.08_{c-l}	1.59
V_4 (Sigantung)	6.65_{b}	11.58_{a}	8.18_{ab}	3.05_{c}	1.93_{c-i}	6.28
V_5(Wijaya)	1.15_{c-l}	0.80_{e-l}	0.75_{f-l}	0.38_{i-l}	1.58_{c-l}	0.93
V_6 (Sapade)	2.73_{cd}	2.00_{c-h}	2.73_{c-j}	0.45_{g-l}	0.50_{g-l}	1.68
V_7 (Sret)	4.00_{c-e}	1.20_{c-l}	0.53_{g-l}	0.33_{j-l}	0.55_{h-l}	1.32
V_8 (Bara)	1.25_{c-l}	1.10_{c-l}	0.25_{kl}	0.75_{f-l}	0.30_{kl}	0.73
V_9 (Genie)	1.43_{c-l}	1.10_{c-l}	1.55_{c-l}	1.15_{c-l}	0.95_{d-l}	1.24
V_{10} (Hanna 08)	1.25_{c-l}	1.70_{c-k}	1.85_{c-j}	0.50_{g-l}	0.55_{g-l}	1.17
Mean	2.39	2.53	2.06	1.00	0.89	1.77

[1] The numbers followed by different letter notation on the same columns and rows are significantly different based on Duncan Multiple Range Test at 5% level.

Based on calculation of stress sensitivity index (Table 5) by comparing data of plant height character and root length of cayenne pepper under control condition and recorded known on high character of planlet of Spicy varieties (V2) and Green Cakra varieties (V3) is rather tolerant to salinity stress, long root character of Green Cakra varieties (V3) tolerant to salinity and spicy varieties (V2) is rather tolerant to salinity stress. Crop tolerance is the ability of plants to grow and complete the life cycle and able to provide results on the stress condition (Wibowo *et al*, 2016). The mechanisms of plant resistance to stress vary between species and varieties from the most vulnerable to most resistant levels.

However, based on the percentage of sprout or normal planlet, Sigantung variety showed the highest normality (100%) while the varieties of Pedas and Cakra Hijau showed 75% abnormality and normal planlet only 25%. Therefore, for further field studies the three varieties (Pedas, Cakra Hijau and Sigantung) can be used as testable varieties for salinity resistance.

Table 4: Number of planlet leaf with treatment of varieties of cayenne pepper and NaCl concentration *in vitro*.

Varieties	Salinity (ppm NaCl)					Mean
	G_0 (0)	G_1 (2.500)	G_2 (5.000)	G_3 (7.500)	G_4 (10.000)	
	……sheet[1]……					
V_1 (Tetra Hijau)	2.25_{d-g}	1.75_{d-h}	1.18_{d-h}	0.50_{e-h}	0.00_{h}	1.20
V_2 (Pedas)	2.50_{c-f}	2.75_{c-e}	1.51_{d-h}	1.00_{d-h}	0.25_{gh}	1.40
V_3 (Cakra Hijau)	2.00_{d-g}	3.00_{cd}	1.35_{d-h}	1.50_{d-h}	0.00_{h}	1.60
V_4 (Sigantung)	8.00_{a}	7.00_{ab}	5.75_{ab}	3.25_{b-d}	1.50_{d-h}	5.10
V_5 (Wijaya)	2.25_{d-g}	1.75_{d-h}	1.75_{d-h}	0.00_{h}	0.25_{gh}	1.20
V_6 (Sapade)	3.00_{cd}	2.00_{d-g}	3.33_{d-g}	0.50_{e-h}	1.25_{d-h}	2.02
V_7 (Sret)	4.67_{cd}	1.50_{d-h}	0.50_{e-h}	0.50_{e-h}	0.00_{h}	1.43
V_8 (Bara)	2.25_{d-g}	3.25_{b-d}	0.00_{h}	0.50_{f-h}	0.00_{h}	1.20
V_9 (Genie)	1.25_{d-h}	2.25_{d-g}	0.00_{h}	0.00_{h}	0.50_{f-h}	0.80
V_{10} (Hanna 08)	1.27_{d-h}	1.25_{d-h}	0.50_{e-h}	0.50_{e-h}	0.25_{gh}	0.75
Mean	2.94	2.65	1.63	0.83	0.40	1.69

[1] The numbers followed by different letter notation on the same columns and rows are significantly different based on Duncan Multiple Range Test at 5% level.

Table 5: Selection of tolerable, slightly tolerant and susceptible salinity genotypes based on stress sensitivity index values.

Varieties	High planlet			ISC[1]	Description
	Control	10.000 ppm	Difference		
V_2 (Pedas)	4.03	1.28	-2.75	0.91	somewhat tolerant
V_3 (Cakra Hijau)	3.28	1.08	-2.20	0.90	somewhat tolerant
V_4 (Sigantung)	9.70	1.93	-7.78	1.07	susceptible
Varieties	Length of roots the planlet			ISC[1]	Description
	Control	10.000 ppm	Difference		
V_2 (Pedas)	2.43	1.28	-1.15	0.76	somewhat tolerant
V_3 (Cakra Hijau)	1.35	1.08	-0.28	0.30	tolerant
V_4 (Sigantung)	6.65	1.93	-4.73	1.51	susceptible

[1] Plants are said to be tolerant if ISC <0.5, rather tolerant if 0.5 <ISC <1, and susceptible if ISC> 1 (Riduan *et al.,* 2005)

4 CONCLUSIONS

The type of cayenne pepper varieties tested, the concentration of NaCl and the interaction both influence the growth character of plantlet height, root length, number of leaves, normal plantlet percentage and percentage of plantlet not grow. Of the ten tested varieties, three varieties were obtained which showed normal growth sprouts if cultured in medium up to 10,000 ppm NaCl, namely Spicy, Green and Sigantung varieties. These three varieties can be used as the selected genotype for testing salinity resilience in the field.

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