



Determination of Salinity Tolerance on Cayenne Genotypes Based on Leaf Damage Symptoms

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Abstract. *Capsicum* sp. Has been reported as a salt-sensitive crop. Therefore, using salt-tolerant varieties will be a wise solution for maintaining crop production in coastal areas. Source of saline-tolerance controlling genes are needed to develop saline-tolerant genotypes. The objective of this study was to evaluate the tolerance of cayenne accessions to salinity stress during the vegetative phase based on the leaf damage symptoms. The experiment was carried out using a hydroponic technique with a solution of macro and micronutrients AB mix supplemented by 6000 ppm NaCl. A total of 20 genotypes of cayenne pepper were evaluated in a completely randomized design with three replications. Each experimental unit consisted of two plants grown in polybags with sand media. To determine the tolerance level of plants to salinity stress, the tolerance index (TI) was calculated based on the score of leaf symptoms. The results showed that genotypes A21 and A32 exhibited better vegetative growth performance in leaf greenness, number of leaves, and stem diameter compared to other genotypes. There was no saline-tolerant genotype found. All genotypes showed abnormal symptoms on the leaves. However, the sensitivity index indicated that genotypes A21 and A32 were classified as moderately tolerant with the mildest symptoms.

Keywords: *Capsicum* · Nutrient-culture · Salt-stress · Leaf-indicator

1 Introduction

Cayenne is one of the most favored hot seasoning spices of Indonesian culinary. An upsurge in population, simultaneously with depletion of fertile soil, has forced efforts to increase national cayenne production on suboptimal land, including the use of coastal areas. The soil in coastal areas has high salinity, which inhibits plant growth. Many reports mention that salinization mostly due to high evaporation lifting the salt from the soil solution to the soil surface. Most of the water on earth contains about 30 g of sodium chloride per litre [1]. It also occurs in coastal areas influenced by seawater.

In coastal areas, salinization of the soil is due to the use of salt-contaminated water for irrigation. Salt contamination of the fresh water is triggered by inflow of sea water to the upland aquifer due to uncontrolled ground water extraction [2]. The other factor increasing soil salinity is extended seawater flood as the sea level rises [3].

High salinity had adverse effects on plant growth and yield [1]. Salinity stress influences physiological processes, such as water deficit symptoms due to increasing soil osmotic potential [4]. It also triggers ion toxicity as Na^+ and Cl^- highly accumulate in the plant tissue [5], which causes ionic imbalances and restrains nutrient uptake [6]. Salinity stress also limits gas exchange which influences stomata closure and restrains CO_2 supply to the leaves [7].

To counteract the adverse effects of salt in soil solution, plants employ a variety of features. The most important feature is osmotic alteration; to preserve turgor [8]. Plants achieve this in part by excluding Na^+ and Cl^- , especially from leaves, and relying on organic solutes for osmotic adjustment ('ion exclusion') and by accumulating enough Na^+ and Cl^- to balance those in the soil solution while simultaneously maintaining ionic regulation in various cell compartments ('tissue tolerance') [9].

Agronomic and environmental engineering approaches to overcome the problem of salinity in coastal areas have indeed been exhausted; the only approach to minimize the impact of saline land on crop production is to breed greater salt tolerance crops for cultivation [9]. However, cayenne is considered sensitive to salinity stress [7]. Therefore, exploring sources of gene(s) controlling tolerance to salinity in many cayenne populations is a very strategic approach. The objective of this study was to determine salinity tolerance on cayenne based on leaf damage symptoms.

2 Materials and Methods

The experiment was carried out in a wick system hydroponic with sand as the planting media. The sand was put into polybags with dimensions of 35 cm high and 30 cm in diameter. At the center bottom of each polybag, a flannel fabric wick was set through the media. The polybag was placed on a plastic bucket, 30 cm in diameter, containing the nutrient solution. Three weeks old seedlings were set up in the hydroponic system without salinity stress.

Hydroponic nutrients consisting of macro and micronutrients were prepared from a commercial AB mix ("Hydro J" of Jingga-Ag Inc.) previously dissolved as the stock solution. The formulation of hydroponic nutrients followed the methods of Resh [10]. In addition to the macro and micronutrients, NaCl at a concentration of 6000 ppm was added to the hydroponic nutrient solution as the saline stress treatment. The stress treatment was carried out 25 days after transplanting.

A total of 20 cayenne genotypes (Table 1) were arranged in a completely randomized design with three replications, and each experimental unit consisted of 2 plants. At six weeks after saline stress treatment, plant height, leaf greenness, number of leaves, and stem diameter were measured to evaluate plant performances. Normality and homogeneity test of the data were performed by the method of Kolmogorov-Smirnov and Bartlett, respectively. Analysis of variance (ANOVA) was conducted to determine the effect of treatments on the dependent variables. For variables significantly affected by treatments, DMRT at $\alpha = 5\%$ was conducted to compare the mean values among treatments.

Table 1. List of genotypes

Genotype code	Origin
A02	Bengkulu2, Indonesia
A03	Lampung, Indonesia
A04	Taiwan1
A05	Taiwan2
A07	Bangka, Indonesia
A11	Temanggung1, Indonesia
A13	Bengkulu3, Indonesia
A14	Temanggung2, Indonesia
A15	Temanggung3, Indonesia
A16	Bengkulu4, Indonesia
A17	Bengkulu5, Indonesia
A18	Taiwan3
A21	Thailand1
A25	Bogor2, Indonesia
A26	Magelang, Indonesia
A28	Bengkulu7, Indonesia
A29	Bengkulu8, Indonesia
A30	Bengkulu9, Indonesia
A31	Rawit Bangkok, Indonesia
A32	Bengkulu10, Indonesia

Table 2. Score and leaf symptoms of cayenne due to NaCl stress

Score	Observed symptoms
1	Normal leaves, no symptoms
3	Nearly normal leaves, but leaf margin slightly rolled upward with yellowish color
5	Leaf margin rolled upward with yellowish color spread over leaf surface
7	Leaf margin rolled upward with yellowish color spread over leaf surface and necrotic
9	Almost all plants dead or dying

The genotype response to salinity stress was observed visually on the leaf symptoms. The intensity of the symptoms was translated into the symptom scores (Table 2). Scoring was performed according to the standard evaluation system used to rate the visual symptoms of salt toxicity injury on rice [11] with modification.

The tolerance level of plants to salinity stress was calculated based on the proportion score of the leaves of each plant. The resulting scores were then converted to stress sensitivity indices (SSI) as suggested by Popoola et al. [12].

$$SSI = \frac{\sum i(n \times v)}{N \times 9} \times 100\% \quad (1)$$

where n = number of plants in each score, v = the score of leaf symptoms, N = total number of the observed plants. The salinity tolerance was classified as highly tolerance (SSI = 0%), tolerance (SSI = 1 – 10%), moderately tolerance (SSI = 11–25%), susceptible (SSI = 26 – 50%), and highly susceptible (SSI ≥ 50%).

3 Results

The cayenne genotypes exhibited varied responses to NaCl stress. The variation in response to salinity was most probably due to genetic properties of the germ-plasms evaluated. The analysis of variance (ANOVA) revealed that the genotype had a highly significant effect on stem diameter and leaf greenness, a significant effect on the number of leaves, but no effect on plant height (Table 3). This data demonstrated that genotype performance against NaCl stress varied greatly. Genotype diversity can be due to genetic factors as they are from disparate regions. The coefficient of variation (CV) was relatively high indicating the influence of non-treatment factors was significant.

3.1 Performance of Cayenne Genotypes on NaCl-Stressed Media

In this study, the cayenne genotypes were subjected to stress of 6000 ppm NaCl equivalent to 102.5 mM. Observations at the age of 6 weeks after transplanting showed that all tested genotypes lessened in growth. Vegetative growth of chili pepper showed varied performance [13]. Saline stress decreased plant height by more than 50% in genotypes A03, A04, A07 A11 and A26. The genotypes with less than 20% decrease in plant height were A13, A18 and A25. The variation of cayenne pepper plant height is in line with the variation in the number of leaves. The taller the plant, the higher the number of the leaves, with a correlation coefficient of 68%. Genotypes A13, A17 and A29 appeared taller with more leaves than other genotypes.

Table 3. The mean square value of genotype, error and calculated F of ANOVA

Variable	Genotype	Error	F _{cal.}	Probability	CV (%)
Plant height ^T	1.5261	0.9170	1.6642 ^{ns}	0.0867	21.71
Leaf greenness ^T	7.1576	1.8183	3.9365 ^{**}	0.0001	24.81
Number of leaves	35.5395	18.1333	1.9599 [*]	0.0364	22.51
Stem diameter	7.8835	1.7288	4.5602 ^{**}	0.0000	26.84

^{**}: significantly difference at P < 0.01, ^{*}: significantly difference at P < 0.05, ns: not significant, T = analysis on transformed data with $\sqrt{(y + 0.5)}$

Leaf greenness is a variable that describes the severity of symptoms due to NaCl stress. At high NaCl concentrations, cayenne plants showed wilting symptoms, starting from the leaf margins. NaCl stress until 6 WAT caused yellowish leaves with necrotic spots. Discoloration indicates the occurrence of damage to chlorophyll, disrupting photosynthesis. To estimate the chlorophyll content of the leaves, the greenness of the leaves was measured using SPAD (Soil Plant Analysis Development) meter. SPAD-502 has been proved to be a reliable tool for the non-destructive measurement of total foliar chlorophyll concentration and content across a range of plant ages, growing conditions, and genotypes [14]. The measurement of the greenness of the leaves revealed little variation. Genotypes A02, A16, A26, A28, A29, and A32 had higher greenness values than the others, although the values of some genotypes were not significantly different. The stem diameter of the A32 genotype was greater than that of the other genotypes. A32 had a relatively high variable value of leaf greenness and number of leaves in addition to stem diameter. When all variables were considered, the genotypes that performed best under 6000 ppm NaCl stress were A29 and A32 (Table 4).

Table 4. Performance of cayenne genotypes at 6000 ppm NaCl stressed for six weeks

Genotypes	Control	6000 ppm NaCl stressed			
	Plant Height (cm)	Plant Height (cm)	Number of Leaves	Leaf Greenness (unit)	Stem Diameter (mm)
A02	23.83 ^a	14.97 ^{bcd}	20.33 ^{a-d}	40.13 ^a	6.29 ^{bc}
A03	51.17 ^a	22.66 ^{abc}	17.33 ^{b-e}	30.17 ^{ab}	4.30 ^{cde}
A04	16.08 ^a	7.40 ^d	12.17 ^e	7.20 ^d	2.95 ^f
A05	20.17 ^a	11.08 ^{cd}	14.00 ^{de}	10.77 ^{cd}	3.10 ^{ef}
A07	52.31 ^a	23.68 ^{ab}	20.67 ^{a-d}	38.18 ^{ab}	5.23 ^{b-e}
A11	48.04 ^a	22.79 ^{abc}	17.50 ^{b-e}	26.91 ^{ab}	4.86 ^{b-e}
A13	36.14 ^a	31.37 ^a	22.17 ^{ab}	37.44 ^{ab}	4.16 ^{cde}
A14	38.71 ^a	25.48 ^{ab}	18.50 ^{a-e}	23.57 ^{ab}	4.27 ^{cde}
A15	40.83 ^a	23.42 ^{abc}	21.83 ^{ab}	35.61 ^{ab}	5.09 ^{b-e}
A16	26.00 ^a	14.74 ^{bcd}	21.17 ^{abc}	43.38 ^a	5.37 ^{bcd}

(continued)

Table 4. (continued)

Genotypes	Control	6000 ppm NaCl stressed			
	Plant Height (cm)	Plant Height (cm)	Number of Leaves	Leaf Greenness (unit)	Stem Diameter (mm)
A17	34.96 ^a	23.83 ^{ab}	23.17 ^{ab}	38.38 ^{ab}	5.51 ^{bcd}
A18	26.17 ^a	22.03 ^{abc}	19.00 ^{a-e}	17.97 ^{bc}	3.90 ^{def}
A21	22.21 ^a	16.90 ^{a-d}	16.50 ^{b-e}	37.18 ^{ab}	3.11 ^{ef}
A25	27.75 ^a	23.94 ^{ab}	19.33 ^{a-d}	38.10 ^{ab}	6.75 ^b
A26	43.46 ^a	15.67 ^{bcd}	18.50 ^{a-e}	41.23 ^a	6.57 ^b
A28	50.54 ^a	26.13 ^{ab}	20.17 ^{a-d}	44.87 ^a	4.79 ^{b-e}
A29	47.58 ^a	27.48 ^{ab}	25.33 ^a	45.34 ^a	6.62 ^b
A30	25.21 ^a	14.72 ^{bcd}	14.67 ^{cde}	26.40 ^{ab}	3.15 ^{ef}
A31	16.13 ^a	15.81 ^{bcd}	13.83 ^{de}	30.48 ^{ab}	3.94 ^{def}
A32	29.29 ^a	17.01 ^{bcd}	22.17 ^{ab}	44.20 ^a	8.98 ^a

Note: numbers followed by the same letters are not significantly different

3.2 Tolerance of Cayenne Genotypes to NaCl Stress

Visual observation of leaf damage symptoms due to NaCl stress has long been developed for rice plants [15]. Determination of the level of NaCl tolerance in rice plants based on the severity of symptoms in leaves has been formulated by IRRI guidelines [11]. In broadleaf plants such as cayenne, information on the rating of leaf damage symptoms due to NaCl stress has not been formulated to determine the tolerance level of plants.

With NaCl stress of 6000 ppm, all plants in this study showed symptoms of growth abnormalities that could be observed in the leaves. At 6 weeks under stress, several plants had already died. Survived plants showed variations in leaf symptoms. The mildest symptom was the leaf margin curling up. The most severe symptoms were the leaves turning brown coloration and falling. The gradation of the severity of cayenne leaf damage symptoms due to NaCl stress was displayed in Fig. 1.

Table 5. Tolerance level of cayenne genotypes based on symptoms of leaf damage under stressed at 6000 ppm NaCl

Genotypes	Sensitivity Index (%)	Category
A02	67.16	highly susceptible
A03	69.45	highly susceptible
A04	100.00	highly susceptible
A05	95.68	highly susceptible
A07	49.69	susceptible
A11	85.49	highly susceptible
A13	79.97	highly susceptible
A14	89.08	highly susceptible
A15	48.25	susceptible
A16	54.83	highly susceptible
A17	64.32	highly susceptible
A18	86.15	highly susceptible
A21	23.75	moderately tolerant
A25	65.87	highly susceptible
A26	49.30	susceptible
A28	59.68	highly susceptible
A29	58.67	highly susceptible
A30	95.77	highly susceptible
A31	77.87	highly susceptible
A32	23.06	moderately tolerant

The symptom scores on the leaves obtained were used to determine the genotype sensitivity index. No accession was tolerant to stress NaCl 6000 ppm for 6 weeks. Genotypes A21 and A32 were the two accessions showing mild symptoms with severity index (SI) values of 23.75% and 23.06%, respectively, and they were classified as moderately tolerant to NaCl. Fifteen accessions had SI values of 50% or higher, so they were classified as highly susceptible. The other two accessions, A07 and A15, were susceptible with SI values of 49.69 and 48.25, respectively (Table 5).

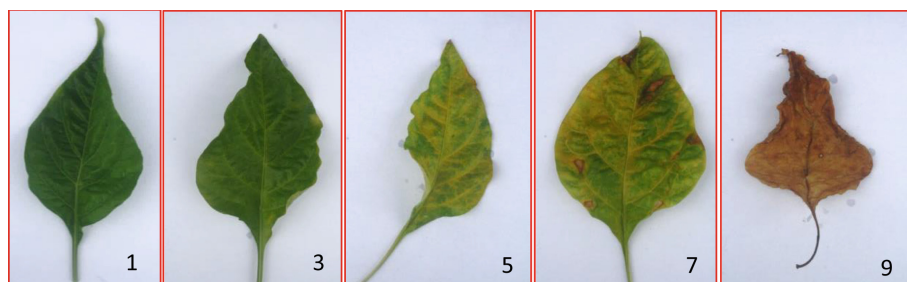


Fig. 1. Score of leaf symptoms on NaCl toxicity on cayenne leaves.

4 Discussion

Chili pepper is one highest economic value vegetable crops in Indonesia [16]. This plant is generally classified as sensitive to salinity [17]. The tolerance level to salinity stress depends upon genotype and salt concentration [18]. At a concentration of 40 mM NaCl, most genotypes could tolerate the salinity, and there was no significant effect on growth. At a concentration of 80 mM NaCl or higher, all the plants tested experienced significant growth disturbances [19]. The sensitivity level of chili plants was also influenced by the plant growth stage. At the vegetative stage (6 weeks after transplanting), the plant height, the leaf area, and the dry weight significantly decreased at salinities higher than 25 mM NaCl. The plant height reduction in these conditions was almost as high as 50%. The vegetative stage is the most sensitive phase compared to the seedling and generative phases. Growth inhibition in the seedling phase occurred at salinities higher than 50 mM NaCl [20].

The stress of 6000 ppm NaCl or the equivalent of 102.5 mM NaCl resulted in decreased growth in all chili plants. Reduction in plant height, leaf greenness and stem diameter were due to disruption of plant physiological processes. High NaCl concentration triggers lower leaf water potential, decreased leaf relative water content, and reduced turgor. Plants will close their stomata and limit CO₂ assimilation to reduce the rate of photosynthesis [21].

Reduced plant turgor at high NaCl concentrations resulted in wilting, starting from the leaf margins. Young leaves are more sensitive and wilt easily. Potassium ion (K⁺) plays an important role in maintaining intracellular turgor pressure. It is transported to plant cells against concentration gradients via K⁺ transporters and membrane channels. During salt stress, Na⁺ ions compete with K⁺ for transporters by increasing the concentration of Na⁺ in the soil because they share the same transport mechanism, which reduces K⁺ uptake [22].

Symptoms of wilting and curling on leaves of chili sensitive to NaCl stress began to appear at 2 WAP. Continued stress caused the leaf color to turn yellowish to white, starting from the leaf margin. Discoloration extended to the center of the leaf at 4 WST. The proportion of the number of discolored leaves, as a sign of chlorophyll damage, indicated the sensitivity level of the plant. The maximum chlorophyll fluorescence of rocket was significantly lower than the control plants at a concentration of 100 mmol/L NaCl or higher [23]. Furthermore, the NaCl stress level decreased the net photosynthetic

rate. At the age of 6 WAP all plants showed symptoms of necrosis with varying volumes. Symptoms of necrosis in leaves are the behavior of Programmed Cell Death (PCD) as a last-ditch effort to survive. PCD is a physiologically and genetically regulated process that removes damaged cells in response to abiotic and biotic stress [24]. Cell death in rice roots was found to be well-regulated during salinity stress, suggesting that the dead cells may have a role in preventing the influx of excess Na⁺ ions into the inner parts of roots and shoots, resulting in salt exclusion [25].

In our study, there was variability in the growth performance of genotypes grown in NaCl stressing media until six weeks after transplanting. Some genotypes showed better growth than the others, such as A13, A28 and A29. However, the good growth of these genotypes, indicated by plant height and number of leaves, did not correlate with their tolerance characteristic. On the other hand, some genotypes with mediocre performance exhibited better tolerance to NaCl stress, such as A21 and A32. Genetics make up of each genotypes plays an important role in controlling this phenomenon.

Salinity stress of 6000 ppm NaCl for 6 weeks caused significant growth retardation in the cayenne plant. Based on the symptoms of leaf damage, cayenne genotypes of A21 and A32 exhibited the mildest damage symptoms and were classified as moderately tolerant genotypes.

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Authors' Contributions. R organized the experiment and prepared manuscript for publication. MS designed the salt stress treatment. WP set up the hydroponic system. CH was responsible for data management and analysis. YS was responsible for plant maintenance during the experiment and data collection. All authors read and approved the final manuscript.

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