Employing STI to Determine Saline Tolerant Cayenne Genotypes

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Abstract. Saline tolerant genotypes are required in the development of saline adaptive cayenne cultivars. Salinity tolerance was reported to be polygenic controlled. Evaluation of saline-tolerant genotypes involving many characters simultaneously might increase the success of selection. This study aimed at evaluating the tolerance of cayenne pepper accessions to salinity stress employing the stress tolerance index (STI). A total of 20 genotypes were grown using a wick-system hydroponic. Each polybags containing dirt-free sand media were placed on a plastic container containing AB mix nutrients. The media was connected to the nutrient solution by a wick of flannel fabric. NaCl stressing media was prepared by fortifying the AB mix nutrient with 6000 ppm NaCl. A control media was also prepared without supplementing NaCl into the AB mix nutrients as a comparison in evaluating the decrease in growth and yield. The experiment was carried out in a greenhouse with a completely randomized design with three replications. Each experimental unit consisted of two plants. To determine the level of plant tolerance to salinity stress, STI value was calculated which involved 13 cayenne growth and yield variables. The results showed that the tolerant genotypes based on average STI were A25_Bogor2, A26_Magelang and A29_Bengkulu8. Genotypes that were moderately tolerant were A02_Bengkulu2, A07_Bangka, A11_Temanggung1, A13_Bengkulu3, A16_Bengkulu4, and A32_Bengkulu10. The remaining 10 other genotypes are classified as sensitive.

Keywords: Cayenne, Nutrient-culture, Salt-stress.

1 Introduction

Saline soils are abundant in dry climates due to the impact of high evaporation [1]. The salinity of coastal lands is also caused by seawater intrusion, which is depends on vegetation and season [2]. In Indonesia, saline agricultural land is mainly found in coastal area, which covers at least 12 020 Mha of the total agricultural land (100 700 Mha) [3]. High salinity had adverse effects on plant growth and yield [1]. Among abiotic stresses, high salinity stress is the most severe environmental stress. The loss of agricultural production due to the effect of salinity is estimated to be around 25% to 50% of the total irrigated land exposed to salt [4].

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Salinity stress increases the osmotic potential of the soil causing damage to root function, other organelles, and cellular processes [5]. Na+ and Cl- ions that enter and accumulate in plant tissues also trigger ion toxicity [6]. This condition induces leaf senescence, abscission, or death [7]. Chilies are sensitive to salinity stress, especially during the flowering or fruit development phase [8]. The approach to minimizing the impact of saline land on crop production is to breed for tolerant cultivars [9]. In Indonesia, breeding for saline-adaptive chilies has not been developed much so until now it is still difficult to obtain saline-tolerant chili seeds. Therefore, chili varieties that can adapt to saline environments are important for researchers.

Saline tolerance trait is known polygenic controlled. Various genes are regulated, the products of which are involved either directly or indirectly in plant protection. Effective selection for environmental stress tolerant plants depends on the breeder's ability to find a compromise between yield, yield loss, and yield stability [10, 11]. Many genes contribute to regulating salinity tolerance such as genes encoding osmolytes, ion channels, receptors, calcium signaling components, and several other regulatory signaling factors or enzymes [12]. Therefore a selection of saline-tolerant plants should involve many characters.

The level of tolerance of a genotype to saline stress can be calculated by various formulas. The stress-tolerant index (STI) value is better selection criterion for saline tolerance than the stress sensitivity index (SSI). The stress tolerance index is calculated based on the yield loss rate in relative to normal conditions [13]. High STI values under conditions of high salinity are known to have a positive correlation with plant height, total and productive tillers, biomass and yield of rice plants under normal conditions [14]. The stress tolerance index has been used successfully to identify chili genotypes with the best response under salinity stress conditions [15, 16].

This study aimed at selecting 20 cayenne genotypes that were treated with 6000 ppm NaCl stress using the STI method.

2 Materials and Methods

The experiment was conducted in the greenhouse of Dept. Crop Production, Faculty of Agriculture, The University of Bengkulu. The cayenne genotypes, in a total of 20 genotypes, were evaluated in a completely randomized design. Each treatment was repeated 3 times and each experimental unit consisted of 2 plants. Sixteen cayenne genotypes were collected from various regions in Indonesia (A02_Bengkulu2, A03_Lampung, A07_Bangka, A11_Temanggung1, A13_Bengkulu3, A14_Temangung2, A15_Temangung3, A16_Bengkulu4, A17_Bengkulu5, A25_Bogor2, A26_Magelang, A28_Bengkulu7, A29_Bengkulu8, A30_Bengkulu9, A31_commercial variety Rawit Bangkok, and A32_Bengkulu10). The other four accessions were introduced genotypes (A04_Taiwan1, A05_Taiwan2 A18_Taiwan3, A21_Thailand1). NaCl stress was applied to the hydroponic nutrient solution in a concentration of 6000 ppm [17]. The macro and micronutrients, from a commercial AB mix (“Hydro J” of Jingga-Ag Inc.) were applied to the hydroponic media following the methods of Resh (2022) [18]. The chilies seeds were first sown in celled trays until a pair of fully opened leaves emerge, then, they were transplanted into a dirt-free sand substrate which had been washed from salt contamination and other debris. The substrate was placed in polybags connected to the nutrient solutin in the containers using flannel wicks. The plants were maintained in NaCl-stress condidition from transplanting to six weeks old. Plants were taken care for following the commercial chili growers and observed for their vegetative and generative growth until harvest. Two control plants per genotype, grown without NaCl stress, were treated equally. The dichotomous height, no of leaves, no of branches, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, canopy area, fruit length, fruit diameter, no of fruits, and fruit weight/plant were measured to calculate the value stress tolerance index (STI).

The value of STI was calculated by the formula of Osuna-Rodríguez et al. [15]:

$$\text{STI} = \frac{(Yp.Ys)}{(\bar{Y}p)^2} \tag{1}$$

Where STI, Ys, Yp and $\overline{\text{Yp}}$, were Stress Tolerance Index, the observed value in stress condition, the observed value in non-stress condition, the mean value over all genotypes evaluated in non-stress condition.

Tolerance was categorized in relative over all genotype under study and classified into three classes, i.e. tolerance, moderately tolerance, and sensitive, respectively. The class interval was calculated by the following formula:

$$\text{Interval} = \frac{(STIh - STIl)}{3} \tag{2}$$

Where: STIh was the highest STI and STIl was the lowest one.

The salinity tolerance was classified as

Tolerance = STIh - interval $<$ X $\leq$ STIh

Moderately tolerance =STIh - 2 x interval $<$ X $\leq$ STIh - interval

Sensitive = STIl $\leq$ X $\leq$ STIh – 2 x interval

3 Results

3.1 Performance of Cayenne Genotypes in NaCl-stress Media

Cayenne plants experienced significant growth inhibition at 6000 ppm NaCl stress or equivalent to 102.5 mM. The decrease in yield was greater than in vegetative growth. The decrease in growth was represented by the first dichotomous height and shoot dry weight, while the decrease in yield was represented by the fruit weight /plant. Saline stress reduced the growth of some genotypes by more than 50%. More severely affected genotypes with dichotomous height reduced by higher than 50% were A03, A04, A11 and A26 The smallest decrease in dichotomous height was in A13, which was 34.4%.

At the end of the experiment, the shoot dry weight was measured. The interference with NaCl stress was more evident in the ability of the plants to accumulate dry matter.

A decrease of more than 50% of plant dry weight occurred in almost all genotypes except A11, A15, A25, A29, and A32. The most severe disturbance was on the ability to bear fruit. Fruit weight per plant was reduced by more than 50% in all genotypes except A21. Genotypes A04, A05, A14 and A30 were even unable to produce fruit as the flowers and young or immature fruits fallen. Genotype A21 genetically set a small number of fruits so that the fruit weight per plant was also low, however, 6000 ppm NaCl stress only reduced fruit weight 19.9%.

Table 1. Decrease in growth of cayenne genotypes at 6000 ppm NaCl stress media.

Genotype	First Dichotomous Height (cm)			Shoot dry weight (g)			Fruit weight/ plant (g)		
	Control	6000 ppm NaCl	Decrease (%)	Control	6000 ppm NaCl	Decrease (%)	Control	6000 ppm NaCl	Decrease (%)
A02	26.50	14.97	43.5	45.86	18.30	60.1	120.83	43.01	64.4
A03	47.20	22.67	52.0	72.49	6.38	91.2	150.74	44.65	70.4
A04	21.50	7.40	65.6	36.39	1.49	95.9	228.66	0.00	100.0
A05	19.50	11.08	43.2	55.79	1.98	96.5	201.16	0.00	100.0
A07	45.00	23.68	47.4	84.82	38.73	54.3	184.30	45.24	75.5
A11	48.04	22.79	52.6	44.68	39.98	10.5	233.63	31.95	86.3
A13	47.85	31.37	34.4	69.91	11.62	83.4	168.68	35.33	79.1
A14	41.80	25.49	39.0	76.01	4.64	93.9	42.35	0.00	100.0
A15	40.83	23.42	42.6	38.99	20.11	48.4	247.40	54.07	78.1
A16	26.00	14.74	43.3	114.15	26.99	76.4	134.80	29.56	78.1
A17	34.96	23.83	31.8	65.11	22.19	65.9	84.10	27.43	67.4
A18	31.50	22.03	30.1	50.00	18.82	62.4	123.15	12.12	90.2
A21	13.50	6.90	48.9	73.42	2.00	97.3	38.83	31.09	19.9
A25	47.80	23.95	49.9	75.58	46.48	38.5	183.64	64.32	65.0
A26	43.46	15.67	63.9	170.61	42.52	75.1	280.62	39.32	86.0
A28	50.54	26.13	48.3	46.70	12.33	73.6	296.25	36.10	87.8
A29	45.50	27.48	39.6	36.49	34.50	5.5	509.86	57.95	88.6
A30	20.50	14.72	28.2	49.50	2.80	94.3	429.66	0.00	100.0
A31	26.13	15.81	39.5	63.90	2.75	95.7	37.57	2.45	93.5
A32	29.29	17.01	41.9	64.41	61.13	5.1	85.77	7.91	90.8

3.2 Tolerance of Cayenne Genotypes to NaCl Stress

Evaluation on plants in selection for tolerance to environmental stress has long been developed by researchers. Fernadez has presented the STI formula to estimate for plant tolerance to drought stress based on the relative decrease in plant performance under stress conditions compared to that under normal conditions. This formula is still used in screening for environmental-stress tolerance. This formula is known as effective as Mean Productivity (MP), Geometric Mean Productivity (GMP), and Yield Index (YI) [19, 20].

At NaCl-stress of 6000 ppm, all plants in this study showed symptoms of growth reduction. At 6 weeks after transplanting, all plants of genotype A04, the sensitive one, started to lose their leaves and two weeks later they died. Genotype A04, visually, showed the most severe growth disturbance. Almost all variables had the STI value of 0 except for dichotomous height, shoot fresh weight, and shoot dry weight, which was also very small. Therefore, the average STI value of the A04 genotype was the lowest (0.01). The genotype with the highest STI value was A26 (0.92). The contribution of the high STI value is from the variable shoot fresh weight and root fresh weight.

Based on the average STI value over all variables observed, the salinity-tolerance categories were determined. The level of tolerance was divided into three classes, namely tolerance, moderately tolerance, and sensitive. The class interval was obtained from the highest STI minus the lowest STI divided by 3. Our data revealed that the calculated class interval was 0.3. The tolerance category was attributed to genotype with the STI value in the range of 0.62 - 0.92, medium tolerance was between 0.32 - 0.62, and sensitive was below 0.32.

Genotypes A25, A26, and A29 were three genotypes which had the highest STI values, i.e. 0.84, 0.92, and 0.75, respectively, and they were classified as tolerant. The seven genotypes classified as medium tolerant were A02, A07, A11, A13, A15, A16, and A32. The rest ten genotypes with STI values <0.31 were classified as sensitive. Furthermore, based on the STI value of fresh fruit weight per plant, genotype A29 had the highest STI value of 0.83. The accessions Bengkulu were more adaptive to saline stress eventhough they had a lower average STI than A25 and A26 (Table 2).

Table 2. Decrease in growth of cayenne genotypes at 6000 ppm NaCl stress media.

Genotype	Dichotomous height	No of leave	No of branch	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Canopy area
A02	0.32	0.85	0.14	0.35	0.08	0.19	0.10	0.02
A03	0.86	0.24	0.36	0.17	0.03	0.11	0.01	0.48
A04	0.13	0.00	0.00	0.02	0.00	0.01	0.00	0.00
A05	0.17	0.04	0.02	0.05	0.02	0.03	0.02	0.00
A07	0.85	0.74	0.64	0.36	0.69	0.77	0.44	0.52
A11	0.88	0.36	0.20	0.31	0.40	0.42	0.25	0.23
A13	1.20	0.17	0.09	0.31	0.17	0.19	0.14	0.17
A14	0.85	0.09	0.07	0.06	0.10	0.08	0.03	0.00
A15	0.76	0.28	0.24	0.13	0.63	0.18	0.30	0.20
A16	0.31	0.81	0.51	0.75	0.67	0.72	0.51	0.33
A17	0.67	0.58	0.34	0.22	0.28	0.34	0.26	0.51
A18	0.55	0.59	0.39	0.21	0.17	0.22	0.14	0.08

A21	0.07	0.10	0.04	0.04	0.05	0.03	0.03	0.01
A25	0.92	1.16	0.77	1.02	0.79	0.82	1.03	0.30
A26	0.54	0.66	0.39	2.11	2.03	1.70	1.61	0.28
A28	1.06	0.32	0.17	0.10	0.05	0.13	0.07	0.16
A29	1.00	0.23	0.58	0.19	1.24	0.29	1.55	0.13
A30	0.24	0.06	0.03	0.04	0.03	0.03	0.01	0.00
A31	0.33	0.10	0.05	0.12	0.14	0.04	0.07	0.01
A32	0.40	1.45	0.50	0.68	0.50	0.92	0.27	0.86

Table 2. continued.

Genotype	Fruit length	Fruit diameter	Fruit weight	No of fruit	Fruit weight/ plant	Average STI	Category
A02	0.37	1.08	0.39	0.18	0.15	0.32	moderately tolerance
A03	0.20	0.11	0.03	1.13	0.19	0.30	sensitive
A04	0.00	0.00	0.00	0.00	0.00	0.01	sensitive
A05	0.00	0.00	0.00	0.00	0.00	0.03	sensitive
A07	0.68	0.44	0.08	0.58	0.23	0.54	moderately tolerance
A11	0.43	0.34	0.03	0.27	0.21	0.33	moderately tolerance
A13	0.91	0.49	0.76	0.12	0.17	0.38	moderately tolerance
A14	0.00	0.00	0.00	0.00	0.00	0.10	sensitive
A15	0.90	0.74	0.09	0.29	0.37	0.39	moderately tolerance
A16	0.54	1.01	0.08	0.05	0.11	0.49	moderately tolerance
A17	0.15	0.19	0.01	0.20	0.06	0.29	sensitive
A18	0.26	0.18	0.12	0.16	0.04	0.24	sensitive
A21	0.52	0.29	0.32	0.07	0.03	0.12	sensitive
A25	0.57	1.63	1.28	0.31	0.33	0.84	tolerance
A26	0.50	1.25	0.13	0.51	0.31	0.92	tolerance
A28	0.48	0.42	0.09	0.07	0.30	0.26	sensitive
A29	1.10	0.52	0.66	1.45	0.83	0.75	tolerance
A30	0.00	0.00	0.00	0.00	0.00	0.03	sensitive
A31	0.18	0.12	0.02	0.00	0.00	0.09	sensitive
A32	0.42	1.13	0.04	0.03	0.02	0.55	moderately tolerance

4 Discussion

Chili pepper is a vegetable crop plant that can grow well in various regions in Indonesia [21]. However, it growth and yield are greatly decreased when it is grown in high salinity soil [22]. Sensitiveness of the plant to salinity stress is greatly depend on the phase of plant growth and stress duration [23]. The fast growing vegetative phase is more sensitive than the seedling and generative phases [17].

In this study stress of 6000 ppm NaCl resulted in decreased growth and yield of all the chili genotypes tested. Disruption of plant physiological processes might be the factor responsible for the reduction in vegetative growth rate and yield components. Growth disturbance occured because high NaCl concentration in the media trigger a lower water potential in the leaves, lowering the relative water content of the leaves, and consequently, reducing plant turgor. Under these conditions the plant closes its stomata and limits CO2 assimilation to reduce the rate of photosynthesis [24].

Sensitive plants showed wilting symptoms due to lessening plant turgor. The one responsible for maintaining intracellular turgor is potassium ion (K+). These ions are transported to the plant cell against the concentration gradient via K+ transporters. Therefore, young leaves are more sensitive and easily to wilt. At high Na+ concentrations, these ions compete with K+ for transporters because they share the same transport mechanism. Thus, it reduces the absorption of K+ and decreases cell turgor [6]. In this study, NaCl stress caused wilting and curling symptoms in young leaves of the sensitive chili genotype A04 from 2 WAP. Leaves turn yellow to white and eventually fall. It was reported that high NaCl causes chlorophyll fluorescence so that the leaves turn pale [25]. Symptoms of necrosis and leaf fall are the behavior of Programmed Cell Death (PCD) as an effort to survive under stressful conditions. PCD is regulated physiologically and genetically in response to abiotic and biotic stress [26].

NaCl stress causes growth disturbance in surviving plants. Plant response varies depending on genetics. Each genotype has a different response to NaCl-stress condition manifested in all the observed variables. Therefore, determining the tolerance level to NaCl stress based on one or a few variables becomes inaccurate. Evaluation of genotypes for environmental stress has been developed by many researchers [27]. Yassin et al. [28], using various methods to determine tolerant plants and using confirmed STIs with other methods. In this study, 13 vegetative and generative variables were used to determine the STI value. High STI variations were obtained based on the average STI of all variables. Genotypes A25, A26, and A29 were the three accessions with the highest STI values and they were classified as saline tolerant genotypes. Therefore, these three genotypes can be used for future breeding materials.

5 Conclusion

Salinity stress of 6000 ppm NaCl caused a significant decrease in growth and yield of cayenne pepper plants. Based on the STI values of the growth variables and yield components, the cayenne pepper genotypes A25, A26 and A29 showed the highest STI values and were classified as tolerant genotypes.

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