Assessment of salinity tolerance on chili pepper genotypes

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Abstract. Population diversity is necessary in selecting salinity tolerant plants. The objective of the study was to evaluate the tolerance of chili pepper (*Capsicum* sp.) to salinity stress. The evaluation was carried out artificially in a greenhouse. Twenty-four accessions of chili were grown on hydroponic media with stress treatment of 3000 ppm and 6000 ppm NaCl. As a comparison, the control was planted without NaCl. Nutrients were given from the AB mix nutrient solution containing macro and micronutrients. The study used a completely randomized design with 3 replications. To evaluate the ability of the genotype to adapt to NaCl stress, the difference between control and observations at 6000 ppm NaCl stress was calculated. The results showed that the growth of chili pepper genotypes under NaCl stress varied widely. There was an interaction between the genotype and the stress level of NaCl on leaf greenness and the number of stomata. In general, the stress of 3000 ppm NaCl significantly reduced chili performance starting from 6 weeks after transplanting. The stress of 6000 ppm NaCl caused some sensitive plants to die at 8 weeks after transplanting. The tolerant genotypes at 6000 ppm NaCl stress were A10, A21, and A33.

1 Introduction

Chili was originally a wild plant, which belongs to the family of Solanaceae. This plant originated from South America and then spread to the Americas, Europe and Asia [1]. Chili is popular because of its pungency and flavor [2, 3]. The characteristic pungency and flavor of chili fruit provides a unique hotness combined with salt and other spices for flavor enhancement. Capsaicinoids, carotenoids, vitamins, flavonoids such as anthocyanins are present as the major phytochemicals in chili pepper fruits [4]. Capsaicinoids contain capsaicin which makes chili peppers spicy [5]. The capsaicin in chili peppers is also used to prevent cardiovascular disease, cancer, and neurological disorders [6]. In general, fresh chilies contain 0.1-1.0% capsaicin which can be found in the seeds, skin, placenta, and flesh of the fruit [7].

Cayenne pepper has a higher level of spiciness than chili. The production of cayenne pepper in Indonesia has always increased since 2011. In 2020 the production of cayenne

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pepper reached 1508404 tons. However, in 2021 there will be a decline in production of 8.09%. to 1386447 tons. In that year, Indonesia imported 27851.98 tons of cayenne pepper in the first semester [8].

Increased production of chili can be done by expanding the planting area. However, the agricultural land available in Indonesia is mostly marginal. Marginal land is poor in nutrients. The land can still be used for agricultural development but the yield potential is low. The low productivity of the land is because the physical, biological and chemical properties of the soil do not support the plant [9].

Including marginal land is saline land. Of the various saline stresses, NaCl stress has the most adverse effect on plants [10]. Usually saline land is found on the coastal. The entry of sea water into the mainland due to uncontrolled groundwater extraction also causes salinization of fresh water [11]. Another factor that increases soil salinity is rising sea levels due to climate change [12, 13]. Under these conditions, the soil pH becomes high so that it is not suitable for plant growth and development [14].

Plant growth and yield were disturbed on saline land [15]. Salt levels above 4.0 dS/m affect physiological processes. The symptoms caused are symptoms of a water deficit due to the increased osmotic potential of the soil [12]. The accumulation of Na+ and Cl- ions also causes plant tissue toxicity [16] and inhibits nutrient absorption [17]. Salinity stress also causes limited gas exchange in the stomata thereby inhibiting the supply of CO2 to the leaves [18].

Plants have a vary of mechanisms to counteract the detrimental effects of salt in soil solutions. In general, plants carry out osmotic changes to maintain turgor [19]. The first mechanism involves removing Na+ and Cl-, mainly from leaves, and relying on organic solutes for osmotic adjustment. The second mechanism involves collecting Na+ and Cl- to balance them in the soil solution while maintaining ionic regulation in various cell compartments [20]. This study aimed to evaluate 24 accessions of chili pepper grown at various levels of salinity.

2 Materials and methods

Experiments were carried out in nutrient culture. A nutrient solution medium consisting of macro and micronutrients from a commercial AB mixture. The formulation of hydroponic nutrients followed the methods of Resh [21] with modifications. The study used a Completely Randomized Block Design with two factors and three replications. The first factor is the accession of cayenne pepper and the second factor is the concentration of NaCl. A total of 24 accessions of cayenne pepper from several regions in Indonesia and introductions from various countries were evaluated for their performance on NaCl-stressed media. Three stress levels i.e. control (without stress), 3000 ppm NaCl and 6000 ppm NaCl were used for the evaluation of cayenne pepper up to 8 weeks after transplanting.

The performance of each accession was evaluated at 8 weeks after transplanting (WAT). Observation variables included plant height, number of leaves, leaf area, leaf greenness, number of stomata, and percent of stomata open. To determine the effect of treatment on the observed variables, an analysis of variance (ANOVA) was performed. Meanwhile, to compare the average value between treatments, the LSD test was carried out. The reduced performance of chili pepper under the stress presented descriptively.

3 Results

The chili genotype evaluation in this study used stresses of 3000 and 6000 ppm NaCl or equivalent to 51.25 and 102.5 mM. As a comparison, chili is also grown without stress.

Chili accessions showed varying responses to NaCl stress. The difference in response was indicated by the interaction of chili accession with NaCl concentration on the variable leaf greenness. and the number of stomata. Genotypes and NaCl concentration had a very significant effect on all variables observed but the percentage of stomata open on NaCl concentration.

3.1 Effect of treatment interaction on vegetative growth of chili

Without NaCl stress, the chili genotype that had the highest leaf greenness was A37, while the lowest were A21 and A24. The leaf greenness of A41 was not significantly different from A37. Stress at 3000 ppm NaCl changed the ranking of the genotypes so that the highest level of leaf greenness was A39. Meanwhile, at the stress of 6000 ppm NaCl, A13, A20, and A39 had the highest greenish leaf values The chili leaves treated with NaCl stress began to yellowish at 6 WAT. This can be seen from the lower leaf greenness value at higher NaCl stress. In the stress of 3000 ppm NaCl, the highest leaf greeness values were A39 and A43 (Table 1). There was an interaction between the treatments tested on the number of stomata. The genotype with the highest number of stomata in the control treatment was A41. There were three genotypes that actually had few stomata, namely A18, A21, and A32 with the number of stomata 15.3, 15.0, and 15.0 respectively (Table 2).

Table 1. Leaf greenness of chili genotypes in normal and saline-stressed conditions.

Geno types	Control		3000 ppm NaCl		6000 ppm NaCl		Geno types	Control		3000 ppm NaCl		6000 ppm NaCl	
A04	36.3	Ae-h	26.3	Bdef	23.5	Cb-e	A30	32.5	Ahi	24.5	Bef	26.3	Ba-d
A07	34.9	Ah	27.1	Bc-f	18.7	Cfgh	A31	35.6	Afgh	28.4	Bcde	24.2	Ca-e
A10	40.1	Ac-g	26.7	Bc-f	27.5	Babc	A32	37.8	Ad-h	24.0	Bef	21.4	Cd-g
A13	40.4	Ac-f	28.3	Bcde	29.4	Ba	A33	37.1	Ad-h	28.4	Bcde	23.3	Cb-f
A15	41.6	Acde	30.4	Bbcd	26.7	Cabc	A37	49.1	Aa	26.7	Bc-f	24.1	Ca-e
A18	32.4	Ahi	22.4	Bf	27.7	Cab	A38	37.5	Ad-h	34.2	Bab	15.3	Ch
A20	35.7	Afgh	27.4	Bcde	29.2	Ba	A39	36.7	Ae-h	36.8	Ba	28.8	Bab
A21	29.0	Ai	22.8	Bf	20.2	Ce-h	A40	35.2	Agh	22.5	Bf	21.2	Bd-g
A24	28.5	Ai	28.1	Bcde	25.5	Ba-e	A41	47.2	Aab	24.5	Bef	24.3	Ba-e
A25	37.7	Ad-h	27.4	Bcde	22.7	Cc-f	A42	40.9	Ac-f	29.3	Bb-e	21.4	Cd-g
A28	43.9	Abc	31.7	Babc	22.0	Cc-g	A43	42.0	Abcd	36.3	Ba	20.3	Cefg
A29	43.6	Abc	30.7	Bbc	29.0	Ba	A44	41.4	Acde	22.2	Bf	17.2	Cgh

Note: Numbers followed by the same capital letter on the same line or the lowercase letters in the same column were not significantly different according to LSD test α=5%.

Table 2. Number of stomata of chili genotypes in normal and saline-stressed conditions.

Geno types	Control		3000 ppm NaCl		6000 ppm NaCl		Geno types	Control		3000 ppm NaCl		6000 ppm NaCl	
A04	33.0	Bde	33.0	Bef	34.7	Ac	A30	19.0	Ahi	14.3	Bl	13.0	Bkl
A07	41.3	Abc	36.0	Bde	24.3	Cefg	A31	25.3	Af	20.0	Bijk	16.3	Cijk
A10	44	Ab	30.3	Bfg	20.0	Chi	A32	15.0	Bi	16.7	Akl	13.3	Ckl
A13	40.3	Abc	42.0	Bbc	41.7	Bb	A33	24.0	Afg	24.7	Ahi	16.3	Bij
A15	34.3	Acd	27.3	Bgh	26.0	Bdef	A37	27.7	Cf	30	Bfg	32.3	Ac
A18	15.3	Ci	17.0	Bkl	21.0	Agh	A38	28.3	Bef	25.3	Chi	31.0	Acd
A20	19.7	Bgh	15.7	Cl	22.0	Afgh	A39	16.3	Bhi	19.3	Ajk	11.0	Cl
A21	15.0	Ci	16.7	Bkl	30.7	Acd	A40	16.7	Bhi	17.7	Akl	15.0	Cjkl
A24	26.3	Bf	24.7	Bhi	27.7	Ade	A41	51.0	Aa	46.7	Ca	49.7	Ba
A25	19.3	Ahi	20.7	Aij	15	Bjkl	A42	41.7	Bbc	44.7	Ab	29.7	Cd
A28	24.0	Bfg	32.3	Aef	15.7	Cijk	A43	15.7	Bhi	17.7	Akl	7.3	Cm
A29	27.3	Bf	38.7	Acd	16.0	Cijk	A44	27.7	Af	23.7	Cij	18.3	Bhij

Note: Numbers followed by the same capital letter on the same line or the lowercase letters in the same column were not significantly different according to LSD test α=5%.

3.2 Effect of genotype and NaCl on vegetative growth

The response of chili genotypes to NaCl stress based on plant height was almost the same between observations of 4, 6, and 8 WAT. Genotypes A07, A28, A29, A31, and A41 were significantly higher than other genotypes. However, the high plants were not always accompanied by a large number of leaves. The highest number of leaves from the 5% level LSD test was A20. Genotypes with very few leaves were A10, A18, A25, and A39. The four chili peppers had fewer than 8 leaves (data not shown). The effect of NaCl can be seen from 4 WAT. The higher the concentration of NaCl, the lower the plant height and number of leaves. The decrease in plant height was very significant with values of 43.63 cm, 29.50 cm, 21.07 cm respectively at concentrations of NaCl 0 (control), 3000 ppm and 6000 ppm. The same phenomenon occurred in the number of leaves 20.92, 14.15, 10.26 respectively at control, 3000 ppm and 6000 ppm NaCl.

Tolerance performance in chili at the stress of 6000 ppm NaCl was calculated by comparing the difference between the values of the decrease with the control at 8 WAT. The decrease in chili vegetative performance based on plant height, number of leaves, and leaf greenness ranged from 10 to 80%. There was a consistent decrease in plant height with a decrease in the number of leaves. The genotypes that were more tolerant with the percentage reduction in plant height, number of leaves, and leaf greenness were less than 40% were A10, A21, and A33. Several genotypes also showed good performance, namely A28 and A30. Meanwhile, the genotypes that were sensitive with a percentage decrease of more than 70% even died at 8 WAT were A4, A18, A25, and A41 (Table 3).

Table 3. Percent decrease in plant height, number of leaves, leaf greenness and percent of live plants.

Geno types	Plant Height	Number of Leaves	Leaf greenness	Live plants	Geno types	Plant Height	Number of Leaves	Leaf greenness	Live plants
A04	59.95	80.25	35.26	67	A30	20.81	45.94	19.08	100
A07	55.15	36.73	46.42	100	A31	47.22	33.08	32.02	100
A10	32.00	17.20	31.42	100	A32	56.31	49.59	43.39	100
A13	44.27	47.72	27.23	100	A33	38.71	31.11	37.20	100
A15	67.50	44.31	35.82	100	A37	51.86	53.13	50.92	67
A18	78.54	81.31	14.51	0	A38	72.97	46.88	59.20	67
A20	42.22	55.67	18.21	100	A39	54.68	61.86	21.53	100
A21	12.90	-22.70	30.34	100	A40	86.54	50.00	39.77	100
A24	35.79	54.94	10.53	100	A41	55.39	44.94	48.52	33
A25	75.10	61.54	39.79	67	A42	46.28	59.58	47.68	100
A28	33.50	39.19	49.89	100	A43	70.16	73.21	51.67	67
A29	54.49	72.12	33.49	100	A44	52.52	50.26	58.45	100

4 Discussion

The response of chili plants to saline stress varies. It is known that most of the genotypes can tolerate salinity at a concentration of 40 mM NaCl. However, at concentrations of 80 mM NaCl or more, all the plants showed significant growth disturbances [22]. In this study, the highest stress was treated with 6000 ppm or the equivalent of 102.5 mM NaCl. The decrease in plant height, number of leaves, and leaf greenness are caused by disruption of plant physiological processes. A high concentration of NaCl triggers a decrease in leaf water potential, and a decrease in turgor because plants close stomata to reduce the rate of photosynthesis [23].

The percentage of stomata open decreased in almost all genotypes but was not significantly different. We found a high variation in the number of stomata among genotypes. Some genotypes also exhibited less percentage of open stomata in the stress treatment of 6000 ppm NaCl. The closing of stomata is the result of a decrease in plant turgor. In general, plants showed symptoms of wilting due to a decrease in turgor followed by yellowish leaves. The element responsible for regulating plant turgor is potassium. Water and solutes enter plant cells against the concentration gradient through membrane channels by K^+ transporters. If the soil contains Na^+ ions, it competes with K^+ and reduces the uptake of K^+ by plants [24].

Physiological disturbances due to NaCl stress that can be seen is a decrease in the leaf greenness. The longer exposure to stress up to 8 WAT causes yellowish leaves and even necrotic spots. Color changes indicate the occurrence of damage to chlorophyll which results in the disruption of photosynthesis. Leaf chlorophyll content was thought to measure leaf greenness using SPAD (Soil Plant Analysis Development). SPAD-502 is accurate for measuring total leaf chlorophyll concentration and content across various plant ages, growing conditions, and genotypes [25]. Measurement of leaf greenness in the treatment of 6000 ppm NaCl showed slight variation in the range from 24.02 to 34.39 Genotypes G29 and G39 had higher leaf greenness scores than the others, i.e. 34.39 and 34.09, respectively. Both genotypes also had a small number of stomata, i.e. 27.33 and 15.56, respectively (data not shown). However, there is no consistent correlation between the number of stomata and the leaf greenness.

Several observed showed varying responses to NaCl stress. Determination of tolerant genotypes based on decreased growth is more responsible because it eliminates the influence of genotype. The genotypes that were tolerant with a percentage decrease of less than 40% in plant height, number of leaves, and leaf greenness were A10, A21, and A33. While the sensitive genotypes with a percentage decrease of more than 70% even died at 8 WAT were A4, A18, A25, and A41.

5 Conclusion

Genotypes classified as tolerant with an average decrease in performance of less than 40% were A10, A21, and A33. While the sensitive genotypes with a percentage decrease of more than 70% were A4, A18, A25, and A41.

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