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Evaluation of soybean (*Glycine max* L.) under different salinity stress on seedling growth and biochemical responses

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Abstract The effects of NaCl concentrations (0 mM, 40 mM, 80 mM, and 120 mM) on soybean growth, physiology, and biochemical were investigated. Increasing salinity decreased plant height, root length, leaf number, leaf greenness, biomass, and chlorophyll content, while proline levels increased. There were significant interactions between cultivars and NaCl concentrations in almost all parameters. At 120 mM, plant death was found in Chiang Mai 84-2 and Chiang Mai 60 cultivars, in contrast, the cultivar Nakhon Sawan 1 exhibited no plant death at any NaCl concentration, indicating its suitability for salinity stress.

Keywords: Soybean, Sodium chloride, Salt stress, Proline content, Chlorophyll content

Introduction

Soybeans (*Glycine max* (L.) Merr.) are considered one of the world's most important crops which are rich in protein, lipids, and various minerals for use in food, feed, and many industrial materials. The increasing of population, food demand, healthy focusing, and Argo-industry and farming development make the demand of soybean increase accordingly (Hasan *et al*., 2015). In addition to food and feed, soybeans have various other uses by the root nodule of soybean can provide nitrogen fixation. In addition to this environmental benefit, soybeans are cultivated for various purposes, including animal feed, fast-growing crops, and renewable fuel production. However, the environmental influences soybean production such as abiotic stress and biotic stress. Salinity is one of abiotic stress due to accumulation of soluble salts, including chlorides and sulfates of sodium which reduce water uptake, promote the toxicity of ions such as sodium (Na^+) and chloride (Cl^-) and osmotic pressure inside the plant body due to accumulated salts (Munns, 2005; Absari and Kuswanto, 2019). The land affected by salt was estimated about 1 billion hectares of land out of the 13 billion hectares in the

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world and more than 3% of agriculture areas are seriously threatened by salinization (FAO, 2021; Al-Tawaha *et al*., 2021). Salinity stress continues to grow and is severe due to global warming and drainage problems in irrigated areas (Hassani *et al*., 2021). Hence, the development of highly salt-tolerant plants is necessary to support the increasing nutritional demands.

Soybean exhibits sensitivity to varying levels of salt stress throughout its growth stages, from seed germination to later developmental phases. Zhang *et al*. (2019) reported that the damages to soybean were caused by high levels of salt including high osmotic stress, water loss, homeostasis and ion imbalances and morphology changes such as leaf chlorosis, necrosis and scorching. Salt tolerance during the seed germination and post-germination stages is crucial for soybean survival and growth. High salt concentrations particularly affect seed germination by reducing radicle and lateral root development, as well as the overall germination rate. (Simaei *et al*., 2012; Zhou *et al*., 2023). In the soybean seedling stage, mostly play severe leaf necrosis and even mortality in salt-sensitive soybeans, and only a moderate growth reduction in salt-tolerance soybeans which are caused by the accumulation of more chloride ions in the stems and leaves (Abel, 1969). Moreover, soybean yield components include root fresh and dry weight, shoot fresh and dry weight, total fresh and dry weight, plant height, root length, leaf number, and leaf area of soybean seedlings also decrease which is affected by salt stress (Hamayun *et al*., 2010; Dolatabadian *et al*., 2011; Egbichi *et al*., 2014; Kang *et al*., 2014; Klein *et al*., 2015). In terms of induced Oxidative Stress, the increasing ROS production such as lipid peroxidation influences the antioxidant system and causes oxidative damage (Egbichi *et al*., 2013). Soybean seedling suffers from high salt stress caused a decrease in chl *a*, chl *b*, chl (*a*+*b*), and carotenoid (Car) contents (Sheteawi, 2007). In this study, soybean was evaluated the root morphology, growth stage of soybean under different salinity level of physiological and biochemistry of soybean under salinity stress.

Materials and methods

This experiment was conducted from May to July 2024 at School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok. The study was designed in a 3x 4 factorial in CRD with three replications. The first factor involved three soybeans (*Glycine max* (L.) Merrill) cultivars consisting of Chaimai 84-2, Chaimai 60, and Nakornsawan 1, which are commonly grown in Thailand. The second factor was the the concentration of Sodium chloride (NaCl) 0 mM (control) 40 mM, 80 mM, and 120 mM. All three soybean cultivar seeds were first grown in the seedling tray with peatmoss then after five days the soybean seedling were transplanted into a plastic box for salinity stress treatment. There were 36 plants for each treatment in the plastic box. NaCl was added to the nutrient solution at diference concentration, twelve plastic boxes were mixed salt and nutrient solution and three plastic boxes were mixed water and nutrient solution (control) the total amont of solutions in each box is 2.4 lites.

Data collection

The styrofoam with holes was placed above each solution. Growth parameter was observed within 2 weeks included plant height (cm) measured from the cotyledon to the tip of shoot, length of root (cm) measured from the base of the stem to the tip of the root, leaf number measured by counting the fully opened leaves, The greeness of leaf were measured by SPAD Chlorophyll Meter at three points average upper, middle, and lower of plant, the percentage of plant dead were count, fresh weight per plant (g) dry weight per plant (g) were measured after placing them into a hot air oven at 70 ºC for 48 hours.

Determination of proline content and chlorophyll content

Proline contents was determining biochemical parameters of soybean seedlings salt stress according to the method described Bates *et al.* (1973) which was modified. Approximately 0.4 g of fresh soybean leaf tissue was homogenized in 8 ml of 3% aqueous sulfosalicyclic acid and left at room temperature for 30 minutes then filtered through Whatman No. 1 filter paper. In a test tube, 1 ml of the supernatant was combined with 1 ml of acid-ninhydrin and 1 ml of glacial acetic acid. The mixture was boiled in a water bath for1 1 hr at 100°C allowed to cool ice for 10 min. The reaction mixture was added 2 ml toluene then vortex for 15-20 second and the absorbance was measured at 520 nm with a SP-V1000 Spectrometer (Onilab, USA). The total proline concentration against a toluene blank was calculated by comparison with the standard curve using L-proline at 520 nm wavelength. Three replicates were analyzed. Chlorophyll 'a' 'b' and total chlorophyll of each soybean leaves were extracted with 80% acetone and determined according Arnon's method (1949), and the absorbance was measured at 645 and 663 nm.

Statistical analysis

The data obtained from the experiment were used to analyze statistical variance analysis (Analysis of Variance; ANOVA). Differences in means were compared using Fisher's Least Significant Difference (LSD) through the R-program version 4.0 (R Core Team, 2021).

Results

Effect of salinity stress to soybean growth

Sodium Chloride concentrations had varying effects on plant dead, with the highest concentration (120 mM) leading to plant death following NaCl treatment. The results showed that the three evaluated cultivars had a significant impact on soybean growth, as indicated in Table 1. At 3 days after transplanting, the plant height of Chiang Mai 84-2 was significantly higher compared to the other cultivars. However, no significant differences were observed among the cultivars at 8 and 14 days after transplanting. Soybean plants treated with high concentrations of NaCl (120 mM) exhibited significantly lower plant height at 3, 8 and 14 days after transplanting. There were interactions between cultivars and NaCl concentrations, with increased NaCl levels halting the growth of soybean plant height.

Table 1. Effect of sodium chloride rate on height of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	Height (cm.)			
	0 days	3 days	8 days	14 days
Cultivar (A)				
Chiang Mai 84-2	7.46	11.85^{a}	12.90	22.05
Chiang Mai 60	6.24	9.33^{b}	12.45	19.97
Nakhon Sawan 1	6.35	7.90^{b}	12.19	22.76
F-test	ns	*	ns	ns
Sodium chloride rate (B)				
0 mM	6.77	11.73^{a}	17.23^{a}	36.12^{a}
40 mM	6.80	10.95^{ab}	15.73^{a}	29.10^{b}
80 mM	6.20	9.05b^{c}	11.92^{b}	17.68^{c}
120 mM	6.97	7.03^{c}	5.18^{c}	3.47^{d}
F-test	ns	*	*	*
A x B				
Chiang Mai 84-2 x 0 mM	7.56^{ab}	15.96^{a}	20.06^{a}	40.06^{a}
Chiang Mai 84-2 x 40 mM	9.00^{a}	11.06^{bc}	16.90^{abc}	31.67^{bc}
Chiang Mai 84-2 x 80 mM	6.46^{ab}	11.46^{bc}	14.67^{bcd}	16.50^{ef}
Chiang Mai 84-2 x 120 mM	6.83^{ab}	8.90^{bcde}	n.d.	n.d.
Chiang Mai 60 x 0 mM	5.06^{b}	10.40^{bcd}	13.93^{cd}	29.63^{cd}
Chiang Mai 60 x 40 mM	6.33^{ab}	12.23^{b}	15.10^{bcd}	27.23^{cd}
Chiang Mai 60 x 80 mM	5.93^{ab}	7.83^{cde}	12.07^{de}	23.00^{de}
Chiang Mai 60 x 120 mM	7.10^{ab}	6.86^{de}	8.73^{f}	n.d.
Nakhon Sawan 1x 0 mM	7.16^{ab}	8.83^{bcde}	17.70^{ab}	38.67^{ab}
Nakhon Sawan 1x 40 mM	5.06^{b}	9.56^{bcd}	15.20^{bcd}	28.40^{cd}
Nakhon Sawan 1x 80 mM	6.20^{ab}	7.86^{cde}	9.03^{ef}	13.57^{f}
Nakhon Sawan 1x 120 mM	7.00^{ab}	5.33^{e}	6.83^{f}	10.40^{f}
F-test	*	*	*	*

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

After 3 days transplanting, there were no significant differences in root length among the cultivars (Table 2). However, Nakhon Sawan 1 showed higher significantly of root length compared to the other cultivars at 8- and 14-days post-transplanting. Regarding the effect of NaCl concentrations, the root lengths of soybean under 120 mM NaCl at 8 and 14 days after salt-stress treated were significantly when compared to those under 0 mM. Interactions between cultivars and NaCl concentrations were significant at 3 and 14 days after salt-stress treated, with root length decreasing as NaCl concentration increased. However, NaCl concentrations did not affect root length at 8 days post-transplanting.

Table 2. Effect of sodium chloride rate on root length of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	Root length (cm.)		
	3days	8days	14days
Cultivar (A)			
Chiang Mai 84-2	8.68	6.83^{b}	6.48^{b}
Chiang Mai 60	7.45	8.75^{ab}	8.89^{ab}
Nakhon Sawan 1	7.58	9.16^{a}	11.11^{a}
F-test	ns	*	*
Sodium chloride rate (B)			
0 mM	7.57	10.25^{a}	12.67^{a}
40 mM	8.92	10.32^{a}	11.04^{ab}
80 mM	8.07	7.76^{ab}	7.97^{b}
120 mM	7.05	4.66^{b}	3.61^{c}
F-test	ns	**	*
A x B			
Chiang Mai 84-2 x 0 mM	7.80^{ab}	9.93	7.66^{bc}
Chiang Mai 84-2 x 40 mM	8.90^{ab}	9.93	11.26^{abc}
Chiang Mai 84-2 x 80 mM	10.23^{a}	7.46	7.00^{bcd}
Chiang Mai 84-2 x 120 mM	7.80^{ab}	n.d.	6.16^{bcd}
Chiang Mai 60 x 0 mM	7.50^{ab}	10.00	12.50^{abc}
Chiang Mai 60 x 40 mM	7.40^{ab}	11.03	8.80^{bc}
Chiang Mai 60 x 80 mM	7.43^{ab}	6.66	8.10^{bc}
Chiang Mai 60 x 120 mM	7.50^{ab}	7.33	n.d.
Nakhon Sawan 1x 0 mM	7.43^{ab}	11.03	17.86^{a}
Nakhon Sawan 1x 40 mM	10.46^{a}	10.00	13.06^{ab}
Nakhon Sawan 1x 80 mM	6.56^{ab}	9.16	8.83^{bc}
Nakhon Sawan 1x 120 mM	5.86^{b}	6.66	4.66^{cd}
F-test	*	ns	*

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

Significant differences were observed between the cultivars of soybean for the number of leaves after transplant at 8 and 14 days. Chiang Mai 84-2 showed the highest significantly as compared to among cultivars in 8 and 14 days after transplantation. Under high concentration (120 mM), the number of leaves showed the lowest significantly in soybean was transplant at 3, 8 and 14 days. There were interactions between cultivars and NaCl concentrations when the concentrations of NaCl increased the number of leaves decreased significantly. All cultivars showed the number of leaves under low NaCl concentration (40 mM) were not significant different when compared to control of all cultivars (Table 3).

Table 3. Effect of sodium chloride rate on number of leaves of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	Number of leaves		
	3days	8days	14days
Cultivar (A)			
Chiang Mai 84-2	3.66	4.91[a]	7.83[a]
Chiang Mai 60	3.00	3.58[ab]	7.00[ab]
Nakhon Sawan 1	3.00	3.58[b]	6.50[b]
F-test	ns	*	*
Sodium chloride rate (B)			
0 mM	4.66[a]	7.00[a]	11.33[a]
40 mM	3.66[a]	4.89[b]	10.00[b]
80 mM	2.55[b]	3.22[c]	6.44[c]
120 mM	2.00[b]	1.67[d]	0.67[d]
F-test	*	*	*
A x B			
Chiang Mai 84-2 x 0 mM	5.00[a]	8.00[a]	14.00[a]
Chiang Mai 84-2 x 40 mM	4.00[ab]	7.00[a]	11.00[b]
Chiang Mai 84-2 x 80 mM	3.67[ab]	4.66[bc]	6.33[de]
Chiang Mai 84-2 x 120 mM	2.00[b]	n.d.	n.d.
Chiang Mai 60 x 0 mM	5.00[a]	5.00[b]	10.00[bc]
Chiang Mai 60 x 40 mM	3.00[ab]	3.33[bcd]	10.00[bc]
Chiang Mai 60 x 80 mM	2.00[b]	3.00[cd]	8.00[cd]
Chiang Mai 60 x 120 mM	2.00[b]	3.00[cd]	n.d.
Nakhon Sawan 1x 0 mM	2.00[b]	8.00[a]	10.00[bc]
Nakhon Sawan 1x 40 mM	4.00[ab]	4.33[bc]	9.00[bc]
Nakhon Sawan 1x 80 mM	2.00[b]	2.00[d]	5.00[e]
Nakhon Sawan 1x 120 mM	2.00[b]	2.00[d]	2.00[f]
F-test	*	*	*

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

After 3- and 8-days transplantation, the leaf greenness of Chaing Mai 60 showed higher significantly as compared to among cultivars, but the leaf greenness of Nakhon Sawan 1 was higher significantly as compared to among cultivars after 14 days transplant. High NaCl concentrations (120 mM) affected to decrease the leaf greenness in 3, 8 and 14 days after transplantation. Interactions between cultivars and NaCl concentrations were significantly when the concentrations increased, the leaf greenness decreased (Table 4). No significant difference was observed among the soybean cultivars for the fresh weight of leaves. However, a significant difference was found in the fresh weight of roots, with Chiang Mai 60 showing the lowest value compared to the other cultivars (Figure 1.)

Table 4. Effect of sodium chloride rate on leaf greenness of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	SPAD		
	3days	8days	14days
Cultivar (A)			
Chiang Mai 84-2	26.50[b]	20.24[c]	16.34[c]
Chiang Mai 60	28.80[a]	29.95[a]	19.32[b]
Nakhon Sawan 1	25.13[b]	28.01[b]	25.31[a]
F-test	*	*	*
Sodium chloride rate (B)			
0 mM	27.62[a]	29.36[ab]	28.48[a]
40 mM	28.43[a]	31.01[a]	24.38[b]
80 mM	27.54[a]	27.83[b]	21.46[c]
120 mM	23.67[b]	16.06[c]	6.96[d]
F-test	*	*	*
A x B			
Chiang Mai 84-2 x 0 mM	28.70[ab]	28.80[b]	27.00[abc]
Chiang Mai 84-2 x 40 mM	28.33[ab]	29.66[ab]	25.00[abc]
Chiang Mai 84-2 x 80 mM	27.50[ab]	22.50[c]	13.36[e]
Chiang Mai 84-2 x 120 mM	21.50[d]	n.d.	n.d.
Chiang Mai 60 x 0 mM	28.13[ab]	30.46[ab]	28.66[ab]
Chiang Mai 60 x 40 mM	31.33[a]	31.96[ab]	24.83[bcd]
Chiang Mai 60 x 80 mM	28.70[ab]	33.20[a]	23.80[cd]
Chiang Mai 60 x 120 mM	27.06[ab]	24.16[c]	n.d.
Nakhon Sawan 1x 0 mM	26.03[bc]	28.83[b]	29.80[a]
Nakhon Sawan 1x 40 mM	25.63[bcd]	30.16[ab]	23.33[cd]
Nakhon Sawan 1x 80 mM	26.43[bc]	29.03[b]	27.23[abc]
Nakhon Sawan 1x 120 mM	22.43[cd]	24.03[c]	20.90[d]
F-test	*	*	*

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

The fresh weights of leaves and roots did not significantly differ between the 40 mM NaCl treatment and the control, but a significant difference was observed when soybean was treated with 120 mM NaCl, which resulted in the lowest fresh weight for both leaves and roots. There were significant interactions between cultivars and NaCl concentrations; as the NaCl concentration increased, the fresh weight of leaves and roots decreased. However, the fresh weight of leaves and roots for all cultivars, except Nakhon Sawan 1 at 40 mM, did not significantly differ from the control at 0 mM NaCl. For the dry weight of leaves and roots, Nakhon Sawan 1 was significantly higher compared to the other cultivars. A significant difference was observed in the dry weight of leaves and roots across different NaCl concentrations in soybeans. Under high concentrations (80 and 120 mM), the dry weight of leaves and roots was significantly lower compared to those under control and low concentrations (40 mM). There were significant interactions between cultivars and NaCl concentrations, with increasing NaCl levels leading to a significant decrease in leaf and root dry weight. Additionally, Chiang Mai 84-2 and Nakhon Sawan 1 showed plant death after being treated with high NaCl concentrations (Figure 1).

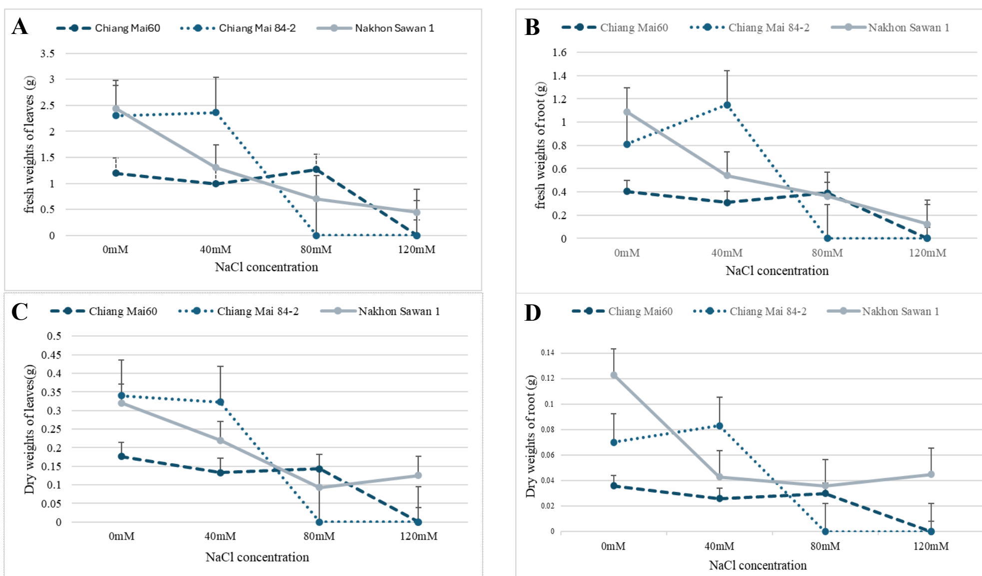

Figure 1. Effect of sodium chloride rate on fresh/dry weights of leaves and roots of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1: A = The fresh weights of leaves, B = The fresh weights of root, C = The Dry weights of leaves, D = The Dry weights of root.

Effect of salinity stress to proline contents

The proline contents are shown in Table 5. The levels of proline in young leaves (16.308 µg/g) and mature leaves (17.532 µg/g) of Nakhon Sawan 1 were highest significantly as compared to among of cultivars. The high NaCl concentration at 80 mM significantly affected proline contents in both young leaves and mature leaves which showed the high proline contents as compared to control. Interactions between cultivars and NaCl concentrations were significantly when the concentrations increased, the proline contents also increased.

Table 5. Effect of sodium chloride rate on the proline content in 3rd leaf and 5th leaf of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	Proline (µg/g FW)	
	3rd leaf	5th leaf
Cultivar (A)		
Chiang Mai 84-2	2.540^{c}	3.146^{c}
Chiang Mai 60	10.726^{b}	13.862^{b}
Nakhon Sawan 1	16.306^{a}	17.532^{a}
F-test	*	*
Sodium chloride rate (B)		
0 mM	2.543^{c}	1.481^{c}
40 mM	7.007^{b}	6.213^{b}
80 mM	20.021^{a}	26.847^{a}
120 mM	n.d.	n.d.
F-test	*	*
A x B		
Chiang Mai 84-2 x 0 mM	3.269^{de}	1.078^{e}
Chiang Mai 84-2 x 40 mM	4.351^{de}	8.362^{c}
Chiang Mai 84-2 x 80 mM	n.d.	n.d.
Chiang Mai 84-2 x 120 mM	n.d.	n.d.
Chiang Mai 60 x 0 mM	2.758^{de}	1.755^{de}
Chiang Mai 60 x 40 mM	9.418^{c}	3.920^{cde}
Chiang Mai 60 x 80 mM	20.002^{b}	35.912^{b}
Chiang Mai 60 x 120 mM	n.d.	n.d.
Nakhon Sawan 1x 0 mM	1.602^{e}	1.609^{de}
Nakhon Sawan 1x 40 mM	7.253^{cd}	6.357^{cd}
Nakhon Sawan 1x 80 mM	40.062^{a}	44.630^{a}
Nakhon Sawan 1x 120 mM	n.d.	n.d.
F-test	*	*

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

Effect of salinity to chlorophyll contents

The results showed 3 cultivars were significantly affects on chlorophyll a, chlorophyll b and total chlorophyll contents (Table 6). The chlorophyll a (0.58 mg/g), chlorophyll b (8.080 mg/g) and total chlorophyll (8.662 mg/g) levels of Chiang Mai 84-2 had the lower significant than among of cultivars. For the effect of NaCl concentrations, the ranges of chlorophyll contents were 0.284-1.060 mg/g. Chlorophyll b contents ranged between 4.252-14.449 mg/g. and total chlorophyll contents ranged from 4.537 to 15.509 mg/g. The levels of chlorophyll a (0.58 mg/g), chlorophyll b (8.080 mg/g) and total chlorophyll (8.662 mg/g) were significantly lower in NaCl concentration rates of 120 mM when compared to 0, 40 and 80 mM. There were interactions between cultivars and NaCl concentrations when the concentrations of NaCl increased chlorophyll contents decreased significantly.

Table 6. Effect of sodium chloride rate on chlorophyll a, chlorophyll b and total chlorophyll content of Chiang Mai 84-2, Chiang Mai 60 and Nakhon Sawan 1

Treatment	Chlorophyll (mg/g)		
	Chlorophyll a	Chlorophyll b	Total chlorophyll
Cultivar (A)			
Chiang Mai 84-2	0.58^{b}	8.080^{b}	8.662^{b}
Chiang Mai 60	0.750^{a}	10.201^{ab}	10.952^{ab}
Nakhon Sawan 1	0.795 ^{a}	11.293^{a}	12.088^{a}
F-test	*	*	**
Sodium chloride rate (B)			
0 mM	1.060^{a}	14.449^{a}	15.509^{a}
40 mM	0.781^{b}	10.874^{b}	11.655^{b}
80 mM	0.284^{c}	4.252^{c}	4.537^{c}
120 mM	n.d.	n.d.	n.d.
F-test	*	*	**
A x B			
Chiang Mai 84-2 x 0 mM	1.185^{a}	16.022^{a}	17.208^{a}
Chiang Mai 84-2 x 40 mM	0.558^{d}	10.884^{bc}	8.778^{cd}
Chiang Mai 84-2 x 80 mM	n.d.	n.d.	n.d.
Chiang Mai 84-2 x 120 mM	n.d.	n.d.	n.d.
Chiang Mai 60 x 0 mM	0.950^{b}	12.349^{ab}	13.299^{abc}
Chiang Mai 60 x 40 mM	0.804^{c}	10.884^{ab}	11.688^{bcd}
Chiang Mai 60 x 80 mM	0.495^{d}	7.371^{cd}	7.867^{cd}
Chiang Mai 60 x 120 mM	n.d.	n.d.	n.d.
Nakhon Sawan 1x 0 mM	1.045^{b}	14.975^{ab}	16.021^{a}
Nakhon Sawan 1x 40 mM	0.982^{b}	13.517^{ab}	14.499^{ab}
Nakhon Sawan 1x 80 mM	0.358^{e}	5.386^{d}	5.745^{d}
Nakhon Sawan 1x 120 mM	n.d.	n.d.	n.d.
F-test	*	*	**

ns = non-significant, * = significantly different at 95%, ** = significantly different at 99%
n.d. = no data due to plant dead

Discussion

Salinity negatively impacts seed germination, plant growth, and development, leading to substantial reductions in crop yields globally (AbdElgawad *et al*., 2016). The findings revealed that the highest NaCl concentration resulted in the lowest rate of plant growth in all cultivars. There were notable interaction effects between cultivars and NaCl concentration on plant growth and biomass in seedling growth stage. In summary, NaCl concentrations of 40 mM, 80 mM, and 120 mM resulted in a reduction of plant height, root length, number of leaves, leaf greenness, fresh biomass and dry biomass when compared to the 0 mM concentration. The highest concentration of 120 mM NaCl at 14 days after treated gave the plant death in Chiang Mai 84-2 and Chiang Mai 60 at 80 mM NaCl compared to non-salinity treated, plant growth in all cultivars decreased, fresh and dry biomass was reduced especially Chiang Mai 84-2 cultivar. The data showed that each cultivar showed a different growth response. Better performance was revealed by the Nakorn Sawan 1 cultivar than other cultivars. Omara and El-Gaafarey (2018) found that salinity treatment at 120 mM led to a 45% reduction in cowpea plant height. Similar reductions in plant height due to increasing NaCl concentrations have also been observed in soybean (Manyanon and Banharn, 2016), mungbean (Alharby *et al*., 2019). Findings from several studies have demonstrated that rising salt stress results in decreased plant performance (Amirjani 2010; Kadri *et al*., 2021; Togatorop *et al*., 2023). Salt stress hampers plant height growth by lowering turgor pressure in expanding tissues, resulting from reduced water potential in the plant roots. The root system is the first part of the plant to detect salt stress, which hinders plant growth both immediately by causing osmotic stress due to water deficiency and gradually by leading to salt-induced ion toxicity from nutrient imbalances in the cytosol (Acosta-Motos *et al.,* 2017).

Plants generally employ different mechanisms in response to salinity stress, depending on the level and duration of the stress. In the early stage of salinity stress, the plant's ability to absorb water through its root’s decreases, while water loss from the leaves increases due to salt-induced osmotic stress. This hyperosmotic stress leads to several physiological changes in plants, including membrane damage, nutrient imbalances, ROS detoxification and disruptions in photosynthesis. (Ma *et al.,* 2020). Photosynthetic pigments like chlorophyll a and chlorophyll b are essential for photosynthesis. Under salinity stress conditions, net photosynthesis significantly declines as the levels of chlorophyll a, chlorophyll b, and total chlorophyll (a+b) decrease in soybean plants (Anjum *et al*., 2013). Similarly with this study, chlorophyll a, chlorophyll b, and total chlorophyll (a+b) decreased as NaCl concentration increased across all cultivars. Particularly, Chiang Mai 84-2 cultivar had the lowest chlorophyll pigment levels and showed a more rapid decline under higher salinity stress compared to the other two cultivars. In response to salinity, plants produce osmoregulatory compounds as proline (Pro) under various abiotic stresses to sustain metabolic activity and enhance osmotic balance. These osmoregulators enhance the chances of plant survival under salt stresses by maintaining cellular homeostasis (Abbaspour, 2012). The results of present study showed that increasing NaCl concentrations leade to increase of proline content. Nakhon Sawan 1 cultivar is obviously show that increasing salinity levels caused significant increases in soybean leaf proline content as compared with non-salinity stress plants. On the other hand, the Chiang Mai 84-2 cultivar produced the lowest proline content, which caused soybean death as NaCl concentrations increased to 80 mM and 120 mM. An increase in proline levels in soybeans under salt stress was also reported by Sadak *et al*. (2020).

This study evaluated the salt tolerance of different soybean cultivars at the seedling growth stage to identify cultivars highly suited to salinity stress. Nakhon Sawan 1 was identified as a suitable cultivar for salinity stress, as no plant death occurred at any NaCl concentration. Furthermore, the salt tolerance of each cultivar provides valuable information for soybean breeding programs focused on developing high salt-tolerant cultivars.

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