International Journal of Agricultural Technology 2023 Vol. 19(4):1919-1928
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effect of different salinity stress on seedling growth in long bean (*Vigna sinensis* L.) genotypes

Togatorop, E. R.*, Sari, D. N. and Handayani, S.

Faculty of Agriculture, University of Ratu Samban, Arga Makmur, Bengkulu, Indonesia.

Togatorop, E. R., Sari, D. N. and Handayani, S. (2023). Effect of different salinity stress on seedling growth in long bean (*Vigna sinensis* L.) genotypes. International Journal of Agricultural Technology 19(4):1919-1928.

Abstract The response of long bean genotype in different NaCl concentrations (0 mM, 70 mM, 140 mM and 210 mM) was investigated. The highest concentration of NaCl gave the highest percent of plant death in all genotypes. The increasing concentration of NaCl decreased the plant height, leaf number, leaf chlorophyll, length of root, fresh biomass and dry biomass. Interaction between genotypes and NaCl was significant in parameters plant height, length of root and fresh biomass. The results showed the LC_{50} of the long bean genotypes was ranged between 93 mM to 210 mM. Under salt stress, the genotypes Metro super, Salsa, Branjangan, and Pujangga 2 showed better growth performance.

Keywords: Biomass, Lethal concentration, Salt, Solution, Tolerant

Introduction

Long bean (*Vigna sinensis* L.) is a commodity vegetable that are widely consumed and favored by the Indonesian people. It has benefits for human healthcare due to its content of vitamins, nutrition, minerals, protein, cellulose and amino acid (Togatorop *et al*., 2020; Toppo and Sahu, 2020; Wang *et al*., 2021). National long bean production in 2016-2020 decreased by an average of 7.228,25 tons per year (BPS, 2022). The decrease in production was caused by fluctuations in the supply of long bean from each area. Bengkulu province is a long bean producing area, but long bean cultivation is still concentrated in the highlands, which is increasingly limited in land area. On the other hand, there are still many lowland farmers who produce low long bean production due to limited superior seeds and land capability. Bengkulu lowlands include coastal land that has saline soil. According to (Karolinoerita and Yusuf, 2020) coastal land has a high concentration of dissolved salts in the soil, so the buildup of salt affects the ability of plant roots to absorb nutrients and water. Plants cultivated in saline soil cannot develop fully because of the high NaCl content which is toxic (Sukarman and Purwanto, 2018; Wijayanti, 2014). In addition, salinity

* **Corresponding Author:** Togatorop, E. R.; **Email:** eny28torop@gmail.com

affects the photosynthesis process which results in reduced leaf growth and even results in crop failure (Abeer *et al*., 2015; Omara and El-Gaafarey, 2018; Romadloni and Kurniawan, 2018). Utilization of coastal areas can be a solution for increasing long bean, but coastal areas are identified with high salinity conditions.

Salinity is the main factor inhibiting the development of crops (Fuskhah *et al*., 2019). Salinity is abiotic stress caused by the environment due to the presence of dissolved salts in high concentrations. Salinity causes toxic effects and inhibits plant growth due to the osmotic pressure of excessively accumulated salts into the plant body (Absari and Kuswanto, 2019). In Indonesia, saline soil conditions are often found in coastal areas. Conditions in saline soils make it difficult for plants to take up nutrients in this form of dissolved ions in the water to be restricted, causing a decrease in fresh weight and water content in plants (Arteaga *et al*., 2020; Karolinoerita and Yusuf, 2020). Salinity stress in the soil is induced by dissolved salt content. Sodium chloride (NaCl) is the most widespread salt in the soil. Increases salt content in the soil also occurs mainly due to high evaporation, and direct exposure to seawater (Kouam and Mandou, 2017).

Some research results showed that saline conditions in cultivated land caused inhibition of the photosynthesis process, low weight of pods and empty pods of cowpea (Abeer *et al*., 2015), inhibited rooting, seed size and reduced chlorophyll contents of mungbean (Dutta and Bera, 2014; Taufiq and Iswanto, 2018; Uswah *et al*., 2018), reduced number of seeds and weight of soybean seeds (Purwaningrahayu, 2016). According to Junandi *et al*. (2019) in salinity stress conditions, plants will carry out a defense mechanism through salt avoidance to prevent deficit water and a high tolerance mechanism to avoid high salt concentrations from entering the plant tissues. Salinity stress in the soil is induced by dissolved salt content. Sodium chloride (NaCl) is the most widespread salt in the soil. Increases salt content in the soil also occurs mainly due to high evaporation, and direct exposure to seawater (Kouam and Mandou, 2017). It is important to develop new varieties of high yielding long beans that are resistant to salinity stress. The seedling stage is the most sensitive phase to salt stress (Ravelombola *et al.*, 2019). The observed long bean genotype begins with the NaCl concentration screening test. There is a collection of several long bean genotypes that have been cultivated in the Bengkulu province area. Therefore, the aim of this research was observed the response of long bean genotype in different NaCl concentrations in the seedling phase.

Materials and methods

This research was conducted from August to September 2022 at the Green House Balai Pengkajian Teknologi Pertanian (BPTP) Bengkulu. The materials used were seedling tray, vermicompost, NaCl, soil, plastic box, styrofoam and SPAD meter. Planting materials used were eight genotypes of long bean namely Viperoo, Metro Super, KP3, Kanton Tavi, Borneo, Salsa, Branjangan and Pujangga 2. All genotypes were treated by salinity concentration of NaCl. Concentration of NaCl treatment given to long bean genotypes were 0 mM (control), 70 mM, 140 mM and 210 mM. This research used a randomized complete block design with three replications. There were 48 plants for each treatment in the plastic box, nine plastic boxes were mixed salt and nutrition solution and three plastic boxes were mixed water and nutrition solution (control). Each of these solutions is two liters. Rectangular styrofoam with holes designed was placed above each solution. All seeds were first grown in the seedling tray with mixed soil and vermicompost in a ratio of 1:1. After five days the surviving plants were transplanted into a plastic box for salt screening. Growth plant was observed within 2 weeks and the number of dead plants per treatment was calculated. Growth parameters included plant height (cm) measured from the bottom to the growing point, leaf number (pieces) measured by counting the fully opened leaves, leaf chlorophyll measured using a SPAD meter, length of root (cm) measured from the base of the stem to the tip of the root, fresh biomass (g) and dry biomass (g) measured by scaling all parts of the plant.

LC_{50}=was analyzed using curve expert 1.3 software. Effects of salinity on plant growth were evaluated using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

Results

NaCl concentration gave a different effect on percent plant death for all long bean genotypes. In all genotypes, the concentration of NaCl at 70 mM, 140 mM and 210 mM showed a higher percent of plant death than the concentration of 0 mM (Figure 1). The percent plant death of genotypes Viperoo, Metro Super, KP3, Kanton Tavi, Borneo, Salsa, Branjangan and Pujangga 2 is described by Polynomial Fit with the equations sequently:
$y = -3.996E - 0.15 + 0.269x + 0.002x^2 - 1.117x^3$, $y = -8.659E - 0.15 + 0.497x - 4.922x^2 - 5.344x^3$, $y = -1.776E - 0.15 - 0.380x + 0.014x^2 - 4.907x^3$, $y = 5.551E - 0.16 + 0.452x - 0.004x^2 + 1.554x^3$, $y = 3.308E - 0.14 - 0.638x + 0.011x^2 - 3.449x^3$, $y = 3.330E - 0.15 + 0.359x - 0.002x^2 + 8.260x^3$, $y = 1.511E - 0.14 + 0.240x - 0.0003x^2 + 1.943x^3$, $y = 4.2859E - 0.14 - 0.469x + 0.008x^2 - 2.380x^3$.

Curve-fit analysis showed that the LC_{50} value of Viperoo = 125.93, Metro Super = 118.23, KP3 = 93.454, Kanton Tavi = 210, Borneo = 155.793, Branjangan = 195.977 and Pujangga 2 = 193.191, while the Salsa genotype only reached LC_{20} value that was 102.354. Genotype KP3 had the lowest LC_{50} value.

The result showed 8 genotypes evaluated had a significant effect on the parameters of plant height, leaf number, leaf chlorophyll, length of root, fresh biomass and dry biomass. The average of growth performance shown in Table 1. Plant height of genotypes Borneo, Salsa and Pujangga 2 were higher than among the genotypes, with the each average were 16.98 cm, 19.94 cm and 16.05 cm not-significantly different to genotypes Viperoo, Metro Super and Branjangan. The lowest plant height was identified in the genotypes KP3 and Kanton Tavi, with the average were 12.67 cm and 13.15 cm. Genotype Metro Super was having the highest leaf number with an average 4.48. Leaf chlorophyll varied from 16.11 to 27.45.

Table 1. Effect of genotypes on plant height (PT), leaf number (LN), leaf chlorophyll (LC), length of root (LR), fresh biomass (FB), dry biomass (DB)

Genotypes	**Parameters**					
	PT	LN	LC	LR	FB	DB
Viperoo	15.73 ab	3.73 b	22.73 b	6.05 d	1.87 ab	0.26 a
Metro Super	15.76 ab	4.48 a	25.60 ab	6.84 cd	2.10 a	0.25 a
KP3	12.67 c	3.88 ab	16.11 c	3.53 e	0.97 d	0.06 b
Kanton Tavi	13.15 c	2.52 d	25.98 ab	5.92 d	1.51 c	0.19 a
Borneo	16.98 a	3.63 bc	27.45 a	7.43 bc	1.69 bc	0.21 a
Salsa	16.94 a	2.85 d	26.02 ab	8.71 a	2.01 ab	0.25 a
Branjangan	14.88 b	2.93 d	26.16 a	8.24 ab	1.73 bc	0.23 a
Pujangga 2	16.05 ab	3.00 cd	26.93 a	7.72 abc	1.95 ab	0.27 a

Note: Means followed by the same letter in the same column for each genotype, are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Genotype Borneo was having the highest leaf chlorophyll with the average was 27.45 not significant to genotypes Metro Super, Kanton Tavi, Salsa, Branjangan and Pujangga 2. The lowest leaf chrolophyll was identified in the genotypes Viperoo and KP3, with the average were 22.73 and 16.11. Genotypes Salsa, Branjangan and Pujangga were having length of root more longer than genotypes Metro Super and Borneo, with the average ranged 6.84 cm - 8.71 cm. The shortest length of root found in genotypes KP3, Kanton Tavi and Viperoo with the average were 3.53 cm, 5.92 cm and 6.05 cm. The average fresh biomass off all genotypes ranged between 0.97 g - 2.10 g and the dry biomass ranged between 0.06 g - 0.27 g. The research showed genotype KP3 seems to be the lowest growth from among the genotypes.

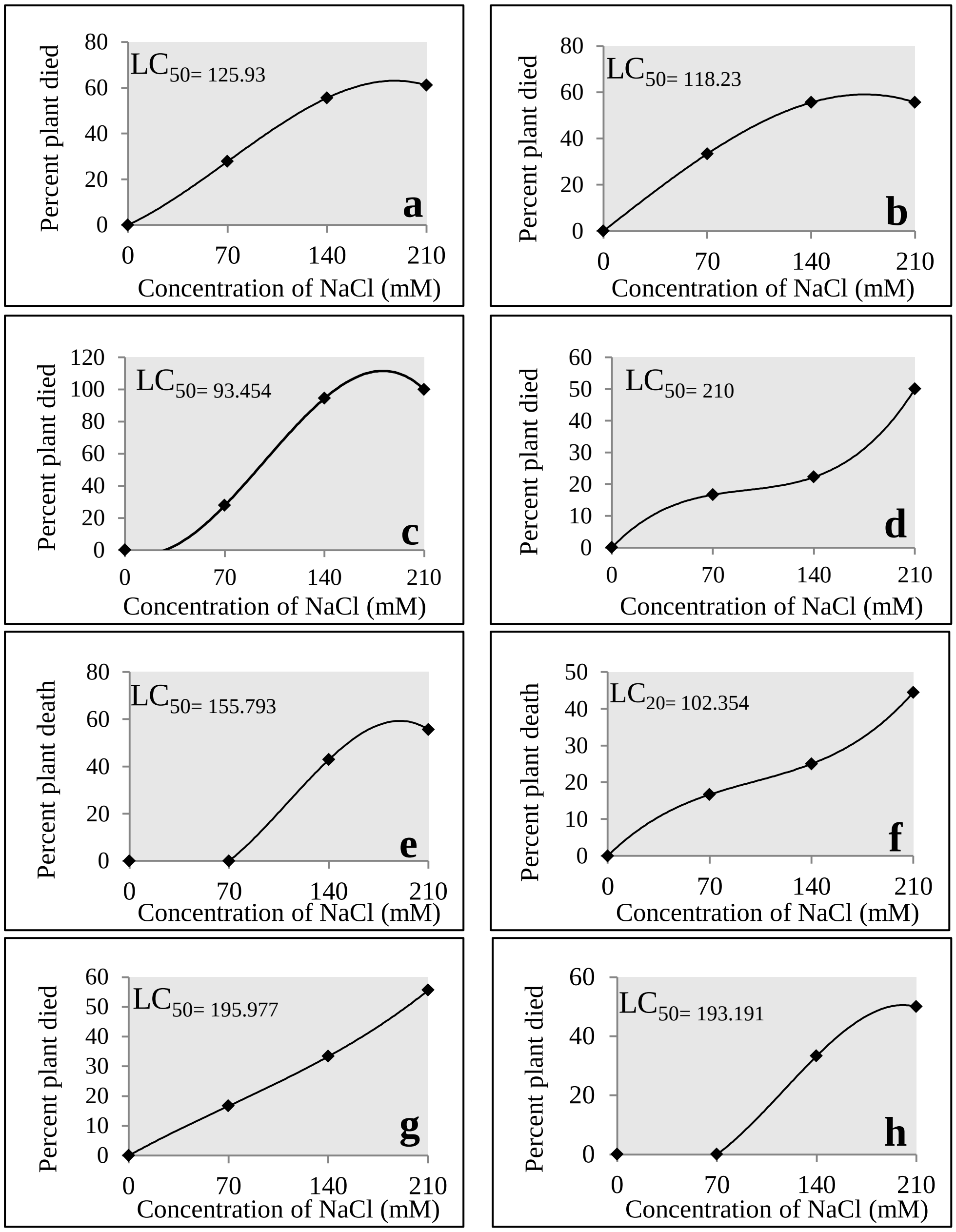

Figure 1. LC_{50} and LC_{20} values of long bean after NaCl treatment. Genotypes: (**a**)Viperoo, (**b**) Metro Super, (**c**) KP3, (**d**) Kanton Tavi, (**e**) Borneo, (**f**) Salsa, (**g**) Branjangan, (**h**) Pujangga 2

The effect of NaCl concentration for the growth parameters is performed in Table 2. The plant height ranged between 13.27 cm - 17.11 cm, leaf number ranged between 2.40 - 4.68, leaf chrolophyll ranged between 19.58 - 29.58, length of root ranged between 5.77 cm - 8.04 cm, fresh biomass ranged between 1.12 g - 2.87 g and dry biomass ranged between 0.15 g - 0.34 g.

Table 2. Effect of NaCl concentration on plant height (PT), leaf number (LN), leaf chlorophyll (LC), length of root (LR), fresh biomass (FB), dry biomass (DB)

NaCl	**Parameters**					
	PT	LN	LC	LR	FB	DB
0 mM	17.11 a	4.68 a	29.58 a	8.04 a	2.87 a	0.34 a
70 mM	15.95b	3.70 b	26.51 b	7.31 a	1.64 b	0.19 b
140 mM	14.75 c	2.77 c	22.83 c	6.10 b	1.28 c	0.16 b
210 mM	13.27 d	2.40 d	19.58 d	5.77 b	1.12 c	0.15 b

Note: Means followed by the same letter in the same column for each genotype, are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

The plant height (13.27 cm), leaf number (2.40), leaf chlorophyll (19.58), length of root (5.77 cm), fresh biomass (1.12 g) and dry biomass (0.15 g) were significantly lower values in concentration of 210 mM as compared with concentration 0 mM, 70 mM and 140 mM. The highest plant height (17.11 cm), leaf number (4.68), leaf chlorophyll (29.58), length of root (8.04 cm), fresh biomass (2.87 g) and dry biomass (0.34 g) was shown on concentration of 0 mM. Moreover, the dry biomass was identified not-significant between concentrations 70 mM, 140 mM and 210 mM. The increasing level of NaCl decreased the plant height, leaf number, leaf chlorophyll, length of root, fresh biomass and dry biomass.

Interaction between genotypes and NaCl was significant in parameters plant height, length of root and fresh biomass. Figure 2 showed the highest plant height on concentration 0 mM was observed in genotypes Viperoo, Metro Super, Borneo, Salsa, Branjangan and Pujangga 2 (16.80 cm - 21.04 cm), whereas the shortest were the genotypes KP3 and Kanton Tavi (14.22 cm - 14.83 cm). After NaCl treatment, a concentration of 210 mM showed reducing the plant height. Even though it was observed with the highest NaCl concentration, the genotypes with the highest plant height were Viperoo, Borneo, Salsa and Branjangan.

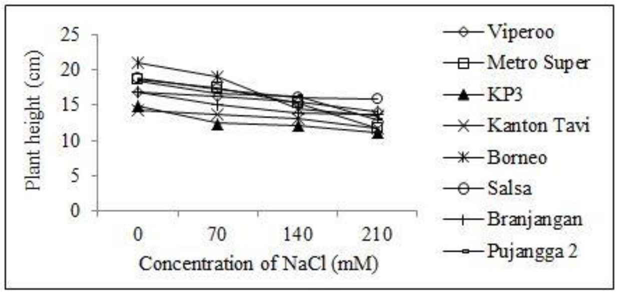

Figure 2. Interaction effect of NaCl concentration and long bean genotypes on plant height

The shortest length of root was obtained at concentration 210 mM is shown in Figure 3. The lowest length of root was shown in genotype KP3, whereas the genotypes Salsa, Branjangan and Pujangga 2 had the longest length of root.

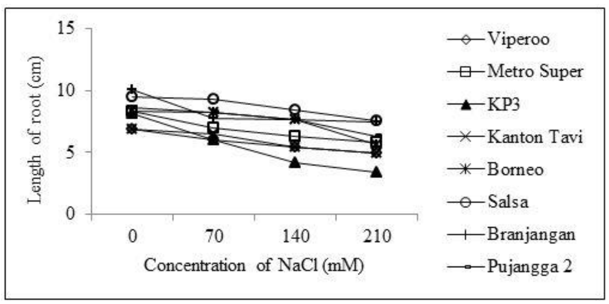

Figure 3. Interaction effect of NaCl concentration and long bean genotypes on length of root

It showed the fresh biomass of all genotypes in all NaCl concentration treatments ranged from 0.43 g to 3.07 g (Figure 4). The lowest fresh biomass was shown in genotype KP3 and the highest was in genotype Metro Super. Fresh biomass at 0 mM concentration was higher than among concentrations.

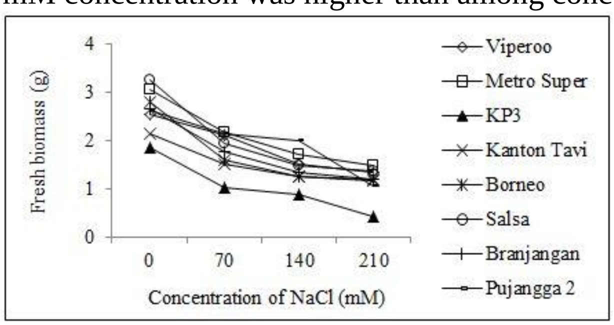

Figure 4. Interaction effect of NaCl concentration and long bean genotypes on fresh biomass

Discussion

Results indicated the highest concentration of NaCl gave the highest percent of plant death in all genotypes. In general the concentration of NaCl that caused 50 % genotypes seedlings was ranging 140 mM to 210 mM. Based on the result from the Curve-fit analysis genotype KP3 was the most sensitive with the lowest LC_{50} value (93.454), while genotype salsa had the highest LC_{50} value (210). LC_{50} values of genotypes Viperoo, Metro Super, Kanton Tavi, Borneo, Branjangan and Pujangga 2 ranged between 102.354 - 195.977. There were significant interaction effects between genotypes and concentration of NaCl on plant height, length of root and fresh biomass. The concentration of NaCl at 210 mM were reduced plant height in all genotypes ranged between 16.23 % - 37.50 %. Likewise on length of root (20.45 % - 57.95 %) and fresh biomass (44.53 % - 76.63 %). Different results reported by Kadri *et al*. (2021), that the salinity level at 256.7 mM was reduced length of root over 80%. Omara and El-Gaafarey (2018) reported that treatment of salinity at 120 mM reduced plant height of cowpea by 45.44 %. The reduced plant height caused to the increasing concentration of NaCl also occurs in soybean (Purwaningrahayu, 2016) and mungbean (Alharby *et al.*, 2019), salt stress reduces plant height growth by reducing turgor pressure on growing tissues due to low water potential in plant roots. The root system is the first part of the plant to sense salt stress, which prevents plants growth both directly by causing osmotic stress from a lack of water and over time by causing salt induced ion toxicity from an imbalance in cytosol nutrients (Acosta-Motos *et al*., 2017).

The treatments genotypes and concentration of NaCl significantly affected on all observed parameters. This results shown that the effect of concentration NaCl on long bean genotypes can be seen from the growth of plant height, length of root and fresh biomass. Salinity affects the development of plant tissue that and also condition physiologicalof the plant. The data showed that each genotype showed a different growth response. Better performance was revealed by the genotypes Metro super, Salsa, Branjangan, and Pujangga 2 than by other genotypes. The genetically varied varieties resistant to salt stress found within the cowpea genotypes can be of substantial utility for development of germplasm for salinity breeding programs (Win and Oo, 2015). In general, concentration of NaCl at 70 mM, 140 mM and 210 mM reduce plant height, leaf number, leaf chlorophyll, length of root, fresh biomass and dry biomass as compared to the concentration 0 mM. Similar result was reported by Ravelombola *et al*. (2019) and Gogile *et al*. (2013), that concentration NaCl at 200 mM decreasing plant height, length of root, chlorophyll content and stem biomass on cowpea seedlings. The result from many research showed that the lowest performance indicated with increasing salt stress (El-Beltagi *et al*., 2013; Kadri *et al*., 2021). According to Nunes *et al*. (2019), several varieties plant responses at different salt concentrations revealed that their optimum viability was in non-saline environments, indicating to be extremely sensitive to high salinity during growth. Screening is one method to evaluation effect of different salinity stress on seedling growth in long bean (*Vigna sinensis* L.) genotypes. In conclusion, the LC_{50} of the long bean genotypes was ranged between 93 mM to 210 mM. The genotypes Metro super, Salsa, Branjangan, and Pujangga 2 showed better growth performance. Therefore, the NaCl LC_{50} will be used for further field experiment.

Acknowledgements

The authors sincerely thank Ministry of Education, Culture, Research, and Technology for funding the research project National Competitive through the 2022 Novice Lecturer Research Scheme. Grant Number 154/E5/PG.02.00.PT/2022, 1417/LL2/PG/2022, 53/VII.a-3/VI/2022.

References

Abeer, H., Abd_Allah, E. F., Alqarawi, A. A. and Egamberdieva, D. (2015). Induction of salt stress tolerance in cowpea [*Vigna unguiculata* (L.) Walp.] by arbuscular mycorrhizal fungi. Legume Research-An International Journal 38:579-588.

Absari, E. U. and Kuswanto, K. (2019). Respon beberapa genotip kacang tunggak (*Vigna unguiculata* L.) terhadap cekaman salinitas. Plantropica: Journal of Agricultural Science 4:57-67.

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J. and Hernandez, J. A. (2017). Plant responses to salt stress: adaptive mechanisms. Agronomy 7:18.

Alharby, H. F., Al-Zahrani, H. S., Hakeem, K. R. and Iqbal, M. (2019). Identification of physiological and biochemical markers for salt (NaCl) stress in the seedlings of mungbean [*Vigna radiata* (L.) Wilczek] genotypes. Saudi journal of biological sciences 26:1053-1060.

Arteaga, S., Boscaiu, M., Prohens, J. and Vicente, O. (2020). Role of active transport of potassium to leaves in the mechanisms of tolerance to salinity in common bean (*Phaseolus vulgaris* L.). Notulae Scientia Biologicae 12:447-459.

[BPS] Badan Pusat Statistik. 2022. Produksi Tanaman Hortikultura. Jakarta: Badan Pusat Statistik. Retried from https://www.bps.go.id.

Dutta, P. and Bera, A. K. (2014). Effect of NaCl salinity on seed germination and seedling growth of mungbean cultivars. Legume Research 37:161-164.

El-Beltagi, H. S., Mohamed, H. I., Mohammed, A. H. M. and Mogazy, A. M. (2013). Physiological and biochemical effects of Î³irradiation on cowpea plants (*Vigna sinensis*) under salt stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41:104-114.

Fuskhah, E., Purbajanti, E. D. and Anwar, S. (2019). Test of the resistance of rhizobium bacteria to salinity for the development of food legume plants in coastal areas. In IOP Conference Series: Earth and Environmental Science 250:012044.

Gogile, A., Andargie, M. and Muthuswamy, M. (2013). The response of some cowpea (*Vigna unguiculata* (L.) Walp.) genotypes for salt stress during germination and seedling stage. Journal of Stress Physiology & Biochemistry 9:73-84.

Junandi, J., Mukarlina, M. and Linda, R. (2019). Pengaruh cekaman salinitas garam NaCl terhadap pertumbuhan kacang tunggak (*Vigna unguiculata* L. Walp) pada tanah gambut. Jurnal Protobiont 8:101-105.

Karolinoerita, V. and Yusuf, W. A. (2020). Salinisasi lahan dan permasalahannya di Indonesia. Jurnal Sumberdaya Lahan 14:91-99.

Kadri, A., Chaabena, A., Abdelguerfi, A. and Laouar, M. (2021). Influence of salinity on germination and early seedling root growth traits of alfalfa (*Medicago sativa* L.) landraces collected in Southern Algerian oases. Agriculture and Natural Resources 6:976-985.

Kouam, E. B. and Mandou, E. L. T. M. S. (2017). Effects of salinity stress (NaCl) on growth attributes and some nutrient accumulation in cowpea (*Vigna unguiculata*). Current Botany 8.

Nunes, L. R. D. L., Pinheiro, P. R., Pinheiro, C. L., Lima, K. A. P. and Dutra, A. S. (2019). Germination and vigour in seeds of the cowpea in response to salt and heat stress. Revista Caatinga 32:143-151.

Omara, A. and El-Gaafarey, T. (2018). Alleviation of salinity stress effects in forage cowpea (*Vigna unguiculata* L.) by Bradyrhizobium sp. inoculation. Microbiology Research Journal International, 23:1-16.

Purwaningrahayu, R. D. (2016). Karakter morfofisiologi dan agronomi kedelai toleran salinitas. Iptek Tanaman Pangan 11:35-48.

Ravelombola, W., Qin, J., Weng, Y., Mou, B. and Shi, A. (2019). A simple and cost-effective approach for salt tolerance evaluation in cowpea (*Vigna unguiculata*) seedlings. HortScience 8:1280-1287.

Romadloni, A. and Kurniawan, P. W. (2018). Pengaruh beberapa level salinitas terhadap perkecambahan kacang hijau (*Vigna radiata* L) varietas Vima 1. Jurnal Produksi Tanaman 6:1663-1670.

Sukarman, M. A. and Purwanto, S. (2018). Modifikasi metode evaluasi kesesuaian lahan berorientasi perubahan iklim. Jurnal Sumberdaya Lahan, 12:1-11.

Taufiq, A. and Iswanto, R. (2018). Evaluasi toleransi genotipe kacang hijau terhadap cekaman salinitas. Jurnal Agronomi Indonesia 46:269-275.

Togatorop, E. R., Sari, D. N., Sari, D. N. and Susilo, E. (2020). Characterization of 14 yard long bean (*Vigna sinensis*) genotypes on lowland of bengkulu. Jurnal Hortikultura Indonesia 11:202-211.

Toppo, S. and Sahu, S. (2020). Studies based on performance of different genotypes of yard long bean (*Vigna unguiculata* ssp. Sesquipedalis (L.) Verdic.). Journal of Pharmacognosy and Phytochemistry 9:1810-1812.

Uswah, I. A., Herman, H. and Roslim, D. I. (2018). Analisis ketahanan 10 genotipe kacang hijau (*Vigna radiata* L.) asal provinsi Riau terhadap cekaman salinitas. Dinamika Pertanian 34:55-60.

Wang, Y., Maltais-Landry, G., Rathinasabapathi, B., Sargent, S. A. and Liu, G. (2021). Growth and yield responses of pot-grown long bean and luffa to nitrogen rates. Agriculture 11:1145.

Wijayanti, W. (2014). Keragaan 29 galur kacang tanah (*Arachis hypogaea* L.) pada kondisi salin. Vegetalika 3:40-51.

Win, K. T. and Oo, A. Z. (2015). Genotypic difference in salinity tolerance during early vegetative growth of cowpea (*Vigna unguiculata* L. Walp.) from Myanmar. Biocatalysis and Agricultural Biotechnology 4:449-455.

(Received: 22 September 2022, accepted: 18 June 2023)