Effect of salinity on phenotypic soybean mutant character 
(*Glycine max* (L.) Merr)

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Abstract. Get saline-tolerant soybean seeds can be produced using soybean mutant seeds that are induced by chemical or physical mutations. This study used soybean mutant that has previously been chemically induced by colchicine which is expected to produce plants that able to adapt to saline soils. This field experiment was conducted on January-March 2020 in Faculty of Agriculture, Universitas Sumatera Utara. The experiment was arranged in a Randomized Block Design with one treatment that are levels of salinity (0; 2; 4; 6 dS/m) with ten replicates for each treatment. The data were analyzed statistically using ANOVA and then following by the DMRT test at 5% level. The results of this study show that increasing salinity can decrease phenotypic characters, were root length, root volume, root dry weight, canopy dry weight, and length of stomata. However, we discovered that soybean mutant can well grow at S3 (4 dS/m) as following the parameters were plants height (21.77 cm), stem diameter (2.90 cm), amount of stomata (42.67 stomata), and width of stomata (15.76 µm). Moreover, we discovered that the chlorophyll content of the soybean mutant increases until at 4 dS/m (7.67 g/ml).

1. Introduction
Soybean is one of the three main commodities in Indonesia besides paddy and corn [1]. Even so, the high demand for soybeans is not offset by adequate domestic soybean production, so that Indonesia is always dependent on soybean imports. Strategy as an effort to increase soybean production is expanding the planting area by utilizing marginal soil which is saline soil. Saline soil has excessive salt content, sodium (Na) and chloride (Cl), so that it can inhibit seed germination, plant growth, and production.

Plant growth and development is inhibited due to excessive accumulation of Na and Cl in the cytoplasm, causing changes metabolism in cells [2]. Increasing the concentration of Na in plant tissue can increase oxidative stress, which causes damage to the structure of chloroplasts and is related to chlorophyll loss [3]. Salinity stress can cause nutrient imbalance [4], which reduce nitrogen (N) and phosphorus (P) content in all plant tissue [5]. Increased salinity in soybean plants reduces plant height, total biomass, and leaf yield quickly experiences premature loss (senescence). Antioxidant activity of catalase and peroxidase decreased at 100 and 200 mM NaCl [6]. To get saline-tolerant soybean seeds can be produced using soybean mutant seeds that are induced by chemical or physical mutations.
Mutation is one of the techniques in plant breeding are used to improve or develop the genetic of plants and to increase genetic diversity. This study used soybeans that had chemically induced by colchicine which is expected to produce polyploidization in plants. Polyploidization is the process of multiplying the number of chromosomes, increasing cell volume and the content of compounds in an individual cell. Polyploid organisms themselves are organisms with cells that have more than two complete sets of chromosomes or genomes [7]. Grobogan variety is one of five soybean varieties that could grow and produce in saline soil [8].

Based on the background that has been described, this research aims to investigate the response of soybean seeds mutant (was given induced colchicine before) on phenotypic character that is expected to produce plants or able to adapt to saline soils.

2. Materials and methods
In this experiment, mutant soybean seeds from Grobogan variety that has previously been induced by 0.04% colchicine. The material genetics were used as much as 156 seeds. This field experiment was conducted on January-March 2020 and was carried out in the plastic house Faculty of Agriculture, Universitas Sumatera Utara. The experiment was arranged in a Randomized Block Design with one treatment that are levels of salinity (0; 2; 4; 6 dS/m) with ten replicates for each treatment with sampling (10 plants per replicate). The data were analyzed statistically using Analysis of Variance (ANOVA) and then following by the Duncan’s multiple range test (DMRT) at a 5% level.

The saline soil was accessed from Percut Sei Tuan, Deli Serdang Regency, North Sumatera. The salinity soil level was 6.14 dS/m. To adjust the salinity levels according to treatments, some water and sea water were added. The measurement of salinity level were carried out in Laboratory of Chemical Soil, Faculty of Agriculture, Universitas Sumatera Utara. This research started with land preparation, planting, maintenance, fertilizing (fertilizing using fertilization recommendations and was same to all treatments), and phenotype parameters analysis. The observation on plant height and leaf number were measured at 2 to 4 week after planted (WAP). The observation on canopy dry weight, root dry weight, root length, root volume, and stem diameter were measured when plant at the final reproductive phase.

The number, length, and width of the stomata were measured by microscopic observation (objective 400x). The leaves that were used as research samples were completely open in 2 WAP. The surface of the epidermis under the leaves is smeared with nail polish, allowed to dry for 5-10 minutes to keep the stomata open. Then glue the transparent solution on top of the dry nail polish and lightly press it so that it is perfect. Then lift the solatip slowly until the nail polish is lifted and glue it to the glass object. Leaves preparation were observed under a microscope using an optilab (Zen Lite) program.

The observation of chlorophyll content was done on 2 WAP at the Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Sumatera Utara. The method used in calculating the amount of chlorophyll a, b and total were according to Wintemans and de Mots method [9].

3. Results and Discussion
3.1 Effect of salinity on plants height character
Data presented in Figure 1 showed that levels of salinity significantly increased plant height compared with other treatment plants. Treatment S3 (4 dS/m) with an average plant height of 21.77 cm was significantly different from treatment S2 (2 dS/m) and S4 (6 dS/m). Plants were capable of growing plants up to a salinity level of 6 dS / m. Plant height growth continues until the end of the vegetative period. This is in accordance to Wistiani [10] that mutant plants had induced by colchicine can increase plant growth because it is able to work with hormones that can spur plant growth, also the same as the research of Sartika and Basuki [11]. In addition, the growth of cayenne pepper varieties was more tolerant which shown by plants able to reduce plant growth less [12].
3.2 Effect of salinity on the number of leaves character

The highest number of leaves at 2 WAP, 3 WAP, and 4 WAP, respectively, was found at 0 dS/m (3.90), 2 dS/m (9.00; 15.00) treatments as shown in Figure 2. Treatment S2 (2 dS/m) had the highest mean number of leaves compared to other treatments in the last week of observation. Generally, the number of leaves had no significant effect.

**Figure 1.** Effect of salinity level on plant height at 2, 3, and 4 WAP. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%

**Figure 2.** Effect of salinity level on the number of leaves at 2, 3, and 4 WAP. Results are the mean of ten replication ± SD.
Salinity treatment can further inhibit leaf growth. Plants will reduce the formation of leaves so that plants do not expend large amounts of energy in the process of growth and development. Plants reduce leaf growth rate in order to reduce leaf transpiration rate because plants reduce water absorption [13]. In addition, it is caused by the low water potential in the environment so that the water in plants comes out and is associated with turgor pressure in plants. The decrease in water potential in the environment by salinity conditions causes water in the cells to come out and results in difficulty of obtaining water for plants and also inhibits expansion by plants [14-15]. Salinity stress is an abiotic stress that can affect plant productivity and quality [16]. The growth of roots, stems and leaves is reduced due to metabolic imbalance caused by poisoning with Na\(^+\), Cl\(^-\) ions, osmotic stress, nutrient deficiency and oxidative stress.

3.3 Effect of salinity on stem diameter character
The highest average stem diameter was at 0 dS/m (2.90 cm) and the lowest at 6 dS/m (2.43 cm) (Figure 3) and also the increasing salinity caused decreases in stem diameter. The growth in the diameter of the chili plants also decreases as the salinity was added [17]. Shorter stems was caused by high cell osmotic so that cell volume and cell potential also decrease. Salinity stress affected osmotic dehydration so that the cell volume and osmotic as well as cell potential decrease [18,19]. Other studies have also stated that plant growth such as stem diameter and growth of other olive plants has decreased [20].

![Figure 3](image3.png)

**Figure 3.** Effect of salinity level on stem diameter. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%.

3.4 Effect of salinity on root length and root volume characters
The observed parameters of root length and root volume with the highest mean were at S1 (0 dS/m), namely 14.66 cm and 7.38 ml respectively. Based on Figure 4, it showed that the root length and root volume respectively decreased along with the increase in salinity levels, and soybean mutants were not able to inhibit the decrease in root length and volume. This is due to the toxic effects and the high accumulation of Na\(^+\) and Cl\(^-\) ions in plant roots. The accumulation of ions that accumulate in plant roots will lead to inhibition of normal plant root cell division, thus inhibiting the formation of lateral roots and elongation of the main roots to absorb nutrients [21]. Rosmayati et al. [16] stated that the growth of roots, stems, and leaves is reduced due to metabolic imbalances caused by poisoning with...
Na\textsuperscript{+}, Cl\textsuperscript{−} ions, osmotic stress, nutrient deficiency, and oxidative stress. Besides, according to Kaydan et al. [22] it can interfere with plant metabolism which results in inhibition of meristematic activity and cell enlargement which will require a lot of energy.

![Figure 4](image_url)

**Figure 4.** Effect of salinity level on root length and root volume. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%.

![Figure 5](image_url)

**Figure 5.** Effect of salinity level on root dry weight and canopy dry weight. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%.

### 3.5 Effect of salinity on root dry weight and canopy dry weight characters

Figure 5 showed that shoot dry weight and root dry weight respectively decreased along with increasing salinity levels and colchicine administration was not able to inhibit the decrease in root length and volume. The highest canopy dry weight and root dry weight were found in treatment S1 (0 dS/m) with 3.26 g and 0.95 g. The same result was obtained by [23] which states that the dry weight of
the roots and plants were significantly different in 1500 ppm of NaCl treatment compared to the control.

It was because the higher soil salinity levels can affect the growth of root biomass and plant canopy due to the salinity of soil chemical elements such as K⁺, Na⁺, Ca²⁺, Cl⁻, and Mg²⁺. High Na⁺ and Cl⁻ in plants poison plants which will interfere with the absorption of other nutrients such as Ca²⁺, K⁺, N, and P ions are absorbed into plant tissue which results in inhibiting metabolism in plants [19,24]. An increased in salinity levels also results in stomata closure so that CO₂ supply decreases and results in a decrease in the photosynthetic process. A decreased in the photosynthetic process results in a decrease in plant canopy dry weight where canopy dry weight is an accumulation of the results of the photosynthesis process [25,26].

### 3.6 Effect of salinity on stomata number, length, and width characters

The number of stomata, stomatal length, and stomatal width were significantly different from other salinity treatments (Figure 6). The three characters have the highest average in S3 treatment (4 dS/m), which were 42.67; 28.14; and 15.76 µm. Soybean mutants are presumed to be able to inhibit stomatal closure so that the photosynthesis process in plants can run optimally until the treatment are 4 dS/m. However, the decrease in the number, length, and width of stomata was observed at the 6 dS/m treatment. It ensues to reduce the CO₂/O₂ ratio in the leaves. Stomatal closure is carried out by plants, so that plants can reduce the CO₂/O₂ ratio of leaves and inhibit CO₂ fixation, thus, the concentration of reactive oxygen species (ROS) such as superoxide radicals (O²⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH⁻) and singlet oxygen (¹O₂) increases [27,28].

![Figure 6. Effect of salinity level on number of stomata, length and width. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%](image)

### 3.7 Effect of salinity on chlorophyll content character

Based on results, plants with the highest total chlorophyll content were found at S3 (4 dS/m) (Figure 7). Though it was not significantly different at S2 (2 dS/m), the total chlorophyll content tends to increase in line with higher salinity level. The increasing of salinity, which was categorized as rather high based on [29], has caused the decrease of chlorophyll content of S4 (6 dS/m). A decrease in the chlorophyll index inflicts a decrease in photosynthesis. The same outcome was also reported by [26] that the total leaf chlorophyll content of several genotypes/varieties of soybean plants decreased with
increasing of NaCl concentration. This is fathomed that salinity makes it difficult for CO$_2$ and RuBp to bind and the closure of the stomata by decreasing the amount of potassium in the cells, causing disrupted photosynthesis [17]. The accumulation of Na$^+$ ions causes the absorption of N and Mg$^{2+}$ elements to form chlorophyll being inhibited, thus, a decrease in the absorption of these elements which results in disturbed chlorophyll formation and inhibited photosynthesis [30].

Furthermore, plants were able to inhibit the decrease in chlorophyll content degradation by up to 4 dS/m by aiming at an increase in leaf chlorophyll content. It might be that soybean mutants can help plants to tolerate high salt content in the soil. In accordance with the research of Soeparjono [31] which stated that the treatment of gamma-ray mutagens in Limon orange plants was in vitro salinity. The same thing was also found by Ahire and Auti [32] who stated that chemical and physical mutagen treatment can increase the chlorophyll content in soybean plants.

![Figure 7](image)

**Figure 7.** Effect of salinity level on chlorophyll content. Results are the mean of ten replication ± SD. The same letters in the same parameter show not significantly different in the DMRT at a level of 5%

### 4. Conclusions
The results of this study showed that as increasing salinity decreased phenotypic characters, such as root length, root volume, root dry weight, canopy dry weight, and length of stomata. Furthermore, we discovered that soybean mutant could grow at S3 (4 dS/m). Moreover, we discovered that the chlorophyll content of the soybean mutant increased at 4 dS/m.

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### References
[1] Badan Pusat Statistik 2015 *Produksi Kedelai Menurut Provinsi (Ton)* (Jakarta: Badan Pusat Statistik)
[2] Yuniati R 2004 *Makara Sci.* 8 21–4
[3] Khosravinejad H F R and Farbondia T 2008 *Pak. J. Biol. Sci.* 11 2438–42
[4] Hu Y and Schmidhalter U 2005 *J. Plant Nutr. Soil Sci.* 168 541–9
[5] Hirpara K D, Prakash J R, Ashish D P and Amar N P 2005 *Anales de Biol.*, 27 3–14
[6] Amirjani M R 2010 *Am. J. of Plant Physiol.* 5 350–60
[7] Can S 2012 *Sci. China Life Sci.* 55 301–11.
[8] Rahmawati N and Rosmayati 2010 *Penapisan Varietas Keladi* (*Glycine max* L. *Merril*) *Toleran Cekaman Salinitas*. Program Doktor Ilmu Pertanian. Fakultas Pertanian (Medan: Universitas Sumatera Utara).
[9] Wintermans J F G M and De M A 1965 *Biochimia et Biophysica Acta* 109 448–53
[10] Wistiani I A J 2014 *Tesis Induksi Mutasi Kromosom dengan Kolkisin pada Tanaman Kesuna Bali* (*Allium sativum Linn*) dan Analisis DNA dengan Marka RAPD (Denpasar: Universitas Udayana)
[11] Sartika T V and Basuki N 2017 *J. Produksi Tanaman* 5 1669–77
[12] Zhani K, Hermans N, Ahmad R and Hannachi C 2013 *J. of Stress Physiology and Biochemistry*. 9 209–28
[13] Carillo P, Mastrolonardo G, Nacca F and Fuggi A 2005 *Funct. Plant Biol.* 32 209–19
[14] Yiu J C, Tseng M J, Liu C W and Kuo C T 2012 *Sci. Horticulturae*. 134 200–9
[15] Pessarakli, M. 1999. *Handbook of Plant and Crop Stress*. (Arizona : Marcel Dekker Inc).
[16] Rosmayati, Rahmawati N, Astari R P and Fachrina W 2015 *J. Pertanian Tropik*. 2 132–9
[17] Amira M S 2015 *Int. J. of Agri. and Crop Sci.* 8 573–6
[18] Taufiq A, Kristiono A and Harnowo D 2015 *Jurnal Penelitian Pertanian Tanaman Pangan*. 34 153–64.
[19] Purwaningrahayu R D and Taufiq A 2017 *J. Bio. Indonesia* 13 175–88
[20] Kchaou D, Larbi A, Gargouri K, Chaieb M, Morales F and Msallem M 2010 *Sci. Horticulturae* 124 306–15
[21] Bagdi D L and Bagri G K 2015 *J. of Environmental Bio*. 37 573–6
[22] Kaydan D, Okut N and Yagmur M 2007 *Tarim Bilimleri Derg* 13 114–9
[23] Bastomi M Y 2018 *Efek Cekaman NaCl Terhadap Pertumbuhan Dua Varietas Cabai Rawit* (*Capsicum frutescens L.*). Fakultas Sains dan Biologi. (Malang: Universitas Islam Negeri Maulana Malik Ibrahim)
[24] Lubis M 2008 *Pertumbuhan dan Kandungan Protein Jagung di Bawah Cekaman NaCl*. (Yogyakarta: Universitas Negeri Yogyakarta)
[25] Dolatabadian A, Modarresanavy S A M and Ghanati F 2011 *Notulae Sci. Bio.* 3 41–5
[26] Aini N, Yamioka W S D, Syekhfini, Purwaningrahayu R D and Setiawan A 2014 Prosiding Seminar Nasional Lahan Suboptimal (Palembang) 319–25
[27] Lee D H, Kim Y S and Lee C B 2001 *J. Plant Physiol.* 737–45.
[28] Gratao P L, Polle A, Lea P J and Azevedo R A 2005 *Funct. Plant Biol.* 32 481–94.
[29] Jones J B 2002 *Agronomic Handbook Management of Crops, Soil, and Their Fertility*. (New York: CRC Press)
[30] Putri P H, Susanto G W A and Taufiq A 2017 *Toleransi genotipe kedelai terhadap salinitas. Penelitian Pertanian Tanaman Pangan* 1 pp 3
[31] Soeparjono S 2014 *Prosiding Seminar Nasional Pemuliaan*. Universitas Jember.
[32] Ahire D and Auti S 2015 *Int. J. Bioassays* 4 4235–40