



## Chromosome status and yield characteristics of soybean (*Glycine max* L. (Merr)) in saline soil as affected by induced mutation

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### Abstract

To fulfill the demand of soybeans, the expansion of the planting area can be one of the choices through the conversion of marginal land by utilizing suboptimal land, such as saline soil. One of the plant breeding techniques to obtain tolerant plants to salinity is the use of chemical mutations, such as colchicine. The study aimed to reveal the effect of salinity on the characters of soybean plants (mutated with colchicine) and the effect of colchicine on the number of chromosomes and ploidy level. This study was conducted from January to March 2020 and carried out in the plastic house of Faculty of Agriculture, Universitas Sumatera Utara. The experiment was arranged in a Randomized Block Design with salinity levels (0 dS.m<sup>-1</sup>, 2 dS.m<sup>-1</sup>, 4 dS.m<sup>-1</sup>, 6 dS.m<sup>-1</sup>) as treatments and consisted of ten replications in each treatment. The result showed that the soybean plants were not successfully induced by colchicine at a concentration of 0.04 % with soaking duration of 10 hours, as indicated by the number of chromosomes and analysis of ploidy level. The soybeans that had been induced were planted in saline soil. There were significant effects observed on the productive branch, number of filled pods, and the weight of seeds per plant. Colchicine treatment did not successfully affect the number of chromosomes of soybeans but had an impact on the chromosome pattern. In addition, the salinity treatment of 4 dS.m<sup>-1</sup> significantly affected the number of filled pods compared to the treatment of 6 dS.m<sup>-1</sup>.

## INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the important food commodities, which is the basic ingredient of various food in Indonesia. Nutritional content, especially protein, in soybeans is high enough so that it is needed by the community to comply with their daily protein needs (Tidke et al., 2015). Saline soil is a suboptimal soil that can be used as a planting area for soybean plants.

Ministry of Agriculture Republic Indonesia (2015) states that the national demand for soybeans in 2020 is estimated at 2.87 million ton per year. To be able to fulfill the demand for soybeans, expansion of the planting area can be done. Limited agricultural land

can be overcome by utilizing suboptimal land such as saline soil.

Saline soil is soil containing large amounts of Na<sup>+</sup> and Cl<sup>-</sup> salts, which interfere with plant growth. The stress of salinity on plants can be caused by several factors such as ion concentration, duration of stress implementation, plant species, cultivars, plant phase growth, plant organs, and environmental conditions (Deinlein et al., 2014; Martinez-Ballesta and Carvajal, 2014).

Soybean plants can be categorized as sensitive to salinity, affected from the germination to generative phase. Salinity can affect plant morphology, ion transport, biochemistry, and physiology (Abbasi et al., 2015). Currently, the availability of soybean

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seeds that are tolerant to saline soil is still limited. One of the plant breeding techniques that we can do is mutation. Soybean seeds that are tolerant to saline soils can be produced using chemical mutations.

One of the plant breeding techniques that can be done to produce plants that are tolerant to salinity stress is chemical mutations, one of which is using colchicine. Colchicine ( $C_{22}H_{25}O_6N$ ) is an alkaloid derived from the tubers and seeds of the *Colchicum autumnale* L. plant. Colchicine is a poison, especially in plants, which affects the dividing nucleus. Colchicine binds to tubulin protein, thereby preventing the formation of spindle threads. Colchicine prevents the role of microtubules in the separation of chromosomes. Hence, it is called an antimitotic mutagenic agent (Leung et al., 2015; Manzoor et al., 2019).

Polyploid plants that have experienced volume enlargement are expected to increase the content of compounds in their cells related to oxidative stress so that they can also increase the ability of plants to adapt to soil salinity. The study aimed to study the effect of salinity on the characters of soybean plants that have been mutated with colchicine and the effect of colchicine on the number of chromosomes or the ploidy level.

## MATERIALS AND METHODS

This study was carried out using soybean plants cultivar Grobogan that had been previously mutated with colchicine. There were five varieties that are able to adapt to salinity, namely Grobogan, Anjasmoro, Bromo, Cikurai, and Detam 2. However, their production is very low. Among those five varieties, Grobogan, Cikurai, and Detam 2 can produce seed pods, while Anjasmoro and Bromo only produce pods (Rosmayati et al., 2015).

In this experiment, 156 seeds of Grobogan variety that had previously been induced by 0.04 % colchicine were used. The field experiment was conducted from January to March 2020 in the plastic house of Faculty of Agriculture, Universitas Sumatera Utara. The experiment was arranged in a Randomized Block Design with salinity levels ( $S_1= 0 \text{ dS.m}^{-1}$ ,  $S_2= 2 \text{ dS.m}^{-1}$ ,  $S_3= 4 \text{ dS.m}^{-1}$ ,  $S_4= 6 \text{ dS.m}^{-1}$ ) as treatments, consisting of ten replications within each treatment (ten plants in each replication). The data were analyzed using ANOVA (Analysis of Variance) and followed by the DMRT test (Duncan's Multiple Range Test) at  $\alpha= 5 \%$ . The saline soil was derived

from Percut Sei Tuan, Deli Serdang Regency, North Sumatera. The soil's salinity level was  $6.14 \text{ dS.m}^{-1}$ , measured using conductivity meter. The salinity levels were adjusted to the treatments by adding sea water and water to increase and decrease the level, respectively. The yield characteristics observed include productive branch, the number of filled pods, and the weight of seeds per plant. The observation on the chromosome status was made on the number of chromosomes and the ploidy level, measured when the plant at the final reproductive phase.

The seeds were immersed in colchicine with a concentration of 0.04 % for 10 hours. Soaking was done in a beaker glass starting from 7 AM. Wardhani and Wiendi (2015) suggested that the soybean seeds are immersed in colchicine at 7 AM since the metaphase in the mitosis of soybean cells takes place at 10 AM. For control treatment, soybean seeds were not immersed in colchicine solution.

The analysis of the ploidy level in soybeans was carried out using the flow cytometry method. Leaves from control plants were placed in a petri dish, added with 500  $\mu\text{L}$  of extraction buffer, and then the leaves were chopped with a razor blade. Furthermore, the extract was filtered, and the filtrate was added with 2 mL of staining buffer. After that, the incubation was carried out for 10 minutes. The test tube containing the sample was inserted into the flow cytometry device. In a flow cytometry tool, the cell nucleus containing the chromosomes was identified by firing a laser beam. The glowing rays were then captured by the detector on the flow cytometry tool, which were then stopped so that the analysis results data could be returned to the computer in the form of the curve (Suda et al., 2007)

Cytology tests to count the number of chromosomes were carried out using the soybean's roots. The metaphase in soybean occurs at 10.00 PM – 10.15 PM. Chromosomes were observed under a microscope with a magnification of 1000 $\times$ .

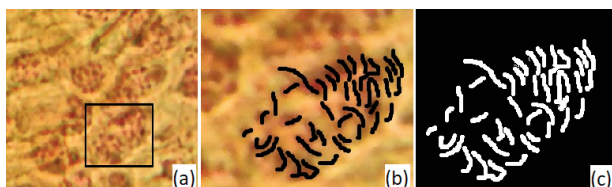
## RESULTS AND DISCUSSION

### Number of chromosomes

The number of soybean chromosomes in each treatment is 40 (Table 1). From these results, it can be seen that soybean plants did not experience a change in the number of chromosomes. The

**Table 1.** The number of chromosomes

Soybean	The number of chromosomes
Non-mutated soybean	40
Mutated soybean	40



**Figure 1.** a) control cell nucleus, b) control chromosomes at a magnification of 1000x, c) enlarged control chromosome



**Figure 2.** a) mutant cell nucleus, b) mutant chromosomes at a magnification 1000x, c) enlarged mutant chromosomes

**Table 2.** The results of the analysis of ploidy level using flow cytometry

Treatment	Mean	Ploidy level	Histogram of flow cytometry
Unmutated soybean	110,475	Diploid	
Mutated Soybean	105,355	Diploid	

colchicine concentration and soaking time in this study were not able to affect the changes in the number of chromosomes (Figure 1).

Based on Figure 2, the colchicine treatment with a concentration of 0.04 % and a soaking time of 10 hours could not reach polyploidy in plants. This is due to the inappropriate concentration and soaking time. Colchicine causes fatal effects on plant cells, resulting in cell death and damage in plant tissue

(Mostafa and Alhamd, 2016). However, the correct concentration and duration of soaking time can induce polyploidy in these plants.

Dhooghe et al. (2011) state that chromosome multiplication depends on the colchicine concentration given. Furthermore, the results were also exceptionally influenced by the duration of imbibition and plant genotype (Slusarkiewicz-Jarzina et al., 2017; Mangena, 2020). To further confirm the effect of colchicine

toward ploidy level, flow cytometry was used.

**Analysis of the ploidy level**

Flow cytometry can gauge DNA collection of the nuclei. Furthermore, flow cytometry can estimate the ploidy level better than counting chromosomes by microscope, which is traditional technique. Takahira et al. (2011) identified the ploidy level of canola using leaf tissue with small leaf sample (2 mg to 8 mg). The results (Table 2) show the DNA peaks of each treatment. The peak seen in control soybeans amounted to one on channel 100, defined as the standard for diploid cells.

In addition, the peak in the control plant was higher than in the soybean plants induced with colchicine, which can be seen from the mean value of the treatment. The peaks in the mutant plants were lower than those in the control plants as indicated by the mean value. The mean value for control plants was 110,475, and the mean value for the mutant plants was 105,355. The same results were obtained by Niu et al. (2016), discovering that there were two

types of ploidy level in *Jatropha curcas*, which were tetraploid and octoploid. There was a decrease in the number of cells (apoptosis) caused by the formation of proteins in the cells and a decrease in the process of endocytosis and exocytosis due to the influence of colchicine (Zhou et al., 2017).

**Productive branch**

Table 3 showed that the salinity level of 4 dS.m<sup>-1</sup> resulted in the highest number of productive branches, which was not significantly different compared to the treatment without salinity levels (0 dS.m<sup>-1</sup>). The application of colchicine is thought to be able to protect cells from reactive oxygen compounds and free radicals that interfere with the function of chloroplasts by producing antioxidant compounds in chloroplasts (Afzal et al., 2005), which can reduce the negative impact of high salt concentrations, protecting the function of chloroplasts and reducing the reactive oxygen species (ROS). Thus, the plants can photosynthesize well, thereby supporting increased production, especially the number of productive

**Table 3.** Effects of salinity on the productive branch characters, number of filled pods, and weight of seeds per plant

Treatments	Productive branch (branch)	Number of filled pods (pod)	Weight of seed (g per plant)
S1 (0 dS.m <sup>-1</sup> )	5.25 b	9.50 b	4.01 b
S2 (2 dS.m <sup>-1</sup> )	2.67 ab	1.00 a	0.87 ab
S3 (4 dS.m <sup>-1</sup> )	6.00 b	8.33 b	3.31 b
S4 (6 dS.m <sup>-1</sup> )	2.33 a	1.33 ab	0.67 a

Remark: Means followed by the same letters in the same column are not significantly different based on the DMRT at α= 5 %.

branches. The low number of productive branches at the salinity treatments of 2 dS.m<sup>-1</sup> and 6 dS.m<sup>-1</sup> had a relationship with low plant height. Hakim (2012) stated that plant height and productive branches had a positive correlation with the number of fertile nodes, filled pods, and seed yield. Salinity stress can cause oxidative damage through production reactive oxygen species (ROS) that can destroy fat, protein, and amino acid, thereby impairing growth of plant and production (Rishi and Sneha, 2016).

**Number of filled pods**

The treatment of 4 dS.m<sup>-1</sup> salinity level could still have a fairly high number of pods when compared

to control (0 dS.m<sup>-1</sup>) and other salinity treatments (Table 3). This is presumably because plants have been able to reduce the number of reactive oxygen compounds due to salinity stress so that plants can perform photosynthesis better so that they can form and fill pods by producing antioxidants produced by chloroplasts.

The increase in the number of chloroplasts can indirectly increase the photosynthetic process in the leaves for the formation of assimilates and hoarding of plant photosynthetic products (Ismail et al., 2018). Likewise, photosynthesis is important biochemical pathways to convert solar energy into chemical energy for growth and production (Parihar et al., 2015). The

low number of filled pods at the salinity levels of 2 dS.m<sup>-1</sup> and 6 dS.m<sup>-1</sup> were related to low plant height as well. Hakim (2012) states that plant and branch height have positive relationship with the number of fertile nodes, filled pods, and seed yield. Agam et al. (2020) discovered that gamma-mutated black soybean planted under salinity stress ranging from 2.00 dS.m<sup>-1</sup> to 6.67 dS.m<sup>-1</sup> decreased the number of pods per plant to 96 % than under optimum condition.

#### Weight of seeds per plant

The S3 treatment (4 dS.m<sup>-1</sup>) had the highest seed weight per plant compared to salinity levels of 2 dS.m<sup>-1</sup> and 6 dS.m<sup>-1</sup> (Table 3). However, it was not significantly different from the treatment without salinity (0 dS.m<sup>-1</sup>). This can occur due to the antioxidant content produced in chloroplasts in plants, which should be reduced due to saline stress, becomes relatively insufficient. This was in accordance with the statement by Afzal et al., (2005), mentioning that antioxidants such as ascorbic acid produced in chloroplasts can reduce the negative impact of high salt concentrations. The increase in the number of chloroplasts indirectly causes an increase in the rate of photosynthesis and an increased rate of photosynthesis, which can produce more photosynthate (the result of photosynthesis), so that larger and more tubers or fruit can be produced as a storage place for photosynthate (Rachmawati et al., 2009).

The treatments of S2 (2 dS.m<sup>-1</sup>) and S4 (6 dS.m<sup>-1</sup>) had low seed weight per plant (Table 3) because the plants had not been able to protect the chloroplast function so that they had increased ROS compounds in plants that could interfere with the photosynthesis process. According to Frederick et al. (2001), it has an impact on the ability to photosynthesize so that the growth rate of growth decreases between the R1 and R5 phases, thereby reducing the number of pods and the number of seeds, which results in the decreased production.

Subramanyam et al. (2012) reported that plants with salinity tolerance genotypes might be effective in using osmotin as a transcription regulator for enzymes coding genes that play a role in salinity tolerance. In addition, it was reported that the salinity tolerant genotype plants could form 32–35 pods per plant with seed weights of 10.3 g per plant to 12 g per plant at 20 dS.m<sup>-1</sup>.

## CONCLUSIONS

Colchicine in soybean did not successfully affect the number of chromosomes but had an impact on the pattern based on the analysis of ploidy level. The salinity treatment of S3 (4 dS.m<sup>-1</sup>) had no significant effect on the number of filled compared to the treatments of S1 (0 dS.m<sup>-1</sup>) and S4 (6 dS.m<sup>-1</sup>). However, the salinity treatment of S3 (4 dS.m<sup>-1</sup>) showed significantly different effect on the number of filled pods compared to the S4 treatment (6 dS.m<sup>-1</sup>). However, the salinity treatment of S3 (4 dS.m<sup>-1</sup>) did not show significant difference in the productive branches and seed weight per plant compared to S1 (0 dS.m<sup>-1</sup>) and S2 (2 dS.m<sup>-1</sup>) treatments.

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## REFERENCES

- Abbasi, G.H., Akhtar, J., Ahmad, R., Jamil, M., Anwar-ul-Haq, M., Ali, S., and Ijaz, M. (2015). Potassium application mitigates salt stress differentially at different growth stages in tolerant and sensitive maize hybrids. *Plant Growth Regul.*, 76, pp. 1–15.
- Afzal, I., Basra, S.M.A., and Iqbal, A. (2005). The effects of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *J. Stress Physiol. and Biochem.*, 1(1), pp. 6–14.
- Agam, M.G.S.A.S., Kusmiyati, F., and Herwibawa, B. (2020). Diversity analysis in soybean (*Glycine max* (L.) Merrill) mutant lines grown in saline soils using agronomic traits and RAPD markers. *IOP Conf. Ser.: Earth Environ. Sci.*, 482, pp. 012017.
- Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., and Schroeder, I. J. (2014). Plant salt-tolerance mechanisms. *Trends in Plant Sci.*, 19(6), pp. 371–379.
- Dhooghe, E., Van Laere, K., Eeckhaut, T., Leus, L., and Van Huylenbroeck, J. (2011). Mitotic chromosome doubling of plant tissues in vitro. *Plant Cell Tiss. Organ Cult.*, 104, pp. 359–373.



- Frederick, J.R., Camp, C.R., and Bauer, P.J. (2001). Drought-stress effects on branch and main-stem seed yield and yield components of determinate soybean. *Crop Sci.*, 41(3), pp. 759–763.
- Hakim, L. (2012). Komponen hasil karakter morfologi penentu hasil kedelai. *J. Penelitian Pertanian Tanaman Pangan*, 31(3), pp. 73–179.
- Ismail, M., Yudono, P., and Waluyo, S. (2018). Tanggapan dua kultivar kedelai (*Glycine max* L.) terhadap empat aras salinitas. *Vegetalika*, 7(2), pp. 16–29.
- Suda, J., Kron, P., Husband, B.C., and Trávníček, P. (2007). Flow cytometry and ploidy: Applications in plant systematics, ecology and evolutionary biology. In: J. Dolezel, et al., eds., *Flow Cytometry with Plant Cell*. 1<sup>st</sup> ed. Weinheim: WILEY-VCH Verlag GmbH and Co. KGaA, pp 103–130.
- Leung, Y.Y., Hui, L.L.Y., and Kraus, V.B. (2015). Colchicine-update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum.*, 45(3), pp. 341–350.
- Martinez-Ballesta, M.D. and Carjaval, M. (2014). New challenges in plant aquaporin biotechnology. *Plant Sci.*, 217–218, pp. 71–77.
- Mangena, P. (2020). In vivo and in vitro application of colchicine on germination and shoot proliferation in soybean (*Glycine max* (L.) Merr.). *Asian J. Crop Sci.*, 12(1), pp. 34–42.
- Manzoor, A., Ahmad, T., Bashir, M. A., Hafiz, I. A. and Silvestric, C. (2019). Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. *Plants*, 8(7), pp. 194.
- Ministry of Agriculture Republic Indonesia. (2017). Strategi pencapaian swasembada kedelai 1<sup>st</sup> ed. Bogor: Kementerian Pertanian Republik Indonesia, pp. 45–50.
- Mostafa, G.G. and Alhamd, M.F.A. (2016). Detection and evaluation the tetraploid plants (*Celosia argentea*) induced by colchicines. *Int. J. Plant Breed. Genet.*, 10(2), pp. 110–115.
- Niu, L., Yan-Bin, T., Mao-Sheng, C., Qiantang, F., Yuling, D., Huiying, H., and Zeng-Fu, X. (2016). Identification and characterization of tetraploid and octoploid *Jatropha curcas* induced by colchicine. *Caryologia: Int. J. of Cytology, Cytosystematic and Cytogenetics*, 60(1), pp. 58–66.
- Rishi, A. and Sneha, S. (2016). Antioxidative defence against reactive oxygen species in plants under salt stress. *Int. J. Curr. Res.*, 5(7), pp. 1622–1627.
- Rosmayati, Rahmawati, N., Astari, R.P., and Wibowo, F. (2015). Analisa pertumbuhan vegetatif kedelai hibridisasi genotipa tahan salin dengan varietas anjasmoro untuk mendukung perluasan areal tanam di lahan salin. *Jurnal Pertanian Tropik*, 2(17), pp. 132–139.
- Parihar, P., Singh, S., Singh, R., Singh, V.P., and Prasad, S.M. (2015). Effect of salinity stress on plants and its tolerance strategies; a review. *Env. Sci. And Pollution Research*, 22(6), pp. 4056–4075.
- Tidke, S.A., Ramakrishna, D., Kiran, S., Kosturkova, G., and Ravishankar, G.A. (2015). Nutraceutical potential of soybean: Review. *Asian Journal of Clinical Nutrition*, 7(2), pp. 22–32.
- Ślusarkiewicz-Jarzina, A., Pudelska, H., Woźna, J., and Pniewski, T. (2017). Improved production of doubled haploids of winter and spring triticale hybrids via combination of colchicine treatments on anthers and regenerated plants. *J. Appl. Genet.*, 58(3), pp. 287–295.
- Subramanyam, K., Arun, M., Mariashibu, T.S., Theboral, J., Rajesh, M., Singh, N.K., Manickavasagam, M., and Ganapathi, A. (2012). Overexpression of tobacco osmotin (TBOSM) in soybean conferred resistance to salinity stress and fungal infections. *Planta*, 236, pp. 1909–1925.
- Takahira, J., Cousin, A., Nelson, M.N., and Cowling, W.A. (2011). Improvement in efficiency of microspore culture to produce doubled haploid canola (*Brassica napus* L.) by flow cytometry. *Plant Cell Tiss. Organ Cult.*, 104(1), pp. 51–59.
- Wardhani, M. and Wiendi, N.M.A. (2015). Induksi mutasi genetik melalui penggandaan kromosom kedelai (*Glycine max* (L.) Merr.) varietas wilis tanggamus dengan kolkisin secara in vitro. *Prosiding Simposium Seminar Bersama PERAGI-PERHORTI-PERIPI-HIGI mendukung kedaulatan pangan energi yang berkelanjutan*, pp. 274–280.
- Zhou, K., Fleet, P., Nevo, E., Zhang, X., and Sun, G. (2017). Transcriptome analysis reveals plant response to colchicine treatment during on chromosome doubling. *Sci. Rep.*, 7(8503), pp. 1–11.