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Mahameru Soybean (*Glycine max*) Cultivar, High Salinity Tolerant

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Abstract

Mahameru cultivar is high salinity tolerant cultivar. The previous study result showed Mahameru cultivar could tolerate 140 mM NaCl, but Cilacap Coast salinity levels often reaching 200 mM NaCl. A research of salinity stress on Mahameru cultivar at 200 mM NaCl have not conducted yet. Therefore to conduct the research of Mahameru at high salinity stress to obtained high salinity tolerant soybean cultivar. The observed variables are anatomy (epidermis thickness, the density of stomata and trichomes, palisade thickness) physiology (the dry weight of roots and canopy, the content of chlorophyll a and b) Production (whole pod, total filled pod, total empty pod, weight per one-hundred beans). The salinity treatment was 0, 50,100, 150, 200 mM NaCl given at three days before planting and twenty-one days after planting. The data of anatomy and physiology was taken at forty-five days after planting. The production data was taken when soybean plants turned brown. The result indicates that salinity affects anatomy characteristic of leaf, higher the salinity increasing epidermis thickness and the density of stomata and trichomes. Salinity affected the content of chlorophyll a and b. Higher the salinity increased the content of chlorophyll a and b. Salinity did not affect soybean production. Based on this study Mahameru cultivar is resistant to salinity up to 200 mM NaCl. The benefit of this research help to enhance national soybean production with utilization coastal land for soybean planting Mahameru cultivar.

How to Cite

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the food sources that contain vegetable protein. The need of soybean in Indonesia is increasing every year, related to industrial food growth such as tempe, tauco, and soybean milk (Widiati *et al.*, 2014). Indonesian soybean production still cannot fulfill the domestic need. The increasing and stabilizing of soybean product in Indonesia face many limitations. One of the limiting factors is the biotic factor is pod borer (*Etiella zinckenella* Treitschke) infestation that can cause soybean yield decreased (Kuswantoro *et al.*, 2017). The other reason behind low soybean production is the unavailability of agricultural land to plant soybean. Agricultural land to plant soybean that still not used yet is coastal land or saline land (Zulfi *et al.*, 2014). The problem in using the coastal land for agricultural purpose is that coastal land has high salinity level. The high level of salinity is one of the abiotic factors which can inhibit the growth of plants (Farid and Sjahril, 2006). Soybean plants that planted at 100 mM NaCl salinity stress causing an increase in cuticle thickness and the number of trichomes, an increase in roots and stem xylem thickness (Makbul *et al.*, 2011). El-Rodeny & El-Okkiah (2012) state that soybean planted in high salinity stress will undergo changes such as decreased cortex thickness and increased root xylem thickness, reduced stem cortex thickness and increased stem phloem and xylem thickness, and increased leaf mesophyll thickness. Salinity causes a decrease in gross weight of soybean crops (Amirjani, 2010). Soybean crops that planted on 50 mM NaCl to 150 mm NaCl salinity stress will have it is chlorophyll a and b decreased, higher the salinity lower the chlorophyll a and b value (Sheteawi, 2007; Ghassemi-Golezani & Minoo, 2011). The soybean that planted at 100 mM NaCl salinity stress it is stomata density decreased (Atabayeva *et al.*, 2013). The soybean that planted at 60 nM NaCl salinity stress decreased in roots and canopy dry weight (Rastegar *et al.*, 2011).

The research was conducted to test Mahameru cultivar (Figure 1) could resist high salinity stress level up to 200 mM NaCl observed by it is anatomy, physiology, and production aspect. This research about Mahameru cultivar was never conducted before. The benefit of this study is to help Indonesia Government to fulfill domestic soybean demand using saline land as agricultural land for soybean crops planting.

METHODS

This research was done March to July 2017 at Structure and Development of Plants Laboratory and Plant Physiology Laboratory greenhouse of Biology Faculty University of Jenderal Soedirman Purwokerto, Central Java, Indonesia. The observed variables were anatomical characteristics such as leaf's upper surface epidermis and lower surface epidermis thickness, palisade thickness, mesophyll thickness, stomata thickness, and trichromatic thickness (Figure 2). The physiological characteristics such as roots dry weight, canopies dry weight, and chlorophyll a and b value. Chlorophyll a value formula: chlorophyll a (µg/l) = 12.21 (A663) – 2.81 (A646), chlorophyll b value formula: chlorophyll b (µg/l) = 20.13 (A646) – 5.03 (A663). The fresh leaves were cut small, weighed 0.2g, mashed in a mortar, then 20 ml of acetone 80% was added. The result centrifuged for ten minutes at 400rpm, and supernatant absorbent observed at 646 and 663 wavelengths (Porra, 2002). The productivity characteristics observed such as a total pod, total filled pod, total empty pod, weight per one-hundred beans. The production data was taken when soybean crops 90% mature and the pod turned brown.

The experimental design used in this research is a Completely Randomized Design (CRD). The treatment given are NaCl concentration namely 0 mM, 50 mM, 100 mM, 150 mM, 200 mM. The treatment is done five times as replicates. The anatomy and physiology observation used the same crops, and production observation used different crops. Twenty-five samples prepared for anatomy and physiology observation, the other twenty-five samples prepared for production observation, total samples used were fifty soybean crops. The media used is not hollowed plant's pot sized 35 x 40 cm. The NaCl treatment is applied before the seed is planted. Each plant's pot filled with 4,5kg soil and 0,5kg goat dung fertilizer. For anatomy and physiology observation is done at day 45th and for production, observation is done until the seed is mature on the crops. The crop maintenance procedure is watering the crops with aquades, and NPK fertilizer is given at day 21st. The preparation of anatomical observation done by making leaf transverse cut with paraffin method (Sass, 1958). The roots and canopies dry weight are obtained by covering them with papers and dried in the oven at the temperature of 70°C for 48 hours then weighed. The result then tested with ANOVA with 95% and 99% confidence interval and followed by Honest Sig-

nificant Difference (HSD) test.

RESULTS AND DISCUSSION

Leaf anatomical

Stomatal length and width

The stomatal length of leaf's lower epidermis at 0 mM NaCl concentration was 23.50 µm, and at 200 mM NaCl concentration was 18.00 µm. The stomatal width of leaf upper epidermis at 0 mM NaCl concentration was 11.00 µm and at 200 mM NaCl concentration was 9.50 µm. The stomatal length on leaf upper epidermis at 0 mM NaCl concentration was 20.26 µm and at 200 mM NaCl concentration was 18.50 µm. The stomatal width of leaf upper epidermis at 0 mM NaCl concentration was 9.00 µm and at 200 mM NaCl concentration was 9.26 µm. The impact of salinity concentration effect on the stomatal length on leaf lower epidermis analysis showed a significant difference ($p>0.01$), it means that salinity concentration had affected the stomatal length. The impact of salinity concentration effect on the stomatal width on leaf lower epidermis and upper epidermis, stomatal length on upper epidermis analysis result showed no significant difference ($p<0.05$), it means that salinity concentration had not affected the stomata.

The salinity treatment affected a stomatal length of the lower epidermis. Higher the salinity, the average stomatal length of lower epidermis decreased. The stomatal length of the leaf lower epidermis decreased from 23.50 µm at 0 mM NaCl concentration to 18.00 µm at 200 mM NaCl concentration. The soybean crops at 200 mM NaCl concentration try to survive the harsh salinity concentration by shrinking it is the lower surface stomatal length to decrease evaporation (Table 1). The decrease of stomatal length happened because of Na+ and Cl- ions that inhibit water absorption at the roots. The excessive amount of Na+ and Cl- ions in the soil will inhibit water absorption then will decrease soybean cell osmotic potential (Makbul *et al.*, 2011).

The salinity stress will decrease the stomatal size of lower and upper epidermis of soybean crops (El-Rodeny & El-Okkiah, 2012). The Na+ and Cl- ions will affect water potential in the roots area and will induct the forming of Reactive Oxygen Species (ROS) that can destroy cell membrane that contains fats, proteins, and amino acids (Amirjani, 2010). Salinity stress decreases the stomatal size of the epidermis (Dolatabadian *et al.*, 2011). The decrease in the stomatal size is in response to the reduced water availability as an adaptation. The stomatal size is significantly affected by water availability, at 80% – 100% water availability there is no difference in soybean cultivar stomatal size, but at 40% - 60% water availability there is the difference soybean cultivar stomatal size (Widiati *et al.*, 2014). It is an adaptation by soybean crops in response to decreased water availability. The decrease in stomatal size is a response to salinity stress that inhibits water absorption by root that will disturb hormone regulation then inducted stomatal size changes.

However, salinity treatment did not affect the stomatal width of the lower epidermis, and stomatal length and width of the upper epidermis ($p<0.05$). The stomatal length of upper epidermis at concentration 0 mM NaCl was 20.20 µm and at 200 mM was 18.50 µm (Table 1). This result indicates that Mahameru cultivar can survive at 200 mM NaCl salinity level.

Stomatal and Trichomatal Density

The stomatal density (Figure 3) of lower epidermis at 0 mM NaCl was 14.00/mm2, at 200 mM NaCl was 7.4/mm2. The stomatal density of leaf upper epidermis at 0 mM NaCl was 6.40/mm2. The trichromatic density of leaf lower epidermis at 0 mM NaCl was 1.80/mm2, at 200 mM NaCl was 2.80/mm2. The trichromatic density of leaf upper epidermis at 0 mM NaCl was 1.40/mm2, at 200 mM NaCl was 2.60/mm2. The result of salinity treatment analysis showed that salinity concentration gave significant difference ($p>0.05$) to stomatal density on the leaf lower and upper epidermis of Mahameru cultivar soybean. However, the salinity did not provide

Table 1. The Average of Stomatal Length and Width of Leaf Upper and Lower Epidermis

Treatment	Stomatal length on the lower epidermis	Stomatal width of the lower epidermis	Stomatal length on the upper epidermis	Stomatal width of the upper epidermis
S0	23.50± 2.24	11.00 ±1.63	20.26± 0.58	9.00 ±1.37
S1	19.50± 2.09	9.50 ± 1.11	19.50 ±1.12	9.00 ±1.37
S2	18.00 ±2.09	11.00 ±1.36	19.00 ±1.37	10.00 ±1.77
S3	22.50± 1.77	11.00± 1.36	21.50± 2.85	10.50 ±1.12
S4	18.00± 3.26	9.50 ± 1.11	18.50 ±2.85	9.26 ±1.69

a significant difference ($p<0.05$) on trichromatic density on leaf lower epidermis and upper epidermis.

Higher the salinity make the stomatal density of leaf lower epidermis decreased. The highest stomatal density of leaf lower epidermis was 14.40/mm2 at 0 mM NaCl concentration; the lowest stomatal density was 7.40/mm2 at 200 mM NaCl concentration (Table 2). The decrease in stomatal density on leaf lower epidermis is an adaptive response from soybean crops that grow in saline condition to lower the evaporation from stomata. The decrease in stomatal density on leaf lower epidermis is happened because of Na+ and Cl- ions that toxic to the crops decreasing K+ and Ca+ ions stability then cell osmotic potential decreased. The balance of K+ and Ca2+ ions in the cell can affect the cell osmotic potential and caused the growth of soybean crop inhibited (Tunçturk *et al.*, 2008).

High concentration of salinity inducted the changes in the anatomical character of soybean crops (El-Rodeny & El-Okkiah, 2012). The salinity treatment analysis in stomatal density on leaf showed a significant difference of value ($p>0.05$). Higher the salinity, the stomatal density of Mahameru cultivar soybean leaf lower epidermis decreased. The highest stomatal density on leaf upper epidermis was 6.40 mM2 at 0 mM NaCl concentration; the lowest stomatal density was 5.00/mm2 at 200 mM NaCl concentration (Table 2). Decreases in the stomatal density on upper leaf epidermis is happened because of ROS accumulation on cells. Salinity stress can induce exceeded ROS forming that can destroy the cell (Baniaghil *et al.*, 2013).

The trichromatic density of leaf lower epidermis did not show a significant difference in salinity treatment ($p<0.05$). The average value of the trichromatic density of leaf lower epidermis showed an increased value by increasing salinity level. The value was 1.80/mm2 at 0 mM NaCl and 2.80/mm2 at 200 mM NaCl. The highest value level of trichromatic density on leaf lower epidermis was 3.00/mm2 at 150 mM NaCl. Increased in the trichromatic density on leaf lower epidermis is an adaptive ability to prevent the exceeding loss of water to survive in the saline condition. The soybean crops that grow on saline land had it is trichromatic density increased (Dolatabadian *et al.* 2011). High salinity inhibits the plant's growth causing shorter roots, smaller central cylinder and parenchyma's cortex, and lighter roots and canopies dry weight (Gabriel *et al.*, 2011).

The salinity treatment did not significantly affect trichromatic density on Mahameru cultivar leaf upper epidermis. The average value trichromatic density on leaf upper epidermis was 2.40/mm2 at 0 mM NaCl and increased to 2.60/mm2 at 200 mM NaCl (Table 2). There is an increase in trichromatic density on soybean leaf upper epidermis from 1.40/mm2 to 1.77/mm2 (Juwarno and Samiyarsih, 2017).

Leaf Section

Based on the research the upper epidermis thickness was 9.50 µm at 0 mM NaCl and 9.50 µm at 200 mM NaCl. The lower epidermis thickness was 8.00 µm at 0 mM NaCl and 10.00 µm at 200 mM NaCl. The palisade thickness was 30.00 µm at 0 mM NaCl and 26.50 µm at 200 mM NaCl. The mesophyll thickness was 66.50 µm at 0 mM NaCl and 49.50 µm at 200 mM NaCl. The salinity treatment showed a significant difference in the lower epidermis thickness value ($p>0.01$), and to the mesophyll thickness value ($p>0.05$). However, the salinity treatment did not show a significant difference in the upper epidermis thickness and the palisade thickness ($p<0.05$). Higher the salinity level than the Mahameru cultivar leaf lower epidermis thickness increased. The average value of lower epidermis thickness at 0 mM NaCl was 8.00 µm and increased to 10.00 µm at 200 mM NaCl.

The increase in lower epidermis thickness is related to prevent the loss of water in Mahameru cultivar crops. The thicker leaf lower epi-

Table 2. The Average of stomatal and trichromatic density on leaf upper and lower epidermis (total/mm2).

Treatment	Stomatal density of lower epidermis	Stomatal density of upper epidermis	Trichomatal density of lower epidermis	Trichomatal density of upper epidermis
S0	14.40±2.90	6.40±1.90	1.80± 0.40	2.40± 0.50
S1	12.20 ±1.50	4.40 ±1.30	2.60 ±0.50	1.60 ±0.50
S2	9.60 ±1.70	3.60 ±1.30	2.40 ±0.90	2.00 ±0.70
S3	8.60 ±0.50	4.40 ±0.90	3.00 ±1.00	2.40 ±1.10
S4	7.40 ±1.10	5.00 ±0.00	2.80 ±0.80	2.60 ±0.50

Table 3. The average of leaf lower epidermis thickness, upper epidermis thickness, palisade thickness, and mesophyll thickness (μm).

Treatment	Upper Epidermis Thickness	Lower Epidermis Thickness	Palisade Thickness	Mesophyll Thickness
S0	9.50 ±1.12	8.00 ±1.12	30.00± 6.12	66.50± 8.59
S1	9.50 ±1.12	9.00 ±1.37	29.50 ±6.22	59.50 ±4.81
S2	10.00±1.77	9.00 ±1.37	37.50 ±12.25	66.50 ±13.18
S3	9.00 ±1.37	7.50 ±0.00	38.00 ±8.91	67.00 ±11.37
S4	9.50 ±1.12	10.00 ±0.00	26.50 ±4.87	49.50± 6.71

dermis is relevant to it is function as a water reservoir. High salinity condition causing water potential decreased and K+ and Ca2+ ions became unstable.

The salinity stress causing cell osmotic potential decreased that will inhibit soybean crop growth (El-Rodeny & El-Okkiah, 2012). The average value of soybean leaf epidermis at 0 mM NaCl was 9.06 μm and decreased to 8.87 μ atn 80 mM NaCl; there is a decrease in leaf epidermis thickness (Juwarno & Samiyarsih, 2017). The saline condition changes soybean crops anatomical characteristics such as decreases in the leaf epidermis thickness (Dolatabadian *et al.*, 2011).

The result showed that salinity treatment did not give a significant difference ($p<0.05$) to Mahameru cultivar leaf upper epidermis thickness. The upper epidermis thickness value at 0 mM NaCl and 200 mM NaCl was 9.50 μm. This result showed that Mahameru cultivar could survive at high salinity condition. The saline condition decreases soybean leaf epidermis thickness (Hameed *et al.*, 2013). The salinity stress decreases barley (*Hordeum vulgare* L.) leaf upper and lower epidermis thickness (Atabayeva *et al.*, 2013). The decreases in leaf epidermis thickness are caused by ROS production in chloroplast, mitochondria, and peroxisome that destroy growth cell lipids membrane.

The result showed that salinity treatment did not give a significant difference ($p<0.05$) in Mahameru cultivar leaf palisade thickness. The leaf palisade thickness at 0 mM NaCl was 30.00 μm and decreased to 26.50 μm at 200 mM NaCl (Table 3). This stated that Mahameru cultivar could resist salinity level-up to 200 mM NaCl. The Na+ and Cl- did not affect Mahameru cultivar growth. The soybean crops that grow on salinity stress condition had it is leaf palisade thickness decreased (El-Rodeny & El-Okkiah, 2012). Na+ and Cl cause the decreases in thickness level of leaf palisade- that toxic to soybean crops and will inhibit it is growth. The palisade thickness increased on sweet potato from 74.94 μmat 0 kg/hectare fertilizer application to 92.50 μm at 200 kg/hectare (Juwarno *et al.*, 2009).

The result showed that salinity treatment did give a significant difference ($p>0.05$) to Mahameru cultivar leaf mesophyll thickness. The decreases in Mahameru cultivar leaf mesophyll epidermis is caused by the lack of Ca2+ and Mg2+ ions as a constituent element of plant cell wall, disintegrating the plant cell wall and disturbed nutrient transportation.

The soybean crop that exposed to the saline condition it is anatomical characteristic such as mesophyll thickness and trichromatic density will change as an adaptive mechanism to salinity (Dolatabadian *et al.*, 2011). The anatomical change in plants is an adaptation in response to the lack of water and nutrition to survive (Gabriel *et al.*, 2011). The Mahameru cultivar soybean mesophyll thickness at 0 mM NaCl was 66.50 μm and sharply decreased to 49.50 μm at 200 mM NaCl (19.72 %). The soybean crops that exposed to salinity will have their growth inhibited (Farid & Syahril, 2006). The decrease of mesophyll thickness is happened because of ROS production on chloroplast, mitochondria, and peroxisome that destroy growth cell lipids membrane.

Physiology

The roots dry weight at 0 mM NaCl was 0.51, at 200 mm NaCl was 0.51 g. The canopies dry load at 0 mM NaCl was 3.71 g, at 200 mm NaCl was 3.12 g. The chlorophyll a value at 0 mM NaCl was 15.81 μg/l, at 200 mm NaCl was 17.94 μg/l. The chlorophyll b value at 0 mM NaCl was 5.09 μg/l, at 200 mm NaCl was 7.19 μg/l.

Figure 1. Cultivar Mahameru at 0 mM NaCl, 60 days after planting.

The salinity treatment did not give a significant difference ($p<0.05$) in the roots dry weight. The root dry weight at 0 mM NaCl was 0.51 g, and so do the root dry weight at 200 mM NaCl (Table 4). This result showed that Mahameru cultivar could survive in saline condition up to 200 mM NaCl. The soybean crops that grow in salinity stress will have their roots, and canopies dry weight decreased. Soybean crops inhibited growth are happened because of Na+ and Cl- that toxic to plants accumulated (Kondetti *et al.*, 2012). The soybean crops that treated at 50, 100, dan 200 mM NaCl salinity concentration will decrease in gross and dry weight, that is happened because of Na+ and Cl- ions accumulation in plant and affect it is growth (Amirjani, 2010)

The salinity treatment gave a significant difference ($p>0.05$) in the value of Mahameru cultivar chlorophyll a. Higher the salinity level will increase chlorophyll a value. The average of chlorophyll a value at 0 mM NaCl was 15.81 µg/l and increased to 17.94 µg/l at 200 mM NaCl (Table 4). The salinity treatment at 50 and 100 mM NaCl will decrease photosynthesis pigment (chlorophyll a and b) (Sheteawi, 2007). Salinity stress causing the value of chlorophyll a and b in soybean crops decreased (Sofalian *et al.*, 2013). The soybean crops that grow on salinity stress will have their chlorophyll a and b decreased (Makbul *et al.*, 2011). Soybean crops inhibited growth are happened because of Na+ and Cl- that toxic to plants accumulated causing changes in soybean physiological characteristic (Kondetti *et al.*, 2012).

The salinity treatment at 50, 100, dan 200 mM NaCl causing a decrease in roots and canopies dry weight because of Na+ and Cl- accumulation in the plant (Amirjani, 2010). The result of this research shows that the Mahameru cultivar chlorophyll a and b value was increasing in saline condition, in contrary with other soybean cultivars that showed decreases in the chlorophyll a and b value (Makbul *et al.*, 2011).

Production

The total of pods at 0 mM NaCl was 19.60/crop, at 200 mM NaCl was 17.60/crop. The total of filled pods at 0 mM NaCl was 18.40 /crop, at 200 mM NaCl was 17.60/crop. The total of empty pods at 0 mM NaCl was 1.20 /crop, at 200 mM NaCl was 0.0/crop. The weight of one-hundred bean at 0 mM NaCl was 11.99 g, at 200 mM NaCl was 12.42 g. The result showed that salinity treatment did not give a significant difference ($p<0.05$) at total pods, total filled pods, total empty pods, and the weight of one-hundred bean of Mahameru cultivar. The average value of total pods and total filled pods was decreased as salinity stress increased (Table 5).

The decrease in total pods of Mahameru cultivar soybean was happened because of accumulation of Na+ and Cl- ions resulted in osmotic stress. The soybean crops that planted on saline condition will have it is growth inhibited (Kondetti *et al.*, 2012). The exceeding amount of Na+ and Cl- will disturb Ca2+ and Mg2+ stability and resulted in cell disintegration, lower the production of chlorophyll, and decrease pods production. The soybean crops that planted on saline condition will have their photosynthate decreased becau-

Table 4. The average value of canopies dry weight (g), roots dry weight (g), chlorophyll a and chlorophyll b (µg/l).

Treatment	Canopies dry weight	Roots dry weight	Chlorophyll a	Chlorophyll b
S0	3.71 ±1.62	0.51±0.26	15.81 ±1.35	5.09±0.45
S1	3.50 ±0.57	0.59 ±0.21	15.06 ±0.93	5.44 ±0.40
S2	2.73 ±1.87	0.39 ±0.32	16.79 ±2.18	6.05 ±0.99
S3	2.72 ±1.03	0.36 ±0.17	13.71±2.76	5.52 ±1.16
S4	3.12 ±0.67	0.51 ±0.23	17.94± 2.07	7.19 ±1.14

Table 5. The average value of total pods, filled pods, empty pods, and weight of one-hundred beans.

Treatment	Total Pods	Total Filled Pods	Total Empty Pods	Weight of one-hundred beans
S0	19.60 ±2.20	18.40±2.30	1.20± 1.30	11.99± 3.31
S1	18.40 ±2.30	16.60 ±2.90	1.80 ±2.90	15.68 ±1.77
S2	17.80 ±1.50	16.20 ±1.10	1.60 ±1.50	13.28 ±2.08
S3	17.20 ±2.30	16.20 ±1.60	1.00 ±0.70	13.06 ±1.73
S4	17.60 ±3.20	17.60 ±3.20	0.00 ±0.00	12.42 ±0.71

se of osmotic stress and decreased water potential at roots (Sofalian *et al.*, 2013). The essential components such as chlorophyll and, lipid membranes, and protein will destroy by ROS (Weisany *et al.*, 2011). Salinity stress will decrease total soybean pods produced (Simbolon *et al.*, 2013).

The salinity treatment did not affect total filled pods produced by Mahameru cultivar. There is a decrease in total filled pods produced because of salinity stress. The total filled pods produced at 0 mM NaCl was 18.40 and decreased to 17.60 at 200 mM NaCl. The soybean that planted on salinity stress has it is total filled pods produced decreased. This happened because of chlorophyll a and b value decreased and inhibited growth. The soybean that planted on saline condition will have it is total filled produced been reduced (Kisman, 2010). The soybean that planted in drought stress will have it is total filled pods declined (Kobraee *et al.*, 2014).

The total empty pod's production is decreased because of salinity treatment. The average of total empty pods produced decreased from 1.20 pods at 0 mM NaCl to 0.0 pods at 200 mM NaCl (Table 5). The increase in total empty pods of Mahameru cultivar soybean was happened because of accumulation of Na+ and Cl- ions resulted in osmotic stress. Total empty pods are one of the production components that determine the production of soybean under salinity stress (Zulfi *et al.*, 2014). The total empty pods increased because of decreased chlorophyll value resulted in decreased bean production in plants (Ghassemi-Golezani & Minoo., 2011). The decrease of chlorophyll value is happened because of lacked Mg2+ ions as conformation element of chlorophyll.

The average weight of one-hundred beans did not affected by salinity treatment ($p<0.05$). The average weight of one-hundred beans is increasing (Table 5). The past research result showed that weight of one-hundred beans is decreased in salinity stress condition. Salinity stress causing water potential decreased, low cell integrity, and inhibited growth. This is happened because of exceeded ROS produced in soybean crops that exposed to salinity stress. ROS destroy cell membrane, chloroplast membrane and mitochondria membrane resulted in the inhibited growth of plants (Saad-Allah, 2015).

The leaf anatomical Mahameru cultivar figure.

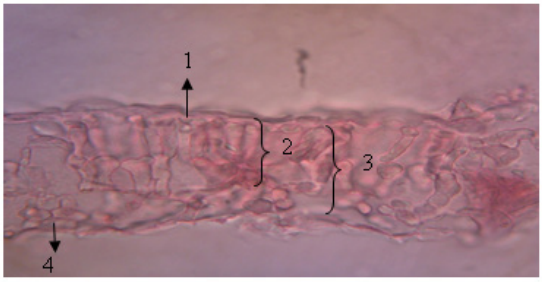

Figure 2. Cross section leaf Mahameru cultivar magnified 400 X; 1. Upper Epidermis; 2. Palisade; 3. Mesophyll; 4. Lower Epidermis.

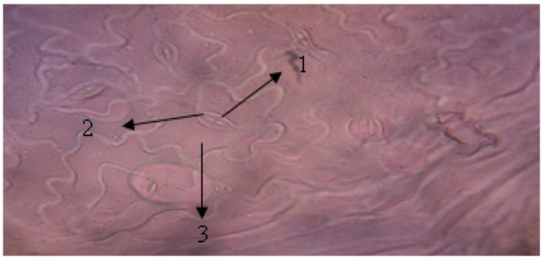

Figure 3. Leaf transverse section Mahameru cultivar magnified 400 X; 1. Stomatal pore; 2. Guard cell; 3. Epidermis cell.

The Mahameru cultivar can cultivate at high salinity up to 200 mM NaCl for enhance soybean productivity in Indonesia.

CONCLUSION

Mahameru cultivar anatomical characteristics were affected by salinity stress. Higher the salinity concentration, epidermis thickness, stomatal and trichromatic density increased. Mahameru cultivar physiology characteristics were

affected by salinity stress. Higher the salinity concentration, chlorophyll a and b value increased. The salinity stress did not affect Mahameru cultivar production value. This indicates that Mahameru cultivar can survive at 200 mM NaCl.

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REFERENCES

Amirjani, M. R. (2010). Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. *American Journal of Plant Physiology*, *5*(6), 350-360.

Arshi, A., Ahmad, A., Aref, I. M., & Iqbal, M. (2010). Calcium interaction with salinity-induced effects on growth and metabolism of soybean (Glycine max L.) cultivars. *Journal of Environmental Biology*, *31*(5), *795-801.*

Atabayeva, S., Nurmahanova, A., Minocha, S., Ahmetova, A., Kenzhebayeva, S., Aidosova, S., Nurzhanova, A., Zhardamalieva, A., Asrandina, S., Alybayeva, R. & Li, T., (2013). The effect of salinity on growth and anatomical attributes of barley seedling (Hordeum vulgare L.). *African Journal of Biotechnology*, *12*(18), 2366-2377.

Baniaghil, N., Arjanesh, m.H., Ghorbanli, M., Syahbazi, M. (2013). The effect of plant growth promoting Rhizobacteria on growth parameter, antioxidant enzymes, and microelement of Canola under salt stress. *Journal of Applied Environmental and Biological Science*, *3* (1), 17-27.

Dolatabadian, A., Sanavy, S. A. M. M., & Ghanati, F. (2011). Effect of salinity on growth, xylem structure and anatomical characteristics of soybean. *Notulae Scientia Biologicae*, *3*(1), 41.

El-Rodeny, WM, & El-Okkiah, AFS (2012). Physiological and anatomical changes in Glycine max L. Under salinity stress. *Egypt. Journal Botany* 2nd *International Conference*, 37-50.

Farid, M., and R. Sjahril. (2006). Defence soybean mechanism on salinity and drought based on morphology character. *Buletin Penelitian Unhas Makasar*. *9* (2), 146-153.

Gabriel, C., Julio, C.R., Leonardo, I.O., Juan, M.A., Mariel, G.P. (2011). Salinity induced anatomical and morphological changes in Chloris guyana Kunth roots.Argentina, *Biocell*, 35 (1), 9-17.

Ghassemi-Golezani, K., & Taifeh-Noori, M. (2011). Soybean performance under salinity stress. In *Soybean-Biochemistry, Chemistry and Physiology*. InTech. 631 – 642.

Hameed, M., Ashraf, M., Naz, N., Nawaz, T., Batool, R., Ahmad, M.S.A., Ahmad, F., and Hussain, M. (2013). Anatomical adaptations of *Cynodon dactylon* (L.) Pers. From the salt range (Pakistan) to salinity stress. II. Leaf anatomy. *Pakistan Journal Botany*, *45* (S1), 133-142.

Juwarno, Sumarsono, Samiyarsih, S. (2009). The effect of nitrogen fertilizer dosages on anatomical character of *Ipomoea batatas* L. Leaf. *A Scientific Journal Biosfera*, *26* (1), 30-34.

Juwarno, J., & Samiyarsih, S. (2017). Anatomical and Molecular Responses of Soy Bean (Glycine Max (L.) Merr.) Due to Salinity Stresses. *Molekul, 12*(1), 45-52.

Kisman, (2010). Morphological character of soybean as adaptation marker on drought stress. Agroteksos. 20 (1), 23-30.

Kobraee, S., Shamsi, K., & Vaghar, M. S. (2014). Dry matter production and allocation in soybean shoots under drought and micronutrient treatments. *Current Biotica*, *8*(1), 13-20.

Kondetti, P., N. Jawali, S.K.Apte and M. G. Shitole. (2012). Salt tolerance in Indian soybean (Glycine max (L.) Merill) varieties at germination and early seedling growth. *Annals of Biological Research, 3*(3), 1489-1498.

Kuswantoro, H., Bayu, M. S. Y. I., Baliadi, Y., & Tengkano, W. (2017). Resistance of Advanced Soybean Lines to Pod Borer (*Etiella zinckenella* Treitschke). *Biosaintifika: Journal of Biology & Biology Education*, *9*(2), 317-324.

Makbul, S., Güler, N. S., Durmu□, N., & Güven, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress. *Turkish Journal of Botany*, *35*(4), 369-377.

Nawaz, K., Hussain, K., Majeed, A., Khan, F., Afghan, S., & Ali, K. (2010). Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. *African Journal of Biotechnology*, *9*(34), 5475-5480.

Porra, R. J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynthesis Research*, *73*(1-3), 149-156.

Qu, Y. N., Zhou, Q., & Yu, B. J. (2009). Effects of Zn 2+ and niflumic acid on photosynthesis in Glycine soja and Glycine max seedlings under NaCl stress. *Environmental and experimental botany*, *65*(2), 304-309.

Rastegar, Z., & Kandi, M. A. S. (2011). The effect of salinity and seed size on seed reserve utilization and seedling growth of soybean (Glycin max). *Int. J. Agron. Plant Prod*, *2*(1), 1-4.

Rozbeh, F., M. Adel, and A. Ali. (2015). Effect of salt stress on seedling growth and ion homeostasis of soy bean (*Glycine max*) cultivars. *Journal of Scientific Research and Development*. *2* (5) : 118-121.

Saad-Allah, K. M. (2015). Impact of sea salt stress on growth and some physiological attributes of some soybean (Glycine Max L.) varieties. *Iranian Journal of Plant Physiology*, *6*(1), 1559-1571.

Sass, J. E. (1958). Botanical microtechnique. *Botanical*

microtechnique., (3rd ed).

Sheteawi, S. A. (2007). Improving growth and yield of salt-stressed soybean by exogenous application of jasmonic acid and ascorbic. *International Journal of Agriculture and Biology (Pakistan)*. *9*(3), 473 – 478.

Simbolon, R. R., Kardhinata, E. H., & Husni, Y. (2013). Evaluasi Toleransi Tanaman Kedelai (Glycine max (L.) Merrill) Generasi M3 Hasil Radiasi Sinar Gamma Terhadap Salinitas. *Agroekoteknologi*, *1*(3), 590-603.

Sofalian, O., Miandoab, P. B., Asghari, A., Sedghi, M., & Eshghi, A. (2013). Relationship between salt tolerance related physiological traits and protein markers in soybean cultivars (Glycine max l.). *Cercetari agronomice in Moldova*, *46*(4), 47-56.

Tunçturk, M., Tunçturk, R., & Yasar, F. (2008). Changes in micronutrients, dry weight and plant growth of soybean (Glycine max L. Merrill) cultivars under salt stress. *African Journal of Biotechnology*, *7*(11), 1650-1654.

Triyani, A., Suwarto, S. Nurchasanah. (2013). Soybean (*Glycine max* L. Merril.) genotive tolerance on salinity concentration at vegetative phase. *Agronomic Journal*, *13* (1), 1-9.

Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., & Ghassemi-Golezani, K. (2011). Physiological responses of soybean ('Glycine max'L.) To zinc application under salinity stress. *Australian Journal of Crop Science*, *5*(11), 1441.

Widiati, R., Musa, Y., Ala, A., & Bdr, M. F. (2014). Stomatal Performance Of Soybean Genotypes Due To Drought Stress And Acidity. *International Journal of Scientific & Technology Research*, *3*(11), 270-275.

Yuniati, R. (2004). Soybean (*Glycine max* (L.) Merrill) genotype screening on NaCl for planting at saline area. *Makara Sains*, *8* (1), 21-24.

Zulfi, A.L.S., Rosmayati., Isman N. (2014). Strain selection of soybean (*Glycine max* (L.) Merrill) of f4 generation on saline soil. *Journal online Agrotechnology*, *2* (4), 1287-1295.