Physiology response of fourth generation saline resistant soybean (Glycine max (L.) Merrill) with application of several types of antioxidants

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Abstract. Antioxidant applications are expected to reduce the adverse effects of soil saline. This research was conducted in plastic house, Plant Tissue Laboratory Faculty of Agriculture and Plant Physiology Laboratory Faculty of Mathematic and Natural Science, Universitas Sumatera Utara, Medan also in Research Centers and Industry Standardization, Medan from July-December 2016. The objective of the research was to know the effect of various antioxidant treatments with different concentrations (control, ascorbic acid 250, 500 and 750 ppm; salicylic acid 250, 500 and 750 ppm; α-tocopherol 250, 500 and 750 ppm) on fourth generation soybean physiology in saline condition (Electric Conductivity 5-6 dS/m). The results of this research showed that the antioxidant type and concentration affected not significantly to physiology of fourth generation soybean. Descriptively the highest average of superoxide dismutase and peroxide dismutase was showed on ascorbic acid 250 ppm. The highest average of ascorbate peroxidase was showed on α-tocopherol 750 ppm. The highest average of carotenoid content was showed on ascorbic acid 500 ppm. The highest average of chlorophyll content was showed on α-tocopherol 250 ppm. The highest average of ratio of K/Na was showed on salicylic acid 250 ppm.

1. Introduction
Soybean is one of the kind of plant nuts used as food source of energy and protein [1]. Efforts to increase soybean production can be done with intensification and extensification. Efforts that can be taken in the intensification is to plant high yielding varieties and apply the optimal package of technology. As for extensification efforts taken is to open a new agricultural area [2]. The saline soil is one of the largely untapped land for cultivation, due to toxic effects and increased root osmotic pressure resulting in disruption of plant growth [3].

one of the causes of plant damage in stressful conditions is occurrence of oxidative stress caused by accumulation of reactive oxygen species (ros) such as oxygen (O₂), hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and hydroxyl radicals (oh⁻), due to inhibition of the process photosynthesis by closing stomata causing damage oxidative properties of photosynthetic [4].

some of the antioxidants that can be applied to plants are ascorbic acid, salicylic acid and α-tocopherol. application of ascorbic acid 500 ppm can help morphology and physiology of soybean to grow and produce better in the soil [5]. salicylic acid applied to soybean in saline soils can increase chlorophyll and superoxide dismutase (sod) [6]. the application of α-tocopherol may increase the activity of wheat leaf enzymes in saline soils [7].
the objective of the research was to know the effect of various antioxidant treatments with different concentrations on fourth generation soybean (glycine max (L.) merrill) physiology in saline condition (ec 5-6 ds/m).

2. Materials and methods
Field experiment was conducted on July-December 2016 in plastic house Faculty of Agriculture, Universitas Sumatera Utara. The experiment was arranged in a non factorial randomized block design with ten treatments (control, ascorbic acid 250, 500 and 750 ppm; salicylic acid 250, 500 and 750 ppm; α-tocopherol 250, 500 and 750 ppm) with four replications for each treatment. The research started from land preparation, planting, application of antioxidant, maintenance, fertilizing and physiology parameters analysis.

The antioxidants were applied at 2 weeks after planting (stadium V), to pod filling period (stadium R), with intervals of 1 week. To determine the volume of spraying, every week spray volume calibration was done on plant leaves by using hand sprayer. The application time was at 07.00 AM. The antioxidants solvent was dissolved by stirrer with aquades according to predetermined concentrations.

The observation on SOD, POD, APX, chlorophyll content, carotenoid content and ratio of K/Na were measured when the plants at stadium V. The physiology parameters analysis include SOD, peroxide dismutase (POD), ascorbate peroxide (APX) and carotenoid content was held in Plant Tissue Laboratory Faculty of Agriculture, Universitas Sumatera Utara. Chlorophyll content observation was held in Plant Physiology Laboratory Faculty of Mathematic and Natural Science, Universitas Sumatera Utara. Ratio of K/Na observation was held in Research Centers and Industry Standardization, Medan.

The data were analyzed statistically using F-test and then following by orthogonal contrast test at 5% level.

3. Results and Discuss
Data presented in Table 1 shows that application of several types of antioxidants affected not significantly increased SOD, POD and APX compared with untreated plants.

Table 1. Effect of application of several types of antioxidants on SOD, POD and APX in soybean in saline soil

| Treatment       | SOD (unit/mg protein) | POD (unit/mg protein) | APX (µM/min/mg protein) |
|-----------------|-----------------------|-----------------------|-------------------------|
| A0 (Control)    | 4860.69               | 0.17                  | 0.50                    |
| A1 (Ascorbic Acid 250 ppm) | 5213.31               | 1.16                  | 0.50                    |
| A2 (Ascorbic Acid 500 ppm) | 4626.46               | 0.33                  | 0.56                    |
| A3 (Ascorbic Acid 750 ppm) | 2312.50               | 0.16                  | 0.35                    |
| A4 (Salicylic Acid 250 ppm) | 1775.86               | 0.07                  | 0.46                    |
| A5 (Salicylic Acid 500 ppm) | 2558.40               | 0.09                  | 0.43                    |
| A6 (Salicylic Acid 750 ppm) | 1323.05               | 0.10                  | 0.24                    |
| A7 (α-tocopherol 250 ppm) | 4888.42               | 0.11                  | 0.80                    |
| A8 (α-tocopherol 500 ppm) | 1695.73               | 0.10                  | 0.74                    |
| A9 (α-tocopherol 750 ppm) | 3201.41               | 0.10                  | 0.84                    |

Data in Table 1 indicated treatment of ascorbic acid 250 ppm increased the activity of SOD and POD. This suggests that ascorbic acid is capable enhancing plant physiology against salinity stress. Ascorbic acid is an antioxidant with preventing and reducing the toxic effects of ROS by donating electrons to ROS, such as single oxygen, radical superoxide, radical hydroxyl; regenerate radical
tocopherol for membrane protection; as well as electron donors for APX activities [8]. Ascorbic acid has many benefits to plant physiology in protein synthesis, as an antioxidant, enzyme cofactor and as a signal cell modulator in a variety of important physiological processes, including cell wall biosynthesis, secondary metabolites and phytohormone, stress physiology, photoprotection, cell division and growth [9].

Antioxidant treatment had no significant effect on POD enzyme activity. The application of salicylic acid in P. sativum in saline soil did not have a significant effect on POD enzyme activity. This is caused by the hydrogen peroxide (H$_2$O$_2$) produced by plants not only dispart by peroxidase enzyme but also catalase enzyme (CAT) and APX [10].

In APX observation, α-tocopherol 750 ppm can increase the activity of APX compared with other antioxidants. Decrease in APX activity occurs on the antioxidant salicylic acid 750 ppm. The decrease in APX activity is relate to the abundance of H$_2$O$_2$ accumulation as substrate enzyme APX, so the plant is unable to reduce the negative effects caused by H$_2$O$_2$. The APX enzyme uses ascorbic acid as electron receptors to reduce H$_2$O$_2$ into water and monodehydroascorbate (MDHA) [11].

**Table 2.** Effect of application of several types of antioxidants on chlorophyll content and carotenoid content in soybean in saline soil

| Treatment                  | Chlorophyll Content (mg/g) | Carotenoid Content (mg/g) |
|----------------------------|-----------------------------|----------------------------|
| A$_0$ (Control)            | 4.91                        | 0.31                       |
| A$_1$ (Ascorbic Acid 250 ppm) | 4.45                        | 0.34                       |
| A$_2$ (Ascorbic Acid 500 ppm) | 4.07                        | 0.41                       |
| A$_3$ (Ascorbic Acid 750 ppm) | 4.09                        | 0.37                       |
| A$_4$ (Salicylic Acid 250 ppm) | 4.90                        | 0.32                       |
| A$_5$ (Salicylic Acid 500 ppm) | 4.21                        | 0.32                       |
| A$_6$ (Salicylic Acid 750 ppm) | 4.38                        | 0.30                       |
| A$_7$ (α-tocopherol 250 ppm) | 4.95                        | 0.29                       |
| A$_8$ (α-tocopherol 500 ppm) | 4.40                        | 0.31                       |
| A$_9$ (α-tocopherol 750 ppm) | 3.95                        | 0.33                       |

On observation of chlorophyll content, the application of α-tocopherol 250 ppm was more effective in increasing total chlorophyll compared with α-tocopherol 750 ppm. Decrease in chlorophyll in the saline soil occurs due to disruption of chlorophyll formation in chloroplasts. Growth of plants in environments with high salt levels causes plants to hyperosmotic effects. As a result, interference with membrane function, metabolic toxicity, disturbance of the photosynthesis process, may even lead to crop death [12]. Salinity in soybeans can cause a decrease in green color on the leaves.

α-tocopherol in resolve the harmful effects of salt stress relate with the stability and protection of photosynthetic pigments from oxidative damage. α-tocopherol 250 ppm is more effective in increasing the amount of chlorophyll in soybeans. Increased chlorophyll indicates more active photosynthetic activity and will increase crop production. Salinity through increased osmotic pressure causes reduction of water uptake and metabolic disorders and physiological processes is under its influence. This causes decreased production by decreasing the amount of leaf chlorophyll [13].

In the observation of carotenoids, ascorbic acid 500 ppm can increase the physiology of soybean to salinity with high carotenoid content. The effect of ascorbic acid on reducing the harmful effects of salt stress is thought to result from activating several enzyme reactions in the metabolic process. Ascorbic acid can trigger the action of enzymes in the metabolism process of plants which will further increase the rate of photosynthesis where if the process of photosynthesis increases then the resulting assimilate is sufficient to be distributed to the seeds so the quality of the beans produced also increases [11].
In observation of ratio of K/Na, salicylic acid 250 ppm can increase ratio of K/Na. Whereas, low ratio of K/Na indicates that antioxidant treatment has not been effective in improving soy physiology in saline soils. The physiology of soybean in saline soils can be seen from decreasing salt levels through the soil's ability to exchange Na ions to K. High salt levels in the soil affect plant growth through four mechanisms i.e. (1) elevated levels of salt cause osmotic stress, (2) inhibition in K⁺ absorption which is the main nutrient of the plant, (3) Na⁺ ions at elevated levels are toxic to cytosolic enzymes and (4) high salt concentration stimulates oxidative stress and cell death [14].

The soil's ability to convert K ions to Na will decrease the Na content to the plants. Physiological responses of soy genotype to salinity stress can be (1) prevention of ion transfer from roots to other parts of the plant, (2) not accumulate many salts in leaves and stems, and (3) have better osmotic adjustability in plant cells. The physiology of soybeans on salinity is also related to the regulation of stable moisture content in the canopy and the accumulation of soluble saccharides, dissolved proteins, amino acids, and K⁺ and Ca⁺ ions for osmotic adjustment [15].

### 4. Conclusions
The results of this research showed that the treatment of antioxidant type and concentration affected not significantly to physiology of fourth generation soybean. The best ascorbic acid concentration was obtained at 250 ppm to improve soybean physiology in saline soil.

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**Table 3.** Effect of application of several types of antioxidants on ratio of K/Na in soybean in saline soil

| Treatment                  | Ratio of K/Na |
|----------------------------|---------------|
| A₀ (Control)               | 0.21          |
| A₁ (Ascorbic Acid 250 ppm) | 0.20          |
| A₂ (Ascorbic Acid 500 ppm) | 0.27          |
| A₃ (Ascorbic Acid 750 ppm) | 0.29          |
| A₄ (Salicylic Acid 250 ppm)| 0.34          |
| A₅ (Salicylic Acid 500 ppm)| 0.23          |
| A₆ (Salicylic Acid 750 ppm)| 0.30          |
| A₇ (α-tocopherol 250 ppm)  | 0.21          |
| A₈ (α-tocopherol 500 ppm)  | 0.22          |
| A₉ (α-tocopherol 750 ppm)  | 0.31          |
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