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The Foliar Application of Exogenous Antioxidant for increasing Drought Tolerance in Soybean

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Abstract : The objective of the research was to identify strategies for increasing soybean production in the dry lands through exogenous antioxidant role, in order to increase soybean production in the dry lands and to identify the role of exogenous antioxidants to changes in reactive oxygen species soybean on drought stress. The research used a randomized block design with 2 factors and 3 replications. The first factor was drought stress treatment namely 80%, 60% and 40% of field capacity. The second factor was foliar application of antioxidant exogenous consisted of without antioxidant ; salycilic acid 500 ppm, ascorbic acid 500 ppm, α-tocopherol 500 ppm and chitosan 500 ppm. The results suggest that increased drought stress (80-40% of field capacity) increased the total soluble protein, superoxida dismutase (SOD), peroxidase (POD) and H_2O_2. Foliar application antioxidant increased the total soluble protein, SOD, POD and H_2O_2 as compared to without the application of antioxidants.
Keywords : antioxidant exogenous, foliar application, soybean.

Introduction

Soybeans have a strategic potential in food security as a source of protein and high-quality functional foods for human needs. One of the benefits of soybean seed is that it contains secondary natural metabolite compounds, including phenolic acid, lignin, and isoflavones. These compounds are thought to have multi-health benefits for humans and plants. For example, isoflavones and phenolics are reported to have health benefits as antioxidants against heart disease and cancer.[1,2,3,4]

Drought stress is a major constraint to the production and yield stability of soybean. Drought stress can affect plant growth process, both from the anatomical, morphological, physiological and biochemical[5]. Based on the biochemical aspects, drought stress can cause cell damage to crops due to the oxidative stress caused an increase in the accumulation of free radicals such as reactive oxygen species (ROS) in plants. ROS accumulation hampering the process of photosynthesis with the closing of stomata, causing oxidative damage to photosynthetic organelles. Free radicals are reactive in the plant tissue that can lead to damage in several ways that break the chains of protein, fat and membrane damage react with DNA causing cell mutation[7,8]. However, ROS tolerant crops will perform an adaptation by producing compounds that are antioxidants. Natural antioxidant in plants generally in the form of phenolic compounds or polyphenols which can be a class of flavonoids, cinnamic acid derivatives, coumarin, tocopherol, and polyfunctional organic acids[9].

An increase in ROS in plants by drought causes oxidative stress which result in damage to the cell by oxidation of lipids and nucleic acids. To cope with oxidative stress, plants have mechanisms to increase resilience through the formation and activity of antioxidant enzymes such as glutathione peroxidase (GPx), Glutathione Reductase (GRX), superoxide dismutase (SOD) and other antioxidant compounds that can save crops from ROS. Damage Oxidative stress occurs when there is an imbalance between the ability of antioxidant enzymes and toksifikasi ROS[10, 11, 12]

One approach to increase oxidative stress tolerance in soybean plants on drought stress is the foliar application of exogenous antioxidants. Application of antioxidants is expected to prevent / reduce the activity of ROS caused by drought, so the plants are more tolerant and as an indicator is the increased activity of SOD which served to protect the function of chloroplasts[13, 14]. Several types of exogenous antioxidants that can be used include ascorbic acid, salicylic acid and chitosan[15, 16.]

The antioxidant activity are widely known on aspects of biomedical, nutritional and environmental protection [17, 18], but there is still little knowledge about the role of exogenous antioxidants in reducing the effects of drought stress on soybeans. Based on the above background, the study aims to identify the role of exogenous antioxidants to changes in reactive oxygen species and antioxidant enzymes in soybean under drought stress.

Material and Methods

Research was conducted in kassa house of Faculty of Agriculture University of Sumatera Utara (Indonesia) from July to November 2016. Analysis of ROS and antioxidant enzymes do in Tissue Culture Laboratory of the Faculty of Agriculture USU Medan. Analysis of protein content is done at the Laboratory of Food Technology, Faculty of Agriculture USU.

Experimental design and crop management

Treatments were arranged in a Factorial Randomized Block Design with two factors and three replications. The first factor is exogenous antioxidant treatment consists of (1). Without exogenous antioxidants (2). Chitosan 1 mg / ml (3). 500 ppm of ascorbic acid and (4). Salicylic Acid 500 ppm and (5). α-tocopherol. The second factor is treatment of drought stress namely 80%, 60% and 40% of field capacity.

Preparation of antioxidant exogenous refered to standard procedures. Chitosan, ascorbic acid, methyl jasmonate and salicylic acid are product of Sigma Aldrich. Chitosan is a linear amino polysaccharide of glucosamine and N-acetylglucosamine units and is obtained by alkaline deacetylation of chitin extracted from the exoskeleton of crustaceans such as shrimps and crabs, as well from the cell walls of some fungi[19,20]. Autoclaved stock solution of 120^{o}C for 20 minutes, and sterile distilled water to obtain a final concentration of chitosan solution 1 mg/mL. Antioxidant exogenous, salicylic acid, ascorbic acid and methyl jasmonate dissolved in distilled water and diluted to concentrations (500 ppm). The determination of the concentration of salicylic acid refers to research [21.]

The soil for the study were taken from dry land Tungtungan Village, Medan. Before planting liming with dolomite 500 kg / ha and incubated for 3 weeks. A total of 10 kg of air-dry soil put in a poly bag size 30 cm x 40 cm. Poly bag had previously been covered with plastic. Soybean varieties used Wilis. Selection of the cultivar referring to previously experiment conducted by Hasanah[22]. Soybean planted in poly bags. Foliar application of exogenous antioxidant carried out in accordance concentrations on 2 week after planting (WAP) to R6 with an interval of once a week.

Determination of soil water content to determine the weight of air-dry soil that will be incorporated into the poly bag carried by the drying method [23], while the determination of water content at field capacity (FC) was conducted using a hydrometer Bouyoucos[23.] Treatment 80% of FC done since the time of planting until harvest. In the treatment of 60% and 40% of FC, the application of water initially as much as 80% of FC performed each up to 4 WAP, after that, the plant in poly bag watered with a certain volume once a day to maintain water levels in the poly bag until it reached each treatment % of FC by the gravimetri method. P and K fertilizer application at a dose of 150 kg ha^{-1} P_2O_5 and 75 kg K_2O ha^{-1} for all crops at planting time. N fertilizer application at dose of 50 kg ha^{-1} Urea was given half the dose of N fertilizer at planting time and the rest at 4 WAP. Weeding is done manually by removing the weeds in accordance with the conditions of the field. Harvesting is done after soybean showed the harvest criteria that the skin pods has brownish, stems and leaves have dried up at the age of 82 days after planting.

Parameters observed was total soluble protein content with units mg Bovine Serum Albumine (BSA)/g sample, reactive oxygen species (H_2O_2) and antioxidant enzymes (superoxide dismutase/SOD) and peroxidase (POD). The determination of total soluble protein content refers to the procedure described by Bradford[24] with units mg Bovine Serum Albumine (BSA)/g sample, SOD refers to procedures by Giannopolitis and Ries[25] and POD refers to Pinto[24] determination of H_2O_2 refers to Sergiev[26]

Statistical data analysis

Data were subjected to analysis of variance (ANOVA) for comparison of means. A combined analysis of variance was done to evaluate isoflavones affected by growing season. Means were separated using Duncan's Mutiple Range Test at the 0.05 probability level.

Result and Discussion

Total Soluble Protein

Based on Table 1 showed that the interaction between drought stress and antioxidant exogenous application have significantly effect on the total soluble protein. Increased drought stress (80% - 40% of field capacity/FC) tends to lower total soluble protein. In general, antioxidant treatment of chitosan increase the protein content than other treatments. Effect of drought stress treatment 80% of FC and antioxidants chitosan significantly increased the protein total soluble protein, while 40% of FC treatment and chitosan produced low total soluble protein.

Table 1. Total soluble protein of soybean with application of antioxidant exogenous and drought stress

Drought stress (K) (% of field capacity)	Antioksidan (A)					Mean
	Without antioksidan (A0)	Salycilic acid (A1)	Ascorbic acid (A2)	α-tocophero l (A3)	Chitosan (A4)	
	…………………… mg BSA/g…………………..					
80 (K1)	58.09abc	50.30bc	74.98abc	97.88abc	152.51a	86.75
60 (K2)	73.86abc	34.50c	96.24abc	73.99abc	113.22abc	78.36
40 (K3)	84.59abc	131.93ab	36.99bc	51.44bc	32.34c	67.46
Mean	72.18	72.24	69.40	74.44	99.36	

Different letters represent significant differences at Duncan's Mutiple Range Test ($p < 0.05$)

Protein is a complex organic compound weights high molecular polymer of an acid monomers amino are connected to each other by peptide bonds. Plants absorb nutrients and then distributed to the entire parts of the plant to the leaves so the plants form proteins and make reforms catabolism). Plants experience drought stress will increase proline content that contributes to dehydration tolerance in a way protects the protein and membrane structure. In this mechanism, there synthesis and accumulation of organic compounds that can lower thereby reducing the osmotic potential of water potential in the cell without limiting enzyme function and maintain cell turgor. Some of the compounds which plays a role in cell osmotical adjustments include osmotic sugar, proline and betaine, protein dehidrin[27]. The reduction in the total soluble proteins detected in the plants under water stress is due to probable increase of the protease enzyme activities, in which these protease enzymes promote the breakdown of the proteins and consequently decrease the protein amount present in the plant under abiotic stress conditions [28, 29, 30.] In inadequate conditions to the plant the pathway of protein breakdown is active, because the plant uses the proteins for the synthesis of nitrogen compounds as amino acids that might be auxiliary for the plant osmotic adjustment[31].

Peroxidase Enzyme (POD)

Base on Table 2 showed that drought stress, antioxidant exogenous application and the interaction between drought stress, antioxidant exogenous application not signicantly effect on the peroxidase enzyme. Increased drought stress (80% - 40% of FC) tends to increase peroxidase enzyme. Salicylic acid antioxidant treatment tends to increase the content of peroxidase enzymes than other treatments, whereas without the antioxidant treatment tent to reduce the content of peroxidase. Effect of drought stress treatment 40% of FC and chitosan tent to increase the peroxidase enzyme, while 80% of FC treatment and chitosan produce the lower of peroxidase enzyme.

Antioxidant enzymes of soybean were increased in response to different concentration of Salycilic Acid (SA). It was found that increased SOD activity was accompanied by increase in catalase (CAT) and peroxidase (POD) because of high demands of H_2O_2 quenching. It was cleared that increment in SOD and POD simultaneously affect each other. First line of defense was provided by SOD against the cellular due to environmental stress along with its major superoxide scavenger[32]. Salicylic acid was also found to enhance the activities of antioxidant enzymes such as peroxidase (POD), superoxidase dismutase (SOD) and catalase (CAT), when sprayed exogenously to the drought stressed plants of tomato[33].

Table 2. Peroxydase enzyme content of soybean with application of antioxidant exogenous and drought stress

Drought stress (K) (% of field capacity)	Antioksidan (A)					Mean
	Without antioksidan (A0)	Salycilic acid (A1)	Ascorbic acid (A2)	α-tocophero l (A3)	Chitosan (A4)	
	…………………… unit/protein……………..					
80 (K1)	0.64	1.28	0.74	0.47	0.32	0.69
60 (K2)	0.70	1.58	0.84	0.59	0.41	0.82
40 (K3)	0.57	0.51	0.82	1.97	1.97	1.17
Mean	0.63	1.12	0.80	1.01	0.90	

Superoxide dismutase (SOD)

Base on the Table 3 showed that drought stress, antioxidant exogenous and the interaction between them has no significantly effect on the enzyme of SOD. Increased drought stress (80% - 40% of FC) tends to increase the SOD enzyme. The α- tocopherol antioxidant treatment tends to increase the content of SOD than other treatments, whereas the chitosan antioxidant treatment tends to reduce the content of SOD. Effect of drought stress treatment 40% of FC and antioxidants of α- tocopherol increased the SOD, while 60% of FC treatment and chitosan produce low SOD enzyme.

Tabel 8. SOD enzyme of soybean with application of antioxidant exogenous and drought stress

Drought stress (K) (% of field capacity)	Antioxidant exogenous (A)					Mean
	Without antioksidan (A0)	Salycilic acid (A1)	Ascorbic acid (A2)	α-tocopherol (A3)	Chitosan (A4)	
	…………………… unit/protein …………….					
80 (K1)	0.38	0.97	1.08	0.85	0.43	0.74
60 (K2)	1.36	0.90	0.67	1.43	0.22	0.91
40 (K3)	0.62	0.28	2.13	2.62	1.28	1.39
Mean	0.79	0.72	1.29	1.63	0.64	

Among enzymatic mechanisms, SOD plays an important role, and catalyzes the dismutation of two molecules of superoxide into O_2 and H_2O_2; the first step in reactive oxygen species scavenging systems. A study utilizing two rapidly drought-stressed clones of *Coffea canephora*, proposed that drought tolerance might, or at least in part, be associated with enhanced activity of antioxidant enzymes[34.] Alpha-tocopherol is the major vitamin E, this antioxidant deactivates photosynthesis-derived reactive oxygen species, and prevents the propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. Alpha-tocopherol levels change differentially in response to environmental constraints, depending on the magnitude of the stress and species-sensitivity to stress. Alpha-tocopherol (vitamin E), which is hydrophobic and associated with membranes, is one of the most important components of cellular defense against oxidative injury. It reacts with superoxide, lipid peroxy radical, and singlet oxygen and terminates the chain reactions of lipid peroxidation. A significant increase of -tocopherol concentration is observed in several plant species when they are subjected to drought. Highly significant correlations were observed between stress tolerance and tocopherol concentration (Spearmans rank correlation coefficient r =0.731)[35, 36, 37, 38]

H_2O_2 content

Base on Table 4 showed that the drought stress and antioxidant exogenous application have significantly effect on H_2O_2. Increased drought stress (80% - 40% of FC) increased H_2O_2. Treatment antioxidant ascorbic acid increased the content of H_2O_2 than other treatments, whereas without the antioxidant treatment reduced the content of H_2O_2. Effect of drought stress treatment to 40% and the antioxidant ascorbic acid tent to increase the content of H_2O_2, while 80% KL treatment and without antioxidant exogenous tent to decrease the produce of H_2O_2 content.

Table 9. H_2O_2 content of soybean with application of antioxidant exogenous and drought stress

Drought stress (K) (% of field capacity)	Antioxidant exogenous (A)					Mean
	Without antioxidant (A0)	Salycilic acid (A1)	Ascorbic acid (A2)	α-tocophero l (A3)	Chitosan (A4)	
	…………………… unit ……………..					
80 (K1)	0.059	0.127	0.174	0.059	0.112	0.106b
60 (K2)	0.150	0.153	0.146	0.166	0.116	0.146ab
40 (K3)	0.111	0.130	0.232	0.156	0.158	0.157a
Mean	0.107e	0.137b	0.184a	0.127d	0.129c	

Different letters at the same row or coloumn represent significant differences at Duncan's Mutiple Range Test ($p < 0.05$)

Ascorbic acid acts as a cofactor for several enzymes and regulates the phytohormone-mediating signaling processes[39], and many physiological processes in plants[40], also modulates the tocopherol synthesis, which protects the plant from several environmental stresses[41]. Salicylic acid (SA) is an important signaling molecule in plants, which helps to regulate plant resistance against drought stress. Hydrogen peroxide (H_2O_2), acts as signaling molecule and assistant in triggering stress resistance mechanism. Exogenous application of salycilic acid helps in improving the resistance against drought stress in soybean[42, 43].

Conclusion

Increased drought stress (80-40% of FC) increased the total soluble protein, SOD, POD and H_2O_2. Foliar application antioxidant increased the total soluble protein, SOD, POD and H2O2 as compared to without the application of antioxidants.

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