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The role of silicon on content of proline, protein and abscisic acid on soybean under drought stress

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Abstract. Silicon is the second most abundant in the soil and Silicon could be considered as an essential element in many crops to enhancing growth and alleviating biotic and abiotic stresses. Silicon present exclusively and absorbed by plants in the form of silicic acid. Drought stress that occurs in plants inhibit of several metabolic processes in plant tissue, so that the availability of sufficient water in the soil becomes very important in plant cultivation. Plants have different adaptability to drought conditions, one of the efforts to increase plant resistance in conditions of limited water availability can be done with physiological engineering through controlling levels of proline, protein and abscisic acid, by giving silicon as an element that is known to increase power plants against drought. The results showed that silicon had closely related to decreased content of proline and abscisic acid, an increased in silicon concentration tended to decrease proline ($R^2 = 0.94$) and abscisic acid ($R^2 = 0.95$), and applying a higher silicon increased crude protein ($R^2 = 0.95$).

1. Introduction

Drought is an important abiotic limiting factor related to groundwater availability and this condition affects almost all metabolic processes of plants including pigment levels, photosynthetic activity, osmotic balance and membrane integrity [1,2] Lack of water (commonly referred to as drought) is an abiotic stress, which negatively affects the appearance of the plant if it is under prolonged water deficit conditions [3,4]. The decrease in groundwater content generally ranges from field capacity to the point of permanent wilting, the decrease in groundwater content is due to the thinning of the water layer which causes water tension to increase so that water absorption by plants decreases. Decrease in groundwater content that lasts until the point of wilting causes the rate of growth and photosynthesis to decrease.

Plant adaptation mechanisms to overcome drought stress are transpiration control responses [5,6] and cell osmotic regulation [7]. In this mechanism, there is synthesis and accumulation of organic compounds which can reduce the osmotic potential, thereby reducing the water potential in cells without limiting enzyme function and maintaining cell turgor. Several compounds that play a role in the osmotic adjustment of cells include osmotic sugars, proline and betaine, dehydrin proteins [8–10].



Silicon is a second abundant amounts in the layers of the earth's crust, but generally the Si element is present in the form of bonds with other compounds and is insoluble. Silicon (Si) is considered as a nutrient that is not essential but is present in abundance in the soil. Silicon is a chemical metalloid element and has the effect of being able to mitigate the side effects of drought [11–13], increasing stomatal conductance [14]. The addition of Si in soybean cultivation increases endogenous GA levels, reduces levels of abscisic acid (ABA) and proline [15], ABA and proline are compounds that play a role in the movement of the stomata holes. In drought stress conditions will increase the content of ABA [4] and proline [16]. Based on the results of several studies, it is known that silicon can increase plant resistance to drought stress. [17,18]

Silicon is thought to be involved in fortification which affects the oxidation of cell membranes and causes protection of various functions of plant organs that experience drought. Silicon also seems to be part of osmolyte regulation in cells under drought stress conditions [19] Silicon has the ability in controlling the damage from biotic stress caused by the attack of fungi and bacteria, Silicon allegedly had an important role in reducing decreasing in growth due to abiotic stress and resulted in the protection of various plant organs function that is experiencing drought conditions [20] The aim of this study was to evaluate the role of silicon in changes in levels of proline, protein and abscisic acid in soybean under drought stress conditions.

2. Methods

This research was carried out in Greenhouse, the planting material used was soybean cultivar Grobogan, soybean plants were grown in pot media and drought conditions were simulated by eczema using Poly Ethylene Glycol (PEG 6000) at a concentration of 15% which was equivalent to - 0.67 MPa [21] Applications of silicon at various concentrations of 0 ml l⁻¹ (control), 5, 10 and 15 ml l⁻¹ are given after the plants are 14 days after planting and then every seven days.

Proline was determined according to the method described [22] which was modified. Approximately 0.5g of fresh or frozen plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 2 filter paper. Two ml of filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hr at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Bausch and Lomb Spectrometer 7 IO'. Appropriate proline standards were included for calculation of proline in the sample.

Content of protein were measured using the Kjeldahl method [23] This method is a simple method for the determination of total nitrogen in amino acids, proteins, and nitrogen-containing compounds. The sample is digested with sulfuric acid and catalyzed with a suitable catalyst to produce ammonium sulfate. After strong liberation of the alkali, the ammonia formed is steam distilled quantitatively into an absorbent solution and determined by titration.

Analysis of *Abscisic Acid*, made through extraction of sample plants and continued with the analysis of the content of the ABA using Gas Chromatography. The observation is done at the age of 50 days. A method of analysis that will is a method of Monteiro. Leaf samples of the plants used are leaves to 2 and 3 of the top and from plants aged 50 days after planting. Furthermore, the data from laboratory analysis were statistically tested using correlation regression and Anova.

3. Results

Correlation analysis between the silicon concentration and levels of proline and abscisic acid showed that there was a strong negative relationship ($r = - 0.972$ and $r = 0.975$, respectively), but the silicon concentration showed a positive correlation to crude protein levels ($r = 0.974$). This means that the increasing concentration of silicon has a role in decreasing the levels of proline and abscisic acid (ABA), but the increasing concentration of silicon that is applied tends to increase protein levels. Furthermore, through the regression test, it showed that the silicon concentration determined

significantly the levels of proline, protein and abscisic acid ($R^2 = 0.945, 0.950$ and 0.952 , respectively: Figure 1).

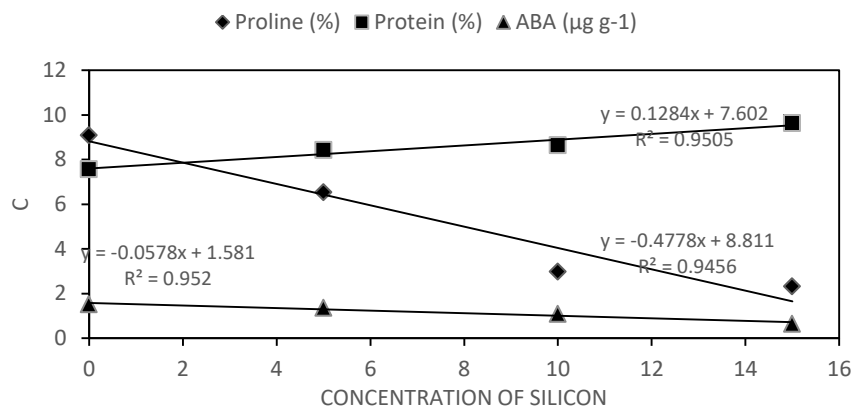


Figure 1. Correlation between concentration of silicon and content of proline, protein and abscisic acid

The increase of silicon concentration showed that silicon plays a role in plant metabolic processes including regulating plant adaptation to biotic and abiotic stresses [24]. Proline is an amino acid compound that is synthesized from the phosphorylation of glutamate. The glutamine trajectory is the primary route for proline biosynthesis in drought-stressed conditions [25,26]. Proline accumulation is the result of an increase in free amino acids when plants are exposed to stressful environments, such as drought, high salinity, and too low or too high temperatures. Active plants produce various kinds of metabolites and defense systems to survive, for example osmoprotectants such as proline, glycine betaine, mannitol, and sugars as compounds for tolerance to drought stress and salinity [27] Figure 2 showed that each increase silicon concentration that was applied shows a decrease content of proline of soybean.

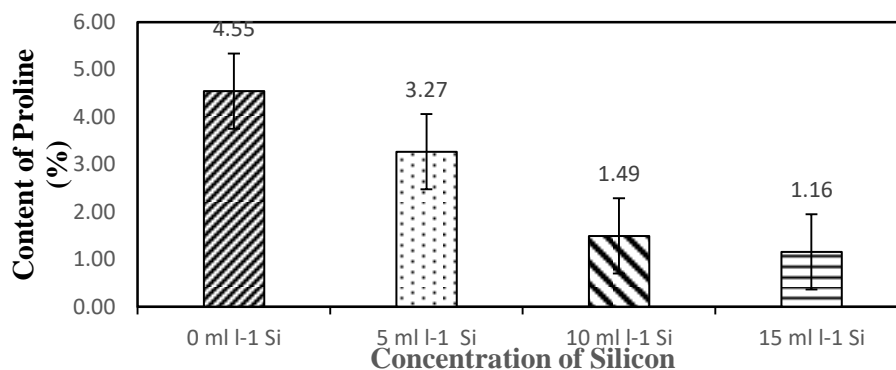


Figure 2. Content of Proline resulting from the treatment at various concentration

Plant tolerance to drought stress resistance physiologically related to changes in metabolic activity, which is indicated by changes in the accumulation of proline in leaf tissue. Plants that experience drought levels of proline tend to increase. Increased leaf proline content in soybeans with higher drought stress is closely related to the large role of proline as an osmoregulator. Excessive production of these compounds results increased tolerance to drought stress in plants. [28,29].

Evaluation of the soy protein content by the application of silicon with varying concentrations showed a different response to proline. Figure 3 explains that each increase in silicon concentration tends to increase the protein content of soybean leaves.

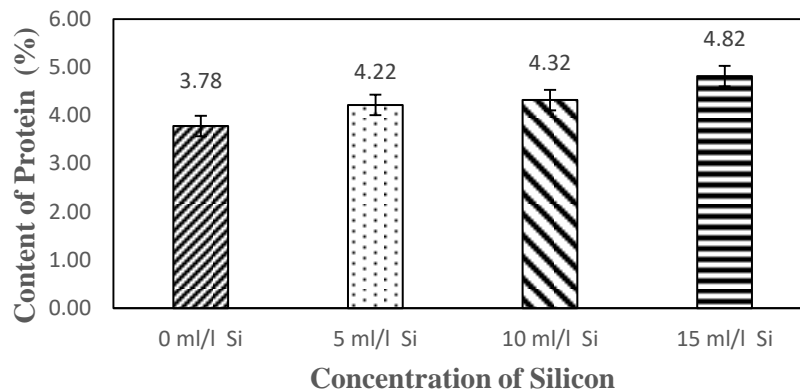


Figure 3. Content of Protein resulting from the treatment of silicon at the various concentration

Plants in drought stress conditions will increase the content of proline which plays a role in dehydration tolerance by protecting protein and membrane structure [10]. In this mechanism, there will be synthesis and accumulation of organic compounds which can reduce the osmotic potential, thereby reducing the water potential in cells without limiting enzyme function and maintaining cell turgor. Some compounds that play a role in the osmotic adjustment of cells include osmotic sugars, proline and betaine, dehydrin proteins. Drought can induce changes in changes in oxidative reactions in photosynthetic pigments including protein, lipid synthesis and affect the activity of several photosynthetic enzymes [30].

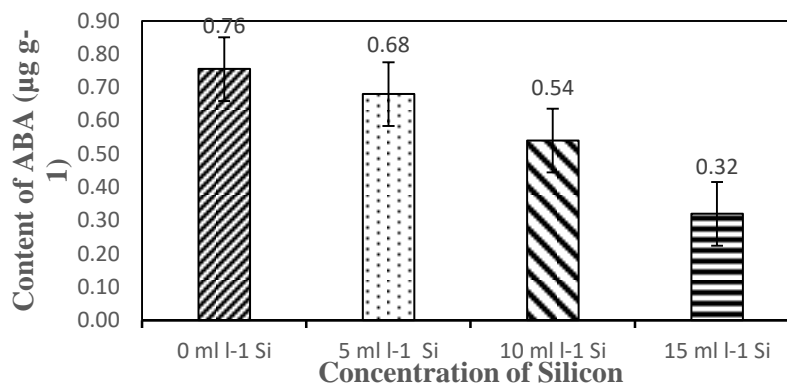


Figure 4. Content of Abscisic Acid (ABA) resulting from the treatment of silicon at the various concentration

Figure 4 above explains that every increase in the concentration of silicon caused the content of ABA to decrease. Physiologically, plants experienced drought have an increase in the ABA (Abscisic Acid) biosynthetic process. ABA is a hormone that is synthesized in roots, translocated to leaves, and may also be synthesized by the guard cells themselves [31]. ABA found in leaves, especially in guard cells, controls the stomata closure process [32,33]. High levels of intensity of drought and longer period of that plant suffered, ABA accumulation and result in the flow of potassium gets into the body of the plant is disturbed because the ABA inhibits the proton pump, whose potassium (cations flow K⁺) into the guard cells, this condition affects regulatory system opening of the stomata [34,35]. The occurrence of the process increased the biosynthesis of Abscisic Acid (ABA) on the plant, which suffered a drought caused by the process of oxidation in protoplast occurs continuously in conditions of water shortage [36].

4. Conclusion

The application of silicon at several concentrations showed that the increase in silicon concentration had a strong effect on content of proline, protein and abscisic acid (ABA). Each addition of silicon concentration causes a decrease in proline and ABA levels but tends to increase crude protein content. In general, the treatment of silicon concentration of 15 ml l⁻¹ had a large effect on almost all observed variables.

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