Silicon-mediated accumulation of oil and antioxidant in Soybean

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Abstract. The purpose of this study was to examine the effects of silica application on the protein, oil, fatty acid, phenol and isoflavone content in soybean seeds that experience water deficiency stress. This study used a factorial completely randomized design. The first factor was the watering interval (3, 6 and 9 day intervals). The second factor was the dosage of silica application (0; 31.25 and 62.50 SiO₂ / polybag or equivalent doses of 0, 125 and 250 kg SiO₂ / ha). This study concluded that the higher and longer duration of drought stress was reflected in the longer watering interval caused a decrease the number of filled pods, number and yield of seeds, and oil content, but increased content of protein, total of phenolic and isoflavon of soybean seeds. Application of silica under drought stress conditions reduced the reduction in the number of filled pods, seed quantity and yield, and oil content compared to without silica application. The content of oil, total of phenolic and isoflavones of soybean seeds can be inducted through the application of silica. The application of silica improves the quality of soybeans as a functional food.

1. Introduction
Soybean seeds contain protein and amino acids, oils and fatty acids, sugars, minerals [1][2][3] and natural secondary metabolites such as phenolics and isoflavones [4]. Phenolic and isoflavones have natural antioxidant properties which exhibit various physiological properties such as anti-allergic, antiatherogenic, anti-inflammatory, antimicrobial, antithrombotic [4] and can protect against degenerative diseases in humans, such as coronary heart disease, osteoporosis, stroke, and cancer [4]. Because of its phenolic and isoflavone content, soy is an important functional food.

Soybeans are planted after rice, so they often experience drought stress. Drought stress is one of the causes of decreased soybean seed yields [5][6], due to a decrease in photosynthesis, fewer grains and smaller sizes. Drought stress causes changes in protein content, content and composition of fatty acids and antioxidants of soybean seeds. Drought stress is one of the causes of decreasing soybean protein content [7][8][9]. However, drought stress in the reproductive phase (flowering phase) increases the protein content of soybean seeds [7][8].

Drought stress also causes a decrease in fatty acid content [9], and changes in the content and composition of fatty acids [1]. Drought stress during a critical period, that is the formation and filling seeds period, reduce the quantity and quality of soybean oil [10]. According to [11], drought stress...
decreases palmitic, linolic and linoleic acids but increases stearic and oleic acids. Drought stress produces ROS as a result of oxidative stress which can be destructive macromolecules such as DNA, proteins and lipids which continues to result in cell death. Plants will form an antioxidant defense system in response to drought stress [12], by increasing the production of enzymatic and non-enzymatic antioxidants [13][14] as well as secondary metabolites compounds and protein, such as sugar and proline [14] phenolic compounds [14][15][16] and flavonols, organic acids, lipophilic, carotenoids, and ascorbate [17].

Plants increase the production of phenolic and flavonoids antioxidants in response to abiotic stress, including drought stress. However, drought stress will reduce the yield, protein and fatty acids content of soybean seeds. Phenolic and flavanoid production can be induced through the application of elicitors, among them salicylates (SA) and isonicotinic acid (INA) [18] and silica [19]. Several research results show that the application of silica can reduce the adverse effects of drought stress and increase soybean seed yields [20][21], protein and fatty acid content and antioxidant content in soybean seeds. namely the phenolic content [22].

This study examines the application of silica in increasing seed yield and the content of protein, oil, fatty acids, phenols and isoflavones in soybean seeds as an effort to achieve quality functional food ingredients.

2. Materials and methods

This study used a factorial completely randomized design. The first factor was the watering interval, namely 3, 6 and 9 day intervals. The second factor was the dosage of silica application, namely 0; 31.25 and 62.50 SiO2 / polybag or equivalent doses of 0, 125 and 250 kg SiO2 / ha. Every treatment combination was repeated five replications, so there were 45 experimental units. Every experimental unit consisted of two edamame plants. The parameters observed were the number of filled pods, number and yield of seeds, content of oil, protein, total of phenolic and isoflavan of soybean seeds. The data obtained were then analyzed by analysis of variance to determine the effect of the treatment and continued with Duncan's Multiple Range Test at the 5% level.

The total phenol content was determined by colorimetric method, using Folin-Ciocalteu reagent and calculated based on the standard curve of gallic acid, referring to the method developed by [23]. The total flavonoid content was determined by colorimetric aluminum chloride method and calculated based on the standard of quercetin curve, referring to the method developed by [23] and [24]. Protein content in seeds was analyzed by estimating the N content in the seeds by the micro-Kjeldahl method which has been modified by [4]. The oil content of soybean seeds was estimated by Soxhlet extraction method which has been modified by [4].

3. Results and Discussion

3.1 Soybean Seeds

The results of variance analysis and continued Duncan's test showed that the application of silica at watering intervals of 3, 6 or 9 days were increased the number of filled pods, number of seed and soybean seed yield (Table 1). Application of silica at a dose of 31.25 g SiO2/polybag or the equivalent dose of 125 kg SiO2 / ha increases the number of filled pods, the number of seeds and soybean seed yield per plant, except the number of filled pods at watering intervals for 3 days was increased with a dose of 62.50 g SiO2/polybag or the equivalent dose of 250 kg SiO2/ha.

Drought stress due to longer watering intervals causes a decreased number of filled pods, number of seeds and yield of soybean seeds per plant. This matter because drought stress decreases the water status of plants, chlorophyll content, closing the stomata so that occur inhibited of photosynthetic, inhibited of flowering and inhibited of seed development which continues to decrease crop yields. Result of research previously reported that drought stress reduced the water status of plants and relative leaf water content [25][28], chlorophyll content [26], closing of stomata, photosynthetic inhibited [26], inhibited of flowering, filling and development of seed [27] thereby reducing the size and number of seeds [25][28] and reduced of crop yields [28].
Table 1. Number of filled pods, number of seeds and yields of soybean seeds that experience drought stress and silica application.

| Watering Interval (days) | Dosage of silica (kg SiO$_2$/ha) | Number of filled pods (pods/plant) | Number of seeds (seeds/plant) | Seed Yield (g/plant) |
|-------------------------|----------------------------------|-----------------------------------|-------------------------------|---------------------|
| 0 (control)             | 28.67                            | bc                                | 51.33                        | c 10.26 c           |
| 3                       | 125                              | ab                                | 59.76                        | b 12.48 b           |
|                         | 250                              | a                                 | 65.94                        | a 13.92 a           |
|                         | 0                                | e                                 | 34.67                        | e 7.56 d            |
| 6                       | 125                              | d                                 | 43.83                        | d 9.74 c            |
|                         | 250                              | cd                                | 45.10                        | cd 9.95 c           |
|                         | 0                                | g                                 | 22.63                        | g 4.27 f            |
| 9                       | 125                              | f                                 | 28.35                        | f 5.89 e            |
|                         | 250                              | ef                                | 30.46                        | ef 6.45 de          |

The numbers within a column followed by the same letter are not significantly different in Duncan's test at the 5% level.

Application of silica was able to increase the number filled of pods, the number of seeds and the yield of soybean seeds per plant. That were because the application of silica can increased water uptake and dissolved nutrients, thereby increasing the water status of plants. Increasing the water status of plants increases the relative leaf water content and leaf water potential, and increases the cell turgidity which affects the stomata openings. The wider open stomata will facilitate the exchange of CO2 and increase the content of CO2 in leaf cells. The increase in leaf water content will guarantee chlorophyll synthesis, therefore the leaf chlorophyll content increases due to the application of silica. Increase in leaf water content, CO2 content in leaf cells and leaf chlorophyll content will increase the rate of photosynthetic and assimilate yield, which in turn increases the number of filled pods, the number of seeds and the yield of soybean seeds per plant. Several of research results previously reported that, whether plants under drought stress conditions or not, the application of silica increased the relative leaf water content and leaf water potential [28][29]. Increased water content due to silica fertilization correlates with increased conductance and stomatal openings [29] as well as exchange of CO2 and CO2 content in leaf cell spaces [28], increasing leaf chlorophyll content [29], giving strength to leaf uprightness [27] and increasing the rate of photosynthesis [29].

Some of research results previously reported that, drought stress causes inhibited of flower formation, pollen and stigma become brittle, causing pollination failure [30][31], and if there was pollinated will be followed by miscarriage a few days later [32] there was an inhibited in the formation of seeds [32]. Inhibited in the formation of flower, pollination and seed formation cause a decrease in the number of filled pods, number and size of seeds and seed yield. The results of this study are in line with some result of research previously reported that, drought stress reduced the number of filled pods, number and size of seeds and yield of seeds.

Silica application was able to minimize the bad effects of drought stress, to minimizes the reduction in the number of filled pods, number and size of seeds and yield seeds [7][8][9]. The same condition on intensity and duration of drought stress, the application of silica was able to increase the number of filled pods, number and size of seeds and seed yield [8][10].

3.2 Protein and Oil Content of Soybean Seeds.

The results of variance analysis and continued Duncan's test showed that the application of silica, at both 3, 6 and 9 days watering intervals, increased the protein content and oil content of soybean seeds (Table 2). Application of silica at a dose of 31.25 g SiO$_2$/polybag or the equivalent dose of 125 kg SiO$_2$/ha increased the protein content and oil content of soybean seeds.
Increased drought stress with watering intervals of 6 days increased the total protein content of soybean seeds, but more severe drought stress caused a decrease in the total protein content of soybean seeds. The increase in seed protein content with an increase in the 6-day watering interval was related to the number of seeds produced, seed size and seed moisture content. According to [7] it shows that drought stress causes a decrease in the number of seeds produced, seed size and seed moisture content so that the protein content of seeds increases. Kumar et al. [33] and Rotundo and Westgate [35] also reported that there was an increase in the protein content of soybean under drought stress compared to controls.

Table 2. Total protein content and total oil content of soybean seeds under drought stress and silica application.

| Watering Interval (days) | Dosage of silica (kg SiO2/ha) | Total protein content (%) | (mg/grain) | Total oil content (%) | (mg/grain) |
|--------------------------|-------------------------------|---------------------------|------------|----------------------|------------|
| 0 (control)              |                               | 35.16 c                   | 70.28 c    | 2.11                 | 4.21 c     |
| 3 125                    |                               | 36.51 b                   | 76.23 b    | 2.36                 | 4.92 ab    |
| 250                      |                               | 37.65 b                   | 76.48 b    | 2.67                 | 5.64 a     |
| 6 0                      |                               | 36.25 b                   | 79.61 b    | 1.53                 | 3.36 de    |
| 125                      |                               | 38.98 a                   | 86.61 a    | 1.89                 | 4.15 c     |
| 250                      |                               | 39.21 a                   | 86.48 a    | 1.96                 | 4.36 bc    |
| 0 125                    |                               | 34.11 d                   | 64.37 d    | 1.09                 | 2.06 f     |
| 250                      |                               | 35.95 c                   | 74.70 b    | 1.38                 | 2.87 e     |
| 9                        |                               | 36.39 b                   | 79.15 b    | 1.47                 | 3.20 e     |

The numbers within a column followed by the same letter are not significantly different in Duncan's test at the 5% level.

The decrease in soybean protein content due to increased severe drought stress due to longer watering intervals was associated with nitrogen absorption inhibition. Drought stress causes a decrease in the stability and integrity of the root cell membrane which leads to a decrease in root permeability. Decreasing stability, integrity and permeability of roots causes a decrease in the ability to absorb nutrients or nutrients dissolved in the soil. According to Kristanto [28] it was reported that drought stress causes root damage, decreases the integrity and stability of root cell membranes, leaks electrolytes in the root cell membranes and reduces the ability to absorb nitrogen, phosphorus and potassium nutrients. Reduced nitrogen uptake reduces the synthesis and protein content of soybean seeds [7][8]. The decrease in protein content due to drought stress is related to proteolytic activation so that the degraded protein which results in increased total dissolved nitrogen and an increase in proline content [30]. Lower seed protein content due to drought stress was also reported by Carrera et al. [19]. Differences in soybean protein content due to drought stress depend on the cultivar [1], the growth phase and the duration and intensity of drought stress [19].

Application of silica increases the protein content of soybean seeds associated with increased root nodulation. Increased root nodulation correlates with nitrogen uptake and protein synthesis. Several previous researchers reported that the application of silica increased root nodulation [30] increased the amount and dry matter of root nodules, as well as nitrorgen fixation and nitrogen uptake [30][36]. The increase in nitrogen uptake increases the decrease in the synthesis and protein content of soybean seeds [8]. According to Shwethakumari and Prakash’s research [22], it was reported that the application of silica might have involved in the biosynthesis of cell wall components there by enhanced the protein content of soybean. Increased drought stress decreased the total oil content of soybean seeds. The application of silica at each watering interval increased the oil content of soybean seeds or when compared to control (3-day watering interval without silica application), silica was able to reduce the decrease in soybean oil content. Drought stress caused a decrease in soybean oil content, which was positively correlated with a decrease in seed yield. Drought stress inhibits the process of
fatty acid biosynthesis which leads to reduced oil yield and changes in seed oil composition [37][38][39]. Drought stress inhibits the process of fatty acid synthesis, integration and stability of fatty acids which causes a decrease in fatty acid content and changes in fatty acid composition. Changes in the composition of soybean oil that experienced drought stress were a decrease in palmitic, linoleic and linolenic acids but an increase in stearic and oleic acids [40][41]. Drought stress causes the accumulation of reactive oxygen species (ROS) in excess, causing oxidative damage to cell structures, cell metabolism, and macromolecules, such as membrane lipids, nucleic acids, and fatty acids [21]. Inhibition of fatty acid synthesis and damage to fatty acids that have been formed causes a decrease in oil content. A decrease in oil content in seeds due to drought stress was also reported by Bellaloui and Mengistu [1] and Rotundo and Westgate [35].

Drought stress decreased soybean oil content in each watering treatment at intervals of 3, 6 and 9 days. The increase in soybean oil content was related to the role of silica in modulating fatty acid synthesis. According to the results of research by Shwethakumari and Prakash [22] it was reported that the application of silica increased the metabolism of nucleic acids which in turn resulted in a higher oil content in the seeds. Some researchers previously reported that application of silica increased the oil content of soybean seeds [1][35][22]. Maleki et al. [10], stated that oil content has a significant positive correlation with seed weight.

3.3 Total phenol and isoflavone content of soybean seeds

The results of the analysis of variety and continued Duncan's test showed that the application of silica, both at the watering interval of 3, 6 and 9 days, increased the total phenol content and total isoflavone (Table 3). Application of silica at a dose of 31.25 g SiO2/polybag or the equivalent dose of 125 kg SiO2/ha increased the total phenol content and total isoflavones at the 6 and 9 days watering intervals, while at the 3 day watering interval, the total phenol content and total isoflavone increased by doses 62.50 g SiO2/polybag or the equivalent dose of 250 kg SiO2/ha.

Table 3. The total phenol and isoflavone content of soybean seeds under drought stress and silica application.

| Watering Interval (days) | Dosage of silica (kg SiO2/ha) | Total phenol content (%) | Total phenol content (µg/grain) | Total isoflavone content (µg/g) | Total isoflavone content (µg/grain) |
|--------------------------|-------------------------------|--------------------------|-------------------------------|-------------------------------|-----------------------------------|
| 0,0                      |                               | h 138.77                 | h 767.73                      | f 153.45                      | f                                 |
| 3                        | 125                           | gh 164.85                | gh 816.92                     | de 170.77                     | e                                 |
|                          | 250                           | fg 207.94                | fg 864.85                     | b 189.90                      | cd                                |
|                          | 0,0                           | c 241.83                 | c 869.24                      | b 197.06                      | bc                                |
| 6                        | 125                           | b 455.73                 | b 906.12                      | a 199.91                      | ab                                |
|                          | 250                           | a 587.76                 | a 935.84                      | a 206.45                      | a                                 |
|                          | 0,0                           | ef 241.96                | ef 798.06                     | ef 150.58                     | f                                 |
| 9                        | 125                           | d 328.92                 | d 825.43                      | cd 171.46                     | e                                 |
|                          | 250                           | bc 401.84                | bc 854.27                     | bc 180.89                     | d                                 |

The numbers within a column followed by the same letter are not significantly different in Duncan's test at the 5% level

The increase in drought stress due to watering at 6 day intervals increased the total phenol content and total flavonoids. The increase in stress was more severe with the 9-day watering interval, reducing the total phenol content and total flavonoids, although the values were still higher than at the 3-day watering interval. The total phenol and flavonoid content of soybean seeds is influenced by environmental stress, including drought stress, especially in the phase of seed formation and development [6]. Drought stress encourages plants to synthesize secondary metabolites in the form of phenolic compounds that show antioxidant properties [42] associated with plant antioxidant defense
systems that play a role in scavenging ROS [42] and preventing lipid peroxidation, protein denaturation and DNA damage [13]. The results of this study are in line with the results of the study by Weidner et al. [43], Ma et al. [31] and Król et al. [13], that the accumulation of phenolic compounds such as phenolic acids and flavonoids is triggered by drought stress. However, drought stress was more severe, the total phenol content and total flavonoids of soybean seeds decreased. Decrease in phenol and flavonoid content due to exposure to drought stress with intensity and long duration causes an increase in plant temperature and a decrease in plant water status which results in leaf chlorophyll damage, stomata closure and no exchange of CO2 into leaf cell spaces. Chlorophyll damage and low CO2 content in leaf cells cause inhibition of photosynthesis. The low yield of photosynthesis causes the low synthesis of primary and secondary metabolite compounds, including phenols and flavonoids. The decrease in total phenol content and total flavonoids of soybean seeds due to exposure to severe drought stress was reported by [6].

Drought stress causes a decrease in the total phenol content and total flavonoids of soybean seeds. The application of silica increases plant fitness and resistance and increases these content, both in drought stress and watering. The application of silica increases the absorption and deposition of silica in plant tissue which provides strength to plant cells and tissues, increases the proline and lignin content associated with plant strength, resistance and fitness [28]. Silica modulates the metabolism of phenolic compounds [44][45] and isoflavonoids [46].

4. Conclusion
The higher of intensity and duration of drought stress, which was reflected in the longer watering interval, led to a decreased in the number of filled pods, the number and yield of seeds, and oil content, but increases protein, total phenol and total flavonoid content in soybean seeds. Silica application at soil on soybean plants, was experiencing drought stress, namely watering intervals of 6 and 9 days or not drought stress, namely the watering interval of 3 days, increasing the number of filled pods, number and yield of seeds, oil content, protein content, total phenol and total flavonoids of soybean seeds. However, more severe drought stress caused a decrease in all parameters. Application of silica under drought stress conditions minimizes the reduction in number of filled pods, the number and yield of seeds, and oil content compared without silica application. The oil content, total phenol and total flavonoids of soybean seeds can be induced through silica applications. The application of silica improves the quality of soybeans as food functional.

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