

The effect of nitrogen, sulfur and silicate fertilizer application on growth, yield, and biochemical content of vegetable soybean pod (*Glycine max* L. Merr.)

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Abstract

Optimized fertilizer management, focusing on nitrogen, sulfur, and silica, plays a crucial role in agriculture production. This study aimed to investigate the effects of administering sulfur and silica on the growth, production yield, and biochemical contents of vegetable soybean. The investigation was conducted in factorial randomized block design with two factors and three replications. The first factor was the nitrogen (46% N) fertilizer dosage (N1 = 50 kg/ha and N2 = 100 kg/ha), while the second variable was the dosage with the following sulfur-silica soluble compound (2.3% S – 10% Si) fertilizer ratios (P0 = 0 ml/L (control), P1 = 1 ml/L (23 mg/L S and 100 mg/L Si), P2 = 1.33 ml/L (30,7 mg/L S and 133 mg/L Si), and P3 = 2 ml/L (46 mg/L S and 200 mg/L Si). The results indicated that applying 2 ml/L S-Si fertilizer along with 100 kg/ha of nitrogen can lead to the most favorable results in number of branches, number of leaves, number of pods, pod weight per plant, weight of 100 seeds and plant biomass weight. The fertilizer dose comprising of 50 kg/ha of N and 9.2-40 mg/plant of S-Si clearly demonstrated the highest protein content, measuring 110.6 mg/g. S-Si fertilizer dosages, exhibiting a linear escalation in the 11S/7S globulin ratio within vegetable soybean seeds. The peak 11S/7S ratio was observed in the treatment of 100 kg/ha of N - 2 ml/L of S-Si. The study results indicate that a combination of nitrogen fertilizer and sulfur-silicon yields the best vegetable soybean pod yield, improving soybean protein nutritional quality. Therefore, sulfur and silica treatments should be standardized in vegetable soybean management.

Keywords: vegetable soybean, nitrogen, sulfur, silicate, protein 11S/7S.

Introduction

Soybean is a leguminous plant extensively cultivated in Asia (Bennetau-Pelissero, 2018). Vegetable soybean [*Glycine max* (L.) Merr.], is an agricultural commodity derived from high-protein soybean cultivars harvested at the R6 growth stage (green seeds fill fresh pods, with the beans in the pods reaching 80% maturity). Vegetable soybean is commonly consumed in its fresh or frozen form (Mahoussi et al., 2020). The increasing popularity of vegetable soybean is primarily due to its nutritional value and health benefits. Nair et al. (2023) reported a significant increase in cultivation area over the past 15 years, driven by the rising demand for vegetable soybean in both domestic and international markets.

The nutritional value of vegetable soybean consists mainly of protein, fiber, starch, and sugars. Vegetable soybean is highly nutritious due to its high-quality protein content with isoflavones and its role as a good source of dietary fiber when incorporated into a diet (Johnson et al., 1999; Xu et al., 2012). Given its complex nutritional benefits, vegetable soybean has significant potential for further development (Zeipiņa et al., 2017). This development is needed in terms of productivity, morphology, taste, and nutritional content,

as increasing the quality and quantity of vegetable soybean plant production will enhance the market value of vegetable soybean and meet the growing demand.

Vegetable soybean, characterized by its sweetness and milder flavours, is a high-productivity, shorter-lived plant-based protein source that requires further development. However, studies have not shown a direct correlation between yield and sulfur (S) fertilization. Only a limited number of publications have investigated variations in seed composition resulting from S availability under various field conditions (De Borja et al., 2021).

Jiang et al. (2020) and Brooks et al. (2023) have emphasized the crucial role of fertilization management in vegetable soybean production for increasing productivity, with nitrogen being the most essential nutrient for protein content, photosynthesis, DNA, and amino acids, thus enhancing pod yield.

Sulfur and silica are crucial for vegetable soybean production as they aid in root nodule formation, tiller growth, and metabolism. The sulfur increases the dry pods and seeds of peanuts, a process facilitated by phosphate and lime.

Tandon (1995) reported that applying sulfur to acidic, dry land increased legume yields, resulting in an increase seed protein content of up to 34%. The addition of sulfur enhances nitrogen uptake in plants due to the synergistic relationship between sulfur and nitrogen. The application of 100 kg sulfur/ha has increased nitrogen uptake by legume plants by 40 kg/ha (Schnug et al., 1993).

Furthermore, silica is considered to increase the vegetable soybean productivity, with the yield of treated soybeans using silica fertilizer increasing by 21% compared to those not receiving the same treatment (Tripathi et al., 2021). This synergistic combination enhances the uptake of sulfur and nitrogen required by peanuts and effectively increases pod and seed dryness in peanuts (Wigena et al., 2001). Based on this background, our study aims to investigate the effects of sulfur and silica application on the growth, production yield, and physiological/biochemical traits of vegetable soybean.

For both human and animal production, soybean is a crucial source of protein. However, since soybean seed protein is the sole source of diet-related protein, it lacks S-containing amino acids such as methionine and cysteine. Amino acids cysteine (Cys) and methionine (Met), which are fundamental building blocks of proteins, contain sulfur (S), as do various coenzymes and secondary plant products derived from these amino acids. These types of amino acids, as stated by Haneklaus et al. (2007), account for approximately 70% of the sulfur content in plants.

Vegetable soybean contains both 11S protein and 7S globulin and is more nutrient-dense due to its higher content of amino acids containing sulfur compared to 7S globulin. Protein quality is determined by the ratio of 11S to 7S globulin. Only 2 to 3 cysteine groups in 7S globulin limit the number of disulfide bonds that can form. In contrast, 11S globulin contains 6-37 sulfhydryl and disulfide groups per mole of protein (Kinsella, 1979). 11S globulin consists of both acidic and basic subunits with a molecular weight (Mw) ranging from 27 to 37 kDa, whereas 7S globulin comprises of six isomers, each consisting of three distinct protein subunits: α' , α , and β , with molecular weights of 80, 76, and 53 kDa, respectively (Thanh and Shibasaki, 1976).

According to previous research by Slameto and Sri Trisnowati (2000), the effect of ZA fertilizer as a source of sulfur on vegetable soybean, respectively, showed that the dose of 150 kg of ZA (Zwavelzuur Ammonium) had the most significant impact on protein content, yield and growth. The results indicated a tendency of increasing vegetable soybean yield with higher doses of S fertilizer.

Vegetable soybean belongs to the Leguminosae family of plants. Nitrogen fertilizer serves as a starter for vegetable soybean plants, and the nitrogen dose is determined based on recommendations from the Jember regional agricultural service. Through mutualistic symbiosis with Rhizobium bacteria in root nodules, Leguminosae plants can naturally provide nitrogen nutrients. Meanwhile, the quantity of sulfur silica fertilizer is selected based on recommendations from the fertilizer manufacturer (Kosifarm Co., Korea) and the results of soil analysis, where indicates a low sulfur (S) content.

Research on the use of S-Si nitrogen fertilizer on vegetable soybean is essential to enhance both the production and quality of vegetable in the East Java Province of Indonesia. The objective of the research is to determine the impact on growth, yield, and biochemical content of vegetable soybean in relation to the application of nitrogen and the concentration of sulfur-silica fertilizer.

We hypothesize that the application of a specific dose of nitrogen fertilizer and a particular sulfur-silica concentration will increase growth, yield, protein content, and also affect the organic compound content of vegetable soybean.

Result and discussion

The growth and yield of the vegetable soybean

Based on the results of the analysis in Table 1, the application of the highest amount of nitrogen fertilizer showed the most significant effect on the number of pods. Specifically, the application of 100 kg/ha of nitrogen and 2 ml/L of sulfur-silica fertilizer showed the best treatment with the highest average number of pods per plant. The formation and filling of pods were primarily influenced by the availability of nutrients, water, and sunlight (length of irradiation). Sunlight serves as a crucial source of energy for pod formation through photosynthesis (Hanafiah et al., 2023). Nitrogen was administered in the form of urea due to its role in enhancing crop yields, as it has been shown to linearly increase yields in vegetable soybean plants (Brooks et al., 2023). According to Arachchige (2020), initially, plants absorb nitrogen from the soil, accumulating it in stems and leaves before it is transported to the pod shells. The older the pods, the more nitrogen is absorbed into the seeds. Vegetable soybean plants require sufficient nitrogen because their protein content is high, ranging from 35-45% (Salvagotti et al., 2009).

Plant organ development is influenced by nitrogen provision and water requirements. The variable of plant biomass weight exhibited significantly different results with the application of nitrogen fertilizer, while the addition of sulfur-silica did not yield significantly different outcomes. In the provision of nitrogen elements, the highest treatment was observed in the N2 treatment (100 kg/ha) with an average of 80 grams per plant. The effect of nitrogen provision can facilitate cell division during the growth phase, as it provides the necessary proteins for efficient growth of plant parts such as stems, leaves, branches, and other components. Therefore, nitrogen provision can enhance overall plant weight (Conceição et al., 2018).

Vegetable soybean production also depends on the number of branches. The number of branches yielded significantly different (p -value < 0.001) results when sulfur-silica was applied, while it did not exhibit significant differences (p -value > 0.005) in response to nitrogen fertilizers or the combined application of nitrogen and sulfur-silica fertilizers. The sulfur-silica treatment with the highest number of branches was observed in the P3 treatment, which received a dosage of 2 ml/L and had an average of 5.5 branches. Weekly silica application demonstrated a positive impact on branch development, attributed to the sufficient silica content. According to Timotiwu et al. (2018), adequate silica in rice plants can fortify plant tissues, leading to stronger and sturdier stems and stalks. The application of silica is expected to enhance plant growth, particularly in terms of vegetable soybean branch development. More branches are anticipated to result in a greater number of vegetable soybean pods and increased productivity in vegetable soybean plants. The abundance of branches is also influenced by the availability of nitrogen elements for nutritional support during the vegetative phase and the presence of sulfur (S) as a constituent of amino acids crucial for chlorophyll and protein synthesis. According to Hasnelly et al. (2021), optimizing nutrient levels is essential for

achieving maximum plant growth when supplementing nutrients to plants.

The application of nitrogen and sulfur-silica elements produced significantly different results in terms of the number of pods, pod weight per plant, and the weight of 100 seeds (Table 1). Applying sulfur-silica fertilizer linearly increased vegetable soybean yields at 50 kg/ha or 100 kg/ha of fertilizer, as these three elements are interrelated in terms of plant productivity. Nitrogen aids in growth and biomass accumulation, while sulfur improves growth parameters, physiochemistry, and yield and quality components of various crop plants (Shah et al., 2022). Silica is essential for maintaining plant metabolic homeostasis under various stress conditions (Khan et al., 2023). Proper dosages of sulfur and silica are crucial, as a deficiency of these elements can hinder plant productivity. Nitrogen is particularly an important macronutrient in both the vegetative and generative phases. During the vegetative phase, plants require nitrogen to support their growth (Brooks et al., 2023). Improved vegetative growth in vegetable soybean plants enhance the photosynthesis process, resulting in increased production of photosynthates. The accumulation of photosynthates from the vegetative phase to the generative phase is stored as carbohydrates in the form of seeds or pods. Higher photosynthate levels lead to heavier pods (Manurung et al., 2018).

Sulfur plays a pivotal role in the process of pod filling because vegetable soybean seeds are rich in protein. Sulfur, along with nitrogen, constitutes the essential building blocks of protein, particularly methionine, cystine, and cysteine amino acids (Narayan et al., 2022; Yu et al., 2021). In this context, Tandon (1995) reported that application of sulfur to an acidic, dry-land may increase legume yield, resulting in a remarkable increase in seed protein content of up to 34%. Moreover, sulfur application enhances nitrogen uptake in plants due to the synergistic relationship between sulfur and nitrogen. For instance, the application of 100 kg of sulfur/ha increases nitrogen uptake by leguminous plants up to 40 kg/ha (Schnug et al., 1993). This synergistic combination improves sulfur and nitrogen uptake by vegetable soybean plants and their pods.

Biochemical content of vegetable soybean pods

Silica plays a crucial role in accelerating photosynthesis rates and enhancing plant resistance to both biotic (pests and diseases) and abiotic (drought, salinity, alkalinity, and extreme weather) stresses (Zargar et al., 2019). Applying silica fertilizer to the soil can enhance the soil's water absorption capacity and increase the availability of essential nutrients like phosphorus and potassium required for flower, pod, and seed formation in soybean plants. Hussain et al. (2021) suggested that spraying silica on leaves aims to compensate for water scarcity. High sulfur availability in the soil also enhances sulfur uptake by seeds (Pagani et al., 2011). This underscores the importance of sulfur in enhancing nutrient absorption, particularly in seeds. Notably, the interaction of nitrogen and sulfur-silica fertilizers yielded significantly different results. This suggests that nitrogen fertilizers supply vegetable soybean plants with nutrients during their vegetative phase, ensuring nutrient availability from the vegetative to generative phases. Meanwhile, sulfur-silica fertilizers impact the generative phase, particularly seed formation, and weight, making them suitable for increasing vegetable soybean seed

weight.

As Sapre and Vakharia (2016) highlighted, silica in plants inhibits oxidative compounds that cause stress and increases antioxidant enzymes, optimizing plant growth and production. Preventing pests is essential because they can significantly impact plant growth by causing diseases and damaging crops. By implementing effective prevention measures, we can safeguard plants and promote healthy growth. This can be achieved by applying silica fertilizer during cultivation to strengthen plant parts, particularly pods and seeds; thus, preventing a decline in plant productivity due to pests (Mansyur et al., 2021). Additionally, silica fertilization positively affects the number of pig bean pods (Kardoni et al., 2013).

The protein content is one of the essential components of vegetable soybean. Protein from vegetable soybean exhibits equal quality when compared to protein from animal products (Medic et al., 2014). Based on the graph, the Nitrogen and S-Si treatments have distinct effects on vegetable soybean protein content. Fertilizer treatment with an N dose of 50 kg/ha and an S-Si dose of 2 ml/L showed the highest protein content at 110.6 mg/g, while the fertilizer treatment with an N dose of 50 kg/ha and an S-Si dose of 1.33 ml/L exhibited the lowest protein content at 105.4 mg/g. The highest protein content was observed in the lowest doses of S-Si because sulfur (S), suggesting a crucial role in protein formation (Aulakh, 2003). Nitrogen had no impact on the protein content of vegetable soybean because the organic nitrogen (N organic) associated with N from fertilizer treatments rendered N fertilizer treatment unnecessary, as N organic or nitrogen from fixation proved sufficient for vegetable soybean (Ferreira et al., 2015).

In this study, the 11S/7S ratio demonstrated a linear relationship between protein content and the weight of 100 vegetable soybean seeds. However, this relationship exhibited an insignificant influence (Figure 2). Nevertheless, in the study by Kim et al. (2008), it was reported that the 11S/7S ratio had a significant linear impact on the yield of soybean curd while forming a quadratic relationship with the hardness of soybean curd. Elevated level of 11S globulin has contributed to a higher nutritional quality of soybean protein (Guo et al., 2022). Protein constituted the predominant component synthesized during soybean seed formation (Hai Ngoc et al., 2021). Moreover, protein content increased linearly throughout the soybean seed development process. Additionally, this study indicated that the 11S/7S ratio displayed a negative linear correlation with reducing sugar content. However, its effect was insignificant (Figure 2). Moreover, Wang et al. (2018) reported that glucose negatively correlated with protein content in soybean seeds.

The reducing sugar content of vegetable soybean due to N and S-Si treatment showed different responses. The highest reducing sugar content was 112.8 mg/g from the treatment using 100 kg/ha of N and 9.2-40 mg/plant of S-Si. The lowest reducing sugar of 32.3 mg/g resulted from the fertilizer treatment using 100 Kg/Ha of N and 2 ml/L of S-Si. Based on the data, the highest N doses resulted in the highest reducing sugar content of vegetable soybean. Karaivazoglou et al. (2007) reported that using N can increase reducing sugar concentration of the cured product. In addition, the use of N in plant growth may reduce carbohydrates (monosaccharide or disaccharide) for amino acid biosynthesis in plant metabolism (De Wilde et al., 2006).

Table 1. Results of the effect of addition of N and S-Si Fertilizers on soybean growth and yield.

Treatment	Number of Branches	Number of Leaves	Number of Pods	Number of Pod Weight Per Plant (g)	Weight of 100 Seeds (g)	Plant biomass Weight (g)
50 Kg N ha ⁻¹						
P0	3±0	43±1.00	30±0.58 d	61.3±2.52 d	82.7±1.53 d	54.7±4.04
P1	4±0	42±2.65	35±0.58 c	105.3±5.03 c	87.3±1.53 c	57±4
P2	5±1	41±3.06	39±0.58 b	114.0±4.36 b	96.0±2.65 b	60.3±14.18
P3	5±0.6	45±1.15	44±0.58 a	126.3±4.93 a	108.3±3.06 a	66±5.29
100 Kg N ha ⁻¹						
P0	3±0.6	52±2.65	35±0.58 d	70.7±3.06 d	86.3±1.53 d	76±2.65
P1	4±0	53±2.31	41±1.00 c	123.3±4.51 c	91.3±2.52 c	80.3±17.24
P2	5±0.6	54±3.51	47±1.00 b	136.3±5.13 b	111.0±3.00 b	81.3±3.51
P3	6±0.6	56±2.08	56±1.73 a	152.0±3.61 a	115.3±3.51 a	82.3±0.58
F Statistics (0.05)						
N	1.29 Ns	5.03*	441.80 **	100.98**	51.29**	24.85**
S-Si	23.76**	1.59*	410.33 **	299.38**	152.28**	0.82 ns
N x S-Si	0.14 ns	0.34 ns	13.00 **	5.75**	6.23**	0.13 ns

P0 = 0 S-Si mg/plant. P1 = 9.2-40 S-Si mg/plant. P2 = 12.2-53 S-Si mg/plant. and P3 = 18.4-80 S-Si mg/plant. ns = not significant. * = significant (p<0.05). ** = significant (p<0.001). Numbers followed by different letters indicate significance in the treatments of nitrogen and sulfur-silica fertilizer combinations based on Duncan's test results (p ≤ 0.05).

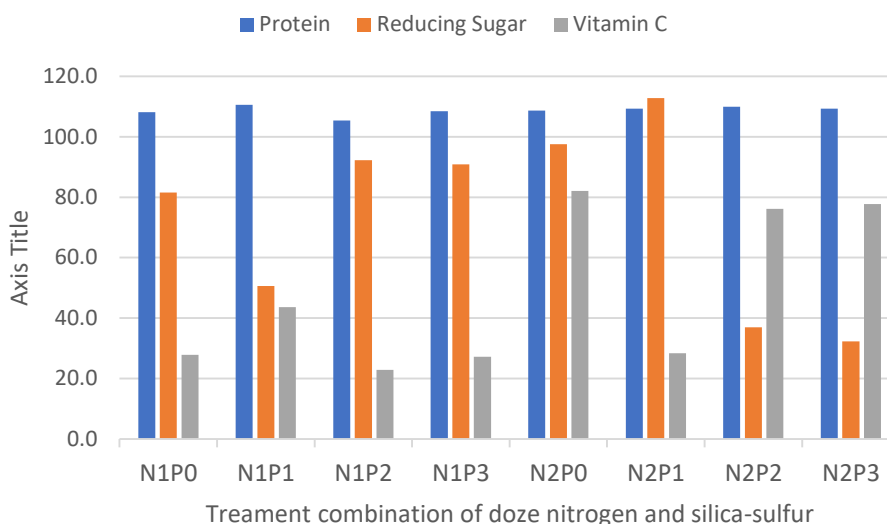


Figure 1. Protein, reducing sugar and vitamin C content of edamame seed treated by nitrogen and sulfur-silica composition.

Table 2. 11S glycinin, 7S β-conglycinin content and 11S/7S ratio of edamame seeds treated by nitrogen and sulfur- silica composition.

Treatment	11S %	7sS%	Ratio 11S/7S
N1P0	14.2	8.3	1.7
N1P1	17.5	9.4	1.9
N1P2	19.6	9.8	2.0
N1P3	21.8	7.7	2.8
N2P0	17.7	6.8	2.6
N2P1	26.2	7.9	3.3
N2P2	22.5	8.7	2.6
N2P3	25.3	7.3	3.5

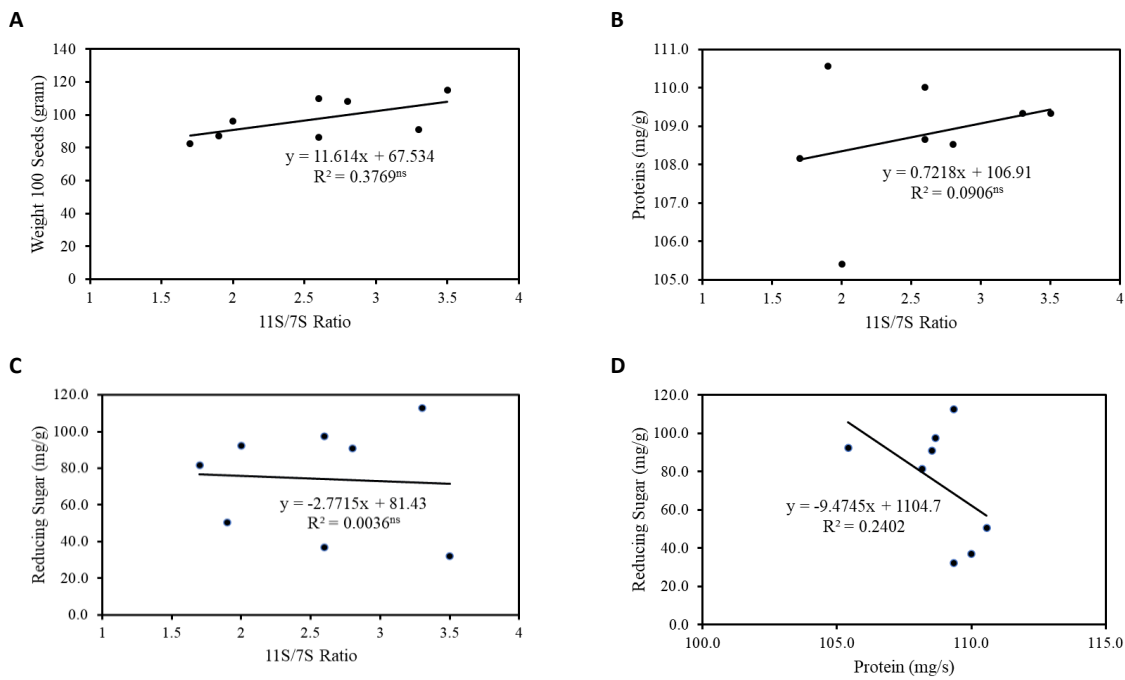


Figure 2. The relationship between a) 11S/7S ratio and weight of 100 seeds. b) 11S/7S ratio and protein. c) 11S/7S ratio and reducing sugar. and d) protein and reducing sugar of edamame seed. ^{ns} = not significant.

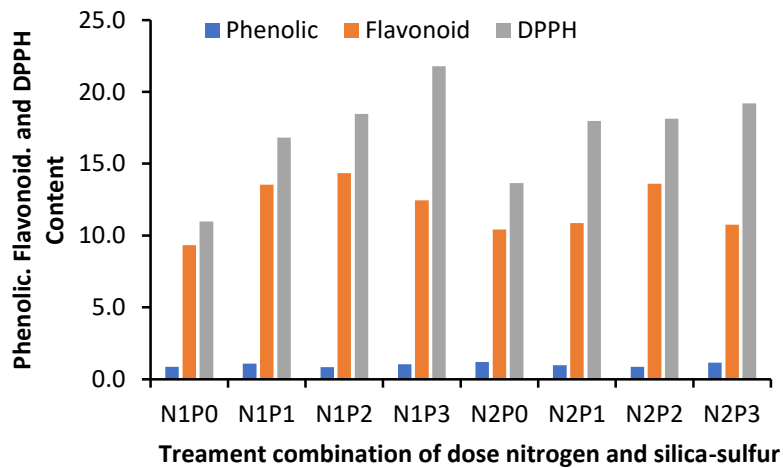


Figure 3. The effect of nitrogen and sulfur fertilization on phenolic, flavonoid content and DPPH percentage of edamame seeds.

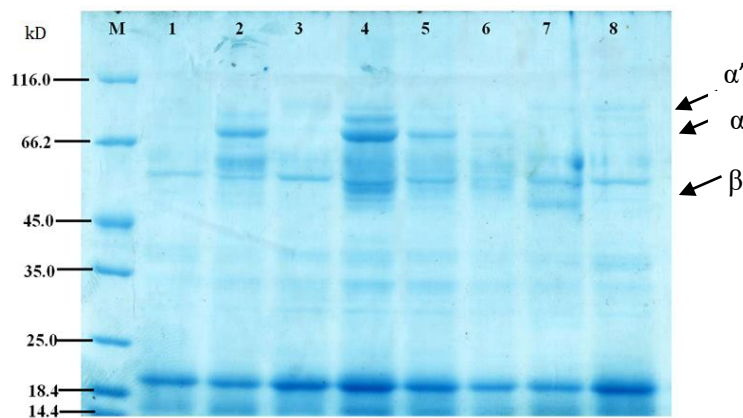


Figure 4. Profile of 7S β-conglycinin of edamame seeds treated by nitrogen and S- Si fertilizer. α'. α. and β indicate subunits of 7S globulin (β-conglycinin), M marker; 1. N1P0 = N 50 Kg/Ha - 0 mg/plant; 2. N1P1 = N 50 Kg/Ha - S-Si 9.2- 40 mg/plant; 3. N1P2 = N 50 Kg/Ha - S-Si 12.2-53 mg/plant; 4. N1P3= N 50 Kg/Ha- S-Si 18.4-80 mg/plant; 5. N2P0= N 100 Kg/Ha- 0 mg/plant; 6. N2P1= N 100 Kg/Ha - S-Si 9.2-40 mg/plant; 7. N2P2= N 100 Kg/Ha - S-Si 12.2-53 mg/plant; 8. N2P3= N 100 Kg/Ha - S-Si 18.4-80 mg/plant.

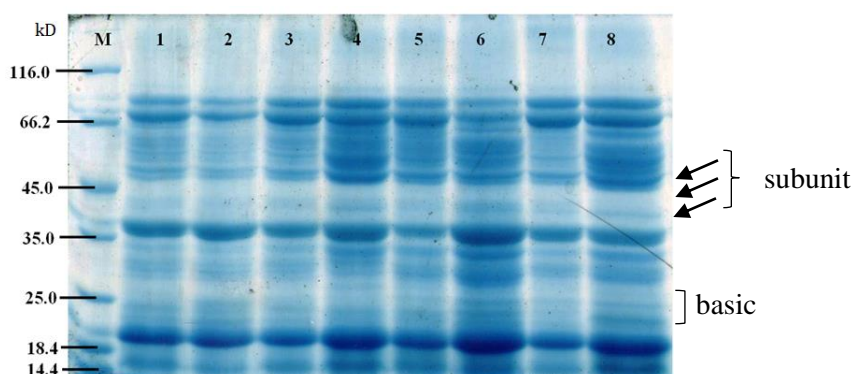


Figure 5. Profile of 11S globulin & glycinin of edamame seeds treated by nitrogen and S-Si fertilizer. Subunit and basic indicate polypeptides of 11S globulin (glycinin). M marker; 1. N1P0 = N 50 Kg/Ha - 0 mg/plant; 2. N1P1 = N 50 Kg/Ha - S-Si 9.2- 40 mg/plant; 3. N1P2 = N 50 Kg/Ha - S-Si 12.2-53 mg/plant; 4. N1P3= N 50 Kg/Ha- S-Si 18.4-80 mg/plant; 5. N2P0= N 100 Kg/Ha- 0 mg/plant; 6. N2P1= N 100 Kg/Ha - S-Si 9.2-40 mg/plant; 7. N2P2= N 100 Kg/Ha - S-Si 12.2-53 mg/plant; 8. N2P3= N 100 Kg/Ha - S-Si 18.4-80 mg/plant.

Vitamin C content by fertilizer treatment showed different responses. The highest vitamin C content of 82.1 mg/g was found in the treatment using 100 kg/ha of N and 0 mg/plant of S-Si. Meanwhile, the lowest Vitamin C content of 22.9 mg/g was obtained from the treatment using 100 kg/ha of N and 1.33 ml/L of S-Si. The highest phenolic content of 1.2 mg/g was shown after 2 fertilizer treatments, such as 100 kg/ha of N and 0 ml/L S-Si and 100 kg/ha of N and 2 ml/L of S-Si. The lowest phenolic content of 0.8 mg/g was shown after the treatment using 50 kg/ha of N and 1.33 ml/L of S-Si. Previous findings reported that increasing S fertilizer can increase the phenolic content of wheat, but it also depends on the variety of the plant (Tian et al., 2021). Sulfur also has an essential role for plants to cope with environmental stress and plant biochemical content (Adib et al., 2020).

Fertilizer treatment using 50 kg/ha of N and 1.33 ml/L of S-Si showed the highest flavonoid content of 14.3 mg/g. The lowest flavonoid content of 9.3 mg/g was obtained from the treatment using 50 kg/ha of N and 0 ml/L of S-Si. The highest DPPH percentage of 21.8% was shown after applying 50 kg/ha of N and 2 ml/L of S-Si, and the lowest DPPH percentage of 11% was shown after applying 50 kg/ha of N and 0 mg/plant of S-Si.

The results of electrophoresis of 7S β -conglycinin and 11S glycinin, using Thanh and Shibasaki's protocol (Thanh and Shibasaki, 1976), showed that nitrogen and sulfur-silicon (S-Si) fertilizer doses resulted in different protein profiles in vegetable soybean seeds (Figure 4 and Figure 5). The nitrogen fertilizer dose of 100 kg/ha provided the highest 11S/7S ratio compared to a nitrogen dose of 50 kg/ha (Table 2). Additionally, S-Si fertilizer doses linearly increased the globulin 11S/7S ratio in vegetable soybean seeds. The highest 11S/7S globulin ratio was observed in the N2P3 treatment (N 100 kg/ha - S-Si 2 ml/L), which was 3.5.

Sulfur is an essential nutrient required by plants for protein synthesis, especially during seed development (Narayan et al., 2022). High levels of 11S globulin contribute to the higher nutritional quality of soybean protein (Guo et al., 2022). Under sulfur deficiency conditions, sulfur-rich proteins such as 12S globulin, glycinin, and 2S albumin experience a decrease, while there is an accumulation of 7S globulin (β -conglycinin) during soybean seed development (Mondal et al., 2022)."

Sharma and Sharma (2014) found that 31.25 kg/ha of nitrogen and 20 kg/ha of sulfur fertilization could increase the 11S/7S ratio in soybean seeds during the physiological maturity stage. Burkitbayev et al. (2021) also reported that

sulfur-containing agrochemicals in the form of powder and soluble increase glycinin (globulin 12S) protein and result in the highest growth and grain yield values in soybean.

There is currently no information available regarding the effect of silica fertilizer on the accumulation of Seed Storage Proteins (SSPs). However, silica fertilizer has been shown to significantly enhance soybean vegetative growth under drought-stress conditions (Sah et al., 2022). Nakagawa et al. (2018) reported that drought stress reduces the activity of genes encoding glycinin proteins such as GmGy1, GmGy2, GmGy4, and GmGy5, particularly GmGy4 during soybean seed filling. Additionally, drought stress also leads to a decrease in the content of β -conglycinin, resulting in a decline in protein levels. Henriot et al. (2019) also reported that drought stress and sulfur deficiency can reduce yield and accumulation of 11S glycinin in pea seeds. Based on these conditions, silica fertilizer indirectly regulates protein composition during vegetable soybean seed formation.

The amount of amino acids-containing sulfur is minimal in all soybean types. The sulfur-containing amino acids, regarded as the first limiting amino acids of soybean (Peter et al. 1998; Coates et al. 1985), are known to be insufficient in soybean. Amino acid composition in protein and nitrogen solubility, in general, determine the nutritional and functional properties (Kinsella, 1979). To boost the amount of sulfur-containing amino acids in soybeans, numerous breeding techniques, including genetic engineering, are now being used. Additionally, increasing S supply may generate seeds by utilizing improved management procedures that contain increased S-containing proteins.

Materials and methods

Materials and experimental design

The vegetable soybean seeds used are the Ryoko variety. The nitrogen fertilizer used is prilled urea with a content of 46% Nitrogen (N). The Sulfur-Silica (S-Si) fertilizer used is Oboki's fertilizer by Kosifarm Co (Korean) in liquid form with a chemical composition of 2.3% S and 10% Si. This study employed a randomized block design model with two factors and three replications. The first factor was the dose of nitrogen (N) fertilizer (N1 = N 50 Kg/Ha and N2 = N 100 Kg/Ha), and the second was the dose of sulfur-silica (S-Si) fertilizer (P0 = 0 ml/L (control), P1 = 1 ml/L (23 ml/L S and 100 ml/L Si), P2 = 1.33 ml/L (30,7 ml/L S and 133 ml/L Si), and P3 = 2 ml/L (46 ml/L S and 200 ml/L Si).

The planting was carried out with a spacing of 20 x 40 cm², with each bed containing 12 holes, and each hole was filled with 2 seeds to a depth of 2-3 cm. Prior to planting, the beds were mulched to suppress weed growth. Sticking was performed to replace seeds that did not germinate, dead seeds, and unhealthy plants on the 10th Day After Planting (DAP). Subsequently, the soil was thoroughly irrigated, with four applications: during planting, at the time of fertilizer application, at the onset of flowering, and during pod filling. Additional watering occurred as needed when the soil became dry.

Fertilizer treatment

Fertilization was done twice: with basic fertilizer and fertilizer treatment. Basic fertilization was carried out three times: at the beginning of planting using 4.5 kg/bed of cow manure, 150 kg/ha of nitrogen fertilizer, and 100 kg/ha of potassium, which was repeated twice. The first repetition was given on the 14th day after plantation (DAP), and the second was on the 30th DAP. Fertilization treatment was carried out with nitrogen fertilization and S-Si fertilization. Two doses of nitrogen fertilization were given (50 kg/ha and 100 kg/ha). Nitrogen applications needed to be converted to urea. The dose of urea in treatment N1 was 108 kg/ha, and the dose of urea in treatment N2 was 217 kg/ha. Nitrogen fertilizer application was given in stages, with two applications at the beginning of planting and on the 14th DAP, by perforating the soil in a circular shape on the edge of the plant with a depth of 2 cm and inserting nitrogen fertilizer, then covering it again with soil. Sulfur-silica fertilization was done by the foliar spray technique. With the required volume of liquid fertilizer of 400 ml per plant from the beginning of planting to harvest time. The spraying was done in the morning at the ages of 15, 25, 35, 45, 55, and 65 DAP. Meanwhile, the harvesting was done on the 70th DAP.

Growth and yield observation

Various growth and yield parameters were the number of leaves, number of branches, number of pods, weight of pods per plant, weight of 100 seeds, and fresh biomass weight. The number of leaves was observed on the 30th DAP by counting all the leaves from the bottom to the top. The number of branches was observed on the 32nd DAP by counting the plant branches. The number of pods was observed during harvesting by counting the total pods in each plant. Pod weight per plant was observed when harvesting by weighing the pods from 3 plant samples. The weight of 100 seeds was observed when harvesting by weighing the weight of 100 seeds in 3 plant samples.

Determination of biochemical in pod samples

The 7S and 11S globulins were purified from soybean pods according to the methods of Thanh and Shibasaki (1976). With a few minor adjustments, the 7S and 11S globulin fractions were separated from defatted soybean flour using Thanh and Shibasaki's (22) technique. Defatted soybean flour is extracted for 1 hour at room temperature with 0.03 M Tris buffer (pH 8.0) and 0.01 M mercaptoethanol. With 2N HCL, whole soybean (WS) protein extract was brought to pH 6.4. Centrifugation was used to separate the 11S globulin, and the extract was dialyzed at pH 6.4 for three hours at 4°C. By lowering the pH to 4.8, a crude 7S globulin fraction was extracted from the supernatant that resulted in this supernatant. The 7S globulin was produced, rinsed in Tris buffer (pH 6.2), and then dispersed in the standard buffer by

adding 2 N NaOH until the pH of the standard buffer reached 7.

To assess the quantity of dissolved protein, the Bradford (1976) method was modified. Samples of 950 µl were used, 5 µl of sample, 45 µl of distilled water, and 15 minutes of incubation. To determine the absorbance at a wavelength of 595 nm, a spectrophotometer was employed. Bovine serum albumin (BSA) is the reference standard for determining the amount of soluble protein in a sample in terms of mg BSA/g. Reducing sugars were measured using the Nelson-Somogyi method. The sample was mixed with phosphate buffer, DNS, and boiling water, then cooled and absorbance was measured using a spectrophotometer. The glucose concentration was determined using absorbance as the x-value. While vitamin C was measured using UV-spectrophotometric analysis, vitamin C concentration was measured using titration against 2,6-dichlorophenolindophenol. Samples were homogenized, filtered, and centrifuged. Ascorbic acid was tested against the solution until it turned pink, and the amount of vitamin C was measured as mg/g of fresh weight.

The study determined total phenolics (TPC) content using a modified Folin-Ciocalteu colorimetric method as described by Singleton et al. (1999). TPC was quantified using a calibration curve with gallic acid equivalent (GAE) standards and expressed in milligrams of GAE/g FW. The method involved mixing gallic acid solution or extracts with reagents and sodium carbonate.

The study assessed flavonoid content using a colorimetric assay method, originally described by Zhishen et al. (1999). The method involved combining a diluted extract with NaNO₂, Al(NO₃)₃, NaOH, and NaOH, then diluted and absorbance readings at 510 nm. The total flavonoid content was determined using a calibration curve.

The determination of antioxidant activity (DPPH) was conducted using the method proposed by Gálvez et al. (2005). This method involved evaluating the antioxidant activity of a sample extract in methanol by employing DPPH solution and measuring absorbance at a wavelength of 517 nm. The mixture was incubated for 20 minutes.

Statistical analysis

The analysis of growth and yield data for vegetable soybean was conducted using Analysis of Variance (ANOVA), followed by a post hoc test Duncan's Multiple Range Test (DMRT) (one-way) to assess the significance of interactions between nitrogen and sulfur-silica fertilizer treatments. Meanwhile, the biochemical data for vegetable soybean subjected to treatments were described in a descriptive manner. Linear regression analysis was used to determine the interaction of the 11S/7S ratio on weight of 100 seeds, protein, reducing sugar. In addition, regression analysis was carried out on protein and reducing sugar parameters in vegetable soybean pods.

Conclusion

It can be concluded that Sulfur and Silica treatments should be introduced into production as a standard treatment in vegetable soybean management. It can help farmers to increase the yield of crops.

References

- Adib SS, Dehaghi MA, Rezazadeh A & Naji A (2020) Evaluation of sulfur and foliar application of Zn and Fe on yield and biochemical factors of cumin (*Cuminum cyminum* L) under irrigation regimes. *Journal of Hermed Pharmacology*. 9(2): 161-170.
- Arachchige PSP, Rosso LHM, Hansel FD, Romundo B, Torres AR, Ciampiti IA, Jagadish SVK (2020) Temporal biological nitrogen fixation pattern in soybean inoculated with *Bradyrhizobium* Agrosystems, Geosciences & Environment. 1-10.
- Aulakh MS (2003) Crop responses to sulfur nutrition In *Sulfur in plants*. Springer, Dordrecht. 341-358.
- Bennetau-Pelissero C (2018) *Plant Proteins from Legumes In Reference Series in Phytochemistry* New York: Springer International Publishing.
- Bradford MMA (1976) Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.
- Burkitbayev M, Bachilova N, Kurmanbayeva M, Tolenova K, Yerezhepova N, Zhumagul M, Mamurova A, Turysbek B, Demeu G (2021) Effect of sulfur-containing agrochemicals on growth, yield, and protein content of soybeans (*Glycine max* (L) Merr). *Saudi Journal of Biological Sciences*. 28(1): 891-900. <https://doi.org/10.1016/j.sjbs.2020.11033>
- Brooks K, Reiter M, Zhang B, Mott J (2023) Vegetable soybean yield and quality response to nitrogen and sulfur fertilizers. *Agronomy*. (Basel, Switzerland) 13(7): 1865 <https://doi.org/10.3390/agronomy13071865>
- Coates JB, Medeiros JB, Thanh VH, Nielsen NC (1985) Characterization of the subunits of B-conglycinin Arch *Biochem Biophys* 243: 184-194
- Conceição GM, Martin TN, Brunetto G, Backers RL, Andrade FFD, Beche M (2018) Contribution of nitrogen derived from mineral supplementation for soybean seedlings *Agrotecnologia*. 42(1).
- De Borja R, Rosso AF, Davidson LHM, Kovács DP, Purcell LC, Below FE, Casteel SN, Knott C, Kandel H, Naeve SL and Carciochi W (2021) Sulfur fertilization in soybean: A meta-analysis on yield and seed composition. *European Journal of Agronomy*. 127: 126285
- De Wilde T, De Meulenaer B, Mestdagh F, Govaert Y, Vandeburie S, Ooghe W, Verhé R (2006) Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003. *Journal of Agricultural and Food Chemistry*. 54(2): 404-408.
- Ferreira AS, Balbinot Junior AA, Werner F, Zucareli C, Franchini JC, Debiassi H (2016) Plant density and mineral nitrogen fertilization influencing yield, yield components and concentration of oil and protein in soybean grains. *Bragantia*. 75.
- Gálvez M, Martín-Cordero C, Houghton PJ and Ayuso MJ (2005) Antioxidant activity of methanol extracts obtained from *Plantago* species *J Agr Food Chem*. 53: 1927-1933
- Guo B, Sun L, Jiang S, Ren H, Sun R, Wei Z, Hong H, Luan X, Wang J, Wang X, Xu D, Li W, Guo C, Qiu L-J (2022) Soybean genetic resources contributing to sustainable protein production. *Theoretical and Applied Genetics*. 135(11): 4095-4121 <https://doi.org/10.1007/s00122-022-04222-9>
- Haneklaus S, Bloem E, Schnug E (2007) Sulfur and plant disease. *Mineral Nutrition and Plant Disease*. 101-18.
- Hanafiah DS, Lubis K, Haryati, Setiada H, Damanik GM, Limbong MS, Silaen FR, Joshua, Lestami A (2023) Assembly of soybean genotypes developed through Three-Way cross. *SABRAO Journal of Breeding and Genetics*. 55(3): 940-950. <https://doi.org/10.54910/sabrao202355328>
- Henriet C, Aimé D, Térézol M, Kilandamoko A, Rossin N, Combes-Soia L, Labas V, Serre R-F, Prudent M, Kreplak J, Vernoud V, Gallardo K (2019) Water stress combined with sulfur deficiency in pea affects yield components but mitigates the effect of deficiency on seed globulin composition. *Journal of Experimental Botany*. 70(16): 4287-4304 <https://doi.org/10.1093/jxb/erz114>
- Hussain S, Mumtaz M, Manzoor S, Shuxian L (2021) Foliar Application of Silicon Improves growth of soybean by enhancing carbon metabolism under shading conditions. *Plant Physiology and Biochemistry*. 159(1): 43-52
- Johnson D, Wang S, Suzuki A (2000) Vegetable soybean: A vegetable soybean for Colorado. *Energy (Kcal)*. 582: 573
- Jiang GL, Katuuramu DN, Xu Y, Ren S, Rutto LK (2020) Analysis and comparison of seed protein, oil, and sugars in vegetable soybean dried using two oven-drying methods and mature soybeans. *Journal of the Science of Food and Agriculture*. 100(10): 3987-3994.
- Karaivazoglou NA, Tsotsolis NC, Tsadilas CD (2007) Influence of liming and form of nitrogen fertilizer on nutrient uptake, growth, yield, and quality of Virginia (flue-cured) tobacco. *Field Crops Research*. 100(1): 52-60.
- Kardon F, Jalil S, Seyed Mousavi SJ, Parande S (2014) Effect of salinity stress and silicon application on yield and component yield of faba bean (*Vicia faba*). *International Journal of Agriculture and Crop Sciences*. 6(12): 814-818.
- Khan I, Awan SA, Rizwan M, Brestic M, Xie W (2023) Silicon: an essential element for plant nutrition and phytohormones signaling mechanism under stressful conditions. *Plant Growth Regulation*. 100(2): 301-319 <https://doi.org/10.1007/s10725-022-00872-3>
- Kim S-L, Koo H-M, Chun S-C, Kim J-T, Kim M-Y, Chi H-Y, Kim E-H, Kim H-B, Kim M-J, Seo B-R, Kang E-Y, Seo S-H, Chung I-M (2008) Characterization of 7S and 11S globulins in soybean varieties differing in seed size and their effects on the properties of soybean. *Curd Food Sci Biotechnol*. 17(1): 135-143.
- Kinsella JE (1979) Functional properties of soy proteins. *J Am Oil Chem Soc*. 56: 242-258.
- Mahoussi KAD, Eric EA, Symphorien A, Florent J-B Q, Flora, JC, Achille EA, Clement A, Brice S (2020) Vegetable soybean, vegetable soybean: Research, production, utilization and analysis of its adoption in Sub-Saharan Africa *Journal of Horticulture and Forestry*. 12(1): 1-12 <https://doi.org/10.5897/jhf20190604>
- Manurung DSR, Hasanah Y, Sipayung R (2018) Growth Response and production of soybean (*Glycine max* (L) on application of phosphorous fertilizer and rhizobium inoculation. *Agricultural Research*. 1(3): 289 - 294.
- Medic J, Atkinson C, Hurburgh CR (2014) Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society*. 91(3): 363-384
- Mondal S, Pramanik K, Panda D, Dutta D, Karmakar S, Bose B (2022) Sulfur in seeds: An overview. *Plants*. 11(3): 450 <https://doi.org/10.3390/plants11030450>
- Nair RM, Boddepalli VN, Yan M-R, Kumar V, Gill B, Pan RS, Wang C, Hartman GL, Silva e Souza R, Somta P (2023) Global status of vegetable soybean. *Plants*. 12(3), 609 <https://doi.org/10.3390/plants12030609>

- Nakagawa ACS, Itoyama H, Ariyoshi Y, Ario N, Tomita Y, Kondo Y, Iwaya-Inoue M, Ishibashi Y (2018) Drought stress during soybean seed filling affects storage compounds through regulation of lipid and protein metabolism. *Acta Physiologiae Plantarum*. 40(6). <https://doi.org/10.1007/s11738-018-2683-y>
- Narayan OP, Kumar P, Yadav B, Dua M, Johri AK (2022) Sulfur nutrition and its role in plant growth and development. *Plant Signaling and Behavior*. <https://doi.org/10.1080/1559232420222030082>
- Nguyen HN, Kambhampati S, Kisiala A, Seegobin M, Joseph Neil R (2021) The soybean (*Glycine max* L) cytokinin oxidase/dehydrogenase multigene family; Identification of natural variations for altered cytokinin content and seed yield. *Plant Direct*. 5(2).
- Pagani M, Agustin JD, Echeverria C, Hernan MJ (2011) Performance of sulfur diagnostic methods for corn. *American Society of Agronomy*. 2 413- 421.
- Peter JS, Pack NC, Shibles RM (1998) Effects of nitrogen source and timing of sulfur deficiency on seed yield and expression of 118 and 78 seed storage proteins of soybean. *Field Crop Res*. 59: 1-8
- Sah SK, Reddy KR, Li J (2022) Silicon enhances plant vegetative growth and soil water retention of soybean (*Glycine max*) plants under water-limiting conditions *Plants*. 11(13): 1687. <https://doi.org/10.3390/plants11131687>
- Salvagotti F, Specht JE, Cassman KG, Walters DT, Weiss A, Dobermann A (2009) Growth and nitrogen fixation in high-yielding soybean: Impact of nitrogen fertilization. *Agronomy*. 101(4): 958-970
- Sapre SS, Vakharia DN (2016) Role of silicon under water deficit stress in wheat: (Biochemical perspective): A review. *Agricultural Reviews*. 37(2): 109 – 116.
- Schnug E, Haneklaus S, Murphy D (1993) Impact of sulfur fertilization on fertilizer nitrogen efficiency. *Sulfur in Agric*. 17: 8- 12.
- Shah SH, Islam S, Mohammad F (2022) Sulfur as a dynamic mineral element for plants: a review. *Journal of Soil Science and Plant Nutrition*. 22(2): 2118–2143. <https://doi.org/10.1007/s42729-022-00798-9>
- Sharma A, Sharma S (2014) Effect of nitrogen and sulfur nutrition on yield parameters and protein composition in soybean [*Glycine max* (L) Merrill]. *Journal of Applied and Natural Science*. 6(2): 402–408. <https://doi.org/10.1031018/jansv6i2435>
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*. 299: 152–178.
- Slameto, Sri Trisnowati (2000) The Influence of ammonium sulfate fertilizer dosage and application timing on yield and protein quality of vegetable soybean (*Glycine max* L Merr). Thesis Faculty of Agriculture, Gadjah Mada University, Yogyakarta.
- Tandon HLS (1995) Sulfur Research and Agricultural Production in India Fertilizer Development and Consultation Organization, New Delhi 19: 3–8.
- Thanh VH, Shibasaki K (1976) Major proteins of soybean seeds. A straight forward fraction and their characterization. *J Agr Food Chem*. 24: 1117-1121.
- Tian W, Wilson TL, Chen G, Guttieri MJ, Nelson NO, Fritz A, Li Y (2021) Effects of environment, nitrogen, and sulfur on total phenolic content and phenolic acid composition of winter wheat grain. *Cereal Chemistry*. 98(4): 903-911.
- Timotiwu PB, Agustiyansah, Ermawati (2018) The effects of foliar boron and silica through the leaves on soybean growth and yield. *Agricultural Studies*. 6(3): 34-48.
- Tripathi P, Na CI, Kim Y (2021) Effect of silicon fertilizer treatment on nodule formation and yield in soybean (*Glycine max* L). *European Journal of Agronomy*. 122: 126172.
- Tao W, Xu P, Chen Zh, Zhouab X, Wang R (2018) Alteration of the structure of rice proteins by their interaction with soy protein isolates to design novel protein composites. *Food & Function*. 8. <https://doi.org/10.1039/C8FO00661J>
- Wigena Putu GP, Rachim A, Santoso D, Saleh A (2001) Effects of lime to sulfur-sulfate transformation on oxic dystrodepts and its relationship to peanut production. *Indonesian Soil and Climate Journal*. No:19
- Xu Y, Sismour E, Pao S, Rutto L, Grizzard C (2012) Textural and Microbiological Qualities of Vegetable Soybean (Vegetable soybean) Affected by Blanching and Storage Conditions *J Food Process Technol*. 3: 165 doi: 10.4172/2157- 71101000165
- Hasnelly S, Yasin, Agustian, Darmawan (2021) Response of Growth and Yield of Soybean (*Glycine max* L Merrill) to the Method and Dose of Leachate Liquid Organic Fertilizer Application *Planta Tropika: Journal of Agro Science*. 9(2): 109-115.
- Yu D, Lin T, Sutton K, Lord N, Carneiro R, Jin Q, Zhang B, Kuhar T, Rideout S, Ross J, Duncan S, Yin Y, Wang H, Huang H (2021) Chemical compositions of vegetable soybean genotypes grown in different locations in the US. *Frontiers in Sustainable Food Systems*. 5 <https://doi.org/10.3389/fsufs2021620426>
- Zargar SM, Mahajan R, Bhat JA, Nazir M, Deshmukh R (2019) Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. *3 Biotech*. 9(3): 1-16.
- Zeipina S, Alpina I, Lapse L (2017) Insight in vegetable soybean yield and quality parameters: A review. *Agricultural Sciences (Crop Sciences, Animal Sciences)*. 2: 40 - 45
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals *Food chemistry*. 64(4):555-9