

Growth and Production of Soybean (*Glycine Max* L.) Due to Urea Fertilization, *Rhizobium* sp. Inoculation and Phosphate Solubilizing Bacteria in Saline Soil

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Abstract. *The study aimed to examine the dose of urea fertilizer, to examine the inoculation dose of Rhizobium sp., and the doses of phosphate solubilizing bacteria, as well as study the interaction between the two in soybean cultivation in saline soils. The research was conducted from July to November 2021 at the Greenhouse and chemical analysis at the Ecology and Plant Production Laboratory, Faculty of Animal and Agriculture, Diponegoro University, Semarang. The research design used was a factorial 3 x 4 Completely Randomized Design (CRD) with 3 replications. The first factor is the dose of urea fertilizer with N0 = 100%, N1 = 90%, N2 = 80%. The second factor is the inoculation dose of Rhizobium sp. and phosphate solubilizing bacteria with the information P0 = no inoculum (control), P1 = 3 g/seed, P2 = 6 g/seed, P3 = 9 g/seed. Based on the research that has been done, it can be concluded that urea fertilization and Rhizobium sp. and phosphate solubilizing bacteria showed a significant effect on the character of plant height and early flowering but had no significant effect on the number of leaves and wet weight of pods produced in saline soil.*

Keywords: *Bacteria, Soybean, Rhizobium sp., Saline, Urea*

1. Introduction

Soybean production has decreased every year. Based on data from the Central Bureau of Statistics (BPS) soybean production in 2013 was 807,570 tons/ha and decreased by 4.22% of the total soybean production in 2012 [1]. The decline in soybean production is expected to occur due to the narrowing of the land area used for soybean cultivation due to land damage and the increasing number of lands with marginal conditions such as peatlands, swamps, and land with high salinity levels. The area of saline land in Indonesia continues to increase with an overall value of around 440,300 ha which has the criteria for slightly saline land of 304,000 ha and land with saline criteria of 140,400 ha [2]

The utilization of saline soil is one solution to increasing soybean growth and production in Indonesia. Saline soil conditions are generally found on the soil in coastal locations. The main condition in soil salinity is the high salt content, especially NaCl and this adversely affects plant growth and productivity and can result in a decrease in production yields [3]. Efforts to improve saline soil can be done in various ways, one of which is fertilization. Applying fertilizer to saline soil can use urea-type fertilizer. Urea fertilizer is a type of inorganic fertilizer that has a nitrogen content of 46% in its constituent materials [4]

The use of inorganic fertilizers can cause soil damage. Therefore, other methods are needed to optimize soybean productivity on saline soils and minimize soil damage. One of the efforts to increase the productivity of saline soil can be done by presenting inoculum in the form of a combination of *Rhizobium* sp. and phosphate

solubilizing bacteria. The addition of *Rhizobium* sp. plays a role in fixing free N₂ in the air into the form of NH₄⁺ or NO₃⁻ which can be absorbed by plants [5]. *Rhizobium* sp. contributes to the growth of legumes. *Rhizobium* sp. is a bacterium that can symbiotically with legume plants to produce root nodules that play a role in fixing nitrogen [6]. Solubilizing bacteria can provide P, which is organic phosphate with other elements such as Ca, Al, and Fe with acid so that P is available for plants [7]. The use of organic matter reduces the use of chemical fertilizers. The use of organic bacteria can fix N so that it can reduce the use of inorganic N fertilizers [8]. The study aimed to examine the dose of urea fertilizer that was most suitable for the growth and yield of soybeans and to examine the inoculation dose of *Rhizobium* sp. and the dose of phosphate solubilizing bacteria that was most appropriate for the growth and yield of soybeans, as well as examining the interactions that occurred between the dose of urea fertilizer and the dose of inoculated bacteria *Rhizobium* sp. and phosphate solubilizing bacteria in soybean cultivation in saline soil.

2. Research Method

Research has been conducted at the Greenhouse Agrotechnopark, Faculty of Animals and Agriculture Science, Diponegoro University, and chemical analysis at the Laboratory of Ecology and Plant Production, Faculty of Animals and Agriculture Science, Diponegoro University, Semarang. The research has been carried out from July 2021 to Februari 2022. The materials used in this study were soybean seeds of the Anjasmoro variety, isolates of phosphate solubilizing bacteria (BPF), and isolates of *Rhizobium* sp. saline tolerance of 20,000 ppm NaCl, soil samples, manure, Urea, KCl, and TSP. Ingredients for 1 liter of Yeast Mannitol Agar (YEM) + Congo Red (CR) media in the form of 0.5 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 20 g NaCl, 10 g Mannitol, 1 g yeast extract, 20 g bacto agar, 2.5 ml congo red 1%. Ingredients for 1 liter of Pikovskaya media are 10 g glucose, 5 g Ca₃(PO₄)₂, 0.5 g (NH₄)₂SO₄, 0.2 g KCl, 0.1 g MgSO₄·7H₂O, 0.1 g MnSO₄, 0.1 g FeSO₄, 0.5 g yeast extract, and 15 g of agar, NA media material consisted of 2.8 g of NA and 100 ml of distilled water. The tools used in this study were polybags measuring 35 cm x 35 cm for planting soybean seeds, scissors, nameplates to mark each polybag, stakes and ropes to keep soybean plants from collapsing, solatip to cover the soil when it is about to be sterilized, barrels for soil sterilization, stove as a heater, autoclave for sterilizing laboratory equipment, analytical balance to weigh materials, shaker to homogenize solution, Erlenmeyer and test tube for the solution and liquid media, stirrer for mixing media making materials, a petri dish for media storage, ose for mixing media. taking bacterial solates, Bunsen for sterilization of ose, laminar air flow for propagation, micropipette for taking solution, oven for drying, object glass, and cover glass.

Experimental design. This research was carried out experimentally using a 3 x 4 factorial Completely Randomized Design (CRD) method with 3 replications. The first factor was the dose of urea fertilizer with N₀ = 100%, N₁ = 90%, N₂ = 80%. The second factor was the inoculation dose of *Rhizobium* sp. and phosphate solubilizing bacteria with information P₀ = no inoculum (control), P₁ = 3 g/seed, P₂ = 6 g/seed, P₃ = 9 g/seed. This research begins with the preparation stage in the form of making YEMA+CR and Pikovskaya media, propagation and rejuvenation of *Rhizobium* sp. sp bacteria, and making double inoculum of *Rhizobium* sp. sp bacteria. and phosphate solubilizing bacteria. In addition, the preparation of soybean seeds was also carried out, soybean seeds are mixed with inoculum, *Rhizobium* sp., and BFF according to the treatment dose. The seeds selected in this study were using superior and saline-resistant varieties of soybeans, namely the Anjasmoro variety. Furthermore, the preparation stage was carried out two weeks before planting by sterilizing the soil first using the steaming method. The sterilized soil was as much as 6 kg/polybag with a polybag size of 35 cm x 35 cm. Then seeding was carried out in ordinary soil and 7 days after sowing, the plant seeds were transplanted into saline soil to observe their growth. Fertilization treatment was carried out using a single Urea fertilizer supplemented with a companion fertilizer, namely TSP fertilizer, and KCl was applied 2 times to soybean plants. The first fertilization was carried out after the plants were 21 days after planting with a dose of the total dose. The second fertilization was done when the first flowers appear on the main stem. Fertilization with fertilizer was done circularly at a distance of 10 cm from the plant stem. Observations were made once a week by observing the predetermined parameters. Harvesting was carried out when the plant was 11 MST (Sunday After Planting) by picking the pods and then

dismantling all parts of the soybean plant and then placing them in a plastic bag for further observation. Parameters observed were plant height, number of leaves, early flowering time, number of pods, and wet weight of pods.

3. Results and Discussions

General Condition of Research Site

The soil used for the study was taken from ex-pond land in the Tugurejo Village area, Tugu District, Semarang City, Central Java. Location is located in (Figure 1).

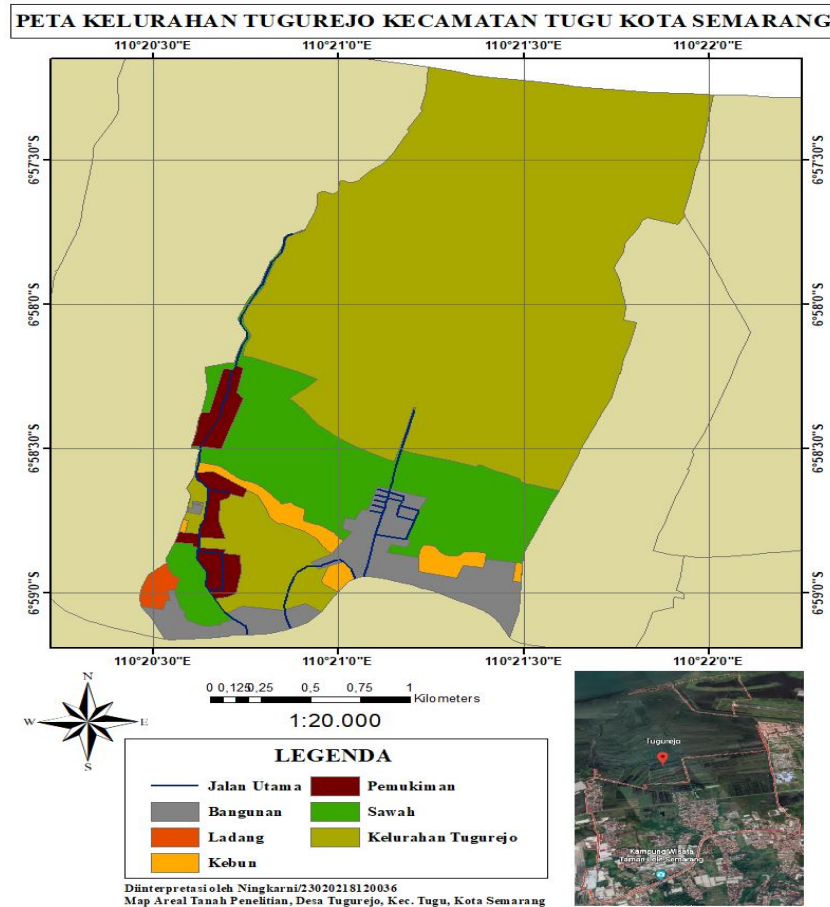


Figure 1. Location of Research Land Retrieval, Tugurejo Village, Tugu District, Semarang City.

Based on Figure 1. Tugurejo Village is one of the villages located in the peninsula bay of Semarang City which is dominated by fish ponds, open land, and some agricultural land. The location in illustration 1 is an interpretation of the point where the saline soil took that was used for the study. During a prolonged dry season, the soil in the area mentioned above is prone to high salinity because the evaporation rate is too high with low rainfall. The opinion of [9] is that in the dry months the salinity is higher.

The soil in the Tugurejo location is dominated by alluvial soil types and some regosols. Soil characteristics consist of the analysis of chemical properties including the availability of macronutrients (Nitrogen, Phosphorus, Potassium, Salinity, pH) as well as analysis of soil physical properties including sand fraction, dust fraction, and clay fraction as indicators in determining soil texture. Soil characteristics can be seen in Table 1.

Table 1. Characteristics of Chemical and Physical Properties of Research Soil

Characteristics	Value	Criteria
N (%)	0,25	Medium*
P ₂ O ₅ (%)	0,17	Very low*
K ₂ O (%)	0,57	Very low*
Salinity (dS/m)	11,65	Very high*
pH H ₂ O	6,61	Slightly Alkaline*
Sand Fraction (%)	50	Sandy Clay*
Dust Fraction (%)	60	
Clay Fraction (%)	40	

*Soil Analysis Result Assessment Based on Soil Research Institute, 2009

Based on the table above, the initial soil conditions and before being mixed with other materials had a Nitrogen content of 0.25% and classified as moderate and a Phosphorus content of 0.17, and a Potassium content of 0.57 and classified as very low and poor physical properties. According to [10] the characteristics of alluvial soil are heavy clay texture with low N content and organic matter and slightly alkaline pH content. Based on the results of the electrical conductivity analysis, the sample soil has a very high salinity content. This is supported by the opinion of [11] that the results of soil analysis with a value of 8-16 dS/m fall into the high salinity class.

One effort to improve soil content is to add organic fertilizer at the beginning as a basic fertilizer mixture. The results of the analysis of saline soil after experiencing the addition of cow manure can be seen in Table 2.

Table 2. Characteristics of Chemical and Physical Properties of Research Soil After Adding Cow Manure

Characteristics	Value	Criteria
N (%)	0,29	Medium*
P ₂ O ₅ (%)	0,18	Very low*
K ₂ O (%)	0,58	Very low*
Salinity (dS/m)	9,26	Very high*
pH H ₂ O	6,83	Slightly alkaline*
Sand Fraction (%)	50	Sandy Clay*
Dust Fraction (%)	60	
Clay Fraction (%)	40	

* Soil Analysis Result Assessment Based on Soil Research Institute, 2009

Based on Table 2. above, it can be seen that the results of the analysis of saline soil after adding cow manure increased the nutrient content and the value of Electrical Conductivity became lower even though the overall soil condition was still classified as unfavorable criteria. This is supported by the opinion of [12] that the addition of cow manure can increase the accumulation of silica and decrease the electrical conductivity in the soil. The salinity of the soil used is classified as having very high salinity criteria. This is to the opinion of [13] that soil can be categorized as having saline content if it has passed the Electrical Conductivity test and produces a value of more than 4 dS/meter. This is also supported by the opinion of [14] that the salinity content of 8.19 dS/m belongs to the high salinity criteria.

Plant Height

The results of the analysis of variance in Table 3. show that there is an interaction between the urea fertilization treatment and the application of *Rhizobium* sp. and phosphate solubilizing bacteria both had an effect ($P < 0.05$) on the plant height of soybean varieties of Anjasmoro. Duncan's test results are presented in Table 3.

Table 3. The Average Yield of Soybean Plant Height Due to urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria

<i>Rizobhium</i> sp. and Phosphate Solubilizing Bacteria	Urea fertilizer			Average
	Fertilized 100%	Fertilized 90%	Fertilized 80%	
	-----cm-----			
Addition of bacteria 0 g/seed	88 ^{ab}	88,5 ^{ab}	76,1667 ^{bc}	84,22
Addition of bacteria 3 g/seed	74,25 ^{bc}	87,1667 ^{ab}	76 ^a	79,14
Addition of bacteria 6 g/seed	59,3333 ^c	75,6667 ^{bc}	97,8333 ^{ab}	77,61
Addition of bacteria 9 g/seed	90,8333 ^{ab}	77,6667 ^{ab}	88 ^{ab}	85,50
Average	78,10 ^a	82,25 ^a	84,5 ^a	

Explanation:

- Figures followed by different superscripts on the data showed significantly different results according to Duncan's Test ($P < 0.05$)
- Numbers followed by the same superscript on the mean line are not significantly different according to Duncan's test ($P > 0.05$)

Based on the results of Duncan's test, it was found that the treatment of urea fertilization and the application of *Rhizobium* sp. effect on soybean plant height (Table 1). Soybean plant height in each treatment was not significantly different, this study showed that there was no variation in the character of soybean plant height due to urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria. 80% urea fertilization equivalent to 0.41g/polybag gave the highest yield of 84.5cm while 100% urea fertilization or equivalent to 0.52g/polybag gave the lowest yield of 78.10cm. According to the opinion Lingga and Marsono (2006) of [15] which states that excess nitrogen content in plants can be the cause of nutrient imbalances so that plant growth is disrupted. Soybean plants require a small amount of urea fertilizer. According to the opinion of the [16] which states that soybeans require a small dose of nitrogen, which is only about 25kg/ha in land conditions.

The application of double inoculation of bacteria, namely *Rhizobium* sp. and phosphate solubilizing bacteria had no significant effect on plant height yields. The bacterial inoculation dose of 9g/seed gave the best results on soybean plant height according to Table 3. In the opinion of Zulaikah and [17] which stated that the administration of *Rhizobium* sp. able to increase soybean plant height. This is in line with the opinion of [18] that the P element in phosphate solubilizing bacteria is able to increase the growth of plant cells so that it will strengthen the stem and then the plant height will be formed.

Based on Table 3. it is known that there is an interaction between urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria on soybean plant height. The highest soybean plant height

was obtained at 80% urea fertilization or equivalent to 0.41g/polybag fertilization and the application of double bacterial inoculation of *Rhizobium* sp. and phosphate solubilizing bacteria with a dose of 6g/polybag which is 97.83cm. According to [19] that inoculation of *Rhizobium* sp. bacteria and N contribution can stimulate the growth of roots, stems and leaves.

Number of Leaves

The results of the analysis of variance (Table 4.) showed that there was no interaction between urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria on the number of soybean leaves. Treatment of urea fertilization and application of *Rhizobium* sp. and phosphate solubilizing bacteria had no significant effect ($p > 0.05$) on the number of leaves of soybean plants. Duncan's test results are in table 4.

Table 4. The Average Yield of Soybean Plant Leaves Due to urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria

<i>Rizobhium</i> and Phosphate Solubilizing Bacteria	Fertilized 100%	Urea fertilizer Fertilized 100%	Fertilized 100%	Average
-----Petioles -----				
Addition of bacteria 0 g/seed	4,75	5,41	5,38	5,18
Addition of bacteria 3 g/seed	4,89	4,38	5,33	4,76
Addition of bacteria 6 g/seed	4,24	4,30	4,57	4,37
Addition of bacteria 9 g/seed	4,65	4,96	4,79	4,80
Average	4,56	4,76	5,02	

Explanation: There are no numbers followed by superscripts in the average because there are no significantly different results in Duncan's Test

Based on the results of the Duncan test that has been carried out, it shows that the results of the analysis do not show any effect of urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria on the number of leaves of soybean plants. 100% urea fertilization or equivalent to 0.52g/polybag gave the lowest average number of leaves of 4,556. This is presumably because the nitrogen content that is too high in the soil can interfere and slow down plant growth. In line with the opinion of Pasaribu and Suprpto (1993) in the research of [20] that the N content due to fertilization that is too high can inhibit seeding and reduce the amount of tethered N so that plant growth is inhibited. However, this is not in line with the research of Lakitan (1996) in the study of [21] that nitrogen nutrients are the most influential nutrient on leaf growth and development.

Soil treatment without the application of *Rhizobium* sp. and phosphate solubilizing bacteria gave the highest yield on the number of leaves of soybean plants, namely 5.181. This is back to the opinion of [17] which states that the addition of *Rhizobium* sp. can increase plant height, wet weight and number of leaves of soybean plants. This is also not in line with previous research from [22] which stated that the higher the P uptake, the higher the number of leaves on soybean plants.

Flowering Time

The results of the analysis of variance (Table 5) showed that there was no interaction between the urea fertilization treatment and the application of *Rhizobium* sp. and phosphate solubilizing bacteria on the early flowering time of soybean plants. Urea fertilization treatment had an effect ($P < 0.05$) on the initial flowering time, while the application of *Rhizobium* sp. and phosphate solubilizing bacteria had no effect ($P > 0.05$) on the early flowering time of soybean plants. Duncan's test results are presented in Table 5.

Table 5. Early Flowering Time of Soybean Plants Due to Urea Fertilization and Application of *Rhizobium* sp. And Phosphate Solubilizing Bacteria

<i>Rizobhium</i> and Phosphate Solubilizing Bacteria	Urea Fertilizer			Average
	Fertilized 100%	Fertilized 90%	Fertilized 80%	
	-----Week-----			
Addition of bacteria 0 g/seed	4,67	4,67	5,00	4,78
Addition of bacteria 3 g/seed	4,67	4,67	5,00	4,78
Addition of bacteria 6 g/seed	4,33	4,67	5,00	4,67
Addition of bacteria 9 g/seed	4,67	4,00	5,00	4,56
Average	4,58 ^a	4,50 ^b	5,00 ^b	

Explanation: There were no numbers followed by superscripts in the mean line, indicating that there were no significantly different results according to Duncan's test ($p < 0.05$).

Based on the results of the Duncan test, it was found that the urea fertilization treatment had an effect on the early flowering time of soybean plants (table 3). The early flowering time of soybean plants with 100% fertilization or equivalent to 0.52g/polybag was significantly different from 90% and 80% fertilization or equivalent to 0.46g/polybag and 0.41g/polybag, but 90% urea fertilization was not significantly different from fertilization urea 80%. Fertilization was carried out using a combination of Urea, TSP and KCL fertilizers with a dose of TSP fertilizer of 4 g/polybag and KCL fertilizer of 2.13 g/polybag as well as urea fertilizer according to treatment. Fertilization is done when the plants are 21 days old after transplanting and the second fertilization is done when the first flowers appear. This is in line with Suryanti's 2009 research on the research of [23] which states that the best time for N fertilization is 3 weeks after planting and at the beginning of flowering. According to [24] stated that at the beginning of flowering the bacterial content of *Rhizobium* sp. and less active phosphate solubilizing bacteria work to fix nitrogen in the soil so that additional fertilization is needed so that plant needs for nutrients are met so that plant production activities can run well.

The application of various doses of bacteria did not affect the early flowering time of soybean plants. In general, in this study, soybean plants grown in saline soil experienced faster flowering times than those grown in ordinary soil. This is in accordance with the opinion of [25] which states that soil salinity can play a role in accelerating the time of flower emergence. High salinity conditions of 8dS/m made the two bacteria *Rhizobium* sp. and phosphate solubilizing bacteria cannot live and adapt. This is in line with [5] that salinity stress has a negative impact on microorganisms in it. In addition, soil salinity that is too high is also an obstacle to the formation of root nodules on soybeans. This is in line with the opinion of [5] that inoculated bacteria are

expected to survive and reproduce under salinity stress, but the inoculation will experience infectibility so that the ability to form nodules in legumes will decrease.

Pods Wet Weight

The results of the analysis of variance (Table 6.) showed that there was no interaction between the urea fertilization treatment and the application of *Rhizobium* sp. and phosphate solubilizing bacteria on wet weight of soybean pods. Provision of urea fertilizer and application of *Rhizobium* sp. and phosphate solubilizing bacteria had no significant effect ($P > 0.05$) on the wet weight of soybean pods in saline soil. Duncan's test results are presented in Table 6.

Table 6. Wet Weight of Soybean Pods Due to Urea Fertilization and Application of *Rhizobium* sp. And Phosphate Solubilizing Bacteria

<i>Rizobhium</i> and Phosphate Solubilizing Bacteria	Urea Fertilizer			Average
	Fertilized 100%	Fertilized 90%	Fertilized 80%	
	-----gram-----			
Addition of bacteria 0 g/seed	1,17	1,13	1,09	1,13
Addition of bacteria 3 g/seed	3,24	3,42	3,28	3,32
Addition of bacteria 6 g/seed	3,26	3,22	3,29	3,26
Addition of bacteria 9 g/seed	3,24	3,19	3,28	3,24
Average	2,73	2,74	2,74	

Explanation: There were no numbers followed by superscripts in the mean line, indicating that there were no significantly different results according to Duncan's test ($p < 0.05$).

Based on the results of Duncan's test, it was found that the treatment of urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria did not significantly affect the wet weight of soybean pods. The average wet weight of soybean pods due to urea fertilization was 2.74 and showed no significant difference between urea fertilization with doses of 100%, 90% or 80%. The low wet weight production of soybean pods is thought to be influenced by greenhouse environmental conditions that have too high temperature and humidity, causing excessive evaporation so that the water content contained in the plant continues to decrease and causes low wet weight of pods. According to [26] the wet weight of soybean pods is influenced by the water content of the skin of the pods and soybean seeds. The climatic conditions in the greenhouse also affect the yield of the total wet weight of the pods. According to a statement from [27] which states that unpredictable climatic conditions cause soybean plant growth to be less than optimal so that production decreases.

4. Conclusions and Suggestions

Urea fertilization has a significant effect on the yield of plant height and early flowering time of soybean plants. Treatment of urea fertilization as much as 80% or equivalent to 0.41g/polybag gave the best results for plant height and early flowering time of soybean plants. While the application of *Rhizobium* sp. and phosphate solubilizing bacteria did not significantly affect soybean yield and production. While the interaction treatment

between urea fertilization and the application of *Rhizobium* sp. and phosphate solubilizing bacteria had a significant effect on the high yield of soybean plants.

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