IOP Conference Series: Earth and Environmental Science

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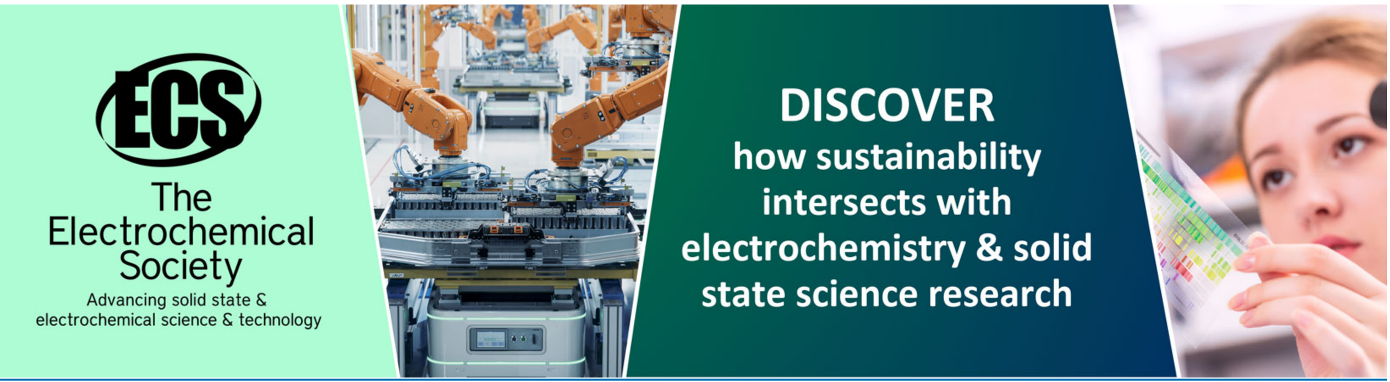
To cite this article: Y Hasanah *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1241** 012033



IOP Conf. Series: Earth and Environmental Science 1241 (2023) 012033 doi:10.1088/1755-1315/1241/1/012033

The role of Molybdenum in relation to *Rhizobium* sp. in increasing biological Nitrogen fixation and soybean growth

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Abstract. Soybean is a legume that can form a mutualism symbiosis with *Rhizobium* bacteria to bind N. Increasing the effectiveness of N fixation by Rhizobia requires the role of micronutrient Molybdenum (Mo), because Mo is needed for nitrate reductase enzyme for NO_3^- assimilation. The objective of the research was to evaluate the role of Mo and *Rhizobium* sp. inoculation in the growth of soybean and increase the root nodules number. A factorial randomized block design was used in the research. The first factor was inoculation of *Rhizobium* sp. (0; 5 and 10 ml/plant), and the second factor was the application of Mo (0; 200; 400 and 600 g/ha). The research result showed that inoculation of *Rhizobium* sp. up to 10 ml/plant increased plant height, number of leaves and leaf area. Application of molybdenum up to 400 g/ha was able to increase the number of nodules formed. There was no significant interaction between *Rhizobium* sp. inoculation and application of Mo in all observed variables.

1. Introduction

Soybean (*Glycine max* L. Merrill) is the main legume which is a source of food, vegetable protein used for the food industry and public consumption. In addition, soybean act as a functional food because of the main secondary metabolites in soybean which is highly beneficial for health, namely isoflavones [1-2]. The role of isoflavones is preventing heart damage, degenerative diseases (prostate cancer, atherosclerosis, colon cancer and diabetes mellitus), osteoporosis, reducing menopausal syndrome and anti-tumour [3-5].

Soybean is one of the legumes that can symbiotic mutualism with *Rhizobia* bacteria to bind N. In this symbiosis Rhizobia get a place to live in root nodules, while soybean plants themselves get N from fixationby bacteria. This symbiosis produces root nodules containing Rhizobia bacteria which are able to fix N from the atmosphere. Rhizobia inoculation is expected to fulfil the nitrogen needs of soybean plants and reduce the use of inorganic nitrogen fertilizers. In general, biological nitrogen fixation as part of nitrogen input to support plant growth has decreased due to intensification of inorganic fertilization [6-7].

In increasing the effectiveness of N fixation by Rhizobia, the role of the micronutrient Molybdenum (Mo) is needed, because Mo is needed for the nitrate reductase enzyme for NO_3- assimilation. If the concentration of Mo in the soil is low or around 0.7 ppm it can inhibit the formation of root nodulesand interfere with N fixation carried out by rhizobia bacteria [8]. The inhibition of Rhizobium fixation will reduce the availability of N for plants, thereby disrupting the growth and production of soybean plants [9]. Molybdenum (Mo) is a micronutrient that plays an important role in plant growth and development, is indispensable in the biosynthesis of molybdate-dependent enzymes. Molybdoenzymes include nitric reductase, aldehyde oxidase, xanthine oxidase, sulphite oxidase, and nitrogenase. This enzyme plays an important role in the process of nitrogen, carbon and sulphur metabolism in plants. Naturally, the concentration of Mo in soil is 0.2-0.6 mg/kg soil, whereas metal-rich soils can contain 10–100 mg Mo/kg soil [10].

The results of previous study proved that on red-yellow podzolic soils the application of 150 g Mo/ha increased the growth and yield of soybeans, with the plant height of 70.00 cm and the number of branches of 8.42/plant, the number of seeds of 160.50 seeds/plant and seed dry weight of 13.43 g/plant. Application of Mo 150 g/ha produced the biggest seed dry weight, significantly different from without Mo, but not significantly with application of Mo 50 and 100 g/ha [11].

Based on the background, the objective of the research was to evaluate the role of Molybdenum in increasing the effectiveness of N fixation by *Rhizobium* sp. and soybean growth and can be utilized in a sustainable agriculture system as an environmentally friendly innovation.

2. Materials and methods

2.1. Site of the research and materials

This research was carried out at the research field of Faculty of Agriculture, Universitas Sumatera Utara, Medan, from July to September 2022. The tools used in this study were hoes, buckets for watering, analytical scales, ovens, containers for soaking seeds, meter for plant height, markers, calculators, and other research support tools. The materials used in this study were soybean seeds of Anjasmoro variety, N fertilizer from Urea (45% N), P_2O_5 from SP-36 (36% P_2O_5) and K_2O from KCl (60% K_2O), ammonium molybdate fertilizer as a source of molybdenum, rhizobium inoculants (collection of the Soil Biology Laboratory of Faculty of Agriculture, Universitas Sumatera Utara), polybags, and other supporting materials.

2.2. Experimental design and crop management

A factorial randomized block design was used in the research with 2 factors and 3 replications. The first factor was application of *Rhizobium* sp. (0; 5 and 10 ml/plant) and the second factor was application of Molybdenum (0; 200; 400 and 600 g/ha).

The planting land used was first cleaned of weeds in the area by using a hoe. After that, plots were madewith a length of 110 cm and a width of 110 cm. Top soil is prepared as a planting medium, the top soil is air-dried and sieved, then 10 kg of top soil is put into the polybag. Then the polybags are arranged based on the distance between the polybags, which is 40 cm x 20 cm.

Soybean seeds of the Anjasmoro variety were planted in polybags provided at a depth of 1.5-2 cm. Soybean seeds planted amounted to 2 seeds/polybag. *Rhizobium* sp. was applied within a week after planting, or after the emergence of roots on soybean plants, *Rhizobium* sp. application was given accordingto a predetermined dose with a rhizobium population density of 10^8.

Molybdenum fertilization is given in the form of Ammonium Molybdate according to a predetermine dose at 1 week after planting (WAP). Into 10 ml of water added ammonium molybdate then sprinkled around the roots of the soybean plants.

Watering is carried out in the morning or evening according to land conditions, if it rains, there is no need for watering. The basic fertilization applied was 25 kg Urea/ha given a half dose at the planting time and the rest at 4 WAP, 100 kg/ha SP-36 was given at the beginning of planting, and 75 kg/ha KCl was givenat the beginning of planting. Weeding is done manually by removing weeds around the plant and using asmall hoe. Harvesting is done when the plant has shown physiological maturity, namely brownish yellowpods. Harvesting is done by picking ripe pods.

2.3. Analysis of data

The analysis of data is using analysis of variance and continue with Duncan's Multiple Range Test at the $\alpha = 5\%$

3. Result and discussion

3.1. Plant height

Application of *Rhizobium* sp. up to 10 ml/plant increases plant height. This phenomenon showed the role of *Rhizobium* sp in plant growth as indicated by the increase in plant height 4-6 WAP (Table 1).

Previous researchers also stated the same thing that the inoculation of *Rhizobium* sp. increased plant height compared to non-inoculated plants. The effectiveness, efficiency and compatibility of the inoculants used will determine the success of the symbiosis of bacteria with their host plants. The ability of each inoculant differs depending on the conditions of the inoculant and is unable to compete with indigenous microbes [12].

Table 1. Plant height 2-4 WAP of soybean with application of *Rhizobium* sp. and Molybdenum.

WAP	Molybdenum (g/ha)	*Rhizobium* sp. (ml/plant)			Mean
		R0 (0)	R1 (5)	R2 (10)	
		..........cm..........			
2	M0 (0)	14.75	14.42	14.58	14.58
	M1 (200)	14.92	13.42	14.17	14.17
	M2 (400)	15.00	15.92	15.46	15.46
	M3 (600)	14.33	15.00	14.67	14.67
	Mean	14.75	14.69	14.72	
3	M0 (0)	18.75	20.17	20.58	19.83
	M1 (200)	20.33	19.58	20.42	20.11
	M2 (400)	19.17	21.50	20.90	20.52
	M3 (600)	18.58	21.25	19.92	19.92
	Mean	19.21	20.63	20.45	
4	M0 (0)	25.42	29.75	28.42	27.86
	M1 (200)	26.67	25.75	31.42	27.94
	M2 (400)	27.58	27.92	32.75	29.42
	M3 (600)	25.08	27.58	31.25	27.97
	Mean	26.19b	27.75ab	30.96a	
5	M0 (0)	30.42	37.83	38.08	35.44
	M1 (200)	32.08	32.75	41.92	35.58
	M2 (400)	32.50	35.42	42.17	36.69
	M3 (600)	30.42	36.42	41.67	36.17
	Mean	31.35c	35.60b	40.96a	
6	M0 (0)	34.08	46.33	46.67	42.36
	M1 (200)	35.83	42.75	50.50	43.03
	M2 (400)	39.25	46.83	51.42	45.83
	M3 (600)	34.50	47.42	51.08	44.33
	Mean	35.92c	45.83b	49.92a	

Note: The same letters behind the numbers at the same time of observation are not significantly different according to Duncan's Multiple Range Test at α =5%.

3.2. Number of leaves
Inoculation of *Rhizobium* sp. up to 10 ml/plant increased the number of leaves at 3-5 WAP compared to without *Rhizobium* sp. Application of Mo, interaction between *Rhizobium* sp. and the application of Mo had no significant effect on the number of leaves 2-6 WAP (Table 2).

The increase in the number of leaves by increasing the inoculation of *Rhizobium* sp. until 10 ml/plant related to the role of *Rhizobium* sp. in increasing the availability of N for soybean plants. For plants, N plays a very important role in vegetative growth, one of which is increasing the number of leaves. This is in line with the statement of Igiehon *et al.* [13] that N is very important in the vegetative growth of plants.

Table 2. Number of leaves 2-6 WAP of soybean with application of *Rhizobium* sp. and Molybdenum.

WAP	Molybdenum (g/ha)	*Rhizobium* sp. (ml/plant)			Mean
		R0 (0)	R1 (5)	R2 (10)	
		..........leaves..........			
2	M0 (0)	2.33	2.50	2.67	2.50
	M1 (200)	2.42	2.50	2.75	2.56
	M2 (400)	2.58	2.92	2.92	2.81
	M3 (600)	2.50	2.75	2.50	2.58
	Mean	2.46	2.67	2.71	
3	M0 (0)	3.75	4.58	4.58	4.31
	M1 (200)	4.08	4.42	5.25	4.58
	M2 (400)	4.50	5.25	4.92	4.89
	M3 (600)	4.17	4.92	4.50	4.53
	Mean	4.13b	4.79a	4.81a	
4	M0 (0)	4.75	6.00	6.58	5.78
	M1 (200)	5.58	6.08	6.42	6.03
	M2 (400)	5.83	6.33	6.25	6.14
	M3 (600)	6.00	6.42	6.50	6.31
	Mean	5.54b	6.21a	6.44a	
5	M0 (0)	7.17	7.58	8.17	7.64
	M1 (200)	7.00	7.83	8.17	7.67
	M2 (400)	7.67	7.67	8.33	7.89
	M3 (600)	7.58	7.92	8.00	7.83
	Mean	7.35b	7.75ab	8.17a	
6	M0 (0)	10.42	10.58	11.67	10.89
	M1 (200)	10.75	11.08	11.50	11.11
	M2 (400)	10.83	11.75	11.83	11.47
	M3 (600)	11.08	11.08	11.08	11.08
	Mean	10.77	11.13	11.52	

Note: The same letters behind the numbers at the same time of observation are not significantly different based on Duncan's Multiple Range Test at α =5%.

3.3. Leaf area
Inoculation of *Rhizobium* sp. increased leaf area, while application of molybdenum and the interaction between rhizobium and application of molybdenum are not significant effect on the leaf area (Table 3).

Increased *Rhizobium* sp. inoculation increased leaf area. this is related to the role of *Rhizobium* sp. in increasing N availability for plants. Nitrogen is the main component constituent of proteins. chlorophyll. enzymes. hormones and vitamins and play an active role in plant vegetative growth including an increase in leaf area. In line with previous research that N element plays a very important role in the formation and growth of vegetative organs (stems, leaves and roots), so that increasing the availability of N will increase the area of plant leaves which affects its assimilation. The N fixation process produces N which is released from the nodules in the form of urea (allantoin and allantoic acid) which is then translocated to the leaves and used for chlorophyll biosynthesis [14].

Table 3. Leaf is of soybean with application of *Rhizobium* sp. and Molybdenum.

Molybdenum (g/ha)	*Rhizobium* sp. (ml/plant)			Mean
	R0 (0)	R1 (5)	R2 (10)	
	..........cm^2..........			
M0 (0)	105.95	105.95	105.95	105.95
M1 (200)	97.97	97.97	97.97	97.97
M2 (400)	106.20	106.20	106.20	106.20
M3 (600)	107.90	107.90	107.90	107.90
Mean	104.50a	140.27b	149.63c	

Note: The same letters behind the numbers are not significantly different based on Duncan's Multiple Range Test at α =5%.

3.4. Number of nodules

Application of molybdenum had a significant effect on increasing the number of root nodules, while *Rhizobium* treatment and the interaction between rhizobium and molybdenum are not significant effect the number of nodules (Table 4).

Increasing the application of Mo up to 400 g/ha increases the number of root nodules. This is related to the role of Mo in the formation of root nodules, because Mo is a specific micronutrient for plants that form root nodules by N-fixing bacteria (*Rhizobium* sp.). In N fixation, Mo plays a very clear role in relation to Mo in the cofactor 'FeMoCo' which is at the nitrogen reduction center in the cofactor, at least for most nitrogenases [15]. The nitrogenase enzyme is a catalyst for the process of N fixation in the symbiosis of mutualism between legumes and rhizobia. The fixation process is controlled by the nitrogenase enzymes (Mo-Fe protein) and nitrogenase reductase (Fe protein). The nif gene expression level is positively correlated with nitrogenase activity [16].

Table 4. Number of nodules of soybean with application of *Rhizobium* sp. and Molybdenum.

Molybdenum (g/ha)	*Rhizobium* sp. (ml/plant)			Mean
	R0 (0)	R1 (5)	R2 (10)	
	..........cm^2..........			
M0 (0)	6.00	13.00	20.00	13.00b
M1 (200)	9.33	14.67	22.33	15.44ab
M2 (400)	14.33	20.00	25.67	20.00a
M3 (600)	13.33	18.00	23.67	18.33a
Mean	10.75	16.42	22.92	

Note: The same letters behind the numbers are not significantly different according to Duncan's Multiple Range Test at α =5%.

4. Conclusion

Inoculation of *Rhizobium* sp. up to 10 ml/plant increased plant height, number of leaves and leaf area. Mo applications up to 400 g/ha increased the number of root nodules. There was no significant interaction between *Rhizobium* sp. inoculation and application of Mo in this study.

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Acknowledgement

This research is part of the Universitas Sumatera Utara TALENTA Strategic/Thematic Research Grant. The authors would like to thank for Universitas Sumatera Utara-Research Institute for funding this research.