IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

Inoculation of rhizobium bacteria and nutrient of seawater to increase soybean production and quality as food

Inoculation of rhizobium bacteria and nutrient of seawater to increase soybean production and quality as food

E Fuskhah*, A Darmawati

Faculty of Animal Husbandry and Agricultural Sciences Diponegoro University Tembalang Campus, Semarang 50275 Indonesia

Corresponding author: eny_fuskhah@yahoo.com

Abstract. Seawater is abundant natural resources not fully utilized even though it contains nutrients needed by plant like N, P, K, Ca, and Mg. On the other hand rhizobium capable of forming effective root nodules of association with legumes and fix nitrogen. The research aim was to get information the benefit of seawater and rhizobium inoculation for soybean as food. The research held in green house of Animal Husbandry and Agriculture Faculty, Diponegoro University Semarang. Seawater was taken from Semarang Marina Beach. Soybean was local bean of Grobogan. The arranged design was completely randomized design with factorial design 4 x 2 in 4 replications. First factor was salinity level of seawater in a row L0, L1, L2, and L3 was EC 0, 1, 2, and 3 ds/m. Second factor was Rhizobium inoculation, R1 = without rhizobium inoculation, and R2 = with Rhizobium inoculation. The parameters were seed production, fresh weight production of straw, crude protein and crude fiber production of soybean seed. Based on variant analysis, there was no interaction seawater salinity level and rhizobium inoculation againt to all of the parameters. Seawater level and rhizobium inoculation tended to increase production and quality of soybean. The best result was treatment L2R2, that is EC 2 ds/m with rhizobium inoculation. The seed soybean production was 3191.25 kg/ha equal to 3,2 ton/ha, with 42.54 % crude protein content of seed, and 13.87 % crude fiber content of seed.

Keywords: rhizobium bacteris, nutrient seawater, soybean, quality food

1. Introduction

Soybean (*Glycine max* L.Merill) is a highly nutritious food commodities as a source of vegetable protein and low in cholesterol. Soybean is also an important food commodity after rice and maize. Consumption of soybean in the form of fresh or in processed form can improve nutrition. Soybean is one of the most important legume crops in the world representing a major component of the diet of food-producing animals and humans [1]; [2]. In Indonesia, many processed soybean for a wide variety of foods, such as bean sprouts, soy milk, tofu, bean curd, soy sauce, oncom, tauco, tempeh, and soy flour. In addition, it is also widely used as an animal feed ingredient. Soybean contains about 20% oil on dry matter basis with 30-50% of protein [3]. It also has superior amino acid profile and its protein has great potential as a major source of dietary protein and can play an important role to solve malnutrition problems [4]. Soybean has been recognized as one of the premier agricultural crops today, thus it is the best source of protein and oil and has now been recognized as a potential supplementary source of nutritious food [5].

Soybean is found in Family *Fabacea* [6]. The plant can also be used as soil fertility improvements if used as soil cover crop. Soybean can be grown as a sole crop, intercropped, or mixed with important cereals such as maize, sorghum and millets [7]. A temperature of 26.5 - 30°C appears to be the optimum for most of the varieties and a well drained and fertile loam soils with pH of 6.0 - 7.5 are most suitable for the cultivation of soybean [8]. Soybean is a legume plant has the ability mutualistic symbiosis with Rhizobium sp roots that grow in the area. The presence of these bacteria causes the formation of nodules that is capable of fixing free nitrogen from the air so that it can supply the crop of N. Crop nitrogen requirement by nitrogen fixation occurs not at the beginning of the vegetative phase and the end of the reproductive period, but occurred after the formation of nodules that occur because rhizosphere colonization and infection rhizobia in legume root [9]. The result of this symbiosis is expected to increase the production of forage crops.

Rhizobium inoculation is a significant technology for the manipulation of rhizobia for improving crop productivity and soil fertility. Rhizobium inoculation can lead to establishment of large rhizobia in the rhizosphere and improved nodulation and nitrogen fixation even under adverse soil conditions [10]. Soybean rhizobium inoculation is the process of applying rhizobium inoculants to the soybean seed before planting in order to increase the nitrogen fixation and nodulation of the soybean roots. Inoculating soybean provides adequate number of bacteria in the soybean root zone, so that effective nodulation will take place [8]. Research of Fuskhah *et al.* [11] showed that the use of Rhizobium inoculant of 20-60 g / kg of seed combined with phosphorus fertilization can increase dry matter forage of *Centrosema pubescens* Benth. The ability to fix nitrogen can reduce the cost of artificial fertilizer N, so the application of Rhizobium inoculation in legumes is very important to stimulate nitrogen fixation.

On the other hand, the seawater is so abundant in Indonesia turned out to contain many ions, including ions that plants need. The content of elements needed by plants such as magnesium (Mg), calcium (Ca) and potassium (K) in the seawater is high enough [12]. This shows that the seawater can be an alternative source of nutrients or nutrients for plants. The average concentration of dissolved salts in seawater around 3.5%, depending on the location and rate of evaporation [13]. Among these salts, the concentration of sodium (Na) and chloride (Cl) is dominant and there in large numbers resulting in high salinity [14]. Therefore, in use, the seawater need to be diluted in advance to reduce the salinity until the threshold that does not harm the plant. The seawater is in practice widely used to irrigate crops tolerant to salinity (halophyte) in areas near the coast. Turi (*Sesbania grandiflora*), according to research of Fuskhah *et al*. [15] resistant to high levels of NaCl, up to 4000 ppm NaCl equivalent to EC (electrical conductivity) 7.5 ds/m still showed an increase in production. It is suspected halophyta turi including plants that are resistant to high salinity levels.

The research aim was to get information the benefit of sea water and rhizobium inoculation for soybean as food. The study hypothesis was there was interaction between nutrients of sea water and Rhizobium bacteria inoculation to increase the production and quality of soybean.

2. Materials and Methods

The research was conducted in the green house of Ecology and Plant Production Laboratory of Animal Husbandry and Agriculture Faculty, Diponegoro University Semarang in 2015. Sea water as sample was taken from Semarang Marina Beach. Soybean which choosen was local bean of Grobogan. The arranged design was completely randomized design with factorial design 4 x 2 in 4 replications.

*Materials***:** sea water, water hyacinth, Rhizobium isolate that isolated from soybean, Grobogan soybean seed, EC meter, pot, fertilizer N, P and K, 95% ethanol, sublimat 0.1%, distilled water, yeast media mannitol agar (YMA), congo red (CR), brome thymol blue (BTB), glucose peptone agar (GPA), brome cresol purple (BCP), NaCl, aluminum foil, rubber bands, cotton, plastic, test tubes, tweezers, erlenmeyers, rod stirrer, petri dishes, ose, oven, autoclave.

*Methods***:** A total of 32 pots filled with 10 kg soil and sterilized. Soybean seeds prepared. Basic fertilizer used fertilizer N, P, and K respectively with a dose of 100 kg N / ha, 150 kg P2O5 / ha and 100 kg K2O / ha. The sea water is used for watering with the corresponding dilution dose treatment. The experimental design used was completely randomized design pattern 4 x 2 factorial with four replications. The first factor was salinity level of sea water :

L0 = no seawater (fresh water) and without mulch of water hyacinth (control)
L1 = seawater level 1 ds/m and 8 tons / ha of water hyacinth mulch
L2 = seawater level 2 ds/m and 8 tons / ha of water hyacinth mulch
L3 = seawater level 3 ds/m and 8 tons / ha of water hyacinth mulch
The second factor was the Rhizobium bacteria inoculation treatment :
R1 = without Rhizobium bacteria inoculation
R2 = with Rhizobium bacteria inoculation

Isolation of Rhizobium :

Nodule samples taken from Grobogan soybean root nodules. Nodules collected, then performed the sterilization, isolation, identification according to Vincent [16].

a. Collection of nodules
Soybean root nodules collected by cutting the root nodules of 0.5 cm on each side of the nodule.
b. Sterilization of nodules
Nodules soaked in a solution of 95% ethanol for 5-10 seconds, then soaked in a solution of the sterilizing sublimat 0.1% for 3-5 minutes, then washed five times with sterile water.
c. Isolation of Rhizobium
Sterile nodules destroyed with tweezers, then fluid nodules grown in medium YMA + CR plates, incubated for 3-5 days at room temperature (28^{o}C)
d. Identification of Rhizobium
Colonies were grown separately and subsequently identified as Rhizobium bacteria grown on medium identification, using slant medium YMA + CR, YMA + BTB, and GPA + BCP. Colonies grow well, white, and red color of the medium remains that the media YMA + CR. Colonies grow well, the medium color changed from green to yellow at BTB + YMA media, and the colony grows not good and the color of the medium remains are purple on media GPA + BCP. Next, tested with dye of Gram. Rhizobium was negative Gram. All Rhizobium obtained collected. Pure culture obtained can then be saved as a stock culture of Rhizobium.

The parameters observed were seed production, fresh weight production of straw, crude protein and crude fiber production of soybean seed. The data obtained were analyzed using ANOVA and to know the difference between treatments was followed by Duncan's Multiple Range Test [17].

3. Results and Discussion

Soybean production

Results of analysis of variance showed that there was no interaction between seawater treatment and rhizobium bacteria inoculation against the seed production (Tables 1).

Table1. Average of seed production of Grobogan soybean on various level of seawater watering and rhizobium bacteria inoculation

Seawater Salinity levels	Rhizobium inoculation		Average
	R1 (without)	R2 (with inoculation)	
	------------ (kg /ha) -----------		
L0 (without seawater)	1900.00[b]	2599.38[ab]	2249.69
L1 (EC 1 mmhos / cm)	2381.88[ab]	2371.25[ab]	2376.56
L2 (EC 2 mmhos / cm)	2791.25[ab]	3191.25[a]	2991.25
L3 (EC 3 mmhos / cm)	2043.75[b]	2705.00[ab]	2374.38
Average	2279.22	2716.72	

Remarks: The average numbers followed by the different letter are significantly different ($p<0.05$)

Table 1, results of analysis of variance showed that there was no interaction between seawater treatment and rhizobium bacteria inoculation against the seed production of Grobogan soybean. However, based on Duncan's multiple range test, there are differences on some combination of treatments. Treatment R2L2 significantly different with R1L0 and R1L3, while R2L2 not significantly different between the R2L0, R2L1, R2L3, R1L1, R1L2. The highest seed yield indicated by R2L2 treatment, treatment with Rhizobium inoculation and watering seawater EC 2 ds/m is equal to 3191.25 kg / ha or 3.2 ton / ha followed by R1L2, R2L3, R2L0, R1L1, R2L1, R1L3 and R1L0 each of 2791.25; 2705.00; 2599.38; 2381.88; 2371.25; 2043.75; 1900.00 kg / ha. R1L0 is the lowest result that is treatment without Rhizobium inoculation and without watering it with seawater.

Watering with seawater in soybean tend to increase seed production of Grobogan soybean compared with no watering with seawater. Seawater contains large amounts of dissolved salts, with about 3.5% by weight [13]. These salts contain high amounts of Na, K, Ca and Mg with about 10.556, 380, 400, 1300 mg/l respectively. Given the high content of cations, sea water can be used as a nutrient source for plants including plants that are sensitive to high salt levels (glycophyte plants) [12]. Sodium is known as the additional elements that are profitable and for some types of plants it can replace some of the functions K [18]. Natrium influence will be very large when K supply to plants is inadequate [19]. This element can reduce the impact caused by a deficiency of K but can not fully replace the function of K [19].

Seawater has also been studied for its effect on cereal germination, and oil seed crops in India [20]. It was found that cereal crops such as wheat, barley, rice and maize were more tolerant to high salinity compared with oil seed crops such as sesame, peanut, sunflower and mustard. Membrane transport both at the root and translocation within the plant is the main characteristic of plants tolerant to high salinity [21].

Rhizobium bacteria inoculation was also tend to increase seed production of Grobogan soybean. This study is in line with [11] in *Centrosema pubescens* Benth that show increased production in plants inoculated with Rhizobium in line with the increase in the amount of inoculum. Soybean plants in India is increasing the percentage of harvest is between 25.75% to 52% with Rhizobium inoculation compared with controls. *Cajanus cajan* 19.47% increase crop yields in plants inoculated with Rhizobium and *Cicer arietinum* increase the harvest of 20.94% on inoculated with Rhizobium [22].

Soybean is a legume plant has the ability mutualistic symbiosis with Rhizobium sp that grow in the rhizosphere. The presence of these bacteria causes the formation of nodules that is capable of fixing free nitrogen from the air so that it can supply the crop needs of N. Plant roots provide nutrients and carbohydrates for energy bacteria and bacteria provide nitrogen compounds fixed [23]. The result of this symbiosis is expected to increase the production of seed.

Soybean plants more tolerant to seawater compared to peanuts, sunflower and other grains. Various minerals contained in sea water include chlorine (Cl) role is to enhance and improve the quality and quantity of crop production, the elements Na and K assist in the formation of proteins, sugars and carbohydrates, the elements Mg helps the formation of carbohydrates, amino acids, vitamins, sugars and fats, as well as elements Ca contribute to reinforcing rods also help pollinate plants and the acceleration of the growth of the seeds.

Soybean production is influenced by various factors such as the results of N fixation by Rhizobium. Besides Rhizobium bacteria capable of fixing N also can keep the soil fertile, so the plants will easily grow and good evolve. Plant underserved N supply will grow stunted and the leaves turn yellow. Plants with less nitrogen will grow up with a lot of yellow leaves and easy to fall in with the plants become stunted and have a limited root system. Rhizobium association with soybean plants can increased production.

Soybean Quality

Results of analysis of variance showed that there was no interaction between seawater treatment and rhizobium bacteria inoculation against the quality parameters, namely the crude protein and crude fibre contents of soybean seed (Tables 2 and 3).

Table 2. Average of crude protein content of Grobogan soybean seed on various level of sea water watering and rhizobium bacteria inoculation

Seawater Salinity levels	Rhizobium inoculation		Average
	R1 (without)	R2 (with inoculation)	
	----------- (%) -----------		
L0 (without seawater)	41.80^{ab}	41.99^{ab}	41.89
L1 (EC 1 ds/m)	42.01^{ab}	42.69^{a}	42.35
L2 (EC 2 ds/m)	41.32^{ab}	42.54^{a}	41.93
L3 (EC 3 ds/m)	40.56^{b}	42.32^{ab}	41.44
Average	41.42^{b}	42.38^{a}	

Remarks: The average numbers followed by the different letter are significantly different ($p<0.05$)

Table 2, results of analysis of variance showed that there was no interaction between seawater treatment and rhizobium bacteria inoculation against the crude protein production of Grobogan soybean. However, based on Duncan's multiple range test, there are differences on some combination of treatments. Treatment R2L1, R2L2 significantly different with R1L3, while not significantly different with the others. The highest crude protein production indicated by R2L1 treatment, treatment with Rhizobium inoculation and watering seawater EC 1 mmhos / cm is equal to 42.69% followed by R2L2, R2L3, R1L1, R2L0, R1L0, R1L2 and R1L3 each of 42.54; 42.32; 42.01; 41.99; 41.80; 41.32; 40.56 %. R1L3 is the lowest result that is treatment without Rhizobium inoculation and watering with seawater EC 3 ds/m.

Seawater-based agriculture has been developed especially to plants belonging halophytic planted near the beach where lack of clean water. Various studies provide great opportunities in the cultivation of plants that produce foliage halophytic especially for feed and seed use sea water. The halophytic plants were irrigated with sea water has a high nutritional and easily digested. Seeds of plants accumulate salt halophytic not exceed glycophytic plants and contains protein and oil content is high, although irrigated with sea water with a high salt content.

Rhizobium inoculation has been able to increase crude protein content of soybean significantly. Soybean rhizobium inoculation is the process of applying rhizobium inoculants to the soybean seed before planting in order to increase the nitrogen fixation and nodulation of the soybean roots. Increased levels of crude protein in the soybean seed treatment with Rhizobium bacteria inoculation showed that the bacteria Rhizobium quite effective. Rhizobium inoculant addition of 20 ml / pot by the number of Rhizobium 2 x 10^9 cells / ml already meet the standards. Inoculating soybean provides adequate number of bacteria in the soybean root zone, so that effective nodulation will take place. It thus lead to increased crude protein content. On the other hand, crude fibre content of soybean as Table 3.

Table 3. Average of crude fibre content of Grobogan soybean on various level of sea water watering and rhizobium bacteria inoculation

Seawater Salinity levels	Rhizobium inoculation		Average
	R1 (without)	R2 (with inoculation)	
	------------ (%) -------------		
L0 (without seawater)	15.49	13.37	14.43
L1 (EC 1 ds/m)	14.52	15.12	14.82
L2 (EC 2 ds/m)	14.05	13.87	13.96
L3 (EC 3 ds/m)	15.65	14.20	14.92
Average	14.93	14.14	

Table 4, results of analysis of variance showed that there was no interaction between seawater treatment and rhizobium bacteria inoculation against the crude fiber production of Grobogan soybean. The tendency of increase in crude fiber content on the treatment by the addition of sea water is possible due to the high content of K, Ca, Mg in seawater [24]. The nutrient for plants, among others, to work harden the stem of the plant and stimulates the formation of seeds, the plants rapidly generative phase, so the crude fiber content is high. Potassium (K) serves to strengthen the body of the plant, raising straw and wooden parts of plants, so that the leaves, flowers and fruits are not easily fall.

Rhizobium inoculation has not been able to decrease production of crude fiber of soybean seed significantly. Factors that led to persistently high crude fiber content of soybean that is the sea water salinity stress high salt content NaCl resulting in a lack of effective processes N2 fixation by Rhizobium bacteria. The salt stress causes a decrease in N2 fixation activity which impact on the nodule respiration [25]. Salt stress will reduce nodule formation by inhibiting the initial process of symbiosis with the host plant. Inhibition of the formation of nodules by salinity is associated with decreased colonization of Rhizobium and reduced the formation of root hairs [26].

4. Conclusions

The results showed there was no interaction between seawater salinity level and rhizobium inoculation againt to production and quality of soybean seed. Rhizobium inoculation increased production and quality of soybean seed. The best result was treatment L2R2, that is EC 2 ds/m with rhizobium inoculation. The seed soybean production was 3191.25 kg/ha equal to 3,2 ton/ha, with 42.54 % crude protein content of seed, and 13.87 % crude fiber content of seed.

Acknowledgements
The authors are grateful to the Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education of Indonesia for providing fund to conduct this study.

References

[1] Hapgood F. 1987. The prodigious soybean. National Geographic, 172: 66–91.
[2] Friedman M., and Brandon D. 2001. Nutritional and health benefits of soy proteins. Journal of Agricultural and Food Chemistry, 49: 1069–1086.
[3] Kwarteng JA. and Towler MJ . 1994. West Africa Agriculture. A textbook for schools and colleges, by Macmillan Publication Press Limited, pp 144-116.
[4] Ruhul AAKM, Jahan SRA, Karim MF, Hasanuzzaman M. 2009. Growth dynamics of soybean (*Glycine max* L.) as affected by varieties and timing of irrigation. American-Eurasian Journal of Agronomy 2: 95-103.
[5] Wilcox JR, and Shibles RM. 2001. Interrelationships among seed quality attributes in Soybean. Crop Sciences. 41(1):11-14.
[6] Shurtleff W., and Aoyagi A. 2007. History of Green Vegetable Soybeans and Vegetable Type Soybeans. Lafayate California

[7] Mmbaga ET, and Friesen D . 2003. Adoptable maize/legume systems for improved maize production in northern Tanzania. African Crop Science Conference Proceedings, Vol. 6.649-654.

[8] Lamptey S., Ahiabor B D K., Yeboah S., Asamoah C. 2014. Response of soybean (*Glycine max)* to rhizobial inoculation and phosphorus application. Journal of Experimental Biology and Agricultural Sciences. 2(1) : 72-77

[9] Beck, D.P., J.Wery, M.C. Saxena, and A.Ayadi. 1991. dinitrogen fixation and nitrogen balance in cool season food legumes. Agronomy Journal 83: 334-341.

[10] Peoples MB, Herridge DF, Ladha JK. 1995. Biological nitrogen-fixation: an efficient source of nitrogen for sustainable agricultural production. Plant Soil 174: 3-28

[11] **Fuskhah, E**, E.D. Purbayanti, F. Kusmiyati, dan R.T. Mulatsih. 1997. The effect of Rhizobium sp inoculation and phosphorus on the degree of catalysis of the nitrogenase enzyme in *Centrosema pubescens* Benth. Research Journal. Diponegoro University Research Institute. IX(34): 19-25

[12] Yufdy, M.P. dan A. Jumberi. 2011. Utilization of Sea Water Nutrients to meet Plant Needs. Http://www.dpi.nsw.gov.au. Access date 7 March 2011.

[13] Brown, J., A. Colling, D. Park, J. Phillips, D. Rothery, and J. Wright. 1989. Ocean Circulation. New York. Pergamon Press.

[14] Pickard, G.L, and K. O. Emery. 1990. Descriptive Physical Oceanography, Pergamon Press.

[15] **Fuskhah, E.**, R. D. Soetrisno, S. Anwar, F. Kusmiyati. 2007. Resistance of Rhizobium Bacteria and Feed Leguminous Plants to Stress Salinity in the Area of the North Coast of Central Java. Competitive Grant Research Report, Directorate General of Higher Education, Ministry of National Education.

[16] Vincent, J.M. 1970. A Manual for The Practical Study of The Root Nodule Bacteria. IBP Handbook No. 15, Blackwell Scientific Publ., Oxford.

[17] Steel, R.G.D. and J.H.Torrie. 1990. Principles and Procedures of Statistic. John Wiley and Sons, New York.

[18] Marschner H. 1995. Mineral nutrition of higher plants. Second Edition. 889pp. London: Academic Press

[19] Mills, H.A. and J.B. Jones, Jr. 1996. Plant Analysis Handbook 11. Micro - Macro Publishing, Athens, GA.

[20] Reddy, M. P., and Iyengar, E. R. R. 1999. Crop responses to salt stress : Seawater application and prospects, Handbook of plant and crop stress. Marcel Dekker Ink. New York, pp 1041-1068.

[21] Rains, D.W. and S.S.Goyal. 2003. Strategic for managing crop production in saline environment: an overview. Global and Integrative Perspective. Goyal, S.S., S.K.Sharma, D.W.Rains, (Eds). The Haworth Press Inc, New York.1-10.

[22] Rao, N.S. Subba. 1995. Soil Microorganisms and Plant Growth. Third Edition. 335 pp Science Publishers, Inc.

[23] Handayanto, E. dan K. Hairiah. 2007. Soil Biology, Healthy Soil Management Platform. Pustaka Adipura. Yogyakarta.

[24] **Fuskhah, E.**, dan **A. Darmawati**. 2014. Utilization of Seawater as Nutrients and Water Hyacinth Mulch in Soybean Plants Inoculated with Rhizobium Bacteria. Competitive Research Report for Year 1.

[25] Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews. Dec. 1999, p 968-989.

[26] Swaraj, K. And N.R. Bishnoi. 1999. Effect of salt stress on nodulation and nitrogen fixation in legume. J. Indian Exp. Biol. 37(9) : 843