ISOLATION AND CHARACTERIZATION OF NODULE BACTERIA FROM MUNGBEAN AND INVESTIGATION ITS TO DROUGHT WATER STRESS ON SOYBEAN PLANT

Nur Amin
Department of Plant Protection, Faculty of Agriculture, Hasanuddin University, Indonesia
*Corresponding author: nuramin_62@yahoo.com

ABSTRACT
Among the environmental stress factors the most widely limiting for crop production on a global basis is water. Water stress has been found to decrease productivity of most plant particularly soybean. The aims of the study are isolation and propagation of nodule bacteria from root of mungbean by using YEMA media, characterization of nodule bacteria by using YEMA + Congored and the investigation of drought water treatment. The result indicated that nodule bacteria from root of mungbean do not absorbs red colour on YEMA medium and the coloni white cloudy like milk and the form is spherical colonies with a convex surface. Characterization showed as Rhizobium sp. The investigation of drought water stress indicated that, there are significant difference on the treatment of 50 ml water/1000 g soil of drought water stress toward the growth i.e. plant hight, number of leaves and nodule formation on soybean plant.

Keywords: Nodule Bacteria, Rhizobium, Drought water stress, Soybean.

1. INTRODUCTION
Indonesia has the potential of agricultural land marginal so is relatively widespread, but has not been utilized and managed properly. Agricultural land marginal has been identified as an area used for agriculture or livestock grazing and agroforestry. In Indonesia agricultural land marginal include upland, distinguished by altitude (lowland and upland). Dry land area that allows for the development of agriculture reached 75.1 million ha. The development of soybeans on dryland is very possible. Soybean is a major source of vegetable protein for most Indonesia's population. Indonesia's economy has soybeans as a major source of raw materials for industry tofu, tempeh, and animal feed in the form of soybean cake. The total requirement of soybean Indonesia in 2012 reached 2.3 to 2.5 million tons per year, on the other hand the number of Indonesian soybean production is only about 800,000 tons per year. Thus, to meet the needs of soybean, the Indonesian government should be import. Target in 2014 Indonesia self-sufficient in soybeans, for which the government targets to acquire new land primarily dry land [1].

The ability of Rhizobium sp. to survive at a low water potential in soil has been established by many studies in which viability was assessed by determining colony forming ability on agar plates with a high water potential [2]. Very few studies have addressed either the efficiency of Rhizobium growth at low water potential [3]. or growth recovery after a rapid water potential increase brought about by a rewetting process [4]; [5]. A significant turnover of microbial biomass during rewetting of field soils has been established [6]. It is unknown what impact a rapid water potential change might have on soil Rhizobium populations both under free-living conditions and during the period of establishment of the symbiosis.

Keeping in view numerous manifestations of a beneficial action of Rhizobium bacteria on plants, present investigation involves isolation and characterization of nodule bacteria Rhizobium from roots of mungbean. This study was based on the hypothesis that these microbes Rhizobium which are nitrogen fixing and growth promoting exhibit the ability to survive and tolerate moisture stress, should they be implied in imparting tolerance to host plant.
To achieve this goal, two experiments were conducted. Experiment 1 are isolation and propagation of nodule bacteria from root of mungbean by using YEMA media, characterization of its by using YEMA + Congored and experiment 2 was aimed to investigation of drought water stress.

2. MATERIAL AND METHODS
Research was conducted at the Laboratory and Greenhouse of the department of plant protection, Faculty of Agriculture, Hasanuddin University, Makassar Souh Sulawesi, Indonesia. The laboratory research included isolation and propagation of nodule bacteria from root of mungbean by using YEMA media, characterization of its by using YEMA + Congored. The investigation of Greenhouse treated of drought water stress.
2.1. Laboratory Research

2.1.1. Isolation and Purification of Nodule Bacteria

The sources of inoculum nodule bacteria were made from Mungbean planting on areas of Gentungan district, Gowa regency, Province of South Sulawesi, Indonesia. The isolation of nodule bacteria were carried out as described by [7]. Intact roots were surface sterilized by shaking them in 0.1% HgCl2 solutions for 10 min, dried with tissue paper and followed by 5-6 washings with sterilized distilled water. One gram of surface sterilized root of plants was crushed and decimal dilutions (10X) were prepared. Nodule bacteria were splited and placed on congo red medium, then incubated at room temperature until the grow of colonies. The purification of the nodule bacteria were purified and maintained on yeast extract mannitol, YEM, slants [7].

2.2. Characterization of The Nodule Bacteria

Nodule bacteria that have been taken with a needle Ose pure and entered into aquadest sterile (5 ml), and then shaken using a vortex, pipetted 0.1 ml included in petridish YEMA containing media, and leveled with a spatula, and then incubated at room temperature. Separate colony grows well selected and planted in the media YEMA slant in a test tube (as a pure culture). Characterization of nodule bacteria grown on selective media were prepared by modifying the basic medium with the addition of dye Congo Red [8], then observed growth and change color.

2.3. Investigation of Drought Water Stress on Soybean Plant in Greenhouse

This study uses soybean seed a local varieties Willis. Soybean seeds were planted in a polybag (size 10x15 cm), where each polybags filled with 4 soybean seed. Soybean seeds soaked first into sterile distilled water for 2 hours before being planted into polybags. After that, the seeds were stocked directly into a polybag, each polybag filled 4 soybean seeds. After one week selected the best seed, while others deprived of polybags. Nodule bacteria were identified as Rhizobium isolate, propagated in the medium of Yema. Application of Rhizobium on soybean nodule were done at 14 days after planting, by pouring the 2 holes near the rhizosphere. Rhizobium concentration used was 10^5 cfu/ml by 5 ml each hole, where the inoculation is done only once.

The investigation of drought water stress is conducted with five levels of water, namely:

- WSO = Water Stress 0 ml/1000 g soil
- WS1 = Water Stress 5 ml/1000 g soil
- WS2 = Water Stress 50 ml/1000 g soil
- WS3 = Water Stress 75 ml/1000 g soil
- WS4 = Water Stress 100 ml/1000 g soil

where each treatment was the provision of water once every day and performed at 17.00 pm. The investigation was repeated 4 times. The Parameter of this investigation are plant height, the number of leaves and the number of nodule. The Observations were made in the afternoon every other day for 4 weeks.

2.4. Statistical Analysis

For the investigation of drought water stress, ANOVA was also performed to determine the effects of plant height, the number of leaves and nodule formation. The percent data were arcsine-transformed before being subjected to ANOVA. When significant differences were detected, means were separated using Tukey’s test at 5% probability level.

3. RESULT AND DISCUSSION

3.1. Isolation and Characterization of Nodule Bacteria

The characterization of nodule bacteria of mungbean plants indicated as Rhizobium sp., which by using media YEMA + Congo Red growing white milk because it does not absorb the color red. Other characteristics of the nodule bacteria is the forms spherical colonies with a convex surface (Figure 1). This is in accordance with [9], that Rhizobium in culture of medium YEMA + Congored was white milky or cloudy, and form colonies with a rounded surface such as dome or cone. Rhizobium isolate in YEMA medium in forms of convex elevation [10]. The author [11] suggested that the differences between colonies Allorhizobium, Rhizobium, and Sinorhizobium can be detected from the shape and color of the colonies, as well as the production of polysaccharides. Rhizobium colonies have a spherical shape, convex (convex), 2-4 mm diameter, high extracellular polysaccharide production, gummy (mucilaginous), and mostly yellow in the middle.
Soil bacteria of the genera *Rhizobium*, *AzoRhizobium* and *BradyRhizobium* (collectively referred to as rhizobia) are involved in interaction with leguminous plants to form N2-fixing nodules. Rhizobia have also been found to be capable of colonizing roots of non-legumes as efficiently as they colonize their legume hosts [12]. These organisms are characteristically able to invade the roots hairs of temperate-zone and some tropical- zone leguminous plants and incite production of nodules. Rhizobia have great potential to nitrogen fixers. They are rods, cocci, gram negative; colonies are circular, convex, semitranslucent, raised and mucilaginous, usually 2-4mm in diameter within 3-5 days on yeast man nitol-mineral salt agar media. Commonly pleomorphic under adverse conditions [13]. Cells contain plasmids, including large, naturally-occurring plasmids of 90-22 x 106 daltons [14]. The nitrogen-fixation (Nif) genes also appear to be plasmid-borne [15].

The present study found that *Rhizobium* sp. grow less than 2 days. The genus rhizobium can be distinguished by the growth rate which the growth rate of the time needed to establish of a colony, there is growing quite fast, less than 3 days as *Rhizobium*, *Sinorhizobium*, and *Allorhizobium* [16]. There are currently classified as growing, between 4-5 days, such as *Mesorhizobium* and *AzoRhizobium*, but there is also a relatively slow-growing bacteria, more than 6 days, such as *Bradyrhizobium*.

3.2. Investigation of Drought Water Stress on Soybean Plant in Greenhouse

3.2.1. Effect on Plant Hight

The means of plant height of soybean at 50% treatment (WS2) showed statistically significant difference with the treatment of control (WS0) or treatment with 25% (WS1), but did not show significant differences by treatment with 75% (WS3) and 100% (WS4) (Table 1). This indicates that simply by giving 50% of water from the minimum requirements with the use of Rhizobium isolate has been able to increase the growth of soybean plants after treatment with Rhizobium isolate. The growth of some strains of Rhizobium isolate greatly influenced by high salinity and low water potential conditions of the cropping land [17]. The developmental stage of a cultivated plant has its influence on multiplication of Rhizobium. Developmental stage of a cultivated plant determines, to a sufficient degree, the number of Rhizobium occurring in a crop [18]. Increased growth rate and metabolism of bacteria is probably related with the development of root system, photosynthetic activity and the amount of exudates associated with this and produced by plants [19].

Table 1. Means of Plant Height of Soybean (cm)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS0</td>
<td>33bc</td>
<td>32b</td>
<td>6c</td>
<td>6c</td>
</tr>
<tr>
<td>WS1</td>
<td>28b</td>
<td>28b</td>
<td>28b</td>
<td>29b</td>
</tr>
<tr>
<td>WS2</td>
<td>43c</td>
<td>43c</td>
<td>43c</td>
<td>44c</td>
</tr>
<tr>
<td>WS3</td>
<td>41c</td>
<td>41c</td>
<td>42c</td>
<td>43c</td>
</tr>
<tr>
<td>WS4</td>
<td>43c</td>
<td>45c</td>
<td>46c</td>
<td>46c</td>
</tr>
</tbody>
</table>

Means followed by the same letter (superscript) for each treatment are not significantly different at P = 0.05 according to Tukey’s test, n = 5
3.2.1. Effect on Number of Leaves
The number of leaves in the treatment of WS2 (50 ml/1000 soil) is always higher than the other treatments (Figure 1). Maximum leaves in the treatment of WS2 indicated that the optimum growth and development of soybean plant and may be attributed to the symbiotic relationship of rhizobium (bacteria) with the roots of leguminous crops, which fix the atmospheric nitrogen into the roots of soybeans and thus the number of leaves plant was increased.

![Figure 1. Number of Leaves of Soybean Plant](image)

3.2.1. Effect on Number of Nodule Plant1
The number of nodule plant1 in each treatment and control have no statistically different, while the level is low. The number of nodule plant1 in the treatment of WS2 (50 ml water/1000 soil) is higher than the other treatments, which 18 nodule plant1 (Table 2). The low number of nodule plant1 showed that the root formation is not well developed. Rhizobium isolates require foods for photosynthesis of soybean plants as an energy source for the formation of root nodules. The water-stress effect have studied at different stages of N2 fixation in cowpea plants and verified the adaptive physiological response to stress. They found a positive interaction between water stress and development stages of N2 fixation. Water stress applied at the stage of 15-30 days gave the most negative interference, indicating it is possibly a critical period of water stress for cowpea. In our study, the water stress was applied from day 10 to day 30 of growth.

The treatment of WS2 (50 ml water/1000 g soil), indicated of the role of Rhizobium isolate as an stimulus to grow of the soybean plant though not much. This is evidenced by the formation of the structure of pods on the plants treated by WS2 at 3 weeks of observation or five (5) weeks after planting. Basically the use of Rhizobium isolate is to increase crop yields, but in achieving optimum outcomes is influenced by several factors such as the number of microorganisms in the soil, pH, soil structure, content of certain chemical elements and temperature [20].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Nodule Plant1</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS0</td>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>WS1</td>
<td>7</td>
<td>Low</td>
</tr>
<tr>
<td>WS2</td>
<td>18</td>
<td>Low</td>
</tr>
<tr>
<td>WS3</td>
<td>8</td>
<td>Low</td>
</tr>
<tr>
<td>WS4</td>
<td>10</td>
<td>Low</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS
This study shows that there was a treatment of WS2 (50 ml water/1000 g soil) treated with Rhizobium isolate, the optimum level for the growth of soybean plant i.e. plant height, number of leaves and nodule formation on soybean plant.
REFERENCES


