INFLUENCE OF MOLYBDENUM IN ASSOCIATION WITH RHIZOBIUM ON ENHANCED BIOLOGICAL NITROGEN FIXATION, GROWTH AND YIELD OF SOYBEAN UNDER DRIP IRRIGATION SYSTEM

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ABSTRACT

Soybean (Glycine max) is one of the most important legume crops which fix atmospheric nitrogen in symbiotic association with bacteria through nodules. A study was conducted to investigate the effect of molybdenum and pre-inoculation of Rhizobium on biological nitrogen fixation and yield of soybean under drip irrigation system. A total of six treatments were used in this experiment which included control, 1 kg of Mo/ha, 1.5 kg of Mo/ha, 10 kg of Mo/ha, Rhizobium isolate UPMR020, UPMR020 + 1 kg of Mo/ha, UPMR020 + 1.5 kg of Mo/ha, UPMR020 + 10 kg of Mo/ha. Application of 1kg of Mo/ha along with bacterial inoculum produced the highest (51) nodules per plant. This treatment also produced the highest nodule’s weight (199 mg/plant) and plant dry weight (72 g/plant). Application of 1 kg of Mo/ha in association of UPMR020, produced significantly high yield (29.46 g/plant) compared to control which produced only 9.15 g/plant, thus indicating the potential of this treatment to be used for soybean with drip irrigation system.

Key words: Glycine max, Growth, Molybdenum, Nodulation, Rhizobium, Yields

INTRODUCTION

Soybean is a multipurpose agricultural crop. It is a major source of edible oil, protein and animal feed. Soybean protein supplies almost all essential amino acids for building and repairing tissues in human being. Soybean oil, free from cholesterol and saturated fatty acids, contains a large amount of lecithin and a fair amount of fat-soluble vitamins. Lecithin is an important constituent of all organs of human body and especially of nervous tissue, heart and liver.

Plants need huge amount nitrogen compared with other nutrients as nitrogen goes into the structure of DNA and RNA. Chlorophyll contains four nitrogen atoms. Almost 70% leaf nitrogen goes into the structure of photosynthetic stains, enzymes, nucleotide, and amides (Meyer et al., 1973; Araghi-Sooreh and Hatami-Lorzini, 2012). The symbiotic nitrogen fixation is the most important in global nitrogen because it contributes to increase the agricultural production to different farming systems (Bordeleau and Prevost, 1994). The biological nitrogen fixation is estimated about 140 mt, where the symbiotic fixation is about 112 mt, and the rest is free nitrogen fixation (Beck et al., 1993). In symbiotic association, microsymbiont needs a symbiotic living host macrosymbiont, either plant or animal (Bordeleau and Prevost, 1994). Effective symbiosis between Rhizobium and legume plants depends on supply of molybdenum accumulated in nodules during nitrogen fixation. The amount of molybdenum needed for active nitrogen fixation is different depending on legumes, climatic conditions, soil, and application method (Hagstrom and Berger, 1973; Mohamed et al., 2011). In soybean plants, enough molybdenum can be accumulated when plants are provided with molybdenum. When sodium molybdate was mixed with inoculants immediately

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before application to legume seed at planting time, both nodulation and yields were increased (Burton and Curley, 1966). Although Rhizobium bacterium is effective in nitrogen fixation, it is affected by soil elements. Molybdenum is one of the elements which influence nitrogen fixation through its effect on increasing and decreasing number of nodules (Dakora, 1985; Wankhade et al., 1992).

Drip irrigation is an efficient method of supplying water to plants. It is the most beneficial way to irrigate crops, compared with other methods such as sprinkler irrigation. Five times more water was used in soybean for sprinkler method than in drip plot (Wang et al., 2000). Drip irrigation not only conserves water but also maintains soil profile at a higher temperature, favorable for plant emergence and development. Soil temperature remains significantly higher in drip than in sprinkler plot, which leads to a higher emergence rate and enhance seedling growth. Therefore, the specific objective of this study was to investigate the effect of molybdenum and pre-inoculation of Rhizobium on nitrogen fixation, growth and yield of soybean plants under drip irrigation system.

**MATERIALS AND METHODS**

Soybean seed variety AGS190 was obtained from Crop Science Department, Faculty of Agriculture, Universiti Putra, Malaysia (UPM). The Rhizobium strain UPMR020 was provided by Agrotechnology Laboratory, Faculty of Agriculture, UPM. The strains was subcultured in 100 ml Erlenmeyer flasks with yeast extract mannitol agar and shaken continuously for 48 h (150 rpm, 28°C). Soybean seeds were left in inoculum for 4 h before planting in pots.

Pots were prepared by taking 20 kg soil/pot and calcium carbonate was added 2 weeks before planting to raise soil pH. Three fertilizers i.e. 40 kg of N/ha as ammonium sulfate (1.60 g/pot), 120 kg of P/ha as triple super phosphate (2.20 g/pot) and 100 kg of K/ha as potassium sulfate (1.40 g/pot), were added before planting. Triple super phosphate and potassium sulfate were once again added before flowering period. Molybdenum in liquid form was added in 4 levels 0, 1, 1.5, and 10 kg of Mo/ha as ammonium molybdate (0, 0.10, 0.16, 1.08 g/pot) after 1 week of planting. Six seeds were planted in each pot and after 2 weeks only 3 plants were allowed to grow. Drip irrigation was used for all pots with a discharge 2 L water/h. After 16 weeks, plants were harvested (2 plants/pots) and plant parts segmented into stems, branches and leaves were digested to determine the contents of total N, Mo, P, and K in plant and oil content in seeds. Other parameters like the average number of nodules, weight of nodules, yield, and number of pods, weight of pods, weight of 100 seeds and plant dry weight were recorded from the plants dried in oven at 65°C for 48 h to show the effect of molybdenum and bacterial inoculation under drip irrigation on soybean plants.

**Experimental design and determination of growth parameters:** The experiment was laid out in a randomized complete block design (RCBD) with three replications. The treatments were: 1. Control, 2. 1 kg of Mo/ha, 3. 1.5 kg of Mo/ha, 4. 10 kg of Mo/ha, 5. UPMR020, 6. UPMR020 + 1 kg of Mo/ha, 7. UPMR020 + 1.5 kg of Mo/ha and 8.UPMR020 + 10 kg of Mo/ha. The parameters recorded were number of nodules/plant, weight of nodules, plant dry weight, molybdenum and potassium contents in plant and yield.

**Statistical analysis:** The data was subjected to analysis of variance (ANOVA) and tested for significance using Least Significant Difference (LSD) by PC-SAS software (SAS Institute, Cary, NC, 2001).

**RESULTS AND DISCUSSION**

**Number of nodules:** The Figure 1 shows that bacterial inoculation had significant effect on nodulation of soybean plants. Application of sole molybdenum did not show any effect on nodulation. However, molybdenum along with Rhizobium showed significant effect on nodulation. The significantly highest number of nodules (51 nodules/plant) was found in plants receiving 1kg of Mo/ha along with bacterial inoculum. The numbers of nodules decreased with further increase in molybdenum rates. In inoculated treatments, addition of 10 kg of Mo/ha produced only 11 nodules/plant which was lower than sole inoculum.

**Weight of nodules:** Addition of molybdenum along with Rhizobium had significant effect on weight of nodules of soybean plant. In inoculated treatments, lower rate of molybdenum (1 kg of Mo/ha) boosted up the weight of nodules but increased rates further reduce the weight of nodules. The significantly
highest weight of nodules (199 mg/plant) was found in plants treated with Rhizobium along with 1 kg of Mo/ha and it was the least in non-inoculated plants receiving 10 kg of Mo/ha (Figure 2).

**Plant dry weight**: Lower rate of molybdenum and Rhizobium inoculation had significant effect on plant dry weight. The significantly highest plant dry weight was found in plants receiving 1 kg of Mo/ha along with bacterial inoculum followed by the plants receiving only 1 kg of Mo/ha. On the other hand, the significantly lowest plant dry weight was found in plants receiving 10 kg of Mo/ha followed by the plants in control treatment (Figure 3).

**Molybdenum content in plant**: The molybdenum content in plants increased with the increase of molybdenum rates irrespective of inoculation or non-inoculation with Rhizobium. However, molybdenum content in inoculated plants was higher than that of non-inoculated plants. At the end of experiment, molybdenum content was 0.81, 1.20, 2.33 and 3.22 µg/g for 0, 1, 1.5 and 10 kg of Mo/ha in non-inoculated plants, whereas it was 1.13, 1.98, 2.06 and 3.24 µg/g in inoculated plants (Figure 4).

**Potassium content in plant**: The inoculated plants contained higher potassium than that of un-inoculated plants (Figure 5). Addition of bacterial inoculation and molybdenum had significant (Pd < 0.01) effect on potassium content in plants. The significantly highest amount of potassium content was found in inoculated treatment where 10 kg of Mo/ha was applied followed by 1.5 and 1.0 kg of Mo/ha. On the other hand, the least amount of potassium content was found in plants under control treatment followed by the plants treated only with Rhizobium.
**Yield:** Addition of 1 kg/ha of Mo increased the yield both in inoculated and non-inoculated plants. However, increased rates of Mo i.e., 1.5, 10 kg of Mo/ha, further decrease the yield both in inoculated and non-inoculated treatments. The inoculated plants gave higher yield than that of un-inoculated plants. The significantly lowest yield of 6 g/plant was obtained from plants receiving of 10 kg of Mo/ha followed by the plants under control treatment which gave 9.15 g/plant. The significantly highest yield (29.46 g/plant) was obtained from the plants receiving 1 kg of Mo/ha along with bacterial inoculum (Figure 6).

**DISCUSSION**

Application of 1 kg of Mo/ha along with bacterial inoculation increased the number of nodules, weight of nodules, plant dry weight, molybdenum content, potassium content and yield than that of other treatments. These results were in line with the study conducted by Gault and Brockwell (1980) who found that the addition of molybdenum increased the root nodules in berseem and Medicago. Also, Wankhade et al. (1992) observed that addition of molybdenum at different levels increased the number of nodules in peanut. They added two levels of molybdenum with bacterial inoculation which increased the weight of dry nodules after 30, 45, 60 and 75 days of planting.

The above-mentioned results were in conformity with those of Bhuiyan et al. (2008) who found that adding molybdenum at a rate of 1 kg of Mo/ha with phosphorus and bacterial inoculation significantly increased the growth of mungbean, the number of nodules, the dry matter production as well as grain yield compared with un-inoculated control. Adding 1 kg of Mo/ha with bacterial inoculation, increased nodules in soybean, which may perhaps due to the fact that Rhizobium requirements of Mo are more than requirements of plant (Gupta, 1997; Schwamberger and Sims, 1991). Adding lime to soil raised pH which in turn decreased Mo adsorption. The high pH increases negative charge of soil components and anion adsorption. Molybdenum can also be more available by the increase in hydroxyl ions (Ribera et al., 2010).

Adding 10 kg of Mo/ha was more than bacterial need, which implies that molybdenum became toxic for bacteria, affected plant growth and physiology and led to weak growth and development in plant and bacterial nodules. Plant dry weight increased until harvesting period, which gave the highest weight by the effect of bacterial inoculation. Similar results were recorded by Bailey (1988) who found increase in soybean dry weight as a result of bacterial inoculation with plant age. The increase in plant dry weight as a result of adding molybdenum has been confirmed by many researches on different legume plants. Significant increase in Berseem and Medicago dry weight treated with molybdenum was observed in two studies (Sherrell, 1984; Doerge et al., 1985).
The increase in molybdenum concentrations was higher under 10 kg of Mo/ha with or without bacterial inoculation. This was due to the increase in molybdenum for plants and genetic ability of soybean like many other legumes in accumulating molybdenum in its tissues more than needed. The effect of adding molybdenum on the increase in soybean content of this element was in accordance with Sherrell (1984) on berseem, Demooy (1970) on soybean and Ahirichs et al. (1963) on Medicago. The increase in potassium content in plant resulted from adding Mo because there was positive relationship between Mo and K, and adding high levels of Mo increased potassium uptake from soil (Ujwala Ranade-Malvi, 2001). The experiment showed an increase in soybean yield under bacterial inoculation, and the results were in agreement with those of Ciafardini and Lombardo (1991) on soybean inoculated with bacteria. On the other hand, adding 1 and 1.5 kg of Mo/ha increased soybean yield with or without bacterial inoculation. Meanwhile, 10 kg of Mo/ha decreased plant yield, which could be caused by the toxic effect of this level for bacteria as it might have been more than the requirement of plant as well as bacteria. The decreased efficiency of nitrogen fixation and defect caused in nutritional balancing inside plant was reflected in plant growth and yield. Hence, adding of 1 kg/ha molybdenum contributes to better growth and higher plant yield, especially in inoculated plants. The results were in accordance with the studies carried out on berseem and soybean (Demooy, 1970; Gault and Brockwell, 1980) and on green gram and peanut (Wankhade et al., 1992; Jat and Rathore, 1994).

**CONCLUSION**

Bacterium Rhizobium, UPMR020 was an efficient inoculum in nitrogen fixation process. Application of 10 kg of Mo/ha gave the lowest results, which implied that this level could become toxic for both bacteria and plant. This experiment indicated that UPMR020 formed the most effective combination with 1 kg of Mo/ha for optimum growth, nodulation and yield of soybean under drip irrigation system.

**REFERENCES**


