**Harnessing the Power of Beneficial Microbes to Enhance Productivity in Soybeans**

**Mary Baker**

**February 20, 2017**

**Table of Contents**

1. Abstract
2. Research Question
3. Hypothesis
4. Literature Review
5. Materials
6. Methods
7. Results
8. Statistical Analysis
9. Discussion
10. Conclusion
11. References

**Abstract**

The purpose of this research was to determine the effect of phytohormones and nitrogen on soybeans. Phytohormones regulate plant growth and nitrogen is necessary for plant growth and is often the nutrient limiting plant growth. Legumes are able to associate with Nitrogen-fixing bacteria known as Rhizobia and use the nitrogen fixed by this bacteria as their source of nitrogen. In exchange for nitrogen, plant provide energy in form of carbohydrates to these bacteria-costly to the plant. When legumes have access to readily available nitrogen, plant prefers to use this available nitrogen and so nodulation is reduced of legumes, but in this research we wanted to understand if application of Auxin can reduce the negative impact of nitrogen fertilizer on nodulation. The rationale for this research question is that application of Auxin can increase plant growth and increase demand for nitrogen. So, we hypothesize that auxin-treated plants, may maintain high level of nodulation even when there is some free nitrogen available.

Two sets of seeds were infected with *Bradyrhizobium japanicum,* but only one set was inoculated with Plant Growth Promoting Rhizobia (PGR). The seeds were plants in a controlled environment and after three weeks, the plants were sprayed with 0%, 50%, and 100% nitrogen, After 30-35 days, the plants were harvested. Unfortunately, none of plants developed any nodules in this experiment. But we found that, except for in one test (+PGR root weights), an application of 100% nitrogen to the leaves is the most effective method to increase weights. This supports the background research. Each average weight shows that 50% nitrogen application hinders growth. In conclusion, the null hypothesis “there will be no difference in shoot weight when PGR is present” can be rejected. In order to reject the other null hypotheses, more research needs to be conducted.

**Research Question**

Question one

Does application of a plant growth regulator, auxin, increase plant growth?

Question two

Does Application of auxin affect nodulation of soybean?

Question three

Does an infusion of nitrogen affect soybean plant growth?

Question four

Do plants treated with auxin maintain higher nodulation in presence of available nitrogen?

**Hypothesis**

Test one- Null

1. There will be no difference in nodulation when PGR is present.
2. There will be no difference in nodulation when PGR is not present.

Alternative

1. When PGR is applied to the seed, the nodulation will increase independent of nitrogen level.
2. When there is no PGR applied to the seed, the nodulation will increase.

Test two- Null

1. There will be no difference in root weight when PGR is present.
2. There will be no difference in shoot weight when PGR is present.
3. There will be no difference in root weight when PGR is not present.
4. There will be no difference in shoot weight when PGR is not present.

Alternative

1. When PGR is present, there will be a difference in root weight.
2. When PGR is present, there will be a difference in shoot weight.
3. When PGR is not present, there will be a difference in root weight.
4. When PGR is not present, there will be a difference in shoot weight.

**Literature Review**

When a farmer or grower plants a soybean, the key to their success is the formation of nodules on the soybean. This formation occurs due to the infection with symbiotic bacterial known collectively as Rhizobia. Rhizobia, enter the host root, soybean plant, without being killed because of an elaborate chemical communication that help plants recognize them as beneficial rather than pathogenic. Rhizobia infects the root hairs of the plant and form structures known as nodules, where they fix atmospheric nitrogen to organic nitrogen using carbohydrates from plant as source of energy. The plant and bacteria have developed this mutually beneficial relationship where the bacteria provides the plant with organic nitrogen in exchange for carbohydrates. After one week from planting, nodules will begin to form, and the nodules house the bacteria and provide favorable condition for them to engage in a process known as biological nitrogen fixation. The nodules are visible to the naked eye in one week; when they are young, they are a grey/white color, but once the Nitrogen fixation has begun, the nodules will turn pink or red. Active Nitrogen fixation occurs at stages V2 and V3; then, the highest Nitrogen fixation happens during R5 and R6 (Krupke, P. C.).\* (Stages are divided into Vegetative (V) and Reproductive R) Rhizobia obtains energy from carbohydrates. During the nodulation process, the Nitrogen is converted into useful ammonium which is absorbed by the plant.

We want to know if, in order to increase the Nitrogen Fixation, phytohormones can be applied. Phytohormones are chemicals that regulate plant growth; they are found within the plant but at low concentrations. Further research will be discussed and reviewed to assess the limitations and factors that affect the Biological Nitrogen Fixation in Soybeans.

Stresses affect nodulation. At the University of Hawaii, a study was conducted to determine the effect of salinity on nodule formation. When the NaCl, salinity, was applied to the plant during early stages, the nodules’ weights and growth was negatively affected. The total shoot N and the concentration of N declined as the amount of NaCl was increased. This is a negative effect on the Biological Nitrogen Fixation which is the opposite outcome wanted. However, a delayed application resulted in an increase in nodule number, nodule mass, and total N found in shoot; this result a more positive outcome. This experiment shows that nodule formation is affected by the NaCl concentration. When NaCl was introduced before inoculation of Rhizobia, it reduced the formation of nodules in every stage. This observation could be very essential for farmers to address. If their fields’s soil are high in NaCl, this could negatively affect the amount and strength of nodulation which would result in lower yield rates. (Singleton, P. W)

The Kettering Research Laboratory conducted an experiment that observed how the exposure of light on cotyledons affected the Rhizobium infection and nodulation formation. one hundred seedlings were grown in three different light settings: full light, low light, and dark. The plants grow in full light had 50% the amount of nodules as the ones grown in the dark. The plants grown in low light had 57% the amount of nodules as the ones grown in the dark. It was unclear whether the light was affecting the cotyledons or the emerging radicals; therefore, plants were germinated in the dark, and, then, exposed to light. The plants that were kept in the dark seven days after inoculation were considered nutrient deprived due to lack of photosynthesis. Prior to the introduction of the bacteria, it is best to grow the plants in the dark in order to increase the amount of nodules. (Malik, N. S.) In 2003, an experiment that determined the effect of nitrate placement in hydroponically grown soybeans. The plants were grown in a two layered system by separating the lower and upper roots. Four treatments were given- 0/0, 0/5, 5/0, 5/5 where 0 was applied to lower roots and 5 to the upper roots, etc. It was found the only nitrate accumulation was only found on plants that came in contact with the 5mm. (Yashima, H.)

It was discovered that the use of the signal molecule genistein helps to aid in the production of nodules. The use of the signal molecule helps to decrease stress on the plant from salinity, acidity, and suboptimal root zone temperature. The plants were grown under controlled greenhouse conditions; the soybeans were planted in sand and inoculated after one day. They were harvested 20, 40, and 60 day after inoculation. In most cases, the use of genistein was able to enhance the growth and development of nodules. The use of this biochemical could really improve the growth and health of soybeans in a non-controlled environment such as a farmer’s field. (Miransari, M.)

Phytohormones applied to plants may change the communication between Rhizobium and the host. They encourage the plant to take up more nitrogen. A new Plant Growth Promoting Rhizobia (PGPR) strain was extracted from soybeans. The, the bacterial mechanisms of plant growth promotion were evaluated. This strain, LL2012, by itself, was able to increase the amount of known phytohormones, auxin, gibberellins and salicylic acid. When the strain LL2012 was introduced together with Rhizobia, they were able to significantly improve nodulation (Masciarelli, O.). Based on this we hypothesized that application of auxin together with the rhizobia may also enhance nodulation and plant growth, even in presence of nitrogen fertilizer.

Further research is extremely important in this field. The increased output of nodules produced higher yields and healthier plants. The discovery of new and improved phytohormones-based products may enhance biological nitrogen fixation. This research will aid farmers across the world.

**Materials**

* Grow cart
* Six grow lights
* 3 plastic trays deep enough to hold 1-2 inches of water
* Washed play sand
* 84 12 oz. styrofoam cups
* Sharpie marker
* Seeds (all seeds need to be inoculated with *Bradyrhizobium*)
  + +auxin
  + -auxin
* Hoagland's Solution
  + With no nitrogen
  + with 50% Nitrogen
  + With 100% Nitrogen
* Spray bottles
* Water
* Microgram balance
* Computer with excel capabilities
* Space heater- optional
* Scissors

**Methods**

Test One

1. Obtain materials
2. Set up grow cart and lights
3. Labeled 84 styrofoam cups in sets of 14
   1. +PGR N0
   2. +PGR N50
   3. +PGR N100
   4. -PGR N0
   5. -PGR N50
   6. -PGR N100
4. Poke three holes 2mm in diameter in the bottom of each cup
5. Fill each cup with sand
   1. Make sure the sand is not cold, allow it to warm up to room temperature
6. Plants 3 seeds 1.5 cm deep
7. Record planting date
8. Fill trays with 1-2 inches of water
   1. The cups take up the water by capillary action
9. Temperature range should be between 68°F-72°F
10. After germination, prune plants down to one plant per cup
11. Three weeks after planting date, begin applying fertilizer (N0, N50, N100)
    1. Mix Hoagland’s powder with 2 liters of water
    2. Fully wet all of the leaves
    3. Spray plants once a week for three weeks
12. After 30-35 days, harvest plants
13. Rinse the plant’s roots in water carefully
14. Cut stem from root
15. Separate nodules from 1’’ around the stem and record their number and measure fresh weight
16. Separate nodules that are in lower roots and record their number and measure fresh weight
17. Measure the weight of the above ground parts and record
18. Run statistical analysis

Test two

1. Complete steps 1-13 from test one
2. Measure the fresh weight of the root and record
3. Measure the fresh weight of the shoot and record
4. Run an ANOVA on the data to determine statistical significance

**Results**

Test one

No results- for unknown reason(s), plants failed to establish nodules.

Test two

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **(+) PGR NO (grams)** | | **(+) PGR N50 (grams)** | | **(+) PGR N100 (grams)** | |
|  | **root** | **shoot** | **root** | **shoot** | **root** | **shoot** |
|  | **0.254** | **0.938** | **0.138** | **0.383** | **0.372** | **1.053** |
|  | **0.152** | **0.089** | **0.204** | **0.322** | **0.082** | **0.313** |
|  | **0.157** | **0.682** | **0.235** | **0.178** | **0.142** | **0.46** |
|  | **0.344** | **0.749** | **0.242** | **0.247** | **0.161** | **1.071** |
|  | **0.118** | **0.418** | **0.195** | **0.265** | **0.147** | **0.595** |
|  | **0.104** | **0.551** | **0.133** | **0.271** | **0.238** | **0.288** |
|  | **0.177** | **0.106** | **0.166** | **0.187** | **0.055** | **0.656** |
|  | **0.171** | **0.785** | **0.115** | **0.122** | **0.188** | **0.312** |
|  | **0.235** | **0.685** |  |  |  |  |
|  | **0.375** | **0.721** |  |  |  |  |
|  | **0.24** | **0.104** |  |  |  |  |
| **Average** | **0.2115** | **0.5298** | **0.1785** | **0.246875** | **0.173125** | **0.5935** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Min PGR N0** | | **Min PGR N50** | | **Min PGR N100** | |
|  | **root** | **shoot** | **root** | **shoot** | **Root** | **shoot** |
|  | **0.128** | **0.317** | **0.141** | **0.653** | **0.108** | **0.707** |
|  | **0.125** | **0.157** | **0.128** | **0.181** | **0.163** | **0.689** |
|  | **0.275** | **0.086** | **0.165** | **0.433** | **0.179** | **0.328** |
|  | **0.407** | **0.823** | **0.242** | **0.852** | **0.247** | **0.677** |
|  | **0.195** | **0.812** | **0.194** | **0.143** | **0.127** | **0.533** |
|  | **0.251** | **0.714** | **0.101** | **0.137** | **0.224** | **0.781** |
|  | **0.179** | **0.222** | **0.137** | **0.169** | **0.252** | **0.628** |
|  | **0.192** | **0.341** | **0.165** | **0.147** | **0.107** | **0.165** |
|  |  |  | **0.124** | **0.131** | **0.99** | **0.085** |
|  |  |  |  |  | **0.186** | **0.182** |
|  |  |  |  |  | **0.194** | **0.295** |
| **erage** | **0.219** | **0.434** | **0.1552** | **0.3162** | **0.2525** | **0.4609** |

When auxin was applied, average root weights showed that an application of 0% of nitrogen (complete elimination of nitrogen) produced the highest weight. When 100% of nitrogen was applied, it produced the lowest root weight. 100% application of nitrogen average weight was only 5 hundredths of a gram lower than the 50% plants as shown in table one. The application of 100% nitrogen produced the highest shoot weights in the +PGR plant types. The plants treated with 50% nitrogen had the lowest shoot weights by more than half. -PGR average root weights showed that 100% nitrogen application produced the highest weight while 50% of nitrogen application was the lowest by more than half. In the average shoot weights, the treatment of 100% nitrogen again produced the highest weights, but 0% application average weight was only 2 hundredths of a gram less.

**Statistical Analysis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| (+) PGR Roots ANOVA |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| Groups | Count | Sum | Average | Variance |  |  |
| 0 | 11 | 2.327 | 0.211545 | 0.00771 |  |  |
| 0.5 | 8 | 1.428 | 0.1785 | 0.002292 |  |  |
| 1 | 8 | 1.385 | 0.173125 | 0.00972 |  |  |
| ANOVA |  |  |  |  |  |  |
| Source of Variation | SS | df | MS | F | **P-value** | F crit |
| Between Groups | 0.008439 | 2 | 0.004219 | 0.628262 | **0.542066** | 3.402826 |
| Within Groups | 0.161182 | 24 | 0.006716 |  |  |  |
| Total | 0.16962 | 26 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| (+)PGR Shoot Anova |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| Groups | Count | Sum | Average | Variance |  |  |
| 0 | 11 | 5.828 | 0.529818 | 0.093178 |  |  |
| 0.5 | 8 | 1.975 | 0.246875 | 0.007001 |  |  |
| 1 | 8 | 4.748 | 0.5935 | 0.101733 |  |  |
| ANOVA |  |  |  |  |  |  |
| Source of Variation | SS | df | MS | F | **P-value** | F crit |
| Between Groups | 0.558941 | 2 | 0.27947 | 3.961978 | **0.032594** | 3.402826 |
| Within Groups | 1.692915 | 24 | 0.070538 |  |  |  |
| Total | 2.251855 | 26 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| (-)PGR Root ANOVA |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| Groups | Count | Sum | Average | Variance |  |  |
| 0 | 8 | 1.752 | 0.219 | 0.008504 |  |  |
| 0.5 | 9 | 1.397 | 0.155222 | 0.001802 |  |  |
| 1 | 11 | 2.777 | 0.252455 | 0.062381 |  |  |
| ANOVA |  |  |  |  |  |  |
| Source of Variation | SS | df | MS | F | **P-value** | F crit |
| Between Groups | 0.047404 | 2 | 0.023702 | 0.849234 | **0.439714** | 3.38519 |
| Within Groups | 0.697748 | 25 | 0.02791 |  |  |  |
| Total | 0.745152 | 27 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| (-) PGR Shoot ANOVA |  |  |  |  |  |  |
| SUMMARY |  |  |  |  |  |  |
| Groups | Count | Sum | Average | Variance |  |  |
| 0 | 8 | 3.472 | 0.434 | 0.091103 |  |  |
| 0.5 | 9 | 2.846 | 0.316222 | 0.072393 |  |  |
| 1 | 11 | 5.07 | 0.460909 | 0.064671 |  |  |
| ANOVA |  |  |  |  |  |  |
| Source of Variation | SS | df | MS | F | **P-value** | F crit |
| Between Groups | 0.111963 | 2 | 0.055982 | 0.751 | **0.482244** | 3.38519 |
| Within Groups | 1.86357 | 25 | 0.074543 |  |  |  |
| Total | 1.975534 | 27 |  |  |  |  |

|  |  |  |
| --- | --- | --- |
| (+) PGR Shoot T-test: 0%-50% |  |  |
|  | 0 | 0.5 |
| Mean | 0.529818 | 0.246875 |
| Variance | 0.093178 | 0.007001 |
| Observations | 11 | 8 |
| Hypothesized Mean Difference | 0 |  |
| df | 12 |  |
| t Stat | 2.926782 |  |
| **P(T<=t) one-tail** | **0.00634** |  |
| t Critical one-tail | 1.782288 |  |
| **P(T<=t) two-tail** | **0.01268** |  |
| t Critical two-tail | 2.178813 |  |

|  |  |  |
| --- | --- | --- |
| (+) PGR Shoot ANOVA T-Test: 50%-100% |  |  |
|  |  |  |
|  | 0 | 1 |
| Mean | 0.529818 | 0.5935 |
| Variance | 0.093178 | 0.101733 |
| Observations | 11 | 8 |
| Hypothesized Mean Difference | 0 |  |
| df | 15 |  |
| t Stat | -0.4375 |  |
| **P(T<=t) one-tail** | **0.333991** |  |
| t Critical one-tail | 1.75305 |  |
| **P(T<=t) two-tail** | **0.667983** |  |
| t Critical two-tail | 2.13145 |  |

|  |  |  |
| --- | --- | --- |
| (+) PGR Shoot T-test: 0-100% |  |  |
|  |  |  |
|  | 0 | 1 |
| Mean | 0.529818 | 0.5935 |
| Variance | 0.093178 | 0.101733 |
| Observations | 11 | 8 |
| Hypothesized Mean Difference | 0 |  |
| df | 15 |  |
| t Stat | -0.4375 |  |
| **P(T<=t) one-tail** | **0.333991** |  |
| t Critical one-tail | 1.75305 |  |
| **P(T<=t) two-tail** | **0.667983** |  |
| t Critical two-tail | 2.13145 |  |

Alpha Level= 5%

Based on the ANOVA, auxin increase in shoot weights was statistically significant. Therefore, the null hypothesis “there will be no difference in shoot weight when PGR is present” can be rejected. The t-test shows statistical significance between the 0-50% nitrogen. . Due to the condition of this experiment, some plants were dried more than other plants. We suspect that this might have affected the plants in the 50% Nitrogen treatment more than others and have caused a decline in the weight of these plants. Otherwise, we expected to see a gradual increase in shoot weight with increase in Nitrogen application.

**Discussion**

Overall, except for in one test (+PGR root weights), it is determined that an application of 100% nitrogen to the leaves is the most effective method to increase weights. Plants treated with auxin showed an even greater increase in shoot weight and that can lead to even higher demand for nitrogen. Each average weight shows that 50% nitrogen application hinders growth. There was significance increase in shoot weight when auxin was applied. This shows using phytohormones we can induce plant growth. This in turn can increase the demand for nitrogen and so we think that we can keep high level of nitrogen, while benefiting from supplementing some nitrogen fertilizer.

Although there was no significant difference in the other parameters, based on the average weights from the tests, it can be determined that inoculating seeds with phytohormones will increase the average root and shoot weights. In order to show significance, more research will need to be conducted.

There were a few limitations that could have affected the results. Based on the health of some of the roots, the could have been an excess of water given to the plant which would have decreased the health of the overall plant. Also, the temperature range needed to stay between 68°F-72°F and a space heater was used to increase the temperature. However, the heater did not distribute the heat effectively, and some of the plants were in temperature near 76°F which could have dried the plants out. It seemed as though some of the stem were very long and spindly which caused the stems fall over which could have affected the amount of light they received. If the nitrogen could have been applied directly after germination, it could have increased plant growth and overall health.

**Conclusion**

In conclusion, the null hypothesis “there will be no difference in shoot weight when PGR is present” can be rejected. In order to reject the other null hypotheses, more research needs to be conducted.

**References**

Flynn, R. (n.d.). Nitrogen Fixation by Legumes. Retrieved November 23, 2016, from http://aces.nmsu.edu/pubs/\_a/A129/

Krupke, P. C. (n.d.). Soybean Growth Stages | Pests | Integrated Pest Management | IPM Field Crops | Purdue University. Retrieved November 23, 2016, from https://extension.entm.purdue.edu/fieldcropsipm/soybean-stages.php

Malik, N. S., Pence, M. K., Calvert, H. E., & Bauer, W. D. (1984). Rhizobium Infection and Nodule Development in Soybean Are Affected by Exposure of the Cotyledons to Light. *Plant Physiology,* *75*(1), 90-94. doi:10.1104/pp.75.1.90

Masciarelli, O., Llanes, A., & Luna, V. (2014). A new PGPR co-inoculated with Bradyrhizobium japonicum enhances soybean nodulation. *Microbiological Research,* *169*(7-8), 609-615. doi:10.1016/j.micres.2013.10.001

Miransari, M. (2016). *Environmental stresses in soybean production*. Retrieved November 23, 2016, from https://books.google.com/books?id=0LJ0BgAAQBAJ&pg=PA243&lpg=PA243&dq=experiments on soybeans nodulation process&source=bl&ots=u\_I4KxVg00&sig=Rc4dPlaUnlKrxjwoq912mL8xKr0&hl=en&sa=X&ved=0ahUKEwjUh8LS6L3QAhUOx2MKHWMCBD0Q6AEIUDAG#v=onepage&q=experiments on soybeans nodulation process&f=false

Nagata, M., & Suzuki, A. (2014). Effects of Phytohormones on Nodulation and Nitrogen Fixation in Leguminous Plants. *Advances in Biology and Ecology of Nitrogen Fixation*. doi:10.5772/57267

Ruark, M. (n.d.). *Nitrogen and Soybeans*. Reading. Retrieved November 23, 2016, from http://www.soils.wisc.edu/extension/area/2009/Nitrogen\_And\_Soybeans\_Ruark.pdf

Singleton, P. W., & Bohlool, B. B. (1984). Effect of Salinity on Nodule Formation by Soybean. *Plant Physiology,* *74*(1), 72-76. doi:10.1104/pp.74.1.72

Yashima, H., Fujikake, H., Sato, T., Ohtake, N., Sueyoshi, K., & Ohyama, T. (2003). Systemic and local effects of long-term application of nitrate on nodule growth and N2fixation in soybean (Glycine max[L.] Merr.). Soil Science and Plant Nutrition, 49(6),