Final Report on Internship in India: June-August 2013

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1. Introduction

I am a senior for the upcoming school year and graduating in 2014 from Waukee High School. While I have lived in Iowa for most of my life I am a suburban girl. I grew up in a rapidly expanding district that was safe and clean. Much of my family works in agriculture, my dad and my uncle are plant breeders, and my grandpa was a soil scientist. When I turned 14 I began working for DuPont Pioneer pollinating corn in the summer. It was hard work and long hours and the only hands on exposure I had had to agriculture but I found myself fascinated by the concept of crossing corn to get the breeds that are the best in different situations. This exposure has resulted in a deeper interest in agriculture and the good it can do throughout the world.

My experiences with the World Food Prize started out a little frantic. Sophomore year Mrs. Delaney sent out an email inviting us to write a paper in order to participate in the Iowa Youth Institute. Of course I wanted to go it was an incredible experience to get to communicate with knowledgeable people in fields from agriculture and food security. The only catch was we needed to write a five page research paper in two nights due to the late notice. I was lucky enough to be able to pull something together in time for the Iowa Youth Institute which was an incredible experience.

With great luck I was chosen as one of 80 Iowa students to go to the Global Youth Institute this past year in 2012. There I talked to many incredible scientist, politicians, and world leaders. I watched an old friend and tennis partner present her research that she had done as a Borlaug-Ruan Intern over the summer and was compelled to ask her about the experience in Costa Rica. After talking with Lizzy I was determined to apply for the internship.

I was ecstatic to find out I had been provided with the opportunity for an internship but slightly taken aback when I found out where I would spend my summer. India. I have never had much exposure to India in any way and it was definitely going to be out of my comfort zone. Through my time here I realized that perhaps the greatest gift I had been given in terms of this internship was the fact that I stayed in India. It forced me to be out of my comfort zone and face things that I otherwise could have remained ignorant to.

2. AVRDC

2.1 History and Mission

AVRDC-The World Vegetable center is a non-profit organization whose mission is “The alleviation of poverty and malnutrition in the developing world through the increased production and consumption of nutritious and health-promoting vegetables.” AVRDC has expanded to a global scale since it was founded in 1971. Headquartered in Taiwan, The World Vegetable Center has regional offices in Sub-Saharan Africa, Central, East and South Asia. AVRDC in Taiwan is home to the largest public collection of vegetable germplasm with more than 59,500 accessions that conserves genetic diversity. The germplasm is available for distribution in order to improve crops. Unlike other organizations AVRDC not only develops improved lines of vegetable crops but also follows all the way through to improved consumption. The four themes that AVRDC follows to ensure this are germplasm, breeding, production, and consumption.

AVRDC also works to promote the livelihood of women as women are often responsible for the producing and selling of vegetables.

2.2 Legume Breeding Program

During my internship at AVRDC I worked under the supervision of Dr. Ramakrishnan M. Nair, legume breeder, on mungbean as part of the legume breeding program. The program included four legumes—mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*), vegetable soybean (*Glycine max* (L.) Merrill), vegetable cowpea (*Vigna unguiculata* (L.) Walp.), and yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis*] —but focuses on mungbean and vegetable soybean. My internship centered on mungbean, which is the cheapest source of protein for vegetarians and contains high levels of folate and iron. The program focuses on developing disease and stress tolerant varieties of mungbean while also producing high yield. The major activities during my internship were; reviewed strategies for improved nitrogen fixation in mungbean, conducted a pilot experiment on the effects of rhizobial inoculation on mungbean growth and performed artificial hybridization on mungbean crop in the greenhouse.

3. Work Related Studies

3.1 Mungbean Cross Pollination

A routine part of my day at AVRDC was to perform cross pollinations in mungbean. This process begins with emasculation of the plant in the evening and the pollination the following morning. Emasculation involved gently gripping the plant bud with your thumb and forefinger and then taking a small needle and carefully slicing down the back of the bud. Then the standard petal and wing petals are held with your thumb as you carefully remove each of the 10 anthers with the tip of the needle. Once this is complete the bud is closed to protect it until pollination. The following morning when pollen is shedding pollination must be done. This process begins with the selection of an appropriate male parent from which the flower is removed. Once again the petals are held back, and the keel surrounding the pistil is opened with the needle. Then the pollen is gently rubbed off on the stigma of the previously emasculated female parent.

Although easy in concept cross pollinations are challenging to perform because mungbean is a highly self pollinated crop. Often the dress of emasculation and pollination on a bud will be too great and it will ultimately drop. I was very frustrated with the low success rate in the crop since corn pollinations are very easy and effective. By the completion of my internship however I managed to get crossed pods with three different cross combinations. The first two pods were both PAU 911 x ML 1628. PAU 911 is moderately resistant to Mungbean Yellow Mosaic Disease (MYMD) and ML 1628 is resistant to MYMD therefore the object of the cross is to develop cultivars with strong resistance to MYMD. Another successful cross was NM 92 x VC 6510-151. NM 92 is moderately resistant to MYMD and the male parent, VC 6510-151 is resistant to Powdery Mildew Disease thus the cross is intended to improve lines with resistance to both MYMD and Powdery Mildew Disease. The final cross Harsha (susceptible to both MYMD and Powdery Mildew Disease) x ML 818 (Moderately resistant to MYMD and Powdery Mildew Disease) is intended to develop high yielding lines with improved disease resistance.

3.2 Field Advanced Breeding Trials

While I was working the rainy season crop was being sown and evaluated. For mungbean this included F_2 and F_3 families (early generation) of various crosses. I experienced the field work first hand when I went to the field to help. Before sowing started ridges were made in the soil and then we had to plot out the field with stakes. This process involved making a line that began as the basis on which all other measurements relied. Pythagorean's Theorem was used to ensure that the remainders of the plots were the appropriate size and then once they were staked out, lime was applied for marking on the soil. Sowing was done completely by hand. After the seed was counted and organized we went to the field and a row was made on the top of the ridge. The seeds were then spaced 5 cm apart according to a rope that had been marked accordingly. The seeds were pushed into the earth about an inch and then the soil was then gently pushed back on top of the seed. I also got a chance to observe the segregation of three trials after sowing. In the field we looked at each individual plant in the trials of F_3 population 2011 VR1, and F_2 populations 2012 VR4 and 2012 VR6. We took careful observation on whether or not the plant germinated, and the hypocotyl color (green or purple). The color was used to determine if the cross was a true cross because when one parent is purple and the other is green the trial should segregate in a ratio of three purple to one green (as purple was dominant over green). Once the data was collected I entered it into an excel document and then compared the observed and expected values using the chi square statistic and determined that since the chi square values were not significant ($p<0.05$) all three trials individually segregated according to the 3:1 ratio appropriately.

3.3 ICRISAT Genebank

AVRDC has its own genebank with the largest public collection of vegetable germplasm in the world and is located at the AVRDC headquarters in Taiwan. Luckily since AVRDC's South Asia office is located on ICRISAT campus I was able to visit the ICRISAT Genebank. It is home to more than 120,000 accessions of chickpea, pigeon pea, groundnut, sorghum, and pearl millet as well as six small millets gathered from 144 different countries. Several varieties conserved in genebank have now disappeared from the natural habitats of Asia and Africa. The genebank capture the genetic diversity within a species including the wild varieties. The genebank has two types of storage for either long term or short term storage which differs in temperature and humidity. The genebank is also doing several interesting projects using the diversity found in wild accessions that can greatly improve a specific trait in a crop such as the time in which it takes to grow.

4. Review of Literature: Strategies for Improved Nitrogen Fixation in Mungbean

4.1 Overview

Nitrogen is an essential compound necessary for all organic life. It is necessary to produce amino acids and proteins required for growth of organisms. Although nitrogen makes up most of the Earth's atmosphere its common form is practically inert and unavailable for use because a triple bond is formed between two nitrogen molecules. Therefore nitrogen must be converted to another form, and while there are several ways to do this, nitrogen fixation by legume symbioses is the most plentiful. This process occurs when rhizobia carries out a reaction using the enzyme nitrogenase to convert diatomic nitrogen into ammonia. The reaction occurs in root nodules of legumes where bacteria cause the proliferation of host plant cells. Since the enzyme is inactivated by the presence of oxygen these nodules contain leghaemoglobin that reduces the amount of free oxygen. Once converted the nitrogen is available for the legumes use or may remain in the soil to nurture the following crop[8].

This process is important as it can affect many different areas of crop production as well as the environment. Inadequate nitrogen can be a limiting factor in organic processes and can result in a lower yield. The nitrogen fixing ability is important to the soil quality and also can help to prevent land erosion. For this reason leguminous crops are often used in crop rotations with grain crops. High levels of nitrogen fixation can also reduce the need for other nitrogen fertilizers that can have harmful effects on the environment. These artificial fertilizers allow the growth of algae blooms, which can potentially lead to dead zones, and create air pollution in the form of nitrous oxide.

Mungbean is an important crop in South and Southeast Asia. This pulse or grain legume crop offers high nutritional value as it is high in protein, starch, calcium, vitamins, and zinc. In areas where vegetarianism is common or for where people cannot access meat, mungbean and other legumes offer essential nutrients. Mungbean is consumed by boiling whole dried seeds or split seeds, as *dhal* (porridge), bean sprouts, bean paste, or processed into high value noodles.Like other legume crops mungbean fixes nitrogen, which --as previously stated-- is important to crop production both long and short term and can reduce the harmful impact of farming on the environment. Although the nitrogen fixing ability is present in mungbean research suggests that as crop improvement for yield reduced this ability overtime[5]. Because this process of nitrogen fixation is important, strategies for improved nitrogen fixation are outlined in this review.

4.2 Selection and Breeding

Host plant Selection

One approach to increasing nitrogen fixation is through standard plant breeding practices. Genetic variability is a key to potential for plant breeding; according to Miller and Fernandez "response to selection is proportional to the amount of genetic variability present in a population"[14]. Research at Texas A&M University showed through the screening of 97 mungbean accessions that more variability for nitrogen fixation may be present within the species. Mungbean lines resistant to climatic factors such as drought and excessive moisture also have the potential to be bred for increased biological nitrogen fixation.

While further understanding of genetic factors affecting nitrogen fixation needs to be gathered, breeding of pigeon pea (*Cajanus cajan)* and common bean (*Phaseolus vulgaris*) to enhance nodulation and nitrogen fixation has been successful using pure line selection and inbred backcross breeding suggesting that these would potentially be effective in mungbean breeding[13]. Quantitative Trait Loci (QTL) mapping of the legume *Lotus japonicus* showed that many important characteristics for nitrogen fixation were controlled by the same locus and were co-localized with QTLs for seed mass [19]. Analysis of mungbean yielding similar results could provide information to expedite the standard breeding process. Successful breeding for increased nitrogen fixation has been done in common bean by Bliss at the University of Wisconsin. This was accomplished using an inbred backcross method that allowed the development of a hybrid line with good agronomic traits as well as increased yield and nitrogen fixation thus resulting in the release of 5 lines of common bean[11].While the potential for increased nitrogen fixation through breeding is good not much experimentation has been done in the area to indicate how beneficial it could prove. While nitrogen is essential to plant growth heavy application of nitrogenous fertilizers reduce the nodulation in mungbean[20].

Several factors should be considered in an effort to increase biological nitrogen fixation including genetic resistance to soil nitrates, and selection of lines with promiscuous nodulation. Promiscuous nodulation is defined as cultivars that effectively fix nitrogen with indigenous strains[3, 11]. A 2003 report on the genetics of promiscuous nodulation in soybean using strains of rhizobia for cowpea showed that non-promiscuity was dominant over promiscuity and also that promiscuous lines were often different in leaf color intensity that those that were not[10]. Research also showed that promiscuity is control by a small number of genes at few loci[10]. Promiscuous lines would reduce the need for rhizobia inoculation, reducing the need for nitrogenous fertilizers by farmers with fewer resources[10, 11]. Although experimentation and breeding have increased nitrogen fixation in common bean that yield well in low soil N, Bliss suggests an emphasis on plants with the ability to fix nitrogen in the presence of soil nitrates[11]. Most studies are conducted in low nitrogen conditions since when nitrogen is not readily available for plant use the legume must then produce it through biological nitrogen fixation[2]. Mutant soybean plants showed a lack of regulation of nodulation in high nitrogen soils, resulting in good nodulation even in the presence of high nitrates; this quality would increase nitrogen fixation even at high nitrate levels if it can be reproduced in mungbean cultivars[7].

Rhizobia Strain Selection

Improvement of biological nitrogen fixation can also focus on the other half of the symbiotic relationship: the rhizobia strain. Rhizobia inoculation significantly increased nodulation over a control without rhizobia[2], and inoculation has been shown in many studies to increase nitrogen fixation. In addition to plant breeding, research needs to be conducted on rhizobia strains that are most effective in improved nitrogen fixation[5, 15]. These rhizobia strains need to be able to effectively compete with the native rhizobia present in soil as well as fix ample nitrogen[4]. Additionally the ideal symbiotic relationship relies on high nitrogenase activity[9]. A study comparing 10 strains of rhizobia concluded that the mungbean varieties M1, Niftal, and GMBS 1 were effective at increasing yields because they highly competitive against indigenous strains and also induced high nitrogenase activity[9]. If naturalized rhizobia is effective at fixing nitrogen fixation then inoculation of an additional strain may be unnecessary. The relationship between rhizobia and the specific cultivar has significant impact on the potential nitrogen fixation.

Strain x Host x Environment

Host x Strain specificity is a challenge as it is difficult to identify combinations that are effective and well suited to each environment. The two approached to this challenge are identifying highly efficient combinations of rhizobia strain matched with suitable host plants to produce the highest potential biological nitrogen fixation. The alternative is to ignore the specific relationship and to experiment using strains of rhizobia that are known to be highly effective[11]. These rhizobia strains should be either native strains or strains that will be used as inoculants and that are effective with a wide range of genotypes[11]. This approach is more practical because it allows for several varieties to be identified at the same time and success is more likely. Chickpea (*Cicer arietinum)* cultivars have shown significant differences when inoculated with the same strain rhizobia indicating that the host x strain specificity can have a substantial impact on nitrogen fixation. Host x strain interactions may be important in nitrogen fixation when rhizobia is inoculated in order that the host plant prevents the less effective naturalized rhizobia from nodules and allows for nodulation with the effective rhizobia strains[11]. Five of these nodule restricting genes have been identified in soybean and exploited to only allow nodulation of effective rhizobia strains[11].

The environment also plays a role in the potential of nitrogen fixation as the mungbean cultivar must be well adapted to conditions including soil pH, temperature, soil medium, and rainfall. The ideal environment for nitrogen fixation is in soil with very low soil nitrogen as the additional and readily available nitrogen inhibits the nodulation and fixation process[5]. Environmental factors make host x strain specificity more challenging because different combinations are needed for each region to suit the differing environment. Salinity also can drastically affect the nodulating ability. In an experiment on soybeans even a small amount of NaCl significantly retarded the growth and nodulation of the plant [18]. Mungbean cultivars well adapted to the environment are essential to high biological nitrogen fixation and this can be increased even more through the inoculation of a highly compatible, highly competitive and effective strain of rhizobia.

4.3 Management and Cultural Practices

Current practices

Like other traits of crops such as yield and resistance to pests, biological nitrogen fixation relies in part on how the crop is cultivated. This includes what fertilizers are applied, how the crop is planted, and what crop was previously sown in the field. While farming practices vary in each region of India it is common that very small amounts or no fertilizer is applied to fields. Very few areas have suitable irrigation or in areas with the correct infrastructure sufficient water is not available. Seed treatments, including seed priming and rhizobia inoculation, are very uncommon. Also the use of improved varieties is very low: farmers commonly use local cultivars[16]. Only about 10% of the area on which mungbean is grown in India uses a form of rhizobia inoculation[17]. In some regions of India, such as Punjab, fertilizers used in excess may contribute to poor rhizobia distribution in soils, other crop field management techniques may also lead to decreased distribution of rhizobia including the burning of straw [17]. 2002 and 2003 experiments from Punjab Agricultural University (PAU) showed that both tillage and no-tillage treatments allow for better nodulation than tillage with crop residue [17].

Ideal Nitrogen Fixing Practices

Increasing nitrogen fixation in mungbean can also be done, in part, through the management of the crop. This includes fertilizers, seed priming, and also sowing of the plant in particular systems. Although nitrogen is required for the growth of plants it is only produced when there is no available soil nitrogen, thus conserving energy in high nitrate soils. In essence if nitrogen is available to the plant it will use it; if not the plant undergoes biological nitrogen fixation to gather this necessary nutrient. There are several crop management techniques that promote nitrogen fixation keeping this principle in mind. The first is to reduce the amount of nitrogenous fertilizer applied to the crop[5]. This has been extensively studied and the best rates of fertilizer application have been determined. While there is some discussion over the need for a "starter dose" of nitrogenous fertilizers to promote healthy growth the consensus is clear that high amounts of nitrogen impede the symbiotic nitrogen fixing ability. While some studies show that a starter dose of nitrogen is helpful and drastically increases crop yield[2] other studies state that the use of any nitrogen fertilizer inhibits nitrogen fixation significantly and, therefore, should not be recommended under most circumstances[5]. Certain cropping systems may also catalyze the nitrogen fixation process. Recommended systems include intercropped and solid seeded fields[5]. Both these systems increase the legumes competition for the available soil nitrogen, thus promoting nitrogen fixation since nitrogen is essential to plant growth and yield.

Micronutrients have also been shown to increase the nodulation and nitrogen fixation of mungbean. In a recent study, molybdenum, zinc, and iron in combination with rhizobia inoculation were observed at two different levels of each micronutrient. Each of the three nutrients was found to have significantly greater nodulation over both the control and only rhizobia inoculation[1]. A 2011 article also showed the benefits of priming seeds before sowing. The research compared several types of micronutrient treatments and found all treatments increased biological nitrogen fixation over the control and that phosphorus application at 0.6% in the form of KH2PO4 yielded the highest levels of biological nitrogen fixation[21]. Seed priming also had a positive effect on other desirable traits including yield and nodulation in this study; it is also easy and cost effective making it a viable option for resource poor farmers [21]. Another study yielded similar A PAU report for 2003 suggests that the best inoculation method of rhizobia is to use a liquid suspension of the inoculants over a carrier based treatment method [17]. Carrier based inoculation can be very sensitive to stresses including inappropriate pesticides and fertilizers, when the seed coat is fragile, and toxins emitted from the seed itself that can all result in lower biological nitrogen fixation [17].

4.4 Accessibility for Farmers

Distribution and timing

Not only does the rhizobia strain impact its effectiveness but the conditions that rhizobia is packaged in and transported under also have a significant impact on nitrogen fixation[4]. Better packaging of rhizobia needs to be developed and utilized so that it is cost effective yet protects the rhizobia until the time of seed treatment. Rhizobia inoculants are not readily available on the shelf like other fertilizers and pesticides [17].Where inoculants are common the extension of the importance of biological nitrogen fixation and the demonstration of the technology are often missing.

Studies done at PAU also show that the timing of sowing during the Kharif (rainy season) also impacts the biological nitrogen fixation, suggesting that crops sown in May and June do not nodulate as much as those sown in July or April due to the increased temperature [17]. The study also found that while the April sown crops nodulate effectively the nodules contained less leghaemaglobin than that of the July sown crops [17]. The timing of seed treatments are also important because studies suggest that the seed coat may excrete a substance that is toxic to rhizobia thus seed treatment done 7 days prior to sowing has a greatly reduced number or rhizobia than those planted immediately [17].

Availability

While many studies have shown the importance of rhizobia inoculation on nitrogen fixation and other agronomic traits the availability of quality inoculants can be a problem for farmers. A 2003 study carried out in Australia reported that the current inoculation technology is not effective in increasing biological nitrogen fixation, thus the inoculants available were not effective at competing with the naturalized soil rhizobia[12]. While fertilizers and pesticides are readily available on the shelf for farmers inoculants are not [17]. Since different rhizobia strains are needed for the different legumes, sometimes cultivars, and for individual regions it is much more difficult to produce. There is no "one size fits all" for rhizobia inoculants. Also while the fermentation tanks required for culturing rhizobia is fairly simple the infrastructure must be assembled and an appropriate local inoculant carrier must be determined in each region of developing countries[4].

4.5 Conclusion

Biological nitrogen fixation is a very unique and important to leguminous crops. The process uses atmospheric dinitrogen and fixes it in the soil in a usable form of nitrogen that allows for plant growth in both the legume and the succeeding crop. Because this process is so valuable this review looks into ways to improve the biological nitrogen fixation in mungbean, a staple legume in India and Southeast Asia that is high in nutrients and easy to digest. Strategies for improved nitrogen fixation include selection and breeding, improved crop management, and increased farmer availability. Breeding of host plants that have promiscuous nodulation combined with selection of rhizobia strains that are effective at competing with naturalized rhizobia are keys to this improvement. Proper crop management should be done to ensure low levels of nitrogen in soil are maintained because the need for nitrogen drives biological nitrogen fixation. Phosphorus application along with other micronutrients can also increase the biological nitrogen fixation in mungbean. Increased nitrogen fixation can also be accomplished, in part, through increased accessibility to farmers including better rhizobia packaging and infrastructure that allowed farmers to purchase the necessary inoculants easily. Several strategies have been suggested for improved biological nitrogen fixation and the implementation of one or more could prove extremely useful in this endeavor.

5. Experiment on the Effect of Rhizobia Inoculation on Mungbean Growth

5.1 Abstract

In this experiment two factors, namely, variety (two levels: Harsha and SML 668) and rhizobia inoculation (three levels: the first was rhizobia inoculated seeds with phosphate fertilizer in sterilized soil (T1); the second (T2) was the control which comprised of uninoculated seeds, and application of both nitrogenous and phosphate fertilizer; and the third (T3) level was uninoculated seeds in soil collected from the field, with phosphate fertilizer) were evaluated. Therefore, a randomized complete block design was adopted with 6 treatments. Three seeds were initially sown in each pot and seeds were re-sown if each pot did not yield two plants. After 40 days observations were recorded for plant height, root length, and number of nodules. An analysis of variance (ANOVA) was conducted to determine if significant differences were present due to rhizobia inoculation/variety and/or both. Variety main effect and the variety x inoculation interaction were not significant while the inoculation main effect was significant. The plants that were grown on soil collected from the field (T3) showed significantly higher

plant growth (plant height and root length) and produced more number of nodules per plant compared to T1 and T2. This indicated the presence of naturalized soil rhizobia in the field, which were able to nodulate well with mungbean. Also, this preliminary study showed that the rhizobial inoculum used was not effective on the two mungbean varieties tested.

5.2 Introduction

Since the green revolution there has been an increased drive in the production of food in an attempt to feed the world's growing population. This effort has been largely based on cereal production although recently there has been increasing awareness of malnourishment that grain alone does not --and cannot-- alleviate. AVRDC- The World Vegetable Center focuses on improving and promoting vegetables including leguminous species to provide the under-nourished with the nutrients needed for a quality life. In South, East and Southeast Asia mungbean (*Vignaradiata*(L.) R. Wilczek var. *radiata*) is an important legume. Mungbean is a good source of protein and vitamins, among other essential nutrients. Like other legumes mungbean fixes nitrogen in the soil due to a symbiotic relationship with rhizobia -- bacteria that lives in the soil. The focus of this experiment is to determine the effect of rhizobia inoculation on mungbean growth. The hypothesis is that mungbean inoculated with rhizobia has more nodule growth than both mungbean in sterile soil, and mungbean in field soil with natural rhizobia present. This was chosen because other studies have suggested that natural rhizobia strains are not the most effective in biological nitrogen fixation and that without inoculated rhizobia present nitrogen fixation cannot occur. The ability to fix nitrogen in the soil is a very important trait of legumes because it promotes growth of the legume crop as well as the succeeding crop and reduces the need for nitrogen fertilizer. For this reason legumes such as mungbean are often part of a cereal crop rotation to increase the yield of the following crop and to improve soil quality.

5.3 Materials and Methods

Mungbean seeds of two varieties, Harsha and NM 94, were sown in pots (at a rate of 3 seeds pot^{-1}) in a greenhouse on June 14, 2013. Five replications were carried out with each variety for three different rhizobia levels. The first level (T1) was rhizobia treated seeds sown in sterilized soil with P_2O_5 (Single Super Phosphate) applied at a rate of 1.40 g pot^{-1}. The second level (T2) was carried out with untreated seeds sown in sterilized soil with P_2O_5 (Single Super Phosphate) applied at a rate of 1.40 g pot^{-1} and nitrogen, in the form of urea, applied at 0.24 g pot^{-1}acted as the control. The third level (T3) consisted of untreated seeds sown in red field soil from ICRISAT Campus, Patancheru India and P_2O_5 (Single Super Phosphate) applied at a rate of 1.40 g pot^{-1}.

The experiment was carried out in a complete randomized block design with 5 replications. After germination seeds were resown to maintain at least 2 plants per pot. After being grown for 40 days in the greenhouse where weeding and watering was performed as needed measurements for plant height, root length, and number of nodules was taken per plant. Plant height (cm) was measured from the base of the plant to the top of the center stem. After plants were carefully uprooted from the soil and rinsed in water, root length was determined (in cm) by measuring from the base of the plant to the root tip. Nodules were counted on thoroughly rinsed roots by observing and, when necessary, removing them from the roots to count accurately. Observations were recorded on 2 plants in each replication. Data was analyzed using analysis of variance (ANOVA) at 5% probability level and results are presented below.

5.4 Results and Discussion

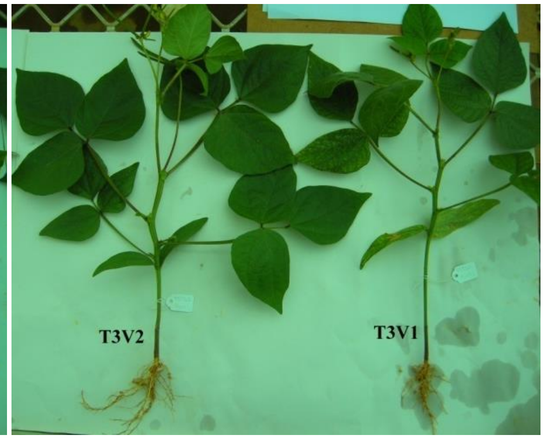
The analysis of variance (ANOVA) showed that there were no significant (p>0.05) differences between the mungbean varieties, Harsha and NM 94. for plant height, root length, and nodulation ability. When plant height was compared according to rhizobia level, the third level (T3) using field soil was significantly (P< 0.05) greater than level one (rhizobia treated seeds, T1) and level two with untreated seeds, T2. Root length/plant and number of nodules/plant also showed the third level (T3) of rhizobia to be the significantly (P< 0.05) greater than the other two levels (Table 1). The variety x inoculation interaction was not significant (p>0.05) which suggested that there was no difference between the varieties in their response to the three rhizobial levels.Altogether plants sown in field soil had significantly greater growth in all three categories than those sown in sterilized soil. This could be due to a greater available supply of nutrients in the field soil compared to the sterilized soil, Also, the higher nodulation shown by the plants grown in the soil collected from the field could be attributed to a more effective natural rhizobia strain than the inoculant used. Field soil could also contain other microorganisms that encourage plant growth. Helpful follow ups of the study could be to determine the soil composition from the field and to investigate what crop was previously sown where the soil was gathered from.

Table 1; Plant growth parameters at different rhizobial inoculation levels

Rhizobia Level	Plant Height (cm)	Root Length (cm)	No. of Nodules
T1	18.234^{a}	6.470^{a}	0.550^{a}
T2	16.240^{a}	7.575^{a}	0.000^{a}
T3	35.160^{b}	9.645^{b}	52.300^{b}

*Values in the same column with the same letter are not significantly different

Figure 1 & 2; Uprooted plants representing each treatment in replication 5



5.5 Conclusion

Nitrogen fixation is vital to farmers as the cost of nitrogenous fertilizers is on the rise and many are unable to afford it, reducing the yield of mungbean crops. Additionally the application of such fertilizers can run off the fields causing environmental damage. Both of these problems can be addressed simultaneously when biological nitrogen fixation is increased reducing the need for nitrogenous fertilizers. The results of this experiment can be used as a preliminary study for rhizobia inoculation and highlights the need for compatible strain of rhizobia to be inoculated with the crop in the absence of effective naturalized strains.

6. Reflection

I'll be the first to admit that this internship was not a walk in the park. In fact it's probably one of the most challenging things that I have done. But it was probably the best thing that I have ever done for myself and the things I have learned will stick with me for years to come. Saying that this internship is the best thing I have done for myself is true, although selfish. Through this experience I have been shown a world first hand that I knew existed but pretend to ignore. It seemed easier to me to pretend there weren't such widespread problems in the world than to actively work toward alleviating them. Although the World Food Prize Institutes discussed these issues there seeing it firsthand made it sink in finally.

It is heartbreaking to see big teary eyed children walking on the streets taping on car windows begging for money. To look them in the eye and not be able to help them. Ignoring the suffering of the people lying in makeshift tents sleeping on the sides of the streets next to piles of trash and stray dogs. It's selfish of me. While we may not be able to go give them money or a home right away we all can be working towards helping to reduce the hunger and malnutrition and to increase income and food security. My time spend at AVRDC has been to develop high yielding, nutritious, resistant crops than can serve small farmers in feeding their families and also to earn a small profit off of the crop.

My research also addresses sustainable agriculture with an emphasis on nitrogen fixation that reduces the need for nitrogenous fertilizers and helps promote growth of the next crop. This information impacts the quality of the arable lands and protects lakes and rivers from runoff nitrogen and reduces the potential for algae blooms which can be detrimental to ecosystems.

Internal reflection shows that this internship made me much more independent, increased my confidence, and gave me a more worldly view of the world. But more importantly it showed me how lucky I am to be given everything that I have been and that I should use these opportunities to help those who haven't been so lucky and not to remain ignorant. I have been shown why we need to be proactive on the fight against hunger and down the path to increase food security.

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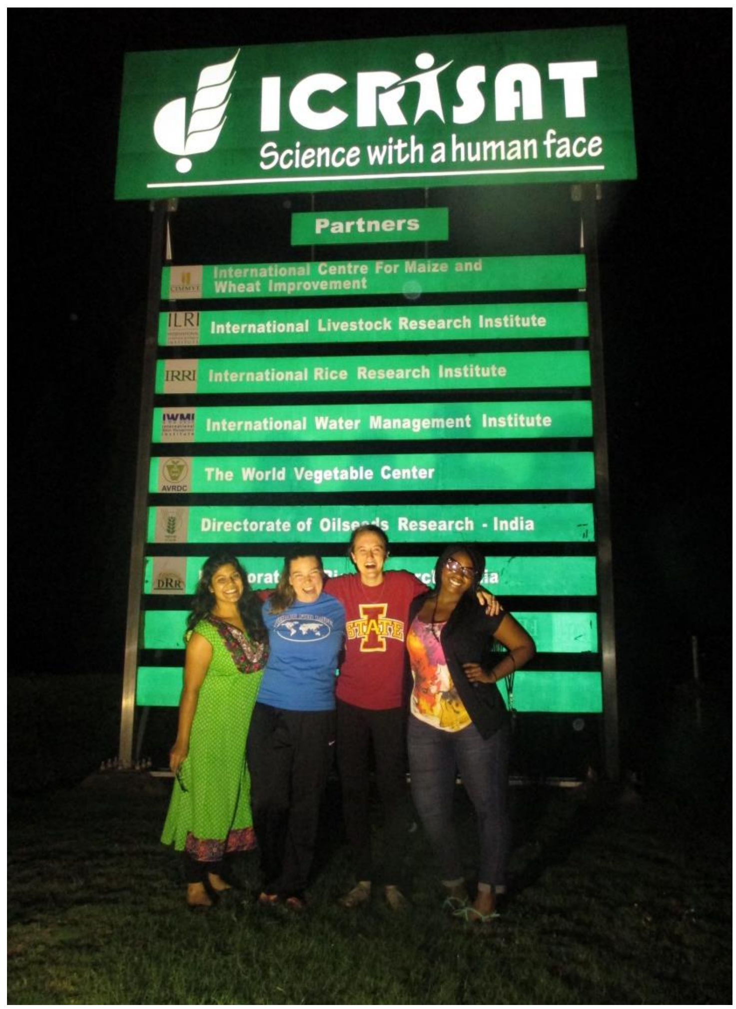
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