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THE EFFECT OF GENETIC VARIATION IN ROOT HAIR LENGTH OF COMMON BEAN (*PHASEOLUS VULGARIS*) ON THE ACQUISITION OF POTASSIUM FROM A LOW POTASSIUM ENVIRONMENT

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ABSTRACT

The purpose of this project was to study the effect of genetic variation of root hair length in common bean (*Phaseolus vulgaris*) on the acquisition of potassium from low potassium environments. Preliminary experiment one tested two growth media mixtures and a fertigation regime to determine how to best induce potassium stress. The media mixture with 10% sub-soil, 30% medium grade sand and 60% coarse perlite had more stable results with larger variation in shoot dry weight and therefore was chosen to be used for the remainder of the experiments as opposed to the media mixture of 20% sub-soil, 30% sand and 50% perlite. In preliminary experiment two the amount of potassium (K) in the nutrient solutions was reduced for the low and medium K treatments. This was done because the K stress in preliminary experiment one was not severe. The K treatments had no effect on shoot dry weight or root hair length in preliminary experiment two and therefore preliminary experiment tree was set up to test a new fertigation regime. Instead of continually adding K to the pots at varying concentrations, as was done in the first two experiments, the K treatments were regulated based on molar amounts of K added to each pot. The phosphorus (P) supplied to the plants was decreased to determine whether adequate P caused root hairs to show no variation in length. Preliminary experiment three was compromised by poor management of K levels and therefore the results were inconclusive. Preliminary experiment four was similar to preliminary experiment three and tested a lower P level and whether supplying plants with K only once at the beginning of the experiment was a better fertigation regime. The adequate/high K treatment had significantly longer root hairs than the medium and low K treatment but no significant effect on shoot dry weight was observed. A root hair survey was done to determine which genotypes to select for the final experiment. While root hair length did not vary much between genotypes (root hair length ranged from 0.33mm to 0.54mm) significant differences were observed. The recombinant inbred lines selected for the final experiment with longer root hairs included DG 13, 37, and 47 and those chosen with shorter root hairs included DG 32, 52, and 67. The final experiment included a control treatment of adequate P and K and included three K treatments with a reduced level of P. All low P treatments received 1.0mmol of P total. The low K treatment received 0.75mmol K and the medium K treatment received 1.50mmol K. The high K treatment was fertigated with a quarter strength Epstein solution (with no P) which supplied the plants with 1.50mM K. The K treatments had no effect on shoot dry weight, leaf area, root hair length or root system length even though the K

shoot concentration was significantly affected by the K treatments. This can be explained because K follows Liebig's Law of the Minimum in which case the inadequate P caused the varying K treatments to have no effect on growth since P was the nutrient limiting plant growth (Rubio et. al 2003). The P treatment had a significant effect on plant growth parameters but no effect on root hair length. The shoot dry weight of the low P treatments was reduced by 50% compared to the high P treatments. The root to shoot ratio had a significant increase from 0.19 under adequate P to 0.29 under low P. This shows the importance of P as a plant growth regulator. Plants that were supplied with low P but adequate K had a lower K shoot concentration than those supplied with adequate P and K. This was not expected since low P suppressed plant growth and increased the root to shoot ratio which seems as though it would cause high K shoot concentrations. It is thought that the low P affected K uptake because it reduced shoot growth and ultimately the supply of carbohydrates produced through photosynthesis. With fewer carbohydrates there is less energy in the form of ATP to actively take up K^+ ions from the external environment. Comparing the low K to the medium K treatment, the low K treatment took up 68% of the 0.75mmol K supplied, while the medium K treatment took up 42% of the 1.50mmol K supplied, indicating that the plants growing under more severe K deficiency conditions were more efficient in K acquisition. Further experiments are needed to determine the best way to induce K stress and why no significant differences were observed in root hair length.

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Chapter 1: Literature Review

Root hairs are essential in plant nutrient acquisition and account for up to 90% of total nutrient uptake (Föhse et al. 1991). Potassium is essential for plant growth but its availability in the soil is often sub-optimal. Potassium is generally lost from the soil by leaching in sandy soils and also through the export of crops. Potassium (K) is important in regulating many processes in plants and is essential for stress tolerance. Through osmoregulation, K is able to influence water transport throughout the plant, turgor pressure in the cells, cell elongation, and opening and closing of the stomates. Potassium interacts with various enzymes to allow their activation and can also affect pH by neutralizing compounds within the cell. Photosynthesis is affected by the levels of K in a plant because of its role in stomatal opening and closing, and is involved in regulating adenosine triphosphate (ATP) production which is an essential part of photosynthesis. Many plant functions depend on K and adequate levels of K promotes vigorous and productive crop production. Phosphorus has been studied extensively in the Pennsylvania State University lab that this experiment was conducted in and therefore this study will take into consideration what is known about phosphorus and potassium to gain a better understanding of potassium and its interaction with plants in the soil. While potassium reserves will not be used up as soon as phosphorus reserves, developing plants that are more efficient at K acquisition will allow for more profitable agriculture and help farmers in developing countries who cannot afford K fertilizer (Rengel and Damon 2008).

Potassium is found in the soil in three different pools: unavailable, slowly available, and readily available. The unavailable form of K makes up 90-98% of the K in soil and is found in primary minerals. The slowly available K in soil accounts for about 1-10% of K in soil and is generally fixed in interlayers of clay minerals such as 2:1:1 \clays. The K that is readily available to plants is found in the soil solution at 0.1-0.2% and also as exchangeable K on clay particles at 1-2%. As plants take up K from the soil solution more K is released from the exchange sites on clay particles into the soil solution for up-take (Rehm and Schmitt 2002; Brady and Weil 2010). The clay content in the soil influences K availability. In soils with lower clay content there is lower cation-exchange capacity and therefore the soil has a lower capacity to replenish K to the depletion zone created by the roots as they take up K (Marschner 1995).

Plants get their nutrients from the soil in three different ways. One way is through root interception. As roots grow through the soil they will come into contact with nutrients. Roots only occupy about 1-3% of the soil that they grow in and therefore only acquire between 6 and

10% of the required N, P and K through root interception. Nutrients also become available to plants through mass flow. Mass flow is the vertical movement of water in soil; either upward because of evaporation or downward because of gravity pulling excess water down. This flow of water in the soil allows for plants to acquire anions such as NO_3^-, Cl^-, and SO_4^{2-} as well as some cations such Ca^{+2}, Mg^{+2}. Together root extension and mass-flow can provide the plant with many nutrient requirements, but nutrients such as P, K, Zn, Fe are not supplied to the plant in adequate amounts through mass flow. A third way to acquire nutrients is through diffusion. As plants take up nutrients a depletion of ions is created in the near vicinity of the root, called the depletion zone. This zone creates an ion concentration gradient and the nutrients in the soil will slowly diffuse from an area of higher nutrient concentration to the depletion zone, an area of lower nutrient concentration. The majority of the P and K that a plant needs are acquired in this fashion. The rate of diffusion of P and K through the soil is described by the diffusion coefficient, which is dependant to the soil type. The driving force for diffusion is the concentration gradient that is created by the roots when the uptake of ions by the root is greater than the supply of nutrients by mass flow (Barber et al. 1963). The gradient formed is dependent on the rate of uptake by the plant as well as the mobility of the nutrient in the soil (Jungk 2001). The diffusion coefficient (D1) of K^+ in water is $2.0x10^{-9}$ m^2/s and for $H_2PO_4^-$ is $0.9x10^{-9}$ m^2/s. In soil the effective diffusion coefficient for K^+ is between 10^{-11} m^2/s and 10^{-12} m^2/s , and for $H_2PO_4^-$ is between 10^{-12} m^2/s and 10^{-15} m^2/s. In soil, ions only move through the pore spaces that are filled with water and therefore water content in the soil affects nutrient mobility and acquisition.

Since plant roots must be in contact with the soil solution in order to take up nutrients, the surface area of the roots are essential for nutrient uptake. The majority of plant species have root hairs which facilitate the process of nutrient uptake by increasing root surface area that is in contact with the soil. Root hairs grow from root epidermal cells and generally begin to form immediately behind the zone of root elongation in a zone that is between 1-4 cm in length (Jungk 2001). The main function of root hairs is to transfer nutrients from the soil solution into the plant. As root hairs respire they also release CO_2 into the soil solution which then reacts with water to form protons and bicarbonate ions. The protons acidify the soil solution near the root surface which promotes liberation and transfer of cations such as Mg^{+2} and K^+ into the cells of the root hairs. Root hairs are especially important in soils where nutrient concentration is low and adequate amounts of nutrients are not supplied to the plant by mass flow (Brady and Weil 2010).

According to the laws of diffusion, root hairs are more efficient in nutrient acquisition than the root cylinder (Jungk 2001). Because of their large surface area and small radius, root

hairs can access larger volumes of soil at a lower cost than would be achieved by increasing root diameter. The perpendicular growth of the root hairs also aids the plant in acquisition of nutrients that it would otherwise be unable to take up (Föhse *et al.* 1991). The acquisition of nutrients that are more tightly bound to the soil, such as phosphorus and potassium, are influenced by root hair length. For example, plants with long root hairs have a higher acquisition of P in low P soils than plants with short root hairs (Itoh and Barber 1983). Root hair formation is influenced by genetic and environmental factors. Mineral nutrient availability influences root hair formation and characteristics, especially nitrate (N) and phosphate nutrients. Under low N conditions, at concentrations below 1000 μ*M*, root hair length and density were found to increase. The factor of increase depended on the species of plant and a correlation between root hair length and N plant content was found (Foehse and Jungk 1983). In *Arabidopsis thaliana*, low P availability greatly changed the anatomy of roots and root hairs. Under low P availability the density of root hairs was five times greater than that under high P conditions (Ma et al. 2001a). As P levels in soil increase root hair length decreases logarithmically (Bates and Lynch 1996). Miguel also found that as P levels increase root hairs are shorter and there is no variation in root hair length between genotypes with long and short root hairs, but under low P levels variations are observed between long and short root hair genotypes (2004). The distance from the root tip to where root hairs start to form decreased and the number of epidermal cell files that "bear hairs" increased under low P availability. All of these root hair traits function together to make P acquisition more efficient (Ma et al. 2001b).

The effect of root hairs on P acquisition in common bean (*Phaseolus vulgaris*) has been studied. There is a positive correlation between common bean genotypes with long root hairs and shoot P concentration and content (Miguel 2004). No studies have been conducted on the relationship between root hair length and K acquisition in common beans or how genetic variation of root hair length with in one species is related to the efficiency of K acquisition. Since the diffusion coefficient of K in soil is higher than that of P, K acquisition might be less dependent on root hairs than P. Effective K diffusion could be similar to that of P in soils that have a high K buffering power which would make K acquisition more dependent on root hair characteristics (Claassen and Jungk 1984). But since nitrate, which is mobile in the soil, has been found to increase root hair length and allow for more N acquisition, K most certainly will also be affected by root hair length. Studies with multiple plant species that have various root hair cylinder volumes have shown that K uptake is related to the volume of the root hair cylinder, and therefore also root hair length (Jungk *et al. 1982*). Root hair length, but not density, increases

when pea (*Pisum sativum*), red clover (*Trifolium pretense*), lucerne (*Medicago sativa* L.), barley (*Hordium vulgare*), rye (*Secale cerale*), perennial ryegrass (*Lolium perenne* L.), and oilseed rape (*Brassica napus oliefera*) are grown in low K soils compared to higher K soils; which indicates that there is a relationship between K availability and root hair characteristics (Hogh-Jensen and Pedersen 2003). Hogh-Jensen and Pedersen found that those species of plants with longer root hairs also had a larger accumulation of K because of the increased root surface from longer root hairs, not because of the increase of the length of the entire root system. Gahoonia et. al (2006 and 2007) found that chickpea and lentil genotypes with prolific root hair formation had higher K shoot concentration compared to genotypes with shorter and less dense root hairs. All this suggests that it is likely to find a relationship between root hair length and K acquisition in common bean.

This study investigates whether natural genetic variation in root hair length within one species, common beans, plays a significant role in K acquisition in a low K environment. It is hypothesized that common bean genotypes with longer root hairs will be beneficial for the acquisition of K from low K environments than genotypes with shorter root hairs. It is expected that bean genotypes with longer root hairs will have increased plant growth and K shoot content than those with shorter root hairs.

Chapter 2: Methods to Induce Potassium Stress

Preliminary Experiment 1

Materials and Methods

The experiment consisted of two media mixtures, three nutrient solutions with varying potassium concentrations, and three replications for a total of 18 plants. The growth medium contained red soil (C horizon from a limestone-derived silt loam; fine, mixed, semiactive, mesic Typic Hapludalf), course perlite, and medium grade sand. The first growth medium treatment had 10% red sub-soil, 30% medium grade sand, and 60% coarse perlite. The second treatment had 20% red sub-soil, 30% medium grade sand, and 50% coarse perlite by volume. A total of 63L of medium was made for each treatment. Nine 7.0L pots, with a diameter of 23 cm and height of 20cm, were filled to the rim with the first medium treatment and another nine 7.0L pots were filled to the rim with the second medium treatment. Care was taken to insure uniform bulk density.

The nutrient solutions were prepared based on the Epstein Nutrient Solution. First, one liter of each stock solution was made. To make the Epstein stock solutions the amounts of each compound from Table 2.1 were weighed and each mixed with 1.0L of DI water. To make the micronutrient stock solution, the amounts from Table 2.2 were weighed out and added to 1L of DI water. The micronutrient stock solution was adjusted from the original Epstein solution so that it did not contain any potassium (K). Calcium chloride ($CaCl_2$) was used instead of potassium chloride (KCl) to supply the Cl^-. From the stock solutions the three nutrient solutions to be used on the plants were made.

Table 2.1 Stock Solutions for Epstein Nutrient Solutions		
Stock Solution	Molecular Weight	Amt. for 1 L (g)
1M KNO_3	101.11	101.11
1M $Ca(NO_3)_2$	236.15	236.15
0.5M $MgSO_4 \cdot 7H_2O$	246.48	246.48
1M $NH_4H_2PO_4$	115.03	115.03

Table 2.1 The amount of each compound needed to make 1L of the appropriate concentration stock solution

Table 2.2 Micronutrient Stock Solution		
Micronutrient Stock Solution adjusted for no K	Molecular Weight	Amt. for 1 L (g)
$CaCl_2$	110.98	2.77
H_3BO_3	61.84	1.546
$MnSO_4\ H_2O$	169.01	0.338
$ZnSO_4\ 7H_2O$	287.55	0.575
$CuSO_4\ 5H_2O$	249.68	0.125
$(NH_4)_6Mo_7O_{24}\ 4H_2O$	235.86	0.618
Fe-NaEDTA (Kodak)	367.1	18.36

Table 2.2 The amount of each compound to be added to 1L of water to make the micronutrient stock solution

The three nutrient solutions used to fertilize the plants were prepared in such a way to keep all nutrient concentrations the same while only varying the K concentration. The first solution had the highest K concentration at 1.5mM K. The second solution had a medium K concentration at 0.375mM K and the third solution had a low K concentration at 0.1875mM K. These nutrient solutions were prepared according to the amounts listed in Table 2.3. For the medium and low nutrient solutions, which were adjusted for K concentration, the $Ca(NO_3)_2$ concentration was adjusted to keep the $NO3^-$ concentration the same as in the high K solution. The $MgSO_4$ concentration was also adjusted to keep the Ca:Mg ratio the same in all three solutions, which was 2:1 . The concentration of all nutrients in each solution are listed in table 2.4.

Table 2.3 Nutrient Solution with Varying K Concentrations (1L)			
	High K	Medium K	Low K
Stock Solution	(ml)	(ml)	(ml)
1M KNO_3	1.50	0.36	0.19
1M $Ca(NO_3)_2$	1.00	1.56	1.84
0.5M $MgSO_4\ 7H_2O$	0.50	0.78	0.92
1M $NH_4H_2PO_4$	0.50	0.50	0.50
Micronutrient Solution	0.25	0.25	0.25
H_2O	to 1L	to 1L	to 1L

Table 2.3 The amounts of each stock solution needed to make 1L of nutrient solution with varying K concentrations

Table 2.4 Total Concentrations in Millimoles(mM) of Macronutrients in Solutions for Preliminary Experiment 1			
Nutrient	Low K	Med K	High K
K	0.188	0.375	1.500
NO3	3.508	3.495	3.000
NH4	0.501	0.501	0.501
N total	4.008	3.996	3.501
PO4	0.500	0.500	0.500
Ca	1.666	1.566	1.006
Mg	0.828	0.780	0.250
SO4	0.829	0.781	0.251

Total Concentrations in Micromoles(µM) of Micronutrients in Solution for Preliminary Experiment 1	
Nutrient	
Mn	0.500
Cu	0.125
Fe	12.500
Zn	0.500
B	6.250
Mo	0.875

Table 2.4 This table shows the concentration of all nutrients in the low, medium, and high K level nutrient solutions used in preliminary experiment 1; the concentration of the microelements are also given.

Once the pots were filled with the prepared soil they were soaked with DI water. Three seeds of common bean (*Phaseolus vulgaris*) were planted in each pot on 3 September 2011. The seeds were from a recombinant inbreed line (RIL) #76, which is a product of a cross between TLP19 and B98311 generated by Jim Kelly (Michigan State University). Once the cotyledons were open the weaker two of the three seedlings were removed by cutting them off at the soil line. Three pots were used per K treatment for each medium mixture. The plants received their treatments in a randomized block treatment and were grown in greenhouse A section 18 at The Pennsylvania State University (40°47'29"N77°51"31"W). The average day temperature was 26°C and the average night temperature was 22°C. Beans were watered with their appropriate nutrient solution to maintain at least 80% of water holding capacity, which was typically 400ml

twice a week for the first two weeks and 400ml three times a week for the last weeks. The pH of the nutrient solution was adjusted to 6.0 using 1M HCl each time the plants were watered, but this started only two weeks after planting the seeds. On the 3 and 10 of October (day 30 and 37 after planting) the leaf length and width of the middle trifoliate leaf was measured and a leaf area proxy was calculated by multiplying the length times the width. On 3 October the length of each internode was recorded and the average internode length was calculated. The xylem pressure potential of each plant was measured using the Model 615 Pressure Chamber Instrument (PMS Instrument Company). These pressure measurements were taken and recorded pre dawn on the 13 of October (day 40). The plants were also harvested on the 13 of October (day 40). Shoots were washed in a 0.33mM HCl solution, rinsed in DI water, dried with paper towels, and then dried in a 60° oven for four days. Roots were washed and one representative adventitious root and basal root were removed from each plant and placed in a vial with 50% ethanol. A 5cm section of these roots, 10 cm from the basal end were photographed through a dissecting light microscope using a Nikon SMZ-U camera for root hair length and density and it will be determined which root type (adventitious or basal) and which section is most appropriate for root hair analysis. The rest of the root system was placed in the drying oven. After four days in the drying oven the dry weight of the shoots and roots were measured. The root to shoot ratio was calculated by dividing the root dry weight by the shoot dry weight. The total length of the root system for plants grown in low and high K levels in the 10% field soil media mixture was calculated by first determining the specific root length and then multiplying the specific root length by the dry weight of the root system. The specific root length was calculated by dividing the length of one section of root (as determined by scanning the root and using WinRHIZO to determine the length of that section) by the dry weight of that section of root. This was done for an adventitious and basal root from each treatment. The average of the basal and adventitious specific root length was used to calculate the length of the entire root system of each plant.

Results

The F-Test in Microsoft Excel was used to analyze the shoot dry weight and determine whether there was a significant difference between the treatments. The only two treatments that were compared and found to be significantly different ($p<0.01$) were the 10% soil LK and 10% soil HK treatments.

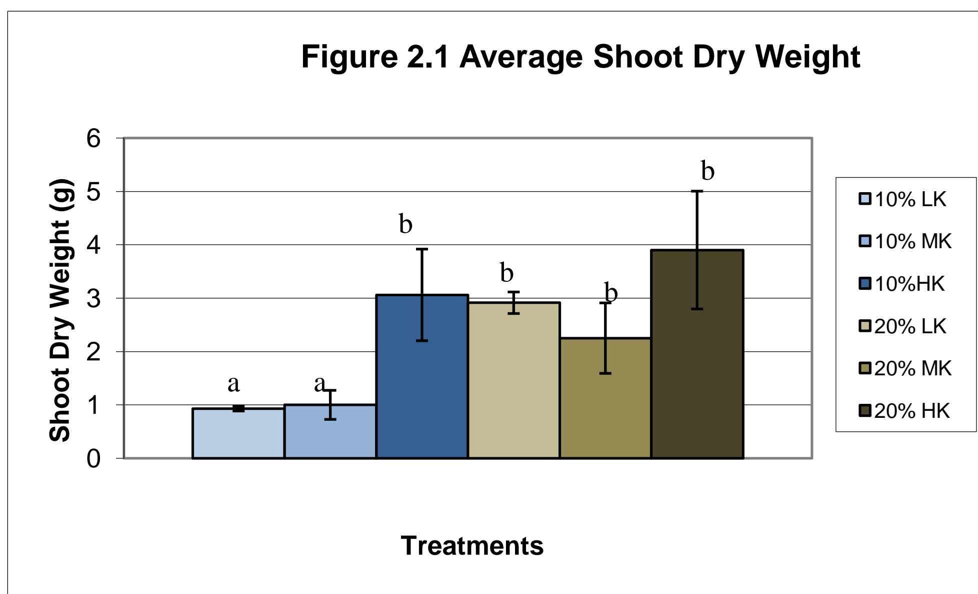

Figure 2.1 Shoot dry weight for low, medium, and high levels of K and two media mixtures are shown. Values shown are means of 3 replications and standard error bars are included.

It was determined using the F-test that there is a significant difference ($p<0.01$) between the average calculated length of the root system of plants grown in low K versus high K in 10% field soil media mixture. The average length in meters of the root systems are shown below (Figure 2.2).

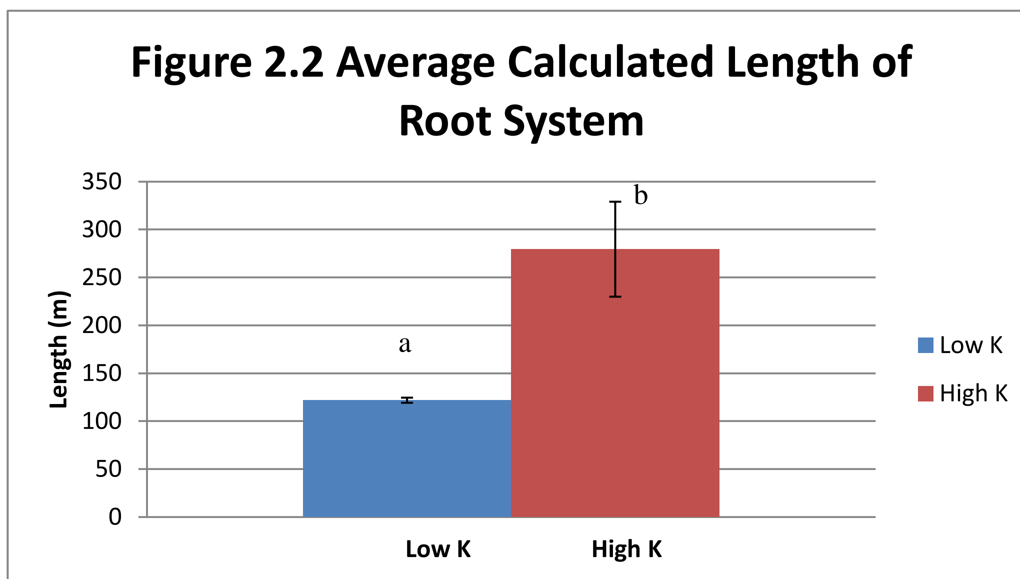

Figure 2.2 Average calculated length of root system under low and high K levels and 10% field soil media mixture.

When comparing the root to shoot dry weight ratios for all treatments there was no significant difference ($p<0.05$) found between any of the treatments.

Discussion

Results from preliminary experiment 1 could have been comprised by the problems faced with the pH of the nutrient solutions. The pH of the nutrient solutions was 7.6 and this was not adjusted to 6.0 until two weeks after seeds were planted. The plants that received the low and medium K nutrient solutions did not show the levels of K deficiency in their leaves that was expected. Overall, the plants did not look as healthy as expected and this is most likely due to the high pH.

From the shoot dry weight data it was concluded that the 10% field soil mixture gives more stable and accurate results and therefore the rest of the data that was analyzed mainly focused on the 10% field soil media mixture and not the 20%. Also since there was no difference in shoot dry weight between the low and medium K treatments only the low and high K level treatments were analyzed for the length of the root system.

As for the root to shoot dry weight ratio, there was no significance difference between any of the treatments unlike phosphorus stress where the root to shoot ratio would increase under phosphorus stress. This indicates that under K stress both the root and the shoot system dry weight are effected in a similar fashion.

When analyzing the calculated length of the root system, it was found that the plants grown in low K levels have significantly less length of root that those grown under high K levels. Since the root to shoot ratios are similar it follows that the shoot dry weight of plants grown under low K is significantly less than those plants grown under high K.

Preliminary Experiment 2

Materials and Methods

After analyzing some of the data from preliminary experiment 1, preliminary experiment 2 was started. The experiment consisted of three nutrient solutions with high, medium and low K levels. Nutrient solution ingredients and the nutrient concentrations are listed in Table 2.5 and Table 2.6. The high K nutrient solution remained the same as that in preliminary experiment 1, but the medium K was reduced to a concentration of 0.1875mM K, and the low K was reduced to a concentration of 0.09375mM K. The same methods were used to prepare these nutrient solutions as were used in preliminary experiment 1. These levels of K were chosen

because there was no significant difference between the low and medium K solutions used in preliminary experiment 1 and because symptoms of K stress did not appear very severe. By decreasing the low K treatment to 0.09375mMk it is thought that the K stress on the plant will be greater and the effect of low K would be able to be observed and measured more easily.

Only one soil type was used in preliminary experiment 2; the soil containing 10% red sub-soil, 30% medium grade sand, and 60% course perlite. This soil mixture was chosen because it has the smaller standard error in preliminary experiment 1 and seemed to be more stable and give accurate results. Two genotypes were used. DOR 364 is a small red bean from the Meso-American gene pool and is from a widespread breeding line. DOR 364 is known to have short roots hairs. G19833 is a large Andean land race and has been categorized as having long and dense root hairs (Yan et al. 1995). These genotypes were chosen in order to observe the efficiency of beans with long and short root hairs in soils with low K and determine whether root hair length is beneficial for K acquisition in soils with low K. The experiment included four replications for a total of 24 plants each grown in a 7L pot. The plants were grown in the same greenhouse and under the same conditions that the plants from preliminary experiment 1 were grown. The pH of the nutrient solutions were adjusted from the start of the experiment to 6.0. The seeds were planted 19 Oct. 2011 and harvested six weeks later on 29 Nov. 2011.

Table 2.5 Nutrient Solution with Varying K Concentrations (1L)			
	High K	Medium K	Low K
Compound	(ml)	(ml)	(ml)
1M KNO3	1.50	0.19	0.09
1M Ca(NO3)2	1.00	1.66	1.70
0.5M MgSO4 7H2O	0.50	0.83	0.85
1M NH4H2PO4	0.50	0.50	0.50
Micronutrient Solution	0.25	0.25	0.25
H2)	to 1L	to 1L	to 1L

Table 2.5 The amount of each compound needed to make 1L of the appropriate concentration stock solution

Table 2.6 Total Concentrations in Millimoles(mM) of Macroelements in Solutions for Preliminary Experiment 2			
Nutrient	Low K	Med K	High K
K	0.094	0.188	1.500
NO3	3.500	3.508	3.000
NH4	0.501	0.501	0.501
N total	4.001	4.008	3.501
PO4	0.500	0.500	0.500
Ca	1.709	1.666	1.006
Mg	0.852	0.828	0.250
SO4	0.853	0.829	0.251

Total Concentrations in Micromoles of Microelements in Solutions for Preliminary Experiment 2	
Nutrient	
Mn	0.500
Cu	0.125
Fe	12.500
Zn	0.500
B	6.250
Mo	0.875

Table 2.6 This table shows the concentration of all nutrients in the low, medium, and high K level nutrient solutions used in preliminary experiment 2; the concentration of the microelements are also given.

Measurements that were taken for this experiment include; leaf area proxy weekly, leaf appearance rate (LAR) weekly, photosynthesis and stomatal conductance at two week intervals, xylem pressure potential before harvest, shoot K content at harvest, leaf area at harvest, shoot dry weight at harvest, specific root length, total root dry weight and length, root hair length and density.

The leaf area proxy was calculated by multiplying the length of the middle leaflet of each trifoliate that is longer than 20mm, by the width at the widest part of the same middle trifoliate leaflet. The leaf area measurements were taken on 4, 11, and 28 Nov. 2011. The LAR was calculated by counting the number of trifoliate leaves each week which have a middle leaflet longer than 20mm and plotting the data over time. The specific root length was measured by scanning the root sample with the EPSON scanner and then analyzing the picture with

WinRHIZO Pro to determine the specific root length. The photosynthesis and conductivity were measured using the Li-COR LI 6400 on 11, 17, and 29 Nov 2011. The xylem pressure potential was measured using the Model 615 Pressure Chamber Instrument (PMS Instrument Company) on 29 Nov. 2011 just before harvesting.

Results

There was no significant difference in xylem pressure potential between any of the nutrient levels within the DOR364 plants or within the G19833 plants. There were also no significant difference within the same nutrient levels between the two different genotypes.

Table 2.7 Average Leaf Water Potential and Standard Error

Genotype	K Level	AverageLeaf Water Potential (-Bar)	Standard Error
DOR364	Low	1.69	0.2366
DOR364	Med	2.00	0.4082
DOR364	High	1.56	0.4828
G19833	Low	1.25	0.0000
G19833	Med	1.13	0.3146
G19833	High	1.38	0.0722

Table 2.7 The average xylem pressure potential reported in negative bar and the standard deviation and standard error.

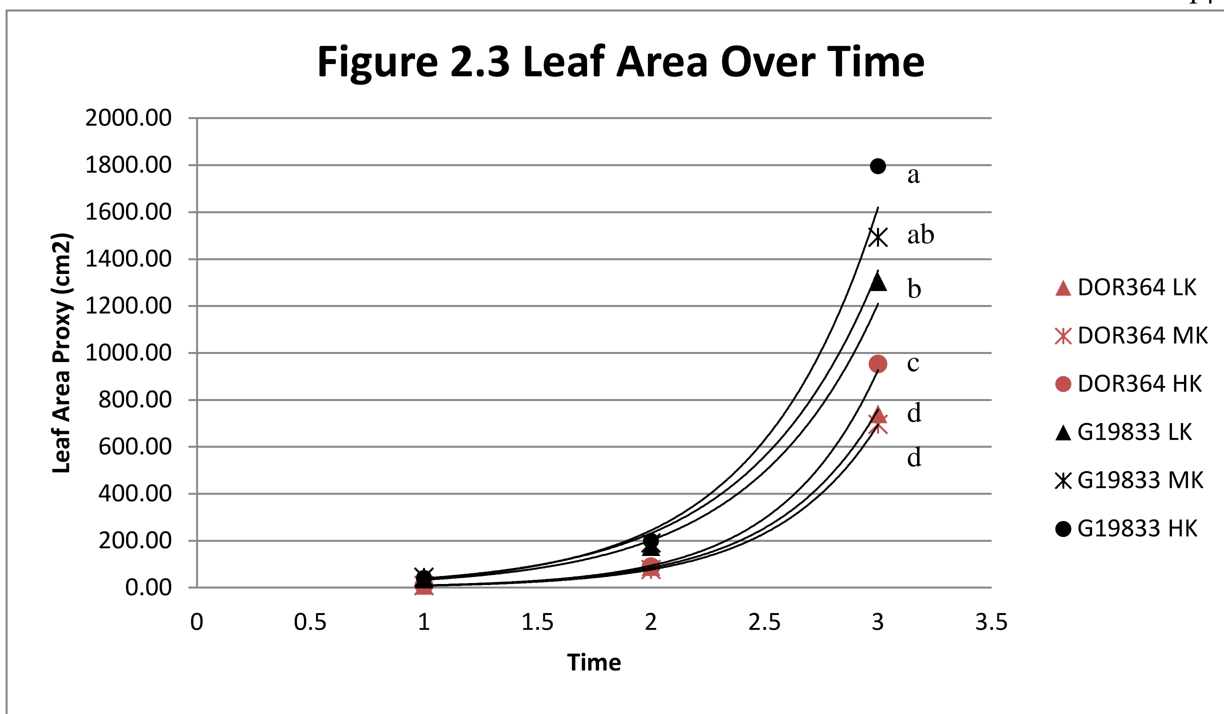

Figure 2.3 The average leaf area proxy plotted for each treatment over time. Exponential trendlines where fitted to each set of data points.

All DOR 365 plants regardless of nutrient level used had lower leaf area than G19833 plants on 28 Nov, the day before being harvested. Using the T-test ($p<0.05$) there is a significant difference between the final leaf area (leaf area on 28 Nov) of DOR 364 plants grown in low versus high K level solutions. There was also a significant difference between plants grown in medium versus high K level solutions. There was no significant difference between leaf area of plants grown in low versus medium K level solutions. As for the G19833 plants there was a significant difference between final leaf area for plants grown in low versus high K level solutions, but no difference between low versus medium or medium and high.

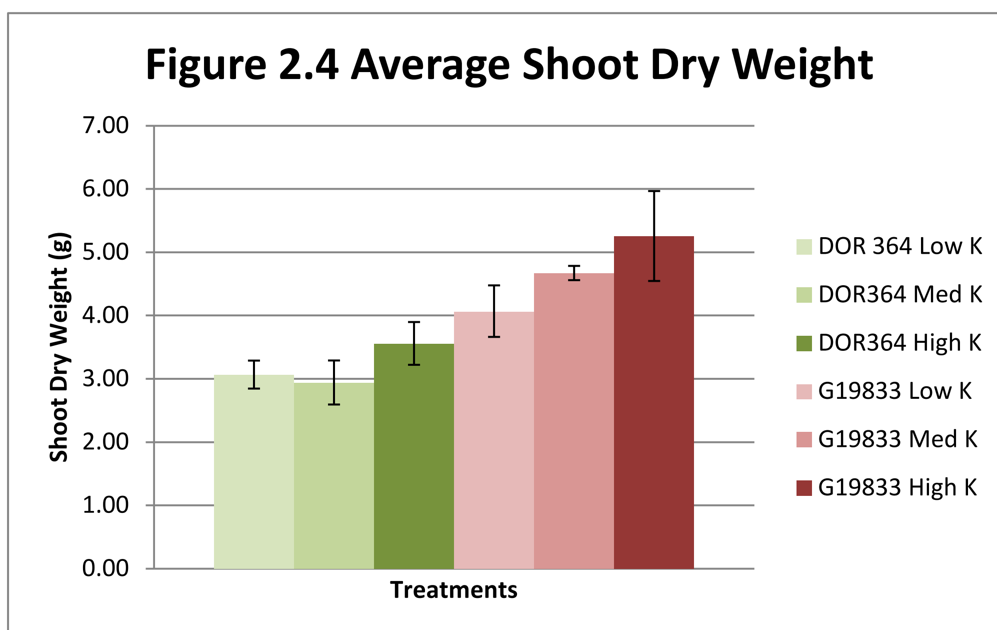

Figure 2.4 Average shoot dry weight for low, medium and high K treatments of DOR364 and G19833 plants.

While none of the K treatments had a significant effect on shoot dry weight there was still a trend that is noticeable (Figure 2.4). The G19833 plants had a larger shoot dry weight than the DOR364 plants. The average shoot dry weight for G19833 under the high K treatment was 5.25g and under the low K treatment was 4.07g. The average shoot dry weight for DOR364 under high K was 3.56g and under the low K treatment was 3.06g.

Discussion

There was no significant difference between the plant water potential for any of the treatments in preliminary experiment 2. It was expected that the K deficient plants would have a higher leaf water potential because plants with lower K levels cannot regulate their stomates as efficiently as plants with adequate K levels and therefore the K deficient plants may have a lower transpiration rate (Roa and Roa 1983). The results of no significant differences could be because all the plants were watered with equal amounts of nutrient solution and the K stress was not severe enough to affect the leaf water potential significantly.

The significant difference in leaf area between DOR364 and G19833 plants grown in low vs high K levels indicated that the level of K stress in the low K level nutrient solution was severe enough to give noticeable difference. Since leaf area of the G19833 plants grown in medium K were not significantly different from those grown in the high K solution, but in DOR364 it was different could indicate that DOR364 plants are more susceptible to K stress. This would make

sense because DOR364 has shorter root hairs than G19833 and may not be as efficient in K acquisition as G19833 plants with longer root hairs.

Preliminary Experiment 3

Introduction

In preliminary experiment 2 there were no significant differences seen in the root hair lengths of different genotypes (DOR364 and G19833) that were expected to have phenotypic differences in root hair length when grown under low, medium and high K environments. The plants in preliminary experiment 2 were fertigated with nutrient solution at each watering event and therefore it is thought that there was not sufficient K stress on the plants that were grown under low K levels. The plants in preliminary experiment 2 also received adequate levels of P, which would cause their root hairs to be shorter since it has been observed that low P stimulates root hair elongation (Bates and Lynch 1996).

The method used to give the plants the nutrients they require was adjusted. Instead of watering the pots with nutrient solution at each watering event, the pots only received 2 liters of nutrient solution after the germinated seeds were transplanted into the pots. After that they receive DI water with no added nutrients. The nutrient solution used also contained less P so that the root hairs would not be short in response to high levels of P. The purpose of preliminary experiment 3 was to determine what amount of moles of nutrients needs to be added to the pots of media in order to create an environment with low P levels. The P deficiency has to be great enough to prevent all root hairs from being short. The purpose of preliminary experiment 3 was also to determine adequate levels of K stress where differences in plant growth and root hair growth can be observed in response to these lower levels of K. Preliminary experiment 4, although only a small pilot study, was done in response to preliminary experiment 3 and was aimed at determining a proper fertigation regime.

Genotypic variation in root hair length are important because by selecting genotypes with favorable root hair characteristics, such as longer and denser root hairs, farmers may be able to increase their yields simply by growing these genotypes with superior root hair characteristics. Root hairs are essential for nutrient acquisition and longer root hairs increase the surface area of the root that comes into contact with the soil solution. By increasing root hair length a plant's nutrient acquisition will be more efficient. This has been seen in the acquisition of P in common bean. There is a positive correlation between common bean genotypes with long root hairs and

shoot P concentration and content (Miguel 2004). To test the effect that differences in root hair length has on K acquisition from low K environments, it is important to grow genotypes that show variation in root hair length. If genetic variation in root hair length and its effect on nutrient acquisition is better understood, improving the genetics of common bean can take place by selecting genotypes with desirable traits. The third part of this paper describes the phenotyping of various common bean genotypes. This was done to help with the selection of the genotypes to study for evaluation of the effect of root hair length on potassium acquisition.

Materials and Methods

Preliminary Experiment 3 took place in The Pennsylvania State University's horticulture greenhouse A section 21 (40°47'29"N77°51"31"W). The media mixture used consisted of 10% red subfield soil (C horizon from a limestone-derived silt loam; fine, mixed, semiactive, mesic Typic Hapludalf), 60% perlite, and 30% medium grade sand by volume. This media mixture was determined by preliminary experiment 1 to have less variable results than other tested mixtures. Once the pots were filled with the media mixture, each pot was watered with DI water until water started dripping out the bottom. The common beans (*Phaseolus vulgaris*) were first germinated in a germination chamber at 28 degrees Celsius and transplanted at 3 days of age into 7 liter pots filled with moist media. Two genotypes were used in this study; DOR364 and G19833. Nutrient solutions were prepared according to the concentrations listed in table 2.8 and 2.9. All nutrient solutions contained the microelements presented in table 2.10 in the specified amounts. The study had two phosphorus levels, four potassium levels, and two replicates for each genotype and nutrient treatment. Both phosphorus levels were lower than what is expected for a plant to use, in order to create a phosphorus deficiency that will encourage root hair growth. Phosphorus is 0.2% of a plant's dry weight varying slightly with plant species, the growing conditions and the time at which the tissue sample is taken (Market 1992). If the average weight of a DOR 364 plant is about 4.2g and of a G19833 plant is about 6.5g (weights from preliminary experiment 2), then the millimoles (mM) of P that the plant requires is between 0.27 and 0.42mM.

Calculation:

Given: P shoot content = 0.2%

Weight of plant = 4.2 g and 6.5g

Molecular Weight of P = 30.9738

(0.002)(6.52g) = 0.0084gP

0.0084gP (1 mole P/30.9738gP)(1000millimoles P/ 1mole P) = **0.27 millimoles P**

(0.002)(4.2g) = 0.013gP

0.013gP (1 mole P/30.9738gP)(1000millimoles P/ 1mole P) = **0.42 millimoles P**

The P levels were thus chosen to be 0.15mM and 0.50mM. Since P is fairly immobile ($H_2PO_4^-$ having a diffusion coefficient of $0.9x10^{-9}$ m^2/s) in the soil, not all of the P would be available for uptake by the plant and cause a deficiency in P.

The high potassium treatment was given an ample amount of potassium as not to create any potassium stress. The medium, low and extra low potassium treatments were adapted from the Epstein nutrient solutions. The medium K treatment had a 1/4 strength K, the low K had a 1/8 strength K, and the extra low had 1/16 strength K. Plants were watered with 1 L of appropriate nutrient solution the day after transplanting and two days after that.

Table 2.8 Millimoles of Nutrient in 1L of Solution for Lower Phosphorus Treatment

Nutrient	HK	MK	LK	ELK
K	12.0	1.5	0.8	0.4
PO_4	0.15	0.15	0.15	0.15
Ca	8.0	8.0	8.0	8.0
Mg	2.0	2.0	2.0	2.0
SO_4	2.0	2.0	2.0	2.0
NO_3 from KNO_3	12.0	1.5	0.75	0.375
NO_3 from $Ca(NO_3)_2$	16.0	16.0	16.0	16.0
NH_4 from $NH_4H_2PO_4$	0.15	0.15	0.15	0.15
H_2NCONH_2	3.53	8.78	9.15	9.34
Total N	35.2	35.2	35.2	35.2

Table 2.8 Millimoles of nutrients in 1L of solution at various K levels that the plants under the lower phosphorus treatment received. K treatments; 12.0millimoles K is high K (HK), 1.5millimoles K is medium K (MK), 0.8millimoles K is low K (LK), and 0.4 millimoles K is extra low K (ELK).

Table 2.9 Millimoles of Nutrient in 1L of Solution for Higher Phosphorus Treatment				
Nutrient	HK	MK	LK	ELK
K	12.0	1.5	0.8	0.4
PO_4	0.50	0.50	0.50	0.50
Ca	8.0	8.0	8.0	8.0
Mg	2.0	2.0	2.0	2.0
SO_4	2.0	2.0	2.0	2.0
NO_3 from KNO_3	12.0	1.5	0.75	0.375
NO_3 from $Ca(NO_3)_2$	16.0	16.0	16.0	16.0
NH_4 from $NH_4H_2PO_4$	0.50	0.50	0.50	0.50
H_2NCONH_2	3.35	8.60	8.98	9.16
Total N	35.2	35.2	35.2	35.2

Table 2.9 Millimoles of nutrients in 1L of solution at various K levels that the plants under the higher phosphorus treatment received. K treatments; 12.0millimoles K is high K (HK), 1.5millimoles K is medium K (MK), 0.8millimoles K is low K (LK), and 0.4millimoles K is extra low K (ELK).

Table 2.10 Micromoles of Microelements in 1L of Nutrient Solution	
Element	Micromoles
Mn	0.500
Cu	0.125
Fe	12.500
Zn	0.500
B	6.250
Mo	0.875

Table 2.10 Micronutrients and amounts that were added to all nutrient solutions that were used in preliminary experiment 3.

After each pot received its 2 liters of nutrient solution, watering was continued every other day with DI water using drip rings connected to a tub of water and a pump. Pots were watered until they started leaching out at the bottom (about 5 minutes). Leachate was collected each week. The leaf length and width of the middle leaflet longer than 2cm of each trifoliate was measured each week. From this data a total leaf area proxy was calculated by multiplying the length and the width and adding up the values for each plant.

About 25 days after planting, all plants were showing signs of stress such as slow growth and chlorosis, even the plants under the high K and higher P treatments. Therefore twenty-eight days after planting the higher P treatment plants received 0.5millimoles of PO_4 and 35.2 millimoles of N each, and the lower P treatment plants only received 35.2 millimoles of N. See Tables 2.11 and 2.12 below for the nutrients that were added.

Table 2.11 Millimoles of Nutrients added (1L per pot) to the High Phosphorus Treatment Plants

Nutrient	Millimoles
PO_4 from NH_4PO_4	0.50
N as H_2NCON_2	34.7
N from NH_4PO_4	0.50
Total N	35.20

Table 2.11 Millimoles of nutrients that were added to the higher P treatment plants because of the stunted growth and sign of N deficiency.

Table 2.12 Millimoles of Nutrients added (1L per pot) to the Low Phosphorus Treatment Plants

Nutrient	Millimoles
N as H_2NCON_2	35.20

Table 2.12 Millimoles of N added to the lower P treatment plants because of the signs of N deficiency.

Forty-three days after imbibitions, the shoots and roots were harvested. The shoots were dried in an oven at 60 degrees Celsius and after reaching a constant mass, the shoot dry weight was measured. One adventitious and one basal root sample were collected from each plant, but no further analysis was done with the roots.

Results

Although some data was collected the results from this experiment cannot be fully relied upon because of an initial mix-up when applying the nutrient solutions. The problem was corrected, but it is not certain if the treatments received the exact nutrient solutions as intended.

Discussion

The experiment was compromised by poor management of K levels and therefore the results are inconclusive. It can still be concluded from preliminary experiment 3 that fertigating the plants only once at the beginning of the experiment does not supply the plant with an adequate amount of nutrients that it would need to grow for the remainder of the experiment. It

was also determined that five minutes of watering with the drip ring irrigation system is too long of a time because the pots reach field capacity and then start leaching out at the bottom of the pots. This could also have contributed to the poor growth of the plants because much of the nutrients may have leached out from the soil.

Preliminary Experiment 4

Materials and Methods

Because of the poor growth of all the plants in preliminary experiment 3, a small study was done where the plants received P and K in solution form at the initial watering (nutrient concentrations in Table 2.13), but then for the remainder of the experiment were fertigated with nutrient solution that contained no P or K (concentrations in Table 2.14), except for the high K treatment which received 1.5mmoles of K at each watering throughout the entire growing period of the experiment. In this way, P and K are limited but all other nutrients will be available to the plants. Plants were grown in the same fashion as described for preliminary experiment 3 and were grown in the same greenhouse. Seeds from L88 population RIL #57 were used. There were three K treatments; high, medium and low. And the P level was increased to 1.0mmoles per pot, which is more than what was given to each plant in preliminary experiment 3, but still limiting to the plant.

Table 2.13 Millimoles of P and K at Initial Watering			
Nutrient	HK	MK	LK
PO_4	1.0	1.0	1.0
K	1.50	1.50	0.75
Milliliters of Stock Solution to Make 1L of Nutrient Solution for Initial Watering			
1M $NH_4H_2PO_4$	1.0	1.0	1.0
1M KNO_3	1.5	1.5	0.75

Table 2.13 Nutrient solution used at the first watering of the plants. This was the plant's only supply of P and K for the experiment, except for the high K treatment plants which received 1.5mmoles of K at each watering. High K treatment is HK, medium K treatment is MK, and low K treatment is LK.

The shoots and roots were harvested 48 days after imbibition. The shoots were dried and weighed for shoot dry weight. The roots were excavated and washed with a hose and one representative basal and adventitious root were removed from each plant and placed in a vial with 75% ethanol. Three pictures were taken in the 10-15 cm section from the basal end of the root to be used for root hair length analysis. The pictures were taken through a dissecting light microscope using a Nikon SMZ-U camera at 4.0X zoom. The pictures and Image J were used to measure the root hair length. Five root hairs were measured from each picture and the measurements from each picture were grouped together to get an average root hair length for each treatment.

Table 2.14 Millimoles of Nutrient in 1L of Solution (-P and -K)	
Element	Millimoles
Ca	1.0
Mg	0.25
SO4	0.25
NO3 from Ca(NO3)2	2.0
H2NCONH2	2.0
Total N	4.0
Milliliters of Stock Solution to Make 1L of Nutrient solution (-P and -K)	
Stock Solution	Milliliters
1M Ca(NO3)2	1.0
1M H2NCONH2	1.0
0.5M MgSO4 7H2O	0.50
micronutrient stock (-K)	0.25

Table 2.14 Nutrient solution to water the plants after the initial watering. It contained no P or K.

Results

Although the low K plants did show some signs of stress in the form of leaf chlorosis, no significant difference in shoot dry weight was found between any of the treatments.

A significant difference in root hair length of basal root samples was observed between low and high K treatments ($P<0.01$) and between medium and high K treatments ($P<0.05$) (Figure 2.5).

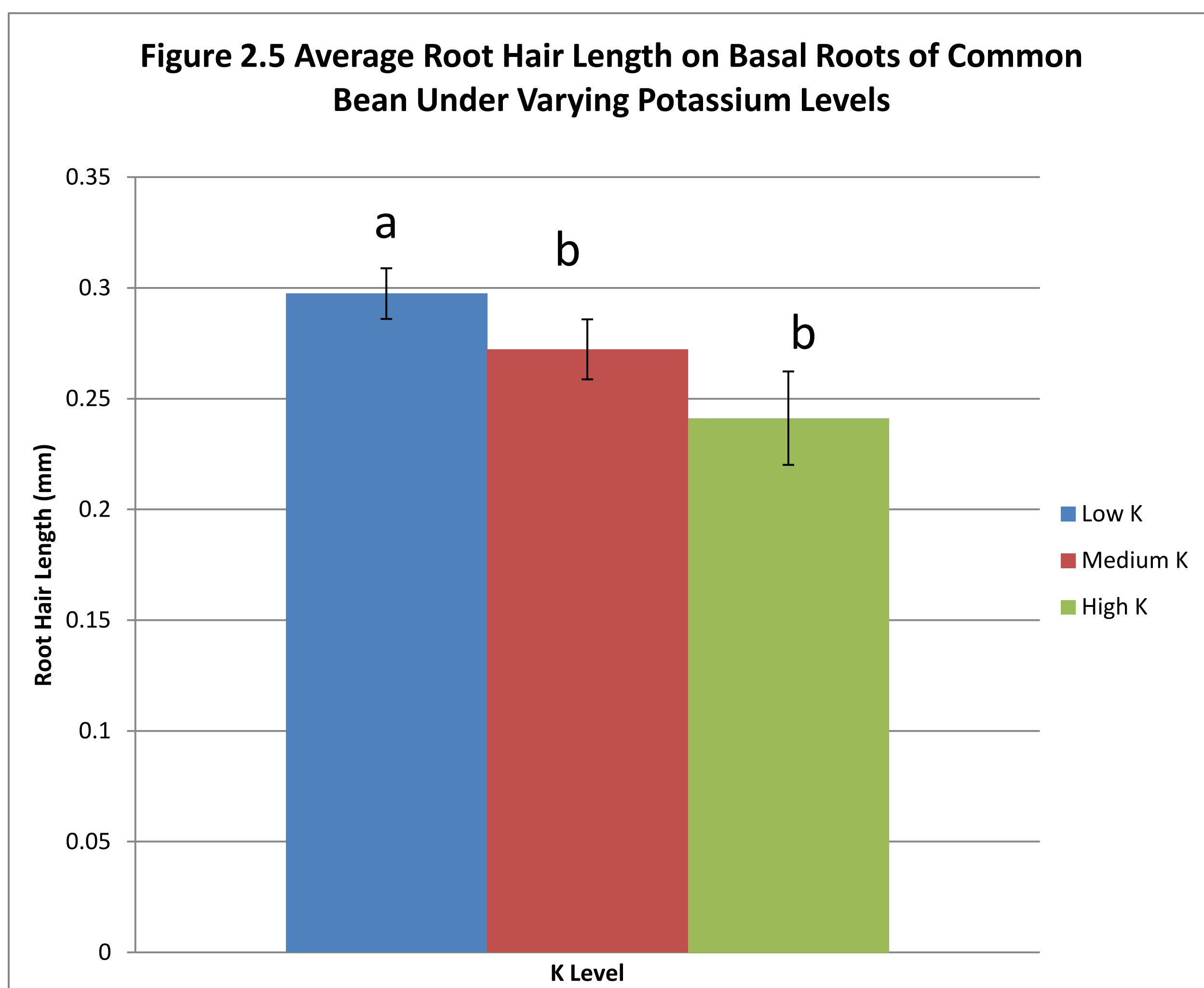

Figure 2.5 Average root hair length at low, medium and high potassium levels. The root hair length of those plants grown under low K were significantly longer than those grown under medium and high K levels ($P<0.05$).

No significant differences in root hair length were observed for root hairs on adventitious root samples.

The average root hair length of root hairs on basal roots from preliminary experiment 4 was 0.27mm and the average root hair length of root hairs in basal roots from preliminary experiment 2 was 0.21mm. There is a significant difference in average root hair length ($P<0.01$) between the two experiments.

Discussion

It was noted that the root hair lengths measured all seem to be shorter than what have been observed by other researchers. The average root hair length for preliminary experiment 4 was 0.27mm. Similar root hair lengths were observed in preliminary experiment 2; the average

being around 0.21mm. Miguel (2004) observed root hair lengths between 0.5 and 0.85mm under low P conditions and an average length of 0.34mm under high P levels. These differences could be due to many factors. Miguel conducted his studies in the field and in the greenhouse. For the studies that were done in the greenhouses a different media mixture was used; one containing sand, vermiculite and soil at the ratio of 4:3:3. This study used a media mixture of sand, perlite, and soil at a ratio of 3:6:1. Other factors that could influence root hair length include soil temperature and the growing conditions in the greenhouse. Roots are very responsive to their environment and therefore differences in root hair length should not be surprising. There is also a possibility that the washing of the roots with a hose after excavation damaged the root hairs, therefore in the final experiment more care will be taken when harvesting the root samples for root hair analysis.

From preliminary experiment 4 it was concluded that the level of P stress was adequate to prevent all root hairs from being similar in length and showing little variation. The root hair lengths were significantly longer in preliminary experiment 4 compared to preliminary experiment 2. Although the difference is significant, it is not a very large increase (from 0.21mm in preliminary experiment 2 to 0.27mm in preliminary experiment 4). It must also be noted that the same genotypes where not used in both experiments, therefore the comparison is not as valid as if they had been the same genotypes.

Since plants under low K had significantly longer root hairs than those under high K, it can be concluded that root hair length is responsive to K levels in the soil. Plasticity in root hair length has been observed in Arabidopsis and maize in response to P levels in the media. As availability of P decreases root hair length of plastic genotypes increases (Zhu 2010 and Bates and Lynch 1996). Hogh and Pedersen found that pea (*Pisum sativum* cv. Nitouche), lucerne (*Medicago sativa* L. cv. Daisy), barley (*Hordium vulgare* cv. Jolante), rye (*Secale cerale* cv. Motto), perennial ryegrass (*Lolium perenne* L. cv. Pimpernel), and oilseed rape *(Brassica napus oliefera* cv. Star) responded to low K conditions by increasing root hair length which also led to increases in K content in the shoots (Hogh and Pedersen 2003). Identifying genotypes that show K plasticity could be even more beneficial than those that simply always have long root hairs. Since there was no significant difference between the root hair length of the plants treated with medium K versus high K, it seems that inducing a medium K stress is difficult and that the plants are either K stressed therefore have shorter root hairs, or the plants are not K stressed and have longer root hairs.

It was determined that analyzing root hair length from basal roots is better than using adventitious roots because differences were observed in root hair length on basal roots but not on adventitious roots. Different root classes behave differently. For the final experiment, only basal root samples were collected for root hair analysis. The amount of P, 1.0millimoles per pot, provided enough P stress that all the root hairs were not short and with little variation as what was seen in preliminary experiment 2. This same amount of P was applied to the pots for the final experiment.

Root Hair Survey

Materials and Methods

In order to determine which genotypes to grow for the final experiment, 14 different recombinant inbreed lines from the parent cross DOR364 x G19833 were phenotyped for root hair length. The genotypes selected were those used by Miguel (2004) and a couple new genotypes were selected. They included: DxG 6,13,27,32,36,37,47,52,53,66,67,82 and 83. Three seeds from each genotype were rolled up in germination paper and placed in a beaker with 0.5M $CaSO_4$. Seeds were placed in the germination chamber (at 28 degrees Celsius) for four days and were then moved to a growth room with 8 hours of light for another four days. On the ninth day pictures of the root hairs were taken using a dissecting light microscope and a Nikon SMZ-U camera at 4.0X zoom. Three representative areas on a basal root were selected and photographed per plant. Five root hairs were measured for each picture and then averaged.

Results

Significant differences in root hair length were observed. The average root hair length for each genotype can be seen in Figure 1.2. DG19 had the longest root hair length at 0.54mm and DG 67 had the shortest at 0.33mm.

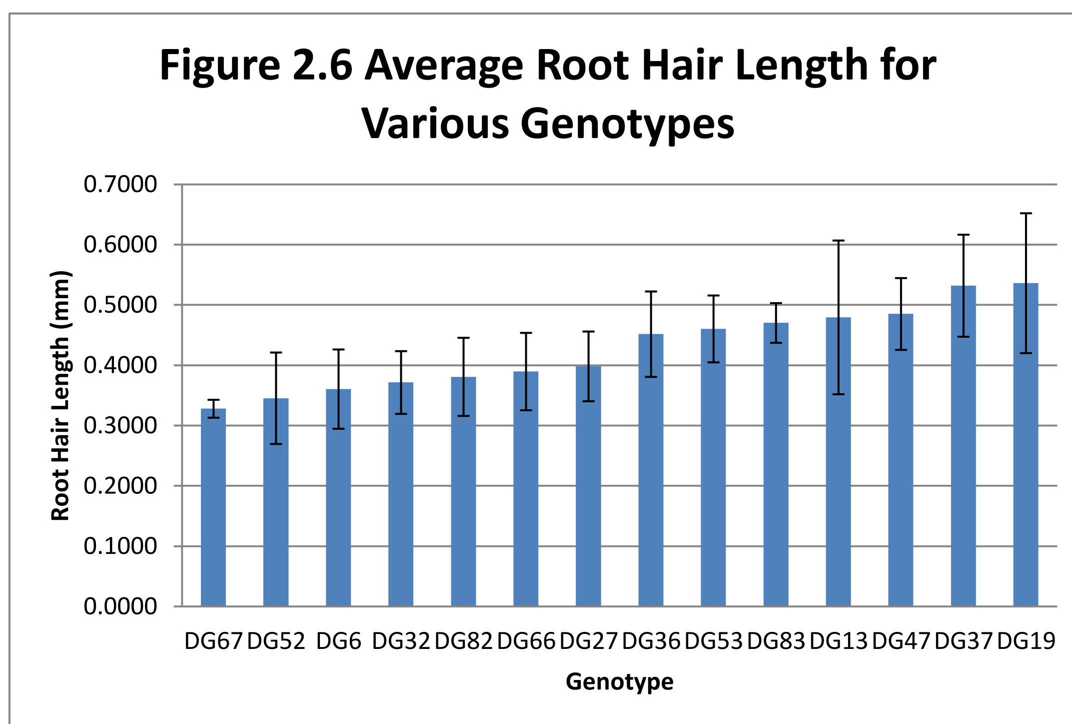

Figure 2.6 Average root hair length for the various genotypes tested. Significant differences were observed at $P<0.05$ and $P<0.01$.

Many phenotypes did not agree with the phenotypes reported by Miguel in his thesis. Some genotypes such as DG19 and DG47 which were found in this study to have shorter root hairs, were found by Miguel to have the longest root hairs. The histograms (figure 2.7 and 2.8) show that he observed the various genotypes to have longer root hairs in general. The majority of the genotypes tested in this study had an average root hair length between 0.0 and 0.5mm but Miguel's mostly fell in the range of 1.0-1.5mm. Although this study did not test exactly the same genotypes as Miguel did, 9 out of the 14 genotypes tested were the same.

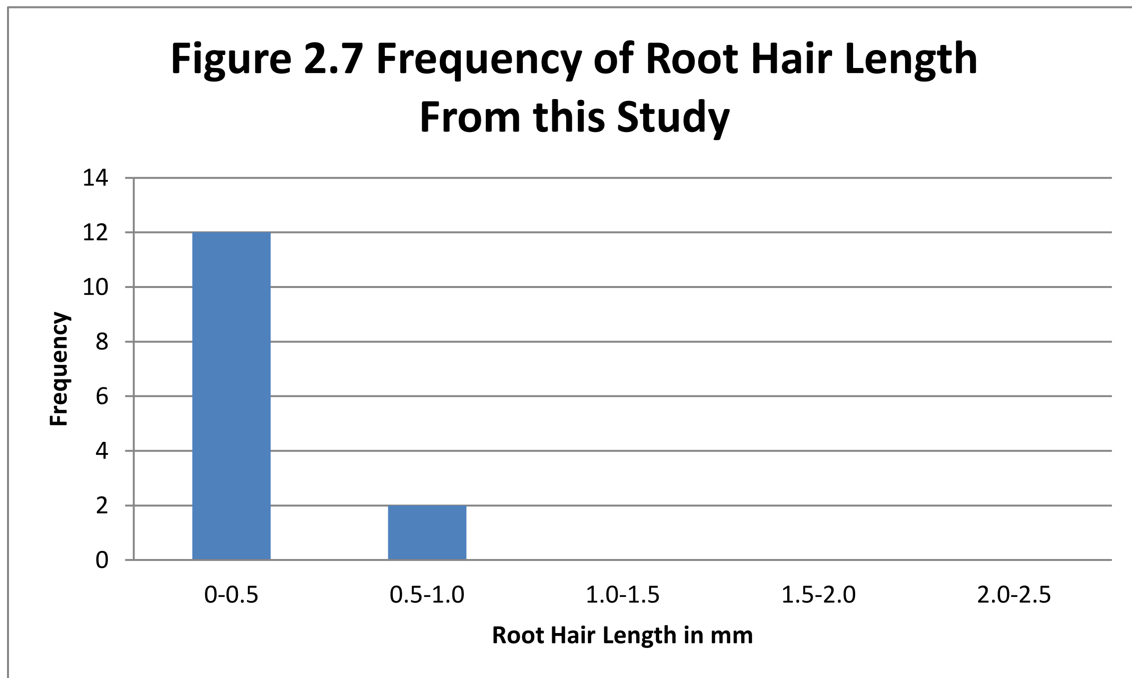

Figure 2.7 Majority of genotypes from this study had root hair lengths in the range of 0.0-0.5mm.

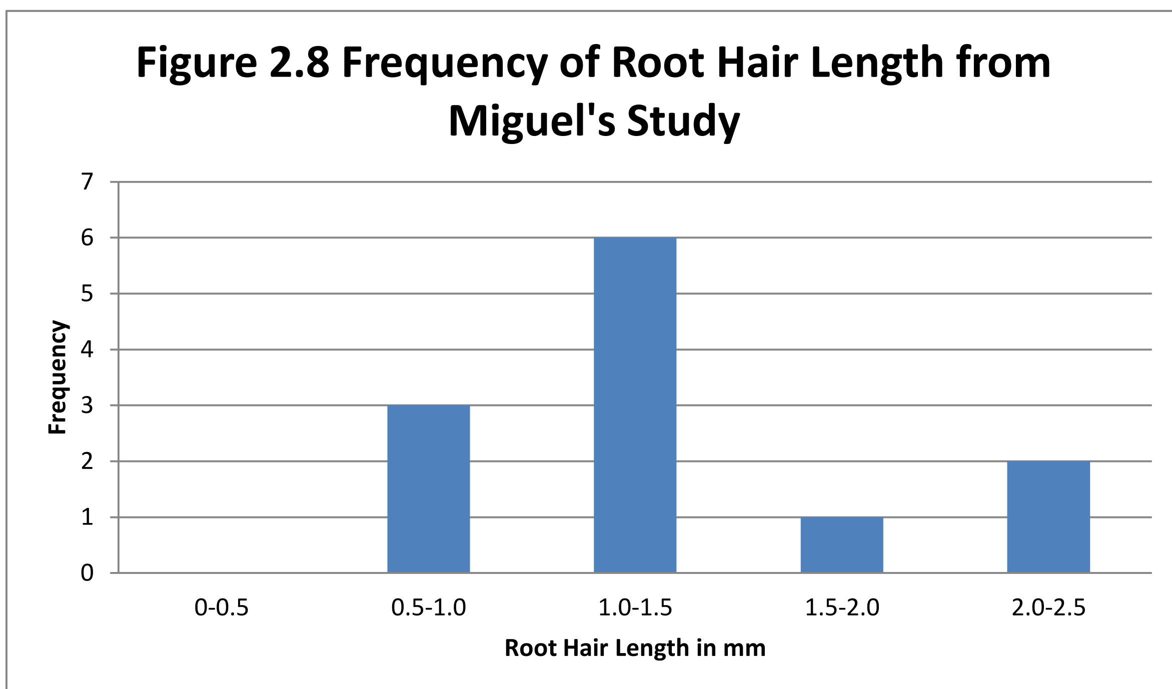

Figure 2.8 The frequency of genotypes used by Miguel (2004) fell into the longer ranges of root hair length.

Discussion

The differences in root hair length observed between the same genotypes tested in this study and in Miguel's study show how variable root hair length can be. A given genotype may not always express the same phenotype. The phenotype is influenced by genetics but also many other environmental factors. Because of the variability observed in root hair length it seems that selecting a specific common bean genotype to get a desired root hair length is more difficult than what was expected. It is important for there to be differences in root hair length once the plants are harvested, which will hopefully also correlate with or be related to the shoot K content and shoot dry weight. Three genotypes which were observed (in this study) to have longer root hairs were selected (DG 13, 37, 47) and three which were observed to have shorter root hairs were selected (DG 32, 52, 67). These six genotypes were chosen to be used the final experiment.

Chapter 3: Final Experiment

Introduction

The final experiment was conducted to test affect of genotypic variation in root hair length on the acquisition of potassium (K) under low K conditions. In preliminary experiment four there was a significant difference in root hair length when the plants were grown under lower P conditions to prevent root hair from being short due to adequate P. Because this was seen in preliminary experiment four, it was decided that a lower P level would be used in the final experiment as well to encourage root hair growth and variation. The experiment included six genotypes identified in the root hair survey. It was expected that those genotypes with longer root hairs would perform better under K stress conditions than those genotypes with shorter root hairs. It was also expected that root hair length would be affected by the potassium treatments. The low K treatment plants are expected to have the longest root hairs after six weeks of growth, followed by the medium K treatment with a medium root hair length, and the high K treatment was expected to have the shortest root hair length. More variation was expected to be seen in root hair length than in the previous studies since P suboptimal in this trial. A control treatment with high P and high K was included in this study and it is expected that it will have a shorter root hair length than the treatment with low P and high K.

Methods and Materials

This study was conducted in greenhouse A section 20 at The Pennsylvania State University (40°47’29”N77°51”31”W). The plants were grown in 10L pots with a top diameter of 25.5cm and a depth of 22cm. The growth media mixture used consisted of red soil (C horizon from a limestone-derived silt loam; fine, mixed, semiactive, mesic Typic Hapludalf), course perlite, and medium grade sand at a ratio of 1:6:3, respectively (as determined in preliminary experiment 1). The media mixture was made based on volume. The pots were first wrapped in duct tape (to reduce solar heating of the root environment), placed on raised benches in the greenhouse, and filled to the rim with media and care was taken to insure uniform bulk density. Each pot was watered with 2 liters of deionized (DI) water before planting. Six recombinant inbreed lines (RIL) of common bean (*Phaseolus vulgaris*) were used for this experiment. The RILs are from the parent cross between DOR364 and G19833. The RILs selected were: DG 13, 37, and 47 as having longer root airs and DG 32, 52, 67 were selected as having shorter root hairs. They were selected based on the observed root hair lengths from the root hair survey.

The selected seeds from the six genotypes were pre-germinated in a growth chamber at 28°C. The seeds were rolled up in germination paper and placed in a glass beaker with a 0.5mM $CaSO_4$ solution. After two days in the chamber the seedlings were transplanted to the prepared pots in the greenhouse.

Table 3.1First Watering: Millimoles of Nutrient per L of Solution		
Nutrient	(LP,HK) (LP,MK) (HP,HK)	(LP,LK)
KNO_3		
K	1.5	0.75
NO_3	1.5	0.75
$NH_4H_2PO_4$		
PO_4	1.0	1.0
NH_4	1.0	1.0
$Ca(NO_3)_2$		
Ca	1.0	1.0
NO_3	2.0	2.0
$MgSO_4$		
Mg	0.25	0.25
SO_4	0.25	0.25
H_2NCONH_2		
N	0.0	0.75
Total N	4.5	4.5

Table 3.1 Explanation of nutrient quantities for each treatment.

The experiment was set up in a randomized block design. The experiment had four replicates, each on a separate bench in the greenhouse (referred to as a block) and each one was planted two days after the other. The six genotypes were each grown under a lower P conditions with the three different K levels (high (LP,HK), medium (LP,MK) and low (LP,LK)). A control treatment was included with non-limiting P and non-limiting K (HP,HK). Each block contained 24 plants (six genotypes and four nutrient treatments)

The nutrient solution used to supply the plants with the appropriate nutrients and to control the K levels for the various treatments were prepared based on the Epstein Nutrient Solution. All plants were hand watered with a specific nutrient solution for the first watering. The plants that received the low K treatment were watered with 1 L of a ¼ strength Epstein solution that contained an adjusted amount of P and K. The plants that received the medium and high K treatment and the control plants were watered with 1 L of ¼ strength Epstein solution, adjusted for P and K (see Table 3.1).

After the plants were fertigated once with the above nutrient solution, they were fertigated with the following nutrient solution three times a week for the rest of the experiment. The plants under the high K treatment and the control plants were fertigated with a ¼ strength Epstein nutrient solution with no P. The control (HP,HK) plants were give 3.5g of triple superphosphate ($Ca(H_2PO_4)_2 H_2O$) per pot 23 days after planting. The plants under the low and

Table 3.2 Millimoles of Nutrient per L of Solution		
Nutrient	(LP,HK) (HP,HK)	(LP,LK) (LP,MK)
KNO_3		
K	1.5	0.0
NO_3	1.5	0.0
$NH_4H_2PO_4$		
PO_4	0.0	0.0
NH_4	0.0	0.0
$Ca(NO_3)_2$		
Ca	1.0	1.0
NO_3	2.0	1.0
$MgSO_4$		
Mg	0.25	0.25
SO_4	0.25	0.25
H_2NCONH_2		
N	0.5	2.0
Total N	4.0	4.0

Table 3.2 Millimoles of nutrient in one liter of nutrient solution used for the regular fertigation regime

medium K treatments were fertigated with a ¼ Strength Epstein solution with no K or P (see table 3.2). Each block had its own irrigation system through which the nutrient solution was given to the plants. The set up for each block consisted of two 32 gallon trash cans with a pump connected to tubes with drip rings for each pot. At each fertigation event the pumps were run for 4 minutes allowing approximately 0.5L of nutrient solution to be delivered to each pot.

Starting 15 days after imbibition, leaf area measurements were taken for each plant once a week. The length and width of the middle leaflet of each trifoliate was measured in millimeters. A proxy for the leaf area was calculated for each plant each week by multiplying the length by the width and adding up all these areas for each plant. Two leachate samples per nutrient treatment were collected per block, 29 days after imbibition and 41 days after imbibition. The leachate was filtered through a MAGNA Nylon 0.45micron filter. To measure the K concentration in the leachate, the filtered leachate was then diluted by a factor of five. The diluted leachate was analyzed for K concentration using the Perkin Elmer Analyst 100 AA-Atomic Absorption Spectrometer (AAS). The AAS was used in atomic emission mode to test for K concentration and the procedure adjust by Bob Snyder was followed. The samples that had a concentration higher than the standard curve were diluted again by a factor of three and tested again for K concentration. The K concentration was reported in mg of K^+ per liter.

Harvest occurred 42 days after imbibition for block 1 and 2, 41 days after imbibitions for block 3, and 40 days after imbibition for block 4. The shoot was cut at the soil line, rinsed in DI water, then in a 0.33mM HCl solution, and rinsed again in DI water before being dried in a 60 degree Celsius oven. Once the shoots reached a constant weight they were weighed for shoot dry mass, and ground at mesh 40 (0.4mm).

The roots were carefully excavated and two representative basal root samples were collected per plant and stored in 75% ethanol in a refrigerator. Extra care was taken when collecting the root samples for root hair length analysis. The root samples were removed from the root system and dipped in a bucket of DI water to remove media particles. The rest of the root system was washed with a hose and dried in the oven at 60 degrees Celsius until a constant mass was attained. The roots were then weighed for root dry mass. Of the two basal root samples collected, one was used to analyze root length. The length of the root sample was obtained by scanning the basal root using an EPSON scanner and then using WinRhizo to determine the total length. Based on the weight of the root sample and the length of the root sample, the specific root length was calculated by dividing the sample root length by the dry weight of that sample. The root length of the entire root system was calculated based on the specific root length and the dry weight of the root system. The second basal root sample was used for root hair analysis. Three pictures were taken in the 10-15 cm section from the basal end of the root to be used for root hair length analysis. Images were obtained using a Nikon SMZ-U camera at 4.0X zoom mounted on a dissecting light microscope. Image J was used to measure the root hair length. Five root hairs were measured from each image and the measurements from each image were grouped together to calculate an average root hair length for each plant. The basal root samples used for root length and root hair length analysis were dried in the oven after analysis and the weights were added to the root system dry mass.

After being ground, 50mg ±5mg of the shoot powder was weighed out in a glass vial and the weight recorded. The samples were ashed in a Thermolyne Type 4800 Furnace for 12 hours at 495°C. Three samples of peach leaves were included to use as a standard reference. The peach leaves used were 1547 Peach Leaves, U.S. Department of Commerce, National Institute of Standard s and Technology, Gaithersburg, MD 20899. The theoretical K concentration in the peach leaves was 2.43±0.03 wt. percent. After ashing and cooling the samples to room temperature, 500µl of 1N HCl was added to each sample. A Method Blank was prepared in the same way but without any ashed plant material. The acid was allowed to digest the ash for 15 minutes. Then 17.5ml of Millipore-filtered water was added to each sample. Finally 2.0ml of 1% Lanthanum chloride hexahydrate solution was added to each sample for a final volume of 20ml. The samples were capped and shaken. Each sample was filtered through a MAGNA Nylon 0.45micron filter and then analyzed for K concentration using the AAS. All samples were diluted by a factor of fifty. The samples were analyzed using the AAS in emission mode and at the wavelength set to 766.5nm. The percent of K in the shoots was calculated by dividing the AAS

value, which was in mg K^+/L, by 1000 to get the amount of K in the plant sample. The total amount of K in the whole plant was calculated from the shoot dry weight using cross multiplication. The K content was recorded in percent by weight.

Statistical analysis of the data included the Student T-test and analysis of variance (ANOVA). A combination of Excel and R were used to carry out the statistical analysis.

Results

The leaf area was significantly greater at 28DAP and at 35DAP for the HP,HK treatment compared to all other treatments ($P<0.01$). At 14 and 22DAP there was no significant difference between the HP,HK treatments and the other treatments. There was no significant effect of the K treatments on the leaf area at any point in time.

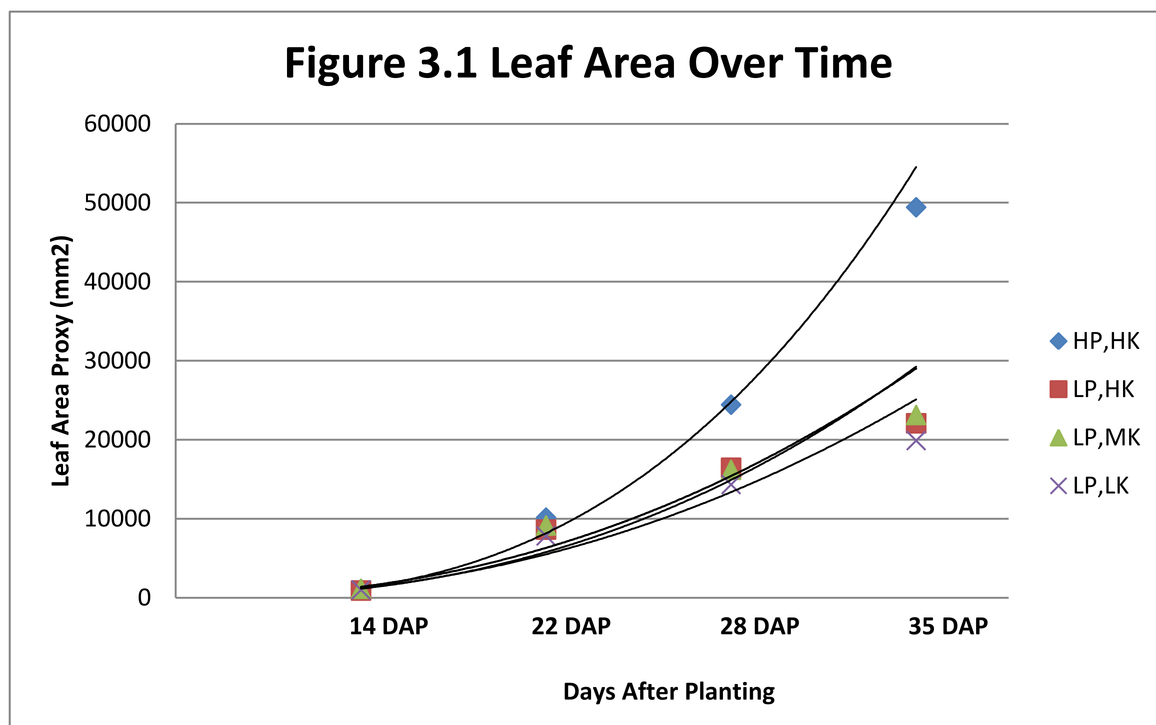

Figure 3.1 The average leaf area 14 days after planting until 35 days after planting. The HP,HK treatment had the greatest increase in leaf area.

The growth curves shows that the HP,HK treatments growth increased over time while the LP,HK treatment decreased slightly over time (Figure 3.2).

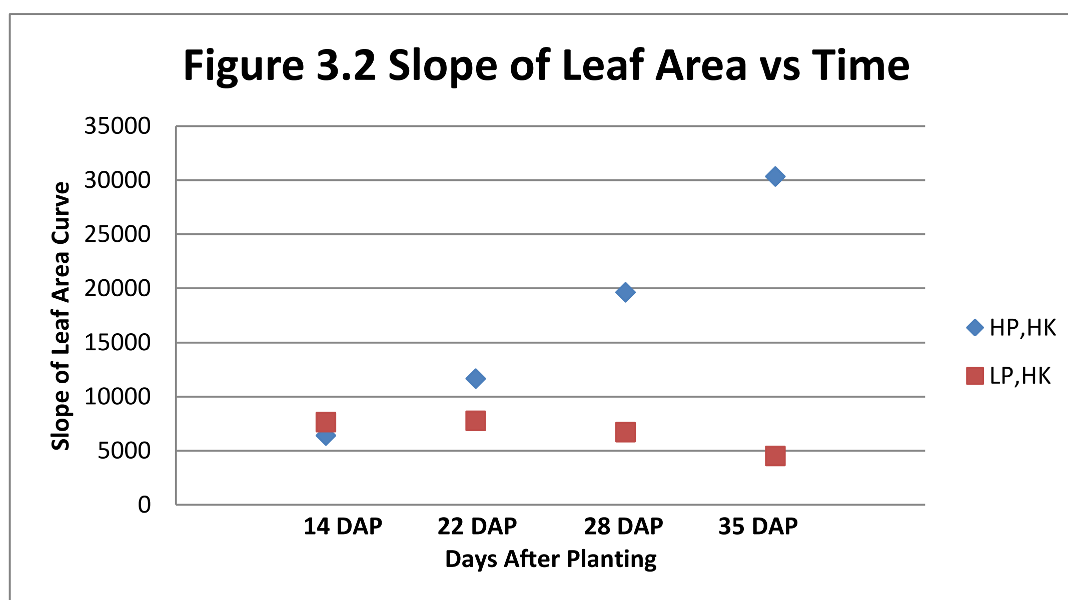

Figure 3.2 Slope of the leaf area curve versus time. The growth rate increased for the HP,HK treatment but decreased for the LP,HK treatment

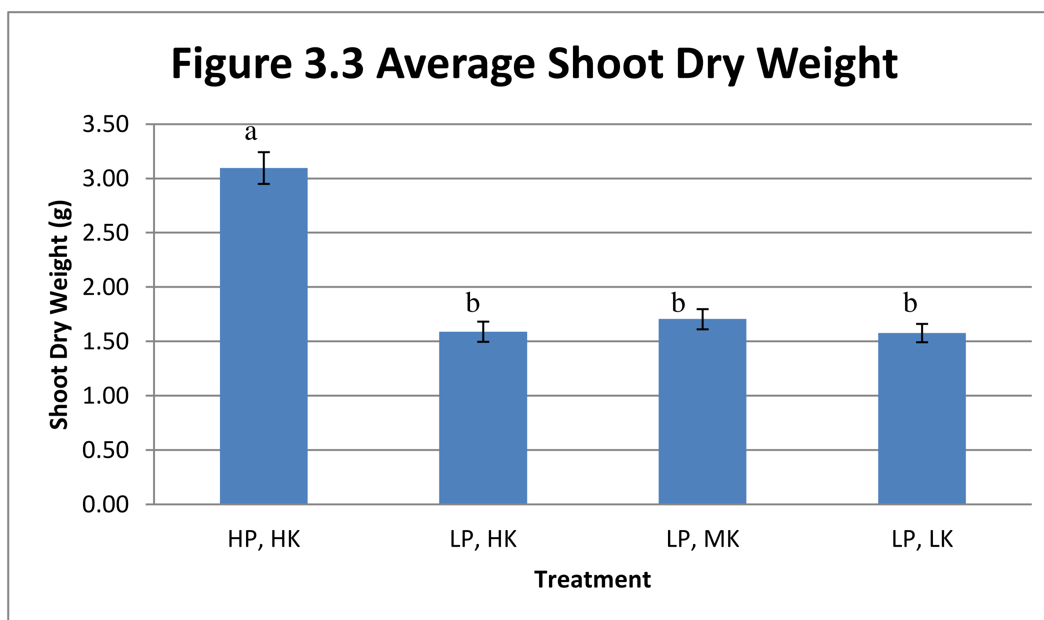

Figure 3.3 The LP,HK and the, LP,MK along with the LP,LK treatments did not receive adequate P and had a 50% decrease in shoot dry weight compared to the HP,HK treatment which had adequate P and K.

The HP,HK treatment plants had a significantly larger shoot dry weight than any of the other treatments. There was a 50% reduction in the shoot dry weight for the treatments that did not receive adequate P. The K treatments had no significant effect on shoot dry weight.

Table 3.3 ANOVA Table for Shoot Dry Weight (g) Based on Treatment					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	39.27	13.089	118.945	<2e-16
Treatment excluding control	2	0.237	0.1186	1.992	0.147

Table 3.3 Analysis of variance results for shoot dry weight based on treatment including and excluding the control (HP,HK) treatment.

The genotype had a significant effect on shoot dry weight. DG13 had the smallest average shoot dry weight at 1.4g and DG52 had the largest at 2.3g. The results were significant including and excluding the control (HP,HK) treatment in the analysis. The interaction between genotype and treatment was not significant.

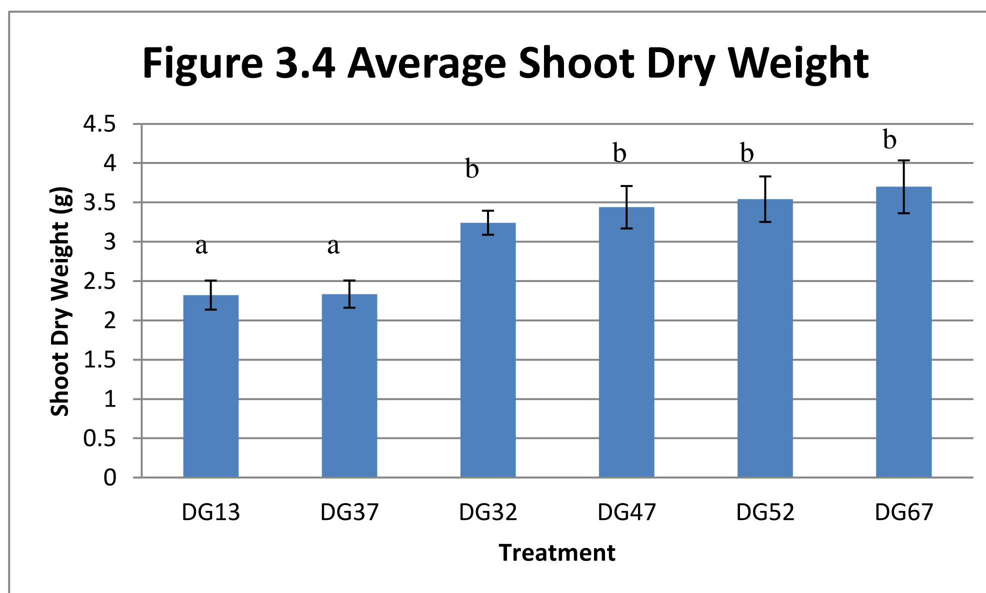

Figure 3.4 Average shoot dry weight was significantly affected by genotype. Standard error bars are included. (P<0.01).

Table 3.4 ANOVA Table for Shoot Dry Weight (g) Based on Genotype					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Genotype including control	5	13.38	2.676	24.320	5.01E-14
Genotype excluding control	5	7.39	1.4781	24.3837	1.37E-12
Treatment: Genotype including control	15	2.04	0.136	1.234	0.269
Treatment: Genotype excluding control	10	0.501	0.0501	0.842	0.591

Table 3.4 Analysis of variance results for shoot dry weight based on genotype including and excluding the control (HP,HK) treatment.

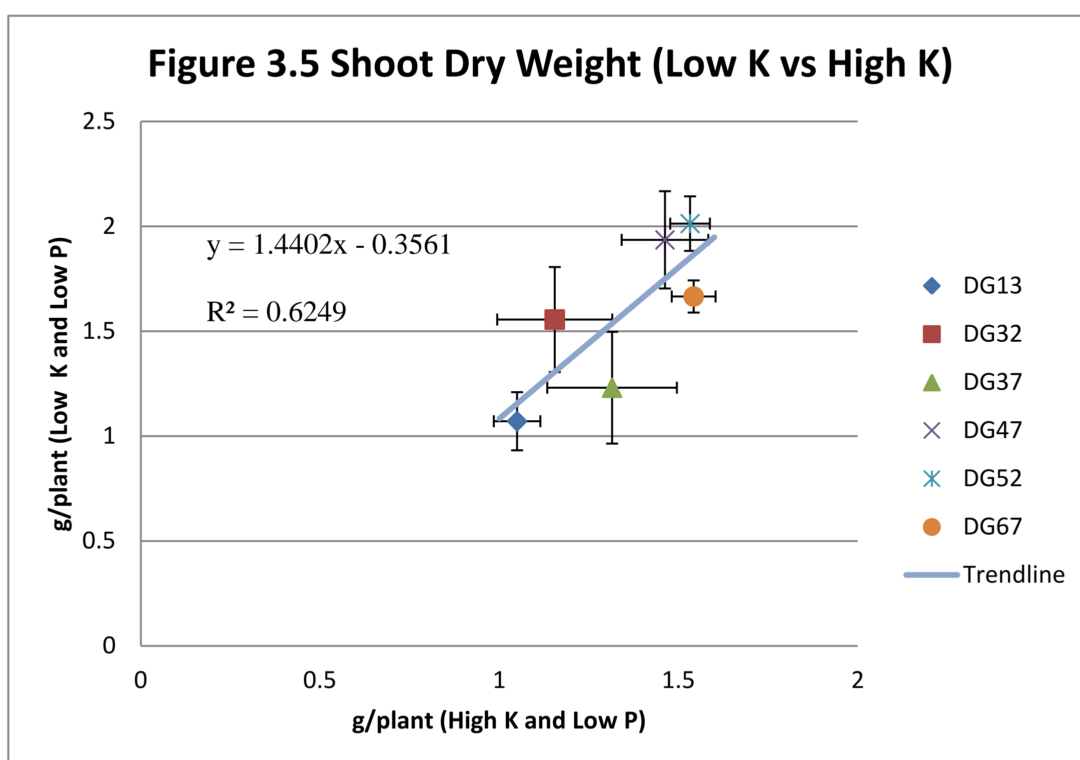

Figure 3.5 Shoot dry weight of LP,LK treatment versus LP,HK treatment. Standard error bars are included. The coefficient of determination is 0.62.

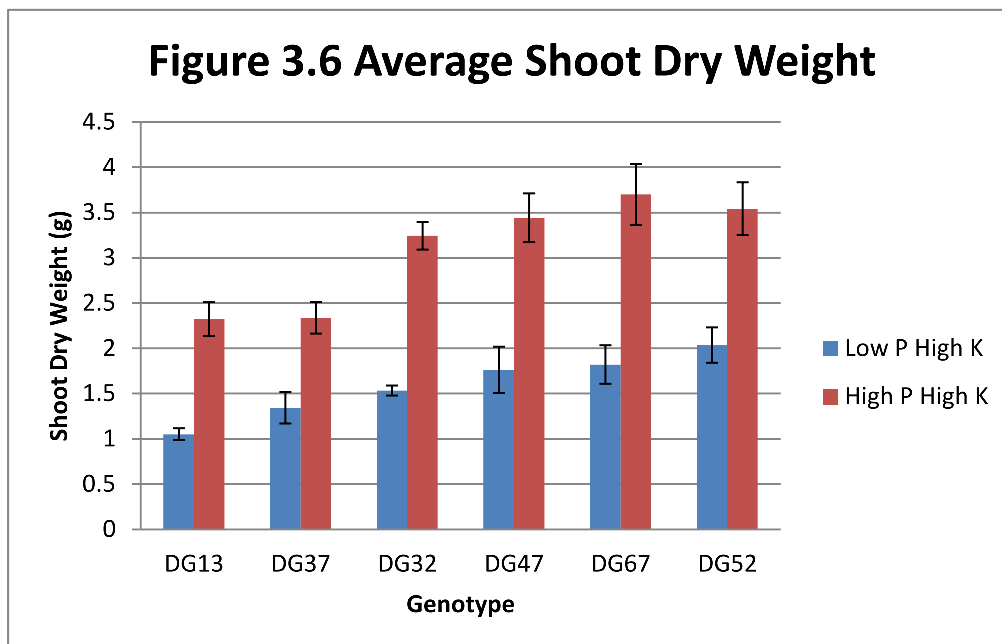

Figure 3.6 Comparing average shoot dry weight of various genotypes under low and high P treatment. Standard error bars are included.

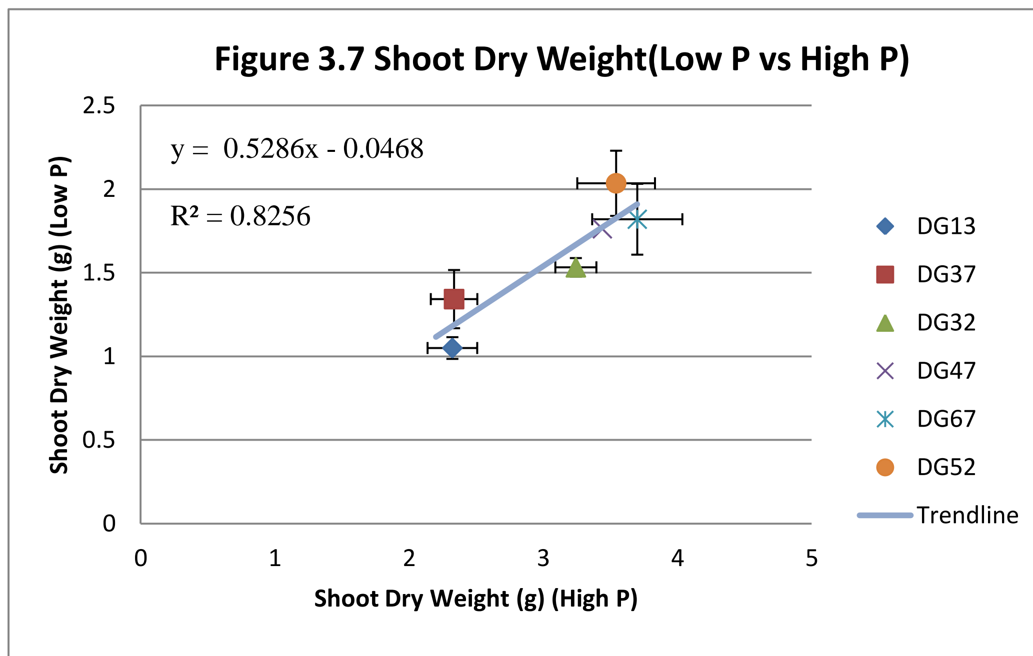

Figure 3.7 Correlation between shoot dry weight for low P treatment vs high P treatment. Standard error bars are included. The coefficient of determination is 0.83.

The nutrient treatments had no significant effect on root hair length including and excluding the control (HP,HK) treatment. Genotype did have a significant effect on root hair length when the HP,HK treatment was excluded from the analysis. The range of root hair lengths was between 0.197mm for DG67 and 0.252mm for DG13. There was no significant interaction between treatment and genotype.

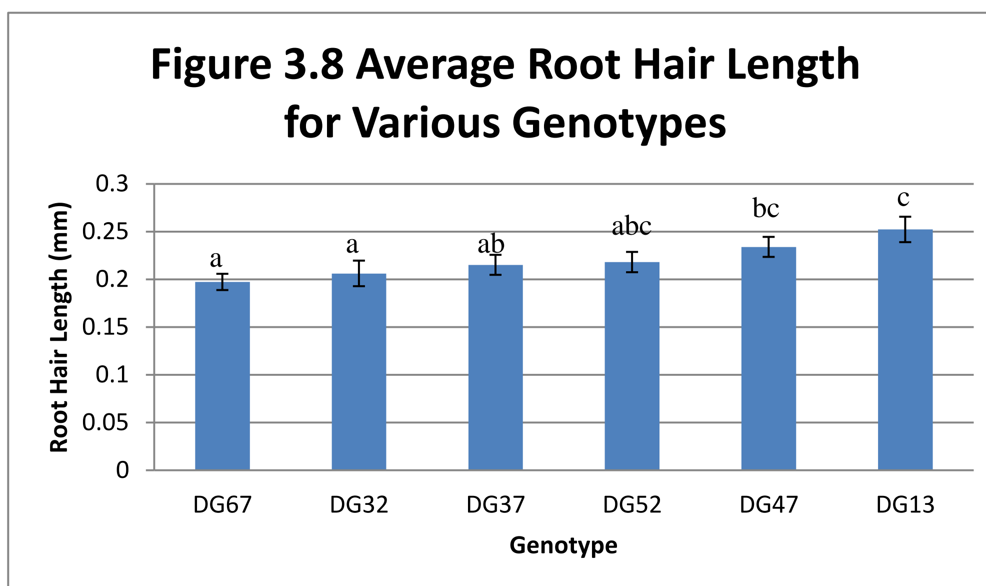

Figure 3.8 Root hair length for various genotype with significant differences were observed but there was little variation. Standard error bars are included. ($P<0.05$)

Table 3.5 ANOVA Table for Root Hair Length (mm)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	0.00562	0.001875	0.790	5.04E-01
Treatment excluding control	2	0.00110	0.000548	0.348	0.7078
Genotype including control	5	0.02516	0.005032	2.12	0.0733
Genotype excluding control	5	0.02364	0.004729	3.003	0.0188
Treatment: Genotype including control	15	0.02881	0.001921	0.809	0.6638
Treatment: Genotype excluding control	10	0.00937	0.000937	0.595	0.8106

Table 3.5 Analysis of variance results for root hair length based on genotype and nutrient treatment including and excluding the control (HP,HK) treatment.

The treatments had a significant effect on the root length of the plant only when the HP,HK treatment was included in the analysis. The HP,HK plants had an average root length of

123.2m while the LP,HK plants had a root length of 89.8m, the LP,MK plants 93.1m and the LP,LK plants 85.3m (Figure 3.9)

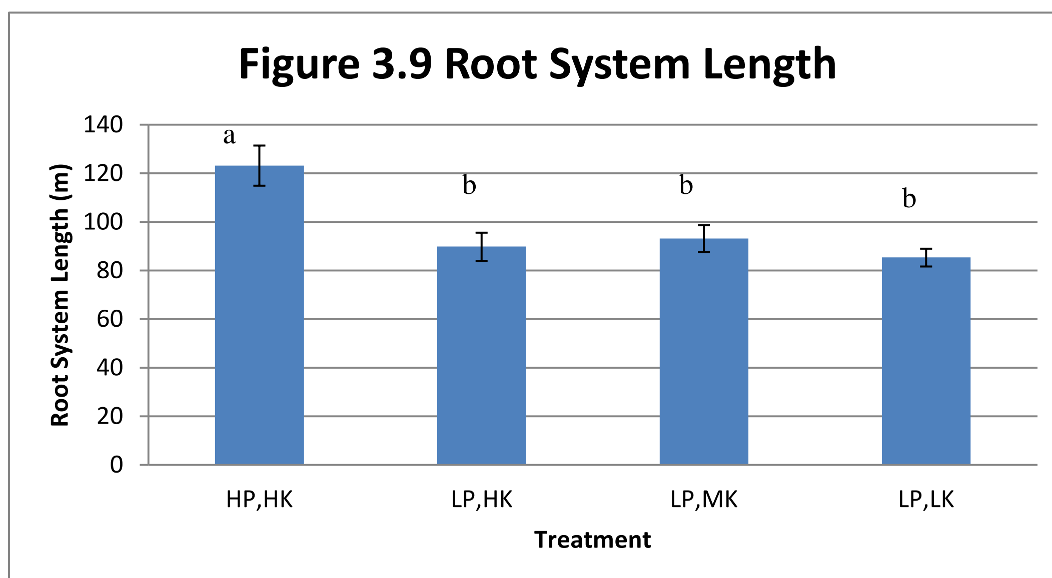

Figure 3.9 Root system length under the four nutrient treatments. The HP,HK treatment had a significantly longer root system than the other three treatments with low P. ($P<0.01$).

Root length was slightly affected by genotype excluding the control (HP,HK) and was significantly affected including the control. Under high P conditions there was more variability in root system length. DG32, 67, and 47 tended to have longer root system lengths compared to DG37, 52, and 13 under HP,HK conditions. Under low P conditions there was less variability.

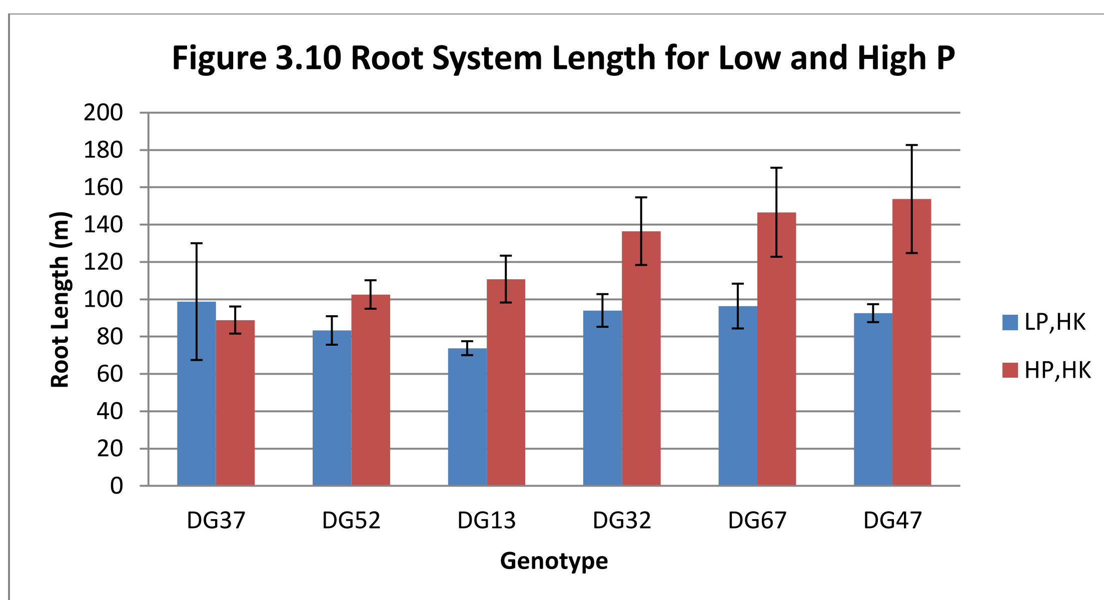

Figure 3.10 Root system length for various genotypes under low and high P treatments. Standard error bars are included.

Table 3.6 ANOVA Table for Root System Length (m)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	21243	7081	10.210	1.2e-05
Treatment excluding control	2	742	370.9	0.703	0.5000
Genotype including control	5	14395	2879	4.151	0.00235
Genotype excluding control	5	6033	1206.6	2.286	0.0597
Treatment:Genotype including control	15	9154	610	0.880	0.58854
Treatment:Genotype excluding control	10	3860	386.0	0.731	0.6917

Table 3.6 Analysis of variance results for root system length based on genotype and nutrient treatment including and excluding the control (HP,HK) treatment.

The K treatments had no significant effect on root dry weight to shoot dry weight ratio, but the HP,HK which had adequate P levels had a significantly lower root to shoot ratio compared to the three K treatments.

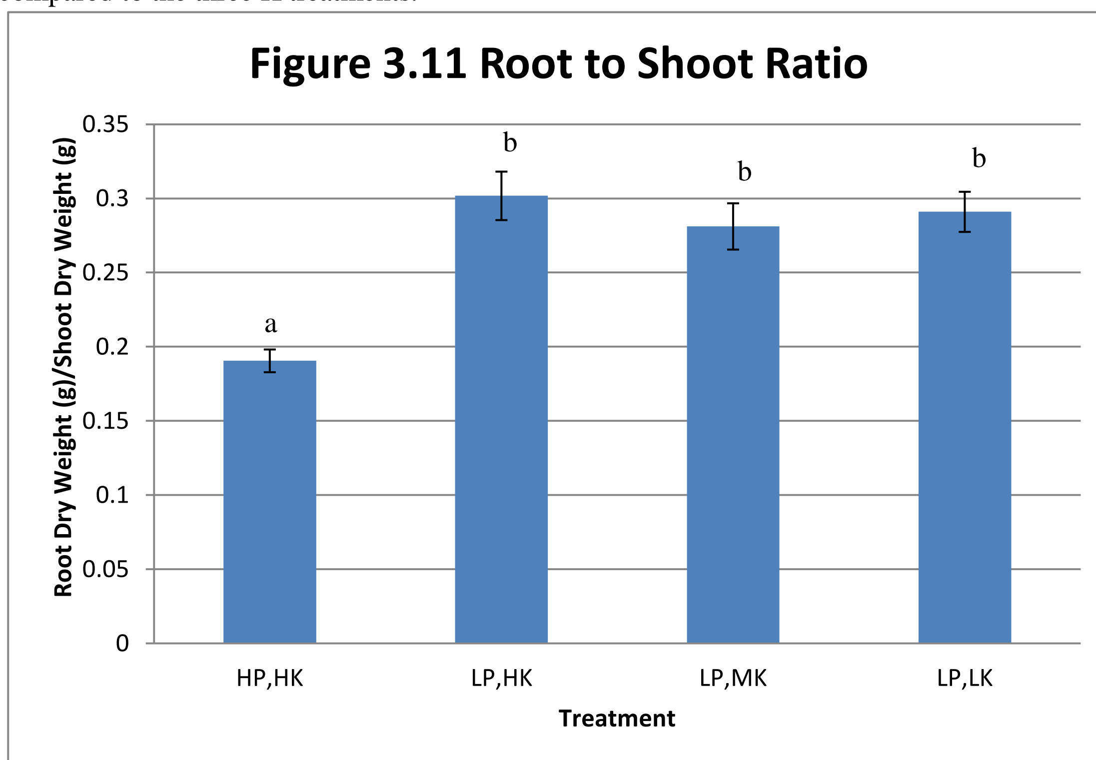

Figure 3.11 Average root to shoot ratio for each treatment. Error bars are included and significance is indicated ($P<0.01$).

Table 3.7 ANOVA Table for Root to Shoot Dry Weight Ratio (g)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	0.18787	0.06262	37.737	1.50E-14
Treatment excluding control	2	0.00510	0.00255	1.232	0.300
Treatment: Genotype including control	15	0.04204	0.00280	1.689	0.0737
Treatment: Genotype excluding control	10	0.01915	0.00191	0.926	0.518

Table 3.7 Analysis of variance results for root to shoot ratio based on nutrient treatment including and excluding the control (HP,HK) treatment.

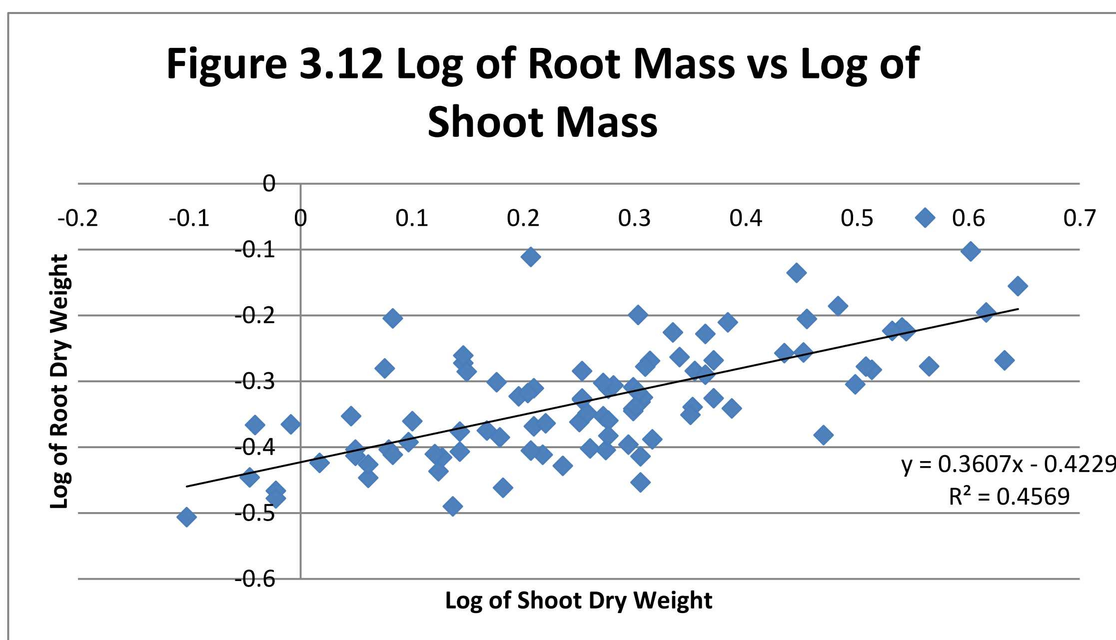

Figure 3.12 Log of Root dry weight versus log of shoot dry weight

The average root dry weight was significantly ($p<0.001$) larger in the HP,HK plants compared to those with inadequate P treatments (H, M, and L). The average root dry weight for the HP,HK plants was 0.57g, for the LP,HK treatment 0.45g, for the LP,MK treatment 0.46g, and for the LP,LK treatment 0.43g. There was no significant effect of potassium treatment on the root dry weight.

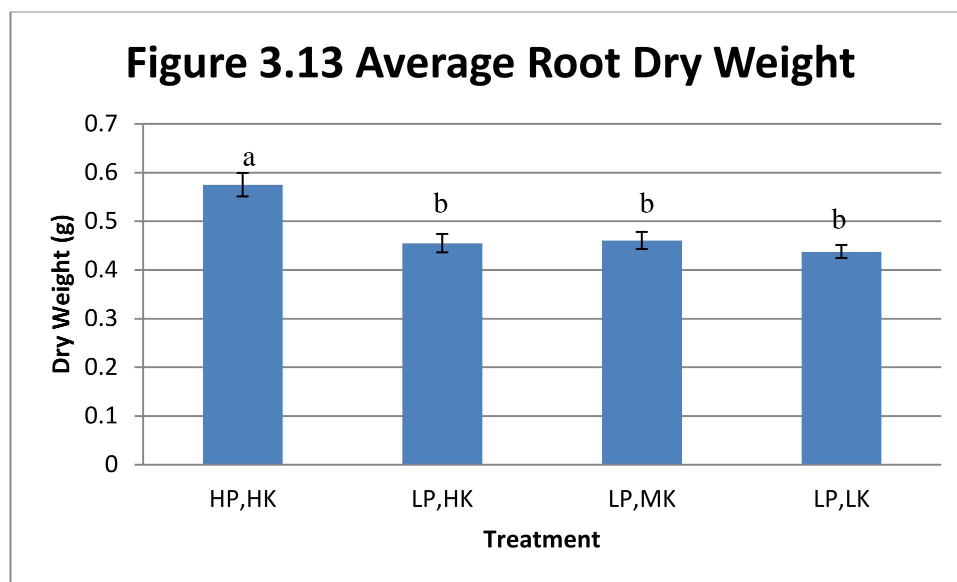

Figure 3.13 Average root dry weight for each nutrient treatment. The HP,HK had the largest root dry weight. Error bars are included and significance indicated (P<0.01).

There was some correlation between root length and root dry weight; 54% of the variation in root system length is explained by the root dry weight. DG67 was one genotype that deviated most from the trend. The average RDW for DG67 was 0.63g and the root system length was 96m.

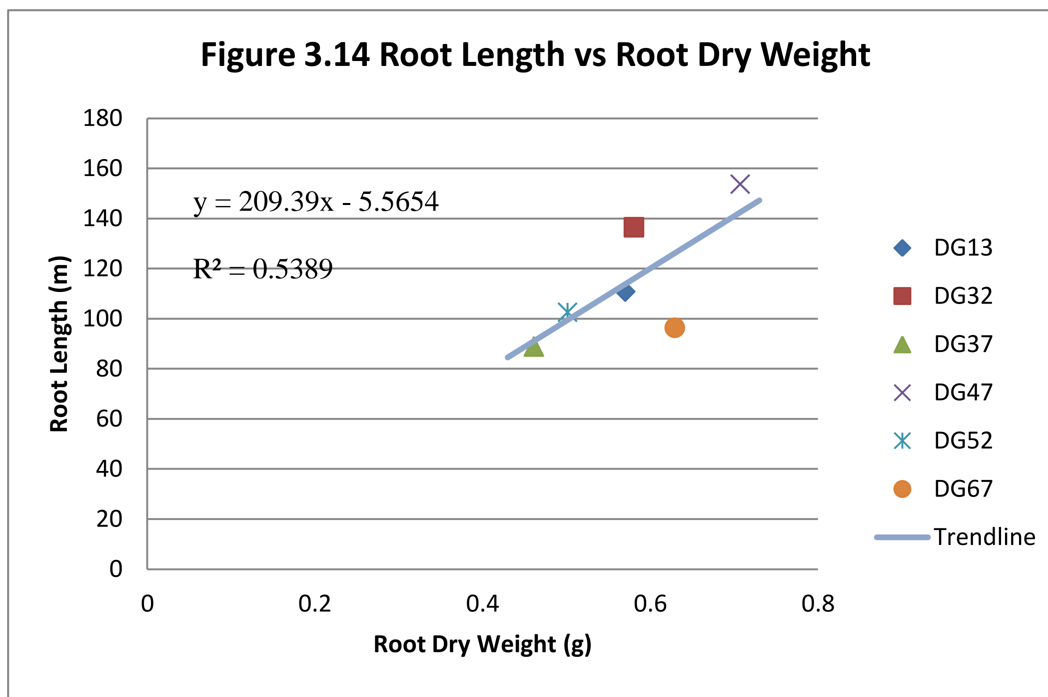

Figure 3.14 Correlation between root dry weight and root system length. The coefficient of determination is 0.54.

Leachate was collected 30 days after planting and 41 days after planting. The K concentration in the leachate collected 30 days after planting (DAP) was significantly affected by the K treatments. The low K treatment had an average K concentration of 1.59mg K^+/L, the medium K treatment 2.9059mg K^+/L, the high K treatment 4.30mg K^+/L, and the HP,HK treatment had a concentration of 4.63mg K^+/L (Figure 3.15). The K concentration in the leachate collected 41 DAP of the low and medium K treatments decreased to 0.76 mg K^+/L and 0.80mg K^+/L, respectively. The K concentration for the high K treatment was 4.73 mg K^+/L, slightly higher than what it was 30DAP. The K concentration for the HP,HK went down to 2.33 mg K^+/L 41 DAP (Figure 3.16)

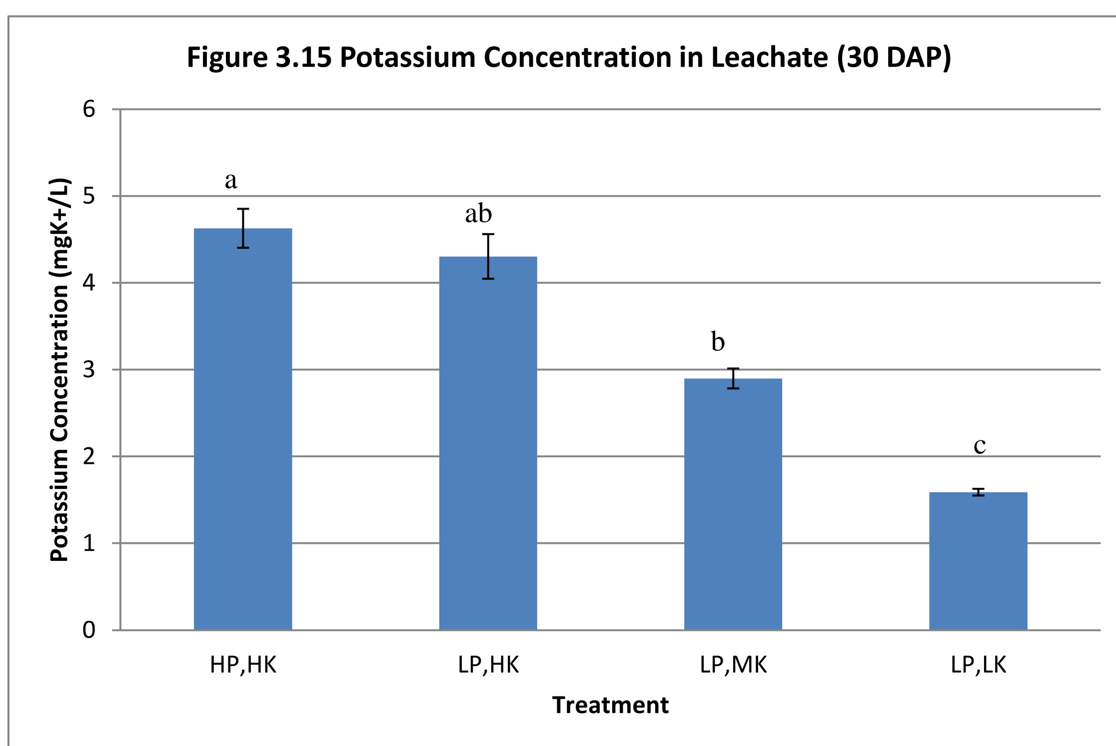

Figure 3.15 Potassium concentration of leachate 30 days after planting. Significance at P<0.01.

Table 3.8 ANOVA Table for Potassium Concentration in Leachate (mgK$^+$/L)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	90.3	30.10	4.553	0.00628
Treatment excluding control	2	56.99	28.496	5.013	0.0112

Table 3.8 Analysis of variance results for potassium concentration of leachate collected 30 DAP for various nutrient treatments including and excluding the control (HP,HK) treatment

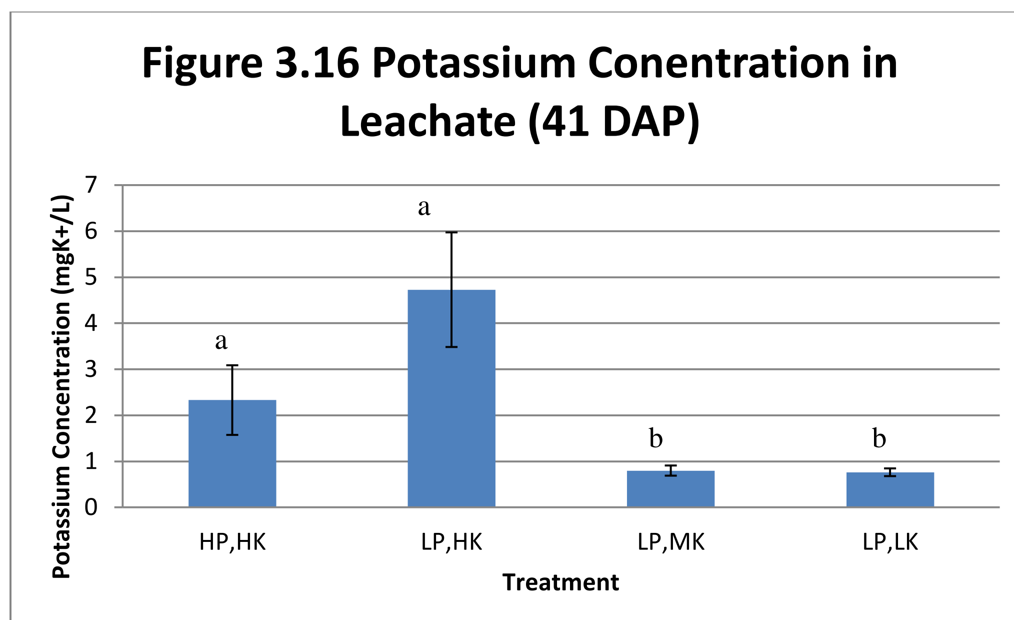

Figure 3.16 Potassium concentration in leachate 41 days after planting.

Potassium treatment had a significant effect on the potassium content in the shoots. The percent of K in the shoots by weight is shown in Figure 3.17. The low K treatment had 1.3% K, the medium K treatment had 1.5% K, the high K treatment had 2.4% K, and the HP,HK treatment had 2.9% K.

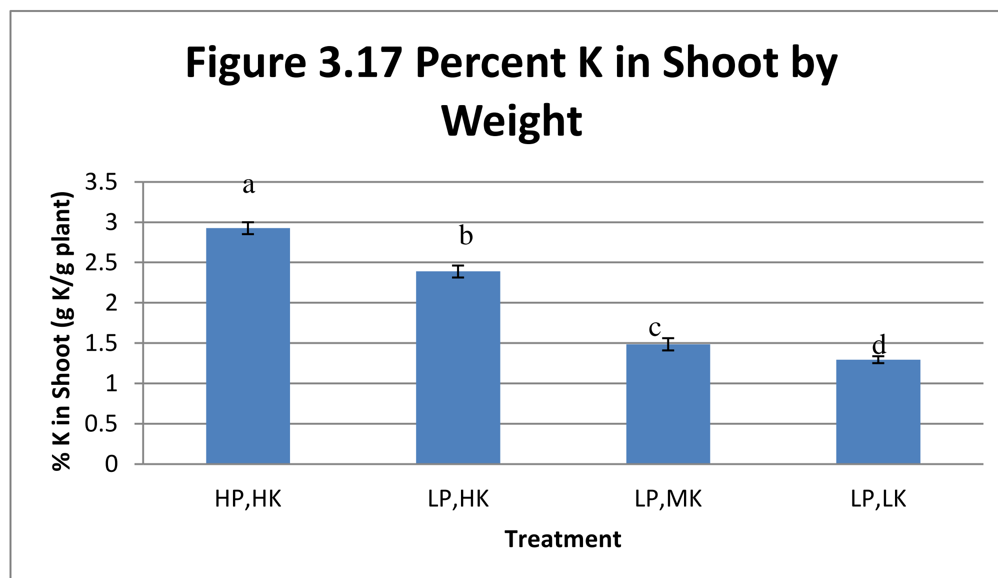

Figure 3.17 Treatment had a significant effect on the percent of K in the plant. The % K by weight ranged from 1.3% in the low K treatment to 2.9%K in the HP,HK treatment.

Table 3.9 ANOVA Table for % K in Shoot by Dry Weight					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	39.87	13.291	181.467	<2e-16
Treatment excluding control	2	14.400	7.200	144.305	<2e-16
Genotype including control	5	1.35	0.270	3.689	0.0057
Genotype excluding control	5	1.187	0.237	4.757	0.00156
Treatment:Genotype including control	15	1.72	0.115	1.568	0.1115
Treatment:Genotype excluding control	10	1.258	0.126	2.521	0.01780

Table 3.9 Analysis of variance results for percent K in the shoot based on genotype and nutrient treatment including and excluding the control (HP,HK) treatment.

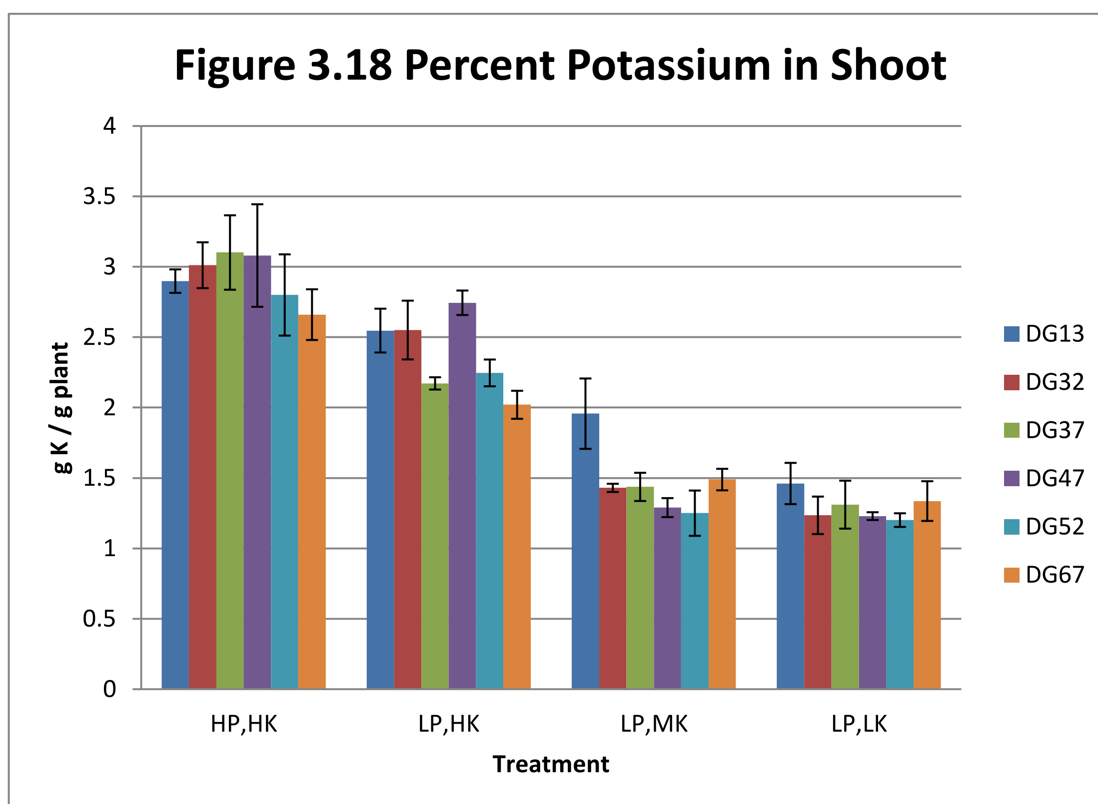

Figure 3.18 Percent K in shoot for each genotype and treatment. Standard error bars are included.

The amount of K supplied to the plant in the low K treatment was 0.75mmol of K per pot, in the medium K treatment was 1.5mmol per pot, and the high K treatment received 1.5mmol with each liter of nutrient solution added to the pot. The total K added to the pots over the 41 day growing period was roughly 8mmol K per pot. Figure 3.19 shows the amount in millimoles of K supplied and the amount taken up by the plant. The HP,HK treatment plants took up an average of 2.3mmol K, the LP,HK treatment 1.0mmol K, the LP,MK 0.63mmol K, and the LP,LK treatment took up 0.52mmol K.

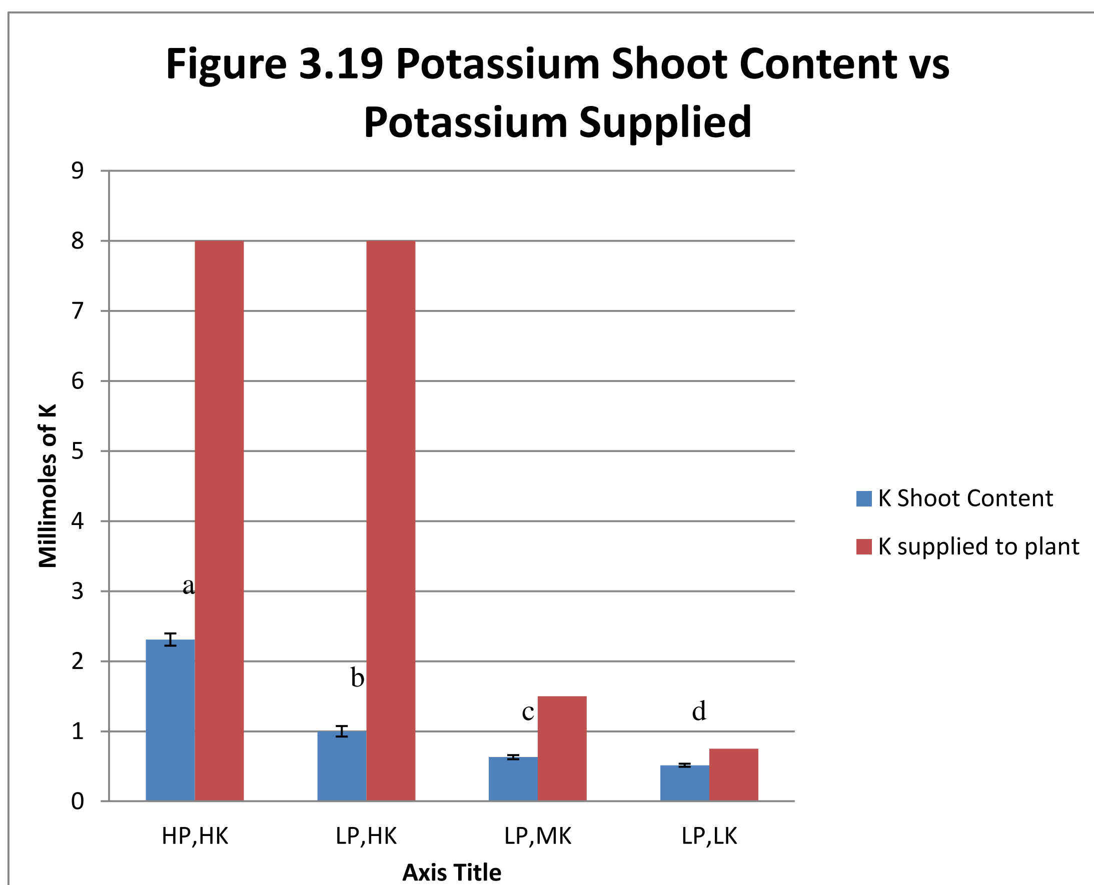

Figure 3.19 Amount (in mmoles) of K taken up by the plants compared to the amount of K (in mmoles) supplied to the plants. Standard error bars included for the K shoot content. Significance indicated (P<0.01).

Table 3.10 ANOVA Table for Potassium Content in the Shoot (mgK/plant)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment including control	3	0.0707	0.023565	403.65	<2e-16
Treatment excluding control	2	0.003610	0.0018051	43.154	6.53e-11
Genotype including control	5	0.00423	0.000845	14.48	<3e-09
Genotype excluding control	5	0.001374	0.0002748	6.570	0.000134
Treatment:Genotype including control	15	0.00213	0.000142	2.43	0.00797
Treatment:Genotype excluding control	10	0.000690	0.0000690	1.651	0.125600

Table 3.10 Analysis of variance results for K content in shoot based on genotype and nutrient treatment including and excluding the control (HP,HK) treatment.

The K content in the plants correlated with the shoot dry weight. As shoot dry weight increased the grams of K per plant increased (Figure 3.20). Figure 3.20 does not include the trendlines for each treatment, but the trendline equations and coefficient of determination are shown in table 3.11. The LP,HK treatment had the steepest slope, followed closely by the HP,HK treatment. The LP,MK and LP,LK both had significantly less steep slopes than the other two treatments (Table 3.11). The coefficient of determination was between 0.70 and 0.83 for all treatments except for the LP,MK treatment which had a coefficient of determination of 0.36.

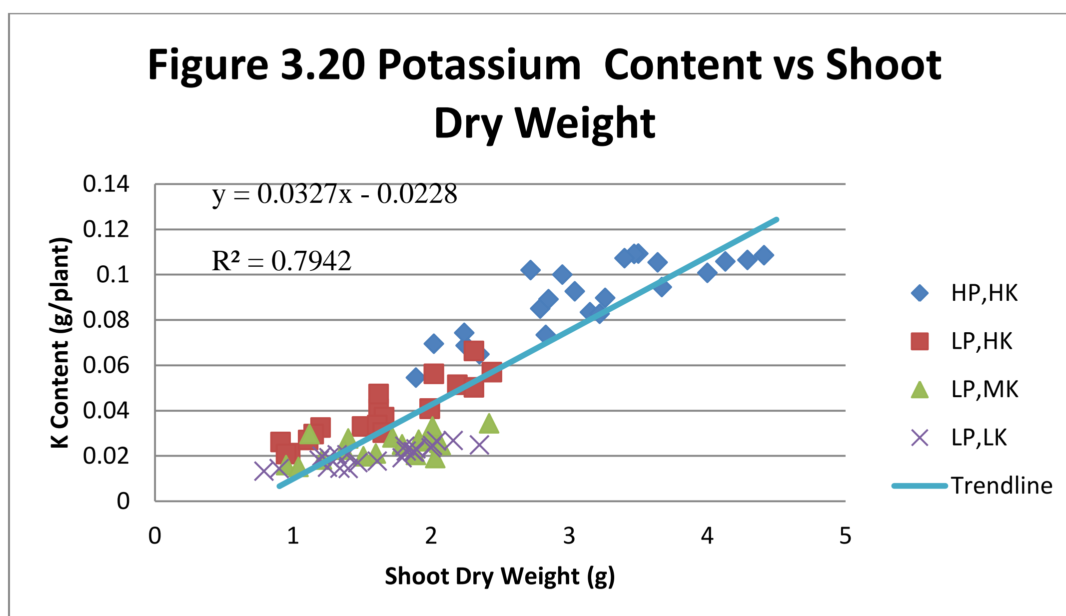

Figure 3.20 As shoot dry weight increases the K content in the plants increased. The trendline for all data points is included. The coefficient of determination is 0.79.

Table 3.11 Trendline Information for Each Treatment Comparing K Content vs Shoot Dry Weight		
Treatment	trendline equation	Coefficient of Determination (R^2)
HP,HK	y = 0.0194x + 0.0296	0.7021
LP,HK	y = 0.0234x + 0.0007	0.8135
LP,MK	y = 0.0079x + 0.0111	0.3598
LP,LK	y = 0.0085x + 0.0066	0.7318

Table 3.11 The trendline equation and coefficient of determination for each treatment data set.

Discussion

Since there was no significant effect of the low, medium, and high K treatment on shoot dry weight (SDW), it is thought that the inadequate P caused low, medium, and high K treatments to be insignificant because the plants were P stressed. A study by Rubio et. al (2003) found that plants respond differently to multiple nutrient disorders and do not necessarily follow Liebig's Law of the Minimum or the Multiple Limitations Hypothesis. The Law of Minimum states that plant growth is limited by the single scarcest nutrient while the Multiple Limitations Hypothesis states that "optimum plant behavior results from balancing resource costs and benefits so that all resources limit plant growth simultaneously" (Rubio et. al 2003). According to Rubio et. Al (2003), if a plant has a P deficiency, supplying the plant with adequate K would not allow for increased growth. In other words, the interaction between K and P follows the Law of Minimum. In this experiment P was the nutrient that limited growth. Growing under low P, the plants were not responsive to the various K treatments. As seen in figure 3.11, the LP,LK,and the LP,MK along with the LP,H K treatments all had a higher root to shoot ratio than the HP,HK treatment which had adequate P and K. The larger root to shoot ratio was understandable because under low P root growth is enhanced (Gutschick 1993; Nielsen et al. 2001). More resources were used for root growth, which explains why the shoots dry weight of the low P plants had a 50% shoot dry weight reduction compared to the HP,HK plants. Root growth was enhanced at the cost of shoot growth. The larger root system could have allowed for better K acquisition which further decreased the effect of the low K treatment on shoot performance. Another possibility as to why the K treatments had no effect could be due to the form of N used in the nutrient solution. Britto and Kronzucker (2008) found that the transporters used for K uptake are sensitive to ammonium and that the ammonium ions will act as competitors to the K^+ ions making it more difficult for the uptake of K+. Since urea, which form ammonium hydroxide in water, was used to supply N, it

could have interfered with K uptake. The ammonium ions readily undergo nitrification in the soil and this would reduce the competition of ammonium, but it is still a possibility.

Figure 3.5 shows the relationship between shoot dry weight for LP,LK and LP,HK treatment plants. About 62% of the change in the g/plant of the LP,LK treatment is due to the variation in the g/plant for the LP,HK treatment. As for the LP,HK and HP,HK treatments, only 82% of the variation (Figure 3.7) in g/plant of the low P treatment can be explained by the variation in the high P treatment. This demonstrates that the plant growth of the various genotypes is more closely related to the shoot size when under P stress than under K stress. Although the coefficient of determination for the LP,LK versus LP,HK was 62%, there was no significant difference in shoot dry weight between LP,LK and LP,HK plants as seen in Figure 3.3, making it difficult to make further conclusions. When comparing the low P vs high P treatments, the genotypes can be divided into two groups; genotype DG37 and 52 which both had a SDW reduction of 42% under low P, and another group including DG13, 32, 37, 47 and 67 which had an average SDW reduction of 52% under low P. The genotypes which had only a 42% reduction in SDW could be better suited for growth in areas of low P compared to those genotypes which had a 52% reduction. The effect of genotype on shoot dry weight under the LP,HK treatment was only slight significant and therefore conclusions are not strongly supported. DG37 and DG52 had average RHLs at 0.22mm and 0.23mm (DG13 had the longest average RHL of .25mm), so the smaller reduction in SDW is not due to longer RHL. Genotype had a slight affect on root system length. DG 37 and 52 had the shortest root system length at 83m and 87m (DG67 had the longest at 117m) in the HP,HK treatment. DG37 had the second smallest average SDW while DG52 had the largest average SDW. This may mean that DG37 and 52 somehow performed better than the other genotypes under low P since shoot growth was not reduced as much. DG52, which had the largest average SDW and had a smaller reduction in SDW under low P, may be a good genotype to use in low P environments. The increase in root to shoot ratio was average for DG52 under low P (compared to high P) but DG37 had the largest increase in root to shoot ratio under low P. This means that DG37 did respond to the low P as expected which would also mean that it would do well in low P environments since it responds well by adjusting its root to shoot ratio.

The root system length was significantly shorter for those treatments that did not receive adequate P (Figure 3.9). Comparing only the root system length does not give a proper understanding of how low P affects plants. While it seems that the root system length is shorter under low P instead of longer, this is because the overall plant growth is reduced. The root to

shoot ratio shows that under low there are more roots in comparison to shoots (Figure 3.11). The root systems of the low P treatments are shorter than the high P treatment, but relative to shoot size they are longer.

When graphing root length versus root dry weight (RDW) (Figure 3.14) it is noted that DG67 had a root length smaller than what would be expected for its RDW. This would be explained by larger root diameter. Roots with a larger diameter is not necessarily good for low P or K conditions because forming longer thinner roots will allow for more soil exploration and acquisition of immobile nutrients such as P and K.

Although no significant difference was found in the root hair length for various K treatments or even between the low P and adequate P plants, these results may have been confounded. The root hair length was measured in a section of root 10 to 15cm from the base of a basal root. This section of root was generally closer to the base of the plant than the tip of the basal root and therefore considered to be older or formed earlier in the life of the plant. The root hairs that were formed on this section of root that was analyzed for root hair length may have grown under adequate P conditions. The reason that P could have been adequate was because all plants received 1.0mmole of P on the fourth day after planting the germinated seeds into the pots. The 1.0mmole of P was the only P that the low P plants received. The 10-15cm section of root may have formed during the time when P was still available to the root and therefore those root hairs on the section of root would be short. It is known that P availability regulates root hair length and under low P root hairs are significantly longer than under adequate P conditions (Bates and Lynch 1996). While the 5cm section of root was saved, the rest of the basal root was discarded and therefore was not analyzed for RHL closer to the tip of the root. It is thought that the RHL might be longer closer to the tip since these roots were more likely to have been formed under low P conditions. If the root hairs were indeed longer it could explain, along with the larger root system, why the low K treatments had no effect on shoot dry weight.

In this experiment six different genotypes were used. As seen in the Root Hair Survey section, there was not a very strong correlation between root hair lengths of the genotypes that were phenotyped for root hair length in this experiment compared to the work done by Magalhaes Miguel (2004). Root hair length is a phene that is controlled by many environmental factors as well as many genes (Lynch and Brown 2008). The average root hair length measured in the final experiment was 0.22mm compared to 0.43mm in the root hair survey. This difference is most likely due to the differences in growing conditions and the age of the plant at which the measurements were taken. The root survey was done on seedlings grown in germination paper in

a germination chamber while the final experiment measurements were on 41 day old plants grown in the greenhouse. There was a slight correlation between genotypes and RHL when comparing the results from the root hair survey and the final experiment. Genotype DG 67 had the shortest RHL in both studies, DG13 and DG47 were longer in both studies, DG 32 was short in both studies, DG52 was relatively shorter in the root hair survey but relatively longer in the final experiment, and DG37 was relatively long in the root hair survey but intermediate in length in the final experiment. The results may have been more accurate had the RHL been measured closer to the tip of the basal root. The variation in RHL in the root hair survey and in the final experiment was small. Although genotype did have a significant effect on RHL the range was only between 0.197mm to 0.252mm for the final experiment. There was too little variation in RHL of the root hair survey and the final experiment to make any conclusions about correlation of RHL between the two studies. In future experiments a whole basal root should be collected and RHL measured along the whole root in order to determine where the best location is for measuring RHL.

To better understand why the K treatments had no effect, the leachate which was collected 30 days after planting and 41 days after planting (DAP), was analyzed for K concentration. A significant difference was seen in the K concentration of the leachate depending on the K treatment (Figure 3.15 and 3.16). The K concentration in the leachate collected 30 DAP correlated with the K treatment (low, medium, and high) meaning that the K treatments were indeed different. The possibility that the K treatments were done incorrectly can be excluded. The decrease of K concentration in the leachate from 30 DAP to 41 DAP is expected for the LP,LK and LP,MK treatments since no additional K was added to the pots. There was a drop in K concentration in the leachate collected 41 DAP for the HP,HK treatment. It seems that the HP,HK plants may have used more K during week five and six of growth. The drop in K concentration for the HP,HK treatment can be explained by the increased growth rate. While plant leaf area increased over time for all treatments (Figure 3.1), the growth rate of only the HP,HK treatment increased over time (Figure 3.2). The increased growth rate of the HP,HK plants would explain why the K concentration in the leachate would be lower 41 DAP; the HP,HK plants used more K because of their vigorous growth. The growth rate was constant for the first three weeks for the HK plants but actually decreased after week three. The slowed growth explains why there was not a significant drop in K concentration of the leachate for the LP,HK treatment; the LP,HK plants did not have the same K requirement as the HP,HK plants whose growth rate was increased over time.

All treatments had an effect on the grams K per grams of shoot (Figure 3.17). The range of %K in the shoots was between 1.3% (for the LP,LK treatment) and 2.9% (for the HP,HK treatment). A K concentration below 1% was K deficient and K concentrations above 6% have excess or toxic amounts of K (Munson 1998). Since the range of K in the plants was between these values, it further indicates that the plants were not under K stress. The % K in the shoots correlated with the K concentration in the leachate. The HP,HK treatment had the highest % K in the shoots and the highest concentration of K in the leachate. The HP,HK treatment, which received the same amount of K as the LP,HK treatment, had a significantly larger % K in the shoots than the HK treatment. This is unexpected because the LP,HK plants had a larger root to shoot ratio. The larger relative root system would be expected to be helpful for K acquisition for the LP,HK plants compared to the HP,HK. Since the LP,HK plants were smaller than the HP,HK it would also be expected that the concentration of K in the LP,HK plants would be higher. The HP,HK treatment did have a higher K concentration in the leachate than the LP,HK treatment. While this could explain the high percent K in the HP,HK shoots, the difference in leachate concentration between the HP,HK and LP,HK treatment was not significant. The %K increase as related to the increase in shoot dry weight also indicates that the LP,HK was more efficient than the HP,HK treatment (Figure 3.20 and Table 3.11). It is a possibility that the LP,HK treatment was not as efficient at K uptake because of the low P conditions.

K uptake is dependent on the electrochemical potential gradient formed across the plasma membrane of the cell. The gradient is dependent on two things; the K concentration of the soil solution compared to the K concentration inside the cell and the electrical potential across the plasma membrane of the cell. If the concentration of K is low outside of the cell then K uptake will generally be an active process requiring energy in the form of ATP and if the K concentration is high enough it can be a passive process (Britto and Kronzucker 2008). However, the cytosol K concentration is so high (ranging from 40-200mM) it is unlikely that K will passively enter the cell. For this reason K transport into the cells is more dependent on the electrical charge separation across the membrane. The electrical potential gradient is dependent on the hydrogen ions that the cells pump out into the rhizosphere. This pumping of ions requires ATP. It is known that P is a plant growth regulator and for this reason it also has an effect on the acquisition of carbon and other nutrients (Rubio et. al 2003). As seen in this experiment, the low P conditions reduced the shoot growth, and because of this reduction the supply of carbohydrates produced by photosynthesis was also reduced. With fewer carbohydrates, less ATP is produced and therefore K uptake could be affected since it indirectly requires ATP. This could explain

why the HK,LP treatment had a lower %K than the HK,HP treatment even though it had a higher root to shoot ratio. It is expected that the low P would have more of an effect on K uptake in those treatments with low K since K can be passively transported if the concentrations are high enough outside the cell. It could also be that the root to shoot ratio is not as significant of a player in K acquisition as the total root mass. The HK,HP treatment had a significantly larger root dry weight than all the low P treatments (Figure 3.13) and also had the greatest percent of K in its shoots.

The K content increase as related to the increase in shoot dry weight was significantly less for the LP,MK and LP,LK treatments compared to the LP,HK and HP,HK treatments (Figure 3.20). The slopes of the trendlines for the LP,MK and LP,LK were about three times smaller than the LP,HK and HP,HK treatments. This suggests that the low K conditions made the plants less efficient at taking up K or that they took up K at a slower rate. It has been found that under K stress plants will trigger the expression of high affinity transporters to take up K. They also up-regulate K^+ channels (Ashley et. al 2006). This is done in order to acquire as much K as possible even thought the external K concentrations were low. It seems that despite a possible increase in K transporters the plants were still not as efficient under low K as under higher K conditions. This could simply be because the K was not as available to the plants. Comparing the amount of K supplied (in millimoles of K) to the amount of K taken up by the plants (in millimoles of K), it is found that the LP,MK treatment took up 42% of the 1.5mmol K supplied to it while the LP,LK took up 68% of the 0.75mmol K supplied. The plants that received the least amount of K were more efficient than those that received slightly more (Figure 3.19). This was also seen when comparing the slopes in table 3.11. The LP,LK treatment had a steeper slope than the LP,MK, meaning that it was more efficient at K uptake. It is possible that the 0.75mmol K treatment up-regulated K transporters and K channel expression more than the 1.5mmol K treatment and therefore was more efficient in taking up K.

Conclusion

Although this study did not have K treatment effects or much variation in root hair length, completing the study allowed me to analyze the results and make some conclusions, as well as determine what should be changed for future experiments. The study also helped me to better understand that K follows Liebig's Law of the Minimum; the nutrient that is most limiting will determine the growth of the plant and therefore the potassium treatments had no effect on growth.

Although the goal was not to observe P affects, the low and high P treatments affected the root to shoot ratio of beans considerably and not all genotypes respond in the same way to the low P treatment. Under low P root growth is stimulated at the expense of shoot growth. It was not expected that the HP,HK treatment would have a higher K concentration than the LP,HK treatment, but this shows that K uptake is affected by P supply. In a P deficient environment there is less shoot growth and therefore fewer carbohydrates produced. The reduced amount of carbohydrates means there is less energy in the form of ATP to actively take up K^+ ions.

Throughout the whole project I learned many things, including the importance of planning prior to implementing a study and thinking thoroughly through everything and writing out wthat needs to be done, saves one from making mistakes. Not only is knowing what I am doing important, but reading the literature and understanding what other scientist have found is just as essential. Another thing I learned is that working hard in the moment is crucial even though it may seem painful or a waste of time in that moment. That hard work sometimes saves time in the future and it leaves one with no regrets for not trying harder. This is especially important when setting up the actual experiment and collecting data. There are many things that once done, cannot be undone; once the plants are harvested it is difficult to go back and take more measurements.

I have learned that a proper fertigation regime is crucial to nutrient studies. Understanding how nutrients move in the soil, how plants interact with the nutrients and take them up all determines how nutrient stresses can be induced. Inducing various levels of K stress has been a challenge in this project and proved to be more difficult than I expected. For future studies I would not limit P but instead lower the K concentration even more. I would do a preliminary trial with no potassium added to the growth medium to determine whether the K in the soil is not sufficient for plant growth. As simple as it may sound, I learned the importance of getting treatment and phenotypic differences; without them it is difficult to make conclusions. I would make certain that I had treatment differences before conducting a larger scale experiment.

When analyzing for RHL the position of the root used to select root hairs for analysis is important. RHL will be influenced by the nutrients that are available at the time of root hair formation. I would like to measure the RHL at various positions along the basal roots to determine where the best place is for taking root hair samples. This would also allow me to see whether the P that was supplied to the plants early on in their growth affected the RHL at a certain position of the basal root.

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Hortco Greenhouses and Garden Center — Summer 2011

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