GENETIC VARIABILITY FOR LOW PHOSPHOROUS TOLERANCE IN COWPEA

A Thesis

by

TULLE WAYNE ALEXANDER

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Chair of Committee,	J. Creighton Miller, Jr.
Co-Chair of Committee,	Bir B. Singh
Committee Member,	Kevin M. Crosby
Head of Department,	R. Daniel Lineberger

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ABSTRACT

As a result of rising fertilizer prices and environmental concerns, efforts are being made to develop crop varieties with better nutrient acquisition and use efficiencies to ensure higher yields and sustainability, especially in the semi-arid tropics and sub-tropics where soils are inherently low in nitrogen and phosphorus. Cowpea does not require additional nitrogen fertilizer because of its ability to biologically fix nitrogen, but it needs phosphate application. However, preliminary studies have shown that some cowpea genotypes have the ability to extract bound phosphorus from low-P soils and from rock phosphate.

Therefore, a project was initiated at Texas A&M University to develop high yielding cowpea varieties with enhanced acquisition and efficient utilization of phosphorus from low-P soils and rock-P. This study was conducted to screen 12 selected cowpea varieties under low-P soil, with rock- phosphate application. One-kg pots were filled with 1000 g of low-P soil (< 4 ppm) collected from Nacogdoches, TX, and amended with five phosphorus treatments – no added phosphorus, 200, 400, and 600 ppm rock-phosphate and Normal-P (Hoagland's solution). Pots with No-P and rock-phosphate treatments were treated with a modified (P-free) Hoagland's solution. The Normal-P treatment received unmodified Hoagland's solution. Pots were arranged in a completely randomized design with three replications and planted with three seeds which were thinned to a single plant per pot after emergence. Pots were watered every second day to field capacity with reverse-osmosis purified water. The experiment was terminated 42 days after planting and dry weights of plants from each pot were recorded.

Major varietal differences were observed for biomass production in the low-P and rock-P treatments. Some of the promising cowpea varieties are IT97K-1069-6, IT98K-476-8, TX 2028-1-3-1 and Big John which performed well regardless of phosphorus treatment. California Blackeye #50, Dan Ila, IAR-48, and IT00K-1148 performed poorly in low-P soils, but exhibited significant growth response with addition of rock-P.

ACKNOWLEDGEMENTS

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My sincerest appreciation goes to Mr. Douglas Scheuring for teaching me everything I know about farming and for being one of the most genuinely good people I have ever had the opportunity of knowing.

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INTRODUCTION AND LITERATURE REVIEW

Cowpea (*Vigna unguiculata* L. Walp.) is a crop of worldwide importance that is produced for human as well as animal nutrition. Cowpea is significant because of its ability to yield under adverse conditions and because of its high protein content in both seed and biomass. It is one of the major food and forage crops in the arid tropics, especially western Africa, where drought and nutrient deficiency are the main limiting factors to production (Padulosi and Ng, 1990; Singh et al*.*, 1997). Cowpea is consumed by hundreds of millions worldwide and is grown on over 14 million hectares. In western Africa alone, five million tonnes are produced on nine million hectares annually. Yields experienced by subsistence farmers in sub-Saharan Africa are considerably poorer than yields obtained in more developed areas, due primarily to a lack of available inputs.

As a legume, cowpea forms a symbiotic relationship with *Rhizobium* bacteria species, and as such, significant production can be made with relatively little external mineral nitrogen input. After drought, nutrient deficiencies are the major limitations to crop production in western Africa (Bationo et al., 1998). Additionally, the scarcity of phosphorus (P) fertilizers is the primary factor constraining legume production in the arid tropics (Krasilnikoff et al., 2003). Phosphorus is an important nutrient because it is involved in photosynthesis, energy transfer, DNA replication, and nitrogen fixation. Phosphorus deficiency interferes with efficient nitrogen fixation, because the symbiosis is reliant upon ample available ATP. Under low-P conditions, the availability of fixed atmospheric nitrogen is reduced (Schulze et al., 2006). Conventional sources of P are rare or unavailable in much of western Africa. Rock phosphate (rock-P), a poorly soluble, mined product containing calcium phosphate, which is available to farmers in western Africa, contains 25-30% P_2O_5 (Trompette et al., 1980*)*. The application of rock-P as a soil amendment increases available P in the root zone and increases grain yield in cowpea crops, but only to a minor extent due to rock-P's poor solubility (Muleba and Coulibaly, 1999). The objectives of this study were to identify genetic differences for tolerance to low-P soil and responsiveness to P from rock-P. This information will help develop cowpea lines with capacity to perform well under low-P conditions, with rock-P addition. This work is important in understanding plant response to environment, and improving cowpea yields in areas of low nutrient availability.

Adaptations to Low Phosphorus and Poorly Soluble Phosphate

For sustainable cropping, researchers must identify and exploit P acquisition mechanisms to improve P acquisition efficiency (Tesfaye et al., 2007). Legume species have been shown to acquire P from poorly soluble forms of phosphate (Kamh et al., 1999). Multiple adaptation mechanisms to low-P conditions have been discovered. Research suggests that root architectural traits significantly influence P uptake (Lynch and Beebe, 1995). To increase P uptake, plants tend to change physiology and biochemistry. Changes in root morphology include increases in root length, root hair density, and the prevalence of lateral roots (Zhang et al., 2009). Many plants, including legumes, exude organic acids in order to increase the mobility of bound phosphates (Neumann et al., 1999). Organic acid exudation allows plants to solubilize bound forms of phosphate and increase uptake through roots (Zuniga-Feest et al., 2010). Legumes are known to exude piscidic, and to a greater extent, citric acids (Ae et al., 1993; Keerthisinghe, 1998).

Cowpea plants have developed adaptations to increase the amount of available P. Organic acids exuded by cowpea roots increase reactivity and solubility of rock-P, which increases the amount of P in soil as an available nutrient (Kpomblekou and Tabatabai, 1994). Increases in length and density of root hairs increase the volume of soil able to be exploited by roots (Vesterager et al., 2006). A significant source of cowpea's low-P tolerance is increased use efficiency. In experiments where plants are grown in P deficient soils, cowpea plants with highest mass were those with the lowest P uptake (Sanginga et al., 2000; Alkama et al., 2009).

Screening Cowpea for Tolerance to Low-P

Some previous work has been done to identify cowpea lines with high performance in low-P and rock-P systems. Vesterager et al. (2006) screened varieties of pigeonpea for tolerance to low-P and explored mechanisms responsible for phosphorus uptake. In that study, cowpea was used as a control. It was noted that cowpea exhibited greater biomass and root hair production, but pigeonpea demonstrated greater rate of P uptake. Krasilnikoff et al. (2003) saw significant genetic variation among lines for P acquisition and root morphology. The root changes identified led to increased soil volume explored, but there was no consensus that increased soil exploration led to increased P uptake. Saidou et al. (2007) screened cowpea lines for low-P tolerance and for response to rock-P addition. Significant genetic variability was noted for low-P tolerance and response to rock-P. Jemo et al. (2006) screened cowpea lines under rock-P and superphosphate treatments to identify lines with superior nitrogen fixation potential. It was noted that N fixation improved with P addition and that there was significant genetic variability for N fixation and P uptake. Two lines were identified that had superior N fixation under low-P.

MATERIALS AND METHODS

Plant Materials and Growth Medium

Twelve cowpea lines were used for the experiments. Seven lines were selected from IITA germplasm, based on previous performance in low-P systems in Nigeria (Saidou et al., 2011). Five lines were selected from U.S. breeding programs. These are elite, high performing lines, with ideal growth habit for US production systems (e.g. high yield, early maturing, dwarf-type upright plant architecture). Table 1 presents the lines used.

African Lines	US Lines
Aloka – Niger Republic	Golden Eye Cream - TAMU
Dan Ila – Northern Nigeria	TX2028-1-3-1-0 - TAMU
IAR-48 – Ahmad Bello Univ., Nigeria	Big John – Texas Farmer Selection
IT97K-1069-6 – IITA, Nigeria	California Blackeye #46 – U.C. Riverside
IT98K-476-8 – IITA, Nigeria	California Blackeye #50– U.C. Riverside
IT98K-1092-1– IITA, Nigeria	
IT00K-1148– IITA, Nigeria	

Table 1. Cowpea lines used in this study.

Since travel to Africa is cost prohibitive, a suitable soil analogue that could be obtained locally was used. Soils found in pine forests near Nacogdoches, TX have similar characteristics with those typical of western Africa (i.e. sandy, kaolinitic mineralogy, acid pH). These soils have low plant available (Bray method) P, with the remaining P comprising insoluble Fe-P complexes bound to soil particles (Bray and Kurtz, 1945).

The specific soil used had a pH of 5.5, 6.0 ppm plant available P and 86.01 ppm total P (plant available plus bound forms).

Experimental Design

The experiment was designed to identify and quantify genetic variation between cowpea lines with respect to low-P tolerance and to acquisition efficiency of P from rock-P. The experiment was conducted in the greenhouse using pots on benches in a completely randomized design (CRD) with three replications, five treatments, and 12 lines, totaling 180 individual pots. Five experimental treatments were used in this experiment – a low-P treatment with no additional P other than that supplied by the planting medium (termed ‘No-P’, soil from Nacogdoches), three treatments of rock-P addition (200, 400, and 600 ppm), and one treatment with a highly soluble and plant available source of P (termed ‘Normal-P’). This experiment was carried out in a greenhouse in College Station, TX, where light, temperature, and humidity could be closely controlled, and in which there was low environmental variation.

Executing the Experiment

Pots were situated on trays to prevent dripping and subsequent loss of the finely ground rock-P. Plants were irrigated to field capacity by applying water and nutrient solution to the tray underneath and allowing the water to be wicked upwards. Field capacity was determined by filling pots with soil and irrigating to saturation. After the pot drained completely, the pot was weighed to determine the amount of irrigation to apply to reach field capacity. At each watering throughout the experiment, plants were weighed and an appropriate amount of water was applied to again reach field capacity.

The pots were prepared by weighing 1000 g soil into a disposable polyethylene bag, and the appropriate amount of rock-P, if any, was added. The appropriate initial nutrient solution and water was added to the bag and the soil was homogenized by hand until the soil moisture was well distributed. No-P and rock-P treatments were initially fertilized with a modified (P-free) Hoagland's solution while the Normal-P treatment was initially watered with unmodified Hoagland's solution (Hoagland and Arnon, 1938). The soil was then placed into pots for planting. Pots were planted with three handpicked seeds and, after three days, duplicate plants were removed so that a single plant was growing in each pot. The plants were allowed to grow for 42 days (the expected time of flowering) after which time the experiment was terminated and the plants harvested and measured. During the growing season, the pots were watered at a frequency dictated by the rate of evapo-transpiration. Plants were watered to field capacity with deionized water, and P-negative nutrient solution was applied weekly to prevent confounding data as a result of a deficiency in non-P nutrients.

Cotyledon Clipping Experiment

To determine what effect, if any, seed size had on results from the initial experiment, the original experiment was repeated with modification. The experiment was planted in duplicate and only one rock-P treatment (600 ppm) was used. In one set, the cotyledons were removed immediately after emergance, to lessen the effects that seed borne P would have on terminal performance. After clipping cotyledons, all practices from the initial experiment were repeated.

Harvesting and Statistical Analyses

At the time of harvest, plants were uprooted gently by washing away the sandy soil so as to reduce root loss. Plant height was measured, and the plants were then cut into foliage and root sections which were dried and weighed separately. After plants were dried and weighed, tissue samples were ground in a Wiley mill and mineral analysis performed.

Data was analyzed via JMP and SAS 9.2 Enterprise Guide (SAS Institute, 2011). Analysis of variance (ANOVA) was performed and Student’s t-tests were used to rank lines and quantify differences between experimental treatments. Analyses to determine the effect that genotype has on low-P tolerance, as well as the ability to acquire P from rock-P, were performed and a categorization of lines developed.

RESULTS

No-P Treatment

In comparing the mean shoot masses for the No-P treatment between lines, Big John, IT98K-476-8, and TX2028-1-3-1-0 had the highest mean dry mass with 1.04, 0.94, and 0.88 g, respectively. Lines IT98K-1092-1, IAR48, and Golden Eye Cream were the poorest performers with 0.39, 0.45, and 0.50 g mean dry shoot mass, respectively (Fig. 1). To assess low-P tolerance, masses for No-P treatment were compared with masses from the Normal-P treatment, within genotype. Lines IT98K-1092-1, Dan Ila, Golden Eye Cream, IAR-48, and IT00K-1148 showed statistically significant differences between shoot masses of plants from No-P and Normal-P treatments (Fig. 2). Lines with the greatest root mass were TX2028-1-3-1-0, Golden Eye Cream, and Big John with 0.47, 0.38, and 0.33 g dry root tissue, respectively. Lines with the least root mass were IT98K-1092-1, California Blackeye 50, and Aloka, with 0.019, 0.20, and 0.21 g dry root tissue, respectively. The line IT98K-1092-1 was the only line that showed a significant difference between mean root masses of the No-P treatment and the Normal-P treatment.

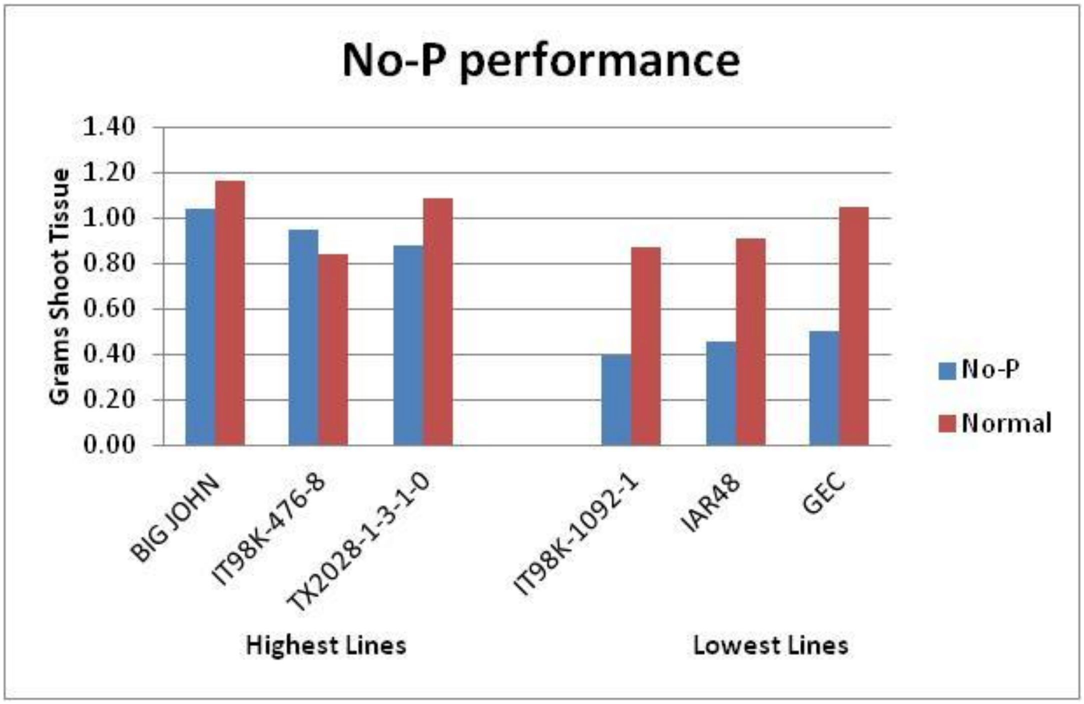


Figure 1. Lines with highest and lowest mean shoot masses in No-P treatment.

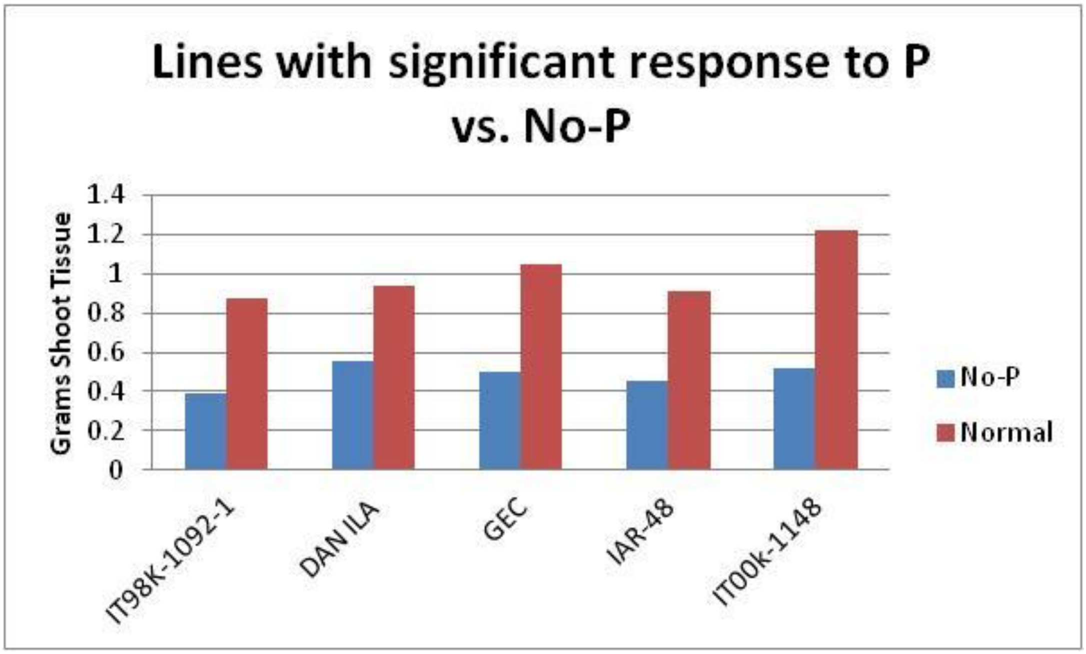


Figure 2. Lines with significant response to phosphorus addition.

Rock Phosphate Treatment

Upon observing performance among lines in the 600 ppm rock-P treatment, Big John, California Blackeye 50, and TX2028-1-3-1-0 exhibited the largest shoot masses, with 1.29, 1.17, and 1.04 g dry shoot biomass, respectively. Lines IT98K-1092-1, Golden Eye Cream, and Aloka exhibited the poorest performance, with 0.54, 0.64, and 0.72 g, respectively (Fig. 3). Lines California Blackeye 50, Dan Ila, IAR-48, and IT00K-1148 had statistically significant positive slope with respect to rock-P dose; as rock-P dosage increased, yield followed. Golden Eye Cream, Aloka, and IT98K-1092-1 demonstrated no significant increase in biomass as rock-P was added, and all rock-P treatments were significantly poorer than the respective Normal-P treatment. Figure 4 shows the data for Golden Eye Cream, which is representative of lines with no significant response to rock-P. Lines with the greatest root biomass in the 600 ppm treatment were TX2028-1-3-1-0, IAR48, and IT00K-1148 with 0.50, 0.50, and 0.43 g dry root tissue, respectively. Lines Golden Eye Cream, Big John, and Aloka displayed the poorest root growth with 0.27, 0.29, and 0.29 g dry root tissue, respectively. Lines IT97K-1069-6, IT98K-1092-1, and Dan Ila demonstrated significant increase in root dry mass as rock-P was added. No line had significantly different root masses between Normal-P and Rock-P treatments. Data for shoot and root mean masses for all treatments are presented in Tables 2 and 3, respectively. The ANOVA table and table of effect tests for combined experiment are presented in Tables 4 and 5, respectively. Line and phosphorus treatment were significant determinants of shoot mass, whereas the interaction was not significant.

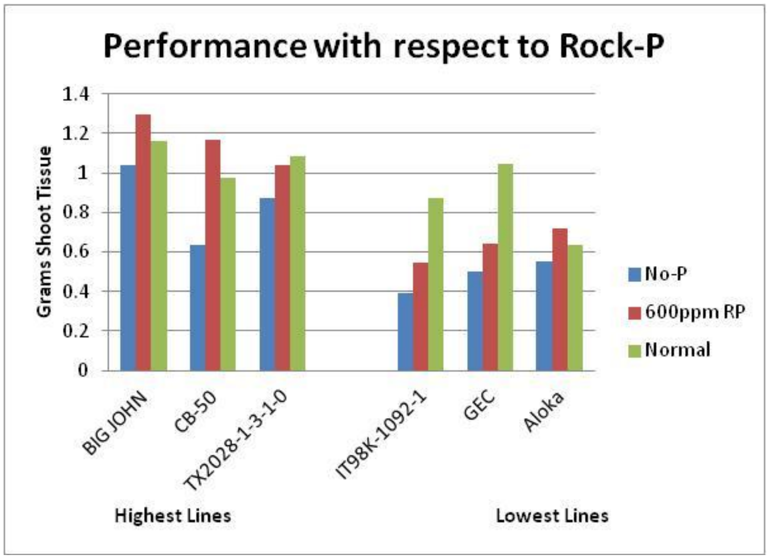


Figure 3. Highest and lowest mean shoot masses in 600 ppm RP treatment.

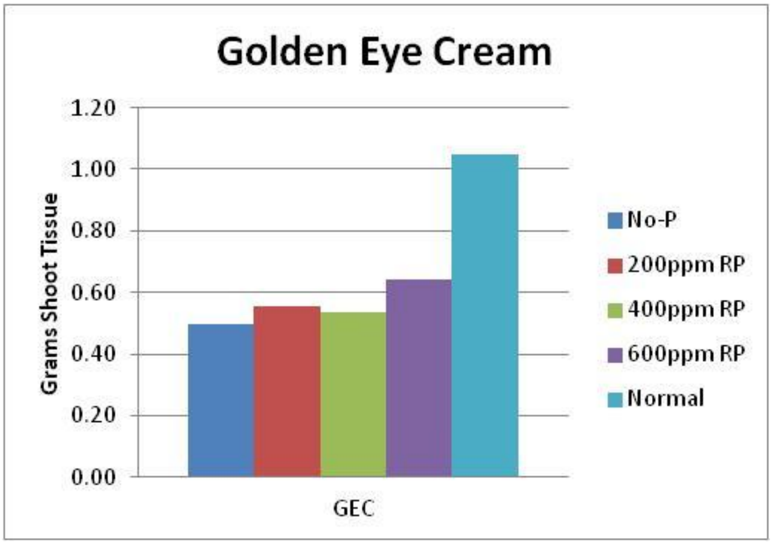
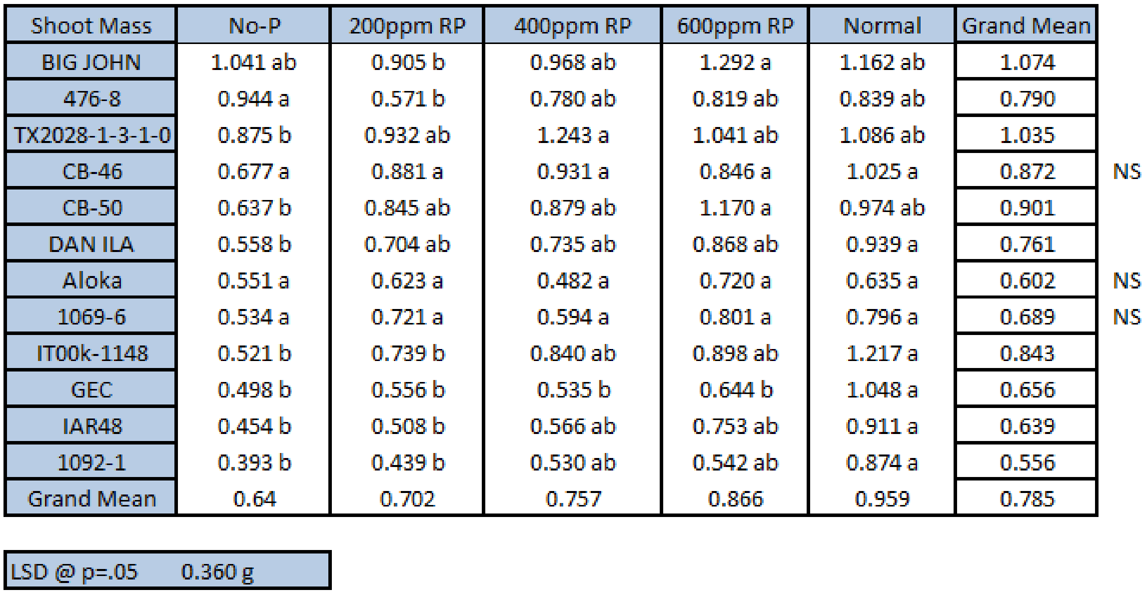


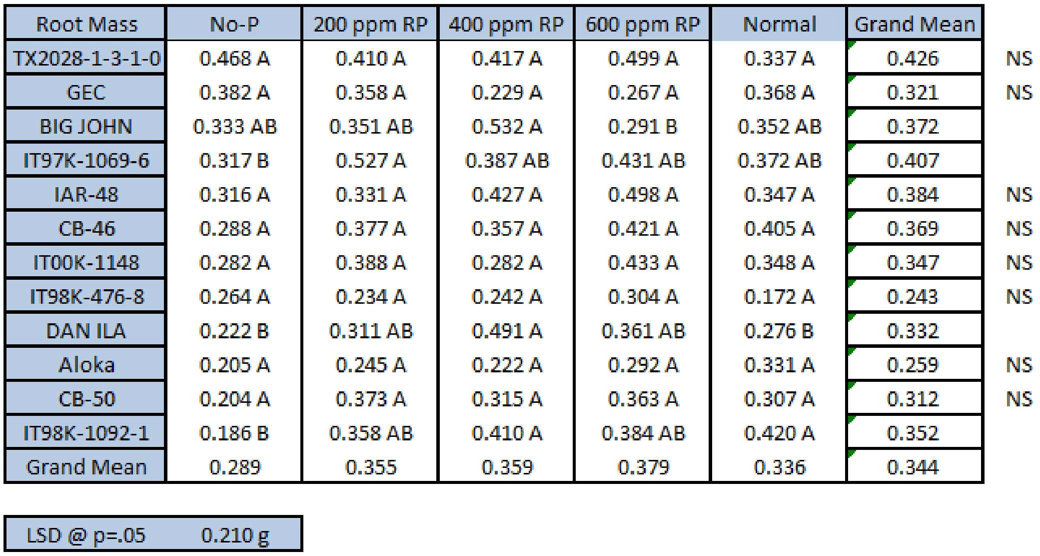
Figure 4. Golden Eye Cream performance. Golden Eye Cream shows yield depression under No-P treatment and no response to rock-P addition



Shoot Mass	No-P	200ppm RP	400ppm RP	600ppm RP	Normal	Grand Mean	
BIG JOHN	1.041 ab	0.905 b	0.968 ab	1.292 a	1.162 ab	1.074	
476-8	0.944 a	0.571 b	0.780 ab	0.819 ab	0.839 ab	0.790	
TX2028-1-3-1-0	0.875 b	0.932 ab	1.243 a	1.041 ab	1.086 ab	1.035	
CB-46	0.677 a	0.881 a	0.931 a	0.846 a	1.025 a	0.872	NS
CB-50	0.637 b	0.845 ab	0.879 ab	1.170 a	0.974 ab	0.901	
DAN ILA	0.558 b	0.704 ab	0.735 ab	0.868 ab	0.939 a	0.761	
Aloka	0.551 a	0.623 a	0.482 a	0.720 a	0.635 a	0.602	NS
1069-6	0.534 a	0.721 a	0.594 a	0.801 a	0.796 a	0.689	NS
IT00k-1148	0.521 b	0.739 b	0.840 ab	0.898 ab	1.217 a	0.843	
GEC	0.498 b	0.556 b	0.535 b	0.644 b	1.048 a	0.656	
IAR48	0.454 b	0.508 b	0.566 ab	0.753 ab	0.911 a	0.639	
1092-1	0.393 b	0.439 b	0.530 ab	0.542 ab	0.874 a	0.556	
Grand Mean	0.64	0.702	0.757	0.866	0.959	0.785	

LSD @ p=.05	0.360 g

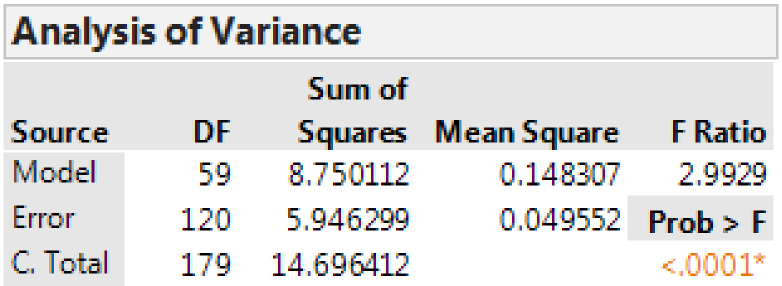
Table 2. Mean shoot masses. Mean shoot masses for all treatments arranged according to No-P performance. Letters indicate significance groups within columns. Means not sharing the same letter within a column are significantly different from one another. NS – not significant.



Root Mass	No-P	200 ppm RP	400 ppm RP	600 ppm RP	Normal	Grand Mean	
TX2028-1-3-1-0	0.468 A	0.410 A	0.417 A	0.499 A	0.337 A	0.426	NS
GEC	0.382 A	0.358 A	0.229 A	0.267 A	0.368 A	0.321	NS
BIG JOHN	0.333 AB	0.351 AB	0.532 A	0.291 B	0.352 AB	0.372	
IT97K-1069-6	0.317 B	0.527 A	0.387 AB	0.431 AB	0.372 AB	0.407	
IAR-48	0.316 A	0.331 A	0.427 A	0.498 A	0.347 A	0.384	NS
CB-46	0.288 A	0.377 A	0.357 A	0.421 A	0.405 A	0.369	NS
IT00K-1148	0.282 A	0.388 A	0.282 A	0.433 A	0.348 A	0.347	NS
IT98K-476-8	0.264 A	0.234 A	0.242 A	0.304 A	0.172 A	0.243	NS
DAN ILA	0.222 B	0.311 AB	0.491 A	0.361 AB	0.276 B	0.332	
Aloka	0.205 A	0.245 A	0.222 A	0.292 A	0.331 A	0.259	NS
CB-50	0.204 A	0.373 A	0.315 A	0.363 A	0.307 A	0.312	NS
IT98K-1092-1	0.186 B	0.358 AB	0.410 A	0.384 AB	0.420 A	0.352	
Grand Mean	0.289	0.355	0.359	0.379	0.336	0.344	

LSD @ p=.05	0.210 g

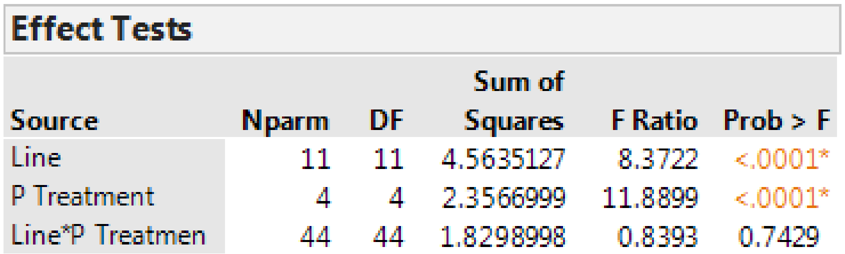
Table 3. Mean root masses. Mean Root masses for all treatments arranged according to No-P performance. Letters indicate significance groups within columns. Means not sharing the same letter within a column are significantly different from one another.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	59	8.750112	0.148307	2.9929
Error	120	5.946299	0.049552	Prob > F
C. Total	179	14.696412		<.0001*

Table 4. ANOVA table. Analysis of variance for combined experiment.



Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Line	11	11	4.5635127	8.3722	<.0001*
P Treatment	4	4	2.3566999	11.8899	<.0001*
Line*P Treatmen	44	44	1.8298998	0.8393	0.7429

Table 5. Effect tests for combined experiment. Line and treatment are strongly significant, whereas the interaction is not significant.

Shoot Dry Mass Phosphorus Analysis

With the exception of Aloka, all lines followed the same pattern; there were no significant differences in shoot P concentration in the No-P and rock-P treatments, but the Normal-P treatment had significantly higher shoot P concentrations. Figure 5 provides shoot P concentration for Aloka and Figure 6 provides data for IT97K-1069-6, which has a trend representative of the remaining ten lines. There was, however, a strong negative correlation between shoot mass and shoot P concentration; across the experiment better performing plants tended to have lower P concentration. The correlation statistic is presented in Table 6 and data for tissue phosphorus concentration are summarized in Table 7.

Cotyledon Clipping and Seed Size

Seed size was a significant predictor of shoot performance and was mildly correlated with shoot mass (correlation coefficient = 0.21). Clipping cotyledons had no significant effect on any treatment when compared to the un-clipped control with cotyledons intact. Table 8 summarizes results from cotyledon clipping experiment.

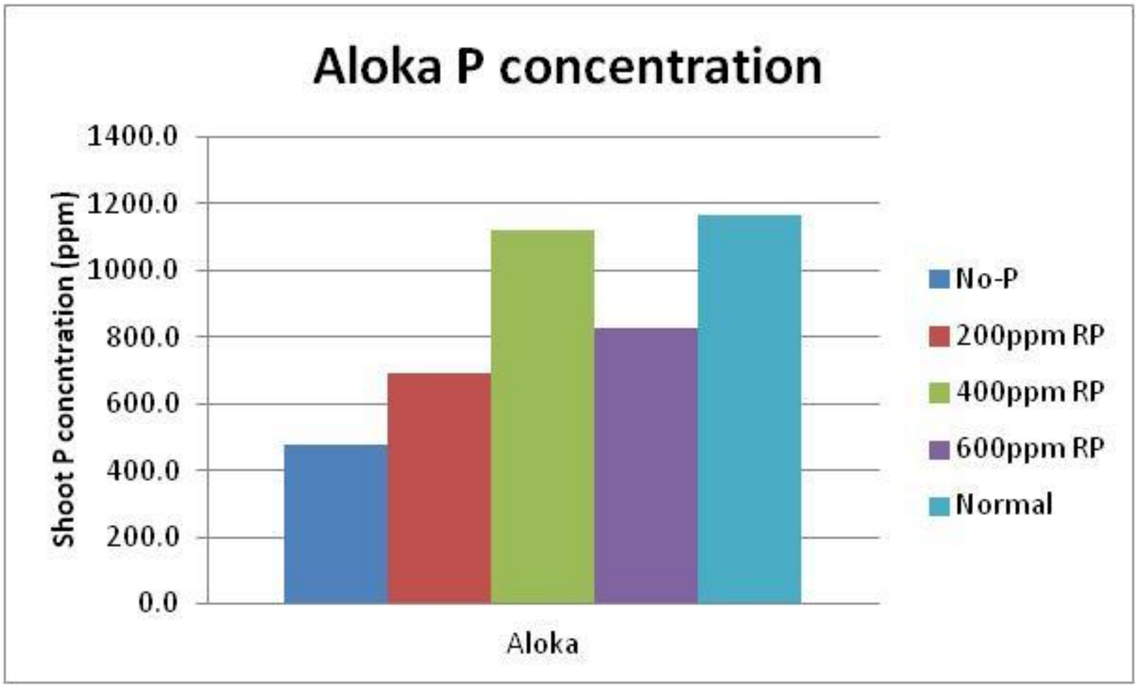


Figure 5. Shoot P concentrations for Aloka.

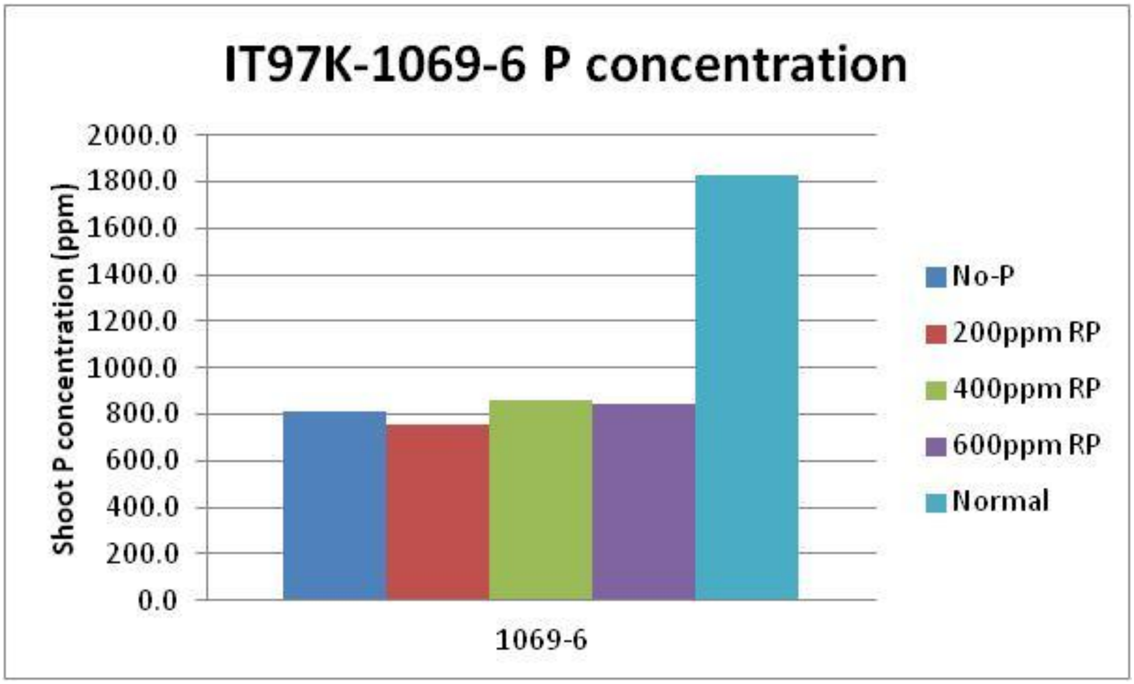
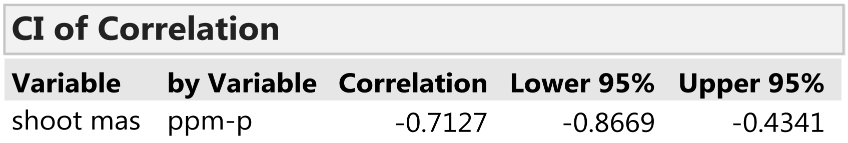


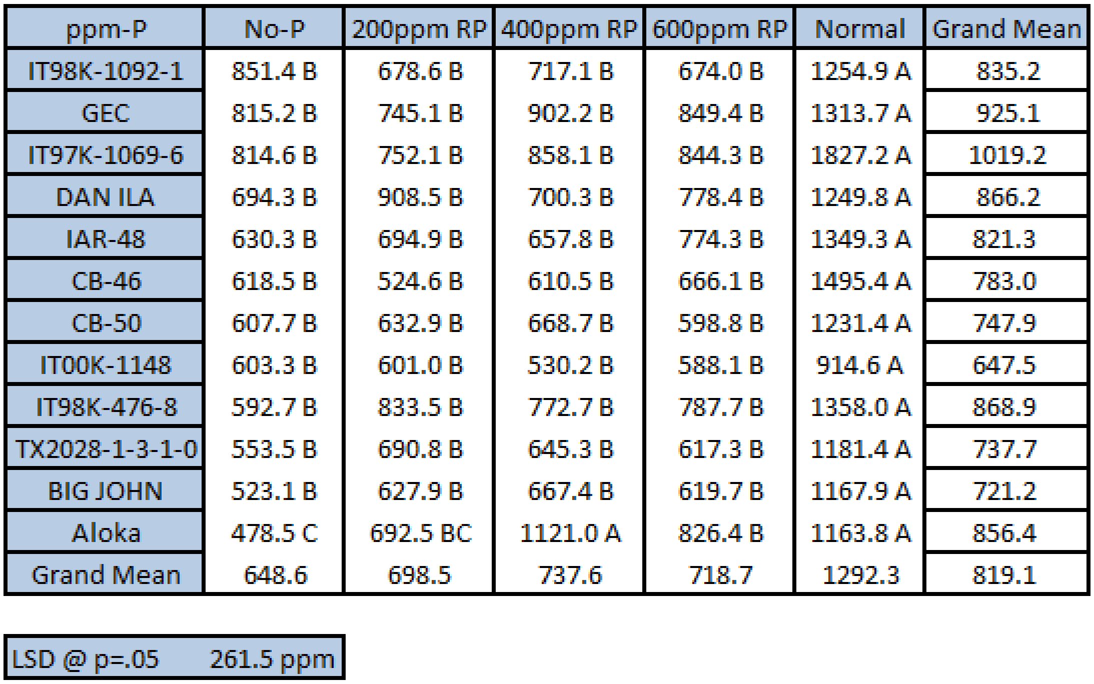
Figure 6. Shoot P concentrations for IT97K-1069-6.



CI of Correlation

Variable	by Variable	Correlation	Lower 95%	Upper 95%
shoot mas	ppm-p	-0.7127	-0.8669	-0.4341

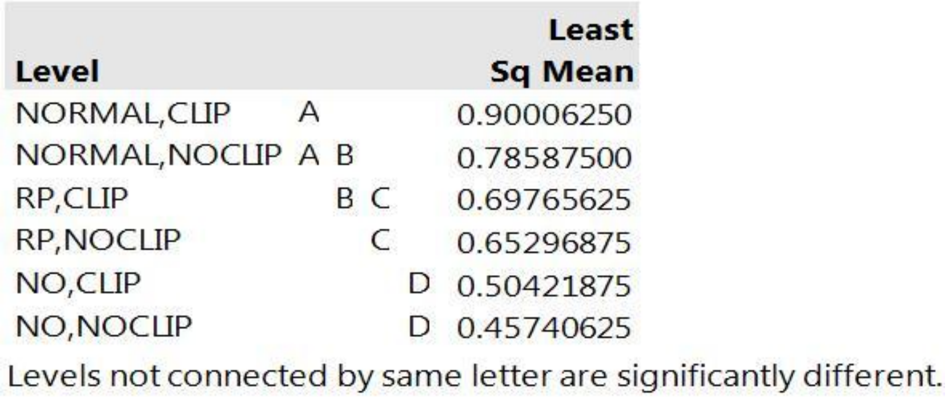
Table 6. Correlation of shoot mass vs. shoot P concentration.



ppm-P	No-P	200ppm RP	400ppm RP	600ppm RP	Normal	Grand Mean
IT98K-1092-1	851.4 B	678.6 B	717.1 B	674.0 B	1254.9 A	835.2
GEC	815.2 B	745.1 B	902.2 B	849.4 B	1313.7 A	925.1
IT97K-1069-6	814.6 B	752.1 B	858.1 B	844.3 B	1827.2 A	1019.2
DAN ILA	694.3 B	908.5 B	700.3 B	778.4 B	1249.8 A	866.2
IAR-48	630.3 B	694.9 B	657.8 B	774.3 B	1349.3 A	821.3
CB-46	618.5 B	524.6 B	610.5 B	666.1 B	1495.4 A	783.0
CB-50	607.7 B	632.9 B	668.7 B	598.8 B	1231.4 A	747.9
IT00K-1148	603.3 B	601.0 B	530.2 B	588.1 B	914.6 A	647.5
IT98K-476-8	592.7 B	833.5 B	772.7 B	787.7 B	1358.0 A	868.9
TX2028-1-3-1-0	553.5 B	690.8 B	645.3 B	617.3 B	1181.4 A	737.7
BIG JOHN	523.1 B	627.9 B	667.4 B	619.7 B	1167.9 A	721.2
Aloka	478.5 C	692.5 BC	1121.0 A	826.4 B	1163.8 A	856.4
Grand Mean	648.6	698.5	737.6	718.7	1292.3	819.1

LSD @ p=.05	261.5 ppm

Table 7. Mean shoot P concentrations for all treatments. Letters indicate significance groups within columns. Means not sharing the same letter within a column are significantly different from one another. NS signifies no statistically significant differences within the respective line.



Level					Least Sq Mean
NORMAL,CLIP	A				0.90006250
NORMAL,NOCLIP	A	B			0.78587500
RP,CLIP		B	C		0.69765625
RP,NOCLIP			C		0.65296875
NO,CLIP				D	0.50421875
NO,NOCLIP				D	0.45740625

Levels not connected by same letter are significantly different.

Table 8. Means and significance groups for cotyledon clipping experiment. LSD @ p=.05 is 0.116 g.

DISCUSSION AND CONCLUSIONS

Categorizing Lines

Based on the results, lines were determined to be rock-P responsive or non-responsive, and/or low-P tolerant or susceptible. These determinations were made by comparing performance in one treatment relative to other treatments. Lines that showed a significant improvement in biomass yield from No-P to Normal-P were deemed ‘Susceptible, whereas lines with no significant improvement were ‘Low-P Tolerant’. Figure 7 presents photos of low-P tolerant and susceptible lines. Similarly, lines with no improvement correspondent with rock-P addition were ‘Non-Responsive’ and lines with significant biomass yield response to rock-P addition were determined to be ‘Responsive’. Figure 8 shows photos of a responsive and a non-responsive line representative of their respective groupings. A combined determination can then be made for each line, identifying it as tolerant/susceptible and responsive/non-responsive. For example, Golden Eye Cream, Aloka, and IT98K-1092-1 were determined to be susceptible and non-responsive. These lines had poor biomass yield in the No-P treatment and all rock-P treatments, but had a significant increase in biomass yield as soluble P was added. California Blackeye #50, Dan Ila, IAR-48, and IT00K-1148 were deemed low-P susceptible but rock-P responsive. These lines had poor yield in No-P coupled with significant response to rock-P. The low-P tolerant group comprised IT97K-1069-6, IT476-8, Big John, California Blackeye #46, and TX2028-1-3-1-0. These lines performed equally well irrespective of P treatment. Identifying rock-P responsiveness in lines that are low-P tolerant becomes difficult, as tolerant lines present no significant difference between No-P and Normal-P treatments; a response to rock-P is masked by low-P tolerance. For example, Big John demonstrated roughly equal performance irrespective of treatment. Identifying true rock-P responsiveness in lines that are low-P tolerant was not possible with the current study. Table 9 categorizes lines into tolerance and responsiveness groups.

Figure 7. Photos of Low-P Tolerant and Susceptible Lines. Low-P tolerant lines (TX2028-1-3-1-0, Big John, and IT98K-476-8, left) and low-P susceptible lines (IT98K-1092-1, Aloka, and Dan Ila, right).

Rock Phosphate Responsive

Non-Responsive

Figure 8. Photos of Rock-P Responsive and Non-responsive Lines. Rock phosphate responsive line (Dan Ila, top) and non-responsive line (Aloka, bottom).

	Rock Phosphate Responsive	Non-Responsive
Low-P Susceptible	California Blackeye #50 Dan Ila IAR-48 IT00K-1148	Golden Eye Cream Aloka IT98K-1092-1
Low-P Tolerant	IT97K-1069-6 IT98K-476-8 Big John California Blackeye #46 TX2028-1-3-1-0	

Table 9. Tolerance / responsiveness categories. List of lines and their respective tolerance and responsiveness categories. Responsive lines are those with a statistically significant positive response to added rock phosphate

Interpreting Root Mass Data

No conclusions could be drawn based on root data. Root mass and shoot mass were not significantly correlated. The correlation coefficient was 0.135 with a confidence interval of -0.012 and 0.276. The lack of significance in root data is probably due to the method of root extraction. Plants were grown in a natural soil, and washing of roots to remove all soil residues proved impossible. Since root masses were relatively small, any soil residue greatly impacted results.

Shoot Phosphorus Concentration

There was a significant negative correlation (correlation coefficient = -0.713) between shoot mass and shoot P concentration. These findings are in agreement with Furlani et al. (2002), who found that in screening experiments with soybeans, shoot dry matter was highly negatively correlated with P concentration, and that low-P concentrations correlated with plants that were highly P use efficient. These results suggest that P use efficiency may be a more important mechanism for low-P tolerance than acquisition mechanisms.

Effect of Seed Size on Shoot Mass

After examination, it was hypothesized that seed size may be a significant predictor of shoot production, since as seed size increases more nutrients are stored within the seed. To test this, seeds were milled and analyzed for total P s concentration. There was no significant correlation between seed P concentration and shoot performance. Seed size was mildly correlated with shoot mass (correlation coefficient = 0.21). To determine what effect seed size had and to mitigate any influence that seed size may have on performance, the original experiment was repeated in which a duplicate set had cotyledons removed immediately after germination. Clipping the cotyledons had no significant effect on any treatment, and if anything, improved performance. These results may conflict with Yan et al. (1995) who found that large seeded varieties perform significantly better than lines with smaller seeds. The effect of seed size on performance may be explained by active breeding. This relationship is either direct, in which breeders have selected for large seeds, which imparts low-P tolerance, or the relationship is spurious. If the relationship is spurious, both low-P tolerance and seed size are predicted by a third variable, in this case breeding. Breeders simultaneously select for multiple traits and seed size is an important trait. Larger seeded lines may be benefiting from overall improved genetics. In either scenario, selecting for increased seed size may directly or indirectly select for low-P tolerance.

Summary and Future Work

The objectives of this study were to identify genetic differences for tolerance to low-P soil and responsiveness to rock-P addition, and this study adequately addressed the original objectives. The study was designed to categorize cowpea lines based on their performance in low-P and rock-P conditions; the current methodology could not elucidate the mechanisms or inheritance of tolerance or responsiveness. Future studies will use the determinations made in this study to breed for improved performance, understand mechanisms, and determine how these traits are inherited. Future work should include genetic studies and the development of mapping populations to identify the genes involved.

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