ROOT ETIOLATION AS A STRATEGY FOR

PHOSPHORUS ACQUISITION IN COMMON BEAN

A Thesis in

Horticulture

by

Laurie Morrow de la Riva

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2010
The thesis of Laurie Morrow de la Riva was reviewed and approved* by the following:

Jonathan P. Lynch
Professor of Plant Nutrition
Thesis Advisor

Kathleen M. Brown
Professor of Postharvest Physiology
Graduate Program Officer for Horticulture

Richard P. Craig
Professor Emeritus of Plant Breeding and J. Franklin Styer Professor Emeritus of
Horticultural Botany

*Signatures are on file in the Graduate School
ABSTRACT

It has been proposed that roots should favor primary growth (elongation) over secondary growth (thickening) in response to low phosphorus availability, in order to increase soil exploration, a process termed “root etiolation” by analogy with shoot responses to low light intensity. The objectives of this study were to evaluate the role of root etiolation in phosphorus acquisition under sub-optimal phosphorus conditions as an adaptive trait and to confirm genotypic variation for this response. Two common bean (*Phaseolus vulgaris* L.) genotypes known for their differing efficiency under low P, DOR 364 and G19833, were grown in sand culture in a greenhouse at two rates of buffered P availability. Twenty-seven days after planting, basal root axis length was longest under low P. Root and stele cross sectional areas increased over time under high P but not under low P. Low P root and stele cross sectional areas ultimately measured only 50% and 30% of high P, leading to a significantly higher stele to root area ratio under high P at basal and middle segments of the basal root. Reduced secondary development was also observed as reduced xylem vessel development under low P. These developmental differences were not observed to be due to allometry. Older sections of the basal root axis under high P respired almost 70% more per unit length than under low P. DOR 364 had significantly larger specific root length than G19833, especially under low P. This genetic difference was also apparent in significantly reduced root cross sectional area for DOR 364 under low P. Our results verify that roots etiolate under phosphorus stress and that etiolated roots are metabolically ‘cheaper’ due to their reduced respiration per unit length. There also appears to be a genetic component to root etiolation that could be included in a breeding program for plants better adapted to low phosphorus soils.
# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................... v

LIST OF TABLES .............................................................................................................. ix

ACKNOWLEDGEMENTS ................................................................................................. x

CHAPTER 1. INTRODUCTION ......................................................................................... 1

CHAPTER 2. MATERIALS AND METHODS ...................................................................... 6

   Root Anatomy ............................................................................................................. 8
   Tissue Analysis .......................................................................................................... 8
   Statistical analysis .................................................................................................... 8

CHAPTER 3. RESULTS ..................................................................................................... 11

   Whole Plant Responses to Phosphorus Availability .................................................. 11
   Basal Root Anatomy Responses to Phosphorus Availability ....................................... 12
   Genotypic Differences in Anatomy .......................................................................... 13
   Cost / Benefit of Anatomical Differences under Low Phosphorus ............................. 15
   Genotypic Differences for the Cost / Benefit ............................................................ 15

CHAPTER 4. DISCUSSION ............................................................................................... 39

REFERENCES .................................................................................................................. 48
LIST OF FIGURES

Figure 1. Diagram of basal root and segment locations for sectioning and respiration measurements .................................................................10

Figure 2. Plants grown under low P exhibited classic symptoms of P deficiency: reduced shoot growth, smaller leaves, shorter leaf internodes (39 days after planting) ...............17

Figure 3. Shoot dry weight (g) is significantly less under low P than high P at 27 and 39 days after planting (DAP). At 39 DAP under low P genotype DOR 364 is significantly smaller than G19833 (bars are 1 standard error of the mean, n = 4) .........................18

Figure 4. Shoot tissue P (mg P per g DW) is significantly less under low P than high P at 15, 27 and 39 days after planting (DAP) (bars are 1 standard error of the mean, n = 4).......18

Figure 5. Root to Shoot dry weight ratio is significantly greater for plants grown under low P than high P at 27 and 39 days after planting (DAP). There is also a significant P treatment x genotype interaction 39 DAP (DOR 364 has more extreme values, bars are 1 standard error of the mean, n = 4) ........................................................................19

Figure 6. Shoot to root relationship under low P is not significantly different allometrically from high P (regression line equations HP: (1/root DW) = 1.66 (1/shoot DW) + 0.336, $R^2 = 0.942$; LP: (1/root DW) = 1.27 (1/shoot DW) + 0.316, $R^2 = 0.458$; data were pooled for 3 harvests and 2 genotypes, n = 24) ........................................................................19

Figure 7. Basal root axis length is significantly greater under low P at 27 days after planting (DAP) but significantly less under low P at 39 DAP (bars are 1 standard error of the mean, n = 4) ........................................................................20

Figure 8. Specific Root Length (SRL) (root length per root dry weight, m/g) is significantly greater under low P and greater for genotype DOR 364 at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, n = 4). Other significant differences are noted in Table 3 .................................................................20

Figure 9. Basal root density (g/cm$^3$) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations. There is also a significant genotype x P interaction for the basal segments 39 DAP as DOR 364 is denser than G19833 under HP but less than G19833 under LP. Additionally for the basal segments, there is a
significant genotype response at 15 DAP (G19833>DOR 364) and significant P response 27 DAP (HP>LP) (bars are 1 standard error of the mean, n = 4). ..........21

Figure 10. Basal root anatomy changes significantly under low phosphorus in bean. 39 days after planting plants grown under HP are both larger and more developed. This is obvious in DOR 364 all along the root and in G19833 at the basal and apical ends. All cross sections are the same scale, bar is 100 um ...........................................22

Figure 11. Root cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, n = 4). Other significant differences are noted in Table 5 .........................23

Figure 12. Low P root cross sectional area (CSA) as % of high P is another way to represent the same data contained in Figure 11. Low P root CSA is divided by HP root CSA from the same rep (bars are 1 standard error of the mean, n = 4).................................23

Figure 13. Stele cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations. At 27 DAP there is a similar significant P response for basal segments and a significant P x genotype interaction for apical segments (DOR 364 has more extreme values than G19833) (bars are 1 standard error of the mean, n = 4)..................................................................24

Figure 14. Low P stele cross sectional areas (CSA) as % of high P is another way to represent the same data contained in Figure 13. Low P stele CSA is divided by HP stele CSA from the same rep (bars are 1 standard error of the mean, n = 4).................................24

Figure 15. Stele to root cross sectional area (CSA) ratio is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations and less for G19833 for middle and apical locations. Stele:root ratio was also significantly less for low P at 27 DAP for basal segments. Genotype effect is significant for apical segments at all harvest dates (bars are 1 standard error of the mean, n = 4)...........25

Figure 16. There is an allometric relationship between tissue DW and root cross sectional area of basal segments under HP ($R^2 = 0.642$) however the tissue DW does not successfully predict root CSA under LP ($R^2 = 0.105$). The line shown for LP is not significant (data pooled for harvest dates and genotypes, only basal segment data used for root CSA, n = 24). .................................................................25
Figure 17. There is an allometric relationship between tissue DW and stele cross sectional area of basal segments under HP ($R^2 = 0.527$) however the tissue DW does not successfully predict stele CSA under LP ($R^2 = 0.095$). The line shown for LP is not significant (data pooled for harvest dates and genotypes, only basal segment data used for stele CSA, $n = 24$).

Figure 18. Xylem cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, $n = 4$).

Figure 19. Phloem cross sectional area (CSA) is significantly less for genotype DOR 364 at 27 days after planting (DAP) for basal segments while phloem CSA is significantly less for DOR 364 and low P at 39 DAP for apical segments. Phloem CSA could not be measured and data are therefore missing for the most developed (basal segments, 39 DAP, HP) and least developed (apical segments, especially DOR 364) locations (bars are 1 standard error of the mean, $n = 4$).

Figure 20. Xylem to Phloem cross sectional area (CSA) ratio has a significant interaction between P treatment and genotype at 15 and 39 days after planting (DAP) for middle segments (low P < high P, G19833 has more extreme values than DOR 364). Xylem: phloem CSA ratio is significantly less under low P at 27 DAP for basal and apical segment locations (bars are 1 standard error of the mean, $n = 4$, although many missing, see note on Figure 19).

Figure 21. Sum of the 4 largest xylem vessel radii$^4$ (as per Hagen-Poiseuille law: transport capacity of a pipe increases with the fourth power of the pipe radius) is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations. Additionally xylem vessel radius$^4$ is significantly less under LP and for DOR 364 for basal sections at 15 and 27 DAP (bars are 1 standard error of the mean, $n = 4$).

Figure 22. Root respiration rate per dry weight is only significantly different at 39 days after planting (DAP) for basal segments with a P x genotype interaction (DOR 364 has more extreme values than G19833) (bars are 1 standard error of the mean, $n = 4$).

Figure 23. Root respiration rate per length is generally less under low P. There is a significant P treatment x genotype interaction at 39 days after planting (DAP) for the basal and
middle segment locations with DOR 364 having more extreme rates than G19833
(bars are 1 standard error of the mean, n = 4). Other significant differences are noted
in Table 9.

Figure 24. Root respiration rate per volume is significantly less under low P at 27 and 39 days
after planting (DAP) for middle segment locations. At 39 DAP there is a significant
interaction at basal locations for P treatment x genotype with G19833 having more
extreme rates than DOR 364 (bars are 1 standard error of the mean, n = 4).

Figure 27. Basal root axis length significantly correlates with shoot tissue P under low P at 15 (p
= 0.004) and 27 (p = 0.019) days after planting. This suggests that while the basal
root axes are still actively elongating under LP (see Figure 7), lower levels of P in the
shoot predict longer basal root axes (adjusted $R^2 = .733$ at 15 DAP, adjusted $R^2 =
0.565$ at 27 DAP, genotypes pooled, n = 8). Measures do not correlate at 39 DAP or
under HP.

Figure 28. Cross sectional area of 4 largest xylem vessels ($\mu m^2$) show the interesting
developmental trend for 1 late metaxylem vessel to develop before the others.
Although observed at both high P and low P and for both genotypes, at 39 days after
planting it is consistently apparent in DOR 364 under low P. This deserves further
investigation.
LIST OF TABLES

Table 1. Whole Plant Analysis of Variance ..................................................................................30
Table 2. Whole Plant Descriptive Statistics ..................................................................................30
Table 3. Segment Density, Specific Root Length, Volume/Length Analysis of Variance ..............31
Table 4. Segment Density, Specific Root Length, Volume/Length Descriptive Statistics ..............32
Table 5. Root Cross Sectional Area, Stele Cross Sectional Area Analysis of Variance ..............33
Table 6. Root Cross Sectional Area, Stele Cross Sectional Area Descriptive Statistics ..............34
Table 7. Xylem Cross Sectional Area, Phloem Cross Sectional Area Analysis of Variance ........35
Table 8. Xylem Cross Sectional Area, Phloem Cross Sectional Area Descriptive Statistics .........36
Table 9. Respiration Rate Analysis of Variance .........................................................................37
Table 10. Respiration Rate Descriptive Statistics ......................................................................38
ACKNOWLEDGEMENTS

I want to express gratitude to Dr. Jonathan Lynch and Dr. Kathleen Brown for initially stimulating me as an undergraduate, later employing me in their labs, guiding me as an active graduate student, and finally patiently waiting for me to write as my life led me away from the lab and university. I have deep appreciation for Professor Emeritus Richard Craig for his teaching and confidence in me.

Special thanks go to Lab Manager Robert Snyder and Lynch and Brown Lab members who showed me how things were done in the lab, especially to Melissa Ho and Ivan Ochoa. I am grateful to Ruth Halderman at the Electron Microscope Facility of the Huck Institute of the Life Sciences for her assistance with microscopy. I would also like to acknowledge the Department of Horticulture Walter Thomas Scholarship and Bean/Cowpea CRSP for funding this research.

Lastly, an enormous debt of gratitude is owed to my family. My parents, David and Linda Morrow, provided encouragement and support in addition to countless hours of childcare. Unfortunately my father did not live to see the completion of my degree. My husband Miguel has given me unconditional love, understanding and technical support at critical times. Finally this process would not have been what it was without the arrivals of Samuel David, Andres Gabriel, and Joshua Miguel, three blessings that gave me the title of “Mom” before “MS.” They too have sacrificed during this process.

SOLI DEO GLORIA!
CHAPTER 1. INTRODUCTION

In most of the United States of America and other industrialized nations it is difficult to appreciate the importance of food security because we have never been hungry. People who experienced the Great Depression, World War II and its aftermath are being replaced by younger generations who have only known times of abundance and the problems of over consumption rather than food security.

Food security is a complex problem with many interrelated components but obviously agriculture plays a significant role. The Green Revolution of the mid Twentieth Century revolutionized wheat and rice production in the developing world through the combination of breeding dwarf varieties that resisted lodging under high fertility and the application of chemical fertilizers. Although these innovations positively impacted agricultural production for those with access to improved seed and fertilizers, a half century later food security is still an issue (Lynch 2007). Many subsistence farmers are such because the only land they have access to has poor fertility. Without the resources to improve the soil, they reap poor harvests and continue in their cycle of poverty and subsistence agriculture.

Common bean (*Phaseolus vulgarus* L.) is a key protein source and staple food in much of Latin America and Africa. Production is limited to a great extent in the developing world by low soil phosphorus availability (Lynch 1995). The same resource-poor farmers who depend on beans for their calories and nutrients are the ones who are unable to pay for yield-boosting chemical fertilizers. Fortunately there is heritable diversity in common bean germplasm for yield responsiveness under low fertility (Lynch and Beebe 1995; Whiteaker, Gerloss *et al.* 1976).

Of all the nutrients necessary for plant growth, nitrogen and phosphorus are quantitatively the most important. Nitrogen is the nutrient needed in greatest quantities but it can be fixed from the
unreactive divalent form in the air ($N_2$) to the biologically critical form $NH_3$ by *Rhizobium* bacteria or the energy intensive Haber-Bosch process. Phosphorus however is a non-renewable resource that cannot be synthesized from the air. It is found naturally as either inorganic phosphorus rock or in organic matter. Phosphorus is frequently bound tightly to the soil particles or is located near the soil surface where it is deposited in organic litter. The available phosphorus is relatively immobile in the soils, as opposed to nitrogen that readily moves by mass flow. (Burech, Smithson *et al.* 1997; Lynch 2005; Marschner 1986; Sahrawat, Abekoe *et al.* 2001; Smithson and Giller 2002) The World Phosphate Institute analyzed 500 soils collected from 42 tropical countries and classified 65% as acutely deficient in P. Only 8% were considered not deficient (Sahrawat, Abekoe *et al.* 2001). As there is an urgent need to develop crops that are more efficient at acquiring inorganic P from the soil and/or at using P more efficiently (Lambers, Shane *et al.* 2006), a root system that is better able to access and utilize P would provide a tangible improvement in that direction (Lynch 2007).

Plants have a variety of root adaptations to low P availability. Roots can form mycorrhizal associations, effectively increasing root surface area, and modify the rhizosphere favorably for P uptake through root secretions (Marschner 1986). Some plant species have the ability to form root clusters that are highly effective at acquiring inorganic P, a trait proposed as a potential key to new crop development with superior P acquisition (Lambers, Shane *et al.* 2006). As phosphorus is relatively immobile in the soil, roots extract what is available and then must grow into new sources. Root architecture, or the special configuration of a root system, provides the framework for locating those sources (Lynch 2005). Common bean basal roots have been shown to respond to low P by becoming more horizontal, thereby growing where P is found in most natural soils (Bonser, Lynch *et al.* 1996). The proliferation of adventitious roots, growing from underground hypocotyls, is another strategy for locating P in topsoil (Miller, Ochoa *et al.* 2003). There can also be consequences with increased adventitious rooting, decreasing basal lateral and taproot growth as root architecture adapts to P localization (Walk, Jaramillo *et al.* 2006). Borch (Borch, Bouma *et al.*
reported that while main and lateral root length was unchanged under low P in bean, lateral root number and density decreased significantly. Root hairs become longer and denser in response to low P and have been shown to increase P uptake (Bates and Lynch 1996; Fohse, Claassen et al. 1991; Ma, Bielenberg et al. 2001). Root anatomy changes under low P availability with the formation of cortical aerenchyma that was disproportionally correlated to reduced root respiration (Fan, Zhu et al. 2003).

Root growth is important for P acquisition, but root consumption of carbon is a primary constraint to the growth of P-stressed plants. It follows that plants with root systems that use less C in acquiring P or that acquire more P per unit C used will have superior performance in low-P soils (Lynch and Beebe 1995). Beans respond to low P availability by allocating more resources to root growth. This can result in an increased root: shoot ratio with the associated carbon costs of roots (Lynch 2005; Lynch and Brown 2006). Nielsen (Nielsen, Eshel et al. 2001) demonstrated that low P common beans used a significantly higher fraction of their daytime net carbon assimilation on root respiration compared with medium and high P grown plants. Efficient genotypes had lower rates of root respiration and allocated a larger fraction of their biomass to root growth (Nielsen, Eshel et al. 2001). Anatomical root traits that affect the respiratory cost of constructing and maintaining roots have been suggested to enhance phosphorus acquisition at minimal root carbon cost (Fan, Zhu et al. 2003). Root cortical aerenchyma was also implicated in increased drought tolerance by reducing root metabolic costs thereby permitting greater root growth (Zhu, Brown et al. 2010).

Thinner roots are associated with more efficient nutrient uptake. Increasing phosphorus had a negative effect on the root length/root weight ratio, or the specific root length (SRL), and root radius increased 30% with increasing P fertilizer on Carex coriacea, a sedge (Powell 1974). Eissenstat (Eissenstat 1992) reported plants with a larger SRL may have an advantage over plants that do not due to a reduced carbon cost to produce root length. In a study of root morphology and response
of 10 temperate pasture species to P and nitrogen stress, Hill (Hill, Simpson et al. 2006) observed that most species decreased root diameter, decreased root mass density (dry mass per volume), and increased specific root length in response to P deficiency. A decreasing root diameter and an increasing root tissue mass density can have contrasting effects on SRL (Wahl, Ryser et al. 2001). *Dactylis glomerata* and *Brachypodium pinnatum*, grasses endemic to nutrient poor soils, produced finer roots with a higher tissue mass density when the phosphorus or nitrogen supply was low (Ryser and Lambers 1995). *D. glomerata* root diameter increased 40% with increasing P and consistently high N. In a more recent study of 19 perennial grass species, root tissue mass density did not correlate to cross sectional area (Wahl and Ryser 2000). Lateral root diameter was reported to decrease under low P in water hyacinth (*Eichhornia crassipes*) (Xie and Yu 2003) and maize (*Zea mays* L.) (Zhu and Lynch 2004). When phosphorus was only provided to a short segment of barley seminal root the first order lateral root diameter (but not axes) in the P-enriched zone increased significantly over high P control (Drew and Saker 1978). On the other hand, Mollier and Pellerin (Mollier and Pellerin 1999) reported no significant effect of P deprivation on the diameter of maize roots at the basal end, but apical diameters were slightly reduced by P deprivation. Fohse (Fohse, Claassen et al. 1991) found no correlation between P influx and root radius in bean and six other species grown in pots with soil.

Low phosphorus availability appeared to reduce secondary root growth and decrease % stele area measured from basal root cross sections of common bean (Fan, Zhu et al. 2003). It was proposed that roots should favor primary growth (elongation) over secondary growth (thickening) in response to low phosphorus availability, in order to increase soil exploration, a process termed “root etiolation” by analogy with shoot responses to low light intensity (Fan, Zhu et al. 2003; Lynch 2007; Lynch and Brown 2006).

In this study, 2 common bean genotypes contrasting for P efficiency were grown under differing P levels and destructively harvested 3 times. Upon harvest, basal root axis lengths were measured.
Three short segments excised from basal root axes (basal, mid point, apical) were analyzed for root respiration then segments were hand sectioned and observed under the microscope to compare secondary development. It was hypothesized that suboptimal P would reduce basal root diameter, secondary development, and respiration compared with sufficient P. The objective of this study was to evaluate the role of root etiolation in phosphorus acquisition under sub-optimal phosphorus conditions as an adaptive trait and confirm genotypic variation in this response.
CHAPTER 2. MATERIALS AND METHODS

Seeds of common bean (*Phaseolus vulgaris* L.), genotypes DOR 364 and G19833 (obtained from CIAT, Cali, Colombia, in 2002), were surface sterilized with a 10% bleach solution for 60 seconds followed by scarification with a razor blade. These genotypes differ in root development under phosphorus stress (Fan, Zhu et al. 2003). Seeds were germinated in rolls of low P brown germination paper (Anchor Paper Co., St. Paul, MN) saturated with 0.5 mM CaSO$_4$ in darkness for 24 to 36 hours at 28°C. Two germinated seeds were transplanted into 5.75 l pots containing silica sand mixed with phosphorus-doped alumina (5.0 g Al-P/l sand) to maintain P availability at 1 µM (mean desorption 1.0166 µM, SE = 0.0318) for low P and 111 µM (mean desorption 111.501 µM, SE = 2.721) for high P (Lynch, Epstein et al. 1990). Pots were placed in a temperature-controlled greenhouse on the Penn State campus at University Park, PA (4049’N, 7749’W) beginning on May 7, 2004. There were 4 repetitions blocked in time with planting dates 2-3 days apart. Seedlings were thinned to one plant per pot after emergence. Plants were fertigated once or twice daily as required with a volume sufficient for pots to readily drain using a solution consisting of 1.5 mM KNO$_3$, 1.2 mM Ca(NO$_3$)$_2$, 0.4 mM NH$_4$NO$_3$, 0.025 mM MgCl$_2$, 0.005 mM Fe-EDTA, 0.5 mM MgSO$_4$, 0.3 mM K$_2$SO$_4$, 0.3 mM (NH$_4$)$_2$SO$_4$, 0.0015 mM MnSO$_4$, 0.0015 mM ZnSO$_4$, 0.0005 mM CuSO$_4$, 0.00015 mM (NH$_4$)$_6$Mo$_7$O$_24$, and 0.0005 mM Na$_2$B$_4$O$_7$. Plants in the high P treatment received 100 µM KH$_2$PO$_4$ through the first harvest (15 days) and then the rate was increased to 300 µM thereafter while low P plants received 1 µM KH$_2$PO$_4$ throughout the experiment as well as extra K$_2$SO$_4$ at a rate which similarly increased after the first harvest in order to replace the additional K provided to the high P treatment.

Plants were harvested at 15, 27 and 39 days after transplanting (DAP) the imbibed seeds. Entire plants were harvested by immersing the pots in water and carefully teasing the roots from the sand. Harvested plants were placed in DI water and immediately transported to the lab for oxygen uptake measurements. Once in the lab, roots were untangled and separated. The shoot, primary root and
adventitious roots were removed and set aside for dry weight measurements. The basal roots, defined as the roots that emerge from the base of the hypocotyl in bean, were carefully separated and removed. One representative basal root per plant was measured for root axis length and preserved in ethanol (25%) for anatomical evaluation. Two basal root axis measurements were omitted (one each DOR 364 low P and high P at 39 DAP) due to broken/missing tips. Five representative basal roots (or all remaining basal roots, depending upon root number) per plant were selected for oxygen consumption measurements that were taken immediately. Any remaining basal roots were saved for dry weight measurement. Root samples for oxygen measurements were prepared as follows. Three segments were removed from each basal root by cutting 2-2.5 cm from the two ends of the root (termed “basal” from the oldest part of the root and “apical” from the tip) and from the mid-point (“middle”) of the basal root (Figure 1). Lateral roots were removed, leaving only the main axis of the basal root for measurement. Root segments cut from each of the 5 selected basal roots were combined based upon region of origin. Rarely, the axis tips appeared to be missing (broken) or discolored and thus not used, resulting in less than 5 segments for that region. The 5 segments from each region were placed in a 3.2 ml chamber with oxygenated MES buffer (1mM CaSO$_4$ and 5 mM MES adjusted to pH 5.5 with KOH) and measured with an oxygen electrode (Oxygraph OXYG1 Hansatech Instruments Ltd. Norfolk, England) for about 10 minutes. Oxygraph software (version 1.16, Hansatech Instruments Ltd.) was used to analyze the rate of oxygen consumption. Temperature was maintained at 25°C with a circulating water bath. The oxygen consumption rate was recorded between 1 and 9 minutes. In rare cases the first minute was not sufficient for the rate to stabilize so the average rate between 2 and 10 minutes was recorded. Root segment length was measured on a flatbed scanner with WinRHIZO software (WinRHIZO Pro version 2002c, Regent Instruments Inc., Quebec, Canada). Segments were dried at 60°C for 2-3 days and weighed.
Root Anatomy

Three root segments of similar size and from the same locations as in the oxygen measurement were excised from basal roots and preserved in 25% ethanol. Free hand cross sections of root segments were stained with dilute toluidine blue (0.05%), which differentiates between lignin and cellulose; lignin stains blue-green while cellulose stains purple or red-violet (McCully and Canny 1988). Cross sections were examined under an Olympus light microscope (brightfield optics) at 10x and images were captured using a digital camera. Occasionally due to cross section size, images were also captured at 4x or 20x. Images were analyzed using Image J (version 1.32j National Institutes of Health, USA) software. Where possible, measurements were made from images captured at 10x magnification. Both stele and total root cross sectional area were measured by using the closest fitting ellipse with the imaging software. In cases with a degraded cortex (older, basal sections) total root area measurements were calculated using the freehand line function. Xylem area, xylem vessel area, and phloem area measurements were also calculated using the freehand line function. The four largest xylem vessels were measured from each cross section. Although the vessel sizes were measured as area (μm²), they were assumed to be circular and their radii were calculated from the area. Based upon the Hagen-Poiseuille law, each of the 4 radii were raised to the fourth power and summed for that cross section. Data from two root tips were omitted (one each DOR 364 LP and HP at 39 DAP) due to broken/missing tips.

Tissue Analysis

Dried shoot tissue was ground and analyzed for P content (Murphy and Riley 1962). Basal-most root segments from harvest 2 (27 DAP) were analyzed for C and N using an elemental analyzer (Fisons EA 1108E).

Statistical analysis

Data were analyzed using Minitab software (Minitab 15, Minitab Corporation) using General Linear Models. Data were tested for homogeneity of variance and transformed where necessary
prior to testing for significance. Data were analyzed separately for each harvest date (15, 27, 39
DAP) and segment location (basal, middle, apical). Interactions among harvest dates, among
segment locations and harvest date x segment location were not analyzed. However, data were
pooled in some cases by combining genotypes and by combining harvest dates. Significance
was determined at p-value less than or equal to 0.05, unless otherwise noted.
Figure 1. Diagram of basal root and segment locations for sectioning and respiration measurements
CHAPTER 3. RESULTS

Whole Plant Responses to Phosphorus Availability

Plants in the low P treatment expressed symptoms of P deficiency including smaller overall stature and leaf size and shorter internodes than high P plants (Figure 2). Shoot dry weight (DW) was significantly lower for LP than HP at 27 and 39 days after planting, with P treatment differences increasing greatly at later harvests (Figure 3, Table 1, Table 2). Shoot size increased over the 3 harvests dates except for DOR 364 under LP that decreased in size between 27 and 39 DAP. Low P plants had significantly less shoot tissue P content than HP (Figure 4). Root mass was also lower under the LP treatment, but not to the same extent as shoot mass. There was a significantly greater root: shoot ratio under LP than under HP 27 and 39 DAP (Figure 5). At later harvest dates, the difference between high and low P root: shoot ratio increased. However, the root to shoot relationship under low P is not significantly different allometrically from high P (Figure 6). The basal root axis length increased with time, with the exception of LP root lengths, which failed to increase between 27 and 39 DAP (Figure 7). LP plants actually had the longest basal axis length at 27 DAP (significant at p = 0.051).

The two genotypes differed only slightly in terms of whole plant comparisons. At 15 days after planting G19833 had significantly more shoot dry weight and a higher concentration of shoot tissue P than DOR 364, however at 39 DAP there was a significant P treatment x genotype interaction where DOR 364 had the extreme values for shoot DW (Figure 3, Figure 4). G19833 consistently had larger root DW than DOR 364 regardless of treatment and harvest date. Both genotypes reacted similarly to the P conditions in terms of root: shoot ratios (Figure 5).
Basal Root Anatomy Responses to Phosphorus Availability

Low phosphorus significantly increased the specific root length (SRL) (length/dry weight) of basal roots for all segments at 27 and 39 DAP (except for middle at 27 DAP) (Figure 8, Table 3, Table 4). SRL increased for each segment location along the root from the base to the tip.

Root density (g/cm$^3$) for basal segments under LP increased much slower and was lower than HP (significantly lower at 27 and 39 DAP) (Figure 9). In the middle sections there was a similar trend yet less pronounced (significantly larger at 39 DAP). The smallest densities were found at the tips, although P differences were marginal (at 39 DAP p-value = 0.062).

Images of cross sections cut from the basal, middle and apical segments revealed a dramatic difference based on P availability as low P cross sections were smaller and less developed than high P (Figure 10, Table 5, Table 6). Root cross-sectional area increased over time under HP in the basal and middle segments while varying little under LP (Figure 11) which led to an decreased ratio of LP to HP cross sectional area (CSA) over time (Figure 12). Low P root CSA was significantly smaller than HP at 27 and 39 DAP for basal segments and at 39 DAP for middle segments. Root CSA under LP consistently decreased over time for apical segments with the treatments significantly different at 27 and 39 DAP. The stele cross sectional area responded similarly to high and low P as the root cross sectional areas (Figure 13, Figure 14). Differences in P treatment were significant at 27 (basal, apical segments) and 39 (basal, middle, apical segments) days after planting. By 39 DAP stele CSA was consistently smaller under LP. It follows that the stele to root cross sectional area ratio (stele CSA / root CSA) would reveal similar treatment differences with LP ratios smaller than HP for basal and middle sections (Figure 15). Low P ratios remained steady over time while HP increased. Treatments were significantly different at 15 (middle), 27 (basal) and 39 (basal, middle) DAP. There was no P difference at the tip. Although an allometric relationship exists between total tissue DW and both root CSA and stele CSA under high P ($R^2 = 0.642$ root CSA and $R^2 = 0.527$ stele CSA), there was not enough evidence to suggest that total tissue DW
predicts root CSA and/or stele CSA under low P ($R^2 = 0.105$ root CSA and $R^2 = 0.095$ stele CSA). It follows that changes in root cross sectional area and stele cross sectional area under low P were not just allometric (Figure 16, Figure 17).

The xylem and phloem cross sectional areas were also quantified. Trends in xylem CSA measurements closely mimicked stele CSA measurements with smaller xylem CSA under LP (Figure 18, Table 7, Table 8). Differences due to P were significant at 27 (basal segments, $p = 0.053$ for apical) and 39 (basal, middle, apical segments, $p = 0.057$ for apical) DAP. Phloem areas were difficult to impossible to quantify in the most mature (27 and 39 DAP) basal segments as well as in some of the apical segments. Low P phloem CSA were significantly smaller than high P for apical segments at 39 DAP. At the middle segment location at 39 DAP plants grown under LP exhibited marginally smaller phloem areas than their HP counterparts ($p$-value = 0.079) (Figure 19). The xylem to phloem ratio (xylem CSA / phloem CSA) data at 39 DAP were also incomplete due to lack of phloem data. Plants grown with deficient P had significant less xylem to phloem CSA at 15 (middle), 27 (basal, apical) and 39 (middle) DAP (Figure 20). At 39 DAP apical segments under LP appeared to have a larger xylem to phloem ratio than HP, but the difference was not significant. Measurements of the sum of the 4 largest xylem vessel radii to the fourth power revealed significantly smaller vessels under LP from both the basal (15, 27, 39 DAP) and middle (39 DAP) locations on the root (Figure 21).

**Genotypic Differences in Anatomy**

Specific root length (SRL) was one trait with significant genotypic variation. As most clearly seen at 39 DAP, DOR 364 consistently had greater SRL than corresponding G19833 root segments under LP (Figure 8, Table 3, Table 4). There was a significant P treatment x genotype interaction for basal (27, 39 DAP) and middle (39 DAP) segments at later harvest dates. DOR 364 tended to have the more extreme values in SRL, except for apical segments. At the apical end of the root DOR 364 and G19833 had a similar pattern in SRL over time with DOR 364 having significantly
greater SRL at 27 and 39 DAP.

Root densities (g/cm$^3$) were more divergent under high and low P for DOR 364 than for G19833 with significant P treatment x genotype interaction at 39 DAP for basal segments (Figure 9). There was also significant genotypic difference in root density at 15 DAP for basal sections when DOR 364 was less dense then G19833 regardless of treatment.

Just as G19833 was usually larger overall than DOR 364, G19833 root cross sectional areas were usually larger than DOR 364 (Figure 11, Table 5, Table 6). Genotypic differences were significant at 15 (basal, middle, apical), 27 (basal, middle) and 39 (middle) DAP. The genetic differences are easily visualized as LP/HP (Figure 12). At later harvest dates LP root CSA was a significantly smaller percentage of HP for DOR 364 compared with G19833. The downward trend with time was much sharper for DOR 364. Stele cross sectional areas followed that same general pattern although to a lesser extent (Figure 13, Figure 14). There were no significant differences in genotype for stele CSA, but over time DOR 364 LP as a percentage of HP had a steeper downward trend. The only significant genotypic differences for the stele/root ratio were consistently found on the apical segments (15, 27, 39 DAP) (Figure 15). G19833 apical segments under HP had the smallest ratios, even though they individually measured the largest at 39 DAP.

G19833 had significantly larger xylem CSA on the basal and middle segments at 15 and 27 DAP (Figure 18, Table 7, Table 8). G19833 appeared to have larger phloem areas than DOR 364 (differences significant at 27 (basal) and 39 (apical) DAP) (Figure 19). There was a significant P treatment x genotype interaction at 15 DAP for basal segments (more extreme values for G19833). The xylem: phloem ratio also had a significant P x genotype interaction for the middle segments (15 and 39 DAP), G19833 again having the extreme values (Figure 20). G19833 had a significantly smaller xylem: phloem ratio than DOR 364 at 15 DAP on the apical segments. The sum of the 4 largest xylem vessel radii to the fourth power had some significant genotypic variation
for the basal (15 and 27 DAP) and middle (27 DAP) segments (Figure 21). While G19833 vessels increased in size over time, DOR 364 increased under HP but decreased under LP.

**Cost / Benefit of Anatomical Differences under Low Phosphorus**

Root respiration rate per g dry weight revealed that although respiration changed over time and at different sections of the root, P treatment and genotype made little difference (treatment differences were significant at one location apiece at 27 (middle) and 39 (basal) DAP) (Figure 22, Table 9, Table 10). That was not the case when the respiration rate was calculated per cm length. The most dramatic difference was for the basal segments of the root where the LP treatment had significantly lower respiration rates per unit length than HP at 27 and 39 DAP (Figure 23). The rate increased significantly over the three harvest dates for HP, but decreased slightly for LP. At the middle segment location, respiration under LP was also significantly lower at 27 and 39 DAP. At the apical end of the roots, the respiration rate decreased for both treatments over time, but rate was significantly higher for HP than LP at 15 DAP. The general trends for respiration rate per length mimicked those for root and stele cross sectional areas. When calculated for root segment volume (Figure 24), respiration rate varied little for the basal segments (LP significantly lower than HP at 39 DAP). However, for middle segments there were P differences with a significantly lower rate for LP following 15 DAP. While HP rates per volume remained fairly consistent over time, they decreased for LP. For apical segments respiration decreased for all treatment combinations over time, but HP was usually higher at each harvest date (significant at 15 DAP).

**Genotypic Differences for the Cost / Benefit**

Significant genotypic differences for respiration rate per dry weight occurred at 15 DAP (basal – p-value = 0.052) as well as a significant interaction between genotype and P treatment at 39 DAP (basal) (Figure 22, Table 9, Table 10). Root respiration per DW was greater for DOR 364 at 15 DAP, but at 39 DAP DOR 364 had the extreme values at low and high P. The respiration rate per unit length was significantly different for genotype at 15 (basal, middle) and 27 (middle) DAP with
G19833 respiring more than DOR 364. The basal segments had a significant genotype x P treatment interaction with DOR 364 having the highest and lowest rates at 27 DAP (Figure 23). For the apical segments G19833 had a higher respiration rate per unit length than DOR 364 for each treatment, with rates generally decreasing over time as the plants matured (at 27 DAP p-value = 0.084). Unlike the basal end, for the apical segments there was a minimal difference for DOR 364 between HP and LP. Respiration rate per unit volume (Figure 24) varied little between genotypes (at 15 DAP p-value = 0.069 middle). There was a significant genotype x P treatment interaction at 39 DAP for basal sections as G19833 had the highest (HP) and lowest (LP) rates. At the apical end this was replicated at each harvest date though genotypic differences were not significant.
Figure 2. Plants grown under low P exhibited classic symptoms of P deficiency: reduced shoot growth, smaller leaves, shorter leaf internodes (39 days after planting)
Figure 3. Shoot dry weight (g) is significantly less under low P than high P at 27 and 39 days after planting (DAP). At 39 DAP under low P genotype DOR 364 is significantly smaller than G19833 (bars are 1 standard error of the mean, n = 4).

Figure 4. Shoot tissue P (mg P per g DW) is significantly less under low P than high P at 15, 27 and 39 days after planting (DAP) (bars are 1 standard error of the mean, n = 4).
Figure 5. Root to Shoot dry weight ratio is significantly greater for plants grown under low P than high P at 27 and 39 days after planting (DAP). There is also a significant P treatment x genotype interaction 39 DAP (DOR 364 has more extreme values, bars are 1 standard error of the mean, n = 4).

Figure 6. Shoot to root relationship under low P is not significantly different allometrically from high P (regression line equations HP: (1/root DW) = 1.66 (1/shoot DW) + 0.336, R² = 0.942; LP: (1/root DW) = 1.27 (1/shoot DW) + 0.316, R² = 0.458; data were pooled for 3 harvests and 2 genotypes, n = 24).
Figure 7. Basal root axis length is significantly greater under low P at 27 days after planting (DAP) but significantly less under low P at 39 DAP (bars are 1 standard error of the mean, n = 4).

Figure 8. Specific Root Length (SRL) (root length per root dry weight, m/g) is significantly greater under low P and greater for genotype DOR 364 at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, n = 4). Other significant differences are noted in Table 3.
Figure 9. Basal root density (g/cm³) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations. There is also a significant genotype x P interaction for the basal segments 39 DAP as DOR 364 is denser than G19833 under HP but less than G19833 under LP. Additionally for the basal segments, there is a significant genotype response at 15 DAP (G19833>DOR 364) and significant P response 27 DAP (HP>LP) (bars are 1 standard error of the mean, n = 4).
Figure 10. Basal root anatomy changes significantly under low phosphorus in bean. 39 days after planting plants grown under HP are both larger and more developed. This is obvious in DOR 364 all along the root and in G19833 at the basal and apical ends. All cross sections are the same scale, bar is 100 um.
Figure 11. Root cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, n = 4). Other significant differences are noted in Table 5.

Figure 12. Low P root cross sectional area (CSA) as % of high P is another way to represent the same data contained in Figure 11. Low P root CSA is divided by HP root CSA from the same rep (bars are 1 standard error of the mean, n = 4).
Figure 13. Stele cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations. At 27 DAP there is a similar significant P response for basal segments and a significant P x genotype interaction for apical segments (DOR 364 has more extreme values than G19833) (bars are 1 standard error of the mean, n = 4).

Figure 14. Low P stele cross sectional areas (CSA) as % of high P is another way to represent the same data contained in Figure 13. Low P stele CSA is divided by HP stele CSA from the same rep (bars are 1 standard error of the mean, n = 4).
Figure 15. Stele to root cross sectional area (CSA) ratio is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations and less for G19833 for middle and apical locations. Stele:root ratio was also significantly less for low P at 27 DAP for basal segments. Genotype effect is significant for apical segments at all harvest dates (bars are 1 standard error of the mean, n = 4).

Figure 16. There is an allometric relationship between tissue DW and root cross sectional area of basal segments under HP ($R^2 = 0.642$) however the tissue DW does not successfully predict root CSA under LP ($R^2 = 0.105$). The line shown for LP is not significant (data pooled for harvest dates and genotypes, only basal segment data used for root CSA, n = 24).
Figure 17. There is an allometric relationship between tissue DW and stele cross sectional area of basal segments under HP ($R^2 = 0.527$) however the tissue DW does not successfully predict stele CSA under LP ($R^2 = 0.095$). The line shown for LP is not significant (data pooled for harvest dates and genotypes, only basal segment data used for stele CSA, $n = 24$).

Figure 18. Xylem cross sectional area (CSA) is significantly less under low P at 39 days after planting (DAP) for each of the segment locations (bars are 1 standard error of the mean, $n = 4$).
Figure 19. Phloem cross sectional area (CSA) is significantly less for genotype DOR 364 at 27 days after planting (DAP) for basal segments while phloem CSA is significantly less for DOR 364 and low P at 39 DAP for apical segments. Phloem CSA could not be measured and data are therefore missing for the most developed (basal segments, 39 DAP, HP) and least developed (apical segments, especially DOR 364) locations (bars are 1 standard error of the mean, n = 4).

Figure 20. Xylem to Phloem cross sectional area (CSA) ratio has a significant interaction between P treatment and genotype at 15 and 39 days after planting (DAP) for middle segments (low P < high P, G19833 has more extreme values than DOR 364). Xylem: phloem CSA ratio is significantly less under low P at 27 DAP for basal and apical segment locations (bars are 1 standard error of the mean, n = 4, although many missing, see note on Figure 19).
Figure 21. Sum of the 4 largest xylem vessel radii**: (as per Hagen-Poiseuille law: transport capacity of a pipe increases with the fourth power of the pipe radius) is significantly less under low P at 39 days after planting (DAP) for basal and middle segment locations. Additionally xylem vessel radius* is significantly less under LP and for DOR 364 for basal sections at 15 and 27 DAP (bars are 1 standard error of the mean, n = 4).

Figure 22. Root respiration rate per dry weight is only significantly different at 39 days after planting (DAP) for basal segments with a P x genotype interaction (DOR 364 has more extreme values than G19833) (bars are 1 standard error of the mean, n = 4).
Figure 23. Root respiration rate per length is generally less under low P. There is a significant P treatment x genotype interaction at 39 days after planting (DAP) for the basal and middle segment locations with DOR 364 having more extreme rates than G19833 (bars are 1 standard error of the mean, n = 4). Other significant differences are noted in Table 9.

Figure 24. Root respiration rate per volume is significantly less under low P at 27 and 39 days after planting (DAP) for middle segment locations. At 39 DAP there is a significant interaction at basal locations for P treatment x genotype with G19833 having more extreme rates than DOR 364 (bars are 1 standard error of the mean, n = 4).
Table 1. Whole Plant Analysis of Variance

<table>
<thead>
<tr>
<th>Harvest Date</th>
<th>ANOVA</th>
<th>Total DW</th>
<th>Shoot DW</th>
<th>Shoot P (log)</th>
<th>Basal Axis Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>15 DAP</td>
<td>Genotype</td>
<td>10.94</td>
<td>0.009</td>
<td>14.04</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>P Trt</td>
<td>1.48</td>
<td>0.255</td>
<td>2.74</td>
<td>0.132</td>
</tr>
<tr>
<td></td>
<td>Geno * P Trt</td>
<td>3.48</td>
<td>0.095</td>
<td>4.62</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>1.46</td>
<td>0.290</td>
<td>3.44</td>
<td>0.065</td>
</tr>
<tr>
<td>27 DAP</td>
<td>Genotype</td>
<td>17.97</td>
<td>0.002</td>
<td>4.07</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>P Trt</td>
<td>37.42</td>
<td>0.000</td>
<td>70.06</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Geno * P Trt</td>
<td>4.48</td>
<td>0.063</td>
<td>1.22</td>
<td>0.298</td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>0.08</td>
<td>0.544</td>
<td>0.97</td>
<td>0.449</td>
</tr>
<tr>
<td>39 DAP</td>
<td>Genotype</td>
<td>31.03</td>
<td>0.000</td>
<td>22.56</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>P Trt</td>
<td>166.42</td>
<td>0.000</td>
<td>125.98</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Geno * P Trt</td>
<td>30.79</td>
<td>0.000</td>
<td>22.99</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Rep</td>
<td>1.16</td>
<td>0.376</td>
<td>1.00</td>
<td>0.435</td>
</tr>
</tbody>
</table>

Table 2. Whole Plant Descriptive Statistics

<table>
<thead>
<tr>
<th>Harvest Date</th>
<th>Genotype</th>
<th>P Treatment</th>
<th>Total tissue DW (g)</th>
<th>Shoot DW (g)</th>
<th>Shoot tissue P (mg/g)</th>
<th>Basal root main axis length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Error</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>0.6278a</td>
<td>(0.068)</td>
<td>4</td>
<td>0.4043a</td>
<td>(0.051)</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.6523a</td>
<td>(0.036)</td>
<td>4</td>
<td>0.4107a</td>
<td>(0.034)</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>1.2362b</td>
<td>(0.080)</td>
<td>4</td>
<td>0.8646b</td>
<td>(0.080)</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.8650ab</td>
<td>(0.174)</td>
<td>4</td>
<td>0.5560ab</td>
<td>(0.123)</td>
</tr>
<tr>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>3.2960a</td>
<td>(0.578)</td>
<td>4</td>
<td>2.3940b</td>
<td>(0.449)</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.8782b</td>
<td>(0.051)</td>
<td>4</td>
<td>0.5242a</td>
<td>(0.019)</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>4.6720a</td>
<td>(0.373)</td>
<td>4</td>
<td>3.2670b</td>
<td>(0.279)</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>1.4290a</td>
<td>(0.145)</td>
<td>4</td>
<td>0.7700a</td>
<td>(0.120)</td>
</tr>
<tr>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>11.5970c</td>
<td>(0.548)</td>
<td>4</td>
<td>9.1220c</td>
<td>(0.354)</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.7633a</td>
<td>(0.090)</td>
<td>4</td>
<td>0.4036a</td>
<td>(0.058)</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>11.8510c</td>
<td>(0.697)</td>
<td>4</td>
<td>8.6260c</td>
<td>(0.495)</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>1.7160b</td>
<td>(0.115)</td>
<td>4</td>
<td>0.9083b</td>
<td>(0.068)</td>
</tr>
</tbody>
</table>
Table 3. Segment Density, Specific Root Length, Volume/Length Analysis of Variance

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>ANOVA</th>
<th>Density F</th>
<th>Density p</th>
<th>SRL F</th>
<th>SRL p</th>
<th>Volume/Length F</th>
<th>Volume/Length p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>58.39</td>
<td>0.000</td>
<td>50.20</td>
<td>0.000</td>
<td>32.25</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>1.59</td>
<td>0.239</td>
<td>1.48</td>
<td>0.255</td>
<td>3.87</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.03</td>
<td>0.857</td>
<td>0.10</td>
<td>0.763</td>
<td>0.66</td>
<td>0.437</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>13.53</td>
<td>0.001</td>
<td>3.02</td>
<td>0.087</td>
<td>9.60</td>
<td>0.004</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>4.63</td>
<td>0.060</td>
<td>31.71</td>
<td>0.000</td>
<td>3.35</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>29.64</td>
<td>0.000</td>
<td>93.30</td>
<td>0.000</td>
<td>27.52</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.93</td>
<td>0.360</td>
<td>18.42</td>
<td>0.002</td>
<td>0.57</td>
<td>0.468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.00</td>
<td>0.184</td>
<td>2.06</td>
<td>0.176</td>
<td>3.12</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.00</td>
<td>0.953</td>
<td>37.34</td>
<td>0.000</td>
<td>2.71</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>54.00</td>
<td>0.000</td>
<td>362.23</td>
<td>0.000</td>
<td>128.66</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>8.74</td>
<td>0.016</td>
<td>74.04</td>
<td>0.000</td>
<td>18.04</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.42</td>
<td>0.744</td>
<td>3.26</td>
<td>0.074</td>
<td>2.30</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>4.38</td>
<td>0.066</td>
<td>8.06</td>
<td>0.019</td>
<td>2.56</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.62</td>
<td>0.452</td>
<td>0.04</td>
<td>0.839</td>
<td>0.68</td>
<td>0.430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.67</td>
<td>0.435</td>
<td>0.92</td>
<td>0.364</td>
<td>0.15</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>12.78</td>
<td>0.001</td>
<td>2.26</td>
<td>0.150</td>
<td>10.52</td>
<td>0.003</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.08</td>
<td>0.779</td>
<td>79.63</td>
<td>0.000</td>
<td>13.95</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>1.60</td>
<td>0.242</td>
<td>2.29</td>
<td>0.164</td>
<td>0.32</td>
<td>0.583</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.31</td>
<td>0.285</td>
<td>1.07</td>
<td>0.327</td>
<td>0.02</td>
<td>0.879</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.08</td>
<td>0.969</td>
<td>5.19</td>
<td>0.024</td>
<td>1.37</td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.02</td>
<td>0.898</td>
<td>9.53</td>
<td>0.013</td>
<td>2.87</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>18.47</td>
<td>0.002</td>
<td>31.09</td>
<td>0.000</td>
<td>3.03</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>4.20</td>
<td>0.071</td>
<td>16.62</td>
<td>0.003</td>
<td>4.73</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.62</td>
<td>0.620</td>
<td>1.27</td>
<td>0.342</td>
<td>3.33</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>1.75</td>
<td>0.218</td>
<td>1.05</td>
<td>0.332</td>
<td>0.05</td>
<td>0.821</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>4.51</td>
<td>0.063</td>
<td>0.23</td>
<td>0.646</td>
<td>2.11</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.41</td>
<td>0.265</td>
<td>0.33</td>
<td>0.581</td>
<td>0.03</td>
<td>0.866</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>5.42</td>
<td>0.021</td>
<td>0.24</td>
<td>0.863</td>
<td>3.36</td>
<td>0.069</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.00</td>
<td>0.983</td>
<td>5.12</td>
<td>0.050</td>
<td>10.47</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.52</td>
<td>0.491</td>
<td>9.28</td>
<td>0.014</td>
<td>17.13</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.76</td>
<td>0.406</td>
<td>0.01</td>
<td>0.914</td>
<td>1.78</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>3.47</td>
<td>0.064</td>
<td>0.99</td>
<td>0.442</td>
<td>1.60</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.12</td>
<td>0.738</td>
<td>5.77</td>
<td>0.043</td>
<td>3.88</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>4.87</td>
<td>0.055</td>
<td>8.90</td>
<td>0.017</td>
<td>2.15</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.07</td>
<td>0.795</td>
<td>0.19</td>
<td>0.678</td>
<td>0.03</td>
<td>0.862</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>3.06</td>
<td>0.084</td>
<td>0.48</td>
<td>0.707</td>
<td>0.26</td>
<td>0.851</td>
</tr>
</tbody>
</table>
Table 4. Segment Density, Specific Root Length, Volume/Length Descriptive Statistics

<table>
<thead>
<tr>
<th>Segment Date</th>
<th>Genotype Treatment</th>
<th>Density (g/cm³)</th>
<th>SRL (m/g)</th>
<th>Volume/Length (cm³/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>Standard Error</td>
</tr>
<tr>
<td>15 DAP Basal</td>
<td>DOR 364, HP</td>
<td>0.250 ± 0.012</td>
<td>23.80 ± 1.84</td>
<td>0.00172 ± 0.00017</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.264 ± 0.024</td>
<td>25.22 ± 2.12</td>
<td>0.00156 ± 0.00015</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.323 ± 0.020</td>
<td>12.20 ± 1.05</td>
<td>0.00263 ± 0.00029</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.333 ± 0.018</td>
<td>14.60 ± 2.41</td>
<td>0.00224 ± 0.00034</td>
</tr>
<tr>
<td></td>
<td>DOR 364, HP</td>
<td>0.412 ± 0.021</td>
<td>6.62 ± 1.36</td>
<td>0.00413 ± 0.00075</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.310 ± 0.017</td>
<td>19.59 ± 1.34</td>
<td>0.00167 ± 0.00004</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.431 ± 0.010</td>
<td>5.37 ± 0.68</td>
<td>0.00457 ± 0.00066</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.360 ± 0.021</td>
<td>10.36 ± 0.51</td>
<td>0.00273 ± 0.00020</td>
</tr>
<tr>
<td></td>
<td>DOR 364, HP</td>
<td>0.595 ± 0.040</td>
<td>1.77 ± 0.50</td>
<td>0.01171 ± 0.00253</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.308 ± 0.009</td>
<td>21.36 ± 1.67</td>
<td>0.00156 ± 0.00016</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.511 ± 0.022</td>
<td>2.54 ± 0.23</td>
<td>0.00811 ± 0.00131</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.389 ± 0.022</td>
<td>8.12 ± 0.71</td>
<td>0.00322 ± 0.00009</td>
</tr>
<tr>
<td>27 DAP Basal</td>
<td>DOR 364, HP</td>
<td>0.214 ± 0.008</td>
<td>29.91 ± 0.65</td>
<td>0.00157 ± 0.00008</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.201 ± 0.026</td>
<td>31.95 ± 1.77</td>
<td>0.00163 ± 0.00016</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.234 ± 0.031</td>
<td>26.61 ± 1.34</td>
<td>0.00172 ± 0.00026</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.233 ± 0.027</td>
<td>25.30 ± 3.29</td>
<td>0.00187 ± 0.00033</td>
</tr>
<tr>
<td>39 DAP Basal</td>
<td>DOR 364, HP</td>
<td>0.257 ± 0.007</td>
<td>26.91 ± 0.99</td>
<td>0.00145 ± 0.00007</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.254 ± 0.023</td>
<td>27.31 ± 1.20</td>
<td>0.00155 ± 0.00008</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.278 ± 0.009</td>
<td>18.61 ± 1.11</td>
<td>0.00197 ± 0.00018</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.244 ± 0.011</td>
<td>20.74 ± 1.43</td>
<td>0.00203 ± 0.00018</td>
</tr>
<tr>
<td>15 DAP Middle</td>
<td>DOR 364, HP</td>
<td>0.346 ± 0.032</td>
<td>14.52 ± 2.40</td>
<td>0.00214 ± 0.00020</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.195 ± 0.023</td>
<td>33.09 ± 2.51</td>
<td>0.00162 ± 0.00017</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.300 ± 0.017</td>
<td>16.42 ± 1.36</td>
<td>0.00208 ± 0.00016</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.247 ± 0.014</td>
<td>19.30 ± 1.38</td>
<td>0.00213 ± 0.00012</td>
</tr>
<tr>
<td>27 DAP Middle</td>
<td>DOR 364, HP</td>
<td>0.195 ± 0.028</td>
<td>41.74 ± 3.48</td>
<td>0.00131 ± 0.00017</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.177 ± 0.036</td>
<td>41.25 ± 2.03</td>
<td>0.00155 ± 0.00029</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.245 ± 0.024</td>
<td>33.68 ± 4.51</td>
<td>0.00132 ± 0.00022</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.179 ± 0.027</td>
<td>38.97 ± 6.79</td>
<td>0.00163 ± 0.00027</td>
</tr>
<tr>
<td>39 DAP Middle</td>
<td>DOR 364, HP</td>
<td>0.247 ± 0.032</td>
<td>40.61 ± 4.18</td>
<td>0.00107 ± 0.00015</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.242 ± 0.035</td>
<td>59.91 ± 6.80</td>
<td>0.00073 ± 0.00007</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.221 ± 0.018</td>
<td>24.98 ± 3.29</td>
<td>0.00198 ± 0.00038</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.267 ± 0.053</td>
<td>45.76 ± 9.89</td>
<td>0.00095 ± 0.00013</td>
</tr>
<tr>
<td>15 DAP Apical</td>
<td>DOR 364, HP</td>
<td>0.291 ± 0.050</td>
<td>52.09 ± 9.54</td>
<td>0.00081 ± 0.00022</td>
</tr>
<tr>
<td></td>
<td>DOR 364, LP</td>
<td>0.218 ± 0.033</td>
<td>83.21 ± 9.55</td>
<td>0.00054 ± 0.00012</td>
</tr>
<tr>
<td></td>
<td>G19833, HP</td>
<td>0.259 ± 0.009</td>
<td>32.96 ± 5.04</td>
<td>0.00128 ± 0.00025</td>
</tr>
<tr>
<td></td>
<td>G19833, LP</td>
<td>0.213 ± 0.027</td>
<td>57.70 ± 10.20</td>
<td>0.00092 ± 0.00017</td>
</tr>
</tbody>
</table>
Table 5. Root Cross Sectional Area, Stele Cross Sectional Area Analysis of Variance

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>ANOVA</th>
<th>Stele CSA</th>
<th>Root CSA</th>
<th>Stele/Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>3.59</td>
<td>0.091</td>
<td>14.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.36</td>
<td>0.564</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.81</td>
<td>0.211</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.88</td>
<td>0.486</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>1.83</td>
<td>0.209</td>
<td>8.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>15.96</td>
<td><strong>0.003</strong></td>
<td>8.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.18</td>
<td>0.683</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.27</td>
<td>0.150</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.66</td>
<td>0.438</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>50.74</td>
<td><strong>0.000</strong></td>
<td>47.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.44</td>
<td>0.261</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.52</td>
<td>0.276</td>
<td>1.37</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.16</td>
<td>0.700</td>
<td>5.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.36</td>
<td>0.563</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.08</td>
<td>0.778</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.24</td>
<td>0.153</td>
<td>3.36</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>2.23</td>
<td>0.170</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.99</td>
<td>0.346</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td><strong>0.02</strong></td>
<td>0.905</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.69</td>
<td>0.582</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>2.47</td>
<td>0.150</td>
<td>12.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>23.01</td>
<td><strong>0.001</strong></td>
<td>9.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.00</td>
<td>0.946</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.74</td>
<td>0.554</td>
<td>1.36</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.39</td>
<td>0.548</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.14</td>
<td>0.715</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>2.17</td>
<td>0.174</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.15</td>
<td>0.164</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>1.72</td>
<td>0.222</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>5.19</td>
<td><strong>0.049</strong></td>
<td>5.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>8.38</td>
<td><strong>0.018</strong></td>
<td>6.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.88</td>
<td>0.487</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.11</td>
<td>0.748</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>3.06</td>
<td>0.118</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.33</td>
<td>0.583</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.33</td>
<td>0.805</td>
<td>1.71</td>
</tr>
</tbody>
</table>
### Table 6. Root Cross Sectional Area, Stele Cross Sectional Area Descriptive Statistics

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>Genotype P Treatment</th>
<th>Stele CSA ($\mu m^2$)</th>
<th>Root CSA ($\mu m^2$)</th>
<th>Stele/Root CSA ($\mu m^2/\mu m^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Error</td>
<td>n</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>0.1198 a (0.011)</td>
<td>4</td>
<td>0.415a (0.046)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.1441 a (0.024)</td>
<td>4</td>
<td>0.437a (0.031)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.2248 a (0.049)</td>
<td>4</td>
<td>0.684b (0.106)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.1618 a (0.031)</td>
<td>4</td>
<td>0.582ab (0.078)</td>
</tr>
<tr>
<td>Basal</td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>0.3861 b (0.097)</td>
<td>4</td>
<td>0.6864ab (0.097)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.1121 a (0.023)</td>
<td>4</td>
<td>0.1121a (0.023)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.4439 b (0.099)</td>
<td>4</td>
<td>0.4439b (0.099)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.2223ab (0.024)</td>
<td>4</td>
<td>0.2223ab (0.024)</td>
</tr>
<tr>
<td>Basal</td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>1.7540b (0.649)</td>
<td>4</td>
<td>2.263b (0.642)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0965 a (0.020)</td>
<td>4</td>
<td>0.365a (0.049)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>1.5860b (0.487)</td>
<td>4</td>
<td>2.022b (0.484)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.1877a (0.034)</td>
<td>4</td>
<td>0.549a (0.071)</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>0.0874 a (0.015)</td>
<td>4</td>
<td>0.389a (0.043)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0834a (0.009)</td>
<td>4</td>
<td>0.452a (0.029)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.0963 a (0.021)</td>
<td>4</td>
<td>0.535a (0.126)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0848 a (0.011)</td>
<td>4</td>
<td>0.614a (0.089)</td>
</tr>
<tr>
<td>Middle</td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>0.0926 a (0.013)</td>
<td>4</td>
<td>0.093a (0.013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0762 a (0.006)</td>
<td>4</td>
<td>0.076a (0.006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.1436 a (0.055)</td>
<td>4</td>
<td>0.144a (0.055)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0991 a (0.014)</td>
<td>4</td>
<td>0.099a (0.014)</td>
</tr>
<tr>
<td>Middle</td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>0.2280bc (0.085)</td>
<td>4</td>
<td>0.704ab (0.090)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0703a (0.016)</td>
<td>4</td>
<td>0.358a (0.055)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.2900c (0.048)</td>
<td>4</td>
<td>1.142b (0.198)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0948 ab (0.011)</td>
<td>4</td>
<td>0.746ab (0.098)</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>0.0528a (0.007)</td>
<td>4</td>
<td>0.396a (0.022)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0661a (0.008)</td>
<td>4</td>
<td>0.475a (0.025)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.0679a (0.009)</td>
<td>4</td>
<td>0.597a (0.043)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0600a (0.009)</td>
<td>4</td>
<td>0.528a (0.098)</td>
</tr>
<tr>
<td>Apical</td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>0.0698b (0.009)</td>
<td>4</td>
<td>0.070b (0.009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0324a (0.002)</td>
<td>4</td>
<td>0.032a (0.002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.0394ab (0.010)</td>
<td>4</td>
<td>0.039ab (0.010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0439ab (0.005)</td>
<td>4</td>
<td>0.044ab (0.005)</td>
</tr>
<tr>
<td>Apical</td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>0.0487a (0.022)</td>
<td>4</td>
<td>0.305ab (0.097)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>0.0137a (0.004)</td>
<td>4</td>
<td>0.086a (0.022)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>0.0968a (0.032)</td>
<td>4</td>
<td>1.037b (0.419)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>0.0171a (0.001)</td>
<td>4</td>
<td>0.141ab (0.018)</td>
</tr>
</tbody>
</table>
Table 7. Xylem Cross Sectional Area, Phloem Cross Sectional Area Analysis of Variance

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>ANOVA</th>
<th>Xylem CSA</th>
<th>Sum of 4 largest vessel radii</th>
<th>Phloem CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>5.14</td>
<td><strong>0.050</strong></td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.80</td>
<td>0.394</td>
<td>10.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>2.44</td>
<td>0.152</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.38</td>
<td>0.312</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>5.63</td>
<td><strong>0.042</strong></td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>10.34</td>
<td><strong>0.011</strong></td>
<td>10.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.86</td>
<td>0.378</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.09</td>
<td>0.171</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>1.41</td>
<td>0.265</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>37.65</td>
<td><strong>0.000</strong></td>
<td>17.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.04</td>
<td>0.335</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.52</td>
<td>0.275</td>
<td>0.61</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>5.16</td>
<td><strong>0.049</strong></td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.08</td>
<td>0.789</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>3.88</td>
<td>0.080</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.86</td>
<td>0.495</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>5.35</td>
<td><strong>0.046</strong></td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>2.37</td>
<td>0.158</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.05</td>
<td>0.832</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.10</td>
<td>0.398</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>2.85</td>
<td>0.126</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>25.09</td>
<td><strong>0.001</strong></td>
<td>8.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.01</td>
<td>0.943</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.21</td>
<td>0.888</td>
<td>0.85</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.00</td>
<td>1.000</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>1.19</td>
<td>0.304</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.72</td>
<td>0.418</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.40</td>
<td>0.306</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.16</td>
<td>0.701</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>5.15</td>
<td><strong>0.053</strong></td>
<td>3.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>3.14</td>
<td>0.114</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.09</td>
<td>0.409</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.58</td>
<td>0.477</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>5.53</td>
<td><strong>0.057</strong></td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.11</td>
<td>0.749</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.43</td>
<td>0.324</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 8. Xylem Cross Sectional Area, Phloem Cross Sectional Area Descriptive Statistics

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>Genotype P Treatment</th>
<th>Xylem CSA (µm²)</th>
<th>Sum of 4 largest vessel radii^4 (µm)</th>
<th>Phloem CSA (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Error</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Error</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Standard Error</td>
<td>n</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>29.654 a</td>
<td>(4.644)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>37.011 a b</td>
<td>(8.993)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>89.215 b</td>
<td>(20.373)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>48.235 ab</td>
<td>(15.057)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>116.066 ab</td>
<td>(32.670)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>30.189 a</td>
<td>(5.692)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>146.051 b</td>
<td>(30.003)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>98.584 a</td>
<td>(13.758)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>1,290.296 b</td>
<td>(645.493)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>29.818 a</td>
<td>(8.854)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>1,192.500 b</td>
<td>(395.422)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>81.209 a</td>
<td>(16.945)</td>
<td>4</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>12.232 a</td>
<td>(450)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>16.238 a</td>
<td>(1.275)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>26.357 a</td>
<td>(6.677)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>18.683 a</td>
<td>(3.494)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>19.601 a</td>
<td>(3.328)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>14.424 a</td>
<td>(925)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>41.790 a</td>
<td>(17.950)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>20.684 a</td>
<td>(2.772)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>56.163 bc</td>
<td>(18.421)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>13.241 a</td>
<td>(4.367)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>83.678 c</td>
<td>(13.001)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>20.103 ab</td>
<td>(4.153)</td>
<td>4</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>3.178 a</td>
<td>(380)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>3.835 a</td>
<td>(323)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>3.465 a</td>
<td>(487)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>3.547 a</td>
<td>(138)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>3.124 a</td>
<td>(190)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>1.805 a</td>
<td>(113)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>2.611 a</td>
<td>(553)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>2.114 a</td>
<td>(108)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>4.122 a</td>
<td>(1.895)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>1.654 a</td>
<td>(983)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td>6.025 a</td>
<td>(2.080)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td>1.332 a</td>
<td>(435)</td>
<td>4</td>
</tr>
</tbody>
</table>

^4: Significance codes: a, b, c, d, e, f (within a row indicate significant difference)
Table 9. Respiration Rate Analysis of Variance

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest Date</th>
<th>ANOVA</th>
<th>Resp/DW</th>
<th>Resp/Length (log)</th>
<th>Resp/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>5.02</td>
<td>0.052</td>
<td>38.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>1.05</td>
<td>0.331</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>3.05</td>
<td>0.115</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.70</td>
<td>0.574</td>
<td>7.63</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>3.49</td>
<td>0.095</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.17</td>
<td>0.689</td>
<td>66.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.00</td>
<td>0.344</td>
<td>7.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>2.79</td>
<td>0.102</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.99</td>
<td>0.347</td>
<td>6.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>30.04</td>
<td>0.000</td>
<td>232.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>41.37</td>
<td>0.000</td>
<td>11.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>5.99</td>
<td>0.016</td>
<td>1.07</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>1.71</td>
<td>0.224</td>
<td>10.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.25</td>
<td>0.627</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.02</td>
<td>0.889</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.43</td>
<td>0.734</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.28</td>
<td>0.611</td>
<td>12.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>15.21</td>
<td>0.004</td>
<td>22.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.90</td>
<td>0.369</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>7.57</td>
<td>0.008</td>
<td>15.16</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.04</td>
<td>0.849</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>2.77</td>
<td>0.131</td>
<td>30.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.27</td>
<td>0.614</td>
<td>5.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.60</td>
<td>0.258</td>
<td>0.44</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>Genotype</td>
<td>0.08</td>
<td>0.780</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.17</td>
<td>0.693</td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.13</td>
<td>0.729</td>
<td>6.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.21</td>
<td>0.886</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>Genotype</td>
<td>0.08</td>
<td>0.783</td>
<td>4.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>1.52</td>
<td>0.253</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>0.07</td>
<td>0.791</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>1.84</td>
<td>0.217</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>Genotype</td>
<td>0.03</td>
<td>0.876</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P Trt</td>
<td>0.00</td>
<td>0.996</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geno * P Trt</td>
<td>1.13</td>
<td>0.316</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rep</td>
<td>0.59</td>
<td>0.636</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Table 10. Respiration Rate Descriptive Statistics

<table>
<thead>
<tr>
<th>Segment</th>
<th>Harvest</th>
<th>Genotype</th>
<th>P Treatment</th>
<th>Resp/DW (µmolO₂/g/min)</th>
<th>Resp/Length (µmolO₂/cm/min)</th>
<th>Resp/Volume (µmolO₂/cm²/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td></td>
<td></td>
<td>Mean Standard Error n</td>
<td>Mean Standard Error n</td>
<td>Mean Standard Error n</td>
</tr>
<tr>
<td>Basal</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.5559a (0.037) 4</td>
<td>0.2356a (0.014) 4</td>
<td>138.55a (7.95) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.5317a (0.034) 4</td>
<td>0.2182a (0.032) 4</td>
<td>139.20a (12.50) 4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.4217a (0.029) 4</td>
<td>0.3518b (0.031) 4</td>
<td>137.50a (18.20) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.5151a (0.029) 4</td>
<td>0.3811b (0.063) 4</td>
<td>172.00a (15.70) 4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.3331a (0.053) 4</td>
<td>0.5320c (0.065) 4</td>
<td>135.30a (17.80) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.3140a (0.053) 4</td>
<td>0.1588a (0.020) 4</td>
<td>95.20a (12.10) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td></td>
<td>0.2422a (0.032) 4</td>
<td>0.4512bc (0.010) 4</td>
<td>103.50a (11.60) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td></td>
<td>0.2761a (0.015) 4</td>
<td>0.2674ab (0.016) 4</td>
<td>99.56a (9.05) 4</td>
</tr>
<tr>
<td>Middle</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.1556a (0.026) 4</td>
<td>0.9750c (0.111) 4</td>
<td>89.70ab (10.20) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.2937c (0.011) 4</td>
<td>0.1392a (0.008) 4</td>
<td>90.60ab (5.41) 4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.2187b (0.006) 4</td>
<td>0.8849c (0.090) 4</td>
<td>111.88b (6.57) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.2076b (0.019) 4</td>
<td>0.2615b (0.032) 4</td>
<td>80.78a (8.80) 4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.5608a (0.045) 4</td>
<td>0.1872a (0.013) 4</td>
<td>119.92a (9.89) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.5811a (0.056) 4</td>
<td>0.1831a (0.020) 4</td>
<td>113.32a (7.14) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td></td>
<td>0.6266a (0.047) 4</td>
<td>0.2353a (0.011) 4</td>
<td>150.40a (32.40) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td></td>
<td>0.6632a (0.061) 4</td>
<td>0.2817a (0.058) 4</td>
<td>152.10a (15.00) 4</td>
</tr>
<tr>
<td>Apical</td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.4392b (0.077) 4</td>
<td>0.1648bc (0.031) 4</td>
<td>111.80b (16.80) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.2843a (0.040) 4</td>
<td>0.1053a (0.017) 4</td>
<td>70.00a (14.80) 4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.3921ab (0.037) 4</td>
<td>0.2165c (0.035) 4</td>
<td>108.49b (9.33) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.2977a (0.043) 4</td>
<td>0.1490ab (0.027) 4</td>
<td>71.40a (8.86) 4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>0.3996a (0.052) 4</td>
<td>0.2925b (0.049) 4</td>
<td>135.60c (15.10) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.3539a (0.053) 4</td>
<td>0.1053a (0.011) 4</td>
<td>67.04a (9.78) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td></td>
<td>0.4127a (0.024) 4</td>
<td>0.2533b (0.013) 4</td>
<td>122.82bc (4.21) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td></td>
<td>0.3252a (0.036) 4</td>
<td>0.1701ab (0.018) 4</td>
<td>80.60ab (10.20) 4</td>
</tr>
<tr>
<td></td>
<td>15 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>1.4259a (0.074) 4</td>
<td>0.3450a (0.017) 4</td>
<td>279.90ab (46.80) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>1.4199a (0.088) 4</td>
<td>0.3436a (0.005) 4</td>
<td>248.40ab (49.00) 4</td>
</tr>
<tr>
<td></td>
<td>27 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>1.4340a (0.126) 4</td>
<td>0.3639a (0.007) 4</td>
<td>357.80b (56.30) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>1.3440a (0.124) 4</td>
<td>0.2956a (0.019) 3</td>
<td>234.30a (28.20) 4</td>
</tr>
<tr>
<td></td>
<td>39 DAP</td>
<td>DOR 364, HP</td>
<td>G19833, HP</td>
<td>1.0800a (0.300) 4</td>
<td>0.2733a (0.078) 4</td>
<td>254.60a (75.30) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR 364, LP</td>
<td>G19833, LP</td>
<td>0.8840a (0.102) 4</td>
<td>0.1527a (0.024) 4</td>
<td>210.10a (34.60) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td></td>
<td>1.1940a (0.140) 4</td>
<td>0.4964a (0.069) 4</td>
<td>258.70a (19.30) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td></td>
<td>0.9050a (0.318) 3</td>
<td>0.3140a (0.135) 4</td>
<td>179.30a (39.80) 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, HP</td>
<td></td>
<td>0.6500a (0.163) 4</td>
<td>0.1470a (0.048) 4</td>
<td>171.90a (33.30) 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G19833, LP</td>
<td></td>
<td>0.7978a (0.078) 4</td>
<td>0.0856a (0.013) 4</td>
<td>172.80a (32.00) 4</td>
</tr>
</tbody>
</table>

38
CHAPTER 4. DISCUSSION

In this study we observed that in common bean, phosphorus availability influences secondary growth and there appears to be a genetic component to this trait. Basal roots grown under low P had root and stele cross sectional areas an average of 50% and 40% the size of those grown under high P by the final harvest date. The reduction in root and stele CSA under low P was not due to an allometric relationship with reduced total plant tissue mass under low P. Basal root segments under LP had only 30% the respiration per unit length as HP at 39 DAP. These observations support the hypothesis that root etiolation is a component of efficient phosphorus acquisition as proposed in Fan (Fan, Zhu et al. 2003). This phenomenon is analogous to shoot etiolation where shoots sacrifice girth for length in their search of light.

The influence of phosphorus on specific root length (SRL) and root to shoot ratios has been reported multiple times, especially in common bean. This study again confirmed increased SRL (g/m) and higher root: shoot under LP. Rather than report SRL for the entire basal root or root system as is typically done; it was measured at three distinct points along the basal root axis (basal, middle and apical segments). The segments used to measure SRL were short and lateral roots were removed, resulting in an explicit measure of SRL as opposed to when SRL is measured for an entire root system or entire class of roots. As expected, SRL increased for the segments along the root from the base to the tip. Consistent with other reported results, in this study low P-grown basal roots were less dense (g/cm³) (Hill, Simpson et al. 2006), were thinner (Hill, Simpson et al. 2006) and had less secondary development as measured in % stele area (Fan, Zhu et al. 2003) than their HP counterparts.

Basal roots are known to be an important component of P accumulation in bean (Beebe, Rojas-Pierce et al. 2006; Bonser, Lynch et al. 1996). A typical way to measure root exploration into new soil is through overall root length where neighboring roots could be competing for the same resources; however in this case the length of the major basal root axes were measured. As a major
root axis grows, it provides the framework for the initiation and elongation of new secondary (and likewise later order) lateral roots. At 15 DAP basal root axes of DOR 364 grown under low P were longer than the other genotype x P treatment combinations (Figure 7). By 27 DAP low P basal root axes under LP were significantly longer than those on the HP plants. Perhaps due to severe P-stress at 39 DAP, low P basal root axes did not continue to increase in length and axes under HP were significantly longer. Hill (Hill, Simpson et al. 2006) reported a similar growth response for entire root systems of 10 different species where root length was shortest at 0 applied P, longest at 4.5 or 10.5 mg P per pot, and then intermediate at higher levels of applied P. When correlating shoot tissue P with the basal root axis length under LP, axis length increased with decreasing tissue P while the axes were still elongating (15 and 27 DAP) (Figure 25). Meanwhile under HP, there was a slight, though not significant, positive correlation. This suggests that even before the plants appeared to be P-deficient (shoot DW, Figure 3) at 15 DAP, they contained less P in their shoots and were responding by sending out longer basal root axes. The correlation is significant at 15 DAP (adjusted $R^2 = 0.733$) and at 27 DAP (adjusted $R^2 = 0.565$), but not significant at 39 DAP, suggesting that as P-stress continues, there just are not enough resources for basal root axis elongation.

While low P basal root axes were elongating at a similar or greater rate than high P, developmental differences were becoming apparent inside the roots. With few exceptions, root cross sectional areas (CSA) were significantly smaller under low P than high P at 27 and 39 DAP, regardless of segment location. At later harvests, these thinner LP roots further varied from HP by generally having significantly smaller steles, smaller stele to root CSA ratios, smaller xylem CSA and xylem vessels. As illustrated in Figure 15 for basal and middle segment locations on the root, while the HP steles were proportionally growing, the LP steles proportionately remained about the same size. This suggests that low phosphorus availability delayed development and decreased secondary growth.
In addition to decreasing relative stele size, low P availability affected the development of xylem vessels with one late metaxylem vessel having the tendency to grow larger in cross sectional area than the rest. This developmental pattern was most apparent in the middle segments of the root axis but also observed at the final harvest date for DOR 364 under LP at all 3 segment locations. Although observed under both high and low P availability, it was both more striking and more common under LP. At 39 DAP when the plants were the most P-deprived the effect was only apparent under LP (and not HP), leading to the speculation that phosphorus does in fact play a role in this developmental pattern. One larger xylem vessel has great implications for water transport. Due to the Hagen-Poiseuille law (transport capacity of a pipe increases with the fourth power of the pipe radius), there is not a linear relationship between vessel cross sectional area and vessel transport capacity and as such many smaller vessels are required to transport the same volume of liquid as one large vessel. However, Pate (Pate, Jeschke et al. 1995) reported that vessel flow in woody Proteaceae species roots increased to a much lesser extent than the r^4-based increase. Wahl and Ryser (Wahl and Ryser 2000) suggest that a higher number of vessels with a relatively smaller diameter per vessel per root cross sectional area in slow-growing species might reflect a higher protection of vessel functioning. With a smaller volume: surface ratio, narrow vessels are associated with a lower probability for embolisms. Small vessel size is associated with grass species endemic to drought-prone habitats (Wahl and Ryser 2000; Wahl, Ryser et al. 2001). It is more efficient as far as transport capacity is concerned to increase vessel diameter rather than producing more vessels in one root or by producing more roots (McCully and Canny 1988). Wahl (Wahl, Ryser et al. 2001) suggests that at high nutrient supply plants have a relatively larger leaf area than at low fertility and thus a greater xylem cross sectional area can be understood in terms of a need for an increased transport capacity at high nutrient supply. Developing one larger vessel at the expense of several at the same time could be a classic case of putting all, or certainly most, of ones eggs in the same basket. A cavitation in the principle vessel through the middle of a basal root could pose problems for the plant. Further exportation is needed into the role of phosphorus in xylem vessel development.
One of the key suppositions of this study was that thinner roots with less secondary development would be “cheaper” to grow and maintain. Although root respiration per dry weight varied little regardless of P status, respiration measurements per unit length were higher under HP, suggesting that thicker roots have a higher respiratory “cost.” It is interesting to note that respiration per mass decreased over time and varied along the root, but there was little difference for phosphorus treatment and a negligible difference for genotype. However, in agreement with HP/LP anatomical differences over time and length of the root, P differences in respiration per length were most notable and consistent at 39 DAP and at the basal-most end of the basal root where LP had lower respiration per length than HP. Thus it appears that it is not so much the mass of different kinds of cells that affect respiration, but rather the root thickness per unit length that determines respiration (thicker, more developed roots respire more). This has great implications for a P-stressed plant as less carbon resources are being depleted for root secondary growth and development and more can be devoted to soil exploration.

While plants grown under HP are known to be larger than those deprived of phosphorus it is important to look at allometric relationships to see if the measured differences between the two P treatments were strictly based upon the slower growth of LP plants. Allometry is the relationship between the size of an organism and the size of any of its parts (Niklas 1994). In a comparison of shoot dry weight (DW), while all plants were about the same size at 15 DAP, HP plants increased 10 to 20 times in size whereas LP basically maintained the size of their shoots (Figure 3). Allometric relationships were compared by correlating of the inverse of total plant DW with the log or inverse of various root measurements and comparing the slopes of the regression lines. Different data transformations were necessary in order to assure similar variances in the data. Under LP the correlations were poor regardless of genotype, root measurement or root segment location (basal, middle, apical). However, in many cases under HP and specifically at the basal end there was a strong relationship between total tissue DW and the different root measurements taken in this study. For example using total tissue DW as a predictor for root cross sectional area
(inverse transformation used for both variables), there was a strong correlation under HP for basal segments with greater root CSA with larger shoot size (Figure 16). Conversely, there was no significant relationship between total tissue DW and root CSA under low P. As there was only one regression line slope (HP), there can be no comparison of regression line slopes. A similar finding resulted from the comparison of the inverse of total tissue DW with the inverse of stele CSA (Figure 17). These graphs illustrate that the difference in measured values between P-deprived and P-sufficient plants was not just due to their differences in plant size. Basal roots grown without sufficient P actually have less secondary development then those grown with sufficient P even when taking into account the differences in plant development and overall size.

Although thinner roots may be more efficient at locating limited, immobile soil nutrients, they also appear to have negative consequences. If thinner roots are always better than thick roots, then one could assume that all plants would have thin roots. Eissenstat (Eissenstat 1992) discussed costs and benefits of constructing roots of small diameter, noting that thicker roots may be more reliant on mycorrhizae and longer lived than thinner roots. Just as etiolated shoots have less strength, etiolated roots could also be weak. Thinner roots could be less successful under rocky conditions as they have less strength. With increased surface area per number of cells, they could be more susceptible to attack by pathogens. Etiolated roots may be shorter lived (Eissenstat 1992). Root sections with one principle xylem vessel would be more vulnerable to cavitations than roots with several large, developed xylem vessels. Thus greater efficiency in P foraging could come at an overall cost to the plant.

While both common bean genotypes studied displayed an etiolation response to a low P root environment, some differences emerged between them, suggesting a genetic component to root etiolation that has implications for plant breeding. Although DOR 364 has traditionally been labeled P inefficient and G19833 P efficient (Beebe, Rojas-Pierce et al. 2006; Lynch and Beebe 1995; Nielsen, Eshel et al. 2001), it is reasonable that an improved variety like DOR 364 could have some traits that are favorable under low P conditions (Beebe, Rojas-Pierce et al. 2006). Alternatively, as a
P-inefficient genotype, DOR 364 might have been under more severe stress. Although in many cases DOR 364 and G19833 had similar values for a variety of measurements under HP, DOR 364 had more extreme values than G19833 under LP for most measurements taken (SRL, density, root CSA, stele CSA, xylem CSA, sum of 4 largest xylem vessel radii to the fourth power, and respiration per unit length). Of particular note, P-deprived DOR 364 had the lowest respiration per unit length all along the root axis and at all three harvest dates (Figure 23). DOR 364 exhibited more plasticity for the root etiolation than G19833, exhibiting the trait under LP but not under HP. This is not a genotype that always has thin roots; rather it is a trait that is expressed when the environmental conditions dictate. In a study of quantitative trait loci (QTL) for root architectural traits using the same bean genotypes (Beebe, Rojas-Pierce et al. 2006), contrasting genotypic effects on specific root length were observed. It was suggested that greater specific root length contributed to P accumulation in G19833, but that DOR 364 also contributed to greater SRL by a different mechanism. It was suggested that DOR 364 tends to express greater P accumulation per unit root length than G19833. DOR 364 could be achieving that through increased root etiolation. Recombinant inbred lines (RILs) produced by crossing DOR 364 and G19833 exist. Additional study using RILs in addition to DOR 364 and G19833 parents could increase our understanding of a genotypic component to root etiolation.

At the apical end, there was a consistent and significant genotype effect for stele: root area with DOR 364 remaining larger than G19833 regardless of P treatment. Could there be some value in developing the tip faster under LP? It is interesting to note that the smallest ratios at the apical end belonged to G19833 under HP (in stark contrast to the basal end where both genotypes at HP were much larger than LP and where G19833 had a higher ratio than DOR 364). The size of the xylem vessel elements at the tip may be a key to understanding apical development under low P (Figure 26). Under LP stress at 39 days after planting, DOR 364 had 1 large late metaxylem vessel while G19833 had 4 of a similarly small size. This could be further studied as it was neither apparent at 15 nor 27 DAP where both genotypes had vessels of similar size at LP. Hand sectioning at the
apical end was the most difficult, resulting in poorer quality images and consequently measurements from the images. Furthermore it is the region with the most developmental changes within the shortest distance. Additional investigation into the role of phosphorus on the development of the root apex with improved sectioning technique could yield interesting results.

In conclusion, we have shown that longer, thinner, less developed basal roots occur as a response to low phosphorus in bean and that there appears to be a genetic component to this phenomenon which also reduces the carbon cost of soil exploration. This should be useful to plants growing in soils of suboptimal P. This “root etiolation” is analogous to how shoots etiolate in response to low light conditions and can be considered an adaptive trait by reducing the metabolic costs of root expansion into soil less depleted in phosphorus. Genotypic variation in this response suggests that root etiolation may be a useful trait for use in plant breeding programs for low fertility soils. It would be interesting to investigate the presence of root etiolation in bean genotypes traditionally used under the Costa Rican slash/mulch “frijol tapado” system where seeds are broadcast into weeds that are subsequently cut down leaving a mulch for the bean plants to grow up through. The high organic content and assumed low soil bulk density could make it easier for thinner roots to be successful and could thus be an interesting source for genetic material with greater tendency to etiolate. Other root types (primary, adventitious, lateral) could also be studied for the presence of this trait as its advantages would not be restricted to basal roots.
Figure 25. Basal root axis length significantly correlates with shoot tissue P under low P at 15 (p = 0.004) and 27 (p = 0.019) days after planting. This suggests that while the basal root axes are still actively elongating under LP (see Figure 7), lower levels of P in the shoot predict longer basal root axes (adjusted $R^2 = .733$ at 15 DAP, adjusted $R^2 = 0.565$ at 27 DAP, genotypes pooled, n = 8). Measures do not correlate at 39 DAP or under HP.
Figure 26. Cross sectional area of 4 largest xylem vessels (μm²) show the interesting developmental trend for 1 late metaxylem vessel to develop before the others. Although observed at both high P and low P and for both genotypes, at 39 days after planting it is consistently apparent in DOR 364 under low P. This deserves further investigation.
REFERENCES


