Introduction

Root hairs are extensions of trichoblasts, specific root epidermal cells, that enable plants to more effectively extract soil moisture and nutrients by increasing the effective surface area of plant roots. In addition, they aid in stabilization and actively alter the rhizosphere chemistry and biology (Hofer, 1996). Root hairs develop in the maturation zone of the root tip (Taiz and Zeiger, 2006). Individual root hair growth is rapid (1μm min^{-1}) (Grierson and Schiefelbein, 2002) and short lived (cytoplasmic disintegration 2 – 3 days, vital staining 1 – 3 weeks) (Johnson et al., 2001). In general, functional roots and root hairs are those in the most distal regions of the root system (Hofer, 1996) due to suberization (Taiz and Zeiger, 2006) of roots in the proximal region. Nutrient uptake per unit of root length declines with root age, suggesting that the older proximal root tissues lose function while younger root sections more actively take up nutrients and water (Gao et al., 1998). The dysfunction of roots in the proximal region is an essential survival mechanism to plants in nutrient limited environments. If roots in the proximal and distal regions were equally permeable to water in a water abundant, nutrient deficient environment, distal region roots would be "short circuited" and lose the ability to take up water and nutrients (Taiz and Zeiger, 2006). As roots remove nutrients from soil, nutrient depletion zones develop in the rhizosphere and distal region roots are responsible for seeking out nutrient rich soil regions (Taiz and Zeiger, 2006).

Since water and nutrient uptake is most active in the root hair zone where the root is most permeable (Segal et al., 2008a), there exists a need to observe root hairs to better understand their importance and function. In addition, characterization of species differences in root hairs may lead to development of more nutrient and water efficient crops and improved nutrient uptake and biophysical modeling. Although characterization of crop root systems has led to more efficient methods of nutrient management, since their discovery, interest in root hairs has outweighed the ability to observe them *in situ* leading to gross oversimplification of their importance. Early nutrient uptake models did not include root hairs perhaps because quantification was not possible (Claassen and Barber, 1976), but the role of root hairs in nutrient uptake was recognized early on due to disagreement between predicted and actual nutrient uptake and was later included in nutrient uptake quantification (Itoh and Barber, 1983). However, as stated in Roose and Schnepf (2008), disagreement between predicted and actual uptake does is not always due to absence of a root hair parameter in the model. Recent advances in modeling allow inclusion of multiple parameters associated with root hairs in nutrient uptake studies (Ma et al., 2001b). The elusive challenge is to capture the dynamic nature of root hairs owing to the soil environment while reducing the mathematical tedium (Roose and Schnepf, 2008).

Early methods of root hair measurement involved excavating root hairs grown in field soil or culturing root hairs in laboratories, followed by light or electron microscopy. Excavation of root hairs likely results in damage to root hairs and erroneous measurement especially when quantifying root hair density (RHD). Observing and measuring root hairs using light and florescent microscopy is tedious due to the 3-dimensional nature of root hairs and their single focal plane (excluding confocal microscopy). Scanning electron microscopy requires dehydration/fixation with glutaraldehyde, formaldehyde, paraformaldehyde, and/or osmium tetrachloride, that distort root hair shape and orientation. Additionally, root hairs produced from solution culture and/or artificial growth media may not reveal *in situ* development characteristics (Mackay and Barber, 1984; Ma et al., 2001a; Gahoonia and Nielsen, 2003). Rhizotrons, mini

rhizotrons, and magnetic resonance imaging (MRI) devices are cost prohibitive in many cases and may not be capable of resolving root hairs (Pan, et al. 1998; Segal et al., 2008b). Inexpensive and accurate means of observing the rhizosphere are needed to properly determine the effective role of plant root hairs in the soil environment.

High resolution desktop scanners are capable of yielding high quality *in situ* root images at low cost (less than $150 per scanner), and may provide insight to soil environmental effects on root development and nutrient uptake efficiency. The objectives of the present research are to 1) evaluate the capability of high resolution scanners to distinguish crop species differences in root hair morphology, and 2) determine if crop species differ in root hair responses to soil water availability.

Materials and Methods

Ritzville silt loam (Coarse-silty, mixed, superactive, mesic Calcidic Haploxerolls) was collected, sieved to pass a 20 mm mesh screen, wetted with tap water to 0.11 or 0.20 g H_2O g $soil^{-1}$, homogenized and placed in 36 x 34 x 23.5 cm Rubbermaid® oblong plastic containers with Canon® CanoScan LiDE 600 F flatbed scanners (less than $150 per scanner) that had been sealed with DAP ® silicone rubber sealant. Two scanners were placed in each plastic container and slightly sloped to ensure primary axial root interception with scanning surfaces would occur (Figure 1 diagram). Soil was packed to a bulk density of 1.0 to 1.2 g cm^{-3} with an average of 1.1 g cm^{-3} for all treatments. Thus volumetric water was determined to be 0.12 and 0.22 cm^3 H_2O cm^{-3} soil. Soil water potential was determined for the two levels of water using a True Cell® 5 bar pressure plate to be -63 and -188 kPa for 0.20 g H_2O g $soil^{-1}$and 0.11 g H_2O g $soil^{-1}$ treatments, respectively.

The experiment was arranged in a randomized complete block, split plot factorial design with treatments that consisted of four crop species, canola (*Brassica napus*), camelina (*Camelina sativa*), flax (*Linum usitatissimum*), and lentil (*Lens culinaris*), at two levels of water availability (-63 and -188 kPa), and four replicates. The experiment was conducted twice, each time with two replicates with a total of four replicates. Each scanner represents an experimental unit or subplot, each plastic container holds two scanners and two species, each group of two plastic containers (four species at one water availability level) represents a plot, and each replicate represents a block where variation in replicates is assumed additive thus variation between experiments is also assumed additive. Seeds were germinated under moist paper towels and grown without the addition of water throughout the remainder of the 9-day experiment. Plants were grown under artificial lighting (400 μmol m^{-2} s^{-1}) with a 12-hour photoperiod and 420, 490, 540, 590, and 620 nm as the most intense wavelengths.

Rhizospheres were scanned daily at 4800 dots per inch (dpi) and image files were transferred onto a laptop portable PC with Canon software. Images with visible primary axial root tips were utilized to measure maximum root hair length (RHL) and density using Adobe® Photoshop ® CS3 Extended. Root hair lengths were estimated maximum distance from the base of the root hair to the terminus without regard to root hair tortuousity, although tortuous root hairs were uncommon. Root hair density represents the total number of root hairs 360 degrees around a primary axial root over approximately 1 mm of primary axial root length. It is assumed that 180 degrees or ½ of root hairs will grow parallel to the scanning surface and be scanned due to restriction of root hair growth toward the scanner. The remaining 180 degrees or ½ of root

hairs would not be scanned due to soil interference. Therefore, estimation of RHD was accomplished by counting root hairs on one side of primary axial roots and assumed to represent ¼ of root hairs per unit length of primary axial root. Each data point represents an average of nine observations for each experimental unit. Root surface area (RSA) was calculated for each data point as the primary axial root surface area plus the root hair surface area over 1 cm of primary axial root. Primary axial roots and root hairs were assumed to be cylinders where primary axial root length was 1 cm and RHL was equal to the product of observed RHL and RHD. Root and root hair radii were averages of each species.

Violations in the general linear model assumptions of constant and normally distributed error variance were present for RHD and RSA; therefore response variables were transformed using Box-Cox exponential transformation. After transformation, analysis of variance was performed using SAS statistical software with the Proc GLM Contrast and Proc Mixed procedures (SAS Institute, 1991).

The Richards growth model was used to fit root hair growth due to the sigmoidal appearance of the root hair geometry (RHG). The Richards model is as follows:

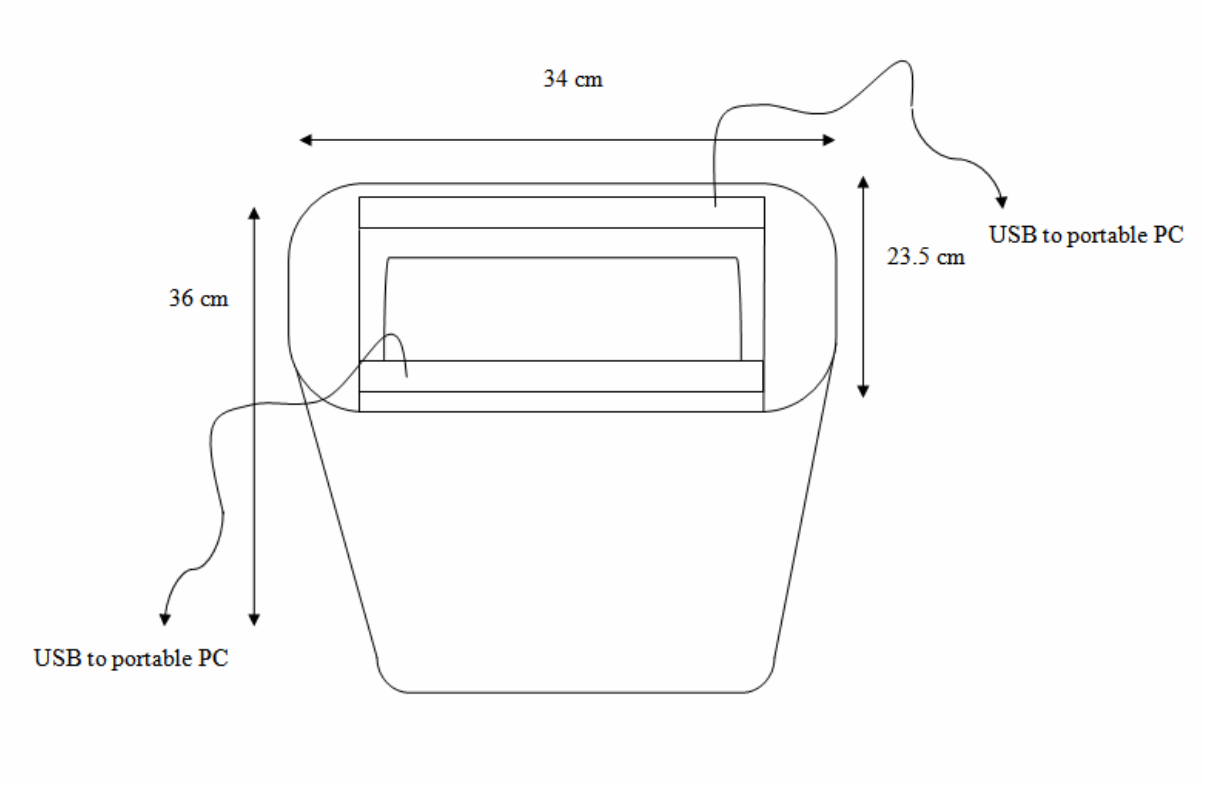
$$Y = A / (1 + B * e^{(-C * X_1)})^{1/D}$$

where:
Y – RHL
X_1 – Distance from root tip
A – Theoretical Maximum RHL
B – Growth Lag
C – Slope factor
D – Growth Lag

Determination of A, B, C, and D was performed using least squares estimates using Proc NLIN (SAS Institute, 1991). The Richards model was validated by comparing parameter A with actual measured maximum RHL (Table 2).

By utilizing existing personal computers and open source image analysis software, supplies, not including labor, for this method of image acquisition and analysis could be obtained for approximately $2400 (the cost of 16 scanners).

Figure 1: Diagram of scanners in plastic containers. Each scanner represents one experimental unit.



Results and Discussion

High resolution images were obtained (Figure 2 photo) and successfully utilized to estimate maximum RHL and RHD. Overall, lower moisture decreased RHL, RHD, and RSA ($p < 0.05$) (Table 1). Also noteworthy is the difference among species for RHL, RHD ($p < 0.0001$) and RSA ($P<0.001$) (Table 1). Whereas canola and camelina had the greatest RHL, they also had the lowest RHD. We found a slight inverse relationship between RHL and RHD ($R^2 = 0.33$), however, previous studies revealed almost no relationship (Itoh and Barber, 1983; Schweiger et al., 1995). Root surface area has been found to respond differently to water availability. Meisner and Karnok (1992) with peanuts (*Arachis hypogaea*) and Metcalf et al. (2008) in a rainforest system found results similar to this study, however, RSA response to water availability is not clear in other forest systems (Joslin et al., 2000). Root surface area differences due to species was striking and showed no trend with that of RHL or RHD. Lentil had the greatest RSA followed by canola and camelina then flax. Further investigation of effective RSA may be needed to determine the impact of overlapping zones of depletion in the rhizosphere.

Figure 2: High resolution (4800 dpi) images of (a) canola, (b) camelina, (c), flax, and (d) lentil root system including root hairs.

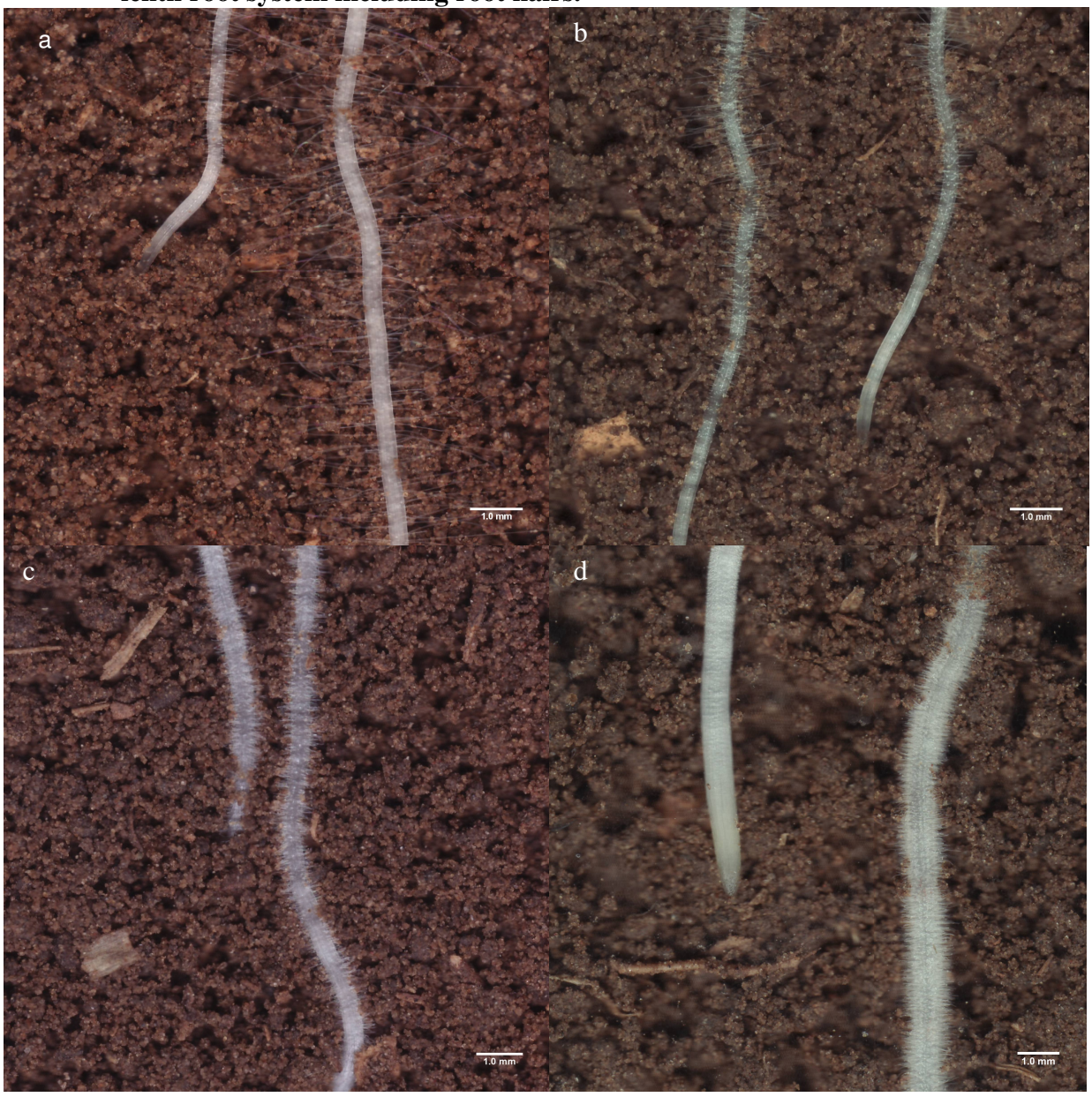


Table 1: Root hair length (RHL), root hair density (RHD), and root surface area (RSA) as affected by species and water potential.†

	RHL (μm)			RHD (root hairs mm^{-1})			RSA (cm^2 cm^{-1})		
Species	-188 kPa	-63 kPa	Mean	-188 kPa	-63 kPa	Mean	-188 kPa	-63 kPa	Mean
Canola	1007	1452	1230 a	132	157	144 a	1.38	2.27	1.76 bc
Camelina	607	1165	886 a	152	219	181 a	0.74	1.86	1.14 ab
Flax	191	224	207 b	328	328	328 b	0.79	0.94	0.86 a
Lentil	350	261	306 b	514	595	553 c	3.10	2.88	2.99 c
Mean	539 a	776 b		232 a	278 b		1.21 a	1.80 b	

ANOVA

Source of Variation	df		df		df	
Water (W)	1	*	1	*	1	*
Species (S)	3	****	3	****	3	***
W x S	3	NS	3	NS	3	NS

*, ***, **** Significant at the 0.05, 0.001 and 0.0001 probability levels, respectively.

NS Not significant at the 0.05 probability level.

† Within columns or row for each table section (RHL, RHD, or RSA), means followed by the same letter are not significantly different according to Tukey HSD (0.05).

Pairwise comparison contrast of available water effect on individual species showed no significant difference at the experimentwise error rate (α=0.05) for RHL, RHD, or RSA (Table 1); however, given the variability of RHD in lentil and RHL in canola and camelina, a larger controlled experiment may enable clearer detection of differences among species due to water availability.

Water availability had a distinct effect on RHG and distance from tip to root hair initiation zone, most notably in camelina (Figure 3). At high water availability, root hair elongation of all species developed in a sigmoidal pattern, whereas at low water availability the initiation zone shifted toward the root tip and the pattern was more hyperbolic. This effect was most evident in camelina, but present in all species. Previous studies have shown root hairs initiate at the end of the root elongation zone (Schneider et al., 1998), which illustrates the stunting of cell elongation owing to water stress in our findings. Root hair length near the initiation zone was more variable in low water, again most notably in camelina and less pronounced with other species. Root hair length variability decreased with increasing distance from the root tip for both water treatments, but the variability in length of canola root hairs was almost none at low water.

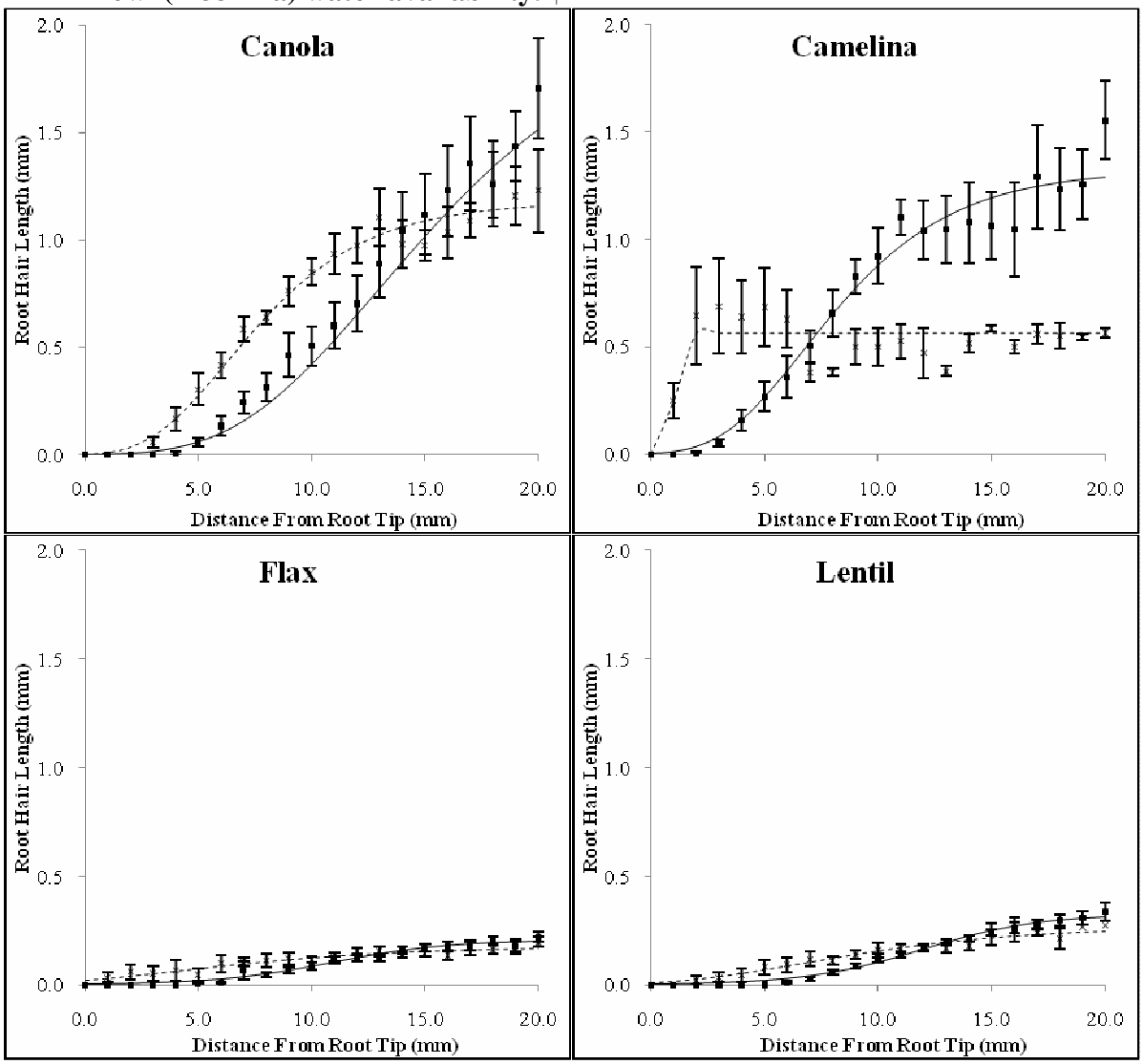
Table 2: Root hair growth fitted to the Richards model for canola, camelina, flax and lentil at high (-63 kPa) and low (-188 kPa) water availability.

Treatment	----------Coefficient----------				Sum of Squares	R^2
	A	B	C	D		
Canola (-63 kPa)	2.10	1.0E-04	0.16	1.4E-05	10.83	0.89
Canola (-188 kPa)	1.18	1.5E-05	0.29	2.4E-06	5.12	0.94
Camelina (-63 kPa)	1.32	4.8E-05	0.27	7.6E-06	12.04	0.89
Camelina (-188 kPa)	0.56	5.1E-02	6.50	9.5E-05	6.62	0.78
Flax (-63 kPa)	0.21	7.4E+01	0.39	1.0E+00	0.21	0.90
Flax (-188 kPa)	0.18	1.3E-05	0.18	5.9E-06	0.34	0.80
Lentil (-63 kPa)	0.33	1.1E+02	0.39	1.0E+00	0.21	0.95
Lentil (-188 kPa)	0.27	2.7E-05	0.19	7.7E-06	0.22	0.87

Previous laboratory experiments show RHL is influenced by water stress which is linked to abscisic acid (ABA) production (Bibikova and Gilroy, 2003). Others have concluded that root hair length, number, and geometry are influenced by soil environment, especially soil N and P, and plant species (Barber, 1995; Ma et al., 2001a; Ma et al., 2001b; Gahoonia and Nielsen, 2003). Furthemore, root hair development may be a survival mechanism in low fertility environments (Gahoonia et al., 2006). However, Mackay and Barber (1985) found that soil P affected root hair length to a lesser degree than did soil water in maize. Our findings support some of the earlier experiments, but further research is needed to elucidate the combined role of soil, water, and nutrients on root hair development and to determine how root hair development may affect drought tolerance. This improved method of *in situ* root-soil imaging has utility in many areas of soil science research including mycorrhizae and other soil mesofauna and flora

observation, root turnover and carbon storage which will enable better exploration of the soil environment.

Figure 3: Root hair geometry for canola, camelina, flax, and lentil at high (-63 kPa) and low (-188 kPa) water availability. †



† Solid lines and squares indicate high water availability (-63 kPa) and dashed lines and crosses indicate low water availability (-188 kPa). Lines represent least squares fit using the Richards growth model. Points and error bars represent mean and standard error of actual data, respectively.

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