EVALUATION OF SWITCHGRASS (*Panicum virgatum* L.) ROOT CHARACTERISTICS AS INFLUENCED BY ROW SPACING AND CULTIVAR

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CHAPTER 1

1.1 INTRODUCTION

Increase in carbon dioxide (CO_2) and greenhouse gas concentrations along with energy security emphasize the need for alternate transportation fuels from bioenergy feedstocks (Mc. Laughlin et al., 2002). However, using crop residues for the ethanol production is raising concerns as it results in competition for land (Blanco and Lal, 2007). Switchgrass (*Panicum viragtum* L.) is considered an important herbaceous bioenergy feedstock because of its high biomass yield potential, ability to grow on marginal land with poor soil quality (Wright and Turhollow, 2010). Furthermore, it can promote soil quality by enhancing soil organic carbon (SOC) (Frank et al., 2004; Liebig et al., 2005). Increased SOC helps to mitigate the emissions of anthropogenic CO_2 (Lal, 1997). Along with deep fibrous root system which extends to 3.3 m below soil surface (Ma et al., 2000), switchgrass also helps to improve soil and water quality by filtering pollutants like leached nutrients (N, P). Moreover, switchgrass has higher water and nitrogen use efficiencies and derives some additional N supply through fixation (Parrish et al., 2005). The deep and extensive roots of switchgrass can transfer C (carbon) into soil, which improves soil quality. The SOC content up to the depth of 30 cm was higher under switchgrass than under cultivated crops (Liebig et al., 2005). Even though the major source of soil C input is root biomass, the mechanisms underlying the changes of SOC and the availability of N are difficult to predict. Morphological parameters of individual root and the entire root system are influenced by genetic variability and environmental conditions, the parameters are potential indicators of the mineral nutrient uptake by plants on different soils. Parameters such as root length, average diameter, root weight, and surface area were used to determine quantity and functional size along with predicting responses to the environmental changes. Root length density (RLD) (cm cm^{-3} soil) (Majdi, 2000), Root weight density (RWD) (mg $cm^{-3)}$ (Leuschner et al., 2004) (Wood et al., 2000), average diameter (mm), specific root length (SRL) (cm mg^{-1}) (Ostonen et al., 2007) are important parameters in evaluating root water and nutrient uptake along with environmental changes.

Among crop management practices, row spacing and cultivar selection are the most basic criteria for optimizing biomass production. However, the effects of residue, environmental conditions, N fertilization, row spacing and cultivar differences on root biology are not fully understood. Since root biomass in bioenergy crops is a major source of soil C input, it is important to understand how management practices modify different root parameters. Therefore, different root characteristics of switchgrass need to be evaluated for sustainable production of bioenergy feedstock. The overall goal of this study is to evaluate different root characteristics and their distribution and their relationship to above ground biomass production.

Specific objectives of this research are:

(1) To evaluate switchgrass root characteristics as influenced by row spacing.

(2) To identify differences for root characteristics among switchgrass cultivars.

(3) To determine the relationship between switchgrass above ground biomass and belowground root biomass.

1.2 Literature Review

1.2.1. Soil organic carbon (SOC)

The concentration of atmospheric carbon dioxide (CO_2) has increased from 280 ppm in 1850 to a current 392 ppm (NOAA/ ESRL, 2012). The changes in concentration of the greenhouse gases (GHGs) including CO_2 in the atmosphere impact the energy balance and directly lead to climate change (IPCC, 2007). Mainly, C is found to be stored in five major areas like oceans (38400 Pg), geologic sites (4,130 Pg), pedologic sites (2,500 Pg), atmosphere (760 Pg) and biota (560 Pg) (Lal 2008). Soil (pedology) consists of both organic and inorganic C pools with each comprising of 1550 and 950 Pg, respectively (Batjes, 1996). The knowledge of C dynamic in soil is important to lessen emission of GHGs since terrestrial ecosystem is the largest of the C pools. Through photosynthesis process plants assimilate 120 Pg yr^{-1} of atmospheric C, of which half is returned to the atmosphere by plant respiration and other the remainder is retuned through soil respiration. Among the contributors for increase in atmospheric CO_2 concentrations, fossils fuels are consider as major contributor at the rate of 8.7 Pg C yr^{-1}. In addition, deforestation and erosion results in the C emission at the rate of 1.6 and 1.2 respectively (Lal, 2008).

The SOC is an alternative form for soil organic matter (SOM) which is an important determinant of soil quality. SOM is widely recognized for its role on soil biological factor (provision of substrate and nutrients for microbes), chemical (buffering and pH changes) and physical (stabilization of soil structure) properties. The C sequestration process is a bioprocess that can offset anthropogenic CO_2 emissions with numerous ecosystem positive aspects like supplying nutrients, buffering and soil pH, water movement and various soil processes (Lal, 1997; Nissen and Wander, 2004). Generally, SOC sequestration depends on inherent and dynamic soil properties. Inherent properties such as texture and depth and dynamic properties such as structure, mineralogy, tillage, and residue removal determine the process of SOC sequestration (Lal, 1997). However, the C in the system is mainly dependent on inputs like biomass-C and other influential factor like climate, partitioning among components and net production (Wood, 2000). The inherent and dynamic properties of soil determine the attainable levels of SOC sequestration between potential and actual level (Field et al., 2008).

1.2.2. Root derived C on SOC sequestration

Photosynthetically fixed C is generally found to be transferred to soils as plant litter, exudates from roots and in non- agricultural ecosystems the majority of C input is derived from roots (Gregory, 2006). The root:shoot ratio is very important to SOC pool due to higher contribution of roots to SOC cycle. Johnson et al., (2007) showed that above and below ground plant tissues helps in residue decomposition due to differences in chemical composition. The SOC was found to be 1.5 times greater when derived from roots than from shoots due to the presence of complex C compounds in roots (Balesdent and Balabane, 1996). The aliphatic compounds mainly resulting from roots are more resistant to degradation than those of shoots (Crow et al., 2009). Similarly, Rasse et al., (2005) found that mean residence time of C from roots to be 2.4 times greater than that derived from shoot. It was also showed that stability may be due to the presence of other mechanism like root distribution with depth, lower surface area for degradation reaction (aggregation) , chemical reactions with different metallic ions which helps to increase derived C and their protective measure. Similarly, root exudate (rhizodeposition) is another source of C resulting from root (Johnson et al., 2006). Rhizodeposits are consumed by microorganism that are finally converted into recalcitrant forms of C since it contains more decomposable compounds with short residing time (Tisdall, 1996; Kuzyakov, 2002; Gregory, 2006). Microbial process and rhizodeposits helps in aggregation in protecting SOC against decomposition. These indicate the need for additional research to understand the roles of various root characteristics which influence the nutrient translocation from root to shoot and root to soil.

1.2.3. N- mobilization

Nitrogen is the most limiting element in plant growth and development (Gruber and Galloway, 2008). Both NH_4^+ and NO_3^- are the forms of N available to plants through mineralization process of SOM from microbes, N source of fertilization or deposition (US DOE, 2008). Thus, application of N to the plant will enhance CO_2 fixation through the photosynthetic mechanism. N-alteration during SOM mineralization and Ctransformations is similar because of the elemental association of C:N compounds produced by plants and microbes (McGill and Cole, 1981). Thus, C and N are closely associated from photosynthesis to decomposition. High rate of atmospheric-N deposition and CO_2 emission is major a concern (Reay et al., 2008; Heimann and Reichstein, 2008; Vitousek et al, 1997). Response of SOC with elevated CO_2 and N and their interactions with other elements is yet to be known which ultimately makes it difficult to predict the impacts of plant productivity and SOC pools (Van Groenigen et al., 2006).

With the application of N- fertilizer, SOC concentration was found to be elevated (Johnson and Curtis, 2001; Christopher and Lal, 2007). Application of appropriate doses of N ultimately helps to increase SOC and soil N with incorporation of plant residue (Gregorich et al., 1996). Similarly, positive response to SOC was found with increase in plant residue followed with appropriate supply of N (Alvareze and Lavado, 1998).

In contrast to the linear relationship of SOC and N fertilization, Khan et al., (2007) reported that with the application of inorganic N fertilizer, SOC concentrations decreased. The concentration of SOC decreased most prominently in plots receiving heavy doses of fertilizer even though there is higher residue input with course of time. Khan et al., (2007) also concluded that heterotrophic decomposition of SOC along with the N addition results in declining SOC concentrations. Similarly, no linear response was observed in SOC and N application rates (Bradford et al., (2008). Within different rates of N- application that range from 0-100 kg N ha^{-1} yr^{-1}, the root derived sequestered C was found higher at 30 kg N ha^{-1} yr^{-1}. Bradford et al., (2008) concluded that application of higher N rates (100 kg N ha^{-1} yr^{-1}) will lower the mineral associated C and increase organic matter associated fraction.

In the photosynthesis mechanism, the enzyme used for C- fixation, Rubisco and other enzymes, account for more than 50% of total leaf N (Chapin et al., 2002). When N limits plant production, N application increases leaf N concentration leading to higher photosynthetic rates resulting in higher yield (Hyvonen et al., 2007). Thus addition of N in grassland enhances higher productivity and C input in soil (Baer and Blair, 2008). However, the response of belowground root biomass to the application of N is not apparent as found in above ground biomass. With the application of N, biomass partitioning can be altered in plants (Thornley, 1972). The partitioning theory states that plants distribute the fixed element to those organs which enhances the uptake of limiting elements. Thus, alleviation of nutrient deficiency through external input like N fertilization can encourage plant to provide large portion of C to aboveground parts in comparison to belowground root biomass. Altered biomass allocation or partitioning due to N- fertilization enhances biomass production along with coarse root (>2.5mm) but results in lower fine root production (Oren et al., 2001; Iivonen et al., 2006; Nilsson and Wiklund, 1995). Application of N fertilizer results in decrease of soil respiration which is attributed to reduced fine roots production (MacGill et al., 2004; Olsson et al., 2005). On the other hand higher N application results in faster turnover than lower production of fine roots (Raich and Nadelhoffer, 1989).

1.2.4. Switchgrass as Bioenergy feedstock

Biomass has been more recently considered as a renewable energy source in both developed and developing countries (Sagar and Kartha, 2007). The use of renewable biofuels is mandated to increase from 18 billion liters to 136 billion liters by the US's Energy Independence and Security Act of 2007 (Energy Independence and Security Act, 2007). As ethanol production from corn ethanol is reaching its blending wall, lignocellulosic ethanol production technology is being made more efficient. Increased proportion of CO_2, a greenhouse gas in the atmosphere can be reduced by enhancing the use of potential biofuels instead of fossil fuels and also be a source of income for farmers (Lemus and Lal, 2005; Liu et al., 2010). According to Scharlemann and Laurance (2008), non-food plants (switchgrass, trees, algae) can benefit environment compared to ethanol produced from food sources (corn, sugarcane). Switchgrass has been selected as potential bioenergy feedstock for its high biomass production and cost effective growth characteristics because of its low input high diversity (LIHD) (McLauglin, 1992; Bransby et al., 1998). Switchgrass has higher potential for C sequestration from atmosphere as well as improves soil quality via its root system (Wood et al., 1996). The CO_2 emission from the use of switchgrass as an energy crop is 1.9 kg C GJ^{-1} while from fossils fuels is 13.8, 22.3, and 24.6 for natural gas, petroleum and coal, respectively (Turhollow and Perlack, 1991). Switchgrass with its deep fibrous root system (Sladden et al., 1991) has major proportions of fine roots, main pathways of nutrients, have larger contact with volume of soil per unit root volume (McCully, 1999). Any increase in C sequestration by switchgrass will be due to increased root biomass rather than increased C concentration

(Ma et al., 2000). Deep root system of switchgrass enhances pollutant filtering and increase SOC (Sartori et al., 2006; Tolbert et al., 2002).

1.2.5. Characteristics of switchgrass

U.S. Department of Energy (DOE) selected switchgrass as a promising herbaceous energy crop by evaluating 34 different species (McLaughlin and Walsh, 1998; Bransby et al., 1998). Being perennial and native in its habitat, it is distributed in a wide range of environments and can be grown with minimal management on marginal land with poor growth conditions (Sanderson et al., 2006). Moreover, switchgrass has C_4 photosynthetic mechanism that entitles higher N and water use efficiency and sometimes results in N-fixation with bacteria.

There are two ecotypes of switchgrass, lowland and upland. Lowland ecotypes produce more biomass, are taller and with thicker stems than upland cultivars (Parrish and Fike, 2005). Upland cultivars are more drought tolerant than lowland cultivars. The average production of switchgrass above ground biomass ranged from 12-21 Mg ha^{-1} across 13 states in US (McLaughlin, 2005).

The deep and fibrous root system of switchgrass benefits in transferring C which improves soil quality (Ma et al., 2000 a). SOC distribution is found to be high over the entire soil profile under switchgrass plots than under cultivated lands (Liebig et al., 2005). Ma et al., (2000 b) reported that switchgrass root extend up to the depth of 3.3 m below soil surface with 65-80% of roots found mainly in the top 0.3 m. In addition, the establishment of switchgrass benefits in C mineralization, microbial biomass C, C turnover and other several soil properties (Wood et al., 2000).

Biofuels are not the final option for solving the problems like energy scarcity, C sequestration, but the sustainable production of biofuel could be an intermediate option to enhance renewable fuel sources. Therefore, basic necessity is a sustainable method of cultivation for all the changes associated with soil quality and higher production of bioenergy crops.

1.2.6. Root Characteristics

Root system is known to have a high degree of plasticity in response to different soil condition. Root architecture is a fundamental characteristic of plant production especially in an environment with limited availability of water and nutrient (Lynch, 1995). Higher root biomass and higher root to shoot ratio may be advantageous to plants in the acquisition of nutrients and water in any environment (Rogers et al., 1994). On the level of the individual root and the entire root system, various morphological parameters, which are influenced by genetic variability and environmental conditions, have been used as potential indicators of the mineral nutrients of plant on different soils. These parameters include root length, average diameter, root weight, and surface area that are used to determine quantity and functional size along with predicting responses to the environmental changes. Root length density (RLD) (cm cm^{-3} soil) (Majdi, 2000), root weight density (RWD) (Mg $cm^{-3)}$ (Leuschner et al., 2004) (Ma et al., 2000 b), average diameter (mm), specific root length (SRL) (cm mg^{-1}) (Ostonen et al., 2007) are important parameters in evaluating root pattern on crop water and nutrient uptake along with environmental changes. The RLD is often used to characterize the root system (Beshart et al., 2009) and is an important parameter in crop growth simulation and in evaluating consequences of root pattern on crop water and nutrient uptake (Zhuang et al., 2001). It is mainly influenced by crop genotype, growth phase, soil depth and water and nutrient availability (Robinson, 1994; Sattelmacher et al., 1993), elemental toxicity like with aluminum and manganese (Williams et al., 1984) and soil properties like structure, strength, bulk density and texture (Jones, 1983). Furthermore, RLD is modified by the crop response to environmental factors.

Similarly, RWD is an important parameter to observe the accumulation and translocation of nutrients (Ma et al., 2000). RWD is mainly influenced by nutrient availability, soil types and different cultivation practices (Ma et al., 2000 b). Variations in root biomass production in different soil type will be an important indicator for site selection and for C sequestration by switchgrass (Ma et al., 2000 b). SRL is the most frequently used parameter to measure fine roots since it characterizes the economic aspects of root systems and is an indicative of environmental changes. Fitter (1991) used length/ mass ratio as an index of root benefit to root cost where benefit is proportional to the resource acquisition and cost as construction and maintenance (Eissenstat, 1997). High SRL (long and thin root) is equivalent to thin leaves, are less expensive to produce (Withington et al., 2006). Fine root (<0.5mm-2mm) distribution is mainly responsible to for nutrient and water uptake while coarse root (>2mm) is responsible for the spread and stability of fine roots and for nutrient transport (Ostonen et al., 2007).

1.2.7. Root Measurement

Analysis of root parameters is always time consuming and often inaccurate due its fine distribution (Van Tienderen, 1990). There are various methods to determine root parameters. Line intersect method was first introduced by Newman (1966) and later it was modified by Marsh (1971) and Tennant (1975) in which roots are randomly dispersed over gridded surface, in which gridline intersections is counted and later converted into manual calculation. It has higher error probability since it doesn't account for overlapping and with same assumption of random distribution of root. Later, electronic methods were used for the image acquisition of root system like video camera (Ottman and Timm, 1984), Optical sensor (Arsenault et al., 1995). Minirhizotron observations is a non-destructive method but has disadvantage since it is a time consuming process of translating qualitative to quantitative data sets (Hendrick and Pregitzer, 1996). The image analysis system RHIZO is beneficial to use since it identifies area of root overlap and make corrections. It also provides figure of root length on the basis of diameter and provides different types of image like grayscale, skeleton type and other digital image with different resolution (Arsenault et al., 1995).

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CHAPTER 2

Evaluation of Switchgrass Root Characteristics as Influenced by Different Row Spacing

Abstract

Biofuels that replace fossil fuels have the potential to reduce greenhouse gases in the atmosphere. Switchgrass (*Panicum virgatum*), a dedicated energy crop, has higher potential for carbon sequestration as well as improves soil quality via its root system. Switchgrass cultivation practices like row spacing may differ in biomass production and sustainability due to differences in root distribution and biomass. However, information on switchgrass root growth and its distribution is extremely limited. The objective of this study was to analyze switchgrass root characteristics and their distribution over the entire soil profile (0-1.1 m). Measurement of root parameters was carried out with an image analysis system (winRHIZO) with grey level image type with 100 dpi resolution. Root length (RL) was found to be higher at narrow spacing (19.05 cm) by 24.86% at August, while it was in wider spacing (76.2 cm) at December by 26% at upper depth(0-0.1 m). However, root length density (RLD) was observed to be 42% higher at lower depths ranging from 0.2-1.1 m, under narrow spacing (19.05 cm), at the end of growing season. Root weight density (RWD) was significantly higher in December harvest than in August harvest over entire soil profile at higher spacing by 28.4%. Similar results were also

observed for average diameter. Greater increase in average diameter by 29%, 41% and 12.5% at upper depth in 19.05 cm, 38.1 cm and 76.2 cm row spacing, respectively, was recorded by the end of growing season. However, specific root length (SRL) was higher by 42% with 19.05 cm spacing than 76.2 cm spacing and highly correlated with average diameter (R^2=0.96). More than 75% of total root was of fine roots (0-2 mm diameter), and were more in lower spacing during growing season while higher in wider spacing at end of season. No any significant relation was observed in root biomass at different spacing practices but above ground biomass was significantly higher (15000 kg ha^{-1}) in 76.2 cm spacing than with narrow spacing (11000 kg ha^{-1}). Variation in switchgrass root characteristics owing to different cultivation practices will be an important determinant in C sequestration as well as in soil quality improvement.

2.1. Introduction:

Increased concentration of CO_2, a potent greenhouse gas, in the atmosphere can be reduced by replacing biofuels with fossil fuels. Switchgrass has high potential for C sequestration from atmosphere as well as improves soil quality via its root system (Ma et al., 1996 a). CO_2 emissions from the use of switchgrass as an energy crop is 1.9 kg C GJ^{-1} while from fossils fuels it is 13.8, 22.3, and 24.6 kg C GJ^{-1} for natural gas, petroleum and coal respectively, (Turhollow and Perlack, 1991). Switchgrass (*Panicum virgatum* L.) with its deep fibrous root system (Sladden et al., 1991) has major proportions of fine roots, the main pathways of nutrients which have larger contact with volume of soil per unit root volume (McCully, 1999). Any increase in C sequestration by switchgrass is due to increased root biomass rather than increased C concentration (Ma et al., 2000). Root systems are associated with high scale of agility in their development in response to heterogeneity of the soil. Plant root system is very crucial for above ground biomass, fluxes of energy, nutrients cycling, anchoring the plant in soil, and to absorb water and nutrients. On the level of the individual root and the entire root system, various morphological parameters, which are influenced by genetic variability and environmental conditions, have been used as potential indicators of the mineral nutrients of plant on different soils. These parameters include root length, average diameter, root weight, surface area, used to determine quantity and functional size along with predicting responses to the environmental changes. However, measuring all this parametres are tedious and time consuming. Root length density (RLD) (cm cm^{-3} soil) (Majdi, 2000), Root weight density (RWD) (Mg cm^{-3}) (Leuschner et al., 2004) (Wood et al., 2000), average diameter (mm), specific root length (SRL) (cm mg^{-1}) (Ostonen et al., 2007) are important parameters in evaluating root pattern on crop water and nutrient uptake along with environmental changes. Variations in growth and N demand may be influenced by plant competition for the nutrients and physiological basics and the root system size. Sanderson and Reed (2000) reported that plant increased in dry weight when plant row spacing is increased and also they found that plants with full root systems grown together or at close distance may remove more N. However, switchgrass root distribution pattern in different row spacing and different soil layers is yet unknown. The objective of my study was to evaluate different root characteristics with their distribution pattern in different row spacing and how their distribution pattern influences the above ground biomass.

2.2. Materials and Methods:

2.2.1. Field Setup:

This experiment was conducted at an experimental field in Stillwater (Agronomy Farm), Payne County, Oklahoma. According to the Field and Research Service Unit (FRSU) of Oklahoma Agriculture Experiment Station the soil type is Easpur loam (Fine-loamy, mixed, superactive, thermic Fluventic Haplustoll) with 0 to 1 percent slope and occasional flooding. Climatic variations from hot summer to cool weather and occasional drops due to cold surges, relatively uniform precipitation (peak in spring) and infrequent snowfall are major characteristics of Payne County (Henley et al., 1987).

Alamo, a cultivar of switchgrass was used in the study. The plant stands were established from seed in April 2009 with three different row spacing of 19.05, 38.1 and 76.2 cm where these row spacings were regarded as different treatments 1, 2, and 3, respectively. The experimental design was randomized complete block design with 3 replications, each replication being a large plot of 10 m wide x 15 m long with 20 m alleys in between two replications. No fertilizer was applied. The plots were maintained with no application of irrigation, herbicides and other minimum management practices. For the evaluation of root morphological characteristics, the root biomass was harvested at peak growth and after senescence, i.e. in the month of August (1st harvest) and December (2nd harvest) of 2010. Harvest implies the root sampling period.

The root biomass samples were taken from in-between row and on the row from east to west of each plot. The GPS coordinates and the layout of the field plots are shown in the Table 1.1.

2.2.2. Sample collection:

A tractor mounted hydraulically powered soil core sampler was used to collect the root samples from the depth up to 0.1m. The core sampler with a diameter of 74 mm was used to collect the root samples. After collecting cores, the samples were separated according to the soil depth layer that ranges from 0-1.1m. From each core, five sections were separated into 0-0.1 m, 0.1-0.2 m, 0.2-0.4 m, 0.4-0.8 m, and 0.8-1.1 m. Each depth sample was placed in a plastic bag with required label. The root samples were carried to cold storage ($4^{o}C$) so that the root samples in the bags remained fresh until washed.

The samples were carried out from the cold storage to the lab where samples were washed to get clean root samples. The soil attached to the root samples were washed with cold tap water. The sieve with small pore size (< 1 mm) was used to wash the samples so that fine roots can be collected without any loss. The washed root samples were transferred to clean plastic bags with required label and placed in the refrigerator until measurement. No separation was made between live and dead roots.

2.2.3 Measurement and analysis:

The root measurements were carried out to determine the modified root morphology due to row-spacing treatments. The image analysis system, WinRhizo software (ver.5.0.

Reagent Instruments, Quebec, Canada), which has the capability of analyzing images acquired from a flatbed scanner (LA 2400, Epson) was used to measure the root parameters. The root parameters measured include root length, average diameter, surface area, root volume and distribution of root length according to their diameter. Before measuring the samples, the system was calibrated with the resolution of 100 dpi (Dots per inch) in grey level image and roots were measured with the same resolution. The root length of the samples at each depth were collected in order to determine the root length density (RLD) (cm cm^{-3}).The RLD was calculated as root length divided by soil volume of specific depth.

RLD= RL/Vol. where, RL= Total Root length of specific depth and Vol. = Total soil volume of specific depth

Similarly, specific root length (SRL) (cm mg^{-1}) was calculated as total root length divided by root weight for each depth. After measurement of root samples, they were oven dried for 24 hours at 70^0C. Then, dry weight was taken in order to calculate root weight density (RWD) (mg cm^{-3}). The RWD was calculated as

$$RWD = RDW / (\pi * CR^2 * CL)$$

Where, RDW= Root dry weight, CR= Core radius, CL= Core length.

Similarly, Root mass (RM) in kg ha^{-1} was calculated as follows:

$$RM = RWD * CL * 100$$

Where, 100 is used as a conversion factor of area and mass.

Distribution of root length according to their diameter was expressed in percentage. The roots were classified on the basis of diameter classes as coarse roots (> 2mm diameter) and fine root (<2 mm diameter).

The GLM procedure using the SAS package release 9.2 (SAS Institute, 2002) was used to estimate for all the main and interaction effects on RL, RLD, RWD, average diameter, SRL and RM. The data were analyzed with two way ANOVA model to evaluate the treatment effect (row spacing) and harvesting time effect for each depth. Treatment differences were compared with LSD at probability (*P*) value at 0.05 level of significance.

2.3. Result and Discussion

2.3.1. Root Length:

Roots of switchgrass were found to extend up to 1.1 m below surface. Root length (RL) was found to be higher in the surface layer i.e. 0-0.1 m but consequently decreased with depth up to 1.1 m below surface in all row spacing (19.05, 38.1 and 76.2 cm). The impact of row spacing on RL is shown in Table 1.2, the interaction of row spacing was not significant ($P=0.05$) with harvesting period, but was significant ($P=0.05$) with soil depth. At peak growth stage (August-1st harvest) RL was higher in narrow spacing (19.05 cm) by 24.86% than wider spacing (76.2 cm) as shown in Figure 1.1. It can be attributed to higher competition for nutrients that produces more fine roots in the surface layers. Switchgrass with its fibrous root system has major proportions of fine roots, the main pathways of nutrients, and therefore has larger contact with volume of soil per unit root volume (McCully, 1999). Similar relationship was found at the end of growing season (December-2nd harvest), decreasing with depth. The RL increased by 26% with wider spacing at 2nd harvest when compared to 1st harvest. In contrast, 24.7% decrease in RL was recorded at the depth of 0-0.1 m in narrow spacing (Figure 1.1). So in narrow spacing roots continue to elongate deeper as fine roots at surface degrade. Wider row spacing has enough exploration volume so it explores the surface layers rather than deeper layers. At lower depth (0.8-1.1 m) RL increased by 50% in narrow spacing but at higher spacing (76.2 cm) no change was recorded.

2.3.2. Root Length Density:

The distribution of roots in soil is necessary to ascertain their effect on water and nutrient uptake by plants which could be achieved by the analysis of root length density (RLD). Root length per unit soil volume i.e. RLD was found to be higher at the upper soil depth in all spacings and was significant *(P=0.05)* with soil depth. However, no significant interaction was observed with harvest frequency and row spacing. Similar observations were made by De Silva (1998). RLD was higher by 24% in narrow spacing (19.05 cm) at upper depth (0-0.1 m) compared to wider spacing RLD. About 83% of total RLD was found to be concentrated in the top 0.2 m depth (Figure 1.2). During the growing season nutrient and water absorption is mainly carried out from the surface layers. However, at 2nd harvest RLD was higher by 24.7% in wider row spacing at upper depth. At lower depths, (0.2-1.1 m) lower spacing resulted in a higher RLD of 42% compared with wider row spacing. It may be due to the fact that RLD is mainly affected by root exploration space, as less space creates more competition for uptake of nutrients and water so that fine roots are more at upper depth. Increase in RLD results in high nutrient uptake due to increased exploration of soil by the roots (Barber and Silberbush, 1984). But at the end of the growing season roots use available resources for elongation of roots rather than accumulation of nutrients. At soil depth of 0.8 to 1.1 m RLD was higher by 49% in 2nd harvest than 1st harvest at lower spacing but was equal at higher spacing at both harvests. In general, RLD was found to be higher during the growing season rather than at the end of the season due to the flushes of new roots occur in spring which is similar to the observations of Atkinson (1980).

2.3.3. Root Dry Weight:

Impact by different row spacing on root dry weight is shown in Table 1.4. Higher row spacing has higher root weight at the upper surface i.e.0-0.2 m but with increasing depth lower spacing had higher root weight. Root weight is significantly correlated with harvest frequency and soil depth (Table 1.4) but no such relationship was observed with row spacing (*P=0.05*). As expected, root weight declined with soil depth as observed by Mengel (1974). Root weight was found to be higher in row spacing 76.2 cm by 28.4% than 19.05 cm spacing at upper depth. From the depth of 0.1-0.2 m the root weight decreased sharply and continued to decrease with depth. While considering harvesting frequency, root weight was found to be higher with wider row spacing in both harvests at surface. Root weight was significantly higher in 2^{nd} harvest than in 1^{st} harvest in all soil profiles (Figure 1.3). It implies that higher root exploration volume will result in higher root weight density which is related to nutrient and water absorption by root. But here, with decrease of root length density in 2^{nd} harvest at narrow spacing it resulted in the increasing root weight at the same depth. It implies that with less exploration volume, it will degrade fine roots which are mainly used for nutrient absorption in the form of carbohydrate or protein at the end of growing season and it mainly results in the accumulation of nutrients in the upper depths while an elongation of roots rather than accumulation is found in lower profile. This finding is similar to the result of Ma et al., (2000 b), which showed wider spacing has higher RWD in which they assume exploration volume is directly related with RWD and presence of fine roots (<2.0mm diameter) is inversely related with RWD (Atkinson 1998).

2.3.4. Average Diameter:

The evaluation of average diameter at different row spacing is shown on Table 1.5. Average diameter was significant with harvest frequency and soil depth but did not differ significantly (*P=0.05)* between different row spacing. Though we observed RLD was higher in narrow spacing but the average diameter was higher in wider spacing by 28.57% at upper depth. Higher average diameter was observed in wider row spacing in the entire soil profile. By the end of growing season (December) there is more increase in average diameter by 29%, 41% and 12.5% at the surface in 19.05 cm, 38.1 cm and 76.2 cm row spacing, respectively (Figure 1.4) compared to 1st harvest i.e. in August harvest. At the end of growing season, RLD decreased at narrow spacing which implies that loss of fine roots and decrease in accumulation and storage of nutrients but with high exploration volume it can produce more roots which results in more RLD and less increase in average diameter.

2.3.5. Specific Root Length

Specific root length at different row spacing over the entire soil profile is shown in Table 1.6. Higher root length and lower root weight will results in higher SRL and vice versa. Narrow spacing has higher SRL at upper depth by as much as 41% compared to wider spacing. It may be due to the presence of higher fine root (<0.5mm) fraction, which increases RLD but lowers RWD. In 2nd harvest SRL was found to be reduced over entire soil profile compared with 1st harvest. This is due to increased root weight and simultaneous decrease in root length. As we found that RLD was 49% higher at lower depth i.e.0.8-1.1 m in 2nd harvest which implies that there is simultaneous increase in root length and root weight so there is 29% decrease of SRL compared to 1^{st} harvest and greatly correlated with average diameter (R^2=0.96) (Figure 1.6). Similar results were evident in other studies (Bartsch, 1987; Leuschner et al., 2004; Ostonen et al., 2007) which showed that average diameter and root distribution are highly correlated with SRL.

2.3.6. Root distribution according to the diameter:

Root distribution (%) according to the diameter over the soil profile at different row spacing and harvesting frequency is shown in Figure 1.6. In 1^{st} harvest (peak growth, August) fine roots (0-0.5 mm) were highly distributed in the upper depth in narrow spacing but low in wider spacing i.e. <50% which implies that higher RLD in 19.05 cm and 38.1 cm row spacing than 76.2 cm. In 2^{nd} harvest narrow spacing resulted in decreased RLD which is a result of decrease in fine roots that accounted for less than 40%, similar result was found with row spacing of 38.1 cm. But row spacing 76.2 cm showed higher proportions of fine roots, which resulted in higher RLD and RWD. More than 75% of total root is comprised of fine roots (0-2 mm diameter), so larger presence of fine roots results in higher SRL. Since SRL is strongly dependent on the fine root classes (Ostonen, 1998).

2.3.7. Above ground biomass vs. below ground biomass:

The effect of row spacing on biomass yield of above ground and below is shown Figure 1.7. Higher yield was recorded with in wider row spacing which is significantly *(P=0.05)* higher than narrow spacing but no significant *(P=0.05)* difference was observed from row spacing 38.1 cm with other spacing. Around 15000 kg ha^{-1} of aboveground biomass was recorded from the 76.2 cm spacing compared to 11000 kg ha^{-1} in the 19.05 cm spacing (Figure 1.8). However, no significant difference in root biomass among different row spacing similar to the findings of Ma et al., (2000 a) was recorded in the current study. The root biomass ranged from 6000 kg ha^{-1} to 8000 kg ha^{-1} from narrow to wider spacing.

2.4. Conclusion:

In conclusion, wider row spacing allowed for greater root biomass and above ground biomass. In addition, roots in wider row spacing, compared to narrow spacings, demonstrated higher RLD, RWD and average diameter after senescence. The Study also concluded that switchgrass has greater fine root fractions during active growing season and in narrow row spacing higher in narrow spacing which is mainly responsible for effective nutrient and water uptake.

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APPENDIX 1

Overall Row spacing (chapter 2) Figures and Tables in Appendix 1

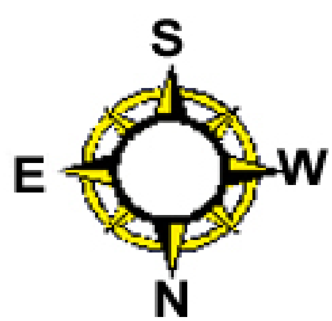
Appendix: 1

Table. 1.1: Plot plan for the Row Spacing Trial

Plot Size Harvested 10 ft. Wide X 30 ft. Long

3	15"	30"	7.5"
2	30"	7.5"	15"
1	7.5"	15"	30"
	plot 1	plot 2	plot 3

Planting Date: 5/15/2009
Variety: Alamo
Fertilized: 75 lbs./A using 46-0-0 6/3/2009
Treatments: 7.5",15", 30" Row Spacing
Treatment 1 = 7.5"
Treatment 2 = 15"
Treatment 3 = 30"
Plot Size: 20ft wide X 30ft long
Alley's: 20 ft
Design: RCB with 3 treatments and 3 reps



For the 7.5" rows we harvested 20 rows in the 12ft cut
For the 15" rows we harvested 10 rows in the 12ft cut
For the 30" rows we harvested 5 rows in the 12ft cut

Table 1.2: Root Length (RL) as influenced by row spacing

	Soil Depth	Row Spacing(cm)			
		19.05	38.1	76.2	Mean
First	0-0.1 m	2385.48^{a}	2027.34	1792.36^{a}	2068.39^{a}
	0.1-0.2 m	1062.84^{b}	976.32	1107.72^{b}	1048.96^{b}
	0.2-0.4 m	634.57^{cb}	610.22	532.59^{c}	592.46^{c}
	0.4-0.8 m	712.87^{cb}	586.22	622.75^{cb}	640.62^{c}
	0.8-1.1 m	338.04^{c}	447.06	333.26^{c}	372.79^{c}
	Mean	1026.76	929.43	877.74	
Second	0-0.1 m	1794.68^{a}	1681.94a	2403.71^{a}	1960.11^{a}
	0.1-0.2 m	792.67^{b}	713.50^{b}	866.15^{b}	790.77^{cb}
	0.2-0.4 m	824.99^{b}	896.84^{b}	432.79^{b}	718.20^{cb}
	0.4-0.8 m	1072.34^{b}	959.72^{b}	700.90^{b}	910.99^{b}
	0.8-1.1 m	683.65^{b}	583.25^{b}	367.91^{b}	544.93^{c}
	Mean	1033.66	967.05	954.29^{b}	

Means having different bold superscript letters in the same row are significantly different at *P=0.05* for either row spacing or soil depth.

Table 1.3: Root length density (RLD) as influenced by row spacing

	Soil Depth	Row Spacing(cm)			
		7.5"	15"	30"	Mean
First	0-0.1 m	3.93[a]	3.34[a]	2.95[a]	3.40[a]
	0.1-0.2 m	1.75[b]	1.61[b]	1.82[b]	1.73[b]
	0.2-0.4 m	0.52[c]	0.50[c]	0.44[c]	0.49[c]
	0.4-0.8 m	0.29[c]	0.24[c]	0.26[c]	0.26[c]
	0.8-1.1 m	0.19[c]	0.25[c]	0.18[c]	0.20[c]
	Mean	1.34	1.19	1.13	
Second	0-0.1 m	2.95[a]	2.77[a]	3.96[a]	3.23[a]
	0.1-0.2 m	1.30[b]	1.17[b]	1.43[b]	1.30[b]
	0.2-0.4 m	0.68[cb]	0.74[cb]	0.36[c]	0.59[c]
	0.4-0.8 m	0.44[cb]	0.39[cb]	0.29[c]	0.37[c]
	0.8-1.1 m	0.37[c]	0.32[c]	0.20[c]	0.30[c]
	Mean	1.15	1.08	1.25	

Means having different bold superscript letters in the same row are significantly different at *P=0.05* for either row spacing or soil depth.

Table 1.4: Root dry weight (RDW) as influenced by row spacing

	Soil Depth	Root Dry Weight (mg)			
		19.05	**38.1**	**76.2**	**Mean**
First	**0-0.1 m**	1.77 ^{a}	1.61^{a}	2.79^{a}	2.06^{a}
	0.1-0.2 m	0.69 ^{b}	0.62^{b}	1.04^{b}	0.78^{b}
	0.2-0.4 m	0.35 ^{b}	0.42 ^{b}	0.27^{c}	0.35^{c}
	0.4-0.8 m	0.31 ^{b}	0.27 ^{b}	0.25^{c}	0.28 ^{c}
	0.8-1.1 m	0.15 ^{b}	0.16 ^{b}	0.12^{c}	0.14 ^{c}
	Mean	0.66	0.61	0.90	
Second	**0-0.1 m**	2.72^{a}	3.54 ^{a}	4.20^{a}	3.49^{a}
	0.1-0.2 m	1.21^{b}	0.75 ^{b}	0.54 ^{b}	0.83^{b}
	0.2-0.4 m	0.75^{cb}	0.54 ^{b}	0.55 ^{b}	0.62^{cb}
	0.4-0.8 m	0.87^{cb}	0.50 ^{b}	0.51 ^{b}	0.63^{cb}
	0.8-1.1 m	0.37^{c}	0.34 ^{b}	0.23 ^{b}	0.31^{c}
	Mean	1.18	1.14	1.20	

Means having different bold superscript letters in the same row are significantly different at *P=0.05* for either row spacing or soil depth.

Table 1.5: Average Diameter as influenced by row spacing

	Soil Depth	Average Diameter(mm)			
		19.05	**38.1**	**76.2**	**Mean**
	0-0.1 m	0.65^{b}	0.72^{a}	0.91^{a}	0.76^{a}
	0.1-0.2 m	0.72^{ba}	0.75^{a}	0.85^{a}	0.77^{a}
First	**0.2-0.4 m**	0.75^{ba}	0.75^{a}	0.76^{a}	0.76^{a}
	0.4-0.8 m	0.79^{a}	0.75^{a}	0.77^{a}	0.77^{a}
	0.8-1.1 m	0.73^{ba}	0.77^{a}	0.75^{a}	0.75^{a}
	Mean	0.73	0.75	0.81	
	0-0.1 m	0.91^{a}	1.22^{a}	1.04^{ba}	1.05^{a}
	0.1-0.2 m	0.93^{a}	0.88^{b}	1.10^{a}	0.97^{ba}
Second	**0.2-0.4 m**	1.02^{a}	0.74^{b}	0.99^{ba}	0.92^{bac}
	0.4-0.8 m	0.84^{a}	0.70^{b}	0.76^{b}	0.76^{c}
	0.8-1.1 m	0.77^{a}	0.81^{b}	0.82^{ba}	0.80^{ba}
	Mean	0.89^{a}	0.87	0.94	

Means having different bold superscript letters in the same row are significantly different at *P=0.05* for either row spacing or soil depth.

Table 1.6: Specific root length (SRL) as influenced by row spacing

Harvest	Soil Depth	Specific Root Length (Cm Mg^{-1})			
		19.05	**38.1**	**76.2**	**Mean**
	0-0.1 m	1.17^{ba}	0.94^{b}	0.48^{c}	0.89^{d}
	0.1-0.2 m	1.11^{b}	1.18^{ba}	0.79^{bc}	1.04^{cd}
First	**0.2-0.4 m**	1.48^{ba}	1.08^{ba}	1.46^{ba}	1.45^{cb}
	0.4-0.8 m	1.63^{ba}	1.64^{ba}	1.85^{a}	1.74^{b}
	0.8-1.1 m	2.21^{a}	2.04^{a}	2.02^{a}	2.34^{a}
	Mean	1.52	1.38	1.32	
	0-0.1 m	0.49^{b}	0.35^{b}	0.43^{a}	0.42^{c}
	0.1-0.2 m	0.49^{b}	0.71^{ba}	1.20^{a}	0.82^{bc}
Second	**0.2-0.4 m**	0.84^{b}	1.23^{a}	0.59^{a}	0.95^{b}
	0.4-0.8 m	0.93^{b}	1.43^{a}	1.03^{a}	1.24^{ba}
	0.8-1.1 m	1.6^{a}	1.26^{a}	1.21^{a}	1.46^{a}
	Mean	0.87	1.00	0.89	

Means having different bold superscript letters in the same row are significantly different at *P=0.05* for either row spacing or soil depth.

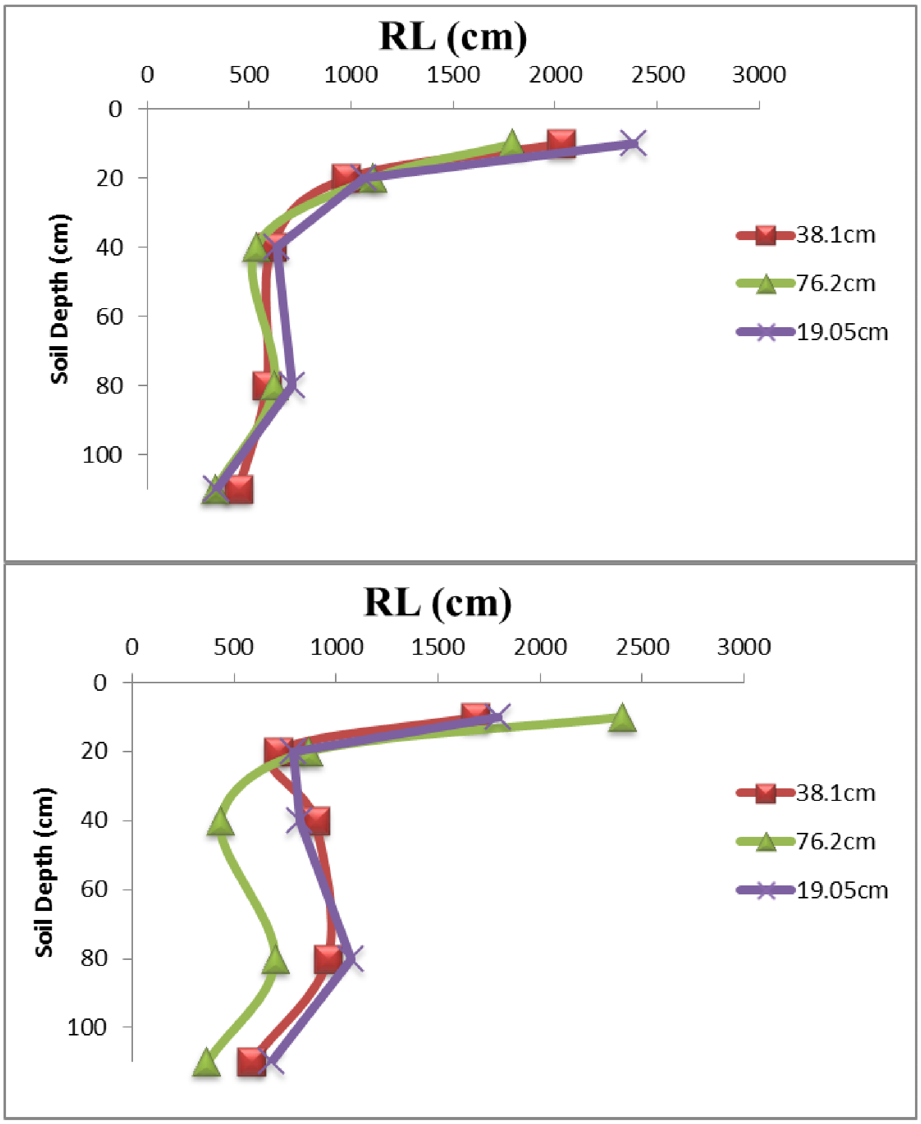
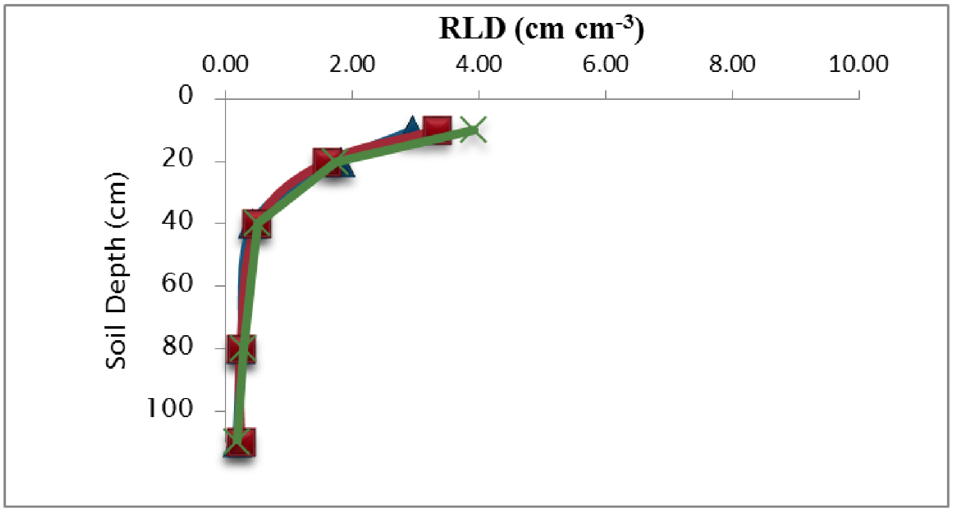


Figure 1.1: Mean root length density (RLD) at different row spacing of (Top) First root harvest (August) (Bottom) Second root harvest (December)



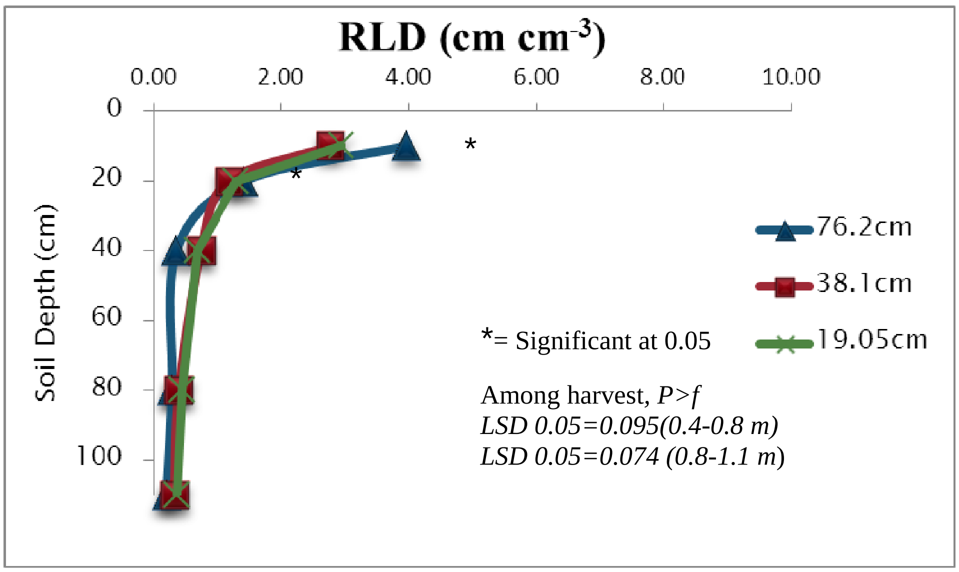
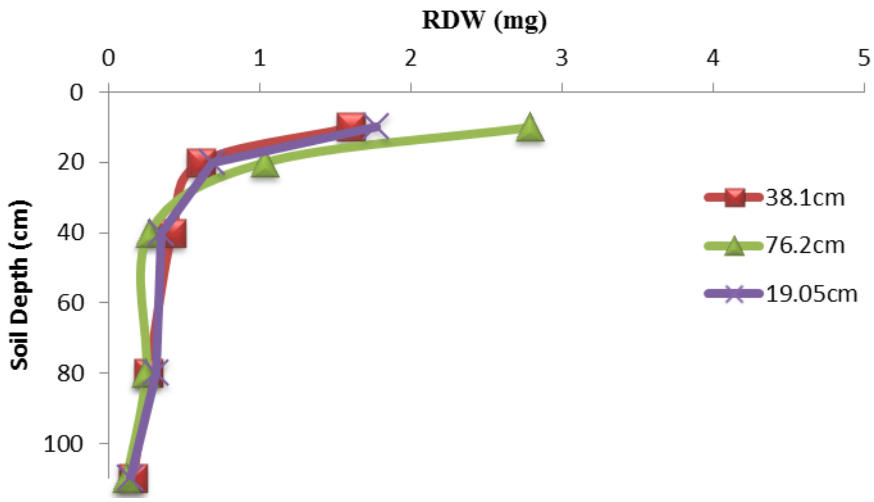


Figure 1.2: Mean root length density (RLD) at different row spacing of (top) First root harvest(August) (bottom) Second root harvest (December)



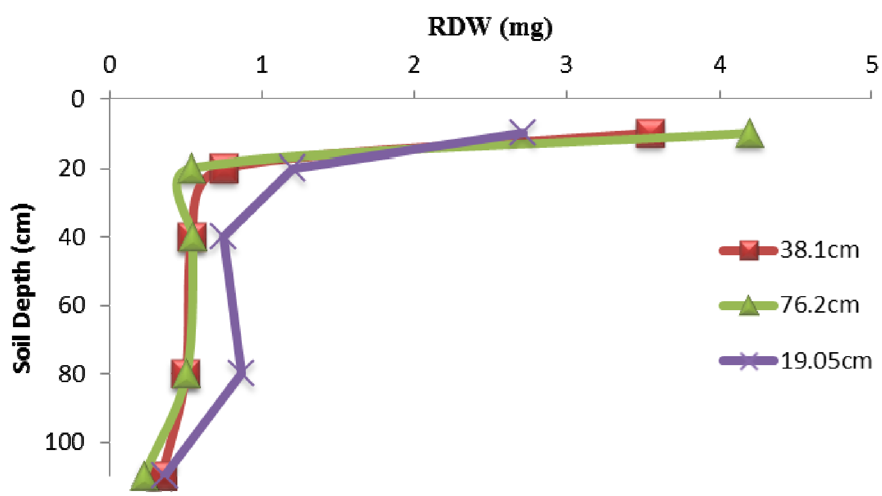


Figure 1.3: Mean root dry weight (RDW) at different row spacing of (Top) First root harvest (August) (Bottom) Second root harvest (December)

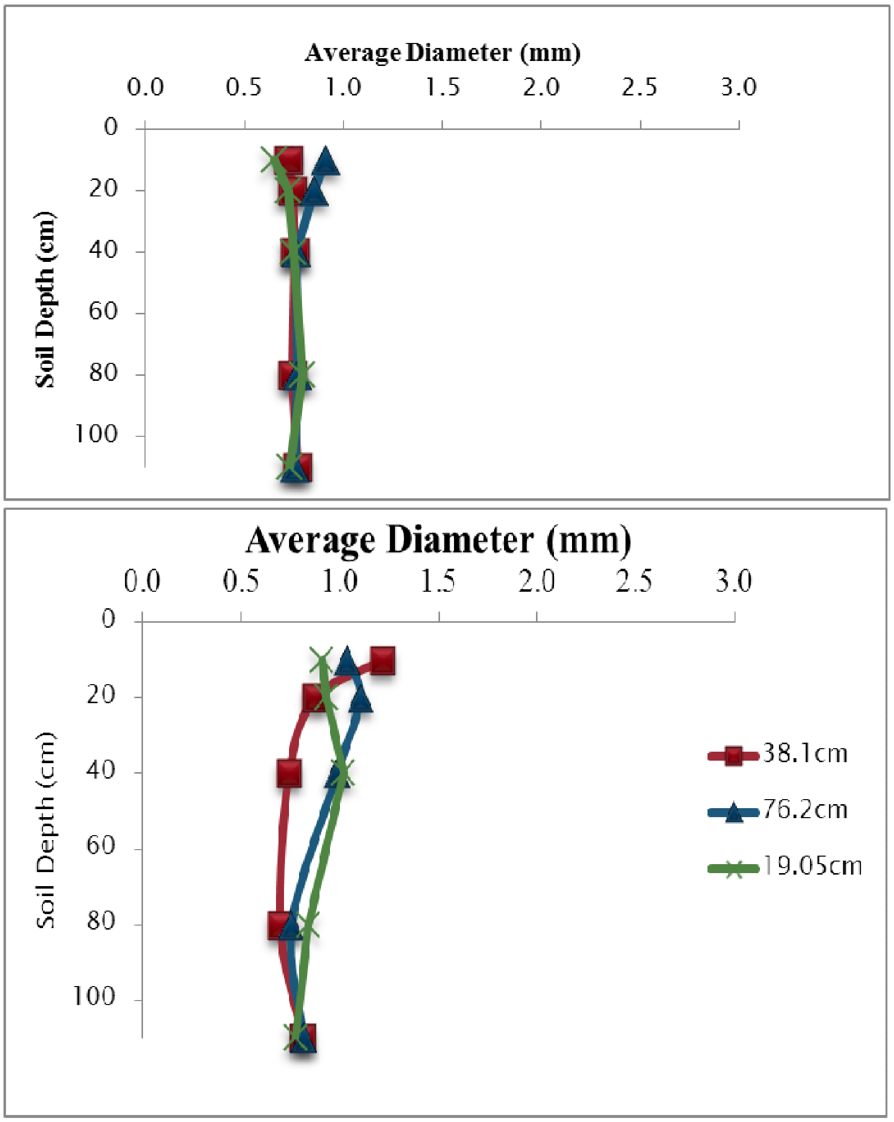


Figure 1.4: Average diameters at different row spacing of (Top) First root harvest (August) (bottom) Second root harvest (December)

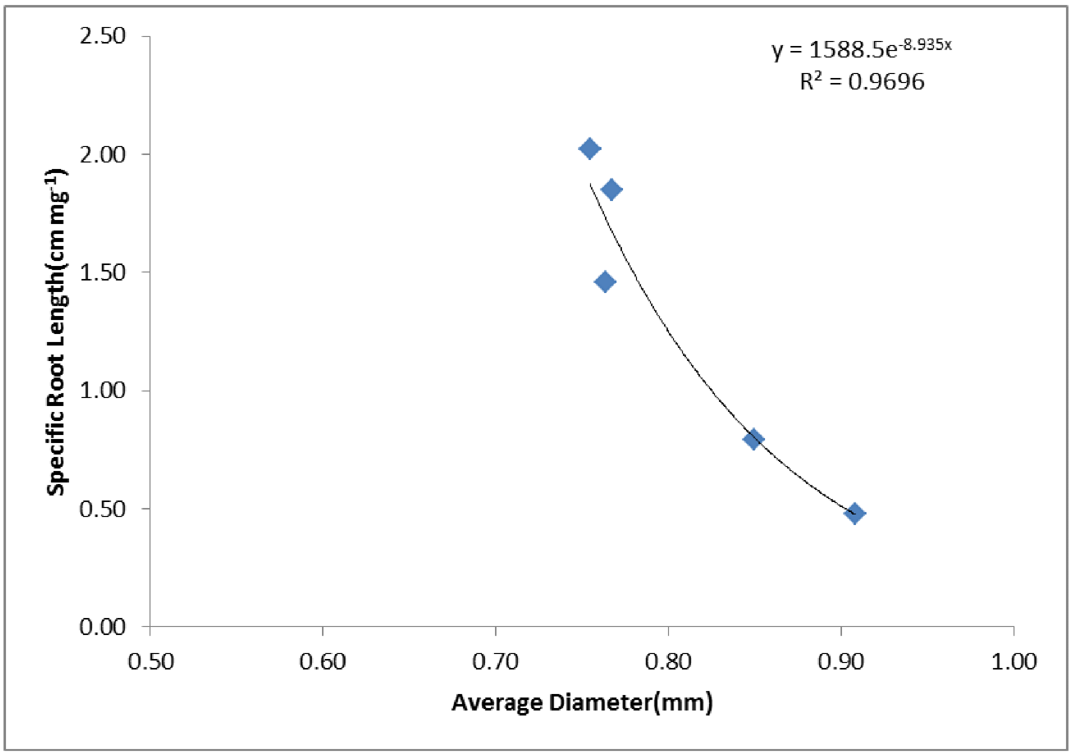


Figure 1.5: Correlation between Specific root length (SRL) and Average diameter

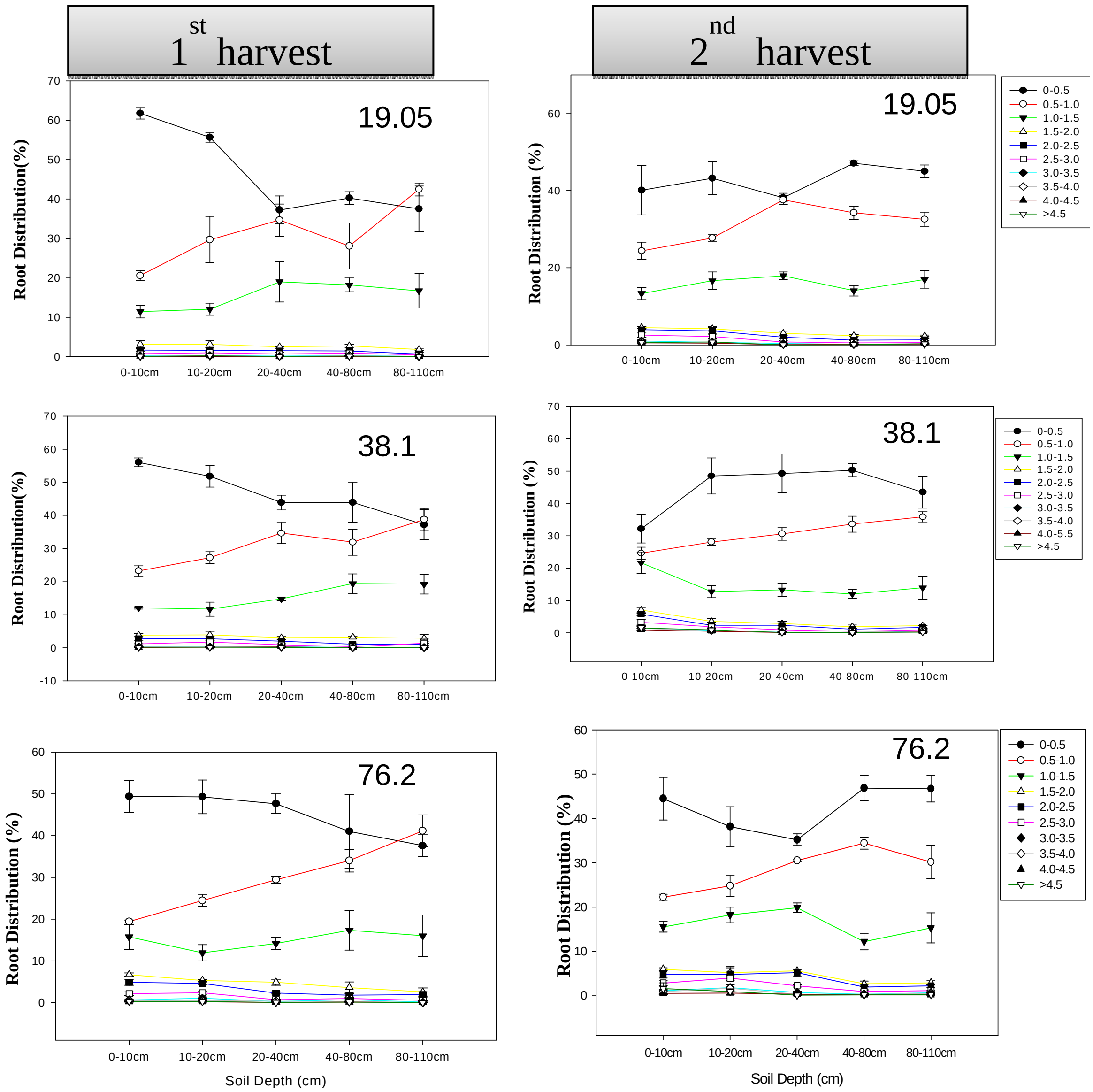


Figure 1.6: Root distribution (%) according to the diameter influenced by different row spacing on different harvest (1st harvest- August and 2nd harvest- December)

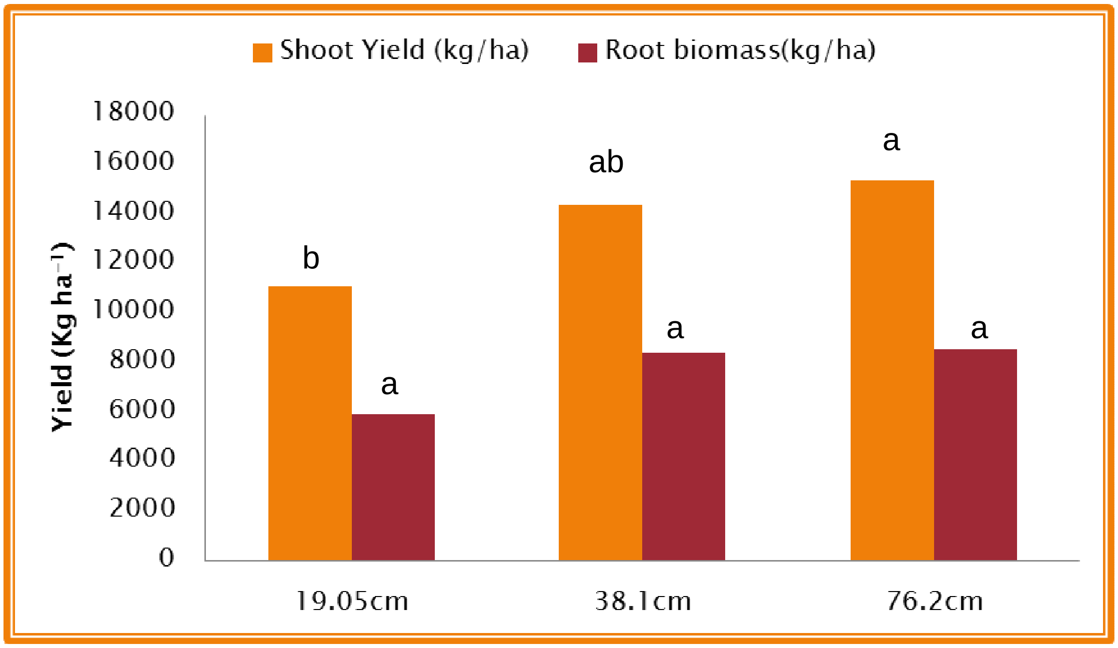


Figure 1.7: Comparison of mean aboveground biomass (kg ha^{-1}) and below ground root biomass (kg ha^{-1}) in different row spacing.

CHAPTER 3

EVALUATION OF SWITCHGRASS ROOT CHARACTERISTICS AS INFLUENCED BY CULTIVAR DIFFERENCES

Abstract

Switchgrass (*Panicum virgatum L.*) has been selected as a potential bioenergy feed stocks for its high biomass production and cost effective management. The individual root and the entire root system, various morphological parameters, which are influenced by genetic variability and environmental conditions, have been used as potential indicators of plant performance on different soils. The objective of the study is to evaluate different root characteristics and their distribution pattern as influenced by cultivar difference (lowland and upland cultivars) at peak growth and after senescence. Root length density (RLD) at the surface (0-0.1m) was 87% at peak growth compared to 78% RLD after senescence. Lowland cultivars were lower in RLD and root weight density (RWD) in comparison to upland cultivars by 39%. Lowland cultivars had higher average diameter than upland cultivars over entire soil depth from 0 to 1.1 m. The average aboveground biomass and below ground root biomass of lowland and upland cultivars were 15000 and 4000 kg ha^{-1}, and 8 and 7 kg ha^{-}1, respectively. Fine root (0-2.0 mm diameter) proportion was found to be high among upland cultivars by more than 150 cm compared to lowland

cultivars while 15% coarse root (2.0->4.5 mm diameter) distribution was found among lowland cultivars in compared with upland cultivars.

3.1. Introduction:

Biomass has been considered as an important and more recently considered as a renewable energy source in both developed and developing countries (Sagar and Kartha, 2007).The use of renewable biofuels increases from 18 billion liter to 136 billon liter by the US's Energy Independence and Security Act of 2007(Energy Independence and Security Act, 2007).Thus, lignocellulosic ethanol production technology are being made more efficient. Increased proportion of CO_2, a greenhouse gas in the atmosphere can be reduced by enhancing the use of potential biofuels instead of fossil fuels and also be a source of income for farmers (Lemus and Lal, 2005; Liu et al., 2010). According to Scharlemann and Laurance (2008), non-food plants (switchgrass, trees, algae) can benefit environment than ethanol produce from food sources (corn, sugarcane). Switchgrass (*Panicum virgatum*) has been selected as potential bioenergy feedstocks for its high biomass production and cost effective growth characteristics because of low input high diversity (LIHD) (McLauglin, 1992; Bransby et al., 1998). Two ecotypes of switchgrass are found i.e. lowland and upland. Lowland cultivars are taller, have thick stems and produce high aboveground biomass than upland cultivars while upland cultivars are more drought tolerant (Wullschleger et al., 2010). Along with its deep fibrous root system which can extended up to 3.3 m below soil surface (Ma et al., 2000), also switchgrass helps to improve soil and water quality by enhancing soil organic carbon(SOC) and filtering pollutants like leached nutrients (N, P). Moreover, switchgrass has higher water use efficiency and capture additional N through fixation (Parrish et al., 2005). The deep and extensive roots of switchgrass can transfer C into soil, which can enhance soil quality. Under switchgrass the SOC distribution in a soil profile was found higher than cultivated crops up to the depth of 30cm (Liebig et al., 2005). The mechanisms underlying in the changes of SOC and the availability of N are difficult to predict overall effect. Since major source of soil C input is root biomass of bioenergy crops. On the level of the individual root and the entire root system, various morphological parameters, which are influenced by genetic variability and environmental conditions, have been used as potential indicators of the mineral nutrition of plant on different soils. These parameters include root length, average diameter, root weight, surface area, used to determine quantity and functional size along with predicting responses to the environmental changes. Root length density (RLD) (cm cm^{-3} soil) (Majdi, 2000), Root weight density (RWD) (Mg $cm^{-3)}$ (Leuschner et al., 2004) (Ma et al., 2000 b), average diameter (mm), specific root length (SRL) (cm mg^{-}1) (Ostonen et al., 2007) are important parameters in evaluating root pattern on crop water and nutrient uptake along with environmental changes. However, study on different root characteristics and their distribution pattern are very limited. The objective of this study will be to 1) evaluate different root characteristics and their distribution pattern influenced by switchgrass cultivars (lowland and upland cultivars) at peak growth and after senescence, and 2) determine the relationship between aboveground biomass and belowground biomass.

3.2. Materials and methods

3.2.1 Field Set up

This experiment was conducted on an experimental field at Stillwater at Agronomy Farm, Payne County, Oklahoma. According to the Field and Research Service Unit (FRSU) of Oklahoma Ag Experiment Station the soil type is easpur loam (Fine-loamy, mixed, superactive, thermic Fluventic Haplustoll) with 0 to 1 percent slope with occasional flooding. Climatic variations from hot summer to cool weather and occasional drops due to cold surges, relatively uniform precipitation (peak in spring) and infrequent snowfall are major characteristics of Payne County (Henley et al., 1987).

Ten 10 cultivar of switchgrass (3 lowland and 7 upland cultivars) were used in the study. The plant stands were established in 2009 with row spacing of 15 inch. The experimental design was randomized complete block design with 3 replications, each replication being a large plot of 20 ft. wide x 30 ft. long with 20 ft. alleys in between two replications. For the evaluation of root morphological characters and distribution pattern, the samples were taken from different 10 cultivars at peak growth and after senescence at the month of August and December respectively. No fertilizer has been applied to this plot. The plot management included no application of irrigation, herbicides and pesticides. The root biomass samples were taken from the middle of the row from east to west of each plot. The GPS coordinates and the layout of the field were shown in the **Table 2.1**

3.2.2. Sample collection:

The root samples were collected in August and December, 2010, from each block. The tractor mounted hydraulically powered soil core sampler was used to uproot the root samples from the depth up to 1.1 m. The core sampler, diameter of 74 mm was used to uproot the root samples. After uprooting the entire core was separated. Five depths 0-0.1 m, 0.1-0.2 m, 0.2-0.4 m, 0.4 -0.8 m, 0.8-1.1 m. Each depth sample was placed in separate plastic bag with required label. The root samples were collected from each plot from on the row and in-between the row in separate plastic bags with label was then carried to cold storage so that root samples in the a bag remains as it was harvested until washed. The samples were carried out from the cold storage to the lab where samples were washed to get clean root samples. The soil attached to the root samples were washed with tap cold water. The sieve of small pore size (< 1 mm) was used to wash the sample so that fine roots can be collected without any loss. Then, the washed root samples were collected in clean plastic bags with required label and placed in the refrigerator until measurement. Both live and dead roots were used.

3.2.3. Measurement and analysis:

The roots measurement was carried out to determine the root morphology as affected by switchgrass cultivars. The image analysis system, WinRhizo software (ver. 5.0. Reagent instruments, Quebec, Canada) which has the capability of analyzing images acquired from a flatbed scanner (LA 2400, Epson) was used to measure the root parameters. The root parameters include root length, average diameter, surface area, root volume and distribution of root length according to their diameter. Before measuring the samples, the system was calibrated with the resolution of 100 dpi (dots per inch) in grey level image and was measured with the same resolution. The root length of the samples were collected in order to determine the root length density (RLD) (cm cm^{-3}).The RLD was calculated as root length divided by soil volume of specific depth.

RLD= RL/Vol

Where, RL= Total Root length of specific depth and Vol = Total soil volume of specific depth

Similarly, specific root length (SRL) (cm mg^{-1}) was calculated as total root length divided by root weight for each depth. After measurement of root samples the root samples were oven dried for 24 hours at 70^0C. Dry weight was taken in order to get root weight density (RWD) (mg cm^{-3}). The RWD was calculated as

RWD= RDW/ ($\pi * CR^2 * CL$)

Where, RDW= Root dry weight, CR= Core radius, CL= Core length.

Similarly, Root mass (RM) in kg ha^{-1} was calculated as follows:

RM=RWD * CL *100

Where, 100 is used as a conversion factor of area and mass.

Distributions of root length according to their diameter were expressed in percentage. The roots were classified on the basis of diameter classes as coarse roots (> 2mm diameter) and fine root (<2mm diameter).

The GLM procedures using the SAS package release 9.2 (SAS Institute, 2002) was used to estimate for all the main and interaction effects on RLD, RWD, Average diameter, and root biomass. The data were analyzed with two ways ANOVA model to evaluate the effect of cultivars and harvesting frequency for each depth. The significant differences were observed at significance level of $P=0.05$ in the ANOVA.

3.3. Result and Discussion:

3.3.1. Root Length Density:

Root length density (RLD) distributions are shown in Table 2.2 and Table 2.3 for lowland and upland cultivars. Upland cultivars (Blackwell, Southlow and Cave-in-rock) have higher RLD at surface i.e. 0-0.1m in both harvest (August and December) than low land cultivars (Alamo, Kanlow and Carthage) as shown in Figure 2.1. However, Alamo has higher RLD in 2nd harvest than in 1st harvest as shown in Figure 2.1. There is no gain of RLD in other cultivars in the 2nd harvest i.e. at the end of growing season. There is no significant difference (*P=0.05*) among the harvest of RLD at the depth of 0-0.1 m (Figure 2.2). While at lower depth i.e. 0.1-0.2 m RLD was significantly higher in 1st harvest than 2nd harvest among upland cultivars but was higher in 2nd harvest among lowland cultivars (Figure 2.2). At soil depth 0.2-0.4 m, there was no any significant difference (*P=0.05*) between cultivars, but higher RLD for lowland cultivars Alamo and Kanlow was recorded at the end of growing season. The upland cultivar Cave-in-rock had lower RLD in lower depth at the end of growing season than at peak growth. The 2nd harvest RLD was significantly higher at the depth of 40-110cm among cultivars than 1st harvest. Lowland cultivars had lower RLD in comparison to upland cultivars by 39% over entire soil depth but has increased RLD in lower depth, also higher RLD was observed in 2nd harvest of lowland cultivars. About 87% of RLD was found in upper depth i.e.0-0.1 m while 83% of RLD was recorded during 2nd harvest at the same depth. Similarly, several studies found bulk of RLD in the upper layers (Tufekcioglu et al., 1999; Xu et al., 2010;

Ma et al., 2000; Monti et al., 2009). There was 31% increase in RLD in deeper depth i.e. 0.8-1.1 m at the end of growing season (Figure 2.1). Similarly, Xu et al., (2010) reported continuous root growth throughout the season. Higher RLD was observed at growth stage, it may be due to the increase of fine roots. Frank et al., (1994) reported that during peak growth stage soil respiration increases and simultaneously increased respiration will increase fine roots and soil organic carbon (Tufekcioglu et al., 1999).

3.3. 2. Root Weight Density (RWD):

Root weight density influenced by different lowland and upland cultivars at different harvest over entire soil profile is shown in Table 2.4 and 2.5, respectively. RWD was found to be decreased with subsequent depth (Mengel et al., 1974). Upland cultivars (Southlow, Cave-in-rock and Blackwell) have higher RWD in surface i.e. 0-0.1 m than lowland cultivars (Alamo, Kanlow and Carthage) (Ma et al., 2000 b). At the same depth, all cultivars had higher, by more than 50% RWD in 2nd harvest (Figure 2.3). It may be due to the translocation of nutrients from canopy to the crown/root at the end of growing season. Several authors indicated that at the end of growing season there will be the translocation of nutrients and non-structural carbohydrates from canopy to root systems (Tufekcioglu et al., 1999). Similarly, during growing season about 50% of the carbohydrate will be lost through the soil respiration (Frank et al., 1994) since soil respiration increases during growing season. In all soil depth from 0-1.1 m RWD is significantly higher in 2nd harvest than in 1st harvest (Figure 2.4). RWD of lowland cultivars increases by 52% while that of upland cultivars increases up to 40 % in 2nd

harvest over the entire soil profile but lowland cultivars have a lower RWD than upland cultivars (Table 2.4 and 2.5). Lowland cultivars did not produce more root biomass in the surface which accounts for high percent of the root mass, even though higher root biomass is found in deeper depth (Ma et al., 2000 a). With subsequent depth lowland cultivars increases RWD while upland cultivar Cave-in-rock at the depth of 0.2-0.4 m was found lower in 2nd harvest, it may be due to lower RLD.

3.3. 3. Average Diameter:

The average diameter of different switchgrass cultivars at different harvest from the depth of 0-1.1 m is shown in Table 2.6 and 2.7, respectively. Average diameter was found significantly higher in 2nd harvest than 1st harvest in all soil profile ranges from 0-110cm (Figure 2.6). Lowland cultivars had higher average diameter than upland cultivars in all soil depth from 0 to 1.1 m (Figure 2.5). Average diameter of lowland cultivars was 0.8-1.2 mm while it was 0.8-1.0 mm for upland cultivars (Table 2.6 and 2.7). Similarly, in 2nd harvest i.e. at the end of growing season, lowland cultivars had roots with higher average diameter than upland cultivars. It may be due to the translocations of nutrient from canopy to the roots which leads to the accumulation and increase in diameter. About 50% of nitrogen fixed will be translocated to the root from the above ground biomass during senescence (Garten et al., 2010). Lowland cultivar produces higher aboveground biomass with more tiller number than upland cultivars. More number of rhizomes is found on the lowland cultivar which produces less fine roots and more coarse roots (Ma et al., 2000).

3.3. 4. Above ground biomass Vs. Below ground biomass:

The aboveground biomass and below ground root biomass of lowland and upland cultivars are shown in Table 2.8 and 2.9, respectively. Lowland cultivars have higher above ground biomass than upland cultivars. The lowland cultivar Alamo produced around 15000 kg ha^{-1} whereas upland cultivar Southlow, Cave-in-rock, Blackwell about 8000 kg ha^{-1} (Figure 2.7). Similarly, in comparison with belowground root biomass upland cultivar Blackwell and Cave-in-rock has higher root biomass i.e. about 7000 kg ha^{-1} while lowland cultivars results about 4 kg ha^{-1} (Figure 2.7). A significant higher correlation found between aboveground biomass and root biomass among upland cultivars (R^2=0.75) while no such relations was found among lowland cultivars (Figure 2.7). Bransby et al., (1998) and Ma et al., (2000 b) found the similar results in which high yielding aboveground biomass of lowland cultivars did not correlate with root biomass.

3.3. 5. Root distribution according to the diameter:

Root distribution according to the diameter is shown in Figure 2.8. The distribution of root length (cm) is measured according to the root diameter that ranges from 0 - >4.5mm.The fine root ranges from 0-2 mm diameter while coarse root ranges from 2.0 - >4.5 mm diameter. In 1st harvest, the distribution of very fine root (0-0.5mm) is found higher in upland cultivar which is near to 800 cm of total length (Figure 2.8). Higher proportions of fine roots contribute to increase RLD, as we have found that upland cultivars have higher RLD than low land cultivars. In the 2^{nd} harvest the fine root proportions was found to be increased by more than 150 cm of total root length among low land cultivars while upland cultivars had decreased fine root proportions but as a whole upland cultivars had higher fine root proportions than lowland cultivars. Similarly, in the coarse root distribution lowland cultivars had higher diameter than upland cultivars. Around 15% increase of coarse root diameter (2.0-2.5mm) was found among lowland cultivars in 2^{nd} harvest while more than 25% increase in coarse roots of diameter 2.5-3.0mm was found in upland cultivars (Figure 2.8). The higher fine roots during growing season might be due to the translocation of nutrients from roots to canopy, which expenses more energy in soil respiration and produces more fine roots while at the end of growing season, the process will be the other way around (Tufekcioglu et al., 1999). Also, Hartnett (1989) reported that switchgrass maintained rhizomes interconnections among stems for long time so that it can produce finer root biomass.

3.4. Conclusion:

Cultivar differences were evident for root parameters evaluated in the study. Lowland cultivars had significantly lower value for all the root parameters compared to upland cultivars. The root biomass of lowland cultivars was only 57% of the root biomass of upland cultivars. In contrast, the aboveground biomass of lowland cultivars was 80% higher than upland cultivars. Hence, differences in lowland and upland cultivars for root traits can be exploited for breeding varieties with enhanced C sequestration.

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APPENDIX 2:

Overall Varietal trial (chapter 2) Figures and Tables in Appendix 2

Table 2.1: Plot plan of cultivar trial.

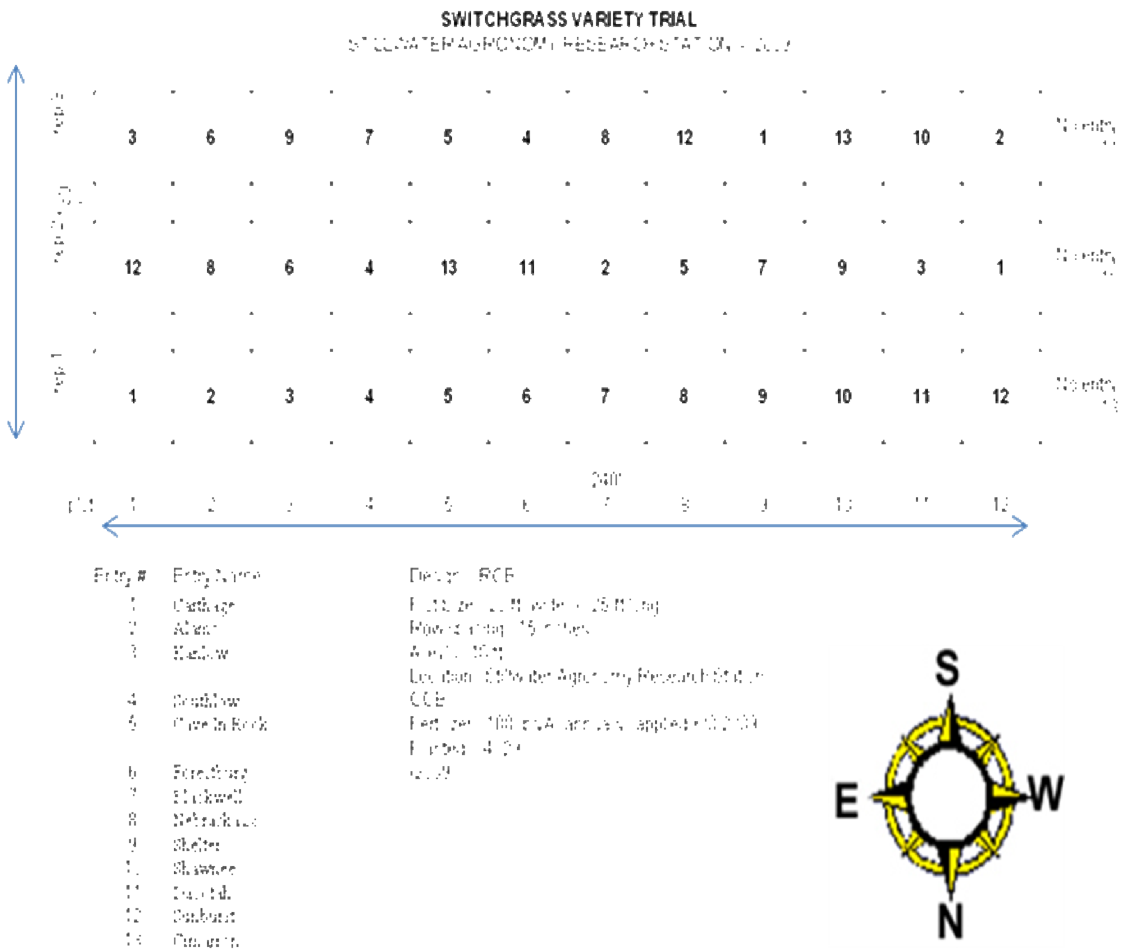


Table 2.2. Mean RLD of lowland cultivars at different harvest at different soil depth. A B C Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Lowland cultivar		
		Carthage	Alamo	Kanlow
		Root Length Density (cm cm^{-3})		
First	0-0.1 m	3.06^{A}	2.04^{A}	2.57^{A}
	0.1-0.2 m	0.63^{B}	0.46^{B}	0.62^{B}
	0.2-0.4 m	0.26^{CB}	0.24^{B}	0.4^{B}
	0.4-0.8 m	0.31^{CB}	0.2^{B}	0.28^{B}
	0.8-1.1 m	0.1^{C}	0.15^{B}	0.08^{B}
Second	0-0.1 m	2.51^{A}	2.94^{A}	1.77^{A}
	0.1-0.2 m	1.03^{B}	0.63^{B}	0.57^{B}
	0.2-0.4 m	0.64^{B}	0.67^{B}	0.29^{B}
	0.4-0.8 m	0.57^{B}	0.4^{B}	0.46^{B}
	0.8-1.1 m	0.31^{B}	0.36^{B}	0.3^{B}

Table 2.3. Mean RLD of upland cultivars at different harvest at different soil depth. A B C D Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Upland cultivar						
		Shelter	Southlow	Sunbrust	Cave-in-Rock	Forestburg	Blackwell	Nebraska28
		Root Length Density (cm cm^{-3})						
First	0-0.1 m	3.98^{A}	4.59^{A}	4.06^{A}	4.09^{A}	3.11^{A}	5.61^{A}	3.89^{A}
	0.1-0.2 m	1.06^{B}	2.05^{B}	0.99^{B}	1.28^{B}	0.99^{B}	1.17^{B}	1.43^{B}
	0.2-0.4 m	0.49^{B}	0.76^{CB}	0.56^{B}	0.89^{CB}	0.48^{B}	0.54^{CB}	0.54^{C}
	0.4-0.8 m	0.32^{B}	0.28^{CB}	0.24^{B}	0.48^{C}	0.14^{B}	0.29^{C}	0.24^{DC}
	0.8-1.1m	0.05^{B}	0.18^{B}	0.04^{B}	0.23^{C}	0.04^{B}	0.19^{C}	0.09^{D}
Second	0-0.1 m	3.58^{A}	4.75^{A}	2.91^{A}	3.71^{A}	2.92^{A}	4.11^{A}	3.26^{A}
	0.1-0.2 m	1.03^{B}	1.55^{B}	0.67^{B}	0.94^{B}	0.8^{B}	1.01^{B}	0.89^{B}
	0.2-0.4 m	0.57^{B}	0.92^{CB}	0.35^{B}	0.44^{CB}	0.51^{CB}	0.6^{B}	0.6^{B}
	0.4-0.8 m	0.39^{B}	0.42^{C}	0.33^{B}	0.36^{C}	0.43^{C}	0.44^{B}	0.41^{B}
	0.8-1.1m	0.38^{B}	0.39^{C}	0.34^{B}	0.44^{CB}	0.33^{C}	0.51^{B}	0.33^{B}

Table 2.4. Mean RWD of lowland cultivars at different harvest at different soil depth. $^{A\ B\ C}$ Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Lowland cultivar		
		Carthage	Alamo	Kanlow
		Root Weight Density (mg cm^{-3})		
First	**0-0.1 m**	2.79^{A}	2.62^{A}	2.07^{A}
	0.1-0.2 m	0.62^{B}	0.45^{B}	0.94^{A}
	0.2-0.4 m	0.13^{B}	0.14^{B}	0.36^{A}
	0.4-0.8 m	0.09^{B}	0.08^{B}	0.12^{A}
	0.8-1.1 m	0.02^{B}	0.07^{B}	0.05^{A}
Second	**0-0.1 m**	3.38^{A}	4.93^{A}	3.45^{A}
	0.1-0.2 m	0.55^{B}	0.85^{B}	5.38^{A}
	0.2-0.4 m	0.29^{B}	0.89^{B}	0.39^{B}
	0.4-0.8 m	0.25^{B}	0.56^{B}	0.21^{B}
	0.8-1.1 m	0.09^{B}	0.31^{B}	0.11^{B}

Table 2.5. Mean RWD of upland cultivars at different harvest at different soil depth. A B C D Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Upland Cultivar						
		Shelter	Southlow	Sunbrust	Cave-in-Rock	Forestburg	Blackwell	Nebraska28
		Root Weight Density (mg cm^{-3})						
First	0-0.1 m	3.61^{A}	3.72^{A}	3.37^{A}	4.25^{A}	3.12^{A}	4.96^{A}	2.85^{A}
	0.1-0.2 m	0.56^{B}	0.88^{B}	0.44^{B}	0.72^{B}	0.56^{B}	0.9^{B}	0.69^{B}
	0.2-0.4 m	0.18^{B}	0.25^{B}	0.17^{B}	0.38^{CB}	0.21^{B}	0.27^{B}	0.23^{C}
	0.4-0.8 m	0.07^{B}	0.08^{B}	0.07^{B}	0.14^{CB}	0.05^{B}	0.06^{B}	0.05^{C}
	0.8-1.1 m	0.01^{B}	0.1^{B}	0.01^{B}	0.06^{C}	0.01^{B}	0.06^{B}	0.11^{C}
Second	0-0.1 m	7.97^{A}	10.05^{A}	3.93^{A}	7.56^{A}	3.56^{A}	7.12^{A}	3.91^{A}
	0.1-0.2 m	0.68^{B}	1.82^{B}	0.54^{A}	1.1^{B}	0.54^{A}	1.04^{B}	0.52^{B}
	0.2-0.4 m	0.42^{B}	1.14^{CB}	0.2^{A}	0.28^{B}	0.23^{A}	0.52^{B}	0.14^{B}
	0.4-0.8 m	0.24^{B}	0.4^{CD}	0.14^{A}	0.16^{B}	0.13^{A}	0.24^{B}	0.12^{B}
	0.8-1.1 m	0.28^{B}	0.23^{D}	0.15^{A}	0.14^{B}	0.12^{A}	0.3^{B}	0.09^{B}

Table 2.6. Mean average diameter of lowland cultivars at different harvest at different soil depth. $^{A\ B\ C}$ Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Lowland cultivar		
		Carthage	Alamo	Kanlow
		Average Diameter(mm)		
First	**0-0.1 m**	0.72^{A}	0.85^{A}	0.73^{CB}
	0.1-0.2 m	0.76^{A}	0.85^{A}	1.02^{A}
	0.2-0.4 m	0.63^{A}	0.76^{A}	0.85^{B}
	0.4-0.8 m	0.58^{A}	0.69^{A}	0.76^{CB}
	0.8-1.1 m	0.55^{A}	0.76^{A}	0.62^{C}
Second	**0-0.1 m**	0.84^{A}	1.01^{A}	0.87^{BA}
	0.1-0.2 m	0.81^{A}	0.84^{A}	1.16^{A}
	0.2-0.4 m	0.73^{A}	0.89^{A}	1.13^{A}
	0.4-0.8 m	0.7^{A}	0.96^{A}	0.68^{B}
	0.8-1.1 m	0.64^{A}	0.81^{A}	0.61^{B}

Table 2.7. Mean average diameter of upland cultivars at different harvest at different soil depth. A B C Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil Depth	Upland cultivar						
		Shelter	**Southlow**	**Sunbrust**	**Cave-in-Rock**	**Forestburg**	**Blackwell**	**Nebraska28**
		Average Diameter (mm)						
First	**0-0.1 m**	0.68^{A}	0.7^{A}	0.65^{A}	0.74^{A}	0.71^{A}	0.72^{A}	0.64^{A}
	0.1-0.2 m	0.64^{BA}	0.58^{B}	0.63^{BA}	0.59^{B}	0.7^{A}	0.68^{BA}	0.62^{A}
	0.2-0.4 m	0.61^{BA}	0.57^{B}	0.61^{BA}	0.6^{B}	0.65^{A}	0.6^{BA}	0.64^{A}
	0.4-0.8 m	0.53^{B}	0.59^{B}	0.66^{A}	0.59^{B}	0.61^{A}	0.51^{B}	0.65^{A}
	0.8-1.1 m	0.55^{B}	0.56^{B}	0.48^{B}	0.59^{B}	0.57^{A}	0.54^{B}	0.6^{A}
Second	**0-0.1 m**	1.02^{A}	0.96^{A}	0.84^{A}	0.96^{A}	0.76^{A}	0.94^{A}	0.87^{A}
	0.1-0.2 m	0.8^{B}	0.58^{C}	0.64^{A}	0.7^{B}	0.63^{BA}	0.75^{B}	0.75^{BA}
	0.2-0.4 m	0.7^{B}	0.66^{CB}	0.65^{A}	0.62^{B}	0.66^{BA}	0.71^{B}	0.67^{B}
	0.4-0.8 m	0.8^{B}	0.71^{B}	0.67^{A}	0.61^{B}	0.64^{BA}	0.65^{B}	0.63^{B}
	0.8-1.1 m	0.69^{B}	0.61^{CB}	0.68^{A}	0.61^{B}	0.59^{B}	0.69^{B}	0.59^{B}

Table 2.8. Mean root biomass of lowland cultivars at different harvest at different soil depth. $^{A\ B\ C}$ Means with same bold letter are not significantly different at *P=0.05*.

Harvest	**Soil Depth**	**Lowland cultivar**		
		Carthage	**Alamo**	**Kanlow**
		Root biomass (kg ha^{-1})		
	0-0.1 m	2792.14^{A}	2621.21^{A}	2073.15^{A}
	0.1-0.2 m	616.43^{B}	451.02^{B}	938.43^{A}
First	**0.2-0.4 m**	250.32^{B}	271.27^{B}	725.60^{A}
	0.4-0.8 m	346.26^{B}	316.49^{B}	467.56^{A}
	0.8-1.1 m	59.55^{B}	219.45^{B}	135.64^{A}
	0-0.1 m	3376.59^{A}	4929.25^{A}	3452.68^{A}
	0.1-0.2 m	550.27^{B}	851.32^{B}	5380.27^{A}
Second	**0.2-0.4 m**	575.63^{B}	1784.23^{BA}	776.33^{B}
	0.4-0.8 m	1000.19^{BA}	2220.92^{BA}	854.62^{B}
	0.8-1.1 m	284.51^{B}	915.27^{B}	336.34^{B}

Table 2.9. Mean root biomass of upland cultivars at different harvest at different soil depth. A B C Means with same bold letter are not significantly different at *P=0.05*.

Harvest	Soil depth	Southlow	Cave-in-rock	Forestburg	Blackwell	Nebraska 28	Shelter	Sunbrust
		Root biomass (kg ha^{-1})						
First	0-0.1 m	3719.17^{A}	4250.70^{A}	2926.67^{A}	4963.07^{A}	2845.80^{A}	3605.96^{A}	3373.28^{A}
	0.1-0.2 m	879.99^{B}	719.72^{B}	556.52^{A}	903.51^{B}	686.64^{B}	562.40^{B}	442.20^{B}
	0.2-0.4 m	506.53^{B}	765.30^{B}	421.25^{A}	532.26^{B}	466.83^{B}	359.49^{B}	338.54^{B}
	0.4-0.8 m	322.00^{B}	577.84^{B}	186.00^{A}	233.05^{B}	213.20^{B}	266.86^{B}	262.45^{B}
	0.8-1.1 m	301.42^{B}	191.14^{B}	30.14^{A}	191.88^{B}	316.85^{B}	39.70^{B}	16.54^{B}
Second	0-0.1 m	10051.84^{A}	7556.71^{A}	3561.85^{A}	7116.35^{A}	3910.32^{A}	7972.81^{A}	3927.96^{A}
	0.1-0.2 m	1823.20^{B}	1099.80^{B}	539.61^{B}	1038.05^{B}	518.29^{B}	682.60^{B}	539.24^{B}
	0.2-0.4 m	2285.61^{B}	561.66^{B}	455.06^{B}	1049.81^{B}	288.18^{B}	833.67^{B}	402.50^{B}
	0.4-0.8 m	1615.88^{CB}	622.68^{B}	503.58^{B}	941.74^{B}	490.35^{B}	957.18^{B}	578.94^{B}
	0.8-1.1 m	682.23^{C}	424.92^{B}	366.84^{B}	904.25^{B}	269.80^{B}	834.77^{B}	438.89^{B}

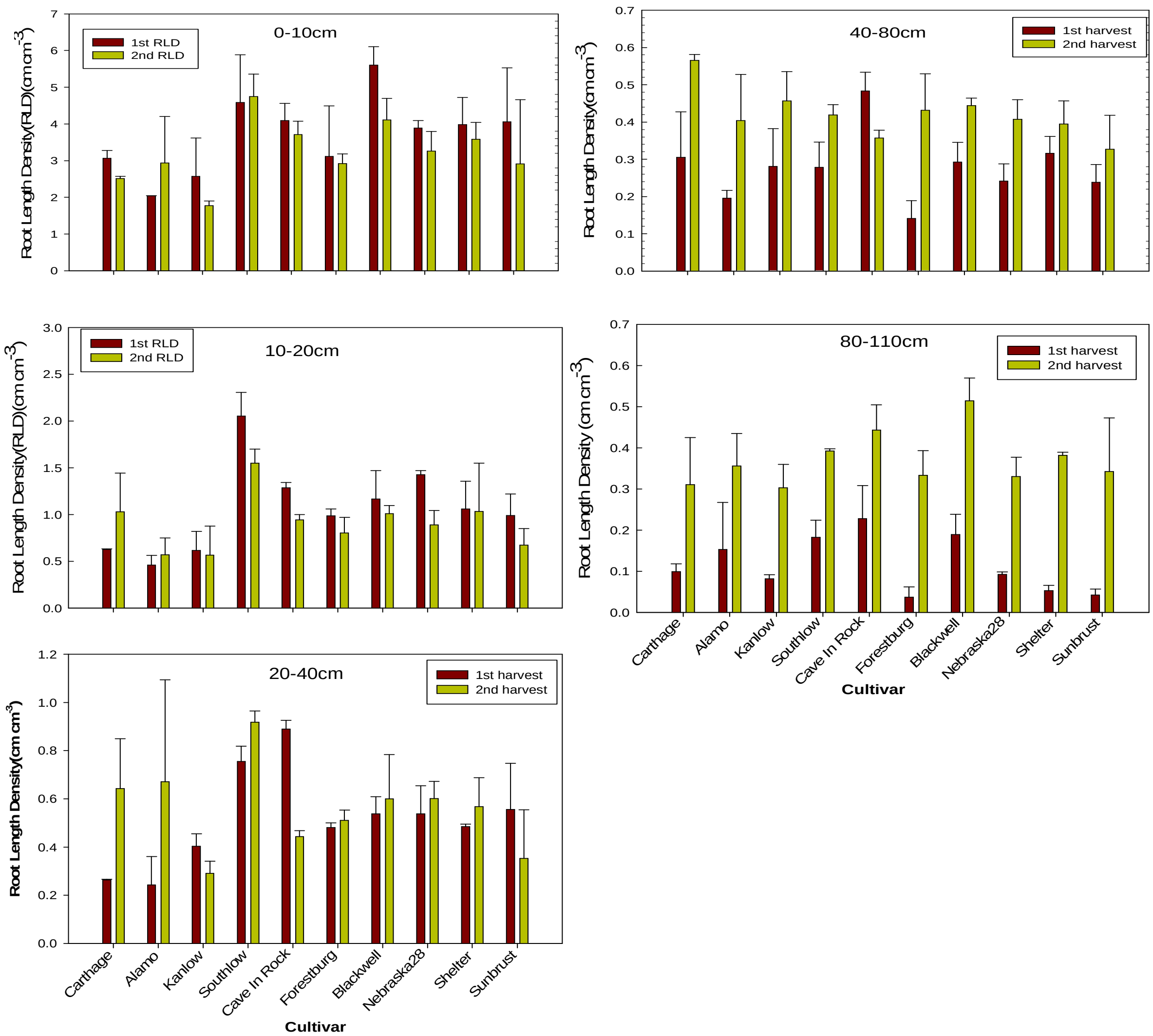


Figure 2.1. Root length density at different harvest all over soil profile. Error bar represents standard error mean (category mean).

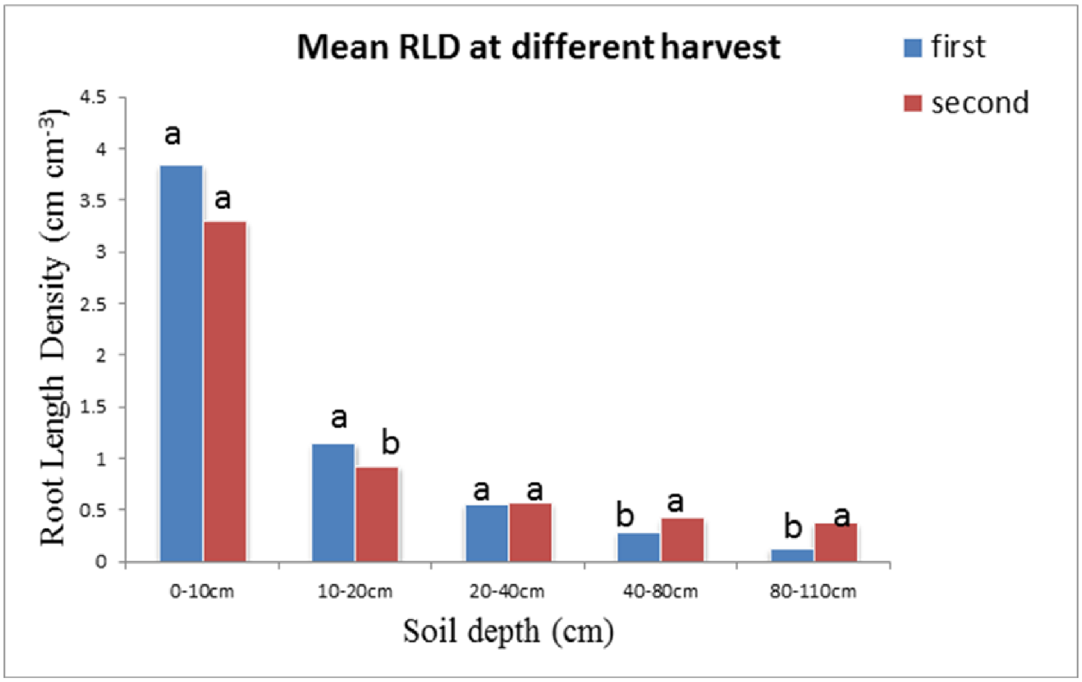


Figure.2.2. Mean root length density (RLD) at different harvest among different cultivars at *P=0.05*at different soil depth.

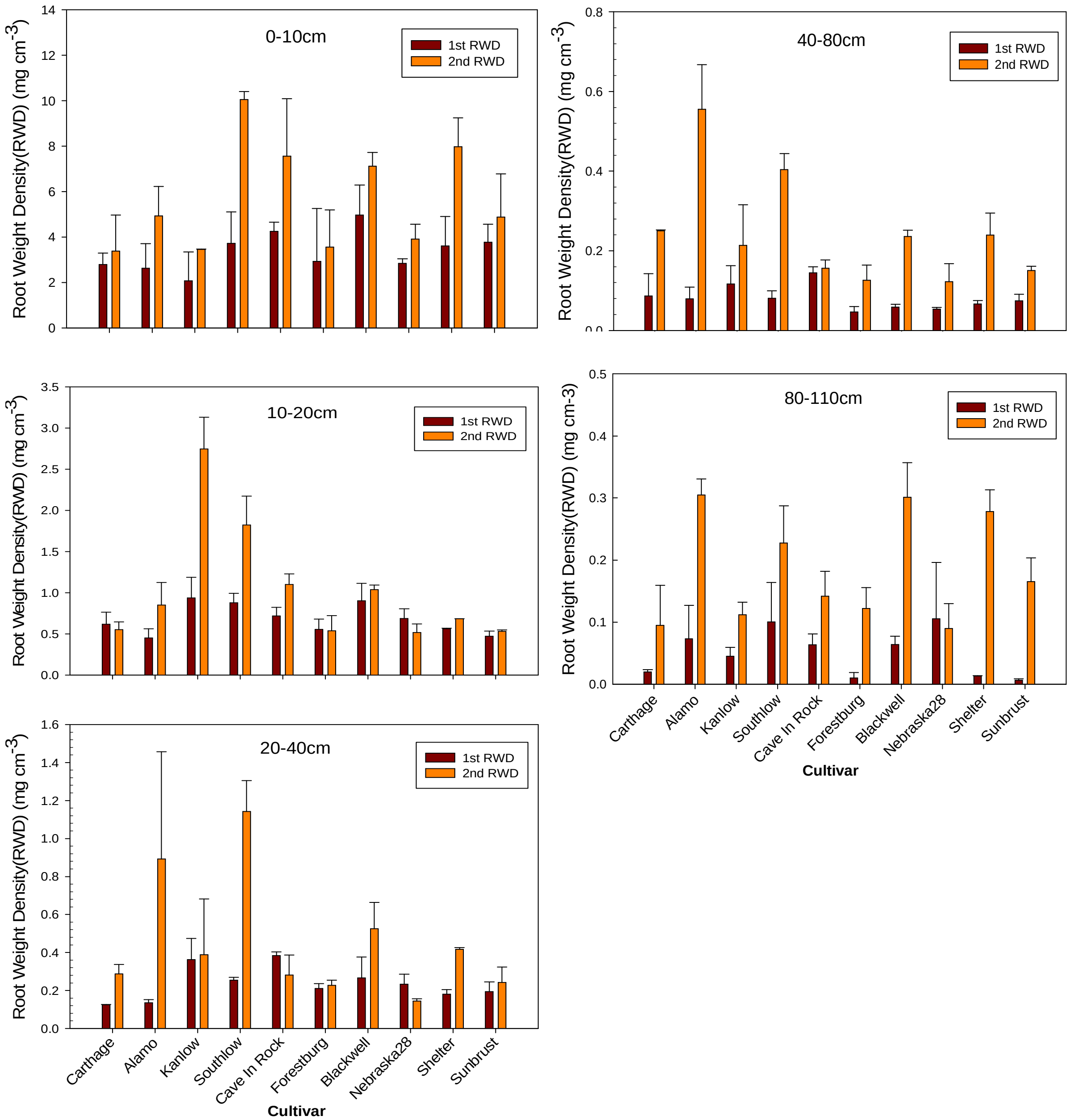


Figure 2.3. Root weight density at different harvest all over soil profile. Error bar represents standard error mean (category mean).

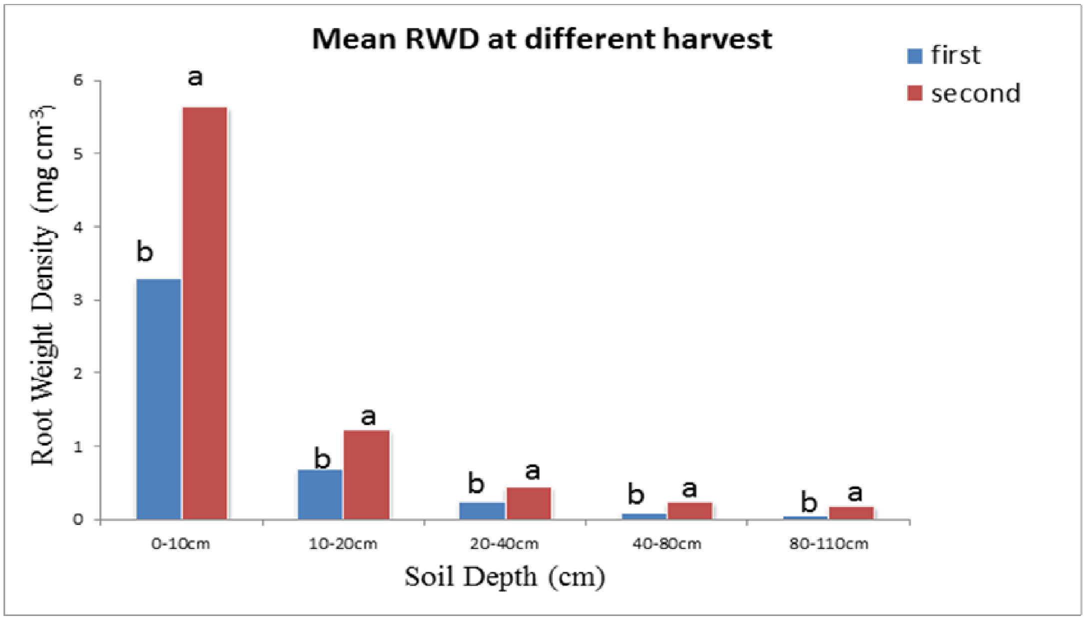


Figure.2.4. Mean root weight density (RWD) at different harvest among different cultivars at *P=0.05*at different soil depth.

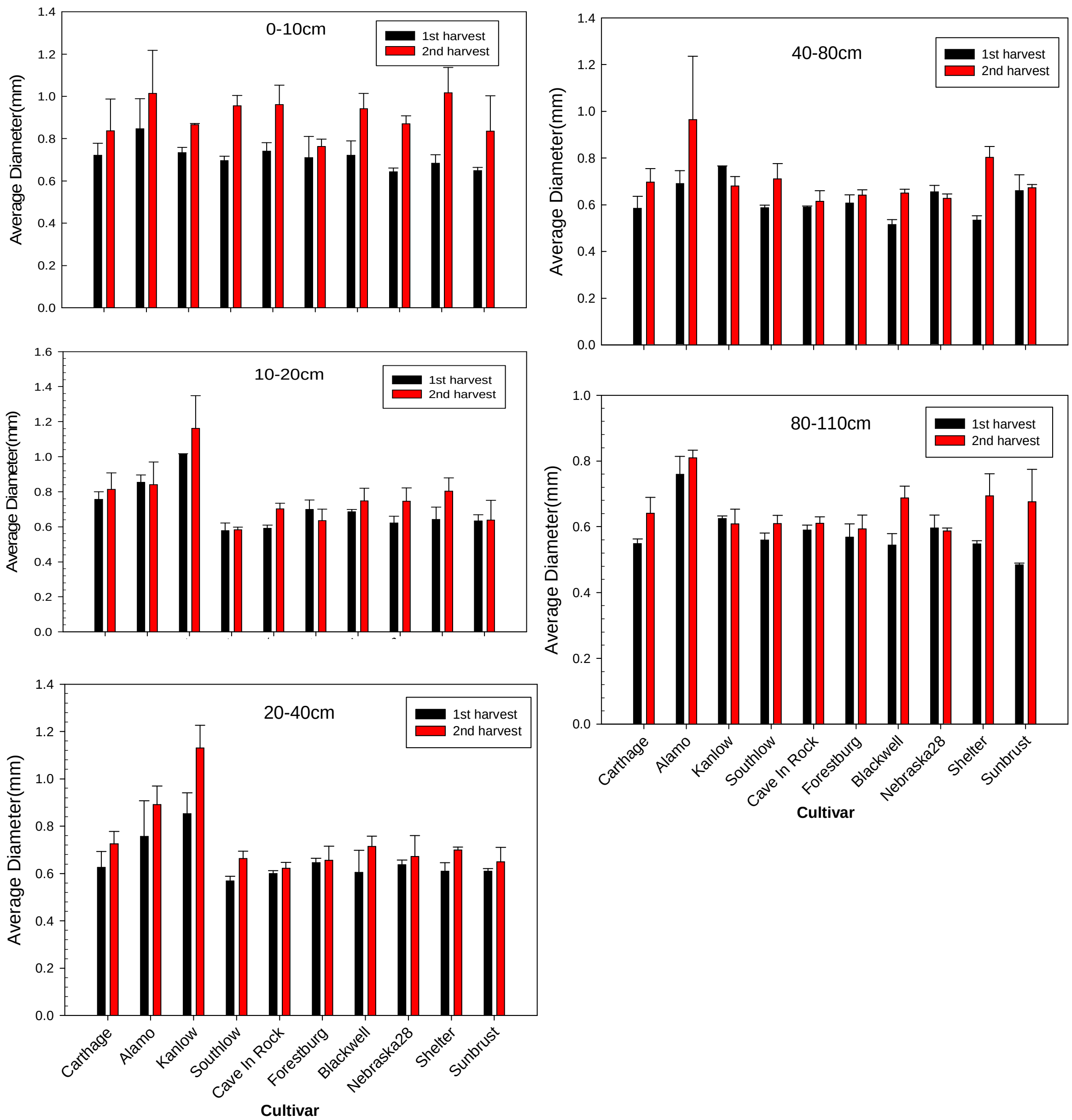


Figure 2.5. Average diameter at different harvest all over soil profile. Error bar represents standard error mean (category mean).

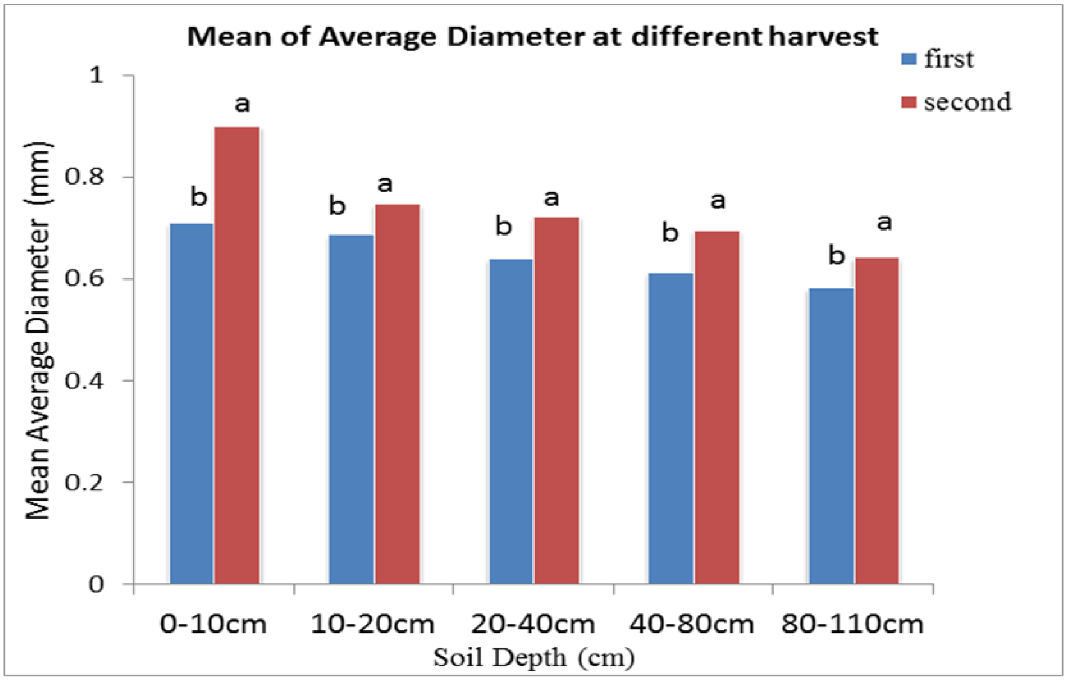


Figure.2.6. Mean average diameter at different harvest among different cultivars at *P=0.05* at different soil depth

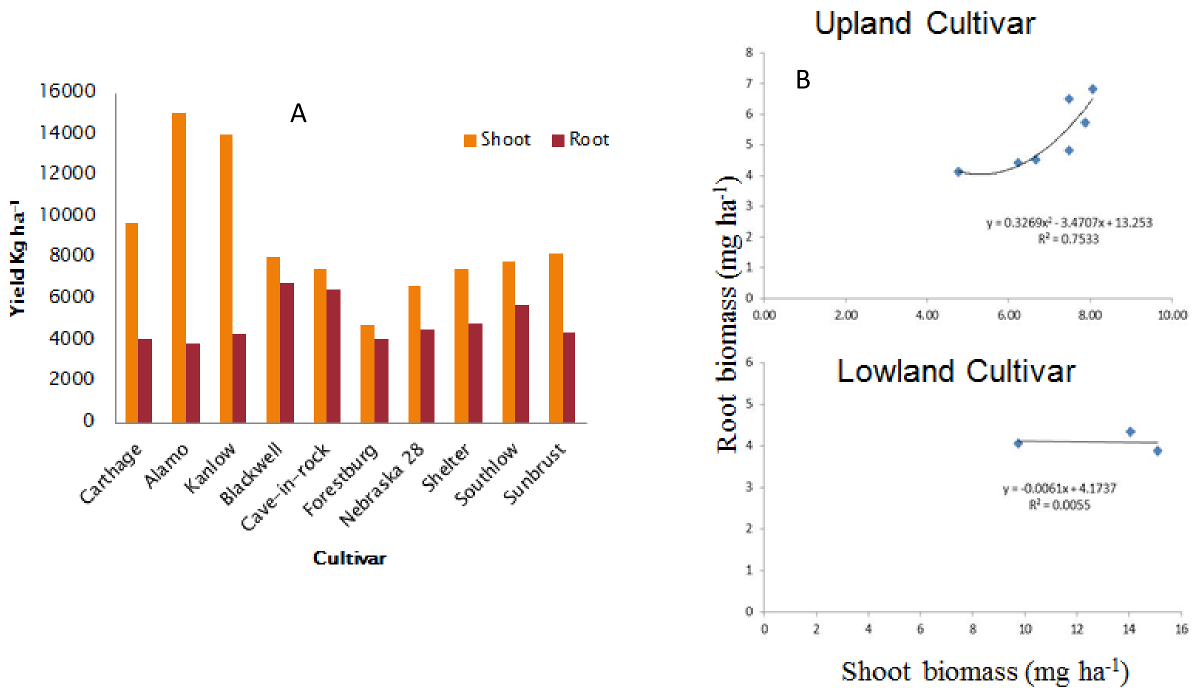
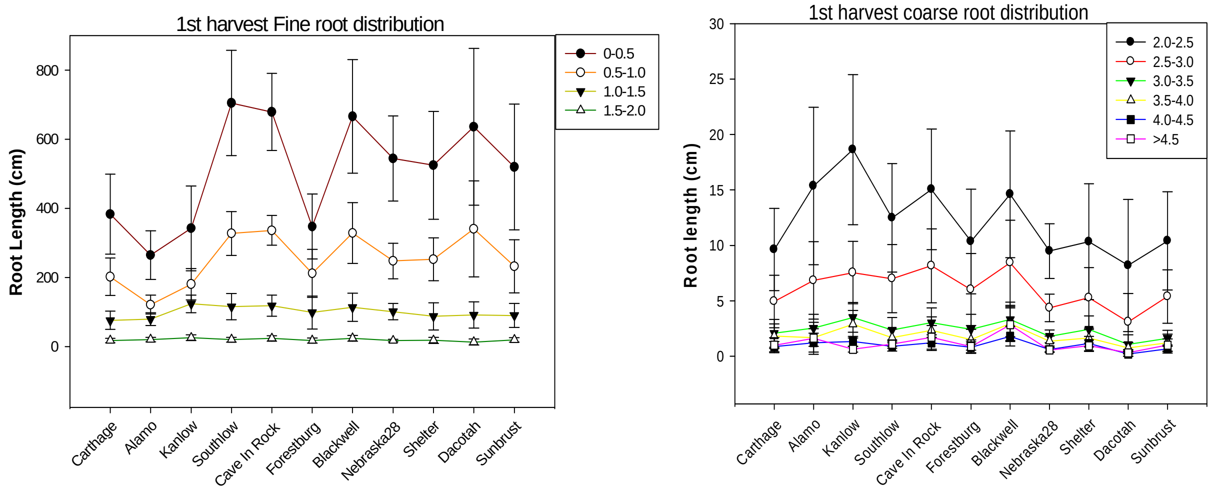


Figure: 2.7. Comparison between aboveground and belowground biomass of (A) different switchgrass cultivars and (B) relationship between shoot and root biomass of upland and lowland cultivars.

(A)



(B)

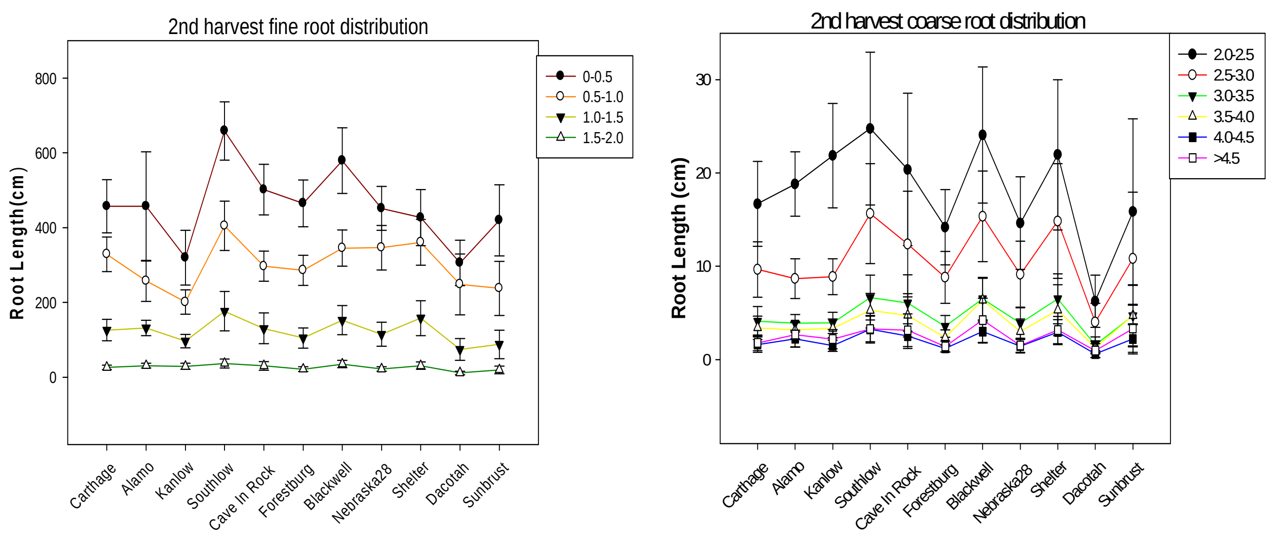


Figure: 2.8. Fine root (0.05 mm diameter) and Coarse root (2.5- >4.5mm diameter) distribution among cultivars at different harvest (A) at peak growth (August root harvest) and (B) at the end of the season (December root harvest).

VITA

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Pages in Study: 88 Candidate for the Degree of Master of Science

Major Field: Plant Science

Scope and Method of Study:

Switchgrass, a model herbaceous energy crop has high potential for C sequestration from atmosphere and also improves soil quality via its deep fibrous root system. The individual root and the entire root system, various morphological parameters, which are influenced by genetic variability and environmental conditions, have been used as potential indicators of water and nutrient uptake. Studies on switchgrass root characteristics and their distribution pattern are very limited. Root samples were taken with hydraulic powered core and measurement of root parameters were carried out with an image analysis system (winRHIZO ver. 5.0). The objectives of the study were to 1) evaluate switchgrass root characteristics as influenced by row spacing at peak growth and after senescence, 2) identify differences for different root characteristics among switchgrass cultivars at peak growth and after senescence and 3) determine the correlation between switchgrass above and below ground root biomass.

Findings and Conclusions:

Wider row spacing (76.2 cm) had higher root length density (RLD) and root weight density (RWD) by the end of the growing season (December) while narrow spacing (19.05 and 38.1 cm) had increased RWD though lower in RLD. RWD and average diameter were found higher due to the decrease in fine roots proportions by the end of the growing season. Higher RLD was observed in upland cultivars than in low land cultivars. Similarly, RWD and average diameter increased in all layers of soil profile by the end of growing season. Above ground biomass of upland cultivars was positively correlated with below ground root biomass but no such relationship was observed among lowland cultivars.

In conclusion, switchgrass demonstrated plasticity of root growth in response to row spacing. Lowland cultivars have higher above ground biomass while upland cultivars have higher root biomass. Root biomass is highly influenced by the distribution of fine roots.

ADVISER'S APPROVAL: Dr. Vijaya Gopal Kakani